Oxidation in foods and beverages and antioxidant applications

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Edited by
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Oxidation and protection of red meat

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Abstract: This chapter discusses the basis for lipid and protein oxidation in fresh and processed red meat products. These processes lead to quality degradation, and a variety of antioxidant strategies have been developed to minimize and/or prevent this quality loss. The chapter provides an overview of the field's status to date.

Key words: red meat, lipid oxidation, myoglobin oxidation, protein oxidation, antioxidants.

1.1 Introduction and compositional considerations

1.1.1 Red meat defined

Red meat includes the postmortem muscle of mammalian species. The degree of redness is proportional to the haem protein content of meat and is dependent on the specific muscles involved, the species, and age of the animal from which the meat was derived. Meat is composed of myofibres which contain the contractile apparati critical to proper functioning *in vivo*. For simplicity sake, myofibres can be classified physiologically as slow, fast and intermediate, or biochemically as oxidative, glycoclytic or oxidative/glycolytic. Slow, oxidative myofibres are characterized by a higher fat content, a slower contraction speed, oxidative metabolism, and greater myoglobin and mitochondrial concentrations. Fast, glycolytic myofibres contain less fat and more glycogen, contract more rapidly, rely on anaerobic metabolism and contain less myoglobin and mitochondria. Intermediate fibres contain elements that are intermediate between fast and slow

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myofibres. Muscles that contain a greater proportion of red myofibres than white ones will contain more myoglobin and be more red in appearance. Traditionally, the primary species that yield red meat are cattle, pigs, sheep, goats and deer. However, within these species there can be significant differences in the degree of redness of different muscles and this is most readily observed in pork. For example, within a pig carcass, the longissimus muscle contains more white fibres and is considered more white than the psoas muscle. For the purposes of this chapter we will focus on oxidation and protection of meat from the common mammalian livestock species (i.e., cattle, sheep, pigs).

The substrates in meat that influence susceptibility to oxidation are derived from the animal's diet. It is important to note that the extent to which nutrition can influence the concentration of nutrients capable of accelerating or delaying oxidation depends, in part, on the animal's nutritional physiology. Specifically, pigs are monogastric animals, while cattle, sheep and goats are ruminants. The fat of monogastric animals is more readily altered by dietary fat composition.

Considerable understanding of oxidation in meat can be obtained by consulting related studies of tissue oxidation in the medical literature. Some caution needs to be observed when doing this. The conversion of muscle to meat during the antemortem to postmortem transition is characterized by many biochemical changes (Greaser, 1986). Cellular integrity is lost with time and a number of physico-chemical changes occur that affect oxidation in meat. For example, mitochondrial morphology is lost with time postmortem (Cheah and Cheah, 1971, 1974; Tang *et al.*, 2005) and this is accompanied by changes in oxygen consumption capacity. The accessibility of oxygen to fatty acids in membranes and production of radical intermediates would both be affected by these changes.

Oxidation of meat lipids, specifically unsaturated fatty acids in triacylglycerols and phospholipids, and of cholesterol, is a critical concern. The generation of peroxides, radical species and secondary oxidation products has implications for flavour, colour and loss of myofibrillar protein functionality. Protein oxidation generally leads to decreased functionality relevant to processed meat texture and water-holding capacity. All oxidative reactions generally result in compromised sensory quality and an undesirable sensory experience for the consumer. Antioxidant mediation of oxidative events has been adopted strategically to lessen the undesirable effects of oxidation reactions in red meat. Delivery of antioxidants has been accomplished by dietary intervention and ingredient addition, and resulted in shelf-life extension. In addition, packaging technologies that alter the atmosphere in which meat resides has also been employed to minimize the undesirable consequences of oxidation.

The goal of this chapter is to provide a summary review of relevant studies published in the literature. Our emphasis will be on the more applied aspects of red meat oxidation because the fundamental aspects are covered in earlier sections of this book. The reader should recognize that results from studies of oxidation very much depend on the experimental conditions employed by specific investigators. Space limitations preclude us from recounting specific

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details of conditions for all of the work cited and we encourage the reader to carefully consult the original investigations for these critical components when considering related work.

1.2 Lipid oxidation in red meat

1.2.1 Substrates for lipid oxidation

Triacylglycerols, phospholipids and cholesterol are the three major substrates for lipid oxidation in red meat. The fatty acids esterified to meat triacylglycerols and phospholipids can be saturated or unsaturated. In general, oxidative susceptibility is directly proportional to the degree of unsaturation in the constituent fatty acids. Selected nutrient profiles including fatty acids are presented in Table 1.1. Phospholipids are the major components of cell membranes and sub-cellular organelles in meat. They contain two fatty acids and the fatty acid at the sn-2 position is commonly unsaturated. The oxidation of fatty acids in phospholipids, more so than in triacylglycerols, has been attributed as the cause of sensory quality deterioration in foods (Pearson *et al.*, 1977). As noted previously, the fatty acid profile of fresh meat from monogastric animals is more easily manipulated than that from ruminants. The production of comminuted red meat products can utilize raw meat materials differing in fatty acid unsaturation to achieve products with a specific level of unsaturated fat.

The process of lipid oxidation in red meat leads to the production of a complex mixture of primary and secondary oxidation products that reflect the degree and location of unsaturations in the fatty acid substrates (Belitz *et al.*, 2004). Aldehydes and ketones are produced in measurable quantities and are responsible for many of the odours and flavours associated with rancidity in red meat (Pearson *et al.*, 1977). The most well-known secondary product of lipid oxidation in red meat is malondialdehyde (MDA). Some products of lipid oxidation are sufficiently reactive that they bind with other macromolecules. Previous research has suggested that MDA is predominantly complexed with protein in foods (Piche *et al.*, 1988; Giron-Calle *et al.*, 2002). α,β-Unsaturated aldehydes are reactive products of lipid oxidation (Witz, 1989) and in particular, 4-hydroxy-2-nonenal (HNE) is very reactive. HNE is produced from oxidation of linoleic acid in membranes (Pryor and Porter, 1990) and has been identified in beef and pork (Munasinghe *et al.*, 2003; Sakai *et al.*, 1995, 1998, 2004, 2006).

The fundamental bases of lipid oxidation have been addressed in the literature and are not discussed in this chapter. However, a critical consideration when assessing oxidation as a function of fatty acid unsaturation is that of methodology. Methods may be qualitative or quantitative, and can focus on consumption of oxygen or the production of primary or secondary products from the fatty acid substrate. The commonly used thiobarbituric acid (TBARS) assay (Fernandez *et al.*, 1997) is more sensitive to the generation of 3-carbon secondary oxidation products, specifically MDA, than to other oxidation products. Also, the profile of oxidation products can yield different chromophores in

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Table 1.1 Selected nutrient profile of red meats (value per 100 g; USDA, 2008)

Nutrient	Beef[a]	Pork[b]	Lamb[c]	Goat[d]	Venison[e]	Veal[f]	Buffalo[g]
Proximate							
Water (g)	69.38	71.98	71.17	75.84	73.57	75.18	76.30
Energy (kcal)	173	152	160	109	120	120	99
Protein (g)	19.05	21.80	20.12	20.60	22.96	19.97	20.39
Total lipid (g)	10.19	6.48	8.20	2.31	2.42	3.89	1.37
Ash (g)	0.98	0.98	0.90	1.11	1.16	1.02	1.05
Carbohydrate, by difference (g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fibre, total dietary (g)	0.0	0.0	–	0.0	0.0	0.0	0.0
Sugars, total (g)	–	0.00	–	–	0.00	–	–
Minerals							
Iron, Fe (mg)	2.16	0.77	1.59	2.83	3.40	0.88	1.61
Copper, Cu (mg)	0.069	0.058	0.147	0.256	0.253	0.108	0.151
Vitamins							
Vitamin C, total ascorbic acid (mg)	0.0	0.3	–	0.0	0.0	0.0	0.0
Lipids							
Fatty acids, total saturated (g)	4.330	2.240	3.533	0.710	0.950	1.170	0.460
Fatty acids, total monounsaturated (g)	4.380	2.930	3.242	1.030	0.670	1.250	0.420
Fatty acids, total polyunsaturated (g)	0.380	0.700	0.320	0.170	0.470	0.400	0.270
Cholesterol (g)	59	55	66	57	85	83	46
Phytosterols (g)	0	–	–	–	–	–	–

[a]Beef, rib, shortribs, separable lean only, choice, raw, trimmed to 0″ fat; [b]Pork, fresh, loin, centre rib (chops or roasts), boneless, separable lean only, raw; [c]Lamb, Australian, imported, fresh, rib, separable lean only, trimmed to 1/8″ fat, raw; [d]Goat, raw; [e]Game meat, deer, raw; [f]Veal, rib, separable lean only, raw; [g]Game meat, buffalo, water, raw.

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the TBARS test. Sun *et al.* (2001) reported on the generation of yellow chromophores (A_{max} @ 450 nm) from the reaction of TBA with specific aldehydes known to be generated by lipid oxidation. Thus, the TBARS assay is not a good method to compare oxidation in meats with significantly different fatty acid profiles. Investigations concerned with the effects of lipid oxidation in meat products, rather than *in vitro* systems, benefit from inclusion of sensory panels to complement objective laboratory analyses of lipid oxidation. In fact, the TBARS assay cannot be used reliably to predict rancidity unless it is correlated with sensory analyses.

A third class of lipids found in red meats, in addition to triacylglycerol and phospholipid, is the steroid alcohol, cholesterol. It is found only in animal-based food products and is capable of being oxidized. In general, cholesterol oxidation products (COPs) are absent from fresh meat but have been identified in processed meats. For example, Paniangvait *et al.* (1995) reported that seven species of COPs were identified in processed meats while fresh meat contained only trace amounts or lacked them completely. The presence of COPs was reported in freeze-dried pork at concentrations of approximately 460 ppm (Park and Addis, 1987) and 177 ppm (Csallany *et al.*, 1989). In a study on the development of COPs, Engeseth and Gray (1994) reported initial low concentrations of COPs in raw and cooked beef round steak of 1.4 ppm and 3.1 ppm, respectively. Following storage at 4 ºC for 4 d, COPs levels increased, particularly in cooked beef, from 3.1 to 17.3 ppm. In raw meat, COPs increased from 1.4 to 5.9 ppm. The effects of cooking and storage on the production of COPs were also reported in buffalo meat (Rao *et al.*, 1996) and mutton (Kowale *et al.*, 1996). The process of pre-cooking meat for subsequent preparation by consumers or the food service industry (e.g., frying) prior to consumption can lead to formation of COPs (Larkeson *et al.*, 2000). Not surprisingly, inclusion of antioxidants in marinated pork (Lee *et al.*, 2008), and the exclusion of oxygen from packaging atmospheres via vacuum packaging was reported to prevent cholesterol oxidation in cooked turkey, pork, and beef patties (Du *et al.*, 2001).

Cholesterol oxidation in processed foods is influenced by the presence of unsaturated fatty acids in triacylglycerols and phospholipids (Liu *et al.*, 1994; Osada *et al.*, 2000). Cholesterol was unstable in the presence of unsaturated fats; however, it was relatively stable at 100 ºC (Osada *et al.*, 1993). Lipid radicals formed during the processing and storage of foods can accelerate the oxidation of cholesterol and produce cholesterol oxidation products (COPs) (Paniangvait *et al.*, 1995).

1.2.2 The role of oxygen

Oxygen is necessary for lipid oxidation to occur in meat. Both triplet and singlet forms of oxygen have been implicated. The diffusion of oxygen into intact cuts of red meat is relatively slow while incorporation of oxygen into comminuted meat products occurs readily during meat processing. Oxygen can be limited in,

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or eliminated from, meat-containing packaging environments in order to minimize lipid oxidation (McMillin, 2008; Brandon *et al.*, 2009).

It is important to note that a stable red colour of fresh meat is dependent on the presence of a saturating quantity of oxygen (i.e., sufficient to saturate ferrous myoglobin present). Complete elimination of oxygen from fresh meat packages minimizes lipid oxidation and growth of aerobic psychrotrophs but it also leads to a low proportion of myoglobin in the oxygenated form (i.e., oxymyoglobin, OxyMb) and a purplish colour that is not desirable. Incomplete removal of oxygen from packaging can also be problematic by favouring brownish metmyoglobin (MetMb) formation (Faustman and Cassens, 1990).

1.2.3 Pro-oxidants in red meat

Transition metals, particularly iron, can serve as strong pro-oxidants. Red meat is a rich source of dietary iron and is generally not limiting when other conditions for lipid oxidation are favourable. Both haem iron and nonhaem iron enhance lipid oxidation in meat (Ahn and Kim, 1998). Red veal semimembranosus muscle that contained 10.9 μg Fe/g meat demonstrated greater lipid oxidation than white veal with a significantly lower concentration of iron (i.e., 8.7 μg Fe/g) (Faustman *et al.*, 1992). Iron may be complexed in low molecular weight compounds, or associated with haem (e.g., myoglobin) or non-haem proteins (e.g., transferrin) (Decker and Xu, 1998). A low molecular weight fraction obtained from the cytosol of pork and beef was associated with greater lipid oxidation than a high molecular weight fraction (Yin and Faustman, 1994). In addition to endogenous sources, transition metals may enter into meat during processing via contact with ferrous metal equipment surfaces or ingredients.

The redox state of transition metals is as important to catalytic potential as is the concentration of transition metal present. The reduced state of metal ions is more catalytically active and the presence of reducing agents (e.g., ascorbic acid) can serve to maintain the catalytic potential/pool and thus exacerbate metal-catalyzed oxidation of both proteins and lipids (Decker *et al.*, 1993; Min *et al.*, 2008). Gorelick and Kanner (2001) reported that the effect of ascorbic acid on iron-induced oxidation is concentration-dependent.

Haemoglobin and myoglobin can be potent accelerators of lipid oxidation in muscle foods (Baron and Andersen, 2002; Richards and Dettmann, 2003) and their effect is concentration-dependent (Ramanathan *et al.*, 2009). Haem-containing proteins actively enhance lipid oxidation with iron in the ferric state, whereas non-haem iron appears to be more active in the ferrous state (Greene and Price, 1975). Both MetMb and OxyMb catalyzed lipid oxidation in a myoglobin-liposome model system with OxyMb more effective than MetMb at equimolar concentration (Chan *et al.*, 1997). The authors of this study suggested that hydrogen peroxide, presumably generated during OxyMb oxidation, played an important role in mediating OxyMb and lipid oxidation. Bou *et al.* (2008) recently reported that the extent to which OxyMb enhanced lipid oxidation was dependent on the extent of its heat-induced denaturation. The oxidation of

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OxyMb to MetMb was considered responsible for increased lipid oxidation, but denaturation (> 75 ºC) led to decreased catalytic potential. The authors attributed the latter observation to loss of solubility and greater ability of exposed amino acid side chains to quench free radicals.

Classically known as 'warmed-over flavour' (WOF), WOF is a lipid oxidation-based off-flavour that develops following the cooking of meat (Pearson and Gray, 1983). The heat treatment used in preparation of meat disrupts membranes, denatures iron-containing proteins releasing iron, and accelerates diffusion of reactants such that subsequent oxidation in stored meat leads to off-flavour development (Pearson and Gray, 1983; Gray and Pearson, 1987). Investigators have sought to differentiate between the sources of iron and their relative impact on lipid oxidation (Rhee and Ziprin, 1987; Han *et al.*, 1995) and WOF development in meat (Igene *et al.*, 1979). Yancey *et al.* (2006) recently described livery flavour development in cooked beef and reported a positive correlation between total iron concentration and this flavour defect.

1.2.4 Influence of antemortem feeding

The fatty acid composition of muscle and adipose tissue of meat influences nutritional value and quality parameters, including shelf life and flavour (Wood and Enser, 1997; Wood *et al.*, 2003). Specifically, it determines the firmness/oiliness of adipose tissue, and the oxidative stability of muscle, which in turn affects flavour and colour. In general, meat with greater concentrations of unsaturated fatty acids is more susceptible to lipid oxidation than meat with lesser concentrations (Hogberg *et al.*, 2002; Lund *et al.*, 2008).

Dietary manipulation of the fatty acid composition of muscle and adipose tissue in live animals is more readily achieved in monogastric than ruminant livestock species (Wood and Enser, 1997; Kouba *et al.*, 2003). Pigs fed diets high in fishmeal/fish oil (Monahan *et al.*, 1992; Apple *et al.*, 2009) will yield meat with fat that contains relatively high concentrations of long chain *n*-3 polyunsaturated fatty acids (PUFAs) typically associated with marine fish species. This can lead to pork flavour that is 'fishy'. Ruminant fats are not altered by diet as rapidly or to the same extent as that of monogastrics. Biohydrogenation of unsaturated fatty acids readily occurs in the rumen and this complicates the use of dietary manipulation for altering the fatty acid profile of beef and lamb meat (Schaefer, 2000).

Several research groups have compared the effects of concentrate and grass silage diets on fatty acid composition of red meat species (Enser *et al.*, 2000; Scollan *et al.*, 2001; Teye *et al.*, 2006; Warren *et al.*, 2008a,b). These feeds can markedly change the fatty acid composition of both neutral lipids and phospholipids (Warren *et al.*, 2008a). In general, meat from ruminants fed grain-based diets contain greater amounts of linoleic acid and lower amounts of *n*-3 fatty acids compared to animals fed grass-based diets (Wood *et al.*, 2003; Dannenberger *et al.*, 2007; Leheska *et al.*, 2008). Diets enriched in fish oil led to increased tissue concentrations of long chain PUFAs (Elmore *et al.*, 2000), while

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linseed oil (Elmore *et al.*, 2000; Wachira *et al.*, 2002) resulted in increased linoleic acid in intramuscular fat. Meat fat obtained from lambs fed palm oil-containing diets demonstrated high levels of palmitic and oleic acids (Castro *et al.*, 2005). Several studies have examined the effect of feeding linseed/flaxseed oils on 18:3 *n*-3 concentrations in pork, beef and lamb in an effort to reduce the ratio of *n*-6:*n*-3 fatty acids below 4.0 (Enser *et al.*, 2000; Nute *et al.*, 2007). A linseed oil-enriched diet fed to pigs increased the deposition of 18:3 *n*-3 in adipose and muscle tissues, particularly within muscle phospholipids (Enser *et al.*, 2000). Kouba *et al.* (2003) reported that feeding pigs a 6% crushed linseed diet increased the content of *n*-3 PUFA in plasma, muscle and adipose tissues which resulted in a greater PUFA:SFA ratio. They reported greater TBARS values for pork from pigs fed the linseed diet than in pork from control pigs. No effect of the linseed diet on vitamin E concentration in the muscle of pigs fed for 20 or 100 days was noted, but lower muscle vitamin E content in pigs fed the linseed diet for 60 days was observed. Additional reports of lowered levels of vitamin E in porcine tissues containing high PUFA levels have been published (Wang *et al.*, 1996; Leskanich *et al.*, 1997).

Despite the process of biohydrogenation that occurs in the rumen, studies have shown that dietary *n*-6 and *n*-3 PUFA can be increased in adipose and muscle tissues of cattle and sheep and that the n-6:n-3 ratio may be manipulated (Noci *et al.*, 2005; Nute *et al.*, 2007; Warren *et al.*, 2008a,b). Larick and Turner (1989, 1990) reported changes in the fatty acid profile in neutral and phospholipid fractions of beef as a function of forage grazing versus feedlot-administered concentrates. Warren *et al.* (2008a) reported that cattle preferentially incorporated 18:2*n*-6 fatty acids from a grain-based concentrate diet, and 18:3*n*-3 fatty acids from a grass silage diet. Increased 18:3*n*-3 in muscle phospholipids of lambs fed linseed oil or fish oil/marine algae was also reported (Nute *et al.*, 2007). Dietary linseed oil (*n*-3 PUFA rich) in combination with either *n*-9 monounsaturated fatty acids (MUFA; olive oil), or *n*-6 PUFA (sunflower oil), decreased the *n*-6:*n*-3 fatty acid ratio of pig muscle. However, partial replacement of *n*-9 MUFA by *n*-3 PUFA in pig diets resulted in greater lipid oxidation in pork (Rey *et al.*, 2001). Pork from the pigs fed sunflower and linseed oils had significantly higher TBARS values throughout 9 days of refrigerated storage compared to that from animals fed olive and linseed oils.

Diets (e.g., grazing) that increase PUFA may also provide more antioxidants (e.g., α-tocopherol) that will accumulate in the muscle and protect meat fatty acids from lipid and/or protein oxidation (Petron *et al.*, 2007; Santé-Lhoutellier *et al.*, 2008a,b). Warren *et al.* (2008b) observed lower plasma and muscle levels of α-tocopherol, increased lipid oxidation and lesser colour stability in loin steaks from steers fed concentrate diets than those fed grass silage. Greater lipid oxidation in meat from the concentrate-fed steers was linked to high PUFA concentrations and relatively low levels of α-tocopherol. Descalzo *et al.* (2005) reported a three-fold increase in TBARS in beef from grain-fed steers when compared with beef from pasture-fed steers; the authors concluded that natural antioxidants in pasture compensated for the high level of PUFAs and protected

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meat lipids against oxidation. Lower TBARS values were observed in meat from lambs fed concentrate diets than pasture-fed lambs during initial storage (0 day) followed by a six-fold increase in TBARS on the seventh day of refrigerated storage under gas permeable film (Sante-Lhoutellier *et al.*, 2008b). They reported significantly greater concentrations of α-tocopherol in pasture-fed lambs (6.42 mg/g) compared to concentrate-fed lambs (1.61 mg/g). Lipid oxidation increased during storage to a greater extent in cooked and raw minced meat from lambs fed concentrate-based diets than those provided with an herbage diet (Luciano *et al.*, 2009).

Many researchers have attributed the effect of concentrate feeding (relative to pasture feeding) on increased lipid oxidation to lower concentrations of tocopherols in animal tissues (Larrain *et al.*, 2008; Warren *et al.*, 2008b; Sante-Lhoutellier *et al.*, 2008b). However, not all studies support this contention. Ponnampalam *et al.* (2001) demonstrated that lipid oxidation was not affected by supplementation with PUFAs and Petron *et al.* (2007) reported that lamb colour stability and lipid oxidation were not significantly influenced by the botanical composition of the pasture on which lambs were fed. When linseed oil, fish oil or a combination of the two were fed to beef animals with supra-nutritional concentrations of vitamin E, the fish oil diet produced meat with a reduced vitamin E concentration and higher TBARS than the other diets (Vatansever *et al.*, 2000). Fish oils are rich in long chain PUFA and any accumulation of these would increase the relative oxidative susceptibility of muscle tissues. Lambs fed fish oil, marine algae and a combination of the two produced meat that was oxidatively less stable, presumably due to a lower vitamin E content of the muscle (Nute *et al.*, 2007).

While pasture diets have generally led to improved lipid stability of meat from ruminants, a positive effect of pasture diets on maintaining colour stability of red meat has been reported more often in beef (Vestergaard *et al.*, 2000; Insani *et al.*, 2008; Warren *et al.*, 2008b) than in lamb (Sante-Lhoutellier *et al.*, 2008b; Petron *et al.*, 2007). Recently, Luciano *et al.* (2009) reported lower a* (redness) values and greater MetMb levels in minced meat from lambs fed concentrate-based diets than that from animals fed an herbage diet.

1.2.5 Influence of postmortem processing

Particle size reduction and restructuring

The mechanical processes employed in meat processing such as chopping, mixing, grinding, massaging and tumbling can be detrimental to meat lipid and colour stability. Tissue disruption and reduction of meat particle size provides a greater surface-to-volume ratio for reaction with oxygen. Destruction of endogenous reducing systems by the grinding process in meat has been reported (Kropf *et al.*, 1985; Madhavi and Carpenter, 1993). Mincing also provides a significant deteriorative stress for meat by disrupting cellular compartmentalization and releasing pro-oxidants (Gray *et al.*, 1996). The tumbling process employed for whole muscle cuts can cause cellular damage that is magnified

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with increased tumbling time (Lawlis *et al.*, 1992), and was reported to promote lipid oxidation in pre-cooked roast beef in the absence of any antioxidant (Cheng and Ockerman, 2003).

Salt

Salt (NaCl) is used in processed meat products to inhibit the growth of micro-organisms, add flavour and extract salt-soluble proteins (Cheng *et al.*, 2007). Unfortunately, the addition of NaCl to meat products has been reported to enhance lipid oxidation (Rhee and Ziprin, 2001). NaCl enhances rancidity development in cured pork (Ellis *et al.*, 1968) and in raw and cooked beef, both during cooking and subsequent storage (Chen *et al.*, 1984). Several investigators have reported that the addition of NaCl to ground beef enhanced myoglobin oxidation (Trout, 1990; Torres *et al.*, 1988; Sørheim *et al.*, 2009). The basis for the well documented pro-oxidant effect of NaCl in meat products is not well defined but may involve the presence of trace metal impurities present in salt that catalyze lipid oxidation. Some investigators have suggested that NaCl can lead to iron release from proteins (Kanner *et al.*, 1991) and/or that chloride ions can combine with iron and enhance its catalytic activity (Osinchak *et al.*, 1992).

The postmortem injection of carcasses with calcium solutions has been used to improve tenderness of beef and lamb. Lawrence *et al.* (2003) reported a pro-oxidant effect of calcium chloride and calcium lactate in beef longissimus muscles. In addition, calcium chloride injection has induced muscle darkening (Wheeler *et al.*, 1993) and caused faster discolouration in beef loins than untreated controls (Kerth *et al.*, 1995).

n-*3 Fatty acid addition*

There has been considerable interest in fortifying food products with *n*-3 PUFA because of the health benefits associated with these nutrients (Connor, 2000). The highly unsaturated nature of these fatty acids predisposes them to oxidation (Moghadasian, 2008). Direct addition of fish oil to dry fermented sausages at relatively high levels (i.e., 10.7 g/kg) resulted in increased TBARS values compared to controls (Muguerza *et al.* 2004). However, no statistical difference in cholesterol oxidation products (7-ketocholesterol) was observed. Lee *et al.* (2005) reported on an effective antioxidant mixture for minimizing oxidation in ground beef fortified with an *n*-3 fatty acid emulsion (Djordjevic *et al.*, 2004). Using this approach, investigators were able to produce oxidatively stable fresh pork sausages and restructured ham products (Lee *et al.*, 2006).

Cooking and storage

The development of lipid oxidation is more rapid in cooked meat than in raw products. Heating denatures iron-containing proteins (Schricker and Miller, 1979) and disrupts membranes. It also deactivates enzyme-based antioxidant defence systems in muscle (Igene *et al.*, 1979).

Lipid oxidation occurs during both cooking and subsequent storage of meat products. Keller and Kinsella (1973) observed increased TBARS values on

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cooking up to 70 ºC; further increases were observed when cooked samples were stored for 36 days at −18 ºC. Oxidation in refrigerated cooked pork was greater with longer cooking times, slower cooking rates and higher cooking temperatures (Kingston *et al.*, 1998). Wills *et al.* (2006) reported that the type of cooking process (e.g., ohmic- vs. impingement-cooked) influenced lipid oxidation in ground beef patties. Interestingly, the heating of meat to temperatures up to 80 ºC increased lipid oxidation, while retorting meat (> 100 ºC) reduced lipid oxidation (Chen *et al.*, 1984).

Curing

Nitrates and nitrites are critical ingredients in the production of cured red meat products worldwide. Their primary purpose is to prevent botulism but they have also been demonstrated to yield the characteristic colour of cured meats and to inhibit lipid oxidation (Gray *et al.*, 1981; Honikel, 2004). The reduction of nitrate to nitrite and subsequent formation of nitric oxide (NO) from nitrite are necessary prerequisites for most meat curing reactions (Moller and Skibsted, 2002); NO can react directly with haem iron to form mono- and dinitrosylhaemochrome. This step along with the formation of nitroso-compounds that have antioxidant activity is believed to be the basis for observed antioxidant activity (Kanner *et al.*, 1984; Pegg and Shahidi, 2000).

During dry-curing of hams and fermented sausages, lipids are progressively altered through both lipolysis and oxidation (Gandemer, 2002). Peroxide values reach their highest levels 2 to 4 months after initiation of the drying process, while TBARS values and volatile components show a continuous increase during several months of dry-cured ham processing. At the end of the drying and ripening process, the general oxidation level in muscle and adipose tissues tends to decrease (Hinrischen and Pedersen, 1995; Ruiz *et al.*, 1999).

The concentrations of TBARS were significantly greater ($p < 0.05$) in dry fermented sausages with added nitrite as compared to those with added nitrate during the entire ripening process from day 14, indicating that lipid oxidation was retarded by the presence of nitrate (Marco *et al.*, 2006). In contrast, Navarro *et al.* (2001) reported that the peroxide index and TBARS levels were greater in dry sausages with added nitrate than in samples with nitrite.

Irradiation

The process of irradiation utilizes high energy to inactivate microorganisms. Unfortunately, lipid oxidation can be enhanced via free radical reactions induced by the irradiation process (Ahn *et al.*, 2000; Jo and Ahn, 2000; Du *et al.*, 2002). Several studies reported that free radicals can also react with myoglobin or haemoglobin resulting in discolouration of irradiated meat samples (Jo *et al.*, 1999; Kamarei *et al.*, 1979). The extent of impact is dependent on irradiation dose, presence of oxygen, fat content and fatty acid profile, and presence of antioxidants (Hampson *et al.*, 1996; Ahn *et al.*, 1998; Jo *et al.*, 1999; Jo and Ahn, 2000). For example, pork patties from L. dorsi muscle (> 6.5% fat) had greater TBARS and contained more propanal and pentanal after irradiation than

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did patties from psoas (1.8% fat) and rectus femoris (2.4% fat) muscles (Ahn *et al.*, 1998). However, Houben *et al.* (2000) found no difference in TBARS of irradiated lean (< 1% fat) versus fat (> 20% fat) minced beef.

Volatile compounds may be produced as a result of irradiation of meat and these are responsible for characteristic off-odours that may occur (Brewer, 2009). An increase in lipid peroxidation products (especially hexanal and trans-4,5-epoxy-(E)-2-decenal) in combination with a loss of desirable meaty odorants (4-hydroxy-2,5-dimethyl-3(2H)-furanone and 3-hydroxy-4,5-dimethyl-2(5H)-furanone) resulted in development of warmed over flavour of cooked, refrigerated beef (Kerler and Grosch, 1996).

High pressure processing

The exposure of meat products to high pressures has been employed to inactivate microorganisms and ensure food safety (Rastogi *et al.*, 2007). A primary means by which it exerts its effect is through the destruction of biomembranes. This physical disruption also leads to greater susceptibility of meat lipids to oxidation (Cheah and Ledward, 1995) and release of iron from molecular complexes (Cheah and Ledward, 1997). Cheah and Ledward (1996) reported that lipid oxidation was catalyzed in pork at pressures of approximately 300 MPa and greater at room temperature. Application of the pressure in both oxygen- and nitrogen- containing environments was effective in enhancing lipid oxidation in ground pork as lipid oxidation was greater than in controls when meat was subsequently stored aerobically.

1.3 Meat protein oxidation

The proteins of postmortem muscle are generally divided into three categories. Myofibrillar proteins provide the cytoskeletal structure of myofibres and comprise the contractile apparatus that characterizes muscle tissue. They are salt-soluble and play an important role in determining the tenderness of fresh meat and texture of processed meats. The most abundant myofibrillar proteins in meat are myosin, actin, tropomyosin, troponin, titin, and α-actinin. Sarcoplasmic proteins are those located in the myofibre cytoplasm. They are considered water-soluble and are largely involved in energy metabolism. Myoglobin is a haem-containing sarcoplasmic protein that binds and stores oxygen and imparts red colouration to livestock meats. Collagen is the most abundant protein in mammals and comprises most of the connective tissue protein in these animals.

Oxidation is generally associated with undesirable consequences in food, but beneficial effects can also occur. Protein oxidation may be associated with off-flavour development (Zakrys *et al.*, 2008), discolouration (Estevez and Cava, 2004), undesirable textural changes (Estevez *et al.*, 2005), loss of essential amino acids (Berlett and Stadtman, 1997), decreased water-holding capacity (Huff-Lonergan and Lonergan, 2005), decreased emulsification capacity (Parkington *et al.*, 2000) and decreased tenderness (Rowe *et al.*, 2004b; Zakrys

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et al., 2008). Conversely, protein oxidation may lead to enhanced susceptibility to digestive proteolysis (Xiong, 2000), increased cross-linking reactions that improve the formation of protein gels (Liu and Xiong, 1996; Parkington *et al.*, 2000) and decreased susceptibility to proteolytic activity (Morzel *et al.*, 2006). An excellent review of the effects of protein oxidation on muscle food quality has been published by Xiong (2000).

Iron and copper catalyze protein oxidation and exposure of myofibrillar proteins to these metal ions generates increased carbonyl concentrations, a marker consistent with protein oxidation (Decker *et al.*, 1993). Haem iron may also participate in protein oxidation reactions. A ferric-ferryl redox cycle can occur as a result of exposure of Mb to peroxide and this has led to the formation of Mb radicals that can cross-link with other proteins (Reeder *et al.*, 2002).

Reactive oxygen species are generated through several different mechanisms, including lipid oxidation, and these readily react with proteins producing protein radicals. These can then react with adjacent molecules, often other proteins, to propagate the radical chain reaction or to form cross-linked species. Sulfur-containing amino acids (i.e., cysteine, methionine) are especially susceptible but many amino acids are affected (Table 1.2).

Carbonyls are a common product and are measured to track the progression of protein oxidation. Significant increases in carbonyl content were reported in lamb meat during refrigerated storage for 7 days (Sante-Lhoutellier *et al.*, 2008a). Martinaud *et al.* (1997) reported that during aging of meat for 10 days, carbonyl content of myofibrillar proteins increased, while concentrations of sulfhydryl groups decreased. Myosin appeared to be the most susceptible protein. Buttkus (1967) demonstrated that MDA was capable of reacting with myosin and binding to constituent histidine, arginine, tyrosine and methionine residues.

Protein oxidation precipitated by free radical reactions can lead to scission of the protein or aggregation through any of several mechanisms. Interestingly and

Table 1.2 Oxidation of amino acid side chains (adapted from Poon *et al.*, 2004)

Amino acid	Oxidation product(s)
Arginine	glutamic semialdehyde
Cysteine	Cys-S-S-Cys and Cys-S-S-R disulfides
Glutamic acid	4-hydroxyglutamic acid; pyruvate α-ketoglutaric acid
Histidine	aspartic acid; asparagine; oxo-histidine
Leucine	3- and 4-hydroxyleucine
Lysine	2-aminoadipic semialdehyde
Methionine	methionine sulfoxide; methionine sulfone
Phenylalanine	2-,3-,4-hydroxyphenylalanine
Proline	glutamic semialdehyde; pyroglutamic acid; 2-pyroglutamic acid; 2-pyrrolidone; 4-hydroxyproline
Threonine	2-amino-3-ketobutyric acid
Tryptophan	N-formylkynurenine; kynurenine; 2-,4-,5- and 6-hydroxyvaline
Tyrosine	3,4-dihydroxyphenylalanine; di-tyrosine crosslinks
Valine	3-hydroxyvaline

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as noted previously, the outcome of this can be undesirable (e.g., loss of protein solubility and functionality) or desirable (improved gel network formation). The factors affecting the balance between two outcomes are complex (Xiong, 2000).

1.3.1 Factors that affect protein oxidation in red meat

In postmortem muscle, proteins are more vulnerable to oxidation than proteins in antemortem muscle. This is due to tissue degradation that results in greater susceptibility of membrane lipids to attack, release of iron and depletion of endogenous antioxidants (Xiong, 2000). A variety of factors affect protein oxidation in meat. For example, Srinivasan *et al.* (1996) reported that more acidic pH values (i.e., pH 5.5. vs. 7.0) in beef heart surimi favored protein oxidation and carbonyl formation. They attributed this to the fact that haem-catalyzed oxidation was favoured by greater hydrogen ion concentration at lower pH values. Protein oxidation susceptibility in pork is affected by the concentration of the oxidizing species (Park *et al.*, 2007) and pig genetics (Ramírez and Cava, 2007). The process of freeze-drying has been associated with oxidation-mediated cross-linking of myoglobin (Nakhost and Karel, 1984).

In general, higher cooking temperatures favour protein oxidation. A study of cooking effects on meat protein oxidation reported that cooked meat had significantly greater concentrations of protein carbonyl groups than raw meat (Santé-Lhoutellier *et al.*, 2008a). Astruc *et al.* (2007) recently reported that protein oxidation that resulted from cooking was localized to cell peripheries and suggested that membrane proteins were primarily involved. Heating also favours the gelation process induced by protein oxidation (Parkington *et al.*, 2000).

Irradiation is an effective process for ensuring food safety; however, the radiolysis of protein can accelerate protein oxidation. Researchers reported that irradiated beef steaks have greater carbonyl concentrations and lower protein solubilities (Rowe *et al.*, 2004a). Oxidation of sarcoplasmic proteins in irradiated beef occurred to a lesser extent when beef was obtained from vitamin E-supplemented cattle. Rowe *et al.* (2004b) demonstrated that oxidation induced by irradiation treatment partially inactivated μ-calpain leading to decreased myofibrillar protein breakdown (30-kDa degradation product of troponin T) and beef tenderness.

Oxygen is a critical component of protein oxidation reactions and an important consideration in packaging of red meats. High oxygen concentrations accelerated protein oxidation in beef steaks (Zakrys *et al.*, 2008) and ground beef (Lund *et al.*, 2007a). Pork stored in modified atmosphere packaging demonstrated greater loss of protein thiols and myosin heavy chain disulfide crosslinks with higher concentrations of oxygen (Lund *et al.*, 2007b). However, carbonyl content was no different between treatments. Myofibril fragmentation, an indication of proteolytic activity, occurred to a lesser extent in the high oxygen atmosphere. The relative effects of the oxidizing conditions presented by elevated oxygen on proteolytic enzymes versus the protein substrate could not be determined from the study design.

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Antioxidant addition to muscle foods through dietary or ingredient approaches has been used to improve the quality of red meat. The addition of exogenous antioxidants to liver patés enhanced the oxidative stability of proteins (Estévez *et al.*, 2006). Inclusion of antioxidants has also improved the functionality of myofibrillar proteins relative to the texture of surimi prepared from bovine hearts. Cardiac muscle contains very high concentrations of cytochromes and myoglobin and is thus iron-rich which provides a significant oxidative challenge. Xiong, Decker and co-workers published several papers that demonstrated significant improvement in the functionality of myofibrillar proteins from cardiac muscle when antioxidants were included in the washing and isolation processes (Wan *et al.*, 1993; Xiong *et al.*, 1993; Srinivasan *et al.*, 1996). Subsequent work by Parkington *et al.* (2000) suggested that antioxidants in the washing process could be adjusted to allow some limited oxidation to improve gel-forming ability of beef heart surimi.

Antioxidants may also be 'delivered' to muscle food products via dietary supplementation of meat-producing livestock. Haak *et al.* (2006) reported no significant effect of dietary vitamin E in combination with linseed or soybean oils on protein oxidation in pork. However, several reports have demonstrated lowered levels of protein oxidation in beef from cattle supplemented with dietary vitamin E. Vitamin E supplementation was hypothesized to contribute to calcium-activated protease (i.e., calpains) mediated tenderness by protecting the latter from oxidation in calcium-treated beef (Harris *et al.*, 2001). Calpains (both m- and μ-) are cysteine proteases and require reducing conditions to protect the critical active site cysteine residue. Rowe *et al.* (2004b) reported that non-irradiated longissimus beef steaks from cattle supplemented with vitamin E contained more markers of postmortem proteolysis than dietary controls and suggested that this resulted from antioxidant protection of the endogenous calpain proteases.

1.3.2 Oxidation in haem proteins

Myoglobin is the major haem protein in meat. It contains 153 amino acids and haem which is comprised of an iron molecule centrally chelated within a protoporphyrin ring. The iron molecule may be present in a ferrous (+2), ferric (+3) or ferryl (+4) state. Four of its six coordination sites keep it bound within the protoporphyrin ring while the fifth site anchors it to a histidine residue of the myoglobin apoprotein and the sixth residue binds any one of several possible ligands. Ferrous myoglobin is the 'active' physiological redox form of the protein *in vivo*. It also is present in the cut surfaces of meat where exposure to the atmosphere allows the deoxygenated ferrous Mb (DeoxyMb) to bind oxygen and form OxyMb. OxyMb provides a desirable cherry-red appearance to meat. While myoglobin is subject to the same oxidation reactions as described previously for all proteins, the discussion of its oxidation in this chapter refers to the conversion of ferrous (+2) to ferric (+3) haem iron and results in the haem protein's inability to bind oxygen at the normal pH of meat (*ca.* 5.6). Ferric myoglobin contains

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water at the sixth ligand position and is referred to as MetMb; it imparts an undesirable brownish red colour to meat. Ferryl (+4) myoglobin can be produced under significant oxidation pressures; it is very reactive, generally short-lived and is more of a concern from its potential to catalyze or enhance lipid oxidation than to negatively impact meat colour (Baron and Andersen, 2002). Redox cycling between ferric and ferryl states can decompose preformed lipid peroxides and accelerate lipid oxidation (Moore *et al.* 1998).

The oxidation of ferrous myoglobins to ferric myoglobin is thermodynamically favourable (Livingston and Brown, 1981) and results in fresh meat discolouration. However, the rate and extent of haem iron oxidation in myoglobin are affected by a number of intrinsic and extrinsic factors; several extensive reviews of these factors have been previously published (Faustman and Cassens, 1990; Renerre, 1990). Interestingly, discolouration rates are muscle-specific (O'Keeffe and Hood, 1982; McKenna *et al.*, 2005; Jeong *et al.*, 2009).

The ultimate pH (pH_U) of postmortem, post-rigor muscle is generally accepted to be approximately 5.6. However, muscle-specific differences do exist and have been reported to range between 5.54 and 6.2 (Tarrant and Sherington, 1980), and 5.69 and 5.93 (McKenna *et al.*, 2005). Ferrous myoglobin oxidation is much faster at lower pH than higher pH values (Gotoh and Shikama, 1974; Ledward *et al.*, 1986). Alteration of pH_U can result from antemortem stress (Juncher *et al.*, 2001), or postmortem, post-rigor processing (Stetzer *et al.*, 2007) and this has implications for OxyMb oxidation and meat colour stability.

As with all chemical reactions, ferrous myoglobin oxidation is accelerated with eleveated temperature. While a rule of thumb for chemical reactions generally states a Q_{10} of approximately 2, it has been reported that the Q_{10} for OxyMb oxidation is 5 (Brown and Mebine, 1969). Lower storage/display temperatures appear to favor redox stability of ferrous myoglobin as indicated by maintenance of red colour in beef (Jeremiah and Gibson, 2001; Pietrasik *et al.*, 2006) and pork (Hansen *et al.*, 2004).

The predominant redox form of myoglobin in meat (or solution) is highly dependent on partial oxygen pressure (pO_2). This phenomenon was first described by Neill and Hastings (1925) and shows that low non-zero pO_2 values (approximately 4 mmHg) favour the formation of MetMb over either of the ferrous forms. This has led to packaging strategies that completely exclude oxygen (e.g., vacuum-packaging) or provide saturating conditions (e.g., high oxygen MAP) in an attempt to minimize MetMb formation (Faustman and Cassens, 1990). Oxygen consuming reactions in meat can reduce tissue pO_2 and lead to redox instability of ferrous myoglobins. These include mitochondrial and sub-mitochondrial activity, aerobic bacterial growth and lipid oxidation. Chan *et al.* (1998) demonstrated that spoilage levels of aerobic bacteria consumed sufficient oxygen to facilitate OxyMb oxidation *in vitro*. Monahan *et al.* (2005) recently suggested that oxygen consumption resulting from lipid oxidation could contribute to low-pO_2 induced OxyMb oxidation.

Cheah and Cheah (1971, 1974) were among the first investigators to report that mitochondria and sub-mitochondrial particles have the ability to consume

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oxygen in postmortem muscle. The rate/extent of oxygen consumption declines with breakdown of mitochondrial morphology and time postmortem (Tang *et al.*, 2005). Cornforth and Egbert (1985) utilized the mitochondrial inhibitor, rotenone, to demonstrate the significant effect that mitochondrial activity has on pre-rigor meat colour. O'Keeffe and Hood (1982) provided convincing evidence that oxygen consumption in meat was a primary determinant of myoglobin redox instability and meat discolouration.

1.3.3 Effect of lipid oxidation on myoglobin oxidation

The process of lipid oxidation and its consequences for protein function in general (Haurowitz *et al.*, 1941), and for haem protein redox stability in particular (Koizumac *et al.*, 1973; Szebeni *et al.*, 1984; Yin and Faustman, 1993; Chan *et al.*, 1997; O'Grady *et al.*, 2001) has been previously investigated in model systems. These studies have demonstrated that oxidation of haem proteins from a ferrous to ferric form is correlated with lipid oxidation and that manipulation of experimental conditions intended to selectively affect one of these processes leads to a similar response in the other. For example, inclusion of the lipid soluble antioxidant, α-tocopherol, within a myoglobin : liposome system slowed both lipid oxidation and OxyMb oxidation (Yin *et al.*, 1993). Microsomal preparations from skeletal muscle have also been used to study oxidation related phenomena in muscle-based foods (Chan *et al.*, 1996). The advantage of using microsomes is that they are membrane fractions from the tissue of interest and thus their fatty acid profile may be more relevant to the *in vivo* condition. OxyMb oxidation was greater in the presence of microsomes isolated from tuna than from pork and beef (Yin and Faustman, 1994). Lipid oxidation followed the same trend which was attributed to the greater degree of lipid unsaturation present in tuna compared to bovine and porcine muscle.

Lipid oxidation and discolouration were noted to be linked in meat, and Greene (1969) was one of the first investigators to document this. Several subsequent reports have added support to this observation (Greene *et al.*, 1971; Lin and Hultin, 1977; Faustman *et al.*, 1989; Balentine *et al.*, 2006).

The mechanistic basis for enhanced haem protein oxidation by oxidizing lipids is not known but primary and secondary products of lipid oxidation could be responsible. Chan *et al.* (1997) studied the effects of oxidizing lipids on OxyMb oxidation. Incubation of OxyMb in dialysis sacs (mw cutoff 500 Da) placed in solutions of fresh and oxidized liposomes revealed that TBARS were measurable within the dialysis sacs during incubation. The concentration of TBARS and MetMb formation increased with the extent of oxidation of the lipid solution in which the sacs were incubated. Inclusion of α-tocopherol within liposomes delayed MetMb formation and the appearance of TBARS in the dialysis sacs.

Secondary products of lipid oxidation (e.g., aldehydes, ketones; Esterbauer *et al.*, 1991) are less reactive than primary products but readily react with specific functional groups on proteins and alter the normal functionality/redox stability

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Table 1.3 Summary of studies that have reported on the effects of secondary lipid oxidation products on specific proteins

Protein	Lipid oxidation products	Result	Reference
Bovine serum albumin	2-Octenal	Formation of octenal : histidine adducts	Alaiz and Giron (1994)
Glucose-6-phosphatase	α,β unsaturated oxo-compounds	Inhibition of enzyme activity	Jørgensen *et al.* (1992)
Glucose-6-phosphate dehydrogenase	HNE	70% less in enzyme activity	Szweda *et al.* (1993)
Glutathione peroxidase	Methylglyoxal	Enzyme inhibition	Park *et al.* (2003)
Haemoglobin	MDA	Formation of Hb : MDA adducts	Kautiainen *et al.* (1989)
$NADP^{+}$-dependent dehydrogenase	HNE, MDA, lipid hydroperoxides	Potential dysregulation of NADPH levels	Yang *et al.* (2004)
Oxymyoglobin	α,β unsaturated aldehydes; HNE	Enhanced OxyMb oxidation in the presence of aldehydes	Chan *et al.* (1997), Faustman *et al.* (1999a), Suman *et al.* (2006)
Thioredoxin and thioredoxin reductase	HNE	Enzyme inhibition	Fang and Holmgren (2006)

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of these proteins (Grimsrud *et al.*, 2008) (Table 1.3). Unsaturated aldehydes (i.e., α,β-unsaturated aldehydes) appear to be more reactive than their saturated counterparts and effectively decrease the redox stability of OxyMb (Faustman *et al.*, 1999a). To date, 4-hydroxynonenal (HNE), an α,β-unsaturated aldehyde that is a product of linoleic acid oxidation, has been demonstrated to consistently decrease bovine and porcine myoglobn redox stability *in vitro* through covalent binding to histidine residues in the protein (Alderton *et al.*, 2003; Suman *et al.*, 2007). It appears that the effect of HNE may be species-specific depending on the number and location of nucleophilic histidine residues (Suman *et al.*, 2006). The covalent attachment of reactive aldehydes to myoglobin may alter the haem protein's conformation sufficiently to alter the haem environment and predisposing to greater redox instability (Livingston and Brown, 1981).

1.3.4 Role of myoglobin oxidation in lipid oxidation

As noted previously, haem proteins can serve as pro-oxidants. Interestingly, the process of OxyMb oxidation to MetMb may enhance lipid oxidation via the generation of superoxide anion with subsequent dismutation to hydrogen peroxide and/or the production of ferryl myoglobin, a potent accelerator of lipid oxidation (Chan *et al.*, 1997; Baron and Andersen, 2002). Baron *et al.* (1997) studied the initiation of lipid oxidation in linoleic acid emulsions by several different myoglobin species and reported that the presence of lipid peroxides was more crucial than the redox state of haem proteins in the initiation process. Investigators have reported that the combination of hydrogen peroxide and MetMb forms an 'activated' MetMb complex which is extremely pro-oxidative towards lipid (Harel and Kanner, 1985).

Alteration of myoglobin can enhance its ability to catalyze lipid oxidation. Kristensen and Andersen (1997) used heated linoleic acid emulsions containing myoglobin to study thermal-induced denaturation of myoglobin concurrent with lipid oxidation. They demonstrated that immediately below the T_D, myoglobin structural changes were induced that paralleled pro-oxidative activity toward linoleic acid. Lynch and Faustman (2000) reported that MetMb previously reacted with oxidation products of unsaturated fatty acids, specifically α,β-unsaturated aldehydes, was more pro-oxidant towards lipids than control MetMb. Considerable interest has been recently expressed in the potential for haem release from haemoglobin and myoglobin to subsequently enhance lipid oxidation. It appears that the met- form of haem proteins releases haem approximately 60$\times$ more readily than ferrous forms (Hargrove and Olsen, 1996). Grunwald and Richards (2006a,b) utilized specific Mb mutants to demonstrate the relationship between MetMb formation and haem release, and extended the observations to lipid oxidation. A Mb mutant with high haem affinity (V68T) was a poor promoter of lipid oxidation while a second mutant with low haem affinity (H97A) readily promoted lipid oxidation; WT Mb which has a haem release rate intermediate between these mutants was also intermediate in its pro-oxidant effect. The majority of work published to date has

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focused on haem proteins and lipids from fish and so the relevance to red meats remains undetermined.

1.4 Antioxidant mediation of red meat oxidation

Lipid and/or protein oxidation may be minimized by increasing the antioxidant concentration of red meat, utilizing modified atmosphere packaging or by production of antioxidant compounds (e.g., Maillard reaction products) during processing.

1.4.1 Endogenous antioxidants

There are a variety of endogenous antioxidant enzymes and compounds found in muscle and that serve to protect against oxidation *in vivo* (Decker and Mei, 1996). For example, enzymes include glutathione peroxidase, catalase, and superoxide dismutase (Decker and Xu, 1998). Antioxidant compounds include glutathione (Tang *et al.*, 2003), carnosine (Chan and Decker, 1994) and α-tocopherol (Schaefer *et al.*, 1995).

The two groups of dietary antioxidants that have received the most research attention to date are the carotenoids and vitamin E. Representative compounds in each of these are provided naturally in forages or can be supplemented in feed concentrates. The carotenoids (e.g., β-carotene) are fat-soluble compounds that can accumulate in fat and produce coloured fat in the carcasses of red meat species (Prache and Theriez, 1999; Dunne *et al.*, 2009). In the United States, yellow fat is considered undesirable relative to white fat and is discriminated against. Additionally, carotenoids have not been demonstrated to provide consistently significant protection against lipid oxidation in red meat.

The dietary supplementation of vitamin E (i.e., α-tocopheryl acetate) to meat-producing animals has been consistently shown to increase α-tocopherol concentrations in muscle and fat subsequently obtained from these animals (Faustman, 2004). This has led to decreased lipid oxidation in beef (Faustman *et al.*, 1989), veal (Igene *et al.*, 1976), pork (Buckley and Connolly, 1980, Guo *et al.*, 2006), and lamb (Wulf *et al.*, 1995; Strohecker *et al.*, 1997). Faustman *et al.* (1999b) identified products of α-tocopherol in meat consistent with the peroxy-radical scavenging activity of this fat-soluble antioxidant.

Interestingly, α-tocopherol appears capable of increasing the redox stability of myoglobin in selected red meats (Faustman *et al.*, 1989). OxyMb stability is improved by α-tocopherol *in vitro* (Yin *et al.*, 1993) and in beef (Arnold *et al.*, 1992) in a concentration-dependent manner. The redox stabilizing effects of elevated tissue concentrations of α-tocopherol on bovine (Schaefer *et al.*, 1995; Buckley *et al.*, 1995; Faustman *et al.*, 1998) and ovine myoglobins (Wulf *et al.*, 1995; Guidera *et al.*, 1995; Strohecker *et al.*, 1997; Lauzurica *et al.* 2005) are well documented in the literature. However, unlike beef, a colour-stabilizing effect of α-tocopherol has not generally been observed in

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pork (Asghar *et al.*, 1991; Hoving-Bolink *et al.*, 1998; Cannon *et al.*, 1996; Phillips *et al.*, 2001a).

One hypothesis for the basis of the protective effect of α-tocopherol towards OxyMb states that α-tocopherol delays oxidation of lipid and subsequent release of secondary oxidation products which are pro-oxidative towards OxyMb (Faustman *et al.*, 1998). This would represent an indirect effect of α-tocopherol in maintaining acceptable beef colour and is consistent with the known function of α-tocopherol as a lipid-soluble antioxidant. The fatty acid profile of pork is more unsaturated than that of beef or lamb, and thus it would be expected to generate secondary products of lipid oxidation that could affect the redox stability of myoglobin. Recent reports suggest that species-specific differences in myoglobin primary structure (Suman *et al.*, 2007) and sarcoplasm composition (Ramanathan *et al.*, 2009) could explain the lack of an effect in pork.

Arnold *et al.* (1993) established an effective concentration of 3.3 μg α-tocopherol/g beef muscle for minimizing lipid and myoglobin oxidation (Fig. 1.1).

1.4.2 Exogenous antioxidants and processing

Muscle-based foods contain a variety of antioxidant enzymes and metabolites that have the potential to mediate the oxidation process (Decker and Mei, 1996). Most attempts to reduce lipid and pigment oxidation in meats have focused on exogenous (i.e., ingredient) addition of pure synthetic and/or natural antioxidant molecules/mixtures, animal-derived proteins (Elias *et al.*, 2008; Wang *et al.*, 2008) and antioxidant-containing plant materials. The active antioxidant components may function as free radical scavengers, reducing agents and/or chelators of catalytic metal ions.

Free radical quenchers

Free radical scavengers delay or inhibit lipid oxidation by reacting with free radicals generated during the initiation or propagation steps. Synthetic phenolic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ). It is understood that the inclusion of BHA and BHT in ground beef patties led to significantly reduced lipid oxidation when compared to controls. BHA and BHT treatments had at least a two-fold lower lipid oxidation value when compared to untreated controls or other natural antioxidants (i.e., Fenugreek extracts) (Hettiarachchy *et al.*, 1996). In contrast, the addition of BHA and BHT (100 ppm each) to a coating batter subsequently applied to ground pork patties that were deep fried did not result in antioxidant protection when compared to control patties during 35 days of storage at 4 ºC (Biswas *et al.*, 2004).

Combining antioxidant strategies can be especially effective. Many investigators have reported complementary effects (additive or synergistic) of antioxidants with different functional attributes (McBride *et al.*, 2007). Ansorena and Astiasaran (2004) demonstrated that combining BHA and BHT with

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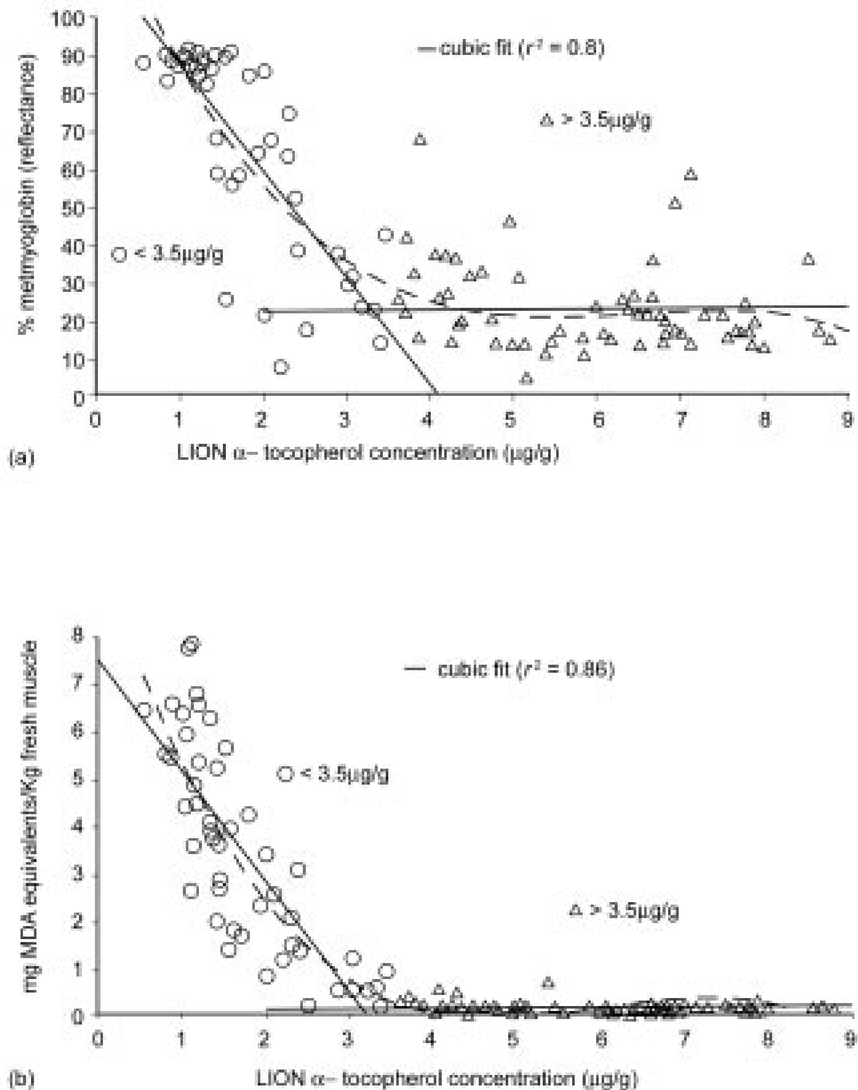

Fig. 1.1 Relationships between longissimus α-tocopherol content and surface metmyoglobin accumulation (a) or lipid oxidation (b) on day 12 of display (from Arnold *et al.*, 1993 with permission).

vacuum packaging of dry fermented pork sausages minimized lipid oxidation during storage at 4 ºC for 5 months. Irradiated ground beef produced with BHA plus BHT with either ascorbate, erythorbate and trisodium phosphate individually or in combination exhibited significantly lower TBARS and greater redness values than control samples during nine days of simulated retail display (Duong *et al.*, 2008). The role of natural (cloves, cinnamon, ascorbic acid and

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maillard reaction products (MRPs)) and synthetic (BHA, TBHQ and PG) antioxidants in controlling WOF and non-haem iron release in cooked and refrigerated stored meats from lamb, beef and pork was tested by Jayathilakan *et al.* (2007a). They observed susceptibility to lipid oxidation in the order pork > beef > sheep, and the order of antioxidant activity for the natural antioxidants was MRPs > cloves > ascorbic acid > cinnamon; for synthetics it was TBHQ > BHA > PG.

α-Tocopherol is a natural radical-scavenging phenolic antioxidant that may be delivered through dietary approaches or ingredient addition. Ingredient addition provides the potential for a more cost-effective approach for incorporating α-tocopherol into meat products. The use of α-tocopherol as a processing ingredient to delay lipid oxidation and stabilize meat colour has demonstrated inconsistent results. Aksu and Kaya (2005) used BHA and α-tocopherol each at 50 and 100 mg/kg diced beef in the preparation of cooked and sliced kavurma. Improved colour stability and decreased TBARS values in the products occurred in the following order, 100 mg BHA > 100 mg α-tocopherol > 50 mg BHA = 50 mg α-tocopherol > control, during storage under vacuum at 4 ºC for 300 days. Crackel *et al.* (1988) reported that mixed tocopherols combined with ascorbyl palmitate and citric acid were as effective as TBHQ in retarding lipid oxidation in restructured beef steaks over 12 months frozen storage. Miles *et al.* (1986) found that α-tocopherol effectively controlled oxidation of restructured pork during refrigerated storage at 4 ºC for 16 days.

Contrary to these studies, tocopherols at concentrations up to 1000 mg/kg meat had little effect on lipid oxidation in processed pork products stored at −18 ºC for 37 weeks (Channon and Trout, 2002). A similar lack of antioxidant effect of α-tocopherol was reported by Vara-Ubol and Bowers (2001) in which inclusion of the antioxidant (0.03% wt/wt) to ground, cooked pork failed to inhibit the formation of hexanal during six days storage at 4 ºC. The same authors reported that sodium tripolyphosphate (STP; 0.2–0.3%) was more effective than α-tocopherol; a combination of α-tocopherol with 0.2% STP did yield enhanced antioxidant activity relative to controls (no antioxidant) and α-tocopherol or STP alone.

Directing the added antioxidant to or near the origin of oxidation sites is critical to efficacy and likely explains the basis for inconsistent results when tocopherols are added as ingredients (Wills *et al.*, 2007). This was demonstrated convincingly by Mitsumoto *et al.* (1993) when they compared the antioxidant activity of α-tocopherol delivered through dietary means and when added exogenously. These investigators achieved relatively high levels of α-tocopherol in the longissimus muscle of cattle through dietary supplementation and then compared the progression of lipid oxidation in ground beef of control cattle in which exogenous α-tocopherol was added in an oil carrier to achieve an equivalent antioxidant concentration. The results clearly demonstrated that the dietary approach was superior in antioxidant effectiveness (Fig. 1.2). The authors attributed this to the proper positioning of α-tocopherol within the muscle tissues biomembranes by the living animal, a goal that could not be

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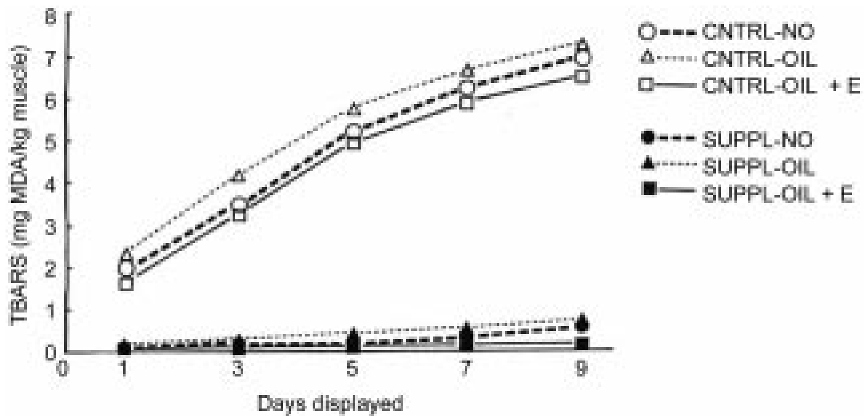

Fig. 1.2 Relationship of dietary vitamin E supplementation × postmortem vitamin E treatment × day on 2-thiobarbituric acid reactive substances (TBARS) values. MDA = malonaldehyde. CNTRL-NO = no addition to unsupplemented beef; CNTRL-OIL = treatment with oil of unsupplemented beef; CNTRL-OIL + E = treatment with oil and vitamin E of unsupplemented beef; SUPPL-NO = no addition to vitamin E-supplemented beef; SUPPL-OIL = treatment with oil of vitamin E-supplemented beef; SUPPL-OIL + E = treatment with oil and vitamin E of vitamin E-supplemented beef (from Mitsumoto *et al.*, 1993 with permission).

assured by the exogenous addition of α-tocopherol as an ingredient. Raghavan and Hultin (2004) used a non-red meat (i.e., fish muscle) model to demonstrate the partitioning of added δ-tocopherol between neutral and polar lipid fractions as a function of carrier used. Ethanol was more effective than canola oil in obtaining a greater relative concentration of antioxidant in the polar lipid fraction. Wills *et al.* (2007) subsequently hypothesized that vitamin E applied to muscle foods in an oil carrier would associate with the neutral lipid fraction (triacylglycerols) instead of the biomembrane's polar lipid fraction and be less effective. They demonstrated that delivery of δ-tocopherol using a polar carrier (i.e., ethanol) significantly reduced the TBARS values in cooked ground beef.

Reducing agents

The addition of reducing agents (e.g., ascorbic acid, erythorbic acid and their salts), improves colour stability and product storage life. Ascorbic acid at low levels (up to 250 ppm) catalyses the development of lipid oxidation, whereas at higher levels (500 ppm) is considered to inhibit the reaction, possibly by altering the relative concentration of ferrous and ferric iron, or by acting as oxygen scavenger (Sato and Hegarty, 1971; Igene *et al.*, 1985). The ability of ascorbic acid to delay lipid oxidation is attributed to its ability to scavenge oxygen, to regenerate the activity of primary antioxidants and to inactivate pro-oxidants (Bauernfeind and Pinkert, 1970; Bruno and Traber, 2006). The ingredient addition of ascorbic acid or ascorbate has led to reduced lipid oxidation (Shivas *et al.*, 1984; Mitsumoto *et al.*, 1991; Realini *et al.*, 2004) and improved colour stability (Greene *et al.*, 1971; Mitsumoto *et al.*, 1991; Realini

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et al., 2004) in beef. Hood (1975) demonstrated that injection of sodium ascorbate (500 mL 50% w/v, pH 7.2) into cattle immediately prior to slaughter led to improved colour stability of beef subsequently obtained from the treated animals. The reducing activity of ascorbic acid appears to improve muscle colour stability via metmyoglobin reduction (Lee *et al.*, 1999). Unlike vitamin E, the dietary supplementation of vitamin C has not demonstrated any antioxidant advantage in red meat subsequently obtained from treated animals (Gebert *et al.*, 2006).

Phillips *et al.* (2001b) reported that the addition of erythorbic acid to ground beef patties at 0.04% and 0.06% (w/w) concentrations resulted in prolonged red colour in patties cooked to internal end point temperatures of 60, 66, 71 or 77 ºC from the raw state; an undesirable consequence leading to premature browning (Killinger *et al.*, 2000). The addition of sodium erythorbate, erythorbic acid, sodium ascorbate, ascorbic acid and ascorbyl palmitate to ground beef at a concentration of 2.3 mM to fresh ground beef significantly decreased lipid oxidation and increased the total reducing activity relative to controls during refrigerated and frozen storage (Sepe *et al.*, 2005). Mancini *et al.* (2004) found that treating beef lumbar vertebrae with 1.5% or 2.5% ascorbic acid minimized lumbar vertebrae discolouration, with the 2.5% ascorbic acid treatment most effective through five days of display. Grobbel *et al.* (2006) reported that 1.25% ascorbic acid was as effective as 2.5% ascorbic acid in preventing bone marrow discolouration in beef lumbar vertebrae. More recently, Mancini *et al.* (2007) reported that combining citric acid and ascorbic acid had no synergistic effect on retention of desirable colour of beef lumbar vertebrae.

Ascorbic acid and its salts and isomers have been combined with other functional antioxidants. An antioxidant combination containing radical quencher (0.2% w/w, rosemary extract), sequestrant (0.5% w/w, sodium citrate) and reductant (1000 ppm sodium erythorbate) incorporated into *n*-3 PUFA fortified fresh pork sausages significantly reduced TBARS and lipid hydroperoxides, and stabilized meat colour during refrigerated (4 ºC) and frozen (−18 ºC) storage (Lee *et al.*, 2006). The addition of vitamin C with lactic acid and clove oil as a dipping marinade for buffalo meat steaks did not result in any additional inhibition of lipid oxidation compared to lactic acid and clove oil alone (Naveena *et al.*, 2006). However, vitamin C inclusion helped stabilize buffalo meat colour during storage. A mixture of rosemary, ascorbic acid, sodium lactate and red beet root extract significantly reduced lipid and pigment oxidation of fresh pork sausages during storage at 2 ºC for 20 days (Martinez *et al.*, 2006).

Metal chelators

Phosphate is commonly added to injected whole muscle processed red meats for its ability to improve water-holding capacity and cooked product yields. It also can act as a chelator to greatly decrease the catalytic activity of metal ions and potentially minimize lipid oxidation (Love and Pearson, 1971; Trout and Dale, 1990). Reports of no effect of phosphates on red meat oxidation have also been published (Akamittath *et al.*, 1990; Stika *et al.*, 2007).

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The addition of 2% EDTA was hypothesized to chelate non-haem iron released on cooking which led to significant reductions in meat lipid oxidation (Igene *et al.*, 1979). Experiments with ground pork demonstrated that the sequestering agents catechol, EDTA, diethylenetriamine pentaacetic acid, sodium pyrophosphate, and to a lesser extent sodium tripolyphosphate, lowered fat oxidation and improved sensory quality of stored cooked meat products (Shahidi *et al.*, 1986).

Darmadji and Izumimoto (1994) observed that the addition of chitosan (1%) led to a 70% decrease in TBARS values of meat after three days at 4 ºC. The mechanism of inhibition was suggested to be related to chelation of free iron released from meat haemoproteins during heat processing or storage. The rate of lipid oxidation in fresh pork sausages was significantly decreased by addition of 0.5 to 1.0% chitosan, while samples containing both chitosan and nitrites (150 ppm) showed the lowest MDA values during 28 days storage at 4 ºC (Soultos *et al.*, 2008).

Natural source antioxidants

The use of natural ingredients, especially of plant origin, in red meat products has increased in recent years. Current research suggests that most fruits, vegetables, nuts, cereals and spices contain bioactive phytochemicals that contain natural antioxidants with the potential for minimizing oxidation in fresh and processed red meat products (Shahidi *et al.*, 1992). The polyphenols are a class of phytonutrients with noted health benefits. Significant polyphenols include tannins, flavonoids and anthocyanins (Balasundram *et al.*, 2006). It is important to note that it is difficult to compare the efficacy of different preparations of antioxidant-containing plant materials. Each plant (and its extracts) can contain a large variety of bioactive compounds with antioxidant activity. In order to compare the relative effectiveness of different plant materials, the relevant constituent antioxidants must be standardized in some manner. Differences in cultivars, geography and climate, soil conditions and many other factors make such comparisons very challenging.

Dietary fibres with significant antioxidant activity from citrus fruits (Fernandez-Lopez *et al.*, 2004), wheat bran (Vitaglione *et al.*, 2008), oat bran (Persson *et al.*, 2004), and rice bran (Choi *et al.*, 2009) have been incorporated in the formulations of meat products including ground meat and sausages. Buffalo meat loaves, breakfast sausages, fermented sausages, restructured beef steaks and beef patties have been prepared with natural antioxidants from plant-derived ingredients such as carrots (Devatkal *et al.*, 2004), onion and leek (Fista *et al.*, 2004), tomato peel and seeds (Calvo *et al.*, 2008) and cabbage (Chu *et al.*, 2002).

Antioxidant activity has been reported for walnut components (Serrano *et al.*, 2006), grape seed and bearberry extract in raw and cooked pork (O'Grady *et al.*, 2008); plum concentrates and powder in precooked roast beef (Nunez de Gonzalez *et al.*, 2008); cherry and apple fruits in ground beef patties (Britt *et al.*, 1998), and green tea extract in fresh pork sausages (Valencia *et al.*, 2008). In a comprehensive report, McCarthy *et al.* (2001) reported the antioxidant activities

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of aloe vera, fenugreek, mustard, tea catechins and ginseng in cooked pork patties. Tea catechins are potent natural antioxidants and have exhibited greater antioxidant efficacy compared to vitamin C in cooked or raw beef (Mitsumoto *et al.* 2005). Han and Rhee (2005) investigated antioxidant properties of white peony, red peony, sappanwood, Mountain peony, rehmania, and angelica in ground, raw and cooked chevon and beef during refrigerated storage. Significant reductions in lipid oxidation and discolouration were recorded in ground chevon and beef patties, respectively. In a recent study, Hernandez-Hernandez *et al.* (2009) evaluated the antioxidant effects of rosemary and oregano extracts on TBARS and colour of model raw pork batters. They observed greater antioxidant activity for rosemary extracts compared to oregano extracts and attributed the higher antioxidant effect of rosemary to the presence of high concentrations of carnosic acid and carnosol, and unidentified active compounds. They further reported that oregano extracts containing high concentrations of phenols, mainly rosmarinic acid, efficiently prevented colour deterioration.

Lemon and orange extracts were reported to exert antioxidant effects in beef meat balls (Fernandez-Lopez *et al.*, 2004), and pine bark extracts were found to be suitable antioxidants in food systems (Vuorela *et al.*, 2005). The antioxidant effect of rosemary, marjoram, caraway, sage, basil, thyme, ginger and clove were reported in fresh pork sausages, raw and cooked minced meat patties and raw buffalo meat steaks (Abd El-Alim *et al.*, 1999; Naveena *et al.*, 2006; Georgantelis *et al.*, 2007). Antioxidant activity of 10 spices (allspice, black pepper, cardamom, cinnamon, clove, coriander, cumin, ginger, nutmeg and rose petals) commonly used in the formulation of fermented meat sausages were evaluated for their antioxidative properties. Clove followed by rose petals and allspice were found to exhibit the greatest antioxidant index when used in a dry form (Al-Jalay *et al.*, 1987).

There have been many reports of the antioxidant effectiveness of rosemary (*Rosmarinus officinalis L.*) extracts in red meat products. The antioxidant activity of rosemary extracts has been associated with the presence of carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which act primarily as radical scavengers (Basaga *et al.*, 1997; Zheng and Wang, 2001). Effective concentration ranges of 500–1000 mg rosemary/kg in beef steaks (Stoick *et al.*, 1991), 200–1000 mg/kg in various foods (Shahidi *et al.*, 1992), 1000 mg/kg in precooked-frozen sausage (Sebranek *et al.*, 2005) have been reported.

Rosemary powder alone (1000 ppm) and rosemary with ascorbic acid (500 ppm) incorporated within ground beef patties showed decreased metmyoglobin formation and lipid oxidation relative to ascorbic acid (500 ppm), taurine (50 mM), carnosine (50 mM), or their combinations (Sanchez-Escalante *et al.*, 2001). Djenane *et al.* (2002) showed that when used in combination with vitamin C (500 ppm), rosemary (1000 ppm) was more effective in delaying myoglobin oxidation and lipid oxidation when sprayed on beef steak surfaces than combinations of taurine (50 mM) and vitamin E

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(100 ppm). Balentine *et al.* (2006) evaluated the timing of application of rosemary extract and concluded that optimal redness values, oxymyoglobin content and low TBARS values were achieved when rosemary was added as a pre-grinding treatment to beef.

Maillard reaction products resulting from the reaction between reducing sugars and amines have been reported to exert antioxidant activity (Manzocco *et al.*, 2001; Benjakul *et al.*, 2005). These can be formed in vitro and subsequently applied to meats (Jayathilakan *et al.*, 1997) and are effective antioxidants (Jayathilakan *et al.*, 2007a,b).

Packaging

Packaging strategies, specifically use of modified atmospheres, have been used to minimize lipid oxidation and meat discolouration (Jakobsen and Bertelsen, 2000; McMillin, 2008), often in combination with various ingredient technologies (Lund *et al.*, 2007a; Mohamed *et al.*, 2008). Packaging impregnated with antioxidant ingredients has proved efficacious (Tovar *et al.* 2005; Nerin *et al.*, 2006; Camo *et al.*, 2008) Vacuum packaging effectively removes oxygen from the meat environment and thus minimizes the opportunity for lipid oxidation to occur. The production of purge can be undesirable in some packaged meat products and the exclusion of oxygen also leads to purplish red deoxymyoglobin predominating on cut meat surfaces. Consumers have noted that this colour differs from the normal red (i.e., OxyMb) and questioned its relevance to quality. Modified atmosphere packages containing relatively high concentrations of oxygen (e.g., 80%) help saturate myoglobin and prolong colour shelf-life. Unfortunately, the abundant oxygen also favors aerobic microbial growth and lipid oxidation, especially in ground meat products (McMillin, 2008).

The recent use of carbon monoxide (CO) to provide a stable red colour to packaged fresh meats has received considerable research attention. Carbon monoxide binds to haem iron in myoglobin very tightly and provides a colour almost identical to that of OxyMb. COMb in modified atmospheres was reported to be without effect (Wilkinson *et al.*, 2006) or to improve oxidative stability of treated meat (Laury and Sebranek, 2007).

1.5 Future trends

Red meat is a nutrient dense food that is susceptible to lipid and protein oxidation. Significant deterioration in meat quality can occur as a result of these processes and a variety of strategies have been developed to minimize their impact. As nutritional considerations continue to evolve in foods, especially those that emphasize functionality with the potential to improve human health (e.g. alteration of fatty acids profile to one that is more unsaturated), antioxidant strategies will need to keep pace. There is a need to better identify the bioactive molecules in plant materials and quantify their effective concentrations so that accurate comparisons of relative efficacy can be achieved. Processing and

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packaging innovations will continue to be necessary for maximizing oxidative stability as distances between food production and consumption continue to increase.

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2

Oxidation and protection of poultry and eggs

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Abstract: This chapter presents an overview of the subject of lipid and protein oxidation of poultry and eggs. It also examines the effect of oxidation on sensory quality including flavor, color and texture attributes, on the nutritional quality and on the shelf-life of raw and cooked poultry and poultry products. The most important strategies to protect poultry meat and eggs against oxidation, such as meat choice, addition of primary and secondary antioxidants, use of dietary antioxidants, addition of nitrites and nitrates, smoking and packaging, are also considered.

Key words: lipid oxidation, protein oxidation, protection of poultry and eggs, sensory quality of poultry products, nutritional quality of poultry products.

2.1 Introduction

The consumption of poultry meat has increased in both developed and developing countries in recent years. In the US, the per capita poultry meat consumption has increased from 40.8 pounds in 1980 to 56.3 pounds in 1990, and to just over 100 pounds in 2002 (National Chicken Council, 2002). Overall, per capita consumption of red meat and poultry has not changed significantly, but when beef, pork and poultry are examined separately, beef appears to be losing market share to poultry (USDA, 2000). Chicken and turkey were by far the most widely consumed poultry species amounting to approximately 80.5 and 17.7 pounds, respectively (National Turkey Federation, 2002). Consumer

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concerns focused mainly on taste as the decisive criterion, but indifferent consumers were strongly price-oriented (Resurreccion, 2003). Differentiation by taste, healthiness, cholesterol, calorie content, synthetic ingredients, convenience, animal welfare, and process characteristics such as organic production have been also reported by other authors (Grunert *et al*., 2004).

Owing to this increased consumption, world production of poultry increased from just 15 000 tonnes in 1970 to 74 000 tonnes in 2002 (FAO, 2003). With 63 000 tonnes produced in 2002, chickens remained the predominant species, among other turkey, ducks and geese that presented production figures of 5200, 3000 and 2000 tonnes, respectively. While in 2002 the amount of turkey, ducks and geese represented the same proportion of the total poultry production as in 1970, turkey proportion appeared to decrease slightly and palmiped production slightly increased, possibly because turkey production occurs mainly in Europe and North America while production of palmipeds is generally more common in Asia and South America. It is predicted that poultry will become the overall meat of choice by 2020 (Bilgili, 2002).

The continued growth and competitive nature of the poultry industry has been attributed to a variety of factors, some of which relate to the improvements in intensive production and processing, while others include the more recent development of a wide range of value-added, further processed products that meet both direct consumer demand and the rapid expansion of fast food outlets. Poultry products are universally popular, because they are not often subject to cultural or religious constraints and the meat itself is perceived as wholesome, healthy and nutritious, being relatively low in fat and with a more desirable unsaturated fatty-acid content than other meats. Most importantly, high quality poultry products are available to many people at affordable prices, although production costs vary widely around the world. In the USA, less than 10% of all chicken are sold as whole carcasses (Thornton and O'Keefe, 2002), while the majority of carcasses are cut up, deboned or further processed and the meat is either portioned, sliced, ground, flavored, marinated, coated or cooked. Sales of turkey parts including whole breasts, breast cutlets and tenderloins, and legs, as well as ground turkey have also increased in recent years as consumers look for fast and healthy food choices. Turkey is reportedly consumed at least biweekly by about one half of USA consumers, with lunchmeat, ground turkey and sandwiches being some of the most popular turkey items (National Turkey Federation, 2002).

As the consumption of poultry meat rises worldwide, the industry will remain responsive to the demands of consumers, both in the range and nature of the products that are developed, whereas quality will continue to be the watchword. Consumers define quality according to their own perceptions, goals and personal preferences, but, in practice, what the consumers actually want to know in relation to product quality include the scientifically measurable characteristics of color, flavor and texture. Skin color appears to be critical for the marketing of fresh whole birds or cut portions, while meat flavor and texture can only be appreciated when the product is consumed. One of the main factors limiting the

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quality and acceptability of meat and meat products is lipid oxidation, a process that leads to deterioration of many quality characteristics such as color, flavor, texture, nutritive value, and safety of foods. Poultry meat is particularly susceptible to lipid oxidation due to its high content in polyunsaturated fatty acids. It is generally agreed that lipid oxidation in muscle foods begins immediately after slaughter in the highly unsaturated phospholipids fraction in subcellular membranes. In most cases, fresh meat has acceptable stability against oxidative processes, but processing operations such as freezing and subsequent thawing, mechanical deboning, mincing, restructuring, addition of salt, and refrigeration may lead to increased oxidation due to destruction of cellular structure and functions. Exposure to light or irradiation will further accelerate oxidation, the degree of which, however, depends on the inherent antioxidative capacity of the meat. Cooked or pressure-treated meat and meat products are even more susceptible to oxidation, since endogenous antioxidant enzymes may denaturate and lose their activity, while iron-containing proteins may become a source of catalytic iron. In such meat products, dietary antioxidants, post slaughter antioxidant additives, or food packaging techniques must be utilized to control oxidation, thereby enhancing meat quality parameters such as color, flavor and texture.

This chapter presents an overview of the subject of lipid and protein oxidation of poultry and eggs. It also examines the effect of oxidation on sensory quality including flavor, color and texture attributes, on the nutritional quality and on the shelf-life of raw and cooked poultry and poultry products. The most important strategies to protect poultry meat and eggs against oxidation, such as meat choice, addition of primary and secondary antioxidants, use of dietary antioxidants, addition of nitrites and nitrates, smoking and packaging, are also considered.

2.2 Oxidation of poultry meat and eggs

Oxidation is a leading cause for quality deterioration during processing and storage of muscle foods. Poultry and poultry products are particularly prone to oxidative processes in lipids and proteins due to the relatively high concentrations of unsaturated lipids, pigments, metal catalysts, and various other oxidizing agents.

2.2.1 Lipid oxidation

Lipid oxidation is a complex process whereby various reactive oxygen species including free radicals and other non-radical promoters of oxidation react with the unsaturated fatty acids. In the case of free radicals, the attack begins by the removal of a hydrogen atom generally adjacent to a double bond of a polyunsaturated fatty acid (PUFA), leading to a lipid radical ($L^{\bullet}$), which, in the presence of oxygen, generates a lipoperoxyl radical ($LOO^{\bullet}$). However, the

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peroxidative action of the non-radical singlet oxygen ($^1O_2$) is initiated by its direct addition to a fatty acid double bond, leading to $LOO^•$ production. The latter is able to abstract a new hydrogen atom from an adjacent fatty acid. As a result, $LOO^•$ is reduced to a lipid hydroperoxide (LOOH) and generating a new radical $L^•$. This process, known as lipoperoxidation in live systems and as oxidative rancidity in food, represents an oxidative chain reaction, which in the absence of antioxidants becomes autopropagative leading to the production of LOOH (Fellenberg and Speisky, 2005). These LOOH are easily decomposed into various small molecular weight aldehydes, ketones, alcohols and lactones, some of them being potentially cytotoxic to living organisms. Accumulation of these compounds in poultry meat can affect its sensory attributes (Higgins *et al.*, 1999; Ruiz *et al.*, 2001).

Poultry meat and eggs, by virtue of the fact that they contain unsaturated lipids and prooxidant components, are particularly susceptible to lipid oxidation. Among the various lipid fractions, the polar phospholipids present in the cell membranes of meat and eggs contain the highest proportion of unsaturated fatty acids and, for this reason, as opposed to neutral lipid fractions, are primarily responsible for lipid oxidation. This implies that even low-fat poultry meat samples are susceptible to lipid oxidation since fat reduction essentially reflects a reduction in triacylglycerides while the phospholipids fraction is much less affected (Monahan, 2000). Another consequence is that species differences in susceptibility to lipid oxidation are largely determined by the level of PUFA present in meat. Thus, the extent of lipid oxidation in cooked meat is directly related to the level of unsaturated lipids, with susceptibility to oxidation decreasing in the order chicken > pork > beef > lamb (Rhee *et al.*, 1996).

Lipid oxidation in raw meat can also be related to its heme iron content, with beef showing a greater tendency to oxidize than poultry and pork. The iron contained in the heme group of some proteins has been proposed as the major initiator of the oxidative decomposition of PUFA. This is of importance since chicken leg and breast contain 0.67 and 0.24 mg hemoglobin/g, respectively (Kranen *et al.*, 1999) and, in consequence, when the birds are slaughtered, the biochemical processes that turn the muscle into meat allow hemoproteins to control the lipoperoxidative processes that significantly accelerate the deterioration of meat (Andersen and Skibsted, 1991; Alayash *et al.*, 2001). In addition, lipid oxidation increases with decreasing pH in meat postmortem (Tichivangana and Morrissey, 1985). Therefore, variations in muscle pH within and between species cannot be also ignored (Rhee *et al.*, 1996).

The iron released from heme following cooking may also be responsible for the rapid oxidation of cooked meat during storage (Kristensen and Andersen, 1997). However, recent findings have cast some doubt on the iron release theory (Monahan *et al.*, 1993). A negligible increase in free iron at the expense of heme iron has been found in myoglobin solutions heated up to 90 ºC. In the same study, the prooxidant activity of myoglobin was found to increase at around its thermal denaturation temperature of 60–70 ºC, but to decrease at higher temperatures. This decrease can lead to the conclusion that the prooxidant

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activity of heme iron in cooked meat exceeds that of free iron. Other studies have also shown that ferritin, when heated, might promote lipid oxidation (Decker and Welch, 1990). However, hemeosiderin, another major iron-containing protein fraction which accounts for as much as 60% of total iron in chicken (Hazell, 1982) did not appear to play a significant role in lipid oxidation (Apte and Morrissey, 1987).

Since the oxidation process begins immediately after slaughter, the biochemical changes that accompany the conversion of muscle to meat give rise to conditions where the process of lipid oxidation is no longer tightly controlled and the balance between prooxidative and antioxidative factors favors oxidation. The endogenous antioxidant defense mechanisms available to the cell in the live animal still function during the early postmortem phase but their effectiveness diminishes with increasing postmortem time (Sies, 1986; Lee *et al.*, 1996). In the postslaughter phase, it is highly unlikely that these defense mechanisms still function. Therefore, the rate and extent of oxidation in poultry meat is mainly influenced by postslaughter events such as the rate of early postmortem pH, carcass temperature, and the processing procedures where any disruption of muscle membrane integrity by mechanical deboning, mincing, restructuring, or cooking alters cellular compartmentalization. In such meat products, added antioxidants, dietary antioxidants, or food packaging techniques must be utilized to control oxidation, thereby enhancing meat quality parameters such as color, flavor and texture.

Muscle also contains several non-enzymic components capable of controlling lipid oxidation. These include the lipid soluble α-tocopherol and β-carotene, and water soluble compounds such as ascorbic acid, sulphydryl-containing proteins and histidine-containing dipeptides (Chan and Decker, 1994). Tocopherols and β-carotene terminate the lipid oxidation reaction by acting as free radical quenchers, whereas ascorbic acid and other reducing components in meat serve to regenerate lipid-soluble antioxidants (Packer *et al.*, 1979). The histidine-containing dipeptides carnosine and anserine are considered to act as either metal chelators or free radical scavengers (Chan and Decker, 1994).

2.2.2 Protein oxidation

Until recently, the fate of proteins in postmortem muscle exposed to oxidative stress was mostly unknown. Recent studies in the biomedical sciences have led to the discovery that many intracellular and membrane proteins in muscle can be readily modified by reactive oxygen species (Butterfield and Stadtman, 1997). Modification changes in oxidized proteins included amino acid destruction, decrease in protein solubility due to protein polymerization, loss of enzyme activity, formation of amino acid derivatives including carbonyls, and increases in protein digestibility (Uchida *et al.*, 1992; Agarwal and Sohal, 1994). As in the living tissues, reactive oxygen species can cause meat proteins to polymerize, degrade and interact with other muscle constituents to produce complexes. Recent studies have shown that these physicochemical modifications are leading

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causes for the alterations in gelation, emulsification, viscosity, solubility, and hydration in muscle foods (Decker *et al.*, 1993; Srinivasan and Xiong, 1997; Wang *et al.*, 1997).

Protein oxidation is a slower and less extended process than lipid oxidation affecting amino acid residues. Mechanical actions, which disrupt the cellular walls, bring various endogenous prooxidants in direct contact with proteins in the presence of molecular oxygen, thereby making proteins vulnerable to attack by reactive oxygen species. The sites of free radical attack on proteins include both the amino acid side chains and the peptide backbone.

In general, all amino acids with reactive side chains such as sulphydryl and amine groups, imidazole and indole ring, and thioether, are particularly susceptible to oxidation initiated by oxidizing lipids and their products. Thus, cysteine, methionine, lysine, arginine, histidine, tryptophan, valine, serine and proline residues are common targets of reactive oxygen species generated via lipid oxidation (Gardner, 1979). However, all amino acids are not equally susceptible to this attack. Cysteine is possibly the most susceptible amino acid residue as it is usually one of the first to be oxidized. During refrigerated or frozen storage of a myofibrillar protein concentrate, as much as 33% of the total sulphydryl content was lost (Srinivasan and Xiong, 1997; Wang *et al.*, 1997), whereas during meat aging, the sulphydryl content gradually decreased while the protein carbonyl content increased (Martinaud *et al.*, 1997). Other workers found decreased oxidation of protein sulphydryls and reduced formation of protein carbonyls in muscle from turkeys fed vitamin E (Mercier *et al.*, 1998). Methionine, the other sulfur-containing amino acid, is also readily oxidized to form methionine sulfoxide derivatives (Vogt, 1995).

Destruction of amino acid residues in muscle proteins can also take place independently of lipids. Site-specific metal-catalyzed protein oxidation occurs widely and has been proposed to take place via hydroxyl radicals ($^{\bullet}OH$), which are produced from H_2O_2 at specific iron-binding sites on proteins (Stadman and Oliver, 1991).

Carbonyl derivatives are the most important oxidation by-products of the free radical attack on proteins. As the levels of carbonyls are highly indicative of protein oxidation, they are widely used for the assessment of the extent of protein oxidation. Some workers found that the concentrations of carbonyls in Fe/ascorbate-oxidized turkey muscle increased when compared with the non-oxidized control, coinciding with a 24% reduction in the ϵ-amino content of the oxidized samples (Xiong and Decker, 1995). Protein carbonyls were also rapidly produced during storage of water-washed chicken muscle mince when compared with samples washed with propyl gallate and tripolyphosphate (Liu and Xiong, 1996).

Protein carbonyls can be generated by direct oxidation of amino acid side chains, fragmentation of the peptide backbone, reaction of proteins with reducing sugars and binding of proteins to non-protein carbonyl compounds (Xiong, 2000). Thus, oxidation of the side chains of arginine, lysine, proline and threonine can yield carbonyl derivatives, whereas deamination reactions, in

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particular, may be a major route for the production of protein carbonyls. In addition to the direct attack on protein side chains or peptide bonds, carbonyls can also be produced by reaction of products of lipid oxidation such as 4-hydroxy-2-nonenal, with either the ϵ-amino group of lysine, the imidazole moiety of histidine or the sulphydryl group of cysteine. Proteins can also react with reducing sugars to produce Schiff base derivatives that can be rearranged to form ketoamine derivatives (Stadman and Berlett, 1997).

Whether they are derived from proteins or originate from non-protein substances, protein carbonyls are highly reactive groups. Cross-linking between protein molecules through carbonyl-amino Schiff base contributes to polymerization, aggregation, and in some cases, insolubilization of oxidized muscle proteins, especially in concentrated meat products (Xiong and Decker, 1995). Protein-protein cross-linked derivatives in meat can also occur by oxidation of cysteine sulphydryl groups to form disulfide linkages, by complexation of two oxidized tyrosine residues, by interaction of an aldehyde group in one protein with the ϵ-amino group of a lysine residue in another protein, by cross-linking of two ϵ-amino lysine in two proteins through a dialdehyde, and by condensation of protein free radicals (Decker *et al.*, 1993; Srinivasan and Xiong, 1997).

2.3 Effect of oxidation on the sensory quality of poultry meat and eggs

Sensory evaluation has proven to be invaluable to the muscle foods industry. Sensory evaluation is a tool that if used properly can solve problems before they arise. Major sensory attributes can be considered tom be the flavor, the color and the texture of the muscle food.

2.3.1 Flavor

Poultry meat flavors result from interactions among proteins, nucleic acids, lipids, reducing sugars and other components found in meat. Raw poultry meat has very little flavor, but once cooked, characteristic cooked, roasted and meaty flavors are developed through interactions of the thermally induced lipid oxidation reactions, the Maillard reaction, and other heat dependent reactions.

The main components of meat that promote the onset of lipid oxidation are heme, non-heme iron, unsaturated fatty acids and phospholipids. In cooked meat, oxidative reactions quickly initiate, first within the phospholipids fraction, and within hours result in development of detectable off-flavors. The phospholipids appear to play a major role in the generation of cooked meat flavor since the characteristic flavor of cooked meat disappears in case phospholipids have been previously removed from the meat (Mottram and Edwards, 1983). The phospholipids significantly contribute to the flavor of cooked meat through thermally induced lipid oxidation and interaction of lipid with Maillard reaction

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products (Mottram and Whitfield, 1995). However, development of warmed-over flavors is not solely a result of lipid oxidation since protein degradation reactions have also contributed toward this development (Saint Angelo and Bailey, 1987).

'Warmed-over flavors' (WOF) is a common term that originally referred to the rapid development of lipid-derived oxidized flavor in refrigerated cooked meat products. In meat, this distinctive off-flavor can appear within a few hours of thermal processing, but it is most noticeable when refrigerated cooked meat and meat products are reheated. When meat is ground, chopped or cooked, membranes are disrupted releasing cell content, and membrane phospholipids, which are most prone to oxidation due to their high content in polyunsaturated fatty acids, are exposed to oxidation. Research has shown that subcutaneous fat from meat produced about 50 volatile compounds during WOF development, whereas intramuscular lipids generated more than 200 (Pegg and Shahidi, 2007). Consequently, the process of WOF development begins within several hours of cooking the meat product compared to regular lipid oxidation, which can take several days to develop.

Although WOF was initially recognized as a result of a cascade of oxidation events in cooked meat, the original definition has been expanded to also include fresh meat that has been stored in a freezer. Even though the flavor related to stored fresh meat is somewhat different from the characteristic WOF of reheated meats, the flavor compounds involved are qualitatively the same but present at different concentrations. Now, it is generally accepted that any process involving disruption of the integrity of muscle tissue, such as grinding, deboning, restructuring, freezing or cooking, will enhance the development of WOF. Thus, in an effort to better describe the overall increase in off-flavor notes in meat, the term 'meat flavor deterioration' has also been proposed instead.

The major reaction in the formation of off-flavors is a free radical chain reaction referred to as autoxidation. Even though hydroperoxides, the primary products of lipid oxidation, are odorless and tasteless, their degradation leads to formation of a complex mixture of low molecular weight compounds with distinctive flavor (Shahidi *et al.*, 1986). A variety of hydrocarbons, aldehydes, enals, ketones, and sulfur compounds is included among the secondary products of lipid oxidation that contribute to poultry flavor. The carbonyl compounds are important flavor constituents yielding a rancidity flavor to poultry, whereas the sulfur compounds formed during cooking from the sulfur amino acids cysteine, cystine and methione, are major flavor constituents yielding a chicken flavor.

Gasser and Grosch (1990) identified 16 principal compounds in chicken broth, including 2-methyl-3-furnathiol, methional and 2-trans-nonenal. The compounds 2-trans-4-trans-decadienal (fatty), and γ-dodecalactone (tallowy) predominated in the broth. Hexanal, produced during oxidation of unsaturated fatty acids, was the most common volatile detected in both breast and thigh broiler meat throughout a 15-day storage period at 4 ºC (Ajuyah *et al.*, 1993). However, not all flavors derived from oxidation give rise to unpleasant off-flavors. Short chain aldehydes with unsaturation at the 2-position have been

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described as sweet and pungent, while longer chain analogues have been described as sweet, fatty, and green.

In the case of cooked meat, low molecular weight aldehydes such as pentanal, hexanal and trans,trans,-2,4-decadienal as well as oxocompounds, like the very potent trans-4,5-epoxy-2-decenal, are believed to be partially responsible for WOF development during storage (Konopka and Grosch, 1991). Pippen and Nonaka (1963) reported that when chicken and turkey meat were cooked in a nitrogen atmosphere, fewer carbonyls and smaller amounts of those produced were detected compared to turkey meat cooked in an air atmosphere. Furthermore, rancid chicken meat contained the same volatiles but in larger quantities than fresh chicken meat. By 1984, more than 300 compounds had been identified as components of cooked chicken flavor (Maarse, 1984). Further work by Noleau and Toulemonde (1986) listed 197 components from roasted chicken flavor. The list included 27 hydrocarbons, 17 alcohols, 47 aldehydes, 33 ketones, 14 acids, 6 esters, 22 bases, 10 sulfur compounds, 3 halogens, 2 acetals, 1 nitrile, 5 phenols and 10 furans. Taylor and Larick (1995) identified 318 volatiles in cooked chicken fat but a trained flavor profile sensory panel detected no meaty flavor and little cooked flavor in samples. Panel detection of chicken fat flavor was correlated to compounds such as pentanal, hexanal, heptanal, and 2-octene, and chicken fat flavor was correlated to compounds such as 1,1,1-trichloroethane, 4-methylactane, and 2-heptanone.

Saint Angelo *et al.* (1988) identified volatile compounds responsible for WOF as hexanal (grassy), propanal (alcoholic), pentanal (pungent), nonanal (soapy), 2,2-octadione and 2-pentylfuran. Johnson and Civille (1986) and later Byrne *et al.* (2002) found a decrease in the meaty and sweet notes on cooked refrigerated chicken meat, and an increase in WOF notes such as cardboard, linseed oil, rancid, and sulfur/rubber. Byrne *et al.* (2002) identified a variety of other compounds including 3-methyl-butanal (malty), 2-methylbutanal (roasted corn), 3-methylthiopropanal, dimethyl sulfide (garlic), dimethyl tetrasulfide (cabbage), 1-octen-3-ol (mushroom), 2-heptenal (almond). Further, Ruenger *et al.* (1978) found in reheated samples two compounds that identified as heptaldehyde (harsh) and n-nona-3,6-dienal (fishy). Ruenger *et al.* (1978) also reported that WOF in cooked turkey was detected equally by sensory panelists in thigh and breast meat, contradictory to Wilson *et al.* (1976) who reported that thigh meat developed WOF to a greater extent than breast meat.

Various factors including poultry species, muscle type, thermal processing, chilling method, irradiation and storage, can affect the flavor of poultry. Chicken has fewer tendencies to develop oxidized flavors than turkey, because the higher level of vitamin E in chicken fat retards oxidation. Oxidation proceeds faster in chicken thigh meat than in breast meat, as the darker meat contains more lipid and heme iron.

Fresh meat used in product formulation shows less of a tendency to develop WOF than older meats. Mechanically deboned chicken meat is particularly susceptible to rancidity due to mixing of the meat with bone marrow and cellular constituents during deboning, in particular the heme pigments (Froning and

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Johnson, 1973). Mixing of meat tissue with air at this point will also contribute to accelerated oxidation (Dawson *et al.*, 1990). Oxidation of mechanically deboned chicken meat results in off-flavors that will carry through to the final product making oxidized meat less valuable.

Thermal processing may also have a significant effect on flavors in chicken and turkey. No difference in flavor was detected by sensory panelists for turkey cooked to 77 ºC in conventional, convection or microwave ovens (McNeil and Penfield, 1983). An end-point temperature of 77 ºC, however, resulted in insufficient flavor development in turkey rolls according to sensory panelist evaluations (Cremer 1986). Chicken breast sous vide processed at 94 ºC had fewer sulfur containing volatiles and higher quantities of alcohols and hydrocarbons compared to those processed at 77 ºC (Turner and Larick, 1996). Meaty cooked turkey flavor scores were highest for turkey cooked at 135 ºC compared to samples cooked at either 105 or 163 ºC according to Cremer (1986). However, Brown and Chyuan (1987) reported higher off-flavor characteristics in turkey cooked at 135 ºC. Chicken flavor intensity of breast was greater for chicken sous vide processed at 80 ºC than for samples prepared at the same temperature using a cook-chill system (Church and Parsons, 2000). Chicken flavor was maintained in sous vide processed product during storage at less that 5 ºC, while increased off-flavor development was noted in stored cook-chill processed breasts.

Cooking methods, such as boiling, roasting, frying, and pressure-cooking, vary in the heating conditions and thus, variably affect overall meat flavor. It has been estimated (Spanier *et al.*, 1990) that when meat is heated at above 70 ºC, the Maillard reaction predominates with Maillard reaction products contributing to reduction of lipid oxidation and off-flavor formation due to their antioxidative properties (Specht and Baltes, 1994). At elevated temperatures, however, lipid oxidation also increases due to enhanced myoglobin degradation and release of free iron (Spanier *et al.*, 1990, 1992). Sensory evaluation has shown increasing intensity of bloody, metallic and sour flavor as the final internal temperature of the meat increases (Heymann *et al.*, 1990).

Refrigeration processes can also influence the cooked flavor of poultry and poultry products, but the main effects are from storage rather than chilling or freezing. In relation to chilling, conflicting results have been obtained. Commercially processed, dry-chilled broilers were found to have a subtle but detectable flavor advantage over conventional immersion-chilled broilers (Hale *et al.*, 1973). Other workers found no effect of chilling on meat flavor (Zenoble *et al.*, 1977), whereas Ristic (1982) reported that water chilling of broilers produced a more favorable flavor than air chilling for both leg and breast meat. Cryogenic chilling systems might have an effect on flavor as Lillard (1982) stated that chilling systems using liquid nitrogen or carbon dioxide resulted in improved flavor. Using three different chilling and thawing methods on turkeys, Brodine and Carlin (1968) reported that none of these methods had any effect on either flavor or juiciness of cooked breast or thigh meat.

Freezing itself appears to have no effect on flavor of poultry meat. However, Brunton *et al.* (2002) stated that cooked turkey breast was particularly susceptible

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to lipid oxidation-mediated off-flavor development during refrigerated storage. Compared to liquid nitrogen-cooled turkey breast, the levels of a number of unsaturated carbonyl compounds were much higher in freshly cooked air-cooled samples and showed large increases in the chilled meat during storage.

Exposure of meat to medium or high levels of irradiation has frequently been associated with off-flavor development. This is mainly the result of lipid oxidation that can be accelerated by irradiation (Kim *et al.*, 2002a; Nam *et al.*, 2002). Dimethyl disulfide and dimethyl trisulfide, described by sensory panelists as sulfurous and foul, have been identified as two of the compounds contributing to off-flavor (Du *et al.*, 2001). Other volatile compounds identified in irradiated poultry include 3-methyl butanal, 2-methyl butanal, cis-3-nonenal, and trans-6-nonenal. Although irradiation has often been associated with off odors, higher levels of fresh chickeny, bloody, and sweet aromas in raw chicken and chicken flavor in cooked chicken were detected by 14 trained panelists in irradiated samples compared to non-irradiated chicken (Hashim *et al.*, 1995).

Similar to heat processing, small radiation doses can result in pasteurization whereas a high dose will result in the so-called 'commercial sterilization' or 'radappertization', similar to the situation in heat-treated canned food. Therefore, it is generally recommended that meat irradiated at medium to high levels be vacuum packed, whereas a sub-freezing temperature is also recommended to minimize off-flavor formation. High-level radappertization allows the product to be stored at room temperature without spoilage. For radappertization, a mild heat treatment at about 70–77 ºC is usually applied in order to inactivate proteolytic and lipolytic enzymes, which can cause flavor and texture deterioration during storage (Josephson, 1983).

2.3.2 Color

Poultry is supplied in the market either before or after skinning, complete or in pieces such as breast, thigh and legs. In all cases, lacking the appropriate color results in failure in merchandizing. Acceptance of poultry meat, meat products and eggs by the consumer is mainly associated with color, since this appearance is related to freshness, eating quality and flavor.

The relationship between poultry color and functional characteristics is frequently of considerable interest to poultry processors because lighter broiler breast meat is typically associated with a low pH, reduced water-holding capacity and decreased emulsification capability (Fletcher *et al.*, 2000; Qiao *et al.*, 2001). In addition, darker breast meat has been reported to have a shorter shelf-life (Allen *et al.*, 1997).

Poultry skin color generally ranges from cream-colored to yellow, while raw meat typically has a pink to reddish color. When cooked, however, leg and thigh muscles appear dark due to the high quantity of myoglobin, while breast is referred to as white meat. The color depends largely on the composition of the feed used, as the level of dietary carotenoids (e.g., xanthophylls) which are deposited in the fat is highly associated with skin pigmentation (Perez-Vendrell *et al.*, 2001).

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The effects of color changes during storage are less critical but still important for possible effects on product uniformity and consumer acceptance (Petracci and Fletcher, 2002). During the first four hours postmortem, skin and meat color changes are more pronounced, whereas the color continues to change but at a slower rate, for up to 12 to 24 hours postmortem. During the first eight days of storage, color changes vary depending on processing or storage conditions.

Meat color can be expressed in terms of color differences using the international Commission of Illumination system (CIE-Lab), which is based on the lightness (L*), the redness (a*) and the yellowness (b*) coordinate. Chicken breast meat color can be divided into three classes, the dark (L* < 47), the normal (L* = 47–51), and the light (L* > 50) (Woelfel *et al.*, 2002; Holownia *et al.*, 2003). Since the main factor determining poultry color change is the ultimate pH, color parameters for normal meat at pH = 5.99 have been considered as a* = 1.4, L* = 49.2 and b* = 10.3, whereas for dark meat at pH = 6.04 as a* = 5.6, L* = 39.9 and b* = 9.08. The establishment of a cut-off value of 2.72 for parameter a* provides a reliable instrumental method to differentiate between dark- and normal-colored poultry meat (Boulianne and King, 1998).

Processing procedures such as cooking and chilling/freezing affect poultry color. In chickens, color lightness of thigh meat heated to 70, 80 and 90 ºC increased with the temperature while redness decreased (Heath and Owens, 1992). Also, chilled storage of cooked chicken at 4.4 ºC caused a shift in the a* value from red to green while frozen storage caused the chicken to darken and become more red and yellow over time (Heath and Owens, 1992). In turkeys, breast meat roasted at 105 ºC was rated less brown compared to meat reheated to 163 ºC (Cremer and Richman, 1987). L* values for refrigerated poultry fillets decrease as storage proceeds for 2 to 4 days postmortem, suggesting considerable drip losses; further decreases in L* values are related to meat shrinkage (Galobart and Moran, 2004).

Color of poultry meat is also highly dependent on temperature during processing. Higher temperatures during deboning and storage or delays in postmortem chilling of chickens and turkeys are associated with lighter meat colors (Molette *et al.*, 2002; Alvarado and Sams, 2002). Also, delays in turkey carcass chilling produced lighter, redder and yellowier meat than carcass immediately refrigerated (Rathgeber *et al.*, 1999).

The pale poultry meat, which is frequently defined as having an 'L' value of > 53, is often due to a defect known as pale, soft and exudative (PSE). It is generally accepted that PSE meat is closely associated with rapid glycolysis resulting in accelerated rigor mortis development and low pH (Woelfel *et al.*, 2002). This combination may promote muscle protein and consequent loss of protein functionality, denaturation leading to paler meat color, decreased water-holding capacity and softer texture. McKee and Sams (1998) reported that carcass temperatures higher than 20 ºC in turkey resulted in lighter meat with higher drip loss and cook loss. Oxidative conditions accelerate postmortem glycolysis in turkeys, producing PSE meat (Strasburg and Chiang, 2003).

Irradiation can also exert a significant effect on poultry meat color. Increases

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in visual pinkness and/or in objective redness monitored by a* values have been noted in both raw and cooked chicken and turkey meat (Bagorogoza *et al*., 2001; Du *et al*., 2001, 2002a,b; Nam *et al*., 2002). The increased redness was generally stable during refrigerated or frozen storage under both anaerobic and aerobic conditions, but it was less intense in aerobic conditions (Du *et al*., 2002c). The red color of turkey breast meat has been attributed to a decrease in oxidation/reduction potential and binding of carbon monoxide to the sixth ligand of myoglobin (Nam and Ahn, 2002). Although it was considered unusual by sensory panelists, the red/pink color of irradiated chicken was rated appealing and acceptable (Abu-Tarboush *et al*., 1997).

2.3.3 Texture

Texture is a complex set of characteristics. One of the most important textural characteristics in meat is tenderness, which is defined as the ease with which a piece of meat can be cut and chewed. Juiciness, a characteristic related to the fat and moisture content of meat, and the water-holding capacity, are other important textural characteristics in meats. The impact of protein oxidation on textural quality of processed meat, especially for products that are subjected to comminuting and restructuring, is of particular concern.

A major consequence of protein oxidation is the formation of protein aggregates through both noncovalent and covalent intreractions. An increased exposure of nonpolar residues resulting from oxidatively induced unfolding of protein molecules can lead to hydrophobic association of proteins. Hydrogen bonds also contribute to the formation of protein aggregates as well as protein-lipid complexes in oxidized systems. On the other hand, protein aggregates formed through free radical chain reactions can be covalently bound; an amino acid residue side chain that is susceptible to free amino attack can be cross-linked with an accessible reactive amino acid residue from another protein. These oxidative modifications of muscle proteins will inevitably cause variations in protein functionality.

In meat and meat products, the most important functional properties of proteins include those that contribute to the textural characteristics and structural properties of cooked products. One of such important functional properties is gelation and meat particle binding, which result from protein–protein interactions and protein matrix–water interactions. Another functional property is emulsification, which is affected by protein–lipid interactions. A third important functional property is hydration or water binding, which is regulated by protein–water interactions. Factors that affect these functional properties, including oxidation, are therefore responsible for either improvement or deterioration in the quality of attributes of cooked meat.

Smith (1987) reported that frozen storage of hand- and mechanically deboned turkey meat increased lipid oxidation, and decreased protein solubility, myosin ATPase activity, and myofibril gel strength. Gels prepared from frozen stored protein consisted of a globular matrix, compared with gels made from fresh

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muscle protein, which possessed a continuous filamentous microstructure and were capable of entrapping more water. In addition, hydroxyl radical-induced deterioration in functional properties of myofibrillar proteins has been reported. Oxidative reactions catalyzed by both iron and copper in the presence of ascorbate severely impaired the gel-forming ability of turkey myofibrils as manifested by weakening of the gel matrix structure. Because of their porosity, gels made from the oxidized protein were able to hold only 33–75% as much water as gels prepared from non-oxidized protein (Decker *et al*., 1993). The losses in the functionality correlated with decreases in protein solubility and structure stability as well as with increased carbonyl groups, and may have resulted from excessive cross-linking of proteins through carbonyl-amine reactions.

Cross-linking among proteins undergoing free radical attack may be the major cause of the decreased protein solubility. Myosin, the most abundant protein in the myofibril assembly, has been found to be very susceptible to oxidizing agents generated during meat processing and storage. In chicken muscle, myosin is readily oxidized by lipid free radicals and forms large insoluble aggregates (Schilder, 1993). Turkey myofibrillar proteins incubated in ferric or copper ion/ascorbate oxidizing systems showed also a 32–36% decreased solubility within 6 hours. Similar to myofibrillar proteins, sarcoplasmic proteins are also highly susceptible to oxidation (Decker *et al*., 1993). Myoglobin, in particular, is readily denatured by lipid free radicals resulting in decreased solubility.

On the other hand, the main problem reported in relation to the process of freezing and thawing is the formation of large protein aggregates within the meat structure, resulting in water displacement, proteins moving closer together, cross-linking, and incomplete rehydration, when the protein-water affinity is the same or less than the protein-protein affinity (Matsumoto, 1980). Yoon (2002) reported no significant texture toughening in frozen chicken breast after 10 months of storage at −20 ºC, suggesting that toughening is not a determinant factor in the quality loss of frozen chicken breast when the samples are treated with 10% trisodium phosphate or sodium tripolyphosphate solution before frozen storage. Improvement of the water-binding ability of chicken meat without ice crystal formation during frozen storage, is most important for preserving the eating quality of frozen chicken breast.

In general, oxidized myofibrillar proteins exhibit functional behavior distinctly different from their antioxidant-treated controls. The exact functionality changes, which can be beneficial or detrimental, depend on the oxidative processes and conditions. However, a total inhibition of lipid and protein oxidation does not always lead to better protein functionality. Yet, antioxidants that facilitate protein-protein interaction usually improve functionality. The conflicting results indicating that muscle proteins exposed to oxidizing environments can exhibit both improved and reduced functionalities are a manifestation of the complexity of oxidative processes in relation to the functional characteristics of meat (Xiong, 2000).

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2.4 Effect of oxidation on the nutritional quality of poultry meat and eggs

The fate of the nutritional quality of poultry meat and eggs under oxidative conditions is of major interest to both the consumer and the producer. Meat and meat products exposed to strong oxidative conditions would conceivably have a decreased quality due to loss of several vitamins including A, C and E, and some essential amino acid residues, particularly lysine and methionine, that are particularly susceptible to attack by reactive oxygen species. It is not known, however, to what degree the amino acid destruction would constitute a considerable nutritional impact.

In addition, the free radical-initiated protein oxidation usually results in enhanced proteolytic susceptibility due to protein unfolding and the increased accessibility of peptide bonds to proteases (Agarwal and Sohal, 1994). Recent digestibility studies with various enzymes including pepsin, trypsin and chymotrypsin, have shown that the digestibility of oxidatively modified myosin, a major fraction of muscle protein, can be increased or decreased, depending on the extent of oxidation and whether a reducing compound is present during the digestion process (Kamin-Belsky *et al*., 1996). Under relatively mild oxidative conditions, myosin exhibits improved enzyme digestibility, provided that the digestion takes place in presence of a reducing agent. Under strong oxidative conditions that cause myosin molecules to cross-link via non-disulfide linkages, the oxidized protein is usually highly resistant to proteolysis by digestive enzymes. Therefore, the influence of oxidation on the digestibility of muscle proteins is determined by the specific conditions under which proteins are modified, as well as the conditions with which proteins are digested.

Although the impact of lipid and protein oxidation on the sensory characteristics of meat and meat products has been considered to be most important to both the consumer and the producer, great attention was given recently to health risks that lipid oxidation might impose. Lipid hydroperoxides and their decomposition products may cause damage to proteins, membranes and biological components of the consumer, thus affecting vital cell function. Products of lipid oxidation have been suggested to be toxic and are believed to lead to deteriorative processes in humans (Ladicos and Lougovois, 1990). Malondialdehyde is one of these products of lipid oxidation that has been suggested to be mutagenic and has also been implicated in the formation of N-nitrosamines (Sanders, 1987). It is not known, however, to what degree the consumption of oxidized food affects human health. The significance of such compounds for human health remains to be established.

Cholesterol is another product that may affect human health as its oxidation can lead to formation of a group of compounds inducing atherogenicity (Addis and Park, 1989). Pure cholesterol is not atherogenic, even in a sensitive animal such as the rabbit (Taylor *et al*., 1979). Cholesterol undergoes oxidation by peroxyl or oxyradicals of neighboring polyunsaturated fatty acids in phospholipid membranes. The close relationship between fatty acid oxidation and

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cholesterol oxidation products (COPs) in many experiments supports this hypothesis.

COPs are normally not found in fresh meat, but have been detected in a large variety of precooked and processed meats, especially during subsequent storage (Park and Addis, 1987; Pie *et al*., 1991). Production of COPs in chicken meat varies with the cooking method and the type of cut. Breast and thigh muscle samples boiled for 4 h contained 20-hydroxycholesterol, cholesterol α-epoxide, and cholestane triol, while deep frying and microwave cooking produced 20-hydroxycholesterol. Roasting did not result in the production of COPs, except in the skin (Chen *et al*., 1993). Irradiation increased COPs in chicken meat and accelerated their formation during storage (Galvin *et al*., 1998b).

Formation of COPs is mainly a problem in products exposed to increased oxidation such as heating, comminuting, exposure to light, and prolonged storage, which for meats are similar to those promoting warmed-over flavor. COPs are also detected at relatively high levels in other processed foods like spray-dried egg powder (Morgan and Armstrong, 1987; Nourooz-Zadeh and Appelqvist, 1987). However, the specific health risks associated with dietary intake of cholesterol oxidation products at the levels present in meat and meat products remain to be assessed.

2.5 Effect of oxidation on the shelf-life of poultry meat and eggs

The establishment of an appropriate cold chain from production to the point of sale ensures that the meat has a shelf-life that is sufficiently long to satisfy consumer needs. Shelf-life can be defined as the period within which the food is safe to consume and/or has an acceptable quality to consumers (Fu and Labuza, 1993). The shelf-life of poultry depends on several factors, including product characteristics, pre-freezing treatments, freezing processes, packaging film and processes, and storage conditions.

One of the principal factors affecting the shelf-life of raw meat is storage temperature. To ensure an adequate shelf-life for fresh, chilled poultry, carcasses must be cooled to 0–2 ºC following grading, weighing and packaging. These handling stages should be completed without delay to safeguard the keeping quality of the product. For chilled poultry meat, there is, within the European Union, a temperature limit that cannot exceed 4 ºC (Barnes *et al*., 1978). This limit relates more to product safety, however, since significantly lower temperatures are actually needed to safeguard shelf-life during cold storage and distribution.

The importance of keeping processed carcasses as cold as possible is demonstrated by a study in which eviscerated turkey carcasses wrapped in an oxygen-permeable film were stored at temperatures between +5 and −2 ºC (Barnes *et al*., 1978). Keeping the carcass at 0 ºC rather than +2 ºC extended shelf-life by more than 7 days. At −2 ºC off-flavors were not detected until

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about 38 days. Barnes and Impey (1975) compared the shelf-life of eviscerated and uneviscerated chicken stored at 4 ºC both wrapped and unwrapped. The unwrapped, eviscerated carcasses had a mean shelf-life of 7.9 days before off-flavors became evident, whereas holding them in polyethylene bags reduced the mean shelf-life to 5.6 days, due to enhanced moisture retention. By contrast, unwrapped eviscerated carcasses showed no sign of spoilage until day 28. Effects of storage on meat texture and flavor in uneviscerated turkey were investigated by Griffiths *et al*. (1984). Although hanging at 4 ºC had little or no effect on meat texture, the flavor of the cooked meat increased in intensity from day 8 onwards, according to a trained taste panel. By day 24, however, some members of the panel showed an adverse reaction to the flavor.

For frozen poultry and poultry products, the main problem seems to be rancidity, and there are many studies about antioxidant incorporation; color and protein denaturation are also problems that can be avoided if the frozen procedure is correctly performed (Fu and Labuza, 1993).

The most obvious means to prevent oxidative deterioration is to remove air. Wrapping raw meat in oxygen-impermeable film prevents metmyoglobin formation and lipid oxidation during storage, if sufficient reducing activity is present in the meat (Pearson *et al*., 1977). Packaging materials used for meat products are usually plastic films, in which polymers with good oxygen-barrier properties are incorporated into polymers with good humidity-barrier and sealing properties such as polyethylene and polypropylene. Since lipid oxidation is the major problem with aerobically packaged irradiated turkey breast, Nam and Ahn (2003a,b) studied the effects of aerobic, anaerobic and double packaging on color, lipid oxidation, and volatiles of irradiated raw turkey breast during refrigerated storage and after cooking. They reported that double packaging combined with antioxidants was more effective in reducing sulfur volatiles and lipid oxidation than aerobic packaging. Pettersen *et al*. (2004) investigated the effects of natural and synthetic antioxidants in packaging material under different packaging atmospheres on the oxidative stability of mechanically deboned turkey meat. They reported that when α-tocopherol was used to manufacture the polyethylene layers in the package material, lipid oxidation was inhibited when meat was stored in such packages under vacuum or modified atmosphere. In addition, packaging material produced by impregnating butylated hydroxyl anisole (BHA) into co-extruded polyethylene film was found to be partially effective in inhibiting lipid oxidation in turkey patties, a finding that may add a new dimension to future packaging and quality control procedures (Dawson and Gartner, 1983).

