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Lipid Oxidation

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16.1. Introduction

Lipid oxidation is one of the most basic chemical reactions that occur in food, generally resulting in deterioration in sensory and nutritional quality. Many reviews of the chemistry of lipid oxidation have been published (Labuza, 1971; Frankel, 1980, 1982, 1985, 1988, 1991, 1998; Larel, 1980; Schaich, 1980; Richardson and Korycka-Dahl, 1983; Porter, 1986; Chan, 1987; Grosch, 1987; Gardner, 1989; Kochhar, 1996; Min and Lee, 1996; St. Angelo, 1996; Belitz and Grosch, 1999; Kolakowska, 2003). Lipid oxidation is essentially a free-radical chain reaction involving initiation, propagation and termination stages. Unsaturated fatty acids are oxidized to form odorless, tasteless hydroperoxides. These are unstable and degrade to yield flavorful carbonyls and other compounds. Frankel (1998) has reviewed recent advances in the understanding of the chemistry of autoxidation. Inhibiting the progress of lipid oxidation in foods, including milk and milk products, is a key factor in maintaining quality and extending shelf-life.

Milk is a complex biological system containing many factors, which may act as antioxidants and/or pro-oxidants. The relative amounts of these factors in milk are influenced by parameters such as the breed, health, nutritional status, and stage of lactation of the cow. Subsequent processing and storage of milk may also exert a profound influence on the progress of lipid oxidation. The objective of this chapter is to review the many factors, both indigenous and exogenous, that influence lipid oxidation in milk, the flavor consequences, and the measurement of lipid oxidation in milk fat.

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Advanced Dairy Chemistry, Volume 2: Lipids, 3rd edition.
Edited by P.F. Fox and P.L.H. McSweeney, Springer, New York, 2006.

16.2. Mechanism of Lipid Autoxidation

The hydroperoxide theory of the oxidation of unsaturated lipids is universally accepted. The fundamental principles were elucidated by the work of Farmer *et al*. (1942), Bolland and Gee (1946) and Bateman *et al*. (1953). The initial step in the autoxidation of unsaturated fatty acids is the formation of free radicals. The formation of the initial free radical to start the oxidation process may be due to factors such as irradiation, metal complexes, enzymes or active oxygen species. In the case of monounsaturated and nonconjugated polyunsaturated fatty acids in milk lipids, the reaction is usually initiated by removal of a hydrogen from the methylene group adjacent to the double bond. The resulting free radical reacts with ground-state molecular oxygen to form a peroxide free radical. This, in turn, reacts with another unsaturated molecule to continue the chain reaction and generate a hydroperoxide. A sequence involving initiation, propagation and termination reactions has been proposed to explain the autoxidation of lipids:

Initiation: $RH \rightarrow R^{\bullet}$ (RH is unsaturated fatty acid)

Propagation: $R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}$

$RO_2^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$

Termination: $2RO_2^{\bullet} \rightarrow O_2 + RO_2R$

$RO_2^{\bullet} + R^{\bullet} \rightarrow RO_2R$

$2R^{\bullet} \rightarrow R - R$

In general, free radical chain reactions proceed with a very low overall activation energy (Waters, 1971). However, in foods, such as butter, the rate of oxidation may be as much a feature of their microscopic structure which affects diffusion of oxygen, as of their chemical composition.

Schaich (1980) proposed the following generalizations:

1. The rate of oxidation is directly proportional to the amount of peroxide produced and at low oxygen pressures, to the concentration of oxygen.
2. As the concentration of oxygen increases, its influence on oxidation rate decreases. At atmospheric pressure, the rate of oxidation is independent of oxygen concentration.
3. In the early stages of oxidation, the concentration of RO_2H may be very low. Initiating events other than the homolytic decomposition of hydroperoxides are critical at this early stage. Once decomposition of RO_2H has occurred, the rate of hydrogen abstraction from

unsaturated lipids by the resultant alkoxy radicals ($RO^{\bullet}$) is in the order of 10^4-10^6 times faster than by peroxy radicals ($RO_2^{\bullet}$) generated in the propagation phase. Thus, the production of peroxides mediates the rate of lipid oxidation, and the stability of peroxides, as affected by food constituents, is a key factor influencing the rate of development of oxidative rancidity.

One key role of pro-oxidant metals (e.g., copper, iron, haem proteins) in promoting oxidative rancidity is their capacity to catalyse the decomposition of pre-formed hydroperoxides to initiate new oxidation chains (Korycka- Dahl and Richardson, 1980). Hydroperoxides are relatively unstable and enter into numerous and complex breakdown and interaction mechanisms responsible for the production of myriad compounds of various molecular weights, flavor thresholds and biological significance (Nawar, 1985; Chan, 1987; Grosch, 1987). In addition to autoxidation, lipid oxidation can proceed along a photooxidation route or a lipoxygenase route. These differ from autoxidation at the initiation stage only.

Food lipids possess an inherent stability to oxidation, which is influenced by the presence of antioxidants and pro-oxidants. After a period of relative stability (induction period), lipid oxidation becomes autocatalytic and rancidity develops. Thus, the typical time-course of autoxidation, as measured by the concentration of hydroperoxides, consists of a lag phase (induction) followed by the rapid accumulation of hydroperoxides, which reaches a maximum and then decreases as hydroperoxide decomposition reactions become more important. The longer the induction period, the more stable the food to oxidation (Lundberg, 1962).

16.3. Oxidation Products and Off-Flavors

Milk is characterized as having a pleasing, slightly sweet taste with no unpleasant after-taste (Bassette *et al.*, 1986). However, its bland taste makes it susceptible to a variety of flavor defects. Autoxidation of unsaturated fatty acids gives rise to unstable hydroperoxides, which decompose to a wide range of carbonyl products, many of which can contribute to off-flavors in dairy products. The principal decomposition products of hydroperoxides are saturated and unsaturated aldehydes (Frankel *et al.*, 1961), with lesser amounts of unsaturated ketones (Stark and Forss, 1962), saturated and unsaturated hydrocarabons (Forss *et al.*, 1961), semialdehydes (Frankel *et al.*, 1961) and saturated and unsaturated alcohols (Hoffman, 1962; Stark and Forss, 1966).

In addition to those carbonyls that are theoretically possible from the degradation of the hydroperoxides of the principal unsaturated fatty acids in

milk, others have been isolated and identified. This suggests that further oxidation of unsaturated aldehydes initially formed, migration of double bonds or isomerization may occur during autoxidation (Weihrauch, 1988). Since milk fat contains many minor unsaturated fatty acids, very many carbonyl products may be produced during autoxidation. Hence, the overall flavor produced during the autoxidation of milk fat is a combination of many flavors imparted by individual carbonyls present at minute concentrations. Patton *et al.* (1959) demonstrated that 2,4-decadienal imparts an oily, deep-fried, off-flavor in aqueous solutions at concentrations less than 0.5 μg/kg.

It is difficult, however, to correlate specific off-flavors in dairy products with specific carbonyls or groups of carbonyls owing to: (1) the multitude of compounds produced; (2) difficulties arising in qualitative analyses of oxidized dairy products; (3) differences in the threshold value of individual compounds; (4) similarity of flavors imparted by individual compounds near their flavor thresholds; (5) a possible additive and/or antagonistic effect, with regard to both flavor and threshold values of mixtures of compounds; (6) the possible existence of a compound or groups of compounds not previously identified; and (7) the difficulties involved in adding pure compounds to dairy products as a means of evaluating their flavor characteristics (Weihrauch, 1988).