Extending product shelf-life with vacuum packaging has shown different effects. When meat is vacuum-packed in an oxygen-impermeable film, any remaining oxygen is soon consumed by residual tissue respiration. Depending on film permeability, oxygen is largely prevented from entering the pack, while the internal CO_2 concentration steadily increases. Shrimpton and Barnes (1960) showed that storing chicken carcasses at 1 ºC in vacuum packs, using an oxygen-impermeable film, increased shelf-life by about 4 days. While the oxygen

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concentration fell rapidly below 7%, CO_2 within the packs increased to more than 9%. Vacuum packaging of duck carcasses in a heat-shrunk, oxygen-impermeable film extended shelf-life at +2 ºC and −1 ºC by more than 50% in each case (Barnes *et al.*, 1979). On the other hand, vacuum packaging of turkey breast and legs in a barrier film delayed off-flavor development from 16 to 25 days for breast and from 14 to 20 days in the case of drumsticks. Ranken (1987) reported that vacuum packaging or controlled atmosphere (CO_2 and N_2) packaging of meat and meat products are both satisfactory means to prevent color and rancidity problems. Vacuum packaging of mechanically deboned turkey was found comparable to packaging in N_2 atmosphere but superior to packaging in CO_2 atmosphere (Dawson and Gartner, 1983). Vacuum packaging also improved the sensory scores of precooked and pre-fried chicken when compared to paper-wrapped or heat-sealed products (Arafa and Chen, 1976).

A more useful approach for retail display purposes is the use of modified atmosphere packaging (MAP), in which the composition of the gaseous environment can be tailored to particular needs. Its application to poultry has been expanded in recent years, with the development of more cost-effective packaging materials. When mixed with air or oxygen, the optimum concentration of CO_2 was 20–30%, which avoided any discoloration (Stanbridge and Davies, 1998). Timmons (1976) described a system in which a quantity of about 30 kg of poultry meat was packaged, evacuated and then back-flushed with CO_2 prior to heat-sealing. The method was supported to give an 18–21 day shelf-life at −2 to +1 ºC. Transferring the meat stored in 100% CO_2 to normal atmosphere under chill conditions, resulted in extended shelf-life, which was intermediate between that in air alone and the shelf-life in CO_2. The residual preservative effect may be due to slow release of CO_2 from the tissues, thereby inhibiting oxidation.

With red meat, an advantage in using MAP is that the attractive red color of the meat is enhanced and maintained by including 80% oxygen in the pack to sustain the oxymyoglobin content. The same approach can be used for poultry meat, although the color of the product is less critical, especially when the skin is present. However, Mead *et al*. (1983) reported that inclusion of 10% or 20% oxygen in gas packs containing 20–30% CO_2 led to the appearance of unpleasant flavors in turkey breast fillets stored at 1 ºC. In contrast, Hotchkiss et al. (1985) found that chicken breast and legs stored at 2 ºC in atmospheres containing up to 80% CO_2 in air presented higher sensory-panel ratings with little effect on eating quality up to 35 days of storage. Gas mixtures containing CO_2 and air were also found to be suitable for duck meat stored at 1% (Mead *et al*., 1986). However, when the air was replaced by nitrogen, the skin developed a waxen or milky appearance.

In a study with chicken meat, Patterson *et al.* (1984) compared the effects of vacuum-packaging and MAP, using 10 or 20% CO_2 in nitrogen. Both methods gave useful extensions of shelf-life, which were more pronounced at 1 ºC than at 4–5 ºC and for breast than leg or thigh portions. Comparing the shelf-life of products stored in vacuum and in modified atmospheres, Pexara *et al*. (2002)

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concluded that the use of modified atmospheres could not extend nor reduce the shelf-life of vacuum packaging.

Storing meat after slaughter represents a challenge to meat processors, retailers and consumers. If proper storage conditions such as refrigeration, or other preservation methods such as irradiation, are not used, the meat will spoil within a matter of hours or days. Poultry irradiation was first approved in 1990 by the FDA to control salmonella and other food-borne bacteria. Irradiation seems to be the best procedure to ensure the microbiological safety of raw meat, but a few meat quality defects have been reported. Irradiated poultry meat and eggs are readily susceptible to lipid oxidation (Katusin-Razem *et al*., 1992). Color changes in irradiated meat occur because of alterations of the myoglobin molecules to the energy input. The potential for iron ionization to exist in various states makes the environment particularly vulnerable to the presence of electron-donating compounds and high-energy irradiation. Generation of stable red or brown pigments that become red over time appears to be due to irradiation-generated reactive oxygen species that become ligands bound by iron under altered reducing conditions (Brewer 2004). Gomes *et al*. (2003) reported that irradiated mechanically deboned chicken meat showed higher a* values (redness) compared to non-irradiated samples from day 4 of refrigeration. In contrast, Nam *et al*. (2003) reported that the redness of meat decreased during 7 days storage, but irradiated meat was more red than the non-irradiated. Liu et al. (2003) reported that oxymyoglobin in chicken breast increased as a result of irradiation. Zhu *et al*. (2003) found that irradiation exerted a significant influence on the flavor of vacuum-packaged turkey ham.

Today, commercially dried poultry meat is used in dry soup mixes. The most economical way of drying is by using hot air. For this purpose small or thin slices of meat are placed on trays and exposed to circulating dry air. Attention should be given to fat oxidation, which is accelerated during hot-air drying, because of the large surface area exposed to oxygen.

2.6 Strategies to protect poultry meat and eggs against oxidation

A survey of commercially cooked poultry deli meats showed that the 'less processed' meats had higher levels of lipid oxidation than their more processed counterparts (Monahan, 2000). Yet, the former would be perceived by consumers as being more natural and healthier, since they contain lower levels of additives, which include antioxidants. With consumer demands for reduced food additives and more natural foods, it is clear that control of lipid oxidation in cooked meats requires the implementation of a number of strategies for maintaining oxidative quality. The most important strategies to protect poultry meat and eggs against oxidation, such as meat choice, addition of primary and secondary antioxidants, use of dietary antioxidants, addition of nitrites and nitrates, smoking and packaging, are briefly considered below.

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2.6.1 Meat choice

The choice of meat is an important factor with regard to the problem of oxidation. Poultry muscle tissues containing high levels of polyunsaturated fatty acids exhibit more susceptibility toward lipid and protein oxidation. Chicken is less apt to develop oxidation products than turkey, because the higher level of vitamin E in chicken fat retards oxidation. This susceptibility is higher for chicken thigh than breast meat, as thigh contains more lipid and heme iron.

Ensuring that high quality meat is used in product formulation is critical. Fresh meat used in products formulation shows less of a susceptibility toward oxidation than older meat. In fresh meat, little enzymatic oxidation has taken place and, thus, only a few compounds that propagate oxidation have been generated even after enzymes have been inactivated by thermal processing. Antioxidant enzymes, including catalase, glutathione peroxidase, and superoxide dismutase, continue to function postmortem to curb lipid oxidation in uncooked meat; however, their efficacy diminishes with increasing age of the meat. Incorporating antioxidants into the product and reducing the time from cooking to plate are other means by which food service operators can minimize oxidation.

The sensory quality of meat is also closely related to bird age at slaughter. Increasing the age of slaughter, meat flavor in broiler chicken and duck enhances, especially for dark meat, as sexual maturation is accompanied by more intense flavor (Baeza *et al.*, 2000). Concurrent changes in the lipid fraction, such as variations in phospholipids or fatty acid composition, would also account for these observations. Increasing age is also associated with darker breast meat in broilers. With increasing age, breast meat of ducks becomes significantly redder and darker, possibly due to increase in heme iron of muscle (Baeza *et al.*, 2002).

In general, the pH of postmortem poultry muscle is ca. 5.8, whereas thigh meat is slightly higher than breast. The pH of turkey breast and thigh is lower compared to the respective muscles in duck and chicken. Low pH enhances the ability of transition metals, hemoglobin and myoglobin to promote lipid oxidation, but microbial growth is favored at elevated pH, so there are limits to the extent that pH can be elevated in turkey muscle to decrease the onset of lipid oxidation during storage (Richards and Hultin, 2000).

The actual cooking method employed for precooked products can also influence the extent of oxidation. One might assume that grilling of meat would exacerbate the potential for oxidation due to the high temperatures to which lipid and protein constituents are subjected, but this is not so. In fact, thermal processes that employ very high temperatures like grilling seem to inhibit oxidation through the formation of Maillard intermediates (Van Ruth *et al.*, 1998). Similarly, conditions that favor browning such as addition of glucose or smoke by-products may help to retard or inhibit oxidation.

2.6.2 Addition of primary antioxidants

Processing of meat through mechanical deboning, grinding, restructuring, or cooking disrupts tissue membranes and allows the catalysts of lipid oxidation to

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react with the unsaturated fatty acids initiating oxidation reactions. To reduce the problem of oxidation in meat and meat products, food-grade antioxidants, when permitted, or ingredients that impart antioxidant properties, are commonly added.

Various compounds can be used as antioxidants. Those that act as free radical terminators by donating hydrogen atoms to free radicals to interupt chain propagation are called primary antioxidants, and are discussed extensively elsewhere in this book. Many of these antioxidants are phenolic in nature and can be distinguished as synthetic or natural. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ) are commonly-used synthetic antioxidants that are effective in inhibiting lipid oxidation in oxidatively labile muscle at very low concentrations and at low expense. Yun *et al*. (1987) presented evidence that TBHQ and BHA were the most effective antioxidants among various compounds tested as alternatives to nitrite. Their usage is strictly regulated in meat products such as sausages, and BHA and BHT are typically used at a legal limit of 0.02% on fat basis (Gray and Weiss, 1988). However, in the past few years, concern about potential health risks associated with these synthetic antioxidants has led to increased interest in natural alternatives (Saint Angelo, 1996).

The most common natural alternative to the use of the synthetic antioxidants is vitamin E, a lipid-soluble free radical scavenger. Vitamin E is the generic name used to describe at least eight naturally occurring compounds that exhibit the biological activity of α-tocopherol. The group comprises α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols.

Other major alternatives are various carotenoids such as β-carotene and lycopene that are also able to act as radical scavenging antioxidants. It has been demonstrated that β-carotene scavenges peroxyl radicals by forming an adduct between β-carotene and the peroxyl radical, yielding a resonance-stabilized carotenoid radical, and not by donating a hydrogen atom as phenolic antioxidants do (Burton and Ingold, 1984). Depending on their concentrations, carotenoids work as prooxidants under some conditions, and as antioxidants under other conditions. The balance between prooxidant and antioxidant behavior is very delicate, and the antioxidant behavior is most pronounced at low oxygen partial pressure (Jorgensen and Skibsted, 1993).

Consumer interest in increasing the use of natural additives in foods has led to extensive research in the identification of new antioxidants of plant origin. The antioxidant activity of numerous herb extracts including rosemary, sage, oregano, tea catechins, and rice hull extracts, have been studied in meat products (McCarthy *et al*., 2001; Kim *et al*., 2003). The antioxidant activity of most of these plant extracts was mainly attributed to the ability of the polyphenolic compounds present in these extracts to quench free radicals. A major limitation of phenolic antioxidants is that they can become ineffective during prolonged heating at elevated temperatures, which occur during the deep fat frying of foods (Gordon and Kourimská, 1995).

Al-Jalay *et al.* (1987) reported the antioxidant activities of 10 dried spices (allspice, black pepper, cardamom, cinnamon, clove, coriander, cumin, ginger,

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nutmeg, and rose petals) in the preparation of a fermented meat sausage. Clove, rose petals, and allspice exhibited the highest antioxidative potential. On the basis of sensory evaluation, Chouliara *et al*. (2007) reported a shelf-life extension of breast chicken meat by *ca.* 3–4 days for samples containing 0.1% oregano essential oil, 2–3 days for samples under modified-atmosphere packaging, and 5–6 days for samples under modified-atmosphere packaging but containing 0.1% oregano essential oil. Lee *et al*. (2006) reported that cranberry juice powder was effective in inhibiting lipid oxidation in mechanically separated turkey; flavonol aglycons were identified as the class of phenolics that best inhibited lipid oxidation, whereas quercetin was identified as the primary antioxidant present (Kathirvel *et al*., 2009).

A variety of other naturally occurring substances with antioxidant activities including protein hydrolysates, and edible products from vegetables, fruits, oilseeds and grains (Rhee, 1987) have also been studied in meat products and some have been commercialized (Bruun-Jensen *et al*.,1996).

In addition, carnosine, a naturally occurring skeletal muscle dipeptide, has been shown to function as a hydrogen donor in the aqueous phase of the muscle tissue to inhibit lipid oxidation (Decker and Crum, 1991, 1993). Honey has been found also to be an effective antioxidant in processed meat. The antioxidant potential of honey was attributed to the presence of reducing sugars participating in Maillard reaction, phenolic constituents and other compounds with antioxidant activity (McKibben and Engeseth, 2002).

2.6.3 Addition of secondary antioxidants

Apart from the primary antioxidants, secondary antioxidants are also used to reduce oxidation in meat and meat products. Secondary antioxidants can include free radical preventors, such as chelators or metal complexing agents, which act by inhibiting the production of radical species (Ladikos and Lougovois, 1990).

Chelators are compounds which complex metal ions in foods to form stable complexes, thereby disabling the catalytic action of transition metal ions in lipid oxidation. Typically, chelators added to processed meat products are various alkaline phosphates. Phosphates are widely used in meat products to increase water-binding but they also function as secondary antioxidants through metal chelation. The mechanism by which phosphates prevent lipid oxidation appears to be related to their ability to sequester free iron ions that are released from the heme moiety of myoglobin during thermal processing. Among the available phosphates, tripoly-, pyro-, and hexametaphosphates are important for preventing rancidity in cured meat products, whereas orthophosphates are not (Sato and Hegarty, 1971). The stability toward oxidation of fresh broiler carcass marinated in sodium pyrophosphate solutions and subsequently subjected to freezing, cooking and refreezing, was found to increase most by 6% SPP followed by 3% SPP plus 3% sodium chloride (Ang and Young, 1987).

Besides phosphates, ethylenediaminetetraacetic (EDTA) and citric acids are common food-grade additives, which help to stabilize metal ions by reducing

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their activity and ability to act as antioxidants. Citric acid can effectively inactivate metal ions by chelation although high concentrations are often required. In cooked meat, the addition of 2% EDTA has been shown to effectively chelate the non-heme iron released during cooking, and thus effectively reducing lipid oxidation (Igene *et al.*, 1979). Similar findings have been reported for cooked ground beef by Sato and Hegarty (1971), who have also demonstrated a surprising antioxidant activity by cupric salts.

Addition of reducing agents such as ascorbic acid, isoascorbic acid and their salts, also improves color stability and product storage life. This activity is enhanced by the addition of citric acid and its salts, which inhibit metal-catalyzed oxidation reactions.

Ascorbate can scavenge free radicals to form a low-energy ascorbate radical (Buettner, 1993). The reduction potential of this radical is lower than that of polyunsaturated fatty acids, thus making them unable to directly promote lipid oxidation. The reduced forms of the transition metals are able to rapidly decompose hydrogen peroxide and lipid peroxides into radicals that can promote lipid oxidation (Decker and Hultin, 1992). Ascorbate also promotes the release of iron bound to proteins such as ferritin and promotes oxidative reactions. Conversely, ascorbate can inhibit the activity of prooxidative forms of myoglobin, such as the ferryl species (Kanner, 1992). Therefore, ascorbate can act as both an antioxidant and a prooxidant, which is largely dependent on their level of addition. Ascorbic acid at low levels (up to 250 ppm) can catalyze lipid oxidation reactions, whereas at higher levels (up to 500 ppm), it is considered to inhibit these reactions, possibly by upsetting the balance between ferrous and ferric iron, or by acting as an oxygen scavenger (Igene *et al.*, 1985).

Ascorbic acid, its sodium salt, and its isomer erythorbate, also function synergistically with other antioxidants and added polyphosphates to give protection to meats against oxidative degradation. Injection of ducks with α-tocopherol or combinations of citric acid, polyphosphates, propyl gallate and BHA, prior to cooking, retards oxidation during cooking, storage and reheating (Klinger and Stadelman, 1975).

2.6.4 Use of dietary antioxidants

The lipid composition of poultry meat is greatly influenced by the lipids present in poultry diets. As the diet becomes richer in polyunsaturated fatty acids, the susceptibility of the meat to oxidative deterioration increases. In this case, dietary α-tocopheryl acetate supplementation is an efficient means to protect fatty acids from oxidation in eggs and in raw and cooked poultry meat.

Cortinas *et al.* (2005) reported that dietary polyunsaturated fatty acids and α-tocopherol supplementation affected lipid oxidation more markedly in cooked chicken meat and cooked refrigerated meat than in raw and raw refrigerated meat of chickens; lipid oxidation increased linearly as the concentration of the polyunsaturated fatty acids in raw meat increased, but this increase was lower with greater dietary α-tocopherol supplementation. On the other hand, Galvin *et*

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al. (1998a) reported that cooked breast and thigh chicken patties from birds fed 800 mg α-tocopheryl acetate/kg diet for 42 days presented higher α-tocopherol levels and decreased lipid and protein oxidation. In agreement to these, Galobart *et al*. (2001) found that dietary supplementation of 200 mg α-tocopheryl acetate/kg diet to hens for forty days, increased α-tocopherol content and decreased lipid oxidation in fresh and spray-dried eggs. For chicken, the literature evidence suggests that the optimal dietary levels of vitamin E to prevent lipid oxidation are in the region 200 mg α-tocopheryl acetate/kg $\times$ 35 d (Galobart *et al*., 2001).

Dietary vitamin E has also been shown to decrease the formation of COPs during the storage of precooked chicken patties. Supplementation with 400 mg vitamin E/kg feed may be necessary to control cholesterol oxidation in cooked meats and cooked irradiated meats. Galvin *et al*. (1998a) observed significantly lower 20-hydroxycholesterol concentrations after refrigerated storage for up to 12 days of cooked ground breast and thigh meat from broilers fed 200 or 800 mg α-tocopheryl acetate/kg feed than from broilers fed a normal diet containing 20 mg α-tocopheryl acetate/kg. Total COPs ranged from 0.10–1.43 and 0.23–4.20 μg/g in breast and thigh meat, respectively. In breast, total COPs formed after 12 days were on average 41 and 69% lower for the 200 and 800 mg/kg groups, respectively, than for the normal group. In thigh, the decreases of the total COPs were 50 and 72%, respectively, at these supplementation levels.

Turkey diets should probably be supplemented with higher levels of α-tocopheryl acetate, at least 300 mg/kg feed $\times$ 90 d, because of the slower uptake and deposition of α-tocopherol in turkeys (Wen *et al*., 1996). Instead, Sheldon et al. (1997) proposed feeding 200 to 300 mg α-tocopheryl acetate for 21 d immediately prior to slaughter. Similarly, Botsoglou *et al*. (2003a) also reported that raw and cooked breast and thigh turkey meat from birds fed 200 mg α-tocopheryl acetate/kg diet for four weeks presented higher α-tocopherol levels and decreased lipid oxidation.

Various carotenoids including carotenes and xanthophylls can be also added to poultry diets to increase the oxidative stability of meat and eggs. In poultry, there is a preferential uptake of xanthophylls over carotenes The efficiency of carotenoids towards phenoxyl radicals, tocopheroxyl radicals and radicals formed by irradiation of carotenoids in chloroform follows the order lycopene > β-carotene > zeaxanthine > lutein > echinenone > canthaxanthine > astaxanthine, with lycopene being the most efficient radical scavenger (Mortensen and Skibsted, 1977).

While in some studies dietary carotenoids had no effect on the oxidative stability of heated and unheated chicken meat (Jensen *et al*., 1998), other studies showed that lipid oxidation was promoted (King *et al*., 1995). In thigh meat that was chill-stored under fluorescent light, β-carotene acted as a prooxidant when the tissue level of vitamin E was low, whereas it acted as an antioxidant at higher concentrations of vitamin E. Ruiz *et al*. (1999) reported that raw and cooked thigh chicken meat from birds fed 15 mg β-carotene/kg showed antioxidant activity whereas at 50 mg β-carotene/kg showed prooxidant activity after 7 days of storage at 4 ºC.

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Dietary supplementation with other carotenoids, such as lycopene to hens, led to eggs with lower oxidation values (Sahin *et al.*, 2008). Similarly, astaxanthine showed antioxidant activity in poultry skin. Conversely, lutein extracted from marigold, showed some prooxidant activity in chicken breast (Koreleski and Swiatkiewicz, 2007). However, zeaxanthine, which is very similar to lutein, and canthaxanthine, did not inhibit fatty acid oxidation in chicken meat or cholesterol oxidation in egg products (Jensen *et al.*, 1998).

Herb and plant extracts can also be used as efficient dietary supplements to increase the oxidative stability of meat and eggs. Raw breast meat from chickens fed 0.56 g/kg sage, thyme or coneflower extracts for 20 days showed oxidative stability comparable to the control (Koreleski and Swiatkiewicz, 2007). However, raw breast and thigh meat from chickens fed 0.5 g/kg rosemary extracts for 21 days showed oxidative stability that was higher than the control and similar to meat from birds dietary supplemented with 40 mg α-tocopheryl acetate/kg diet. Higher oxidative stability was also shown in raw breast and thigh meat from chickens fed 0.04 and 0.08 g/kg Marian thistle extract (Schiavone *et al.*, 2007).

The presence of active compounds in the essential oils of herbs and plants can explain their effectiveness as dietary antioxidants. Raw and cooked breast and thigh meat from chickens fed an essential oil mix (Botsoglou *et al.*, 2004a), or oregano essential oil (Botsoglou *et al.*, 2002a,b, 2003c) for 42 or 38 days, respectively, showed better oxidative stability than the control, but lower than those birds fed 200 mg/kg α-tocopheryl acetate supplement. Similarly, raw and cooked turkey breast and thigh meat stored under different conditions from birds fed oregano essential oil showed better oxidative stability than the control, but lower than those birds fed 200 mg/kg α-tocopheryl acetate supplement (Botsoglou *et al.*, 2003a,b; Papageorgiou *et al.*, 2003; Govaris *et al.*, 2004).

Ground herbs, plants or plant by-products have been also examined as dietary supplements for increasing the oxidative stability of poultry meat. Hen eggs from birds fed ground thyme, oregano, rosemary or saffron showed better oxidative stability than the control but lower than eggs from birds fed 200 mg/kg α-tocopheryl acetate supplement (Botsoglou *et al.*, 1997, 2005). Similarly, raw and cooked turkey breast and thigh meat from birds fed ground rosemary showed delayed lipid oxidation (Govaris *et al.*, 2007; Botsoglou *et al.*, 2007). In addition, raw and cooked breast meat from Japanese quail fed 50 g/kg dried tomato pulp showed better oxidative stability than the control whereas birds fed 100 g/kg showed a prooxidative effect (Botsoglou *et al.*, 2004b).

2.6.5 Addition of nitrites and nitrates

Nitrites and nitrates are unique additives to meat products since they can effectively retard lipid oxidation and WOF formation. Curing of meat with sodium nitrite was originally developed as a means of preservation. Addition of nitrite to meat at 50–500 mg/kg contributed to increases in ham aroma and decreases in off-flavors as detected by a sensory evaluation panel (MacDonald *et*

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al., 1980). Nitrates are mainly used in the preparation of fermented meat products, where slow release of nitrite is required. If nitrates are used, they will be first reduced to nitrites by the microorganisms present in meat.

The mechanism by which nitrites prevent or retard the oxidation of meat lipids is still a matter of discussion (Freybler *et al*., 1993). The nitric oxide generated by nitrites in cured meat forms complexes with heme pigments, which prevents the release of iron from the porphyrin molecules (Kanner *et al*., 1984). Nitrites can also stabilize unsaturated lipids within tissue membranes against oxidation. In addition, nitrites can act as metal ion chelators of any liberated non-heme iron, thereby increasing the shelf-life of meat products. Moreover, nitrites can react with meat to form nitroso and nitrosyl derivatives, which possess antioxidative properties by acting as radical scavengers. S-nitrosocysteine exhibited antioxidative properties in an aqueous linoleate/myoglobin system similar to those of other antioxidants such as butylated hydroxyl anisole; S-nitrosocysteine was also shown to act not only as inhibitor of linoleic acid oxidation but as a hydroperoxide decomposer as well (Kanner, 1979). The high inhibitory effect of added S-nitrosocysteine on lipid oxidation was demonstrated in ground cooked turkey meat as well as in cooked cured turkey meat stored under anaerobic conditions. In the latter case, similar effects were obtained using nitrite (25 ppm) or the corresponding molecular concentration of S-nitrosocysteine, in color development and inhibition of lipid oxidation (Kanner and Juven, 1980). Morrissey and Tichivangana (1985) reported that the antioxidative effects of nitrite were apparent even at 20 ppm, and that nitrite and nitrosylmyoglobin behaved synergistically toward the inhibition of lipid oxidation.

While the curing process contributes to a significant decrease in the concentration of hexanal and other carbonyl compounds, it does not result in the formation of new flavor compounds that would contribute to the cured meat flavor. Nitrite stabilizes the microsomal lipids and heme pigments to reduce lipid oxidation reactions and the resulting off-flavor formation (Ramarathnam *et al*., 1991). Shahidi (1989) reported that nitrite-cured meat reflects the natural flavor of the meat without the flavor compounds contributed by the lipid oxidation reactions.

Nitrite has been shown to eliminate WOF at a level of 220 ppm and to inhibit development of WOF at 50 ppm (Sato and Hegarty, 1971). The addition of nitrite to chicken thigh and breast meat was found to be more effective as a means of controlling oxidized flavor in cooked meat than removal of heme pigments (Igene *et al.*, 1979). Nitrite levels used in processed meat products are very low and usually range from 100 to 150 ppm (Cassens, 1997). Permitted usage levels are controlled by government agencies due to the potential for nitrosamine production, some of which are considered to be human carcinogens.

Over the past few decades, several suggestions have been made for eliminating or reducing nitrite levels in meat products; however, none have gained wide acceptance. Certain consumers react negatively to an ingredient label stating sodium nitrite; they forget, however, that some common natural foods (e.g., celery) are rich sources of sodium nitrite. It is now also widely known that

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during cooking of sausages and other meat products, the levels of nitrite can be substantially reduced due to conversion of nitrite to nitric acid. During storage, there may be further reduction in the amount of detectable nitrite and, by the time the product is consumed, nitrite levels may be as low as 10–30 ppm. Besides, in meat products that will be processed shortly after addition of nitrite, such as frankfurters, reducing agents such as ascorbate and erythorbate are used at a level of about 500 ppm to quickly convert the nitrite into nitric oxide and reduce the chance of nitrosamine formation. In certain products that will be exposed to high-temperature cooking, such as turkey bacon, which is usually fried, lower levels of nitrite are also usually prescribed (Barbut, 2000).

2.6.6 Smoking

Smoking during thermal processing provides unique flavors and also has the potential to decrease the onset of oxidative rancidity during storage. The traditional method of direct smoking with hardwood in a smokehouse is still used today in the production of various meats. However, in the past few decades, commercial natural wood smoke flavorings or liquid smokes have become more popular throughout the world as a convenient way to add smoke flavor and color without having to use a smokehouse. Wood smoke can be generated by the controlled pyrolysis of the major components of wood, namely cellulose, hemicellulose and lignin (Fisher and Scott, 1997).

The active components of smoke include volatile acids that can affect flavor and pH as well as stability of the product; carbonyl compounds that can react with proteins and other nitrogen compounds to develop color; and phenols that are considered to be the main flavor compounds and are also primarily responsible for both antimicrobial and antioxidant activities (Cadwallader, 2007). Many other compounds, including aliphatic and aromatic hydrocarbons, as well as alcohols, and various oxygen- and nitrogen-containing heterocyclic compounds, also contribute to smoke flavor. Before 1988, over 400 compounds had been isolated from wood smoke (Maga, 1988). Moreover, incomplete pyrolysis during smoke generation can result in formation of additional nitrogen oxides that can act as nitrites do in the curing process. Commercial smokers often use chemical smoke to add flavor, or burn sawdust or wood chips to supply a controlled amount of smoke in a high humidity temperature-controlled smoke house.

Smoking is a common practice for frankfurters, deli products, whole birds, roasted products, and dried meats such as jerky. Research has shown that certain smoke constituents can reduce the occurrence of WOF and extend shelf-life when added to fresh, precooked and processed meat. In some cases, the incorporation of smoke ingredients has reduced lipid oxidation by 20 to 39%. Smoke ingredients with strong flavors can help mask WOF, whereas flavorless smoke fractions can be employed at low levels in marination systems to reduce the extent of WOF. Today, smoked poultry products receive only a light application to enhance the exterior color and provide some special flavor notes.

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This means that the smoke is deposited on the surface of the product and may penetrate to a depth of 1–3 mm (Barbut, 2000).

2.6.7 Packaging

Packaging is a physical means to inhibit oxidation in meat and meat products by eliminating their exposure to oxygen and light. Since the packaging environment can significantly impact partial oxygen pressure, a profound effect on the lipid stability of packaged fresh meat can be observed.

In the majority of packaging situations, rigid or expanded plastic trays are used which are over-wrapped with clear plastic films. These thin films are composed of polyvinyl chloride, and have high-oxygen and low-moisture permeability. This type of packaging is the simplest and least expensive means of meat display, and it is microbiologically stable for up to one week in many countries, or until formation of metmyoglobin at the surface becomes important (Renerre and Labadie, 1993).

Packaging can also prevent deleterious color changes. Kim *et al.* (2002b) reported no alteration in L* values of non-irradiated turkey samples under aerobic or vacuum packaging stored for 7 days, whereas a* values increased in aerobic-packaged samples and b* values decreased in vacuum-packaged samples. A 3 kilogray dose resulted in no changes in L* and b* values but higher a* values in both packaging systems.

Vacuum packaging is an excellent alternative for controlled storage because it prevents oxygen interactions at meat surfaces which slows oxidation. During vacuum packing, a transient formation of brown metmyoglobin will occur, after which the purple/red color dominates. One drawback of this retail package is that the purple color may be unacceptable by the consumer. Consequently, there is the possibility of using vacuum-skin packaging with a peelable oxygen-barrier layer and an oxygen-permeable skin-layer that remains after storing. With this problem of unacceptable purple color, vacuum packaging at present is mainly used for bulk packaging. This technique, coupled with nitrogen flushing, can give substantial shelf-life to finished products.

Modified-atmosphere packaging systems were developed using selective, gas-permeable materials and the pack would be flushed with specific gases or gas mixtures. For chilled products, various combinations of gases could greatly extend shelf-life and stabilize product appearance. Other packaging technologies such as oxygen scavengers, can be added to the packaging either as stand-alone or be incorporated into the packaging film. The use of specialized films with specific gas and water permeability is also critical to product appearance, surface drying, shelf-life and quality.

In the last several years, interest in edible films was also examined because of environmental concerns and issues with plastic degradation (Quattara *et al.*, 2000). Edible films composed of modified starch, soy protein or wheat gluten, and containing natural antioxidants were examined as a means to control oxidation in cooked meat products, but some problems related to solubility have

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been reported. The direct addition of antioxidants into meat formulations might be a more effective strategy, but in some cases, such as for whole muscle and some restructured meat products, this procedure is not feasible.

Edible films require modifications to improve their physical and mechanical properties and avoid chemical changes. This can be achieved through the incorporation of plasticizers that reduce the polymer intermolecular forces increasing the flexibility and extensibility of the film. On the other hand, the addition of plasticizers increases the gas, water vapor, and solutes permeability, but decreases film elasticity and cohesion. Edible plasticizers such as mono-, di-, and oligosaccharides, lipids and their derivatives, and polyols have been evaluated (Choi and Han, 2001). An example of edible film is one containing 5–15% of isolate protein in distilled water with added glycerol as plasticizer; a functional additive such as a suitable antioxidant could also be added, adjusting properly the pH.

2.7 Future trends

As the consumption of poultry meat rises worldwide, the industry must remain responsive to the demands of consumers, both in the range and nature of the products developed. Quality will continue to be the main target, and the uniformity of poultry quality is likely to improve further. In addition, there will be technical innovations in processing that will lead to safer products.

The biochemistry of the oxidative and reducing systems present in fresh meat, which affect protein and lipid stability, is now well-described. However, their respective importance in the regulation of the sensory attributes of meat is not completely established. Also, the health implications of consumption of oxidized lipids and cholesterol are receiving increasing attention. To better understand the relationships between protein and lipid oxidation, where free radicals are implicated, more research is needed to gain a fundamental understanding of these oxidative processes.

Cured meat is the type of precooked meat where further knowledge of antioxidant interaction could result in significant improvements of product quality. For non-cured, precooked meats, the effect of packaging on product quality also deserves further attention in relation to the increasing importance of precooked meats. The development of affordable, effective, materials and systems for gas-packing of poultry has opened up considerable opportunities for the marketing of products with an extended shelf-life in the chilled state.

There is now little doubt that altering the concentrations of PUFAs and antioxidants in the live animal is the best way of maximizing oxidative stability. The vast variety of secondary plant metabolites with antioxidative properties has opened up a promising new field of research. Future studies will hopefully identify phytochemicals that may be nutritionally administered to increase the oxidative stability of meat without having adverse effects on other product characteristics or performance and well-being of animals. However, little

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information concerning absorption, metabolism, and antioxidative activity in tissues is readily available for these substances. A better understanding of the metabolic processes in which these substances are involved could make their future application more efficient on a productive and commercial level. Furthermore, most of the dietary strategies demonstrated to be effective for improving fresh meat quality are not in general use due to extra feeding costs, which are difficult to recover in the commercial setting. However, the relative higher added value of processed meats compared to fresh meat could provide an economic advantage to the use of dietary strategies for reducing deterioration during storage, or to improve quality characteristics.

2.8 Sources of further information and advice

An in-depth discussion of basic scientific factors responsible for the quality of muscle foods, with emphasis on sensory attributes, can be found in the book entitled *Handbook of Meat, Poultry and Seafood Quality*, LML Nollet, Ed., Blackwell Publishing Professional, Ames, IA, 2007.

For an up-to-date reference work for both practitioners and students on meat quality and its control during the production and processing of poultry, the book entitled *Poultry Meat Processing and Quality*, GC Mead, Ed., CRC Press, Boca Raton, FL, 2004, should be considered.

A complete guide to the use of dietary antioxidants to improve meat quality while avoiding exogenous food additives or packaging procedures is given in the book entitled *Antioxidants in Muscle Foods*, E Decker, C Faustman, CJ Lopez-Bote, Eds, John Wiley & Sons, New York, 2000.

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3

Oxidation and protection of fish

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Abstract: This chapter discusses the main aspects of fish lipid oxidation and its relevance to the seafood industry. The intrinsic mechanisms involved in fish oxidation will be reviewed, firstly stressing the importance of fish tissue pro-oxidants and reductors upon the process, followed by the influence of external factors, such as processing and storage. Throughout the chapter, the optimal designs for the use of antioxidants in the fish industry are given significant attention. Finally, this chapter will present the effects of lipid oxidation on the loss of nutritional and sensory quality in fish products, and the technologies being developed to inhibit such oxidation. Future trends and sources of additional information are also included at the end of this chapter.

Key words: fish oxidation, rancidity, quality loss, pro-oxidants, antioxidants.

3.1 Introduction

Fish muscle is a particularly perishable material. The factors responsible for the fish muscle's high susceptibility to change are to be found in its high water content, high autolitic enzymatic activity, and the existence of autochthon bacteria flora able to live at low temperatures. Lipid oxidation is one of the most important changes that can affect the fish muscle, which is mainly associated with the high proportion of unsaturated lipids in the tissues, and the presence of large amounts of heme pigments and metallic traces (Richards and Hultin, 2002). During the post-mortem period, fish endogenous antioxidants are rapidly consumed and pro-oxidants activated, conducting the first lipid oxidation products (Hultin, 1994). Additionally, the conditions of storage or processing can significantly affect the apparition, rate and extension of lipid oxidation.

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Oxidation of fish lipid leads to the formation of volatile compounds associated with rancidity, which significantly reduces the shelf-life of fish products, most especially during storage (Frankel, 1998). Furthermore, the reaction between lipid oxidation products and proteins, amino acids or vitamins, can alter the texture of the fish, and reduce its nutritional value through the subsequent loss of essential amino acids. Delaying the oxidative process is important to the entire production and commercialization of these foodstuffs. By slowing the oxidation process down, it is possible to extend the window of opportunity for sales in the marketplace, and reduce the losses caused by rancid or browned fish muscle. Recently, the addition of natural antioxidant substances has become one of the most promising strategies used to prevent or retard such deterioration in frozen and processed fish products.

3.2 Oxidation of fish and fish products

3.2.1 General aspects involved in fish oxidation

As mentioned above, the high proportion of long chain n-3 polyunsaturated fatty acids (PUFA) found in fish is a crucial factor in the muscle's high vulnerability to oxidation. Fish contain significantly higher levels of polyunsaturated fatty acids, particularly eicosapentaenoic (20:5 n-3) and docosahexaenoic (22:6 n-3), than terrestrial animals (Ackman, 1994). It is widely recognized that the lowest bond dissociation energies for the C–H bond of bisallylic methylene positions, together with the resonance stabilization of the radical intermediate formed, facilitates the hydrogen abstraction from PUFA (Gardner, 1989; Koppenol, 1990). Therefore, the positions between adjacent double bonds of PUFA are thermodynamically favored for attack by free radical species in comparison to ordinary methylene positions of saturated fatty acids.

PUFA can be contained in phospholipids, which are more or less constant in fish muscle (0.5–1.0%), or in triglyacylglycerols. Lipid content, i.e. triglyacylglycerols, varies between species, and depends on the sexual maturity of the fish, and its environmental conditions. The lipid level ranges between 0.5 and 2.0% in lean fish, and may even reach to more than 10% in fatty fish. The high susceptibility of muscle from fatty fish to oxidation has been traditionally explained using the total lipid content (Richards and Hultin, 2001). However, several pieces of evidence indicate that phospholipids, which give structure and fluidity to membranes, are the primary lipid substrate for lipid oxidation in muscle food, whereas triglyacylglycerols play a minor role (Gandemer, 1999). The important role of phospholipids as lipid substrate is related to their higher unsaturation compared with neutral lipids, and their close contact with the aqueous phase surrounding the membranes (Hultin, 1994). The cytosol contains an important amount of active substances that promote oxidation, such as active transition metals or enzymes (Buege and Aust, 1978). Research performed in washed cod muscle, a model system essentially composed of phospholipids as lipid source (Undeland *et al.*, 2002; Pazos *et al.*, 2005c), has also highlighted the

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important contribution of phospholipids to fish oxidation. Undeland *et al.* (2002) have observed that the addition of 15% of fish triacylglycerols does not enhance lipid oxidation in washed cod muscle activated by hemoglobin, despite the high amount of hydroperoxides in samples with added oil (1135 μmol/kg muscle). Richards and Hultin (2001) have demonstrated the strong development of lipid oxidation in a fat-reduced washed cod muscle ($\approx$ 0.1%), suggesting that a reduced amount of membrane lipids is enough for the initiation and propagation of lipid oxidation.

In the last decade, variations in the oxidative stability between species have been associated with factors other than the compositional differences in total lipid content, such as type and content of pro-oxidant substances, or the weakening of the antioxidant barriers in post mortem conditions. The rate and extent of oxidation also depends on the technological processes and storage conditions to which fish are subjected. The following sections will indicate how these intrinsic and extrinsic factors modulate oxidation in fish.

3.2.2 Principal pro-oxidants in fish muscle

Heme proteins, transition metals and lipoxygenases are the primary endogenous components that have the ability to promote radical oxidative chain reactions in fish.

Heme proteins

Hemoglobin (Hb) and myoglobin (Mb) are heme proteins implicated in the onset and propagation of oxidation in muscle-based foods (Kanner, 1994). Hb is constituted by four polypeptide chains, with each chain containing one heme group; Mb is a monomer formed by a single polypeptide with a heme group attached. These heme proteins are much more abundant in the fatty fish species, such as tuna, mackerel and herring, than in lean fish (Venugopal and Shahidi, 1996). Indeed, the high content of heme proteins gives a characteristically reddish hue to the muscle of fatty fish, in contrast to the white muscle of lean fish. The proportion of Hb and Mb is roughly equal in dark muscle, whereas Hb is much more abundant in light fish muscle (Richards and Hultin, 2002). The high content of Hb in light fish muscle, which comprises roughly 75–90% of the total muscle (Burt and Hardy, 1992), has contributed to the increasing number of investigations focused on the pro-oxidant mechanisms of fish Hb, and the search for efficient methodologies to avoid its negative effects on the quality and nutritional value of fish products.

The concentration of Hb in light muscle from fatty fish species fluctuates typically from 3 to 12 μmol Hb/ kg muscle (Richards and Hultin, 2002; Larsson *et al.*, 2007). Different studies have demonstrated that these levels of fish Hb can actively increase lipid oxidation in washed fish muscle; a fish model system practically free of hemeproteins. The pro-oxidant activity of Hb is concentration-dependent in the range found in fish muscle (0.06–5.8 μmol Hb/kg muscle), with a noticeable change in the rate and extent of lipid oxidation with the

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increase of Hb concentration in washed cod muscle (Richards and Hultin, 2002). In spite of the fact that the strongest net oxidation is suffered in the presence of higher Hb levels, there is a reduction in the relative pro-oxidant capacity per mol at higher Hb concentrations. Similar concentration-dependence has been observed in a model system based on fish membranes (Pazos *et al.*, 2005c); therefore, Hb seems to be acting as a reagent rather than as catalyst, although the relative efficiency seems to be higher at low concentrations. Accordingly, the reduction of the Hb content by bleeding can improve to some extent the oxidative stability of the muscle of certain fatty fish (Sakai *et al.*, 2006; Richards and Hultin, 2002). Other investigations have shown that bleeding is not sufficient to prevent lipid oxidation (Sohn *et al.*, 2007).

Three principal mechanisms contribute to the high capacity of Hb to activate oxidative processes in muscle-based foods:

1. generation of free radical species (peroxyl, alkoxyl, etc.) via fragmentation of lipid hydroperoxides (Pazos *et al.*, 2008);
2. abstraction of hydrogen atoms from PUFA by hypervalent ferrylHb species ($^{\bullet}$HbFe(IV)=O), which can be formed by the reaction of ferric metHb with hydrogen peroxide (H_2O_2) or lipid hydroperoxides (Kanner, 1994);
3. release from Hb of inorganic iron ions and hemin (oxidized form of heme group) (Grunwald and Richards, 2006b).

Hb cleavages hydroperoxides significantly more rapidly than free ferrous and ferric iron accomplish (O'Brien, 1969). Recent studies have demonstrated differences in the capacity of several fish Hbs to generate hydroperoxide-derived free radicals, and their relative differences in efficiency have been found to be similar to their capacity to promote lipid oxidation in liposomes (Pazos *et al.*, 2009). This result suggests the significant participation of free radicals formed through hydroperoxide-decomposition on the hemoglobin-mediated lipid oxidation. The radical-generating activity in the presence of hydroperoxides does not appear to be significantly influenced by the redox state of Hb, and consequently ferric metHb and ferrous Hb created similar amounts of radicals (Pazos *et al.*, 2008). However, several investigations have indicated that metHb is more highly active in promoting lipid oxidation than ferrous Hb in washed fish muscle and liposomes (Grunwald and Richards, 2006a; Pazos *et al.*, 2009; Maestre *et al.*, 2009b). This finding may be explained by the more efficient generation of oxidizing ferrylHb radicals in the presence of metHb than in the case of ferrous Hb. The reaction of metMb with the lipid hydroperoxides renders principally ferryl species, whereas other Hb species (ferrous, ferric and ferryl Hb) are implicated in the reaction with oxyMb (Reeder and Wilson, 1998). Conversely, the release of free iron should play a minor role in the pro-oxidant activity of Hb, as the addition of chelating agents has a poor efficiency in preventing lipid oxidation triggered by hemoglobin (Maestre *et al.*, 2009a; Grunwald and Richards, 2006a).

It is certain that fish Hb exhibits a much higher ability to activate oxidation compared to Hb from beef, turkey and chicken (Richards *et al.*, 2002a). The

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lower pro-oxidant activity of Hb from terrestrial animals is attributed to the greater proportion of anodic isoforms in fish Hb, which exhibits poor stability at the pH values typically found in fish muscle (5.5–7.0) (Aranda IV *et al.*, 2009). It is now known that anodic isoforms are significantly more pro-oxidant than catodic Hb isoforms (Richards *et al.*, 2002b). Certain investigations have also revealed differences in the activity of fish Hb from species to species, finding a direct correlation between the pro-oxidant activity and redox instability of the corresponding Hb (Richards and Dettman, 2003; Aranda IV *et al.*, 2009; Undeland *et al.*, 2004; Maestre *et al.*, 2009b). In particular, the pro-oxidative behavior of fish Hb has a positive correlation with its susceptibility to metHb formation and hemin loss either in the presence or absence of lipid oxidation byproduct, such as hydroperoxides and aldehydes (Maestre *et al.*, 2009b). These results may suggest that metHb and hemin play a decisive role in the pro-oxidant activity of fish Hb. Hemin possesses a strong facility to produce hydroperoxide-derived free radicals in the presence of relatively low amounts of hydroperoxides (Pazos *et al.*, 2008). The ability of free radicals to destroy the heme ring may explain the poor efficiency of hemin to generate free radicals under high hydroperoxide/hemin molar rations or successive exposures to low amounts of hydroperoxides. Hemin interacts more clearly with lipid hydroperoxides than hemeproteins, due to lower steric impediments and its higher solubility in lipid phases. Consequently, hemin release can significantly contribute to the early stages of oxidation. Differences in the redox stability of fish Hbs have been also associated with variations in the anodic character of Hb (Maestre *et al.*, 2009b).

Transition metal ions

Iron is the primary transition metal implicated in the lipid oxidation of muscle-based foods, although some authors have suggested that copper can also contribute to the process (Hultin, 1994). The inorganic iron is mainly found as ferric ions, although it can be reduced to a ferrous state in the presence of reducers. The term low molecular weight (LMW)-iron has been used instead of free iron because iron seems to be associated with small metabolites, as ATP, ADP, and amino acids, due to the particularly low solubility of ferric iron at physiological pH in the tissue. The contamination of iron during processing, and the release of iron from iron-containing proteins, may increase inorganic iron during processing and storage (Decker *et al.*, 1988; Decker and Xu, 1998).

Two main oxidative mechanisms are ascribed to LMW-Fe:

1. the reaction of ferrous iron with hydrogen peroxide to generate the highly oxidizing hydroxyl radical, also known as Fenton reaction (Kanner, 1994), and
2. the generation of free radicals through lipid hydroperoxide-decomposition.

Both ferric and ferrous ions can participate in the generation of hydroperoxide-derived free radicals, but ferrous is significantly more efficient than ferric in generating free radicals (O'Brien, 1969). Accordingly, the oxidant activity of

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inorganic iron is frequently enhanced by reducing systems that convert the ferric ions into the most active ferrous ions (Soyer and Hultin, 2000; Pazos *et al.*, 2006b). Fish muscle contains enzymatic and non-enzymatic systems with the ability to activate ferric ions. The enzymatic iron-reducing systems of fish muscle are NAD(P)H-dependent, and are mainly found in the endoplasmic and sarcoplamic reticula (Mcdonald and Hultin, 1987; Decker *et al.*, 1988; Soyer and Hultin, 2000). Ascorbate is the most studied non-enzymatic iron-reducing system in fish muscle because ascorbate has a powerful reducing capacity as a consequence of its low reduction potential. Additionally, it is localized in the cytosol and is therefore in contact with most of the LMW-Fe.

Lipoxygenases
Lipoxygenases are iron containing enzymes localized in the cytosol or microsomal fraction (Harris and Tall, 1994). The enzyme catalyses the stereoselective incorporation of molecular oxygen in 1,4-cis-pentadiene structures of PUFA. Lipoxygenases have been localized in skin, gills and muscle of various fish species (German *et al.*, 1986; Medina *et al.*, 1999a; Banerjee *et al.*, 2002). Lipoxygenase-derived volatiles may be important for fresh aroma in fish, but their contribution to off-flavor generation during storage remains doubtful. German *et al.* (1992) noted that lipoxygenases have low stability, as they are deactivated by up to 50% after 3 h at 0 ºC, and completely deactivated with a single freeze-thaw cycle.

3.2.3 Endogenous antioxidants

The antioxidant defense system of fish muscle is able to control oxidation under normal *in vivo* conditions in such a complicated matrix rich in PUFA, which is a substrate prone to develop oxidative degradations, and loaded with pro-oxidant substances. However, the endogenous barrier against oxidation is continuously weakened *post mortem* as oxidative stress progresses (Jia *et al.*, 1996; Undeland *et al.*, 1999). The multifunctional antioxidant system of fish muscle includes two types of compounds: small radical-scavenging substances and enzymes such as peroxidases, catalase and superoxide dismutase, which remove lipid peroxides, and the reactive oxygen species hydrogen peroxide, and superoxide, respectively (Decker *et al.*, 2000). Among the most important endogenous free-radical scavengers of fish muscle, there are lipophilic compounds, such as α-tocopherol and ubiquinol, and water-soluble substances such as ascorbate and glutathione (Fig. 3.1).

Free-radical scavengers or chain-breaking antioxidants can neutralize radicals implicated in lipid oxidation (i.e., lipid alkyl ($R^\bullet$), alkoxyl ($RO^\bullet$) and peroxyl ($ROO^\bullet$) radicals) through the donation of electrons and hydrogen atoms (Frankel, 1998). Therefore, the effectiveness of free radical-scavengers should be dependent on chemical properties such as hydrogen bond dissociation enthalpies (BDE) and reduction potentials since those parameters quantify the thermodynamic facility to transfer hydrogen atoms and electrons, respectively.

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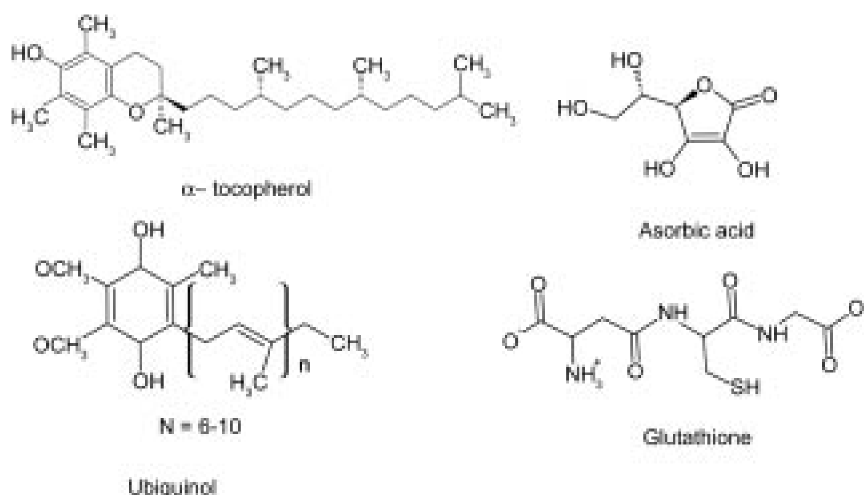

Fig. 3.1 Chemical structures of lipid-soluble and water-soluble antioxidants more relevant in fish muscle.

Compounds with low hydrogen-BDE can more easily transfer hydrogen atoms to free radicals, and substances endowed with minor reduction potentials will be more thermodynamically favored to donate electrons. Similarly, the electron transfer from the endogenous free radical scavengers (α-tocopherol, ubiquinol and ascorbate) to lipid peroxidation radicals is thermodynamically favorable, since the endogenous compound is provided by lower one-electron reduction potentials than lipid peroxides (alkyl, alkoxyl and peroxyl) and the powerful oxidizing hydroxyl radical (Buettner, 1993). For example, the alkyl radical formed in a PUFA by hydrogen atom abstraction has a standard one-electron reduction potential of 0.6 V, proving significantly better than those of α-tocopherol ($E^{o\prime} = 0.5$ V), ascorbate ($E^{o\prime} = 0.28$ V) and ubiquinol ($E^{o\prime} = 0.20$ V) (Table 3.1). Compared to the reduction of alkyl radicals, the electron transfer to peroxyl, alkoxyl and hydroxyl radicals is even more thermodynamically

Table 3.1 Standard one-electron reduction potentials for the most relevant free radicals implicated on lipid oxidation and endogenous antioxidants

Free radical or antioxidant	$E^{o\prime}$ (V)
$HO^{\bullet}$, H^+/H_2O	2.31
$RO^{\bullet}$, H^+/ROH	1.60
$ROO^{\bullet}$, H^+/ROOH	1.00
$PUFA^{\bullet}$, H^+/PUFA-H	0.60
α-Tocopheroxyl$^{\bullet}$, H^+/α-tocopherol	0.50
Ascorbate$^{\bullet}$, H^+/ascorbate	0.28
Semiubiquinone$^{\bullet}$, H^+/ubiquinol	0.20

Source: Adapted from Buettner (1993)

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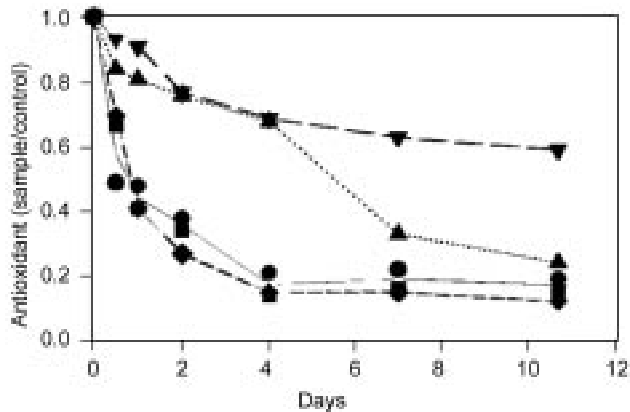

Fig. 3.2 Kinetics of consumption of the endogenous antioxidants in mackerel dark muscle during chilling storage. Results are expressed as the ratio between the antioxidants found in the chilled sample and in non oxidized control samples: ● ascorbate, ■ total glutathione, ▲ α-tocopherol, ▼ ubiquinone-10, ◆ ubiquinol-10. Source: Petillo *et al.* (1998).

favored, since the standard reduction potential is 1.0 V for peroxyl radicals and higher for alkoxyl and hydroxyl radicals, $E^{o\prime} = 1.6\,V$ and $E^{o\prime} = 2.31\,V$, respectively.

As a consequence of this radical-scavenging activity, the endogenous antioxidants are continuously oxidized and finally consumed in post mortem conditions. It is in this state that the natural supply of antioxidants is suppressed. Petillo *et al.* (1998) report different relative rates of consumption for the endogenous antioxidants of mackerel muscle during chilling storage: ubiquinol $\approx$ ascorbate $\approx$ total glutathione > α-tocopherol (Fig. 3.2). Similar tendencies are described under frozen storage, and so, total glutathione exhibits faster depletion than endogenous α-tocopherol in minced mackerel (Erickson, 1993a, 1993b). Ascorbate is also consumed more rapidly than α-tocopherol in chilled horse mackerel muscle (Iglesias *et al.*, 2009). The kinetics of antioxidant loss in fish muscle are in concordance with the antioxidant pecking order predicted by the one-electron standard reduction potentials (Buettner, 1993); α-tocopherol ($E^{o\prime} = 0.5\,V$) possesses much higher reduction potential than ascorbate ($E^{o\prime} = 0.28\,V$) and ubiquinol ($E^{o\prime} = 0.20\,V$), which show similar values (Table 3.1). This antioxidant hierarchy shows that ascorbate and ubiquinol deactivate free radicals in the early steps of oxidation, while α-tocopherol is a later barrier of defense against oxidation. In agreement with the antioxidant pecking order, it is well-documented that the α-tocopheroxyl radical formed during antioxidant actions of α-tocopherol, can be regenerated by ascorbic acid (Packer *et al.*, 1979) and ubiquinol (Mukai *et al.*, 1992). Such cooperative activity between antioxidants seems to explain the antioxidant synergism observed when α-tocopherol and ascorbic acid are used together (Frankel, 1998). This natural redox cycle between endogenous antioxidants can be modified by the addition of exogenous

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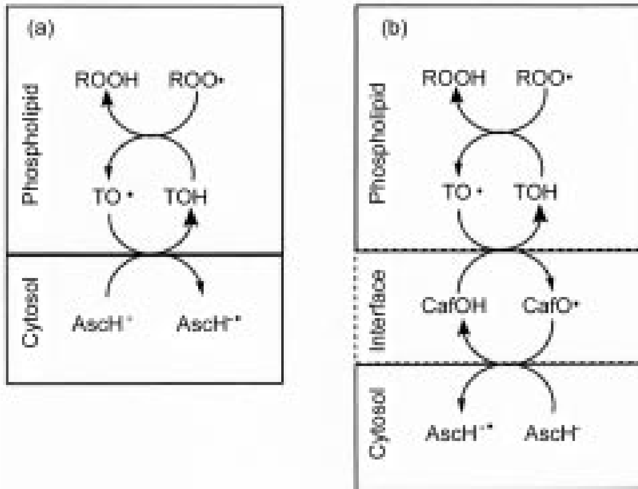

Fig. 3.3 Redox cycles between endogenous α-tocopherol (TOH) and ascorbate ($AscH^-$) of fish muscle in the absence (a) and the presence (b) of exogenous caffeic acid (CafOH).

antioxidant compounds (Fig. 3.3). As an example, the supplementation of caffeic acid, a phenolic compound with a high antioxidant capacity to prevent fish oxidation, appears to directly regenerate endogenous α-tocopherol through the reduction of the α-tocopheroxyl radicals and, at the same time, caffeic acid is repaired by endogenous ascorbate (Iglesias *et al.*, 2009) (Fig. 3.3).

The accumulation of tocopherolquinone, a major oxidation byproduct of tocopherol, has been successfully correlated with the formation of lipid peroxides in fish muscle during chilling and frozen storage (Pazos *et al.*, 2005d). Figure 3.4 shows how the depletion below critical levels of endogenous α-tocopherol produces an exponential generation of lipid peroxides both in frozen fish mince and fillets. This finding highlights the importance of maintaining α-tocopherol levels to protect fish against oxidation.

3.2.4 Extrinsic factors

The oxidation pattern of fish products can be drastically influenced by processing treatments and storage conditions (Decker and Xu, 1998). Therefore, identifying the response of fish muscle to processing and storage treatments is essential in order to optimize shelf-life and quality. The typical effect of common technological treatments will be covered in the following section.

Degree of tissue integrity: whole, fillet, skinning and minced

The rate of oxidation is greater with any loss of muscle integrity. Accordingly, whole fish exhibit greater oxidative stability than in filleted fish (Aubourg *et al.*, 2004, 2005), which in turn is found to be more stable than in minced fish.

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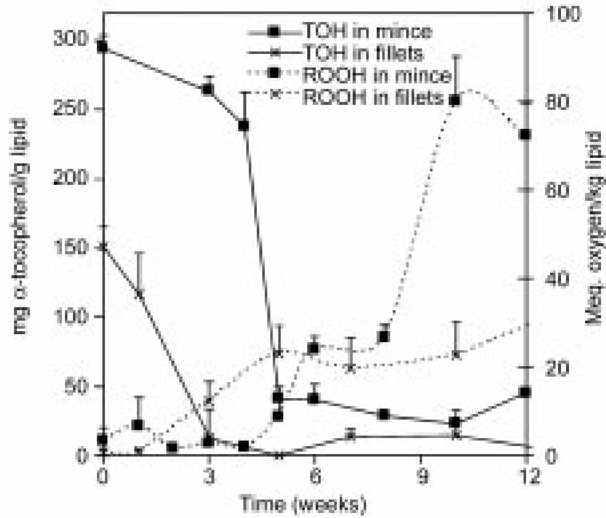

Fig. 3.4 Consumption of α-tocopherol (TOH) and formation of lipid peroxides (ROOH) in horse mackerel fillets and mince during frozen storage.

Mechanical actions such as mincing, and to a lesser extent filleting, disrupt the cellular antioxidative compartmentalization defense mechanism that limits the contact between membrane lipids and pro-oxidants, and also increases exposure of the tissue to oxygen (Undeland *et al.*, 1998a). It has been suggested that these two consequences are responsible for the prominent oxidative instability of mince, and to a lesser extent of fillets. Skin has a protective effect against oxidation on underlying areas, as it limits oxygen accessibility to the tissues; therefore, skinning may accelerate oxidation during storage (Undeland *et al.*, 1998b). The high proportion of dark muscle, elevated lipid content and low level of α-tocopherol in the tissue situated under the skin layer may also contribute to the pro-oxidative effect of skinning.

Washing

Washing is designed to remove constituents of the tissue. It is most extensively employed in the process of making surimi, to enrich the final product with myofibril proteins. The washing process not only removes soluble proteins, but may also remove fat, pro-oxidants, and antioxidants (Eymard *et al.*, 2005). Therefore, the oxidative stability of muscle ultimately depends upon the antioxidant/pro-oxidant balance that remains after the muscle is washed. Eymard *et al.* (2009) have demonstrated that horse mackerel mince is partially oxidized during a washing step with cold distilled water; the resulting washed mince is significantly less stable than unwashed mince during chilled storage. Undeland *et al.* (1998a) have also observed a reduction in the oxidative stability of minced herring during frozen storage, despite the fact that an important

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proportion of redox active metals, such as iron and copper, were removed during the washing treatment. It is thought that washing may promote oxidation in mince because it effectively removes antioxidants from the fish, and since the neutral lipids are more easily removed than polar lipids, phospholipids and free fatty acids are concentrated in the end product. In order to maintain the antioxidant/pro-oxidant balance, investigators have recommended washing with antioxidant solutions (Kelleher *et al.*, 1994).

Washing appears to more effectively improve the preservation of fish fillets. Richards *et al.* (1998) showed that the stability of mackerel fillets obtained in rigor is improved by washing with water. The improvement of oxidative stability by washing has been attributed to the removal of hemoglobin from the fillet surface, whilst the level of endogenous antioxidants remains stable due to the high tissue integrity of fillets.

Freezing

Both enzymatic and nonenzymatic reactions associated with oxidation are significantly decreased at temperatures below −10 ºC (Erickson, 2002). The oxidative stability is particularly extended by frozen storage in the case of lean fish species. It is possible, for instance, to preserve the quality of species such as hake for approximately 12 months at −20 ºC, a typical freezing temperature for the fish industry (Herrero and Careche, 2006). However, frozen storage is not very efficient for preserving fatty fish species from oxidation. For example, horse mackerel fillets stored at −20 ºC maintain satisfactory commercial quality for only 1 month, and the whole fish cannot be preserved for more than 5 months (Aubourg *et al.*, 2004). To achieve a significant increase in shelf-life for fatty fish species, storage temperatures as low as −30 to −40 ºC are necessary. Since the presence of oxygen is vital for oxidation to occur, glazing has often been used to retard oxidation in frozen fish, by placing an ice layer or glaze on the surface of the product to prevent air contact (Erickson, 2002).

3.3 Effects of oxidation on sensory and nutritional quality and shelf-life

Lipid oxidation involves the generation of carbon-centered radicals at bisallylic positions of PUFA, which will react rapidly with molecular oxygen to generate peroxyl radicals (Fig. 3.5). Peroxyl radicals can abstract a hydrogen atom from another PUFA to originate lipid hydroperoxides and new alkyl radicals of unsaturated lipids. The alkyl radicals formed are converted to additional peroxyl radicals in the presence of molecular oxygen, which will repeat the chain radical reactions implicated in the lipid oxidation process. By themselves, lipid hydroperoxides are not considered to be damaging to food quality, but they are very unstable and will break down to volatile compounds responsible for off-flavors and free radial species (alkyl and alkoxyl radicals) that autocatalyzed free radical chain reactions (Frankel, 1998). Furthermore, primary and secondary

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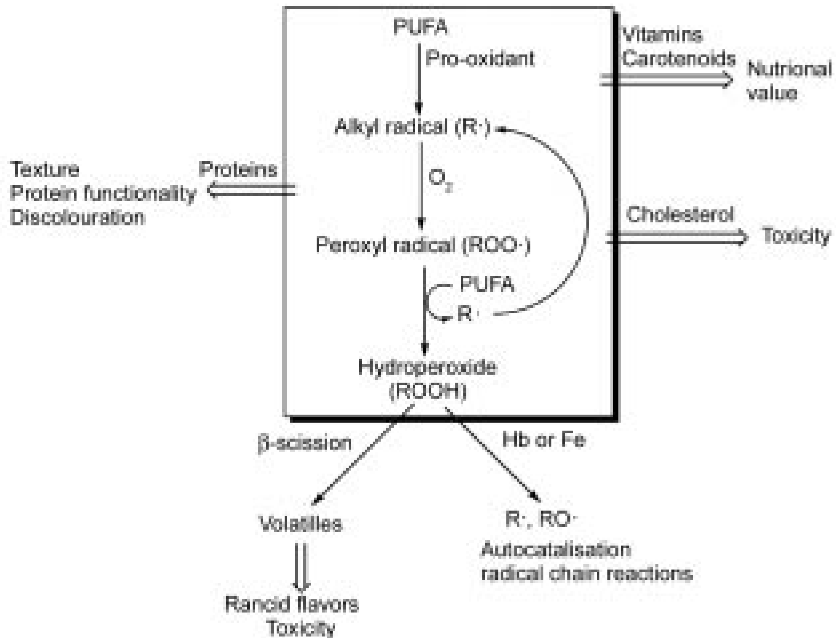

Fig. 3.5 Negative outcomes on sensory, nutritional value and toxicity of fish derived from the production of lipid oxidation byproducts and their interaction with the components of fish muscle.

lipid oxidation byproducts may also react with components of fish muscle, such as proteins, amino acids, vitamins and cholesterol. Such interactions with lipid oxidation byproducts produce degradations, or undesirable modifications of the fish components, and they are notably implicated in the loss of quality linked to oxidation.

The loss of quality in oxidized muscle-based products is generally characterized by flavor and odor deterioration, discoloration, destruction of nutrients and possible formation of toxic compounds (Kanner, 1994). Therefore, an optimum control of oxidation is critical in order to maintain sensory attributes and consumer acceptability, as well as preserve nutritional value and safety in fish products. Figure 3.5 shows the most negative outcomes on sensory, nutritional value and toxicity of fish derived from the production of lipid oxidation byproducts and their interaction with the components of fish muscle. The accumulation of volatiles, or secondary oxidation byproducts, is directly related to the development of flavors and odors associated with rancidity. Aldehydes, hydroperoxides and epoxides are oxidation byproducts with a potentially harmful impact on safety, since different studies suggest the potential toxicity of those substances (Varlet *et al.*, 2007; Kolakowska *et al.*, 2000). Furthermore, under extensive oxidation, the content in PUFA can significantly diminish in fish muscle, consequently reducing its nutritional value (Pazos *et al.*, 2005a). In summary, the interaction of oxidation byproducts with the protein components

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of fish muscle has a negative impact on sensory aspects such as texture and color (Estevez *et al.*, 2006; Suman *et al.*, 2006) and on the functional properties of these proteins (Kolakowska *et al.*, 2000). Oxidation byproducts may also degrade vitamins and carotenoids, reducing the nutritional value of the final fish product. Cholesterol oxidation products are generated under conditions of oxidative stress, and such cholesterol derivatives may be detrimental to the human body (Guardiola *et al.*, 1996).

3.3.1 Oxidative degradation of fish proteins by lipid oxidation by-products

Myofibrillar proteins are the most abundant proteins in fish muscle, constituting 65–75% of the total muscle proteins. Among myofibrillar proteins are included contractile proteins, such as myosin and actin, regulatory proteins, such as tropomyosin and troponin, and other minor proteins. The oxidative modification of myofibril proteins is related to the undesirable modification of textural quality and protein functionality in muscle tissues, such as water-holding capacity reduction and formation of protein aggregates (Xiong, 2000; Torres-Arreola *et al.*, 2007). Sarcoplasmic proteins, or water-soluble proteins, constitute between 20 and 30% of total proteins, and they mostly consist of enzymes involved in the biochemical processes of muscle tissues. The sarcoplasmic proteins hemoglobin/myoglobin are responsible for the reddish muscle of fatty fish species, and their oxidation is the principal cause of muscle discoloration in those fish species (Chaijan *et al.*, 2005). Fish also contains connective tissue proteins, such as collagen, although its content is relatively low in most commercial fish species.

The oxidative damage of proteins may cause backbone cleavage, oxidation of side-chain groups, cross-linking, unfolding, and the formation of further reactive species such as hydroperoxides, carbonyls and 3,4-dihydroxyphenylalanine (DOPA) (Xiong, 2000; Davies, 2005; Hawkins and Davies, 2001). In fish muscle, several investigations have shown similar patterns for lipid and protein oxidation during chilled and frozen storage, suggesting that both oxidative degradations are developed simultaneously (Eymard *et al.*, 2009; Baron *et al.*, 2007; Saeed and Howell, 2002; Tokur and Korkmaz, 2007). However, Andersen *et al.* (2007) have reported a different tendency in salted herring during ripening, since an extensive protein oxidation was detected during ripening storage, but no lipid oxidation. Authors suggested that blood brine and most especially the pro-oxidant activity of Hb are both implicated in the progress of protein oxidation in salted herring. There is evidence that myosin can be directly oxidized by hypervalent heme proteins (Lund *et al.*, 2008), which are formed through the reaction of heme proteins with either hydrogen peroxide or lipid hydroperoxides. Mb-mediated oxidation of myosin generated thiyl and tyrosyl radicals in the myosin structure, and the consumption of cysteine and tyrosine residues. Frederiksen *et al.* (2008) later demonstrated that thiols are important for radical formation and cross-linking of myosin during oxidation with hypervalent Mb.

It has been demonstrated that either radical or nonradical lipid oxidation byproducts are precursors of oxidative damage on proteins (Davies and Dean,

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1997; Esterbauer *et al.*, 1991). Aldehyde compounds generated from the decomposition of lipid hydroperoxides are particularly reactive with proteins and peptides. The decomposition of lipid hydroperoxides produces volatile compounds, such as aldehydes, ketones, alcohols and hydrocarbons in the presence of metallic traces or heme proteins, and under conditions of elevated temperatures. The main mechanism for the formation of aldehydes from lipid hydroperoxides occurs through the homolytic β-scission of one of C–C bonds adjacent to the hydroperoxyl group (Frankel, 1998). The reaction proceeds via the lipid alkoxyl radical, with two surplus electrons of neighboring atoms forming a carbonyl double bond; whereas the second fragment derived from the lipid hydroperoxide remains with an unpaired electron on a carbon atom. Unsaturated aldehydes may suffer further oxidations to produce aldehydes of shorter carbon chain than the parent aldehydes.

Many volatiles with varied reactivity are produced in the course of lipid oxidation. The qualitative and quantitative profile of aldehydes produced during lipid oxidation is highly dependent of the fatty acid carbon length and number of double bonds (Frankel, 1998). Iglesias and Medina (2008) have reported approximately eighty different volatiles compounds in oxidized fish muscle. Within these compounds, 1-penten-3-ol, 2,3-pentanedione and 1-octen-3-ol have exhibited the greatest correlation with the lipid oxidation progress, and are thought to be appropriate markers for lipid oxidation in fish muscle. Volatile aldehydes such as alkanals, 2-alkenal and 2,4-alkadienals have also been identified as lipid oxidation byproducts in fish (Varlet *et al.*, 2007).

Saturated and unsaturated aldehydes appear to interact differently with proteins. Saturated aldehydes tend to form Schiff base adducts with the protein, while α,β-unsaturated aldehydes form mixtures of Schiff bases and Michael addition products (Kautiainen, 1992). Additionally, α,β-unsaturated aldehydes have shown a higher ability to promote the oxidation of oxymyoglobin than their saturated counterparts of equivalent carbon chain length (Faustman *et al.*, 1999). Chaijan *et al.* (2007) have also reported that 2-hexenal causes greater oxidation of fish myoglobin than hexanal. The interaction between fish myosin and aldehydes indicates a similar tendency; the reactivity of aldehydes is directly proportional to the number of carbons and double bonds (Chopin *et al.*, 2007). The reaction of myosin with aldehydes induces a loss in myosin solubility, a decrease in the sulfhydryl groups and the formation of dityrosine. All these modifications increase with the carbon chain length and unsaturation number of the aldehydes. Thanonkaew *et al.* (2006) have also indicated a loss in the solubility and sulfhydryl groups in squid myofibrillar proteins in the presence of aldehydes.

Free radicals derived from lipid oxidation may also cause oxidative modifications of proteins and peptides through free radical chain reactions similar to those for lipid oxidation, which involve initiation, propagation and termination steps (Xiong, 2000). The sites of free radical attack on proteins include both the amino acid side chains and the peptide backbone, and the attack often results in protein polymerization or fragmentation. Proteomic tools have

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been used recently to identify other targets of protein oxidation in fish muscle (Kjaersgard and Jessen, 2004; Kjaersgard *et al.*, 2006; Baron *et al.*, 2007; Kinoshita *et al.*, 2007; Pazos *et al.*, 2010). Proteomics has also been applied to provide markers for post-mortem deterioration in fish muscle (Kjaersgard and Jessen, 2003; Martinez and Friis, 2004).

3.3.2 Interaction of lipid oxidation byproducts with vitamins, carotenoids and cholesterol

Vitamins

Fish is an important source of vitamins the concentration of which depends on species, age and the anatomical part considered. Most vitamins may be damaged under conditions of high lipid oxidation stress. As previously indicated, the progress of lipid oxidation produces a total exhaustion of ascorbate (vitamin C) and α-tocopherol (vitamin E) levels, as a consequence of their free radical scavenging activity. The unsaturated structure of vitamins A and D makes them also susceptible to attacks by free radicals; it is therefore common to see a loss of vitamins A and D in oxidized fish muscle, with a subsequent loss in nutritional value.

Carotenoids

Carotenoids are a very diverse group (> 600 different compounds) of yellow-to-red-color polyenes. Astaxanthin is the most abundant carotenoid pigment in marine animals (Torrissen, 2000). In aquaculture, astaxanthin or canthaxanthin pigmentation is a crucial quality criterion on the flesh of salmonids. Furthermore, carotenoids can function as vitamin A precursors in vitamin A-depleted fish (Schiedt *et al.*, 1985). Carotenoids may react with peroxyl radicals and other free radical species to form carotenoid radicals and other decomposition products (Liebler, 1992).

Cholesterol

Cholesterol is an important member of the neutral lipids class present in the human body, and it is the most prominent sterol found in animal foodstuffs. As an unsaturated alcohol, it may undergo oxidation in conditions of elevated oxidative stress to form a wide range of cholesterol oxidation products (Smith, 1987; Ohshima *et al.*, 1993). 7-Hydroxycholesterol, 7-ketocholesterol, 25-hydroxycholesterol and epimeric epoxide-derivatives are some of the cholesterol oxidation products found in fish products (Ohshima *et al.*, 1993). Lebovics *et al.* (2009) have revealed that the prevention of lipid oxidation in frozen horse mackerel, through the application of a plant extract, effectively inhibits the generation of cholesterol oxides. These cholesterol oxidation products exert detrimental biological activities in the human body, such as carcinogenesis, mutagenicity, atherosclerosis and cytotoxicity (Guardiola *et al.*, 1996).

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3.4 Protecting fish and fish products against oxidation

Considering the problems caused by lipid and protein oxidation, it is clear that the inhibition of lipid oxidation is critical for maintaining an excellent sensorial and nutritional quality of fish and seafood products. Efforts are being made to increase the shelf-life of fish species during storage and processing, and especially in the manufacturing of new taylor-made fish products. *In vivo*, oxidative stability in tissues is controlled by the balance between the endogenous pro-oxidant and antioxidant components (Decker *et al.*, 2000). However, post-mortem, numerous reactions will change the oxidant/reducing balance of fish muscle (Hultin, 1994). The dynamic nature of fish tissues makes it difficult to reduce or minimize lipid oxidation, since the concentration and activity of pro-oxidants and reducing agents can change significantly during storage or processing. Additionally, processing factors such as temperature, light conditions, ingredients or coadjutants can significantly affect the apparition, rate and extension of lipid oxidation.

Innovative approaches are required to preserve quality due to the high vulnerability of the n-3 fatty acids of fish lipids to the effects of oxidation. Procedures aimed at extending the lag phase as long as possible, inhibiting the onset of rancid off-flavors and retarding lipid oxidations have been proposed in recent years. The problem is especially important during fish frozen storage and the manufacture and storage of minced muscle based products.

One of the most important factors for avoiding fish lipid oxidation is the careful control of temperatures. Frozen temperatures retard lipid oxidation but do not completely stop the reaction. Treatments such as ice glasses using salts, phosphates, antioxidants or alginates have been used to prevent surface drying and lipid oxidation during the transportation of fish (Matsumoto, 1979). In some on-board operations, fish are individually placed in plastic bags and vacuum sealed, then frozen in brine freezers (Karmas, 1982). Salt, added to water, creates an excellent medium for freezing large or irregularly shaped seafood, since typical saltwater (23% salt) remains liquid to −6 ºF. The process offers good heat transference, and so the product freezes quickly. In a brine freezer, whole fish are immersed in the mechanically chilled brine. Tuna, swordfish and some shellfish (such as king crab sections) are often brine-frozen. However, studies on small size fatty species such as mackerel, or horse mackerel, immersed in brine solution before freezing have shown a high level of peroxide formation during frozen storage (Aubourg and Ugliano, 2002). Employment of an appropriate antioxidant addition is recommended if salting pre-treatment is needed, in order to avoid a large lipid oxidation development, and ensure an extended shelf-life time.

Other on-board approaches consider the application of new temperature regimes, such as super chilling or chilling with ice slurries. Aubourg *et al.* (2006) have considered the use of a combined ozonized-ice slurry system for the on-board storage of some fish species. Ice slurry systems showed higher quality retention than traditional icing. However, no differences in lipid oxidation could be found between ice slurry and ozonized-ice slurry conditions.

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Chilled storage in ice slurry slows down lipid oxidation in several fish species when compared to traditional flake ice conditions (Losada *et al.*, 2004, 2005). When the quality of fish stored using a combined system consisting of ozone and ice slurry was checked and compared to that of fish stored under ice slurry conditions alone, no effect on oxidation development as a consequence of the presence of ozone was detected (Campos *et al.*, 2005). In addition, the application of vacuum packaging, controlled packaging atmosphere with nitrogen or carbon dioxide, and oxygen absorbers are processes now used widely by the fish product industry to control lipid oxidation (Vermeiren *et al.*, 1999; Sivertsvik *et al.*, 2002). Recent works also suggest that a combination of oxygen absorbers and essential oils could be used for inhibiting lipid oxidation of fish fillets (Mexisa *et al.*, 2009).

Washing with water is also a common practice in fish industry in order to eliminate pro-oxidants (Richards and Hultin, 2002). As has been previously discussed, a washing step will remove some pro-oxidants, such as hemoglobin, but also tissue factors that protect against lipid oxidation. The use of a washing solution with antioxidants can avoid this effect (Richards *et al.*, 1998, Kelleher *et al.*, 1994). Compounds such as ascorbate, used to keep hemoglobin in a reduced state, or chelators such as tripolyphosphates for iron ions have both been successfully added to washing water during the manufacture of mackerel surimi, and chilled and frozen mackerel fillets. An effective inhibition of fish lipid oxidation resulted from these treatments.

Although antioxidant application may inhibit oxidation processes, their direct application to fresh-cut foods is prohibited in many countries. Nevertheless, consumer interest in safe dietary antioxidants is promoting new research into natural products that are known to have antioxidant properties. Bioactive properties, such as the ability to promote healthy conditions and inhibit the development of lipid oxidation in foodstuffs, have resulted in the common application of antioxidant compounds to different foods (Shahidi and Naczk, 1995). In this area, several natural compounds have been effective in delaying lipid oxidation on fish muscle. Pure compounds or natural extracts have been used for supplementing food products made of minced fish muscle or surimi. Vegetable extracts such as those from tea (Ishihara *et al.*, 2000; Tang *et al.*, 2001), rosemary (Vareltzis *et al.*, 1997), olive oil (Medina *et al.*, 1999b), ginger (Fagbenro and Jauncey, 1994) or grape seeds (Pazos *et al.*, 2005a) composed of flavonoids, polyphenols, terpenoids, etc., have successfully inhibited the rancidity of seafood products such as fish patties, fermented fish, canned fish, and emulsified fish. Other natural extracts such as those obtained from materials such as light fish muscle have been also utilized in fish systems (Sannaveerappa *et al.*, 2007). Catechins and their gallate esters (He and Shahidi, 1997), procyanidins (Pazos *et al.*, 2006a), hydroxytyrosol (Pazos *et al.*, 2006a), flavonoids (Ramanathan and Das, 1992), carnosic acid and derivatives (Medina *et al.*, 2003) have also been successfully tested in fish muscle. Some of these components have recently been shown to inhibit the enzymatic activity of lipoxygenase in fish muscle (Banerjee *et al.*, 2002). The group of hydroxycinnamic acids has proven particularly attractive

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as natural antioxidants in seafood, as they are widely distributed in plants and vegetables, and can be obtained at low cost. They have displayed a high ability to inhibit rancidity in chilled and frozen fish dependent of their molecular structure (Medina *et al.*, 2009). The antioxidant efficiency of frozen horse mackerel stored at −10 ºC and −18 ºC was the same as that observed in chilled horse mackerel, confirming the capacity of chilled tests to predict antioxidant effectiveness at lower temperatures.

Some of the most abundant antioxidants in vegetable by-products are polyphenols like proanthocyanidins, flavanol oligomers, with a high number of galloylated and phenolic residues. In recent years, compounds originating from sources such as grape, pine, strawberries, and hazel have been successfully employed to inhibit lipid oxidation in fish products (Pazos *et al.*, 2005a, 2006a). A strong dependence of antioxidant effectiveness on fish muscle has been obtained depending on the presence of one or more catechin units (polymerization degree) and the number of galloylated groups (galloylation degree) set in the polyphenol molecule. A structure–activity relationship study showed that an increase in the degree of polymerization in the polyphenol structure increased the inhibitory potential towards lipid peroxidation. The results of these studies prove the existence of an optimal polymerization degree in the procyanidins framework (Fig. 3.6). The decrease in efficiency observed in the highly polymerized procyanidins is explained by the spatial conformation of these molecules, which does not facilitate the accessibility of hydroxyl groups for scavenging the different radical species (Saint-Cricq De Gaulejac *et al.*, 1999). Equally, electrostatic repulsion among hydroxilic groups can decrease effective metal chelatation.

Additionally, the antioxidant inhibition of procyanidins increased with an increased percentage of galloylation. Different studies have demonstrated that the pyrogallol moiety provides more hydrogen atoms or electrons than the catechol group (Bors *et al.*, 2000). An increase in the number of hydroxyl groups in the galloylated procyanidins enhances their interaction with the polar heads of lipids and the location in the lipid–water interface increases the antioxidant efficiency. The lower polarity of the galloylated procyanidins seems to facilitate this location in lipid membrane systems, such as fish muscle, since the increase in the hydroxyl group facilitates the interaction with the membrane. This fact has been related to their capacity to decrease membrane fluidity (Arora *et al.*, 2000; Sanz and Coll, 1992). Such reduction decreases the mobility of the lipid radicals and delays the lipid deterioration, improving the antioxidant efficiency of galloylated procyanidins in comparison to those homologous procyanidins which are not galloylated.

The addition of antioxidants to the oxidative sensitive sites of fish microsomes and muscle is an important determinant in the effectiveness of their preservation. The selective incorporation of several phenolics into membrane lipids is important because membranes are highly unsaturated phospholipids which contain iron-reducing enzymes and endogenous α-tocopherol: the major membrane antioxidant (Pazos *et al.*, 2006b). Use of a suitable antioxidant carrier or some previous treatment can improve antioxidant incorporation into

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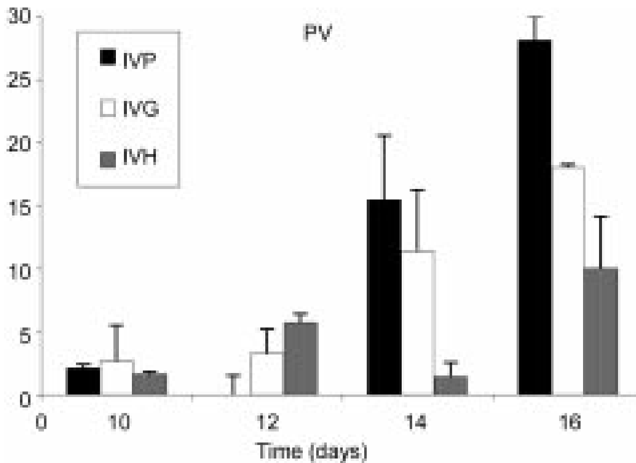

Fig. 3.6 Comparative efficacy of purified fractions from pine bark (IVP, not galloyled), grape pomace (IVG, low galloyzation) and witch hazel bark (IVH, high galloyzation) in chilled fish muscle on the formation of peroxides.

membranes. The incorporation or effective distribution of procyanidins on the fish muscle is improved by the use of a previous washing process on fish fillets prior to antioxidant supplementation (Pazos *et al.*, 2006a), resulting in a significant increase in the antioxidant effectiveness of procyanidins.

The supplementation of effective antioxidant additives into fish muscle has also shown a positive result in the fish endogenous antioxidant system. Pazos *et al.* (2005b) achieved a significant retardation of endogenous α-tocopherol consumption during fish muscle storage through the addition of polyphenols extracted from grape pomace; whereas ubiquinone-10 and total glutathione were significantly less protected. The addition of hydroxycinnamic acids to minced horse mackerel retarded the loss of glutathione and α-tocopherol during chilling and frozen storage as well. Such inhibition has been attributed to the preventative action of the antioxidant, but may also be due to a synergistic or protective effect that leads to a regeneration of oxidized antioxidants (Iglesias *et al.*, 2009). Phenolic acids like caffeic acid regenerated endogenous α-tocopherol from tocopheroxyl radicals, resulting in an antioxidant synergy consistent with the reduction of lipid oxidation observed in the fish muscle supplemented with these antioxidants.

As has been mentioned previously, the activation of hemoglobin to lead methemoglobin and cause liberation of free hemin is considered to be a major catalyzer of lipid oxidation in the fish muscle of fat and semifat fish species, but can be controlled by the addition of reducing agents (Richards *et al.*, 1998). The retardation of hemoglobin autooxidation during post-mortem storage resulted in the delay of lipid oxidation in washed fish muscle models and chilled fish (Undeland *et al.*, 2003). Different antioxidants have been able to inhibit oxidation promoted by hemoglobin and enzymatic NADH-iron and nonenzymic ascorbate-iron in fish membranes (Pazos *et al.*, 2006b). Furthermore, a

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relationship has been identified between the chelating capacity of the exogenous phenolic and the inhibition of oxidation promoted by hemoglobins in fish microsomes. Recent results show a possible loss of iron from the porphyrinic structure, which found the chelating ability to be the major factor involved in the inhibition of oxidation promoted by hemoglobin in cod liposomes (Undeland *et al.*, 2005).

The problem of obtaining an effective distribution of antioxidants into the food can be solved by microencapsulation. Microencapsulation of compounds in carrier matrices can provide protection against degradation, and enhance the stability and flavor of core materials (Kanakdande *et al.*, 2007). In recent years, several studies have been focused on the development of anti-oxidative packaging films coated with microencapsulated vegetables extracts. These novel films are a promising tool to prolong the shelf-life of fish products. In a recent study, Jung and coworkers (2009) have examined the effect of microcapsule-coated film containing volatile horseradish extract for inhibiting rancidity development during fish storage. Covering fish fillets with the horseradish-coated film containing natural antioxidants delayed oxidative discoloration and the formation of oxidative products (Fig. 3.7). Furthermore, the release of

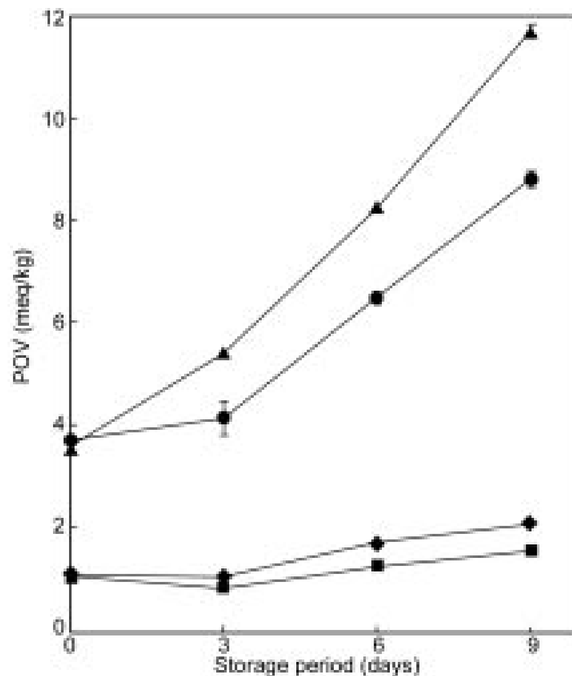

Fig. 3.7 Changes in POV of pork and fish covered with ordinary plastic film and microcapsule-coated film. Results are means of three replicates. Bars indicate standard deviation. ◆ Pork fillet covered with ordinary plastic film, ■ pork fillet covered with microcapsule-covered film, ▲ fish fillet covered with ordinary plastic film, ● fish fillet covered with microcapsule-covered film. Source: Jung *et al.* (2009).

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volatile antioxidants from the microcapsules to the package's atmosphere could be controlled by adjusting the amount of components added for microcapsule formation.

3.5 Future trends

Unlike other muscle foods, fish species are usually harvested in remote locations. The delay between the catch and the landing can be much longer than the time between landing and the shop. In addition to good on-board practices such as suitable levels of hygiene or rapid handling, the control of temperatures in all these processes is critical. The use of ice slurry, antioxidant washing solutions or the addition of reductants and chelatants such as ascorbate and citrate into the slurry solution is now suggested in order to delay lipid oxidation in on-board fish.

In the aquaculture sector, the dietary supplementation of antioxidants is becoming a common practice to extend the shelf-life of postmortem fish. Fish feed often contains a high level of fat. Increasing the tissue level of α-tocopherol (vitamin E), ascorbic acid (vitamin C) or selenium, alone or in combinations, has been shown to successfully extend the shelf life of fish (Baker, 1997; Hemre *et al.*, 1997). This is the case for fish species that are valued for their bright colour, such as farmed Southern Bluefin Tuna (SBT) (*Thunnus maccoyii*). The bright red colour of these species is due to the myoglobin content of its meat, but during storage the myoglobin is oxidized to met-myoglobin and gradually changes from red to brown. The use of feeds fortified with the natural antioxidants dl-α-tocopherol acetate, l-ascorbic acid phosphate and selenium resulted in a significant inhibition of post-mortem discoloration due to oxidation (Thomas and Buchanan, 2006).

New functional seafood products are currently being formulated using these myriad opportunities to enrich fish muscle based products with bioactive antioxidants: antioxidants that can maintain oxidative stability while also contributing to bioactive health promoting properties. Restructured fish products are made from minced and/or chopped muscle plus additional ingredients, to which a new appearance and texture are given. In such products lipid oxidation is a significant problem, since chopped or minced fish muscle suffers rapid lipid oxidation. Effective fish antioxidants such as procyanidins, which can exert a direct preventive effect on the colon epithelial cells by acting as an antioxidant and a selective pro-apoptotic agent, have been proposed as functional ingredients for fish based products. Such food products are believed to be interesting and stable functional foods, offering the combined action of ω-3 fatty acids and natural polyphenols (Medina *et al.*, 2006). Recently, antioxidant dietary fibers such as natural additives that combine the beneficial effects of dietary fiber with natural antioxidants have also been studied in order to formulate new functional foods (Sánchez-Alonso *et al.*, 2007). Simultaneously, ways to incorporate antioxidant compounds from vegetable origin to the farmed

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fish through dietary intervention are now being explored; as in, for instance, pioneer studies into the incorporation of Selenium in farmed fish (Luten *et al.*, 2008).

3.6 Sources of further information and advice

The main researcher groups working on lipid oxidation are:

- Dr Edwin N. Frankel, Department of Food Science and Technology, University of California Davis, CA 95616-8598, USA
- Dr Bruce German, Department of Food Science and Technology, University of California Davis, CA 95616-8598, USA
- Dr Charlotte Jacobbsen, Danish Institute for Fisheries Research, Technical University of Denmark, Denmark
- Dr Ingrid Undeland, Chalmers University of Technology, Sweden
- Ivar Storrø, SINTEF, Applied Chemistry, 7034 Trondheim, Norway
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Oxidation and protection of milk and dairy products

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Abstract: Milk and dairy products are susceptible to oxidation, especially because of photosensitizing molecules such as riboflavin. Although relatively low in antioxidants, milk structure and composition offer some protective mechanisms against oxidation but milkfat modifications, processing and storage conditions increase the risk. Oxidation alters protein structure and fatty acid composition, reduces nutrient value, and degrades sensory quality of milk and dairy products. Light-induced oxidation is the primary concern for most products although modification of milk lipids to increase omega-3 lipids increases the potential for autoxidation. Appropriate selection of packaging materials for dairy products can protect against light-induced oxidation.

Key words: photooxidation, milk, dairy products, packaging, sensory.

4.1 Introduction

Oxidation reactions in milk and dairy products are common mechanisms for off-flavor and off-odor development. These changes result in decreased product shelf-life and reduced nutritional quality as well as reduced functionality for use as dairy ingredients in formulated foods. Compositional factors that influence oxidation of milk and dairy products include fatty acid profile, antioxidant activity within the system, enzyme systems with pro- or antioxidative function, and presence of transition metal ions (Nielsen *et al.*, 2002). Milk proteins and lipids are suceptible to oxidation reactions and altering the fatty acid profile to

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increase unsaturated fats increases the potential for oxidation. Some molecules function as oxidation initiators or as antioxidants. Riboflavin and chlorophyll are examples of photosensitizers in milk, transferring light energy to other molecules and initiating oxidative reactions (Webster *et al.*, 2009; Wold *et al.*, 2005; 2006a; 2006b). Processing, packaging and storage conditions influence shelf-life of milk and dairy products by contributing to or controlling oxidation. Natural antioxidants assist in controlling oxidative reactions but formulation with added antioxidants, atmospheric (light, oxygen) controls, and appropriate packaging selection is needed to protect milk and dairy product quality throughout shelf-life (Duncan and Webster, 2009).

4.2 Oxidation of milk components

The natural milk system is chemically and physically structured to protect nutrients in milk from undergoing oxidation. Characteristically, fresh raw milk is not oxidized, although spontaneous oxidation has been reported (Barrefors *et al.*, 1995). Pasteurized milk, processed from high quality raw milk, does not usually exhibit autoxidation reactions and exhibits low peroxide values (< 5 meq/kg fat) over several days of refrigerated (< 7 °C) storage (Allen, 1994). Light-induced oxidation is the primary concern, which can lead to autoxidation reactions. Storage at low temperatures efficiently preserves saturated fats but is not sufficient to protect polyunsaturated fats against oxidation. Oxidative reactions in dairy products are a concern, especially with products such as extended shelf life (ESL) milk and dry milk products (Madhavi *et al.*, 1996). Changes in composition or physical structure can increase susceptibility to both auto- and light-induced oxidation.

Whole milk comprises approximately 87% water, 3.25% fat and 4% protein. The remaining 5.75% consists of lactose, minerals and other solids. Triacylglycerides make up the majority (97–98%) of fat in milk, while the remaining 2–3% consists of small amounts of di- and mono-acylglycerides, free fatty acids, free cholesterol, esterified cholesterol, phospholipids, and glycolipids. Approximately 70% of fatty acids in milk are saturated. The remaining include monounsaturated (~27%, mostly palmitoleic and oleic acid), polyunsaturated (~4%, mostly linoleic acid) and minor components, such as phospholipids, glycolipids, sterols and fat soluble vitamins (A, D and E) (Fox, 1995).

Since the early 1990s, preharvest bovine nutritional practices and postharvest technologies for increasing unsaturated fatty acids, such as conjugated linoleic acid (CLA) and omega-3 fatty acids, have been studied in order to increase the nutritional profile of milk and dairy products (Allred *et al.*, 2006; Avramis *et al.*, 2003; Baer *et al.*, 2001; Bell *et al.*, 2006; Focant *et al.*, 1998; Gonzalez *et al.*, 2003; Havemose *et al.*, 2006; Jones *et al.*, 2005; Khanal *et al.*, 2005; Kitessa *et al.*, 2004; Kristensen *et al.*, 2004; Lacasse *et al.*, 2002; Nelson and Martini, 2009; Noakes *et al.*, 1996; Ramaswamy *et al.*, 2001; Stegeman *et al.*, 1992). The increased concentration of unsaturated fatty acids increases the

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risk of oxidation occurring in milk fat. Table 4.1 illustrates the composition of milkfat and the modification of unsaturated fats as a function of preharvest approaches. Increasing the concentration of unsaturated fats in dairy systems increases the challenges of protecting against oxidative degradation.

The delicate balance between anti- and oxidative processes in milk is influenced by factors such as bovine nutrition, degree of fatty acid unsaturation, content of transition metal ions, and content of antioxidants such as tocopherols

Table 4.1 Range in fatty acid composition of milkfat as modified by preharvest technologies

Fatty acid[b]	Control	Preharvest modification[a]
	g/100 g fatty acids reported	
$C_{6:0}$	0.87–2.79	0.63–2.4
$C_{8:0}$	0.87–1.63	0.48–1.2
$C_{10:0}$	2.72–4.15	1.26–2.67
$C_{12:0}$	2.88–4.81	1.70–3.02
$C_{14:0}$	10.17–14.53	8.17–11.29
$C_{14:1}$	0.51	0.35–0.58
$C_{15:0}$	1.62	1.06–1.14
$C_{16:0}$	28.83–38.62	21.12–34.1
$C_{16:1}$	1.50–1.82	1.06–1.82
$C_{17:1}$	0.23	0.18–0.20
$C_{18:0}$	9.5–13.1	10.9–13.99
$C_{18:1}$ $t = 11$ (VA[c])	1.04–3.29	1.10–7.81
$C_{18:1}$ *c*-9	17.82–21.0	19.92–26.39
$C_{18:2}$	1.55–4.71	2.16–5.99
$C_{18:3}$ *c*-6,9,12	0.04–0.06	0.01–0.06
$C_{18:3}$ *c*-9,12,15	0.42–0.61	0.41–0.91
CLA[3] *c*-9,*t*11	0.52–0.56	0.61–1.74
CLA *t*-10,*c*12	0.02–0.04	0.00–0.08
Total CLA	0.54–0.61	0.68–1.82
$C_{20:2}$	0.05	0.06
$C_{20:3}$ *c*-8,11,14	0.13–0.17	0.06–0.12
$C_{20:3}$ *c*-11,14,17	0.009–0.010	0.01–0.04
$C_{20:4}$	0.12–0.18	0.08–0.11
$C_{20:5}$ (EPA[c])	0.03	0.04–0.08
$C_{22:4}$	0.02–0.03	0.01–0.02
$C_{22:5}$	0.04	0.05–0.06
$C_{22:6}$ (DHA[c])	0.01–0.02	0.04–0.11
Total n-3 fatty acids	0.52–0.62	0.67–0.82
Total n-6 fatty acids	2.99–5.15	2.98–5.56
n-3:n-6	0.118–0.17	0.121–0.27
Saturated fatty acids	66.7	57.7–60.2
Unsaturated fatty acids	33.3	39.8–42.3

[a] Focant *et al.* (1998); Gonzalez *et al.* (2003); Allred *et al.* (2006); Nelson and Martini (2009).
[b] Expressed as number of carbons: number of double bonds; *c* = *cis* and *t* = *trans*.
[c] VA = vaccenic acid; EPA = eicosapentaenoic acid; DHA = Docosahexaenoic acid; CLA = conjugated linoleic acid; sum of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA isomers.

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and carotenoids (Barrefors *et al.*, 1995; Havemose *et al.*, 2006; Kristensen *et al.*, 2004; Lindmarck-Månasson and Akesson, 2000). Protein type and function also contribute to this balance. Composition and the physical association of molecules in relation to each other influence this balance as well.

4.2.1 Milkfat globule membrane function in protecting milkfat against oxidation

Milkfat globules in raw milk are encapsulated by the native milkfat globule membrane (MFGM). This membrane comprises neutral lipids, phospholipids, proteins, glycoproteins and other minor components. The membrane functions to increase globule stability in the hydrophilic water phase and provide protection of the core lipid (Keenan *et al.*, 1983). The surface of milkfat globules in processed milk is altered by physical disruption (homogenization) and thermal effects (pasteurization) on milkfat and the native MFGM (Bolling *et al.*, 2005; Elling and Duncan, 1996; Elling *et al.*, 1996; Scott *et al.*, 2003b). The natural protection provided by the native MFGM is altered, affecting the susceptibility of milk to oxidation.

Phospholipids, trace minerals and proteins associated with the MFGM play a role in the natural protection or susceptibility of milk to oxidation. Trace minerals (copper, iron, zinc) exist in higher concentration within the MFGM than in the core lipid or the aqueous phase of fluid milk and function as potential catalysts of lipid oxidation in milk (Allen, 1994; Aulakh and Stine, 1971). Model systems containing MFGM materials oxidized rapidly in the presence of copper and ascorbic acid but concentration influenced response (Chen and Nawar, 1991b). Xanthine oxidase and lactoperoxidase are metalloenzymes that increase oxidation and may contribute to the susceptibility of the membrane to lipid oxidation (Allen, 1994; Aurand *et al.*, 1977). The presence of small amounts of copper (Cu^{2+}) can increase effectiveness of the enzyme (Allen, 1994). Some membrane proteins, at high temperatures (95 ºC), reportedly provide an inhibitory effect on oxidation of milkfat possibly due to formation of –SH groups (Chen and Nawar, 1991b).

However, disruption of the membrane by processing increases the proportion and associations of prooxidants in the aqueous phase and in contact with susceptible lipids. Chen and Nawar (1991b) observed that MFGM or isolated membrane components as solids (dry state) protected against oxidation of milkfat at 50 ºC in the absence of water. The absence of water also inhibited pro-oxidative effects of trace metals. The physical state of the system affects oxygen transfer and certain pro-oxidative enzymes are less effective in the absence of water. At 95 ºC, MFGM components, especially non-lipid membrane solids, provided protection against oxidation of milkfat in both aqueous and dry applications.

The exact role of milk phospholipids and proteins is not clear, however, as evidence exists that they participate in both anti- and pro-oxidative activity. Approximately 40–60% of phospholipids at the MFGM surface are unsaturated,

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with one-third being polyunsaturated (Deeth, 1997). There is some indication that milk phospholipids, possibly in conjunction with α-tocopherol, may inhibit oxidative reactions (Chen and Nawar, 1991a). Milk phospholipids reportedly improved the oxidative stability of isolated milkfat trigylderides. At high temperatures (95 ºC), browning of phospholipid products may be important in protecting against oxidation (Chen and Nawar, 1991a). A free amino group in phosphatidylethanolamine (PE) has been identified as functioning as an antioxidant by reacting with carbonyl compounds to form Schiff base reaction products (Chen and Nawar, 1991a; Hussein *et al.*, 1986). The interaction of phospholipids in the milkfat globule membrane with proteins increases antioxidant activity. Chen and Nawar (1991a) suggested that the pro-oxidative activity of dipalmitoylethanolamine (DPE) was probably associated with the formation of a more dispersed membrane structure with more oxygen accessibility.

4.2.2 Milk protein oxidation

Some proteins in milk, especially casein and lactoferrin, are effective at binding metals (Cu^{2+}, Fe^{3+}) and preventing contact between metal ions and the lipid substrate (Allen, 1994). However, casein and whey proteins in the aqueous phase also are susceptible to oxidation reactions, leading to volatile secondary reaction products that contribute to off-aroma and flavor (Bekbölet, 1990; Gilmore and Dimick, 1979), as well as altering enzymatic activity and potentially affecting dairy product fermentation and cheese ripening (Dalsgaard *et al.*, 2007; 2008; Dalsgaard and Larsen, 2009). Protein oxidation can be initiated by heat, light, transition metals, certain food additives, enzymatic activity of the lactoperoxidase system, and the products of enzymatic and non-enzymatic browning (Dalsgaard *et al.*, 2007; Dalsgaard and Larsen, 2009). Milk proteins, peptides and amino acids are susceptible to free radical oxidation and this oxidation occurs relatively quickly (Allen and Joseph, 1985; Aurand *et al.*, 1966; Dalsgaard *et al.*, 2007; Dalsgaard and Larsen, 2009; Davies and Dean, 1997; Dimick, 1982; Østdal *et al.*, 2000). Protein susceptibility to oxidation is highly dependent on peptide or protein structure (Michaeli and Feitelson, 1995; Dalsgaard *et al.*, 2008; Dalsgaard and Larsen, 2009). In model systems including riboflavin, dairy proteins exposed to light may degrade or exhibit structural modifications as quickly as 4 hr with more significant effects occurring over 44 hr or more (Dalsgaard *et al.*, 2007; 2008; Dalsgaard and Larsen, 2009; Gilmore and Dimick, 1979). Conformational and structural changes including cross-linking, fragmentation of covalent bonds, and alterations of amino acids occur (Dalsgaard *et al.*, 2007; Dalsgaard and Larsen, 2009; Østdal *et al.*, 2000). Significant consequences of protein oxidation are polymerization and hydrolysis (Bekbölet, 1990) due to cross linking and breakdown of the primary structure of the protein (Dean *et al.*, 1997). Aldehydes, such as malondialdehyde, formed during moderate lipid oxidation are able to cross-link milk casein proteins (Wiking and Nielsen, 2004). β-casein is modified by forming Schiff bases, increasing susceptibility of the protein to proteolysis by plasmin (Wiking and

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Nielsen, 2004). Aggregation of proteins may occur in highly oxidized milk because of increased production of aldehydes and, as an indirect effect of high degree of lipid oxidation, may decrease the susceptibility of milk proteins to plasmin (Wiking and Nielsen, 2004).

Light energy also affects the susceptibility of milk proteins to aggregation and oxidation. Dalsgaard *et al.* (2007) studied the effects of broad spectrum visible light exposure (400–600 nm, 2200–2600 lx, up to 48 hr) on six different milk proteins with either random coil (α_{S1}- and α_{S2}-mixture, β-, and κ-casein) or globular (α-lactalbumin, β-lactoglobulin, and lactoferrin) structure. Changes in primary structure occurred for all proteins within 4 hr of light exposure as measured by increased carbonyl concentration and dityrosine formation. The casein proteins (random coil structure) were more sensitive to light exposure in the presence of riboflavin. Dityrosine formation, indicating cross-linking of protein structures, was more evident in α- and β-casein, with moderate levels observed in κ-casein and α-lactoglobulin (Dalsgaard *et al.*, 2007; Dalsgaard and Larsen, 2009). Losses of α-helix in α-lactalbumin and β-sheets in β-lactoglobulin were documented as changes in secondary structure for these two globular proteins whereas the casein proteins and lactoferrin did not demonstrate changes in secondary structure. Tertiary structural changes, as estimated by tryptophan emission, were seen for lactoferrin and α-lactalbumin with marginal changes observed for β-lactoglobulin; changes in tertiary structure could not be evaluated for the casein proteins. Changes in quarternary structures, observed as dimerization or polymerization, were observed for α- and β-caseins, α-lactalbumin, and lactoferrin but only slight changes were noted for κ-casein and β-lactoglobulin (Dalsgaard *et al.*, 2007). Globular proteins appeared to have greater oxidative stability than random coil proteins and lactoferrin was the most resistant to oxidation of the globular proteins; one hypothesis for this higher oxidative stability is that greater structural stability, which is reflected in the melting temperatures of globular proteins, may be important.

The carbonyl compound N-formylkynurenine, an oxidation product and a photosensitizer, may contribute to a chain reaction of free radical formation. Lactoferrin undergoing photooxidation had relatively low concentration of this compound, indicating that structural stability at higher levels may be related to relatively slight changes in the primary structure of this protein. Singlet oxygen only interacts with specific amino acids, including tryptophan, histidine, tyrosine, methionine, and cysteine. Most tryptophan residues within globular proteins are buried with the core. Lactoferrin also became more compact (tertiary structure), shielding tryptophan in the core of the protein structure. This, too, is a different behavior than observed for the other globular proteins, which demonstrated 3–10% unfolding.

4.2.3 Riboflavin and other photosensitizers in milk

Milk contains a variety of compounds that act as photosensitizers, most notably riboflavin (Borlet *et al.*, 2001; Sattar and deMan, 1976; Dimick, 1982; Bekbölet,

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1990; Skibsted, 2000). In addition to riboflavin, other known photosensitizers include chlorophyll a and b, protoporphyrin, hematoporphyrin and at least two tetrapyrrol compounds that have been identified with potential photosensitizer function in butter (Wold *et al.*, 2005; 2006a). Riboflavin, found in the aqueous phase of milk, has an average concentration of 1.75 mg/L (Dimick, 1982). Cream and milk have been shown to have measurable amounts of chlorophyll a and b, but only very small amounts of protoporphyrin (Wold *et al.*, 2005). Photosensitizers absorb light of specific wavelengths and initiate free radical oxidation reactions. Fluorescence spectroscopy, a rapid and nondestructive method, has been used to measure photosensitizer breakdown in dairy products, providing an indirect measure of the initiation of the process and an indication of the onset of early lipid oxidation (Veberg *et al.*, 2007). These oxidation reactions ultimately lead to the production of off-odor and flavor compounds.

4.2.4 Vitamin antioxidants in milk

Milk has a much greater potential of undergoing oxidative deterioration than is actually observed (Allen, 1994). In addition to the antioxidative functions of proteins and phospholipids mentioned previously, ascorbic acid (< 20 ppm) and tocopherols (13–35 μg/g milkfat) function as antioxidants in the milk system (Allen, 1994; Barrefors *et al.*, 1995; van Aardt *et al.*, 2004; 2005a; 2005b; 2007). Increasing antioxidants in milk to control oxidized flavor in milk has been attempted by injecting antioxidants in the muscles of dairy cows, adding antioxidants to the feed of dairy cows, or directly adding antioxidants to dairy products (Charmley and Nicholson, 1993; Focant *et al.*, 1998; Jung *et al.*, 1998; van Aardt *et al.*, 2005a; 2005b; 2007). The biggest limitations to adding antioxidants to milk via injection into muscles or addition through diet are low yield and a short duration of elevated antioxidant levels in milk. A limitation to adding antioxidants directly to milk in one large dose is rapid depletion of the antioxidants (Charmley and Nicholson, 1993; Focant *et al.*, 1998; Jung *et al.*, 1998). By adding antioxidants to milk, whether by direct addition or by controlled release from the package, antioxidants will be considered an additive (van Aardt *et al.*, 2005a). Therefore, their use must carry with it labeling requirements.

Although milk contains natural antioxidants, processing and storage deplete these natural resources (Jensen and Nielsen, 1996; Rosenthal *et al.*, 1993). Tocopherol content in milkfat can be increased by supplementation into the bovine diet (Focant *et al.*, 1998) but is reduced by removing milkfat during separation. The concentration of ascorbic acid decreases rapidly during storage, falling to nearly zero within seven days of storage. About 33% of dissolved oxygen is consumed in the process (Allen, 1994). Ultrahigh temperature (UHT) milk is more susceptible to metal or light-induced oxidation because ascorbic acid is depleted early in the shelf-life of the product, especially at warmer (> 7 ºC) temperatures.

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4.3 Processing and storage conditions contributing to oxidation of milk and dairy products

Low temperature storage alone is not enough to protect milk from oxidation (Madhavi *et al.*, 1996). Separation of cream and skim phases, churning, and homogenization disrupt native milk lipid globules and fragment the MFGM, increasing the opportunity for catalysts to intermix with lipids (Allen, 1994). In unheated skim milk, proteolysis is increased by mild oxidation but more extensive oxidation inhibits proteolysis (Igarashi, 1990). Homogenization creates smaller globules, reducing globule size and increasing the surface area by as much as 10 times, which increases the susceptibility of milk to oxidation (Allen, 1994; Bolling *et al.*, 2005; Bradley, 1980; Elling and Duncan, 1996; Elling *et al.*, 1996; Jensen, 2002). Churning increases the contact of copper and iron with milk lipids and susceptibility to oxidation. Drying processes and incorporating additives also can increase potential for oxidation (Allen, 1994). Water used for cleaning and sanitation can be sources of metal ions that initiate oxidation. Copper and other metal plumbing materials can increase the concentration of metal ions in water sources (Dietrich *et al.*, 2003).

The most common oxidation problem for milk and dairy products is a function of light exposure. Both natural and artificial sources of light can cause light oxidation (Bekbölet, 1990; Borlet *et al.*, 2001; Bosset *et al.*, 1995; Koo and Kim, 1971). The practice of displaying milk and dairy products packaged in transparent and/or translucent packaging materials under high intensity fluorescent light exacerbates the problem of oxidation. Exposure to fluorescent lighting plays a major role in the production of oxidized flavor in milk because the majority of milk today is sold through supermarkets and retail establishments and can remain on retail shelves for ten to twenty-one days after processing (Hoskin and Dimick, 1979). Transparent and translucent packaging allows light to reach the food, making it susceptible to photooxidation (Duncan and Webster, 2009; Webster *et al.*, 2009). Milk may be exposed to light between 750 to over 2000 lux for 24 hr a day during the distribution and marketing period. The average light intensity on retail shelves is 2000 lux and the average exposure time is 8 hr (Chapman, 2002). Cool white fluorescent lighting, typically used in refrigerated cases in retail food markets, is not the best type to use with photosensitive foods because it has a large emission band of blue-green light (444 nm), which is very close to the major absorption band of riboflavin (Bosset *et al.*, 1995). Light intensity also plays a role in the rate of off-flavor development. Whited *et al.*, (2002) found that exposure of whole and reduced fat milk to 1000 ± 5% lux light had significantly less oxidized flavor (detected by a trained sensory panel) compared to milk exposed to 2000 ± 5% lux light for the same amount of time and temperature (16 hr at 6 °C).

4.4 Photooxidation of milk and dairy products

Exposure to light is a well-known cause of flavor and nutritional degradation in milk (Bekbölet, 1990; Borlet *et al.*, 2001; Bosset *et al.*, 1995). A number of

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factors, including light source, light intensity, light wavelength, exposure time, and storage temperature affect the rate of light-induced oxidation in foods. Packaging material also affects oxidation rate of foods by controlling the intensity and wavelength of light and oxygen (Bekbölet, 1990; Duncan and Webster, 2009; Hoskin and Dimick, 1979). Controlling the exposure of photosensitizing molecules in milk is important in protecting milk and dairy products from oxidation.

Riboflavin, one of the most studied photosensitizers, is found in high concentrations in the whey fraction of milk and increases the susceptibility of milk to photooxidation (Bekbölet, 1990). Riboflavin can exist in three oxidation states – fully oxidized, radical, and fully reduced. At each oxidation state, there are three conjugate acid-bases. Due to the pH of milk (~6.8), riboflavin has three stable forms that have absorption maxima within the visible range at 400 nm, 446 nm, and 570 nm (Kyte, 1995). Porphyrins and chlorins produce singlet oxygen upon exposure to light (Bekbölet, 1990).

Violet and blue light have been been considered the most harmful to dairy products because riboflavin is a photosensitizer. Riboflavin exposed to ultraviolet or visible light up to 500 nm initiates Type I and Type III photoreactions (Aurand *et al.*, 1977). The implication of other photosensitizers in light-induced oxidation of dairy products indicates that other wavelengths also are of concern (Webster *et al.*, 2009; Wold *et al.*, 2005; 2006a). Protoporphyrin and chlorophyll-like molecules also absorb light in the visible regions. Most photosensitizers are photolabile (Wold *et al.*, 2005; 2006a); for example, riboflavin in whole milk was reduced over 75% within 32 hr when exposed to 2690 lx of fluorescent light (Allen and Parks, 1979). When excess oxygen is present, singlet oxygen (Type II reactions) is generated. Under low oxygen atmosphere, light energy directly degrades sensitive molecules and free radicals (Type I photoreactions) occur.

4.4.1 Effect of photooxidation and oxygen on vitamins in milk

Photooxidation of milk components, especially the vitamins, can be destructive to the nutritional quality of milk. Losses of riboflavin, vitamin A, D, and C, and thiamine because of light exposure of milk have been reported. When riboflavin becomes excited it undergoes one of several transformations:

- the excited electron re-enters the original bonding orbital, giving off energy in the form of light or heat;
- the molecule abstracts hydrogen from the substrate; or
- it transfers its energy to another molecule through sensitization (Rosenthal, 1992).

Theoretically, once these transformations occur, riboflavin goes back to its ground state and is available to be excited again. In reality, however, some riboflavin molecules are destroyed during these reactions and are no longer available for further free radical initiation. Degradation compounds of riboflavin

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include lumiflavin and lumichrome (Huang *et al.*, 2006). As reviewed by Bradley (1980), the rate of riboflavin destruction was found to be proportional to the amount of light coming through the container, the wavelength of light and the presence of riboflavin (Herreid *et al.*, 1952; Sattar *et al.*, 1977a; 1977b). Maniere and Dimick (1975) showed that this rate was greater when riboflavin was in its free form and unassociated with proteins or fat in milk.

Riboflavin and vitamin A degradation by light are inversely related to fat content and loss occurs quickly under storage conditions characteristic of retail display cases. Lower fat content allows more light to penetrate into the milk (Senyk and Shipe, 1981). Plain milk, packaged in clear glass and polyethylene bottles, lost 75% of its riboflavin after 1 hr exposure to direct sunlight while chocolate milk only lost 35% and 45% after 1 and 2 hr exposure, respectively (Paik and Kim, 1976). Whole milk, 2% milk, 1% milk and nonfat milk had vitamin A losses of 18%, 26%, 28%, and 31%, respectively, after 2 hr exposure to 2000 lumens/in^2 when packaged in non-returnable polyethylene containers and losses increased to as much as 57% (nonfat milk) within 4 hr (Senyk and Shipe, 1981).

Packaging modifications such as oxygen barriers, ultraviolet absorbers or pigmentations to control oxygen limits and wavelength effects assist in protecting nutrients from oxidation and degradation. Vitamin A is degraded at wavelengths below 415 nm and limiting exposure to wavelengths below 465 nm greatly reduced vitamin A loss (Sattar *et al.*, 1977a; 1977b). Addition of UV absorbers (Tinuvin 326 and Cyasorb 531) to high density polyethylene (HDPE) pouches significantly protected milk from vitamin A destruction when exposed to fluorescent lighting (190 ± 10 lumens) for 72 hr at 1.5 ºC, holding the loss to only 10% loss. Tinuvin 622, however, did not protect against vitamin A destruction under the same conditions. Tinuvin 326 and Cyasorb 531 absorb light below ~ 400 nm (Fanelli *et al.*, 1985). Mestdagh *et al.* (2005) found a lower amount of vitamin A degradation in milk packaged in PET with UV absorbers than in milk packaged in PET with an oxygen absorber after 22 days storage under 2500 lux fluorescent lighting at 18–25 ºC. However, there was no significant difference in vitamin A loss in these two types of packaging at two months storage.

Oxidation of ascorbic acid to dehydroascorbic acid is accelerated by exposure to light. Vitamin C is stable to heat processing but degrades rapidly when exposed to light and oxygen (Haisman *et al.*, 1992; Woessner *et al.*, 1940). Packaging that provides an oxygen barrier and reduces product exposure to light can help protect vitamin C in milk (Eberhard and Gallmann, 1991; Gliguem and Birlouez-Aragon, 2005; Goussault *et al.*, 1978; Marchet and Forti, 2001). Farrer (1983) found that vitamin C loss in paperboard containers stored in both the light and the dark was 43% after 72 hr. White plastic containers had a vitamin C loss of 100% after 72 hr when stored in the light, but only 25% after 72 hr when stored in the dark. Clear plastic containers showed similar results to white plastic containers. Even with oxygen barrier and photoprotective packaging, vitamin C content will decrease significantly in extended shelf-life products (Gliguem and Birlouez-Aragon, 2005).

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4.4.2 Effect of photo- and autoxidation on volatile chemistry and sensory perception of milk

Good quality milk has a bland, slightly sweet flavor that leaves a clean, pleasant aftertaste (Alvarez, 2009a). Because of the low flavor profile of milk, even small concentrations of volatile compounds can quickly impact the flavor of milk and affect consumer perception. Numerous volatile flavor compounds are produced by photooxidation and autoxidation reactions in milk. Light-induced flavor in milk is widespread and possibly responsible for declining milk sales (Barnard, 1973; Bray *et al.*, 1977; Chapman, 2002; Heer *et al.*, 1995). Barnard (1973) tracked the increase in consumer complaints for light oxidized flavor in milk from 1967, where it accounted for only 6.7% of consumer complaints, to 1970, where it accounted for 24% of complaints. The increase in oxidized flavor coincided with the introduction and increased use of blow-molded plastic milk containers. Over 86% of milk packaged in blow-molded plastic containers tested had light oxidized flavor (Barnard, 1973). By 1982, 53% of samples from plastic containers were found to have moderate to strong light oxidized flavor (White and Bulthaus, 1982).

In 1977, a consumer sensory study found that greater than 73% ($n = 2000$) of panelists were able to detect differences between good quality milk and light oxidized milk (Bray *et al.*, 1977). However, only about 20% of the panelists preferred light oxidized milk to good milk. These panelists indicated that the oxidized milk was sweeter, creamier, more flavorful and/or more familiar in flavor than the unoxidized milk (Bray *et al.*, 1977). By 1995, Heer *et al.* (1995) found that 15 out of 24 panelists (62.5%) could not detect light oxidized flavor. These authors hypothesized that consumers were becoming conditioned to light oxidized flavor because of its increased incidence.

The transition of packaging and increased incidence of light-induced flavor in milk may be affecting milk consumption. Middle-school aged children had significantly lower ($P < 0.0001$) preference for light oxidized milk than both college aged and adult consumers (Heer *et al.*, 1995). Another study found that 34.5% of teenagers could detect light oxidized flavor in milk exposed to 2000 lux fluorescent lighting within one-half hour of exposure. By three hours of exposure, 70.7% detected the off-flavor (Chapman, 2002; Chapman *et al.*, 2002). Teens indicated that they did not like the flavor of light oxidized milk. The average exposure time of milk in retail cases is 8 hr (at an average of 2000 lux light intensity). It is likely, then, that much of the milk teens are served is light oxidized and they will be reluctant to drink it.

Changes in volatile chemistry due to light oxidation occur quickly. Trained sensory panels observe changes in milk flavor within 15–30 minutes and a strong oxidized flavor is found within 16 hr (Chapman *et al.*, 2002; Whited *et al.*, 2002). Untrained consumers were able to detect differences between light-exposed and control milk between 54 minutes and 2 hr using a difference from control method (Chapman *et al.*, 2002). Heer *et al.* (1995) found a threshold level for detection of light oxidized flavor to be 2 hr and 40 min for untrained panelists.

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Off-odor and off-flavor from photooxidation falls into two main categories: light-activated flavor and oxidized flavor. Light-activated flavor arises through the oxidation of proteins and imparts a flavor described as burnt feathers, burnt protein, scorched, cabbage and mushroom flavor (Hansen *et al.*, 1975, Bodyfelt *et al.*, 1988, Alvarez, 2009a). Oxidized flavor arises from the oxidation of lipids and has been described as tasting like wet cardboard, or having a metallic, tallowy and oily flavor (Alvarez, 2009a; Hansen *et al.*, 1975). Many researchers refer to flavor due to photooxidation, regardless of source, as light oxidized flavor.

Proteins appear to be oxidized and produce off-flavor compounds faster than lipids. The first off-flavors that appear in milk, within the first few hours of light exposure, are due to oxidation of protein and are described as burnt feather or burnt protein flavor (Alvarez, 2009a). Allen and Parks (1975) found that methional was produced within 10–15 min when skim milk was exposed to direct sunlight. Methional production was correlated to an increase in broth or potato flavor which changed to a cabbagey, burnt feather flavor upon further exposure to sunlight (Allen and Parks, 1975). Dimick (1982) found that sunlight flavor could be detected even at concentrations of methional as low as 50 ppb. This flavor dissipates within a few days and is replaced by a tallowy or wet cardboard flavor produced by the oxidation of lipids (Aurand *et al.*, 1966).

The amino acids cysteine, methionine, tryptophan and histidine, found in casein and lactalbumin, have been implicated in off-flavor production in milk (Allen and Joseph, 1985; deMan, 1990; Sattar and deMan, 1976). Off-flavors normally connected to oxidation of lipids may originate from increased proteolysis in milk (Wiking and Nielsen, 2004). These amino acids, as well as lysine, undergo substantial oxidation when exposed to peroxidizing lipids forming imidazole, lactic acid, methionine sulfoxide, hydrogen sulfide, and diaminopentane (Jadhav *et al.*, 1996; Macrae *et al.*, 1993). Singleton *et al.* (1963) found that tryptophan and riboflavin formed a complex upon exposure to light which produced flavor typical to protein oxidation in milk; and methionine has been identified as a source of sunlight flavor in milk (Min and Boff, 2002; Patton, 1954).

Methionine, in the presence of riboflavin and light, is converted to methional (β-methyl-mercaptopropionaldehyde) (Allen and Joseph, 1985; Harper and Brown, 1964). The reaction mechanism is similar to the photooxidation of lipids in the presence of riboflavin. Riboflavin transforms to its triplet state upon absorption of light. Triplet riboflavin then oxidizes methionine to methional, and riboflavin itself becomes reduced. Reduced riboflavin is subsequently reoxidized to a flavin radical in the presence of oxygen. The oxygen is reduced to a superoxide radical, which can further react with protein. Dimick and Kilara (1983) determined that methionine sulfoxide formed from methionine in the presence of light, riboflavin, protein and oxygen, produced sunlight flavor. Methional is not always found in light-oxidized milk perhaps because methional is broken down to other volatile compounds.

A number of studies found the production of mercaptan and dimethyl sulfide increased in light oxidized milk (Balance, 1961; Harper and Brown, 1964).

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These compounds were produced from the oxidation of cysteine (Harper and Brown, 1964). Balance (1961) determined that methional broke down into methyl mercaptan (cabbage odor) and dimethyl disulfide (onion, cabbage odor). Forss (1979) felt that sunlight flavor was due to the formation of methanethiol, dimethyl sulfide and dimethyl disulfide. Jung *et al.* (1998) found that cysteine produced a hydrogen sulfide odor when exposed to light, but methionine produced dimethyl disulfide, which has an onion or cabbage odor. The authors concluded that dimethyl disulfide formation was produced through singlet oxygen oxidation of methionine. Dimick (1982) found that methional flavor changed to methyl mercaptan flavor upon further light exposure.

As protein oxidation declines, volatile products of lipid oxidation become more important and are implicated in sensory changes. Exposure of milk to light causes aldehydes and a variety of other minor products, such as ketones, to be produced from lipid oxidation. These compounds are the primary causative agents of off-odor and flavor in milk. Volatile compounds, including acetaldehyde, methyl sulfide, dimethyl disulfide, propanal, n-pentanal, and n-hexanal, heptanal, nonanal, 3-methyl butanal, 2-methyl propanal, 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-nonanone, have been found in milk exposed to light (Bekbölet, 1990; Mehta and Bassett, 1978; Mestdagh *et al.*, 2005; Rysstad *et al.*, 1998; van Aardt *et al.*, 2001; 2005b; Webster *et al.*, 2009). Day and Lillard (1960) concluded that oxidized flavor came about due to a combination of compounds and that combinations of carbonyl compounds below threshold levels were additive and were able to give rise to off flavor.

4.5 Oxidation of dairy products

Understanding free radical formation in dairy products as a function of light, time and temperature and the relationship to sensory changes from oxidative deterioration is confounded by differences in product composition and sensory methods. Table 4.2 summarizes the effect of product composition, packaging and light exposure on oxidation of different dairy products.

4.5.1 Butter

The mild, sweet, clean and pleasant flavor and aroma of high quality butter is easily masked by the volatile by-products of oxidation (Bradley and Smulkowski, 2009). Metallic off-flavor, characterized as a slightly astringent and puckery sensation in the mouth, can be observed as soon as the butter is placed in the mouth and persists after expectoration and characterizes the early stages of metal-induced oxidation. 'Oxidized' sensory characteristics have a cardboardy flavor and a puckery mouthfeel. 'Oily', 'tallowy', 'painty', and 'fishy' sensory terms are applied to more extreme sensations of oxidation and are relatively uncommon in products manufactured under modern processes. The USDA grading system indicates that butter is 'below grade' or is classified

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Table 4.2 Detection of oxidation of dairy products by different sensory methods

Product/treatment	Panel description	Method	Sensory observation	Reference
Milk/cream				
Preharvest dietary modifications with fish oil, soybean oil, palm oil	Trained panelists ($n = 7$); by ADSA methods	9-point intensity interval scale; 1 = none; 3 = barely perceptible; 9 = highly pronounced flavor	Oxidized flavor was low (mean score <2.5) for first 14 days of shelf-life with no differences between control milk and milk with higher unsaturated fatty acid composition	Allred *et al.* (2006)
Preharvest dietary modifications with fish oil	Trained panelists ($n = 12$); training for 5 hr as described by Meilgaard *et al.*, 2007. Reference standards provided.	5-pt scale category scale; 1 = no flavor; 5 = extremely strong flavor	No differences in oxidized flavor between control and modified milk; oxidized flavor intensity was less than 2 ('slight').	Nelson and Martini (2009)
Post harvest modification by addition of fish oil (no antioxidants) or rapeseed+fish oil	Trained panelists ($n = 12$) based on ISO Standards. Descriptors were fishy, rancid, milk and metallic	Continuous intensity scale ranging from 0 to 9;	Fishy odor and taste were noticable within 1 day for samples with peroxide values at 1.5 or above.	Let *et al.* (2005)
Post-harvest modification of cream by incorporation of low-melt and medium-melt fractionated butter oils into reformulated cream	Experienced panelists ($n = 12$) based on past experience in dairy sensory activities. One training session provided for familiarizing with method and quality defects	In/Out Method of Specification; off-flavors at moderate level indicated product was 'out' of specification. Considered acceptable quality if 65% of responses identified the products as 'in' specification	Reformulated cream made with skim phase and low-melt butter oil were 'out' of specification and characterized as oxidized	Scott *et al.* (2003a)

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Milk (2% fat) exposed to light (2000 lx) for up to 8 hr	Trained panel ($n = 10$); trained to detect light-oxidized flavor; consumer panel ($n = 94$) from university community	Semi-ascending paired difference method for determining threshold	Threshold for trained panel was 15 minutes of light exposure; threshold for consumer panel was between 54 minutes and 2 hours	Chapman *et al.* (2002)
Milk (2% fat), UHT pasteurized, exposed to light (1521 lx) for up to 21 days. Overwrap films over glass packaging protected for different wavelengths associated with riboflavin activation	Experienced panel ($n = 14$); trained for 1.5 hr in light-oxidation intensity and validated.	Multi-sample difference test using a ranking system	Overwraps protected against oxidation over time but not to the same degree as a complete light-blocking packaging	Webster *et al.* (2009)
Cheese				
Cheddar cheese; preharvest dietary modifications with fish oil, soybean oil, palm oil	Trained panelists ($n = 7$); by ADSA methods	9-point intensity interval scale; 1 = none; 3 = barely perceptible; 9 = highly pronounced flavor	Oxidized flavor was low (mean score <2.3) for first 14 days of shelf-life; aging slightly increased oxidized flavor	Allred *et al.* (2006)
Cheddar cheese (reduced-fat); postharvest fortification of fish oil into ground cheese curd, which was pressed and vacuum packaged and stored up to 6 months	Trained panelists ($n = 9$–10); reference standards used for training	5-point category scale; 0 = no flavor; 4 = extremely strong flavor	Less than slight (score of 1) oxidized flavor was observed for control and cheeses with up to 71 mg DHA/EPA per serving throughout 6 month storage	Martini *et al.* (2009)

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Table 4.2 *Continued*

Product/treatment	Panel description	Method	Sensory observation	Reference
Asadero cheese; packaged in LDPE film with BHT added and stored for up to 100 days at 5 ºC under fluorescent light	Semitrained panelists ($n = 5$); evaluated for oxidized odor	13 cm unstructured line scale (0 = nonoxidized; 13 = highly oxidized); references for anchors were provided in each session	Cheese packaged in LDPE with 8 mg BHT/g had moderate (scores of 6–8) oxidized odor within 20 days and did not increase; oxidized odor for cheese packaged in control (0 BHT) had higher oxidized odor at 80–100 days (scores approximately 10)	Soto-Cantú *et al.* (2008)
Spreadable processed cheese	Trained sensory panelists ($n = 13$); evaluated for overall sensory quality	9 cm unstructured line scale (0 = excellent; 9 = very poor)	Cheese quality deteriorated during light exposure (1000 lux) over 60 days (10 ºC) compared to control; oxygen and light barrier packaging protected sensory quality	Vercelino Alves *et al.* (2007)
Ice cream				
Vanilla ice cream (10% fat). Fat sources from fresh cream, anhydrous milk fat, low or very high melting fractions of milk fat	Consumer panel ($n = 104$) from university community	Hedonic rating scale with 7 = like very much; 4 = neither like nor dislike; 1 = dislike very much	Fresh products made with low melt and anhydrous milk fat were below score of 4.2 and had peroxide values of 0.7 and 2.7 meq peroxide/kg fat, respectively. Oxidized flavor of fat source affected sensory quality	Abd El-Rahman *et al.* (1997)

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Vanilla ice cream (10% fat). Fat sources from fresh cream or a reformulated cream made with cholesterol-stripped butteroil	Trained panel ($n = 9$). Trained for 8 characteristics with reference standards over 7 1-hr group training sessions and performance validated. 'Cardboard flavor' characterized oxidation	15.2 cm unstructured line scale with anchors (none; extreme)	Ice cream made with reformulated cream was less fresh and had more cardboard flavor than the control and a commercial product	Elling *et al.* (1995)
Butter				
Butter from preharvest CLA-enhanced modified milk (fish oil, sunflower oil)	Untrained panel ($n = 20$) of consumers; experienced panel	Triangle test (consumer); 10-point line scale for texture and flavor attributes with anchors	Difference ($p < 0.001$) between experimental and control butters; CLA-enriched buter had a faster melt rate and lower flavor score	Jones *et al.* (2005)

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as 'grade un-assignable' when metallic, oxidized or more extreme flavor descriptors are appropriate for butter (Bradley and Smukowski, 2009). Low-melt fractions of butter oil, which have more unsaturated fatty acids than medium- or high-melt fractions, and cholesterol-stripped butteroil were responsible for the oxidized flavor of reformulated creams and ice cream (Bolling *et al.*, 2005; Elling *et al.*, 1995; Scott *et al.*, 2003a). Sweet buttermilk components and butter-derived aqueous phase, when used as emulsifying ingredients in reformulated creams, had rich, creamy flavor notes that helped mask the oxidized flavor of the low-melt butteroil whereas skim milk did not contribute those flavor notes (Bolling *et al.*, 2005; Scott *et al.*, 2003a).

Direct quantification of oxidation in milkfat was studied using thermogravimetric analysis (van Aardt *et al.*, 2004). This technique, commonly used to measure oxidative stability of polymers, measures a change in sample weight under controlled atmosphere while temperature increases and the resistance to weight gain is an indication of greater oxidative stability. Butter is highly susceptible to oxidation, being less resistant than most vegetable oils that have higher levels of natural antioxidants (Berger, 1994). Surprisingly, milk fat and olive oil both had low and similar weight increases (0.23%) in comparison to triolein (0.89%) or trilinolein (2.33%), but the temperature at which butter began oxidizing (taking on weight) was lower (100 ºC) than that of olive oil (149 ºC) (van Aardt *et al.*, 2004). Olive oil, which is highly unsaturated (83.5% oleic and linoleic fatty acids) compared to butter (27.4% oleic and linoleic fatty acids), also has a high concentration of α-tocopherol (119 mg/kg) compared to butter with less than 35 μg/g fat.

The International Dairy Federation standard for peroxide value for milkfat is 0.2 meq of oxygen per kilogram of fat (Kaylegian and Lindsay, 1995). Fats with a peroxide level of less than 1 meq/kg fat are generally considered fresh (Hoffman, 1962; Rossell, 1989). The peroxide value increases after refining and storage but peroxide levels less than 10 meq/kg is accepted before off-flavors develop (Rossell, 1989). Fresh butter produced from milk of cows fed a control or a fish oil (2% on dry matter) added diet had peroxide values of 0.34 and 0.45, respectively, within one week of processing (Baer *et al.*, 2001). The peroxide value increased for both products over a 3-month storage time, to 0.65 and 0.98, respectively, indicating that the increase in unsaturated fatty acids associated with the fish oil diet affected the oxidative stability (Baer *et al.*, 2001). This is in contrast to no increase in peroxide values observed in a 3-month storage study of modified butters processed from other preharvest dietary modifications of cows' milk, including sunflower or safflower seeds (Stegeman *et al.*, 1992) or fish oil or extruded soybeans (Ramaswamy *et al.*, 2001). Oxidized flavor, however, was relatively low in both products even after 3 months of storage based on evaluation by three trained panelists (Baer *et al.*, 2001). Butter (2% unsaturated fatty acids) stored for 14 weeks at 20 ºC increased (from 0.1 meq/kg fat) in peroxide value to 1.4 meq/kg fat as compared to a dairy spread with rapeseed oil (9% of unsaturated fatty acids), which increased to 5.4 meq/kg (Christensen and Hølmer, 1996). Lipid oxidation of phospholipids in butter and dairy spread

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increased in a similar manner as the oxidation of total lipids. Butter stored at refrigeration temperature had increased concentrations of several lipid oxidation products including (E)-2-nonenal, (Z)-4-heptenal, and (E,Z)-2,6-nonadienal (Lozano *et al.*, 2007). Butter stored under different light conditions (400–2000 lux) in different packaging materials for 4 days had peroxide values ranging from 0.3 to 5.3 (Emmons *et al.*, 1986).

The degree of unsaturation and extent of oxidation can affect color of milkfat (Gonzalez *et al.*, 2003; Kaya, 2000; Noakes *et al.*, 1996). A higher degree of unsaturation decreased the yellow color, affecting the color of butter, cheese and ice cream (Gonzalez *et al.*, 2003; Noakes *et al.*, 1996). The color of butteroil changed from yellow to light yellow, attributed to the oxidation of chromophors, at peroxide values higher than 10 meq/kg (Kaya, 2000).

Off-flavors in butterfat can carry over to secondary products, affecting product sensory quality and oxidative stability (Abd El-Rahman *et al.*, 1997; Bolling *et al.*, 2005; Elling *et al.*, 1995; Scott *et al.*, 2003a). Butterfat is incorporated into products in a variety of forms including dehydrated butter or milk or cream followed by dehydration, as refined or non-refined butter or as butterfat fractions (Padley, 1994). The use of copper vessels in production of confections increases the risk of oxidation of butter in the formulation. Caramels made with butterfat exhibited a fishy odor (Padley, 1994).

4.5.2 Dried milk powder products

Dried milk powder products are frequently incorporated as ingredients into formulated beverages and food products. Dry buttermilk powder, with a milkfat content of at least 4.5%, is a rich source of phospholipids and mono- and polyunsaturated fatty acids that are highly susceptible to oxidation. High quality milk powders have clean, sweet and pleasant odor and flavor but oxidized products have an odor characterized as wet cardboard or oxidized oils. Extremely oxidized powders smell like aged beef tallow and are descibed as 'tallowy' or 'painty' and are unpalatable (Rankin, 2009). Packaging materials that protect the product against ultraviolet and visible wavelengths, control headspace oxygen and provide an indirect source of volatile antioxidants can help protect dry milk product quality (Rankin, 2009; van Aardt *et al.*, 2007). Storage and processing temperatures, product acidity, metallic salts, and water activity influence the development and rate of oxidation of milk powders. Sour whey powders demonstrated an antioxidative ability at low pH, better than all commonly used antioxidants, in a peanut oil emulsion containing lipid oxidation catalysts, identifying a potential ingredient application (Shon and Haque, 2007).

4.5.3 Cheese

Incidence of autoxidation reactions resulting in metallic flavor notes is relatively rare within Cheddar cheese and occurs primarily within cheeses made from oxidized cheese milk (Partridge, 2009). Oxidized flavor at low to moderate

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levels in Cheddar cheese is more tolerated than it is in other dairy products but at pronounced levels has a definite effect on quality (Partridge, 2009). However, light exposure can increase the incidence of oxidation in cheese.

Measuring primary by-products of oxidation, such as peroxides, may not be effective in predicting oxidation-related sensory changes in cheese whereas measuring free radical production may be an effective method for monitoring rapid initial changes in oxidation as a function of storage conditions. Free radicals, as measured by electron spin resonance (ESR) spectroscopy, were observed in initial stages of light exposure and and a higher rate of transformation of radicals into non-radicals occurred in cheeses exposed to light (Kristensen *et al.*, 2000). Differences in free radical formation between light-exposed and light-protected cheeses were no longer evident by the fourth day of storage. The production of free radicals may result from enzymatic action, catalysis of transition metals, or photooxidation (Skibsted, 2000). ESR documentation of free radical changes were more closely related to sensory changes in Havarti cheese than were peroxide value measures, documenting the change in free radicals within the first 4 d of light exposure when significant sensory changes occurred (Kristensen *et al.*, 2000). Wold *et al.* (2005; 2006b) found that degradation of porphyrins and chlorins, which is related to wavelength of exposure, correlated better than did the degradation of riboflavin to sensory attributes (acidic flavor, sun flavor, and oxidized odor) in cheese. Kristensen *et al.* (2000) also found that riboflavin degradation did not correlate to the introduction of off-flavor in Havarti cheese.

Discoloration of cheese from light-induced oxidation affects consumer acceptance of cheese (Kristensen *et al.*, 2000; Petersen *et al.*, 1999). Photosensitizers (carotenoids, riboflavin, chlorophyll) can initiate reactions that alter cheese color (Kristensen *et al.*, 2000; Peterson *et al.*, 1999). Light intensity may play a role in color change in cheese. Cheese color faded more significantly at light intensities greater than 1600 lx (Deger and Ashoor, 1987).

Colorants may provide some protection from photooxidation or lipid-oxidation of cheeses by absorbing light energy that would otherwise excite riboflavin or other photosensitizers in uncolored cheeses (Petersen *et al.*, 1999). They also may function as free-radical scavengers and quenchers of singlet oxygen (Edge *et al.*, 1997). However, annatto coloring, a water-based colorant used in Cheddar, Gouda and other cheeses, will degrade under intense lighting and may cause 'pinking' defect (Govindarajan and Morris, 1973; Lück, 1973; Partridge, 2009; Peláez and Northolt, 1988; Petersen *et al.*, 1999). Wavelength and system pH may both influence this reaction (Petersen *et al.*, 1999). Annatto was at least five times more sensitive to photobleaching than β-carotene in a model solution (Petersen *et al.*, 1999). Caseinate did not affect photodegradation process of the colorants but the presence of protein may affect the quantum yield because of partial absorption of the light by caseinate. pH effects on color changes were probably due to conformational changes of casein, altering pigment binding or facilitating deactivation of excited carotenoid species. High oxygen transmission rate of cheese packaging film

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and low pH can exacerbate the problem (Hong *et al.*, 1995a; 1995b; Mortensen *et al.*, 2002).

Producers of colored cheeses should be aware of the effect of light on photooxidation of cheese and cheese colorants and sensory quality in order to appropriately select packaging materials and display conditions (Duncan and Webster, 2009; Petersen *et al.*, 1999; Vercolino Alves *et al.*, 2007; Wold *et al.*, 2006b). A high oxygen barrier film and ultraviolet absorbers provide protection against photooxidation of a variety of cheeses. Oxygen concentration may play a role in changes in objective color measurements of cheese, especially the yellowness (b^*) component (Hong *et al.*, 1995a). Yellow color of Cheddar cheese, with and without annatto colorant, decreased with prolonged exposure to fluorescent light, especially when a high oxygen transmission rate film was used for packaging (Hong *et al.*, 1995a; 1995b). Only minor changes in color of sliced Harvarti cheese and packaged under modified atmosphere (25% CO_2, 75% N_2, initial O_2 of 0.4%) in oxygen barrier transparent packaging occurred with exposure to light (1000 lx).

4.5.4 Ice cream

Low storage temperatures of ice cream suggest that oxidation should not be a challenge in this product. The primary oxidation problems are associated with milk and ingredient quality or during mix storage and light-induced oxidation is unlikely to occur in ice cream (Alvarez, 2009b). In addition to fresh milk and cream, sources of milkfat and proteins include processed (concentrated, evaporated, or dried) milk, cream, butter, and protein (whey, casein, caseinates) or buttermilk (Alvarez, 2009b), which may be a source of oxidation flavors or initiators for subsequent oxidation reactions. The oxidized flavor defect in ice cream is described in similar terms as those related to milk, describing the transition from relatively low oxidation characteristics as metallic, astringent or puckery to descriptors for more moderate oxidation sensations such as 'oxidized', 'papery' or 'cardboardy'; 'oily', 'tallowy', 'painty' or 'fishy' are descriptive of extreme oxidation in ice cream (Alvarez, 2009b). Oxidized flavor persists in the mouth after expectoration, suggesting that the product is old or stale, and adversely affecting the sensory experience. This characteristic diminishes repeat sales of the product (Alvarez, 2009b). Elling *et al.* (1995) produced a 10% fat vanilla ice cream by incorporating cholesterol-stripped milkfat in a reformulated cream. The sensory profile of ice cream made with the reformulated cream was characterized with a significantly ($p < 0.05$) higher 'cardboard' and 'less fresh' flavor, which was attributed to the cholesterol-stripped butteroil ingredient.

Ice cream processed using milk with a more unsaturated fatty acid profile by modifiying the bovine diet was relatively stable to oxidation (Gonzalez *et al.*, 2003). Peroxide values of milkfat extracted from ice cream ranged from 2.91 to 4.12 meq/kg. The peroxide values of milkfat from ice cream made from milk from cows fed high-oleic or high-linoleic diets did not differ from products made from control milk.

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4.6 Packaging of milk and dairy products

Packaging materials that provide product visibility, especially for milk, are common because of marketing policies, consumer preference, and sales (Young, 2002). Consumers prefer foods and beverages to be packaged in clear containers where they can see the product (Chapman, 2002; Cladman *et al.*, 1998; Doyle, 2004; Rosenthal, 1992; Sattar and deMan, 1976). The primary plastic packaging materials used for refrigerated milk products are high density polyethylene (HDPE) and PET (Anonymous, 2002). A complete light block is best at preventing against vitamin degradation and protecting sensory quality (Bekbölet, 1990; Senyk and Shipe, 1981; Singh *et al.*, 1975; van Aardt *et al.*, 2001; Webster *et al.*, 2009) but does not permit product visibility. Pigmentation, overwraps or multilayers of packaging material can protect against light oxidation flavor and vitamin loss depending on the intensity of the pigmentation and the color (Table 4.3). Protection of products from ultraviolet and visible wavelengths is important because of the wide range of wavelengths that excite riboflavin and other photosensitizers (Duncan and Webster, 2009; van Aardt *et al.*, 2001; Webster *et al.*, 2009; Wold *et al.*, 2006a; 2006b). Blocking UV wavelengths does not protect against riboflavin destruction (Fanelli *et al.*, 1985) but a number of authors found that blocking wavelengths between ~400 and 500 nm did provide greater protection of riboflavin (Bradley, 1980; Fukumoto and Nakashima, 1975; Luquet *et al.*, 1977; Sattar and deMan, 1973; Sattar *et al.*, 1977b; Senyk and Shipe, 1981; Singh *et al.*, 1975; van Aardt *et al.*, 2001). The major absorption maximum for riboflavin is 446 nm, although it absorbs other wavelengths as well (Kyte, 1995). Milk packaged in HDPE with a carbon black layer (light barrier) and no oxygen barrier was shown to have better protection against light oxidation than HDPE with an EVOH oxygen barrier but no light barrier (Gorgern, 2003). Inhibition of oxygen permeation into a package does not solely protect against degradation. Simon and Hansen (2001) found that milk packaged in oxygen barrier board (EVOH and foil) deteriorated much more slowly than milk packaged in standard or juice boards. The foil-lined board had the added benefit of a light block.

4.7 Future trends

Increased incorporation of omega-3 lipids, functional ingredients that may function as photosensitizers, and minerals fortifications increase the challenges associated with oxidation of dairy products. Reducing oxidation and the sensory and vitamin degradation that occurs along with that oxidation will be especially important for the sale of milk. Consumption of single serve milk has been one section of the fluid milk industry that has been increasing in recent years. It outpaced all other product categories during a 3-year period from 2000 to 2003 – increasing by 45% (Sloan, 2005). Many school districts have been replacing soda vending machines with healthy beverage vending machines, which carry single serve milk. Milk in vending machines may be expected to have a 60-day

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Table 4.3 Effect of product composition, packaging material, light exposure on oxidation of dairy products

Product description	Experimental conditions	Oxidative measure	Other comments	Reference
Milk				
Milk (whole or 2% milk, HTST) packaged in green or clear PET bottles, clear PET with UV-absorbers, HDPE	Exposed to light similar to front of the top row of chill cabinets in supermarket, at 4 ºC	Conjugated diene values increased from absorbance (532 nm) of <0.05 to 0.1–0.15 from green, LDPE, clear PET and to about 0.25 for HDPE over 18 days	Labels over package reduced light exposure by 75% but did not protect against oxidation over the 18 d shelf-life compared to light-blocked controls.	Cladman *et al.* (1998)
Yogurt				
Strawberry yogurt with added omega-3 oil	2 g total fat/272 g yogurt mass; control used milkfat; treatment product fortified with 500 mg algae oil with milkfat; product stored for 22 days at 4 ºC	Lipid hydroperoxides were $\approx$0 μmoles/kg lipid for control and $\approx$2.1 μmoles/kg lipid for omega-3 fortified product		Chee *et al.* (2005)
Milkfat/butter				
Milkfat (and other oils) as carriers for retinyl palmitate	Exposed to light at 4296 lx (400 ft-c), 4 ºC up to 84 h	Peroxide values for coconut oil, butter, corn oil, and peanut oil carriers were approximately 2.5, 3.8, 4.1, and 8.2 meq/kg oil at 48 hr with relatively little increase thereafter.	Peroxide values did not parallel degradation of all-*trans* retinyl palmitate during light exposure.	Zahar *et al.* (1987)
Dairy spread (minimum 80% fat with 75% milk fat; 25% rapeseed)	Stored at 20 ºC for 14 wk	Peroxide values ranged from 0.1 to 1.4 meq/kg for control butter (milkfat) and 0.2–5.4 for dairy spread	Rapid increase in peroxides in dairy spread occurred about week 4	Christensen and Hølmer (1996)

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Table 4.3 *Continued*

Product description	Experimental conditions	Oxidative measure	Other comments	Reference
Cholesterol-reduced butter with added evening primrose oil and phytosterols	Cholesterol was removed by treating with 10% crosslinked β-cyclodextrin (β-CD)	Thiobarbituric acid (TBA) values were 1.2 for control butter, 2.0 for cholesterol-reduced butter, and 3 for cholesterol-reduced butter with evening primrose and phytosterols		Kim *et al.* (2006)
Cheese				
Spreadable processed cheese	Exposed to light at 1000 lux, 10 ºC, up to 60 days	TBA for control (stored in dark) were 0.02–0.04; 0.10 in polyethylene pkg after 17 days; 0.18 in polypropylene pkg after 60 days	Packaging materials were in different shapes and sizes; study also included co-extruded packaging materials and glass	Vercelino Alves *et al.* (2007)
Semi-hard Harvarti cheese, sliced	Exposed to light at 1300 lux, 5.6 ºC, up to 480 hr; modified atmosphere pkg (25% CO_2, 75% N_2); 6 materials with different light transmission, oxygen permeability, water vapor transmission rates	Volatile chemistry of secondary oxidation products affected by transparency of packaging materials and on oxygen transmission rates	Color changes were noticeable between 72–144 hours of light exposure for transparent materials. Odor changes were noted within 12 hr	Mortensen *et al.* (2002)

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(or longer) shelf-life, yet still be packaged in translucent materials, such as PET, and stored under light. In order for school aged children to increase their consumption of milk and improve their health and well being, the milk must taste good. It is imperative that the quality of the milk sold in these vending machines remain high.

Controlling oxidation through preharvest or post-harvest formulation of dairy-based products with antioxidants will increase in importance with extended shelf-life and the emphasis on incorporation of healthy lipids in the diet. Utilizing novel packaging materials that protect traditional and value-added products against light-induced oxidation and promote consumer interest will increase sales. Maintaining sensory quality throughout extended shelf-life of milk is essential. Composition and packaging of dairy ingredients also must be considered in order to protect other food products from oxidative deterioration and degradation of sensory quality.

4.8 Sources of further information and advice

The reader is referred to literature reviews by Bekbölet (1990), Bosset *et al.* (1995) and Borlet *et al.* (2001) for additional information on photochemical effects on milk components. Additional packaging information also may be found in Bosset *et al.* (1995) and Duncan and Webster (2009).

4.9 References and further reading

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5

Oxidation of fish oils and foods enriched with omega-3 polyunsaturated fatty acids

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Abstract: During the last thirty years there has been an increasing interest in omega-3 polyunsaturated fatty acids (PUFA) for nutritional applications. However, due to their polyunsaturated nature omega-3 PUFA are highly susceptible to lipid oxidation, which will lead to formation of undesirable fishy and rancid off-flavours. Such off-flavours can lead to consumer rejection of omega-3 enriched foods. Important issues to address in order to avoid lipid oxidation are: (1) processing conditions and product composition, (2) omega-3 PUFA source and delivery system, and (3) addition of antioxidants. This chapter will summarise our current knowledge about these issues in a range of different omega-3 enriched foods.

Key words: omega-3 fatty acids, fish oil enrichment, antioxidants, mayonnaise, dressing, margarine, dairy products, fitness bar, fish, meat.

5.1 Introduction

During the last thirty years there has been an increasing interest in omega-3 polyunsaturated fatty acids (PUFA) for nutritional applications. This is due to the increasing evidence that omega-3 PUFA have a wide range of nutritional benefits in the human body. The three most nutritionally important omega-3 PUFA are α-linolenic acid (LNA, C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3). The potential health effects of EPA and DHA include reduction of cardiovascular disease risk (Wang *et al.*, 2006; Horrocks and Yeo, 1999; Yaqoob, 2004), anti-inflammatory effects including reduction of symptoms of rheumatoid arthritis

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(Kremer, 2000) and Crohns disease (Belluzzi *et al.*, 1996), and reduced risk of certain cancer forms (Wigmore *et al.*, 1996). Moreover, DHA seems to be important for brain and nervous tissue development in the infant (Cheatham *et al.*, 2006; Jensen *et al.*, 2005; Lauritzen *et al.*, 2005). The evidence for the preventive effect of EPA and DHA on cardiovascular disease is particularly strong (Wang *et al.*, 2006).

EPA and DHA occur in marine organisms, while LNA is mainly found in plant-based foods. The main focus in this chapter will be on the marine omega-3 PUFA and for this reason omega-3 PUFA only refers to EPA and DHA in the following. The current intake of omega-3 PUFA in the industrialised world is lower than recommended by nutritional organisations. One way to increase the intake of omega-3 PUFA could be to substitute part of the vegetable or animal fat by marine lipids in foods like mayonnaise, milk, bread, dressing, spreads, yoghurts, etc. (Jacobsen *et al.*, 2008; Trautwein, 2001). However, due to their polyunsaturated nature omega-3 PUFA are highly susceptible to lipid oxidation, which will lead to formation of undesirable fishy and rancid off-flavours. Such off-flavours can lead to consumer rejection of omega-3 enriched foods. It is thus crucial that lipid oxidation is prevented if such products are to become successful in the market place. Important issues to address are:

- processing conditions and product composition,
- omega-3 PUFA source and delivery system,
- addition of antioxidants.

As for the product composition, the complexity of most of the foods to which omega-3 PUFA are added means that several ingredients in the product may affect the oxidative stability of the omega-3 PUFA enriched product and therefore knowledge about this is essential. Moreover, the processing conditions can also significantly affect the oxidative stability of the final product and this issue needs to be addressed during development of omega-3 enriched foods. With respect to the omega-3 PUFA source and delivery system, both algae and fish oils rich in omega-3 PUFA triglycerides are available on the market and they are supplied as neat oils, microencapsulated powder or emulsified oils. The different sources and delivery systems will behave differently depending on the food systems in which they are incorporated. Regarding antioxidant addition, a wide range of antioxidants are commercially available to prevent lipid oxidation and it can therefore be difficult to select the most efficient one. Lipid oxidation and means to prevent it thus poses a major challenge when developing omega-3 PUFA enriched foods. This chapter will summarise our current knowledge about the above issues in different fish oil enriched foods. Initially, the reactions that will lead to formation of the unpleasant fishy and rancid off-flavours will be summarised.

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5.2 Oxidative flavour deterioration of omega-3 enriched foods

EPA and DHA are highly susceptible to oxidation reactions due to the presence of five and six double bonds in their molecular structure, respectively. Due to the high number of double bonds, oxidation processes may lead to a complex mixture of hydroperoxides and a myriad of volatile, non-volatile and polymeric secondary oxidation products. The structures of some cleavage products are known (Fig. 5.1), but the exact mechanisms for the formation of many of the observed products are not yet completely understood. Omega-3 PUFA though follow the same cleavage mechanisms as those recognised for linolenic acid. Hence, some of the oxidation products that can be expected from autoxidation of omega-3 PUFA are propanal, 2-pentenal, 3-hexenal, 4-heptenal, 2,4-heptadienal, 2,6-nonadienal, 2,4,7-decatrienal, as well as 1-penten-3-one and 1,5-octadien-3-one.

Volatile compounds have different sensory threshold values. Importantly, the human sensory apparatus has a particularly low threshold for volatile off-flavours resulting from oxidation of omega-3 PUFA (Frankel 2005). Thus, 1-penten-3-one as well as (Z)-4-heptenal, (E,Z)-2,6-nonadienal and 2,4,7-decatrienals have been associated with sharp-burnt-fishy off-flavours in oxidized fish oil (Karahadian and Lindsay 1989). However, decatrienals are usually only observed in highly oxidised products and in several studies on fish oil enriched foods this compound was not observed (Venkateshwarlu *et al.*, 2004; Hartvigsen *et al.*, 2000). For further details on the sensory impact of volatile oxidation products from omega-3 PUFA refer to Chapter 6 in Volume 1.

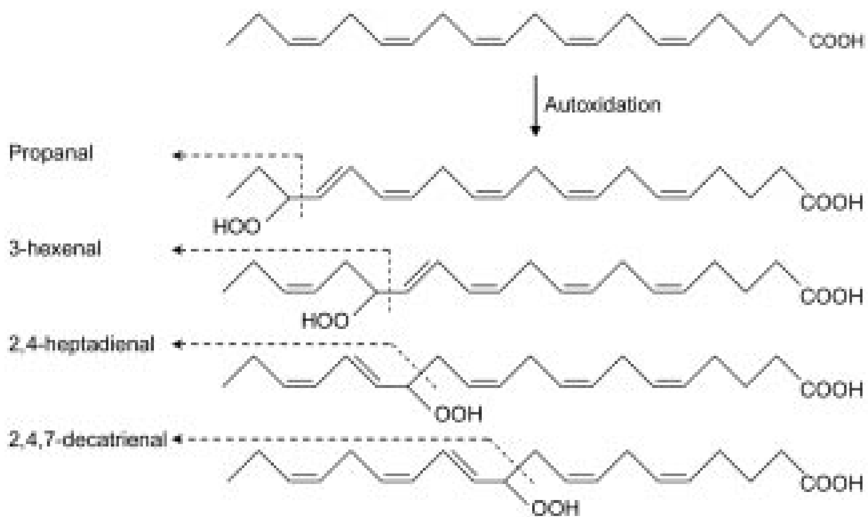

Fig. 5.1 Autoxidation sites associated with major aldehydes expected to form from EPA. Source: Kulås, E. Oxidation of fish lipids and its inhibition with tocopherols. In Kamal-Eldin, A. (ed.) (2003) *Lipid Oxidation Pathways*, Champaign, IL, AOCS Press, 37–69.

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Importantly, oxidation generally occurs faster in emulsions than in neat oils (Frankel *et al.*, 2002) and it may also be expected to occur faster in most other omega-3 PUFA enriched foods than in neat oils. This is due to the mechanical processing that is required for emulsification or mixing, which in some cases necessitates the use of high temperature. It may also be due to other types of heating processes such as baking. Moreover, in many cases, the oil is exposed to air (oxygen) during processing. For emulsions, the increased oxidation rate may also be due to an increased interfacial area as lipid oxidation is thought to be initiated at the interfaces between air, oil and water. Even after refining and deodorisation most fish oils will contain trace levels of peroxides and several food ingredients contain trace levels of metal ions. Therefore, metal catalysed decomposition of peroxides is regarded as the most important driving force for lipid oxidation in many food products.

Owing to the above mentioned reasons special precautions have to be taken to avoid oxidative flavour deterioration of omega-3 PUFA enriched foods. In the following the possible strategies to limit oxidation by choice of ingredients including oil quality and type of emulsifiers, by optimising processing conditions, packaging material and storage conditions, and by addition of antioxidants will be discussed. However, the first step to avoid oxidation in fish oil enriched foods is to ensure that the fish oil that is used for the food product is not oxidised. Means to avoid this are therefore also summarised.

5.3 Prevention of oxidation in fish oil and omega-3 polyunsaturated fatty acid (PUFA) enriched foods

5.3.1 Fish oil and emulsified fish oil delivery systems

The oxidative stability of fish oil decreases like for other oils in the presence of light, high temperature, metal ions and oxygen and therefore exposure to light, oxygen and metal ions should be minimised and the oils should be kept at a low temperature, preferably below 0 °C. Furthermore, the initial quality of the fish oil will determine its shelf life. The quality of the fish oil depends on the quality of the fish used for the fish oil production as well as the processing conditions used for extraction, refining and deodorising the oil. It is beyond the scope of this chapter to discuss these issues in detail, but the reader may refer to Breivik (2007) for further details.

In the industry, fish oil is often protected against oxidation by addition of antioxidants such as tocopherols, citric acid or its esters, ascorbyl palmitate or propyl gallate. Recently, there has also been an increasing interest in using natural antioxidants such as rosemary extracts.

Kulås and Ackman (2001) studied the effect of α-tocopherol (50–2000 mg/kg), γ-tocopherol (100–2000 mg/kg), and δ-tocopherol (100–2000 mg/kg) on the formation and decomposition of hydroperoxides in purified fish oil. The tests were conducted at 30 °C in the dark. Purified fish oil oxidised very rapidly with no apparent induction period. The relative ability of the tocopherols to retard the

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formation of hydroperoxides decreased in the order α-tocopherol > γ-tocopherol > δ-tocopherol at a low level of addition (100 mg/kg), but a reverse order of activity was found when the tocopherol concentration was 1000 mg/kg. Further, the inversion of activity, on the basis of hydroperoxide formation, was subsequently determined to occur for α-tocopherol at 100 mg/kg and for γ-tocopherol at 500 mg/kg, whereas the antioxidant activity of δ-tocopherol increased with level of addition up to 1500–2000 mg/kg. None of the tocopherols displayed any prooxidant activity.

Later Kulås *et al.* (2002) studied the relative ability of α-, γ- and δ-tocopherol (100 or 1000 mg/kg) to influence the distribution of volatile secondary oxidation products in fish oil with particular emphasis on oxidation products expected to be important for adverse flavour formation. The tocopherol type and concentration not only affected the overall formation of volatile secondary oxidation products, but also the composition of this group of oxidation products. Although an active inhibitor of overall volatile formation, α-tocopherol at a high concentration appeared to direct the formation of the more flavour-potent aldehydes, such as heptadienal.

Huber *et al.* (2009) compared the antioxidant properties of naturally occurring flavnols, namely quercetin and quercetin glycosides with common food antioxidants butylated hydroxytoluene (BHT) in fish oil. Glycosylation enhanced the antioxidant activity of quercetin, and quercetin-3-O-glucoside exhibited a better antioxidant activity than BHT in bulk fish oil.

The oxidative stability of fish oil for use in foods can also be improved by microencapsulation. This may not only improve the stability of fish oil before use, but may also improve the oxidative stability of the food in which the microencapsulated product is incorporated as will be illustrated later. Despite the increased oxidative stability obtained by microencapsulation, it may still be necessary to add antioxidants to the fish oil before microencapsulation to prevent lipid oxidation during the microencapsulation process. Serfert *et al.* (2009) thus showed that autoxidation already occurred in the first stages of the microencapsulation process itself, i.e. during emulsification and spray-drying. Moreover, it was possible to obtain an efficient stabilisation using a ternary combination of lipophilic antioxidants, synergistic compounds and a trace metal chelator, e.g. a combination of tocopherols, rich in the delta-derivative and low in the alpha-derivative, with ascorbyl palmitate and lecithin. Trace metal chelation by, e.g. Citrem or lecithin in combination with ascorbyl palmitate proved to be of particular importance in the emulsion, but not during the storage of the microencapsulated oil. In the microencapsulated oil, the addition of rosemary extract rich in carnosic acid to tertiary blends of tocopherols, ascorbyl palmitate and lecithin or Citrem significantly retarded autoxidation.

Another way to increase the oxidative stability of fish oil is to pre-emulsify the oil. If properly designed the pre-emulsification strategy could not only increase the oxidation stability of the fish oil before addition to foods, but may also reduce the amount of stresses to the fish oil such as heat, oxygen and access of light during production of the particular food product. Additionally, the contact

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between the omega-3 PUFA oil and the potential pro-oxidant compounds of the food product during processing is reduced by adding the oil in an already stabilised pre-emulsion as the final step of processing. However, for such a strategy to be successful the emulsion must be designed to have the optimum combination of emulsifier(s), antioxidants and in some cases also stabilisers. Moreover, it is also necessary to take into account the composition and physical properties of the final product to which the pre-emulsion is added. Complete avoidance of exposure of the omega-3 PUFA oil and thus contact with remaining product ingredients in the final product is dependent on the physical stability of the pre-emulsion over time. If the pre-emulsion interacts with other product components, or if diffusion occurs across the emulsion droplet interface, the omega-3 PUFA oil might in time get into contact with the remaining ingredients of the product. Therefore, it is likely that the pre-emulsification strategy may be more suitable for some products than for others as will be illustrated later.

Djordjevic *et al.* (2004a) determined the optimum conditions for producing whey protein isolate (WPI)-stabilised oil-in-water emulsions with a high content of omega-3 PUFA and a low viscosity that could be used for incorporation of omega-3 PUFA in foods. Subsequently, they evaluated the oxidative stability of oil-in-water emulsions (25% oil) stabilised either by casein or WPI. They found that PV was significantly higher in the WPI-stabilised emulsions compared with the casein-stabilised emulsions, but that there was no significant difference in the formation of headspace propanal (Djordjevic *et al.*, 2004b). Moreover, they observed that it was difficult to dissolve casein at low pH, which makes it impractical to use this protein from a technological standpoint. Another problem when using casein was that the viscosity increased steeply at high oil concentrations. Because of these findings they suggested that WPI-stabilised oil-in-water emulsions (pH 3) could be used to produce oxidatively and physically stable omega-3 PUFA delivery systems.

5.3.2 Mayonnaise

Mayonnaise is an o/w emulsion with a high oil content (70–80%) and a low pH (~4) compared to other food systems. The oxidative stability of mayonnaise prepared with fish oil only (70%) and without antioxidants is very poor with a shelf life of only one day at room temperature (Jafar *et al.*, 1994). The shelf life could be increased to 49 days by addition of citric acid or sodium citrate and propyl gallate in the oil phase and EDTA and ascorbic acid in the aqueous phase (Jafar *et al.*, 1994). Hsieh and Regenstein (1991) showed that in mayonnaise made with fish oil only (70%) the use of nitrogen (exclusion of oxygen) retarded lipid oxidation more than addition of TBHQ (0.02%). Despite this improvement in oxidative stability, production of mayonnaise made with fish oil only does not seem to be a suitable strategy. A more realistic strategy is to substitute only part of the vegetable oil with fish oil. Several studies have been carried out in which 20% of the rapeseed oil was substituted with fish oil as will be summarised in the following. The total lipid content in this mayonnaise was 80%.

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Jacobsen *et al.* (2001a) identified the low pH and iron in egg yolk, which is used as an emulsifier, to be the most important factors for lipid oxidation in this product. The following mechanism was suggested to explain this finding: the iron in egg yolk is bound to the protein phosvitin. At the natural pH of egg yolk (pH 6.0), the iron forms cation bridges between phosvitin and other components in egg yolk, namely low density lipoproteins (LDL) and lipovitellin. These components are located at the oil-water interface in mayonnaise. When pH is decreased to 4.0, which is the pH in mayonnaise, the cation bridges between the before-mentioned egg yolk components are broken and iron becomes dissociated from LDL and lipovitellin. Thus, iron becomes more active as a catalyst of oxidation (Jacobsen *et al.*, 2001a).

Jacobsen *et al.* (2000a) also observed that the droplet size of fish oil-enriched mayonnaises influenced the oxidation rate. Thus, mayonnaise with small droplet sizes oxidised faster in the initial part of the storage period than in mayonnaise with larger droplets, whereas no effect of droplet size on oxidative flavour deterioration was observed in the later part of the storage period. The following mechanism to explain these findings was suggested: in the initial oxidation phase, a small droplet size, i.e. a large interfacial area, would increase the contact area between iron located in the aqueous phase and lipid hydroperoxides located at the interface and this would increase oxidation. In the later stage, oxidation proceeds inside the oil droplet and therefore the droplet size is less important.

The ability of a range of different antioxidants to prevent lipid oxidation in this type of fish oil enriched mayonnaise has also been evaluated. Both propyl gallate and gallic acid acted as prooxidants (Jacobsen *et al.*, 1999a; 2001b). The prooxidative effects of propyl gallate and gallic acid were suggested to be due to their ability to reduce metal ions to their more active form, e.g. Fe^{3+} to Fe^{2+}. Likewise, ascorbic acid and ascorbyl palmitate were also shown to act as pro-oxidants, and the proxidative effect of ascorbic acid increased with increasing concentrations (Jacobsen *et al.* 1999b). Additional experiments on the oxidative stability of fish oil enriched mayonnaise with ascorbic acid added indicated that ascorbic acid induced iron release from the oil–water interface into the aqueous phase and this effect of ascorbic acid was strongest at low pH (3.8–4.2). It was proposed by the authors that iron release from the interface was a result of broken ion bridges between iron and egg yolk proteins, whereby iron ions become accessible as initiators for the lipid oxidation. Moreover, in the presence of ascorbic acid or ascorbyl palmitate lipid oxidation was further increased due to its ability to reduce Fe^{3+} to Fe^{2+} that rapidly catalyse lipid oxidation (Jacobsen *et al.* 1999b; 2001a). Furthermore, a combination of ascorbic acid, lecithin and tocopherol (A/L/T) as antioxidants in the mayonnaise did not protect the fish oil against oxidation, but instead promoted oxidation. This finding was probably due to the properties described above for ascorbic acid alone in fish oil enriched mayonnaise (Jacobsen *et al.*, 2000b).

Mixtures of tocopherols were found to have a concentration dependent effect in fish oil enriched mayonnaise. Moreover, their effect also depended on

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whether a water or oil soluble tocopherol mixture was used (Jacobsen *et al.*, 2001c). With an oil soluble tocopherol mixture, sensory analysis and determination of volatile oxidation products showed weak antioxidative effects at lower tocopherol concentrations (≤ 32 mg/kg) with the highest protection at 16 mg/kg. In contrast, prooxidative effects were observed at higher concentrations. Addition of a tocopherol mixture as a water soluble preparation in mayonnaise generally exerted prooxidative effects on fishy flavour. These observations suggest that tocopherol does not have an unequivocal effect on fishy odour and flavour in mayonnaise. The poor effect of tocopherol observed in mayonnaise may be a result of the high levels of tocopherols already present in the oils used in the mayonnaise (oils: rapeseed oil 64% and fish oil 16%). Since tocopherol has only been evaluated as mixtures in mayonnaise, it cannot be ruled out that better effects may be exerted by γ-tocopherol alone as this antioxidant was observed to have antioxidative effects in salad dressing as will be described below. However, rapeseed oil has a very high level of γ-tocopherol, so the question is whether additional γ-tocopherol will have any effect in mayonnaise. Moreover, Jacobsen *et al.* (2001a) suggested that the crucial step in the formation of fishy odour and flavour in mayonnaise is the metal catalysed break down of peroxides from omega-3 PUFA located in the aqueous phase or at the o/w interface. If this is the case, tocopherol can only to a limited extent reduce off-flavour formation by inhibiting deterioration of omega-3 PUFA inside the oil droplets because the reactions between the omega-3 PUFA and the radicals formed from the decomposed peroxides will take place at the o/w interface.

As described above, metal-catalysed oxidation is an important initiation mechanism for lipid oxidation in fish oil enriched mayonnaise and it can therefore be expected that metal chelators would be able to prevent oxidation. However, among the metal chelators evaluated (lactoferrin, phytic acid and EDTA) only EDTA efficiently prevented lipid oxidation and thereby off-flavour formation in this food system (Nielsen *et al.*, 2004; Jacobsen *et al.*, 2001b) (Fig. 5.2). The antioxidative effect of lactoferrin seemed to be concentration dependent, since it had a slight antioxidative activity at low concentrations and showed prooxidant activity at high concentrations (Nielsen *et al.*, 2004). The different antioxidative efficacies of EDTA, lactoferrin and phytic acid were suggested to be due to the different binding constants of the metal chelators to Fe^{2+} or that EDTA might be less sensitive to pH values around 4 than the other metal chelators (Nielsen *et al.*, 2004; Jacobsen *et al.*, 2008).

5.3.3 Mayonnaise-based salads

Recently, the oxidative stability of fish oil enriched mayonnaise-based shrimp and tuna salads were evaluated. Moreover, the influence of different vegetables in shrimp and tuna salads was assessed (Sørensen *et al.*, 2010). Interestingly, a sensory panel could not significantly distinguish the intensity of rancid off-flavour in salads without fish oil from that in salads with fish oil throughout the

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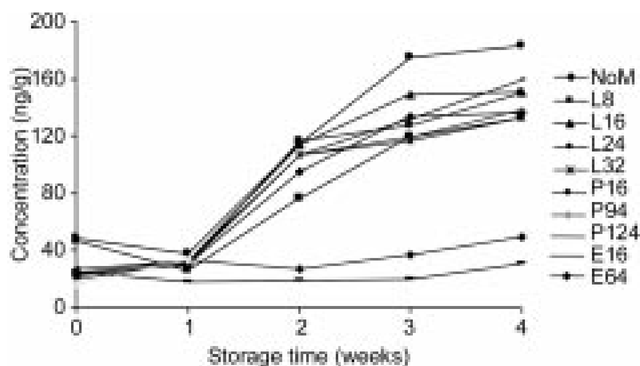

Fig. 5.2 Development of 2-E-pentenal in mayonnaise during storage at 20ºC for up to four weeks. L *Lactoferrin*, P *Phytic acid*, E *EDTA*. Number after antioxidant name indicates concentration in μM. Adapted after Nielsen, N.S., Petersen, A., Meyer, A.S., Timm-Heinrich, M., Jacobsen, C. (2004) The effects of lactoferrin, phytic acid and EDTA on oxidation in two food emulsions enriched with long chain polyunsaturated fatty acids. *J. Agric. Food Chem.* 52, 7690–7699 with permission from ACS Publications.

storage period (57 days) for both salad types, except for tuna salads for which the rancid intensity was higher in the fish oil enriched sample at the very end of the storage period (day 57) only. These results thus indicated that it was possible to add fish oil to these two salad types without compromising the sensory properties if the labelled shelf life was kept below 57 days.

Further, it was found that for the shrimp salads, addition of shrimp had a prooxidative effect, whereas asparagus had an antioxidative effect, which was efficient enough to prevent the proxidative effect of the shrimps in this type of salad. For the tuna salad the influence of ingredients herein seemed more complex and it was not possible to draw clear conclusions on the effect of the ingredients. This was suggested to be due to the fact that an already high concentration of volatiles in the ingredients might have masked any inhibiting effect on the formation of volatiles, which was used as an important parameter for evaluating oxidation (Sørensen *et al.*, 2010).

In this study, the effect of adding 1% oregano, rosemary or thyme to fish oil enriched tuna salad was also assessed. The results showed that the addition of spices increased the oxidative stability of tuna salad and that oregano was the most efficient antioxidant followed by rosemary and thyme.

5.3.4 Dressing

A few studies on fish oil enriched dressing have been reported in the literature (Let *et al.*, 2007a; 2007b). In both studies, the salad dressing was prepared with 25% fat of which 40% was fish oil. Whey protein was used as emulsifier and a mixture of guar gum, xanthan gum and acetylated distarch adipate were added as stabilisers. In the first study, the antioxidative effect of γ-tocopherol, ascorbyl palmitate and EDTA were tested either alone or in combination. The lipophilic

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γ-tocopherol was found to exert an intermediate antioxidative effect on peroxide formation irrespective of the addition level (Let *et al.*, 2007a). However, for the volatiles a concentration dependent effect was observed. The highest protection was obtained with the lowest concentration (22 mg/kg product). A concentration-dependent effect was also observed for ascorbyl palmitate. At low concentrations (5 mg/kg) ascorbyl palmitate slightly reduced oxidation, whereas it acted as a prooxidant in high concentrations (30 mg/kg). In contrast, EDTA was a very efficient antioxidant, which inhibited oxidation by approximately 80% at both concentrations evaluated (10 and 50 mg/kg). Moreover, the efficacy of EDTA could be further improved when used in combination with ascorbyl palmitate and tocopherol (Let *et al.*, 2007a). The strong effects of the metal chelator EDTA and the poor effects of tocopherol and ascorbyl palmitate, which work by other antioxidant mechanisms suggest that metal catalysed oxidation is also very important in salad dressing. The metal content in ingredients used for the salad dressing was not analysed, but on the basis of information available in the literature the iron content was suggested to be relatively low. It may therefore seem surprising that metal-catalysed oxidation is so prominent in a whey protein emulsified salad dressing. Jacobsen *et al.* (2008) suggested that a low pH in combination with even small levels of protein bound metal ions can intensify metal-catalysed oxidation in food emulsions and that this could explain the important role of metal ions in this food system, in which pH is around 4.

In a second study, the effect on lipid oxidation of the delivery system for omega-3 PUFA was evaluated. Omega-3 PUFA were thus added either as neat oil or as an oil-in-water emulsion (50% oil) prepared with whey protein as an emulsifier (Let *et al.*, 2007b). Volatiles and sensory data indicated a better oxidative stability of dressing with neat fish oil compared to the dressing with the fish oil-in-water emulsion (Fig. 5.3). So, in this food system pre-emulsification of the fish oil did not lead to increased oxidative stability. The authors suggested that this finding could be due to increased oxidation in the fish oil-in-water emulsion itself, which was caused by the initial temperature increase (65 ºC, 3 min) during homogenisation of this emulsion. They also concluded that addition of antioxidants before homogenisation of the pre-emulsion may be necessary to improve its oxidative stability.

5.3.5 Margarine

Traditional margarine has a high fat content (~82%) and is a w/o emulsion. Margarine is a solid-liquid emulsion and therefore the diffusion rate of different components, e.g. antioxidants and prooxidants, in this system may be lower compared to a liquid-liquid emulsion such as milk.

Young *et al.* (1990) produced fish oil enriched margarine, in which 20% of the soybean oil was substituted with fish oil. Two different antioxidant mixtures: 150 mg/kg Grindox 117 (ascorbyl palmitate, propyl gallate, and citric acid) and 200 mg/kg Grindox 109 (BHA, BHT, propyl gallate, and citric acid) were evaluated in margarine with and without fish oil enrichment. No significant

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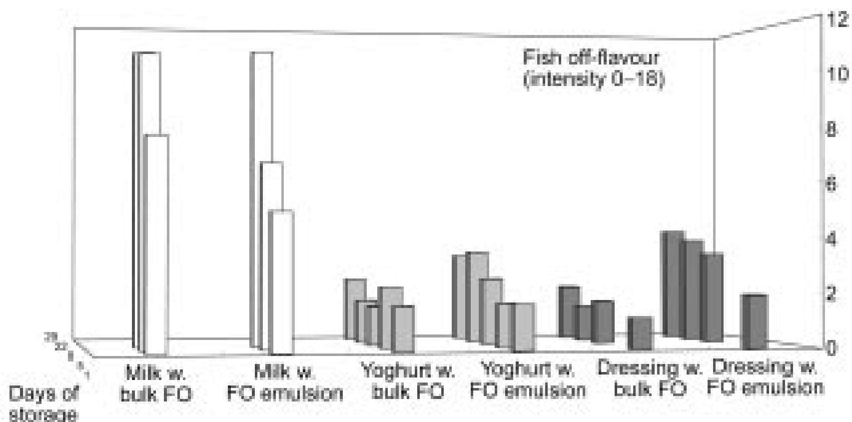

Fig. 5.3 Summarised intensity of fishy odour and flavour of milk, yoghurt and dressing enriched with either neat fish oil or fish oil-in-water emulsion. Average standard deviations were 1.9, 1.3, and 1.4 for milk, yoghurt, and dressing, respectively. From Let, M.B., Jacobsen, C., Meyer, A.S. (2007b) Lipid oxidation in milk, yoghurt and salad dressing enriched with neat fish oil or pre-emulsified fish oil. *J. Agric. Food Chem.* 55, 7802–7809 with permission from ACS Publications.

difference between the two antioxidant mixtures was found. However, preliminary sensory tests indicated a better flavour stability when Grindox 109 (BHA, BHT) was added to margarine, whereas another oxidative stability test indicated that Grindox 177 (ascorbyl palmitate) had stronger antioxidative effect (Young *et al.*, 1990). The oxidative stability was further optimised by adding EDTA (150 mg/kg) to the margarine.

In another study, it was concluded that low calorie spreadable fats (soft margarine and mix of butter and vegetable oil) could be enriched with up to 1% EPA and DHA without significantly affecting the sensory quality (by Kolanowski *et al.*, 2001). The margarine spread may be stored up to 6 weeks and the spread based on butter and vegetable up to 3 weeks without significant decrease of quality. These spreads contained 55% fat and no antioxidants were added.

5.3.6 Milk

In a study by Kolanowski *et al.* (1999), it was not possible to incorporate even low levels of EPA and DHA (0.15% fish oil which equals 0.05% EPA and DHA) into milk without significantly reducing the palatability of the milk and this demonstrated that omega-3 PUFA enriched milk seems to be particular susceptible to oxidation.

Several studies on enrichment of milk with fish oil have been reported by our laboratory to further study and solve this problem. In these studies milk contained 1.5% fat of which either 0.5% (absolute value) or all the oil was fish oil. From these studies it is evident that fish oil enriched milk will oxidise

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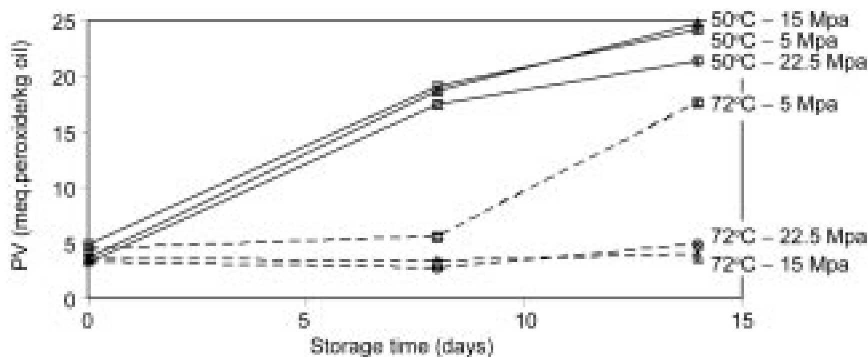

Fig. 5.4 Peroxide values in fish oil enriched milk emulsions homogenised at different pressures and temperatures. Emulsions were stored at 2ºC. From Sørensen, A.D.M., Baron, C.P., Let, M.B., Brüggemann, D., Pedersen, L.R.L., Jacobsen, C. (2007) Homogenization conditions affects the oxidative stability of fish oil enriched milk emulsions: Oxidation linked to changes in the protein composition at the oil-water interface. *J. Agric. Food Chem.* 55, 1781–1789 with permission from ACS Publications.

rapidly if precautions to avoid oxidation are not taken. It was demonstrated that an important precaution is to use fish oil of very high quality, i.e. with a low level of lipid hydroperoxides and secondary oxidation products (Let *et al.*, 2003; 2005a). Let *et al.* (2005a) found that a sensory panel was clearly able to distinguish between milk emulsions produced with fish oil and rapeseed oil mixture with a PV of 0.1 meq O_2/kg and those produced with oils of PV 0.5, 1.0, or 2.0 meq O_2/kg. Throughout storage, the milk emulsion with oil of PV 0.1 meq O_2/kg was perceived as being less fishy and rancid and could not be discriminated from milk without fish oil.

The homogenisation conditions were found to be another important parameter influencing lipid oxidation in this food system (Let *et al.*, 2007c; Sørensen *et al.*, 2007) (Fig. 5.4). Surprisingly, the highest oxidative stability was obtained at high homogenisation pressure (22.5 MPa) and temperature (72 ºC), even though the droplet size was smallest under these conditions (Let *et al.*, 2007c). As previously mentioned for mayonnaise a small droplet size and thereby a large interfacial area may in some cases lead to increased oxidation, but this was not the case for omega-3 PUFA enriched milk emulsions. It was shown by SDS-PAGE and confocal laser scanning microscopy that a temperature increase from 50 ºC to 72 ºC led to an increase in the amount of β-lactoglobulin adsorbed at the oil-water interface and that even more β-lactoglobulin was adsorbed when the pressure was increased from 5 MPa to 22.5 MPa (Sørensen *et al.*, 2007) (Table 5.1). In addition, the level of free thiol groups was increased at the high temperature and pressure (72 ºC and 22.5 MPa), whereas less casein seemed to be present at the oil-water interface with increasing pressure (Table 5.1).

Let *et al.* (2004; 2005b) also demonstrated that oxidative flavour deterioration in omega-3 PUFA enriched milk could be prevented by using a mixture of fish oil and rapeseed oil (1:1). The antioxidative effect of rapeseed oil was proposed to be

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Table 5.1 Relative quantities of β-lactoglobulin, κ-casein, β-casein, α_{s1}-casein and α_{s2}-casein isolated from the MFGM in milk emulsions produced at different homogenisation conditions and reference milk (without oil added and no further homogenisation). The amount is calculated from the protein separation using MOPS running buffer

MFGM sample						
Pressure (MPa)	Temperature (°C)	β-lacto-globulin	κ-casein	β-casein	α_{s1}-casein	α_{s2}-casein
5	50	8.01	16.10	5.07	5.94	5.14
	72	10.17	15.94	3.67	4.28	4.09
15	50	9.36	14.97	4.34	5.21	5.08
	72	14.56	14.17	3.65	4.55	4.59
22.5	50	9.45	14.93	5.18	4.56	4.23
	72	13.72	15.86	4.78	3.68	3.74
Reference	No treatment	4.92	17.95	5.74	5.18	4.19

From: Sørensen, A.D.M., Baron, C.P., Let, M.B., Brüggemann, D., Pedersen, L.R.L., Jacobsen, C. (2007) Homogenisation conditions affects the oxidative stability of fish oil enriched milk emulsions: Oxidation linked to changes in the protein composition at the oil-water interface *J. Agric. Food Chem.* 55, 1781–1789.

due to its high content of γ-tocopherol. In contrast to the findings for salad dressing, Let *et al.* (2007b) observed that the use of the same oil-in-water emulsion as that used for the salad dressing reduced lipid oxidation in milk as compared to addition of omega-3 PUFA in the form of neat fish oil (Fig. 5.3).

The ability of a range of different antioxidants to prevent lipid oxidation in fish oil enriched milk has also been investigated. When a low fish oil concentration (0.5% fish oil) or when a very high quality fish oil (PV < 0.2 meq/kg) was used for supplementation with 1.5% fish oil, EDTA only slightly reduced oxidation. In contrast, EDTA seemed to be more efficient when a high concentration of fish oil of a less good quality (PV 1.5 meq/kg) was used (Let *et al.*, 2005b). The reason for the better effects of EDTA in milk emulsions containing fish oil of a lower quality was most likely that oxidation was much more pronounced in this emulsion and therefore the effect of EDTA was easier to detect. This finding was supported by another study on milk drink containing 5% fat of which 0.5% (absolute value) was enzyme modified fish oil in which EDTA effectively reduced off-flavour formation (Timm-Heinrich *et al.*, 2004).

Similar to the findings reported for mayonnaise, lactoferrin also exhibited a concentration-dependent effect in milk drink prepared with enzyme modified fish oil or sunflower oil (Nielsen *et al.*, 2004). However, even at its optimum addition levels (1000 mg/kg) lactoferrin only slightly reduced oxidation. At other concentrations it even exerted slightly prooxidative effects.

Addition of a combination of α- and γ-tocopherol to omega-3 enriched milk was found to have prooxidative effects (Let *et al.*, 2005b). In contrast, pure α-tocopherol in a concentration of 1.1 mg/kg or γ-tocopherol in concentrations of

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0.8 or 3.3 mg/kg had weak antioxidative effects. Moreover, a much better effect was obtained with γ-tocopherol alone in an intermediate concentration of 1.7 mg/kg. Prooxidative effects of the combination of α-and γ-tocopherol have been suggested to be due to too high a concentration of α-tocopherol in the fish oil enriched milk, resulting from the fact that the fish oil used for the milk systems contained high concentrations of α-tocopherol. Other studies have also shown that α-tocopherol can have prooxidative effects in high concentrations (Huang *et al.*, 1994).

In contrast to the findings described above in mayonaise, ascorbyl palmitate was found to exert a strong antioxidative effect at an addition level of only 1.5 mg/kg in milk with 1.5% fat (Let *et al.*, 2005b). Interestingly, in strawberry flavoured milk drink, which contained 5% fat, ambiguous results were obtained. Thus, ascorbyl palmitate was found to be able to reduce the formation of lipid hydroperoxides and certain volatiles such as heptadienal when added in high concentrations (15 and 30 mg/kg). In contrast, ascorbyl palmitate promoted the formation of hexenal and nonadienal as well as fishy off-flavours when added in concentrations of 7.5 mg/kg and above, but reduced the formation of these two volatiles when added at a lower concentration of 3.75 mg/kg (Jacobsen *et al.*, 2008).

The much stronger antioxidative effects of ascorbyl palmitate in milk with 1.5% fat compared to its effect in the milk drink with 5% fat has been suggested to be due to the different ascorbyl palmitate concentrations applied and it was speculated that it may be possible that ascorbyl palmitate has a stronger antioxidative effect in milk drink if lower concentrations than 3.75 mg/kg are applied and this needs further investigation (Jacobsen *et al.*, 2008). It was also speculated that the different compositions of the two milk systems might have influenced the efficacy of ascorbyl palmitate. Milk with 1.5% fat did not contain any additives, whereas the milk drink contained mono- and diglycerides, carageenan and guar gum, which may interact at the O/W interface. Such interactions may reduce the ability of ascorbyl palmitate to regenerate tocopherol (Jacobsen *et al.*, 2008).

5.3.7 Yoghurt and drinking yoghurt

Kolanowski *et al.* (1999) found that enrichment of flavoured yoghurt with up to 0.3% fish oil (i.e., 0.15% EPA and DHA) resulted in a product with acceptable sensory characteristics and this may suggest that yoghurt could be easier to enrich with omega-3 PUFA than milk.

Jacobsen *et al.* (2006) evaluated the oxidative stability of high fat yoghurt (2.47% fat) of which 1% was pre-emulsified fish oil. Fish oil was preemulsified using either citric acid ester or milk protein as emulsifier before addition to the yoghurt. Both ingredients have been shown to have antioxidant effects in other studies. However, both types of yoghurt were found to have a high oxidative stability and it was therefore not possible to evaluate if citric acid ester (4000 mg/kg) or milk protein exerted any antioxidative effect. Subsequently, the oxidative stability of omega-3 enriched yoghurt and milk was compared and it

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was concluded that yoghurt was much less susceptible to oxidation (Let *et al.* 2007b) confirming the conclusions that could be made from the studies by Kolanowski *et al.* (1999; 2001). In the same study, the effect of adding omega-3 PUFA either in the form of neat fish oil or as a pre-emulsified oil was compared (Fig. 5.3). Similar to the findings for salad dressing it was concluded that addition of pre-emulsified fish oil did not reduce lipid oxidation.

The good sensory stability of omega-3 enriched yoghurt was also confirmed by Chee *et al.* (2005). In this study, a yoghurt mix (2 g fat/100 g) was supplemented with an algae oil emulsion to provide 500 mg omega-3 PUFA per 272 g serving of yoghurt white mass. The emulsion was added to the yoghurt mix either before or after the homogenisation step and prior to pasteurisation. It was then flavoured with a strawberry fruit base and fermented and stored for up to three weeks. A trained sensory panel could distinguish a stronger fishy flavour in both of the supplemented yoghurts after 22 days storage, but a consumer panel rated both control and supplemented samples similarly, as 'moderately liked'.

Recently, it was found that drinking yoghurt, produced from commercial yoghurts (0.5% fat, w/w) mixed with fish oil (1%, w/w), flavour, sugar and stabiliser (pectin) also had a very high oxidative stability (Nielsen *et al.*, 2007). Thus, the yoghurts were found to be stable for at least four weeks when stored at 2 ºC. It was investigated whether one or more of the ingredients were responsible for the high oxidative stability of this product. However, due to a high stability even of plain yoghurt with fish oil added it was not possible to conclude on possible antioxidative effects of the added ingredients (Nielsen *et al.*, 2009). Likewise, addition of citric acid ester (50, 100 and 200 mg/kg) to the drinking yoghurt did not have any effect (Nielsen *et al.*, 2007). Addition of EDTA (50 mg/kg) seemed to have a slight antioxidative effect in drinking yoghurt after addition of metal ions (50 mg/kg iron), even though the presence of this prooxidant did not result in significantly more oxidation (Nielsen *et al.*, 2007).

In order to investigate the mechanisms behind the high oxidative stability, peptides formed during fermentation of yoghurt were recently isolated and fractionated (Farvin *et al.*, 2010a). Subsequently, the antioxidant activity of the peptides were analysed by different *in vitro* assays, including the DPPH radical scavenging activity, Fe^{2+} chelating activity, reducing power and inhibition of oxidation in liposome model system. Overall, the assays showed that the peptides of lower molecular weight had good metal chelating and iron reducing properties, whereas the higher molecular weight peptides were more efficient radical scavengers and exerted a better effect in the liposome model (Farvin *et al.*, 2010a). Further, the low molecular weight peptides were evaluated in fish oil enriched milk and they were shown to exert almost the same antioxidative effect as caseinophosphopetides. It was also observed that the yoghurt contained a considerable amount of free amino acids such as His, Tyr, Thr and Lys, which has been reported to have antioxidant properties (Farvin *et al.*, 2010b). The identified peptides comprised a few N-terminal fragments of αs_1-CN, αs_2-CN, κ-CN and several fragments from β-CN. Almost all the peptides identified contained at least one proline residue. Some of the identified peptides included

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the hydrophobic amino acid residues Val or Leu at the N-terminous of the peptides and Pro, His or Tyr in the sequence which are the characteristic of antioxidant peptides (Farvin *et al.*, 2010b).

It was also speculated whether the bacteria used for fermenting yoghurt would lower the oxygen content and thereby decrease oxidation. Therefore, the oxygen content of the yoghurt was measured and it was found to be lower than that of milk (Farvin *et al.*, 2010a). It was therefore concluded that the higher oxidative stability of yoghurt might be due to the presence of antioxidant peptides and free amino acids formed during fermentation of the yoghurt and that the lower oxygen content of yoghurt, which subsequently reduces the oxidative stress of fish oil incorporated in the yoghurt may also have contributed to the enhanced oxidative stability of fish oil enriched yoghurt compared to fish oil enriched milk.

From these studies it can thus be concluded that fish oil enriched yoghurt is a very suitable product for omega-3 PUFA enrichment from an oxidation point of view, and that no antioxidants are required to protect it from oxidation.

5.3.8 Fitness bars and bread

Oxidation studies on fish oil enriched solid matrices such as breads or bread like products are very scarce, but a few studies have been carried out on lipid oxidation in fish oil enriched energy bars. (Nielsen and Jacobsen, 2009; Horn *et al.*, 2009). In the first study on fish oil enriched fitness bar, the effect of the delivery system (neat fish oil vs. emulsified fish oil vs. microencapsulated fish oil) on lipid oxidation was investigated. Fitness bars enriched with neat fish oil were found to be highly susceptible to lipid oxidation (Nielsen and Jacobsen, 2009). Addition of fish oil as micro-encapsulated powder offered the best protection towards oxidation but pre-emulsification of the fish oil with sodium-caseinate in water or packaging the energy bars in modified atmosphere also improved the oxidative stability. Surprisingly, addition of the metal chelator EDTA (100–2000 mg/kg) to emulsified fish oil decreased the oxidative stability compared to energy bars without EDTA. The authors hypothesised that the EDTA to iron ratio was too low to obtain an antioxidative effect in this system. The concentration of EDTA must be sufficient to chelate all the iron, otherwise preferential chelation of Fe^{3+} over Fe^{2+} takes place, leaving the more active oxidation catalyst Fe^{2+} free to work.

In a second study on fish oil enriched energy bars, another hydrophilic antioxidant, caffeic acid, was tested in concentrations from 3.75 to 15 mg/kg energy bar (Horn *et al.*, 2009). Similar to EDTA, caffeic acid also decreased the oxidative stability. The authors ascribed the prooxidative effect of caffeic acid to its ability to reduce transition metal ions, which are more potent prooxidants than the unreduced form. The hydrophilic nature of caffeic acid was furthermore suggested to lead to a localisation of caffeic acid that would favour reduction of metal ions over radical scavenging.

Two other antioxidants with different polarities/solubilities were tested in the same study, namely the amphiphilic ascorbyl palmitate and the lipophilic γ-

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tocopherol. Ascorbyl palmitate was added in concentrations from 3.75 to 15 mg/kg energy bar and showed less prooxidative activity than the two above-mentioned hydrophilic antioxidants, but still decreased oxidative stability, especially in the highest concentrations (Horn *et al.*, 2009). The authors suggested that the mechanism behind the prooxidative effect of ascorbyl palmitate was the same as that of caffeic acid, i.e. the ability of the 'antioxidant' to reduce transition metals to a more prooxidative form. As mentioned above more or less similar results have been reported for fish oil enriched salad dressing. Thus, a high concentration of ascorbyl palmitate was found to be more prooxidative than a lower concentration in both systems (Let *et al.*, 2007a; Horn *et al.*, 2009). The concentration dependence of ascorbyl palmitate was suggested to exist because the antioxidative mechanism of ascorbyl palmitate is overridden by its ability to reduce transition metal ions when present in a high concentration (Horn *et al.*, 2009).

In contrast to the hydrophilic and amphiphilic antioxidants tested in energy bars, the lipophilic γ-tocopherol (5.5–55 mg/kg energy bar) was shown to exhibit an antioxidative effect when added in concentrations above 22 mg/kg energy bar (Horn *et al.*, 2009). In energy bars, the lipophilic nature of γ-tocopherol was proposed to favour an optimal localisation of the antioxidant in order to carry out its antioxidative effect. In low concentrations, a prooxidative effect of γ-tocopherol was though observed, which is contradictory to other studies on the effects of tocopherols, in which prooxidative effects were found for high concentrations of tocopherols (Huang *et al.*, 1994; Jung and Min, 1992).

Taken together, the prooxidative effects exerted by caffeic acid and ascorbyl palmitate may indicate that metal catalysis of oxidation to some extent is an important factor in energy bars. However, since EDTA was also found to be prooxidative this may suggest that the metal-catalysing mechanism may be different from that observed in systems such as mayonnaise and dressing (Jacobsen *et al.*, 2001a; Let *et al.*, 2007a). The observed strong antioxidative effect of γ-tocopherol suggests that free radical scavengers can reduce lipid oxidation in energy bars. This could indicate that initiation of lipid oxidation by already existing free radicals present in the ingredients used for energy bars may be an important factor. It could therefore be hypothesised that the control of lipid oxidation may be further improved by adding a combination of antioxidants with both free radical scavenging and metal-chelating properties. This deserves further investigation.

Becker and Kyle (1998) evaluated the sensory stability of bread baked with either a regular pharmaceutical grade fish oil made from sand eel, a specialty tuna oil or an algal oil. The taste panel indicated that sensory off-flavours were less likely to be detected in the DHA bread made with algal oils compared to those made with fish oils. Based on these data they suggested that the algal source had a better stability than the fish oils. This proposition was later invalidated by Frankel *et al.* (2002). They showed that the high oxidative stability of the commercial DHA-rich algal oil was lost when the triglycerides was purified to remove tocopherols and other antioxidants. Moreover, an oil-in-

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water emulsion with the same algal oil had a lower oxidative stability than corresponding fish oils.

5.3.9 Fish and meat products

Similar to baked products only few studies have been reported on omega-3 PUFA enriched fish and meat products. Olsen *et al.* (2006) evaluated the antioxidative effect of addition of citric acid (3300 mg/kg) or EDTA (75 mg/kg) to salmon paté containing 14% cod liver oil. The fish oil producer had added α-tocopherol (1075 mg/kg), lecithin (1050 mg/kg) and ascorbyl palmitate (375 mg/kg) to the cod liver oil, which was used in this experiment. Analysis of volatiles by GC-MS as well as sensory analysis suggested that neither citric acid nor EDTA were efficient antioxidants in this food system. In fact, citric acid and EDTA even slightly promoted formation of volatile oxidation products. EDTA had a small positive impact on the sensory perception of the samples, whereas citric acid seemed to slightly increase scores for rancidity. The authors suggested that the poor effect of citric acid was partly to be due to the fact that addition of citric acid reduced pH, which in some cases has been shown to increase oxidation. Likewise, it was suggested that the increased formation of volatiles in samples with EDTA was due to a too low EDTA concentration.

In another study, the antioxidative effect of EDTA was evaluated in cod surimi enriched with 1.5% algal oil (Park *et al.*, 2004). The algal oil was added either as neat oil or as an oil-in-water emulsion. Two different algal oils were evaluated; one without antioxidants added and one with lipid soluble antioxidants (1000 mg/kg tocopherol mixture plus 1000 mg/kg rosemary extract plus 500 mg/kg ascorbyl palmitate). EDTA did not have any effect in cod surimi with neat oil containing lipid soluble antioxidants when evaluated by determination of PV and TBARS. In contrast, an antioxidative effect of EDTA was observed when the algal oil was added to the cod surimi as an emulsion with lipid soluble antioxidants.

Pérez-Mateos *et al.* (2006) also studied the oxidative stability of fish oil enriched surimi in the presence of different antioxidant. In this study, surimi was made from pollock muscle to which menhaden oil or a fish oil concentrate was added, each in two concentrations (7.0 or 11.6% for menhaden oil and 1.7 or 2.8% for the fish oil concentrate). Menhaden oil contained a mixture of tocopherol plus TBHQ added by the fish oil manufacturer, whereas the fish oil concentrate only contained a mixture of tocopherols. Surimi enriched with menhaden oil received better sensory scores than surimi enriched with fish oil concentrate, mainly due to the fact that the latter had a fishy taste. Rosemary (750 mg/kg) or tea extracts (650 mg/kg) were able to partially mask this fishy taste. But both extracts seemed to have weak prooxidative effects in surimi with menhaden oil (Pérez-Mateos *et al.*, 2006).

Lee *et al.* (2005) evaluated the effect of antioxidant combinations in ground beef patties enriched with 500 mg omega-3 PUFA/110 g meat. The effect of BHA (0.1 mg/kg), a tocopherol mixture (0.3 mg/kg) or rosemary (2 mg/kg) as

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well as two antioxidant cocktails (1 mg/kg erythorbate + 5 mg/kg citrate + 0.3 mg/kg toco-mix or 1 mg/kg erythorbate + 5 mg/kg citrate + 2 mg/kg rosemary) were evaluated. TBARS decreased in beef patties containing the rosemary extract or BHA, but increased upon addition of the tocopherol mixture. When the antioxidant cocktails were used a decrease in both TBARS and discoloration was observed. Interestingly, control sample without omega-3 PUFA oxidized more than omega-3 PUFA fortified ground beef patties without antioxidants. This finding was suggested to be due to the antioxidative effect of the tocopherols present in the fish oil itself.

Lee *et al.* (2006a; 2006b) investigated the effect of an antioxidant cocktail in ground turkey enriched with an algal oil emulsion prepared from: 25% algal oil + 2.5% whey protein isolate + 10mM sodium citrate + 0.2% potassium sorbate + 500 mg/kg 70% toco-mix + 100 μM EDTA. Ground turkey contained 500 mg omega-3 PUFA/110 g meat. The antioxidant cocktail consisted of 1000 mg/kg sodium erythorbate + 5 mg/kg sodium citrate + 2 mg/kg rosemary extract. Addition of the antioxidant cocktail to the algal oil fortified product lowered TBARS. In fact, no increase in TBARS was seen over time in the product with antioxidants. Further, there was no difference in sensory scores for overall liking between the control samples without omega-3 PUFA and the ground turkey with omega-3 PUFA and antioxidants. Despite this it was found that the antioxidant cocktail did not prevent a loss of omega-3 PUFA after frozen storage and cooking of the ground turkey. The omega-3 PUFA level was thus reduced to approximately 65% of the original level present in fresh ground turkey.

The effect of the same antioxidant cocktail was also investigated in restructured hams and pork sausages enriched with the same algal oil emulsion and in the same concentration as above (Lee *et al.*, 2006a; 2006b). The antioxidant cocktail appeared to be able to control the level of lipid hydroperoxides in the restructured ham. Moreover, nitrite curing of this product delayed lipid oxidation to such an extent that it was no longer possible to determine any effect of the antioxidant treatment when evaluated by TBARS. However, sensory scores for overall liking were lower for the omega-3 enriched product. For pork sausages, the results were quite similar to those of ground turkey. Thus, addition of the antioxidant cocktail resulted in lower and stable TBARS that did not increase over time and there was no difference in the sensory scores for overall liking between the control samples without omega-3 PUFA and the sausages with omega-3 PUFA and antioxidants.

Valencia *et al.* (2008) investigated antioxidant effects of green tea catechins (200 mg/kg) and green coffee antioxidants (200 mg/kg) in omega-3 enriched pork sausages. In this study, 4.9% fish oil was added in the form of an oil-in-water emulsion prepared with soy protein as emulsifier. Green tea catechins significantly reduced lipid oxidation as evaluated from TBARS, whereas green coffee antioxidants did not have any effect. The authors did not suggest any explanation to the different effects obtained by the green tea catechins and the green coffee antioxidants.

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5.4 Conclusions

As illustrated in this chapter it is possible to develop well tasting omega-3 PUFA enriched products with acceptable shelf life provided that proper precautions to avoid lipid oxidation are taken. Some food products are easier to enrich with omega-3 PUFA than others. Particularly, yoghurt-based products seem to be highly suitable for omega-3 PUFA enrichment due to their content of anti-oxidative peptides. In contrast, milk and mayonnaise are more difficult to enrich with omega-3 PUFA.

It is also clear that in several cases antioxidants such as propyl gallate, tocopherol and ascorbyl palmitate, which traditionally have been used by the food industry, are not very efficient in omega-3 PUFA containing foods. Moreover, the same antioxidant exerts completely different effects in different food systems. Therefore, a better quantitative understanding of the effects of antioxidants in real food systems is necessary.

5.5 Future trends

According to several market analyses, it is expected that the growth in the number of new omega-3 PUFA enriched products will continue in the coming years in different food categories.

This chapter has mainly dealt with EPA and DHA from marine sources. However, with the increased focus on the beneficial effects of omega-3 PUFA in general, products enriched with LNA will most likely also receive more attention from the industry, particularly if the authorities do not allow food producers to discriminate between EPA/DHA on one side and LNA on the other side, as may be the case with the new EU regulation, which will be adopted in 2010.

In the near future the total demand for fish oils for aquaculture and human nutrition is expected to exceed production. This is one of the reasons behind the current efforts to develop plants with a high level of EPA and DHA by genetic engineering. Such genetically modified plant oils are expected to be on the market within the coming 5 to 10 years. Genetically modified plant oils with stearidonic acid (18:4 n-3) are also under development and may be yet another alternative to fish and algae oils. Moreover, there is also an increasing interest in the use of marine phospholipids from krill and other aquatic resources for human nutrition, but more research is needed to elucidate the potential application of these lipids in food products.

Traditionally, the industry has mainly used free radical chain-breaking synthetic antioxidants for the prevention of oxidation in foods. As mentioned above this strategy seems to be less efficient in preventing lipid oxidation in many food systems enriched with omega-3 PUFA. With our increased understanding of the important role of trace metals, emulsifiers and processing conditions in lipid oxidation processes, more efforts will be dedicated to use this knowledge to develop alternative strategies to retard lipid oxidation in real foods

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with omega-3 PUFA oils. One such strategy may be an increased use of both natural metal chelators including plant extracts with both radical scavenging and metal chelating properties. Another strategy may be to design oxidatively stable oil-in-water emulsion delivery systems for each particular food system.

5.6 Sources of further information and advice

The Omega-3 Information Network at:
PO Box 24, Tiverton,
Devon EX16 4QQ, UK.
Tel: +44 (0) 1884-257547, Fax: +44 (0) 1884-242757
E-mail: rayrice@eclipse.co.uk

International Fishmeal & Fish Oil Organisation
2 College Yard, Lower Dagnall Street,
St Albans, Hertfordshire AL3 4P4, UK
E-mail: secretariat@iffo.org.uk

Oils and Fats International
DMG World Media (UK) Ltd
Queensway House, 2 Queensway, Redhill
Surrey RH1 1QS, UK
Tel: +44 (0) 1737 855068, Fax: +44 (0) 1737 855470
Email: anitarevis@uk.dmgworldmedia.com

The Fish Foundation: http://www.fish-foundation.org.uk/references.htm

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6

Oxidation of edible oils

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Abstract: The main reason for the deterioration of fats and oils is the oxidation process. This produces short-chain aroma-active compounds, which are responsible for the development of unpleasant taste and smell, but also for changes in colour, viscosity, density and solubility. This chapter discusses different pathways for the formation of primary and secondary oxidation products and describes some methods which can be used to investigate the oxidative state of fats and oils, including the sensory evaluation. Other topics include the effect of oxidation processes on sensory and nutritional quality, and the influence of oil processing on the oxidative stability of edible oils.

Key words: analysis, autoxidation, free radical chain mechanism, lipid oxidation, processing, refined oils, sensory evaluation, virgin oils.

6.1 Introduction

Edible oils consist of about 96% triacylglycerides, composed of different fatty acids. Some other compounds or groups of compounds, such as free fatty acids, phospholipids, phytosterols, tocopherols, other antioxidants or waxes, can also be found. Fatty acids, free or bound to glycerol are susceptible to oxidative processes resulting in a wide range of volatile and non-volatile degradation products. Therefore one of the major challenges for the oil processing industry is to maintain the high quality of the product after processing until use by the consumer. However, the oxidative stability of edible oils not only depends on conditions during storage, but also the history of the raw material and the processing steps involved.

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Oxidation processes play an important role in the deterioration of fats and oils with rancidity as the main effect. The most characteristic changes which become more and more obvious during the oxidation process are the development of an unpleasant taste and smell, but also changes in colour, viscosity, density and solubility take place. Further consequences include the loss of essential fatty acids, the degradation of vitamins and pro-vitamins, and the formation of odour-intensive compounds. These changes strongly influence the nutritional value and sensory quality of edible oils. The primary stage of the oxidation process produces hydroperoxides. As these hydroperoxides degrade, compounds are formed which are considered to have a certain toxicological potential in higher concentrations. The products of the oxidation process can react with other ingredients in complex composed foods, such as amino acids or proteins, resulting in changes of texture or colour. Therefore oxidation is very important in terms of the palatability, toxicity and nutritional value of edible oils.

6.2 Lipid oxidation

The oxidation process depends on a number of factors and one important task in the production and commercialization of edible oils is to control these factors to a point where no unfavourable changes can be expected over a certain time. The susceptibility of edible oils to oxidation reactions depends on the unsaturation grade of fatty acids, the content and type of antioxidants, temperature, the availability of oxygen, and the presence of light and trace metals.

Oxidation of edible oils begins with the double bonds of fatty acids in the triacylglycerol molecule. The first products of lipid oxidation are hydroperoxides, which are tasteless and odourless and therefore do not significantly impair the sensory quality of the oil (Reindl and Stan, 1982). The problem is that these compounds are generally unstable, resulting in the formation of secondary oxidation products like ketones, aldehydes, alcohols, lactones, hydrocarbons, esters, or others. Since some of these compounds have a very low threshold value for smell or taste, the degradation of the product becomes obvious very quickly (Frankel, 1984; Ladikos and Lougovois, 1990).

The main pathway for the formation of hydroperoxides is autoxidation, but three others are also possible – photo-oxidation, enzymatic oxidation and irradiation. The pathways differ in their formation of radicals, but follow the same subsequent mechanism (see Fig. 6.1).

6.2.1 Autoxidation

The main partners in each reaction are unsaturated fatty acids, either free or bound in the triacylglycerol molecules, and oxygen. Normally, atmospheric oxygen is present as so-called triplet oxygen, $^3O_2$, which contains two unpaired electrons in the ground state. A direct reaction of the triplet oxygen with the fatty acid molecule is not possible in this state because of a spin-forbidden

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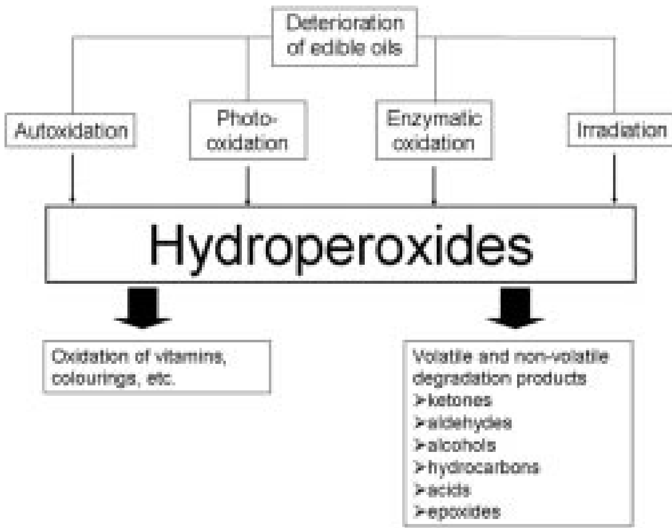

Fig. 6.1 Reactions resulting in oxidative deterioration of edible oils.

reaction, but triplet oxygen can react with fatty acid radicals. The resulting reaction is called autoxidation, a free radical chain reaction first explained by Farmer *et al.* (1942) and Bolland (1949). Figure 6.2 shows the stages of this reaction, which can be divided into an initial phase, propagation, chain branching, and termination. The elimination of a hydrogen atom from the intact fatty acid molecule is necessary to begin the chain reaction. This forms a radical, which is able to react with triplet oxygen. According to Farmer, the methylene groups of the molecule are activated first, then a hydrogen atom is eliminated to

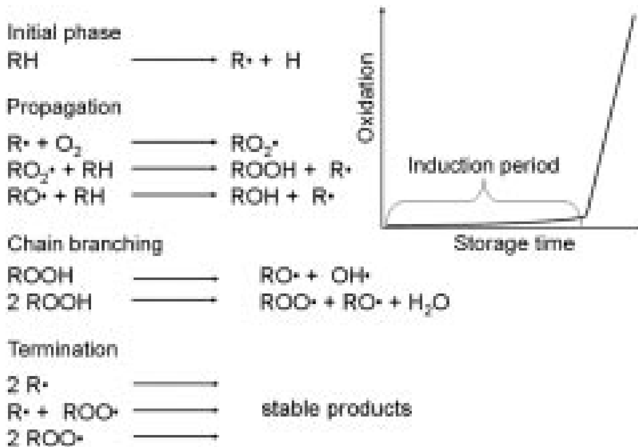

Fig. 6.2 Free radical chain mechanism according to Farmer *et al.* (1942) and Bolland (1949).

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form the free radical. This fatty acid radical is not very stable and reacts with triplet oxygen within a very short time because no activation energy is necessary for this reaction step. A peroxy radical is formed. The reaction between the fatty acid radical and oxygen strongly depends on the solubility of oxygen in the lipid and the resulting availability of oxygen for the reaction. The solubility of oxygen in the lipid decreases with increasing temperature, resulting in the retardation or cessation of the formation of peroxy radicals. If no oxygen is available, other reactions, such as polymerization, take place.

The peroxy radical is also reactive. It abstracts a hydrogen atom from another intact unsaturated fatty acid, resulting in the formation of a hydroperoxide, the primary product of the degradation process of edible oils. After this initial step, the chain reaction continues as the new formed radical combines with triplet oxygen and abstracts hydrogen from another unsaturated fatty acid, forming another hydroperoxide. From this it becomes clear that the autoxidation process accelerates in an exponential function, as each formed radical forms a new radical and one hydroperoxide. However, it takes some time before hydroperoxide formation increases to a level which is detectable. This initial slow phase of hydroperoxide formation, which later accelerates, is called the induction period (see Fig. 6.2).

It is not yet fully understood what causes the initial step of the chain reaction, but it seems that heat, metal catalysts or visible and ultravisible irradiation are responsible. In the presence of heavy metal ions (like Fe^{2+} or Cu^{2+}) an accelerated breakdown of the hydroperoxides takes place, freeing radicals and resulting in an accelerated oxidation process, according to the radical chain mechanism presented in Fig. 6.2.

The susceptibility of fatty acids to hydrogen abstraction strongly depends on the degree of unsaturation and can be explained by the bond strength of the hydrogen of the α-methylene group in the fatty acid molecule. The abstraction of hydrogen from the fatty acid molecule by the peroxy radical is relatively slow, and the peroxy radical always abstracts the most weakly bound hydrogen in the fatty acid molecule. While the bond strength of hydrogen in saturated fatty acids is about 99 kcal/mol, only about 80 kcal/mol are necessary to abstract a hydrogen atom from a methylene group in oleic acid and the abstraction of hydrogen from the doubly allylic methylene group of linoleic acid only requires 69 kcal/mol. A further reduction of the bond strength of hydrogen can be found for linolenic acid, which has two doubly allylic methylene groups in the molecule (40 kcal/mol). Different fatty acid molecules contain different bond strengths of hydrogen, and therefore exhibit significant differences in the relative rate of lipid oxidation. This is given as 1 : 100 : 1200 : 2500 for stearic acid : oleic acid : linoleic acid : linolenic acid on the basis of peroxide formation and 1:40–50:100 on the basis of oxygen uptake (Hsieh and Kinsella, 1989). Thus edible oils with high amounts of polyunsaturated fatty acids are subjected to a fast autoxidation during storage. In contrast, oils that consist mainly of saturated or monounsaturated fatty acids are much more stable against oxidative deterioration (see Fig. 6.3).

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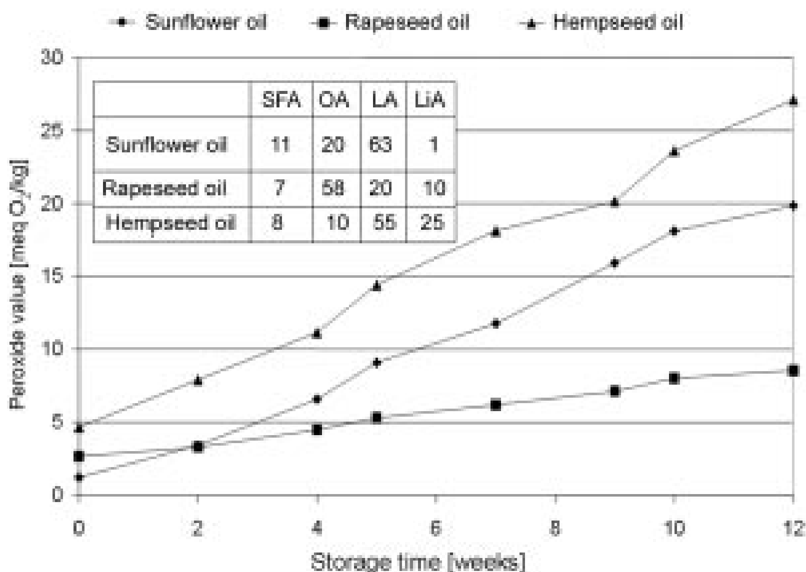

Fig. 6.3 Correlation between fatty acid composition and development of the peroxide value during storage of rapeseed (■), sunflower (◆) and hemp seed oil (▲). (SFA = saturated fatty acids; OA = oleic acid; LA = linoleic acid; LiA = linolenic acid; data expressed as g/100 g).

Following the abstraction of hydrogen, the π-electrons in the pentadienylic radical of the polyunsaturated fatty acid molecule become delocalized, leading to different possibilities for the triplet oxygen to react with the formed radical. In linoleic acid, two different hydroperoxides are formed in positions 8 and 10, but hydroperoxides can also be found in positions 9 and 11. The result is a shift in the position of the double bonds due to resonance stabilization and the formation of conjugated double bounds in oxidized polyunsaturated fatty acids.

The formation of conjugated dienes and trienes from linoleic and linolenic acid during oxidation can be followed by measuring the absorption bands at about 232 nm and 268 nm, respectively. In short-wave UV fresh oils possess a very strongly pronounced absorption band due to the isolated double bonds, with a maximum below 200 nm.

6.2.2 Photo-oxidation

Photo-oxidation is another possible means of activating the fatty acid molecule to continue the free radical chain reaction. In general, two different reactions can take place: with and without sensitizer.

Without sensitizer, fatty acids are activated directly by short-wave UV light, which results in the formation of free radicals able to react with triplet oxygen. If a sensitizer is present in the system, it is necessary to distinguish between two types of photo-oxidation (Foote, 1968) (see Fig. 6.4):

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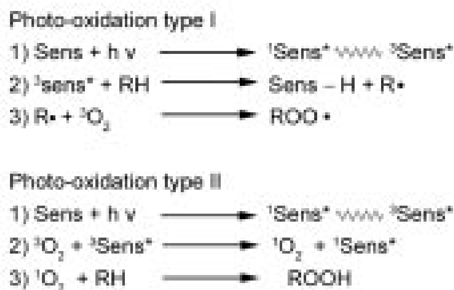

Fig. 6.4 Two different types of photo-oxidation.

- Type I: light activates the sensitizer, which transfers energy to the fatty acid, resulting in the formation of a radical which can react with triplet oxygen (e.g., riboflavin).
- Type II: After activation by light, the sensitizer reacts with triplet oxygen to form reactive singlet oxygen, and which then reacts with the fatty acid molecule (e.g., chlorophyll, pheophytine).

Fatty acids react about 1500 times faster with singlet oxygen than they do with triplet oxygen, and singlet oxygen can react directly with the double bond without further activation of the fatty acid molecule. Thus, the deterioration of edible oils in the presence of light occurs very quickly, without an induction period. Since no radicals are formed in type II reactions, it is not possible to influence the progress of the oxidation process by the use of antioxidants. However, it is possible to inhibit the reaction by quenchers, which are able to accept the activation energy of the light without forming reactive species.

6.2.3 Enzymatic oxidation

The third way for the direct formation of hydroperoxides is enzymatic oxidation by the enzyme lipoxygenase, which belongs to the oxido-reductases group. Lipoxygenases exist in almost all living cells and are able to catalyse the reaction between oxygen and cis,cis-unsaturated fatty acids to form hydroperoxides. The main substrates of lipoxygenases are free fatty acids. Some of them also use triacylglyerides as substrate, but with a lower specificity. Most lipoxygenases are highly specific for the cis,cis-9,12-diene system of fatty acids and, depending on the product specificity of the enzyme, linoleic acid is oxidized either in position 9 (as in lipoxygenase from potatoes) or in position 13 (as in lipoxygenase from soybeans).

6.2.4 Irradiation

The treatment of lipids with ionizing radiation results in the formation of hydroperoxides in two different ways (Mörsel *et al.*, 1991) (see Fig. 6.5):

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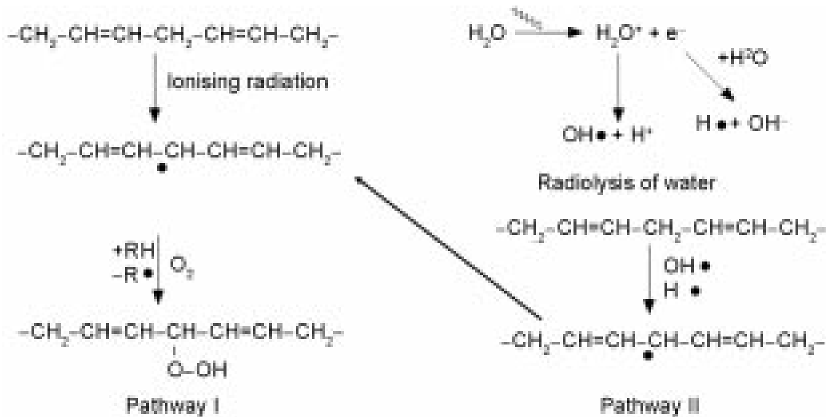

Fig. 6.5 Formation of radicals and hydroperoxides by ionic radiation (RH = unsaturated fatty acid).

1. direct formation of radicals from lipids by abstraction of hydrogen from the allylic methylene group of an unsaturated fatty acid, since the energy of ionizing radiation is about 10^5 times higher than necessary for the abstraction;
2. formation of other radicals (e.g., hydroxyl radicals from the radiolysis of water) which abstract hydrogen from the allylic methylene group. The formed radical of the fatty acid then reacts accordingly to the free radical chain mechanism of a hydroperoxide.

6.2.5 Formation of secondary reaction products

Changes in the quality of edible oils by the formation of hydroperoxides are not directly detectable by the consumer because they are odourless and tasteless. Changes in quality only become obvious after the formation of secondary reaction products with a huge number of aroma-active compounds. Many of these aroma compounds are perceptible in very low concentrations. The type and the amount of aroma-active compounds depend on the fatty acid composition. While hexanal is the main product of the decomposition of linoleic acid, large amounts of *trans*,*trans*-2,4-heptadienal are formed from linolenic acid (see Table 6.1). Aroma compounds formed from linolenic acid also have a very low threshold value. Therefore, the fast deterioration of edible oils with higher amounts of linolenic acid is not only based on the high susceptibility of this fatty acid to oxidation, but also on the formation of degradation products with very low threshold values.

The literature describes many different means of hydroperoxide decomposition (Grosch, 1976; Schieberle *et al.*, 1979; Frankel, 1982; Grosch, 1988; Frankel, 2005a). The main pathway to short-chain volatile compounds is the homolytic β-scission of a carbon-carbon bond to produce oxo-compounds and an alkyl or alkenyl radical. This decomposition results in aroma-active com-

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Table 6.1 Formation of volatile compounds from different fatty acids (μg/g) (Belitz *et al.*, 2004)

Oleic acid		Linoleic acid		Linolenic acid	
heptanal	50	pentane	*	propanal[+]	
octanal	320	pentanal	55	1-penten-3-one	30
nonanal	370	hexanal	5100	(E)-2-butenal	10
decanal	80	heptanal	50	(E)-2-pentenal	35
(E)-2-decenal	70	(E)-2-heptenal	450	(Z)-2-pentenal	45
(E)-2-undecenal	85	octanal	45	(E)-2-henenal	10
		1-octen-3-on	2	(E)-3-hexenal	15
		1-octen-3-hydroperoxide	*	(E)-3-hexenal	90
		(Z)-2-octenal	990	(E)-2-heptenal	5
		(E)-2-octenal	420	(E,E)-2,4-heptadienal	320
		(Z)-3-nonenal	30	(E,E)-2,4-heptadienal	70
		(E)-3-nonenal	30	(Z,Z)-2,5-octadienal	20
		(Z)-2-nonenal	*	3,5-octadien-2-one	30
		(E)-2-nonenal	30	(Z)-1,5-octadiene-3-one	
		(Z)-2-decenal	20	(Z)-1,5-octadiene-3-hydroperoxide	
		(E,E)-2,4-nonadienal	30		
		(E,Z)-2,4-decadienal	250	(E,Z)-2,6-nonadienal	10
		(E,E)-2,4-decadienal	150	2,4,7-decatrienal	85
		trans-4,5-epoxy(E)-2-decenal	*		

* detected but not quantified
\+ one of the main compounds

pounds, which deeply impair the sensory quality of edible oils. The breakdown of hydroperoxides can take place spontaneously or in the presence of metal traces (Frankel, 1982). Two different possibilities of scissions are conceivable, depending on which side of the oxygen-containing carbon atom the cleavage takes place (see Fig. 6.6). Pathway B is energetically favourable, because a resonance stabilized enon or eienon is formed. The accretion of hydrogen or a hydroxyl radical at the radical rest results in the formation of an alkane or a primary alcohol in the further progress of the reaction. Pathway A leads to the formation of an aldehyde and an alkenyl radical, which reacts to form an alkene or saturated aldehyde after the addition of hydrogen or a hydroxyl radical (Grosch, 1987). As a result of this pathway, pentan and hexanal are formed from the 13-hydroperoxide of linoleic acid and 2,4-decadienal from the 9-hydroperoxide. Unsaturated carbonyl compounds can be further oxidized, resulting in high concentrations of hexanal during the oxidation of linoleic acid (Schieberle and Grosch, 1981).

Although the proportion of volatile degradation products is relatively low, the influence of these compounds on the flavour of edible oils is remarkably high due to low threshold values (Frankel, 1982; Grosch, 1988). To evaluate the contribution of volatile degradation products to the total flavour of edible oils, Ullrich and Grosch (1988) developed the determination of flavour units by

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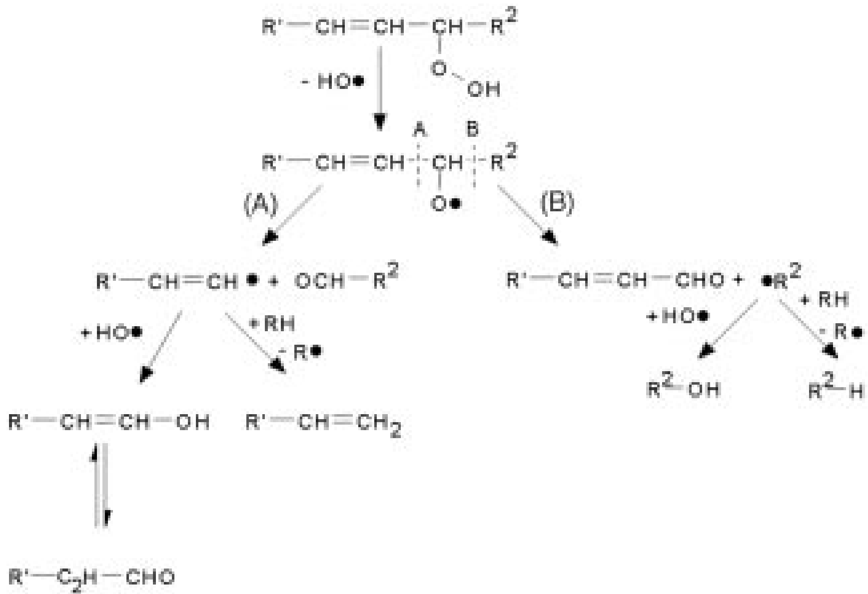

Fig. 6.6 Formation of volatile compounds resulting from the fragmentation of hydroperoxides.

estimating a relative value, such as the flavour dilution factor, which reveals the flavour compounds with the highest contribution to the total flavour (ratio of the concentration to the odor threshold).

6.3 Importance of oxidative processes on the quality of edible oils

Discussing the quality of edible oils means that characteristic features of the oil have to fit with previously defined values for the critical parameters describing the state of the product. This is important for the acceptance of edible oils by consumers, and in the market consumers normally dictate how quality has to be defined for a certain product by their buying behaviour.

The quality of edible oils strongly depends on the oxidative state of the product, because several compounds formed during oxidation are associated with the development of unpleasant taste and smell, which make the product unfit for human consumption. The concentration of volatile and non-volatile aroma-active compounds is directly related to the acceptability of the edible oils.

The assessment of acceptability can be different for the same type of oil, because of differences in the oxidative state of edible oils at the beginning of the storage period. Looking at the life of edible oils from the plant to the consumer, there are several points where oxidation could take place. The oxidative stability and the quality of edible oils in the bottle is determined by the history of the

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product and the resulting oxidative state at the point when the product comes to the consumer, or to the user in industry. The lifespan of edible oil begins when it is still growing in the field, where the oil would normally be protected against oxidative deterioration by the seed hull in the case of rapeseed or sunflower, or by the fruit skin in the case of olives or palm fruits. The use of sound material is absolutely essential for the production of high quality oil. If the protection hull is damaged, enzymatic or autoxidation can take place; but under normal conditions, seeds or fruits are protected against oxidative processes in the field. Factors which can affect oil quality include frost, moisture, dryness, and high temperature. In these conditions, the cells of the material can become damaged, resulting in the activation of different enzymes, such as lipoxygenases and lipases. Frost results in the harvest of green seeds and higher amounts of chlorophyll in the resulting oil. This requires more extensive bleaching because as a virgin oil the product is strongly susceptible to photo-oxidation.

During harvest it is necessary to treat seeds and fruits very carefully, because damage to the protective hull allows for oxidative processes which lower the quality of the edible oil. It is necessary to distinguish between seeds and fruits, because fruits such as olives or palm fruits are much more vulnerable to knocks and pressure than seeds. While intact seeds can be stored over a longer period of time without any loss in quality, the fast processing of fruits is absolutely essential to obtain a high quality product. For seeds, only appropriate storage conditions with a temperature of between 10 and 15 ºC and a moisture content of between 6 and 8% are necessary to maintain the seed quality (Matthäus and Brühl, 2008). In the case of fruits, the degradation of constituents like proteins, carbohydrates or lipids take place directly after harvesting because of enzymatic and microbiological processes. These processes are to a certain extent desirable for the production of olive oil, because its specific aroma is formed. The lipoxygenase pathway, with the resulting oxidative degradation products hexanal, E-3-hexenal, Z-3-hexenal, E-2-hexenal, hexyl acetate, Z-3-hexenyl acetate, hexan-1-ol, E-3-hexen-1-ol, Z-3-hexen-1-ol and E-2-hexen-1-ol, is particularly responsible for the formation of aroma-active volatile compounds during the malaxing step in the production of olive oil (Aparicio *et al.*, 2000). For processing of palm fruits after harvest, it is necessary to avoid such oxidative processes. The enzyme lipase becomes active in palm fruits, resulting in higher amounts of free fatty acids and diacylglycerides. Since free fatty acids are more susceptible to oxidation than fatty acids bound to glycerol, a fast oxidative deterioration takes place.

The production of corn oil or wheat germ oil is a special case, as the germ buds are separated from the endosperm to prevent the flour becoming rancid due to the oxidation of oil in the germ buds. The quality of the resulting oil strongly depends on the corn steeping, germ separation, washing, drying and cooling procedures (Strecker *et al.*, 1990). During storage, the germ buds are subjected to a fast enzymatic degradation of the triacylglycerides, and a subsequent oxidation. Therefore, it is important to produce the oil from the germ buds shortly after separation from the corn, and to store it at a low temperature. A

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lengthy period of transportation and higher temperatures affect the oil quality in terms of the formation of oxidation products and the loss of tocopherols.

During oil processing, oilseeds are either pre-pressed by a screw press with subsequent solvent extraction and refining to remove undesired compounds formed during the processing, or they are de-oiled by a screw press and purified by sedimentation, filtration or centrifugation in order to produce only so-called cold pressed oils.

To avoid oxidative reactions during both types of processing, it is necessary to control access of light, temperature and air as far as possible. This is hugely important for the production of virgin edible oils, because the producer has no chance of refining or steam-washing the oil to remove formed and undesired aroma-active, volatile compounds, and thereby improve the oil quality, after extraction of the oilseed.

In addition to changes of flavour or odour, which become obvious very fast, physical and chemical changes also take place, which can seriously damage the product quality (see Fig. 6.7). Such changes alter the composition, the colour, the viscosity and the nutritional value. Since the human nose is very susceptible to changes of flavour or odour, the oil will normally be rejected long before physical or chemical changes become apparent. Figure 6.8 shows changes in the peroxide value and tocopherol content of rapeseed oil stored in open bottles over a period of 20 weeks. While the peroxide value increases up to 110 meq O_2/kg oil within the storage time, the content of tocopherols is reduced by about one third. Another example of changes in composition during storage is shown in Fig. 6.9, where linoleic acid in a model mixture was stored at 50 °C. Over a period of 360 hours, the content of linoleic acid decreases by about 95%.

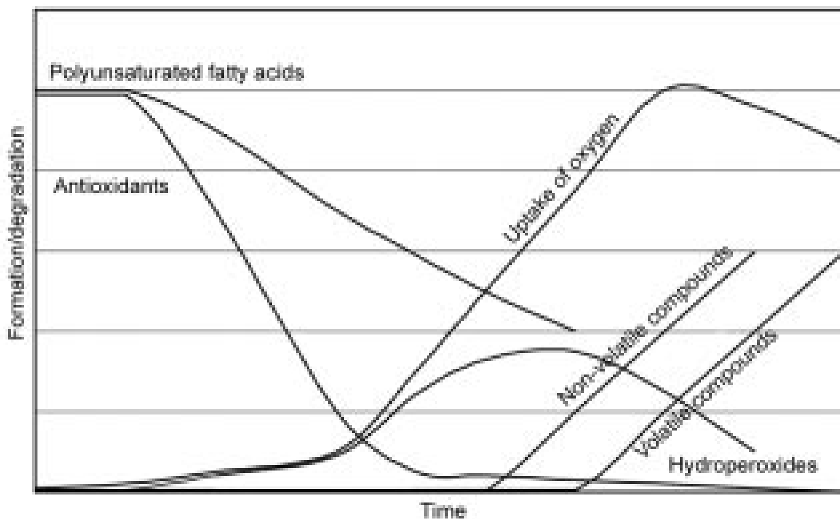

Fig. 6.7 Changes of different parameters during storage of edible oils.

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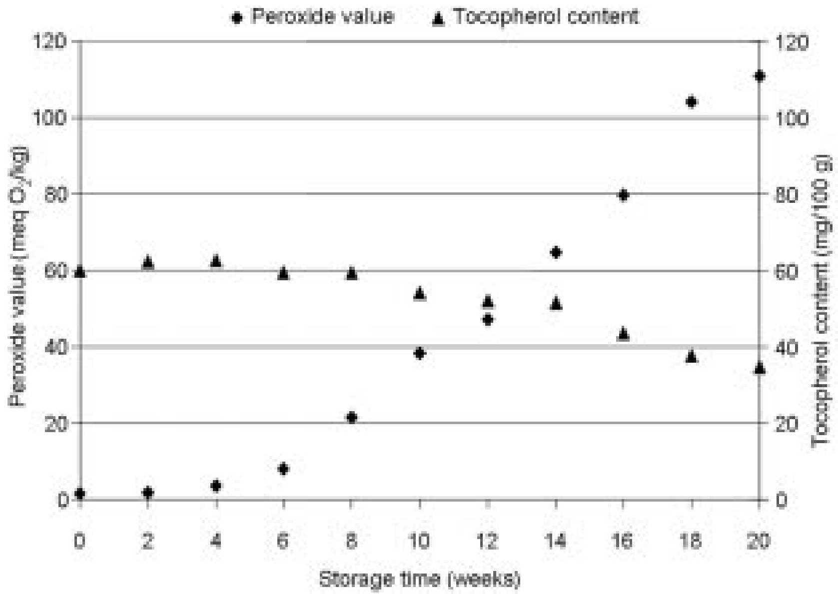

Fig. 6.8 Changes of peroxide value and tocopherol content of rapeseed oil during storage in open vessels at room temperature.

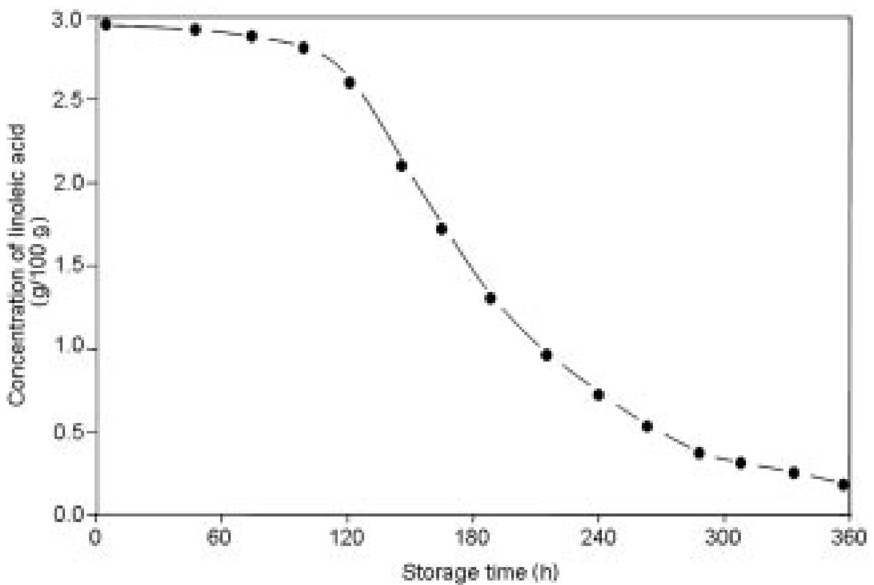

Fig. 6.9 Decrease of the linoleic acid concentration in a model system during storage at 50ºC.

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Owing to its high content of linolenic acid, linseed oil is very susceptible to oxidation and oxidative deterioration is detectable shortly after production. In addition to the oxidation of lipids, another oxidation reaction takes place. Linseed contains several cyclic octapeptides (Morita *et al.*, 1999), which to some extent enter the oil during pressing. If no refining follows the extraction, these cyclic octapeptides remain in the oil and the methionine group of cyclolinopeptide E can be oxidized to form a methionine sulfoxide group, resulting in the development of the bitter taste that is typical of virgin linseed oil after short-time storage (Brühl *et al.*, 2007). Since the exclusion of oxygen from fresh pressed virgin linseed oil by purging with argon prior to storage does not inhibit the development of this bitter taste, a co-oxidation of cyclolinopeptide E by present hydroperoxides is conceivable.

Some derivatives of hydroperoxides, such as hydroxylated fatty acids like 13-hydroxy-*cis*-9-*trans*-11-octa-decadienoic acid, are also described as having a bitter taste, while the corresponding 13-hydroperoxy linoleic acid is not bitter (Biermann and Grosch, 1979). These compounds can be found in legumes and cereals, e.g. oats.