Despite the above difficulties, several specific chemicals have been associated with specific off-flavors in dairy products. Forss *et al.* (1955a,b) reported that *n*-hexanal, 2-octenal, 2-nonenal, 2,4-heptadienal and 2,4-nonadienal are the principal carbonyls contributing to the copper-induced "cardboard" off-flavor in milk. Hall and Lingnert (1986) associated this flavor defect with n-hexanal in spray-dried whole milk. l-Octen-3-one has been associated with a "metallic" off-flavor in dairy products (Stark and Forss, 1962), the metallic off-flavor being reproduced by addition of 1-octen-3-one to milk or cream (Bassette *et al.*, 1986). l-Octen-3-one has a threshold concentration of 1 μg/kg in butterfat (Shipe *et al.*, 1978).

"Creamy" flavors in butter have been associated with 4-*cis* heptenal produced for autoxidation of isolinoleic acid (Begeman and Koster, 1964). "Drier" flavor in foam spray-dried milk has been associated with 6-*trans*-nonenal, which has a flavor threshold in fresh milk of 0.07 μg/kg (Parks *et al.*, 1969). Bassette and Keeney (1960) implicated a homologous series of autoxidation-derived saturated aldehydes, together with products of Maillard browning, in "cereal-type" off-flavors in powdered skim milk. "Staleness" in dry whole milk may be associated with saturated and unsaturated aldehydes (Parks and Patton, 1961). 2,4-Decadienal has been reported to be the principal compound responsible for the off-flavor associated with spontaneously oxidized milk (Parks *et al.*, 1963). Oxidized flavors in sunlight-exposed milk are commonly related to C_6 to C_{11} alk-2-enals

(Wishner and Keeney, 1963). "Fishy" flavor in milk fat is due to a mixture of 1-octen-3-one, the compound associated with metallic flavor, and an "oily" fraction containing *n*-heptanal, *n*-hexanal, 2-hexenal and heptan-2-one (Forss *et al*., 1960a,b). Some 40 volatile compounds were identified in cold-stored cultured butter with a fishy off-flavor, including 4-*cis*-heptenal, 2-*trans*, 4-*cis*-decadienal, 2-*trans,*6-*cis*-nonadienal, 2,2,7-decatrienal, 3-*trans*, 5-*cis*-octadien-2-one, 1-octene-3-one and l-octen-3-ol. "Cucumber" flavor has been associated with 2,6- and 3,6-nonadienal and "mushroom" flavor with l-octen-3-ol (Badings and Neeter, 1980).

Forss *et al*. (1960a,c) compared the qualitative and quantitative distribution of carbonyl compounds in dairy products with "fishy," "tallowy" or "painty" off-flavor. Total content of volatile carbonyl compounds was approximately 10 times greater in the "tallowy" and 100 times greater in the "painty" butterfat than in "fishy" butterfat. "Tallowy" butterfat contained greater amounts of *n*-heptanal, *n*-octanal, *n*-nonanal, 2-heptanone 2-heptenal and 2-nonenal, while "painty" butterfat contained greater amounts of *n*-pentanal and C_5 to C_{10} alk-2-enals.

Flavor threshold values for carbonyl compounds are influenced by factors such as the number of carbon atoms, degree and location of unsaturated double bonds, isomerism, additive and/or antagonistic effects of mixtures of carbonyls and the medium in which the carbonyls exist (Day *et al*., 1963; Meijboom, 1964). Many carbonyls have up to 100 times greater flavor potency in an aqueous medium (e.g., liquid milk) than in a fat or oil (e.g., butteroil). Hence, off-flavor tends to be noticeable at lower concentrations of carbonyl compounds in liquid milk than in butter.

Oxygen and light (particularly ultraviolet wavelengths) exert a synergistic effect on fat degradation with resulting off-flavor and rancidity (IDF, 1982). The sensitivity of cream to light is influenced by heat treatment (e.g., off-flavor develops more rapidly in raw or pasteurized cream than in UHT cream). Free sulphydryl groups, exposed upon denaturation of whey proteins in UHT cream, are thought to inhibit off-flavor development (Bull, 1992). Oxidative deterioration of UHT milk is enhanced by increased levels of dissolved oxygen and increased storage temperature (Jeon *et al*., 1978; Adhikari and Singhal, 1992). The chemical composition of the volatile carbonyl compounds produced due to light-induced lipid oxidation has been reported to be different from that produced due to copper-induced oxidation (Jenq *et al*., 1988).

16.3.1. Spontaneous Oxidation in Milk

Off-flavor due to spontaneous oxidation of milk fat is a troublesome issue because the process and its prevention are not well understood and it tends to occur in otherwise well-managed, high-yielding dairy herds

(Barrefors *et al.*, 1995; Granelli *et al.*, 1998). Dunkley and Franke (1967) classified milk into three categories based upon its susceptibility to oxidation:

1. Spontaneous milk is capable of developing oxidized flavor within 48 h of milking without the presence of contaminating iron or copper. Bruhn *et al.* (1976) reported that 12–20% of raw milk samples are in this category.
2. Susceptible milk does not oxidize spontaneously but does develop oxidized flavor following contamination with iron or copper. Use of noncorrodible dairy equipment has reduced the incidence of copper contamination.
3. Non-susceptible milk does not oxidize even in the presence of iron or copper.

Spontaneous oxidation of milk fat, which has been known for over 60 years (Corbett and Tracy, 1943), is influenced by heredity, stage of lactation and feeding practices (Shipe, 1964). Some cows consistently produce spontaneous milk, others occasionally, and others not at all (Parks *et al.*, 1963). Differences between milk from the different quarters of the same cow may occur.

Aurand and Woods (1959), Astrup (1963) and Aurand *et al.* (1967, 1977) proposed that spontaneous oxidation in milk is directly related to Xanthine oxidoreductase activity. This enzyme has iron, molybdenum and flavin cofactors and is a major component of the fat globule membrane. While some evidence suggests that Xanthine oxidoreductase is involved to an extent in the oxidation of milk lipids (Allen and Humphries, 1977; Hill, 1979; Allen and Wreiden, 1982b), others have reported no relationship between spontaneous oxidation and Xanthine oxidoreductase activity (Smith and Dunkley, 1960; Rajan *et al.*, 1962). King (1958) noted that spontaneous milks had a higher total copper concentration in the fat globule membrane than milks classified as susceptible or resistant. Smith and Dunkley (1960) hypothesized that endogenous copper in milk formed a complex with ascorbate, which was involved in spontaneous oxidation of milk lipids. Aurand *et al.* (1977) suggested that a combination of light, copper and Xanthine oxidoreductase generated singlet oxygen, which then initiated the oxidative process. Hill *et al.* (1977) proposed that oxidation is induced by copper in a pathway involving $^{\bullet}OH$ radicals, while Xanthine oxidoreductase in the fat globule membrane and lactoperoxidase in the milk serum were involved in a secondary pathway generating singlet oxygen with consequent oxidation of milk lipids. Hill (1979) suggested a coupled enzyme system in which lactoperoxidase catalyses lipid oxidation generating aldehyde substrates for Xanthine oxidoreductase that in turn furnishes H_2O_2 for use by lactoperoxidase as an oxidizing agent. Thermal inactivation of these enzymes has been shown to result in greater oxidative stability in milk rich in linoleic acid (Hill, 1979).