6.4 Evaluation of the oxidative state of edible oils

One major problem for food scientists in official or industrial laboratories is the comprehensible and objective assessment of the oxidative state and the oxidative stability of edible oils within the physical or chemical parameters available today. Another difficulty is the correlation of these parameters with the actual perceived quality of the product, which do not always coincide. The sensory evaluation of edible oils plays an especially important role in the assessment of the quality, because international standards like the Codex Alimentarius Standard for Named Vegetable Oils, Codex-Stan 210 (Codex Alimentarius, 2001), but also national standards like the German Guideline for Edible Fats and Oils (Anon., 1997) judge the sensory evaluation as the major parameter for assessing the quality of edible oils. In some cases, physical and chemical parameters give clear indications for an advanced oxidative process, but these results cannot always be confirmed by the sensory evaluation and vice versa. Therefore, the search for key components to describe the oxidative quality of edible oils is an important challenge for research today, and the aim is to find key parameters with a good correlation to the sensory evaluation.

The pathway of the oxidative degradation of edible fats and oils results in many physical changes in the product, such as increased viscosity, changes in composition, and the formation of degradation products. To measure and assess these changes, a wide range of methods is available for describing the oxidative state of edible oils. Additionally, some methods can be applied to find the oxidative stability of the product.

These two points, oxidative state and oxidative stability should be clearly distinguished: the first describes the product at the present time, and the latter

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Table 6.2 Usefulness of different methods for the assessment of lipid oxidation

Method	Sensitivity	Validity
Sensory evaluation	high	high
Volatile compounds (GC)	high	medium
UV absorption	high	medium
Rancimat test	low	medium
Peroxide value	low	low
Anisidine value	medium	low
Totox value	medium	medium
Oxygen uptake	medium	low

tries to give information about the possible behaviour of the product during further storage. In this context, it must be taken into consideration that the measurement of a parameter is one thing, whilst the interpretation and usefulness of that parameter to describe the real situation is quite another task. The assessment of the actual storage behaviour from the obtained results is especially difficult, and misinterpretation can occur.

As well as measuring a specific parameter with a resulting value, it is also important in practice to know how to interpret this value in terms of its consequences for the product. It is necessary to have a limit that can be used for the assessment, but this limit must describe the quality of the product exactly. Unfortunately, only a few limits exist, whilst the interpretation of most other parameters is based on experiences from practice.

Some of the most popular methods for the assessment of the quality of edible oils are described in the next section. In addition to wet-chemical methods, which can be used in most cases as sum parameters, different methods are now available which use HPLC or GLC to determine individual primary and secondary oxidation products. Many different methods exist, but only some of them have importance for practice (see Table 6.2).

6.4.1 Chemical parameters describing the oxidative state of oils

For the food industry, but also for official laboratories, knowledge of the state and the stability of edible oils is important for assessing the quality of products. It is also necessary to know the state of a product in order to minimize the negative effects of lipid oxidation during processing and storage.

Chemical parameters describing the oxidative state of oils can roughly be divided into two groups. The first and smaller group includes methods of measuring primary oxidation products like hydroperoxides or radicals; the second and larger group registers secondary oxidation products. In the case of the second group, methods can gather secondary oxidation products as sum parameter, such as anisidine value or thiobarbituric acid test (TBA) for aldehydes and ketones; or they measure individual compounds responsible for the deterioration of the product, such as hexanal or decadienal by GLC. The aim of all these methods is to describe the quality of the product as realistically as

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possible. However, in most cases a combination of different methods are required to get sufficient and reliable information for the assessment of the quality in terms of the extent of oxidation or the oxidative history of the product. The ultimate criterion for the suitability of any test is its agreement with sensory perception of rancid flavours and odours (Gray, 1978).

Peroxide value

The determination of the peroxide value is the traditional and most used parameter for measuring the primary products of oxidative degradation. From this value, the propagation step of the free radical chain mechanism and the accumulation of hydroperoxides can be followed. However, it is not possible to use the peroxide value alone to judge the quality of edible oils, because hydroperoxides decompose during storage. This decomposition can take place faster than the formation of new hydroperoxides, depending on certain storage conditions such as temperature, light or metal traces. Although the oil has already been damaged by oxidation, and higher levels of degradation products have already formed, the speed of hydroperoxide decomposition can result in falsely low levels of these compounds. In this instance, the peroxide value tells us nothing about the real quality of the product. In order to avoid misinterpreting peroxide values, it is necessary to know the history of the sample. However, the peroxide value is a suitable parameter for measuring the deterioration of quality over time. After the induction period, during which the peroxide value increases slowly, a steep increase indicates that the the oil has gone bad.

In general, the aim of oil production should be to produce oils with peroxide values as low as possible, without the formation of secondary reaction products. A higher peroxide value at the beginning of the storage period has a negative effect on the storage stability of the oil. For refined oils, producers should aim for a peroxide value below 1, better 0.5 meq O_2/kg oil, while the peroxide value for virgin oils can be higher, up to 3 meq O_2/kg oil.

In most cases, the methods for determining peroxide concentration in fats and oils use the reactivity of the bound oxygen with other compounds, like potassium iodide or Fe^{2+}, and the resulting reduction of the hydroperoxides. These methods are known as static methods, because they describe the state of the oil at the present time.

The standard method for determining the peroxide value is based on the method originally described by Lea (1931) and Wheeler (1932) which uses the iodometric titration of delivered iodine by standard sodium thiosulfate solution to a starch endpoint after the reaction of potassium iodide with bound oxygen from hydroperoxides. The amount of delivered iodine is proportional to the concentration of peroxides in the oil (see Fig. 6.10). Different scientific organizations such as the German Society for Fat Science (DGF method C-VI 6a) (DGF, 2008) or American Oil Chemists' Society (AOCS method Cd 8-53) (AOCS, 1992) standardized this method.

The method is empirical and strongly depends upon the design of the experiment and the behaviour of the technician because the oils are susceptible

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Fig. 6.10 Reaction between iodine and lipid peroxides.

to oxygen from the air, light or absorption of iodine by unsaturated fatty acids (Robards *et al.*, 1988). Therefore, to obtain comparable and reliable results it is strongly recommended that the conditions of the method are very precisely maintained. To improve the sensitivity of the method, it is possible to identify the starch-endpoint colorimetrically, or to determine the liberated iodine electrochemical.

According to this method, the peroxide value gives the concentration of peroxides in mmol/kg oil, or it measures the amount of oxygen bound in the unsaturated fatty acid molecules in meq O_2/kg oil. A correlation between the peroxide value and sensory evaluation is not given (Acker *et al.*, 1969), which makes it difficult to assess a product as unfit for human consumption from the peroxide value alone. In some countries, limits for the peroxide value are defined, e.g. Germany with 10 meq O_2/kg oil for virgin oils and 5 meq O_2/kg for refined fats and oils. A limit for virgin olive oil is also defined in the regulation (EWG) 2568/91 (Anon., 1991) as 20 meq O_2/kg oil.

There are other means of measuring the content of hydroperoxides, besides the standard iodometric method. One very sensitive method is the ferric thiocyanate method, which colorimetrically determines hydroperoxides as ferric thiocyanate after the oxidation of Fe^{2+} to Fe^{3+} (Lips *et al.*, 1943; Stine *et al.*, 1954; Pardun, 1976). As the method is much more sensitive than the standard iodometric method, it is possible to measure very low amounts ($< 10^{-2}$ mmol). The peroxide values are 1.5 to 2 times higher than results obtained by the standard method because, accordingly to Grosch and Barthel (1972), one mol of peroxides oxidizes four mol of Fe^{2+}, in contrast to two mol of iodine in the case of the iodometric method.

Another method for the determination of hydroperoxides is based on chemi-luminescence. This is where hydroperoxides react with luminol in the presence of cytochrome C, resulting in the formation of light (Matthäus *et al.*, 1994), measured by a photomultiplier. The method is very fast and easy to handle, and after calibration the integral of the chemiluminescence light curve correlates well with the standard iodometric method.

A direct measurement of hydroperoxides is possible by polarogaphic analysis (Lewis and Quackenbush, 1949; Willits *et al.*, 1953). This method uses the reduction of the hydroperoxides at a mercury electrode. Two electrons are transferred, resulting in the cleavage of the O-O-bond and the formation of a hydroxyl group (Swern and Silbert, 1963). The obtained current-potential curves correlate with the peroxide value. It is also possible to differentiate between different peroxide compounds due to their different half-wave potentials, since the binding energy of the peroxides is different (Willets *et al.*, 1952; Kuta and

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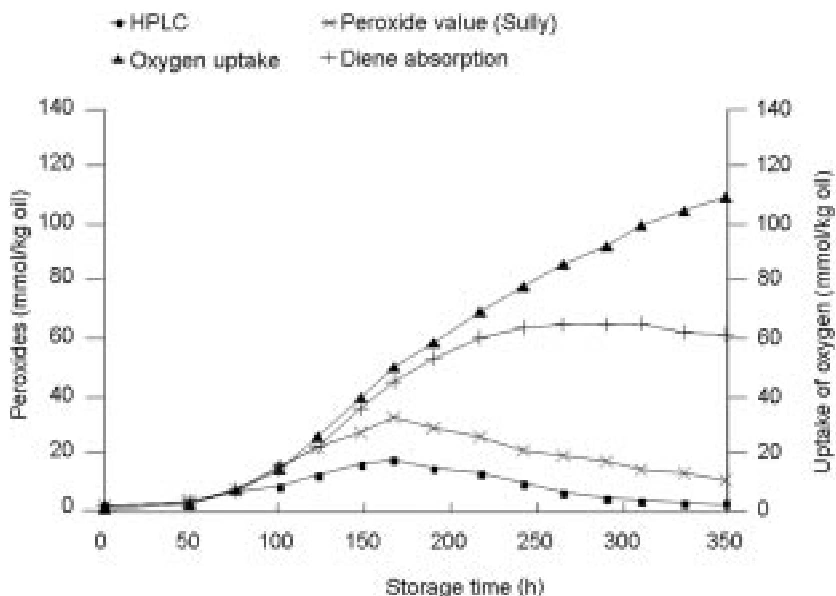

Fig. 6.11 Comparison of different methods for the determination oxygen uptake and peroxide formation of oil during storage.

Quackenbush, 1960). The method described by Lewis and Quackenbusch (1949) uses a mixture of benzene/methanol (1:1), with 0.3 M lithium chloride as conduction salt and a dropping mercury electrode as working electrode. Under this condition, the half-wave potential of lipid hydroperoxides is −775 mV.

The most specific method for the determination of hydroperoxides is the measurement by HPLC. Lipid hydroperoxides have a remarkable UV absorption at 234 nm due to the conjugated diene system, and this fact can be used for their detection. However, it is also possible to detect lipid hydroperoxides using an electrochemical detector (Yamada *et al.*, 1987; Song *et al.*, 1992) or by postcolumn chemiluminescence (Kondo *et al.*, 1993).

Hydroperoxides are often registered as sum parameter without separating the different positions or *cis*/*trans*-isomers, but Tokita and Morita (1985), Kruse (1992) and Koskas *et al.* (1983) described methods for the determination of individual hydroperoxides.

Figure 6.11 show the results of different possible methods of following the formation of peroxides in edible oils during oxidation.

Anisidine value

Hydroperoxides can easily be removed from oil by the use of heat, light or metal trace in the absence of oxygen, resulting in the formation of secondary degradation products. Therefore the determination of the peroxide value alone gives only limited information about the history of the oil. One example is the deodorization step during the refining process, which causes the decomposition

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of hydroperoxides in bad-quality oils and produces the very low peroxide values associated with refined products. Such oils will be accepted by consumers concerned with their taste and smell, but will have a shortened shelf-life because of the oxidative damage that occurred before refining.

In order to follow the termination step of the free radical chain mechanism, or to detect such 'refreshed' oils, it is possible to measure some of the secondary degradation products by the ultraviolet absorption of dinitrophenylhydrazones (DNPH-reaction), by reaction with malonaldehyde (TBA number), or by the use of anisidine (anisidine value).

The determination of the anisidine value is the most popular method and is based on a method developed in 1957 by Holm *et al.* (1957) applying benzidine acetate. Benzidine acetate was criticized as a reactive agent due to its carcinogenic properties, and was replaced by anisidine some years later. Aldehydes, mainly 2-alkenals, 2,4-dienals and, to a limited degree, saturated aldehydes, react with anisidine and form a coloured component which can be measured at 350 nm. Nowadays, the method for determining the anisidine value has been standardized and has been accepted into different method collections. The reaction and the reliability of the results strongly depend on the strict observance of method requirements like reaction time, weighing of the samples, or mixing of the solutions.

According to the definition, the anisidine value is 100 times the absorbance of a solution resulting from the reaction of 1 g oil in 100 mL of a mixture of isooctane and p-anisidine in glacial acetic acid, measured at 350 nm in a 10 mm cell under the conditions described in the method.

The sensitivity of the method is not the same for all types of aldehydes because their response to ultraviolet absorption is different. Reaction products of anisidine with 2-alkenals and 2,4-dienals demonstrate a higher absorption than comparable compounds with saturated aldehydes. The molar absorbance increases by a factor of 4 to 5 if a double bond in the carbon chain is conjugated with the carbonyl double bond (Holm and Ekbom-Olsson, 1972).

A good correlation between increasing anisidine values of salad oils processed from sound soybean oil and decreasing flavour scores was shown by List *et al.* (1974), with a correlation of 0.81 between peroxide value, anisidine value and flavour scores. Owing to its toxicological properties, the search for a replacement for anisidine in the method or the development of new methods is ongoing.

Dubois *et al.* (1996) described a Fourier transform infrared (FTIR) spectroscopy method for the determination of the anisidine value in thermally stressed oils. They calibrated the method with the standard compounds hexanal, *trans*-2-hexenal and *trans,trans*-2,4-decadienal in canola oil, which were considered to be the most representative of the aldehydes. In combination with partial least squares (PLS) calibration technique, it was possible to measure the anisidine value in good agreement with the wet-chemical method. The method was suitable for monitoring the amount of three different classes of aldehydes and AV using the calibration developed, and obtained very good results (Dubois, 1995).

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Quick-tests like the CDR FoodLab method for the determination of anisidine values in oils and fats promise to measure the anisidine value within 2 minutes, but individual standard curves have to be prepared for different types of oil on the basis of the standard method (Brühl, 2008).

In practice, the anisidine value is a valuable parameter for the assessment of the quality and history of edible oils, as secondary oxidative products covered by the anisidine value are not readily removable by standard oil processing steps (Strecker *et al.*, 1990). As Pardun (1974) showed, the anisidine value is suitable for detecting the influence of the age and quality of the raw material on the quality of the resulting oil. While products from fresh and intact seeds showed low peroxide and anisidine (benzidine) values, the resulting values were remarkably higher for oils from bad quality raw material.

Totox value

A valuable parameter to assess the quality of edible oils is the Totox value, which combines the peroxide value and the anisidine value:

$$\text{Totox value} = 2 \times \text{peroxide value} + \text{anisidine value}.$$

The Totox value takes into consideration the actual state of the product (using the peroxide value) as well as the history of the oil (using the anisidine value). Therefore, the significance of the Totox value is much stronger than for the individual values. Although the method for the anisidine value and the calculation of the Totox value have been known for a long time, at the moment no official standard defines a limit for these values to ensure the quality of edible oils. As a rule of thumb, a limit of 10 is used in industry for the anisidine value for good refined oils (Rossell, 1989). Rossell (1989) suggested that since there are only few cases where primary and secondary reaction products are high at the same time, the Totox value should also be below 10 for good quality edible oils. Frankel (2005b) reasoned that good quality oil should have a Totox value of less than 4. A position paper of the German Society for Fat Sciences recommended a Totox value of less than 20 for refined and virgin vegetable oils (DGF, 2006).

For most types of virgin oils on the market, it should be no problem to fulfil a limit of 20 for the Totox value over a shelf-life period of approximately one year. Generally, the peroxide value of fresh virgin oils is a little higher than that of refined oils, but the anisidine value remains low and has no remarkable contribution to the Totox value over the storage period. Higher anisidine values, together with low peroxide values, indicate an unfavourable heat-treatment of edible oils during transportation or storage.

Some authors do not agree with the strong favouring of the primary reaction products in the Totox formula, because there has been no reliable correlation developed between the reduction of the peroxide value and the simultaneous increase of the anisidine value (Strecker *et al.*, 1990). One argument is that this over-assessment of the peroxide value results in the conclusion that the oxidative state of oil can be improved merely through exposure to high temperature, which

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leads to a reduction of the peroxide value and Totox value. For corn oil, the authors observed that the conversion of the peroxide value to the anisidine value during typical oil processing is about 3:1 (each 3 units of the peroxide value to one unit the anisidine value). From this, they propose the so-called oxidation index (Ox-Ind) as peroxide value/3 + anisidine value as a measure for the overall oxidation level.

HPLC method for the determination of secondary oxidation products

One of the most popular methods for the determination of individual secondary oxidation products in edible oils is the reaction of 2,4-dinitrophenylhydrazine (2,4-DNPH) with lipophilic aldehydes, followed by the determination of the coloured 2,4-dinitrophenylhydrazones by HPLC and detection with a UV detector (Seppanen and Csallany, 2001). The determination of some of these compounds such as α,β-unsaturated aldehydes like 4-hydroxy-2-*trans*-nonenal (4-HNE) has a certain toxicological importance as they are highly reactive with thiol and amino groups from biomolecules, such as proteins or enzymes, and inhibit the synthesis of DNA, RNA and proteins (Schaur, 2003).

An advantage of this method is that the formed hydrazones are stable and non-volatile, making the further handling of the compounds easy. However, the method itself is tricky to handle, and the evaluation of the chromatogram can be sophisticated owing to the huge number of different compounds detected. Nevertheless, the method gives reliable and meaningful results for the assessment of edible oils with regard to the formation of the toxicologically important group of α,β-unsaturated aldehydes.

GLC methods for the determination of secondary oxidation products

GLC analysis for the determination of secondary oxidation products gives information about the composition of volatile compounds released from the oil under specified conditions. Thus, either the total content of volatile compounds can be determined, or individual compounds responsible for oxidative deterioration can be used as marker substances to describe the quality of the product. The identification of such compounds, and the correlation with sensory evaluation or other chemical parameters for the assessment of the quality of edible oils, is one of the main aims of research on the analysis of volatile compounds.

Typical key compounds for the assessment of oxidative degradation include hexanal, which is used when the oils under investigation are rich in ω-6-fatty acids, and propanal, which can serve as a reliable indicator when oils high in ω-3 fatty acids are being considered (Shahidi and Wanasundara, 2008). Other compounds that are typical reaction products of oxidative processes in edible oils include other aldehydes like pentenal, 2,4-heptadienal or 2,4-decadienal.

There are other possible methods for determining volatile compounds by GLC, of which Dynamic Head Analysis (also called 'purge and trap') is the most popular. A sample is placed in a heated and sealed vail. This is purged with an inert gas to strip out components that are volatile at the chosen temperature.

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The volatiles are concentrated on an adsorbent trap, such as tenax or charcoal, which is then rapidly heated to desorb the analytes onto a GLC column for analysis. Since volatile compounds are concentrated on the trap and the equilibrium between volatiles in the sample and volatiles in the headspace is continuously shifted into the direction of the headspace, the technique of purge and trap extraction allows greater sensitivity over a static equilibrium process. Using higher temperatures to heat the vessel, the reaction of peroxides and other degradation products can change the initial composition of the analyte. Therefore the formation of new compounds has to be taken into consideration.

There is also a Static Headspace Method in use, where compounds of interest are allowed to become enriched in the headspace of a sealed vail until equilibrium is reached between the sample and the headspace at a defined temperature. Then, an aliquot of the gas phase is injected by a gas-tight syringe into the GLC system. In the early 1990s, a special type of Static Headspace Analysis, known as the Solid Phase Micro Extraction (SPME), was developed by Pawliszyn (1997). In case of SPME, analytes from the headspace (and also from the oil) are adsorbed by a fibre coated with an extracting phase which has been specified for different kinds of analytes prior to thermal desorption in the injection port of a gas-chromatograph.

Another alternative for determining volatile compounds is the direct injection of the sample, either through an injection insert packed with glass wool, or by a temperature controlled external unit. The vaporization of the volatile compounds takes place at an elevated temperature in the injection port, and the compounds are trapped at the beginning of the GLC column. The method is fast and inexpensive, but not as sensitive as the dynamic headspace analysis. Nevertheless, the method can be used for routine analysis in quality control of products.

6.4.2 Measurement and assessment of oil stability

Oil stability is a popular parameter used in industry to obtain some information about the shelf-life that can be expected of edible oils under practically relevant conditions. But the parameter is also useful for comparing the susceptibility of different products to oxidation. The general principle of this parameter is the storage of the oil under certain conditions over a period of time, and the measurement of one or more specific parameters characterizing the oxidative state of the oil until a defined endpoint. In the initial stage of oxidation, only a very slow increase of the specific parameter takes place. This phase is called the induction period. After this period, when the oil is damaged, a sharp increase can be observed. In general, low-quality oils have a shorter induction period than high-quality ones (Sheabar and Neeman, 1988).

Since storage under real-time conditions would be too time-consuming, in most cases storage takes place at an elevated temperature in order to shorten the storage time to a practical length. For the assessment of such results it is important to consider that the transfer of the results to real conditions is

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problematical, as the measurement is performed under accelerated conditions. Therefore, temperatures higher than 60 °C are questionable if it is necessary to draw conclusions about the real shelf-life of the oil, because above this temperature the mechanism of degradation becomes different from when the oil is stored at room temperature. On one side, oxidation becomes more strongly influenced by oxygen availability, but the solubility of oxygen in the oil decreases. Thus, reactions like polymerization and cyclization come to the fore, which are not comparable to the reactions under normal conditions.

It is also possible to compare the products or effects of antioxidants when using higher temperatures. To get reliable information about the shelf-life of edible oils, it is useful to measure oxidative stability at different temperatures, using different parameters for the assessment of the oxidative state (Frankel, 2005c).

Two widely used methods to measure oxidative stability are the Schaal Oven Test and the Active Oxygen Method (AOM), which is the basis for some automated methods like Oil Stability Index or Rancimat method.

The Schaal Oven Test, originally developed by the biscuit and cracker industry for the assessment of their products, involves storing the oil in open jars at 63 ± 0.5 °C or 70 °C, until rancidity is detected by sensory evaluation or measuring the peroxide value. The time from the beginning of the experiment until a significant increase of the appropriate parameter is defined as the induction period of the oil. A disadvantage of this method is that it takes 4 to 8 days, which is a long time for the industry.

While the Schaal Oven Test uses relatively low temperatures, the conditions for the Active Oxygen Method are more drastic because, in this method, air is bubbled through the oil at 98 °C and the peroxide value is measured. The number of hours until the oil sample reaches a peroxide value of 100 meq peroxides/kg of oil is defined as the AOM hours for the oil. This popular method has been standardized in the official AOCS method Cd 12-57 (AOCS, 1992).

Automatic versions of the AOM are available, such as Rancimat test (Metrohm, Herisau, Switzerland) or Oxidative Stability Instrument (Omnion, Inc., Rockland, MA, USA). In contrast to the other methods, both methods measure volatile compounds, secondary reaction products of the oxidation process. These are mainly short-chain organic acids, like formic and acetic acid, which are collected in distilled water, resulting in a rapid change of solution's conductivity when the oil is damaged. Since secondary reaction products are more stable than peroxides measured in the AOM or Schaal Oven Test, the automated methods are more precise and reproducible. Furthermore, the continuous measurement of data points results in a more exact determination of the end point and the resulting induction period.

Prediction of the shelf-life of edible oils from the measurement of the oxidative stability by these various tests is difficult and contains errors. In general, extrapolating from higher temperatures to normal storage conditions is possible, because the shelf-life decreases logarithmically with increasing temperature, and every 10 °C increase in storage temperature results in a halving of

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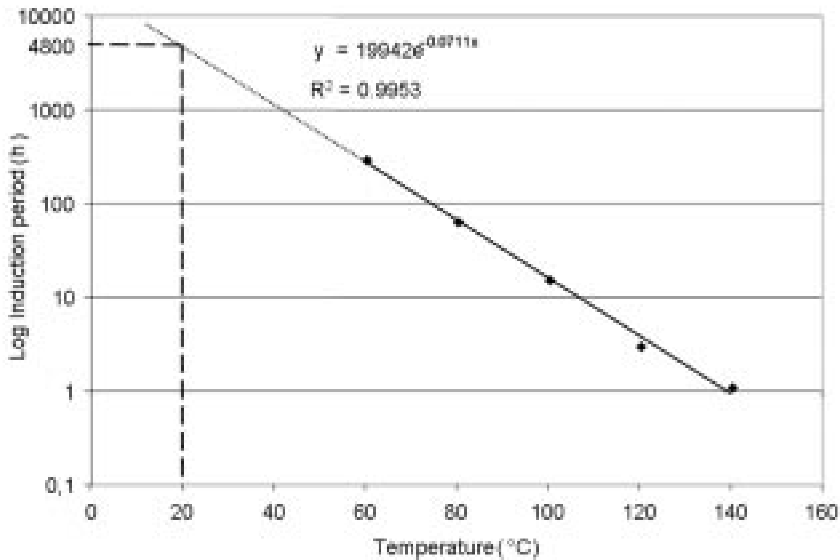

Fig. 6.12 Arrhenius plot for the calculation of the rough shelf-life of virgin rapeseed oil at 20ºC. (Measurement of the induction periods by Rancimat test.)

the shelf-life. Since the reaction conditions under the accelerated storage of Rancimat test or AOM are not directly comparable to normal conditions, this prediction has some errors. Nevertheless, a rough statement about shelf-life at ambient temperature is possible when the measurement of the induction period has been carried out at different temperatures, and the results of the measured induction periods are presented in an Arrhenius plot logarithmically versus the temperature (see Fig. 6.12). The figure shows the results of the measurement of the induction periods of virgin rapeseed oil, measured by the Rancimat test at five different temperatures. Extrapolation of the expected shelf-life of the oil at 20 ºC revealed that the oil would be usable over a period of about 4800 hours or 200 days before oxidative deterioration would become obvious. The correlation coefficient between temperature and induction period was quite good.

6.4.3 Sensory evaluation of edible oils

The most important parameter for the assessment of edible oils is sensory evaluation, because the product has to fit consumer likes, otherwise it has no chance on the market. The sensory assessment can be completed by a consumer panel with a huge number of untrained consumers who assess the product regarding likes and dislikes. The result says nothing about sensory defects of the oil resulting from deterioration, but reflects the consumers' acceptance of and preferences for the product.

On the other hand, the sensory evaluation of edible oils can be carried out by a trained group of tasters, who are familiar with the product and possible perceptions resulting from sound or defective products. In the case of this

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analytical sensory evaluation, the available methods range from simple triangle testing, recognizing differences between two samples, to the more complex descriptive analyses of edible oils.

The tasters of such a panel should use a standardized vocabulary for the description of the perceptions, ensuring that the same term is used for similar sensations. The use of a standardized vocabulary improves the reproducibility and precision of the sensory analysis.

In addition to the description of the sample, this type of sensory evaluation includes a rating system by which the tasters specify the intensity of their perceived sensations. For edible oils, there are two systems available for describing the intensity of the sensations: a scale with distinct values, like school marks; and the use of a continuous scale, i.e. a line of 10 cm length, where the taster puts his tick mark intuitively on the line. The first one is in use for the AOCS Flavour Quality Scale (Waltking, 1982) with a scale from 1 (repulsive) to 10 (excellent) or for the evaluation of virgin oils according to the DGF standard method C-II 1 (07) (DGF, 2008) with a scale from 0 (not perceivable) to 5 (very strong perceivable). The latter system is used for the sensory evaluation of virgin olive oil according to the regulation (EWG) 2568/91 (Anon., 1991).

During the decomposition of the primary products in the oxidation process, a huge number of volatile and non-volatile compounds develop, which have a strong influence on the flavour and the taste of edible oils. Refined oils represent the main product on the oil market. Normally this type of oil is taste- and odourless, which makes the detection of any changes resulting from the formation of aroma-active degradation products by sensory evaluation easy. In this case, sensory analysis gives a good indication for oxidative deterioration. Often, a well-trained panel is more susceptible to rancid sensations than analytical chemistry. Therefore, sensory analysis is very helpful for deciding whether the oil is suitable for human consumption or not. Much more difficult is the sensory evaluation of a product with a certain taste of its own. In the case of cacao butter, or even virgin oils like olive oil or rapeseed oil, which have a strong characteristic smell and taste, the perception of sensory defects resulting from ongoing oxidation processes is complicated. There are a huge number of compounds with a certain importance to the odour, which can have a potential masking effect on the responsible aroma-active compounds.

The contribution of different volatile compounds to a rancid perception, the threshold of these compounds, and their sensory character, can be very different. Therefore, the importance of different compounds for a rancid perception depends not only on the concentration of the compound in the oil, but also on the threshold of perception of these compounds (Pokorny, 1990). For example, the threshold of perception for *trans*-2-octenal is about 7.0 mg/kg, while for *trans*-6-octenal it is 0.015 mg/kg (Meijboom and Jonenotter, 1981). The threshold of perception increases with increasing unsaturation, and is also influenced by the number and type of functional groups in the molecule. Alcohols smell less than the corresponding aldehydes. Additionally, saturated aldehydes give a feeling of power, warmth, resonance, depth, roundness and freshness to products, while

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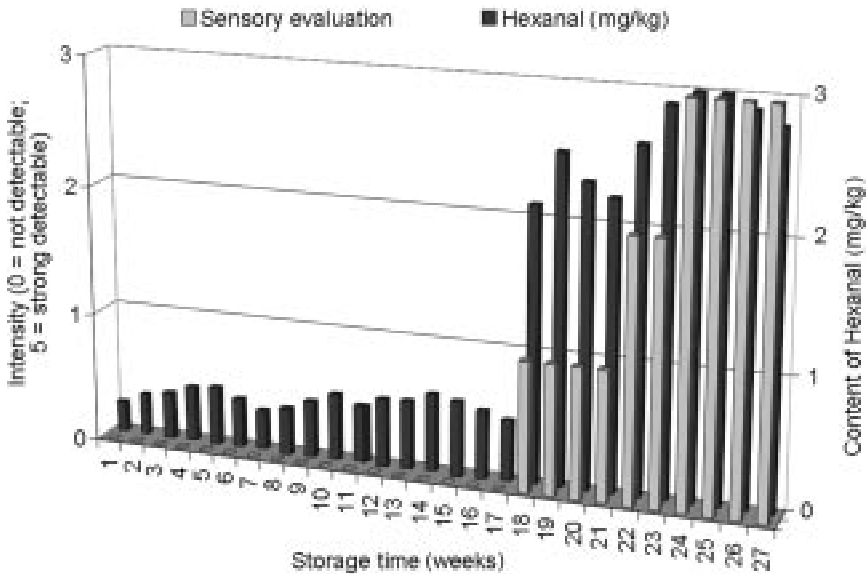

Fig. 6.13 Correlation between sensory analysis and hexanal concentration of rapeseed oil stored over 27 weeks at room temperature.

unsaturated aldehydes, such as 2-enals and 2,4-dienals are characterized by a sweet, fruity, oily and fatty note, and alcohols by a solvent, grassy, green and fatty flavour (Hamilton, 1989). Figure 6.13 shows the correlation between the development of sensory evaluation and the formation of hexanal in virgin rapeseed oil stored over a period of 27 months. While the hexanal concentration remained below 0.3 mg/kg for over a year and a half, no sensory defect was detected by the trained panel. Not until after 18 months did the content of hexanal increase remarkably, and then the panel detected a clear rancid perception.

The aim of analysts is to replace sensory evaluation with more reliable and reproducible methods, whilst providing the same information about the defects and typical attributes of edible oils. Such a tool could be very helpful, especially for process control during production, because sensory evaluation is time-consuming and therefore costly. One attempt to create such an analytical instrument was made by the so-called 'electronic nose' (Lerma-Garcia *et al.*, 2009; Cruz *et al.*, 1998; Bazzo *et al.*, 1998), which consists of several arrays of non-specific sensors that react with volatile compounds from the sample, resulting in a change to the electrical properties of the sensor. These changes are different for different sensors, and the recorded data are used to calculate a result on the basis of a statistical model. By training the instrument with known samples, it is possible to correlate the results of the electronic nose with results obtained from other techniques, e.g. sensory evaluation.

It is possible to teach the electronic nose the differentiation between rancid and not rancid refined oils, as shown in Fig. 6.14. However, if the matrix is more

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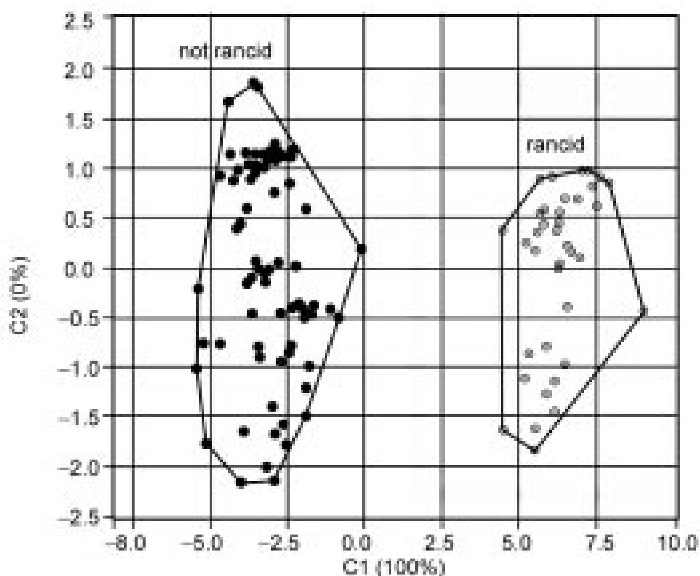

Fig. 6.14 Differentiation of rancid from non-rancid oils by use of an electronic nose.

complex, with more volatile compounds present than in refined oils, the results of the electronic nose become worse because the sensors are not able to recognize specific volatile compounds. Instruments like the electronic nose are not able to take into consideration all perceptions from the sample, unlike the human nose in cooperation with the brain. Therefore, the operating area of such instruments remains limited and they are not able to replace the sensory analysis. It is only a tool to support the sensory analysis in certain questions.

6.5 Effects of oxidation on sensory and nutritional quality of edible oils

6.5.1 Decrease of sensory quality during storage

The main effect that becomes obvious as a result of the oxidation of edible oils is a decrease in sensory quality. In general this negative characteristic of the product is described by the term 'rancid', derived from the Latin word for 'stinky', 'nasty' or 'unfavourable'. Depending on the fatty acid composition of the edible oil and the resulting degradation products, characteristics and perceptions of the rancid sensation can be very different.

In general, hydrocarbons have a significantly higher threshold value than aliphatic carbonyl compounds, which results in these compounds making a smaller contribution to the off-flavour of oxidized edible oils. According to Frankel (1985), the aliphatic carbonyl compounds show the following decreasing order of importance and threshold value: *trans,cis*-2,4-decadienal, *trans,trans*-2,4-decadienal, *trans,cis*-2,4-heptadienal, 1-octen-3-ol, n-butanal, n-hexanal.

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Table 6.3 Flavour quality and odour threshold of some compounds resulting from lipid oxidation

Compound	Flavour quality	Odour threshold in oil (nasal) (μg/kg)
ethanal	fruity, pungent	0.22
propanal	fruity, pungent	9.4
pentanal	pungent, bitter almond	240
hexanal	tallowy, green leafs	320
heptanal	oily, fatty	3200
octanal	oily, fatty	55
nonenal	tallowy	13 500
decanal	orange peel	300
(Z)-3-hexanal	green leafs	1.7
(E)-2-heptanal	oily, fatty, bitter almond	14 000
(E,Z)-2,4-heptadienal	oily, fatty frying odour	4000
(E,Z)-2,6-nondienal	cucumber	4
(E,Z)-2,4-decadienal	frying odour	10
trans-4,5-epoxy-(E)-2-decenal	metallic	1.3
1-penten-3-one	hot, fishy	0.73
1-octen-3-one	mushrooms, fishy	10
(Z)-1,5-octadien-3-one	geraniums, metallic	0.45

Modified according to Grosch (1987) and Belitz *et al.* (2004)

While hexanal and 2,4-decadienal are primary products of the oxidation of linoleic acid, the main degradation products of linolenic acid are 2,4-hetadienal, 3-hexenal and 2,4,7-decatrienal. The contribution of each of the different compounds to the overall sensory evaluation is different. Table 6.3 shows some of the formed degradation products, together with a flavour description. 2,4-decadienal is one of the most important flavour contributors of deep-fat fried products, and is already perceivable in very low concentrations of 1.5×10^{-9} g/L of air. It is also well known as an aroma-compound formed in stored soybean oil. The appearance of 2,4-decadienal in the odour of fried food is one reason for the enormous popularity of such products, because consumers link the enjoyment of eating high-quality and tasty fried food with this typical aroma. For this reason, consumers often do not associate the perception of this odour with oxidized edible oils and, rather than rejecting the product, actually a have a rather positive impression of it. Only when the concentration becomes higher does the positive impression switch to rejection.

Edible oils with higher levels of linolenic acid, such as rapeseed or soybean oil, often produce a fishy off-flavour during storage or heating as a result of the formation of 2,4,7-decatrienal. This fishy smell is the reason that the use of rapeseed oil for frying or deep-fat frying is often declined by the consumer or by industry, although the oil would be suitable from a technical point of view. Why some rapeseed oils form this fishy off-flavour during storage and heating while other rapeseed oils do not show this defect is an open question.

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A typical example of the formation of an off-flavour shortly after production is the so-called 'revision taste' or 'flavour revision' of soybean oil, which results in the typical *green*, *beany* and *grassy* perception of soybean oil at an early stage of storage. Later, these impressions change into *fishy* or *painty*. Crude soybean oil already has this green-beany flavour, but it is removed during refining, resulting in a taste- and odourless product. This flavour returns at a very early stage during storage, at which the degree of oxidation by chemical or physical methods is not yet detectable (Smouse, 1973). Flavour revision takes place at peroxide values below 10. Frankel (1980) mentioned four major causes for this effect, of which he concluded that the oxidation of linolenic acid was the most important precursor. Antioxidants are not effective in inhibiting the revision of soybean oil (Frankel *et al.*, 1959; Mounts *et al.*, 1978). Several other oils also develop specific off-flavour during storage, like 'animal flavour' for lard and tallow, 'fishy' for rapeseed, 'grassy/painty' for rapeseed and linseed, and 'painty/rancid' for palm oil.

6.5.2 Nutritional quality affected by oxidative processes

In the last few decades, a shift took place in the consumption of fats and oils, from animal fats with high amounts of saturated and monounsaturated fatty acids, to vegetable oils consisting of more healthy mono- and polyunsaturated fatty acids. One drawback of this change is that polyunsaturated fatty acids are more susceptible to oxidative deterioration. Not only does this result in rancid off-flavours, but it also impairs the nutritional quality and safety of edible oils. Scientists suspect the formation of oxidation products to be linked with the development of certain diseases, such as carcinogenesis or arthrosclerosis (Staprans *et al.*, 1996; Kanazawa *et al.*, 2002). Such compounds are primary oxidation products like hydroperoxides, which degrade to peroxy, alkoxyl and hydroxyl radicals, or secondary products of lipid oxidation like alkanals (hexanal), alkenals (2-nonenal), α,β-unsaturated aldehydes (acrolein), 4-hydroxy-2-alkenals (4-hydroxy-2-nonenal) or malondialdehyde.

Therefore the question of whether or not oxidized edible oils are harmful to health is justified. A lot of research has been conducted into the investigation of the biological and toxicological effects of oxidized edible oils (Kaunitz and Johnson, 1973; Miller and Landes, 1975; Gabriel *et al.*, 1977; Billek, 1992). In most cases, highly abused frying fats or fractions of enriched oxidized compounds were used in animal trials to assess their effect. The transfer of such results to oxidized edible oils seems to be questionable, because the daily intake of oxidized oils has to be high (10–15%, w/w, of diet) to cause an effect on animals (Kaunitz and Johnson, 1973), which is not realistic in human nutrition.

Cortesi and Privett (1972) described how hydroperoxides present a remarkable toxicity if injected intravenously; but Bergan and Draper (1970) showed that the hydroperoxy group does not survive uptake through the intestine, although damage to the intestinal is possible. There are also indications that dietary lipid peroxidation products could play a role in the pathogenesis of

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atherosclerosis, where they promote endothelial injury, accumulation of plaque and thrombosis (Addis, 1990; Yagi, 1986; 1988).

The presence of free radicals, such as hydroxyl and superoxide radicals, hydrogen peroxide or organic peroxides, either from normal essential metabolic processes in the human body, or from external sources, has been associated with the appearance of different processes, such as aging, oxidation of lipoproteins and carcinogenesis. In vivo peroxyl radicals can change DNA by causing DNA strand breaks, crosslinks between DNA and proteins, and oxidative reactions of the bases of DNA. The oxidation of lipoproteins is known to be responsible for the development of atherogenesis, which is caused by the formation of lipid-laden foam cells and their accumulation in arteries. Also some secondary oxidation products, such as malondialdehyd or 4-hydroxyl-2-*trans*-nonenal (4-HNE), are highly reactive in vivo with other compounds like proteins or DNA. In total, the spectrum of effects on feeding oxidized oils from autoxidation or thermal treatment ranged from diarrhoea, loss of appetite, growth retardation, cardiomyopathy, hepatomegaly, haemolytic anaemia, accumulation of peroxides in the adipose tissue up to deficiency of tocopherols and selen (Sanders, 1989).

In the early 1960s Poling *et al.* (1962) showed that cottonseed oil heated at 60 ºC over a longer period of time resulted in the oil having a lower nutritional value. The animals that were fed with this oil developed a pathologically enlarged liver. The experiment showed that the major enlargement of the liver and the major decrease of the nutritional value occurred within the period of the largest increase of the peroxide value.

The uptake of larger amounts of highly oxidized edible oils seems to be unlikely, because the human nose is a very sensitive detector, capable of recognizing the oxidative deterioration of edible oils long before significant amounts of relevant toxicological compounds are formed. Such products would usually be rejected by the consumer.

Another issue is that edible oils are an important source for essential fatty acids such as linoleic and linolenic acid, and vitamin-E-active compounds like tocopherols or tocotrienols. During autoxidation, these compounds are degraded, which would result in a deficiency of essential fatty acids and tocopherols if oxidized oils were the only source of dietary lipids and vitamin E (Chow, 2000). An increased uptake of oxidized oils also results in an accelerated rate of turnover of vitamin-E-active compounds in order to maintain the immune system of the body. The consequence of this is that there is a greater need for vitamin-E-active compounds, otherwise the balance between attacks of oxidized species from oxidized oil and the antioxidant defence system becomes disturbed (Chow, 1979). Moreover, when using oxidized edible oils for the preparation of protein-rich foods, the availability of amino acids from that food can be reduced by the reaction of lipid degradation products.

Despite these findings, which indicate that the uptake of oxidized material can carry a human health risk if consumed in large quantities, it is not clear yet whether lipid autoxidation products that are received through nutrition in

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realistic amounts really pose a significant risk to human safety. The concentration of the compounds seems to be too low, and the life span of hydroperoxides is very short. Another aspect is that the level at which oxidized edible oils become harmful is not known. Therefore, several authors have concluded that fats used under practical conditions have no toxicological relevance (Chalmers, 1954; Poling *et al.*, 1960; Warner *et al.*, 1962). The general guideline should be to take up as few oxidized oils as possible.

6.6 Effect of processing on the oxidative stability of edible oils

Processing has an important impact on the composition of edible oils in terms of the components which can affect their oxidative stability and shelf-life. In general, there are two different possible methods for the extraction of edible oils from the oil-bearing material. The first is the extraction of the raw material, with or without previous de-oiling, by a screw press to oil content between 15 and 20%, and subsequent solvent extraction, most often by hexane. With an oil yield between 98 and 99%, this process is very economical, and therefore most vegetable oils are produced by this method. The drawback of the method is that, as a result of the extensive extraction conditions, not only are desirable components extracted from the raw material, but also components which deeply impair the quality of the resulting oil in terms of sensory evaluation, colour and storage stability. Therefore, after the extraction of the oil from the raw material, a refining process is necessary to remove undesirable components from the oil in order to make it consumable and storable.

In recent years, an increasing number of small- and medium-sized facilities have been producing not just virgin olive oil, but also other virgin, cold pressed oils using only the process of screw pressing and purifying the oil by filtration or sedimentation. This procedure is not new, indeed the extraction of oilseeds by small presses goes back several hundred years. It was rediscovered by farmers as one possible means of producing high-quality products with an added value, instead of delivering seeds to the large oil mills after the harvest. Pressing by a screw press only is remarkably less efficient than extraction by solvent because, depending on the settings of the screw press, 10–15% of the oil remains in the press cake. On the other hand, fewer undesirable components from the raw material pass over into the oil during pressing and impair the quality of the product. Furthermore, the consumer associates virgin oils with healthy nutrition, and attributes them with a lot of advantages over refined vegetable oils, such as minimal processing, their deep colour and their typical taste and smell.

The oxidative stability and shelf-life of refined and virgin edible oils are rooted in the development and interaction of pro- and antioxidant compounds. The extraction process and the further treatment of the oil strongly influence the content and composition of minor components in the oil. While extensive extraction conditions, together with heat-treatment, result in higher extractability of phenolic and other antioxidative active compounds, such a treatment is also

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conducive to higher concentrations of oxidation products, which could possibly affect the quality of the product. Refining reduces both types of components. Extraction by a screw-press only is much milder with regard to the formation of oxidation products, but on the other hand, more antioxidant active compounds remain in the press-cake. Thus, the processing of edible oils is a balancing act between avoiding oxidation and the removal of antioxidant active compounds from the raw material. The better the producer achieves this, the higher the oxidative stability and the shelf life of the oil will be. Some differences in oxidative stability can arise from differences in the processing of refined and virgin edible oils, but some general aspects have to be taken into consideration during processing to avoid reduced shelf-life and bad quality.

The influence of processing on product quality and shelf-life begins immediately with harvesting, and each step from harvesting to selling may have a certain impact on the quality of the oil. Gupta (2000) summarized the requirements for the formation of free radicals in oilseeds and fruits, which can start the oxidative deterioration of the oils during storage, as:

- poor quality oilseeds
- improper handling and storage of oilseeds
- improper conditions during crushing
- poor handling and storage of the crude oil
- overheating the oil during processing
- undue exposure of the oil to oxygen during processing
- atmospheric bleaching
- deodorization under poor vacuum
- storage and handling of the finished oils without oxygen.

6.6.1 Refined oils

After solvent extraction, crude oil consists of 95–98% triacylglycerides. In addition, it contains phospholipids, free fatty acids, coloured pigments, phyto-sterols, waxes, oxidation products, metal ions, moisture, and aroma components, as well as plant parts and dirt in varying amounts, depending on the conditions of the extraction process. The more extensive the extraction conditions, the higher the proportion of minor compounds, which strongly impair the quality and the shelf-life of the oil. For this reason, it is necessary to purify the crude oil after the extraction process. This process is known as refining. Otherwise, the oil is not acceptable to the consumer. The aim of the refining process after solvent extraction is comprehensive purification of the crude oil, to remove compounds which negatively influence the quality and the shelf-life.

In general, refining is a multistage process, which involves the steps degumming, neutralization, bleaching and deodorization in different variations and performances. It is carried out either as physical or chemical refining. Chemical refining uses alkaline solutions to neutralize free fatty acids during the neutralization step, while physical refining waives neutralization and uses hot water steam during deodorization to remove free fatty acids from the oil.

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Each of the different refining steps removes specific minor components from the oil. Degumming removes phospholipids, proteins and also mucilaginous gums. Alkali refining takes off free fatty acids, phospholipids, metal ions, and chlorophyll, while bleaching reduces the amount of coloured compounds like chlorophyll and carotinoids, metal ions and oxidation products. In the final deodorization step, volatile aroma compounds and free fatty acids, as well as tocopherols, phytosterols, phenolic compounds and carotinoids are removed from the oil. Additionally, the high temperature used for deodorization causes hydroperoxides to partially decompose into short-chain degradation products, which are removed during steam treatment. However, secondary oxidation products which can propagate autoxidation remain in the oil and can result in the fast deterioration of the product during storage. Thus, although the peroxide value is reduced to zero during deodorization, the anisidine value in the oils increases, showing that a low peroxide value of refined oil is not necessarily an indication of high quality. A crude soybean oil, heavily oxidized before deodorization, may have a good flavour, but during storage it develops an oxidized flavour and becomes rancid very rapidly (Gupta, 1994).

At the end of the refining process, the content of minor compounds in the oil has been reduced. While phospholipids, iron, chlorophyll and free fatty acids are almost completely removed from the oil, the amount of tocopherols is reduced between 10 and 60%, and phytosterols between 20 and 50%, depending on the type of oil and the refining conditions (Jung *et al.*, 1989; Ferrari *et al.*, 1996; Ramamurthi, *et al.*, 1998; Shahidi *et al.*, 2005).

Several papers have been published on the comparison of the oxidative stability of vegetable oils from different stages of the refining process (Going, 1968; Kwon and Brown, 1984; Jung *et al.*, 1989; Gordon and Rahman, 1991; Pekkarinen *et al.*, 1998). Owing to the removal of the minor compounds, which can act as pro-oxidants or antioxidants, the refining process has a remarkable effect on the oxidative stability and the shelf life of the oil. In general the refining process causes a decrease in the stability of the refined oil, compared with the crude oil. Rutkowski (1961) described this decrease in the cases of desolventizing, neutralization and bleaching as 18.6%, 34.6% and 52.7%, respectively.

Going (1968) showed that clean-dry crude soybean oil was less susceptible to oxidation than refined and bleached oil, and stated that oil should be stored as crude oil to minimize the deterioration of the oil by oxidation. Furthermore, Going concluded that relatively low levels of oxidation are necessary prior to deodorization to impair the oxidative stability of the finished oil. Similar results were found by Kwon and Brown (1984) who compared the oxidative stability of soybean oil at seven different stages of commercial refining by measuring the weight increase during storage. In this experiment, the oxidative stability of the processed oils decreased in the order crude > degummed > bleached > deodorized. The lowest oxidative stability was found for soybean oil triacylglycerides, purified from minor compounds by column treatment. The authors concluded that the most likely reason for this finding was the loss of

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antioxidant active components, such as phospholipids and tocopherols, during column treatment and refining. Additionally, Kwon and Brown (1984) showed that phospholipids work in synergy with tocopherols to produce an antioxidant effect. Individually, tocopherols and phospholipids improved the oxidative stability of soybean oil triacylglycerides to a certain extent, but had only a small effect. However, the combination of tocopherols and phospholipids in the same concentration strongly improved the oxidative stability of the oil. The higher the concentration of phospholipids, the better the oxidative stability of the oil.

The importance of phospholipids for the oxidative stability of vegetable oils has also been described by Linow and Mieth (1976) who found that the addition of selected phospholipid fractions improved the effect of tocopherol on the oxygen absorption of methlylinolate. Nitrogen-containing phospholipids, like phosphatidylcholine, were especially able to synergize with tocopherols. They also found that the positive effect of phospholipids was not based on the regeneration of tocopherols in the redox-system tocopherol/tocopherylcholine, but rather that phospholipids inhibited the degradation of tocopherols. For concentrations between 0 and 1%, Bratkoska and Niewiadomski (1975) described how phospholipids isolated from rapeseed oil had a prolonging effect on the induction period of refined rapeseed oil. Hildebrand *et al.* (1984) found that the addition of tocopherol and phospholipids (except phosphatidic acid), alone or in combination, increased the stability of soybean oil. They explained this effect in terms of the synergism of phospholipids with tocopherols, rather than by the special ability of phospholipids to bind traces of metal ions.

Phospholipids isolated from coconut oil by column chromatography and added to degummed, bleached and deodorized coconut oil, increased the induction period of the oil in the Rancimat test at 120 ºC from 5.1 to 32.3 hr, a value close to the induction period of crude coconut oil (Gordon and Rahman, 1989). On the other hand, phospholipids can also act as natural surfactants, similar to mono- and diacylglycerides, which reduces the interfacial tension between the contrary materials water and oil, but also air and oil. Thus, higher amounts of phospholipids in the oil during storage in the presence of water can result in hydrolysis of triacylglycerides and the formation of free fatty acids. Gupta (2000) described how a phosphorus content in vegetable oils ≥ 3 ppm results in the formation of free fatty acids during storage. Furthermore, the solubility of oxygen in the oil is improved by the presence of greater amounts of phospholipids.

Jung *et al.* (1989) found that crude soybean oil contains a peroxide value of 2.4 meq/kg, which increased to 10.5 meq/kg during degumming, and 16.5 meq/kg after bleaching. As a result of the high temperature during deodorization, the hydroperoxides were completely decomposed and the peroxide value fell to 0 meq/kg. The increase in the peroxide value after degumming was explained by the moisture content (1–3%) and temperature (70–80 ºC) during processing. The formation of hydroperoxides during bleaching strongly depends on the type and amount of clay used (King and Wharton, 1949). The use of lower concentrations of clay resulted in higher peroxide values. Morrison (1975) mentioned that

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bleaching with 1% activated clay decreased the storage stability, while 3% clay had no effect on the storage stability of sunflower oil.

The effect of the processing steps on the oxidative stability of soybean oil was found in the order crude > deodorized > degummed > refined > bleached (Jung *et al.*, 1989), which was explained by the presence and absence of pro- and antioxidants. While crude oil is relatively low in pro-oxidants like hydroperoxides, the oil contains high values of antioxidants like tocopherols and phospholipids. On the other hand, bleached oil has a high peroxide value and only low amounts of antioxidants.

Farhoosh *et al.* (2009) investigated the development of the peroxide value and carbonyl value of soybean and canola oil during the different refining steps. While PV decreased from 1.89 meq O_2/kg (crude) to 0.64 meq O_2/kg (deodorized) for soybean oil and from 1.94 meq O_2/kg (crude) to 1.78 meq O_2/kg (deodorized) for canola oil, the carbonyl value increased from 2.77 μmol/g to 6.36 μmol/g for soybean oil and 19.9 μmol/g to 26.9 μmol/g for canola oil. The main increase was observed during the bleaching step, probably due to the decomposition of hydroperoxides and the formation of secondary oxidation products.

Today, physical refining, which combines the neutralization and deodorization steps of chemical refining, is internationally more popular because the process is easier to handle and more economic. One drawback is the need for a higher temperature to ensure that sufficient free fatty acids are removed. A high temperature during deodorization leads to the degradation of tocopherols, which are important for the shelf-life of edible oils (Jawad *et al.*, 1983). During the refining of soybean oil, the length of the induction period at 100 ºC decreased significantly with increasing temperature during deodorization (240–300 ºC). The authors assumed that this effect was probably due to the reduction of tocopherols.

Rice bran oil (RBO) is a very rich source of bioactive phyto-chemicals, which include tocopherols and γ-oryzanol (an ester of *trans*-ferulic acid with sterols and triterpenic alcohols, with high antioxidant and radical scavenging properties). During chemical refining γ-oryzanol is removed from the oil to 1.1%, 5.9% and 93.0–94.6%, respectively, with a resulting content of 1.1–1.74%, while physical refined RBO contains 1.63–2.72% γ-oryzanol (Krishna *et al.*, 2001). As a result of alkali-refining, the content of tocopherols and γ-oryzanol is reduced to 34% and 51%, respectively and the order of oxidation stability is: crude >> degummed > bleached = deodorized > alkali-refined (Yoon and Kim, 1994). Furthermore, Mezouari and Eichner (2007) found that crude RBO had better storage stability than full refined oil. The authors explained the good OSI value after 240 days of storage by the large quantity of sterols, γ-oryzanol and tocopherols that remained in the crude oil. They recommended that the conditions of the refining process should be improved to retain a maximum of desirable compounds.

Owing to the high temperature during deodorization, but also during bleaching, refined vegetable oils contain considerable amounts of oligomer

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triacylglycerides (Gomes and Catalano, 1988; Dobarganes *et al.*, 1989; De Greyt *et al.*, 1999; Gomes *et al.*, 2003). Oxidized triacylglycerides can also be found in refined oils. De Greyt *et al.* (1999) found 0.32–2.01% (mean = 1.07%) oligomer triacylglycerides in 19 samples of refined corn, sunflower, soybean, peanut, rapeseed, palm and olive oil and 1.53–4.83% (mean = 3.11%) oxidized triacylglycerides. Similar contents of oligomer triacylglycerides were found by Gomes (1988). De Greyt *et al.* (1999) described the major increase of oligomer triacylglycerides during neutralization and deodorization, while oxidized triacylglycerides increased mainly during bleaching. Gomes *et al.* (2003) described an increase of oligomer triacylglycerides, accompanied by a decrease of oxidized triacylglycerides to 42.2% on average in different industrial refined seed oils. The authors suggested that the decrease of oxidized triacylglycerides was due in part to the occurrence of polymerization reactions. Both parameters could be used for the assessment of the oxidative quality of refined oils, since the level of oxidative degradation in the crude oil corresponds to the percentage of oligomer triacylglycerides found in the refined oil.

In the Rancimat test at 120 °C, the induction period of canola oil decreased during refining from 6.86 h (crude) to 4.33 h (deodorized), whilst that of soybean oil decreased from 5.02 h (crude) to 3.63 h (deodorized) (Farhoosh *et al.*, 2009). The main decrease was found after neutralization and showed no considerable changes during the further refining steps. Similar results were found by Zacchi and Eggers (2008), who showed that the induction period of rapeseed oil decreased remarkably after the degumming and neutralisation steps; only a little decrease was found for the other refining steps. One explanation was that during neutralization antioxidant active phenolic compounds were almost completely removed.

6.6.2 Virgin oils

In contrast to refined oils, the processing of virgin oils is reduced to two steps: the extraction of the oil from the raw material by pressing with a screw press, and the purification of the resulting oil by filtration, sedimentation or centrifugation. The producer has no opportunity to remove unpleasant aroma compounds, oxidation products or other factors that enhance oxidative reactions. The composition and the quality of the virgin oil corresponds to the composition of the raw material. Thus, the compounds responsible for oxidation processes during storage, which are normally removed during the refining process, are present in virgin oils to a greater extent, which generally results in a lower oxidative stability for virgin oils. On the other hand, the fact that no tocopherols or other antioxidant active compounds are removed improves the oxidative stability of virgin oils. Therefore, two reversed aspects have to be taken into consideration when discussing the oxidative stability of virgin oils in contrast to refined oils. The oxidative stability of high-quality virgin oils, produced from sound raw material with the utmost care under optimal conditions, should be higher or comparable to refined oils because, as a result of the careful

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processing, the amount of oxidation-promoting compounds is low, but the concentration of antioxidants like tocopherols is higher. Only when failures were made during production is the oxidative stability of virgin oils remarkably lower.

An important factor, which strongly influences the storage stability and shelf-life of virgin vegetable oils, is the initial content of hydroperoxides after processing. Depending on the processing conditions and the quality of the raw material, oxidation processes as well as the formation of hydroperoxides as primary oxidation products can begin to take place even in the raw material. This results in higher initial peroxide values in the virgin oil. Therefore, in contrast to the processing of refined oils, the producer has no opportunity to reduce the content of hydroperoxides after oil extraction. Satue *et al.* (1995) showed that the oxidative stability of virgin olive oil was significantly lower in comparison to refined, bleached and deodorized olive oil, although virgin olive oils contained higher levels of phenolic compounds. They assumed that the higher initial peroxide value of the virgin oils could be the reason for this finding.

Pekkarinen *et al.* (1998) also assumed that the high initial level of hydroperoxides in virgin rapeseed oil was responsible for their low oxidative stability, in comparison with crude or processed oils. The oils reached a peroxide value of 10 meq/kg during storage in the dark, in the order virgin > superdegummed > steam stripped ≅ crude > refined. During storage under light conditions, the formation of hydroperoxides in virgin oil was much slower over a storage period of 13 days, due to the lower content of chlorophyll in this type of oil, which acts as photo-sensitizer and enhances photo-oxidation.

As important as the initial content of hydroperoxides is the content of antioxidant active compounds, which should be higher in virgin oils than in the corresponding refined ones, since these compounds are to some extent removed during refining. On the other hand, the extensive extraction by heat-treatment and the use of a screw press and solvent results in a better extraction and higher amounts of minor compounds with some antioxidant activity, such as phenolic compounds in solvent extracted crude oils. In a market survey with 21 samples of virgin olive oils, Gutfinger (1981) showed that solvent-extracted olive oils were richer in polyphenols than the virgin oils. He also found a linear relationship between the content of polyphenols and the oxidative stability of the oils during storage at 60 °C. After the removal of polyphenols, the oxidative stability of the oils decreased remarkably.

A similar phenomenon was observed in coconut oil, where temperature had a strong influence on the content of phenolic compounds in the resulting oil. The extraction of coconut oil from coconut milk under hot conditions results in higher contents of phenolic compounds than extraction under cold conditions. This is because hot extraction improves the extractability of the compounds from the raw material (Seneviratne *et al.*, 2009). Temperatures above 100 °C result in a better transfer of the phenolic compounds from the water phase of the coconut milk emulsion into the oil. The phenolic extracts of hot extracted

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coconut oil showed a remarkably higher DPPH radical scavenging activity and inhibition of deoxyribose degradation, as compared with the phenolic extracts of cold extracted coconut oil. The authors did not investigate the influence of the extraction procedure on the initial content of hydroperoxides or free radicals in the resulting oils.

In a storage experiment with olive oils obtained with different extraction technologies (pressure extraction (traditional system), centrifugation without mill waste water recycling (continuous system) and centrifugation with mill waste water recycling), Cercaci *et al.* (2007) found no significant effect of the total sterols obtained from extra virgin olive oil on the oxidative stability of a vegetable model system.

Arranz *et al.* (2008) found a good correlation between the antioxidant activity measured by the DPPH-method and the oxidative stability determined by the Ranicmat method in different nut oils. They ascribed this finding to the tocopherols in the oils, but found that phospholipids also made a contribution. The ranking of antioxidant capacity of nut oils, by both methods, was: pistachio > hazelnut > walnut > almond > peanut.

In order to evaluate the antioxidant activity and to assess the oxidative stability of edible oils, it is important to consider that different parameters respond differently to oxidation, resulting in different results for the trend of oxidation during storage. One example was given by Satue *et al.* (1995) who, when following the oxidation by the peroxide value, showed that α-tocopherol behaved as a pro-oxidant at concentrations >250 ppm in refined olive oil, but was most effective in inhibiting the formation of hexanal. With the peroxide value as the parameter, phenolic extracts from olive oil showed the best effect at 50 ppm, but at 100 and 200 ppm the inhibition of hexanal formation was predominant.

It has also been noted that it is more difficult to detect the oxidative deterioration of virgin oils than that of refined oils because virgin oils have a very strong and dominant odour of their own, which covers rancid sensations. This explains the phenomenon where producers sometimes indicate very long dates of expiry, yet consumers use the oils from a bottle for a long time without noticing the oxidative deterioration.

6.6.3 Oils from heat-treated raw materials

A special phenomenon can be observed in cold-pressed edible oils from roasted seeds, such as nut oils or pumpkin seed oil, which show a higher oxidative stability than corresponding virgin oils from unroasted seeds (Wijesundera *et al.*, 2008). Prior *et al.* (1991) also found a higher oxidative stability of canola press oil after heat treatment of the seeds, which decreased with subsequent refining. They explained this by the appearance of non-triacylglyceride material in crude oil, and stated that the greater the initial quality of the oils (i.e., the lower the content of non-triacylglyceride material) the lower their oxidative stability. A good correlation with the phosphorus content was found in the range between

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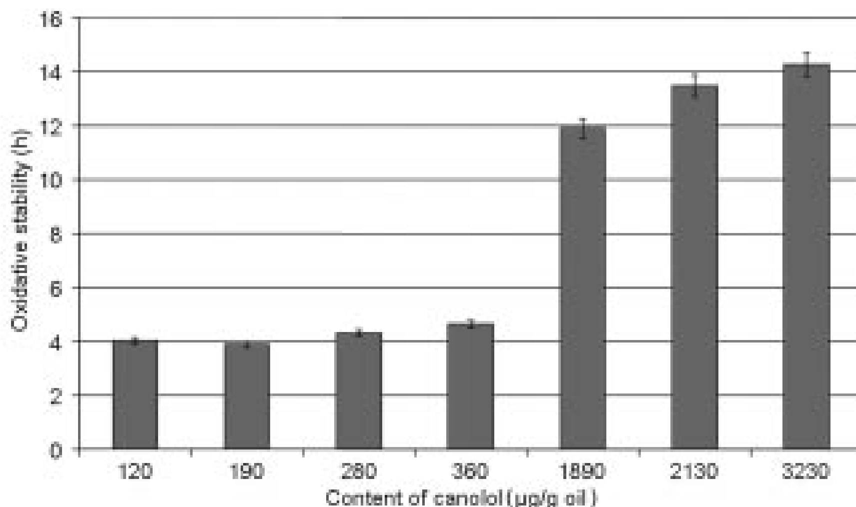

Fig. 6.15 Effect of canolol on the oxidative stability of rapeseed oil (Rancimat test at 120ºC).

0.025 and 0.22% phospholipids. Higher amounts of phospholipids did not further improve the oil stability.

During the roasting process, the intact or crushed seed material is treated by heat between 140 and 160 ºC, resulting in a better availability and extractability of bioactive compounds, as well as the formation of new antioxidants which improve the stability of the resulting oil. Such antioxidants include Maillard reaction products, canolol from the decarboxylation of sinapic acid in rapeseed (Wijesundera *et al.*, 2008) or the generation of sesamol from the degradation of sesamolin during the roasting of sesame seeds (Lee *et al.*, 2009). For example, the heat-treatment of rapeseed leads to a better oxidative stability of the oil in the Rancimat test, which is likely to result from the better extractability of antioxidant active substances during the pressing, and the formation of canolol during roasting (see Fig. 6.15).

6.7 Protection of edible oils against oxidation

During storage of edible oils, the storage stability and the quality of edible oils is strongly influenced not only by factors from outside the oil, but also from factors resulting from the composition of the oil (see Fig. 6.16). The fatty acid composition, as well as the content and the composition of tocopherols, are two major factors that are determined by the type of oil and cannot be controlled by the producer. Factors such as the content and composition of phenolic or minor compounds elude control. The only compounds that can, to a certain extent, be controlled from the outside are catalytic effective compounds like metal traces, as producers can limit the amount of metal that enters the oil during processing

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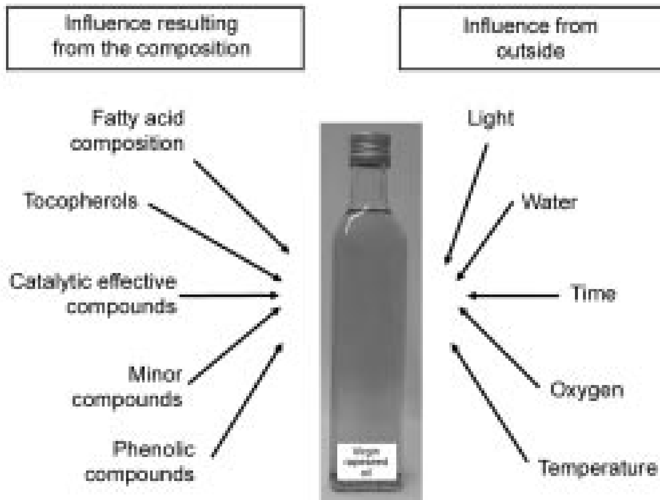

Fig. 6.16 Factors influencing the storage stability and the quality of edible oils.

and from the storage facilities. The only way of maintaining the quality and storage stability of edible oils is to control factors that affect the oil from the outside. Bearing in mind the most important factors influencing autoxidation, producers should aim to control all factors that could potentially enhance the oxidation reaction during processing and storage. These include temperature, light, oxygen availability, time and water.

Another possible method of improving the oxidative stability of edible oils is the addition of antioxidants, which are able to break the free radical chain mechanism of oxidation. Recently, interest has been focused on a new possible means of improving the oxidative stability of edible oils. Through genetic modification, or conventional breeding, it is possible to change the fatty acid composition of edible oils such as rapeseed, soybean or safflower oil and thereby produce so-called high-oleic oils with a remarkably higher oxidative stability. Such oils are used as edible oils, but more commonly for frying.

It is important to remember that any oxidation during processing has an adverse effect on the quality and, to a greater extent, the storage stability of edible oils. Changes in the initial quality of the oil as a result of improper processing are often not immediately apparent, but the effect on storage stability becomes obvious very fast.

Therefore, during processing it is necessary to avoid everything that impairs the oxidative state of the oil, either by the formation of oxidation products, or by removal of antioxidant compounds. Moreover, it is important to remember that the quality of edible oils will decline even if the oil is handled and stored under proper conditions. It is possible to delay oxidative deterioration by appropriate measures, but not to avoid it. Protecting edible oils against oxidation means the extension of the shelf-life, which is an important reason for the industry to undertake enormous efforts to achieve this aim. Various possibilities are conceivable.

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6.7.1 Temperature

The most important factor in terms of accelerating the oxidative degradation of edible oils is the increased temperature. As previously mentioned, for every temperature increase of 10 ºC, the number of oxidative reactions doubles and the storage stability of the oil is halved. Therefore it is recommended that the oil is exposed to a temperature as low as possible. During the production of virgin oils, the flow temperature of the oil during processing is between 30 and 50 ºC, depending on the raw material. Remarkably higher temperatures are used during the production of refined oils, depending on the refining step. If it is necessary to use higher temperatures during processing, it is important to control the exposure oxygen very strictly. Figure 6.17 shows the influence of temperature and oxygen availability on the development of the peroxide value of virgin rapeseed oil. While storage at 6 ºC results in no change of the peroxide value, the degradation was only small when the oil was stored in a sealed bottle at room temperature. A fast increase in the peroxide value can be observed if the oil is stored at room temperature and exposed to the air.

6.7.2 Light

Light is a particular form of energy which is able to accelerate lipid oxidation, even if there are compounds present that can transfer light energy to the substrate directly (riboflavin; photo-oxidation type I) or via formed singlet

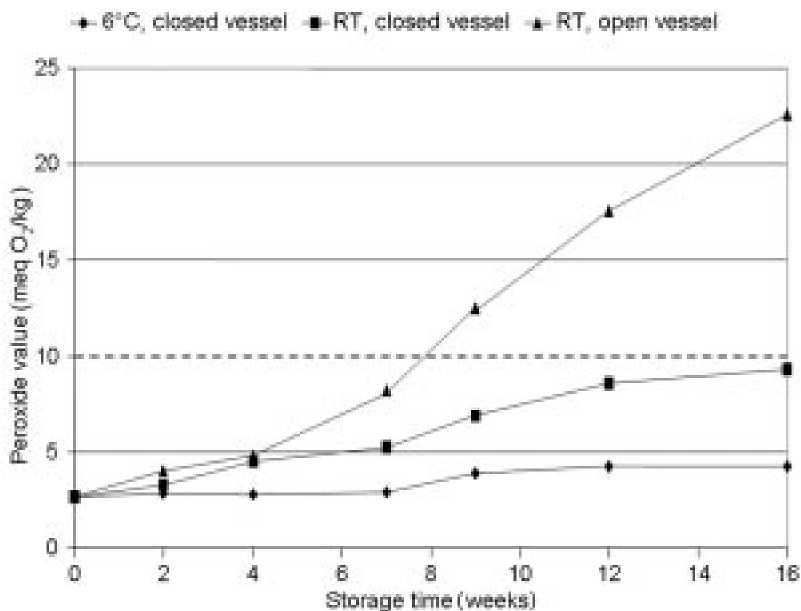

Fig. 6.17 Influence of the storage conditions on the peroxide value of rapeseed oil (RT = room temperature).

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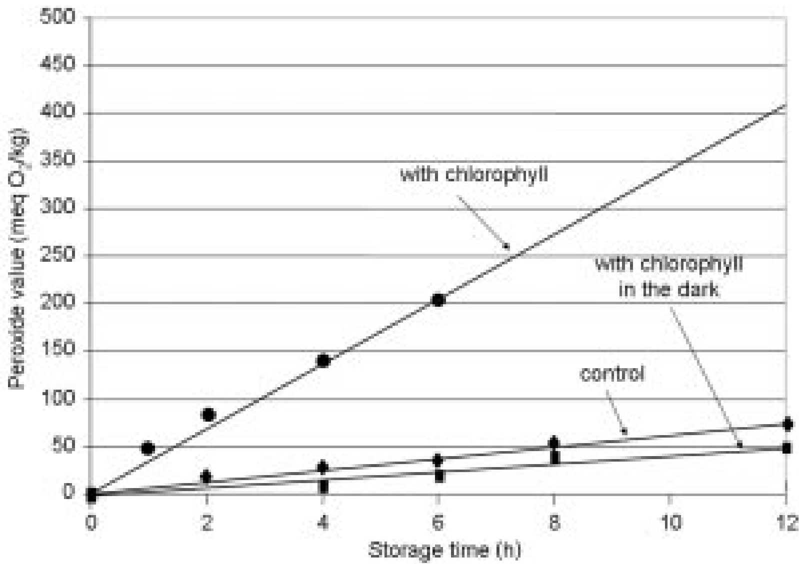

Fig. 6.18 Influence of chlorophyll on the oxidation of distilled soybean esters at 35ºC (modified according to Frankel *et al.*, 1979).

oxygen (chlorophyll, photo-oxidation type II). In both cases, the best protection against oxidation is the exclusion of light from the storage facility by use of an optically opaque container. In the case of processing, light should not be a problem, but it becomes a significant issue when clear glass bottles are used for retail packaging. For the purpose of effective advertising, edible oils (and especially virgin edible oils) are often presented in clear glass bottles in order to attract the consumer's attention to the typical colour of the oil. The presence of chlorophyll or other sensitizers causes the oil to degrade very quickly (see Fig. 6.18). Therefore, it is highly recommended that coloured glass or plastic bottles are used even for virgin oils in order to protect the oil against UV irradiation.

In addition to sensitizers like chlorophyll, virgin oils also contain certain amounts of natural quenchers, such as carotinoids, which are can inhibit the susceptibility of fatty acids to photo-oxidation by deactivating the singlet oxygen or sensitizer.

6.7.3 Oxygen

Oxidation reactions can only take place if oxygen is present. Therefore, it is important to avoid or to limit the contact of the oil with oxygen, not only during storage, but also during processing, as it is here that the basis for the storage stability of the product is determined.

The most crucial steps during processing are when the oil comes into contact with oxygen at an elevated temperature, e.g. deodorization. Here, it is recommended to deaerate the oil at low temperature after deodorization to reduce the

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solubility of oxygen within it. Cooling the oil under nitrogen is also recommended.

In addition to the processing, the transportation and storage of the bulk oil can be a crucial time for the incorporation of air into the oil if improper apparatus is used or poor handling occurs. One important aspect, which should be taken into consideration, is the possible input of air by working pumps during the transportation of oil through pipelines. Another aspect is that storage tanks should always be filled from the bottom to avoid pouring oil through air, which would provide a further opportunity for incorporation to occur. The surface-to-volume ratio of the storage container is also important because peroxide development is correlated directly with this ratio. As the size of the container increases, so too does the peroxide value as the surface-to-volume ratio in bigger containers becomes less favourable (Going, 1968).

It is generally accepted that the headspace of the bottles, tanks or vessels used for the storage of oil should be as small as possible so as to limit the contact of the oil with oxygen. Nevertheless, a certain amount of oxygen is dissolved into the oil. This becomes obvious when a sealed PE bottle of edible oil is stored by a window over a longer period of time as the bottle contracts due to the consumption of the oxygen within it. Another indication of a progressing oxidation in a sealed glass bottle is when on opening the bottle there is a noticeable fizzling of in-streaming air.

Despite knowing the negative effect of oxygen on the quality of edible oils, it is neither economical nor practical to remove air from the product before storage. Oxygen shows a remarkably high solubility in oils (i.e., 3.2 mL/100 mL and 2.9 mL/100 ml in raw soybean oil and sunflower oil, respectively or 1.3 mL/100 mL and 1.9 mL/100 ml in refined soybean oil and sunflower oil, respectively (Aho and Wahlroos, 1967)), which makes its removal from the oil very time-consuming. It takes several hours to remove the oxygen from one litre of oil by purging it with an inert gas. However the stated amount of oxygen in soybean oil is sufficient to raise the peroxide value up to 18, if it completely reacts (Cowan, 1965). Therefore, air is often replaced only from the headspace of the storage facility. Different investigations have shown that nitrogen packaging is very effective at protecting susceptible edible oils against autoxidation and oxidative deterioration (Evans *et al.*, 1973). In all cases, it is important to use containers which are impermeable to air in order to prevent the access of oxygen to the oil from outside.

6.7.4 Metal traces

Metal traces are known to be effective catalysts for the degradation of primary oxidation products and concentrations as low as 0.1 ppm iron and 0.01 ppm copper can accelerate the reaction in soybean oil (Täufel and Linow, 1962). Thus, the presence of metal traces like copper or iron should be minimized. Iron and copper enter the oil naturally during its extraction from the raw material in amounts between 1 and 10 ppm and 0.1 and 0.2 ppm, respectively. Most of this

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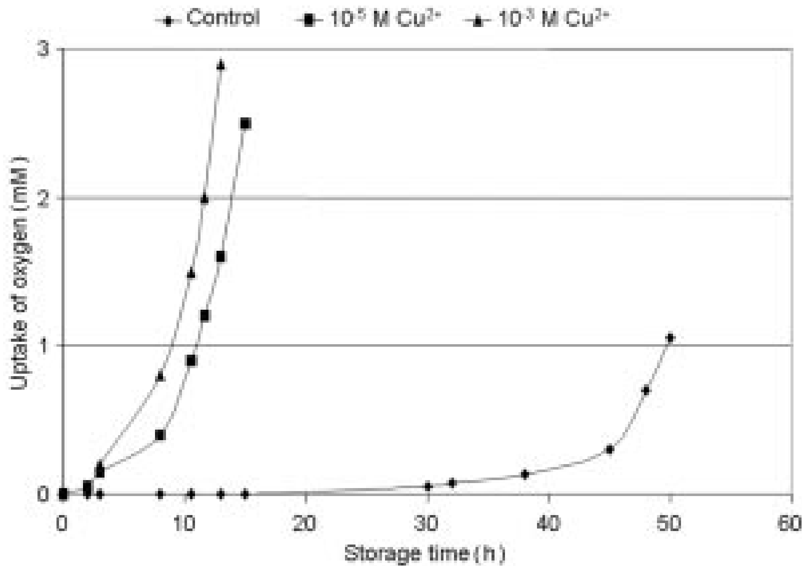

Fig. 6.19 Effect of copper ions on the oxidation of linoleic acid in an emulsion (modified accordingly to Farag *et al.*, 1978).

can be removed during alkali refining. Only in virgin oils does no removal of metal traces take place.

Figure 6.19 shows the effect of different amounts of copper on the oxidation of linoleic acid in an emulsion, with oxygen uptake as the parameter for the progressive oxidation process. The experiment clearly reveals the remarkable effect of copper on the oxidation of linoleic acid (Farag *et al.*, 1978).

It is recommended that stainless steel vessels are used for the transportation and storage of edible oils to avoid the transition of metal ions from the vessel into the oil and enhancing oxidative deterioration. The problem is that this material is very expensive. If large capacities are needed, it is also possible to use low value materials like mild steel, which is coated with an inert material like epoxy or polyurethane resin. To avoid contamination by metals, it is also important that pipelines are made of stainless steel or other inert materials.

The amount of metal traces in the oil can be reduced either by washing or, much more effectively, by using chelating compounds such as citric acid, o-phosphoric acid, ascorbic acid or ethylenediamine tetraacetic acid (EDTA). These compounds act either by coordinating the metals, or by blocking their complex formation with hydroperoxides. It is important to consider that some of the potential inhibitors can also act as pro-oxidants, depending on their concentration, e.g. ascorbic acid becomes a potent pro-oxidant after reducing copper or iron.

Citric acid is sometimes in use as a chelating agent during processing of edible oils, but since its effectiveness decreases at elevated temperatures, the chelating agent is added during the cooling phase of the deodorization process,

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when temperature is below 100 ºC. In some countries citric acid is accepted as a processing aid and therefore does not need to be declared.

6.7.5 Use of antioxidants

A commonly used, easy-to-handle and effective means of delaying autoxidation is the use of antioxidants. The effect of most used antioxidants is based on the ability of such compounds to break the free radical chain mechanism by reacting with the radicals and forming more stable compounds. There are some other types of antioxidants which delay autoxidation by mechanisms other than breaking the free radical chain, such as metal inactivation, deactivation of UV light, destruction of hydroperoxides, synergism, scavenging of oxygen, regeneration of chain breaking antioxidants or quenching of singlet oxygen.

The main prerequisites for the use of a compound or a mixture of compounds as antioxidant are that it is toxicologically harmless, effective even in low concentrations, smell- and tasteless with no influence on the food, soluble in oil with an homogenous distribution, and stable during processing.

The chain breaking mechanism of antioxidants is based on the ability of each molecule to rapidly donate a hydrogen atom to a lipid radical and form a stable product. This results in the formation of an antioxidant free radical (see Fig. 6.20), which should be stable enough not to intervene in the chain mechanism by abstracting a hydrogen atom from an unsaturated fatty acid. The antioxidant activity of molecules with phenolic hydroxyl groups is based on steric hindrance by methyl-, t-butyl or hydroxyl groups in ortho-position to the antioxidative effective hydroxyl group, and on the stabilization resulting from resonance mesomerism in the aromatic ring system. Important for the antioxidant activity of a compound is the velocity with which the reaction between antioxidant and lipid molecule can take place. When using antioxidants, it is important to consider the use of concentrations above a critical value can result in an inversion of the effect (Elmadfa and Thiele, 1982).

Many countries have strict regulations about the application of synthetic or natural antioxidants with regard to the compound and the concentration, and regulatory approvals for most compounds are not available. For this reason, only a small number of antioxidants are usable in practice. Some synthetic anti-oxidants, such as propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluol (BHT) or tert-butylhydroquinone (TBHQ), are permitted in some

Fig. 6.20 Effect of antioxidants to inhibit free radical chain mechanism.

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types of food. However, because synthetic antioxidants are suspected of being toxicologically unsafe, the focus is turning more and more towards natural antioxidants.

The most important natural antioxidants are tocopherols and tocotrienols, which occur naturally in four forms, each, α-, β-, γ- and δ-tocopherol or -tocotrienol, depending on the number and the position of methyl groups in the chromane ring system. The antioxidant activity of tocopherols and tocotrienols is based on their ability to form a tocopherol-tocopherylquinone redox system that is stable enough not to become involved in the further radical mechanism (Trappel, 1972). Since tocopherols react much faster with lipid peroxy radicals (10^4 to 10^9 M^{-1} s^{-1}) than lipid peroxy radicals react with with other lipids (10 to 60 M^{-1} s^{-1}) the efficiency of these compounds in terms of protecting edible oils is very good. The optimal concentration of tocopherols for the protection of edible oils against oxidative deterioration depends on the type of tocopherol, their oxidative stability and the temperature. A rule of thumb is that the lower the oxidative stability, the lower the optimal concentration. While the optimal concentration for α-tocopherol is 100 mg/kg, γ- and δ-tocopherol show optimal antioxidant activity at 250 and 500 mg/kg, respectively (Jung and Min, 1990).

Tocopherols and tocotrienols are natural constituents of edible oils, which give them a natural stability against autoxidation. Relatively high amounts of tocopherols can be found in rapeseed, soybean, sunflower and corn oil, while palm oil contains remarkable amounts of tocotrienols. The content of tocopherols in olive oil is low, but this oil contains higher amounts of phenolic compounds, which are described as having a high antioxidant activity. The content of natural antioxidants in edible oils depends on cultivar, conditions during cultivation, storage conditions and processing.

Other natural antioxidants can be found in sesame oil, which contains a number of lignan compounds such as sesamin, sesamol, sesamolin, sesaminol and sesamolinol, with sesamin as the main compound found in oil from unroasted seeds. These phenolic compounds result in a remarkably high oxidative stability, although the oil contains a high amount of linoleic acid. Oil from roasted sesame seeds is more stable at 60 ºC than corn oil, safflower oil or a mixture of rapeseed and soybean oil (Fukuda and Namiki, 1988).

Although the content of tocopherols is low in virgin olive oil in comparison to other vegetable oils, the oil is relatively stable against oxidation due to the high content of various phenolic compounds. The main antioxidant compounds in olive oil include tyrosol, hydroxytyrosol, oleuropein, caffeic acid and derivatives of tyrosol and hydroxytyrosol (Brenes *et al.*, 1999; Owen *et al.*, 2000; Servili *et al.*, 2004). These phenolic compounds act by scavenging free radicals or chelating metals, mainly in the initial stage of the oxidation (Chimi *et al.*, 1991). For virgin olive oil, Aparicio *et al.* (1999) described a significant correlation of phenols, oleic/linoleic ratio and tocopherols, with oil stability measured by Rancimat. Also the total content of phenolic compounds, linked to a high antioxidant activity, is directly correlated to the oxidative stability when measured by Rancimat (Gutfinger, 1981).

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6.7.6 Vegetable oils with changed fatty acid composition

One critical point for the oxidative stability of edible oils is the fatty acid composition. Oils with higher amounts of polyunsaturated fatty acids are much more susceptible to oxidation than fats and oils consisting of more saturated fatty acids. Kochhar and Henry (2009) showed a linear relationship of high-level monounsaturated fatty acids between 100 times the reciprocal of the induction period (Rancimat test) and the total unsaturated fatty acids obtained as %C18:2 + 0.08 x %C18:1 + 2.08 x %C18:3, while polyunsaturated fatty acid oils exhibited an exponential relationship. From this, they found the order of oxidative stability, determined as induction period by the Rancimat test at four temperatures (90 ºC, 100 ºC, 110 ºC, and 120 ºC), as macadamia oil > rice bran oil ≈ toasted sesame oil > avocado oil > almond oil > hazelnut oil > grape seed oil > walnut oil.

On the other hand, vegetable oils are favoured in nutrition, because of the positive health effects of unsaturated fatty acids. To break this cycle, for some years new plant varieties with significantly higher amounts of oleic acid than the traditional varieties have been available on the market. These new varieties were originally developed for technical applications, but today they are also of interest for human nutrition and the preparation of food, especially if high oxidative stability is desired. These so-called 'high-oleic' oils have the advantage of combining consumer demand for natural vegetable oils and the requirement of the industry for high-stability oils (Kochhar, 2001).

Most of the well-known conventional vegetable oils were subject to different breeding programmes, either by conventional methods, or by genetic techniques. The use of genetic engineering for the breeding of new varieties is problematical in Europe, because the products obtained from these seeds are often not welcomed. Another problem is that it is difficult to produce these varieties with sufficiently good agricultural properties, and with an economically interesting seed yield or oil yield. Therefore, at the moment there are only a few varieties from plants with a high content of oleic acid available on the commercial market.

In addition to high-oleic soybean oil, which has different applications in human nutrition, high-oleic sunflower oil also has a certain importance in the oil market. This oil is available with different amounts of oleic acid, from 75% to more than 90%. Meanwhile some high-oleic rapeseed varieties are also available with more than 70% oleic acid and noticeably reduced amounts of linolenic acid. In particular, this reduction of the high amount of linolenic acid seems to have significantly improved the stability of this type of rapeseed oil in comparison with oil from conventional varieties. Carré *et al.* (2003) showed that a content of more than 1.1% linolenic acid already resulted in significantly higher intensities of fishy and paint-like odours.

6.7.7 What are the legislative regulations?

There are only a few regulations available for the assessment of edible oils that describe the requirements of oils in terms of the oxidative state or the content of

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antioxidants. Most regulations or standards state that edible oils have to be free from rancid odour or taste, e.g. Codex Alimentatrius (2001), German Guideline for Edible Fats and Oils (Anon., 1997), Regulation (EEC) No 2568/91 for olive oil (Anon., 1991). This means that sensory evaluation is the most important parameter for the assessment of edible oils. Even if the chemical parameters do not show any failure, if the oil has a rancid odour or taste it is possible to reject the oil for human consumption. Chemical parameters are only supposed to support or confirm the result of the sensory evaluation, if necessary.

The only chemical parameter in different regulations or standards used for the assessment of the oxidative state of edible oils is the peroxide value. Accordingly to the Codex Alimentarius (2001) the peroxide value of edible oils is limited to 10 meq O_2/kg oil for refined oils, and 15 meq O_2/kg oil for cold pressed or virgin oils. The limitation according to the German Guideline for Edible Fats and Oils (Anon., 1997) is stricter, allowing a maximum peroxide value of just 10 meq O_2/kg oil for virgin oils and 5 meq O_2/kg oil for refined oils. For virgin olive oil, the regulation defines the limit as 20 meq O_2/kg oil in the regulation (EWG) 2568/91 (Anon., 1991).

Owing to the weak points of the peroxide value, the recommendations of the German Society of Fats Sciences (DGF, 2006) suggested replacing the peroxide value with the Totox value, taking into account the formation of secondary degradation products. Some official laboratories already use a Totox value of 10 for refined oils, and 20 for virgin edible oils, in the assessment of samples. The Totox value is also often used in industry as a parameter for the assessment of edible oils, since its significance regarding the oxidative state of the oil is remarkably better than that of the peroxide value.

The use of antioxidants for improving the oxidative stability of edible oils is strictly limited to some compounds and concentrations. In most countries BHA, BHT and gallates are allowed in fats and oils in concentrations between 100 and 400 (BHA), 100 and 400 (BHT) and 50 and 400 gallates, respectively. Only in Germany is the use of synthetic antioxidants for stabilizing edible oils forbidden. Generally, it is not permitted for antioxidants or any other compounds to be added to virgin or cold pressed oils. For refined oils, most countries permit the addition of tocopherols in usual concentrations, ascorbyl palmitate or β-carotene, while the list of permitted antioxidants given in the Codex Alimentatrius (2001) also comprises ascorbyl stearate, propyl gallate (100 mg/kg), TBHQ (120 mg/kg) and dilauryl thiodipropionate (200 mg/kg).

Thus, the assessment of the oxidative quality of edible oils is mainly based on sensory evaluation. The formation of secondary degradation products and detection by sensory evaluation are the most important criteria in the assessment of edible oils. This makes sense since, on the one hand, the human senses are very sensitive to the degradation products of oxidation and, on the other hand, the product has to fit the demands of the consumer, regardless of the chemical parameters. Therefore, the high importance of sensory evaluation for the assessment of edible oils is justified.

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6.8 Conclusion

Oxidative deterioration during storage is one of the major reasons why edible oils become unusable for human consumption. Although any real health hazard seems to be very small, the use of oxidized oils is not recommended and should be avoided. In particular, it is the volatile degradation products, formed in the second step of the oxidation process, that are responsible for the adverse effects on edible oils. These are partly detectable even in very low concentrations, making the oil unusable from a sensory point of view. Today, many steps of the oxidation process have been clarified, yet some questions remain open, e.g. the formation of the initial radical, which causes the free radical chain mechanism. A lot of possibilities are known to slow down the speed of oxidation and to improve the storage stability of edible oils, resulting in a longer shelf-life, which is important for the industry and the consumer. However, it should be clear that it is not yet possible to stop the oxidation process. Once it has started, only the speed of oxidation can be influenced by means of antioxidants, or the prevention of contact with light, temperature, oxygen or metal traces. The application of such avoidance strategies is strictly limited for technological and practical reasons. Recent trends, which try to improve the storage stability of edible oils by changing the fatty acid composition from polyunsaturated towards higher amounts of monounsaturated fatty acids, come with certain disadvantages from a nutritional perspective.

There are a lot of different physical and chemical methods for the assessment of the oxidative state and stability of edible oils available, but at the moment no single method is suitable for making a comprehensive and satisfactory judgement of edible oils. Normally, it is necessary to combine different methods. Therefore the search for one method that is suitable for a comprehensive assessment of the quality of edible oils is an ongoing story. The analysis of the volatile compounds and the search for key compounds are good tools, but still not perfect.

The processing of edible oils has a strong effect on oxidative stability and shelf-life, because it determines the content and the composition of antioxidant and pro-oxidant compounds. While during solvent extraction with heat-treatment, high amounts of antioxidant phenolic compounds pass over into the oil, this content is considerably reduced during the refining process. This results in refined oils having a lower oxidative stability than corresponding crude oils. On the other hand, crude oils are not consumable, which makes the refining process inevitable. The extraction of oil-bearing material by a screw-press only is gentler, resulting in the formation of fewer oxidation products on the one hand, and smaller amounts of antioxidative compounds on the other.

6.9 Sources of further information and advice

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7

Preventing oxidation during frying of foods

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Abstract: Deep frying, one of the most common processes used for food preparation, is widely used by consumers and the food industry. Owing to the presence of oxygen, high temperature and water the frying process involves numerous reactions among which oxidation and polymerization stand out. This chapter is mainly focused on possibilities for preventing oxidation during the frying process with special attention to the influence of oil composition, i.e. degree of unsaturation, natural antioxidative compounds and additives, and frying conditions. Specific aspects of oxidation at high temperatures and low oxygen availability during the frying process, which contribute to modifying physicochemical, sensory and nutritional properties of oils, are reviewed. Also, the most important methods used for the evaluation of changes in oil quality are presented.

Keywords: frying oil, frying variables, thermoxidation, antioxidants, dimethylpolysiloxane, additives.

7.1 Introduction

Deep frying is one of the most common processes of food preparation and it is widely used by consumers and the food industry. In spite of its apparent simplicity, it is a tremendously complex process that involves numerous reactions due to the action of oxygen, high temperature and the water released by the food during heating. The general reactions of the main oil constituents, i.e. triacylglycerols (TG), such as oxidation, hydrolysis, polymerization, cyclization

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and isomerization are well known, although it is not easy to predict the rate of oil degradation due to the high number of variables involved in the process. These variables can be grouped into three categories:

1. those linked to the process itself, such as temperature, length of heating, continuous or discontinuous heating, turnover period, etc.;
2. those associated with the fat or oil used, e.g. degree of unsaturation, initial quality, minor compounds and additives; and
3. those inherent to the food (Jorge *et al.*, 1996a; Dobarganes, 1998; Choe and Min, 2007).

An important aim in frying is the selection of adequate operating conditions to produce high quality foods and, consequently, to maintain a low degradation level in the frying oil. This chapter is mainly focused on possibilities for preventing oxidation during the frying process with special attention to the influence of the oil composition, i.e. degree of unsaturation, natural anti-oxidative compounds and additives, and the frying conditions. Specific aspects of oxidation at high temperature and low oxygen availability during the frying process, which contribute to modifying physicochemical, sensory and nutritional properties, are reviewed. Also, the most important methods used for the evaluation of changes in oil quality are presented.

7.2 Oxidation during the frying process

Lipid oxidation generally proceeds through chain reactions of free radicals that produce hydroperoxides (ROOH) as primary oxidation compounds and a myriad of secondary products formed from hydroperoxide decomposition. Figure 7.1 illustrates the well-known scheme of the oxidation mechanism, where RH represents the TG molecule undergoing oxidation in one of its unsaturated fatty acyl groups.

At room or moderate temperatures, formation of oxidation compounds is relatively slow; the hydroperoxides (ROOH) are the major products formed and their concentration increases until advanced stages of oxidation. Polymerization compounds only become significant in the accelerated stage of oxidation, i.e. after the end of the so-called induction period (Márquez-Ruiz *et al.*, 1996; Márquez-Ruiz and Dobarganes, 2005). However, minor volatile compounds formed during the induction period, in particular carbonyl compounds, are of enormous sensory significance and may contribute to modifying negatively the oil flavor (Frankel, 2005).

The chemistry of lipid oxidation at the high temperatures of food processes like frying is, however, far more complex because both oxidative and thermal reactions are simultaneously involved. At high temperatures, formation of new compounds is very rapid, the oxygen pressure is reduced and, consequently, the initiation reaction becomes important and gives rise to an increase in the concentration of alkyl radicals ($R^\bullet$) with respect to alkylperoxyl radicals

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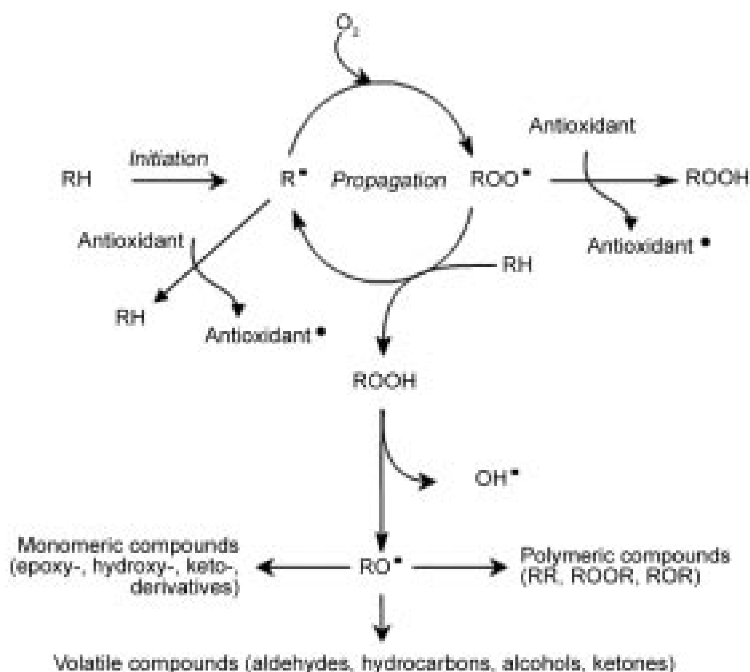

Fig. 7.1 General scheme of autoxidation reactions.

(ROO$^•$). Besides, ROOH decompose rapidly and are practically absent above 150 ºC, indicating that decomposition of hydroperoxides becomes faster than formation (Dobarganes, 1998). As a result, polymeric compounds form from the very early stages of heating through termination reactions that mainly involve alkyl (R$^•$) and alkoxyl (RO$^•$) radicals (Scott, 1965). In addition, due to the low oxygen concentration, significant amounts of non-polar TG dimers (R-R) are also produced (Dobarganes and Márquez-Ruiz, 2007).

Formation of oxidation compounds is delayed by chain-breaking antioxidants which mainly act as hydrogen donors reacting with the alkyl (R$^•$) and alkylperoxyl (ROO$^•$) radicals. While at room temperature the reaction of antioxidants with ROO$^•$ is the most important one due to the rapid reaction of R$^•$ with oxygen, at low oxygen pressure the reactions with both R$^•$ and ROO$^•$ become significant.

A high variety of products of different polarity, stability and molecular weight are generated during the complex process of frying. Three main groups of compounds can be distinguished by molecular weight:

1. TG dimers and oligomers;
2. oxidized TG monomers, and
3. volatile compounds.

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7.2.1 TG dimers and oligomers

The polymerization compounds, dimers and oligomers of TG, are the most specific and abundant compounds in used frying fats (Márquez-Ruiz *et al.*, 1995). Their molecular weights are obviously higher than those of their parent non-altered TG. The main compounds are those containing carbon-carbon links or ether bonding, i.e. carbon-oxygen-carbon links (Dobarganes and Márquez-Ruiz, 2007).

7.2.2 Oxidized TG monomers

Oxidized TG monomers are TG with at least one of their fatty acyl groups oxidized or containing additional oxygen. This group of compounds includes TG containing different oxygenated functions such as hydroxy, keto, epoxy and others, as well as TG with short-chain fatty acyl groups (Velasco *et al.*, 2004a).

Quantitative analysis of mono-functional compounds containing hydroxy, keto and epoxy functions has been reported in a recent publication. Results showed that these three families of compounds constitute major groups within the fraction of oxidized TG monomers (Marmesat *et al.*, 2008a).

TG with short-chain fatty acyl groups mainly form through homolytic β-scission of alkoxyl radicals. Saturated fatty acyl chains of 7 and 8 carbon atoms, n-oxo saturated chains of 8 and 9 carbon atoms, known as core aldehydes, and the same aldehydes oxidized to carboxylic acids are the most abundant structures (Velasco *et al.*, 2004a; 2005).

7.2.3 Volatile compounds

Simultaneously to the formation of short-chain fatty-acid containing TG by β-scission of alkoxyl radicals, volatile compounds are also produced. Thus, the cleavage of a fatty acyl chain generates, on the one hand, a glyceridic product and, on the other, compounds of low molecular weight. As opposed to the glyceridic oxidation products, the volatiles are removed to a large extent from the oil because of the effect of heating.

As previously stated, the oxidation reactions take place in the unsaturated fatty acyl groups attached to the glyceridic backbone and, therefore, the stable final products are TG monomers, dimers and oligomers containing modified and non-modified acyl groups.

Finally, due to the moisture released from the food during heating, hydrolytic reactions give rise to the formation of diacylglycerols and free fatty acids during frying. Free fatty acids accelerate the oxidative degradation and also contribute to a decrease of the smoke point due to their partial volatilization (Kochhar, 2001).

The hydrolytic products, diacylglycerols and free fatty acids, are intermediate compounds in the metabolism of fats prior to the intestinal absorption. Therefore, although these compounds influence the quality of the frying oil, they do not modify its nutritional value. However, oxidative and thermal degradation

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give rise to changes in the fatty acyl chains of TG and so lead to changes in the nutritional value of the oil.

7.3 Assessing frying oil quality

The chemical reactions involved in the frying process generate numerous physical and chemical changes in the oil, a few of which are easily observable. Examples of such changes are an increase in viscosity due to the formation of polymers, foaming also attributed to polymers and to other amphiphilic substances, darkening due to formation of colored reaction products, a decrease in the smoke point due to the release of volatiles and free fatty acids, an increase in acidity as a consequence of hydrolytic reactions, and changes in the organoleptic characteristics due to the development of typical odor and flavor. Therefore, there are many methods based on the measurement of these changes to evaluate the quality of used frying oils. The methods can be classified into two categories: those based on the application of classical analytical indices to evaluate the most remarkable changes, and more complex analytical methods based on the quantitative determination of the total pool or part of the new compounds formed.

The analytical indices more commonly applied include the acidity index, measure of color or UV absorption, smoke point, foam height, viscosity and dielectric constant. Generally, they are of utility in the evaluation of quality of used frying oils, but find a few limitations. In order to evaluate changes, for most of the indices it is necessary to know the value of the initial oil as a reference. Some examples are viscosity, dielectric constant and UV absorption, which can be very variable and only changes with respect to the initial oil are related to degradation. Another limitation is that some indices also respond to components of the food that are released into the oil, such as the determination of color and foaming. Besides, a common aspect for every analytical index is the fact that only partial aspects of such a complex degradation process are evaluated.

Because of rapidity, simplicity, reproducibility and low costs, the analytical indices are, however, very often used when the value of the initial oil is known or when the relation between the measured parameter and the quality of the fried food has been established. As an example, the acidity index, which provides information on the degree of hydrolysis, is one of the most frequently applied indices to control continuous frying processes carried out in the food industry. Owing to the constant characteristics of the continuous frying, the simple application of this index allows one to predict the oil degradation and the quality of the fried food.

The analytical methods based on the quantitative analysis of degradation products do not present the drawbacks associated with the analytical indices and also allow the evaluation of any sample of unknown origin. The two most commonly used methods are the determination of polar compounds and the determination of polymers (IUPAC, 1987; 1992), which provide the total content of the new compounds formed and of polymers, respectively.

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The determination of polar compounds is included in most regulations and recommendations adopted in different countries to evaluate the quality of used frying oils (Firestone, 2007). It consists of the separation of the total pool of degradation products by adsorption chromatography in a packed column using silica gel. The degradation products, including oxidative, thermal and hydrolytic degradation compounds, are separated as a fraction of higher polarity than that of the non-altered TG and are determined by gravimetry. The determination is accurate, reproducible and it does not need complex or sophisticated analytical instrumentation. Its main limitation is that it is a time-consuming determination.

The determination of polymers is another official method included in regulations and recommendations in certain countries. It consists of the direct analysis of the oil by high-performance size-exclusion chromatography with refraction-index detection. The separation of polymers is based on their higher molecular size with respect to the non-altered TG. As outlined above, TG polymers are the most characteristic degradation products formed in frying (Dobarganes, 1998).

Also, both methods can be combined by applying exclusion chromatography to the polar compound fraction obtained by silica column. This possibility is of interest as it allows independent determination of thermal, oxidative and hydrolytic degradations (Dobarganes *et al.*, 2000a).

One important question to be answered is when a used frying oil or fat has to be discarded. In fact, there is no simple answer considering both the complexity of the new compounds formed and the fact that rejection of frying oils is usually based on visual inspection (mainly foaming, smoking, color and odor) or length of use. However, a percentage of total polar compounds around 25% has been established as the upper limit of oil degradation for human consumption by most of the countries that have adopted regulations or recommendations on frying fats and oils (Dobarganes and Márquez-Ruiz, 1998; Firestone, 2007). This level is relatively high as compared to oxidative degradation of oils or food lipids at room temperature, whose levels are expected to increase not more than 5 wt% to be acceptable by consumers. This increase corresponds to a peroxide value of around 100 meq/kg fat, when rancidity is clearly detectable. Still, the limit of degradation established for used frying oils and fats (25% oil) is often surpassed in a significant number of oils and fats in fast food outlets (Dobarganes and Márquez-Ruiz, 1998; Saguy and Dana, 2003). This fact clearly reflects that a better control of frying oil quality is required.

Even though there are adequate analytical methods available to assess frying oil quality, knowing the exact moment when the frying oil has to be discarded according to the present regulations remains as the main problem for the fryer operator when there are no laboratory facilities. Paradoxically, the need of control increases as the laboratory facilities decrease. In this respect, rapid tests to be applied *in situ* have been developed and proved to be useful (Stier, 2004; Marmesat *et al.*, 2007). A few tests are based on chemical reactions between the oil and reactants through consumable kits of easy utilization such as Fritest, and Oxyfritest commercialized by Merck, or Oleotest commercialized by Biomedal,

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Table 7.1 Main analysis for assessing quality of used frying oils

Analysis	Technique/ measurement	Main compounds related	Application
Polar compounds	Adsorption chromatography	Total new compounds	Official laboratories
Polymers	Exclusion chromatography	Polymers	Official laboratories
Polar compound distribution	Adsorption/exclusion chromatography	Polymers, oxidized monomers and diacylglycerols	Official laboratories
Free fatty acids	Acid-base titration	Free fatty acids	Continuous frying industries
Color	Visible absorption	Polymers	Continuous frying industries
Fritest	Visual color	Carbonyl compounds	Restaurants and fast food outlets
Oxyfritest Oleo test	Visual color	Oxidation compounds	Restaurants and fast food outlets
Fry-Check Viscofrit	Viscosity	Polymers	Restaurants and fast food outlets
Testo 265 FOM	Dielectric constant	Total polar compounds	Restaurants and fast food outlets

and others on changes in physical properties of the oil such as viscosity – Fri-Chek (Gertz, 2000) and Viscofrit (Castellón-Arnau, 1999) – or dielectric constant – Testo 265 and FOM 200.

Validation data for rapid tests have been recently reported (Marmesat *et al.*, 2007). Two chemical tests based on carbonyl and non-specified oxidation compounds and two physical tests based on viscosity and dielectric constant were evaluated in a set of 105 used frying oils. The utility of the rapid tests was established by comparing their results with those obtained for the total content of polar compounds and the content of polymers, as they are the most accepted determinations for the evaluation of the quality of frying oils, as outlined above. The main conclusion was that, although these tests still are not widely extended, the application of any of those studied would contribute significantly to improving the quality of used frying fats and oils.

Table 7.1 summarizes the most appropriate methods for assessing frying oil quality in the different sectors.

7.4 Effects of frying on sensory and nutritional quality

Frying is one of the culinary processes that most drastically modifies the sensory and nutritional properties of food. Changes in appearance, texture, color and flavor take place, although an agreeable flavor seems to be the main reason for the

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popularity of fried foods. Volatiles are of paramount importance in relation to the sensory properties of fried foods and arise from several sources. Major compounds come from oxidative reactions which involve the breakdown of frying oil hydroperoxides, as commented above. In addition to the frying oil, other important sources of volatiles include oxidative and thermal decomposition of food lipids, breakdown products of other main constituents of the food, and compounds produced by interaction of food components and/or interaction between food and frying oil. From these different sources, hundreds of volatiles including hydrocarbons, alcohols, ketones, aldehydes, acids, esters, phenols, pirazynes, furans, thiazoles, oxazoles, pyrroles, pyridines, etc., have been identified in different fried products although it is difficult for most of them to know their pathways of formation (Takeoka *et al.*, 1996; Gillat, 2001). Only nitrogen- and sulfur-containing compounds, coming from amino acids and proteins, are clearly related to the food and so justify specific flavors for different fried foods, while the frying oil would add some common organoleptic characteristics to them.

Volatile decomposition products from the frying oil include aldehydes, ketones, alcohols, hydrocarbons, lactones, furans, etc. Among them, 2,4 decadienals seem to be the major contributors to the deep-fried flavor. However, the role of specific compounds in relation to specific flavors is far from being known for several reasons. On the one hand, qualitative composition of volatiles is similar independently of the oil used for frying, as volatiles derived from the unsaturated fatty acids are always present. Even more, the same volatiles contributing to the rancid off-flavor are involved in the positive organoleptic characteristics of fried foods. On the other hand, most of the volatiles are removed during frying due to both the high temperature of the process and the release of steam water from the food during the frying operation. Under these circumstances, no correlation between the amount of volatiles and the level of degradation is expected (Nawar, 1998). Thus, differences in flavor are due to quantitative differences in volatile composition, which in turn depend on the fatty acids undergoing degradation. From oils rich in linoleic acid, hexanal and 2,4-decadienals, obtained from 13 and 9 hydroperoxides, are the major compounds while in high oleic oils high contents of octanal, nonanal, 2-decenal and 2-undecenal coming from 11, 10, 9, and 8 oleic acid hydroperoxide breakdown, respectively, are found (Dobarganes *et al.*, 1986; Nawar, 1998). Volatiles also depend on the quality of used frying oil because, as the degradation progresses, they are also formed from oxidized polyunsaturated and monounsaturated fatty acids.

An interesting series of studies have been carried out by Warner and co-workers on a significant number of soybean, canola, corn and sunflower oils. Conventional oils, partially hydrogenated oils and oils from modified seeds with fatty acid composition differing from the conventional oils were used to prepare French fries and tortilla chips (Warner and Mounts, 1993; Warner *et al.*, 1994; 1997; 2001; Mounts *et al.*, 1994: Warner and Knowlton, 1997; Warner and Gupta, 2003; Warner and Fehr, 2008; Warner, 2009). The aim was to study the relationships between oil composition and sensory quality defined by a trained panel test. Many interesting ideas may be found in these studies, which are

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useful both in the selection of a frying oil for a certain application and in the development of new frying oils as alternatives to hydrogenated oils. They can be summarized as follows:

- Oils containing linolenic acid in significant amounts develop fishy flavor. The content of linolenic acids should be lower than 3% for limiting the development of off-flavors (Mounts *et al.*, 1994; Warner *et al.*, 1994; Warner, 2009).
- A minimum content of linoleic acid, even being more oxidizable than oleic acid, seems to be necessary to obtain the most pleasant flavor due to the formation of 2,4-decadienals in appropriate levels. Linoleic acid should be in the range from 20 to 30% (Warner *et al.*, 1997).
- Oils containing a high percentage of monounsaturated fatty acids show high oxidative stability and good frying performance. However, the quality of deep-fried flavor decreases. This seems to be due to the aldehydes derived from decomposition of oleic acid hydroperoxides, such as 2-decenals and 2-undecenals, that possess negative olfactive notes (Warner *et al.*, 1994; 2001).

To sum up, sensory quality during frying of non-lipidic foods is related to the relative content of the main unsaturated fatty acids of the oil and cannot be deduced from the oil oxidative stability or the levels of polar compounds formed.

As for nutritional changes, they can be defined from two points of view. On the one hand, it is of interest to know how the frying process modifies the food nutritional value and, on the other, how the decrease in frying oil quality may affect the food nutritional properties or biological implications.

Concerning the first point, great changes are expected due to the two main physical changes occurring, i.e. water evaporation and oil absorption, both contributing to modifying the initial food composition by significantly increasing the food energy (Pokorny, 1999; Dobarganes *et al.*, 2000b). As an example, Table 7.2 shows increases in caloric energy after frying of potatoes depending on their gross composition. As can be observed, the energy may increase in the order of 7–8 times for 100 g of food from the raw product to the crisps (chips) and around 300 g of raw potatoes are necessary to produce 100 g of crisps (chips). On the other hand, frying has little impact on the protein or

Table 7.2 Changes in the nutritional value of potatoes depending on their proximate composition

Sample	Lipids (%)	Moisture (%)	Defatted dry matter (%)	Energy (kcal/100 g)
Raw potatoes	< 0.1	80	20	80
Prefried French fries	5	65	30	165
French fries	20	40	40	320
Crisps	40	1	60	600

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mineral content of fried food, whereas the dietary fibre content of potatoes is increased after frying due to the formation of resistant starch. Also, the high temperature and short time of the frying process cause less losses of heat labile vitamins than other types of cooking. For example, the content of vitamin C in French fries is as high as in raw potatoes; thiamine is well retained in fried potato products; and vitamin E is incorporated from the oil (Fillon and Henry, 1998). Even more, in all the foods covered by a thick layer of batter or bread as is the case of prefried vegetables, meat or fish, the major changes attributed to oxygen and temperature take place mainly in the surface of the product (crust) as the oil does not penetrate the food and the temperature inside (core) remains below 100 ºC (Saguy and Dana, 2003).

Food nutritional quality and safety depends largely on the quality of used frying oils. From the nutritional point of view, the non-volatile degradation products of used frying fats and oils are the most relevant since they remain in the oil, are retained in the food, and subsequently ingested. In this respect, oxidized fatty acids are the compounds more suspected of impairing the nutritional and biological properties of fried foods.

Most of the oxidized fats in foods are expected to come from fats and oils heated at high temperature and, more specifically, from used frying fats. However, the intake of oxidized fats is unknown and the oxidation status of the oils used in nutritional studies has not been analyzed so far due to the lack of accurate analytical techniques (Dobarganes and Márquez-Ruiz, 2003).

Highly oxidized dietary fats have been associated with a number of degenerative diseases, particularly with the development of atherosclerosis (Staprans *et al.*, 1996; Chisolm and Steinberg, 2000; Cohn, 2002) and cancer (Kanazawa *et al.*, 2002).

In animal feeding studies, ingestion of oils heated under extreme conditions, far from those used in culinary practices, has often shown detrimental effects such as hepatic oxidative stress and alterations in lipid metabolism, while, in contrast, used frying oils and fats have not shown clear evidence of biological implications but only slight or null effects (Márquez-Ruiz and Dobarganes, 2007).

In general, one of the main problems in most animal feeding studies is that amounts of heated oils in the animal diets and/or conditions for oil preparation are not realistic and, furthermore, may give rise to considerable amounts of compounds practically absent in a normal diet. For example, forced aeration at moderate temperatures generates abundant hydroperoxides, contrary to what is found in used frying oils (Sülzle *et al.*, 2004); or highly unsaturated oils heated over 200 ºC favor formation of cyclic monomers (Sébédio *et al.*, 2007). Also, some deleterious effects of heated oils have been associated with the presence of volatile compounds found in negligible amounts which are more likely to be formed by lipid oxidation *in vivo*, e.g. highly reactive aldehydes such as 4-hydroxy-2-*trans*-nonenal (Seppanen and Csallany, 2002).

Another problem, as already mentioned, is that the oxidation status of the oils used in nutritional studies is so far scarcely known, since it is normally based on

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indirect, non-specific tests that do not allow knowing the content of degradation products, such as peroxide value, conjugated dienes or TBA tests. Identification of the structures and quantification of the oxidized compounds of used frying oils added to diets has been improved in the last years but still remains as a great analytical challenge. Such analytical advances are essential to have evidence of whether normal intakes of oxidized compounds through dietary fried products are sufficient to produce the deleterious effects claimed.

Among the aspects of special concern in the last years, the potential impact of heated fats at the molecular level, particularly on activation of the hepatic peroxisome proliferator-activated receptors (PPAR) implicated in the regulation of lipid and lipoprotein metabolism, stands out (Ringseis *et al.*, 2007; Luci *et al.*, 2007). Also, interesting aspects have been explored taking advantage of recent advances in modern instrumental techniques, such as the bioavailability of triacylglycerols containing epoxy and hydroxy groups (Wilson *et al.* 2002a; 2002b), which are major oxidation compounds in used frying oils (Velasco *et al.* 2004b; Marmesat el al. 2008a); and the analysis of oxidized triacylglycerols in tissues and lipoproteins (Suomela *et al.*, 2004; 2005).

Overall, it is reasonable to conclude that a moderate consumption of oxidized fats under normal culinary practices is safe, but it is also evident that some lipid oxidation compounds are unavoidably ingested and might be harmful in the long term. Therefore, of primary importance is the improvement of the quality control of used frying fats and fried products, particularly in the case of fried foods prepared through discontinuous frying in which the used frying oil can reach a considerable degradation level.

7.5 Preventing oxidation during frying

The main factors influencing oil oxidation during frying can be divided into *intrinsic factors*, namely, the oil composition, which includes the degree of unsaturation, content of free fatty acids and antioxidants, etc.; and *external factors* related to the process conditions and to the food being fried. Once the food to be fried has been decided, becoming a parameter in the process, the variables that can be selected for obtaining the highest fried food quality depend on the frying medium and frying conditions.

7.5.1 Oil composition

Frying fats and oils range from hydrogenated oils designed for specific applications to non-hydrogenated refined oils. Although price, availability and functionality are important variables in the selection of the frying oil or fat, from the technical point of view the suitability of a fat or oil for frying is related to its stability against oxidation. However, due to the negative nutritional implications of partially hydrogenated oils and saturated fats, the situation has changed drastically. The development of genetically modified seeds containing oils with

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fatty acid composition different from that of conventional oils is a promising route to obtain high stability oils with nutritional properties compatible with consumer demands.

A huge number of papers on comparative performance of different oils and fats in the frying process are published every year. However, the results obtained are highly variable and of low utility because of the impossibility of replication for several reasons. Among them, the following stand out:

1. One oil sample is not representative of all the oils of a similar type, as differences in the initial quality and in the contents of minor compounds are important enough to modify significantly the frying performance.
2. Important variables in the frying process, i.e. additives or surface to oil volume ratio, are omitted in the studies. In most of the reports only temperature, length of heating time/frying time, and oil volume are included.
3. No duplicates are carried out of frying experiments and repeatability may be very low in small fryers mainly due to the high number of uncontrolled variables introduced by the food (Rodrigues Machado *et al.*, 2007).

To solve these problems, a standard test to know oil thermostability should be carried out before the application of the oil/fat in frying. Two simple procedures have been proposed to evaluate the performance of fats and oils at frying temperatures in the absence of food. Both procedures allow a strict control of the three most important variables in discontinuous frying, i.e. temperature, length of time, and surface-to-volume ratio. In the first procedure, the oil sample is heated at 180 °C in the heating block of a Rancimat device for ten hours and the content of polar compounds and polymers are determined. The procedure takes some of the advantages provided by the Rancimat test such as standard vessels, temperature correction and temperature homogeneity in all vessels. Repeatability was very good as evaluation of samples in triplicate gave coefficients of variation < 5% for polymers and < 6% for polar compounds (Barrera-Arellano *et al.*, 1997). In the second procedure, 20 g of sample supplemented with 1 g of silica gel containing 10% water is heated in a standard vessel (4 cm i.d.) at 170 °C for two hours. Quantification of polymers is performed and results are expressed as 100/polymer (%). Thus, the higher the value obtained, the more stable the oil will be (Gertz *et al.*, 2000).

Application of either of these two standardized methods would undoubtedly be useful to compare results from different studies, to justify differences in stability between oils and between batches of a same type of oil in discontinuous frying and to check the efficacy of minor compounds influencing stability at high temperature. Also, the method based on heating in a Rancimat device takes advantage of instruments applying standardized heating conditions, such as the Rancimat or Oil Stability Instrument, and so it would contribute to improving reproducibility and to obtaining comparative results of similar utility to those obtained for the prediction of oil stability during storage. In addition, such a test applies the same main conditions of discontinuous frying in small fryers and so it is not an accelerated test.

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Degree of oil unsaturation

Formation of polymers and polar compounds is accelerated by an increase in the degree of unsaturation, although the differences found are much lower or even opposite to those expected (Sebedio *et al.*, 1990; Dobarganes *et al.*, 1993; Warner and Gupta, 2003; Normand *et al.*, 2001; 2006; Barrera-Arellano *et al.*, 2002). This is probably due to the influence of the quality of the unused oil and to the different contents of antioxidants and prooxidants in oils. These small differences are diminished in studies using blends of two oils to modify the degree of unsaturation since the influence of other oil variables is thus partially eliminated (Shu and White, 2004; Steel *et al.*, 2005; 2006; Farhoosh *et al.*, 2009).

Also, studies of interest are focused on the performance of new oils with modified fatty acid composition with respect to conventional oils but maintaining similar unsaponifiable fractions (Dobarganes *et al.*, 1993; Warner and Mounts,1993; Warner and Knowlton, 1997; Xu *et al.*, 1999; Normand *et al.*, 2001; 2006; Guinda *et al.*, 2003; Warner and Gupta, 2003; Shu and White 2004: Matthaus, 2006; Gerde *et al.*, 2007; Marmesat *et al.*, 2008b; Warner and Fehr, 2008). Generally, results demonstrate better performance of oil as the degree of unsaturation decreases, as well as a higher polymers-to-polar compounds ratio, i.e., a higher tendency to polymerization (Marmesat *et al.*, 2008b), when the degree of unsaturation increases. However, unexpected results were also found in this regard (Normand *et al.*, 2001; 2006).