Cytochromes have been reported at low concentrations in the milk fat globule membrane (Gregory *et al.*, 1976; Jarasch *et al.*, 1977). The powerful pro-oxidant properties of ferri-porphyrin proteins, together with their juxtaposition with lipids in the milk fat globule membrane, suggest that cytochromes may play a role in oxidation. Gregory *et al.* (1976) concluded that they do exert a role in milk lipid oxidation.

Clearly, more research is required to clarify the somewhat confused picture regarding the role of enzymes in the oxidation of milk lipids. However, the key factor affecting the susceptibility of milk to oxidation appears to be its relative content and distribution of pro-oxidants and antioxidants. Bruhn and Franke (1971) reported that spontaneous oxidation is directly proportional to the copper content and inversely proportional to the α-tocopherol content of milk. Charmley *et al.* (1991) showed that intramuscular injection of cows with α-tocopherol may overcome a spontaneous oxidized flavor problem caused by low levels of α-tocopherol in milk. In general, milk from pasture-fed cows is less susceptible to oxidation due to a higher content of tocopherols than milk from cows given dry feed (Bruhn and Franke 1971; Urbach, 1989, 1990).

St. Laurent *et al.* (1990) investigated the effects on milk flavor of α-tocopherol supplementation (0, 700 or 3000 IU/day) to a feed consisting of grain mix, hay and pasture in herds with a chronic spontaneous oxidized flavor problem. α-Tocopherol supplementation resulted in improved milk flavor but no relationship was apparent between milk α-tocopherol levels and the extent of flavor improvement. In this study, the flavor problem decreased significantly when the cows subsequently got access to spring pasture.

Barrefors *et al.* (1995) analyzed samples with and without oxidized flavor from two commercial herds. Their data indicated that oxidized milk samples had a higher linoleic acid content in the neutral fat fraction and contained a higher concentration of hexanal. At one of the farms, the concentration of both α-tocopherol and β-carotene were lower in samples that developed off-flavor. They speculated that high-yielding cows fed high amounts of unsaturated fats in their feed needed higher dietary concentrations of α-tocopherol and β-carotene.

16.4. Factors that Affect the Oxidation of Lipids in Milk and Milk Products

A range of environmental and physical factors, processing and storage conditions, and endogenous and exogenous chemical constituents and enzymes have been shown to influence the rate and extent of lipid oxidation in milk and

milk products. These include oxygen, light, endogenous and exogenous metals, antioxidants, ascorbic acid, tocopherols, carotenoids, thiols, proteins and enzymes, browning reaction products, milk fat globule membrane (MFGM) constituents, storage temperature and water activity.

The balance between pro-oxidant and antioxidant factors is critical for the oxidative stability of milk (Stapelfeldt *et al*., 1999; Morales *et al*., 2000). The degree of unsaturation of milk lipids is a factor influencing oxidation. Kristensen *et al*. (2004) obtained milk from cows fed a low-fat diet rich in cereals, which enhanced *de novo* fat synthesis and contained 21.3% unsaturated fatty acids and milk that contained 41.3% unsaturated fatty acids from cows fed a diet rich in soyabean oil. Buttermilk from the more unsaturated milk was less oxidatively stable during 11 days storage at 4°C than buttermilk from the more saturated milk as monitored by hydroperoxide and hexanal production. These workers also monitored during the storage period the levels of fat-soluble antioxidants (α-tocopherol and β-carotene) and water-soluble antioxidants in the serum phase. The fat-soluble antioxidants were not consumed during storage of either of the two buttermilks. Interestingly, however, the antioxidative capacity of the serum phase decreased during storage with a similar time-course for the decrease in both types of buttermilk, suggesting that oxidation is initiated in the serum phase independently of fatty acid composition.

To a certain extent, the composition (including lipid composition) of cows' milk reflects feed composition (Bugaud *et al*., 2001; Ramaswamy *et al*., 2001). Certain feeding regimes have the potential to increase the level of polyunsaturated lipids in milk and the potential for oxidation (Barrefors *et al*., 1995; Charmley and Nicholson, 1995; Hermansen, 1995; Focant *et al*., 1998; Morales *et al*., 2000; Bugaud *et al*., 2001; Timmons *et al*., 2001; Havemose *et al*., 2004).

16.4.1. Oxygen

Lipid oxidation, by definition, requires the presence of oxygen. However, the minimum residual oxygen concentration required for lipid oxidation in food products or in food packages may vary between different foodstuffs. Clearly, products with a large surface area or with a porous structure should theoretically be more predisposed to oxygen exposure and hence oxidation. Different packaging materials will influence oxygen transmission rate as will product to headspace ratio (Mortensen *et al*., 2004). Transmission is also influenced by the partial pressure of oxygen inside and outside the packaging material, by storage temperature and by relative humidity (Robertson, 1993). Reducing the oxygen content in the package headspace will minimize oxidation rate (Hong *et al*., 1995). However, a

low residual oxygen level of 0–5 ml/l headspace has been reported to result in rapid formation of oxidized off-flavor in Havarti cheese (Mortensen *et al*., 2002). The same workers (Mortensen *et al*., 2003) reported a saturation effect at approximately 1% residual oxygen for sliced Havarti cheese.

Oxygen has greater solubility in non-polar than in polar solvents and, hence, is more soluble in liquid milk fat than in whole raw milk. A substantial percentage of the total oxygen in whole milk exists in the fat phase, particularly at higher temperatures (Noll and Supplee, 1941; Timms *et al*., 1982). Oxygen is excluded from the solid fat phase as it crystallizes. However, this may not imply a lower rate of oxidative deterioration at low temperatures. As the temperature of butter is reduced, oxygen excluded from the crystallized fat phase partitions into, and saturates, the liquid fat and aqueous phases of the butter. Thus, oxygen is available to react with the more unsaturated fat in the liquid phase and with phospholipids and pro-oxidants in MFGM fragments. The maximum rate of hydroperoxide production in irradiated butter is observed at $-20^{\circ}C$, which may reflect an increased rate of chain terminations at higher temperatures and reduced propagation reactions at lower temperatures (Hannan and Boag, 1952; Hannan and Shepherd, 1952).

Kinetically, ground-state (triplet-$^3O_2$) oxygen is not very reactive, which obviously restricts the oxidation of food lipids. $^3O_2$ requires ''activation'' to facilitate oxidative reactions. Three principal processes are involved in the activation of oxygen (Fridovich, 1977):

1. Photochemical excitation of an electron in $^3O_2$ to a higher energy state may occur, thereby generating very reactive singlet oxygen, $^1O_2$. Photochemical excitation usually requires the intermediate participation of a photosensitizer (Ranby and Rabek, 1978), although other formation pathways exist. In food systems, the photosensitizer (e.g., riboflavin, chlorophyll, erythrosine) absorbs visible light and transfers energy to $^3O_2$ to generate $^1O_2$.
2. Certain metals may interact with $^3O_2$ to yield singlet-like O_2 (Hanzlik, 1976).
3. Successive, univalent reduction of oxygen may yield reactive oxygen species. This reduction can be effected photochemically, chemically or enzymatically (Korycka- Dahl and Richardson, 1980). A number of enzymes, including Xanthine oxidoreductase in milk, are capable of producing large amounts of superoxide *via* the univalent reduction of oxygen (Fridovich, 1976).