Of special interest are the results obtained on model systems (Barrera-Arellano *et al.*, 1999; Verleyen *et al.*, 2001; 2002) or purified triacylglycerols after passing the oil through an activated alumina column (Lampi and Kamal-Eldin, 1998; Márquez-Ruiz *et al.*, 1999a; Barrera-Arellano *et al.*, 2002; Steel *et al.*, 2005; 2006). After removal of tocopherols and a significant part of other minor compounds, the importance of the fatty acid composition becomes evident.

Table 7.3 shows the levels of polar compounds for six oils of different degree of unsaturation heated at 180 ºC for 10 hours in the absence of food, before and after removal of tocopherols (Barrera-Arellano *et al.*, 2002). As can be observed, after the oil purification the percentage of polar compounds in the initial oils decreased. After heating, the degradation was lower for the natural oils, demonstrating the positive influence of the antioxidants to delay the oil degradation, although low differences were found between mono and polyunsaturated oils. Finally, after purifying the oils, clear differences between mono and polyunsaturated oils were obtained while low differences were found among the oils of the same group, i.e. mono or polyunsaturated oils.

Similarly, Fig. 7.2 shows the effect of alumina purification in six samples of different conventional sunflower oils with similar levels of natural tocopherols. The samples were heated at 180 ºC in the absence of food for different periods of time and the content of polar compounds was determined. Results are expressed as mean values of the six samples. As can be observed, the removal of the antioxidants increased the oils degradation as expected although, even for the same type of oil, the reproducibility increases enormously after alumina purification probably due to lower differences in initial quality.

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Table 7.3 Polar compounds (wt%) in natural and tocopherol-stripped oils, initially and after heating for 10 hours at 180ºC

Sample	Natural oils		Antioxidant-stripped oils	
	Initial	10 hours	Initial	10 hours
Palm olein	9.2	18.4	1.3	16.7
Olive oil	5.3	12.7	0.6	17.0
High-oleic sunflower oil	2.8	15.9	0.5	18.9
Rapeseed oil	4.1	21.3	1.3	23.4
High linoleic sunflower oil	3.1	21.7	0.8	25.8
Soybean oil	3.8	18.6	1.1	26.5

In summary, the selection of an oil of low degree of unsaturation is important to control the degradation in frying and to reduce the relative level of polymeric compounds, although the action of minor compounds should not be underestimated.

Natural minor compounds

Tocopherols and tocotrienols

The relative stability of α-, β-, γ- and δ-tocopherol at high temperature has been studied in detail and there is in practice agreement that α-tocopherol is less stable than δ-tocopherol, while β- and γ-tocopherols degrade at an intermediate rate (Yoshida *et al.*, 1991a; 1991b; 1992; 1993; Gordon and Kourimská, 1995a; Lampi and Kamal-Eldin, 1998).

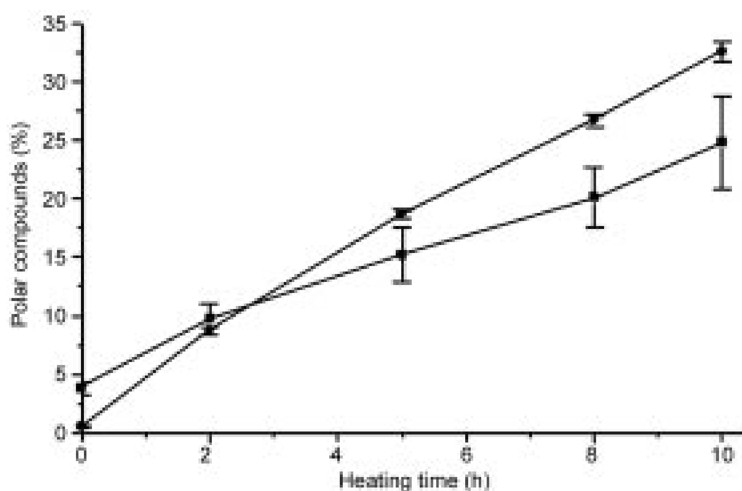

Fig. 7.2 Formation of polar compounds in natural sunflower oil (■) and tocopherol-stripped sunflower oil (●) heated at 180ºC. Error bars express SE ($n = 6$).

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Relative to oil degradation, the loss of tocopherol at frying conditions is, in general, more rapid as the degree of oil unsaturation decreases (Yuki and Ishikawa, 1976; Yoshida *et al.*, 1990; Simonne and Eitenmiller, 1998; Jorge *et al.*, 1996a; 1996b; Wagner *et al.*, 2001). However, the opposite is found at low temperature and around 100 ºC under the conditions used for oxidative accelerated tests (Yuki and Ishikawa, 1976; Martin-Polvillo *et al.*, 2004; Márquez-Ruiz *et al.*, 2008). Thus, a mechanism dependent on temperature seems to be involved in their action (Marinova and Yanishlieva, 1992).

In a study on the influence of the degree of unsaturation on oil degradation during frying, evolution of tocopherols was also analyzed and it was found in all the experiments that not only was the loss of tocopherols more rapid in the least unsaturated oil, but also that tocopherols were exhausted at a lower oil degradation (Jorge *et al.*, 1996a; 1996b). Owing to the difficulties in drawing general conclusions beyond those for the oils used, a series of experiments was designed to determine the influence of tocopherols on the alteration of the lipid substrate avoiding the influence of minor uncontrolled compounds normally present in the oils, which might have an antioxidant or prooxidant effect. Studies were carried out in model systems of standard triacylglycerols (Barrera-Arellano *et al.*, 1999), and in oils of different degree of unsaturation, i.e. soybean, rapeseed, olive, high linoleic sunflower and high oleic sunflower oils, and palm olein (Barrera-Arellano *et al.*, 2002). It was concluded from experiments with natural oils and with tocopherol-stripped oils containing different amounts and types of added tocopherols that, at the point of tocopherol exhaustion, the amounts of polymers and polar compounds were significantly lower in monounsaturated oils as compared to those present in polyunsaturated oils. These results have been reported in different studies either in model systems (Verleyen *et al.*, 2001) or oils (Normand *et al.*, 2001; Verleyen *et al.*, 2002). As an example, Fig. 7.3 shows the loss of tocopherols and the formation of polymers in monounsaturated and polyunsaturated oils. After ten-hour heating tocopherols were exhausted in the monounsaturated oils although the amount of polymers was lower than in polyunsaturated oils, still maintaining a fraction of their initial tocopherols. Also, the good performance of the soybean oil as compared to the conventional sunflower oil stands out, probably due to the higher efficacy of its pool of natural antioxidants containing γ- and δ-tocopherols. Finally, a low difference was found between the degradation of palm olein and soybean oil under the conditions applied in spite of the enormous differences in their degree of unsaturation. All these facts are of interest to insist on the complexity of the changes undergone by oils at frying temperatures and on the importance of the conditions established for replication of studies to obtain consistent general results of wide application.

From these results it is deduced that special attention should be placed to frying operations using monounsaturated oils, as they become unprotected at levels of polar compounds much lower than the limit established in official regulations for discarding fats for human consumption (25 wt%). If the fried food has to be stored, the oxidation at low temperature would be very rapid in the absence of antioxidants (Márquez-Ruiz *et al.*, 1999b).

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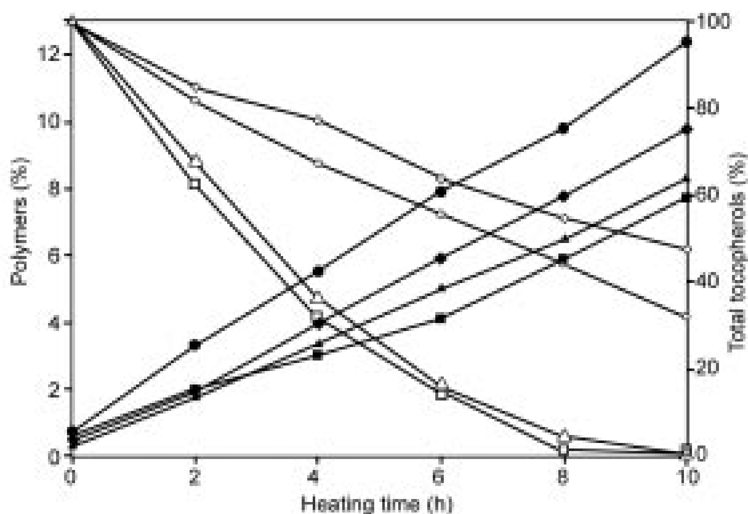

Fig. 7.3 Formation of polymers and loss of natural tocopherols in oils differing in the degree of unsaturation (● sunflower oil; ◆, soybean oil; ▲, palm olein; ■, virgin olive oil; solid symbols, polymers; hollow symbols, tocopherols).

Of special interest are new sunflower lines that contain γ-tocopherol as the major natural antioxidant instead of the α-tocopherol characteristic of conventional sunflower oil (Demurin *et al.*, 1996; Velasco *et al.*, 2004c). This has opened up the possibility of developing modified sunflower oils with novel combinations of fatty acid and tocopherol profiles in search of further improvement of sunflower oil thermal thermostability. In particular, a sunflower line with seed oil characterised by increased levels of both palmitic acid and oleic acid as well as a modified tocopherol profile made up of γ-tocopherol (>95% of the total tocopherols) was found highly stable (Marmesat *et al.*, 2008b). The higher protection given by γ-tocopherol as compared to α-tocopherol independently of the oil used (Lampi and Kamal-Eldin, 1998; Barrera-Arellano *et al.*, 2002) and the present possibilities of new oils of low degree of unsaturation allows foreseeing a future when processor needs and consumer demands will meet.

The effect of tocotrienols has been less studied. In general, the stability of α-tocotrienol was found to be similar to that of α-tocopherol both in palm olein and in purified oils containing both antioxidants added (Simonne and Eitenmiller, 1998; Wagner *et al.*, 2001; Romero *et al.*, 2004; 2007b; Schroeder *et al.*, 2006; Rossi *et al.*, 2007). Paradoxically, the order of stability of the different tocotrienol homologues was different to that found for their tocopherol counterparts, γ-tocotrienol being the least stable (Simonne and Eitenmiller, 1998; Rossi *et al.*, 2007).

Other minor natural compounds

The pool of polyphenols in virgin olive oils, mainly composed by hydroxytyrosol, tyrosol and their derivatives, and lignans (Bendini *et al.*, 2007); of γ-

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oryzanol, i.e. sterol esters of ferulic acid, in rice bran oil (Lerma-Garcia *et al.*, 2009); and of lignans, namely sesamol, sesaminol, sesamolin, etc., in sesame oil (Moazzami *et al.*, 2007; Namiki, 2007) are the three groups of natural antioxidants whose beneficial action in frying has been consistently reported.

The good performance of virgin olive oil in frying as compared to the refined oil is mainly attributed to the presence of polyphenols which are eliminated during the frying process. Interestingly, studies analyzing the evolution of tocopherols and polyphenols during frying report a high rate of degradation for hydroxytyrosol and its derivatives, a similar or lower rate for α-tocopherol and a low degradation rate for tyrosol, its derivatives and lignans (Andrikopoulos *et al.*, 2002; Brenes *et al.*, 2002; Gómez-Alonso *et al.*, 2003; Nissiotis and Tasioula-Margari, 2003; Daskalaki *et al.*, 2009). It is of interest to comment that α-tocopherol is considered the less active antioxidant in frying among its homologues due to its more rapid degradation. However, hydroxytyrosol and its derivatives, considered the most potent group of antioxidants in olive oil contributing to increase enormously the oil oxidative stability, are lost even more rapidly than α-tocopherol. Hydroxytyrosol rapid loss suggests its substrate-independent degradation as a more important reaction than its antioxidant effect. Hence, the effective protection found is probably more related to the group of less active antioxidants present, i.e. lignans and tyrosol and its derivatives, which remain longer at frying temperatures.

There has been a limited number of reports on the good frying performance of sesame and rice bran oil in spite of their wide consumption in populous Asian countries (Valsalan *et al.*, 2004; Krishna *et al.*, 2005). Most of the studies, however, report a significant increase in the oil thermostability, as well as a higher retention of natural tocopherols, in blends of different oils with either sesame or rice bran oil (Chung *et al.*, 2004; 2006; Leonardi, 2005; Sharma *et al.*, 2006; Shing *et al.*, 2007; Khan *et al.*, 2008; Farhoosh and Kenary, 2009). Lignans compounds and γ-oryzanol were highly thermostable. Lignans were more stable than tocopherols although they protected them from degradation (Chung *et al.*, 2006), while the content of oryzanol did not change in practice under usual home frying conditions, irrespective of the initial content in the oil (Krishna *et al.*, 2005).

Because of the positive effect of these groups of phenolic compounds on oil performance, addition of extracts obtained from oils or their by-products to oils of different degree of unsaturation has been proposed and some ingredients and special oils have been commercialized (Kochhar, 2000; 2001; Gertz, 2004). The addition of these natural products to oils continues to be the subject of many studies not only focused on the increase of oil thermostability but also on the retention of compounds of proven health benefits whose intake would increase by means of fried food consumption (Nasirullah and Rangaswamy, 2005; Farag *et al.*, 2007; Hemalatha and Ghafoorunissa, 2007; Lee *et al.*, 2008; Chiou *et al.*, 2009).

Specific phytosterols containing an ethylidene side chain like $\Delta5$-avenasterol, $\Delta7$-avenasterol, fucosterol, vernosterol and citrostadienol constitute

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another group of minor natural compounds whose contribution to delay oil degradation at frying temperature has been reported (Sims *et al.*, 1972; Boskou and Morton, 1976; Gordon and Magos, 1983; Warner *et al.*, 2004). They are normally referred to as antipolymerization agents or polymerization inhibitors although considering the hypothesis for the mechanism of their action they would act as antioxidants, i.e. formation of allyl radicals in a primary carbon atom followed by isomerization to a relatively stable tertiary radical (Gordon and Magos, 1983). Results from more recent studies using purified triacylglycerols and evaluating the formation of polymerization compounds were not so clear. Thus, addition of 0.1% of fucosterol did not affect the rate of polymerization of purified high-oleic sunflower oil triacylglycerols (Lampi *et al.*, 1999). Similar results were obtained when the relationship between the number of double bonds in the sterol molecule (from 0 to 3) and the inhibition of polymerization was studied. Fucosterol, one of the compounds selected, did not protect the oil at a concentration of 0.05% although a slight protective effect was obtained for all the sterols with 2 and 3 double bonds regardless of the presence of the ethylidene group (Winkler and Warner, 2008a). It is also interesting to comment the results concerning sterol retention. Sterols seem to be stable molecules as they are retained in relatively high percentages. However, when the same sterol pool was added to oils of different degree of unsaturation, i.e. purified soybean and high-oleic sunflower oils, the loss of sterols was higher for the less unsaturated oil even if its degradation was significantly lower (Winkler and Warner, 2008b). Thus, the loss of phytosterols seems to follow the same pattern as that commented for tocopherol loss. It would be of interest to go deeper into the minor compounds showing the same pattern, as it could be related to a common mechanism of degradation.

Additives

The addition of minor compounds to increase stability of fats and oils is a common practice in food industry. Among them, citric acid and synthetic antioxidants are used to complex metals and to increase oil oxidative stability, respectively, irrespective of the oil application, while dimethylpolysiloxane and ascorbyl palmitate are added to increase oil thermostability. In this section, the effects of additives other than those present naturally in oils and specifically applied to frying oils are considered.

Dimethylpolysiloxane

Dimethylpolysiloxane (DMPS) is the most important additive in frying, normally added at very low concentrations (1–2 mg/kg). The most interesting information on the action of DMPS is found in the summary of a congress presentation (Rock *et al.*, 1967) and in a brief article (Freeman *et al.*, 1973), whose reading is strongly recommended. The former reported that, at a level of 2 mg/kg, DMPS acted as an antioxidant when samples were thermostatically heated in a fryer, as they deteriorated slower than the controls without the additive. The reverse was found when the oils were heated at the same

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temperature in an oven. The only difference is that in the oven, the temperature is the same in the bulk oil while in the fryer the temperature at the oil-air interface is much lower (Rock and Roth, 1964).

In the study of Freeman *et al.*, all the experiments were carried out by heating the samples on a hot plate or in a fryer, and a tremendous enhancement of oil thermostability was obtained after addition of DMPS. The minimum amount which exerted a protective action corresponded to that forming a monolayer on the oil surface, as stated by decreasing the amount of DMPS until its action disappeared. This concentration obviously depends on the surface-to-oil volume ratio and ranges between 0.05 and 0.06 μg/cm^2. Below this amount, the effect of DMPS dropped off drastically. From these results the authors concluded that the protection is due to the formation of a monolayer of DMPS at the oil surface and hypothesized different possibilities for the precise mechanisms of action of this monolayer. The monolayer may form a barrier preventing the entrance of air or, more probably, the oxidation takes place at the oil–air interface and DMPS presents a relatively inert surface to the atmospheric air. Alternatively, it is proposed that DMPS may inhibit the convention currents in the surface thus impeding the entrance of oxygen (Freeman *et al.*, 1973).

Whatever the exact mechanism of action of DMPS may be, the effect is real and it is important to define the most appropriate frying conditions to obtain the maximum protection when adding DMPS. With this goal, a study was focused on the influence of the main variables in frying, i.e. temperature, length of time, surface-to-oil volume ratio, addition of DMPS and type of heating. The statistical analysis indicated that the main source of error, i.e. the highest percentage of the total variance, was due to the strong interaction between the presence of DMPS and the type of heating. Also, the influence of the surface-to-volume ratio stood out (Jorge *et al.*, 1996a). Later experiments were carried out on discontinuous and continuous laboratory frying as a complementary aid to confirm the results under practical conditions and to define more clearly the circumstances under which DMPS would have a positive action (Jorge *et al.*, 1996b).

Table 7.4 illustrates the great differences found in DMPS effectiveness between simulated continuous and discontinuous frying in two sunflower oils of different degree of unsaturation, i.e. high-oleic and high-linoleic sunflower oils. Results obtained for polar compounds in simulated continuous frying indicated that DMPS exerted no appreciable protective action. Quite in contrast, DMPS was highly effective in discontinuous frying, supporting that oil degradation occurred mainly when the food was not present and the surface was unprotected from the penetration of oxygen. The loss of tocopherols depended on the level of polar compounds although was more pronounced, as commented above, in the oil of lower degree of unsaturation. It can be also observed that the shift to a less unsaturated oil in discontinuous frying was less beneficial to decrease alteration than adding DMPS at low concentration, contrary to what happens in continuous frying.

From these results it was finally concluded that addition of DMPS would not be necessary in continuous (industrial) frying, since the oil surface is disturbed

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Table 7.4 Effect of dimethylpolysiloxane addition on polar compounds and tocopherols in discontinuous and continuous frying

Analysis	Frying system	Without DMPS		With DMPS (2 mg/kg)	
		HOSO	HLSO	HOSO	HLSO
Polar compounds (wt%)	Discontinuous	15.4	18.7	4.9	6.4
	Continuous	6.8	9.5	5.3	8.1
Tocopherols (mg/kg)	Discontinuous	tr.	155	569	584
	Continuous	185	345	417	414

Abbreviations: DMPS, dimethylpolysiloxane; HOSO, High oleic sunflower oil; HLSO, High linoleic sunflower oil. Initial content of tocopherols: HOSO, 650 mg/kg; HLSO, 603 mg/kg.

but well protected by steam water from the fried food. On the contrary, addition of DMPS at very low concentrations has a high positive effect in discontinuous frying and thus, is specifically active during the period in which the oil is not protected by steam water from the fried food. In conclusion, addition of DMPS would be of particular interest in oils used in fried food establishments where the fryers usually remain without food during significant periods of times, i.e. catering services, fast-food outlets, restaurants, etc. (Márquez-Ruiz *et al.*, 2004).

Plant extracts

In the last decade a significant number of studies focused on the positive effects of the addition of plant extracts, mainly herbs and spices, to frying oils have been published following the studies on their antioxidative properties at room temperature (Yanishlieva and Marinova, 2001; Yanishlieva *et al.*, 2006).

The addition of plant extracts seems to have a double objective. On the one hand, plant extracts are used to increase the consumption of natural, healthy compounds in response to consumer demand and, on the other, to delay the oil deterioration by protecting the natural antioxidants, i.e. tocopherols.

Extracts of rosemary (Gordon and Kourimska, 1995a; 1995b; Jaswir and Man, 1999; Man and Tan, 1999; Lalas and Dourtoglou, 2003; Kalantzakis and Blekas, 2006), tea (Naz *et al.*, 2004), sage (Jaswir and Man, 1999; Man and Tan, 1999), oregano (Houhoula *et al.*, 2003; 2004), lavender and thyme (Bensmira *et al.*, 2007), summer savory (Kalantzakis and Blekas, 2006), carob fruit (Bodega *et al.*, 2009), *Curcuma* (Nor *et al.*, 2008a) and others (Shyamala *et al.*, 2005; Nor *et al.*, 2008b) are some examples of the high number of reports on the subject.

In general, the action found is positive because the extracts contain potent antioxidants, which are detailed in an excellent review on the most important herbs and spices (Yanishlieva *et al.*, 2006). However, the protective effect during frying is highly variable even for the same extract because of the inherent characteristics of natural products, joined to the complexity of the frying process. On the one hand, the composition of an extract, normally poorly

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defined, may be quite different depending on the plant variety, extraction method, solvent used for extraction, concentration added, etc. On the other, the antioxidative effect depends on the frying conditions, on the degree of unsaturation of the oil and on the content and chemical structure of other oil minor compounds present exerting a prooxidant or antioxidative action.

Other minor compounds

The effect of a wide variety of specific compounds, either synthetic or purified from natural sources, has been studied. Among them ascorbyl palmitate and synthetic antioxidants, particularly butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA) and tertiary butyl hydroquinone (TBHQ), stand out. The advantage of these studies as compared to those undergone on plant extracts is that the experiments are carried out with individual compounds or combination of them and the action obtained is directly related to the compound studied.

Ascorbyl palmitate (AP) has been the subject of detailed investigations. In some studies, no significant effect was found with respect to the oil without additive at frying temperature (Augustin *et al.*, 1987; Ibrahim *et al.*, 1991). In other reports, the addition of AP retarded the formation of degradation compounds (Andrés, 1984; Gwo *et al.*, 1985; Gordon and Kourimska, 1995b; Satyanarayana *et al.*, 2000; Onal and Ergin, 2002), and increased the stability of tocopherols (Gordon and Kourimska, 1995a), although it was ineffective to protect fried foods during storage (Masson *et al.*, 2002).

Synthetic antioxidants are added to fats and oils to extend effectively their shelf life. However, when their effectiveness was studied at frying temperature, BHA and BHT had a low effectiveness due to their rapid volatilization and decomposition. TBHQ has been reported to be more effective than BHA and BHT (Gordon and Kourimska, 1995b; Allam and Mohamed, 2002).

In the last years new compounds exerting positive action in frying have been the subject of investigations. The addition of individual flavonoids, i.e. pelargoniding, cyaniding, quercetin and myrecetin, was very effective in increasing oil thermostability (Naz *et al.*, 2008). Also, linalyl oleate, containing the ethylidene group of the active sterols, inhibited oil polymerization although similar results were obtained with other terpenyl oleates of different structures (Onal-Olusoy *et al.*, 2005; 2006; 2007). Finally, novel antioxidants derived from TBHQ but with higher solubility, i.e. lauryl TBHQ and lauryl TBQ, have been found to exert stronger antioxidant activity than TBHQ at temperatures higher than 140 ºC (Zhang *et al.*, 2004).

7.5.2 Frying conditions

Apart from an adequate oil, establishing the best operating conditions is necessary to produce high quality foods and, consequently, to maintain a low degradation level in the frying oil. Nevertheless, due to the high number of variables and parameters involved in the process, it is difficult to foresee their effects, as they can be drastically modified by two-way interactions (Jorge *et al.*, 1996a).

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The action of the main variables – length of heating, temperature and surface-to-oil volume ratio – has been deduced from laboratory studies carried out under well-controlled conditions as well as from industrial frying experiments.

With respect to polymer and polar compound formation, the results indicate that the effect of the variables clearly depends on the type of frying, i.e. continuous or discontinuous, and demonstrate the primary importance of the presence of the food in the fryer and of the unused fat added to compensate for that removed as part of the fried food (Sebedio *et al.*, 1991; 1996; Cuesta *et al.*, 1993; Jorge *et al.*, 1996b; Romero *et al.*, 1999; 2007a; Banks, 2007). Such differences are important enough to make proper distinctions between both types of frying processes.

Discontinuous frying

Discontinuous frying is characterized by heating-cooling cycles, and variability of the time period that the oil remains at high temperature without being protected by the food. Under these conditions, oxidative alteration becomes priority and the effect of the variables can be summarized as follows:

Length of heating

The longer the period of heating, the higher the amount of polymers and polar compounds. Also, a higher polymers-to-polar compounds ratio has been reported as the total alteration increases (Dobarganes *et al.*, 1993; Márquez-Ruiz *et al.*, 1995; Marmesat *et al.*, 2007).

Temperature

The action of temperature is drastic beyond 200 ºC. An increase in temperature affects specifically polymer formation. However, the higher the temperature, the lower the solubility of oxygen and, consequently, thermal reactions giving rise to non-polar dimeric linkages are preferentially expected (Dobarganes and Márquez-Ruiz, 2007).

Surface-to-oil volume ratio (S/V)

An increase in S/V favours the entrance of oxygen and thus oxidative reactions and formation of oxidative dimeric linkages at frying temperatures (Jorge *et al.*, 1996a; Fujimoto, 2003).

Continuous frying

In continuous frying, the food is always present in the fryer and the rate of replenishment with unused fat, due to the absorption of the product being fried, becomes the most important variable in the control of the oil quality.

The turnover period defined as the time required for oil usage to equal the total oil volume in the fryer is calculated as follows:

$$\text{Turnover period} = \frac{\text{Oil in the fryer (L)}}{\text{Oil added per hour (L/h)}}$$

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it is normally found that low turnover periods (TP) (< 10–12 h) allow one to maintain a high quality oil in the long term.

In continuous frying, protection of the surface against development of oxidative reactions and the effect of dilution due to the addition of fresh fat modify the action of the variables according to the following pattern.

Length of heating

It has no influence in practice, as the levels of polar compounds and polymers reach a constant value for periods of heating higher than 4 TP (Pérez-Camino *et al.*, 1988; Sebedio *et al.*, 1996).

Temperature

A rise in temperature should increase the amount of polar compounds and polymers but, at the same time, higher temperatures would increase the rate of food production, thus decreasing the turnover period.

Surface-to-oil volume ratio

S/V has apparently a contrary effect to that found in discontinuous fryers, as the oil surface is protected from the entrance of oxygen by the food steam released. Thus, the higher the S/V, the lower the level of polar compounds and polymers formed due to the lower turnover period.

These general considerations give an idea on the complexity of the frying process and on the importance of a careful selection of the equipment and conditions depending on the type of frying and on the products to be fried.

To conclude, it is important to comment that from the high number of studies on the performance of oils in frying and on the action of minor compounds to decrease the oil degradation, more interesting and general information should have been obtained. The main problem is that, with the exception of the collaborative studies for the widely accepted determination of polar compounds and polymers, there is no general reference methodology allowing comparing the results from different research groups. The explanation of the contradictory and unexpected results, as well as the possibility of replicating different experiments, would be easier if researchers in the area agreed initially on the following needs:

1. Application of standard conditions for the experiments in the absence of foods to make comparable the results on oil performance, before carrying out frying experiments. This mainly involves definition of temperature, length of time and surface-to-volume ratio.
2. Selection of common analytical methods to evaluate the oil deterioration. In this respect, standard methods for polar compounds and polymer determination should be applied in all the studies.
3. Selection of conditions to evaluate the action of minor compounds. In this regard, it would be important to agree at least on the substrates used, probably two substrates of different degree of unsaturation would be a good option, and on the concentrations added.

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It has finally to be considered that the advantages of using common tests could be enormous, as real conditions would be applied, and so their results would be more directly related to those obtained in practical frying than when tests using accelerated conditions have to be applied as occurs in oil storage.

7.6 Sources of further information and advice

More information on the different aspects commented in this chapter may be found in specific chapters of books on frying published in the last decade. They are highly valuable for those directly related to the frying process, i.e. researchers, technologists, processors, etc. The following remarks refer to aspects directly related to this chapter or else to information of general interest:

- *Frying of Food*. Eds: D. Boskou and I. Elmadfa. Technomic Publishing Co., Lancaster, PA, 1999. A second edition is in preparation. Of particular interest is the chapter 'Changes of nutrients at frying temperatures' by J. Pokorny.
- *Frying. Improving Quality*. Ed: J.B. Rossell. Woodhead Publishing Ltd., Cambridge, 2001. The chapters 'The composition of frying oil' by S.P. Kochhar and 'Flavour and aroma development in frying and fried food' by P. Gillat are recommended. The former contains interesting information on detrimental compounds formed in frying, whose action has been omitted in this review.
- *Deep-Fat Frying. Fundamentals and Applications*. Eds: R.G. Moreiras, M.E. Castell-Pérez and M.A. Barrufet. Aspen Publishers, Gaithersburg, MD, 1999. It is recommended for those interested in understanding the theoretical principles of the frying process and physical aspects.
- *Deep Frying: Chemistry Nutrition and Practical Applications*, 2nd edn. Ed: M.D. Erickson. American Oil Chemists' Society, Champaign, IL, 2007. Although the book covers interesting chapters on applications, the chapters about the chemistry and nutritional aspects of the new compounds formed are recommended as the only ones dealing with these aspects in detail.
- *Frying Technologies and Practices*. Ed: M.K. Gupta, K. Warner and P.J. White. American Oil Chemists' Society, Champaign, IL, 2004. The book is recommended as it provides very useful information on solutions to the problems found in frying. The chapter 'Role of antioxidants and polymerization inhibitors in protecting frying oils' by K. Warner *et al.*, directly related to this review, is recommended.
- *Advances in Deep Fat Frying of Foods* (Contemporary Food Engineering Series). Ed: S. Sahin and G. Sumnu. Taylor and Francis, Boca Raton, FL, 2009. The chapter 'Flavor changes during frying' by K. Warner is recommended.

Also, the new web page http://www.lipidlibrary.aocs.org/frying/frying.html is recommended. Although it is a work in progress, it is the only scientific web page on frying covering the chemical, analytical and nutritional aspects in detail.

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Other web pages of interest to know the present systems to control the quality of used frying fats *in situ* are the following:

- **Testo 265** (http://www.testo.es/online/abaxx-?$part=PORTAL.ESP.SectorDesk&$event=show-from-menu&categoryid=5570594);
- **FOM 200** (http://www.ebro.de/deutsch/produkte/handmessgeraete/fom200.htm);
- **Fri-check** (http://www.fri-check.de);
- **Viscofrit** (http://www.viscofrit.com);
- **Fritest and oxyfritest** (http://photometry.merck.de/servlet/PB/menu/1168870/1168870.html)
- **Oleotest** (http://www.biomedal.com/bd/es/oleotest.html).

7.7 Future trends

The present objectives in frying are mainly directed to the preparation of healthier fried foods to satisfy consumer demands. In this respect, stable oils of good nutritional properties, reduction of food oil and minimal used frying oil degradation seem to be the most important consumer interests. Thus, in the near future the following targets are expected to be top priority in this field:

1. The development of high stability oils either by breeding selection or by genetic engineering in seeds is expected to continue. Sunflower, rapeseed and soybean oils with lower content of polyunsaturated fatty acids and richer in oleic acid are the main candidates for the substitution of partially hydrogenated oils in frying. Additionally, new seeds with modified tocopherol composition are expected to be developed as a complementary means of increasing oil oxidative stability based on changes in the pool of natural protective compounds.
2. Reduction of oil absorption in fried foods will continue to be a highly active field of research. Application of a special coating, either thin and invisible or thick like a batter, is the most used way for food surface modification. Research is mainly focused on hydrophilic biopolymers, generally polysaccharides, able to reduce the moisture evaporation and/or control the pore characteristics by cross-linking or thermogelling. Given that there is a wide range of possibilities to be applied for fat reduction, which is parallel to the developments of new ingredients to achieve crisper textures, the increase in the consumer acceptance of low-fat fried foods without the use fat replacers could be a reality in the coming years.
3. Development of novel technologies for a better quality of fried foods is also expected. At present, special attention is being paid to the design of food fryers to minimize the turnover period and of vacuum fryers to preserve the food nutrients and to decrease the oil degradation by reducing the oxygen concentration during frying.
4. Finally, the high number of studies and patents reported in recent years on

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new natural additives based on plant extracts with the aim of delaying oxidation in discontinuous frying operations will probably keep on growing. However, the future application of these concentrates to the frying process is not so clear.

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8

Oxidation and protection of nuts and nut oils

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Abstract: Nuts and nut oils are important sources of health promoting bioactive constituents. The quality of nuts and nut oils may, however, deteriorate upon oxidation and hence proper processing, storage and packaging of products is necessary for extending their shelf-life and to minimize loss of their endogenous antioxidants. This chapter provides an account of methods by which oxidative processes could be controlled in nuts and nut oils in order to extend their shelf-life and prime quality during storage.

Key words: nuts, nut oils, oxidation, shelf-life, antioxidants, oxidative stability, processing, packaging, storage.

8.1 Introduction

Nuts may be classified as tree nuts (almonds, walnuts, pecans, pistachios, cashews, hazelnuts, macadamia nuts and Brazil nuts) or ground nuts (peanuts); belonging to the legume family (Coates and Howe, 2007). Nearly 80% of the caloric value of nuts is derived from their lipids, ranging from approximately 568 to 674 kcal/100 g. They are low in saturated (SFA) and high in unsaturated fatty acids, especially monounsaturated fatty acids (MUFA) and to a lesser extent polyunsaturated fatty acids (PUFA) ranging from 31.6% in cashew nuts to 62.4% in pecan, as shown in Fig. 8.1 (USDA national nutrient database for standard reference, 2009; Miraliakbari and Shahidi, 2008b; Ryan *et al.*, 2006). MUFA contribute, on average, approximately 62% to the energy derived from lipids present (Kris-Etherton, 1999). Among nuts, walnuts are a particularly rich

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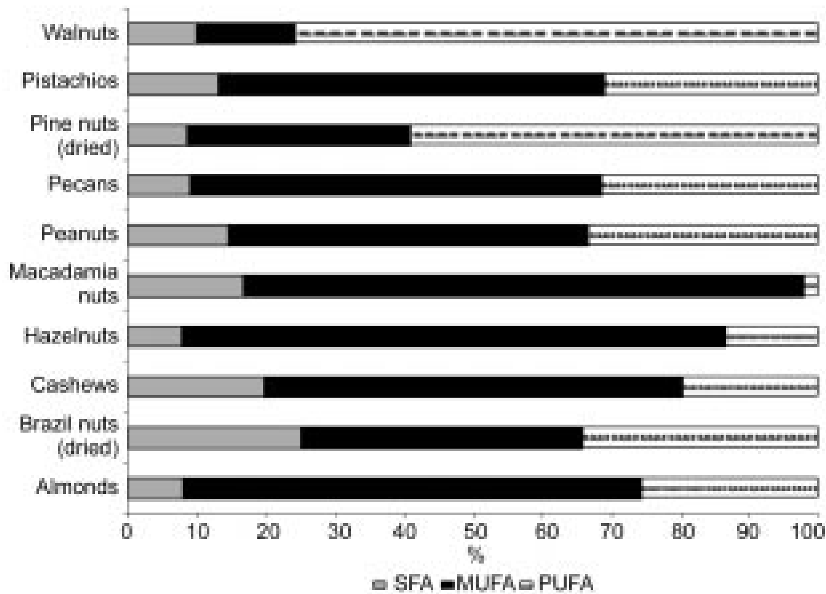

Fig. 8.1 Percentage saturated fatty acids (SFA), monosaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of selected edible nuts. Adapted from USDA national nutrient database for standard reference, http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl (accessed on 20 December 2009).

source of the omega-3 fatty acid (ω-3 FA), α-linolenic acid (ALA) (Nash and Nash, 2008). Numerous studies have documented the beneficial effects of various nuts on serum lipids and lipoproteins and there is increasing evidence that diets that include nuts may be beneficial in decreasing the risk of coronary heart disease (CHD) (Shahidi and Miraliakbari, 2004; Alberta *et al*., 2002; Ellsworth *et al*., 2001; Kris-Etherton *et al*., 1999; 2008; Hu *et al*., 1998). This is believed to result from the particularly high oleic acid content of nuts (Kaijser *et al*., 2000). However, there is emerging evidence that the decreased risk is not solely related to the fatty acid profile, but may also be due to the presence of other bioactive components present in nuts such as vitamin E, fibre, magnesium, potassium and antioxidant phenolic compounds (Acar *et al*., 2009; Maguire *et al*., 2004). The latest investigations on different aspects of tree nut compositions, phytochemicals and health effects have been reported by Alasalvar and Shahidi (2009). Several nut varieties serve as valuable oil crops due to their high oil yield (50–75% w/w), unique flavours and healthful fatty acid composition (Miraliakbari and Shahidi, 2008a). In many parts of the world, such as the Middle East and Asia, tree nuts are cultivated for their use as oil crops and are important sources of energy, essential dietary nutrients, phytochemicals (Bonvehi *et al*., 2000), as well as components of some skin moisturizers and cosmetic products (Madhaven, 2001). Recent investigations have shown that dietary consumption of nut oils may exert even more beneficial effects than consumption of whole nuts, possibly due to the replacement of dietary

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carbohydrates with unsaturated lipids and/or other components present in the extracted oil (Hu and Stampfer, 1999). Shahidi and Miraliakbari (2005) have summarized the characteristics and potential health effects of several tree nut oils and their byproducts, including oils of almond, hazelnut, pecan, walnut, pistachio, Brazil nut, pine nut and macadamia nut, among others.

The development of an unacceptable taste or rancidity is a feature of badly stored nuts or those stored for too long (Kaijser *et al*., 2000). Nuts and nut oils are susceptible to autoxidation due to their high content of unsaturated fatty acids. The Swiss chemist Nicholas-Théodore de Saussure (1746–1845) was the first to observe and report that a layer of walnut oil exposed to air was able to absorb 150 times its own volume of oxygen in one year. He also noted that the oil became more viscous and developed an off-odour (Braconnot, 1815). The lipids involved in the oxidation process contain unsaturated fatty acids such as oleic, linoleic, linolenic and long chain PUFAs; however, other unsaturated lipids present in fats and oils such as cholesterol and other sterols do become oxidized during this process (Uri, 1961). Free radicals are important chain-carrying intermediates in lipid autoxidation, which lead to the formation of hydroperoxides that further degrade to secondary oxidation products such as ketones and aldehydes, among others. The appearance of secondary oxidation products is associated with changes in the odour and flavour of the products, resulting in rancidity. Lipid oxidation negatively affects the flavour, odour, colour, and nutritional value of foods during storage, and this may also limit the utilization of nut oils in processed and fortified foods as well as nutritional supplements (Alasalvar *et al*., 2003a). The oxidative stability of various nuts and nut oils under different conditions has been studied by various researchers (Miraliakbari and Shahidi, 2008a; Alasalvar *et al*., 2003a; Cavaletto *et al*., 1966). Hexanal and other volatile compounds, using headspace gas chromatography (GC), have been used to monitor the oxidative deterioration of fats and oils as well as other lipid-containing foods (Miraliakbari and Shahidi, 2008a; Reed *et al*., 2002; Jensen *et al*., 2001; Lennersten and Lingnert, 1998; Molteberg *et al*., 1995; 1996; Erickson, 1993). In addition, the evaluation of oxidative status is occasionally combined with sensory evaluation, as for roasted peanuts, for which the formation of secondary oxidation products (including pentanal, hexanal and octanal) resulted in loss of peanutty flavour and development of a painty flavour instead (Reed *et al*., 2002). Fourie and Basson (1989) showed that changes in peroxide levels could be used to predict rancidity in nuts instead of using a trained sensory panel. The oxidation of edible fats and oils can be controlled by application of antioxidants, using processing techniques that minimize the loss of tocopherols and other natural antioxidants, inactivation of prooxidant metals and enzymes, minimizing exposure to oxygen, heat and light, hydrogenation of PUFA and the use of an inert gas or vacuum packaging to expel oxygen before long-term storage (Miraliakbari and Shahidi, 2008a).

Beneficial health effects of nuts and nut oils have been well reviewed in the existing literature (Nash and Nash, 2008; Blomhoff *et al*., 2006; Griel and Kris-

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Etherton, 2006; Kris-Etherton *et al*., 2001; Kris-Etherton, 1999); however, information regarding their oxidative stabilities and the various methods to protect them against oxidation during storage is lacking. This chapter provides detailed information on the oxidation of nuts and nut oils. The effects of oxidation on the sensory, nutritional quality and shelflife of nuts and nut oils and the different methods of protecting them against oxidation will also be discussed.

8.2 Oxidation of nuts and nut oils

Lipid oxidation can be defined as the oxygen-dependent deterioration of lipids containing any number of carbon-carbon double bonds. Systematic studies of lipid oxidation have been carried out since the 1940s when it was established that hydroperoxides were the primary products of oxidation (or peroxidation) of unsaturated lipids (Uri, 1961). Lipid hydroperoxides are themselves non-radical compounds, but readily decompose into unwanted secondary products which adversely affect flavour. Lipid oxidation is initiated by heat, light and metal ions, among others; the rate at which fatty acids are oxidized increases with the degree of unsaturation. Lipid oxidation can take place via autoxidation, photosensitized oxidation (photooxidation), thermal oxidation, hydrolytic oxidation and enzymatic oxidation.

8.2.1 Measurement of lipid oxidation in nuts and nut oils

Lipid oxidation can be measured by objective or sensory methods. Sensory methods of assessing lipid oxidation in nuts are widely used, but this is time consuming and taste panels are difficult to maintain. Several objective methods have successfully been employed for evaluating lipid oxidation in nuts and nut oils (Miraliakbari and Shahidi, 2008a) and these include measurement of conjugated dienes, peroxide value, thiobarbituric acid reactive substances, *para*-anisidine value, volatile carbonyl compounds and several spectroscopic methods, including nuclear magnetic resonance (NMR) (Shahidi and Wanasundara, 1998) and Fourier transform infrared (FT-IR) (Shahidi and Wanasundara, 1996) spectroscopies. The conjugated dienes of a sample are quantified by diluting a weighed amount of oil in isooctane and the absorbance is read spectrophotometrically at 234 nm (IUPAC, 1987). The peroxide value (PV) is a measure of the hydroperoxide content of a fat or oil, and is thus a test for primary lipid oxidation products. PV is most commonly quantitated using iodometric titration and is expressed as milliequivalents of active oxygen (peroxide) per kg of lipid (meq/kg) (Shahidi and Wanasundara, 1998). The Australian Macadamia Society indicates a maximum PV of 5.0 meq/kg for oil from raw and roasted kernels while The Southern African Macadamia Grower's Association recommends a maximum PV value of 3.0 meq/kg (Mason *et al*., 2004). The 2-thiobarbituric acid (TBA) test is another convenient method for the measurement of secondary oxidation products (Del Rio *et al*., 2005). Meanwhile, the *p*-anisidine value is

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empirically defined as 100 times the absorbance of a solution resulting from the reaction of 1 g of oil or fat and *p*-anisidine in 100 mL of isooctane, measured at 350 nm in a 1 cm cell (Shahidi and Wanasundara, 1998; AOCS, 1990). Various types of headspace gas chromatographic techniques have been developed to assess the composition of volatiles in oxidized fats and oils (Miraliakbari and Shahidi, 2008a; Guillen *et al.*, 2005; Sanches-Silva *et al.*, 2004). The active oxygen method (AOM), or Swift test, is a common accelerated method for assessing oxidative stability of fats and oils. The method is based on the principle that formation of lipid hydroperoxides is accelerated when lipids are subjected to high temperatures while aerated (Matthaus and Bruhl, 2003). Automated versions of the active oxygen method have been developed, such as the Oxidative Stability Instrument (OSI), Rancimat and Oxidograph. The OSI and Rancimat tests measure changes in conductivity caused by volatile ionic organic acids, automatically and continuously (Liu *et al.*, 2005). The oxidograph test involves heating of an oil sample exposed to air or oxygen, which results in a pressure decrease inside the reaction vessel. The change in pressure is measured electronically by means of pressure transducers and the endpoint of the test is reached when pressure inside the vessel exhibits a marked decrease. Due to the complexity of the chemical processes involved, it is recommended that the progress of oxidation of fats and oils be monitored by more than one method, including at least one test for each of the primary and secondary lipid oxidation products.

8.2.2 Factors affecting oxidation of nuts and nut oils

Nuts are marketed in different commercial forms and as unshelled nuts, shelled kernels and peeled seeds, both raw and roasted. Antioxidants present in the intact pellicle protect the nut kernels against oxidation (Jurd, 1956); however, removal of the shell may damage the kernels and thereby expose the kernels to light and oxygen (Tappel *et al.*, 1957). Most of the studies found in the literature investigate the influence of different factors such as packaging material, temperature, time, roasting, light or irradiation, trace metals and antioxidants on several physical, sensory and chemical parameters of the seeds (Gou *et al.*, 2000; Zacheo *et al.*, 2000; Sattar *et al.*, 1990a; 1990b).

Relative humidity, temperature, light and packaging

The effect of relative humidity (RH) on the quality changes in nuts has been well documented (Irtwange and Oshodi, 2009; Ruiz-Bevila *et al.*, 1999; Maté *et al.*, 1996). Erickson *et al.* (1994) found no consistent difference in oxidative changes for pecans stored at 55 and 65% RH, but Maté *et al.* (1996) found a significant increase in lipid oxidation for walnut kernels stored at 53% RH when compared to those stored at 21% RH. Relative humidity is a more important factor than temperature for storage of hazelnuts (Ayfer, 1973). Quality of raw shelled peanuts can be maintained for at least 1 year at 1 to 5 ºC with moisture contents < 7%, or for 2 to 10 years at −18 ºC and < 6% moisture. Maintaining RH

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between 55 and 70% at 1 to 5 ºC will maintain peanut moisture content at 7 to 7.5% (Maness, 2004).

López *et al.* (1995) did not observe any effect of storage temperature on walnut quality when comparing unshelled walnuts stored in the range of 3–10 ºC. The classical way of storing almonds is to keep them in the shell after natural drying until their consumption or use by the industry (Ruiz-Bevila *et al.*, 1999). In-shell almonds can be stored for up to 20 months at 0 ºC, 16 months at 10 ºC and 8 months at 20 ºC. Shelled nuts can be stored for about half as long as nuts in the shell (about 6 months), and pieces for even less (Labavitch, 2004). In-shell pecans can be stored for 6 months at 22 ºC, 12 months at 0 ºC or 24 months at −18 ºC; whereas shelled pecans can only be stored for 3 months at 22 ºC, 9 months at 0 ºC or 18 months at −18 ºC (Maness, 2004). Mehran and Filsoof (1974) did not detect any flavour difference in almonds after 12 months of storage at room temperature, while macadamia nuts tended to develop off-flavour more rapidly during storage at room temperature. Young and Cunningham (1991) demonstrated that almonds, macadamia nuts and pistachios were acceptable after 6 to 9 months storage at 38 ºC, while cashews, hazelnuts, peanuts, pecans and walnuts were acceptable for only 2 to 5 months when stored at 38 ºC as judged by sensory panels. Fourie and Basson (1989) reported few flavour differences in almonds after 12 months of storage at room temperature. Macadamia nuts tend to develop oxidative rancidity rapidly during storage at room temperature, but can be stored for up to a year at 2 ºC and for 18 months at −18 ºC without becoming rancid. The oxidative stability of nut kernels decreases with an increase in storage temperature.

Senesi *et al.* (1991) found that almonds could be stored for up to 9 months at 4 or 20 ºC without any significant quality loss; the packaging material (low or high oxygen permeability) did not cause any quality changes. Beyond this storage period, however, quality was maintained only by using a low oxygen permeability packaging material and storage at 4 ºC. The oxidation process is likely to be accelerated during retail storage as the kernels are often held at ambient temperature and relative humidity and are also exposed to light that catalyses oxidation (Sattar *et al.*, 1989). Light, depending on its intensity, wavelength, duration of exposure, absorption by the product, presence of sensitizers, temperature and amount of available oxygen may induce oxidation in nuts and nut oils (Özdemir and Devres, 1999b). Jensen *et al.* (2001) demonstrated that storage under light causes pronounced oxidative changes, especially in walnuts stored at 21 ºC, whereas in the dark and storage at 5 ºC no rancid taste was noted during 25 weeks of storage under accelerated storage conditions (50% oxygen). Moisture content affects the stability of both nuts-in-shells (NIS) and kernels. Mason *et al.* (1998) reported that NIS should be dried to 10% moisture content on-farm and then transported to the processor where they should be further dried to a moisture content of 3.5%. The final moisture content of the kernel should be 1.5% or lower (Cavaletto, 1981). Dela Cruz *et al.* (1966) reported good stability of roasted kernels at 1.1% moisture content compared to those at higher moisture levels (1.7 and 1.5%). Mason *et al.* (2004) assessed the

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quality of macadamia kernels (raw and roasted, salted) that were stored for 36 days under conditions similar to those found in the supermarket in bulk retail dispensers (21.7 ºC and 55.5% relative humidity). They recommended that both raw and roasted, salted macadamia kernels should not be stored for more than eight days in supermarket bulk retail dispensers.

Processing, storage and roasting of nuts and nut oils
Several studies have unambiguously demonstrated that storage at high oxygen concentrations results in more pronounced lipid oxidation than storage under low oxygen concentrations (Maté *et al.*, 1996; Ribeiro *et al.*, 1993; Senesi *et al.*, 1991). Postharvest damages to kernels are also a major defect of nuts. The shelling operation results in hidden damage (from the explosion of the oil inside the cotyledons of the cells), broken and damaged nuts. After roasting, damaged and broken nuts may increase to about 10 and 15%, respectively, during transportation of nuts to the factory unless preventive measures are taken (Özdemir and Devres, 1999b). Peroxidase activity in the damaged surfaces brings about off flavours. Pressure in the course of storage and/or transportation should be below 0.9 bars. Excessive pressure damages the cell structure and the enzymes, which increases substrate interaction (Keme *et al.*, 1983). For proper storage of hazelnuts, they should contain 5% moisture after drying and the storage medium should be cool and dry (5–10 ºC and 50–60% relative humidity) (Hadorn *et al.*, 1977). Economical storage is a function of the relative cost of dehumidification, refrigeration, and insulation taken together with the risk of spoilage at high relative humidities and temperatures (Ayfer, 1973).

Nuts are frequently submitted to thermal processing, mainly roasting, to obtain characteristic sensory or texture features (Amaral *et al.*, 2006). Besides extending the range of aromas, textures, and taste of hazelnuts, roasting is also performed to remove the pellicles (seed coats) of the kernels, inactivate enzymes, destroy microorganisms, and reduce water activity (Özdemir *et al.*, 2001; Fellows, 2000). Roasting changes physicochemical properties that might in turn alter the rate of oil oxidation. Without the shell, the kernel experiences faster deteriorative changes that shorten its shelflife. The oxidation of lipids and the rate of rancidity development are highly dependent on the temperature and increases rapidly at higher temperatures (Nogala-Kalucka and Gogolewski, 2000; Senesi *et al.*, 1996; 1991; Sattar *et al.*, 1990b; Harris *et al.*, 1972). The thermal treatment of nuts during roasting inevitably leads to chemical changes mainly determined by the nut composition (moisture, fat, proteins, and carbohydrates) and by the temperature and extent of heating (Fellows, 2000). Optimization of roasting conditions by evaluating the kinetics of the process, moisture and drying characteristics, colour, oxidative stability, sensory characteristics, and microstructural changes, has been extensively described in the literature (Demir *et al.*, 2003; Fallico *et al.*, 2003; Saklar *et al.*, 2003; Demir *et al.*, 2002; Özdemir and Devres, 1999a; 2000a; 2000b). Langourieux *et al.* (2000) reported the influence of roasting parameters on hazelnut aroma compounds, and the volatile compositions of raw and roasted hazelnuts were compared by Alasalvar *et al.* (2003b). Elevated

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drying temperature leads to the formation of carbonyl compounds derived from autoxidation of unsaturated fatty acids (Chiou *et al.*, 1991). Roasting time-temperature combination, temperature of nuts at the exit of the roaster, and exposure time to air prior to packaging and storage significantly influence the shelflife of nuts. Roasting temperature should not exceed 150 ºC. Temperature of hazelnut at the exit of the roaster should be around 20 ºC (Koksal and Okay, 1996; Özdemir and Devres, 1999a). In-shell and unroasted hazelnut kernels can be stored for 24 months with minimal loss of quality at temperatures up to 10 ºC; whereas roasted kernels may only be held for 6 months prior to the development of detectable rancidity when stored at 0, 5 or 10 ºC (Maness, 2004).

Kaijser *et al.* (2000) attempted to identify the parameters that might influence the stability and storage characteristics of selected cultivars of macadamia nuts grown in New Zealand. The stability of the oil measured by the Rancimat test at 110 ºC ranged from 3.6 to 19.8 h. The cultivar that was found to be most unstable also had the highest level of peroxides and linoleic acid. In contrast to macadamia nut oils, other common nut oils heated to 110 ºC had Rancimat induction periods ranging from 3.9 to 7.8 h for walnut oils (Savage *et al.*, 1999) and 15.6 to 25.3 h for hazelnut oils (Savage *et al.*, 1997). Overall, peroxide formation is generally low in whole macadamia nuts as the kernel is protected by a thick pericarp. α-Tocopherol (0.8 ± 1.1 mg/g lipids) and δ-tocopherol (3.5 ± 4.8 mg/g lipids) were the only two tocopherols identified in the extracted macadamia oil. In other nut species, e.g. hazelnuts (Savage *et al.*, 1997) and walnuts (Savage *et al.*, 1999), tocopherols contributed to kernel stability. The major sterols identified in macadamia nuts were sitosterol, Δ5-avenasterol, campesterol, stigmasterol and desmethylsterols (Kaijser *et al.*, 2000). Δ5-Avenasterol has an additional double bond in its side-chain, which gives it an antioxidative property in frying oil (Gordon and Magos, 1983). The study undertaken by Kaijser *et al.* (2000) showed no clear relationship between the stability of the oil and the content of polyunsaturated fatty acids. The authors concluded that it was more likely that the stability of the oil is influenced by factors such as the positions of the individual fatty acids within the triacylglycerol molecules and the presence of tocopherols, caroteneoids, free fatty acids and sterols as suggested by Neff *et al.* (1994).

Oil extraction by supercritical carbon dioxide (SC-CO_2) has been associated with greater thermal oxidative instability of the oils than by solvent or extruder extraction methods (Calvo *et al.*, 1994; List and Friedrich, 1985; 1989). Crowe and White (2003) also observed that SC-CO_2 extracted walnut oils were less stable than pressed walnut oils during accelerated storage in the dark, as determined by PV, headspace analysis by solid-phase microextraction, and sensory methods. However, the SC-CO_2-extracted oils exhibited greater photo-oxidative stability than their pressed counterparts, possibly due to the presence of chlorophylls in the pressed oil. Speciality oils, such as walnut oil, are generally stored in clear glass bottles on store shelves, making them susceptible to photooxidation. The increased stability of SC-CO_2-extracted oils to light-induced oxidation is therefore important. Oxidative stability indices and

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tocopherol contents were, however, significantly ($p < 0.05$) lower in the SC-CO_2-extracted oils than in pressed walnut oils (Crowe and White, 2003).

Nejad *et al*. (2002) studied the effect of various drying methods (sun drying, bin dryer, vertical continuous dryer, vertical cylindrical dryer and funnel vertical dryer) on the quality of pistachio nuts. Different drying methods used did not have any significant ($p > 0.05$) influence on the free fatty acid (FFA), PV and TBA values of lipids in pistachio nuts. Sun drying of hazelnuts is considered best as artificial heat can cause rancidity (Rosa, 1979). Drying temperature affects the sensory attributes of pistachio nuts and its roasted flavour note increased during high-temperature drying (116–138 ºC) (Kader *et al*., 1979). Drying to an appropriate moisture content (4–6% wet basis (wb)) is an important factor ensuring good quality products. Nuts dried to 4% (wb) moisture were rated higher in crispness and sweetness, and lower in bitterness and rancidity than those dried to 6 or 11% (wb) moisture. Nuts at 6% (wb) moisture content also scored higher in sweetness and lower in bitterness and rancidity than those at 11% (wb) (Kader *et al*., 1979). Drying affects the constituents of pistachio but its influence is less than blanching and roasting. It is not advisable to dry hazelnuts at higher than 50 ºC because the rate of rancidity development increases with temperature, resulting in degradation of hazelnut quality. The optimal drying air temperature for hazelnuts is in the range of 40–50 ºC as lower temperatures require a longer processing time and temperatures higher than 50 ºC favour lipid oxidation, especially in shelled hazelnuts (López *et al*., 1997a). The drying of hazelnut at temperatures between 30 and 70 ºC reduces the initial activity of the endogenous lipase, peroxidase and polyphenol oxidase. Higher enzymatic activity is observed in shelled hazelnuts than in their unshelled counterparts, possibly due to a higher water activity of the former than the latter at the same moisture content (López *et al*., 1997b). Bostan (2000) reported that the best method is drying in concrete containers, as nuts in this treatment dry in a short period and hence exhibit high pellicle removal and have good shell and kernel colour.

Short time heat treatments (STHT) based on radiofrequency can be used to control insect pests of concern to international trade of tree nuts (Tang *et al*., 2000). Buransompob *et al*. (2003) investigated the lipid stability of shelled walnuts and almonds as affected by STHT and accelerated storage temperatures; the treatment did not promote rancidity in these nuts. Untreated control and heat-treated shelled walnuts and almond kernels were acceptable after 60 days of storage at 25 ºC or after 20 days of storage at 35 ºC, which is equivalent to storing walnuts and almond kernels at 4 ºC for 2 years. STHT at 55 ºC for 2 minutes or greater retarded the development of oxidative rancidity in shelled walnuts and almonds during distribution and storage.

8.2.3 Oxidative stability of tree nut oils

Miraliakbari and Shahidi (2008a,c) analyzed the lipid composition and minor components of tree nut oils and their antioxidant activity, along with examining the oxidative stability of a number of tree nut oils (oils of almond, Brazil nut,

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hazelnut, pecan, pine nut, pistachio and walnut). Two solvent extraction systems, namely, hexane and chloroform/methanol were compared for yield and other chemical characteristics. Minor components in the nut oils can act as antioxidants and thus in this study the minor components were extracted (stripped nut oil) as described by Abuzaytoun and Shahidi (2006) and Khan and Shahidi (2001; 2002). The oxidative stability of stripped and nonstripped nut oils was studied using accelerated oxidation conditions, under Schaal oven conditions for autoxidation (heated to 60 ºC in a forced air oven for different time periods) and under fluorescent light (photooxidation in a photooxidation chamber with white fluorescent light for different periods).

The formation of conjugated dienes in tree nut oils during a 12-day autoxidation test conducted by Miraliakbari and Shahidi (2008a) is summarized in Table 8.1. In all oils examined, chloroform/methanol-extracted oils and their stripped counterparts were more resistant to the formation of conjugated dienes than their corresponding nonstripped hexane-extracted oils. Chloroform/methanol-extracted pecan oil possessed the lowest level of conjugated dienes among samples examined after 12 days of oxidation (1.2). The stripped oils of walnuts possessed the highest conjugated diene levels among all samples examined; this observation can easily be attributed to the high degree of unsaturation of these oils, in combination with their lack of antioxidative minor components. The peroxide values of tree nut oils subjected to autoxidation are also shown in Table 8.2. The order of oxidative stability, derived using maximum peroxide value levels, was identical to the order obtained using maximum conjugated diene values (pecan oil > pistachio oil > hazelnut oil > almond oil > Brazil nut oil > pine nut oil > walnut oil; chloroform/methanol-extracted oils > hexane-extracted oils; nonstripped oils > stripped oils). Chloroform/methanol-extracted oils containing higher amounts of minor components were more resistant to the formation of secondary oxidation products as reflected in their *p*-anisidine values, when compared to their hexane-extracted counterparts (Table 8.1). Among the samples studied, chloroform/methanol-extracted pistachio oil exhibited the lowest *p*-anisidine value after 12 days of storage under accelerated Schaal oven conditions. Figure 8.2 shows the head-space aldehyde (hexanal) compositions of nonstripped hexane or chloform/methanol extracted tree nut oils during autoxidation. Hexanal and nonanal were the most widely detected headspace volatiles observed in tree nut oils subjected to accelerated oxidation; propanal was present only in walnut oil, which is the only nut oil containing α-linolenic acid, which belongs to the omega-3 family. Hexanal is an oxidation product of linoleic acid, an omega-6 fatty acid (Shahidi and Pegg, 1994). In addition, nonanal is an oxidation product of oleic acid, an omega-9 fatty acid (Ramirez *et al*., 2004). No headspace aldehydes were detected in oil samples prior to the commencement of the stability studies. Chloroform/methanol extracted oils contained lower amounts of headspace aldehydes compared to their hexane-extracted counterparts at each sampling point of the accelerated autoxidation studies. Stripped oils contained 2–4 times the amount of headspace aldehydes compared to their nonstripped counterparts.

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Table 8.1 Conjugated dienes, peroxide value (meq oxygen/kg oil) and *p*-anisidine value in tree nut oils during autoxidation under Schaal oven conditions at 60ºC

Oil	Conjugated dienes		Peroxide value		*p*-anisidine value	
	Storage period (days)					
	0	12	0	12	0	12
Almond oil						
Hexane extracted	0.956	6.451	0.040	0.335	0.120	3.923
Chloroform/methanol extracted	1.744	6.757	0.030	0.164	0.561	3.061
Stripped hexane extracted	2.636	15.914	0.023	0.531	0.267	29.058
Stripped chloroform/methanol extracted	2.270	15.331	0.015	0.458	0.465	24.389
Brazil nut oil						
Hexane extracted	1.768	15.767	0.047	0.661	0.189	8.576
Chloroform/methanol extracted	0.692	3.686	0.030	0.197	0.821	3.327
Stripped hexane extracted	1.301	15.341	0.015	1.992	0.264	19.746
Stripped chloroform/methanol extracted	1.423	14.430	0.023	1.415	0.371	16.048
Hazelnut oil						
Hexane extracted	1.096	7.199	0.031	0.307	0.592	8.262
Chloroform/methanol extracted	1.492	6.131	0.059	0.200	0.288	6.555
Stripped hexane extracted	1.704	12.033	0.015	0.519	0.255	16.428
Stripped chloroform/methanol extracted	1.467	11.092	0.015	0.467	0.343	15.386
Pecan oil						
Hexane extracted	0.502	2.821	0.030	0.158	0.433	7.172
Chloroform/methanol extracted	0.267	1.245	0.023	0.045	0.294	3.243
Stripped hexane extracted	0.945	6.719	0.023	0.346	0.344	11.876
Stripped chloroform/methanol extracted	0.897	6.036	0.015	0.309	0.257	12.686
Pine nut oil						
Hexane extracted	1.216	24.745	0.030	1.317	0.267	17.567
Chloroform/methanol extracted	1.067	7.920	0.016	0.291	0.493	14.029
Stripped hexane extracted	1.509	5.493	0.023	2.305	0.281	29.711
Stripped chloroform/methanol extracted	1.524	5.825	0.015	2.259	0.552	28.549
Pistachio oil						
Hexane extracted	0.481	3.898	0.023	0.116	0.545	1.910
Chloroform/methanol extracted	0.992	1.689	0.015	0.037	0.635	1.731
Stripped hexane extracted	1.145	6.945	0.015	0.438	0.518	10.473
Stripped chloroform/methanol extracted	0.936	6.673	0.015	0.428	0.493	9.361
Walnut oil						
Hexane extracted	0.554	29.998	0.030	2.142	0.230	29.591
Chloroform/methanol extracted	0.535	17.507	0.015	0.334	0.462	24.880
Stripped hexane extracted	0.684	5.965	0.015	4.736	0.193	52.562
Stripped chloroform/methanol extracted	0.693	7.452	0.015	4.629	0.237	48.836

Adapted from Miraliakbari and Shahidi (2008a)

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Table 8.2 Formation of conjugated dienes in tree nut oils during photooxidation. Abbreviations used are the same as those in Fig. 8.2

Oil	Storage period (days)					
	0	2	3	0	2	3
	Nonstripped oils			Stripped oils		
A-H	0.956	10.967	14.257	2.636	27.054	35.170
A-BD	1.744	11.487	14.933	2.270	26.063	33.882
BN-H	1.768	26.804	34.845	1.301	26.080	33.904
BN-BD	0.692	6.266	8.146	1.423	24.531	31.890
HN-H	1.096	12.238	15.910	1.704	20.456	26.593
HN-BD	1.492	10.423	13.550	1.467	18.857	24.514
P-H	0.502	4.796	6.234	0.945	11.422	14.849
P-BD	0.267	2.117	2.751	0.897	10.261	13.340
PN-H	1.216	42.067	54.686	1.509	0.838	1.090
PN-BD	1.067	13.464	17.503	1.524	9.903	12.873
PO-H	0.481	6.627	8.615	1.145	11.807	15.348
PO-BD	0.992	3.971	4.733	0.936	11.344	14.747
W-H	0.554	50.997	66.296	0.684	10.141	13.183
W-BD	0.535	29.762	38.690	0.693	12.668	16.469

[a] Abbreviations used are the same as those in Fig. 8.2.
Adapted from Miraliakbari and Shahidi (2008a)

The photooxidative stability of stripped and nonstripped tree nut oils over 3 days was also examined. Chloroform/methanol-extracted oils were more resistant to conjugated diene formation than hexane-extracted oils under photooxidation. Stripped oils were less resistant to conjugated diene formation than their nonstripped counterparts (Table 8.3). Among nonstripped samples, chloroform/methanol-extracted pecan oil had the lowest levels and hexane extracted walnut oil had the highest level of conjugated dienes after 3 days of photooxidation. Headspace analyses of photooxidized tree nut oils showed that hexanal and nonanal were the most prevalent aldehydes present, while propanal was present only in walnut oil, the latter being the only one containing α-linolenic acid. Figure 8.3 shows the headspace aldehyde (hexanal) compositions of nonstripped hexane or chloroform/methanol extracted tree nut oils during photooxidation. Chloroform/methanol-extracted pecan oil contained the lowest level of total headspace aldehydes among all samples after 3 days of photooxidation. Higher amounts of total headspace aldehydes were detected in autoxidized oils than in photooxidized oils; thus, the oils examined enjoyed reasonable photooxidative stability under the conditions employed. Miraliakbari and Shahidi (2008a) thus concluded that the antioxidative minor components of tree nut oils impart both photooxidative and autoxidative stability to them.

Pokorný *et al.* (2003) compared the oxidative stabilities of a traditional peanut oil (cultiva Virginia, 30.5% linoleic acid) with a modified high oleic

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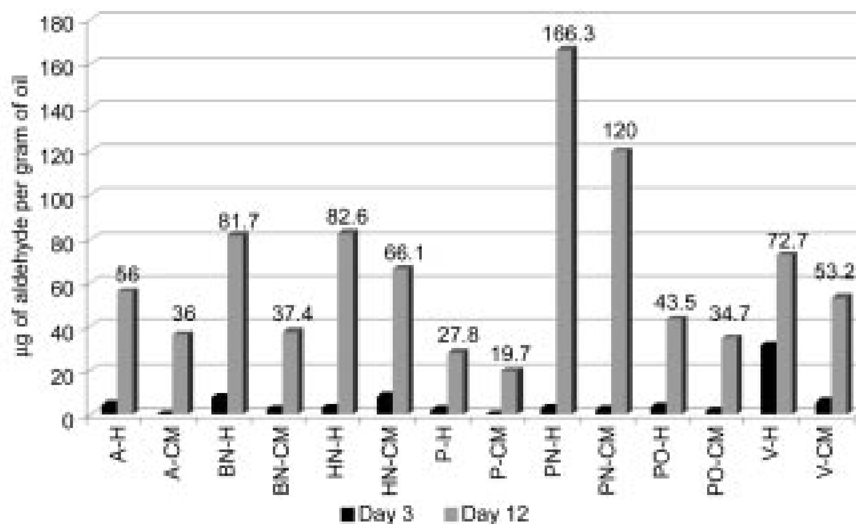

Fig. 8.2 Headspace aldehyde (hexanal) compositions of nonstripped hexane or chloroform/methanol extracted tree nut oils during autoxidation under Schaal oven conditions at 60ºC. Adapted from Miraliakbari and Shahidi (2008a). Abbreviations used: A-H, almond oil, hexane extracted; A-CM, almond oil, chloroform/methanol extracted; BN-H, Brazil nut oil, hexane extracted; BN-CM, Brazil nut oil, chloroform/methanol extracted; HN-H, hazelnut oil, hexane extracted; HN-CM, hazelnut oil, chloroform/methanol extracted; P-H, pecan oil, hexane extracted; P-CM, pecan oil, chloroform/methanol extracted; PN-H, pine nut oil, hexane extracted; PN-CM, pine nut oil, chloroform/methanol extracted; PO-H, pistachio oil, hexane extracted; PO-CM, pistachio oil, chloroform/methanol extracted; W-H, walnut oil, hexane extracted; W-CM, walnut oil, chloroform/methanol extracted.

peanut oil (cultivar SunOleic, 2.7% linoleic acid). Their stabilities were tested under the conditions of Schaal oven test at 40 and 60 ºC, using AOM, Rancimat and Oxipres (oxygen pressure 0.5 MPa) at 100 ºC. The high oleic peanut oil, SunOleic was about 4–8 times more stable than the traditional peanut oil at both 40 and 60 ºC under the conditions of Schaal oven test. The SunOleic peanut oil was also substantially more stable at higher temperatures used in AOM, Rancimat and Oxipres apparatus, which simulate frying conditions (Table 8.3). The difference in stabilities was due to the existing differences in their content

Table 8.3 Oxidative stability of peanut oils. (Adapted from Pokorný *et al.*, 2003)

Method	Virginia peanut oil	SunOleic peanut oil
Schaal test at 40ºC (days)	120	> 850
Schaal test at 60ºC (days)	30	220
Oxipres (h)	11.9	79.8
Rancimat (min)	465	618
AOM (h)	3.74	> 48

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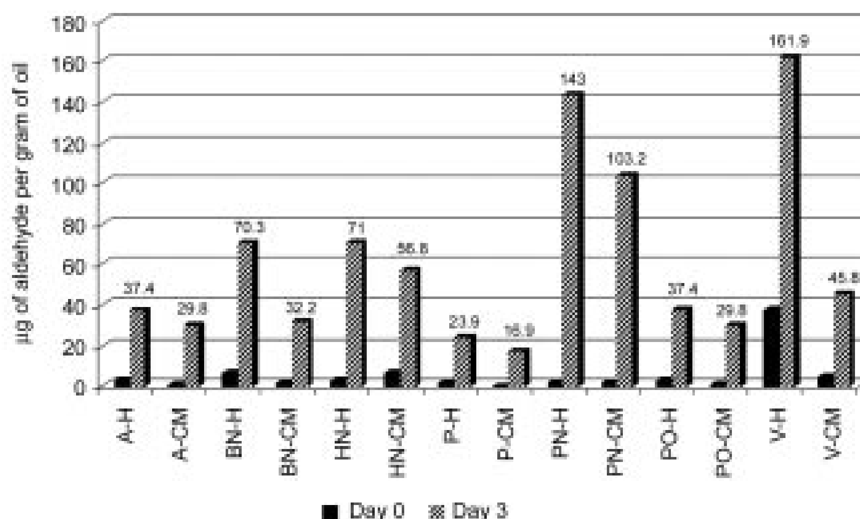

Fig. 8.3 Headspace aldehyde (hexanal) compositions of nonstripped hexane or chloroform/methanol extracted tree nut oils during photooxidation. Abbreviations used are the same as those in Fig. 8.2. Adapted from Miraliakbari and Shahidi (2008a).

of polyunsaturated fatty acids or their respective triacylglycerols. Triacylglycerols containing linoleic acid were oxidized several times faster than triacylglycerols containing only oleic and saturated fatty acids.

8.3 Effect of oxidation on sensory, nutritional quality and shelf-life of nuts and nut oils

Lipid oxidation is a major cause of quality deterioration in nuts and nut oils and affects their sensory attributes, nutritional quality and shelf-life. Consequently, delaying the onset of oxidation could extend the market potential of products of interest. Özdemir *et al.* (2001) reported the effects of roasting of hazelnuts on the amino acid composition, peroxide value, and the contents of thiamine, riboflavin, and free fatty acids. The results showed that roasting significantly affected peroxide value, free fatty acids, thiamine, riboflavine and total amino acid composition of Akçakoca and Giresun hazelnuts. Riboflavin level decreased by almost 30% in Akçakoca hazelnuts and 18% in Giresun hazelnuts. At above 120 ºC, more than 50% of thiamine was lost and the total amino acid levels in the hazelnuts generally decreased as roasting temperature increased. Loss of lysine in the samples of Giresun hazelnuts roasted in two stages (158 ºC for 12 min, 148 ºC for 12 min) was less than 6%, while in Akçakoca hazelnuts, a loss of 31% was noticed when roasted at 126 ºC for 45 min. Amaral *et al.* (2006) investigated the effect of roasting on some nutritional characteristics of lipid fraction of hazelnuts. Hazelnuts were subjected to different time (5, 15, and 30 min) and temperature (125–200 ºC) treatments and analyzed for their moisture

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and crude fat content, as well as determining their phytosterol and fatty acid composition (including trans isomers) by GC-FID, triacylglycerols by HPLC-ELSD, and tocopherols and tocotrienols by HPLC-DAD/fluorescence spectroscopy. Minor changes occurred in the fatty acid and triacylglycerol compositions. As the temperature and roasting period increased, a generally modest relative increase of oleic and saturated fatty acids and a decrease of linoleic acid occurred. Similarly, an increase of triacylglycerols containing oleic acid and a decrease of those containing linoleic acid were found in the roasted samples. Roasting caused a modest decrease of the beneficial phytosterols (maximum 14.4%) and vitamin E homologues (maximum 10.0%) and a negligible increase of the trans fatty acids.

Zacheo *et al.* (2000) studied the changes associated with postharvest ageing in Italian unshelled almonds stored in the dark at 20 ºC and 40% relative humidity for 3 years. They investigated changes in total lipid and protein contents, fatty acid profiles, lipoxygenase activity, hydroperoxide level and tocopherol content. The study confirmed previous reports that unshelled almonds maintained their quality at ambient temperature and did not show any significant chemical and biochemical changes for one year (Senesi *et al*., 1996). On the other hand, roasting carried out in two different ways, either by frying in oil or by a dry process (Young and Cunningham, 1991; Gou *et al*., 2000), promoted changes in the kernels, such as production of volatile compounds (Young and Cunningham, 1991; Gou *et al*., 2000). Harris *et al.* (1972) studied rancidity development in diced unroasted and roasted almonds for up to 6 months at 18 and 38 ºC. Results obtained from a sensory panel demonstrated that diced unroasted and roasted almonds became unacceptable after 6 and 3 months, respectively. Gou *et al.* (2000) studied the influence of oven roasting (two temperatures and six sampling times) on some physico-chemical and sensory properties of *Desmayo Largueta* variety of almond. Rancidity increased with the treatment time up to a maximum value and over-roasting produced antioxidant products due to Maillard reaction, the products of which were absorbed into the oil. Over-roasting also decreased sweetness and increased bitterness and grittiness. The variety of almond can play an important role in the shelf-life of the product. The content of tocopherol, lipids and peroxide values depended on the variety as well as the soil and climate conditions where the nuts are grown. The influence of temperature must be taken into account when shelf-life studies are undertaken. However, accelerated high-temperature treatments result in a different type of change in quality of nuts stored than those at lower temperatures. Consequently, nuts stored under accelerated ageing conditions should be maintained below 43 ºC (Harris *et al*., 1972).

Maskan and Karatas (1998) investigated the changes in fatty acid composition of pistachio nuts stored at 10–30 ºC in the air or under 98% CO_2 and different water activities (0.26–0.33). The fatty acids of pistachios stored in the presence of CO_2 were relatively more stable than those stored under air. The loss of linoleic and linolenic acids depended on both the temperature and water

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Table 8.4 Relative reduction of linoleic and linolenic acids in various conditions. Adapted from Maskan and Karatas (1998)

Fatty acid	Fatty acid loss (%)					
	10ºC		20ºC		30ºC	
	Air	CO_2	Air	CO_2	Air	CO_2
Linoleic	3.98	2.15	4.66	3.02	7.50	9.17
Linolenic	18.24	10.39	32.79	21.02	44.34	50.11

activity in each case; obviously, linolenic acid was more susceptible to oxidation than linoleic acid and hence its relative content decreased more (Table 8.4). Consequently an apparent increase in the relative content of saturated fatty acids, namely myristic, palmitic and stearic and the unsaturated oleic acid was noted. The effect of CO_2 on the stability was more pronounced as the temperature decreased. This may be attributed to the high solubility of CO_2 in the liquid and lipid portion of pistachio nuts (Zhao and Wells, 1995; Calvo *et al.*, 1994) and acting as a barrier at the oil/air interface, and thus reducing the rate of oxygen uptake by the sample (Maskan and Karatas, 1998).

8.4 Protecting nuts and nut oils against oxidation

Extension of the storage period of nuts and their by-products with retention of quality is one of the major needs of the food industry, mainly due to their seasonal availability (Fourie and Basson, 1989). Successful storage will ensure the availability of good quality nuts throughout the year. In order to prevent off-flavour development in nuts, processing techniques should minimize loss of tocopherols and other naturally occurring antioxidants. Thus, different packaging materials, modified atmospheres or refrigeration and application of antioxidants to minimize the oxidation process during storage must be considered.

8.4.1 Using processing techniques that minimize the loss of tocopherols and other natural antioxidants

Oxidative stability of seed oils is greatly affected by their fatty acid composition and minor components such as tocopherols and tocotrienols as well as storage conditions. Both tocopherols and tocotrienols are important antioxidants in stabilizing unsaturated fatty acids in foods and provide an effective protection against oxidative stress together with other antioxidants, such as phenolics, in the human body (Bozan and Temelli, 2008; Lampi *et al.*, 1999). Unrefined oils contain a number of minor components that are partially removed during the refining, bleaching and deodorization that most commercial vegetable oils undergo (Tasioula-Margari and Okogeri, 2001). Some minor constituents can act

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as pro-oxidants such as free fatty acids and hydroperoxides, or as antioxidants including tocopherols, phenols, and possibly phospholipids together with other components (Lee *et al.*, 2002). Nutritionally important antioxidants such as tocopherols and carotenoids generally improve oil stability (Warner and Frankel, 1987). Phytosterols and phytostanols present in vegetable oils have hypocholesterolemic effects and may also exhibit antioxidant activity (Wang *et al.*, 2002). Non-tocopherol phenolics influence the sensory and nutritional characteristics of the oil and possibly improve its stability (Alasalvar *et al.*, 2003a). Miraliakbari and Shahidi (2008a) analyzed the tocopherol content of some tree nut oils and found that hazelnut oil contained the highest tocopherol content (462–508 mg/kg oil), followed by pecan oil (454–490 mg/kg) and then pine nut oil (399–458 mg/kg). α-Tocopherol was the predominant tocol in almond oil (390–439 mg/kg) and hazelnut oil (382–472 mg/kg). Consumption of foods rich in natural antioxidants is protective against certain types of cancer and may also reduce the risk of cardiovascular and cerebrovascular events. These actions of antioxidants have been attributed to their ability to scavenge free radicals, thereby reducing oxidative damage to cellular biomolecules such as lipids, proteins, and nucleic acids (Shahidi *et al.*, 2007; Alasalvar *et al.*, 2006; Wijeratne *et al.*, 2006; Aruoma, 1998).

Tocopherol concentrations have been determined in oils from hazelnut, *Corylus avellana L.* (Özdemir *et al.*, 2001), walnut, *Juglans regia L.* (Kamal-Eldin and Andersson, 1997), and these correlated with their antioxidant activities. In almond, tocopherol concentration plays an important role in protecting lipids against oxidation and thus extending their shelflife (García-Pascual *et al.*, 2003; Zacheo *et al.*, 2000; Senesi *et al.*, 1996). Furthermore, Fourie and Basson (1989) studied variations in tocopherol concentrations in several nuts, and found that almond kernels, with a higher tocopherol concentration than other nuts, had a better storage stability. Rizzolo *et al.* (1994) concluded that almond storage for $\geq$1 year, without quality loss, could only be attained with cultivars that possessed high concentrations of natural antioxidants such as α-tocopherol, suggesting that this homologue is the main component protecting kernel quality (Kamal-Eldin and Appelqvist, 1996) and prolonging storage (Senesi *et al.*, 1996). Fatty acid oxidation becomes only significant after a latent period, during which kernel antioxidants are depleted, as the total concentration of tocopherols decreases during storage (Zacheo *et al.*, 2000). A high concentration of tocopherol has also been shown to be very important in the human diet, due to its vitamin E activity (Kamal-Eldin and Appelqvist, 1996). Consequently, kernel quality may be improved by higher levels of α-tocopherol, due to its lipid stabilizing function and nutritive value as vitamin E, taking into account present consumer trends for foods without synthetic additives (Krings and Berger, 2001). Lavedrine *et al.* (1997) identified α-, β-, and γ-tocopherols in fresh and stored nuts and noted a significant loss after 3 months of storage at 4 °C.

Savage *et al.* (1999) investigated the fatty acid compositions, tocopherols, and stability of 13 of the potentially most useful cultivars of walnuts from

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Europe and the United States that can be grown in the Canterbury region of New Zealand. The Rancimat stability of the oil at 110 ºC and 15 L/min air flow ranged from 3.9 to 7.8 h. Reduced stability of the oil as measured by this method appears to be correlated with higher levels of linoleic acid in the extracted oil. The levels of total tocopherols and the unsaturation in the oil, in a multiple regression analysis, had some relationship ($R^2 = 45.2\%$, $P < 0.001$) with the peroxide value. Owing to the high commercial value of whole walnuts and cold-pressed walnut oil, extreme care needs to be exercised in order to prevent their oxidation. It is important to maintain freshness of products and to use processing techniques that minimize loss of tocopherols and other natural antioxidants.

8.4.2 Using natural or synthetic antioxidants

Control of oxygen availability is a critical factor in minimizing lipid oxidation. The oxygen level can be reduced by vacuum or using modified atmosphere packaging and by using oxygen scavengers such as glucose oxidase (Shahidi and Wanasundara, 1996). These precautions reduce lipid oxidation, especially when combined with the use of antioxidants and low temperature storage in the dark. Both natural and synthetic antioxidants are commonly added to foods to control lipid oxidation. Synthetic antioxidants approved for food use include phenolic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary-butylhydroquinone (TBHQ) and non-phenolics such as ascorbic acid, ascorbyl palmitate and erythorbic acid (Lee *et al*., 1997). Natural antioxidants include carotenoids, ascorbic acid, amino acids and dipeptides, protein hydrolysates, phospholipids, tocols and other naturally occurring phenolic compounds (Kontogiorgis *et al*., 2005). The effectiveness of natural antioxidants from fruits, vegetables, spices, grains and herbs to combat lipid oxidation has been investigated (Shahidi, 1997; 2000a). Rosemary and green tea extracts have been shown to have antioxidant activity in lipids and lipid-containing foods (Shahidi, 2000b; He and Shahidi, 1997; Wanasundara and Shahidi, 1996).

Antioxidants such as BHA, BHT and TBHQ are effective in preventing the oxidation of nuts and nut products such as peanut butter spread, almond spread, or nut oils by delaying the onset of rancidity and increasing shelf-life. Various techniques are employed to add antioxidants to nuts and nut products and these may include:

1. Spraying a dilute antioxidant solution on nut surfaces, which is probably the easiest and most efficient way to deliver antioxidants to nuts. An antioxidant solution is typically diluted with vegetable oil, such as peanut oil, used in the roasting step. The antioxidant solution can be sprayed after the roasting step on nuts on a conveyor belt or in a spray tumbler. Spray tumbling will ensure the most complete coverage of antioxidant on the nut surface.
2. Addition to roasting oil. Antioxidants may be lost or destroyed during this step, as roasting temperatures will be relatively high.
3. Use of antioxidant treated salt or other condiments.

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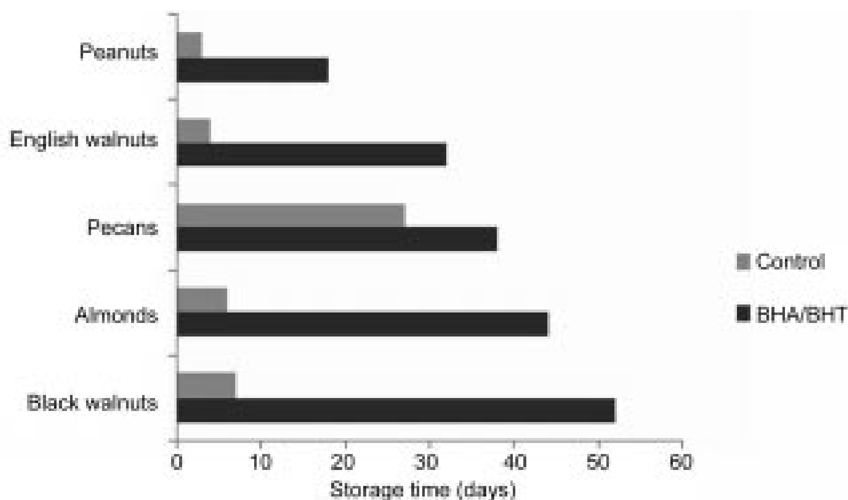

Fig. 8.4 Effect of BHA/BHT treatments on the stability as reflected in the extension of storage time of nut kernels stored at 62ºC. Adapted from Eastman Chemical Company.

4. Addition to coating or glaze. Use of an edible protective coating containing antioxidant, such as a confectioner's glaze used to coat almonds or peanuts.
5. Use of antioxidant treated packaging materials.
6. Addition to nut oils. The required amount of antioxidant is dissolved in a small amount of nut oil and the concentrated antioxidant portion is mixed throughout the entire bulk (Eastman Chemical Company, 2004).

FDA permits the addition of 200 mg/kg (ppm) total antioxidants (based on lipid content) while USDA allows combinations of antioxidants up to the same level on a fat weight basis but limits the use of a single antioxidant to 100 mg/kg of fat weight. However, this level of antioxidants on the surface may result in off-flavour development associated with antioxidants. Therefore, a level of 50–100 mg/kg is recommended. Shelf-life studies of nuts should be conducted to determine the amount of antioxidant required to achieve the desired shelf-life and whether any off-flavours would result from the level of incorporated antioxidants. Figure 8.4 demonstrates the effectiveness of BHA/BHT in improving the shelf-life of various nut products as compared to the control without antioxidants (Eastman Chemical Company, 2004). Thus, use of natural or synthetic antioxidants can minimize oxidation in nuts and nut oils.

8.4.3 Packaging

Appropriate packaging materials and methods are needed to ensure optimum shelf-life of nuts. Physical properties of packaging materials determine interior environmental conditions, in terms of gas composition and relative humidity, which are important for the stability of the products. Control of oxygen levels in

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the packages is critical since many changes are dependent on the redox potential and available oxygen inside the package. Therefore, low water vapour and gas permeable packaging material and vacuum sealing to reduce available oxygen should be employed to prevent rancidity and extend the shelf-life of nuts (Özdemir and Devres, 1999b). Moisture and water vapour exchange between the nuts and their environment can cause physical changes, alter flavour or texture, or promote microbial growth, especially when there is temperature abuse during transportation, storage, and retail display (Özdemir and Devres, 1999b).

Senesi *et al.* (1991) studied the effect of different packaging conditions, transparent and metallized films under vacuum or nitrogen at two storage temperatures (4 and 20 ºC) on the quality and stability of Italian peeled almonds (cv. *Ferraduel*). They found that peeled kernels could be stored up to 9 months without a serious loss in quality when packaged in high barrier packaging, regardless of the storage temperature (48 ºC or ambient). However, in order to maintain the general quality over a longer period (more than 9 months), they proposed the use of metallised film under nitrogen and refrigeration (48 ºC). In another work, Senesi *et al*. (1996) studied the influence of low barrier packaging material combined with refrigeration (2 ºC) on the storage and quality of Italian peeled almond (cv. *Supernova*). They found that it was possible to maintain the quality of peeled almonds up to 12 months in a low barrier material by storing them at refrigeration temperature of 8 ºC.

Borompichaichartkul *et al*. (2009) recommended suitable storage conditions (packaging materials and humidity) at ambient temperatures to maintain superior quality of nuts. The research was conducted at 27–30 ºC, 11–92% RH with two types of packaging material, namely laminated aluminium foil oriented polypropylene/aluminium/polyethylene/linear low-density polyethylene (OPP/AL/PE/LLDPE) and linear low-density polyethylene (LLDPE) under atmospheric pressure. The results showed that the quality of dried macadamia nut in both packaging materials depended on humidity. At relative humidity below 40%, the quality of dried nuts had changed only slightly. In contrast, at above 40% RH, the quality of the dried nuts in LLDPE bag was changed significantly in terms of equilibrium moisture content, water activity, colour and peroxide value when compared to dried macadamia nuts in OPP/AL/PE/LLDPE bags.

Jensen *et al*. (2005) stored peanuts and other products in two experiments, where external factors (light, oxygen concentrations, product-headspace ratios) were varied, and packaging materials with different properties (light transmission and oxygen permeability) used. The oxidative changes in the products were followed by the formation of hexanal as detected by headspace gas chromatography (headspace-GC), free radicals as nominated by electron spin resonance (ESR) spectroscopy, and sensory evaluation. Generally, increased oxygen availability and exposure to light resulted in increased lipid oxidation. Storage in the conventional material (PET with 0–60% transmission of visible light) resulted in a better quality than storage in the more transparent PET, demonstrating that exclusion of light with certain wavelengths was

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sufficient to reduce oxidation. Storage in light also accelerated the rate of oxidation. Improvement of the light barrier of the packaging material could, accordingly, protect the product against light and thereby delay lipid oxidation. However, the effect of the light barrier depended on the colour of the pigment added to the packaging material (Jensen *et al.*, 2005). Incorporation of UV absorbers is also a possibility, but those in common use normally absorb below 400 nm and allow visible light to be transmitted. As visible light also has an effect on the lipid oxidation in the actual products, the packaging materials would have to exclude both ultraviolet radiation and visible light (Lennersten and Lingnert, 1998). Incorporation of pigments as light barrier was found to extend the shelf-life of potato chips from 1 to 10 weeks (Kubiak *et al.*, 1982), a result which supports the finding of Lennersten and Lingnert (1998). Packaging material with a high light barrier (e.g., metallized foil) was thus found to extend the shelf-life of potato chips from 7 days to 5 months compared to a low light barrier (Lennersten and Lingnert, 1998). Light exposure of various nuts (almonds, peanuts, pine nuts and walnuts) stored in amber-coloured glass bottles did not affect the storage stability and has been found comparable to dark storage, in contrast to storage in clear polyethylene (PE), which did not protect the nuts from light-induced lipid oxidation as measured by peroxide value (Sattar *et al.*, 1990a). Walnuts were similarly found to be light sensitive, as exposure to light increased the degree of oxidation, thereby resulting in a significantly reduced sensory quality (Jensen *et al.*, 2001). Comparison of packaging materials with different light transmissions (3, 47, and 87% transmittance) showed, for pecan nuts, a dose-response relationship, where a higher transmission results in larger colour changes (Heaton and Shewfelt, 1976). Polyvinyl chloride is a preferred packaging material because of its high impermeability to oxygen. Coloured or opaque containers are also preferred as they retard photooxidation (Faria and Mukai, 1983).

8.4.4 Application of modified/ controlled atmospheric storage

The modified atmosphere (MA) or controlled atmosphere (CA) has been applied to existing processing methods and storage systems in order to avoid drastic changes in food quality (Johnson *et al.*, 2009). The methods used to generate treatment atmospheres include gas from cylinders, exothermically generated low oxygen atmospheres (GLOA) using combustion to reduce oxygen levels (Storey, 1975), and gas separation systems (Johnson *et al.*, 1998; 2002). Another form of modified atmosphere treatment is the use of low pressure to reduce oxygen tension (Navarro *et al.*, 2003).

Although most of the uses of controlled atmospheres on bulk-stored dried fruits and nuts are for short-term insect disinfestation, MA or CA are often suggested as means to improve shelflife in these products, particularly for tree nuts (Johnson *et al.*, 2009). A common method for controlling the oxidation reaction is to reduce oxygen concentration in the storage atmosphere over the food by vacuum or nitrogen filling for dry or intermediate moisture foods

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(Kacyn *et al.*, 1983) or CO_2 filling, as a biostat, to prevent anaerobic microbial growth and lipid oxidation (Hotchkiss, 1988, Lioutas, 1988). Vacuum packaging or nitrogen-flushed packaging is often suggested to improve the shelf-life of tree nuts. Brecht (1980) proposed 0% oxygen and 100% CO_2 for tree nuts. Low oxygen (> 1 kPa oxygen, 9–9.5 kPa CO_2, 86–89 kPa nitrogen and 1 kPa argon) was as effective as low temperature (18 ºC) in maintaining stability during storage, and caused less off-flavour development than normal atmosphere for both meats and in-shell almonds (Guadagni *et al.*, 1978). The storage behaviour of four varieties of almond ('Marcona', 'Planeta', 'Desmayo', and 'Nonpareil') was investigated at two temperatures (8 and 36 ºC), two packaging atmospheres (air and nitrogen) and two treatments (raw and roasted) for up to 9 months (García-Pascual *et al.*, 2003). Nitrogen packaging resulted in reduced oxygen levels of < 0.25 kPa. No significant differences were observed between air and N_2 packaging for moisture, fat content, peroxide value, α-tocopherol content, and level of aflatoxins. Rouves and Prunet (2002) examined various storage techniques for chestnuts, including low temperature CA (2 kPa oxygen and 5 kPa CO_2 at −1 or 1 ºC). For the varieties 'Marigoule' and 'Bouche de Betizac' water loss was prevented, mold reduced, and taste maintained. The varieties 'Comballe' and 'Marron de Goujonac' did not benefit from CA storage. Ebraheim *et al*. (1994) reported that reduced temperature might be effective in combination with other protective measures such as vacuum packaging in extending roasted kernel shelf-life to one year or more. Keme *et al*. (1983) reported that it was possible to store 'Piernonteses', 'Roman', and 'Akcakoca' hazelnuts at ambient temperature under nitrogen ($\geq$ 99.5 kPa) for prolonged periods of time with a loss of quality comparable to that resulting from storage conditions at low temperatures and controlled RH (3–6 ºC, 50–60% RH). Hazelnut ('Negret') quality was also studied after storage in selected CA conditions (San Martin *et al*., 2001). The shelled and unshelled hazelnuts were stored under different oxygen levels (1, 5, 10, and 20 kPa) at two different temperatures (7 and 25 ºC); the quality of hazelnuts during storage was monitored by determining the peroxide value, acid value, percentage of unsaturated fatty acids and sensory analysis. After one year of storage, none of the storage conditions tested caused significant rancidity. Storage in atmospheres with oxygen levels lower than 10 kPa significantly reduced autoxidation and the low temperature delayed lipid oxidation. In the case of macadamia nuts also, prolonged exposure to oxygen resulted in rancidity, and vacuum packaging or nitrogen flush offered protection from oxygen (Cavaletto, 2004). Shelflife of pecans may be increased by storage in 2–3% oxygen in nitrogen, and less frequently using CO_2 as the balance gas (Maness, 2004). Oxygen transmission rates for packaging materials should be > 0.08 mL/100 cm per 24 h (Dull and Kays, 1988). Vacuum packaging can offer a further benefit of protection from breakage. Pine nuts (pinones), *Araucaria araucana*, stored under different MA (polyethylene bags lined with volcanic dust) and CA (10:5 kPa CO_2:oxygen, and 20:5 kPa CO_2:oxygen) conditions maintained their moisture and starch content, and 20:5 kPa CO_2:oxygen was the optimum CA condition (Estevez and Galletti, 1997). Although pistachios are

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fairly stable when stored in normal air at 20 ºC, storage under reduced oxygen (<0.5 kPa), vacuum packaging or N_2 flushed packaging further improved flavour stability of the pistachios (Labavitch, 2004). Storage stability, oil characteristics, and chemical composition of whole-split pistachio nuts were determined for samples stored in CA (2 kPa air, 98 kPa CO_2), air at the monolayer value at 10, 20, and 30 ºC, as well under ambient conditions (Medeni and Sukru, 1999). Maximum level of oxidation occurred under ambient storage conditions. CO_2 especially improved the storage stability at low temperatures. Using the reaction rate constants of peroxide formation, it was revealed that as temperature increased, the ratio of rate constants (air/CO_2) approached 1, which means that no significant difference existed between air and CO_2 storage at 30 ºC. Shelf-life can be extended by storage under < 1 kPa oxygen. Chun *et al.* (2005) studied the relationship between α-tocopherol content and the oxidative stability of raw and dry roasted peanuts during storage at 21 ºC under air and vacuum. In air, PV (meq/kg) for roasted peanuts reached 47 after 12 weeks, whereas that for raw peanuts was below 2 after 38 weeks. Under vacuum, lipid oxidation was significantly retarded ($P < 0.05$). Tocopherols of raw and roasted peanuts exponentially decreased with increasing PV. After 12 weeks, about 50% of α-tocopherol was lost in roasted peanuts under vacuum compared with about 90% under air. For raw peanuts, more than 70% of each tocopherol remained after 38 weeks under air and vacuum. Cavaletto and Yamamoto (1971) also tested the effectiveness of application of antioxidants BHA/BHT after roasting of macadamia kernels for protecting the kernels packaged in air and under vacuum. Stability of antioxidant-treated kernels was greater than that of untreated kernels, regardless of the vacuum level. Vacuum packaging had no effect on antioxidant treated kernel, but showed some benefit for untreated kernels.

8.5 Future trends

Numerous epidemiological studies have shown that frequent nut consumption is associated with a reduced risk of both fatal coronary heart disease and non-fatal myocardial infarction (Hu *et al.*, 1998; Sabaté, 1999; Nash and Nash, 2008). These studies indicate that increased nut consumption improves health, but the area is wide open for more intensive studies to verify the extent to which the antioxidant content and other bioactive compounds of nuts may contribute to long-term health. However, nuts are seasonal products and their high unsaturated fatty acid profiles make them susceptible to oxidation and deterioration as discussed in the preceding sections. Control of these changes and extension of the shelflife of nuts and nut oils is one of the biggest challenges of the food industry. Proper harvest, post-harvest storage, processing and packaging methods and conditions should be exercised and developed to decrease nutritional loss and textural change, to improve aroma and to extend the shelflife of nuts and their products (Özdemir and Devres, 1998, 1999b). Varieties of nuts

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resistant to rancidity should be selected. Mechanisms of quality, aroma, and colour deterioration in nuts need further research. Methods to economically apply CA and protect product quality are also needed. Thus, introducing simple and effective quality control measurements to reduce economic loss and possible health risks and to increase productivity should be carried out to take full advantage of health benefits of nuts and nut-based products.

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9

Lipid oxidation in emulsified food products

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Abstract: Most lipids in foods exist as colloidal dispersions stabilized by surface active agents that slow down the gravitational separation of oil and water. The surface active agents in emulsion create an oil-water interface that has major impacts on the distribution of the components in foods that impact lipid oxidation. This includes location and reactivity of prooxidative factors such as transition metals, lipid hydroperoxides and minor lipid components and antioxidants such as free radical scavengers and metal chelators. Understanding of how the physical properties of the lipid-oil interface in food emulsions impacts the chemistry of lipid oxidation has lead to new strategies to inhibit oxidation such as creating charged surfaces that electrostatically repel metals and thick interfaces that inhibit lipid–prooxidant interactions. As food formulations continue to move towards inclusion of more polyunsaturated fatty acids and the use of traditional antioxidants becomes more limited due to increased consumer demand for all natural foods, it is important to have a better understanding of the mechanisms of lipid oxidation in foods dispersion so that novel antioxidant technologies can be developed.

Key words: emulsions, lipid oxidation, antioxidants, transition metals, emulsifiers, surfactants, chelators, association colloids.

9.1 Introduction

Many lipid containing processed foods are either oil-in-water or water-in-oil emulsions (McClements, 2005; Friberg *et al.*, 2004). While oxidation is a problem in both, the majority of research has been done in oil-in-water systems.

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Therefore, in this chapter we mainly focus on lipid oxidation in oil-in-water type emulsions, which represents products such as milk, infant formula, salad dressing, mayonnaise, sauces, soups, beverages, cream, and some desserts (McClements and Decker, 2000; Okuda *et al.*, 2005; McClements, 2005; Friberg *et al.*, 2004). As well as the food industry, the cosmetics, pharmaceutical and medical industries also utilize oil-in-water emulsions as a means to encapsulate, protect, and release bioactive lipids in their products. For this reason, the number of studies attempting to understand the physicochemical mechanisms underlying lipid oxidation in oil-in-water emulsions has increased dramatically during the past decade.

Lipid oxidation is a great concern in the food industry because it causes physical and chemical deteriorations, such as losses in important nutrients, formation of potentially toxic reaction products (such as aldehydes and ketones), undesirable changes in appearance and texture, and development of rancidity that shortens product shelf life (Frankel, 1998; McClements and Decker, 2000; Decker and McClements, 2008). Lipid oxidation is favored in oil-in-water emulsions because of the large contact surface between the oxidizable lipid droplets and water-soluble compounds including oxygen and prooxidants, which contribute to the initiation and propagation of oxidation reactions (Frankel, 1998). There are many factors that can potentially influence the rate of lipid oxidation in oil-in-water emulsions: fatty acid composition; aqueous phase pH and ionic composition; type and concentration of antioxidants and prooxidants; oxygen concentration; lipid droplet characteristics such as particle size, concentration and physical state; and emulsion droplet interfacial properties such as thickness, charge, rheology, and permeability (McClements and Decker, 2000). The susceptibility of emulsified lipids to oxidation also depends on the surrounding molecular environment and interactions with other molecules within the immediate vicinity of the droplets.

This chapter aims at summarizing the current knowledge of lipid oxidation in oil-in-water emulsions in order to understand the basic principles of how to prevent lipid oxidation and thereby improve the oxidative stability of emulsified food products. Initially, a brief review of the preparation, properties and characterization of conventional and structured oil-in-water emulsions are discussed. The lipid oxidation mechanisms that occur in bulk oils are then contrasted with those in oil-in-water emulsions. Finally, we highlight various strategies that can be used to retard or prevent lipid oxidation in oil-in-water emulsions.

9.2 Emulsions: preparation, properties and characterization

This section provides an overview of the production and characterization of traditional emulsion that are used in the food and other industries.

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9.2.1 Conventional emulsions

Conventional emulsions consist of two immiscible liquids (such as oil and water) with one of the liquids being dispersed as small spherical droplets in the other liquid (Dickinson and Stainsby, 1982; Dickinson, 1992). The mean droplet diameter in food emulsions ranges from less than 100 nm to greater than 100 μm (McClements *et al.*, 2007). Conventional emulsion can be classified as either water-in-oil (W/O) or oil-in-water (O/W) depending on the spatial arrangement of the two immiscible liquids. Water-in-oil emulsion consists of water droplets dispersed in an oil phase, while oil-in-water emulsion consists of oil droplets dispersed in a water phase. In this chapter we mainly restrict ourselves to discussion of the properties of emulsions that are dispersed in water, i.e., those with an aqueous continuous phase.

Emulsions can be conveniently divided into three different regions: the continuous phase, the interfacial region, and the interior of the droplets (McClements and Decker, 2000). Emulsions are thermodynamically unfavorable systems that tend to break down over time. To create emulsions that are kinetically stable for a reasonable period of time, stabilizers such as emulsifiers or texture modifiers need to be added to prevent gravitational separation, flocculation, coalescence and Oswald ripening (Friberg *et al.*, 2004; McClements, 2005; Dickinson, 1992). The most commonly used emulsifiers in the food and beverage industries are small molecule surfactants (e.g., Tweens, Spans, and esters of fatty acids), phospholipids (e.g., egg, soy or dairy lecithin), surface-active proteins (e.g., casein, whey, egg, and soy) and surface-active polysaccharides (e.g., gum Arabic and modified starch) (McClements, 2005).

Conventional O/W emulsions can be prepared by homogenizing an oil phase and an aqueous phase together in the presence of a water-soluble emulsifier. Wide varieties of homogenizers can be used, including high shear mixers, high pressure homogenizers, colloid mills, ultrasonic homogenizers and membrane homogenizers depending on the characteristics of the materials being homogenized (e.g., product viscosity, interfacial tension, shear sensitivity) and the desired emulsion properties (e.g., droplet concentration, particle size distribution). The desired droplet characteristics can be manipulated by careful selection of homogenizer type, homogenizer operating conditions, and emulsifier. For example, the droplet size of O/W emulsions produced by high-pressure homogenizers can be reduced by increasing the homogenization pressure or number of passes through the homogenizer. The electrical charge on the droplets can be controlled by selecting an appropriately charged emulsifier, which may be positive, neutral or negative and the pH of the continuous phase which can impact emulsifier charge (McClements *et al.*, 2007; McClements, 2005; Walstra, 1993; Walstra, 2003).

9.2.2 Droplet characteristics

Knowledge of the most important properties of the droplets within emulsions is useful for determining the best strategy to control their oxidative stability.

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Droplet concentration
The droplet concentration is usually expressed as the number, mass or volume of droplets per unit volume or mass of emulsion and can be controlled by varying the proportions of the two immiscible liquids used to prepare it (McClements *et al.*, 2007; McClements, 2005).

Particle size distribution
The particle size distribution of an emulsion represents the fraction of droplets falling into different size categories. It is usually represented as either a table or a plot of particle concentration (e.g., volume or number percent) *versus* droplet size (e.g., radius or diameter) (McClements *et al.*, 2007; McClements, 2005).

Droplet charge
The electrical properties of a droplet are usually characterized in terms of its ζ-potential (ζ), which can be conveniently measured (Hunter, 1986). The ζ-potential of a droplet depends on the surface charge density (i.e., the number of charges per unit area), as well as the prevailing environmental conditions (i.e., ionic strength and dielectric constant).

Interfacial characteristics
Each droplet in an emulsion is usually coated by a thin layer of adsorbed material to protect it against aggregation with other droplets. The composition and properties of this interfacial region are defined by the type, concentration and interactions of any surface-active species before, during and after emulsion formation, e.g., emulsifiers, biopolymers, and minerals (Dickinson, 2003).

Physical state
Normally, the lipids in the dispersed phase of an O/W emulsion are liquid. Nevertheless, in some emulsion-like systems the dispersed phase is either partially or fully solidified, e.g. solid lipid particles (McClements *et al.*, 2007; McClements, 2005; Walstra, 2003; Muller and Keck, 2004; Wissing *et al.*, 2004). The nature, location and concentration of the fat crystals within the lipid droplets in an O/W emulsion can be controlled by proper selection of oil type (e.g., solid fat content vs. temperature profile), thermal history (e.g., temperature vs. time profile), the presence of additives (e.g., crystal structure modifiers), emulsifier type, and droplet size (Walstra, 2003; Muller and Keck, 2004; Muller *et al.*, 2000).

9.2.3 Physicochemical properties of emulsions

It should be stressed that any strategy used to retard or inhibit lipid oxidation in emulsions should not adversely affect the bulk physicochemical and sensory properties of the final emulsion product.

Optical properties
Opacity and color are the most important optical properties of emulsions, and can be quantitatively described using tristimulus color coordinates, such as the

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*L*a*b** system (McClements, 2005). The optical properties of emulsions are determined by the droplet size, droplet concentration, and relative refractive index (McClements, 2002a; 2002b; 2005). The lightness of an emulsion tends to increase and the color intensity decrease with increasing droplet concentration and refractive index contrast, and they have a maximum value at a particular droplet size. The optical properties of an emulsion may affect its susceptibility to oxidation. Ultraviolet and visible light can penetrate further into optically clear emulsions than into optically opaque ones, thereby accelerating light-catalyzed lipid oxidation. This has important consequences for the development of transparent beverages containing chemically labile lipids, such as ω-3 fatty acids or carotenoids.

Rheology
Food emulsions vary widely in their rheological behaviors depending on the nature of the food. They may be viscous liquids, visco-elastic liquids, visco-elastic solids, plastics, or elastic solids depending on their composition, structure and interactions (Walstra, 2003; McClements, 2005; Genovese *et al.*, 2007). Generally, when the droplet concentration of an emulsion increases, the viscosity will increase gradually at first and then steeply as the droplets become more closely packed. When the droplet concentration is around 50–60% (for a non-flocculated O/W emulsion), the droplets pack so closely together that the emulsion exhibits solid-like characteristics, such as visco-elasticity and plasticity (McClements, 2005). Flocculated emulsions may exhibit these solid-like characteristics at much lower droplet concentrations due to particle–particle interactions.

Physical stability
Emulsions are thermodynamically unfavorable systems that tend to break down over time because of physicochemical mechanisms such as gravitational separation, flocculation, coalescence and Ostwald ripening (Friberg *et al.*, 2004; McClements, 2005; Dickinson, 1992). One of the most common causes of instability in food emulsions is gravitational separation, which can be either creaming or sedimentation depending on the relative densities of the dispersed and continuous phases. The shelf life of many emulsion-based food products is determined by their physical stability.

9.3 Lipid oxidation mechanisms in emulsions

The oxidation of emulsified lipids is different from that of bulk lipids for a number of reasons: the presence of an aqueous phase containing prooxidants and antioxidants; the presence of an oil–water interface and the partitioning of antioxidants, prooxidants and oxidizable substrates between oil, interfacial, and water phases. The emulsion droplet interface can impact oxidation reactions by attracting or repelling prooxidants and antioxidants through their surface charges

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and by forming a physical barrier that influences the interactions between lipid and water soluble prooxidants (Frankel *et al.*, 1994; McClements and Decker, 2000; Richards *et al.*, 2002). Fritsch (1994) suggested that the impact of oxygen on the rate of lipid oxidation is similar in water-in-oil emulsions and bulk oils due to the direct exposure of the bulk oil to air. However, most oil-in-water emulsions are much more prone to oxidation than bulk oils (Chaiyasit *et al.*, 2007). This is likely due to their large surface areas which expose the lipids to aqueous phase prooxidants.

Many studies indicate that transition metals originating in the aqueous phase are the most common cause of oxidative degradation of emulsified lipids. These water-phase prooxidants are capable of interacting with lipid hydroperoxides located at the droplet surface (Yoshida and Niki, 1992; Mei *et al.*, 1998a; 1998b; Mancuso *et al.*, 2000; Nuchi *et al.* 2001, Dimakou *et al.*, 2007). The interaction of lipid hydroperoxides (ROOH) with both reduced and oxidized forms of transition metals can produce highly reactive peroxyl ($LOO^{\bullet}$) and alkoxyl ($LO^{\bullet}$) radicals (Eqs. 1 and 2) that either attack other unsaturated lipids (LH) within the oil droplets or at the oil-water interface to promote oxidation or in the case of the alkoxyl radical promote β-scission reactions that decompose fatty acids into the low molecular weight volatile compounds that cause rancidity (Frankel, 1998; Decker and McClements, 2008).

$$Fe^{3+} + LOOH \longrightarrow Fe^{2+} + LOO^{\bullet} + H^{+} \qquad 9.1$$

$$Fe^{2+} + LOOH \longrightarrow Fe^{3+} + LO^{\bullet} + OH^{-} \qquad 9.2$$

In general, iron is thought to be the most important transition metal prooxidant as can be seen by the ability of iron binding proteins (e.g., transferrin and lactoferrin) to strongly inhibit lipid oxidation in lipid dispersions (Huang *et al.*, 1999; Mancuso *et al.*, 1999). Ferrous (Fe^{2+}) is much more prooxidative than ferric (Fe^{3+}) due to its higher solubility and reactivity (Halliwell and Gutteridge, 1990). Ferric ions were only found to be effective at decomposing lipid hydroperoxides when they are concentrated at the oil–water interface of a negatively charged emulsion droplet (Mancuso *et al.*, 2000). Since metals are important prooxidants in the oxidative stability of emulsions, minimizing their concentrations is an effective method to decrease oxidation rates (Mei *et al.*, 1998b; Nuchi *et al.*, 2002; Katsuda *et al.*, 2008; Wang and Wang, 2008).

9.4 Characteristics of emulsion droplets that impact lipid oxidation

Three different physical environments can be conveniently defined in oil-in-water emulsions: the lipid inside the emulsion droplets; the interfacial layer surrounding the droplets; and the aqueous continuous phase surrounding the interfacial layer (Fig. 9.1). The chemical composition of the interfacial layer may be fairly complex, and include emulsifiers, antioxidants, minor lipid components (e.g., sterols and triacylglycerol hydrolysis products), biopolymers,

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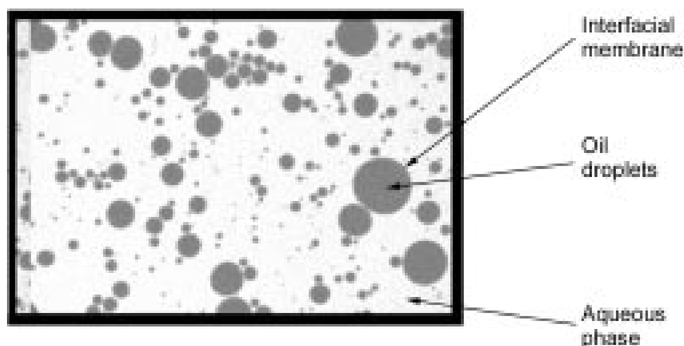

Fig. 9.1 The three regions of an oil-in-water emulsion that can impact lipid oxidation.

mineral ions, and lipid oxidation products (Dickinson and McClements, 1995; McClements and Decker, 2000; Chaiyasit *et al.*, 2007). The thickness of the interfacial layer is usually in the range of a few nanometers (e.g., 1 to 40 nm), and can be controlled by careful selection of emulsifiers and other ingredients. The physical location of the various chemical reactants in emulsions therefore depends on their lipid and water solubility characteristics and surface activities (Hiemenz and Rajagopalan, 1997). For example, polar molecules tend to be located in the aqueous phase, non-polar molecules in the oil phase, and surface active molecules in the interfacial region (McClements and Decker, 2000). A key place to modulate the initiation of lipid oxidation in oil-in-water emulsions is therefore at the oil–water interface since this is the place where lipid- and water-soluble components interact with each other, and where surface-active reactants such as lipid hydroperoxides concentrate (Nuchi *et al.*, 2002). The properties of the interfacial region can be controlled in a variety of different ways to control the lipid oxidation reaction in emulsions (Decker *et al.*, 2005).

9.4.1 Emulsion droplet interfacial area

The interfacial area of an emulsion depends on the droplet concentration and particle size: $A = \phi/6d_{32}$, where A is the interfacial area per unit volume of emulsion, ϕ is the disperse phase volume fraction, and d_{32} is the surface-weighted mean diameter. The size of the droplets in a food emulsion, and therefore the interfacial area, vary in different food products. Droplet diameters can vary from larger than 100 μm in salad dressings and mayonnaise to less than 0.2 μm in cream liqueurs and soft drinks. Since lipid oxidation reactions in emulsions are greatly influenced by surface interactions between metals and hydroperoxides, it would be expected that droplet surface area would be an important factor in oxidation rates (McClements and Decker, 2000; Lethuaut *et al.*, 2002). Nevertheless, studies of the effect of droplet size on lipid oxidation in O/W emulsions are conflicting:

- Some studies have found that the rate of lipid oxidation increased when the surface area increased (Gohtani *et al.*, 1999).

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- Some studies have found that the rate of lipid oxidation increased when the surface area decreased (Hegenauer *et al.*, 1979; Lethuaut *et al.*, 2002; Nakaya *et al.*, 2005; Imai *et al.*, 2008).
- Some studies found that the lipid oxidation rate was fairly independent of surface area (Coupland *et al.*, 1996; Shimada *et al.*, 1996; Osborn and Akoh, 2004; Dimakou *et al.*, 2007; Kiokias *et al.*, 2007; Paraskevopoulou *et al.*, 2007; Sun and Gunasekaran, 2009).

There are a number of physicochemical mechanisms that might influence the effect of droplet size and surface area on the lipid oxidation rate:

1. as the interfacial surface area increases more of the lipid phase is exposed to the surrounding aqueous phase, which should promote lipid oxidation;
2. as the interfacial area increases the partitioning of reactants, pro-oxidants and antioxidants between the oil, water and interfacial regions is altered, which may either promote or retard oxidation;
3. as the interfacial area increases the amount of surfactant at the interface increases, which may decrease the surfactant present in the aqueous phase;
4. more mechanical energy must be supplied to increase the interfacial area of an emulsion containing small droplets during homogenization, which may promote oxidation (Nakaya *et al.*, 2005).

At present, the relative importance of these and possibly other mechanisms is not clearly understood, and further work is required. It is likely that the effects of particle size will depend on the precise nature of the system, e.g., the type and concentration of emulsifiers, prooxidants and antioxidants present. However, research to date suggests that emulsion droplet size and thus interfacial surface area is not a major factor in the oxidative stability of O/W emulsions. This could be due to the extremely large surface area of all of the emulsions used in these studies. These large surface areas could mean that surface area never limits reaction rates.

9.4.2 Droplet charge

The oxidative stability of oil-in-water emulsions depends on the electrical charge on the droplet surfaces (Mei *et al.*, 1998a; 1998b; Mancuso *et al.*, 1999; 2000; Silvestre *et al.*, 2000; Boon *et al.*, 2008). Surface charge determines electrostatic interactions, either attractive or repulsive, between emulsion droplets and charged metals (Mei *et al.*, 1998a; Mancuso *et al.*, 1999; Haahr and Jacobsen, 2008). Droplet charge can be manipulated by selecting appropriately charged emulsifiers (e.g., cationic, anionic or neutral), by using the electrostatic layer-by-layer (LbL) deposition method to deposit charged biopolymers onto oppositely charged droplets (Shaw *et al.*, 2007; Klinkesorn *et al.*, 2005a; 2005b; Djordjevic *et al.*, 2007), or by altering pH that impacts emulsifier charge (Hu *et al.*, 2002; 2003; Djordjevic *et al.*, 2004). Several studies have shown that anionic surfactants (such as sodium dodecyl sulfate, SDS) at droplet surfaces promote lipid oxidation by attracting cationic transition metals to the surfaces (e.g., Fe^{2+}

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or Fe^{3+}), whereas cationic surfactants (such as dodecyl trimethyl ammonium bromide, DTAB) retard lipid oxidation by repelling these transition metals away from the surface (Mei *et al.*, 1998a; 1998b; Mancuso *et al.*, 1999; 2000; Silvestre *et al.*, 2000; Boon *et al.*, 2008).

The impact of droplet surface charge has also been observed in oil-in-water emulsions stabilized by proteins, where the rate of lipid oxidation was faster when the protein-coated droplets were anionic (pH > pI) than when they were cationic (pH < pI) (Donnelly *et al.*, 1998; Mei *et al.*, 1998a; 1998b; Mancuso *et al.*, 1999; 2000; Hu *et al.*, 2002; 2003; 2004b; Trunova *et al.*, 2007; Djordjevic *et al.*, 2008). Hu and coworkers (2002) found that the oxidation of cationic emulsion droplets produced by emulsifying oil with proteins at pH 3.0 varied as a function of protein type. In this experiment, oxidative stability was in the order sodium caseinate > whey protein isolate > soy protein isolate. The density of the cationic charge of the emulsion droplets did not correlate with oxidative stability suggesting that other factors such as droplet interfacial thickness and/or the antioxidant properties of the protein were also involved in the ability of the interfacial proteins to inhibit oxidation at pH 3.0. The impact of negative surface charge on the rate of lipid oxidation in protein-stabilized emulsions was reported by Villiere and coworkers (2005). This study compared stripped sunflower oil-in-water emulsions (30 vol%) stabilized by sodium caseinate (NaCas) or bovine serum albumin (BSA) at pH 6.5. The droplets in the NaCas-stabilized emulsions had a substantially higher negative charge than those in the BSA-stabilized emulsions. Presumably the transition metals ions were more strongly attracted to the surfaces of the lipid droplets in the NaCas-stabilized emulsions. In the presence of EDTA, the oxidation rate was actually lower in the emulsions stabilized by NaCas which was attributed to its higher ability to scavenge free radicals.

Droplet charge also affects the location and activity of antioxidants *via* attractive/repulsive electrostatic interactions. The activity of charged anti-oxidants is often improved when they are located at the surface of charged lipid particles because of electrostatic attraction. An anionic antioxidant (ascorbic acid) was more effective at retarding lipid oxidation in the presence of positively charged lipid micelles (Pryor *et al.*, 1993). A negatively charged antioxidant (Trolox C) had higher antioxidant activity in the presence of positively charged phospholipids (Barclay and Vinquist, 1993), while a positively charged anti-oxidant (spermine) had higher antioxidant activity in the presence of negatively charged phospholipids (Kogure *et al.*, 1993). Mei *et al.* (1999) found that emulsion droplets coated with an anionic surfactant (SDS) were less oxidatively stable than those coated by a non-ionic surfactant (Brij) in the presence of an anionic gallic acid. This phenomenon may be attributed to electrostatic repulsion of the anionic antioxidants away from the anionic droplets thereby making the antioxidants ineffective.