In contrast to triplet oxygen, singlet oxygen is very electrophilic and readily reacts with unsaturated lipids with the formation of hydroperoxides,

which can then decompose homolytically to initiate new free-radical chain reactions. Thus, initiation of oxidation by very low levels of $^1O_2$ is sufficient to generate large numbers of reaction chains involving ground-state oxygen.

Korycka-Dahl and Richardson (1980) listed five possible ways by which $^1O_2$ is generated in dairy products as a result of processing and storage:

1. Chemically, by reaction of residual hypochlorite with hydrogen peroxide:

$$H_2O_2 + {}^{-}OCl \rightarrow {}^1O_2 + H_2O + Cl^-$$

2. Chemically or enzymatically, by reactions involving metalloproteins:
   (i) Protein $- M^{n+} + {}^3O_2 \rightarrow {}^1O_2$-like complex
   (ii) Peroxidase $- OCl^- + H_2O_2 \rightarrow {}^1O_2$
3. Photochemically, *via* riboflavin or another sensitizer.
4. Self-reaction of secondary peroxy radicals:

$$2R{-}HCOO^{\bullet} \rightarrow {}^1O_2 + R{-}HC{=}O + R{-}HCOH$$

5. Oxidation of superoxide by a restricted number of oxidizing agents can lead to $^1O_2$.

16.4.1.1. Superoxide Radicals

The superoxide radical ($O_2^{\bullet-}$) is another reactive oxygen species. Although the superoxide radical may not react readily with unsaturated fatty acids, it is capable of oxidizing phenolic compounds such as tocopherols, thiols and ascorbic acid (Korycka-Dahl and Richardson, 1980). This, in turn, may lead to earlier oxidation of lipids. However, the significance of superoxide as a pro-oxidant in milk is unclear. Superoxide rapidly dismutates in water, yielding hydrogen peroxide which, itself, may be involved in oxidative reactions (Korycka-Dahl and Richardson, 1980):

$$H^+ + HO_2^{\bullet} + O_2^{\bullet} \longrightarrow H_2O_2 + {}^3O_2$$

This reaction is also catalyzed by superoxide dismutase (SOD), which occurs in milk at very low concentrations (Fox and Morrissey, 1981). Hydrogen peroxide can also be formed in milk as a result of microbial metabolism or by reduction of superoxide by ascorbic acid. A mean level of 0.02 mg/l hydrogen peroxide has been reported in milk (Toyoda *et al.*, 1982).

Superoxide may also univalently reduce hydrogen peroxide to yield the extremely reactive, electrophilic oxidant, hydroxyl radical (Schaich, 1980):

$$H_2O_2 + O_2^{\bullet} \longrightarrow HO^{\bullet} + OH^- + O_2$$

However, the significance of this reaction in foods is unclear.

Milk leucocytes have been shown to adhere to the MFGM (Peters and Trout, 1945a,b). The enzymatic generation of active oxygen species by phagocytic activity is another possible source of pro-oxidants (Salin and McCord, 1977; Kanner and Kinsella, 1983).

16.4.1.2. Oxygen Removal

Removal of dissolved oxygen from liquid milk or its replacement by nitrogen reduces the intensity of oxidized flavor (Sharp *et al.*, 1941; Singleton *et al.*, 1963; Schroder, 1982). Available oxygen in the fat should be less than 0.8%, v/v, to prevent the production of a "tallowy" flavor in butteroil (Schaffer *et al.*, 1946). However, the production of all oxidized flavor is not reduced by lowering the concentration of dissolved oxygen. Schroder (1982) reported a reduction in light-induced, but not copper-induced, oxidized flavor development in oxygen-depleted milk. De-aeration to a very low oxygen level was necessary to prevent copper-induced oxidized flavor.

The oxidative stability of whole milk powder can be maintained for an extended period by vacuum treatment or replacement of oxygen with an inert gas (Greenbank *et al.*, 1946; Schaffer *et al.*, 1946). Tamsma *et al.* (1961) reported a statistically significant improvement in storage stability of whole milk powders packed in inert gases containing 0.1% oxygen compared with those packed at 1% oxygen level. Milk powders packed in the presence of glucose oxidase-catalase (oxygen scavenger system) and calcium oxide (dessicant) were comparable in flavor with samples stored in inert gas (Meyer and Jokay, 1960); the enzymes reduced the oxygen level to 0.5% in 1 week. Other workers have used various scavenging systems to deplete the oxygen level in stored milk powders with consequent improvement in keeping quality. One system utilized a mixture of 90% N_2 and 10% H_2 in the presence of palladium, which catalyzed the formation of water from the H_2 and residual oxygen to produce an almost oxygen-free atmosphere in the pack (Abbot and Waite, 1961). An oxygen-absorbing mixture (Na_2SO_3 and $CuSO_4.5H_2O$) enclosed in porous paper pouches has also been shown to be effective (Jackson and Loo, 1959). Oxygen has been depleted to less than 0.001% within 24 h in packed milk powder using a scavenging system consisting of 95% N_2, 5% H_2 and a platinum catalyst (Tamsma *et al.*, 1967).

Oxidized flavor is of minor importance in fermented dairy products such as cheese or yogurt (Wong *et al.*, 1973; Czulak *et al.*, 1974; Korycka-Dahl *et al.*, 1983). Several factors may be involved, including depletion of oxygen by the growth of starter bacteria, the acidic pH of the products, peptides produced by proteolysis, and the formation of antioxidants by microorganisms (Eriksson, 1982).

16.4.2. Light

Many studies have shown that light is very effective in promoting off-flavor development in milk and milk products (Singleton *et al.*, 1963; Hedrick and Glass, 1975; Bray *et al.*, 1977; Sattar *et al.*, 1977a; Bradley, 1980; Nelson and Cathcart, 1984; Bartholomew and Ogden, 1990; Kim and Morr, 1996). Photooxidation of dairy products has been reviewed by Bradley (1980), Bosset *et al.* (1994, 1995), Skibsted (2000), Borle *et al.* (2001), and Mortensen *et al.* (2004). The extent of off-flavor development is a function of the wavelength involved, and the intensity and duration of exposure. Light has been shown to penetrate milk to an appreciable depth (Finley and Shipe, 1971; Newstead and Headifen, 1981).

The water-soluble vitamin, riboflavin, present in milk acts as a potent photosensitizer and has been implicated in the photooxidation of milk fat (Foote, 1976; Aurand *et al.*, 1977; Bekbolet, 1990). Aurand *et al.* (1977) suggested that singlet oxygen is involved based on the inhibitory effects of a singlet oxygen-trapping agent (1,3-diphenylisobenzofuran) or a singlet oxygen quencher (1,4-diazabicyclo-2,2,2-octane) on the oxidation of milk fat catalyzed by Cu^{2+}, enzymes or light. The ability of riboflavin to generate singlet oxygen in milk in its capacity as a photosensitizer has been confirmed by Bradley and Min (1992) and Berliner and Ogata (1997). After photodegradation, riboflavin breaks down to lumichrome and probably formylmethylflavin. Lumichrome is also a strong photosensitizer (Parks and Allen, 1977). Riboflavin has three absorption bands. The band with a maximum between 430–460 nm is the main band responsible for the photooxidation of food, especially milk and dairy products. Riboflavin transfers absorbed energy to other molecules such as dissolved oxygen in milk, thus generating reactive oxygen species. Sattar and Deman (1975) first demonstrated the correlation between duration of light exposure, presence of riboflavin and off-flavor development.

It has also been reported that riboflavin generates superoxide anion in milk exposed to fluorescent light and has been implicated in the destruction of other milk components, such as vitamin C, by light (Spikes and Livingstone, 1969; Korycha-Dahl and Richardson, 1979). The exposure of butter to light has been reported to result in the oxidation of cholesterol, giving rise

to 5-cholesten-3β,7α-diol and the 7β-epimer and, possibly, 6-cholesten-3β,5α-diol (Luby *et al*., 1986a). Potential lipid-derived off-flavor in butter may be reduced by light-barrier packaging such as aluminum foil. Direct exposure to light is the principal factor affecting photooxidation of butter; temperature and duration of storage exert little effect on butter with subsensory levels of light-induced oxidation (Luby *et al*., 1986b).

Light also influences milk flavor due to riboflavin-sensitized effects on milk proteins *via* oxidation of methionine to methional (3-methylthiopropionaldehyde) (Patton, 1954; Tada *et al*., 1971; Sattar *et al*., 1977b) . Other amino acids, besides methionine, may be affected by the presence of light and riboflavin. Allen and Parks (1975) reported that exposure of milk serum to fluorescent light chemically modified 10 amino acids in immunoglobulins. Riboflavin-photosensitized oxidation of milk lipase, resulting in considerable loss of activity (80% loss in 30 min), has been reported in sunlight-exposed milk (Dimick, 1976). Light- and riboflavin-induced changes in cheese have also been reported (Deger and Ashoor, 1987).

Recent work (Wold *et al*., 2005) suggests that dairy products also have natural levels of porphyrins and chlorophylls and that these light-sensitive compounds play an important role in photooxidation of cheese. Fluorescent analysis indicated that photodegradation of porphyrins and chlorophylls correlated closely ($R > 0.9$) with the sensory attribute of oxidized odor.

A number of studies have investigated the effect of packaging materials on the oxidative stability of stored liquid milk. Cladman *et al*. (1998) reported a significantly higher degree of lipid oxidation in milk packaged in high-density polyethylene (HDPE) jugs than in milk packaged in green polyethylene terephthalate (PET) bottles after 7 days of storage, presumably because the green PET reduced light exposure compared to the partly transparent HDPE. Erickson (1997) noted higher levels of lipid oxidation after 8 days storage in milk packaged in HDPE jugs compared to paperboard cartons. Zygoura *et al*. (2004) investigated the effect of packaging materials on the shelf-life of whole pasteurized milk. As expected, more lipid oxidation was observed over a 7-day storage period in clear PET than in pigmented packaging materials. Rysstad *et al*. (1998) reported that UHT milk stored at 6°C under 36 W vertical light was very oxidized in polyethylene containers, while milk stored under the same conditions was only slightly oxidized at 8 weeks in a non-foil paper-based carton and milk stored in aluminum foil cartons had no detectable oxidation. Hexanal production in cream powders stored for 35 weeks at 30°C was reported to be strongly influenced by exposure to fluorescent light and the presence of oxygen in the headspace (Andersson and Lingnert, 1998). Similarly, the intensity of oxidized flavor was greater in ice cream stored under fluorescent light than in the dark (Suttles and Marshall, 1993). Wold *et al*. (2002) used a solid sample

fluorescence technique to quantify the degree of light-induced oxidation in sour cream, goat cream cheese and Jarlsberg cheese and noted that changes in the fluorescence spectrum of light-exposed Jarlsberg were apparent 5–6 mm into the cheese. Few studies have investigated turnover times in fluorescent display cabinets. Haisman *et al.* (1992) reported turnover times of 2–3 days for liquid milk in such cabinets. Turnover times may be longer for plastic-packaged cheeses, which often have a large surface area exposed to light. Studies on Havarti cheese have indicated that an exposure times of < 12 h led to light-induced oxidative quality changes (Mortensen *et al.*, 2002).

16.4.3. Metals

Milk and milk products contain a wide variety of metal ions, including the pro-oxidant transition metal ions, Cu^{2+} and Fe^{3+}. Metal ions capable of undergoing reversible one-electron reductions are important pro-oxidants, which function primarily by decomposing hydroperoxides to generate new reaction chains (Labuza, 1971; Pokorny, 1987). Either the oxidized or reduced metal ion can decompose hydroperoxides to allow the following catalytic cycle to increase the rate of lipid oxidation:

$$M^{n+} + ROOH \rightarrow M^{(n+1)+} + OH^- + RO^\bullet \quad (1)$$

$$M^{(n+1)+} + ROOH \rightarrow M^{n+} + H^+ + ROO^\bullet \quad (2)$$

Thus, a small quantity of an appropriate metal ion can generate large numbers of reaction chains by cycling between the oxidized and reduced forms. Although Fe and Cu in their reduced states are effective reducers of ROOH (Equation l), they are inefficient oxidizers in their higher oxidation states (Equation 2). The oxidation step might be rate-limiting in the catalytic cycle unless alternative mechanisms are available for regenerating the reduced metal (Kochi, 1973). Constituents of foods that reduce Fe^{3+} (or Cu^{2+}) may accelerate the breakdown of peroxides. Ascorbic acid, thiols and transient superoxide may provide reducing equivalents to accelerate these reactions. Interactions between metal ions and reducing groups (e.g., thiols) in milk are undoubtedly complex and may be responsible for inconsistencies and paradoxes in the literature (Yee and Shipe, 1982). Furthermore, ligands associated with transition metals can exert a profound influence on the catalytic properties of the bound metal (Cotton and Wilkinson, 1972; Hanzlik, 1976). The standard reduction potential for Fe^{3+} compared with that of Cu^{2+} suggests that Fe^{3+} is a much stronger oxidizing agent than Cu^{2+}. However, copper in milk is more pro-oxidant than iron (Haase and Dunkley, 1970; Jarrett, 1979; Rao and Murthy, 1987). This anomaly

probably reflects differences between the interactions of the two metals with other milk constituents (e.g., ascorbic acid, thiols, serine phosphate residues). The ligands associated with the metal ions also help to define their reactivities. The distribution of metals in milk and milk products is a complex function of relative solubility products and metal-ligand formation constraints which can, in turn, be influenced by processing, storage and seasonality.