Controlling the electrical charge on emulsion droplets is therefore one of the most important potential means of impacting lipid oxidation in oil-in-water emulsions. If the droplets in an emulsion can be made to be neutral or positive,

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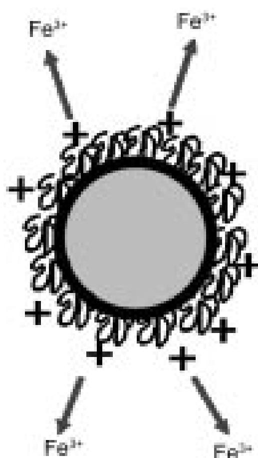

Fig. 9.2 Inhibition of iron-promoted lipid oxidation by a cationic emulsion droplet interface created by using proteins as an emulsifier at pH values below the pI of the protein.

then they are less likely to attract the cationic transition metal ions that frequently catalyze lipid oxidation in emulsions (Fig. 9.2).

9.4.3 Emulsion droplet interfacial thickness

Interfacial thickness can be manipulated by selecting emulsifiers with different molecular dimensions (e.g., molecular weights, conformations, head group sizes, or tail group sizes), or by using the LbL deposition method to deposit one or more biopolymer layers around droplets (Shaw *et al.*, 2007; Klinkesorn *et al.*, 2005a; 2005b; Djordjevic *et al.*, 2007). Multilayer emulsions are discussed in more detail later in this review.

Emulsifiers with large molecular dimensions can be used to form thick interfacial coatings around droplets that may protect against lipid oxidation. For example, the coating could form a barrier that decreases interactions between lipids and hydroperoxides or between lipids and aqueous phase prooxidants, e.g. transition metals (Silvestre *et al.*, 2000; Chaiyasit *et al.*, 2000). The influence of surfactant head-group size on lipid oxidation in salmon oil-in-water emulsions was studied using Brij 76 and Brij 700 as surfactants (Silvestre *et al.*, 2000). The results showed that Fe^{2+}-promoted decomposition of cumene hydroperoxide was lower in emulsions made with Brij 700 (10 times more polyoxyethylene groups than Brij 76), which was attributed to a thicker interfacial layer on the emulsion droplets. The effect of surfactant tail group size has also been studied using Brij-lauryl (12 carbon tail group) and Brij-stearyl (18 carbon tail group) (Chaiyasit *et al.*, 2000). This study suggested that surfactant tail group size played a minor role in lipid oxidation in oil-in-water emulsions, with increasing tail group size slightly increasing oxidative stability.

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9.4.4 Emulsion droplet interface permeability

Different surfactants have different packing properties at the oil-water interface depending on their molecular dimensions, which may impact the diffusion of oxygen, free radicals, and prooxidants through them (Villiere *et al.*, 2005). One might expect that an interfacial layer where the emulsifier molecules were closely packed or cross-linked would provide more resistance to molecular diffusion into or out of the droplets. Kellerby and coworkers (2006a) cross-linked casein on the interface of menhaden oil-in-water emulsions with transglutaminase which resulted in a cohesive interfacial protein layer that could not be removed from the emulsion droplet by Tween 20. Although the interfacial casein was cross-linked, these emulsions did not show increased oxidative stability when compared to untreated emulsions. In another study, O/W emulsions stabilized with β-lactoglobulin were heated to induce disulfide cross-links that produce a cohesive protein interfacial layer. In this case, cross-linking the protein had no impact on the ability of iron to decompose lipid hydroperoxides (Kellerby *et al.*, 2006b). These studies suggest that cohesive protein layers at the emulsion droplet surface do not increase oxidative stability which could be due to the protein interface still being highly porous thus allowing iron to diffuse through the anionic emulsion droplet interface where it can react with lipid hydroperoxides. The impact of the density and packing behavior of small molecular surfactants in O/W emulsions is unknown.

9.4.5 Emulsion droplet interfacial chemical composition

The chemical composition of the interfacial layer surrounding lipid droplets may influence the oxidative stability of food emulsions because of its ability to participate or alter lipid oxidation reactions, e.g., by scavenging free radicals, chelating transition metals, or interfering with hydroperoxide–transition metal interactions (Villiere *et al.*, 2005; Haahr and Jacobsen, 2008). It is therefore possible to control lipid oxidation by controlling the chemical composition of the interfacial layer surrounding the droplets, e.g., by selecting appropriate emulsifiers and/or by adsorbing other materials onto the droplet surfaces. Very little research has been done in this area with the exception of surface active antioxidant compounds which are discussed in more detail below. Rampon and coworkers (2001) reported that adducts between proteins and lipid oxidation products can occur at the emulsion droplet interface during oxidation. Headspace propanal concentrations have also been reported to decrease in protein-stabilized O/W emulsions, again suggesting interactions between lipid oxidation products in proteins at the emulsion droplet interface (Shen *et al.*, 2007). Finally, Leaver and coworkers (1999) found that casein isolated from the interface of an oxidized soybean oil-in-water emulsion exhibited an increased molecular weight which was suggested to be due to casein–lipid oxidation adducts. Since formation of lipid-protein adducts will decrease the volatility of oxidation products (Zhou and Decker, 1999), this could decrease the sensory perception of rancidity but may not actually be decreasing the rates of unsaturated fatty acid deterioration.

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9.5 Influence of antioxidants on lipid oxidation in emulsions

Antioxidants have been defined as 'any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate' (Halliwell and Gutteridge, 1990). In food, the definition of antioxidants was defined by Chipault (1962) as 'substances that in small quantities are able to prevent or greatly retard the oxidation of easily oxidizable materials such as fats'. Addition of antioxidants maintains the nutritional quality and prolongs the shelf life of lipid-containing foods (Halliwell *et al.*, 1995) with compounds that scavenge free radicals and/or chelate prooxidative metals being the most common food antioxidants (Frankel, 1998; Decker and McClements, 2008). The effectiveness of antioxidants in heterogeneous food systems such as emulsions depends on both chemical and physical factors such as overall antioxidant concentration; distribution of antioxidants in the oil, water and interfacial phases; interactions with other food components; and, environmental conditions such as pH, ionic strength and temperature (Frankel *et al.*, 1994; Mei *et al.*, 1998a; Frankel and Meyer, 2000; Xie *et al.*, 2007; Medina *et al.*, 2009). Antioxidants generally work by inhibiting the formation of new radicals and/or reducing the rate at which free radicals are formed. The two most common antioxidants in emulsions are free radical scavengers also known as chain breaking antioxidants and metal chelators.

9.5.1 Free radical scavenging antioxidants in oil-in-water emulsions

The effectiveness of a free radical scavenging antioxidant depends on its chemical reactivity and physical location within an emulsion, e.g. oil, water and interfacial regions (Porter *et al.*, 1989; Porter, 1993; Yi *et al.*, 1991; Koga and Terao, 1994; 1995). This is because an antioxidant should be present at the site of the lipid oxidation reactions to be effective. The effectiveness of antioxidants as a function of their physical location has been described by the antioxidant 'polar paradox' (Porter *et al.*, 1989; Porter, 1993; Frankel, 1998). In this hypothesis, polar antioxidants are more effective in bulk oils and non-polar antioxidants are more effective in oil-in-water emulsions. In bulk oils, polar antioxidants were thought to be more effective since they would accumulate at the oil–air interface. However, recent investigations have shown that due to the low polarity of air, polar antioxidants do not tend to accumulate at oil–air interfaces (Chaiyasit *et al.*, 2007). Therefore, an alternative hypothesis has been proposed to suggest that the increased effectiveness of polar antioxidants is due to their ability to accumulate at the interface of association colloid structures in bulk oils such as reverse micelles and lamellar structures. These association colloids can be formed by minor lipid components such as phospholipids and free fatty acids in the presence of the small amounts of water naturally found in bulk oils (Koga and Terao, 1994; 1995; Chaiyasit *et al.*, 2008).

The antioxidant 'polar paradox' also states that nonpolar antioxidants are more effective oil-in-water emulsions since they are more highly retained in the oil droplet where oxidation is most prevalent. This observation has been

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supported by studies showing that predominantly non-polar antioxidants (α-tocophorol, ascorbyl palmitate, carnosol) are more effective antioxidants than their polar counterparts (Trolox, ascorbic acid, carnosic acid and rosmarinic acid) in oil-in-water emulsions (Frankel *et al.*, 1994; 1996a; 1996b; Hopia *et al.*, 1996; Huang *et al.*, 1996a; 1996b).

Many researchers proposed that the ability of nonpolar antioxidants to be more effective in O/W emulsions was not only due to their ability to be retained within emulsion droplets but also their ability to accumulate at the oil–water interface where oxidation is most prevalent. Most effective antioxidant compounds have structures that allow them to act as surface active agents. In fact, antioxidants such as δ-tocopherol, α-tocopherol, TBHQ and propyl gallate have been found to accumulate at oil-water interfaces as measured by their ability to decrease interfacial tension (Chaiyasit *et al.*, 2007). The association of antioxidants with surfactants such as SDS and Brij has also been observed by NMR and EPR (Heins *et al.*, 2007a; 2007b). Gunaseelan *et al.* (2006) reported that 73% of α-tocopherol is located in the interfacial region of a Brij 30-stabilized octane-in-water emulsion.

Several recent studies have shown that not all antioxidants behave according to the polar paradox hypothesis, indicating that antioxidant activity in complex systems is more complicated than previously assumed (Laguerre *et al.*, 2009). This can be seen from a series of studies on the effectiveness of surface active antioxidant in O/W emulsions. The idea that the effectiveness of antioxidants could be improved by increasing their concentration at the oil–water interface prompted several researchers to synthesize surface active antioxidants by covalently attaching a lipophilic hydrocarbon chain onto antioxidants. Hunneche and coworkers (2008) found that the antioxidant activity of ferulic acid could be improved in O/W emulsions oxidized by metmyoglobin when the ferulic acid was attached to C11 or C12 hydrocarbons through a serine linkage. Medina and coworkers (2009) also reported an increase in the antioxidant activity of hydroxyltyrosol in O/W emulsions when it was esterified to hydrocarbon chains. However, when *p*-hydroxyphenylacetic acid (HPA) was conjugated with either a butyl or dodecyl group, both its retention in the oil droplet and it surface activity increased but its antioxidant effectiveness was less than free HPA in O/W emulsions (Yuji *et al.*, 2007). One potential reason for this could be due to the process of covalently attaching a hydrocarbon chain onto the antioxidant which could decrease its ability to scavenge free radicals. Therefore a subsequent study added C4, C8, or C12 esters of chlorogenic acid (CGA) to O/W emulsions at equal free radical scavenging activity (Sasaki *et al.*, 2010). Even under conditions of equal free radical scavenging activity, the antioxidant esters were not more effective than the free CGA even though they had higher surface activity and retention in the oil droplets. In another study, antioxidant activity in O/W emulsion did not increase when hydrocarbons from C1 to C8 were esterified to CGA. However, C12 CGA ester showed enhanced activity but further increasing the hydrocarbon chain length resulted in a decrease in the ability of the antioxidant to inhibit lipid oxidation even though

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more of these antioxidant oxidant esters where retained in the emulsion droplets (Laguerre *et al.*, 2009).

The overall results of these studies indicate that while the antioxidants polar paradox hypothesis has been a useful way to help understand antioxidant behavior in emulsions, polarity cannot be used to consistently predict the ability of an antioxidant to inhibit lipid oxidation in oil-in-water emulsions. This is likely because most antioxidants do not act alone and many antioxidants often have chemical properties that can promote as well as inhibit lipid oxidation (Alamed *et al.*, 2009). For example, some phenolic antioxidants are able to chelate iron while others like ferulic acid which do not have a galloyl moiety do not bind iron (Alamed *et al.*, 2009). This chelating activity could help explain why water-soluble polyphenols like those found in grape seed extract can strongly inhibit lipid oxidation in O/W emulsions (Hu *et al.*, 2004a). Another possible reason why polarity might not predict antioxidant activity in O/W emulsions is that many antioxidant compounds can participate in redox reactions with iron resulting in the formation of ferrous ions which are stronger pro-oxidants than their oxidized counterpart, ferric ions (Decker and McClements, 2001; Decker and Hultin, 1992; Mei *et al.*, 1999). Such prooxidant activity has been reported for ascorbic acid (Mahoney and Graf, 1986; Decker and Hultin, 1992) gallic acid (Mei *et al.*, 1999), caffeic acid (Sorenson *et al.*, 2008) and lycopene (Boon *et al.*, 2009). Difficulty in predicting antioxidant activity as a function of polarity could also be due to interactions between antioxidants. One example of this type of relationship is ability of ascorbic acid to regenerate oxidized α-tocopherol to reactivate α-tocopherol in biological membranes (Porter, 1993; Buettner, 1993). This could explain why the polar antioxidant propyl gallate was not effective in emulsion containing stripped corn oil (Schwarz *et al.*, 2000) but was effective in oil-in-water emulsions made with commercial corn oil (Alamed *et al.*, 2009). This difference could be due to the ability of propyl gallate to interact with antioxidants naturally found in the commercial corn oil.

The polar paradox is also complicated by the fact that the physical location of antioxidants can also depend on their electrical charge characteristics. Charged antioxidants are more water-soluble than their uncharged counter-parts, and therefore they may be located in the aqueous phase away from the site of action. In contrast, uncharged antioxidants often have low water-solubility, and are therefore located in the oil phase or at the oil-water interface which increases their antioxidant activities (Schwarz *et al.*, 1996; Huang *et al.*, 1999; Mei *et al.*, 1999; McClements and Decker, 2000). The electrical charge, physical location and therefore activity of an antioxidant that is a weak acid or base depends on solution pH (Mei *et al.*, 1999). When the pH is near the pK_a, the charge status and partitioning behavior of antioxidants alters (Wedzicha, 1988). Because of the myriad of physical and chemical properties of antioxidants, it is not surprising that a single hypothesis such as the polar paradox cannot singly predict the ability of a compound to inhibit lipid oxidation in emulsions.

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9.5.2 Transition metal chelators in oil-in-water emulsions

As discussed above, transition metals are strong prooxidants in food emulsions. Their prooxidative activity is related to their ability to react directly with triplet oxygen to form superoxide radical anion (a potential source of free radicals at low pH; Kanner and Rosenthal, 1992), decompose lipid hydroperoxides into free radicals *via* several redox cycling pathways (Halliwell and Gutteridge, 1990) and produce alkyl radicals by abstracting hydrogen from unsaturated fatty acids (Frankel, 1998). The latter pathway is believed to occur very slowly and thus may not be important in promoting lipid oxidation in foods (Reische *et al.*, 1998).

Metal chelators are the main strategy by which the food industry inhibits lipid oxidation due to transition metals. The antioxidant mechanisms of chelating agents include sterically preventing the metal from interacting with the oxidizable lipids and hydroperoxides, preventing metal redox cycling, preferentially binding the oxidized, less reactive state of the metal and decreasing metal solubility (Kanner *et al.*, 1987; Dunford, 1987; Graf and Eaton, 1990; Decker and McClements, 2001). In addition, chelators can inhibit lipid oxidation by changing the physical location of metals so they are not associated with lipids. For example, a study by Cho *et al.* (2003) showed that chelating agents can increase the transfer of iron from inside lipid droplets into the surrounding aqueous phase, thereby reducing the prooxidant activity of iron. EDTA was also able to remove iron from the surface of Tween 20 stabilized emulsion droplets (Mancuso *et al.*, 1999).

Since transition metals are toxic to most living systems, their reactivity is tightly controlled in biological tissues. Control of iron reactivity is primarily accomplished by proteins with highly specialized iron binding sites such as lactoferrin, transferrin, phosvitin and ferritin. Unfortunately, during the processing of foods many of the metal control mechanisms in biological tissues are destroyed and iron is released and iron can originate into the food from avenues such as water or processing equipment. A good example of this is phosvitin. Phosvitin is the major iron storage protein in egg yolk. Iron bound to phosvitin is largely inactive thus protecting the lipid in the egg. However, when egg yolks are used to produce mayonnaise, the low pH environment causes phosvitin to release its iron and promote lipid oxidation (Jacobsen *et al.*, 2001). Therefore, additional iron control is often needed which is accomplished by the addition of food additives such as polyphosphates, flavonoids, organic acids (e.g. citric acid) and ethylenediaminetetracetic acid (EDTA). These chelators can be effective on their own as well as in combinations with free radical scavengers since reduction in metal reactivity will decrease free radical generation and thus spare free radical scavengers from decomposition (Decker and McClements, 2001).

The most common metal chelators in foods contain multiple carboxylic acids such as EDTA, polysaccharides (e.g., pectin and alginate) and citric acid, or contain phosphate groups such as polyphosphates, phosphorylated proteins (e.g., casein and phosvitin) and phytate (Decker, 1998). The effectiveness of chelating

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agents can decrease with decreasing pH as their chelating groups become protonated and lose their metal binding activity. In addition, while many of these compounds have the potential to inhibit metal promoted lipid oxidation, not all are suitable as food additives since some could reduce the bioavailability of essential minerals (e.g., phytate).

9.5.3 Ethylenediaminetetraacetic acid

EDTA is a synthetic antioxidant that contains four carboxylate groups and two amine groups that can form strong complexes with metal ions. EDTA is an extremely potent antioxidant when present at concentration greater than the prooxidant metal concentrations (Mahoney and Graf, 1986; Mei *et al.*, 1998b; Mancuso *et al.*, 1999; Frankel *et al.*, 2002; Alamed *et al.*, 2006). However, when EDTA concentrations are less than prooxidant metals, it can accelerate lipid oxidation presumably by increasing the solubility of the metal without reducing its chemical reactivity (Mahoney and Graf, 1986; Decker, 1998; Jacobsen *et al.*, 2001; Frankel *et al.*, 2002).

9.5.4 Phosphates

Polyphosphates are also widely used as chelating agents in the food industry; however, they do have some limitations in emulsions such as poor chelating efficiency (Hu *et al.*, 2004b), poor stability (Li *et al.*, 1993), and a possibility of adversely affecting protein functionality (Sofos, 1986). In general, the chelating efficiency of phosphates increases with an increasing number of phosphate groups (Sofos, 1986). However, neither sodium tripolyphosphate nor hexametaphosphate were able to inhibit lipid oxidation in fish oil-in-water emulsions at pH 3 or 7. Phosphorylated proteins and peptides originating casein are natural polyphosphates that can be effective antioxidants when used in foods. They have polar domains that contain phosphorylated serine residues such as -SerP-SerP-SerP-Glu-Glu that can form complexes with calcium, iron and zinc (Baumy and Brule, 1988; Bennett *et al.*, 2000). Peptides from hydrolyzed casein are effective at retarding lipid oxidation in oil-in-water emulsions; however, this activity is likely to be due to both metal chelation and free radical scavenging by amino acids in the peptides (Diaz *et al.*, 2003).

9.6 Influence of other emulsion components on lipid oxidation

9.6.1 Influence of minor oil components on lipid oxidation in emulsions

Commercial, refined oils typically containing 0.05–0.70% free fatty acids (Chaiyasit *et al.*, 2007; Jung *et al.*, 1989; Pryor, 1976). Free fatty acids are well known prooxidants in bulk oils (Frankel, 1998) but until recently their impact on oxidation in O/W emulsions was unknown (Waraho *et al.*, 2009). Addition of oleic acid (0–5.0% of oil) to O/W emulsions increases both the negative charge

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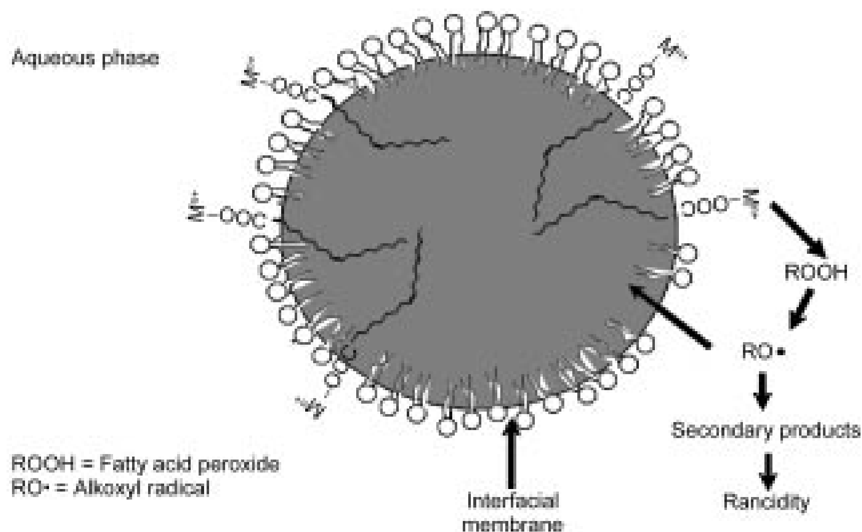

Fig. 9.3 The proposed mechanism for free fatty acid promoted oxidation on emulsified oil droplets. M^{n+} = transition metal.

of the emulsion droplets and the formation of lipid hydroperoxides and headspace hexanal. Methyl oleate did not increase oxidation rates and droplet charge and EDTA strongly inhibited lipid oxidation. These data suggest that free fatty acids are able to migrate to the emulsion droplet surface where they make the droplet anionic allowing attraction of transition metals that promote oxidation (Fig. 9.3).

Phospholipids are widely reported to inhibit lipid oxidation in bulk oils. Their antioxidant activity has been attributed to their ability to chelate metals, increase the partitioning of other antioxidants at the oil–water interface and form complexes with lipid oxidation products that reduce the volatility of the compounds that cause rancidity. When phospholipids are at the interface of the emulsion droplet they generally accelerate lipid oxidation by producing an anionic interface (Shaw *et al.*, 2007; Klinkesorn *et al.*, 2005a; 2005b). The impact of phospholipids in the oil droplet has recently been evaluated in our lab in a Tween-20 stabilized stripped soybean oil emulsion. In this emulsion system, the addition of dioleoyl phophatidylcholine (0.3 μmol/kg oil) to the emulsion did not change the droplet zeta potential suggesting that the higher surface activity of the Tween 20 prevented the anionic phospholipid from accumulating at the droplet surface. However, the dioleoyl phosphatidylcholine did inhibit lipid oxidation as determined by headspace hexanal (Fig. 9.4). The mechanism of this inhibition is currently under investigation.

Phytosterols are also an important minor component of commercial, refined oils. No studies have been conducted to determine if varying phytosterols impact lipid oxidation rates in O/W emulsions. However, Cercaci and coworkers (2007) found that phytosterols oxidize faster in emulsions than bulk oils. This was

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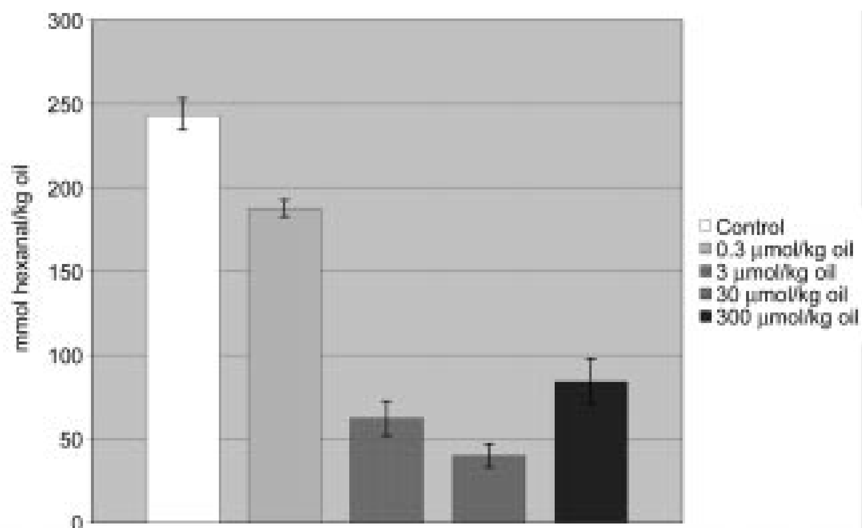

Fig. 9.4 Ability of phosphatidylcholine (0.3 to 300 μmol/kg oil) to inhibit lipid oxidation in stripped soybean oil-in-water emulsions as determined by headspace hexanal concentrations after 8 days of storage at 6ºC in the dark.

postulated to be due to the surface activity of phytosterols which allowed them to concentrate at the oil–water interface where they were more susceptible to oxidation.

9.6.2 Influence of continuous phase proteins on lipid oxidation in emulsions

There is growing interest within the food industry in replacing synthetic food additives with more natural alternatives, which is mainly driven by consumer concerns. Proteins are considered to be natural food additives that are generally recognized as safe (GRAS), which are commonly added to stabilize food because of their ability to adsorb to oil droplet surfaces and prevent droplet aggregation (McClements, 2005). As discussed earlier, these adsorbed proteins form a thin coating around the lipid droplets that can help inhibit lipid oxidation when they are positively charged (pH < protein pI) and can repel cationic transition metals when they form thick interfacial layers that inhibit transition metal–lipid interactions.

When proteins are used to make emulsions, they absorb to the surface of the lipid droplet until the droplet surface is saturated with excess protein partitioning into the continuous phase (Faraji *et al.*, 2004). A number of studies have shown that these continuous proteins (e.g., whey protein isolate, sweet whey, casein, β-lactoglobulin, lactoferrin, and soy protein isolate) are capable of inhibiting lipid oxidation in O/W emulsions (Taylor and Richardson, 1980; Tong *et al.*, 2000; Hu *et al.*, 2003; Faraji *et al.*, 2004; Elias *et al.*, 2005; 2006; 2007). Faraji *et al.* (2004) found that continuous phase proteins had good antioxidant activity at

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pH 7 but not at pH 3. This was found to be due to greater binding of cationic transition metals to anionic proteins at neutral pH. This binding activity can inhibit lipid oxidation by limiting the access of metal to the lipid or decreasing metal reactivity. This study highlighted the importance of solution pH in determining the antioxidant capacity of proteins since metal chelation would only occur when the proteins are anionic.

In addition to metal chelating, proteins contain appreciable amounts of amino acids that act as free radical scavengers, e.g. tyrosine, cysteine, and tryptophan (Taylor and Richardson, 1980; Ostdal *et al.*, 1996). The influence of free radical scavenging by β-lactoglobulin in surfactant-stabilized oil-in-water emulsions was studied by Elias *et al.* (2005). Surface exposed antioxidant amino acids (cysteine and tryptophan) were found to be preferentially oxidized prior to the emulsified lipids, suggesting that they were protecting the lipid from oxidation.

The antioxidant activity of globular proteins is often increased by changing their conformation by thermal processing or enzymatic hydrolysis since this exposes antioxidant amino acid residues that are usually buried in the protein interior (Taylor and Richardson, 1980; Tong *et al.*, 2000; Elias *et al.*, 2005; 2006; 2007; Pena-Ramos *et al.*, 2004; Peng *et al.*, 2009). Taylor and Richardson (1980) found an increase in the antioxidant activity of various milk fractions containing whey proteins after heating. The antioxidant activity of the whey proteins decreased after a sulfhydryl blocker (iodoacetic acid) was added to the emulsions, which suggested that the sulfhydryl groups from whey protein played an important role in the antioxidant mechanism. A later study by Tong *et al.* (2000) on the impact of non-adsorbed whey protein fractions (WPF) on lipid oxidation in salmon oil-in-water emulsions attempted to identify the physico-chemical origin of the protein's antioxidant activity. These workers found that whey proteins could act as free radical scavengers and chelating agents, and that cysteine and tyrosine were particularly important for this function.

Elias and coworkers (2006) found that when whey proteins were subjected to limited enzymatic hydrolysis the antioxidant activity in emulsions increased dramatically. This increase was attributed to the exposure of antioxidant amino acid residues which increased both free radical scavenging and metal chelating activity. These studies suggest that proteins and especially hydrolyzed proteins have excellent potential as food antioxidants. However, many peptide fragments produce strong bitter tastes that could limit their utilization in foods.

9.6.3 Influence of polysaccharides on lipid oxidation in emulsions

Polysaccharides are widely used as functional ingredients in food emulsions because of their ability to thicken, gel, or stabilize them (Matsumura *et al.*, 2003; Kishk and Al-Sayed, 2007). Many polysaccharides have also been found to have antioxidant activity in oil-in-water emulsions, which has been attributed to mechanisms such as free radical scavenging, transition metal binding, and viscosity enhancement (Shimada *et al.*, 1992; 1994; 1996; Matsumura *et al.*, 2003; Paraskevopoulou *et al.*, 2007; Chen *et al.*, 2010). On the other hand, some

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polysaccharide ingredients may increase the rate of lipid oxidation because they contain high levels of transition metal impurities, such as iron (Katsuda *et al.*, 2008).

Xanthan gum was found to act as an antioxidant in soybean oil-in-water emulsions, which was attributed to its ability to bind Fe^{2+} ions at anionic pyruvate sites along the polysaccharide chain (Shimada *et al.*, 1992). Another study by Shimada *et al.* (1996) suggested that the ability of certain polysaccharides to inhibit lipid oxidation in oil-in-water emulsions may be due to an increase in continuous phase viscosity, thus lowering the diffusion rate of oxygen and oil droplet collision probability. Nevertheless, it is important to distinguish between the macroscopic and microscopic viscosities of biopolymer solutions. A biopolymer solution may have an extremely high macroscopic viscosity, but the molecular diffusion of small molecules (like oxygen, free radicals or transition metals) is not restricted because of the large space between the polysaccharides chains in solution. In other words, the microscopic viscosity of the aqueous phase is not greatly increased by the presence of the polysaccharide. Indeed, studies have shown that pullulan and maltodextrin can greatly increase the viscosity of O/W emulsions but do not greatly retard lipid oxidation rate, while the glycoproteins, gum arabic and soluble soybean polysaccharides (SSPS) do not greatly increase the viscosity but have good antioxidant activity (Matsumura *et al.*, 2000; 2003). SSPS was found to suppress the initiation stage and the breakdown of lipid hydroperoxides more efficiently than gum arabic, which was attributed to the presence of more antioxidant amino acids on its protein component. The same group found that pectin had a stronger free radical scavenging activity than SSPS, but that its overall antioxidant activity was less (Matsumura *et al.*, 2003). Tragacanth gum has been shown to have the ability to act as a radical chain-breaker due to its ability to donate hydrogen atoms (Shimada *et al.*, 1992).

While polysaccharides can increase the physical stability of O/W emulsions by increasing viscosity they can also cause emulsion destabilization by causing depletion flocculation. Therefore, if polysaccharides are going to be used to inhibit oxidation, this must be accomplished at concentrations that do not negatively impact the physical stability of emulsions. Using emulsion physical stability as a selection criterion for the upper allowable concentration of polysaccharides (0.1 wt%), Chen and coworkers (2010) investigated the ability of continuous phase low methoxyl pectin, high methoxyl pectin, α-carrageenan and sodium alginate to inhibit lipid oxidation in polyoxyethylene (23) lauryl ether (Brij 35) stabilized O/W emulsions at neutral pH. All polysaccharides were able to inhibit lipid oxidation but low methoxyl pectin was the most effective. This was thought to be due to the higher ferrous binding capacity of low methoxyl pectin since none of the polysaccharides tested were effective free radical scavengers.

Some polysaccharides are surface active, and may therefore be located at the oil-water interface, rather than in the continuous aqueous phase. For example, gum arabic, modified starch and propylene glycol alginate are widely used by

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the food and beverage industries as functional ingredients to emulsify oils (Chanamai and McClements, 2002; Minemoto *et al.*, 2002; Djordjevic *et al.*, 2007; Paraskevopoulou *et al.*, 2007). These polysaccharide emulsifiers generally produce anionic emulsion droplets and therefore can increase oxidation rates. However, the high local concentration of these polysaccharides at the site of the lipid oxidation reaction may enable them to impact lipid oxidation reactions if they contain components that can act as antioxidants.

However, not all carbohydrates act as antioxidants. Some studies from Mabrouk and Dugan (1961), Mabrouk (1964), and Yamaguchi and Yamada (1981) showed that sugars such as pentose, hexose, and reducing disaccharides could act as strong prooxidants in methyl linoleate and linoleic acid in O/W emulsions. Another study from Yamauchi *et al.* (1984) showed that reducing sugars have the ability to reduce transition metal ions from Fe^{3+} to Fe^{2+}, which is a stronger prooxidant, thus accelerating lipid oxidation. On the other hand, the sugar alcohols have been shown to exhibit antioxidant activity in safflower oil emulsions (Sims *et al.*, 1979; Yamauchi *et al.*, 1982).

9.6.4 Influence of surfactant micelles on lipid oxidation in emulsions

Like proteins, when small molecule surfactants are used to stabilize emulsions, they absorb onto the emulsion droplet surface until the interface is saturated with the remaining surfactant partitioning into the continuous phase. Surfactant micelles form in the continuous phase of the emulsions when the concentration of surfactants exceeds the critical micelle concentration or CMC. While continuous phase surfactant micelles are too small to solubilize triacylglycerols they can solubilize smaller lipophilic or amphiphilic components, such as antioxidants and prooxidants, which alters their distribution between the oil, water and interfacial regions (Nuchi *et al.*, 2002, Cho *et al.*, 2002; Richards *et al.*, 2002). Overall, the presence of surfactant micelles in oil-in-water emulsions inhibits lipid oxidation (Richards *et al.*, 2002). Nuchi and coworkers (2002) found that surfactant micelles are able to solubilize lipid hydroperoxides and promote their movement from lipid droplets into the surrounding aqueous phase. The presence of surfactant micelles therefore could inhibit lipid oxidation in the emulsions by preventing the free radicals formed by decomposing hydroperoxides from attacking unsaturated lipids in the droplet core. A study from Cho *et al.* (2002) suggested that surfactant micelles could promote movement of lipid-phase iron out of oil droplets which could inhibit lipid oxidation. In contrast, a study from Richards *et al.* (2002) showed that Brij micelles could solubilize antioxidant out of the emulsion droplet into the continuous phase. Polar antioxidants such as propyl gallate and TBHQ were susceptible to micelle solubilization while the concentration of the non-polar antioxidant BHT in the emulsion droplet was not influenced by surfactant micelles. This study suggested that surfactant micelles could increase the oxidative stability of emulsions by removing antioxidants from the site of oxidation.

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9.7 Controlling lipid oxidation using structured emulsions

A number of researchers have examined the use of structured emulsions to overcome some of the limitations of using conventional emulsions to inhibit lipid oxidation. Some of the potential benefits of these systems are discussed below and their structures are shown in Fig. 9.5.

9.7.1 Multilayer emulsions

Multilayer emulsions are similar to conventional emulsions, but the interfacial layer surrounding the lipid droplets is engineered using a layer-by-layer deposition method. Multilayer oil-in-water (O_M/W) emulsions are composed of oil droplets dispersed in an aqueous medium, with each oil droplet being surrounded by a multilayer interfacial coating. This coating usually consists of emulsifier and biopolymer molecules. A major advantage of using multilayer emulsions is the ability to manipulate the properties of the interfacial layer surrounding the oil droplets, e.g. its chemical composition, charge, thickness, internal structure, permeability, rheology and environmental responsiveness (McClements *et al.*, 2007).

A multiple step process is usually used to prepare multilayer emulsions. A 'primary' oil-in-water emulsion is first prepared by homogenizing an oil and aqueous phase together in the presence of a charged water-soluble emulsifier. The primary emulsion contains small charged oil droplets dispersed in an aqueous continuous phase. A 'secondary' emulsion is created by adding an

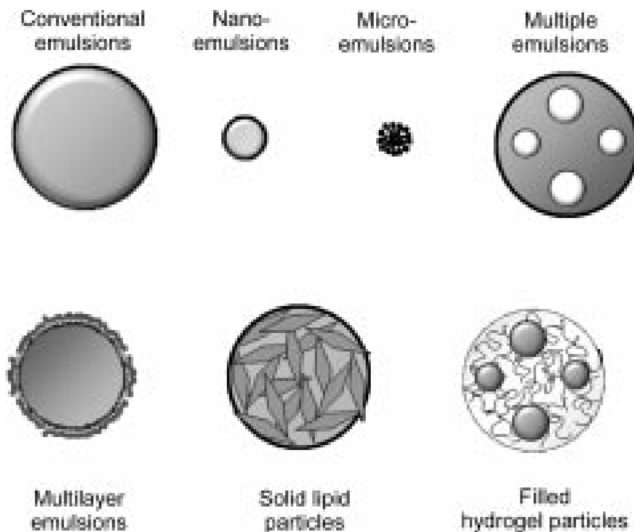

Fig. 9.5 Examples of different kinds of structured emulsion systems that can be utilized in foods.

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oppositely charged polyelectrolyte that adsorbs to the droplet surfaces, thereby producing a two-layer emulsifier–polyelectrolyte coating. The oil droplets can be coated by nano-laminated interfaces containing three or more layers by successive deposition of oppositely charged polyelectrolytes. Each polyelectrolyte layer can be deposited onto the droplet surfaces using either a one- or two-step mixing procedure as described below (McClements *et al.*, 2007):

- *One-step mixing*. An oil-in-water emulsion containing electrically charged droplets is mixed with an aqueous solution of oppositely charged polyelectrolyte molecules, leading to direct absorption of the polyelectrolyte onto the droplet surfaces through electrostatic attraction.
- *Two-step mixing*. An oil-in-water emulsion is mixed with a polyelectrolyte solution at a pH where the polyelectrolyte molecules do not absorb onto the droplet surfaces (e.g., where the droplets and polyelectrolyte are both negative). Then the pH of the solution is adjusted to change the electrical charge on the droplets and/or polyelectrolyte to allow the polyelectrolyte to adsorb onto the droplet surfaces via electrostatic attraction.

Any excess non-adsorbed polyelectrolyte molecules remaining in the continuous phase can be removed by a washing step done between each electrostatic deposition step, e.g. by centrifugation or filtration.

Emulsions stabilized by multiple layers of emulsifiers have the potential to increase the oxidative stability of lipids. As mentioned previously, emulsion droplets with thick interfacial membranes exhibit increased oxidative stability (Silvestre *et al.*, 2000), as do emulsion droplets with a cationic surface (Donnelly *et al.*, 1998; McClements and Decker, 2000). Stabilizing emulsion droplets with multiple layers of emulsifiers have the potential to inhibit lipid oxidation by forming both thick and cationic emulsion droplet interfacial membranes by using two or more emulsifier layers to increase thickness and by using a cationic biopolymer for the outermost layer to produce a positive charge. A number of studies have shown that lipid oxidation can be retarded in multilayer emulsions by making the net charge on the droplets positive (Shaw *et al.*, 2007; Klinkesorn *et al.*, 2005a; 2005b; Djordjevic *et al.*, 2007).

In studies on the oxidative stability of tuna oil-in-water emulsions stabilized by a multilayer system consisting of lecithin and chitosan it was found that emulsion droplets coated with only lecithin oxidized quicker than the combination of lecithin–chitosan as determined by measuring both lipid hydroperoxide and thiobarbituric acid reactive substances (Klinkesorn *et al.*, 2005a). The improved stability of the lecithin–chitosan emulsion compared with the lecithin emulsion is possibly due to cationic repulsion of iron and other prooxidative metals by the positively charged lecithin–chitosan emulsion droplet interfacial membrane. This trend was also seen in an emulsion stabilized by SDS and chitosan where the formation of thiobarbituric acid reactive substance (TBARS) was faster in the anionic SDS-stabilized emulsion compared to a cationic SDS–chitosan multilayer emulsion. Cationic SDS–chitosan layers are also able to inhibit the degradation of flavor oil components such as limonene (Djordjevic *et*

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al., 2007). Addition of pectin to add a third layer on to the emulsion droplets resulted in the formation of an outer anionic interfacial membrane. The oxidative stability of this tertiary emulsion was similar to the secondary, cationic, SDS-chitosan emulsion (Shaw *et al.*, 2008). Katsuda *et al.* (2008) found that anionic emulsions with β-lactoblobulin–citrus pectin multilayer had similar oxidative stability as the cationic emulsions stabilized with β-lactoblobulin alone. These data suggest that by increasing the thickness of the interfacial membrane of the emulsion droplet, it can be possible to overcome the prooxidative effects of an anionic emulsion droplet interface.

9.7.2 Filled hydrogel particles

Filled hydrogel particles consist of oil droplets contained within hydrogel particles that are dispersed within an aqueous continuous phase, and can therefore be referred to as oil-in-water-in-water ($O/W_1/W_2$) emulsions. The concentration, particle size distribution and spatial location of the oil droplets within the hydrogel particles can be varied. Also the properties of the hydrogel particles themselves can be varied, such as their composition, charge, digestibility, stability, permeability, and environmental responsiveness (McClements *et al.*, 2007).

In general, a variety of different methods can be used to form filled hydrogel particles (Pich and Adler, 2007; Chen *et al.*, 2006; Norton and Frith, 2001; Zhang *et al.*, 2007). These include methods based on phase separation of biopolymer solutions, injection of biopolymer solutions into gelling solutions, fragmentation of macroscopic biopolymer gels, and formation of biopolymer gels within double emulsions (McClements *et al.*, 2007). Typically, these methods include mixing a pre-formed oil-in-water emulsion with an aqueous solution containing one or more biopolymers, and then causing the biopolymers to phase separate and/or gel by altering the environmental conditions.

Filled hydrogel particles have been studied as a means of improving the physical and chemical stability of emulsified lipids. For example, Lamprecht *et al.* (2001) created filled hydrogel particles using an aggregative phase separation technique to encapsulate and protect ω-3 fatty acids. They found that the filled hydrogel particles hardened by ethanol were the most stable to lipid oxidation because the biopolymer shell could be formed to help prevent lipid oxidation as well as improve the physical stability of the emulsion. Wu *et al.* (2005) also used filled hydrogel particles to encapsulate fish oils while Weinbreck *et al.* (2004) used them to stabilize flavor oils. Filled hydrogel particles may be designed to retard lipid oxidation by concentrating antioxidant hydrogel molecules (such as proteins or polysaccharides) in close proximity to the encapsulated lipid droplets.

9.7.3 Microemulsions

In some respects microemulsions are similar to nanoemulsions, but in other respects they are quite different (McClements, 2005). Like nanoemulsions,

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microemulsions are comprised of oil, water and emulsifier molecules organized into small lipid particles dispersed within an aqueous continuous phase. In both cases, the lipid particles consist of a hydrophobic core (oil and non-polar parts of emulsifiers) surrounded by a hydrophilic shell (polar parts of emulsifiers and water). In addition, in both nanoemulsions and microemulsions the lipid particles are so small relative to the wavelength of light that they do not scatter light strongly, so that the overall system appears either transparent or only slightly cloudy. Nevertheless, microemulsions can be distinguished from nanoemulsions because the former are thermodynamically stable, whereas the latter are only kinetically stable. In addition, the emulsifier used in microemulsions is nearly always a surfactant (sometimes in combination with a co-surfactant), whereas the emulsifiers used in nanoemulsions may be surfactants, phospholipids or amphiphilic polymers (such as proteins). In microemulsions, the lipid particles consist of a shell of surfactant molecules whose non-polar tails tend to associate with each other within the inner hydrophobic core, while the polar heads are located at the exterior so that they are in contact with water. The major driving force for the formation of this structure is the hydrophobic effect, i.e., the tendency for the system to minimize the contact between oil and water. Oil or other lipophilic molecules present within a microemulsion may form a core at the center of the particle or they may be incorporated between surfactant tail groups.

In bulk lipids the rate of oxidation of fatty acids increases as their degree of unsaturation increases (Decker and McClements, 2008; Frankel, 1998). However, in microemulsions prepared with Tween 20 and free fatty acids, lipid oxidation rates show the opposite trend with oxidative stability increasing as their degree of unsaturation increases (Miyashita *et al.*, 1993; 1994). At present the reason for this observation is unknown, although it has been suggested that it be due to differences in the molecular arrangements of the fatty acids within the micelles. It is possible that the more unsaturated fatty acids are more deeply buried within the hydrophobic interior of the micelles and are therefore less susceptible to attack by aqueous phase prooxidants.

9.7.4 Nanoemulsions

Nanoemulsions are conventional emulsions that contain very small droplets, with the appropriate size range being defined differently by different authors, e.g. 1–100 nm (Mason *et al.*, 2006), 100–600 nm (Bouchemal *et al.*, 2004) or 400–800 nm (Sarker, 2005). In this chapter, we prefer to use a definition where nanoemulsions exhibit distinctly different bulk physicochemical characteristics to conventional emulsions, i.e. when the particle size becomes so small that they do not scatter light strongly and they appear clear, which generally occurs when the diameter is less 50 nm. Nanoemulsions are metastable systems that can be designed to persist for many months or years if they are stabilized appropriately, e.g. by reducing droplet aggregation and Ostwald ripening.

Nanoemulsions can be formed using high or low intensity methods. High intensity methods involve the application of intense mechanical energy to a

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system to break up the disperse phase liquid into smaller portions. The mechanical energy needed to break large droplets into small droplets increases as their initial radius decreases because of the Laplace pressure ($\Delta P = 2\gamma/r$) that tends to oppose particle deformation and rupture. Hence, only mechanical devices that are capable of generating extremely high intensity disruptive forces can be used to form nanoemulsions, such as sonicators and high-pressure homogenizers (especially microfluidizers). Sonicators use high-intensity ultrasonic waves to generate intense disruptive stresses (particularly cavitational, shear and turbulent forces) that break up the droplets (Landfester *et al.*, 2000). In microfluidizers, a premixed emulsion is divided into two streams that are forced through separate microchannels and then made to impinge on each other at high velocity, which generates intense disruptive forces (particularly strong extensional flow) that break up the droplets (Meleson *et al.*, 2004). Low intensity methods rely on the spontaneous phase separation of two immiscible liquids under certain conditions, leading to the formation of a dispersion of one liquid in the other liquid. These methods include the phase inversion temperature, emulsion inversion, and solvent displacement methods. The advantage of low intensity methods is that they require much less mechanical energy to form emulsions; however, they often require the use of organic solvents, synthetic surfactants or co-surfactants.

To the authors' knowledge, no papers have been published on the oxidative stability of nanoemulsions. This would be an important research area since it is possible that the extremely high surface area of nanoemulsions could cause rapid oxidation, which would severely limit the application of this technology in foods.

9.7.5 Solid lipid particles

Solid lipid particle (SLP) emulsions are similar to conventional emulsions because they consist of emulsifier-coated lipid droplets dispersed in an aqueous continuous phase. However, the lipid phase in SLP is either fully or partially solidified. The morphology and packing of the crystals within the lipid phase can often be controlled to obtain particular functional attributes (Saupe *et al.*, 2005; Souto *et al.*, 2004; Uner *et al.*, 2004; Wissing *et al.*, 2004; Wissing and Muller, 2002). As with conventional emulsions, the size and concentration of the lipid droplets can be controlled, as can the nature of the interfacial coating surrounding the lipid phase.

SLP emulsions can be formed using the same methods as for conventional emulsions or nanoemulsions depending on the particle size required, e.g., high or low intensity methods. The main difference is that the lipid phase (or at least a part of it) will be solid rather than liquid at the application temperature. To prepare an emulsion it is therefore necessary to heat the lipid and aqueous phases above the melting point of any crystalline material in the lipid phase prior to homogenization (Saupe *et al.*, 2005; Souto *et al.*, 2004; Uner *et al.*, 2004; Wissing *et al.*, 2004; Wissing and Muller, 2002; Schubert and Muller-Goymann,

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2005). It is then important to always maintain the temperature of the emulsion above the crystallization temperature of the highest melting lipid to prevent any fat solidification within the homogenizer. After formation, the emulsion can then be cooled in a controlled manner to promote crystallization of some or all of the lipids within the droplets.

Solid lipid particles may be able to improve the stability of chemically labile lipophilic components by trapping them inside structured solid matrices so the oxidatively sensitive lipids are protected from aqueous phase prooxidants and oxygen (McClements *et al.*, 2007). However, a study on the influence of carrier lipid physical state on the oxidative stability of octadecane oil-in-water emulsions found that methyl linolenate oxidized more quickly in emulsions containing solid droplets than those containing liquid droplets (Okuda *et al.*, 2005). It was postulated that crystallization of the octadecane caused methyl linolenate molecules to migrate from the interior of the lipid droplets where they were partly protected against oxidation, to the exterior of the lipid droplets where they were more exposed to water-soluble transition metals. If this approach is going to work, then it is important that the polyunsaturated oils are trapped within the interior of crystalline lipids, which may be achieved by controlling carrier oil composition, surfactant type and cooling conditions.

9.7.6 Multiple emulsions

The most commonly used water-dispersible multiple emulsions are water-in-oil-in-water (W/O/W) emulsions, which consist of small water droplets contained within larger oil droplets that are dispersed within an aqueous continuous phase (Garti, 1997a; 1997b; Garti and Benichou, 2004; McClements *et al.*, 2007) (Fig. 9.2). More accurately this type of emulsion should be referred to as a $W_1/O/W_2$ emulsion, where W_1 is the inner water phase and W_2 is the outer water phase, since the composition of the two water phases is usually different. There are also two different interfacial layers in this type of emulsion: the W_1-O layer surrounding the inner water droplets, and the O-W_2 layer surrounding the oil droplets. Therefore, two different types of emulsifier are normally used to stabilize W/O/W emulsions: an oil-soluble emulsifier is used for the inner water droplets and a water-soluble emulsifier is used for the oil droplets.

Usually, W/O/W emulsions are created using a two-step procedure. The first step is to create a W_1/O emulsion by homogenizing water, oil and an oil-soluble emulsifier, and then a $W_1/O/W_2$ emulsion is created by homogenizing the W_1/O emulsion with an aqueous solution containing a water-soluble emulsifier. Similar techniques to homogenize O/W emulsions can be used to produce $W_1/O/W_2$ emulsions such as high shear mixers, high pressure homogenizers, colloid mills, ultrasonic homogenizers and membrane homogenizers (McClements, 2005). However, the homogenization conditions used in the first stage are usually more intense than those used in the second stage, in order to avoid disruption or expulsion of the W_1 droplets formed within the oil phase. The size of the water droplets in the W_1/O emulsion and of the final $W_1/O/W_2$ emulsion

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can be controlled by varying emulsifier type, emulsifier concentration and homogenization conditions. Multiple emulsions have not been widely used in food products because they are highly susceptible to breakdown during processing, e.g., mechanical forces, thermal processing, chilling, freezing and dehydration, or during storage (McClements *et al.*, 2007). Nevertheless, recent advances in understanding the physicochemical basis of the stability of these systems are leading to more applications in foods being implemented.

As with nanoemulsions, very little work has been done on the oxidative stability of multiple emulsions. This could again be an interesting research area since multiple emulsions present an opportunity to incorporate water-soluble antioxidants into the interior of the emulsion droplet.

9.8 Conclusions

Lipid oxidation is a major problem leading to deterioration of polyunsaturated lipids, which needs to be prevented because it would cause undesirable changes in flavor, texture, appearance, and nutritional quality of food products. Since many lipid containing foods are in emulsified form, a thorough understanding of lipid oxidation mechanisms in emulsions should be developed in order to

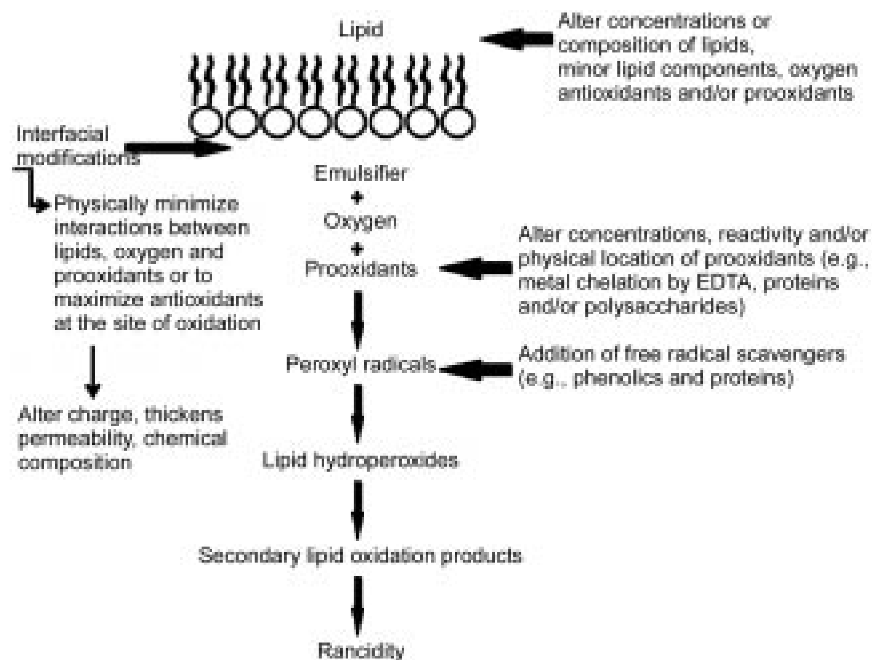

Fig. 9.6 Locations where the chemical and physical properties of oil-in-water emulsions can be altered to impact lipid oxidation reactions.

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develop innovative technologies to solve this problem. Numerous techniques can be applied to inhibit lipid oxidation in oil-in-water emulsions including interfacial engineering to control the composition, thickness or charge of the interfacial layer that separates the encapsulated lipids from the surrounding aqueous phase. The selection of antioxidants is also important to ensure that they are located at the major site of the lipid oxidation reaction. Due to the high susceptibility of emulsified lipids to oxidation, it might be necessary to use combinations of the techniques mentioned in this chapter to effectively retard lipid oxidation and improve the shelf-life, utilization and quality of food emulsion systems. For example, use of a combination of cationic interfacial membrane, emulsion droplet antioxidant (tocopherols) and metal chelators (EDTA) can be an extremely effective way to protect omega-3 fatty acids in emulsions (Djordjevic *et al.*, 2004). A summary of the strategies that can be used to impact lipid oxidation rates in oil-in-water emulsions is shown in Fig. 9.6. Structured emulsions, such as filled hydrogel particles, multilayer emulsions or solid lipid particles, may also be useful in protecting lipids against chemical degradation but further work is needed in this area to evaluate their effectiveness.

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10

Oxidation of confectionery products and biscuits

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Abstract: Both confectionery and biscuit products have, in general, long shelf-lives, often up to one year at ambient. Because of this the fats contained within the products need to have a high oxidative stability. The oxidative stabilities of the types of fats used in confectionery coatings (chocolate and compound coatings) and fillings as well as in nuts and nut pastes and biscuit doughs and creams are discussed along with the effects that changing the fat phase can have on oxidative stability. As well as oxidation, the problem of hydrolytic (or lipolytic) rancidity is discussed. The non-fat components in these products can also have positive and negative effects on their stability, as can the packaging of the product. Ways of improving the oxidative stability of confectionery and biscuit products by means of optimal ingredients, optimal processing and packaging are defined.

Key words: oxidative stability of chocolate, oxidative stability of compound coatings, oxidative stability of nuts, oxidative stability of biscuits, hydrolytic rancidity, lipolytic rancidity, milk crumb, active packaging.

10.1 Introduction

In the context of rancidity and oxidation, both confectionery products and biscuits provide interesting challenges to the food industry, not least because of the long shelf-lives generally associated with these products. An added complication is that they are often composed of a number of different fats and different fat-containing phases each with differing oxidative stabilities. We can divide this sector into a number of parts:

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- chocolate
- compound coatings
- confectionery fillings, including caramels and toffees
- nuts and nut pastes
- biscuits
- biscuit creams.

10.1.1 Chocolate

Chocolate has a closely defined legal composition in many countries. In the EU, for example, it is defined by Directive 2000/36/EC (European Union, 2000). As far as the fat phase is concerned it is composed of cocoa butter, milk fat and, in countries where these are permitted, added non-cocoa vegetable fat. While each of these fats exhibits different oxidative stabilities, other components such as the non-fat cocoa solids in chocolate also have an effect on its oxidative stability. This means that white chocolate, for example, which does not contain these non-fat cocoa solids, can be problematic in terms of its stability.

10.1.2 Compound coatings

Compound coatings are similar to chocolate in many respects apart from the composition of the fat phase (Talbot, 2006, 2009a). In these cocoa butter is replaced to varying extents by other fats which mimic the melting profile of cocoa butter but which are cheaper. Such fats range from cocoa butter equivalents (CBEs), which are very similar to cocoa butter, both chemically and physically, through non-lauric cocoa butter replacers (CBRs) to lauric cocoa butter substitutes (CBSs). The latter two types are distinguished by the presence in lauric CBSs of either palm kernel oil or coconut oil, both of which are rich in lauric acid (C12:0) whereas the non-lauric CBRs are produced from oils and fats rich in C16 and C18 fatty acids (palm oil, rapeseed oil, cottonseed oil, soyabean oil and so on).

10.1.3 Confectionery fillings

Confectionery fillings range from fat-continuous systems based on vegetable fat-based filling fats or nut pastes (e.g., hazelnut paste, peanut butter) or both, through fat-containing, though not necessarily fat-continuous systems such as caramels and toffees to essentially fat-free fillings like fondants and jellies. In the context of oxidative rancidity the fat-free systems will not be considered further.

10.1.4 Nuts and nut pastes

Nut pastes have already been mentioned in the context of fat-continuous fillings but both whole and chopped nuts (hazelnuts, peanuts, almonds, brazil nuts as

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well as more exotic nuts such as macadamia) are widely used both within chocolate products and as decorations.

10.1.5 Biscuits

Biscuits are also often composed of a number of different phases each with a different fat system. At the very basic level a plain biscuit contains a dough fat. Rather than being fat-continuous, the structure of a biscuit is such that these fats are dispersed throughout the product. They have the effect of interrupting the formation of gluten networks that would make the biscuit hard and 'flinty' and also maintain a semblance of an aerated structure in the biscuit by stabilising any air bubbles that are incorporated into the product during mixing. Both of these effects enhance what is termed a 'short' texture within the product. The greater the level of dough fat in a biscuit the 'shorter' is the texture, with products such as shortbread containing high levels of fat (usually greater than 23% and sometimes up to 30%). Most biscuit dough fats are based on vegetable oils (palm oil being the most common) although a 'premium' sector of the market uses butter or butterfat as a dough fat.

10.1.6 Biscuit creams

Biscuit creams are the (usually) fat-continuous filling between two biscuits or between three or four wafers. Although it is possible to use the same fat as in the dough for this application biscuit creams are often based on fats with sharper melting profiles and lower solid fat contents at mouth temperature. Traditionally biscuit creams have been based on palm kernel oil and its derivatives.

Biscuits are, of course, often coated in chocolate or a compound coating and so any comments made later in this chapter relating to these two materials in relation to confectionery also relate to their use on biscuits and wafers.

10.2 Oxidation and hydrolysis of confectionery products and biscuits

Although this book is primarily concerned with oxidation and oxidative rancidity, a second form of rancidity resulting from the hydrolytic breakdown of triglycerides is a significant problem in some confectionery and biscuit products, specifically those containing palm kernel oil or coconut oil. Because of this, hydrolytic rancidity (or lipolytic rancidity as it is sometimes known) will be described and considered in this chapter (see pages 352–3).

10.2.1 Confectionery coatings and fillings

In considering rancidity in confectionery coatings and fillings it is useful firstly to consider the composition of these. As mentioned in the introduction to this

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Table 10.1 Typical chocolate recipes (adapted from Yates, 2009)

	Dark	Milk	White
Cocoa mass	32.50	12.00	
Cocoa butter	15.50	21.40	27.20
Full cream milk powder		20.00	23.00
Sugar	51.38	45.99	49.19
Lecithin	0.60	0.60	0.60
Natural vanilla	0.02	0.01	0.01
Total fat content	33.4	33.5	33.5

chapter we can divide coatings into chocolate (which conforms to the required legislation) and compound coatings. Each of these can be further sub-divided into dark chocolate or coatings (containing no or very little milk fat), milk chocolate or coatings (containing higher levels of milk fat) and white chocolate or coatings (containing no non-fat cocoa solids, i.e. no cocoa powder). In terms of chocolate that generally is the main sub-division. Typical recipes for dark, milk and white chocolate are shown in Table 10.1.

In compound coatings, however, we can further sub-divide depending on the type of fat used in the coating. These are intended to mimic the physical properties, notably the melting profile, of cocoa butter. Some also mimic the chemical composition of cocoa butter, while others have a considerably different chemical composition.

To understand the differences it is necessary to first define the composition of cocoa butter. Cocoa butter is rich in three specific and very similar triglycerides – POP, POSt, StOSt.[1] Not only are these three triglycerides similar in their stereospecific chemical structure having two saturated fatty acid groups on the 1- and 3-positions but they are also similar in terms of their melting points. This gives cocoa butter its sharp melting characteristics.

Compound coatings can be based on CBEs, non-lauric CBRs or lauric CBSs. CBEs are also rich in POP, POSt and StOSt (like cocoa butter) but are produced from a range of vegetable fats (palm oil, shea butter, illipe butter, sal fat, kokum gurgi and mango kernel oil). Non-lauric CBRs have traditionally been produced from partially hydrogenated and fractionated oils such as palm oil, soyabean oil, rapeseed oil, etc. In recent years the move away from hydrogenation of oils because of nutritional concerns regarding *trans* fatty acids (Katan *et al.*, 1994) has meant that a new generation of non-hydrogenated non-lauric CBRs has been produced, often based on combinations of fractions of palm oil. Non-lauric CBRs have some similarity to cocoa butter in terms of their fatty acid contents (containing palmitic, stearic and oleic acids, as well as the *trans* elaidic acid in the hydrogenated types) but differ completely in the distribution of these acids on the triglyceride molecule giving them only a limited compatibility with cocoa

1. POP is 1,3-dipalmitoyl-2-oleoylglycerol; POSt is 1-palmitoyl-2-oleoyl-3-stearoylglycerol; StOSt is 1,3-distearoyl-2-oleoylglycerol.

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Table 10.2 Typical compound coating recipes (adapted from Talbot, 2006)

	CBE-based			Non-lauric CBR-based			Lauric CBS-based		
	Dark	Milk	White	Dark	Milk	White	Dark	Milk	White
Cocoa mass	32.5	20.0		10.0	10.0				
Cocoa butter									
Cocoa powder (10/12% fat)				15.0			14.0	5.0	
Full cream milk powder		20.0	23.0		6.0	20.0		10.0	
Skimmed milk powder					12.0	5.0	7.0	10.0	20.0
Sugar	51.6	42.6	49.6	46.6	43.6	44.6	47.6	45.6	47.6
CBE	15.5	17.0	27.0						
CBR				28.0	28.0	30.0			
CBS							31.0	29.0	32.0
Lecithin	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4

butter. Lauric CBSs are usually based on palm kernel oil after fractionation, hydrogenation or a combination of the two processes. Palm kernel oil contains about 50% lauric acid (Hargreaves, 1988) – hence the description of these fats as lauric CBSs. The fatty acid composition of these products is totally different from that of cocoa butter which means that they are effectively incompatible with cocoa butter. This is because cocoa butter, being a highly polymorphic fat, crystallises in a stable beta crystal form whereas lauric CBSs have a simpler crystal structure and crystallise in a beta-prime crystal form. When the CBSs are mixed with cocoa butter there is 'conflict' between the two types of fat crystal and only at the extremes of composition (i.e., where one or other component is present at greater than about 95%) is there sufficient of one crystal type present to ensure stability. Between these two extremes eutectics are formed resulting in softer products and a greater risk of fat bloom formation. Similar effects are observed when non-lauric CBRs are blended with cocoa butter, albeit to a slightly lesser extent. More detailed information on polymorphism of these fats and their (in)compatibilities is given by Talbot (2006, 2009a, 2009b). Some typical compound coating compositions are shown in Table 10.2.

Unlike chocolate, fat-continuous fillings are not covered by any compositional legislation and so can have a wide range of compositions. Three typical compositions, one with a fat phase of cocoa butter, milk fat and a vegetable fat, the other two also containing a nut paste (with and without added chopped nuts) are shown in Table 10.3. Caramels and toffees are effectively boiled emulsions and typically have an ingredient composition such as that shown in Table 10.4.

Effect of fat phase on oxidative rancidity

The major fat in chocolate is cocoa butter and cocoa butter generally shows a high oxidative stability. This is a result of both its fatty acid composition, i.e.

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Table 10.3 Typical fat-continuous filling recipes

	Chocolate-based filling	Hazelnut-based filling	Hazelnut-based filling with added nuts
Dark chocolate	25	15	15
Milk chocolate	25		
Vegetable fat[a]	50	22.5	22.5
Hazelnut paste		15	15
Full cream milk powder		10	10
Sugar		37.5	32.5
Chopped hazelnuts			5

[a] The vegetable fat used may differ from recipe to recipe

Table 10.4 Typical caramel formulation (adapted from Edwards, 2009)

Ingredient	%
Confectioner's glucose (42DE)	33.74
Full cream sweetened condensed milk	27.78
Brown sugar	22.82
Toffee fat	8.93
Butter oil	5.95
Salt	0.60
Vanilla flavouring	0.18

high in saturates (about 65%) and low in polyunsaturates (about 3%) as well as the presence of natural tocopherols, typically about 11 ppm α-tocopherol, 170 ppm γ-tocopherol and 2ppm α-tocotrienol (Hamilton, 2005) which act as natural antioxidants.

The degree of flavour stability in cocoa butter is often a function of the quality of the cocoa beans from which it was pressed. If these are damaged or of poor quality then the level of free fatty acid and peroxide value can be high and the processor then has little option but to, at least, deodorise the fat and, in some cases, fully refine it. This obviously then removes much of the desirable cocoa flavour along with the undesirable oxidation products.

A second point to bear in mind is that chocolate containing cocoa butter is usually conched. This involves stirring the chocolate while it is heated to temperatures up to 70 ºC, sometimes for several days for plain chocolate. The fact that it withstands this treatment without producing significant levels of rancid off-flavour is testimony to the stability of the basic fat.

Although plain chocolate containing cocoa butter is an important product, many consumers, particularly in the UK and USA prefer milk chocolate, which then brings a second fat, milk fat or butterfat, into the discussion.

Butterfat differs considerably from cocoa butter in its fatty acid composition, although not particularly in the degree of unsaturation. Cocoa butter typically

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contains 35–40% unsaturates depending on origin. Butterfat has a wider range containing, typically, 30–45% unsaturates, again depending on origin, season, animal feed, etc. The main difference is not then in the degree of unsaturation but in the types of saturated fatty acids present in butterfat compared with cocoa butter. There are much shorter chain length fatty acids in butterfat (from as low as C4) than are found in cocoa butter (mainly C16 and C18) and these contribute to a lower stability than is found in cocoa butter. In addition, on its own, butterfat is quite deficient in natural antioxidants – only about 17 ppm of α-tocopherol (Hamilton, 2005) – and care needs to be taken therefore to avoid contact with pro-oxidants such as heavy metals during its use. Fortunately, in chocolate, the relative instability of butterfat is more than counterbalanced by the natural antioxidants which are found in cocoa butter. Milk fat can be introduced into chocolate in a number of ways – as a direct addition of the fat itself, indirectly from the addition of, for example, full-cream milk powders, or as a result of its incorporation in milk crumb (see page 354).

One of the most common methods in the oils and fats industry to define the oxidative stability of fats is the Rancimat Induction Period. Timms and Stewart (1999) define a good quality deodorised cocoa butter as having a Rancimat Induction Period at 120 °C (RIP120) of at least 32 hours. Longer induction periods than this would normally be expected. Indeed Talbot (1996) found RIP120 times of 38 hours with an undeodorised poor quality cocoa butter and of almost 50 hours with a deodorised good quality cocoa butter. Addition of milk fat to these cocoa butters initially gave a slight increase in RIP120 with 10% addition but then a decrease as the level of milk fat increased to 20% of the fat phase. The deleterious effect was greater with the poor quality undeodorised cocoa butter (Fig. 10.1).

As well as building up the fat phase of a chocolate from dark to milk as described above, Talbot (1996) also studied the RIP120 of the fat phase of non-lauric compound coatings. Non-lauric CBRs, as already mentioned, have a

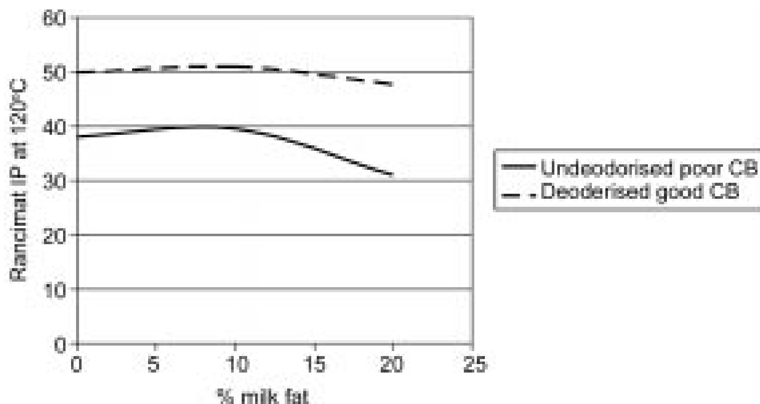

Fig. 10.1 Effect of milk fat on the RIP120 of cocoa butter.

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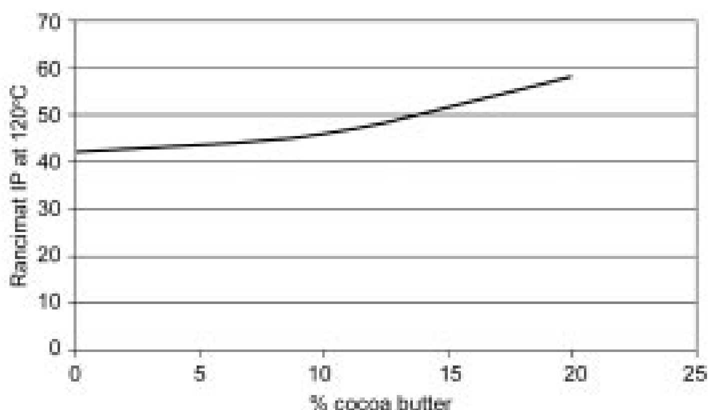

Fig. 10.2 Effect of cocoa butter on the RIP120 of a non-lauric CBR.

limited compatibility with cocoa butter which means that up to 20% cocoa butter can be incorporated into the fat phase of such coatings. Indeed the recipe shown in Table 10.2 for a dark coating based on a non-lauric CBR has a fat phase consisting of 80% CBR and 20% cocoa butter.

The type of non-lauric CBR fat that was studied had been partially hydrogenated and fractionated and was therefore very rich (of the order of 50%) in *trans* fatty acids. It is known that *trans* fatty acids have a higher oxidative stability than the corresponding *cis* acids and so one of the benefits of coating fats such as these is their high oxidative stability. When even the poor quality undeodorised cocoa butter was added to one of these fats the oxidative stability was further enhanced (Fig. 10.2) because of the natural antioxidants that were still present in the cocoa butter.

The other type of compound coating fat is based on palm kernel oil. Palm kernel oil itself, even though it is rich in saturates (83%, Hargreaves, 1988), has a relatively short Rancimat induction period – 12.8 hours at 100 °C has been reported (Rossell, 1994). When used as a compound coating fat, however, the oil is usually fractionated, increasing the total saturates to 91%, or fractionated and then fully hydrogenated, increasing the total saturates to 99% (Hargreaves, 1988) or, more importantly, reducing the unsaturates from 17% down to 9% and 1% respectively. When Talbot (1996) attempted to evaluate the effect on RIP120 of adding cocoa butter to a hydrogenated palm kernel stearine he found it impossible to make a measurement, not because the fat was highly oxidatively unstable, but because under the conditions of the test the shorter chain length fatty acids and triglycerides in the lauric fat atomised and blocked the equipment.

Because of their low level of unsaturates, oxidative rancidity of lauric fats is not a common problem but it should be remembered that these fats have very low levels of natural tocopherols. Coconut oil, for example, typically contains only 25 ppm tocopherols (Gunstone *et al.*, 1986). These low levels will be

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further reduced in the stearine fractions after fractionation so it may be prudent to protect such fats with extra additions of tocopherols. The main issue associated with these fats is that of hydrolytic rancidity (see below).

Effect of fat phase on hydrolytic rancidity

Hydrolytic (or lipolytic) rancidity could be considered something of a side issue in a book about oxidation because it is not oxidative rancidity as such but it is included in this chapter because of its importance in the stability of fats rich in lauric acid (palm kernel oil and coconut oil). Hydrolysis of fats occurs when fat, water and an active lipase are present and will occur with any fat, not just with lauric fats. It is with lauric fats, however, that the main flavour issues arise.

The hydrolysis reaction breaks down the triglycerides in the fat firstly to diglycerides, then to monoglycerides and finally to glycerol. At each stage a molecule of free fatty acid is released (Fig. 10.3). Depending on the chain length of the free fatty acids produced they have different flavour thresholds above which they are apparent as off-flavours (Table 10.5). Also depending on the chain length of the free fatty acids produced the nature of the off-flavour differs. Both palm kernel oil and coconut oil contain about 50% lauric acid so it is this fatty acid that is the main problem. It has a very objectionable soapy off-taste which is apparent at levels as low as 0.07%. Since it is only formed when both water and active lipase are present with the fat then clearly excluding one or other of these components will prevent its formation. Ishido *et al.* (2002) have

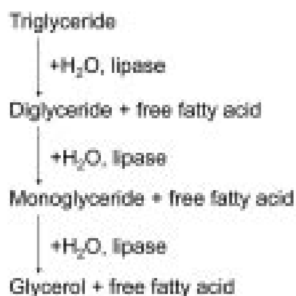

Fig. 10.3 Hydrolysis pathway.

Table 10.5 Flavour thresholds of free fatty acids liberated by hydrolysis (from Loders Croklaan, undated)

Free fatty acid	Flavour threshold (%)
Butyric (C4:0)	0.00006
Caproic (C6:0)	0.00025
Caprylic (C8:0)	0.035
Capric (C10:0)	0.02
Lauric (C12:0)	0.07

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shown that the presence of free fatty acids increases the rate of oxidation of fatty acid esters (in their case they studied linoleic acid with ethyl arachidonate) and so it could be expected that the free acids released by hydrolysis could also promote oxidation in these lauric fats. Strategies for avoiding this are outlined in Sections 10.4.1 and 10.4.2.

Effect of non-fat components

The main non-fat components in chocolate are the non-fat solids from cocoa (i.e., cocoa powder), the non-fat solids from milk (i.e., milk powders) and sugar. High cocoa solids[2] dark chocolate (i.e., chocolate containing greater than 70% cocoa solids) is increasingly being seen as nutritionally beneficial because of the polyphenols, particularly epicatechin, that are found in the non-fat solids of cocoa. These have *in vivo* antioxidant properties such that it has been speculated (Vinson *et al.*, 2006) that two 40 g bars per day of dark chocolate would significantly inhibit atherosclerosis, lower cholesterol levels and, in particular, lower levels of low-density lipoproteins (LDL) and triglycerides in the blood. Vinson *et al.* (2006) also estimate that the average level of consumption of polyphenols from chocolate in the United States is about 100–107 mg/day compared to a total consumption from fruits and vegetables of 588 mg/day. UK consumers eat almost twice as much chocolate as do US consumers and so the average intake of polyphenols will also be higher. Other research has shown that these phenolic components in cocoa inhibit oxidation of low-density lipoproteins by scavenging free radicals or sequestering metal ions. A diet containing 22 g cocoa powder and 16 g dark chocolate resulted in a LDL oxidation lag time about 8% more than with the 'average American diet' (Wan *et al.*, 2001).

Although something that acts as an antioxidant *in vivo* is not necessarily going to have the same antioxidative properties in a food product itself, it is likely that the high oxidative stability of chocolate comes not only from the stability of cocoa butter but is also enhanced by the presence of these components in the non-fat part of cocoa. This then raises the question as to whether there is any difference in the stability of white chocolate, which contains none of these components, and dark and milk chocolate, which contain them to varying amounts.

White chocolate has a particular light-sensitivity in terms of oxidation, especially if stored in a transparent packaging. Under such conditions rancid off-flavours are produced and this is also often accompanied by a colour change. It has been observed that replacing cocoa butter by CBE reduces the likelihood of this photo-oxidation occurring because CBEs contain much less of the photo-sensitiser, chlorophyll than does cocoa butter (Krug and Ziegleder, 1998a,

2. Cocoa solids are a combination of both cocoa butter and cocoa powder. These two ingredients can be included as separate components in chocolate or, more commonly they are sourced together from cocoa mass or cocoa liquor, the ground material from the nibs of roasted cocoa beans.

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1998b). In addition to this, the absence of antioxidant-rich cocoa powder in white chocolate also contributes to a lower oxidative stability. Chlorophyll-catalysed photo-oxidation and absence of cocoa powder would account for the formation of rancid off-flavours but what about the colour changes that can take place on storage of white chocolate? Vercet (2003) is of the opinion that this is due to non-enzymatic browning, which is also a common problem found in the storage of milk powders.

Milk powders are often used in milk chocolate and can be almost fat-free (as in skimmed milk powder) or can be carriers of milk fat (as in full cream milk powder). Milk powder can easily develop cardboard-like off-flavours. How these flavours arise is the subject of some debate and, although a Maillard-type reaction between the sugars and proteins in the milk powder has been suggested, it is more likely to be the result of fat autoxidation. The fact that adding antioxidants or storing milk powder under nitrogen delays the formation of these off-flavours (Hall and Lingnert, 1984) would support the latter hypothesis.

Because of the problems associated with, initially, storing fresh milk and, later, storing milk powders, milk crumb (or chocolate crumb) was developed. Crumb is produced by heating together milk, sugar and cocoa mass and then drying it to give a powder. Crumb was originally developed by Daniel Peters in Switzerland as a means of storing milk from times when it was plentiful to times of shortage. The first UK chocolate product to be made from crumb was Cadbury Dairy Milk in 1905. In the same year Hershey in the United States also produced his first milk chocolate bar based on crumb. These three countries (Switzerland, UK, USA) are still the largest users of crumb in milk chocolate, although other countries have since followed suit. Milk crumb has a typical shelf-life of about 24 months (Haylock and Dodds, 2009).

Although originally developed as a means of stabilising and storing milk, the production and use of milk or chocolate crumb brings other attributes to the chocolate. These are mainly flavour attributes. The process of heating milk, sugar and cocoa mass together imparts caramel-like flavours to the chocolate which account for the distinctive flavours of crumb-based compared with milk powder-based chocolates. The stability of crumb is generally attributed to the presence of antioxidants found in the cocoa mass, although the structure of the product is such that the fat droplets are trapped in an amorphous sugar matrix (Wells, 2009) and may therefore be protected from oxidative attack.

As well as milk powders it is possible, within the legislation of some countries, to add other powdered materials to chocolate such as whey powder or soya protein isolates. (It is important to check the relevant national regulations before producing chocolate with these components as they are not permitted in every country.) Selamat *et al.* (1998) evaluated the effect of soya protein isolates on the oxidative stability of milk chocolate by using increasing levels of soya protein isolates (up to 10% inclusion), to replace mainly full cream milk powder. Chocolate bars were evaluated weekly for up to 10 weeks storage at 21 ºC by peroxide value (PV). The changes seen in the PV are shown in Fig. 10.4. Although the authors claim that replacing full cream milk powder with 10%

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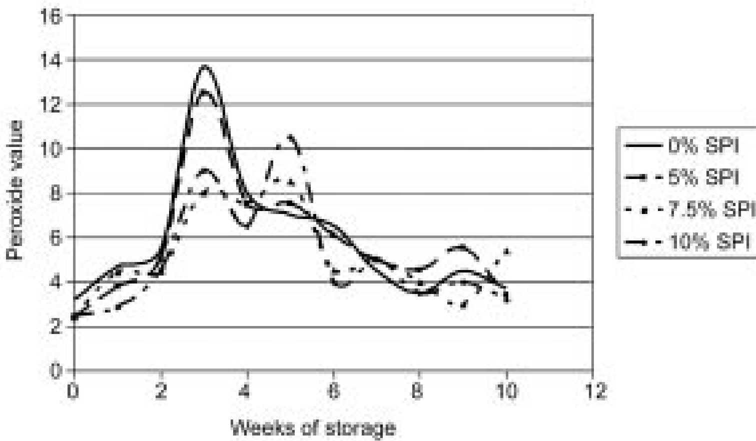

Fig. 10.4 Effect of soya protein isolate on the peroxide value of milk chocolate stored at 21ºC (adapted from Selamat *et al.*, 1998).

soya protein isolate improves the oxidative stability of milk chocolate it is difficult to see this from these results. We see the typical changes in PV of an initial increase as peroxides are formed followed by a decrease as they break down further into aldehydes and ketones. The peak PV (after 3 weeks) is certainly much lower with the addition of both 7.5% and 10% soya protein isolate than in the control or with 5% soya protein isolate but with increasing levels of soya protein isolate a second peak in PV is observed after 5 weeks. Also measured were anisidine values (AV) and Totox values.[3] These were no more convincing, with the peak AV values being higher for chocolates containing soya protein isolates compared with the control. What can be said, though, is that the use of soya protein isolates as partial replacers of full cream milk powder will not adversely affect the oxidative stability of chocolate.

10.2.2 Nuts and nut pastes

Microbial attack and autoxidation are the two main causes of rancidity in nuts, and the storage and handling of nuts is of great importance in ensuring that they retain their delicate and distinctive flavours. Microbial attack can be minimised by storing nuts at a water activity of less than 0.6.

Autoxidation of nuts is generally catalysed either by lipoxygenase, particularly in peanuts, or by metals with many nuts containing significant (around 10 ppm) levels of copper and iron. Roasting nuts reduces their oxidative stability, particularly if the nuts have been chopped prior to roasting. Kinderlerer and Johnson (1991) suggest that hazelnut kernels become rancid on storage

3. Totox values are an arithmetical combination of PV and AV: Totox = 2PV + AV.

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because of the formation of volatile aliphatic aldehydes, in particular hexanal and octanal both of which increased by a factor of ten on storage.

Because of its relatively high oxidative stability it might be expected that coating nuts in chocolate would help to protect them. Reed *et al.* (2000) suggest that this might not actually be the case. They coated two varieties of peanut (one with oleic acid content of 55–65%; the other with oleic acid content of up to 80%) in milk chocolate and white chocolate and stored these under uncontrolled room conditions. They compared them with uncoated peanuts stored at a_w of 0.19 and a_w of 0.60. After 30 weeks, the peroxide value (PV) of the oil extracted from the nuts with the lower level of oleic acid had risen to about 46 meq.O_2/kg oil in uncoated nuts stored at a_w of 0.19 but only to about 15 meq.O_2/kg oil in uncoated nuts stored at a_w of 0.60, suggesting that higher moisture levels helped to protect the nuts against oxidation. The nuts coated in both milk and white chocolate had oil with PV levels of about 30 meq.O_2/kg oil after 30 weeks storage – lower than uncoated nuts stored at a_w of 0.19 but higher than uncoated nuts stored at a_w of 0.60. The suggestion is that rather than protecting the nuts against oxidation, the chocolate coating is protecting them against moisture ingress and that the lack of moisture is accelerating the increase in PV. Much the same effects are seen with the nuts containing higher levels of oleic acid except that the increases in PV are much less (ranging from about 9 meq.O_2/kg oil in uncoated nuts stored at a_w of 0.19 through 3–5 meq.O_2/kg oil in nuts coated in milk or white chocolate down to about 2 meq.O_2/kg oil in uncoated nuts stored at a_w of 0.60).

Many reported studies on the stability of nuts have centred around their stability on storage but prior to use in the end-product. Although many confectionery products do contain either whole or chopped nuts many confectionery fillings contain nut pastes with peanut butter fillings being popular in the USA and hazelnut pastes being popular in Europe. One of the main issues with using these kinds of nut paste in filled confectionery is that the nut oils can migrate from the filling into the chocolate coating resulting in a softening of the coating but also, potentially, in a reduction in the oxidative stability of the coating as the level of unsaturates increases. To give a greater degree of structure to the filling to help minimise this oil migration harder confectionery fats such as cocoa butter are often added to the nut paste. While this is done for structural stability rather than oxidative stability reasons there is an added bonus that using cocoa butter in these centres also improves their oxidative stability.

Figure 10.5 shows the effects on RIP120 of adding up to 50% cocoa butter to peanut oil and hazelnut oil (Talbot, 1996). Although the absolute values differ with each oil, the trends are identical – adding cocoa butter enhances the oxidative stability. Unfortunately, the effects are not additive – if they were then a 50/50 peanut oil/cocoa butter blend would have an RIP120 of 20.5 hours instead of the measured 6.8 hours.

In many nut centres a non-lauric CBR is used in place of cocoa butter, partly because of cost and partly because, in some systems, its functionality can be better and processing of the filling made easier. The effect on RIP120 of peanut

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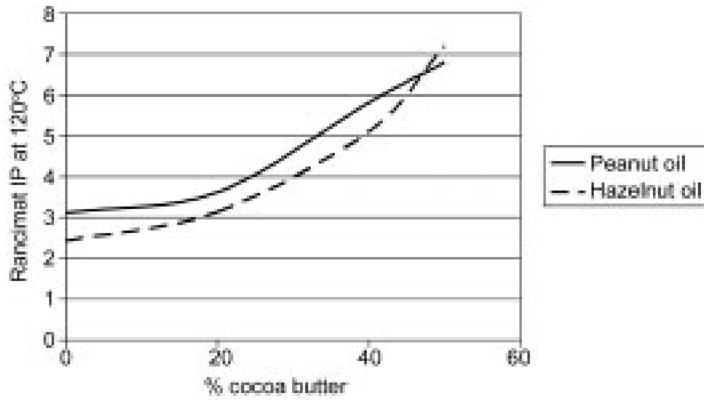

Fig. 10.5 Effect of cocoa butter on the RIP120 of peanut and hazelnut oils.

oil of adding a non-lauric CBR is shown in Fig. 10.6. The effects, both absolute and relative, are very similar to those found when cocoa butter was used to 'structure' the peanut filling.

However, even with the addition of 50% cocoa butter or non-lauric CBR, it is still not possible to achieve long Rancimat induction periods – certainly not as long as those found with the fat phase of the chocolate. Admittedly, such centres are often protected from oxidative attack by the totally enclosing chocolate coating. But what can a manufacturer do to further enhance the oxidative stability of these systems? One thing he can do is to further increase the level of hard fat. This has the disadvantage that the product loses much of its textural

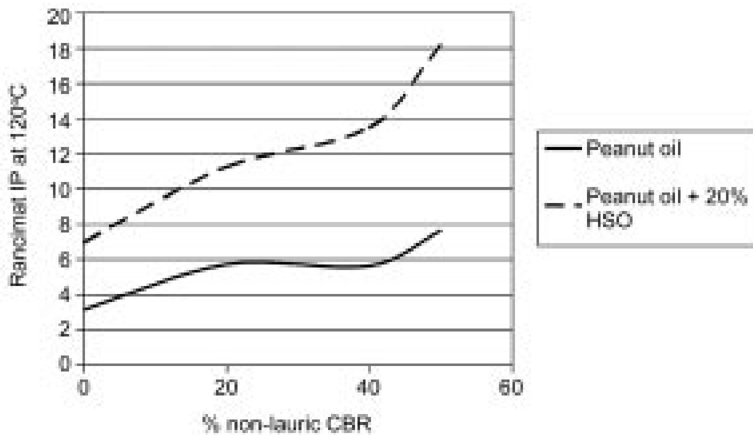

Fig. 10.6 Effect of non-lauric CBRs and high-stability oils on the RIP120 of peanut oil.

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contrast with the outer chocolate and also reduces the degree of nut flavour in the centre. If, however, the peanut filling is extended using a high stability vegetable oil, then the oxidative stability of the centre can be considerably enhanced while retaining the soft texture and, to a large extent, the intensity of nut flavours.

Figure 10.6 also shows the effect of doing this. The dashed line relates to a basic filling oil in which 20% of the original peanut oil has been replaced with Durkex™ 500, a high stability oil produced by Loders Croklaan, Wormerveer, The Netherlands. A direct comparison can be made between systems containing 60% peanut oil. Without the high stability oil the RIP120 is 5.6 hours; with 20% Durkex™ 500 present the RIP120 doubles to 11.2 hours.

10.2.3 Biscuits

Frampton (1994) quotes from a letter sent in 1931 to the UK Biscuit and Cracker Manufacturers' Association commenting that soapy rancidity (resulting from the hydrolysis of coconut oil in both biscuits and biscuit creams) was a common problem in the industry. He goes on to say 'fifty years on, rancidity is not a problem in the UK confectionery and biscuit industry'. Clearly, if that were completely true there would be no call for a chapter such as this. What is true, though, is that great changes have taken place in the biscuit industry to reduce the incidences of oxidative and hydrolytic rancidity in these products.

Much of this improvement had been achieved by a better selection of ingredients as well as by taking the approach of Hazard Analysis Critical Control Point (HACCP). In terms of oils and fats, Frampton highlights three improvements that had taken place in the fifty years following the 1931 letter. It is interesting to contrast these to the situation as it currently is in both the oils and fats and biscuit industries.

The first improvement that Frampton refers to is in hydrogenation, particularly in catalysts that assist in selectively hydrogenating highly unsaturated fatty acids such as linolenic acid (C18:3) to reduce these to levels below 1% and thereby improve oxidative stability. As well as improving oxidative stability of dough fats by removing these highly unsaturated acids, the oxidative stability was further improved by introducing *trans* fatty acids into the fat by hydrogenation. It is now known that *trans* fatty acids contribute to a higher risk of cardiovascular disease (Katan *et al.*, 1994) and, to a large extent, partially hydrogenated fats have been removed from biscuit and confectionery products.

Frampton (1994) also goes on to mention the use of hydrogenated marine oils as biscuit dough fats. These again have largely been replaced (as have animal fats such as beef tallow) in biscuits by vegetable oils. Changes in biscuit dough fats have, to some extent, been taken in a stepwise approach. Firstly, the hydrogenated marine oils referred to by Frampton were replaced by hydrogenated vegetable oils (often blends of unhydrogenated palm oil with up to 20% partially hydrogenated palm oil, rapeseed oil or soyabean oil). In more recent years, these dough fats have been replaced by non-hydrogenated systems,

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usually based on palm oil. In some instances palm oil itself is now used as a biscuit dough fat; in others, blends of palm fractions are used. The elimination of *trans* fatty acids, however, could have implications for the oxidative stability of the dough fat. According to Rossell (1994) palm oil has a Rancimat induction period at 100 ºC (RIP100) of 23.2 hours compared with a hydrogenated rapeseed oil with a slip melting point of 36/38 ºC of 207 hours. Although no information is available on the blend of the two fats, the addition of, say, 15% of the hydrogenated rapeseed oil to palm oil would almost certainly have given a significant improvement in RIP100. Even so, palm oil still has a greater oxidative stability than some of the more traditional biscuit fats such as coconut oil (RIP100 of 16.4 hours) and beef tallow (RIP100 of 9.5 hours).

In his conclusion, Frampton (1994) mentions a new area of risk – not the elimination of hydrogenated fats or *trans* fatty acids but the reduction in saturates. This is now happening with many governmental and international bodies saying that we are consuming too great a level of saturated fat (Food Standards Agency, 2007). One result of this, in the UK, is that the leading supplier of branded biscuit products, McVities, has launched new varieties of some of its plain biscuits containing 50% less saturated fat than before. The saturated fat that has been removed has been replaced by unsaturated fat. This, too, could have implications for oxidative stability.

This, though, considers only one of the improvements that Frampton (1994) refers to. The second one is the use of sequestrants and the reduction of heavy metals in refined oils and fats. It became commonplace for oils and fats processors to add small amounts (usually about 50 ppm) of citric acid to their refined products. This acts as a sequestrant for any heavy metal ions (iron, copper, nickel) that may be remaining in the oil. Sequestrants such as citric acid are still added to refined oils and fats and have, undoubtedly, helped in the stabilisation of lower-*trans* and lower-saturates dough fats.

Thirdly, Frampton (1994) mentions improvements in the refining and storage of oils and fats in terms of the use of activated bleaching earths, changing from mild steel to stainless steel tanks and nitrogen sparging of oils both in land storage tanks and in road delivery tankers. These improvements have continued in the intervening years and have also contributed to good oxidative stability in today's biscuit dough fats.

10.3 Effects of rancidity on sensory quality and shelf-life

As far as sensory characteristics are concerned the main effect of rancidity is on flavour. Chocolates have different flavour profiles, often dependent on the level of non-fat cocoa solids they contain. A high cocoa solids dark chocolate will often have a strong, rich, bitter taste which contrasts markedly with the creamy, milky taste of a fine milk chocolate or with the more caramel flavours in a crumb-based milk chocolate. White chocolate, on the other hand has hardly any cocoa flavour notes, the only cocoa flavour coming from unrefined cocoa butter,

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but it does have strong milky notes. A white compound coating which contains little, if any, cocoa butter will only have milky notes unless extra flavourings are added. Products of this type are often used as the basis of pastel-coloured, fruit-flavoured coatings and bars that particularly appeal to children.

Oxidative rancidity of the fats in chocolate will change these flavour profiles producing unpleasant off-flavours. Subramaniam (2009) monitored the changes in flavour that take place in milk chocolate stored for 75 weeks under different conditions. One of the changes observed was a loss in milk chocolate flavour (on a scale from 0 (no flavour) to 100 (high milk chocolate flavour)). Samples started with a score of 70 but fell to less than half of that if stored at high temperatures or high relative humidities for 13 weeks (see Fig. 10.7). 'Stale' flavours were found to increase (Fig. 10.8) over the same timescale from an original level of 20 (on the same kind of scale) to over 70 after 13 weeks. Not all of these changes will be as a result of oxidative rancidity. Subramaniam suggests that a change in the microstructure of the chocolate from fat-continuous to sugar-protein continuous may also be responsible for the loss of chocolate flavour. Lipases and lipoxidases are thought to be responsible for the production of 3,5-octadien-2-ones in stale milk chocolate (Ziegleder and Stojacic, 1988).

The other area of flavour degradation occurs as a result of hydrolytic rancidity in lauric fats. This, as has already been described, results in objectionable soapy off-flavours which effectively render the product inedible. Steps that can be taken to avoid this are described in Sections 10.4.1 and 10.4.2.

Finally, oxidative and non-enzymic browning are responsible for both flavour and colour changes in white chocolate which can also restrict the shelf-life. Restriction of shelf-life because of flavour changes is obvious; restriction because of colour changes is less so. If a consumer buys a bar of white chocolate

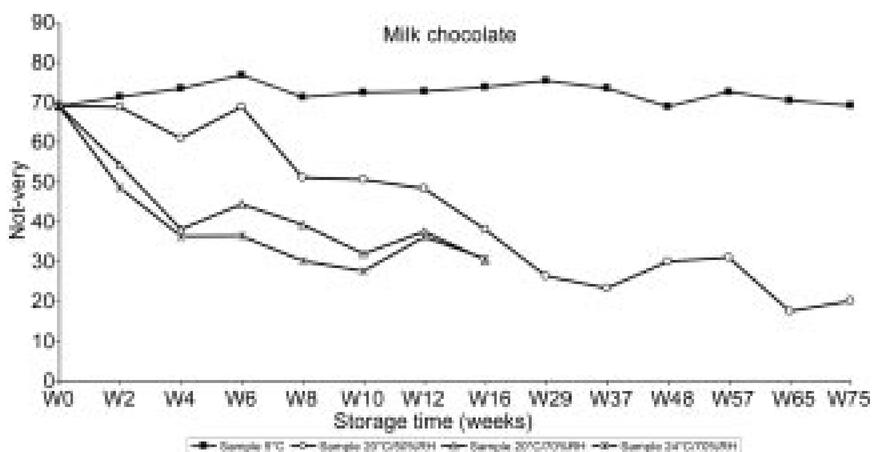

Fig. 10.7 Loss of milk chocolate flavour in a milk chocolate stored under different conditions (from Subramaniam, 2009, with permission from Leatherhead Food Research).

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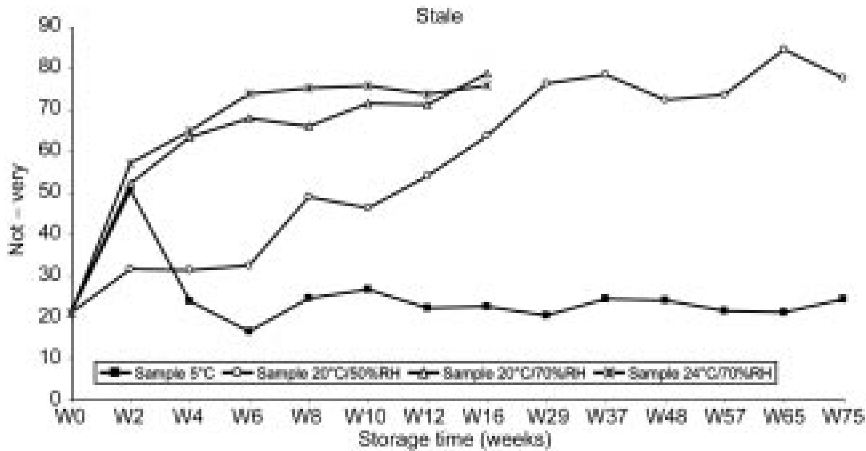

Fig. 10.8 Development of stale flavour in milk chocolates stored under different conditions (from Subramaniam, 2009, with permission from Leatherhead Food Research).

it would need to have undergone a significant degree of browning before they would find it unacceptable. If, however, it was presented alongside a fresher, whiter product on a store's shelf then the difference would be more obvious. The use of opaque packaging (see Section 10.4.3) would avoid both light degradation of the chocolate and also the ability to compare two products side-by-side on the shelf.

10.4 Protecting confectionery products and biscuits against oxidation

The problems of oxidative and hydrolytic rancidity in confectionery and biscuit products have been discussed but what can be done to protect these products against such attack? The easiest answer to that is to avoid the ingredients and situations that will promote rancidity – but that is not necessarily a particularly helpful response. The main areas where steps can be taken to protect products are in:

- ingredient selection
- process changes
- packaging.

10.4.1 Selection of ingredients

One of the most important aspects of ingredient selection lies in using oils and fats that have been well-refined and which contain low levels of heavy metal pro-oxidants, particularly iron and copper. Frampton (1994) proposes limits of maximum 0.05 ppm copper and maximum 1.0 ppm iron. Hamilton (2005)

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proposes even lower limits than these with a maximum copper content of 0.1 ppm and a maximum iron content of 0.1 ppm. Most oils and fats processors fall between the two recommendations and deliver oils with a maximum copper level of 0.05 ppm and a maximum iron content of 0.50 ppm.

All suppliers should deliver oils and fats to a peroxide value specification of maximum 1.0 meq.O_2/kg of oil. Different maximum free fatty acid levels are acceptable depending on the type of fat and its application. Generally speaking a maximum of 0.1% free fatty acid as oleic is acceptable for confectionery fats, dough fats and cream fats. However, because of the low threshold for detection of soapy off-flavours in lauric fats a maximum of 0.05% free fatty acid is more indicative of good quality. A similar free fatty acid specification would be desirable in the case of spray oils because of the greater risk of oxidative attack in such an application. It is known that lecithin has some water-binding properties and, for that reason, it is often added at low levels to lauric fats that are packed in boxes for longer-term storage. This is to remove any water that may go on to hydrolyse the fat phase so reducing the risk of soapy rancidity occurring in the boxed fat.

In terms of the non-fat ingredients in these products it is important, when using lauric fats whether this is in creams, fillings, caramels or biscuits to ensure that other ingredients in the mix are free of active lipase. Lipases are present in raw milk and although they are inactivated by the pasteurisation process it is important to specify that any milk components (powders, etc.) are free from active lipase. This is not to say that lipase-catalysed hydrolysis of fats is always to be avoided. The lipases present in raw milk catalyse hydrolysis and release of the short chain butyric, caproic and capric acids that give a creamy flavour to milk chocolate. Indeed a controlled lipase-catalysed hydrolysis of milk is used to give the flavours associated with some US milk chocolates.

In the area of ingredients it is perhaps relevant to mention the use of antioxidants. Many of the oils and fats used in confectionery contain quite high levels of naturally occurring tocopherols and tocotrienols. The most highly 'protected' of these is probably palm oil which typically contains about 560 ppm of tocopherols and about 420 ppm of tocotrienols (Hammond, 2005). However, it is also possible to add up to 500 ppm of additional tocopherols in the EU and US to further enhance stability.

In terms of biscuit products, particularly savoury biscuits, herb and spice extracts can be used to both give flavour to the product and to impart extra antioxidative effects. Rosemary, sage and oregano are particularly effective when used in this way. Hammond (2005) added various antioxidants to a standard biscuit dough fat blend that had an RIP100 of 54 hours. The addition of 300 ppm of various tocopherols and tocotrienols from soyabean, palm and rice increased the RIP100 to 62–69 hours, but the use of 200 ppm of oregano extract almost doubled the RIP100 to 102 hours. These effects also carried through to biscuits made from the fats. Biscuits made with a standard dough fat lasted for 40 weeks at ambient and 14 weeks at 30 ºC before unacceptable flavours were detected by an expert taste panel. Biscuits made with the standard dough fat

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containing oregano extract lasted for at least 45 weeks at ambient and 21 weeks at 30 ºC before being deemed unacceptable.

The problems of photo-oxidation in white chocolate have been mentioned earlier. Dulebohn and Carlotti (2004) have developed a process in which this is reduced by dispersing a mixture of 1.49 parts of lysine, 1 part of calcium oxide, 2.16 parts of malic acid and 0.72 parts of citric acid. This is added to white chocolate at a level of between 0.1% and 0.5% of the product.

It is also claimed (Sisterna, 2009) that certain sucrose esters normally used as emulsifiers can reduce the degree of oxidation observed in fudges. Fudges made using 0.2% lecithin and 0.4% Sucrosilk® were compared. After 2 months' storage the samples were evaluated by GCMS to determine the levels of hexanal produced as a result of oxidation. The GCMS peaks corresponding to hexanal were much higher in the samples made with lecithin than with the samples made with Sucrosilk®.

10.4.2 Effect of processing

One of the main areas where paying attention to processing conditions can pay dividends in reducing rancidity is in excluding unwanted moisture from lauric-based systems. The use of ingredients without any active lipase has been described in the previous section as a strategy to avoid soapy rancidity in lauric based components. Ensuring the absence of free moisture is another strategy. Moisture can arise in a number of ways, often as condensation. One of the main areas to watch out for is when products using lauric CBS coatings exit the cooling tunnel. Lauric CBS coatings can be cooled very quickly at quite low temperatures (often as low as 5–8 ºC). It is important, though, to ensure that the temperature at which they leave the cooling tunnel is above the dew point in that particular environment, otherwise moisture will condense on the surface of the coating and can cause hydrolysis particularly if it is then trapped within the packaging of the product by packing soon after leaving the cooling tunnel.

Another area where moisture can get trapped in close proximity to a lauric component is in enrobed sandwich biscuits. If the biscuit cream is lauric based and is again cooled quickly, moisture can also condense on the surface. If the product is then enrobed soon afterwards, this moisture can get trapped within the product and cause hydrolysis. Finally, it may seem obvious, but products should not be handled directly because even moisture from the fingers can be enough to result in hydrolysis. Always use gloves when handling these products.

Although the use of stainless steel is more common today there are still storage tanks used which are constructed of mild steel. The iron in these can act as a pro-oxidant. Of greater concern is the use of copper materials, particularly in the confectionery industry. It has been traditional to boil caramels in copper vessels and to 'pan' or coat small pieces in rotating copper pans. Padley (1994) mentions the presence of a fishy taint in caramels that contained 6 ppm of copper but that this only developed if a high level (1000 ppm) of tocopherol was also present. While it is well known that copper acts as a pro-oxidant, Padley goes on

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to question why it is that copper vessels have been used traditionally for boiling confectionery products without any obvious adverse effect and suggests that high levels of copper have an antioxidant effect by autoxidation chain termination.

Various methods have been developed for the protection of nuts, particularly before they are actually used in confectionery or biscuit products. One of these (Patterson *et al.*, 1996) is based on using new varieties of peanut that have higher oleic acid contents and lower linoleic acid levels thus making them more oxidatively stable. Lee and Krochta (2003) have proposed coating peanuts in whey protein isolates to form oxygen barriers and thus reduce oxidation in the nut.

10.4.3 Effect of packaging

However much care is taken with the selection of the correct ingredients or the correct processing, once it leaves the factory the product is very much at the mercy of the conditions under which it is kept. This makes the packaging of the product of vital importance. Packaging of confectionery products can be the subject of a chapter in its own right (if not a book in its own right!) so it is only possible to give an overview here. For more details the reader is referred to Jones (2009).

The main requirements of the packaging material as far as minimising oxidative or hydrolytic rancidity is concerned is for it to be impermeable to both oxygen and moisture. Polyester terephthalate (PET) has a low permeability to both of these, particularly when it is metallised. When combined with low density polyethylene (LDPE) the protection against moisture and oxygen is enhanced (Table 10.6).

Traditionally, aluminium foil with a paper wrap was used for confectionery packaging. This has now largely been superseded by flow-wrapping using flexible films. One of the earliest of these was regenerated cellulose film (RCF), often with an additional coating of, for example, polyvinylidene chloride (PVdC). This was an excellent material for automatic packaging but deteriorated faster than the plastic films that have now largely replaced it.

Table 10.6 Moisture and oxygen permeabilities of polyester terephthalate (PET)-based packaging films (from Anon, 2009)

	Water vapour transmission rate g/m^2, 24 hr, 38°C, 90% RH			Oxygen transmission rate ml/m^2, 24 hr, atm at 25°C		
	Flat	Folded	Crumpled	Flat	Folded	Crumpled
Met PET (12μ)	0.9	1.7	3.4	<1	5.5	16
Met PET/LDPE[a] (50μ)	0.5	0.6	0.7	<1	<1	2

[a] LDPE: low density polyethylene

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Of particular interest in terms of reducing rancidity in confectionery products is the new generation of active packaging. This uses sorbent technology to absorb oxygen and regulate moisture levels inside the pack so as to control both of these before they have the opportunity to adversely affect the product (Anon, 2008).

10.5 Future trends

As always, prediction of future trends is very difficult. I think there will be three, possibly four, main areas in which future improvements are likely to be seen. If we start with the possibility, this is in the area of further improvements in oil quality. The reason I see this as only a possibility is that the quality of products coming out of the oils and fats processors is already generally of a very high quality so any future improvements are likely to be small and difficult to achieve. Nevertheless, if they can be made, they will undoubtedly improve the stability of the refined oils even further.

The three areas with a greater likelihood of happening are:

- the use of natural antioxidants
- protection of inclusions such as nuts
- improved (active) packaging.

There is a general trend in the food industry away from synthetic towards the use of natural ingredients and additives. One of the areas where this is happening in a big way is in antioxidants. Natural antioxidants such as tocopherols have been used for a number of years but antioxidants based on herbs and spices (rosemary, sage and oregano in particular) are being increasingly used. One such additive that is starting to make a big impact in this area is the polyphenols from green tea. It is logical to assume, though, that further natural antioxidants will emerge both from antioxidant manufacturers and as a result of nutritional improvements to foods (i.e., from foods that have *in vivo* antioxidant properties).

Oxidatively sensitive inclusions such as nuts, particularly chopped nuts will benefit from better barriers to protect them from both oxidative and hydrolytic attack. Some of these (mainly based on protein films) already exist but it is likely that further improvements will be made, particularly in barriers made of composite components (protein + fat or protein + hydrocolloids).

Packaging continues to improve and further advances in film technology, especially in active packaging are likely. Also, in this area, there will be a push towards packaging with highly effective oxygen and water barrier properties but which are also recyclable or biodegradable.

10.6 Sources of further information and advice

The oils and fats processors and antioxidant manufacturers are excellent sources of further information and many of them produce information booklets

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summarising the benefits of their products. In terms of textbooks in this area the following are recommended:

- *Industrial Chocolate Manufacture and Use*, 4th Edition. Edited by ST Beckett. Published 2009 by Wiley-Blackwell, Oxford.
- *Science and Technology of Coated and Filled Chocolate, Confectionery and Bakery Products*. Edited by G Talbot. Published 2009 by Woodhead Publishing, Cambridge.
- *Rancidity in Foods*, 3rd Edition. Edited by JC Allen and RJ Hamilton. Published 1994 by Blackie Academic and Professional, Glasgow.
- *Application of Fats in Confectionery* by G Talbot. Published 2006 by Kennedy's Publications Ltd, London.

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11

Oxidation of cereals and snack products

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Abstract: A wide array of cereal and snack food products exists on the market. Many of these products have shelf lives as little as 90 days and up to a maximum of one year. As a result of this variability, designing methods to evaluate shelf life and oxidative stability is very much dependent on the product category. This chapter will cover oxidation products of cereals, the effect of oxidation on sensory quality, factors that affect the antioxidant compounds of cereals, and methods used to protect against oxidation. Information about mechanisms and analytical techniques will not be presented in this chapter.

Key words: cereals, lipid oxidation, lipoxygenase, hexanal, sensory.

11.1 Introduction

Cereal grains such as barley, maize (corn), oats, rice, and wheat make up a large part of the human diet, whereas millet, rye, sorghum and wild rice are other minor cereals consumed by humans. This chapter will focus heavily on the major cereal grains and products made from these grains. A wide array of cereal and snack food products exists on the market. Many of the these products have shelf lives as little as 90 days and up to a maximum of one year. As a result of this variability, designing methods to evaluate shelf life and oxidative stability is very much dependent on the product category. For example, a snack product typically has a shorter shelf life than a breakfast cereal. The literature on the oxidation of cereal and cereal products is not as extensive as that for oilseed. Much of the research on the oxidation of cereals that occurred prior to 1990 dealt with issues such as color loss and hydrolytic rancidity. In fact, much of the research in wheat, for example, dealt with bleaching of carotenoids and not lipid

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oxidation *per se*. Much of the research on rice dealt with hydrolytic rancidity. However, the role of lipoxygenase in oxidation and odor characteristics of cereals has been extensively studied. Thus, this chapter will cover some basic oxidation products of cereals in context to lipoxygenase, the effect of oxidation on sensory quality, factors that affect the nutritional and antioxidant compounds of cereals, and methods used to protect against oxidation. Information about mechanisms and basic analytical techniques will not be presented in this chapter.

11.2 Oxidation of cereals and snack products

11.2.1 Oxidation markers in cereals and cereal products

Understanding the fatty acid profile of the lipids present in cereal can aid in the control of the oxidation that exists in cereals and cereal-based products. Linoleic acid (LA) is the most predominant fatty acid in cereal lipids (Barnes, 1983; Mano *et al.*, 1990; Becker, 2008). Approximately 50–60% of the lipid from corn, barley, and wheat is LA. In addition, palmitic and oleic acids account for approximately 10–25% and 12–22%, respectively. The LA content in rice and oat lipid is approximately 35–40%. Other fatty acids in rice and oat lipid include oleic (35–46%) and palmitic (2–15%) acids.

Frankel and Meyer (2000) demonstrate the necessity for multiple approaches when evaluating antioxidants. Furthermore, they propose that test conditions, end products, and the target of the antioxidant be used for selecting appropriate methodologies to assess antioxidant activity. The multiple approach or selection of the specific method based on the system under investigation can be applied to measuring oxidation (Kanner and Rosenthal, 1992). Cereals and cereal products, or extracts of these materials, typically have a color due to lipophilic pigments such as carotenoids or phenolic compounds. Thus, methods that use spectrophotometry based on UV or titration with color endpoints should be supported with the additional measurements such as volatile analysis. In most cases, volatile profiling of oxidized cereal-based products is the most useful method to determine the shelf life or extent of oxidation. The oxidation products can be measured directly on the product without prior extraction of lipid. The added lipids extraction step can introduce errors into the stability evaluations and in some cases produce inconsistent results.

The production of raw cereal flours causes enzymes such as lipase and lipoxygenase to interact with lipid substrates in the grain (Galliard, 1983). The activation of rice lipase and hydrolytic rancidity has been well documented and is beyond the scope of this review. However, lipoxygenase-promoted oxidation may be the initiator of the oxidation of cereals. Lipoxygenase remains active in raw cereal flours and indirectly causes reductions in carotenoids (Borrelli *et al.*, 1999). Hydroperoxides such as 9-hydroperoxy-octadeca-10t, 12c-dienoic acid, 13-hydroperoxy-octadeca-9c, and 11t-dienoic acid can be formed during the oxidation of LA (Gardner, 1980; Galliard, 1983). Secondary oxidation products that include hexanal, pentane, 2-heptanone, 2-heptenal, and 2-pentylfuran

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(Heydanek and McGorrin, 1981a,b; Molteberg *et al.*, 1996a) are linked to lipoxygenase promoted oxidation. Frankel *et al.* (1981) also observed that hexanal was the predominant secondary oxidation product of the autoxidation and photooxidation of LA. Pentane and 2,4-decadienal were the other major products from LA autoxidation while 2-heptenal and pentane were other volatiles from the photooxidation of LA. Given that LA accounts for the majority of the fatty acids in cereals, volatiles such as hexanal and pentane (Frankel *et al.*, 1981; Frankel, 2005) would be predicted as the major volatiles formed during the oxidation of cereals and cereal products.

Hexanal was a good indicator of the oxidation of raw and processed cereals (Fritsch and Gale, 1977; Ekstrand *et al.*, 1993; Piggott *et al.*, 1991). Work completed on flaxseed fortified pasta showed that volatile markers measured by solid phase microextraction (SPME) were a good predictor of oxidation in pasta (Hall *et al.*, 2005). Klensporf and Jelen (2008) also reported that hexanal was a good indicator of oxidation in a muesli-type breakfast cereal. Paradiso *et al.* (2009) reported that aldehydes were the major volatiles in lab-prepared corn flakes store for one year. Hexanal accounted for 75% of the aldehydes but was not considered a marker for measuring oxidation of flaked cereals.

11.2.2 Oxidation of cereal and cereal-based products: general oxidation and shelf life

Barley and barley products

The uses of barley in foods include breakfast cereals, soup ingredients, and beer, to name a few. The oxidation of barley lipids during beer production has a negative impact on quality. Rutgersson *et al.* (2000) evaluated several hydrothermal processes as a means to increase shelf stability of barley. They reported that all steps involving steeping and thermal processing resulted in increased hexanal levels compared to intact raw barley kernels. The initial increase in hexanal was likely due to lipoxygenase, and subsequent oxidation was due to autoxidation during storage (Rutgersson *et al.*, 2000). In addition, high temperatures during the first steeping phase and low temperature during the second steep phase favored oxidative stability of hydrothermally processed barley.

Kuroda *et al.* (2003) reported that trans-2-nonenal was formed during malting and mashing processes. Lipoxygenase null barley has been shown to produce a beer that is free of the papery or cardboard off-flavor (Skadhauge *et al.*, 2005; Breddam *et al.*, 2005). Lipoxygenase-1 causes the conversion of linoleic acid into 9-hydroperoxyoctadecadienoic acid, which then undergoes degradation to trans-2-nonenal. The lipoxygenase null barley therefore does not produce the trans-2-nonenal and thus the papery or cardboard off-flavor in beer (Skadhauge *et al.*, 2005). Coghe *et al.* (2004) reported that dark malts reduced the hexanal levels in wort. Thus, intermediate compound formed from hexanal could be reduced. For an extensive review on the chemistry of beer aging see Vanderhaegen *et al.* (2006, 2007).

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Corn and corn products

Corn is typically cooked in alkali (i.e., nixtamalization) prior to being used in products such masa, tortillas and snacks. Masa is a paste-like material produced by grinding nixtamalized corn, which is often dried to make dry corn flour. Limited research has been published regarding the oxidation of products made from corn masa.

Lopez-Duarte *et al.* (2009) reported that LA oxidation could be used as a marker of corn flour oxidation. These authors reported that corn masa had a shelf life of approximately 84 days at 25 ºC based on mathematical models. The model was based on data collected from storage studies at temperatures between 45 and 85 ºC. Lopez-Duarte *et al.* (2009) used the peroxide value 2 meq/kg as a criterion for oxidation. They noted that 7 and 36 days were required for the corn masa to reach the 2 meq/kg level at 85 and 45 ºC storage, respectively. The *p*-anisidine between 11 and 14.3 mmol/kg were reported with the highest values being observed at 85 ºC storage condition. These values remained relatively constant for over the course of the 45 ºC storage but rapidly approached 100 mmol/kg in 20, 45, 65, and 85 days at storage temperatures of 85, 75, 65 and 55 ºC, respectively. Lopez-Duarte *et al.* (2009) determined that LA degradation occurred at a rate of 0.30%/day in the corn masa stored at 45 ºC while the rate of 1.47%/day was observed at the 85 ºC storage conditions. The LA degradation is likely the result of autoxidation process as enzymes would likely be inactive during the boiling process used to make corn masa.

Vidal-Quintanar *et al.* (2003) reported that the oxidative stability of tortillas was dependent on storage temperature and initial masa quality. They noted that masa flours with higher initial peroxide values and conjugated dienes also lead to reduced shelf life. However, mathematical modeling indicated that the dry masa flours were oxidatively more stable than the corresponding tortillas (Vidal-Quintanar *et al.*, 2003).

The volatile profile of masa dough indicated very little oxidation occurs during the preparation of masa dough. Karahadian and Johnson (1993) reported hexanal and 2-heptanone values of 27 and 29 ng/g fresh masa dough, respectively. The preparation of tortillas with this dough also had very few oxidation products. In fact, hexanal accounted for 62% of the volatiles in the tortilla. Spray dried masa flours had seven times more hexanal than freshly made masa. Increases in other volatiles associated with oxidation were also observed in spray dried masa flours (Karahadian and Johnson, 1993). Heat processing of corn germ inactivated lipoxygenase but increased the susceptibility of the germ to oxidation during storage (Gardner and Inglett, 1971). Thus, product containing corn germ could be predisposed to oxidation.

Destruction of hydroperoxides was observed in extrusion processing. Konstance *et al.* (2002) reported that peroxide values of raw feed material decreased significantly during the extrusion process of a corn-soy blend. The post extrusion drying temperature did not impact peroxide values of the dried extrudate.

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Oat and oat products

Heydanek and McGorrin (1981b) identified 45 compounds in 3-week old heat treated oat groats. Hexanal, pentanal, 1-pentanol, and 3,5-octadien-2-one were the most predominant compounds and were considered products of autoxidation. Heat processing dramatically improved the storage stability of oat flour. Hexanal and 2-pentylfuran levels were 10 and 36 times higher, respectively, in raw oat flour compared to heat processed oat flours after 42 days of storage (Molteberg *et al.*, 1996a). Other volatiles were also identified and were higher in the raw oat flour. The volatile 1-octen-3-ol was exclusively present in the raw oat flour (Molteberg *et al.*, 1996a).

Molteberg *et al.* (1995) and Lehtinen *et al.* (2003) in contrast found that a mild heat treatment promoted the formation of hexanal. The hexanal levels were 3–4 times higher in the heat processed oats compared to the control (Molteberg *et al.*, 1995). They also noted that storage at 80% relative humidity caused two times more hexanal than samples stored at 30% relative humidity. The oat flour containing a heat (90 ºC, 20 min) treated bran had five times the hexanal levels after 12 months of storage (Lehtinen *et al.*, 2003). The hexanal level was approximately seven times higher in flour containing extruded oat bran compared to the non heat treated bran enriched flour. Oat flour enriched with endosperm had more oxidation than the bran enriched flour (Lehtinen *et al.*, 2003). They proposed that the polar lipid fraction of the oat was more susceptible to oxidation after the heat treatment. Sjovall *et al.* (1997) reported that hexanal, 2-pentylfuran, decane, and nonanal were the major volatiles of the 27 identified in extruded oats. They also noted substantial increases in the hexanal content over the 18-week storage regardless of extrusion conditions. Nonanal levels in the oat extrudate from the high temperature extrusion also increased rapidly after 12 weeks of storage. Unfortified extruded oats were predicted to have a shelf life of approximately 6 to 8 weeks. Viscidi *et al.* (2004) estimated a shelf life of extruded oat cereal to be around 8 to12 weeks.

Rice and wheat

The volatile components of rice and wheat mirror those of other cereals. Shin *et al.* (1986) reported hexanal in the cooked brown rice stored at 5 and 35 ºC for up to 12 months. The amount of hexanal was highly correlated with the amount of oxidized linoleic acid. Piggott *et al.* (1991) reported that insufficient bran removal (i.e., under milling) from rice lead to lower sensory score and lower storage stability. The bran readily undergoes hydrolytic (Ramezanzadeh *et al.*, 1999a) and oxidative degradations (Ramezanzadeh *et al.*, 1999b; Lam and Proctor, 2003) thus supporting the lower sensory scores observed by Piggott *et al.* (1991).

Lam and Proctor (2003) observed dramatic volatile changes in partially milled rice over a 50-day storage. They noted that hexanal and 2-pentylfuran had the greatest impact on odor change at the beginning of storage, whereas 2-nonenal influenced odor change in the late stages of storage. Monsoor and Proctor (2004) reported higher pentanal, pentanol, hexanal, 2-pentylfuran, octanal, and 2-nonenal concentration in broken rice kernels compared to intact

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rice. Hexanal, pentanal, and pentanol were also observed in rice by Suzuki *et al.* (1999). They suggested that lipoxygenase was an important source of the volatiles and that the stale flavor characteristic was due to lipoxygenase. The lack of stale flavor in rice lacking lipoxygenase provided the basis for the involvement of lipoxygenase (Suzuki *et al.*, 1999).

Sjovall *et al.* (2000) reported hexanal, alpha-pinene, 1-hexanol, and 3-carene were the primary compounds formed during the initial weeks of storage of wheat germ. Sensory evaluation indicated that untreated germ was rancid by four weeks and heat treated samples were rancid at 7 weeks. Maeda *et al.* (2009) reported a total of 48 different volatile compounds in wheat. Hexanal, methanol, ethanol, hexanol, 1-octen-3-ol, 3-octen-2-one, (E,Z)-3,5-octadien-2-one, and (E,E)-3,5-octadien-2-one were consider products of the oxidation of polished flour lipids and possibly metabolites of molds.