Schwartz and Parks (1974) noted that awareness of the role of metal ions in the oxidation of milk fat has existed since 1905. It has long been recognized that Cu and Fe are the principal metals involved. Both these metals are normal constituents of milk but may also be present as contaminants: concentrations of Cu and Fe in U.S. milk have been reported to be highest in winter and lowest in summer (Murty *et al*., 1972). Copper is present at a level of 20–400 μg/l and Fe at a level of 100–900 μg/l (Horvat *et al*., 1965; Koops, 1969; Murty *et al*., 1972; Johnson, 1974; Jarrett, 1979). However, as noted above, Cu is the principal catalytic metal in lipid oxidation.

The endogenous copper in milk is derived *via* the bloodstream from the cow's feed (Haase and Dunkley, 1970). It is unclear to what extent the copper content of the feed influences the copper content of milk (Mulder *et al*., 1964; Riest *et al*., 1967; Dunkley *et al*., 1968a). However, the total level of endogenous copper in milk does not appear to be the key factor in spontaneous oxidation. King and Dunkley (1959b) and Samuelsson (1966) reported that oxidation may occur irrespective of copper content above a threshold value of 0.06 μg/kg.

It has been shown that 10–35% of the endogenous copper and 20–47% of the endogenous iron in milk are associated with the MFGM (King *et al*., 1959; Schwartz and Parks, 1974). However, only 2–3% of added copper and virtually no added iron become associated with the MFGM. While endogenous copper and iron in milk are complexed with proteins and are non-dialyzable at the normal pH of milk (King *et al*., 1959), added copper and iron are dialyzable to some extent, suggesting that the interaction of added metals with proteins differs from the endogenous metals. It appears that the juxtaposition of a copper–protein complex with the phospholipids of the MFGM is an important factor in the development of oxidized flavor in liquid milk (Samuelsson, 1966).

Work has also been conducted on the removal of copper from milk. Thiosuccinylated aminoethyl cellulose has been used to remove more than 90% of the copper from milk (Roh *et al*., 1976). Glass-bound trypsin has been used to inhibit metal-induced lipid oxidation (Shipe *et al*., 1972). Further work by Gregory and Shipe (1975) showed that ageing milk before exposure to a metal catalyst reduced the extent of lipid oxidation and

enhanced the apparent anti-oxidative effect of trypsin treatment. The apparent mechanism involves trypsin hydrolysis of milk serum and MFGM proteins, which increases available sites for chelating metals, particularly Cu^{2+}, into non-pro-oxidant complexes.

Enrichment of whole milk before pasteurization with ferrous iron has been reported to give rise to oxidized flavor (Edmondson *et al*., 1971). Aeration before addition of the iron reduced the effect. Kurtz *et al*. (1973), however, reported that milk powder can be fortified with iron in amounts equivalent to 20 mg/l of iron in reconstituted skim milk without development of oxidized flavor.

16.5. Antioxidants

Synthetic antioxidants are used widely in food products to inhibit the progress of lipid oxidation. However, their use in dairy products is prohibited in most countries. Experimental studies of the efficacy of antioxidants such as butylated hydroxy anisole (BHA), hydroxyquinone, dihydroquercetin and gallic acid esters in dairy products have been conducted (Sidhu *et al*., 1975, 1976). Studies on the use of antioxidants in dairy products show that their effectiveness varies in different products. While norhydroguaiaretic acid inhibits the development of oxidized flavor in liquid milk, it promotes autoxidation in milk fat (Hammond, 1970). Tocopherols are very effective inhibitors of spontaneous or copper-induced oxidation in liquid milk (Dunkley *et al*., 1967; King, 1968) but have little effect in whole milk powder (Abbot and Waite, 1965). Other antioxidants that have been shown to exert protective effects are dodecyl gallate in spray-dried whole milk (Abbot and Waite, 1962), ascorbyl palmitate in lactic butter (Koops, 1964) and propyl gallate and quercetin in butteroil (Wyatt and Day, 1965). Anhydrous bovine or buffalo milk fats (ghee) may be stabilized when stored in a hot climate by combinations of phenolic antioxidants (BHA, butylated hydroxy toluene (BHT), propyl gallate (PG)) and ascorbic acid (Helal *et al*., 1976).

Wade *et al*. (1986) reported that BHA and BHT were effective in retarding oxidation of anhydrous milk fat but DL-α-tocopherol acted as a pro-oxidant. Natural antioxidants in betel and curry leaves have also been reported to retard oxidation of anhydrous milk fat (Sharma, 1981; Parmer and Sharma, 1986). Amr (1991) reported that turmeric and wheat grits were as effective as BHA and BHT in controlling oxidative rancidity in sheep's anhydrous milk fat for up to 4 months. However, rosemary, sage, rue and fennel exerted pro-oxidant effects. Quercetin and rutin are reported to be efficient antioxidants in butter (Eriksson, 1987).

Metal-chelating agents, such as citric acid and phosphoric acid, act as synergists in conjunction with antioxidants (Badings, 1960). Salts of ethylenediaminetetraacetic acid (EDTA) have been shown to inhibit oxidation in this manner (Arrington and Krienke, 1954; King and Dunkley, 1959a).

16.5.1. Ascorbic Acid

Ascorbic acid is a very effective scavenger of alkoxy radicals and hence is an effective antioxidant (Niki, 1991; Frankel, 1998). However, under certain circumstances, ascorbic acid exerts a pro-oxidant effect. Andersson and Oste (1994) reported that ascorbic acid concentration in unpasteurized milk was highest in March or August (20–27 mg/l) and lowest in October (12 mg/l). Vitamin C level typically decreases during storage and following heating of milk (Korhonen and Korpela, 1994). Concentrations of ascorbic acid above those in normal milk (approximately 20 mg/l) provide antioxidant protection; however, at the concentrations in milk, ascorbic acid acts as a pro-oxidant. Olson and Brown (1942) showed that ascorbic acid was crucial to the development of oxidized flavor in cream. They reported that cream washed free of ascorbic acid did not develop oxidized flavor when contaminated with copper and stored for 3 days. They postulated that ascorbic acid reduces Cu^{2+} to Cu^{+}, which in turn reduces molecular oxygen to hydrogen peroxide that oxidizes lipids in the MFGM. Pont (1952) reported that addition of ascorbic acid to washed cream, even in the absence of added copper, promoted the development of an oxidized flavor. Krukovsky and Guthrie (1945) and Krukovsky (1955, 1961) showed that added copper did not promote oxidation in milk or butter depleted of ascorbic acid and that oxidation of ascorbic acid-free milk could be initiated by addition of ascorbic acid. In a series of papers (King and Dunkley, 1959a; Smith and Dunkley, 1962a,b,c; Haase and Dunkley, 1969a,b,c) that involved addition of copper ions to milk and model systems, judicious application of specific chelators for copper ions, addition or specific destruction of ascorbic acid in milk and model systems, and the use of reducing agents other than ascorbic acid, the primary function of copper and ascorbic acid in catalyzing lipid oxidation was carefully documented. A specific association between copper and ascorbic acid as the ultimate pro-oxidant was proposed.

However, while some reports (Schwartz and Parks, 1974) indicate a correlation between the oxidation of ascorbic acid and the development of an oxidized lipid flavor, Smith and Dunkley (1962c) concluded that the oxidation of ascorbic acid alone cannot be used as an index of lipid oxidation. They reported that although ascorbic acid oxidation curves for homogenized and pasteurized milk were similar, the homogenized samples had a significantly lower tendency to develop oxidized flavor.

Several workers have shown that a high concentration of ascorbic acid added to liquid milk inhibits oxidation. Chilson (1935) suggested that added ascorbic acid acts as a reducing agent, which is oxidized more readily than milk fat. Bell *et al.* (1962) suggested that addition of L-ascorbic acid to cream produced a medium less conducive to oxidation by lowering the oxidation-reduction potential. Addition of an adequate level of surface-active ascorbyl palmitate to milk products may retard lipid oxidation by orientation at the lipid-aqueous interface where it intercepts free radicals (Badings and Neeter, 1980).

16.5.2. Tocopherols

Vitamin E consists of eight vitamers of which α-tocopherol is the principal one in bovine milk (Lindmark-Mansson and Akesson, 2000). α-Tocopherol acts as a free-radical scavenger. The tocopheryloxy radical formed is relatively stable and can be reconverted to tocopherol by reduction with ascorbic acid. Tocopherols generally act as antioxidants in lipids (Kamaleldin and Appelqvist, 1996). At high concentrations, they may exert a pro-oxidant effect (Hamzawi, 1990), but this is highly unlikely to occur in milk. Milk fat contains approximately 20 μg α-tocopherol/g (Erickson and Dunkley, 1964; Kanno *et al.*, 1968; Bruhn and Franke, 1971; Jensen, 1995). Tocopherol concentrations are at least three-fold higher in lipids of the MFGM than in the core of the fat globule (Erickson *et al.*, 1963). During storage of cream containing added Cu^{2+} and ascorbic acid, total destruction of tocopherols in the MFGM was observed compared with 30% destruction in the butteroil due to the proximity of tocopherols in the MFGM to pro-oxidants and highly oxidizable phospholipids.

The principal factor that influences the α-tocopherol content of milk is the feed of the cow, as influenced by the season of the year. Kanno *et al.* (1968) reported that summer milk produced on green pasture feed averaged 33.8 μg α-tocopherol/g fat, while winter milk produced on dry-lot feeding averaged 21.6 μg α-tocopherol/g fat. Similar findings have been reported by King *et al.* (1967) and Seerless and Armstrong (1970).

The feasibility of increasing the α-tocopherol concentration of milk by supplementation of the feed has been investigated in many studies (Dunkley *et al.*, 1966, 1967; King *et al.*, 1966; St. Laurent *et al.*, 1990; Barrefors *et al.*, 1995; Focant *et al.*, 1998; Granelli *et al.*, 1998). These studies showed that when feed was supplemented with varying levels of α-tocopheryl acetate, the α-tocopherol content of the milk was increased with consequent increased resistance to spontaneous and copper-induced oxidation. King *et al.* (1967) reported that when feed was supplemented to achieve an intake of 1 g α-tocopherol per day per cow, oxidation was effectively controlled in milk

contaminated with 0.1 μg/kg copper. However, other studies have shown no beneficial effect of supplementing the diet with vitamin E (Schingoethe *et al.*, 1979; Charmley and Nicholson, 1995).

Only about 2% of ingested α-tocopherol is actually transferred to the milk (King *et al.*, 1966; Dunkley *et al.*, 1968b; Schingoethe *et al.*, 1979). Consequently, the economics of direct supplementation of feed with α-tocopherol are unfavourable (Bruhn *et al.*, 1976). If protected supplements are fed, however, the potential for transfer to milk is much greater. Goering *et al.* (1976) fed protected safflower supplement to cows and reported a 200% increase in the α-tocopherol content of the milk. Control of oxidized flavor by direct addition of emulsified α-tocopherol to milk can be achieved with only 1% of the amount required by ration supplementation (Weihrauch, 1988).

A significant correlation exists between the α-tocopherol content of milk fat and oxidative stability (Krukovsky *et al.*, 1950). Tocopherol concentration in MFGM lipids shows a closer correlation to oxidative stability than the tocopherol content of butteroil (Erickson *et al.*, 1963). A direct relationship has been observed between tocopherol concentration and the level of copper that can be tolerated by milk (King *et al.*, 1966).

Tocopherols have been reported to act as free-radical scavengers (Terao *et al.*, 1980) but have also been shown to quench $^1O_2$ *via* a charge-transfer quenching mechanism (Yamauchi and Matsushita, 1977; Burton and Ingold, 1981). The ratio of $^1O_2$ quenching rates of α-, γ- and δ-tocopherols were found to be 100:69:38. Each tocopherol molecule can deactivate about 120 molecules of $^1O_2$ before it is destroyed (Zweig and Henderson, 1975).

16.5.3. Carotenoids

Molecules containing conjugated double bonds are oxidized more rapidly than those with the same number of nonconjugated double bonds. Oxidation of β-carotene is very complex and may take several routes. Generally, conjugated double-bond systems favor radical addition rather than abstraction reactions (Scott, 1965). Addition of a methyl radical to conjugated double bonds is very rapid compared with abstraction reactions (Pryor *et al.*, 1972). Carotenoids undergo radical addition reactions leading to bleaching and a variety of compounds characteristic of lipid oxidation in general (Teixeira Neto *et al.*, 1981). However, very electrophilic oxidizing free radicals may abstract an electron from β-carotene to yield a radical β-carotene cation. Epoxides are readily formed at the double bond in the β-ionone ring and such epoxidized carotenoids are commonly found in naturally-occurring pigments (McCormick *et al.*, 1978). Novel oxidation products containing the β-ionone moiety have been implicated in off-flavor

development in dairy products. Oxidation of vitamin A should follow the same patterns as β-carotene. Although the conjugated diene in vitamin D reacts readily with photogenerated $^1O_2$ to form endoperoxides, little is known about the oxidation of vitamins D and K in milk and milk products.

The importance of $^1O_2$ as an initiator of oxidation has increased interest in the prevention of singlet oxygen reactions by quenching to ground-state oxygen ($^3O_2$). Food constituents such as carotenoids, tocopherols and ascorbic acid have been reported to exert this effect (Carlsson *et al.*, 1976; Krinsky, 1979; Matsushita and Terao, 1980; Fakourelis *et al.*, 1987; Warner and Frakel, 1987; Jung and Min, 1991; Mortensen *et al.*, 2001). Quenchers must either be capable of accepting energy from the $^1O_2$ molecule that lies 22.4 kcals above the ground state (energy-transfer quenching) or have the ability to donate electrons to $^1O_2$ (charge-transfer quenching). Quenching of $^1O_2$ by β-carotene is an example of energy-transfer quenching, whereas tocopherols, amines and phenols have been shown to exert a charge-transfer quenching mechanism (Bradley and Min, 1992).