11.3 Effects on sensory and nutritional quality

11.3.1 Sensory

The storage of raw cereal flours has a negative impact on the sensory and nutritional characteristics of the flour. Shearer and Warwick (1983) summarized the effect of storage on wheat flour lipids. Fritsch and Gale (1977) reported that the onset of rancidity in an oat cereal detected by sensory panelists occurred between 5 and 10 ppm hexanal. Warner *et al.* (1978) reported that hexanal and pentanal were good markers for measuring oxidation and were highly correlated to rancidity score measure by sensory panelists. The rancid odor of stored raw oat flour was attributed to a combination of volatiles that included hexanal, 2-pentylfuran, heptenal, and 1-octen-3-ol (Molteberg *et al.*, 1996a). In contrast, these volatiles were not highly correlated to the sensory characteristics of stored heat-treated oat flours. Molteberg *et al.* (1996a) suggested that heat inactivated lipoxygenase, causing fewer initial oxidation products that decomposed into volatiles responsible for the rancid odor. McEwan *et al.* (2005) reported that higher hexanal values in stored oatmeal did not translate into lower sensory scores. Although significant quality reduction occurred in the aged (28 years) oats, 75% of the panelists indicated that they would consume the oats in the form of oatmeal in an emergency situation. McEwan *et al.* (2005) suggested that the volatilization of the hexanal occurred during cooking of the stored oatmeal and therefore would have minimal impact on rancidity.

Vidal-Quintanar *et al.* (2003) reported that sensory scores of masa flour and tortillas stored at temperatures less than 25 ºC did not reach identifiable rancidity. In contrast, samples stored at 45 and 55 ºC had identifiable rancidity at 10 weeks. Iron biglycinate enhanced oxidation of corn meal and porridge resulted in reduced storage stability and sensory quality (Bovell-Benjamin *et al.*, 1999). Hexanal was highly correlated to the rancidity scores. Viscidi *et al.* (2004) reported that quercetin fortified oat cereal had better grassy and painty sensory scores and lower hexanal levels than the control. However, the hexanal

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was not the primary indicator as ferulic fortified oat cereal had hexanal levels that were not significant different from the control, but still had painty and grassy sensory scores better than the control. Guth and Grosch (1993) identified hexanal, 2,3-epoxyoctanal, pentanal, 2,4-nonadienal as the components responsible for the rancid off-flavor in oat extrudates that had been stored one year. Paradiso *et al.* (2008) reported that hexanal was not a good indicator of the oxidation of the rancid sensory perception. They noted that hexanoic acid provided a better indicator of rancidity in stored corn flakes. This conclusion was based on the higher hexanal and lower hexanoic acid levels in the tocopherol containing corn flakes compared to the control. Yet, the tocopherol containing samples had lower (i.e., better) rancidity scores than the control (Paradiso *et al.*, 2008). In a subsequent study, the rancidity of corn flakes stored for one year was highly correlated to 11 different volatile compounds (Paradiso *et al.*, 2009). In contrast to other reports, hexanal was not highly correlated to the poor sensory scores. The use of marker compounds are likely system dependent and thus identification of relationship between sensory characteristics and volatiles in cereals using multivariate statistical tests is needed.

The bitterness of cereal products is believed to be associated with the formation of lipid degradation products that result from lipase. Biermann and Grosh (1979) identified a bitter tasting monoacylglyceride from stored oat flour. However, Molteberg *et al.* (1996a,b) suggested products from lipoxygenase promoted oxidation could also be responsible for the bitterness. Regardless of the source, lipid hydrolysis and oxidation have a negative impact on acceptability of cereal products.

11.3.2 Nutrient and antioxidant components of cereals

Tocopherols

Vitamin E or tocopherols are important biological antioxidants. Maras *et al.* (2004) reported that ready-to-eat cereals and white bread were among the top five sources of vitamin E (α-tocopherol) in the diets of adult Americans. Variation in dietary tocopherols intake was reported, which is likely due to product consumption. The germ is the primary source of tocopherols and toco-trienols (i.e., tocols) in cereals (Barnes, 1983). Dry milling of corn was an effective means to concentrate tocopherol. The germ and bran fractions contained 68 and 4% of the γ-tocopherol, respectively (Moreau *et al.*, 1999). Peterson (1994) reported that germ fraction of barley contained the majority of the tocols. However, the tocols content in rye flour decreased with increasing removal of the outer layers of rye (Michalska *et al.*, 2007). This is supported by the observation of higher tocols in the pericarp fraction compared to the endosperm (Zielinski *et al.*, 2007). The bran fraction of rice also contains high levels of tocols (Aguilar-Garcia *et al.*, 2007). Thus, the level of tocols in cereal-based foods will vary depending on the type of flour used during processing. Whole cereal flour or whole grains have greater levels of tocopherols due to the incorporation of the germ into the flour.

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Hidalgo *et al.* (2009) reported that tocopherol degradation in wholemeal and white flours was a function of time and temperature. They also noted that tocopherols degradation rate was faster in white flour than in wholewheat flour. Nielsen and Hansen (2008a) reported that the mill type influenced tocol content. The white and wholewheat flours from the roller mills lost 31 and 50% of the tocols over 297 days, respectively. In contrast, the white and wholewheat flours obtained from the stone mill lost approximately 34% of the tocols over 297 days (Nielsen and Hansen, 2008b).

The loss of tocopherols during bread baking has been reported. Ranhotra *et al.* (2000) reported that 66% of the vitamin E added to the flour was retained in the breads. No additional loss was observed during the seven day storage. Leenhardt *et al.* (2006) noted similar total losses of vitamin E in bread made with bread and durum wheats. However, they noted that approximately 10% of the vitamin E was lost during kneading while 15–20% was lost during baking.

Malting of barley had little impact on tocol contents (Peterson, 1994). In contrast, hydrothermal treatment caused a 58–80% reduction in barley tocols (Rutgersson *et al.*, 2000). The discrepancy between the reports might be due to methodologies used in the barley treatment. Higher tocols were observed in the spent grain recovered from the mashing process. The higher levels were attributed to reduction in starch content of the malt (Peterson, 1994).

Bryngelsson *et al.* (2002) reported an increased tocols level in oats processed using an autoclave. The initial steaming of the oats caused minor reductions in tocols, but rolling the oat groat did cause a reduction in tocols. A significant reduction in tocols was noted in drum dried rolled oats and wholemeal. Extrusion resulted in a reduction of tocols in wheat, barley, rye and oat. The reduction occurred at all extrusion temperatures (i.e., 120, 160 and 200 ºC) whereby 200 ºC caused the greatest reduction (Zielinski *et al.*, 2001). In contrast, Zadernowski *et al.* (1999) reported that extrusion did not affect tocopherol content in extruded oat flour. Winkler *et al.* (2007) observed that the tocols remained high in corn distillers' grains. Thus, it appears that the method of exposure of the cereal to high temperature can influence tocols retention.

The method of processing also affected tocol levels in rice (Kim *et al.*, 1987). They reported 7 and 21% reductions in tocols after extrusion at 110 and 140 ºC, respectively. Tao *et al.* (1993) reported that microwave processing had little effect on tocols stability whereas gamma irradiation was not recommend for processing rice bran because the tocols were destroyed (Shin and Godber, 1996).

Carotenoids

Carotenoids are vitamin A precursors and have a number of health benefits. Carotenoids exist as carotenes (α- and β-carotene) and xanthophylls (β-cryptoxanthin, lutein and zeaxanthin). Lutein and zeaxanthin have attracted much attention due to the possible role in preventing cataracts (Knekt *et al.*, 1992) and age-related macular degeneration. Thus, the degradation of carotenoids impacts the intake of this category of nutrients.

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Farrington *et al.* (1981) reported the esterification of the lutein during the storage of wheat flour. They also noted that free unsaturated lipids decreased, suggesting that the oxidation of lipid followed by radical quenching by lutein resulted in the increased esterified lutein. Hidalgo and Brandolini (2008) reported that the loss of carotenoids in wholewheat and white flours was similar. They recommended that temperatures not exceed 20 ºC during long-term storage for optimal carotenoid preservation.

Mercier and Gélinas (2001) reported that pure LA was efficient in degrading β-carotene in wheat dough. The combination of slightly oxidized oil, LA and lipase substantially degraded flour pigments (Mercier and Gélinas, 2001). A high correlation exists between the degradation of carotenoids and the presence of lipoxygenase in cereals (Borrelli *et al.*, 1999; Leenhardt *et al.*, 2006). Carotenoids loss was affected by kneading and baking, but not significantly during the fermentation step of bread baking. Leenhardt *et al.* (2006) reported carotenoid losses of 5–66% during kneading. The wholewheat flour lost the most carotenoids (i.e. 66%) while the regular wheat flour lost approximately 40%. About 35–45% of the carotenoids were lost during baking. The degradation of carotenoids improves the functionality of bread dough and is often necessary for white bread production. In contrast, carotenoid degradation is a negative for pasta manufacturers, who want to retain the yellow color.

The role of lipoxygenase and lipid radicals in the bleaching of carotenoids in durum wheat and pasta has been known for nearly 60 years (Irvine and Winkler, 1950; McDonald, 1979; Taha and Sagi, 1987). Matsuo *et al.* (1970) reported that semolina pigment loss was greatest in the presence of LA and mixing under oxygen atmosphere. However, mixing under vacuum reduces the loss of carotenoids. Drying of the pasta has the greatest impact on pigment and was estimated to be responsible for about 75% of the carotenoid reduction (Matsuo *et al.*, 1970). Borrelli *et al.* (1999) reported β-carotene losses of approximately 8% in milled durum wheat and 16% during pasta processing.

The production of masa, tortillas and chips contribute to the reduction of carotenoids from corn. De La Parra *et al.* (2007) reported that masa had between 5 and 65% less carotenoids than the corresponding corn. White corn had the greatest retention (95%) while yellow corn the least (35%). After processing masa into tortillas and corn chips, the losses in carotenoids reached 73 and 83% in yellow corn, respectively. Similar trends were observed in other corn genotypes.

Other compounds

Although phenolics and polyphenolics are not considered nutrients, these compounds do have antioxidant activity. Michalska *et al.* (2007) reported that total phenolic and total flavonoid contents decreased with increased removal of the outer layers of rye. These observations support the general knowledge that phenolic compounds tend to be located in the aleurone and pericarp layers of cereals (Sosulski *et al.*, 1982; Hutzler *et al.*, 1998; Naczk and Shahidi, 2006). Thus, any process that affects these layers, such as milling, will cause a reduction of phenolics in the final product.

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Dimberg *et al.* (2001) also reported that heat caused a reduction in phenolic acids and that alkaline conditions caused greater reductions than acidic conditions. Bryngelsson *et al.* (2002) reported that drum drying substantially reduced phenolic acid while autoclaving enhanced phenolic content. Bryngelsson *et al.* (2002) concluded that the autoclaving process promoted the release of bound phenolic acids. Increasing concentrations of phenolic acids were also observed after the kilning process (Maillard and Berset, 1995). Zielinski *et al.* (2001) reported that free and esterified phenolic acids increased using extrusion. The increase in temperature promoted the release of bound phenolic acids in wheat, barley, rye and oats. In contrast, roasting above 200 ºC significantly reduced phenolic levels (Duh *et al.*, 2001). A 60–65% reduction in total phenolic content was reported in extruded oat flour (Zadernowski *et al.*, 1999). These conflicting reports may be the result of experimental methods.

Goupy *et al.* (1999) observed significant reductions in the flavanol and flavonol contents after malting, whereas phenolic acids were affected to a lesser extent. In contrast, total phenolic content increased between 8 and 66% during the malting process (Maillard *et al.*, 1996). A reduction of bound phenolics and an increase in esterified phenolics was observed during the malting of barley (Dvorakova *et al.*, 2008). Thus, the general conclusion is that the enzymatic activities that occur during malting enhance the release of phenolics, which could then contribute to oxidative stability of finished products.

Processing in general causes reductions in the amounts of anthocyanins. Del Pozo-Insfran *et al.* (2006) noted that the average losses in anthocyanins in blue corn processed into masa, tortillas, and chips were 37, 54 and 75%, respectively. This indicates that processes after masa production can affect anthocyanins. In contrast, De La Parra *et al.* (2007) reported that no additional loss of anthocyanins occurred after masa production. De La Parra *et al.* (2007) reported 46–93% reductions in anthocyanins during the masa production using different corn genotypes. Blue and yellow corns had the greatest and lowest anthocyanin reduction, respectively. Anthocyanin reduction of 49–90% and 35–91% were observed after the tortilla and corn chip production, respectively (De La Parra *et al.*, 2007).

White corn lost approximately 89% of the total phenolics after nixtamalization (Del Pozo-Insfran *et al.*, 2006). Ferulic acid reductions of 24–65% were observed after nixtamalization (De La Parra *et al.*, 2007). No additional losses in ferulic acid or total phenolics were reported in tortillas or chips made from masa. This suggests that the alkaline environment and removal of the pericarp that occurs during nixtamalization may be responsible for the reduction of phenolic compounds.

Oat contains a unique group of phenolic compounds referred to as avenanthramides (Collins, 1989). These compounds have been reported to have anti-atherogenic and anti-inflammatory activities (Ji *et al.*, 2003; Liu *et al.*, 2004; Chen *et al.*, 2004; Nie *et al.*, 2006) and in vivo antioxidant activity (Chen *et al.*, 2004, 2007). Thus, preservation of these in oats further supports the consumption of oat products.

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Reduction of avenanthramides by heating has been documented (Dimberg *et al.*, 2001; Bryngelsson *et al.*, 2002). Steaming and flaking had only a slight effect on the avenanthramides while drum drying caused significant reductions in these compounds (Bryngelsson *et al.*, 2002). Mattila *et al.* (2005) noted that oat flakes contained higher levels of avenanthramides than bran and precooked oat flakes. Dimberg *et al.* (2001) reported that bakery and pasta products contained higher avenanthramides, which they attributed to the release of the bound avenanthramides during processing.

Del Pozo-Insfran *et al.* (2006) reported a strong correlation between anthocyanin losses and loss in antioxidant capacity. Thus, processing methods should be considered critical in preventing significant reductions in antioxidants from cereals. These antioxidants likely contribute to the stability of processed cereal products.

11.4 Protecting cereals and snack products against oxidation

11.4.1 Additives

The addition of traditional synthetic antioxidants has proven effective in controlling the oxidation of cereal products. Almeida-Dominguez *et al.* (1992) reported that corn-based snacks fried in oil containing 0.01% TBHQ combined with high density polyethylene produced the best snack quality. The use of synthetic antioxidants reduced the negative effects of iron fortification in the oxidation of corn porridge (Bovell-Benjamin *et al.*, 1999). The antioxidant BHA and BHT or combination of these with citric acid inhibited hexanal formation in fortified corn meal. Tocopherol was not effective in preventing oxidation of iron-fortified corn meal (Bovell-Benjamin *et al.*, 1999). BHA and BHT were shown to reduce oxidation in breakfast cereals (Butt *et al.*, 2003). A review by Fellers and Bean (1977) demonstrates the success of BHA and BHT in extending the shelf life of wheat based food products.

The addition of phenolic compounds was found to enhance the lipid stability of extruded corn (Camire and Dougherty, 1998). The additions of 200 ppm of cinnamic acid or vanillin protected the extruded corn snack better than the comparable level of BHT. Parker *et al.* (2000) reported that extruded oat lacking the bran fraction had higher levels of alkanal, 2-alkenals, and 2,4-dialkadienals, which are associated with lipid oxidation. The bran contains high levels of antioxidants and since it was lacking in the oat extrudates could not provide protection against oxidation. Viscidi *et al.* (2004) reported that, among phenolic compounds tested, extruded oat containing quercetin had significantly lower hexanal and conjugated diene levels than the control oat cereal at 24 weeks of storage. However, peroxide value in the quercetin fortified oat cereal was not significantly different from the control after 24 weeks of storage.

Camire *et al.* (2007) reported that after 11 weeks of storage extruded corn cereal containing raspberry, cranberry, and concord grape extracts had lower hexanal and pentane levels than the control sample. However, the concord

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grape-containing extrudate was the only sample that had significantly lower total volatiles than the control extrudate, suggesting that specific volatiles should be used as markers for oxidation rather than total volatiles. Klensporf and Jelen (2008) reported that the predominant compound in muesli stored 14 days was hexanal. A red raspberry seed extract reduced the rate of lipid oxidation and the total amount of volatiles. Camire *et al.* (2005) reported that hexanal levels in extruded corn containing various plant extracts were in general lower than the hexanal values in the control extrudate. However, they recommended that volatile analysis might not be the best tool for measuring oxidation products because some of the extracts contain volatile compounds that might confound the volatile analysis.

Paradiso *et al.* (2008) reported that tocopherols were an effective means in extending shelf life of corn flakes. They observed less volatile formation over the course of 360 days. The addition of tocopherols, ascorbic acid or rosemary extracts to corn flake formulas provided mixed results with respect to storage stability. The addition of tocopherols (i.e., 125 and 187.5 ppm) substantially inhibited the formation of volatiles in one-year-old corn flakes (Paradiso *et al.*, 2009). The combination of tocopherol (125 ppm) and ascorbic acid (400 ppm) also inhibited volatile formation but to a lesser extent than tocopherol alone. The corn flakes containing rosemary extract (800 ppm) had more volatiles than the corn sample. The volatile composition in the rosemary extract might be one reason for the higher volatile content in the one-year-old corn flakes. Volatiles such as heptanal, 2-decanal, and lactones were correlated to the rancid sensory scores and were not different between the control flake and the flake containing rosemary extract. The highest tocopherol level did produce flakes that had the best sensory score, indicating the value of tocopherol addition as an antioxidant. The addition of tocopherol to durum wheat semolina also inhibited pigment bleaching (Pastore *et al.*, 2000) further supporting the use of tocopherols as antioxidants in cereals.

The variation in responses to oxidation control in cereal products may be related to the formation of compounds during processing or to the binding of lipids to matrices. Maillard reaction products (MRP) have been documented as antioxidants and are widely known to increase upon heat processing (Lingert and Eriksson, 1981). Rada-Mendoza *et al.* (2004) reported the presence of MRP in cookies, crackers and breakfast cereals, indicating a potential antioxidant source. Wijewickreme and Kitts (1998) reported that α-tocopherol was more effective than MRP in preventing the oxidation of flour dough. The glucose-lysine MRP were more effective than fructose-lysine MRP in controlling oxidation. Wagner *et al.* (2002) reported the MRP from a glucose-glycine model system was an effective antioxidant. In cereals, glycine is readily available and is likely one of the amino acids involved in the production of MRP of baked or high temperature treated cereal products.

MRP were also believed to be responsible for the antioxidant activity of barley malts (Maillard and Berset, 1995; Woffenden *et al.*, 2001, 2002). The inhibition of lipoxygenase is another interesting property of MRP. The inhibition

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of lipoxygenase by melanoidins, caramel and dark malts was observed (Sovrano *et al.*, 2010). They also noted that caramel malts had the highest chain-breaking activity compared to black malts but lower overall lipoxygenase inhibiting activity.

Lehtinen and Laakso (2000) reported that a protein rich fraction from oats reduced the oxidation of linoleic acid. The control of the oxidation was due to the binding of linoleic acid to the protein, making it less accessible to oxidation (Lehtinen and Laakso, 2000). A similar mechanism may explain the reduction in the rate of linoleic oxidation during dough mixing (Chung and Tsen, 1975; Delcros *et al.*, 1998). Thus, the physical inhibition of oxidation in cereal-based products is partly due to an interaction between lipids and protein and potentially the carbohydrates.

The inherent antioxidant components in cereals are also likely to contribute to shelf life extension of cereal-based products. Emmons and Peterson (1999) reported the antioxidant activity of oat extracts. They noted that the groat had better activity than hull extracts. Bratt *et al.* (2003) reported that aven-anthramides in oats contributed antioxidant activity. Since these Dimberg *et al.* (1996) observed a 20% reduction in avenanthramides in oats after heating whereas Dimberg *et al.* (2001) reported higher avenanthramide contents in baked and pasta products. Yu *et al.* (2002) reported the radical scavenging activity of wheat extracts. This report was just one of the many studies that have demonstrated the antioxidant activity of cereals. Thus, the numerous reports suggest that compounds inherent to the cereal likely contribute to the oxidative stability of processed products.

11.4.2 Packaging

The role of packaging on shelf stability of rolled oats in cans was best demonstrated by McEwan *et al.* (2005). They reported the oxidation of oats over a 28-year period. The can seam quality was directly related to the poor sensory quality (McEwan *et al.*, 2005). The ability to maintain low oxygen content in the package was important for maintaining oat stability. Jensen *et al.* (2005) reported that the greatest level of oxidation in oatmeal occurred in packages containing 21% oxygen. Storage of flaked oat cereals (Sakamaki *et al.*, 1988) and extruded oats (Larsen *et al.*, 2003, 2005) in low oxygen permeable packaging prevented oxidation of oat lipid. The inclusion of oxygen absorbers did not significantly improve product stability over packaging only in low oxygen permeable packages. Jensen *et al.* (2005) also reported that oxidation of muesli could be reduced by using light impermeable packaging.

Water activities in the stored oats were between 0.45 and 0.62, which is optimal for maintaining oat quality (McEwan *et al.*, 2005). Ganssmann and Vorwerck (1995) recommended that oats be stored at water activities less than 0.65 to maintain freshness. Sirpatrawan and Jantawat (2008, 2009) reported that polypropylene provide better moisture barrier properties than polyethylene. Although Sirpatrawan and Jantawat (2008, 2009) were interested in moisture

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uptake and loss of crispiness in rice crackers, changes in water activities could ultimately affect oxidation of the rice crackers. The use of mathematical modeling was a useful method for estimating moisture migration and shelf life of cereals and snacks (Sapru and Labuza, 1996; Siripatrawan and Jantawat, 2008, 2009). Predicting moisture migration is important for maintaining water activity. Labuza (1980) described the importance of water activity in oxidative stability of foods. Jensen and Risbo (2007) reported the natural water activity of 0.16 and 0.25 for oatmeal and muesli, respectively. However, increasing oatmeal water activity to 0.23 may improve oxidative stability. Lasekan *et al.* (1996) observed the minimal carbonyl formation in extruded maize at water activities of 0.6–0.8.

Almeida-Dominguez *et al.* (1992) reported that the combination of TBHQ and high density polyethylene (HDPE) packaging was the most effective means for preserving an extruded corn snack. The lower oxygen permeability of HDPE compared to low density polyethylene accounted for differences in the preservation of an extruded corn snack. Corn flour oxidation was lower in ethylene vinyl alcohol packaging compared to polyvinyl film (Márquez and Vidal-Quintanar, 2001). However, modified atmospheric packaging and packing under vacuum had the greatest impact on shelf-life extension of corn flour.

11.5 Future trends

Over the last decade, substantial progress has been made in identifying the volatiles in cereals and cereal-based products. Many of the volatiles identified have been linked to lipids or the oxidation of lipids. The literature clearly supports the role of lipid oxidation in the development of off-flavors; however, the inconsistent correlation between specific volatiles and rancid sensory scores suggests that the oversimplification of selecting one volatile may not be the best answer. Multiple volatiles have been used in some cases but contradict other studies where correlations with one specific volatile were observed. The connection between the rancidity sensory scores and oxidation in cereals has yet to be definitively clarified. This is in part due to the complexity of the cereal. Unlike edible oils that have few antioxidant components, cereals typically contain multiple classes of antioxidants. Under certain conditions, some antioxidants can act as prooxidants, thus confounding the expected lipid stability of cereal products. The trend to produce and consume more whole grains further demonstrates the need to clarify the roles of multiple components in oxidation of cereal products. The assumption that oxidized lipids are solely responsible for the poor sensory attributes of stored cereals may not be completely true. Volatiles generated from the oxidation of proteins via radical reactions with lipid require clarification. Some of these products can produce negative sensory characteristic. In addition, much of the research on cereals has evaluated the volatiles and few studies have considered the impact of non-volatile components on rancidity of cereal products. The contribution of protein-lipid and starch-lipid

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interactions on the stability of lipids and on sensory characteristics requires additional investigations. These physical interactions have been identified as a possible reason for the stability of lipids in cereal products. However, this area of research has not been fully developed. Although a great deal of research has been completed to define the role of oxidation in cereals, understanding the fundamental mechanisms involving multiple components should be clarified in relation to controlling oxidation in cereals and cereal products. The role of packaging in preventing oxidation should be explored further.

11.6 Sources of further information and advice

The intent of the author of this review was to provide the reader with multiple areas of oxidation in cereals including sources of antioxidant components. It is the expectation of the author that readers will seek additional information from the research papers. Many of these references have very good sources of information beyond the information reported in this review. Many researchers fail to look past the internet or web-based databases for reference materials. Sources of information in books pre-dating 1990 have some very good scientific data relevant to oxidation in cereal. In particular, the book entitled *Lipids in Cereal Technology*, edited by P.J. Barnes, provides an excellent summary of lipid knowledge prior to 1983. The role of enzymes in oxidation and general composition of cereals are covered in the book and will serve as a good reference. The book *Lipid Oxidation*, by E.N. Frankel, is another good source of fundamental lipid oxidation information. The book *Autoxidation in Foods and Biological Systems*, edited by Simic and Karel (1980), also provides very good fundamental knowledge regarding enzymes and characterization of lipid oxidation products. Robinson *et al.* (1995) provided a good review on the role of lipoxygenases in the quality of foods. Updated information on enzymes and oxidation can be found in many text books. In a previous book entitled *Antioxidants in Food*, edited by Pokorny, Yanishlieva, and Gordon, the author (Hall, 2001, pp. 159–209) provided a good summary on cereal antioxidants in a chapter entitled ‘Natural antioxidants 1: oilseeds, nuts, cereals, legumes, animal products, and microbial sources’. The review by Vermeiren *et al.* (1999) summarizes packaging technologies suitable for foods.

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12

Oxidative stability of antioxidants in fruits and vegetables

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Abstract: Regular intake of fruits and vegetables has been associated with lower incidences of several diseases. The chapter presents the main phytochemicals contributing to antioxidant potential of fruits and vegetables. The main chemical changes in these compounds due to processing and storage are reviewed, as well as their influence on quality and shelf-life of fruits and vegetables. Furthermore, the most relevant technologies to prevent plant-based products from oxidation are pointed out.

Key words: antioxidants, fruits, vegetables, oxidation, processing.

12.1 Introduction

Frequent consumption of fruits and vegetables has been related to lower risk of cancer, heart disease, hypertension and stroke (Vinson *et al.*, 2001). This beneficial effect is believed to be due, at least partially, to the action of antioxidant compounds, which could reduce oxidative damage in the body. Intake of sufficient amounts of antioxidants is necessary to prevent free radical-induced oxidative stress, which has been associated with several cellular toxic processes including oxidation damage to proteins and DNA, membrane lipid oxidation, enzyme inactivation, and gene mutation that may lead to carcinogenesis (Poulsen *et al.*, 1998).

The major groups of phytochemicals contributing to the total antioxidant capacity of fruits and vegetables include antioxidant vitamins, carotenoids,

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phenolic compounds and glucosinolates (Wang *et al.*, 1996). However, the amounts of these phytochemicals may greatly vary upon several factors related to crop, as well as to growing, handling and storage conditions. In addition, certain conditions during processing may induce a dramatic effect on the antioxidant content of the raw produce. The generation of active oxygen species, which are regular by-products of the plant metabolism, can be substantially increased due to an inappropriate handling, thus leading to a variety of biochemical and physiological injuries that may impair not only the antioxidant potential of the fruit and vegetable commodity but also its physicochemical and sensory characteristics. This chapter provides extensive information on the main phytochemicals contributing to antioxidant potential of fruits and vegetables. The main chemical changes in these compounds due to processing and storage are reviewed, as well as their influence on quality and shelf-life. Furthermore, the most relevant technologies to prevent plant-based products from oxidation are pointed out, with a special stress on minimal processing techniques.

12.2 Antioxidant properties of fruits and vegetables

12.2.1 Antioxidant vitamins

Vitamin C, including ascorbic acid and dehydroascorbic acid, its oxidation product, has many biological activities in the human body. Experimental studies have shown that vitamin C plays an important role in human health, including effects on the immune system and the risk of Alzheimer disease (Sánchez-Moreno *et al.*, 2005). Vitamin C may prevent free radical-induced damage to DNA quenching oxidants (Fraga *et al.*, 1991) that overcome cell dysfunction and decrease low-density lipoprotein induced leukocyte adhesion (Lehr *et al.*, 1995). Vitamin C is one of the most important antioxidants provided by fruits and vegetables. Lee and Kader (2000) reported that fruits and vegetables supply more than 85% of vitamin C in human diets. In general fruits are a good source of vitamin C, which is especially abundant in guava (230–300 mg/100 g), blackcurrant fruits (125.2–151.1 mg/100 mg), strawberries (29–48 mg/100 g) and citrus fruits (30–50 mg/100 mg) (Hägg *et al.*, 1995). Moderately high vitamin C contents have been also reported in kiwi (60 mg/100 g), melon (10–35 mg/100 g), pineapple (12–25 mg/100 g) and banana (10–30 mg/100 g). Not all fruits contain such levels, and apples, pears and plums represent a very modest source of vitamin C (3–5 mg/100 g) (Davey *et al.*, 2000). Vegetables collectively exhibit very variable amounts of ascorbic acid, with the Brassica generally containing the highest vitamin C levels. The content of vitamin C among Brassica vegetables varied significantly between and within their subspecies. Vitamin C levels varied over 4-fold in broccoli and cauliflower, 2.5-fold in Brussels sprouts and white cabbage, and twice in kale, while white cabbage is the poorest source of vitamin C (Podsędek, 2007). Other significant sources include gourds (185 mg/100 g), peppers (120 mg/100 g), potatoes (30 mg/100 g), peas (25 mg/100 g), tomatoes (20–25 mg/100 g) and spinach (51 mg/100 g).

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Onions, lettuce, carrots and root crops have generally much lower levels than the rest of vegetables (Hägg *et al.*, 1994; Davey *et al.*, 2000).

Vitamin E is a fat-soluble vitamin present in cell membranes which traps peroxyl free radicals. The biological activity of vitamin E is exhibited by tocopherols and tocotrienols, especially α-tocopherol. A relationship between low α-tocopherol levels and the development of atherosclerosis has been shown through several lines of evidence (Stampfer and Rimn, 1995). Although vegetables, in addition to fats, oils and cereals, constitute the major source of vitamin E in our diet, very little data are available about tocopherol contents in vegetables. Among cruciferous vegetables, the best sources of total tocopherols and tocotrienols are kale (2.12 mg/100 g) and broccoli (0.82 mg/100 mg). Brussels sprouts (0.40 mg/100 g) and cauliflowers (0.35 mg/100 g) have moderate levels, while cabbage (0.04–0.24 mg/100 g) is characterized by its relatively low amounts (Podsędek, 2007). In addition, high amounts of vitamin E have been determined in spinach (2.03 mg/100 g), asparagus (1.13 mg/100 g), and tomato (0.54 mg/100 ml) (Rickman *et al.*, 2007). In general, fruits do not have significant vitamin E levels with the exception of avocado fruit, whose contents range from 2.1 to 3.2 mg/100 g.

12.2.2 Carotenoids

Carotenoids (carotens and xanthophylls) are yellow, orange, and red pigments present in many commonly eaten fruits and vegetables. In human health their importance is related to their functions as provitamin A, antioxidants, cell differentiation and proliferation regulators, cell-cell communication stimulators, immune function and carcinogen metabolism modulators, and blue light filters. There are many studies showing strong correlations between carotenoids intake with both the improvement of the immune system and the reduction of the incidence of some degenerative diseases such as cancer, cardiovascular diseases, cataract and macular degeneration (Cooper, 2004). Among the 22 vegetable species investigated by Müller (1997), kale (34.8 mg/100 g), red paprika (30.4 mg/100 g), parsley (25.7 mg/100 g), spinach (17.3 mg/100 g), lamb's lettuce (16.0 mg/100 g), carrots (15.9 mg/100 g) and tomatoes (12.7 mg/100 g) were very rich in carotenoids. Among fruits, chokeberry is one of the richest sources of carotenoids, with 4.8 mg/100 g (Heinonen *et al.*, 1989), whereas grapefruit, papayas and nectarines are good sources of carotenoids with more than 2 mg/100 g (Müller, 1997). It is widely known that carotenoids levels in common fruits such as apple, grape, lemon, pear, strawberry, kiwifruit, cherry, pineapple and banana are low (Yano *et al.*, 2005). With regard to individual carotenoids, the best sources of α-carotene are pumpkin (4.8 mg/100 g) and carrots (4.6 mg/100 g). Fruits and vegetables such as sweet potatoes (9.18 mg/100 g), carrots (8.8 mg/100 g), pumpkin (6.9 mg/100 g), apricot (2.5 mg/100 g), red peppers (2.2 mg/100 g), cantaloupe melon (1.6 mg/100 g), broccoli (0.78 mg/100 g), mango (0.4 mg/100 g) and leafy greens are rich in β-carotene. β-Crypoxanthin is found primarily in sweet red peppers (2.2 mg/100 g), Japanese

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persimmons (1.4 mg/100 g), cilantro (0.40 mg/100 g), tangerine (0.48 mg/100 g) and oranges (0.12 mg/100 g). Major sources of lutein are leafy green and green vegetables as broccoli, Brussels sprouts, green beans, peas and zucchini (Holden *et al.*, 1999). Niizu and Rodriguez-Amaya (2005) reported that chicory, lettuce, green pepper, rocket lettuce and cress had lutein (0.77–5.61 mg/100 g), β-carotene (0.27–3.53 mg/100 g), violaxanthin (0.46–3.17 mg/100 g) and neoxanthin (0.31–2.05 mg/100 g) as principal carotenoids.

Lycopene is responsible for the red colour of several fruits and vegetables, including tomatoes, red grapes, watermelon and pink grapefruit. Lycopene constitutes 60–80% of the carotenoids present in tomatoes and tomato products (Clinton, 1998). Many factors can affect the lycopene content in tomatoes such as cultivar, stage of maturity and growing condition. Lycopene content was found to vary from 2.0 mg/100 g to 8.05 mg/100 g in different tomato cultivars grown in Spain (Odriozola-Serrano *et al.*, 2008a). These values are in accordance with those obtained by Nguyen and Schwartz (1999), Thompson *et al.* (2000) and Hernández *et al.* (2007), who reported values ranging from 1.4 mg/100 g to 7.7 mg/100 g. Tomatoes are the main source of lycopene, but also contain neurosporene (2.8 mg/100 g), γ-carotene (2.0 mg/100 g), β-carotene (1.9 mg/100 g), lutein (0.67 mg/100 g), phytofluene (0.51 mg/100 g) and δ-carotene (0.42 mg/100 g) in much smaller amounts (Odriozola-Serrano *et al.*, 2009a).

12.2.3 Phenolic compounds

Phenolic phytochemicals are a large class of natural substances that can be found in many edible plant products which have strong antioxidant activities. In such a capacity they are able to scavenge reactive oxygen species (ROS) generated endogenously and by chemical carcinogens. The content of phenolic compounds in fruits and vegetables, expressed as gallic acid equivalents (GAE), varies considerably among species. A particularly high content of phenolic compounds has been reported in chokeberry (6625 mg GAE/100 g), bilberry (5250 mg GAE/100 g), blackberry (3610 mg GAE/100 g), blackcurrant (3181 mg GAE/100 g), blueberry (1811–4730 mg GAE/100 g), strawberry (3172–4434 mg GAE/100 g), cranberry (120–1765 mg GAE/100 g) and raspberry (1137–1776 mg GAE/100 g) (Szajdek and Borowska, 2008). Zhou and Yu (2006) reported that kale has a high phenolic value of 1630–1880 mg GAE/100 g dw, followed by those of rhubarb (1320 mg GAE/100 g dw), spinach (930–1300 mg GAE/100 g dw) and broccoli (940–1060 mg GAE/100 g dw). Carrot and potato contained less phenolic compounds than other vegetables. Lin and Tang (2007) reported that total phenolic content varied from 144 mg GAE/100 g (bitter melon) to 311 mg GAE/100 g (red onion) when studying 13 varieties of deep-colored fruits and vegetables cultivated in Taiwan.

Phenolic compounds are categorized into different groups depending on their structure and subcategorized within each group according to the number and position of hydroxyl group and the presence of other substituents. The most widespread and diverse group of the polyphenols in fruits and vegetables are the

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flavonols which are built upon C6-C3-C6. In addition, other phenolic compounds such as benzoic acid or cinnamic acid derivates have been identified in fruits and vegetables (Robards *et al.*, 1999). Total contents of flavonols in berries are generally higher than those in the commonly consumed fruits and vegetables. Quercetin is the main flavonol in fruits, found in high concentrations in bog whortleberry (15.8 mg/100 g), loganberry (7.4–14.6 mg/100 g) cranberry (8.3–12.1 mg/100 g), chokeberry (8.9 mg/100 g), sweet rowan (8.5 mg/100 g), rowanberry (6.3 mg/100 g), sea buckthorn berry (6.2 mg/100 g), and crowberry (5.3–5.6 mg/100 g) (Häkkinen *et al.*, 1999a) Moderate levels of quercetin have been reported for orange (0.686 mg/100 g), apricot (0.38 mg/100 g), apple (0.32 mg/100 g), banana (0.29 mg/100 g), black grape (0.24 mg/100 g) and pear (0.21 mg/100 g). Quercetin concentrations lower than 0.15 mg/100 g have been found in green grape, lemon, plum, cherry, melon and nectarine, whereas amounts between 0.14 and 2.19 mg/100 g of myricetin have been reported for cranberry, black currant, crowberry, bog whortleberry, blueberries, bilberry, banana, lemon and orange (Macheix *et al.*, 1990). Kaempferol has been found in gooseberries (1.6 and 1.9 mg/100 g), strawberries (0.5–0.8 mg/100 g), cherry (0.24 mg/100 g), pear (0.21 mg/100 g), black grape (0.18 mg/100 g) and orange (0.18 mg/100 g) (Häkkinen *et al.*, 1999a; Kevers *et al.*, 2007).

Among vegetables, Hertog *et al.* (1992) observed that the quercetin content of onions (347 mg/kg) and the kaempferol concentration of fresh kale (211 mg/kg) were 5–10-fold higher than in most other vegetables. High levels of quercetin have been also detected in kale (110 mg/kg), broccoli (3 mg/kg), fresh French beans (39 mg/kg), and fresh slicing beans (29 mg/kg). Price *et al.* (1998) identified the main flavonol glycosides present in broccoli florets, as quercetin and kaempferol 3-O-sophroside. Three minor glucosides of these aglycons were also detected, namely isoquercetrin, kaempferol 3-O-glucoside and kaempferol diglucoside. The mean kaempferol contents of endive (46 mg/kg), leek (30 mg/kg), and turnip tops (48 mg/kg) were higher than in most other vegetables. Myricetin was only determined in fresh broad bean (26 mg/kg), and luteine was found only in red bell pepper (11 mg/kg) (Hertog *et al.*, 1992). Martínez-Valverde *et al.* (2002) reported variable content of flavonols such as quercetin (0.72–4.36 mg/100 g), kaempferol (0.2–0.12) and naringenin (0.45–1.26 mg/100 g) in different tomato cultivars. Many hydroxycinnamic acids together with conjugates have been reported to occur both in tomato skin and pulp. These phenolics are predominantly present as a family of esters formed between certain hydroxycinnamic acids and quinic acid. Hernández *et al.* (2007) reported that the most abundant hydroxycinnamic acid in five different commercial varieties of tomato fruits was chlorogenic acid, with values ranging from 9 to 14 mg/kg, followed by caffeic acid with amounts varying from 2 to 6 mg/kg, while *p*-coumaric and ferulic acids were found in concentrations lower than 5 mg/kg. Mattila and Hellström (2007) determined the phenolic acid of 45 vegetables consumed in Finland. The highest contents of total phenolic acids were found in red cabbage, carrot, eggplant, Jerusalem artichoke, broccoli, lettuce, basil, spinach, radish and red beet, with contents varying from 11 to

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52 mg/100 g. The main phenolic acid in eggplant, Jerusalem artichoke, lettuce and carrot was caffeic acid, whereas in spinach, radish and red beet ferulic acid was the most abundant compound. The studies on phenolic profiles of Brassica vegetables have been focused mainly on broccoli florets, which are an outstanding source of hydroxycinnamoyl acids. The predominant hydroxycinnamoyl acids were identified as 1-sinapoyl-2-feruloylgentiobiose, 1,2-diferuloylgentiobiose, neochlorogenic acid and 1,2,2′-trisinapoylgentiobiose (Vallejo *et al.*, 2003). Phenolic acids composition in berry fruits is characterized by cinnamic and benzoic acid derivates. Ellagic and *p*-coumaric acids have been described as the two predominant phenolics in strawberries, raspberries and cloudberries. Large quantities of ferulic acid have been found in cranberry and blueberry, significant amounts of *p*-coumaric and ferulic acid have been reported in bilberry, while blackcurrant has exhibited a remarkable content of *p*-coumaric and caffeic acids (Häkkinen *et al.*, 1999b). In apples, the most abundant hydroxycinnamic acid is chlorogenic acid, followed by *p*-coumaroylquinic acid and neochlorogenic acid (Khanizadeth *et al.*, 2008).

Anthocyanins are pigments accounting for the red, violet and blue colour of most fruits. They are, nevertheless, not present in certain fruits (tomato, red pepper) in which the colour is caused by carotenoid pigments. Natural anthocyanin pigments (anthocyanins) are glycosides which release aglycone forms (anthocyanidins) by hydrolysis. Among fruits and vegetables, berries are characterized by containing high anthocyanin levels. Blackberry, cherry, chokeberry, cranberry, blackcurrant and elderberry contain about 1000–10 000 mg/kg. Other fruits also exhibit considerable anthocyanin contents: grape and blood orange exhibit about 500–7000 mg/kg, whereas red cabbage, eggplant, onion and rhubarb about 200–7000 mg/kg (Macheix *et al.*, 1990).

12.2.4 Glucosinolates

Glucosinolates are the major class of secondary metabolites found in Brassica crops. The molecule comprises a β-thioglucoside N-hydroxysulphate, containing a side chain and a β-D-glucopyranose moiety. Glucosinolates are not bioactive compounds until they have been enzimatically hydrolysed to various breakdown products by myrosinases, which are endogenous plant enzymes. Glucosinolates and their concentration vary among the different groups of Brassicaceae. In Brussels sprouts (326.1 mg/100 g dw), cabbage (344.3 mg/100 g dw), cauliflower (333.2 mg/100 g dw), and kale (340.0 mg/100 g dw) one of the predominant glucosinolates was found to be sinigrin. Brussels sprouts also had significant amounts of gluconapin (465.4 mg/100 g dw), glucoraphanin (309.9 mg/100 g dw) and progoitrin (292.2 mg/100 g dw). The most abundant glucosinolates in broccoli are glucoraphanin (320.8 mg/100 g), glucobrassicin (156.6 mg/100 g dw) and progroitrin (Jahangir *et al.*, 2009). Glucosinolates are hydrolyzed by the enzyme commonly known as myrosinase (thioglucohydrolase; E.C. 3.2.1.147) to a variety of compounds (isothiocyanates, nitriles, thiocyanates, epithionitriles, oxazolidines), the composition of which depends

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on pH, metal ions and other protein elements. Some of these products are considered to be very powerful inducers of phase II detoxifying enzymes, while phase I enzymes are reduced. In this way, the initial stage of the carcinogenic sequence associated with DNA damage is blocked and hence these substances are good blocking agents (Bones and Rossiter, 2006).

12.2.5 Antioxidant capacity

Several antioxidant capacity assay methods have been used or developed in recent years to evaluate antioxidant capacity of fruits and vegetables and results may greatly vary depending on the experimental conditions and the specificity of the free radical used (Cao *et al.*, 1993). Tables 12.1 and 12.2 show the antioxidant capacity of fruits and vegetables determined throughout the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), the oxygen radical scavenging absorbance capacity (ORAC) assay and the ferric reducing antioxidant power (FRAP) method. Fruits and vegetables exhibit a wide range of antioxidant capacities. Different ranking order of antioxidant capacity among the fruits and vegetables can be obtained by using the DPPH radical, ORAC or FRAP assays. DPPH assay is not specific to any particular antioxidant component, thus applying to the overall antioxidant capacity of the sample. The ORAC assay measures the relation between the antioxidants and the peroxyl radicals, whereas the FRAP method determines the total reducing capacity of any compounds in the test materials.

The antioxidant capacity determined throughout the radical DPPH of guava and cherimoya is much higher than those reported for other fruits (Table 12.1). Berry fruits such as chokeberry (160.2 μmol Trolox/g), blueberry (16.8–92.9 μmol Trolox/g), bilberry (44.6 μmol Trolox/g), raspberry (18.5–47.6 μmol Trolox/g) and blackcurrant (36.9–93.1 μmol Trolox/g) have been shown to have a particularly high oxygen radical scavenging absorbance capacity. Strawberries, plums and apples exhibit ORAC values similar to those of tropical fruits such as mango and guava. The lowest oxygen radical scavenging absorbance capacity has been observed in watermelon (1.2 μmol Trolox/g), cantaloupe melon and papaya. As can be seen in Table 12.1, the FRAP antioxidant capacity values obtained in berry fruits such as blackberry, blackcurrant, blueberry and raspberry are slightly higher than those determined in guava, plum, strawberry and mango. Red grape, peaches, pears, avocado and pineapple appear to have a very low FRAP antioxidant capacity. Among vegetables, broccoli (18.8 μmol Trolox/g) and spinaches (18.4 μmol Trolox/g) had the highest antioxidant capacity, measured throughout the DPPH radical method. On the other hand, DPPH values in red pepper, yellow pepper, green pepper, garlic and red cabbage have been reported to be higher than those obtained for other common vegetables, such as peas, potatoes, green cabbage, cauliflower, Brussels sprouts, carrot, lettuce, tomato, onion, celery and leek (Table 12.2). Broccoli, cauliflower and artichoke had the highest ORAC values followed by peas, garlic, red cabbage, kale, spinach, potato and green pepper. The oxygen radical scavenging absorbance capacities of tomato (2.16–4.3 μmol Trolox/g), asparagus

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Table 12.1 Antioxidant capacity values of fruits by different analytical methods

	DPPH (μmol Trolox/g)	ORAC (μmol Trolox/g)	FRAP (μmol Fe^2/g)
Lemon	**3.04** (Kevers *et al*., 2007)	**8.43** (Kevers *et al*., 2007)	**6.9** (Stangeland *et al*., 2009)
Orange	**2.24** (Kevers *et al*., 2007)	**13.18** (Kevers *et al*., 2007)	**12.8** (Stangeland *et al*., 2009)
Red grape	**4.43** (Kevers *et al*., 2007)	**17.46** (Kevers *et al*., 2007)	**3.5** (Haleem *et al*., 2008)
Green grape	**3.28** (Kevers *et al*., 2007)	**7.19** (Kevers *et al*., 2007)	**5.19** (Proteggente *et al*., 2002)
Banana	**5.23** (Kevers *et al*., 2007)	**7.83** (Kevers *et al*., 2007)	**5.8** (Stangeland *et al*., 2009)
	11.0 (Miller *et al*., 2000)	**8.1** (Wu *et al*., 2004)	**2.0** (Haleem *et al*., 2008)
Strawberry	**11.0** (Vasco *et al*., 2008)	**10.3–17.8** (Wang *et al*., 2002)	**18.3** (Haleem *et al*., 2008)
	6.83 (Kevers *et al*., 2007)	**24.4** (Proteggente *et al*., 2002)	
		21.1–174.6 (Kevers *et al*., 2007)	
Plum	**16.0** (Vasco *et al*., 2008)	**19.78** (Kevers *et al*., 2007)	**20.57** (Proteggente *et al*., 2002)
	2.24 (Kevers *et al*., 2007)		
Apple	**0.92** (Kevers *et al*., 2007)	**11.39** (Kevers *et al*., 2007)	**8.6** (Haleem *et al*., 2008)
		22.1–42.3 (Wu *et al*., 2004)	
Cherry	**1.80** (Kevers *et al*., 2007)	**20.26** (Kevers *et al*., 2007)	
		33.4 (Wu *et al*., 2004)	
Peach		**7.64** (Proteggente *et al*., 2002)	**3.36** (Proteggente *et al*., 2002)
Apricot	**0.88** (Kevers *et al*., 2007)	**10.27** (Kevers *et al*., 2007)	
		13.1 (Wu *et al*., 2004)	
Kiwifruit	**0.80** (Kevers *et al*., 2007)	**3.6** (Kevers *et al*., 2007)	**6.5** (Haleem *et al*., 2008)
		8.9 (Wu *et al*., 2004)	
Cantaloupe	**0.56** (Kevers *et al*., 2007)	**3.8** (Kevers *et al*., 2007)	
		2.9 (Wu *et al*., 2004)	

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Watermelon	**1.0** (Miller *et al.*, 2000)	**1.2** (Wu *et al.*, 2004)	
Pear	**1.4** (Kevers *et al.*, 2007)	**5.2** (Kevers *et al.*, 2007)	**2.7** (Haleem *et al.*, 2008)
Nectarine	**0.56** (Kevers *et al.*, 2007)	**6.4** (Kevers *et al.*, 2007)	
Mango	**3.1** (Vasco *et al.*, 2008)	**21.0** (Patthamakanokporn *et al.*, 2008)	**16.2** (Stangeland *et al.*, 2009) **6.6** (Haleem *et al.*, 2008)
Papaya		**3.0** (Patthamakanokporn *et al.*, 2008)	**8.9** (Stangeland *et al.*, 2009)
Guava	**30.0** (Vasco *et al.*, 2008)	**18.4** (Patthamakanokporn *et al.*, 2008)	**32.1** (Stangeland *et al.*, 2009)
Cherimoya	**23.0** (Vasco *et al.*, 2008)		
Passion fruit	**0.5** (Vasco *et al.*, 2008)		**7.2** (Stangeland *et al.*, 2009)
Pomegranate			**10.5** (Stangeland *et al.*, 2009)
Avocado		**13.8** (Wu *et al.*, 2004)	**3.4** (Stangeland *et al.*, 2009)
Pineapple		**7.6** (Wu *et al.*, 2004)	**3.3** (Stangeland *et al.*, 2009)
Bilberry		**44.6** (Prior *et al.*, 1998)	
Blackberry		**14.8–22.6** (Jiao and Wang, 2000) **52.4** (Wu *et al.*, 2004)	**50.1** (Haleem *et al.*, 2008)
Blackcurrant		**36.9–93.1** (Moyer *et al.*, 2002)	**57.2** (Haleem *et al.*, 2008)
Blueberry		**16.8–42.3** (Prior *et al.*, 1998) **61.8–92.9** (Wu *et al.*, 2004)	**22.4** (Haleem *et al.*, 2008)
Chokeberry		**160.2** (Zheng and Wang, 2003)	
Cranberry		**8.2–14.1** (Wang and Stretch, 2001)	
Raspberry		**18.5** (Proteggente *et al.*, 2002) **47.6** (Wu *et al.*, 2004)	**20.1** Haleem *et al.*, 2008)

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Table 12.2 Antioxidant capacity values of vegetables by different analytical methods

	DPPH (μmol Trolox/g)	ORAC (μmol Trolox/g)	FRAP (μmol Fe^2/g)
Red pepper	**12.1** (Kevers *et al.*, 2007)	**8.75** (Kevers *et al.*, 2007) **7.1** (Cao *et al.*, 1996)	**6.8** (Haleem *et al.*, 2008)
Yellow pepper	**12.1** (Kevers *et al.*, 2007)	**10.11** (Kevers *et al.*, 2007)	**8.1** (Haleem *et al.*, 2008)
Green pepper	**11.6** (Kevers *et al.*, 2007)	**9.07** (Kevers *et al.*, 2007)	
Chilli pepper			**3.8** (Stangeland *et al.*, 2009)
Spinach	**18.4** (Kevers *et al.*, 2007)	**15.58** (Kevers *et al.*, 2007) **12.6** (Cao *et al.*, 1996)	**10.09** (Proteggente *et al.*, 2002)
Garlic	**13.0** (Miller *et al.*, 2000)	**13.70** (Kevers *et al.*, 2007) **19.4** (Cao *et al.*, 1996)	**19.4** (Cao *et al.*, 1996)
Leek	**1.8** (Kevers *et al.*, 2007)	**6.75** (Kevers *et al.*, 2007)	**4.13** (Proteggente *et al.*, 2002)
Celery	**0.6** (Kevers *et al.*, 2007) **0.5** (Miller *et al.*, 2000)	**6.79** (Kevers *et al.*, 2007) **5.3** (Wu *et al.*, 2004)	
Onion	**0.6** (Kevers *et al.*, 2007)	**7.39** (Kevers *et al.*, 2007) **4.5** (Cao *et al.*, 1996)	**0.8** (Haleem *et al.*, 2008)
Asparagus	**0.72** (Kevers *et al.*, 2007)	**2.96** (Kevers *et al.*, 2007)	
Tomato	**0.8** (Vasco *et al.*, 2008) **0.84** (Kevers *et al.*, 2007)	**2.16** (Kevers *et al.*, 2007) **3.1–4.3** (Wu *et al.*, 2004)	**3.7** (Stangeland *et al.*, 2009) **1.4–3.1** (Haleem *et al.*, 2008)
Frech bean	**0.68** (Kevers *et al.*, 2007)	**5.11** (Kevers *et al.*, 2007)	**2.1** (Stangeland *et al.*, 2009)
Lettuce	**0.56** (Kevers *et al.*, 2007)	**1.84** (Kevers *et al.*, 2007) **1.2** (Cao *et al.*, 1996) **4.2–16.5** (Wu *et al.*, 2004)	**3.19** (Proteggente *et al.*, 2002)

Cucumber	**1.0** (Miller *et al.*, 2000)	**1.6** (Kevers *et al.*, 2007) **0.87–1.12** (Wu *et al.*, 2004)	**0.5** (Cao *et al.*, 1996)
Carrot	**2.0** (Miller *et al.*, 2000)	**2.76** (Kevers *et al.*, 2007) **2.1** (Cao *et al.*, 1996) **3.5–11.5** (Wu *et al.*, 2004)	**6.8** (Miglio *et al.*, 2008)
Zucchini			**27.9** (Miglio *et al.*, 2008)
Kale		**17.7** (Cao *et al.*, 1996)	**4.3–8.3** (Haleem *et al.*, 2008)
Brussels sprouts	**5.0** (Miller *et al.*, 2000)	**9.8** (Cao *et al.*, 1996)	
Broccoli	**18.8** (Kevers *et al.*, 2007)	**23–208** (Ou *et al.*, 2002) **42–137** (Kurilich *et al.*, 2002) **12.3–28.1** (Wu *et al.*, 2004)	**52.2** (Miglio *et al.*, 2008)
Cauliflower	**2.0** (Miller *et al.*, 2000)	**62–152** (Ou *et al.*, 2002) **6.1** (Wu *et al.*, 2004)	**9.0** (Haleem *et al.*, 2008)
Green cabbage	**1.5** (Miller *et al.*, 2000)	**6.94** (Proteggente *et al.*, 2002)	**11.80** (Proteggente *et al.*, 2002)
Red cabbage	**14.0** (Miller *et al.*, 2000)	**18.70** (Proteggente *et al.*, 2002)	**21.24** (Proteggente *et al.*, 2002)
Potato	**3.5–4.0** (Miller *et al.*, 2000)	**10.1–15.3** (Wu *et al.*, 2004)	**1.4** (Haleem *et al.*, 2008)
Radish		**2.9** (Wu *et al.*, 2004)	
Peas	**3.0** (Miller *et al.*, 2000)	**3.04–37.07** (Wu *et al.*, 2004)	**7.04** (Proteggente *et al.*, 2002)
Artichoke		**92.7** (Wu *et al.*, 2004)	

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(2.96 μmol Trolox/g), radish (2.9 μmol Trolox/g), cucumber (0.87–1.6 μmol Trolox/g) are much lower than those determined in red pepper (7.1–8.75 μmol Trolox/g), green pepper (10.11 μmol Trolox/g), Brussels sprouts (9.8 μmol Trolox/g) and green cabbage (6.94 μmol Trolox/g). Regarding the FRAP antioxidant capacity, broccoli has the highest values, whereas zucchini, red cabbage, garlic and spinach exhibit values higher than 10 μmol Fe^{+2}/g. FRAP values for onion and cucumber were relatively low when compared with other vegetables such as potato, fresh beans, tomato, lettuce and chilli pepper (Table 12.2).

12.3 Oxidation processes affecting quality and shelf-life of fruit and vegetable products

Antioxidant compounds such as phenolics, carotenes and ascorbic acid contribute to the sensory properties inherent to fruit and vegetables quality. The organoleptic characteristics of fruits and vegetables may be dramatically modified by the appearance of brown pigments. Changes in the content of antioxidants occur during storage and processing. Sometimes these changes may be also associated with colour degradation processes, which are detrimental to quality. The native amounts of certain phytochemicals such as phenolics and ascorbic acid as well as the incidence of external factors such as temperature and light can greatly impact fruit colour stability during processing and storage. Enzymatic browning is one of the main oxidative phenomena inducing the development of undesirable colour, flavour and loss of nutrients. Such phenomenon is related to the oxidation, in the presence of oxygen, of phenolic compounds by the action of a group of enzymes called polyphenol oxidases (PPOs). The initial step of browning is the enzymatic oxidation of phenols into colourless o-quinones. These quinones are very active entities and will react with other quinone molecules, other phenolic compounds, the amino group of proteins, peptides, and amino acids or aromatic amines, thiol compounds and ascorbic acid, leading to the formation of more intensely coloured pigments.

The first stage of the reaction is reversible and it is for this reason that some reducing agents can prevent enzymatic browning by reducing the colourless o-quinones back to their parent phenols. Nevertheless, once all the substrate of the reaction is oxidized, the o-quinones are no longer reduced, so they can undergo a secondary step which consists of an oxidative irreversible polymerization to yield brown-black melanin pigments. Browning phenomena are caused when, after mechanical operations during processing, enzymes which are liberated from the tissues, come in contact with phenolic compounds. However, several factors may contribute to the development of brown pigments due to the enzymatic browning. Browning susceptibility may be influenced by high phenolic content in fruits as well as high concentrations of active oxidase enzymes, ripeness stage, oxygen availability, and compartmentalization of enzymes and substrates (Nicoli *et al.*, 1994; Rocha *et al.*, 1998). According to

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Soliva-Fortuny *et al.* (2002a), the chloroplasts in mature apple tissues begin to disintegrate, causing a solubilization of PPOs, which would increase the over-sensitivity to browning. In star fruit, browning is related to phenolic and PPO composition, whose content varies upon cultivar (De Almeida *et al.*, 2006). It has been found that the susceptibilities to browning and the phenolic contents in pear fruits of different cultivar were not greatly different, although a significant decrease in phenolic content occurred with delayed harvest times (Amiot *et al.*, 1995). The most common natural substrates for PPO-catalyzed enzymatic browning are chlorogenic acid and its isomer forms, catechin and epicatechin. However, some polyphenol oxidases use other phenols as substrates. In this way, 3,4-dihydroxyphenylethylamine is the major substrate in bananas and grape cathecolase acts mainly on p-coumaryl and caffeoyl-tartaric acids (Dorantes-Alvarez and Chiralt, 2000), whereas 3,4-dihydroxyphenylacetic acid is the best substrate for tomato PPO (Spagna *et al.*, 2005). Refrigeration is a traditional way to slow down PPO activity, since low temperatures are far from optimal for this enzyme. Low temperatures can effectively restrain PPO activity and in turn reduce the oxidation of phenolic substrates to quinones in apple (Leja *et al.*, 2003), eggplant fruit (Concellón *et al.*, 2004), mango (Wen *et al.*, 2006) and strawberry (Chisari *et al.*, 2007). In chilling-sensitive commodities, storage at temperatures below a limiting threshold lead to chilling injury, which frequently leads to browning development. Chilling injury induces membrane damage of organelles such as vacuoles, and the phenolics may become in contact with PPO. In addition, the cells may react to chilling by depositing phenolic compounds in the cell walls, which would then react with PPO already present in the apoplast. PPO activity in two banana cultivars increased during storage more rapidly at 6 ºC than at 10 ºC (Nguyen *et al.*, 2003). On the other hand, PPO activity has been related with colour changes associated with browning and lycopene degradation in tomatoes. In fact, lycopene may reconstitute polyphenols oxidized by the action of PPO (Spagna *et al.*, 2005).

Other enzymes with oxidizing activity are peroxidases (POD) which are involved in the neutralization of active species (Bartosz, 1997). The primary function of peroxidase is to oxidize phenolic compounds to quinones at the expense of H_2O_2. Peroxidase is a widely distributed plant enzyme responsible not only for browning but also for discoloration, off-flavours generation and nutritional damage (Vámos-Vigyázó, 1995). It has been proposed that PPO could act as promoter of POD activity, which could be due to the generation of hydrogen peroxide during the oxidation of phenolic compounds in PPO-catalysed reactions (Subramanian *et al.*, 1999). Location of the enzyme may vary upon fruits. For instance, POD in grapevine fruits is mainly located in the outermost cell layers (skin) and, to a lesser extent, in the mesocarp, where it is located primarily in peripheral vascular trends (Ros-Barceló *et al.*, 2003). In peach fruits, POD is found in both hypodermal and mesocarp tissues, although it was also found in the endocarp of peach fruit (Abeles and Biles, 1991). An increase in POD activity is commonly associated with injury, wound repair and disease resistance. POD can catalyze the oxidation of many kinds of phenols in

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the presence of oxygen, which results in enzymatic browning of harvested fruit, such as pear (Richard and Gauillard, 1997), pineapple (Selvarajah *et al.*, 1998), peach (Stutte, 1989), strawberries (Chisari *et al.*, 2007), apple (Rojas-Graü *et al.*, 2008a) and tomato (Aguilo-Aguayo, *et al.*, 2008). POD played an important role in enzymatic browning of litchi fruit. This enzyme can rapidly oxidize 4-methylcatechol in the presence of H_2O_2, and form brown polymeric pigments (Underhill and Simons, 1993; Gong and Tian, 2002). Reduced oxygen and high carbon dioxide have been proved to effectively control enzymatic browning in fruits. The reduced concentration of oxygen inhibits activity of POD in iceberg lettuce (Ke and Salveit, 1989), melon (Oms-Oliu *et al.*, 2008b) and litchi (Tian *et al.*, 2005).

Anthocynins do not appear to be direct substrates of phenolases, but their degradation is strongly increased by coupled oxidation. Destruction of anthocyanins by reaction with the product of enzymatic oxidation of chlorogenic acid has been determined in eggplant, sweet cherry and Ente plum. The same phenomenon has been also observed in the presence of catechin (Macheix *et al.*, 1990). In apple (Richard-Forget *et al.*, 1992) or in grape musts (Cheynier *et al.*, 1994), the role of caffeoyl derivatives o-quinones in the degradation of anthocyanins and flavonols has been clearly demonstrated. In the presence of caffeoyl tartaric acid quinone, cyanidin-3-glucoside, which is an o-diphenol, was degraded mostly by coupled oxidation whereas non-o-diphenolic malvidin-3-glucoside formed adducts with caffeoyltartaric acid quinone. In solutions containing equimolar amounts of both anthocyanins, both reactions took place but couple oxidation of cyanidin-3-glucoside occurred at a faster rate, partly protecting malvidin-3-glucoside (Malien-Aubert *et al.*, 2001).

On the other hand, carotenoids are highly unsaturated compounds with an extensive conjugated double-bonds system and they are susceptible to oxidation during processing and storage of fruit and vegetables (Shi and Le Maguer, 2000). The chemical oxidation of carotenoids is generally considered to be an autocatalytic free-radical chain reaction that involves induction, propagation and termination stages (Papadopoulou and Ames, 1994). The oxidative reactions take place when carotenoids react with single oxygen or with free radical species, such as peroxyl radicals generated during lipid peroxidation (Miller *et al.*, 1996). The carotenoid radicals are highly resonance stabilized and appear to undergo slow decay to colourless non-radical species. With regard to the enzyme-mediated oxidation of carotenoids, lipoxygenases seem to be the major groups of enzymes involved in carotene degradation. The process is called 'co-oxidation' because the enzyme does not act directly on carotenoid molecules. Co-oxidation occurs when lipoxygenase oxidizes polyunsaturated fatty acids and the oxidation products of enzyme activity (peroxyl radicals) react with carotenoids (Whitaker, 1991). Although the pathway of degradation of carotenoids has not been established, the main changes that occur are isomerization, leading to increased amounts of Z isomers 5,6-epoxide to 5,8-furanoid oxide rearrangement, structural modification by released enzymes, and oxidative cleavage of the polyene chain by free-radical reactions to give shorter-

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chain apocarotenals and ketones as products. Some products of oxidative breakdown such as β-ionone and dihydroactinidiolide make a significant contribution to flavour and aroma (Britton and Hornero-Méndez, 2001). The oxidation of carotenes is accelerated by metal ions, chemical oxidants, and light and it is slowed down by the addition of antioxidants. Losses or changes of carotenoids are small and occur slowly in mature fruits and vegetables. In soft fruits, however, rapid depletion of carotenoids can take place as the tissue collapses and the carotenoids are exposed to oxidizing conditions or degradative enzymes. Regarding fruits and vegetable products, a significant depletion of total carotenoids occurred during the storage of thermally treated orange (Cortés *et al.*, 2006) and tomato juices (Odriozola-Serrano *et al.*, 2009a). In this way, Lin and Chen (2005) reported that lutein in tomato juice was completely degraded in storage for 8, 6 and 6 weeks at 4, 25 and 35 ºC, respectively. Carotenoids would be expected to be stable to freezing or freeze-drying, but losses can occur if oxygen is not rigorously excluded, even with deep-frozen material when this is stored for long periods. Gebcynski and Kmiecik (2007) reported that 12 months of storage caused a moderate loss of β-carotene in frozen vegetables. Biacs and Wissgott (1997) observed that a progressive colour loss, accompanied by a reduction of carotenoid content might take place in frozen tomatoes during storage. These authors attributed the carotenoid bleaching to oxidation reactions.

On the other hand, it has been reported that food processing such as cooking or grinding might increase carotenoids content in treated products (Rao *et al.*, 2006) (Table 12.3). Nguyen and Schwartz (1999) concluded that homogenization and heat treatment disrupt cell membranes and protein-carotenoids complex, making lycopene more accessible for extraction. However, Odriozola-Serrano *et al.* (2009a) suggested that this enhancement in lycopene content might be attributed to the conversion of other carotenoids such as phytoene, phytofluene, ζ-carotene and neurosporene through desaturation, isomerization and cyclization into lycopene.

Ascorbic acid readily oxidizes especially in the presence of trace amounts of transition metal ions and alkali. The first product of this oxidation is the radical

Table 12.3 Lycopene content in fresh and processed tomato products (adapted from Rao *et al.*, 2006)

Tomato product	Lycopene (μg/g of weight)
Fresh tomatoes	8.8–42
Tomato juice	50–116
Tomato sauce	62
Tomato soup (condensed)	79.9
Tomato paste	54–1500
Tomato powder	1126–1264
Pizza sauce	127.1
Ketchup	99–134.4

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Table 12.4 Vitamin C losses in fruits and vegetables processed with several technologies

Product	Vitamic C degradation (%)	Source
Strawberry juice (90ºC, 1 min)	4–5	Odriozola-Serrano *et al.* (2008c)
Carrot juice (90ºC, 1 min)	5	Quitão-Teixeira *et al.* (2009)
Orange juice (90ºC, 1 min)	18	Elez-Martínez *et al.* (2006)
Tomato juice (90ºC, 1 min)	21	Odriozola-Serrano *et al.* (2008b)
Dehydration potato (not reported)	75	Mishkin *et al.* (1984)
Dehydration tomatoes (60ºC, 400 min)	50	Marfil *et al.* (2008)
Dehydration kiwifruits (30–40ºC, 150 min)	12–30	Cao *et al.* (2006)
Frozen sweetcorn (−20ºC)	10	Davey *et al.* (2000)
Frozen green beans (−20ºC)	20	Davey *et al.* (2000)
Frozen broccoli (−20ºC)	30	Davey *et al.* (2000)

monodehydroascorbate, also known as semihydroascorbate, or ascorbate-free radical. Two molecules of monodehydroascorbate will also spontaneously disproportionate to ascorbic acid and dehydroascorbic acid. Dehydroascorbic acid itself is unstable and undergoes irreversible hydrolytic ring cleavage to 2,3-diketogulonic acid in aqueous solution, which possesses no vitamin C activity. Ascorbic acid is a heat-sensitive bioactive compound in the presence of oxygen. Thus, high temperatures during processing can greatly affect the rates of its depletion through an aerobic pathway. Table 12.4 shows the reduction of vitamin C due to different kinds of processing. As can be observed in the table, vitamin C was reduced up to 21% in heat pasteurized juices (90 ºC, 1 min) compared to fresh juices. On the other hand, dehydration processes can be really deleterious to ascorbic acid contents, with losses of 75% in potato (Mishkin *et al.*, 1984), 50% in tomatoes (Marfil *et al.*, 2008) and 12–30% in kiwifruit (Cao *et al.*, 2006). In addition, even mild thermal treatments, such as blanching prior to freezing has been shown to result in high losses of ascorbic acid. Ascorbic acid degradation during freezing processes has been found to be 10% for sweetcorn, 20% for green beans, 30% peas and 30% for broccoli (Davey *et al.*, 2000). The rates of ascorbic acid oxidation and dehydroascorbic acid hydrolysis are influenced by concentration, light, pH, temperature, dissolved oxygen, solvent ionic strength, and the presence of divalent cations as copper and iron. Some works have studied the stability of ascorbic acid of fruits and vegetables during storage. In broccoli (Favell, 1998), yellow pepper, plum, green grape

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(Kevers *et al.*, 2007) and strawberries (Shin *et al.*, 2008) the ascorbic acid content is almost maintained through cold storage. The ascorbic acid content decreased rapidly during the first weeks of storage in red raspberry, blueberries, celery, corn, onion, cabbage (Zee *et al.*, 1991), carrot, green beans, peas (Favell, 1998), apricot, banana, spinach, melon, cherry, citrus, leek (Kevers *et al.*, 2007), green tomato (Moneruzzaman *et al.*, 2008) and potato (Burgos *et al.*, 2009). Vitamin C retention has been used as indicator of shelf-life for chilled juices. It has been considered that juices with 50% of the initial vitamin C are at the end of their shelf-life (Shaw, 1992). The concentration of vitamin C in heat-treated orange juices was reduced by more than 50% after 20 days of storage at 4 ºC (Elez-Martínez *et al.*, 2006), whereas the loss of vitamin C in heat-processed tomato juice was greater than 50% after 28 days of storage (Odriozola-Serrano *et al.*, 2008b). Odriozola-Serrano *et al.* (2008c) reported a reduction of 50% of vitamin C in heat treated strawberry juices during the first 21 days of refrigerated storage. In general, for a wide number of fruits and vegetables it has been observed that the degradation of ascorbic acid during storage appears to follow first-order kinetics (Lee and Chen, 1998; Uddin *et al.*, 2001; Torregrosa *et al.*, 2006; Odriozola-Serrano *et al.*, 2008b). Due to its sensitivity, vitamin C has been also considered as a good indicator of nutritional quality of water soluble vitamins in frozen vegetables. Martins and Silva (2004) reported that, in frozen green beans, ascorbic acid was totally converted to dehydroascorbic acid within the first month of storage. Comparing different frozen green vegetables, Giannakourou and Taoukis (2003) observed that the type of plant tissue significantly affects the rate of vitamin C loss. Frozen spinach was found to be more susceptible to vitamin C degradation through the storage than pea, green bean and okra.

12.4 Protecting fresh and processed fruits and vegetables against oxidation

Several techniques are available nowadays to control oxidative stress-induced injury in fruit and vegetable tissues. The use of antioxidant agents in dips or coatings and modified atmospheres to directly prevent oxidative reactions in fresh vegetable products are methods widely applied.

12.4.1 Antioxidant treatments

As mentioned before, fresh fruits and vegetables processing operations can induce undesirable changes in colour and appearance of these products during storage. Application of antioxidant treatments after peeling and/or cutting is the most common way to control browning of fresh commodities. Most strategies to control browning have focused on theoretical approaches to modulate PPO enzyme activities (Martínez and Whitaker, 1995). Ascorbic acid is the most extensively used to avoid enzymatic browning of fruit due to the reduction of the

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o-quinones, generated by the action of the PPO enzymes, back to their phenolic substrates (McEvily *et al.*, 1992). Ascorbic acid is considered by the US Food and Drug Administration (FDA) a GRAS (generally recognized as safe) antioxidant for being used to prevent browning of fruits and vegetables. In fact, ascorbic acid has long been applied in combination with organic acids and calcium salts to prevent enzymatic browning of several fresh-cut products (Pizzocaro *et al.*, 1993; Gorny *et al.*, 1998; Senesi *et al.*, 1999; Soliva-Fortuny *et al.*, 2001, 2002a, 2002b). However, this treatment may not be completely effective to control enzymatic browning of fresh products, since once the ascorbic acid is completely oxidized to dehydroascorbic acid, *o*-quinones are no longer reduced and darkening may occur due to formation of melanines (Nicolas *et al.*, 1994). In addition, recent works have demonstrated that ascorbic acid causes important oxidative damage in fresh-cut Fuji apples (Larrigaudière *et al.*, 2008). As an alternative to ascorbic acid, several thiol-containing compounds such as cysteine, N-acetylcysteine, and reduced glutathione have been suggested as browning inhibitors to prevent darkening on apple, potato and fresh fruit juices (Rojas-Graü *et al.*, 2006; Molnar-Perl and Friedman, 1990a, 1990b; Friedman *et al.*, 1992). These compounds react with quinones formed during the initial phase of enzymatic browning reactions to yield colourless addition products or to reduce *o*-quinones to *o*-diphenols (Richard *et al.*, 1991). Dips in aqueous solutions containing sulphur-containing amino acids such as N-acetylcysteine and/or glutathione at concentrations around 0.75% have been shown to inhibit browning of fresh-cut pears and apples (Rojas-Graü *et al.*, 2006; Oms-Oliu *et al.*, 2006). Gorny *et al.* (2002) also reported minor changes in the surface colour of Barlett pear slices treated with 2% AA + 1% calcium lactate + 0.5% cysteine (Richard-Forget *et al.*, 1992).

Carboxylic acids (citric acid and oxalic acid) have been also suggested as effective antioxidant agents in fresh fruits and vegetables. Jiang *et al.* (2004) reported that citric acid at concentrations lower than 0.02 M stimulated PPO activity of fresh-cut Chinese water chestnut, but at 0.1 M or higher markedly inhibited the activity. Altunkaya and Gökmen (2008) reported citric acid to be a non-competitive inhibitor of PPO in lettuce. Although there are few reports on antibrowning effects of oxalic acid on fresh fruits, immersing banana and apple slices in oxalic acid solutions effectively reduced browning (Son *et al.*, 2001; Yoruk *et al.*, 2004). In addition, 4-hexylresorcinol has been also proved to have effect on controlling enzymatic browning on cut fruits such as apples and pears (Monsalve-González *et al.*, 1993; Dong *et al.*, 2000; Son *et al.*, 2001; Rojas-Graü *et al.*, 2006; Oms-Oliu *et al.*, 2006). This resorcinol derivative has structural resemblance to phenol substrates and could have a competitive inhibitory effect on PPO activity (McEvily *et al.*, 1992). Its applicability in fresh-cut fruits has been proved, especially when used in combination with reducing agents (Monsalve-González *et al.*, 1993; Luo and Barbosa-Canovas, 1997; Dong *et al.*, 2000; Arias *et al.*, 2008).

Finally, some researchers have proposed that kojic acid [5-hydroxy-2-(hydroxymethyl)-γ-pyrone], a fungal metabolite, produced by many species of

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Aspergillus and *Penicillium* acts as a reducing agent as well as an inhibitor to the PPO enzyme (Chen *et al.*, 1991). The inhibitory mode of kojic acid on PPO is by interfering with the O_2 uptake required for the enzymatic reaction, by reducing *o*-quinones to diphenols to prevent melanin formation via polymerization and/or by combination of the two previous actions (Chen *et al.*, 1991). According to Son *et al.* (2001), the minimal concentration of kojic acid for an effective antibrowning activity on fresh-cut apples was 0.05%, similar to that reported for oxalic acid and cysteine.

12.4.2 Modified atmosphere packaging

The beneficial effects of modified atmosphere packaging (MAP) against oxidation of fresh and fresh-cut commodities have been extensively reviewed by several authors. Because the O_2 is needed for browning reactions, MAP with low O_2 and high CO_2 levels can contribute positively to avoid browning in fresh-cut produce. However, low-O_2 and elevated-CO_2 atmospheres cannot effectively inhibit browning of fresh-cut fruits and vegetables such as apple, banana, pear, potato or artichoke, due to their high phenolic content. As a consequence, the use of MAP systems in combination with antioxidant treatments is necessary to delay browning of several fresh products such as apples (Rojas-Graü *et al.*, 2007a; 2008a), pears (Oms-Oliu *et al.*, 2008a), mangoes (González-Aguilar *et al.*, 2000) and bananas (Vilas-Boas and Kader, 2006).

On the other hand, some authors have reported the effect of MAP on antioxidant constituents of fruits and vegetables. Odriozola-Serrano *et al.* (2008a) reported a good retention of lycopene in fresh-cut tomatoes stored under 5 kPa O_2 + 5 kPa CO_2 atmospheres for at least 14 days at 5 ºC. These authors indicated that lycopene synthesis due to ripening processes, together with low oxidation of carotenoids as a result of the low availability of O_2 in the package headspace, contributed to the higher levels of lycopene throughout storage. Such results were similar to those observed by Perkins-Veazie and Collins (2004) for fresh-cut watermelon stored under similar conditions for 7 days at 5 ºC. In addition, no losses in the total flavonoids content of fresh spinach were reported throughout storage regardless of the packaging atmosphere (Gil *et al.*, 1999). Barth *et al.* (1993) reported significantly higher amounts of ascorbic acid in broccoli stored in MAP than in unpacked broccoli.

According to Soliva-Fortuny and Martín-Belloso (2003), the vitamin C content of fresh-cut pears was kept almost constant throughout storage in the absence of O_2. Ascorbic acid degradation of shredded Galega kale was also more pronounced in air than under hypoxic environments, although almost no differences were found between 1, 2, and 3 kPa O_2 atmospheres (Fonseca *et al.*, 2005). Consistently, the vitamin C content of fresh-cut spinach was found to be better maintained for 7 days under 6% O_2 + 14% CO_2 than in air. However, Gil *et al.* (1999) found that ascorbic acid was transformed to DHA, its concentration being higher in MAP-stored fresh-cut spinach than in air-stored samples after one-week storage. Day (2001) reported that high-O_2 atmospheres did not result

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in a further decrease in ascorbic acid contents in prepared lettuce. However, it has been shown that the vitamin C content in fresh-cut pears packaged under 70 kPa O_2 is rapidly lost in comparison with a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere (Oms-Oliu *et al.*, 2007). Although the availability of O_2 is the main factor affecting vitamin C degradation, high CO_2 levels appear to have a negative effect on the vitamin C content of fresh-cut pears and apples (Soliva-Fortuny and Martín-Belloso, 2003; Soliva-Fortuny *et al.*, 2004). Agar *et al.* (1999) reported that high CO_2 levels stimulated ascorbic acid oxidation and inhibited the reduction of DHA to ascorbic acid. In fresh-cut pears stored under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere, a substantial loss of vitamin C after one week could be related to anoxic conditions reached inside the packages (Oms-Oliu *et al.* 2008a). According to Tudela *et al.* (2002), high CO_2 levels in fresh-cut potatoes increased vitamin C loss by accelerating ascorbate peroxidase-catalyzed oxidation processes. According to this claim, an important increase in peroxidase activity was shown during storage of fresh-cut 'Piel de Sapo' melon packaged under a 2.5 kPa O_2 + 7 kPa CO_2 atmosphere (Oms-Oliu *et al.*, 2008b). Beltrán *et al.* (2005) reported a reduction of phenolic compounds in shredded iceberg lettuce stored under reduced O_2 and elevated CO_2 concentrations (4 kPa O_2 + 12 kPa CO_2). A substantial accumulation of phenolic compounds was reported when fresh-cut lettuce was stored in air, whereas CO_2 levels $>$ 10 kPa led to a reduced phenolic content. According to Mateos *et al.* (1993), exposure to 20% CO_2 reduced the total phenolic content of fresh-cut lettuce due to a decrease in PAL activity as a result of the decrease in cytoplasmatic pH. Nevertheless, Oms-Oliu *et al.* (2008b) reported a higher production of phenolic compounds in fresh-cut melon stored under 2.5 kPa O_2 + 7 kPa CO_2 compared to 10 kPa O_2 + 7 kPa CO_2, air, 30 and 70 kPa O_2 atmospheres, which may be related to an enhanced oxidative stress induced by too-low O_2 and high CO_2 concentrations. In contrast with these results, Odriozola-Serrano *et al.* (2009b) reported an increase of lycopene content in fresh-cut tomatoes stored under high O_2 concentrations (Fig. 12.1). These authors indicated that exposure to $\geq$ 60 kPa of O_2 induced a higher production of lycopene, which may be related to the oxidative stress induced by high ethylene content inside packages.

12.4.3 Edible coatings

As mentioned above, the most common way to control oxidative reactions on fresh commodities is the application of dipping treatments containing anti-oxidants. However, antioxidant agents can also be added into the coating matrix to protect the cut surface against enzymatic browning. In fact, Baldwin *et al.* (1996) found that a carboxymethyl cellulose-based coating with addition of several antioxidants, including ascorbic acid, reduced browning of cut apple more effectively than an aqueous solution of antioxidants. Brancoli and Barbosa-Cánovas (2000) decreased surface discoloration of apple slices with maltodextrin and methylcellulose coatings including ascorbic acid. Pérez-Gago *et al.* (2006) reported a substantial reduction in browning of cut apples when

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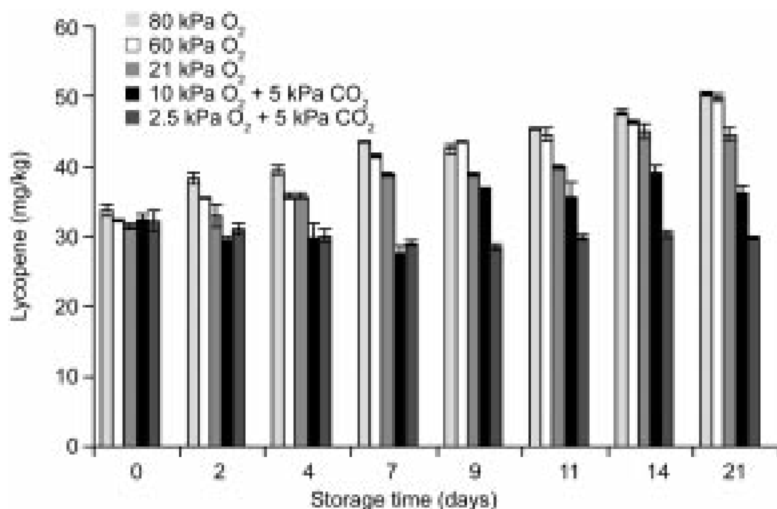

Fig. 12.1 Lycopene content of fresh-cut tomatoes stored during 21 days at 4ºC under different packaging conditions. Data shown are mean ± standard deviation (adapted from Odriozola-Serrano *et al.*, 2009b).

using a whey protein concentrate-beeswax coating containing ascorbic acid, cysteine or 4-hexylresorcinol. Rojas-Graü *et al.* (2007b) proved that alginate and gellan edible coatings can be used to deliver antioxidant agents such as cysteine or glutathione to the surface of fresh-cut apples. Indeed, Rojas-Graü *et al.* (2008b) observed that both alginate and gellan edible coatings containing *N*-acetylcysteine prevented apple wedges from browning during 21 days of storage. Similar results were obtained by Oms-Oliu *et al.* (2008c), who observed that the incorporation of *N*-acetylcysteine (0.75% w/v) and glutathione (0.75% w/v) into gellan, alginate or pectin formulations helped to control enzymatic browning of fresh-cut 'Flor de Invierno' pears, maintaining the initial h° values during two weeks of storage (Fig. 12.2). Olivas *et al.* (2003) prevented fresh-cut 'Anjou' pear wedges from surface oxidation using a methylcellulose-based coating containing ascorbic and citric acid. Similar results were obtained by Lee *et al.* (2003), who studied the effect of carrageenan and whey protein concentrate edible coatings in combination with antibrowning agents on fresh-cut apple slices and observed that the incorporation of ascorbic, citric and oxalic acids was advantageous in maintaining colour during two weeks.

Besides, some researchers have indicated that edible coatings offer the potential to improve the antioxidant properties of fresh produces. Oms-Oliu *et al.* (2008c) maintained the vitamin C and total phenolic content in pear wedges coated with alginate, gellan or pectin edible coatings containing antioxidant agents, which contributed to maintain their antioxidant potential. It was also observed that the use of pectin or alginate coatings may contribute to reduce the wounding stress induced in fresh-cut 'Piel de Sapo' melon (Oms-Oliu *et al.*, 2008d). Chien *et al.* (2007) maintained the ascorbic acid content of sliced red

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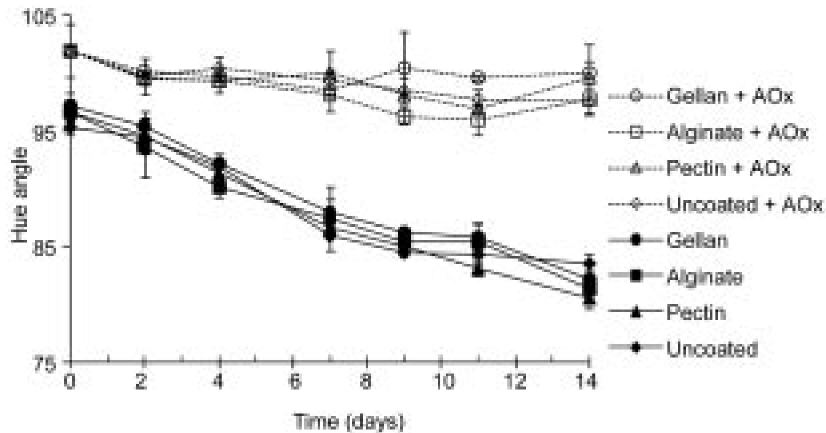

Fig. 12.2 Changes in hue angle of fresh-cut pears coated with gellan, alginate, pectin or uncoated, including or not N-acetylcysteine and glutathione as antioxidant agents – AOx (adapted from Oms-Oliu *et al.*, 2008c).

pitayas (dragon-fruit) coated with low molecular weight chitosan. Li and Barth (1998) improved carotene retention on minimally processed carrots using a cellulose-based edible coating. Serrano *et al.* (2006) maintained total phenolics, ascorbic acid and high retention of total antioxidant activity in table grape coated with *Aloe vera* gel coatings. Tapia *et al.* (2008) formulated alginate and gellan-based edible coatings containing ascorbic acid for fresh-cut papaya pieces. They observed that alginate coatings appeared to perform better on ascorbic acid retention, probably due to slightly better gas barrier properties. The authors also reported that the incorporation of ascorbic acid to the coatings resulted in a substantial increase in the ascorbic acid content of the fresh-cut product, thus helping to preserve the naturally occurring amounts of this compound throughout storage. Similar results have been obtained by Ayranci and Tunc (2004) who reported that the use of citric or ascorbic acids, incorporated as additives into a methylcellulose coating, extensively reduced ascorbic acid losses in whole apricots and peppers.

12.5 Future trends

Fruits and vegetables have a capital importance in human nutrition, either when they are regarded as a food product on their own right or used as key ingredients in processed foods, thus representing the main source of dietary antioxidants. However, studies have shown that the amount of these compounds may substantially vary depending on several factors related to the conditions during crop growing, handling, processing and storage. Future research should be aimed at identifying the mechanisms influencing the biochemistry of the degradation of antioxidant compounds in fruit and vegetable products as a consequence of

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environmental exposure. This would allow us to understand the processes mediating the deterioration or generation of antioxidants in food matrices and to better estimate the impact of processing on the nutritional value of food products.

12.6 Sources of further information and advice

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13

Flavour changes in beer: oxidation and other pathways

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Abstract: Most brewers seek to minimize staling but consumers may not have an adverse reaction to it. The blander the beer, the more pronounced aged character becomes. Diverse flavour changes can occur, especially the development of a papery/cardboard aroma. Precursors include hop bitter acids, alcohols, amino acids and unsaturated fatty acids. Some of these changes may occur upstream in processing but the longest shelf life for beer is achieved by minimizing the oxygen level in the final package and maintaining beer at the lowest possible non-freezing temperature. Prediction of flavour life is not simple: the most reliable procedures are sensory-based ones.

Key words: beer, oxidation, pathways, process.

13.1 Introduction

It is generally considered within the brewing industry that the last major quality challenge to be overcome is instability in the flavour (i.e., flavour change). At the outset, though, it is legitimate to question the extent to which the consumer (as opposed to the brewer) is significantly concerned about such matters. Decisions about beer quality can easily be made when there is an obvious change in expected quality dimensions, for example lack of foam in a beer characterized by robust head or turbidity in a beer that the customer expects to be 'bright'. However, sometimes subtle nuances of flavour change may not be readily apparent to the drinker, even if they are glaring to the brewer. Guinard and colleagues (2000) showed that US consumers presented with branded beers

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will select imported products over domestically brewed ones, regardless of the flavour defects in the older overseas beers that were evident to a trained panel of tasters. Stephenson and Bamforth (2002) showed that subjects served fresh or aged samples of a given brand of beer had an equal preference for the two, although if the same sample of aged beer was presented in separate glasses, one labelled with the name of the brand and the other unbranded, then there was a distinct preference for the unbranded product. The opposite impact was observed when both glasses contained the fresh beer. In other words, the branding has a huge impact on customer preference, overriding nuances of freshness; however, when a beer is stale and the customer has a choice to make, they will select the beer that they *believe* is not the brand they are used to. With a fresh beer, they will select the brand that they *believe* is the one they know.

This study clearly showed that beer freshness is not insignificant. The logical approach of the brewer is to achieve consistency, meaning that the consumer is not going to find different flavours in coincidental purchases of a given brand of beer. Whether the beer should be consistently *fresh* or merely consistent is a moot point. There have been several examples of beers that were stripped of flavours that were judged unacceptable by the brewer, only for the consumer to find less favour in those products (Bamforth, 2004). It has even been suggested that a beer should be deliberately aged to its maximum extent prior to shipping from the brewery, so as to ensure that further changes leading to inconsistency will not occur (Axcell and Torline, 1998).

Most brewers favour the converse approach of avoiding aged characters. Some styles of beer are much more susceptible to perceived flavour change than others, most notably the relatively 'gently' flavoured lager-style products. This is because there is a less robust depth of flavour in such beers as compared to, say, an Irish stout, with intense flavours that mask ageing. Thus we have concepts like the Best Before Date (which in reality is likelier founded on the time before beer will throw a haze rather than change in flavour) and the Born On Date.

13.2 The nature of flavour changes in beer

Reports of the flavour changes occurring in stored beer invariably report the diagram first published by Dalgliesh (1977), but it is important to recognize that this description is nothing more than an approximate summation of the overall changes that occur, and in an ale at that. Dalgliesh (1977) described a decrease in bitterness and an accompanying increase in sweetness but it is not clear whether these are independent phenomena, as perceived by expert tasters, or else a decrease in perceived apparent bitterness because the sweetness increase confuses the taster. Or the converse, with a reduced bitterness manifesting itself as increased sweetness. (There is no argument that the level of bitter acids in beer does indeed decrease during beer storage – see later.) The perception of sweetness may manifest itself in descriptors such as caramel, burnt sugar and toffee-like.

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Dalgliesh (1977) also described the development and subsequent subsiding of a ribes note variously described as blackcurrant buds/leaves or tom cat urine. Following this is the development of a cardboard or wet paper aroma. Meilgaard (1972), however, suggests that there is an ongoing development of cardboard aroma as beer is stored, up to a maximum, with an ensuing decrease.

Other notes described in aged beer include harsh after-bitter and astringent tastes (Lewis *et al.*, 1974), wine- and whiskey-like aroma (Drost *et al.*, 1971), hay or straw-like (Bushnell *et al.*, 2003) and burnt or liquorice (Whitear, 1981). Other notes, such as fruity/estery and floral may decrease in intensity, a phenomenon which is just as much flavour instability as the development of 'new' notes (Bamforth, 1999b; Whitear *et al.*, 1979).

The precise flavour notes observed likely depend on the ageing conditions. Ribes is strongly correlated with package air content (Clapperton, 1976) while higher storage temperatures (of the type used in accelerated ageing experiments) tend to exaggerate cardboard character (Furusho *et al.*, 1999; Kaneda *et al.*, 1995).

13.3 The chemistry of flavour change in beer

13.3.1 Relevant compounds

The flavour of beer changes as a result of both the increase and decrease in the level of substances detectable by taste or aroma and by changes in the levels of substances that may either mask or potentiate the detection of other compounds (Meilgaard, 1975a).

Table 13.1 lists many of the compounds whose level increases during beer storage. Of these classifications, it is the carbonyl compounds that have long attracted the most attention, since the pioneering work of Hashimoto (1966), who reported a substantial elevation in the concentration of volatile carbonyls in stored beer, commensurate with the development of stale character. Highlighted amongst the carbonyl compounds have been acetaldehyde (Engan, 1969) and (E)-2-nonenal (Palamand and Hardwick, 1969; Jamieson and Van Gheluwe, 1970), imparting green apple and cardboard/wet paper notes, respectively. Quickly the latter substance developed a 'reputation' for being the major determinant of stale character in beer, but in many of the studies the conditions used to develop the character were extreme, notably heating at pH 2 and thus very different from what happens in 'real' aging. Nonetheless, using more realistic storage conditions, Wang and Siebert (1974) did demonstrate an increase in (E)-2-nonenal to amounts above the flavour threshold (0.1 ppb). However other carbonyl substances also increase in their levels (Greenhoff and Wheeler, 1981a; 1981b; Hashimoto and Eshima, 1977; Jamieson and Chen, 1972; Stenroos *et al.*, 1976). Referring to the claims reported above that higher 'forced' aging temperatures tend to exaggerate cardboard character in beer, it is pertinent to note the findings of Van Eerde and Strating (1981) that (E)-2-nonenal increases in quantity at 40 ºC in a few days but to a much lesser degree in storage at 20 ºC

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Table 13.1 Compounds formed during beer storage

Class	Compounds
Aldehydes	acetaldehyde
	E-2-nonenal
	E-2-octenal
	E,E-2,4-decadienal
	E,E-2,6-nonadienal
	2-methylbutanal
	3-methylbutanal
	benzaldehyde
	2-phenylacetaldehyde
	3-(methylthio) propionaldehyde
Ketones	E-β-damascenone
	diacetyl
	3-methyl-2-butanone
	4-methyl-2-butanone
	4-methyl-2-pentanone
	2,3-pentanedione
Cyclic acetals	2,4,5-trimethyl-1,3-dioxolane
	2-isopropyl-4,5-dimethyl-1,3-dioxolane
	2-isobutyryl-4,5-dimethyl-1,3-dioxolane
	2-sec butyl-4,5-dimethyl-1,3-dioxolane
Heterocylic compounds	furfural
	5-hydroxymethylfurfural
	5-methylfurfural
	2-acetylfuran
	2-acetyl-5-methylfuran
	2-propionylfuran
	furan
	furfuryl alcohol
	furfuryl ethyl ether
	2-ethoxymethyl-5-furfural
	2-ethyoxy-2,5-dihydrofuran
	maltol
	dihydro-5,5-diemethyl-2(3H)-furanone
	5,5-dimethyl-2(5H)-furanone
	2-acetylpyrazine
	2-methoxypyrazine
	2,6-dimethylpyrazine
	trimethylpyrazine
	tetramethylpyrazine
Ethyl esters	ethyl-3-methylbutyrate
	ethyl-2-methylbutyrate
	ethyl-2-methylpropionate
	ethylnicotinate
	diethyl succinate
	ethyl lactate
	ethyl phenylacetate
	ethyl formate
	ethyl cinnamate
Lactones	γ-nonalactone
	γ-hexalactone
S-compounds	dimethyl trisulphide
	3-methyl-3-mercaptobutylformate

Based on Vanderhaegen *et al.* (2006)

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over 4 months storage. Foster *et al.* (2001), Narziss *et al.* (1999), Schieberle and Komarek (2002) and Vesely *et al.* (2003) observed no meaningful elevation in (E)-2-nonenal concentration as beer is aged, but Lermusieau *et al.* (1999), Liegeois *et al.* (2002) and Santos *et al.* (2003) did. It is certainly the case that agents that scavenge carbonyl compounds such as hydroxylamine (Hashimoto, 1981) and semicarbazide (Bamforth, 2000) remove cardboard notes from beer. It is likely the case that cardboard character can be due to several linear aldehydes (Meilgaard, 1975b). Greenhoff and Wheeler (1981a, 1981b) and Harayama *et al.* (1994) reported that the alkedienals, (E,Z)-2,6-nonadienal and (E,E)-2,4-decadienal, take part in flavour staling. Strecker aldehydes such as 2-methyl-propanal, 2-methyl-butanal, 3-methylbutanal, benzaldehyde, phenylacetaldehyde and methional have also been implicated in staling (Wheeler *et al.*, 1971; Bohmann, 1985; Miedaner *et al.*, 1991; Vesely *et al.*, 2003; Gijs *et al.*, 2002). The ketones β-damascenone (Chevance *et al.*, 2002; Gijs *et al.*, 2002) and 3-methyl-butan-2-one and 4-methylpentan-2-one (Hashimoto and Kuroiwa, 1975; Lustig *et al.*, 1993) are also reported to increase in level in aged beer. The vicinal diketones diacetyl and 2,3 pentanedione increase in beers that have not been properly denuded of the relevant precursors during brewery fermentation and conditioning (Inoue, 1998).

Reference to Table 13.1 highlights other categories of substance that have variously been claimed to increase in level in stored beer: cyclic acetals (Vanderhaegen *et al.*, 2003; Peppard and Halsey, 1982); heterocyclics (Lustig *et al.*, 1993; Madigan *et al.*, 1998; Varmuza *et al.*, 2002; Bernstein and Laufer, 1977; Brenner and Khan, 1976; Shimizu *et al.*, 2001; Vanderhaegen *et al.*, 2003, 2004; Gijs *et al.*, 2002); esters (Bohmann, 1985; Gijs *et al.*, 2002; Lustig *et al.*, 1993; Miedaner *et al.*, 1991; Williams and Wagner, 1978; Schieberle and Komarek, 2002); lactones or cyclic esters (Eichhorn *et al.*, 1989; Gijs *et al.*, 2002); sulphur-containing compounds (Gijs *et al.*, 2000, 2002; Williams and Gracey, 1982; Schieberle, 1991; Tressl *et al.*, 1980).

Mention of the esters reveals an example of a compound that can *decrease* in level during beer storage, thereby reducing the detection of desirable fruity character (Neven *et al.*, 1997; Stenroos, 1973). Probably of even more significance is the decrease in the bitter substances, the iso-α-acids (De Cooman *et al.*, 2000; King and Duineveld, 1999; Walters *et al.*, 1997b). The trans-isomers degrade more rapidly than do the cis-isomers (Araki *et al.*, 2002). Polyphenol levels also decrease during beer storage (Kaneda *et al.*, 1990; McMurrough *et al.*, 1996, 1997) and it is claimed that this relates to the development of harsh and astringent flavours.

13.3.2 Relevant pathways

Oxidation of unsaturated fatty acids

More attention has been lavished on this potential source of carbonyl substances than any other, since it was first suggested that it is the primary mechanism (Dale and Pollock, 1977; Drost *et al.*, 1971; Jamieson and Van Gheluwe, 1970;

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Tressl *et al.*, 1979). Certainly the most abundant fatty acid in barley is linoleic acid, in large part esterified as glycerides (Anness and Reed, 1985). The fatty acids are released by lipases (Baxter, 1984) which are relatively stable and able to act in mashing (Schwarz *et al.*, 2002). However, it is evident that much of the fatty acid is removed either in the glyceride or free form by adsorption on to spent grains and trub in the brewhouse, i.e. physical effects are at least as important as chemical ones (Anness and Reed, 1985).

It has recently been suggested that there is very limited oxidation of unsaturated fatty acids in beer *per se* (Lermusieau *et al.*, 1999; Noel *et al.*, 1999). Nevertheless, Bamforth (1999a) did show that even at the very low levels of linoleic acid surviving into beer, there is ample precursor for the development of carbonyl levels vastly in excess of the flavour threshold. Despite this, the belief remains that oxidation of the unsaturated fatty acids occurs during malting and wort production, leading to the production of oxidized intermediates that enter into the beer to progressively break down through non-enzymic routes to release aging compounds (e.g., Drost *et al.*, 1990). Furthermore it has been suggested that the carbonyl compounds themselves are produced upstream and are bound to either bisulfite (Barker *et al.*, 1983) or as Schiff bases to amino acids or proteins (Noel and Collin, 1995) to be released progressively at the lower pH of beer (Lermusieau *et al.*, 1999). Dufour *et al.* (1999). Kaneda *et al.* (1996) and Lermusieau *et al.* (1999) have cast doubt on the role of bisulfite in this respect.

Unsaturated fatty acids may potentially be oxidized by two routes: an enzymatic route initially catalyzed by lipoxygenase (LOX) and a non-enzymic route involving reactive oxygen species (see Section 13.5.1).

Barley develops two LOX isozymes, LOX-1 and LOX-2, during germination (Baxter, 1982). The former has some limited presence in raw barley (Yang and Schwarz, 1995). The enzymes oxidize fatty acids such as linoleic acid and linolenic acid to hydroperoxy acids (LOOH). Both enzymes are sensitive to heat and are largely inactivated during kilning. The somewhat greater survival of LOX-1 (Yang and Schwarz, 1995) marries with a greater proportion of the 9-LOOH as opposed to the 3-LOOH that develops during mashing at lower temperatures (Walker *et al.*, 1996). Indeed LOX-1 has a broader pH optimum than has LOX-2 and is better able to function at mashing pH's (Doderer *et al.*, 1991). LOOH is produced to a lesser extent at higher mashing temperatures (Kobayashi *et al.*, 1993b) and if the mash pH was lowered from 5.5 to 5.0 (Kobayashi *et al.*, 1993a). (E)-2-nonenal can be formed from 9-LOOH by enzymic and non-enzymic means (Tressl *et al.*, 1979; Kobayashi *et al.*, 1994; Schwarz and Pyler 1984). 9-LOOH is also converted to ketols by hydroperoxide lyase (Kuroda *et al.*, 2003). These ketols are subsequently converted non-enzymically to mono-, di- and trihydroxyacids that in turn break down to produce (E)-2-nonenal. Kuroda *et al.* (2002) suggested that linoleic acid is oxidized to di- and trihydroxy acids by LOX-1 and by a more heat-tolerant peroxygenase.

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Oxidation of higher alcohols

Hashimoto (1972) was the first to propose alcohols as a source of staling aldehydes in beer, a conversion catalyzed by melanoidins. Devreux *et al.* (1981) suggested that light was necessary for this process to occur and also drew attention to inhibition by polyphenols. Nonetheless Andersen and Skibsted (1998) showed that 1-hydroxyethyl derived by the interaction of hydroxyl radicals with ethanol is the most abundant radical species in stale beer, and that it degrades to produce acetaldehyde. It may be that other alcohols indulge in similar reactions to produce longer chain carbonyls.

Strecker degradation of amino acids

Amino acids can react with α-dicarbonyl compounds, such as those formed as intermediates in browning reactions in reactions catalyzed by iron and copper ions. The amino acid is shifted into an aldehyde with one fewer carbon atom (Blockmans *et al.*, 1975; Hashimoto and Kuroiwa, 1975). Polyphenols may have a catalytic role (Blockmans *et al.*, 1975).

Degradation of hop bitter acids

Hashimoto and Eshima (1979) suggested that unhopped beer does not develop stale flavour. Iso-α-acids are degraded in the presence of reactive oxygen species (ROS), with the trans-isomer being rather more susceptible (Kaneda *et al.*, 1989; De Cooman *et al.*, 2000). This degradation may also be potentiated by certain electron acceptors (Huvaere *et al.*, 2003) in the absence of molecular oxygen. Reduced side-chain iso-α-acids, employed in the production of light-resistant beers, are more resistant to breakdown (Jaskula *et al.*, 2007).

Aldol condensation

Aldol condensation reactions in beer were first proposed by Hashimoto and Kuroiwa (1975) with the formation of (E)-2-nonenal by reactions of acetaldehyde with heptanal in a model system incorporating proline (an abundant imino acid in beer) as a catalyst.

Breakdown of glycosides

It has been suggested that compounds such as (E)-β-damascenone are released in beer from glycoside-linkage, wither non-enzymically or through the action of glycosidases, the latter presumably derived from yeast (Gijs *et al.*, 2002; Biendl *et al.*, 2003).

Acetal formation

Cyclic acetals may be produced by the condensation of 2,3-butanediol (produced in the reduction of diacetyl by yeast) and an aldehyde (Peppard and Halsey, 1982).

Maillard reaction

Maillard reactions may account for the development of bready-, sweet and wine-like notes in stored beer (Bravo *et al.*, 2001). Furfuryl alcohol produced by

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Maillard reactions in the kilning of malt and the boiling of wort may condense with alcohol in beer to form the staling substance furfuryl ethyl ether (Vanderhaegen *et al.*, 2004).

Ester formation

Esters can be produced by non-enzymic reactions between alcohols and acids in stored beer. For example, Williams and Wagner (1979) report the development of ethyl 3-methyl-butyrate and ethyl 2-methyl-butyrate. Equally, some existing esters produced in fermentation may be hydrolyzed during beer aging and this may be catalyzed by yeast esterases (Neven, 1997; Horsted *et al.*, 1998).

13.4 The assessment of flavour instability

Procedures are needed to enable maltsters and brewers to establish raw materials and processing and packaging conditions that lead to enhanced shelf life. Such methods might be further classified to those that can be used systematically to evolve materials and processes gradually and those that might be used in real time so that the processing can be adjusted to maximize flavour life. Ultimately the relevant gauge for a flavour-based phenomenon must be organoleptic in nature, and Meilgaard (2001) has drawn attention to the abysmal situation that has long pertained in the reporting of data in the arena of flavour stability:

> 'One would think that the requirements for valid flavour assessment would be well known by now. Yet, out of several hundred papers consulted, only two or three report all or even most of these details.'

One of the challenges is how to accelerate the aging process so as to enable realistic predictions of likely flavour life. Greenhoff and Wheeler (1981a) advocate holding beer at either 60 ºC for 22h or 37 ºC for 3 weeks prior to tasting, claiming that both are a good mimic for 6 months at 18 ºC. Lustig (1996) proposed shaking beer (to simulate transport) followed by 4 days of storage at 40 ºC, suggesting that this is equivalent to 3–4 months at 20 ºC. It should be noted that the nature of flavour changes occurring at different temperatures differs (see above).

Bamforth (2004) has argued that the most meaningful gauge of flavour stability is not the intensity of flavour developed but rather the time taken to develop a stale character.

Various analytical procedures have been proposed as alternatives to tasting protocols. Grisgby and Palamand (1976) and later Cope and Parsons (1983) used thiobarbituric acid to measure carbonyl species produced in forced ageing. Ethylene has also been cited as an indicator of staling potential (Lynch and Seo, 1987). Drost *et al.* (1990) developed the concept of 'nonenal potential' in which samples are heated and the nonenal released measured. As shown above,

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however, nonenal is far from being the only determinant of staling. Furfural has long been used as an ageing marker. Bright *et al.* (1993) developed a model for predicting shelf life based on the measurement of several compounds by solid-liquid extraction and direct desorption onto a gas chromatography column, finding that furfural and phenylethylacetate were amongst the compounds in the model but, interestingly, nonenal was not.

In recent years, great attention has been paid to electron spin resonance spectroscopy as a means to measure free radical species, it having been recognized since the work of Bamforth and Parsons (1985) that primary focus should be on radical reactions in the pursuit of enhanced flavour stability. Pioneering studies were by Ono and colleagues (Uchida and Ono, 1996; Uchida *et al.*, 1996).

13.5 Factors impacting flavour change in beer

13.5.1 Oxygen

Oxygen plays a pivotal role in the staling of beer. It has long been accepted that oxygen levels in final package should be as low as possible. Bamforth's calculations (1999a) showed that even at oxygen levels as low as 0.1 mg/L there is ample oxygen to present problems. There is increasing belief that oxygen ingress earlier in the process can potentiate staling, even oxygen in the malt house (Tressl *et al.*, 1979). The reality is that there are conflicting opinions on this. For as many studies that claim a minimization of oxygen uptake in the brewhouse is beneficial (Narziss *et al.*, 1987, 1989) there are those that suggest that air ingress in the brewhouse is an irrelevance in the context of staling (Hug *et al.*, 1986). Nonetheless, there is undoubtedly a substantial consumption of oxygen throughout the brewing process. It is notoriously difficult to make accurate measurements of how much oxygen is scavenged (Lie *et al.*, 1977); indeed many studies have been made on small-scale equipment with unrealistically high surface area-to-volume ratios that would encourage much greater air ingress than in commercial equipment.

Oxygen may react with wort and beer components in the guise of reactive oxygen species or through enzyme-catalyzed reactions. Apart from lipoxygenase (see earlier), malt contains thiol oxidase (Bamforth *et al.*, 2009) and oxalate oxidase (Kanauchi *et al.*, 2010), both of which will assimilate oxygen. Polyphenol oxidase is irrelevant insofar as it is lost during malting and immediately in mashing (Clarkson *et al.*, 1991). Oxygen may react with thiol groups non-enzymically (Muller, 1997) and via thiol oxidase to produce hydrogen peroxide, which in turn is a substrate for peroxidases (Clarkson *et al.*, 1992) through the action of which polyphenols become oxidized. Studies with $^{18}O_2$ revealed that 60% of oxygen enters into polyphenols, with 35% into volatile carbonyls and the rest into iso-α-acids (Owades and Jakovac, 1966).

The role of reactive oxygen species as determinants of beer staling was first addressed by Bamforth and Parsons (1985) and has since received extensive

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attention (see for example Frederiksen *et al.*, 2008). The nature of these species and how they arise is addressed in the first chapter of this volume. In the context of brewing, the criticality of minimizing activating species, notably the transition metal ions iron and copper, has been highlighted (Bamforth *et al.*, 1993).

13.5.2 Temperature

Apart from oxygen levels, the single most important factor impacting the shelf life of beer is heat. The accelerated ageing regimes described earlier speak to the relevance of Arrhenius' observation that the rate of a chemical reaction increases two- to three-fold for each 10 ºC increase in temperature. Thus a beer that develops clear stale character after around 100 days at 20 ºC, will have aged in around a month at 30 ºC or a day at 60 ºC (Bamforth, 2004). Equally, the shelf life will be enormously increased in refrigerated beer. It is important to stress that the nature as well as the extent of flavour change differs depending on temperature, presumably because of different rates at which the various reactions occur (see earlier; Lustig, 1996). Thus Walters *et al.* (1997b) found that raising the temperature from 0 ºC through 25 ºC to 40 ºC had a disproportionate effect on the loss of iso-α-acids from beer and on the levels of furfural developed.

13.5.3 Antioxidants

The observations of Owades cited above highlight the significance of polyphenols as antioxidants; however, as noted by Irwin *et al.* (1991), there are both pro- and antioxidant polyphenolic species. Apart from acting as radical scavengers (Yuting *et al.*, 1990; Husain *et al.*, 1987; Torel *et al.*, 1986) and as substrates for peroxidases to enable peroxide removal (Clarkson *et al.*, 1989), polyphenols may also function as inhibitors of lipoxygenase (Boivin *et al.*, 1995) and as metal ion chelators (Buggey, 2001). Liegeois *et al.* (2000) and Mikyska *et al.* (2002) suggest that the major benefit of polyphenols may be in the brewhouse. The phenolic acid ferulate may have an antioxidant function in beer (Walters *et al.*, 1997a, 1997b). Melanoidins also are capable of scavenging reactive oxygen species (Hashimoto and Kuroiwa, 1975; Hayase *et al.*, 1986) but here too, they can promote reactions implicated in aging (see earlier, viz. the oxidation of higher alcohols). Melanoidins are also chelating agents, as is phytic acid (Jacobsen and Slotfeldt-Ellingsen, 1983). Sulphur dioxide is also a radical scavenger (Kaneda *et al.*, 1994) and it is claimed to be the most powerful antioxidant in beer (Anderson et al., 2000; these authors suggesting that the polyphenols have no significant radical scavenging ability in beer), but the major benefit of SO_2 in respect of beer flavour stability may be as a binding agent for carbonyls, rendering them in a non-flavoursome form (Barker *et al.*, 1983).

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13.6 The impact of the malting and brewing processes on flavour change in final package

13.6.1 Raw materials

Malt potentially comprises a diversity of staling precursors, substrates for damaging reactions, enzymes capable of both promoting flavour deterioration (most notably lipoxygenase) but also those capable of eliminating reactive oxygen species (peroxidases and superoxide dismutase, Bamforth, 1983), substances which can potentiate the production of reactive oxygen species (e.g., thiol groups in gel proteins) and a diversity of antioxidants. It is no simple matter to 'dissect' malt to establish the relative significance (if any) of some or all of these for flavour deterioration.

There has been much debate about whether lipoxygenase really does play a pivotal role in catalyzing the staling reactions (Vanderhaegen *et al.*, 2006). On the one hand, there are those who fervently believe in its importance (van Waesberghe *et al.*, 2001), even to the most recent suggestions that barleys lacking the gene coding for the enzyme lead to beer with enhanced shelf life (Hirota *et al.*, 2005). Conversely, it has been suggested that the kinetic properties of the enzyme (thermal stability, substrate affinity) make it highly unlikely to act other than in the very initial stages of mashing (Biawa and Bamforth, 2002). If lipoxygenase has a role to play then it is indeed clear that the less well-modified malts (i.e., less lipoxygenase synthesis) with highest practical kilning temperature commensurate with other malt properties subsequently mashed in at the highest possible mashing temperature would be the way to go. It has even been suggested that modified milling regimes that leave the embryo (within which is both linoleic acid and lipoxygenase) intact would lead to ensuing beers with much less staling potential (van Waesberghe, 1997).

More extensively kilned malts have increased antioxidant potential (Chapon *et al.*, 1971). However increased protein modification during malting leads to increased levels of staling substances in beer and higher kilning temperatures lead to elevated levels of staling aldehydes (Lustig, 1996). Therefore Lustig (1996) advocates the avoidance of high thermal load so as to lessen the development of staling substances and their precursors through Maillard and Strecker reactions. Although the general scenario is confused, it may be that adjuncts impact flavour instability. Peppard and colleagues (1983) suggested that rice and corn grits lessen flavour stability whereas wheat flour, barley grits and corn syrup enhance shelf life.

13.6.2 Brewhouse

As discussed earlier, there is no consensus on the importance of minimizing oxygen ingress into wort in brewing as a tool in enhancing flavour life. Trials on a small scale have suggested benefits (Narziss *et al.*, 1987, 1989; Muller, 1995) but this has not always translated onto the commercial scale (O'Rourke *et al.*, 1992). If oxygen does indeed potentiate staling at this stage then it may either be through the non-enzymic or enzymic reactions described earlier. Apart from

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minimizing oxygen levels and maximizing mashing temperatures, it has been suggested that the reduction of mash pH to 5.1 restricts the ability of lipoxygenase to act (Narziss *et al.*, 1993). Regarding reactive oxygen species, then it is known that copper clad brewhouses lead to beers with reduced flavour stability, presumably through the leaching of significant levels of copper ions from the tanks (Narziss *et al.*, 1989; van Gheluwe *et al.*, 1970).

During the kettle boil there is an opportunity to drive off pre-formed staling substances and Buckee and Barrett (1982) showed that more stable beer is produced from wort boiled for 1h as opposed to 30 min. However thermal degradation reactions in kettle and whirlpool generate undesirable flavour compounds (Lustig *et al.*, 1998).

13.6.3 Yeast and fermentation

Yeast has a profound ability to reduce carbonyl compounds (Peppard and Halsey, 1981). The other contribution that yeast makes is through its ability to make sulphur dioxide. Basically anything that impedes yeast's ability to ferment will lead to increased levels of sulphur dioxide in the beer (Ilett, 1995). These factors include clearer worts, reduced wort oxygenation, reduced pitching rate and reduced fermentation temperature. Increased sulphate levels in wort may also serve to increase SO_2. Yeast strains capable of increased SO_2 production have been reported (Korch *et al.*, 1991; Hansen and Kielland-Brandt, 1995). During fermentation there is a decrease in pH. There is a direct relationship between pH decrease and flavour instability, perhaps because at the lower pH's superoxide is predominantly in its protonated perhydroxyl form, which is more damaging (Kaneda *et al.*, 1997)

13.6.4 Downstream and packaging

Downstream of the fermenter the overriding priority is to minimize oxygen pick-up. Practical opportunities include the use of nitrogen or carbon dioxide as a motor gas; blanketing vessels with such gases prior to filling; rigorous deaeration of water (to less than 30 ppb O_2) used for any purpose other than cleaning; rigorous maintenance of pumps and valves. A realistic target for bright beer prior to packaging is 50 ppb and may be even lower with the use of modern membrane technology (Gill and Menneer, 1997). Modern packaging operations incorporate rigorous use of vacuums, CO_2 flushing, tapping and jetting (bottles) or under-cover gassing (cans). Pry-off crown corks offer better shelf life, because twist-off seals allow a greater degree of air ingress between closure and bottle (Hoorens van Heyningen *et al.*, 1987). Crown corks incorporating oxygen scavengers are available (Teumac, 1995). The barrier properties of plastic bottles are improving (Hertlein *et al.*, 1997).

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13.7 The practicality of achieving flavour stability

Consideration of the above makes it evident that beer flavour stability is extremely complicated. The myriad of chemical changes occurring and the prolonged and multi-stage processing with attendant risk of variability all vector towards change and instability. The practical advice to be made to any brewer is to start at the end and work backwards. Unfortunately, few brewers have sufficient control of the packaged beer once it leaves their warehouses. Extremes of temperature, agitation in transportation and unreliable rotation of stock in trade all present huge risks to the flavour life of beer. Anything that lessens the variability (e.g., refrigerated transportation), whilst expensive, adds genuine benefit. At the very least consumer education is key: looking for best before dates or born-on dates, keeping beer refrigerated and so on. Any trick of packaging that would give indication of whether beer had been exposed to undesirable conditions (e.g., excess temperature) would be worthwhile. Working back, it is clear that the packaging operation is key, notably in terms of presenting beer to warehouse with the lowest possible oxygen and transition metal ion content. After that, as we work back through the process there remains a major debate about just how important the various stages and reactions are. It is up to individual brewers to satisfy themselves which process stages seem key in their eyes for their beers and then to ensure raw material and process consistency.

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14

Wine oxidation

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Abstract: This chapter reviews some of the chemistry, outcomes and the practices used to manage oxidation during winemaking and wine aging. The chapter begins by discussing the ways in which oxygen dissolves and is consumed in wine. The chapter then goes on to describe the current theoretical model of oxidation and the impact of oxidation on wine quality. The chapter then discusses some practical means to manage oxidation in wine.

Key words: wine, oxidation, oxygen, phenolic compounds, metals, sulfur dioxide, ascorbic acid.

14.1 Introduction

Wine is distinct from many other foodstuffs, as in many cases, oxidation and oxygen exposure may add value, and could even be required to meet certain quality standards (e.g., Sherry or Madeira wines).

During winemaking, air is encountered in operations such as the crushing of grapes, the transfer of wine from one container to another, filtration, and many other steps. The general outcome of excessive oxygen exposure is an oxidized wine that apart from significant flavour changes, can also be spoiled by the effect of aerobic microorganisms (Ribéreau-Gayon *et al.*, 2006; Boulton *et al.*, 1996). To overcome this problem, many technological solutions have been implemented, and among the most effective are those that minimize the contact of wine with air (e.g., by blanketing wine surfaces with inert gases such as CO_2), prevent the propagation of oxidation reactions and limit the impact of oxidation

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that has already occurred (e.g. with the use of sulfur dioxide). On the other hand, researchers have also noted that moderate oxygen exposure can benefit some wine styles by, for instance, stabilizing colour and reducing astringency and bitterness in red wines (Singleton, 1987; Castellari *et al.*, 1998a; Atanasova *et al.*, 2002).

Currently, the status of wine with respect to oxidation is generally assessed by tasting, with little analytical data to support winemaking decisions; therefore, further research is needed to develop new strategies to better predict and control the requirements of oxygen for a particular wine. The rate of oxidative deterioration does not just depend on the absolute amount of dissolved oxygen, but is complicated by the exact composition of a particular wine, including the phenolic compounds, metal ions, and sulfur dioxide concentrations.

This overview of the chemistry and practices of oxidation in winemaking presents examples of some of the early studies and new developments in this area, and intends to illustrate the complexity of these processes. Insights into possible future practices and research trends are given. Although briefly mentioned, the enzymatic oxidation of musts and wines is beyond the scope of this chapter.

14.2 Oxygen dissolution and consumption in musts and wines

Oxygen can have a major influence on a variety of stages in the winemaking process, as well as in the way wine evolves, both chemically and, thus, sensorially during maturation and aging. Depending on the circumstances, the presence of oxygen can be advantageous or unfavourable; for example, oxygen is used to improve yeast biomass formation and is required for the synthesis of compounds (e.g., sterol) that afford yeast improved tolerance to ethanol during wine fermentation (Lafon-Lafourcade *et al.*, 1979; Fornairon-Bonnefond *et al.*, 2003; Hansen *et al.*, 2001). Conversely, during malolactic fermentation, aeration increases the risk of microbial spoilage and acetification (Fleet, 2003; Bartowsky and Henschke, 2008). In many other instances, however, the presence of oxygen in musts or wines will result in oxidation, where the boundary between detriment and benefit is more complex and difficult to assess.

14.2.1 Oxygen dissolution in musts and wines

The solubilization of oxygen into musts or wines is an equilibrium process that occurs when air or oxygen is brought into contact with the liquid. Oxygen dissolves in wine by diffusion across the wine/air interface in a process that can be accelerated by forced aeration during turbulent flow or splashing, or by the deliberate addition of controlled amounts of air or oxygen gases. In practice, aeration is virtually unavoidable in many instances (e.g., during grape crushing, transferring of must or wine from tank to tank, filtration) and may be performed deliberately when some degree of oxidation is intended to acheive favourable benefits in the final wine, as is the case with hyperoxidation and micro-

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oxygenation treatments. However, most wineries are unable to accurately monitor dissolved oxygen, and often rely on approximate methods. For instance, winemakers often use oxygen probes with insufficient sensitivity, and often adopt measurement procedures that unintentionally introduce air during sampling. This is a situation that will probably change in the near future due to recent developments in this area, such as the introduction of new technologies and approaches to measuring dissolved oxygen, and a series of recently published research papers demonstrating the importance of these measurements in the context of managing oxygen in winemaking (Nevares and del Álamo, 2008; Laurie *et al.*, 2008; Nevares *et al.*, 2009; Anastasova *et al.*, 2008).

The dissolved oxygen concentrations in musts and wines after reaching saturation with air (i.e., the point at which a maximum concentration of dissolved oxygen has been reached) is approximately 9.2 and 8.4 mg/L, respectively, at room temperature and under atmospheric pressure (Singleton, 1987; Cheynier *et al.*, 2002). Nevertheless, considering that the solubilisation process depends mainly on the temperature of the solution, the partial pressure of the gas over the liquid, and the composition of the must or wine acting as solvent, these numbers can vary widely (Chang, 2000).

The most comprehensive data for the solubility of oxygen at different temperatures has been conducted in pure water. For instance, the dissolved oxygen concentration in water saturated with air at sea level increases from 7.6 mg O_2/L at 30 ºC to 10.1 mg O_2/L at 15 ºC, reaching approximately 14.6 mg O_2/L at 0 ºC (Weiss, 1970). Thus, winemakers trying to avoid oxygen pickup have to be particularly careful in protecting the product during operations performed at low temperature, such as crushing of white grapes or tartrate stabilization (Castellari *et al.*, 2004; Waterhouse and Laurie, 2006), where median increases of approximately 1.3 mg O_2/L up to saturation have been reported (Castellari *et al.*, 2004). As far as gas composition is concerned, dissolved oxygen concentrations are ~5 times higher if the must or wine is saturated with pure oxygen instead of air. In a mixture of gases, such as air, the partial pressure of a gas corresponds to the pressure that this gas would exert if it alone occupied the same volume as the mixture (Chang, 2000). Considering that the proportion of oxygen in dry air (0% humidity) is approximately 20.9%, the partial pressure of oxygen at room temperature and atmospheric pressure would be approximately 21 177 Pa (PO_2 = atmospheric pressure × relative amount of oxygen). However, because the amount of oxygen in air varies according to the humidity level, a lower oxygen concentration and partial pressure of oxygen would be expected as we approach an environment saturated with water vapour (20.1% and 20 366 Pa, respectively). Finally, the presence of solutes at high concentrations further decreases these saturation levels. For hydro-alcoholic solutions in particular, such as wines and spirits, the dissolved oxygen concentration decreases as the ethanol content rises up to approximately 30%, but increases rapidly from that point on (Cheynier *et al.*, 2002; Shchukarev and Tolmacheva, 1968).

Oxygen dissolves rapidly in wine if it is splashed or if it undergoes turbulent movement. If a small volume of wine is mixed in the presence of air, it will

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become saturated in approximately 30 seconds (Ribéreau-Gayon, 1933); therefore, winery operations that involve air exposure could result in a rapid increase of the dissolved oxygen concentration. Depending on the particular conditions of the operation performed (e.g., temperature, wine composition), the observed oxygen enrichment effect could vary widely. Crushing and pressing operations, as well as with other practices performed with vigorous agitation in open air, could lead to significant increases in oxygen concentration. For instance, racking operations can increase the level of dissolved oxygen in wines from 0.1 to 1.3 mg O_2/L, and filtration and centrifugation have been observed to give increases between 0.1 and 1.5 mg O_2/L (Vidal *et al.*, 2001; Castellari *et al.*, 2004) Wines treated with small amounts of oxygen during a micro-oxygenation experiment showed concentrations up to 2.4 mg O_2/L versus a non-oxygenated control which had concentrations as low as 4 μg O_2/L (Laurie *et al.*, 2008); bottling operations were also seen to introduce oxygen in wine, with increases up to 0.8 mg O_2/L being the most typical ones (Castellari *et al.*, 2004). Conversely, oxygen dissolution is slow under steady wine conditions (i.e., without mixing), as is the case during barrel aging when the surface area of the wine is low with respect to air exposure. This results in slower dissolution and the distribution of oxygen into layers, ranging from saturation at the interface with the tank headspace down to near zero oxygen concentration below the first 10 to 20 cm of wine volume (Cheynier *et al.*, 2002; Moutounet and Mazauric, 2001; Laurie *et al.*, 2008). The accurate and precise monitoring of dissolved oxygen in winemaking requires the use of specialized meters, as well as precautions that include protecting the wine sample from air/oxygen exposure at all times, and ensuring that the sample temperature is the same as the volume of wine being analyzed (Waterhouse and Laurie, 2006; Laurie *et al.*, 2008).

14.2.2 Oxygen consumption in musts and wines

With time, the dissolved oxygen fraction of musts or wines will be reduced, mainly by its consumption, or reaction, with the biological (e.g, active and non-viable yeast) or chemical fractions of the product (e.g., phenolic compounds and sulfites) (Mazauric and Salmon, 2005; Rosenfeld *et al.*, 2003; Rossi and Singleton, 1966; Singleton, 1987; Silva and Lambri, 2006; Fornairon-Bonnefond and Salmon, 2003). In contrast to its effect on oxygen dissolution, lower temperatures result in lower rates of oxygen consumption (Singleton, 1987).

Must aeration is widely used as a way to favour a correct fermentative process by supporting yeast biomass formation and helping to avoid sluggish or stuck fermentations. *Saccharomyces cerevisiae* uses molecular oxygen to synthesize sterols and unsaturated fatty acids that are required to maintain plasma membrane integrity and cell growth (Lafon-Lafourcade *et al.*, 1979; Ingledew and Kunkee, 1985; Rosenfeld *et al.*, 2003). Also, the activity of oxidative enzymes such as polyphenol oxidase and laccase, that have an activity dependence on oxygen supply, result in the consumption of oxygen in musts (Cheynier *et al.*, 1990; Boulton *et al.*, 1996; Silva and Lambri, 2006). These

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enzymatic reactions are initiated during the crushing of grapes, at which point the grape enzymes mix with their phenolic substrates in an oxygen-rich environment. In some cases, the exposure of must to oxygen is encouraged to promote the enzymatic oxidation of phenolic compounds and the subsequent precipitation of the oxidized phenolic derivatives. Such a process is termed hyperoxidation and is used in certain white wine styles to reduce the concentrations of specific phenolic compounds (i.e., catechins) that may otherwise contribute to detrimental sensory characteristics or increase the propensity of the wine to undergo oxidative deterioration (Schneider, 1998).

The role of phenolic compounds in wine oxygen consumption was established long ago by demonstrating that wines with high phenolic concentrations are capable of consuming more oxygen than those with moderate or low concentrations. A similar effect has been observed after the use of phenolic adsorbents such as activated carbon on wines (Singleton, 1987; Rossi and Singleton, 1966). This is not to say, however, that any given variation in total phenol concentration will result in noticeable changes in the oxygen consuming capabilities of a wine. In fact, individual phenolic substances interact with reactive oxygen species at different rates, which consequently leads to variations in dissolved oxygen consumption (Rossi and Singleton, 1966; Danilewicz, 2003). During oxidation, wine phenolic compounds are eventually either recycled back to their original form if sulfur dioxide and/or ascorbic acid are present, or form substituted derivatives of their original form (e.g., polymers or other addition products, as described below), but in both cases still usually retain much of their original phenolic skeleton. In this regard, the phenolic compounds are able to participate in further oxidation reactions, and as a consequence phenolic compounds are described as having an 'oxidation buffering capacity'. This ability of phenolic compounds to regenerate, in some guise, after oxidation is consistent with the ability of wines with higher concentrations of phenolic compounds to consume larger amounts of oxygen before exhibiting significant sensory changes (Singleton, 1987). In fact, as previously mentioned, it has been well-established that in some instances, and depending on the type of wine (reds more so than whites), the exposure to some oxygen could improve its sensory quality (Singleton, 1987; Boulton *et al.*, 1996; Ribéreau-Gayon *et al.*, 2006).

The chemical consumption of dissolved oxygen, by reaction with organic molecules, requires an activation step that either converts it from its naturally unreactive triplet state (a biradical with two unpaired electrons) to its singlet state, or convert the triplet dioxygen to the hydroperoxyl radical via a 1-electron addition (e.g., by transition metal catalysis) (Singleton, 1987; Gee and Davison, 1984; Bandy and Davison, 1987; Clark *et al.*, 2007). In a typical wine environment, protected from UV light and high temperatures, the activation of oxygen happens most likely with the involvement of transition metals catalysts such as iron and copper (Singleton, 1987; Cheynier *et al.*, 2002; Danilewicz, 2003). However, in a mechanism relevant to poor wine storage conditions, sunlight is also linked to radical formation and reactive oxygen species from photoactive organic acid/metal ion complexes in oxygenated model wine

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systems (Clark *et al.*, 2007), and fluorescent light is known to induce undesirable changes in the flavour of wine (Dozon and Noble, 1989).

14.3 The oxidation of wine constituents

Even though the significance of oxidation in winemaking is well known, current approaches to evaluate, consistently manage, and predict their outcomes are insufficient. Besides a few analytical tools available, the management of oxidation in wine has relied, mainly, on the very subjective sensory evaluation of the product. Lately, a good deal of research on the subject has been performed worldwide, in an attempt to elucidate the very intricate mechanism of wine oxidation, some of which are reviewed in this chapter.

14.3.1 The role of phenolic compounds

Phenolic compounds are important plant secondary metabolites found in fresh and processed fruits and vegetables, including grapes and wine (Haslam, 1998; Shahidi and Naczk, 2003). During winemaking, these substances are mainly extracted from the grapes' skins and seeds, as they contain a much larger concentration of phenolic compounds as compared to the grapes' flesh; however, they could also arise to a lesser extent from contact with toasted oak wood, as some wines are fermented or aged in the presence of oak. In wine, phenolic compounds not only contribute to the colour, astringency, and bitterness of the product, but play an important part in the overall capacity of a wine to withstand oxidation and age properly (Haslam, 1998; Boulton *et al.*, 1996). With respect to health benefits, most of the potential therapeutical value of wine (a good dietary source of phenolic compounds) has been attributed to the presence of phenolic compounds and their radical quenching activity (Scalbert and Williamson, 2000; Soleas *et al.*, 1997) as discussed later. One of the possible protective mechanisms of phenolic compounds is related to their ability to donate a hydrogen atom to free radical species, yielding a relatively stable free radical intermediate that is incapable of propagating oxidation reactions (Salah *et al.*, 1995; Roginsky and Lissi, 2005). However, the beneficial health effects associated with wine polyphenols is most likely not restricted to just their radical scavenging capacity *in vivo*, and may also be attributable to non-radical processes.

The different type and quantity of phenolic compounds in white and red wines rely not only on the basis of grapes' genetic differences, but also on the technological aspect of their cultivation and processing. The concentration of total phenolic compounds, as determined by the method of Folin-Ciocalteau, has been reported to be below 1500 mg/L of catechin equivalents for white wines, and less than 4000 mg/L of catechin equivalents for red wines (Slinkard and Singleton, 1977; De Beer *et al.*, 2004; Ough and Amerine, 1988). Of the various wine compounds that are labile to oxidation (e.g., ethanol, sulfur dioxide, organic acids, etc.), phenolic compounds are the most important for the initiation

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and propagation of oxidation reactions owing to their ease in reacting with reactive oxygen species during processing. In addition to being suitable electron donors at low pH, wine phenolics are known to participate in oxidation reactions and are very diverse in terms of their chemical structure and reducing properties (Danilewicz, 2003). Accordingly, phenolic compounds are considered good antioxidants due to their ability to remain relatively stable following the loss of a hydrogen, specifically by delocalization of the resulting free radical over its aromatic ring, and also by their ability to chelate transition metals (Vanderhaegen *et al.*, 2006). Phenolic compounds with a catechol (di-hydroxy phenol) or a pyrogallol (tri-hydroxy phenol) group are the most readily oxidized (Fig. 14.1). The first group include substances such as caftaric acid, (+)-catechin, (−)-epicatechin, and condensed tannin, while the second set of compounds include gallic acid, (+)-gallocatechin, (−)-epigallocatechin, and their polymers (Singleton, 1987; Danilewicz, 2003; Waterhouse and Laurie, 2006).

Phenolic compounds are more acidic than alcohols, but less acidic than carboxylic acids. In acidic medium, like must or wine (i.e., pH between 3 and 3.9), the equilibrium between the protonated and the anionic form of phenol, (phenolate ion) will be shifted towards the unionized phenol (Fig. 14.2). Considering that metal-catalysed oxidation of phenolic compounds is easier from the phenolate ion than from the unionized phenol (Singleton, 1987; Cheynier *et al.*, 2002), due largely to increased interaction between metal ions and phenolic compounds at higher pH values, precaution should be taken when high pH grapes are processed, or wine deacidification treatments are conducted. Studies carried out at moderately high pH (pH 7 to 9) have shown that phenolic compounds with a pyrogallol group experience oxidation faster than catechol-like phenolics (Inui *et al.*, 2004; Miura *et al.*, 1998). The fate of the oxidised phenolic compound will be discussed in more detail later.

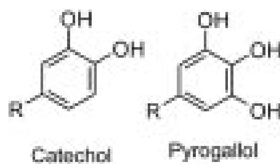

Fig. 14.1 Catechol and pyrogallol groups of oxidisable phenolic compounds.

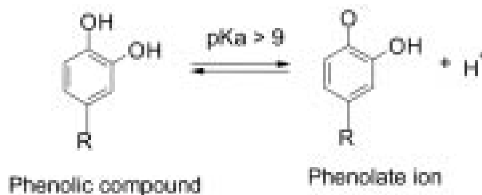

Fig. 14.2 Equilibrium between protonated and deprotonated phenolic compounds.

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14.3.2 The role of metals

Metal ions in wine play a key role in oxidation (Berg and Akiyoshi, 1956; Oszmianski *et al.*, 1996), as they are linked to the catalytic activation of oxygen and the reduction of H_2O_2 into hydroxyl radicals ($HO^•$) (Danilewicz, 2003; Waterhouse and Laurie, 2006). Trace levels of transition metals are ubiquitous in wine, and may arise from the grapes themselves, as well as dust, fungicide residues, and exposure to non-stainless steel winemaking equipment. Typical concentrations of iron and copper in wine have been reported to be between 0.11–3.6 mg/L and 0.9–16 mg/L respectively (Ough and Amerine, 1988; Pyrzynska, 2004). Accordingly, managing the concentration of metals in wine could possibly aid in controlling oxidation reactions and should be further explored. Other research in this area involved speciation measurements of metal ions in wine, that is, the quantification of the largely free, or unbound, form of the metal (i.e., including loosely bound) and forms that are 'bound' to complexes in the wine (Green *et al.*, 1997; Wiese and Schwedt, 1997; Ferreira *et al.*, 2007). Preliminary work has been conducted to investigate which form of copper ions in white wine is more likely to participate in the mediation of oxidation reactions (Clark and Scollary, 2006).

14.3.3 Wine oxidation mechanism

Current hypotheses on the general mechanism of wine oxidation have suggested the following set of reactions (Danilewicz, 2003; Waterhouse and Laurie, 2006): Firstly, molecular oxygen is activated to the hydroperoxyl radical ($HOO^•$) by means of an endogenous metal catalyst (e.g., Fe^{2+}), which are typically present in wines (Ough and Amerine, 1988; Pyrzynska, 2004). This activation step enables the introduction of oxygen, in the form of hydroperoxyl radicals, into the system. These radicals, in turn, react with oxidizable substances such as the catechol or pyrogallol moieties of phenolic compounds, and consequently oxidize them to quinones, while the hydroperoxyl radical is reduced to hydrogen peroxide (Fig. 14.3). Secondly, as in many other biological systems in which metals are present, hydroxyl radicals ($HO^•$) are formed by the transition metal catalyzed decomposition of hydrogen peroxide (Fig. 14.4).

The hydroxyl radical is a powerful oxidant and is suggested to react in a near diffusion controlled manner (Danilewicz, 2003). Other than water, ethanol

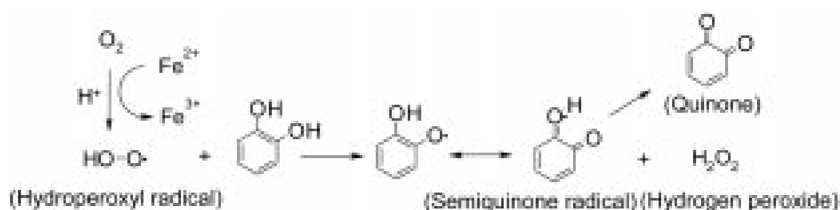

Fig. 14.3 Oxidation of wine phenolic compounds.

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Fig. 14.4 Iron catalysed decomposition of hydrogen peroxide into hydroxyl radical (Fenton reaction).

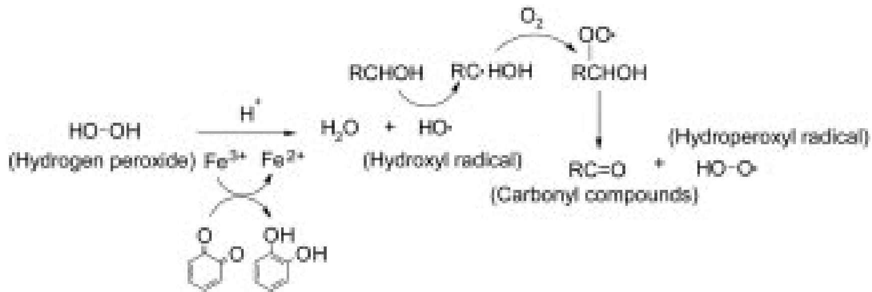

Fig. 14.5 Secondary oxidation reactions in wine.

(~2 M) is the most abundant compound and is thus a prime target of hydroxyl radicals. The hydroxyl radical favours abstraction of an α-hydrogen atom from ethanol (Fig. 14.5) to generate an ethoxy radical which then undergoes a subsequent single electron-loss, either by metal ions or molecular oxygen (Fig. 14.5), to form acetaldehyde. The abstraction of the alternate β-hydrogen atom from ethanol is also known to occur by hydroxyl radicals albeit at lower yields than acetaldehyde. The presence of the 1-hydroxyethyl radical during wine oxidation has been confirmed by electron spin resonance (Elias *et al.*, 2009). The study also provided evidence for the presence of the hydroxyl radical; however, the hydroperoxyl radical could not be identified.

After ethanol, glycerol (~0.2 M) and organic acids, such as tartaric acid (~0.02 M), are the most abundant components in dry table wine (Table 14.1). Of

Table 14.1 Chemical components in wine

Component	Group	Typical range	
		(g/L)	(mM)
Ethanol	Alcohol	60–160	1300–3500
Glycerol	Polyols	1–15	11–160
Glucose	Sugars	tr.–100	tr.–560
Tartaric acid	Acids	1–5	7–33
Sulfate	Anions	0.1–3	1–30
Potassium	Cations	tr.–2.5	tr.–64
Malvidin-3-glucoside	Phenolic compounds	0–1	0–2
Ethyl acetate	Esters	0.05–0.15	0.6–1.7
Proline	Nitrogenous compounds	0.05–0.70	0.4–6
Riboflavin	Vitamins	tr.–0.3	tr.–0.8

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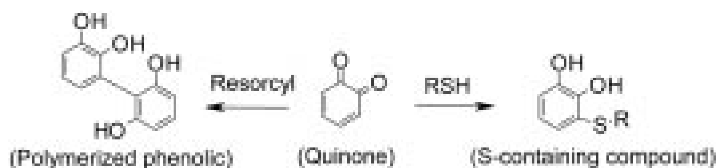

Fig. 14.6 Reactions of oxidation-generated quinones in wine.

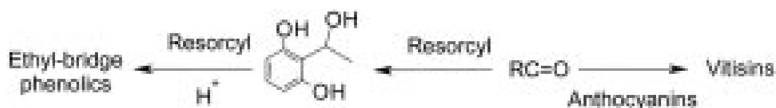

Fig. 14.7 Reactions of oxidation-generated carbonyl compounds in wine.

course, the concentration of sugars in sweet wine will also be similar, if not higher than that of glycerol. Both glycerol and tartaric acid are oxidized by hydroxyl radicals in a similar mechanism to ethanol (Fig. 14.5) to yield aldehydes, such as glyceraldehyde from glycerol and glyoxylic acid from tartaric acid (Laurie and Waterhouse, 2006a; Clark *et al.*, 2007). Although the reaction of hydroxyl radicals with wine components is described as occurring at near diffusion controlled rates, it has yet to be confirmed that this is, indeed, the case for the main wine components in competition for the hydroxyl radical (i.e., 2 M ethanol, 0.2 M glycerol, 0.02 M tartaric acid). Furthermore, it has not been established if the aldehydes resulting from ethanol, glycerol and tartaric acid oxidation are generated at levels proportional to the concentration of ethanol, glycerol and tartaric acid in wine. Other potential oxidation targets of the hydroxyl radical would be fructose or glucose in sweet wines and also malic acid in wines where its concentration is sufficiently high.

In any case, it is the products of the reactions previously described (i.e., quinones and carbonyl compounds) that can further react with other wine compounds such as anthocyanins, sulfur dioxide, sulfur-containing compounds, and others (Figs 14.6 and 14.7), producing many of the organoleptic effects observed during wine oxidation (Danilewicz, 2003; Waterhouse and Laurie, 2006).

14.4 The impact of oxidation on wine sensory features, nutritional and therapeutic value

14.4.1 The impact of oxidation on wine aroma

Wine grapes contain many compounds that only become volatile once fermentation or aging has occurred, thus contributing to the characteristic aromas of wines. Some of the aromatic changes occurring during the aging of young wines are thought to be related to oxidation reactions, initially leading to the loss of flavour intensity, and subsequently to the appearance of aged-wine aromas (Lambropoulos and Roussis, 2007; Singleton, 1987; Elias *et al.*, 2008).

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Wine aldehydes and ketones are mainly produced as by-products of microbial activity and chemical oxidation, though some of these carbonyls might also be extracted from oak barrels during winemaking and aging (Ebeler and Spaulding, 1997; Ough and Amerine, 1988; Ferreira *et al.*, 2002; Elias *et al.*, 2008). As a result, a wide range of concentrations of these compounds is expected depending on winemaking and storage conditions (Culleré *et al.*, 2007; Escudero *et al.*, 2002; de Revel *et al.*, 1999; Flamini *et al.*, 2002). Sweet wines, for instance, have a carbonyl content that surpasses that of dry table wines, presumably due to sugar oxidation (Barbe *et al.*, 2000). The occurrence of these compounds can impact not only the flavour of a wine (Ebeler and Spaulding, 1997), but also its color (Timberlake and Birdie, 1977; Bakker and Timberlake, 1997).

Recently, it has become apparent that the concentrations of several aldehyde compounds in wine provide a good indication of the perceived oxidative spoilage aromas in the wine. Phenylacetaldehyde, methional and (E)-2-alkenals (Fig. 14.8) were all observed to increase in oxidized red and white wines and also in aged red wines (Ferreira *et al.*, 2003; Culleré *et al.*, 2007). The aromas associated with such compounds are 'honey-like', 'potato-like' and dusty/rancid, respectively. However, red wines that were aged without excessive oxidation also contained branched alphatic aldehydes (Fig. 14.8), which were largely absent in the oxidised wines and had the ability to mask the detrimental flavour of the (E)-2-alkenals.

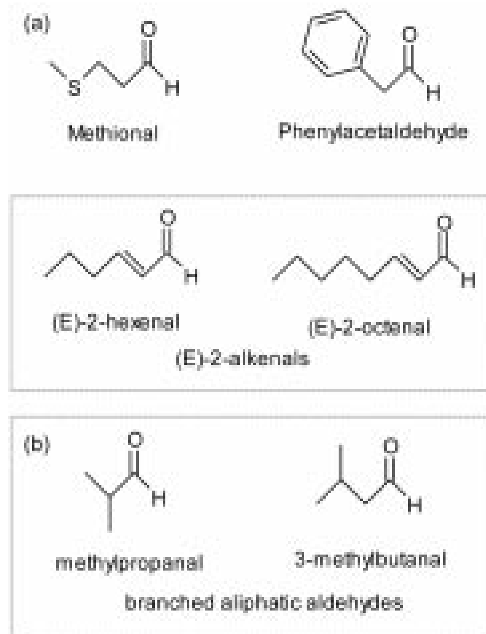

Fig. 14.8 Compounds linked to spoilage aroma in oxidized wine (a), and compounds reported to mask the oxidative spoilage aroma in red wine (b).

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Thiol-based aroma compounds also undergo addition reactions to ortho-quinone compounds, and the aroma profiles of wine may be changed as a consequence. For example, 3-mercaptohexanol, a compound able to contribute fruity aromas to red wine, is significantly depleted by a mechanism resulting from the binding of its thiol group to ortho-quinone compounds (Blanchard *et al.*, 2004). Similarly, the rotten-egg aroma of hydrogen sulfide may decrease after the racking of red wine, partly due to the fact that this process introduces some oxygen to red wine and consequently induces reaction between hydrogen sulfide and ortho-quinone compounds (Waterhouse and Laurie, 2006). Nevertheless, the loss of aromatic intensity could also arise from the direct oxidation of these substances. For instance, oxidation of wine thiols and mercaptans might be catalyzed by trace amounts of metal ions, such as iron and copper (Boulton *et al.*, 1996).

Furthermore, the role of phenolic compounds, glutathione, N-acetyl-cysteine, caffeic acid or sulfur dioxide as inhibitors of aroma losses (i.e., esters, thiols, terpenes) have been reported (Roussis *et al.*, 2005a, 2005b, 2007; Lambropoulos and Roussis, 2007; Garde-Cerdan and Ancin-Azpilicueta, 2007; Sergianitis and Roussis, 2008) and will be further discussed later in this chapter.

14.4.2 The impact of oxidation on wine colour

In white wine, oxidative processes usually culminate in a change of colour, often described as a browning of the wine. The rate of browning is known to correlate with flavanol concentration, specifically (−)-epicatechin (Simpson, 1982; Sioumis *et al.*, 2006), and inversely with free sulfur dioxide concentration (Godden *et al.*, 2001). As flavanols are mainly localized in the skins and seeds of grapes, their concentrations will be higher in white wines produced from heavily extracted grapes. Browning is typically measured by the absorbance intensity of white wine at 420 nm, however the combined measure of yellow (420 nm) and red (500 nm) has been reported as a better indicator of oxidation when comparing different wines (Skouroumounis *et al.*, 2005b).

The exact pigments responsible for the oxidative colouration in white wine have not been determined although model wine studies have shown the ability of glyoxylic acid, formed from the oxidative cleavage of tartaric acid, to undergo electrophilic addition to flavanols, such as catechin or epicatechin, to form yellow xanthylium cation pigments with a maximum of absorbance around 440 nm (Es-Safi *et al.*, 1999a; Labrouche *et al.*, 2005). In model wine studies containing caffeic acid as well as catechin, the yellow xanthylium cations were still produced but an additional type of pigment, with broader absorbance into the 'red' region (~500 nm), was also generated (George *et al.*, 2006). The overall combination of these yellow and red pigments resulted in a solution of orange/brown appearance as the model wine was oxidized. Other yellow pigments are known to be generated from the reaction of ortho-quinone compounds with flavanols, such as (+)-catechin or (−)-epicatechin, followed by intramolecular rearrangement (Guyot *et al.*, 1996).

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Fig. 14.9 Oxidative production of a pyrylium ion moiety.

The colour of red wine generally depends on the age of the wine, with most young red wines having a deep purple/red colour that progresses to an orange/brick red colour during aging. During the aging of red wine, most of the monomeric anthocyanin pigments are transformed into larger molecular weight compounds that are more stable with regard to changes in pH and sulfur dioxide concentration. Many of these transformations are induced by yeast metabolites as a result of primary fermentation; however, others require at least one oxidation step in order to generate the final polymeric pigment. This may involve the oxidative production of carbonyls, such as acetaldehyde (Es-Safi *et al.*, 1999b; Timberlake and Bridle, 1976; Fulcrand *et al.*, 1996; Bakker and Timberlake, 1997; Saucier *et al.*, 1997; Es-Safi *et al.*, 1999a), glyceraldehyde (Laurie and Waterhouse, 2006a, 2006b), glyoxylic acid (Fulcrand *et al.*, 1997; Es-Safi *et al.*, 2000), or pyruvic acid (Fulcrand *et al.*, 1998), and their subsequent reaction with flavonoids, or alternatively the oxidative generation of a pyrylium-moiety (Fig. 14.9) that forms an essential part of the chromophore unit of a pigment. The summary of all the available mechanisms relevant to transformation of anthocyanins during aging is beyond the scope of this chapter, and many can be found in a recent review (Cheynier, 2006). With extensive oxidation, whereby the free sulfur dioxide concentration is significantly depleted, the acetaldehyde generated from ethanol can polymerize flavonoid molecules as shown in Fig. 14.6. In the presence of high concentrations of aldehydes, the red wine flavonoids can polymerize to such an extent that they no longer are soluble, precipitate and result in a loss of colour of the red wine (Somers, 1998; Timberlake and Bridle, 1976).

14.4.3 The impact of oxidation on wine mouth-feel

The modification of the phenolic compounds during wine oxidation is consistent with the changes in astringency and bitterness. For white wine, oxidation often accentuates bitterness on the palate (Margalit, 1997). For red wine, oxidation is attributed to changes in the astringency (Llaudy *et al.*, 2006), which may be beneficial, such as the softening of more harsh and astringent phenolic compounds during aging in oak barrels (Ribéreau-Gayon *et al.*, 2006).

14.4.4 The impact of oxidation on wine nutritional and therapeutic value

The exact composition of wine depends on a variety of factors including grape variety, vintage conditions, wine production techniques and wine age. An

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example of major components, and their typical concentration range, for each of these general groups is outlined in Table 14.1.

Consequently, wine does contain essential nutrients that fall under the categories of the macronutrients (i.e., carbohydrates and proteins) and micro-nutrients (i.e., minerals, vitamins). Of course, the major component of wine, other than water, is ethanol and although this alcohol can be used as a source of energy (typically 7 kcal/g), it is not technically classified as an essential nutrient as it performs no essential function in the body (Insel *et al.*, 2001). Furthermore, the over consumption of ethanol leads to both dramatic health and social problems, and therefore limits the ability of wine to act as a conventional source of nutrition. However, there have been many studies that have shown a link between moderate wine consumption and improved health, such as reduced rates of cardiovascular disease and lowered incidents of some forms of cancer. Such studies have been conducted *in vitro*, *in vivo* animal, via human intervention and epidemiological studies (Logan *et al.*, 2008; Yoo *et al.*, 2008). Numerous results show that either ethanol or phenolic compounds are responsible for the majority of the health benefits observed from the consumption of wine (Stockley and Høj, 2005), and a synergistic role between the two are suggested by some (Zoecklein *et al.*, 1999). The phenolic compounds present in wine are often classified as phytochemicals, rather than nutrients, which are defined as compounds extracted from plants that may possess health protective effects even though they are not essential for life (Wildman and Medeiros, 2000).

The major constituents (i.e., ethanol, glycerol, sugars, and organic acids; see Table 14.1) do not usually decrease significantly during the oxidation of wine, thus limiting a potential loss of nutritional value due to oxidation. Although ethanol is converted to acetaldehyde during wine oxidation, the decrease in ethanol concentration during wine oxidation is usually negligible (George *et al.*, 2006; Wildenradt and Singleton, 1974). The impact of wine oxidation on its less abundant components, such as polysaccharides and nitrogenous compounds, is not known. However, various spoilage aroma compounds can be formed from the oxidation of amino acids such as methionine (Escudero *et al.*, 2000).

Alternatively, it is known that phenolic compounds are significantly affected by wine oxidation, as discussed above, but the consequence of their oxidative transformations on the health benefits of wine is not known. Instead, the majority of studies have followed the concentration changes in phenolic compounds during wine oxidation and/or aging, or the changes in 'antioxidant activity' of wine. Studies have shown the decrease in specific flavanol compounds, such as (−)-epicatechin, and the increase of others, such as gallic acid, during the accelerated oxidation of wine (Simpson, 1982; Kallithraka *et al.*, 2009).

Given the reported multi-facet mode of action for phenolic compounds as antioxidants in the body, including the ability to scavenge radical species, complex metal ions, and act as reductants, the antioxidant activity of wine has similarly been measured using a variety of techniques relevant to these modes of action. For Greek white wines, during an accelerated oxidation process (55 ºC

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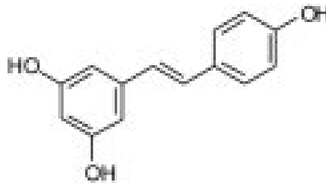

Fig. 14.10 Resveratrol chemical structure.

for 10 days), little change in anti-radical activity but a decrease in the reducing power of the wine was observed (Sioumis *et al.*, 2006; Kallithraka *et al.*, 2009). In this case the anti-radical activity was measured by the scavenging of a 2,2-diphenyl-β-picrylhydrazyl radical by wine and the reducing power was measured by the ability of the wine to reduce iron(III) to iron(II).

Other studies have been conducted on the change in antioxidant status of red and white wines during bottle aging (Larrauri *et al.*, 1999; Pellegrini *et al.*, 2000; Landrault *et al.*, 2001; Zafrilla *et al.*, 2003; Prenesti *et al.*, 2005; Roginsky *et al.*, 2006). As discussed later (Section 14.5.4), it is now recognised that bottle aging is influenced by the amount of oxygen present at bottling and the ingress of oxygen through the specific closure utilised. The combined results of these studies are inconclusive and suggest that the impact of bottle aging on the anti-radical activity of wine is more dependent on the composition/variety of the wine and the type of anti-radical measurement employed. However, a comprehensive study by de Beer *et al.* (2005), taking into account the factors above, provided good evidence for the decrease in anti-radical scavenging of red and white wines during their bottle storage at temperatures ranging from 0 to 30 ºC.

Another wine phenolic compound that has received much attention with its link to health benefits is the stilbene resveratrol (Fig. 14.10). Little work has been conducted on the impact of wine oxidation on the concentration of this compound. However, the oxidation of red grape must, which is dominated by enzymatic oxidation mechanisms, results in a 50% decrease in the resveratrol content of the corresponding wine whilst reductive processing of the grape must lead to an increase (Castellari *et al.*, 1998b). Conversely, other studies have shown that during the bottle aging of wine, the resveratrol concentration remains relatively stable (Jeandet *et al.*, 1995; Yasui *et al.*, 2002).

14.5 Managing oxidation in wine

Managing oxidation is one of the major challenges that winemakers face during wine production and aging. To date, the means to accurately measure dissolved oxygen in wine are expensive and, thus, not broadly used. Similarly, no successful and meaningful approaches on how to measure different degrees of oxidation, or how to consistently produce a certain degree of oxidation in wine have been developed. Therefore, winemakers have, and continue to, rely on

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experience when it comes to deciding how much oxygen and oxidation is required for a particular wine.

The winemaking practices typically used to limit the extent of oxidation include the use of inert gas blanketing as a way to minimize contact with air (e.g., nitrogen and carbon dioxide). Sulfur dioxide is also used to inhibit the propagation of oxidation reactions, while at the same time masking the effects of oxidation by forming complexes with oxidation-derived carbonyl compounds. In contrast, the most common techniques for introducing oxygen and promoting oxidation are those that include any form of aeration or splashing of the must or wine (e.g., pump overs or rackings), or the use of a technique called micro-oxygenation (Schmidtke *et al.*, 2010), in which better control of the oxygen dosing is acheived.

14.5.1 The role of sulfur dioxide and ascorbic acid

Sulfur dioxide is the main agent used for both the chemical and microbiological preservation of wine. At wine pH (3.0 to 3.9), sulfur dioxide is distributed amongst three main equilibrium forms (Fig. 14.11), namely molecular or hydrated sulfur dioxide, bisulfite and sulfite ions. Given the pKa values for these equilibria (Fig. 14.11), it is the bisulfite form that dominates under wine conditions (Fig. 14.11). Although it is the molecular form that is responsible for microbial stability, the bisulfite form (Fig. 14.11) can protect against the oxidation of wine. As discussed earlier, the rate of wine oxidation is higher at elevated pH and consequently sulfur dioxide can better protect wine at lower pH. The subsequent references to sulfur dioxide will include all forms of the compound shown in Fig. 14.11, unless otherwise specified.

The autoxidation of sulfur dioxide in the presence of molecular oxygen requires metal ions, such as copper and iron, to mediate the process (Danilewicz, 2007). However, the main antioxidant role of sulfur dioxide in wine is to remove the reactive species formed after the metal-mediated reaction between molecular oxygen with catechol or pyrogallol containing phenolic compounds (reaction A, Fig. 14.12). That is, the removal of hydrogen peroxide and ortho-quinone compounds (reactions B and C, Fig. 14.12). Although reaction B shows the reduction of the ortho-quinone to a phenolic compound, the bisulfite ion is also known to undergo an addition reaction with ortho-quinone compounds to form a sulfonic-substituted phenolic compound. Recent model wine studies suggest that at typical wine pH, ~38% of ortho-quinones react with sulfur dioxide to form the bisulfite addition product whilst the majority of the remaining ortho-quinones are reduced by sulfur dioxide to their parent phenolic compounds (Danilewicz *et*

Fig. 14.11 The equilibria and pKa values (Margalit, 1997) for molecular sulfur dioxide, bisulfite and sulfite ions.

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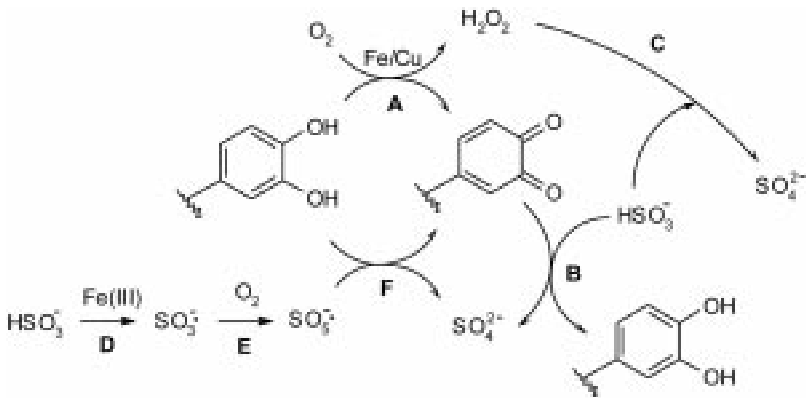

Fig. 14.12 The ortho-quinone and hydrogen peroxide scavenging abilities of sulfur dioxide in wine.

al., 2008). Intriguingly, the presence of phenolic compounds in wine is critical to the behaviour of sulfur dioxide as an antioxidant. This is due to the apparent ability of phenolic compounds to efficiently scavenge reactive sulfur radicals that result from the metal-mediated oxidation of sulfur dioxide by molecular oxygen (reactions D–F, Fig. 14.12). In the absence of phenolic compounds, the sulfur-based radicals shown in Fig. 14.12 can eventually form the sulfate radical, which has similar oxidizing capability as the hydroxyl radical (Danilewicz, 2007).

Sulfur dioxide also has the ability to undergo addition reactions in wine with aldehyde or ketone compounds (Fig. 14.13) (Barbe *et al.*, 2000; Burroughs and Sparks, 1964), thus repressing, for instance, the negative aromatic impact of acetaldehyde. These carbonyl compounds may be generated during wine oxidation, be produced during primary fermentation (e.g., acetaldehyde) or originate in the vineyard (e.g., *Botrytis cinerea* infections). The equilibrium of the addition reaction (Fig. 14.13) will depend on a variety of factors (Ribéreau-Gayon *et al.*, 2000), in particular the nature of the carbonyl compound. For example, the binding of sulfur dioxide to acetaldehyde is strong, as is evidenced by the low dissociation constant of the resulting addition product ($K_d = 2.4 \times 10^{-6}$ M), while the binding of sulfur dioxide to most oxidized sugars is weaker ($K_d = 1.5 \times 10^{-4}$ M) (Ribéreau-Gayon *et al.*, 2000).

In red wine, the efficacy of sulfur dioxide is complicated by its reactivity with anthocyanins, their flavylium cation form, which undergo addition reactions (Fig. 14.14). As a result, the flavylium cation chromophore is lost and the colour of the

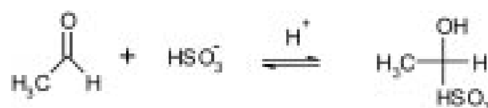

Fig. 14.13 The addition reaction between the bisulfite ion and acetaldehyde.

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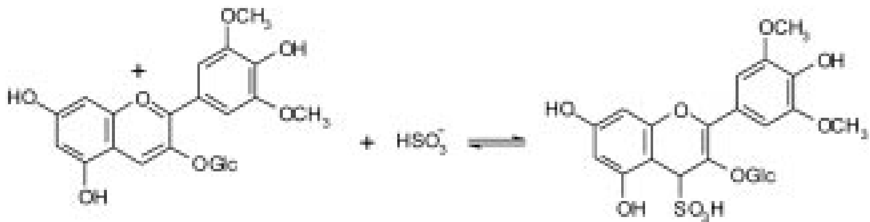

Fig. 14.14 The addition reaction between the bisulfite ion and malvidin-3-glucoside in its flavylium cation form.

red wine is diminished. This also means that it is difficult to gain equivalent sulfur dioxide concentrations in a young red wine to those routinely employed in white wine without a significant loss of the colour of red wine. Fortunately, and as discussed earlier, red wine can absorb higher levels of oxygen than white wine before deleterious effects are apparent and, therefore, a high level of sulfur dioxide is less critical. The binding of sulfur dioxide by anthocyanins also becomes less significant as red wines age due to the conversion of monomeric anthocyanins to either polymeric or substituted-forms that are weak binders of sulfur dioxide (Somers, 1998; Bakker and Timberlake, 1997).

The measurement of sulfur dioxide in wine has traditionally been conducted by iodometric or aspiration/oxidation methods (Ough and Amerine, 1988; Iland *et al.*, 2004). Such techniques for analysis of sulfur dioxide provide a measurement of sulfur dioxide speciation whereby the 'free' and 'total' sulfur dioxide fractions can be quantified, and the 'bound' sulfur dioxide concentration can be determined as the difference between the two. The concentration of total sulfur dioxide in a wine will decrease as the wine is exposed to molecular oxygen due to reactions B and C in Fig. 14.12. This will also lead to a drop in free sulfur dioxide followed by a decrease in bound sulfur dioxide as the equilibrium between 'free' and 'bound' is re-established. The weaker binders of sulfur dioxide, such as keto acid, oxidised sugars and anthocyanins, will be the first to release sulfur dioxide followed by the stronger binders, such as acetaldehyde. The correlation between wine oxidation and decrease in sulfur dioxide concentration was described previously (Laszlo *et al.*, 1978), and has been used as a marker of wine oxidation.

Owing to the allergic reactions that sulfur dioxide could induce in certain consumers and the deleterious sensory impact that sulfur dioxide can have on wine, there is an upper limit of sulfur dioxide that can be added. Although there has been research conducted to find alternative agents to prevent the oxidative spoilage of wine, such as glutathione and/or caffeic acid (Roussis *et al.*, 2007), sulfur dioxide is still the antioxidant of choice for the international wine industry.

The reaction between glutathione and ortho-quinone compounds are well known to occur in the must of grapes (Cheynier *et al.*, 1986), whereby an addition product is formed (Fig. 14.6). If the must of wine is treated in a sufficiently reduced environment, the level of glutathione can be in the order of 20–30 mg/L (du Toit *et al.*, 2007). In this case, it is likely that subsequent

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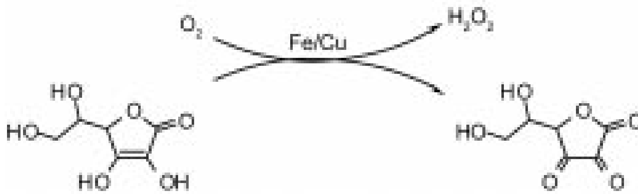

Fig. 14.15 The reaction between ascorbic acid and molecular oxygen to generate dehydroascorbic acid and hydrogen peroxide.

oxidation of the wine will yield ortho-quinones that will, in turn, undergo addition reactions with either sulfur dioxide or glutathione. However, the molar concentration of glutathione will be less than that of typical free sulfur dioxide concentrations (i.e., 0.1 mM vs. 0.5 mM).

In white wine, ascorbic acid is often used as a complementary agent to sulfur dioxide in order to provide added protection against oxidation. Unlike sulfur dioxide, ascorbic acid is much more efficient in scavenging molecular oxygen, but in the process generates dehydroascorbic acid and hydrogen peroxide (Fig. 14.15). The increased efficiency of ascorbic acid to scavenge molecular oxygen, albeit via a metal ion mediated mechanism, is proposed to limit the oxidation of phenolic compounds. However, the scavenging of molecular oxygen by ascorbic acid is not likely to be performed in a single two-electron step in wine and hence transient radical products would exist before the production of hydrogen peroxide and dehydroascorbic acid. Such ascorbic acid-derived radicals have been reported in a variety of reaction media (Laroff *et al.*, 1972; Helliwell, 1996). The reactivity of these intermediate radicals compared to those of the phenolic compounds is not certain, but the beneficial effects observed with ascorbic acid used as a complementary antioxidant, together with adequate amounts of sulfur dioxide, provide good evidence that they may be less detrimental (Skouroumounis *et al.*, 2005b).

It is, therefore, critical to have sulfur dioxide present in combination with ascorbic acid to remove the products formed from the oxidation of ascorbic acid. The sulfur dioxide can scavenge hydrogen peroxide and form addition products with dehydroascorbic acid, or its subsequent degradation products. Indeed, model wine studies have demonstrated that if ascorbic acid is utilized in the absence of sulfur dioxide, it can lead to an increased rate of oxidation reactions (Bradshaw *et al.*, 2003). Erythorbic acid, the diastereoisomer of ascorbic acid, has a similar mode of action to ascorbic acid but still requires the presence of sulfur dioxide and is not a permitted additive in certain European countries.

Clearly, the reactions described seem to be more complicated than originally thought and thus require further attention.

14.5.2 Wine aging in wooden barrels vs. stainless steel tanks

When wines are stored in wooden barrels, aeration occurs mainly during barrel filling, topping off operations, and during barrel opening (Castellari *et al.*, 2004;

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Boulton *et al.* 1996). The same is true for stainless steel tanks, but considering that these are normally much bigger than barrels (~225 L), the extent of the resulting oxidation is much lower. In other words, the level of oxidation observed depends on the ratio between the wine surface area in contact with air and the total volume of wine being held in a given container (Cheynier *et al.*, 2002). The type of oak barrel (e.g., French vs. American) may also impact oxygen ingress rates (Nevares and del Álamo, 2008). Finally, the extraction of highly oxidizable phenolic compounds from oak, including gallotannins and ellagitannins with a high proportion of gallolated phenolic units, is also suggested to enhance the oxidative polymerization of wine phenolic compounds (Nevares and del Álamo, 2008; Vivas and Glories, 1996).

14.5.3 Micro-oxygenation

The micro-oxygenation technique consists of the addition of small and continuous amounts of oxygen by means of either a sparger that produces and distributes the gas in the form of small bubbles (Boulet and Moutounet, 2000), or a pressurized semi-permeable tubular membrane that does not introduce bubbles (Kelly and Wollan, 2003). The technique is mostly used for red wines, for the purpose of accelerating the aging process in a similar way as it would happen in oak barrels, and is in fact frequently combined with oak chips or alternative wood treatments (Llaudy *et al.*, 2006; Perez-Magarino *et al.*, 2009). Empirical observations suggesting effects such as 'softer tannins', colour stabilization, and flavour changes have been widely claimed, but only recently have some of these effects been rigorously studied. However, the reported results have not always been consistent, and vary extensively depending on the particular experimental conditions used at each trial (Atanasova *et al.*, 2002; Llaudy *et al.*, 2006; Perez-Magarino *et al.*, 2007, 2009; Cano-Lopez *et al.*, 2008; Hernandez-Orte *et al.*, 2009). Moreover, it is yet to be demonstrated that the effects of micro-oxygenation are different than those observed after a single 'macro' aeration, provided that the same total amount of oxygen consumption occurs.

14.5.4 Managing oxidation in bottles (shelf-life)

Many white and red wines are produced with the aim of being consumed within the first six to twelve months of market release. However, there certainly are many red wine, and in some cases white wine, styles that greatly improve in quality due to extended bottle aging (i.e., > 12 months).

A means of accurately predicting the optimum length of bottle aging for a particular style of wine would be a major discovery for an oenologist as, currently, winemakers generally recommend the optimum time for a wine to spend in bottle based on experience. Unfortunately, the bottle aging of wine is a complex process impacted by grape/wine chemical composition, oxygen ingress during winemaking and bottling, bottle headspace, closure-type and integrity,

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concentrations of sulfur dioxide/ascorbic acid, storage conditions (e.g., temperature, humidity, light exposure), and oxygen ingress through the bottle closure during storage (Lopes *et al.*, 2006; Skouroumounis *et al.*, 2005a, 2005b; Kwiatkowski *et al.*, 2007; Mas *et al.*, 2002; Brajkovich *et al.*, 2005). Most of these bottle-aging factors either involve oxygen directly (i.e., oxygen ingress) or indirectly, such as the influence of storage temperature on the rate of oxidation reactions. It must be noted that some bottle aging processes do not require oxygen, such as acid catalyzed hydrolyzation or esterification reactions (Clarke and Bakker, 2004).

The majority of trials performed on bottle aging have been conducted on white wines, as they are more prone to exhibiting aroma losses or colour changes due to oxidation (Kontoudakis *et al.*, 2008; Escudero *et al.*, 2002; Ferreira *et al.*, 2002; Singleton and Kramlinga, 1976). Studies in which white wines were bottled and stored under identical conditions, but with different closures, have demonstrated that the closure has a critical impact on the aging. Intriguingly, the oxygen ingress into bottles through cork and synthetic stoppers has a high initial rate in the first 2 days (i.e., 25–45 μL/day and 30–45 μL/day respectively) followed by a lower rate after this time (i.e., 0.1–2.3 μL/day and 6.2–14.7 μL/day respectively between 2 or 12 to 36 months storage) (Lopes *et al.*, 2006). The initial high rate was linked to oxygen diffusion out of the internal structure of the closure whilst the subsequent lower rate was due to diffusion of oxygen through the closure from atmospheric oxygen (Lopes *et al.*, 2007). In comparison, wines sealed with crown seals (otherwise known as ROTE) and technical stoppers had minimal air ingress (1 μL/day), although some significant ingress during bottling/application of the crown seal was also observed.

Recent studies on wine bottle aging have allowed the calculation of oxygen consumed by wine (Dieval *et al.*, 2009). This parameter requires the measurement of the initial dissolved oxygen in the wine, oxygen in the bottle head-space and oxygen ingress through the closure over time. The consumed oxygen displays a similar concentration profile over time to the oxygen ingress into the wine, that is, a high initial rate followed by a slower subsequent rate. In this case, a significant contribution to the high initial rate of oxygen consumption was due to the oxygen in the headspace of the bottle dissolving in the wine and then undergoing reaction.

Consistent with these oxygen ingress rates, white wine bottle trials have shown that after three years, wines sealed with crown seals have been observed to maintain a high intensity of fruit characters, but also generate reduced (e.g., flint-like) characters. Those bottles stoppered with synthetic closures were more oxidized and had less fruit characters, whilst cork closures had higher variability but generally had high fruit intensity (Skouroumounis *et al.*, 2005a, 2005b; Godden *et al.*, 2001). Similar results were observed if other markers of white wine oxidation, such as wine browning and sulfur dioxide consumption, were monitored. Generally, the orientation of the bottle during storage (i.e., vertical vs. horizontal) was not critical. However, one study (Mas *et al.*, 2002) has shown contrasting results to that described above suggesting that the bottling technique

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adopted, wine variety under study and the measurement of dissolved and consumed oxygen are critical in understanding the bottle aging of wine. The important role of the bottle closure in the aging of wine is a likely contributor to the phenomenon of 'random oxidation' of white wine, whereby seemly random selection of wine bottles from a bulk collection may have undergone oxidative spoilage.

14.6 Future trends

Considering that oxygen is ever-present during winemaking and aging, and bearing in mind that, depending on the circumstances, oxidation reactions can have a positive or negative impact on the quality of the wine, winemakers and wine researchers should extend their efforts to understand, measure and gain control on the processes of oxygen consumption and oxidation reactions.

In the near future, we expect to witness more, and better, means of monitoring the amounts of dissolved oxygen and the extent of oxidation in wines, wherein online monitoring alternatives for dissolved oxygen, redox state, and wine composition by *in-situ* probes fixed at each wine tank (e.g., UV-Vis, NIR, cyclic voltammetry, etc.) could become standard.

Also, in view of the awareness and increasing demand for healthy foods, particularly with respect to their antioxidant properties, the use of more natural substances, such as phenolic compounds, as alternatives to the use of synthetic food additives is anticipated. Along those lines, environmental concerns regarding the use of natural materials in the manufacture of oak barrels and corks, as well as their significant cost of production and associate risks of developing microbial taints, could result in a contraction of those markets, offering space for an even larger share to alternative storing and closing systems. Thus, alternative sealing systems that could potentially allow a control oxygen transfer rate will be developed and used accordingly, depending on the oxygen requirements of a given wine type.

14.7 Sources of further information and advice

Books

- *Concepts in Wine Chemistry* by Y. Margalit, 2004
- *Handbook of Enology, The Chemistry of Wine: Stabilization and Treatments* by P. Ribéreau-Gayon *et al.*, 2006
- *Principles and Practices of Winemaking* by R.B. Boulton *et al.*, 1996.

Professional bodies and journals

- American Chemical Society (*Journal of Agricultural and Food Chemistry*)
- American Society for Enology and Viticulture (*American Journal of Enology and Viticulture*)

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- Australian Society of Viticulture and Oenology (*Australian Journal of Grape and Wine Research*).

Research and interest groups
- Groupe Polyphénols (www.groupepolyphenols.com)
- O_2 in wines (www.o2inwines.org).

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15

Use of encapsulation to inhibit oxidation of lipid ingredients in foods

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Abstract: Encapsulation is an effective method to protect unsaturated lipids against oxidation. This chapter discusses the principles of lipid encapsulation. It covers the design of encapsulant systems to improve the oxidative stability of encapsulated lipids during storage and applications of encapsulated lipids in manufactured food products.

Key words: unsaturated lipids, emulsion, encapsulation, oxidation, food products.

15.1 Introduction

Encapsulation involves the isolation of a compound (e.g., drug, bioactive, food ingredient) from its external environment by surrounding it within a secondary material and forming a small capsule. The capsules are typically in the nanometer to micrometer range. The secondary material (i.e., matrix, shell or encapsulant) protects the encapsulated compound (i.e., core or payload) by limiting its access to components, such as oxygen, moisture, acids, and enzymes, which can cause or catalyze its deterioration or degradation. Encapsulation technologies have been traditionally used in the pharmaceutical industry to protect sensitive bioactives and drugs and to target the site of their delivery. In the food industry, encapsulation is used primarily to protect sensitive food ingredients (e.g., flavors, vitamins, omega-3 oils, probiotics) and to enable release of the ingredient at the desired time and site. Recent reviews provide additional information about the underpinning science of and the various

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technologies used to encapsulate traditional food ingredients and bioactives (Augustin and Hemar, 2009; Augustin and Sanguansri, 2008; Gouin, 2004; Madene *et al*., 2006).

Unsaturated lipids (e.g., omega-3 oils) are prone to oxidation, which results in a decrease in their health benefits (e.g., nutritional value, bioactivity) and the development of off-flavors and/or tastes. Encapsulation protects unsaturated lipids against oxidation. The current chapter covers the principles of lipid encapsulation and the design and processing of encapsulated lipid systems to improve the oxidative stability of unsaturated lipids. It discusses the impact of the formulation and processing of encapsulated lipids on core stability, the methods used to assess the quality of ingredients that contain encapsulated lipids and applications of these ingredients in manufactured food products.

15.2 Principles of lipid encapsulation

Encapsulation technology plays an important role to protect and stabilize oxidizable substrates such as fats and oils, and to convert liquid ingredients into convenient powder formats. In the food industry, the use of ingredients that contain micro- and nano-encapsulated oils are an attractive alternative to the direct incorporation of sensitive oils and fats into manufactured food products. For example, one of the interests in the food industry is the use of encapsulation to stabilize sensitive oils such as omega-3 oils which have important health benefits for the consumer but are very prone to oxidation. This is an example of where encapsulation has enabled the development of ingredients to meet the demand of consumers for food products with enhanced nutritional value and quality without compromising the taste of the food products (Drusch and Mannino, 2009).

Encapsulation of lipids and lipid components can be achieved using a number of physical or chemical technologies or processes. These include emulsion and emulsion-based systems, liposome encapsulation, molecular encapsulation using cyclodextrins, coacervation, co-extrusion, and centrifugal extrusion techniques (Gouin, 2004). These primary encapsulation processes have been further improved by using combinations of the principles employed.

15.3 Design of encapsulation systems

Encapsulation is a multi-component technology and a multi-disciplinary approach is required to design any encapsulation system. Consideration of the properties of the core and the encapsulant, and the formulation, encapsulation process and processing conditions is essential to confer the optimum outcome for a desired purpose. The focus in designing systems for the encapsulation of lipids is on the influence of formulation and processing on the oxidative stability of the encapsulated lipids.

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15.3.1 Encapsulation of lipids

Encapsulation of lipids usually involves emulsification of the lipid with an aqueous dispersion of the encapsulant material containing an emulsifier. The formation of a stable oil-in-water emulsion is essential for effective encapsulation of lipids. The emulsion formulation requires subsequent heat treatment or the addition of preservatives to extend its shelf stability. The emulsions can be dried (e.g., spray drying, fluid bed drying, vacuum drying or freeze drying) to convert the micro- and nano-capsules into a free-flowing powder. Spray drying is the most common drying technology employed by the food industry as it has high throughput, is inexpensive, uses equipment that is readily available in the food industry, and produces particles of relatively good quality (Ré, 1998). Further improvements to the properties of the powder capsules can be made by washing the powders with solvents to remove the surface fat or by application of a secondary coating to the powder particles. A high encapsulation efficiency of the core lipid is important to obtain a powder with good flow characteristics and to minimize oxidation of the core lipid.

15.3.2 Encapsulation efficiency

The efficiency of encapsulation is commonly based on the amount of solvent-extractable fat. The ability of the solvent to gain access to and extract the fat depends on many factors, including the location of the oil in the powdered particle (Buma, 1971; Vignolles *et al.*, 2007). The estimation of solvent-extractable fat is an empirical method which also depends on the type of solvent used, the contact time and the ratio of solvent : powder. ESCA (electron spectroscopy for chemical analyses) is a direct measure of the surface fat content of powders. ESCA measures surface fat coverage to a depth of about 10 nm (Fäldt *et al.*, 1993). Using confocal laser scanning electron microscopy Drusch and Berg (2008) concluded that solvent-extractable oil in spray-dried microcapsules is located on or near the surface of the powder particles.

15.4 Processing of dried encapsulated oils

A generalized process for the preparation of dried oil-in-water emulsions is given in Fig. 15.1. As mentioned above, the formation of a stable emulsion is a pre-requisite for effective encapsulation of lipids. Both the lipid component (core) and the type and composition of the encapsulant materials will influence the degree of protection afforded by the matrix to the encapsulated lipid. Any lipid or lipid component can be encapsulated in an emulsifying food-grade matrix material to enable its use in food products. The matrix material (i.e., encapsulant) can be selected from a wide variety of natural or synthetic polymers depending on the micro- and or nano-capsule specification and final application. In addition, the drying conditions can also affect the powder properties and consequently the encapsulation efficiency.

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Fig. 15.1 Generalized process for production of dried oil-in-water emulsions.

15.4.1 Effect of encapsulant material properties

Formulations used to encapsulate lipids are initially optimized to obtain low levels of extractable fat in the dried powder (i.e., high encapsulation efficiency). The primary oil-in-water emulsion intended for drying is usually stabilized by emulsifiers (e.g., low molecular weight emulsifiers such as lecithins or monoglycerides; high molecular weight emulsifiers including proteins or emulsifying gums such as gum arabic), often in combination with a co-encapsulant or a carrier material (e.g., sugars, maltodextrins) (Danviriyakul *et al.*, 2002; Fäldt and Bergenståhl, 1995; Hogan *et al.*, 2001a, 2001b; Rosenberg and Young, 1993). Sufficient emulsifier is required to form a stable oil-in-water emulsion. This is irrespective of the various types of emulsion-based systems such as conventional oil-in-water emulsions, micro- and nano-emulsions, solid lipid nanoparticles, and multi-layered emulsions (McClements *et al.,* 2007) that may be formulated. A stable emulsion is desirable as an increase in the oil droplet size of the emulsion leads to increased levels of solvent-extractable fat, also referred to as 'free' fat or unencapsulated fat (i.e., poorer encapsulation efficiency) and generally to powders of poorer quality (Danviriyakul *et al.*, 2002).

An encapsulant that has often been chosen for lipids and lipid components is protein. This is due in part to the ability of proteins to stabilize the interface and also to enhance the emulsion stability through their viscosity-building properties. Random coil proteins (e.g., casein) and globular proteins (e.g., whey proteins) are both effective for the encapsulation of lipids (Hogan *et al.*, 2001a, 2001c; Rosenberg and Young, 1993). The use of sugars as co-encapsulants with proteins increases the encapsulation efficiency. This has been ascribed in part to the increased hydrophilicity of the encapsulant which limits the accessibility of solvents to the fat (Young *et al.*, 1993). Lower amounts of solvent-extractable fat in dried oil-in-water emulsions were also obtained with higher dextrose equivalent (DE) (i.e., lower molecular weight) of the carbohydrate used in combination with proteins (Danviriyakul *et al.,* 2002; Hogan *et al.*, 2001b). The individual properties of sugars used in combination with proteins can also influence the amount of 'free' fat in the dried emulsions (Fig. 15.2). Lower

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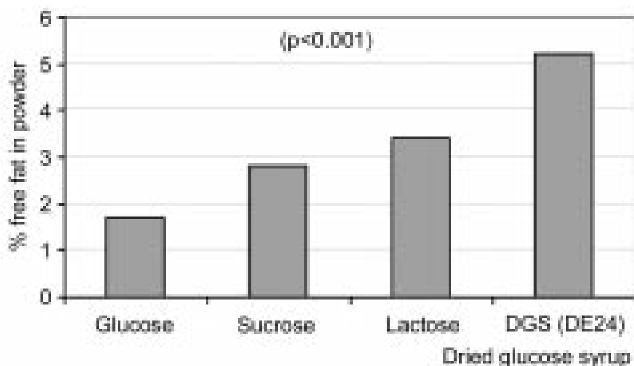

Fig. 15.2 Effect of various sugars used as co-encapsulants with sodium caseinate at 1:2 ratio (by weight) on 'free' fat (solvent-extractable fat) content of dried microcapsules containing 40% fat (dry weight basis) (unpublished results).

molecular weight carbohydrates are able to stabilize proteins during heat treatment and form a denser wall matrix around emulsified fat droplets than carbohydrates with higher molecular weight (Danviriyakul *et al.*, 2002). A range of other matrix materials which have been used for production of dried encapsulated oils include chitosan–maltodextrin mixtures (Klaypradit and Huang, 2008), emulsifying starch (n-octenylsuccinate-derivatized starch) in combination with maltodextrin, glucose syrup or trehalose (Drusch *et al.*, 2006; Drusch and Berg, 2008; Jafari *et al.*, 2008), acacia gum and maltodextrin (Turchiuli *et al.*, 2005) and sugar-beet pectin (Drusch, 2007).

The functional properties of encapsulants may be improved by a pre-processing treatment. For example, heating aqueous mixtures of proteins with reducing carbohydrates to produce Maillard reaction products has been shown to enhance the oxidative stability of microencapsulated oils compared to physical blends of the co-encapsulants (Augustin *et al.*, 2006). Drusch *et al.* (2009) confirmed that encapsulants prepared by heating aqueous protein-reducing sugar mixtures improved the oxidative stability of microencapsulated fish oil but that heating protein-reducing sugar mixtures under restricted moisture conditions did not protect the encapsulated oils against oxidation. Processing of bran extracts prepared using compressed hot water up to 250 °C has shown to produce material with enhanced emulsifying and antioxidative properties. The properties of the pre-processed bran materials vary depending on the treatment temperature (Wiboonsirikul *et al.*, 2008).

15.4.2 Effect of lipid core properties

The physical properties of lipids can affect the distribution of fat within the dried emulsions. Using ESCA, Fäldt and Bergenståhl (1995) found that the surface fat coverage of spray-dried emulsions stabilized by caseinate-lactose blends was influenced by the melting point of the fat. Powders containing intermediate melting points (hardened coconut oil or butterfat) had higher surface fat than those

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containing liquid oil (soybean oil). The use of high melting point hardened rapeseed oil resulted in powders with the lowest amount of surface fat (Fäldt and Bergenståhl, 1995). Other authors found that the type of oil core (fish oil, hardened palm stearin or a mixture of these oils) did not have a marked effect on the solvent-extractable fat of the microencapsulated powders when heated solutions of dried glucose syrup with proteins (whey protein isolate or soy protein isolate) were used as encapsulants (Rusli *et al*., 2006). Given the different methods used to estimate unencapsulated fat and the different encapsulant matrices used, a comparison of encapsulation efficiency between these studies is not possible.

With respect to the fat content of the formulation, it is evident that increasing the fat content beyond a critical level reduces encapsulation efficiency (Hogan *et al*., 2001c; Rusli *et al*., 2006). This critical level is dependent on the materials used to stabilize the liquid emulsion and is related to the properties of the emulsion prior to drying as well as the processes conditions used. Of particular importance is the ratio of the oil:emulsifier in the formulation and the efficiency of the emulsifier used.

15.4.3 Effect of processing conditions

The processing conditions used to manufacture powdered capsules can affect the physical characteristics of the capsules and oxidation of the encapsulated lipid during processing and storage. For example, the use of electrostatic layer-by-layer deposition of emulsifiers around the oil droplets in the emulsion prior to drying may be used to enhance capsule integrity (Klinkesorn *et al.*, 2005). The drying conditions can also impact on the properties of the dried capsules. The powders produced may be agglomerated to improve their wettability (Turchiuli *et al.*, 2005).

In terms of spray drying, a high inlet air temperature can cause particle ballooning (Finney *et al.*, 2002; Walton and Mumford, 1999) and case-hardening, particularly when the wall material has strong film-forming properties. The consequent rupture of atomized droplets is undesirable as it promotes autoxidation of the core material during the drying process (Drusch and Schwarz, 2006). Fang *et al*. (2005) have shown that the rotational speed of the atomizer during spray drying affects the size of the microcapsules. The microcapsules that were prepared at low rotational speed were larger in size than those prepared with the atomizer operating at high rotational speed. Oxidation of the encapsulated lipid in the larger-sized microcapsules proceeded more slowly when both (small- and large-sized) samples were stored at the same relative humidity. However oxidation of the large-sized microcapsules progressed more quickly than the small-sized microcapsules at high relative humidity.

15.5 Lipid oxidation in encapsulated systems

As with bulk oils, lipid oxidation in encapsulated systems is promoted by oxygen, light and the presence of metal catalysts. However, unlike bulk oil

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systems which comprise only the lipid components, the dried emulsion is made up of the matrix components and a heterogeneous distribution of lipids at various locations. The mechanisms which influence fat oxidation in multi-phase systems will therefore be different from those for bulk fats (Frankel, 2005). The complex interplay of factors affecting oxidation and the heterogeneity of the lipid distribution and location results in different states of oxidation in an encapsulated lipid system (Márquez-Ruiz *et al.*, 2003; Velasco *et al.*, 2006).

15.5.1 Measurement of oxidation in encapsulated lipid systems

Lipid oxidation in dried encapsulated oils leads to the formation of a range of oxidation products which results in off-flavors and odors. Irrespective of whether overall oxidation status is measured on the total oil extracted or the whole intact encapsulated system, the result obtained is a composite of the oxidation state of oil droplets with varying states of oxidation.

The traditional method to assess fat oxidation involves the extraction of the total lipid from the dried emulsions and subsequent determination of the levels of primary or secondary products of lipid oxidation. The peroxide value, anisidine value, level of conjugated dienes, estimates of remaining unsaturated fatty acids, and secondary volatile oxidation products such as aldehydes and ketones present in the extracted oil may be measured (Heinzelmann *et al.*, 2000).

Direct methods using headspace analysis has been used to assess oxidative stability of microencapsulated powders. For example headspace hexanal was used to follow oxidation of microencapsulated milkfat (Hardas *et al.*, 2000) while headspace propanal was used for microencapsulated fish oil powders (Rusli *et al.*, 2006). Another direct and fast method to monitor lipid oxidation progress based on high-performance size-exclusion chromatography (HPSEC) has been suggested to quantify primary and secondary oxidation compounds (Márquez-Ruiz *et al.*, 2007). Other methods that have been suggested include the estimation of non-volatile lipid oxidation products including oxidized triglyceride monomers, dimers and oligomers (Velasco *et al.*, 2006).

Bohnert *et al.* (1997) proposed a simplified analytical procedure to analyze long-chain polyunsaturated fatty acids without the need for lipid extraction by direct esterification of lipids from infant formula. The method gives comparable results to standard methods that use lipid extraction, but with less effort. The one-step extraction and methylation method has also been shown to be valid for microencapsulated omega-3 oils in dry powdered foods (Curtis *et al.*, 2008). The extent of oxidation is measured by the depletion of highly unsaturated fatty acids in the sample.

Rapid methods that measure the induction period for oxidation under accelerated conditions have also been employed to assess the oxidative stability of dried oil capsules. One such method is the Oxipres® which measures the oxidation of lipid in heterogeneous products containing oils and fat without the need for extraction of oil from the capsule. The rate of oxygen uptake under conditions of high oxygen pressure at a selected temperature is measured. The

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use of the Oxipres® allows a comparison of the susceptibility of encapsulated lipid systems to oxidation. Experiments carried out on oil–albumin and oil–cellulose mixtures showed the repeatability of the analysis for estimating the induction period of these dry mixtures using the Oxipres® (Trojáková et *al.,* 2000). The authors concluded that the Oxipres® could be used to determine the oxidative stability of dried foods.

Another rapid method that has been examined to assess the oxidation of dried encapsulated lipids is the Rancimat test. In this method the sample is heated under atmospheric pressure at a selected temperature and increased air flow. The induction period for oxidation is sensed by a rapid increase in conductivity due to the capture of the volatile oxidation products in water. This method was originally developed to assess the oxidative stability of bulk oils but it has now been applied to measure the susceptibility of oxidation of dried oil capsules (Velasco *et al.*, 2009).

Most of the objective tests used to measure oil oxidation need to be validated against sensory data. It is possible that similar values for extent of oxidation obtained from objective measurement of oil extracted from the capsule do not necessarily relate to similar taste and flavor profiles of encapsulated lipid system. This is because different encapsulant matrices may bind the products of oxidation to different extents and mask undesirable odors and flavors. Sensory evaluations for odor, flavor and overall acceptability are the ultimate tests of whether the product is acceptable to a consumer.

15.5.2 Factors affecting oxidation of encapsulated lipids

Many factors affect the lipid oxidation rate including the nature of the interface of the encapsulated fat, the concentration of the encapsulants in the aqueous phase, the method of encapsulation, and storage conditions of the capsule (Drusch *et al.*, 2007; Matsuno and Adachi, 1993). In emulsion-based systems, the droplet and interfacial characteristics can affect oxidation of the lipid (McClements and Decker, 2000). It is expected that the interface of the oil droplets within the dried powders and the surface of the powders are also significant factors which determine the rate of oxidation of the oil. This is because the interface of the oil droplet along with the surface of the capsule are the entry points that control access of the encapsulated lipid to oxygen and pro-oxidants, as well as antioxidants. Other factors affecting oxidation include the porosity of the encapsulating matrix, the water activity, the storage temperature and exposure to light during storage, availability of oxygen, and the barrier properties of the matrix (Orlein *et al.*, 2006).

'Free' fat

The oil that is unencapsulated (i.e., not wholly protected) will be more accessible to oxygen. Therefore in systems where access to oxygen is the rate determining factor, the unencapsulated oil will be expected to oxidize at a faster rate than the encapsulated oil.

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Velasco *et al.* (2006) demonstrated that oxidation that occurs in the 'free' oil fraction of dried capsules is characterized by a slow induction period followed by a rapid acceleration of oxidation. In contrast, there is a gradual build-up of oxidation products for the oil that is encapsulated within the dried capsule.

Many authors have found that the level of unencapsulated fat ('free' fat) must be kept low in order to achieve a high level of oxidative stability of the fat. This appears to be the case when the system does not contain antioxidants. For example, in the absence of antioxidants, surface fat is more susceptible to oxidation than encapsulated fat (Baik *et al.*, 2004). The rate of oxidation of the 'free' fat may be reduced by the addition of antioxidants. This can influence the relative rate of oxidation of the 'free' fat and the encapsulated lipid. In fact, oxidation was found to be faster in the encapsulated oil fraction in dried emulsions than in the fraction of oil that was unencapsulated except when the oil that was used for encapsulation was stripped of antioxidants (Velasco *et al.*, 2006).

The overall oxidation status of encapsulated oil is not always related to the amount of unencapsulated fat ('free' fat) because of the heterogeneity of the fat in emulsion-based systems. Hence, solvent-extractable fat ('free' fat) is not a reliable measure of oxidative stability of dried encapsulated lipids (Drusch and Berg, 2008).

State of components in the encapsulated system

The physical state of the components (matrix and core) that make up the encapsulant systems can influence oxidation. Efficient encapsulation of lipid components for oxidative stability requires not only low levels of unencapsulated oil but also depends to a large extent on the ability of the matrix to protect the core from its external environment, which, in turn, is related to the chemical properties of the matrix components.

Materials which have good oxygen barrier properties protect the lipid core from oxidation (Orlein *et al.*, 2006). A matrix that exists in the glassy state is desirable for long-term stability of the encapsulated lipid. The glassy state is often formed when encapsulant systems containing high concentrations of proteins or carbohydrates are dried. The dried encapsulated oil droplets are therefore entrapped within amorphous glassy matrices due to the rapid removal of water. In the glassy state, the diffusion of components is arrested due to the extremely high viscosity of the matrix. The maintenance of the glassy state during storage has been associated with improved stability of encapsulated components (Shimada *et al.*, 1991). Storage of encapsulated powders at high temperature or high humidity results in structural collapse of the matrix or conversion of the glassy state into a crystalline state. When this happens, lipid oxidation is increased (Labrousse *et al.*, 1992). This is possibly due to increased porosity of the matrix or expulsion of the encapsulated oil which increases oxygen accessibility to the oil (Grattard *et al.*, 2002). Oxygen permeation through a glassy matrix has been found to be the rate-determining step for oxidation of lipids within a glassy matrix (Andersen *et al.*, 2000).

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However, it may not be the mobility of the matrix *per se* that arrests the oxidation of encapsulated lipids, as it has been shown that there is no apparent relationship between oxidation rate and the molecular mobility of the matrix, as measured by nuclear magnetic resonance spectroscopy (NMR) (Grattard *et al*., 2002). It has been found that whey protein concentrate (WPC) provides good protection to encapsulated conjugated linoleic acid (CLA) even at high water activities of 0.74–0.90, showing that the WPC-based microcapsule maintained its stability under these conditions (Jimenez *et al*., 2006). Park *et al.* (2005) found that freeze-dried powders with eicosapentaenoic acid ethyl ester (EPE) encapsulated in a blend of maltodextrin (DE2-5) and protein or peptide was more stable to oxidation than EPE entrapped in maltodextrin alone. This observation could not be related to the mobility of the matrix as the addition of protein or peptide did not change the mobility. The increased protection against EPE oxidation with the inclusion of protein or peptide was ascribed to their radical scavenging properties (Park *et al*., 2005).

The physical state of the lipid can also influence oxidation rates. McLean and Hagaman (1992) reported that the oxidation of liposome encapsulated lipids is greater at temperatures below the solid-liquid phase transition temperature of the host lipid. It is well known that the ratio of solid to liquid fat in a bulk lipid is dependent on the fatty acid composition, temperature and also the tempering history of the fat. However, it should be noted that the solid to liquid fat ratio of the oil droplet cannot be predicted from that of the bulk lipid as this may be altered by the addition of emulsifiers (Arima *et al*., 2007). In addition, depending on the type of fat encapsulated, fat crystallized within dry emulsion droplets can undergo polymorphic transformations (Millqvist-Fureby, 2003). Hence encapsulation, through its influence on crystallization behavior of the encapsulated fat, can also influence oxidation of the oil.

Microstructure of encapsulated system

The microstructure of dried emulsions influences the oxidative stability of the oil. This is in part governed by the formulation and the drying conditions. Figure 15.3 shows the structure of powders containing microencapsulated oil at different length scales.

Keogh *et al.* (2001) found that the stability of spray-dried encapsulated fish oil was improved by substituting sodium caseinate with micellar casein or calcium caseinate (i.e., aggregated forms of casein). An increase in the aggregation state of casein resulted in increased vacuole volume. Hence it was not possible to identify whether the reduced oxidative stability was due to the effects of the aggregation of the protein or the increased vacuole volume (Keogh *et al*., 2001).

Addition of antioxidants to the formulation

Oil oxidation may be retarded by adding antioxidants. Many combinations of antioxidants have been examined. It is not only the presence of antioxidants but their partitioning behavior in the encapsulated system that can influence the

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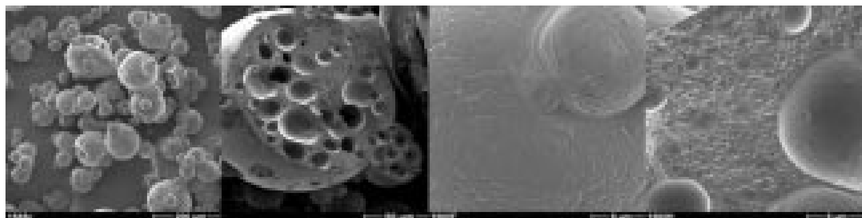

Fig. 15.3 Structure of powder containing microencapsulated oil (omega-3).

oxidation of oils within various parts of the capsule. The partitioning of antioxidants within the encapsulated lipid system will in itself be affected by the nature of the antioxidant used (e.g., their lipophilicity). For example, the lipophilic antioxidant (α-tocopherol) afforded more protection to both surface and encapsulated fats in a dried oil-in-water emulsion than ascorbyl palmitate, which is an amphiphilic antioxidant (Baik *et al.*, 2004).

Owing to the heterogeneous distribution of oil within encapsulated systems, a combination of antioxidants is required for effective stabilization of the encapsulated lipid during production as well as during storage of the final encapsulated oil powders (Serfert *et al.*, 2009). Among the antioxidants found to minimize oxidation of microencapsulated fish oils are tocopherols, citric acid esters of monoglycerides, lecithin, ascorbic acid/ascorbyl palmitate and rosemary extract (Serfert *et al.*, 2009). Different antioxidants exert different protective effects to oils within the encapsulated system.

15.6 Applications of lipid encapsulated systems in food

Lipid encapsulation has found increasing commercial applications in the food industry. The food industry believes that lipid encapsulation can be used to achieve much-needed product differentiation in the marketplace, since it enables the addition of ingredients that cannot normally be added without encapsulation. In addition, encapsulation has the potential to enhance product value.

Encapsulation of lipid components has evolved significantly over the last decade and research has facilitated the enhancement of capsule performance in a number of food systems. Encapsulation has been used to protect lipids and lipid components from oxidation, to act as carriers of lipids and lipid-soluble bioactives, to mask undesirable odors and flavors (e.g., fish oil) and to offer convenience to food manufacturers by supplying powder ingredients that are easier to use and incorporate into food products than their liquid oil versions. The encapsulant materials are usually chosen from ingredients that are already used or present in the final food application, to avoid labelling issues.

15.6.1 Incorporation methods

Lipids and lipid-soluble ingredients can often be incorporated into food or beverages with appropriate formulation and processing. However, options are

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often narrowed down based on stability and taste of the ingredient (e.g., omega-3 oils) in the final food or beverage product. In many cases the type of food or beverage application determines whether an ingredient can be directly added or whether encapsulation is needed for successful incorporation.

15.6.2 Consideration of food manufacturing process

Lipid encapsulation for food applications involves a number of considerations. Candidate technologies are initially identified and the most suitable ones tailored to meet specific encapsulation requirements in foods while keeping an eye on the economics of the technology used to produce the ingredient. For food applications, economics and cost-effectiveness are of the highest importance due to the low margins that consumers are willing to pay for food products containing encapsulated systems. Ingredients that are considered natural or GRAS (generally regarded as safe) are highly favored by the food industry. Further, product safety and bioavailability (optimal absorption) of the encapsulated core within the food product are two important aspects which companies often ask about before they decide on the encapsulation techniques to be used. Therefore a fundamental understanding on tailoring the performance of the capsules to obtain the desired release profiles in the food product as well as deliver a cost-effective formulation for the food product application is essential.

15.6.3 Quality of products containing encapsulated lipids

The development of new food products and beverages containing encapsulated lipids is challenging. Besides the potential technological obstacles encountered during product development, consumer demands need to be considered. Product shelf stability and sensory acceptance have been recognized as key factors to successfully capture market opportunities for functional foods (Siró *et al.*, 2008). Encapsulated lipids are intended to have a shelf life that at least matches, if not exceeds the shelf life of the final food application. Owing to the susceptibility of lipids to oxidation, achieving long shelf life beyond 12 months at ambient temperatures is a challenge for highly unsaturated oils such as omega-3 oils. Hence most of the food product launches containing omega-3 oils are those with shorter shelf life, and often require refrigeration.

The ability of the encapsulant to mask undesirable flavors and odors is another important criterion in order to be able to add the required amount or dose per serving without affecting the sensory acceptance of the final food product. When microencapsulated oils were added to selected dairy products (Table 15.1), they were found to be of equal or superior quality to those fortified with equivalent levels of the oil added as a bulk ingredient (Sharma *et al.*, 2003). Ye *et al.* (2009) showed that the sensory acceptability of a cheese product fortified with encapsulated fish oil was better than those containing bulk fish oil.

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Table 15.1 Sensory acceptability of dairy products containing microencapsulated omega-3 (data from Sharma *et al.*, 2003)

Product	Level of omega-3 added	Control	Fish oil	Encapsulated fish oil powder	Storage time prior to tasting
Plain yoghurt	60 mg omega-3 FA/ 110 g serve	5.6^{b}	6.6^{ab}	8.0^{a}	6 weeks
Drinking yoghurt	60 mg omega-3 FA/ 110 g serve	7.3^{ab}	5.5^{b}	8.1^{a}	8 days
Processed cheese	60 mg omega-3 FA/ 21 g serve	5.9^{ab}	4.3^{b}	6.2^{ab}	24 days
Cheese dip	60 mg omega-3 FA/ 20 g serve	9.4^{a}	6.2^{b}	8.3^{ab}	12 days

FA: Fatty acid
Scale: 1 (dislike extremely) – 15 (like extremely)
Numbers within a row having different superscripts are significantly different

15.7 Future trends

By choosing appropriate matrices, and processing and storage conditions, the lipid or lipid components can be protected from deterioration caused by adverse reactions and/or environmental stresses encountered during the formulation, manufacture and storage of both the encapsulated lipid system, and the final food product into which it is incorporated.

Most of the research to date has been directed at understanding the physical and chemical aspects of lipid encapsulation, the factors that affect oxidative stability of encapsulated lipids and the integrity of the encapsulated systems during storage. As encapsulation has the potential to target the delivery of the encapsulated core to a desired site in the body, the future is in using encapsulated lipid systems to deliver lipids or lipid-soluble bioactives to various sites in the body. Demonstration of the bio-equivalence of encapsulated lipid preparations will be essential if the encapsulated lipid has an intended health benefit. This is now an active field of research.

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16

Antioxidant active food packaging and antioxidant edible films

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Abstract: This chapter reviews antioxidant active packaging for food. It introduces, reviews and comments upon the state of the art, as well as discussing the principles of antioxidant packaging and antioxidant edible films, and the available systems for measuring the antioxidant capacity of these packaging materials. The chapter also includes an overview of several technologies used to produce new active antioxidant materials, emphasizing the advantages and drawbacks of each technology. Finally, it describes several applications that have recently been launched into the market with new antioxidant active packaging materials, and makes some comments about the future trends.

Key words: active packaging, antioxidant packaging, active materials, food protection, food packaging.

16.1 Introduction

16.1.1 What is antioxidant active packaging?

Oxidation is one of the main causes of food deterioration, therefore methods for its prevention are the subject of much research. Antioxidant packaging was the first type of active packaging available. It is still very much in demand, despite the increasing availability of alternative types of active packaging.

Antioxidant active packaging prevents oxidation by either absorbing components contributing to oxidation, such as oxygen or radicals, or by releasing antioxidants inside the packaging.

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Confusion about the definition of antioxidant active packaging can arise, though, as most polymers (for example, polyolefins), and especially those used in food packaging materials, contain synthetic antioxidants. The main role of these antioxidants is to prevent the oxidation of the packaging material itself, increasing its stability over time, not to act on the food within the package. The migration of synthetic antioxidants, such as BHT (2,6-di-tert-butyl-p-cresol), BHA (3-*tert*-butyl-4-hydroxyanisole), Irganox (octadecyl 3-(3,5-ditert-butyl-4-hydroxyphenyl)propanoate), and Irgafos (Di-n-octyl phosphite) from packaging to food is too low to prevent the oxidation of the product (Garde *et al.*, 1998). Therefore the materials in which these synthetic antioxidants are found should not be considered active packaging materials. In fatty foods the level of migration is higher, yet even in this case the antioxidants transferred do not protect the food from oxidation. Furthermore, these synthetic antioxidants are not legally allowed to be added to food, which is why their concentration in the packaging material has such specific migration limits (Directive 2002/72/EC and its amendments; Directive 2004/1/EC; Directive 2004/19/EC; Directive 2007/19/EC; Directive 2008/39/EC).

16.1.2 Principles of oxidation

To understand how antioxidant active packaging achieves its effect, it is essential to consider the oxidation process itself. Food degradation is a complex phenomenon involving a wide variety of reactions. It depends on the type of food and its constituents, such as carbohydrates, lipids, proteins, vitamins and water, and the concentration of these constituents. Many of the reactions involved in food degradation are still unknown. Of the reactions that are recognized, though, lipid oxidation is considered the most important, and for this reason has been the most thoroughly studied (Pokorny *et al.*, 2001).

Lipids are found in most foodstuffs, and they are usually related to the bad odor and bad taste associated with oxidation, as they are responsible for rancidity. The two main groups of lipids are triglycerides and phospholipids. The first group is found in the cells responsible for the storage of fat in animals and plants, while the second group forms part of the cellular membrane. In foodstuffs of animal or vegetal origin, the oxidation of phospholipids in cellular membranes begins the oxidative degradation process.

Lipids spontaneously react with oxygen by means of auto-oxidation. This phenomenon was studied in depth by Ingold (1961) and Denisov and Khudyakov (1987) who analyzed the chemistry and kinetics of several hydrocarbons, and showed auto-oxidation to be a reaction in chain through the formation of free radicals. Such a reaction consists of three main steps: initiation, propagation and termination. This oxidation reaction is mainly catalyzed by transition metals and by the light. There are also several compounds commonly present in food, such as chlorophylls, porphirins, bilirrubin, riboflavin and pheophitines, which enhance photooxidation, and therefore help in the production of radicals. Several enzymes, such as lipoxygenasa, increase and accelerate the oxidation process.

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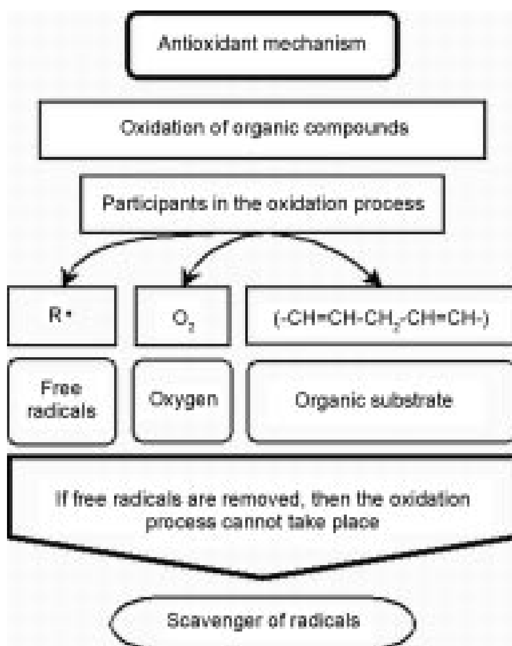

Fig. 16.1 Scheme of oxidation process of an organic substrate.

To summarize: at least three factors are essential for oxidation to occur in an organic substrate:

- the organic substrate (e.g., a foodstuff)
- molecular oxygen
- free radicals.

Figure 16.1 shows a scheme of the reaction. If one of the factors is removed, the reaction does not take place.

16.2 Eliminating molecular oxygen from packages

16.2.1 Traditional approaches

Surprisingly, most strategies to prevent oxidation in foods focus on eliminating molecular oxygen from the package. There are two common systems:

- The first creates a vacuum and then uses a high barrier packaging material to try to prevent the entrance of new oxygen. If the pressure of oxygen is low enough, the limiting step is the diffusion of oxygen through the food layers. The speed of the reaction is then controlled by decreasing the temperature at which the food is stored. However, this method is not as efficient as expected, because frozen water, if present, causes tissues to dry and lipid fractions to separate in natural emulsions, exposing them directly to the air.

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- The second completely removes the oxygen and then employs modified atmosphere packaging (MAP), which also requires a high barrier material.

Combining either of these systems with an oxygen absorber or an active antioxidant material will extend the shelf life of the product. Oxygen absorbers have limited capacity, though, and cannot be used in all applications. Often, oxygen must be present in a packaged food (e.g., fresh meat), while at the same time oxidation must be prevented.

16.2.2 Packaging containing oxygen absorbers

Several formats of oxygen absorbers have been developed and commercialized. They consist of a substance, usually iron powder dispersed or embedded in an inert sorbent, contained in a sachet with a permeable membrane (López-Cervantes *et al.*, 2003). The oxygen permeates through this membrane and reaches the iron. In the presence of moisture the iron powder traps the oxygen and is chemically transformed into iron (III), and oxygen is removed during the chemical reaction. This system has several limitations. Only a small amount of oxygen can be efficiently removed from each piece of packaging. This means that the size of the sachet must be optimized for each pack, taking into account the expected shelf life, the free space inside the pack, and the amount of oxygen that must be removed. The chemical reaction also requires moisture to be effective, which means that the moisture must be supplied either by the food or by the internal atmosphere.

This is an efficient type of active packaging, but it requires an independent sachet together with the food inside the pack, which is not well accepted by the consumer. Furthermore, this is not a true example of an active material, as the active or antioxidant solution is independent of the packaging material.

16.2.3 Other ways to remove oxgyen

There are other ways to remove oxygen by chemical reaction inside packaging, but many of these are not legally permitted, and are not often considered to be true examples of active packaging. For example, CO was extensively used in fresh meat packaging, mixed with other gases in a modified atmosphere. This protected the fresh meat from oxidation, and maintained its red color. The carbon monoxide combines with myoglobin to form carboxymyoglobin, a bright cherry red pigment. Carboxymyoglobin is more stable than oxymyoglobin, the oxygenated form of myoglobin, which can become oxidized to form a brown pigment, metmyoglobin (Sorheim *et al.*, 1999). Using CO, the stable red color can last much longer than in normally packaged meat. Although the use of CO was approved by FDA in 2004 as a safe practice (GRAS), it proved controversial because it can mask spoilage (American Meat Institute, 2008, http://www.meatami.com/ht/a/GetDocumentAction/i/40141. Retrieved May 2009) and there are also concerns about the toxicity of CO. It is no longer permitted in Europe, Canada, Japan or Singapore. This is one reason why active packaging to extend the shelf life of fresh meat is in such high demand.

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16.3 Antioxidant active packaging materials

For a variety of reasons (discussed below), it can be preferable to add antioxidants to the packaging material, rather than eliminate molecular oxygen from foods using barrier materials or oxygen scavengers.

16.3.1 Introduction to antioxidants

Antioxidants can be classified into five different groups according to their mechanism of action:

- Antioxidants which react with free radicals, acting as radical scavengers. They are usually called 'breaking chain' antioxidants, as they break the chain reaction of radicals and avoid the propagation step (Masuda *et al.*, 2002; Amakura *et al.*, 2000). Among them, phenols usually present in essential oils are the most common.
- Antioxidants which decompose peroxides, producing stable substances that are unable to produce radicals, such as tioethers, metionine, tiodipropionic acid, or even some enzymes such as glutation peroxidase. Glucose oxidase is usually added to food, combined with catalase to diminish the concentration of oxygen. However, they have not yet been used in packaging materials, as the price of the material and the stability requirements are too high.
- Antioxidants which react with transition metals to form complexes, and thus avoid the catalytic effect of the metals in the oxidation process. Examples of these antioxidants are citric acid, ascorbic acid, tartaric acid, gluconic acid, oxalic acid, succinic acid and hydroxyglutaric acid as well as oxalate, phosphate and EDTA (ethylendiamintetracetic acid). These antioxidants can be seen as inhibitors of the oxidation process.
- Antioxidants which inactivate the singlet form of oxygen as well as the sensibilizing molecules, dissipating the energy as heat. These are involved in the photooxidation process. Examples of this type are tocopherol and carotenoids.
- Antioxidants which prevent the enzymatic activity required for auto-oxidation. Flavonoids, phenolic acids and galates, which deactivate the lipoxygenasa are examples.

16.3.2 Formation and removal of free radicals

Free radicals are generated from oxygen either from natural sources or from artificial ones. Their production is increased by natural factors, including photochemistry (radiation, mainly in the UV range), endogenous and exogenous molecules, metallic ions or enzymes. Artificial enhancers of free radical production include irradiation, radiolysis pulses, transition metals (having two oxidation states such as iron or copper) and azoderivatives. Once the radicals have been generated, the presence of molecular oxygen produces the peroxide radicals $ROO^{\bullet}$. These react with polyunsaturated lipids, eliminating one hydrogen atom and simultaneously producing other radicals, which react again with oxygen to create other radicals. This way, the chain reaction continues.

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Deactivation of radicals is mainly achieved by the reaction of two lipidic radicals. Free radicals are few in number, their removal is easy, and the process can take place within the packaging material. However, it is difficult to find the right solution for radical removal, as radical scavengers are relatively unknown and very difficult to measure. That is why most researchers still focus on oxygen removal from packaging to prevent oxidation.

If radicals disappear as soon as they are formed inside the packaging, the oxidation process cannot take place. This scavenging process is independent of the amount of oxygen present in the packaging, taking place despite the presence of molecular oxygen. This is useful for products that require oxygen to maintain their properties, such as fresh meat (Nerín *et al.*, 2006).

Among the most efficient radical scavengers, those present in some essential oils have demonstrated a high efficiency, and many well-known antioxidants also act via radical scavenging (Pezo *et al.*, 2008; Prior *et al.*, 2003).

16.3.3 Introduction to antioxidant active packaging

There are several ways to create antioxidant active packaging materials:

- To incorporate antioxidants into the melted polymer, and then extrude the material. Most of the antioxidants are lost or degraded, as the process requires an increase of temperature.
- To produce the active material as a coating on other packaging materials. This is one of the most promising technologies, although some problems still exist.
- To introduce the antioxidant as an internal layer in a multilayer structure. Usually, the antioxidant layer contains an oxygen absorber. This technology is commercially available, but expensive, as it requires a special activation when used in the food industry.
- To apply the coating directly to the food; this requires the use of edible packaging materials. This is another approach in which the applications are more restricted, as not all the foodstuffs can be coated. One disadvantage is the likelihood of over-packaging, as the coated food will still require normal packaging material for health and safety reasons.

All packaging materials may potentially be converted into active antioxidant materials, although the ones most commonly used include polyolefins, (such as PE and PP), PET and PS, either by themselves or combined with others in multilayer structures. Paper and board can also be converted into antioxidant materials to protect food. Even aluminum can be converted into an antioxidant active material through a coating process with an antioxidant layer.

Although most of the antioxidants described above have been used either alone or in combination in foods, most of them cannot be used as antioxidants in active packaging. There are several reasons for this:

- the likely degradation of the active compounds in the production of the active packaging

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- the necessity of direct contact between the compounds and the food requiring protection
- the price of the compounds and their availability
- incompatibility with most of the technologies and materials currently developed for active packaging.

Despite these problems, the first advantage of antioxidant active packaging is that antioxidants added directly to packaging materials will not be eaten. Fewer additives are therefore required for the food to maintain taste and appearance and human consumption of chemicals will decrease. The consumer perception of natural food is very positive, and therefore market and consumer satisfaction is improved. Moreover, when antioxidants are added to packaging materials, they can remain efficient for a longer time, as the material is not eaten and most of the antioxidants remain in the packaging. Consequently, the shelf life of the packaged product is extended. This means that the packaged food can be marketed in a wider area.

16.4 The production of active packaging

16.4.1 Extrusion and multi-layer materials

Several technologies can be used to produce active packaging. The first is *extrusion*, where the active compounds are added to the melted mass of the polymer and then extruded. The final pellets produced are then used to form the container or the packaging material. The main advantage of this technology is that the antioxidants are contained in the material from the very beginning. Therefore they are not perceived by the consumer and will not need to be handled or modified. The food company will use this active material as usual, without modifying its packaging machinery or the conditions in which the food is packaged. Although this is the most attractive solution, the real issue is that most antioxidants cannot withstand this process, as they decompose during manufacturing.

The most significant disadvantage is that the material requires additional protection to avoid losing its antioxidant properties, from the moment in which it is produced in the petrochemical company to the moment in which it is used in the food industry. One solution is to block the antioxidant in the material when it is produced, and to reactivate it when it is required. Several commercially available materials which contain oxygen absorbers apply this blocking and reactivating system, but it is an inconvenience. The antioxidants are reactivated using UV irradiation, which requires a complementary device in the packaging machinery and obviously involves an additional cost. The final result is a multilayer material in which the antioxidant is protected by two layers at both sides of the antioxidant active layer. Several options have been investigated, such as the introduction of alpha-tocopherol or hydroxi-tirosol antioxidants to polyethylene (PE) (Peltzer, 2009) but the protection is not enough. Most of these options have not been commercialized.

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16.4.2 Essential oils in extruded packaging materials

Without any doubt, the most promising antioxidants for active packaging are essential oils. It has been proven repeatedly that essential oils extracted from plants such as rosemary, oregano, dill, basil, ginseng, and green tea, contain a lot of antioxidant compounds, mainly phenols, hydroxyphenols, flavonoids and organic acids such as rosmarinic and carnosic acids (Su *et al.*, 2007; Aruoma *et al.*, 1992; Barclay and Vinqvist, 2003; Bozin *et al.*, 2006; Del Bano *et al.*, 2003; Miura *et al.*, 2002; Nanjo *et al.*, 1999). However, there are still some problems to solve. Firstly, the essential oils contain a lot of volatile compounds, which provide a specific aroma to both the packaging material and to the food. Although the volatile compounds are not responsible for the antioxidant properties (Nerín *et al.*, 2008; Bentayeb *et al.*, 2007a, 2007b), the real issue is that the volatile compounds represent a limitation to their use in food packaging (Gutierrez *et al.*, 2009).

The second aspect to consider with essential oils is their degradation with temperature. When increasing the temperature, as it is required in the extrusion process, up to 90% of the initial amount added to the polymer is lost, making the industrial process financially unworkable.

A third aspect to consider is the composition of the selected essential oil. The composition depends upon the plant, the climate in which the plant has grown, the technology used to dry the plant and the extraction technology used to obtain the essential oil. It is common to have big differences in the composition of the same essential oil from different suppliers. It is therefore important to ensure good quality control before using it as an active agent. For this reason, the availability of a reliable procedure to measure the antioxidant properties is very important.

If other natural antioxidants are selected, such as organic acids, like citric or ascorbic acids, they require direct contact with the food as they do not act as radical scavengers. These acids are also decomposed with high temperatures, and are generally not compatible with synthetic polymers. Furthermore, depending upon their concentration in the system they can also act as pro-oxidants, accelerating the oxidation process (Otero *et al.*, 1997), which is why they are not used as active compounds in extrusion technology.

Polyolefins such as PE or polypropylene (PP), polyesters such as polyethylenterephthalate (PET), and polyamides (PA) can be obtained by extrusion. None of them are commercially available right now with natural antioxidants. However, polylactic acid (PLA) is increasing in both industrial production and interest because it is a biodegradable polymer. Several attempts have been made with PLA and natural antioxidants, but none of them are commercially available yet due to the same problems found with synthetic polymers.

16.4.3 Coating

The second technology to be mentioned is the *coating* process. This consists of applying a liquid dispersion, usually resin and solvent base, on a packaging

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material in its final format. It means that the coating operation is applied at room temperature and only requires a flash of hot air, most of the time no more than 40 ºC, to eliminate the solvent. The coating formulation is therefore anchored onto the material, and cannot be removed. This is a very common process in packaging materials, as varnishes and inks are usually applied to the materials to provide them with thermosealability, color, or other properties. If the coating contains antioxidants as active agents to protect the food, it is a means to incorporate the antioxidants into the packaging material without having the problems previously mentioned. Using this technology and essential oils as natural antioxidants, several antioxidant active food packaging materials have been proposed, patented and industrially obtained (Garcés *et al.*, 2004; López *et al.*, 2007; Nerín *et al.*, 2006, 2008; Bentayeb *et al.*, 2007a). These materials are now available on the market. The whole material is then considered to be a single layer and it has proven to be a very efficient antioxidant material for different kinds of foodstuffs, including fresh meat, dried nuts, fat food and others (Nerín *et al.*, 2006; Gutierrez *et al.*, 2008). The coating process has the additional advantage of being practical for any kind of material, including paper and board, plastics and aluminium or their combinations; either as single materials or on multilayers; and on both flexible semi-rigid and rigid materials. It is also worth mentioning that the polyolefins require surface pretreatment. This can be done by corona treatment or even with atmospheric plasma treatment to condition the surface before applying the coating.

16.4.4 Surface functionalization

The final technology to be mentioned is the functionalization of the surface to anchor the antioxidant, through a chemical or physicochemical interaction. Although this is theoretically possible, it is not available on the market. If the compounds are chemically bonded to the surface, they do not have the chemical groups available to trap the radicals, and the chemical reaction is delayed or impeded.

16.4.5 Antioxidant edible films

There is also great interest in edible polymers as a means of preventing oxidation. In principle, this is an attractive idea, as the food pieces will be completely covered by an edible film which can be directly ingested as part of the food. In other words, it simulates the skin of a fruit which is not peeled. (For reasons of consumer protection, though, the edible coating should not be thought of as the final packaging material. Additional packaging material is required to protect the food from external contamination.) Several requirements must be met when using edible films. The texture of the food should not be changed. Taste and odor are also challenges and the organoleptic properties are easily affected if the edible film contains antioxidants. It is not possible to use essential oils in these cases. Despite these disadvantages there is a lot of research in this area, though

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commercial solutions are very rare. The edible polymers investigated in this context include gliadins, and zeins mainly extracted from cereals such as maize and starch base polymers (Herald *et al.*, 2006; Wang *et al.*, 2003; García *et al.*, 1998, Min and Krochta, 2007).

16.5 Measuring the antioxidant capacity of active packaging

16.5.1 Non destructive procedures

Objective evaluation methods that can support in a quantitative manner manufacturers' data about new active materials are necessary when selecting a new material. They are also important for raw material quality control. Once an active material has been purchased, quantitative evaluation is also required to check if the material remains active after several months of storage.

There are several methods for measuring the antioxidant capacity of food. All of them are complementary, as they provide different values following different mechanisms, and all of them provide a scale of antioxidant capacity as well as quantitative values of antioxidant properties. However measuring the antioxidant properties of a plastic or a material nondestructively is a difficult task. If the material has to be extracted or dissolved, then the integrity of the antioxidants will be modified and their properties cannot be guaranteed to correspond with those originally present in the material. Antioxidant properties should be measured directly in the antioxidant material, as any chemical reorganization or modification will affect the chemical behavior upon which the antioxidant properties are based.

Pezo *et al.* (2006) developed an analytical procedure to demonstrate the radical scavenging properties of new active polymers. It is in fact an indirect procedure, but it has proven to be very efficient for the purpose. The procedure uses salicylic acid as the reagent to produce the final derivatives to be measured. It consists of generating an atmosphere enriched with radicals, which is then carried with a carrier gas through the plastic bag made of the antioxidant material to be measured. The gas current with the remaining radicals, if any, arrives at an impinger where there is an aqueous solution of salicylic acid, wherein the reaction shown in Fig. 16.2 takes place.

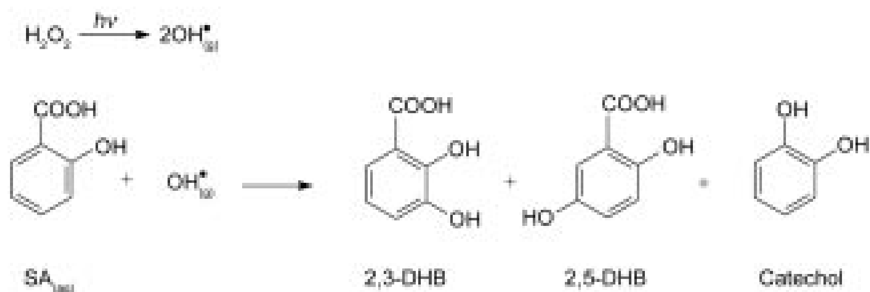

Fig. 16.2 Reaction used to measure the radical scavenger properties.

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The atmosphere enriched in radicals is produced by using a nebulization of hydrogen peroxide and synthetic air as a carrier gas. When there is not a radical scavenger in the plastic bag, all radicals cross the plastic and are carried to the impinger where 2,3-dihydoxybenzene (2,3-DHB) and 2,5-dihydoxybenzene (2,5-DHB) are formed. Only traces of catechol appear. The two isomers are fluorescent and can be separated by HPLC and quantified with high sensitivity using a fluorescence detector. If there are radical scavengers in the material, which means that it behaves as an antioxidant, the radicals are trapped by the material and do not arrive at the impinger. In this case, fluorescent products are not formed. The concentration of the DHB isomers is proportional to the content of radical scavenger. Figure 16.3 shows the scheme of the apparatus used.

Eight different samples or replicates can be measured simultaneously. To maintain the same shape and the same volume of air, several separators of wood were used as the carrier gas containing radicals passed through the plastic bags, as shown in Fig. 16.4. After the reaction, the final solution in each impinger is measured by HPLC with the fluorescence detector. An example of the chromatogram obtained is shown in Fig. 16.4.

As we can see, when the plastic does not contain any antioxidant, the 2,5-DHB peak is very high, as all radicals arrive at the impinger and produce the reaction. Applying this procedure, several antioxidant active packaging materials were measured, and the scale of antioxidant power was established as shown in Fig. 16.4. A detailed description of this procedure can be seen in the paper authored by Pezo *et al.* (2008).

The mentioned procedure is in any case non destructive, which means that the material is measured as it is. For flexible polymers, plastic bags are prepared, thermosealing the edges of the plastic layers. Again the comparison can be done by comparison with a strong antioxidant such as Trolox, but in this case the antioxidant has to be incorporated in the material as well, preferentially using the same technology.

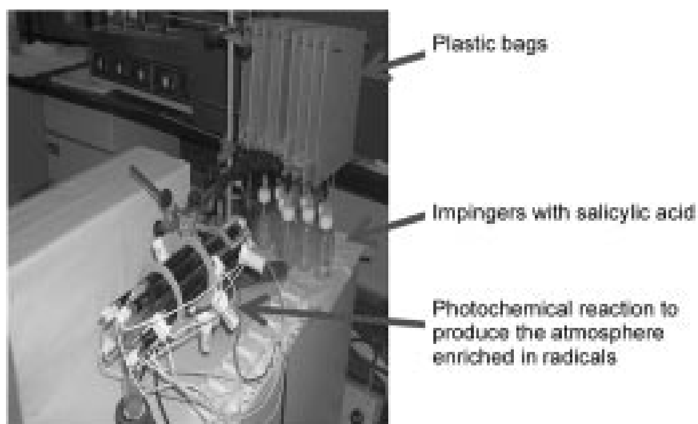

Fig. 16.3 Apparatus used to measure the antioxidant properties.

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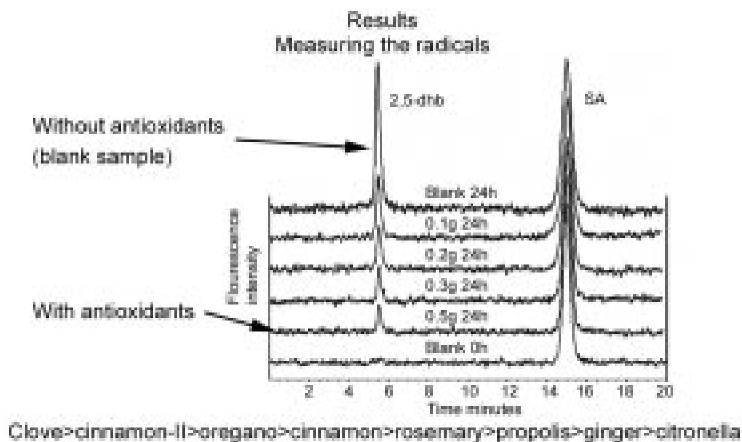

Fig. 16.4 Separation of 2,5-DHB and salicylic acid by HPLC-fluorescence.

Other procedures try to measure the antioxidant capacity using model substances with easily oxidizable compounds. From these we can measure either the compounds produced by the oxidation reaction (Pastorelli *et al.*, 2007), or the evolution of the compounds over time. In such cases, the quantitative scale is relative, and absolute values are not obtained. Comparisons are made with materials containing a strong and well-known antioxidant such as Trolox, which act as references.

16.5.2 Destructive procedures

Destructive procedures which work with a solution, or extraction containing the antioxidants, are abundant. Because they have not been designed to work directly with the material, they require a previous step of sample treatment, and the final result can be different from that obtained with the active packaging. These differences occur because the behavior of the compounds in the plastic is different to that in the solution without the plastic matrix. This means that the interaction with the substrate is very important, as it changes the active points of the molecule, and affects its behavior.

There are several examples of destructive methods that have been applied to a wide range of samples and matrices (Frankel, 1993), including plastic food packaging (Bentayeb *et al.*, 2009). Among them, AAPH (2,2′-azobis(2-amidinepropane)dihydrochloride), DPPH (DiPhenyl-1-PicrylHydrazyl radical) (López Dicastillo *et al.*, 2007; Goupy *et al.*, 2003) and ORAC (Oxygen Radical Absorbance Capacity assay) procedures are the most common. DPPH produces radicals at constant speed and this reaction depends on both the concentration of the initiator and the temperature of the system, while ORAC has been used in liquid simulants to evaluate the antioxidant capacity of EVOH. DPPH$^{\bullet}$ (α,α-difenil-picrylhidracil radical) is able to trap an electron or a hydrogen radical to produce a stable molecule which can only be oxidized with great difficulty. This

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Table 16.1 Indirect methods to measure the antioxidant capacity

Transfer of hydrogen atoms	*ORAC (Oxygen Radical Absorbance Capacity)* *TRAP (Total Radical trapping Antioxidant Parameter)* *crocin bleaching assay*
Transfer of electrons	*Total concentration of phenols* (Folin-Ciocalteu reagent) *TEAC (Trolox Equivalent Antioxidant Capacity)* *FRAP (Ferric ion Reducing Antioxidant Parameter)* *DPPH (Diphenyl-1-picrylhydrazyl)*

radical is violet in methanol solution, but the final molecule produced by the reaction is colorless. It is the change in colour that is measured. ORAC, on the other hand, is based on the capacity of an antioxidant to inhibit the disappearance of fluorescein by reaction with peroxyl radicals.

Another common system is to measure the reaction of thiobarbituric acid (TBARS) with the malondialdehyde produced in lipid oxidation. The product of the reaction is red, and can be measured by visible spectrophotometry at 532 nm. This method is quantitative and provides a sound indication of the antioxidant performance, but measured in the oxidizable model or food rather than in the material.

Other indirect methods used to measure antioxidant properties can be classified according to the mechanism of reaction, either based on transference of hydrogen atoms or on transference of electrons (Huang *et al.*, 2005; Prior *et al.*, 2005). Table 16.1 lists some of the main methods. However, as these methods are not specifically used for active packaging, they will not be discussed here.

16.6 Advantages and disadvantages of the different technologies of antioxidant active packaging

As we have seen, several different types of antioxidant active packaging are available:

- Oxygen absorbers, which are considered to be antioxidants when used in the packaging.
- Antioxidant active packaging containing an antioxidant layer in a multilayer structure, such as commercially available trays. This material is obtained either by coextrusion or by lamination with adhesive.
- Antioxidant active packaging obtained by coating. The coatings mainly contain radical scavengers as antioxidant agents, most commonly essential oils such as oregano, rosemary, and cinnamon.

The use of sachets is not fully accepted around the world. The consumer can mistakenly perceive the independent sachet to be a gift or a condiment, rather than part of the packaging system with a technological function (this is particularly a problem in foods designed for children). Furthermore, the food

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industry has to add the sachet to the product in the packaging line, which adds complexity to the system as well as additional cost. Equally, this is not a universal solution, as the dimensions of the sachet need to be optimized for each package and food. For these reasons, the sachet is not the preferred active packaging option.

If we consider antioxidant materials obtained by extrusion or coextrusion, a food company wishing to use these must evaluate the amount of active material that they will need for the next two or three months (the normal storage time in a company). Because active packaging is not a universal solution, every product would need a different amount of antioxidant or radical scavenger, and consequently it would prove very expensive to invest in sufficient stock of such a wide variety of active materials. Furthermore, we should highlight that the active polymer must be 'activated' and be well protected beforehand to avoid degradation during storage.

Antioxidant active packaging obtained by the coating is the most versatile option. It can be applied directly in the packaging machine, can be designed and used specifically for each product, and even the concentration of the active agents can be optimized properly for each food.

Without any doubt using packaging coated with radical scavengers is much more convenient than using oxygen absorber sachets, as they work in a normal atmosphere, i.e. in the presence of oxygen. They also do not require high barrier materials, special packaging machines, or a modified atmosphere, as these active materials trap the radicals as soon as they are formed in the packaging. Nonetheless, when using essential oils as radical scavengers, organoleptic changes can take place, which may limit their application. The organoleptic changes will depend on the product, the concentration of active compounds and the food, but can be negligible when the food complements the scents supplied by the packaging. This is another reason to study each case in depth.

The stability or shelf-life of active antioxidant materials is another key concern. The food industry requires active materials to be stable during storage for at least two or three months. Some materials that are now available in the market are stable for at least one year. No other degradation compounds have been identified in these active materials containing radical scavengers, even though they contain complex mixtures of compounds found in essential oils.

16.7 Applications of antioxidant active packaging

16.7.1 Commercial availability of antioxidant active packaging materials

Although antioxidant active packaging materials are interesting and attractive, very few are produced on an industrial scale and used in the market.

Oxyguard is an example of an industrial material that acts as an oxygen absorber. Usually Oxyguard is used as part of a multilayer packaging material, covered on both sides by other inactive polymers, for its protection. Oxyguard requires activation once the multilayer packaging material has been produced. It

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is activated using UV irradiation during the food packaging step, so a complementary device is required as part of the packaging machinery.

With regard to radical scavengers, new active materials containing essential oils (Graces and Nerín, 2009) developed by two Spanish companies, Artibal (Sabiñanigo, Spain) and Repsol-Rylesa (Madrid, Spain), have proved to be very efficient in protecting food, and are now commercially available in the market. They do not require additional protection, as they are able to act as radical scavengers in the presence of molecular oxygen. Their stability over time is also very good, with the antioxidant properties of the active material maintained for up to one year.

Several formulations of paraffin coating for paper and board containing essential oils as active agents have also been developed by the Research group GUIA at the University of Zaragoza together with the company Repsol-Rylesa (Spain). Although the coating's most important effect is its antimicrobial property (Rodríguez *et al.*, 2007, 2008), it is worth emphasizing the antioxidant properties provided by this new active material.

16.7.2 Particular foods

Fresh meat

Some foods are more prone to rapid oxidation than others. Antioxidant active antioxidant packaging is particularly important for these foods and is better accepted. Fresh meat is always the first target for active packaging, as its red color disappears in a very short period. To maintain the 'fresh' period the addition of any kind of additive is not permitted. Therefore, active antioxidant packaging is the only solution and among the different approaches explained above, that of radical scavengers (essential oils) introduced into the packaging through a coating process is the best one. The University of Zaragoza (Guia Group, C. Nerín) together with the Company Artibal (Sabiñanigo, Spain) developed an antioxidant packaging ready to use for fresh meat. They studied beef (Nerín *et al.*, 2006), lamb (Camo *et al.*, 2008) and pork, and their success allowed them to offer big meat companies the use of this active antioxidant polymer. This antioxidant polymer contains essential oils as radical scavengers, which are able to chemically trap the radicals produced in the atmosphere within the packaging. Such radicals are usually enhanced by the presence of oxygen and irradiation, such as UV light, both common in the cold display stands of the supermarket. The reaction between the scavengers and the radicals is very fast, so the radicals disappear as soon as they are produced and there is no time for them to initiate the oxidation process in the meat. Consequently, the characteristic red color of fresh meat is maintained even in the presence of molecular oxygen. As mentioned above, this antioxidant polymer is able to act in the presence of molecular oxygen, which is a very important advantage. Other foods can be wrapped with the active polymer without requiring high barrier materials, while still maintaining the antioxidant properties and extending the shelf life.

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Other products
Antioxidant active packaging of fish has not yet been studied. With the exception of salmon and other blue fishes, though, oxidation is not the priority problem with regard to shelf-life extension. However, dried nuts, fatty food, and bakery products with a high fat or butter content are more prone to suffer from oxidation. Several bakery products have been launched into the market (Gutierrez *et al.*, 2008) now using the new active antioxidant materials developed by Artibal (Patent number EP1657181-A1 and Patent number EP1477519) as well as those developed by Repsol in the paper and board sector (Nerín *et al.*, 2007, Patent number WO2007144444-A1).

16.8 Future trends

Currently, there is a great interest in active packaging. Research projects focusing on food packaging are abundant in Europe and the USA, and new proposals often appear in the scientific journals. However, from the industrial point of view there are only a few antioxidant active materials commercially available and produced on an industrial scale.

Commercial plastics companies are making moves, though, to improve the barrier properties of standard polymers. Nanotechnology, in particular the incorporation of nanoparticles into packaging, must be mentioned in this context, as it offers new possibilities to enhance the oxygen barrier properties of materials. Usually, nanoparticles do not directly affect the oxidation reaction or their participants, but they make the diffusion of oxygen through the packaging materials more difficult (Pereira *et al.*, 2007; Krook *et al.*, 2005). The most common compounds used are clays and zeolites at nanometric dimensions which have plates that are able to separate one other (Azeredo, 2009). This type of nanoparticle, when homogeneously distributed through a material such as a polymer, adds tortuosity to the permeation of oxygen, and makes oxygen diffusion much more difficult. Consequently, the material dramatically increases its defense against oxygen. In reality, this is based on a physical mechanism rather than on active packaging, although the final result is the extension of the food's shelf life. However, no matter how attractive such materials are, very few materials containing nanoparticles are currently commercially available. Compared to the traditional and well-known high barrier materials in which the diffusion of oxygen is very low, nanoparticles merely add a physical barrier to oxygen, while low diffusion in other materials is based on solubility and physicochemical interactions between oxygen and the chemical composition of the material.

There will probably be more advances in nanotechnology in the near future, but antioxidant active packaging materials are likely to predominate because of their undoubted advantages.

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