Energy transfer from $^1O_2$ to β-carotene leads to the formation of ground-state oxygen and an excited triplet-state quencher (Seeley and Meyer, 1971; Foote *et al.*, 1974). β-Carotene is known to be one of the most potent quenchers of $^1O_2$, with one molecule estimated to quench 250–1000 molecules of $^1O_2$ (Foote and Denny, 1968; Foote, 1976). The rate of quenching is influenced by the number of conjugated double bonds present. Carotenoids with nine or more conjugated double bonds are efficient quenchers, whereas those with seven or less are not capable of accepting energy from $^1O_2$.β-Carotene has been shown to inhibit chlorophyll-sensitized photo-oxidation of methyl linoleate (Terao *et al.*, 1980). Using a model dairy spread (water-in-oil emulsion), Hansen and Skibsted (2000) showed that β-carotene provided good protection against light-induced lipid oxidation and riboflavin degradation. The protection of the riboflavin was due to the competing absorption of the light by the carotene. Outside the absorption band of the carotene (i.e., < 366 nm), this protective effect disappeared. This indicated that the protective mechanism of the carotene in this case was an absorption (filter) effect of the incident light and not a quenching effect of radical or singlet oxygen.

16.5.4. Thiols

Pasteurization of milk increases its susceptibility to spontaneous (Bergman *et al.*, 1962), copper-induced (Smith and Dunkley, 1962a) and photo-induced (Finley, 1968) oxidation. Postulated explanations generally implicate migration of copper to the cream phase of milk (Sargent and Stine, 1964).

However, an inhibitory effect of high heat treatment on the oxidative deterioration of milk and milk products has been reported by many investigators who have attributed this effect to the activation of thiol groups (Josephson and Doan, 1939; Tamsma *et al*., 1962; Wilson and Herreid, 1969; Schwartz and Parks, 1974; Baldwin and Ackland, 1991; Saidi and Warthesen, 1995; Tong *et al*., 2000). The principal sources of thiols in milk are the fat globule membrane (McPherson and Kitchen, 1983) and the serum proteins, particularly β-lactoglobulin (Larsson and Jenness, 1950; Schwartz and Parks, 1974).

While, historically, thiols resulting from heat treatment of milk have been considered as performing an antioxidant function in milk as univalent reducing agents, peroxide decomposers or as metal ligands, it is also possible that they may exert a pro-oxidant role. Univalent autoxidation of thiol yields thiyl radicals, superoxide and hydrogen peroxide may provide a basis for a pro-oxidant role (Yee and Shipe, 1982). These workers proposed that copper-catalyzed oxidation of thiol groups may generate pro-oxidants in milk. Autoxidation of thiols is known to generate substantial amounts of superoxide anion (Misra, 1974). Yee and Shipe (1982) concluded that thiol groups in milk may be pro-oxidant or antioxidant, depending upon the conditions. Free thiol groups in the presence of copper promoted the oxidation of emulsified methyl linoleate in their model system, whereas free thiol groups in the presence of haem behaved as antioxidants.

Sulphydryl oxidase, an indigenous milk enzyme, has been proposed for the oxidation of thiols in UHT milk to reduce cooked flavor and also thereby to serve as an antioxidant, in conjunction with lactoperoxidase (to destroy the resultant H_2O_2), by obviating pro-oxidants resulting from autoxidation of thiols (Swaisgood and Abraham, 1980).

Stapelfeldt *et al*. (1997a) determined the oxidative stability of high-heat, medium-heat and low-heat whole milk powder under different storage conditions. The sensory quality dropped to an unacceptable level for low-heat powder after 33 days and was paralleled by a decrease of "free" thiol groups to an unmeasurable level. In contrast, medium-heat and high-heat powders retained good sensory quality and the initial level of free thiol groups was reduced by only one-third after 63 days of storage.

16.5.5. Proteins and Enzymes

Caseins possess significant antioxidant activity, which may be related, in part, to their hydrophobic nature (El-Negoumy, 1965; Taylor and Richardson, 1980b; Allen and Wrieden, 1982a; Ericksson, 1982) and orientation of potential antioxidant side-chains of constituent amino acids at the lipid interface. Brunner (1974) reported retardation of lipid oxidation in

homogenized milk when milk fat globules are resurfaced with casein. Caseins can also bind metals to phosphoseryl residues (Manson and Cannon 1978; Hegenauer *et al.*, 1979a,b). Auklakh and Stine (1971) reported that sodium α_{s1}- and β-caseinates bound 2 mol Cu^{2+} per mol protein. The association of peroxidizing lipids with proteins can be especially damaging to the physicochemical properties of the protein (Schaich, 1980). The major whey proteins are considerably less effective as antioxidants than the caseins (Taylor and Richardson, 1980b; Allen and Wrieden, 1982a). Lactoferrin has been shown to inhibit peroxidation induced by Fe^{2+}, presumably by binding Fe^{2+} (Gutteridge *et al.*, 1981; Allen and Wrieden, 1982b). Binding of iron to lactoferrin may decrease the conversion of hydrogen peroxide into hydroxyl radical *via* the Fenton-type reaction (Lindmark-Mansson and Akesson, 2000). Bovine lactoferrin has been reported to inhibit oxidation of ascorbic acid and tryptophan (Bihel and Birlouez-Aragon, 1998).

Lactoperoxidase was strongly pro-oxidant in the presence or absence of added Cu^{2+} or Fe^{3+} in a trilinolein emulsion model (Allen and Wrieden, 1982a) or in high linoleate milk (Hill, 1979). Pasteurization at 72°C/15–20 s had little effect, but following heating at 80°C for 15–20 s, lipid oxidation was greatly reduced. Hill (1979) and Allen and Wrieden (1982a) also showed that superoxide dismutase and catalase exert a strong antioxidant effect when added in their model systems. However, addition of Cu^{2+} (10 μM) with superoxide dismutase to the emulsion was pro-oxidant and might compete with the enzyme for $O_2^{\bullet}$ to convert it to pro-oxidant species such as $OH^{\bullet}$ (Allen and Wrieden, 1982b). Superoxide dismutase has been detected in and isolated from milk (Hill, 1975; Asada, 1976; Korycka-Dahl *et al.*, 1979), but it is apparently present at insufficient levels to provide substantial antioxidant protection (Holbrook and Hicks, 1978; Fox and Morrissey, 1981). However, the observed inhibition by superoxide dismutase of lipid oxidation catalyzed by Xanthine oxidoreductase (Holbrook and Hicks, 1978) may have confounded the hypothesis of Smith and Dunkley (1960) on the importance of Xanthine oxidoreductase in spontaneous oxidation of milk lipids (Section 8.3.1). However, Holbrook and Hicks (1978) were unable to correlate spontaneous oxidation of milk with its content of superoxide dismutase.

Amino acids have been reported to act as antioxidants, pro-oxidants and/or to have no effect on lipid oxidation (Farag *et al.*, 1978; Taylor and Richardson, 1980a). Antioxidant effects have been attributed to: (1) their primary functional groups; (2) chelation of pro-oxidant metals; (3) regeneration of primary antioxidants; or (4) synergism with other food constituents (Chen and Nawar, 1991b). Faraget *et al.* (1978) suggested that protonated amino groups accelerate lipid oxidation while non-protonated amino groups

inhibit oxidation. The antioxidant/pro-oxidant effects of amino acids in emulsion systems may be pH dependent (Riisom *et al*., 1980). Pro-oxidant activity is enhanced at lower pH.

Chen and Nawar (1991b) examined the effects of amino acids and amino acid analogues on fat oxidation in milk. All the amino acids tested (cysteine, tryptophan, lysine, alanine, serine, histidine and tyrosine) significantly prolonged the induction period of lipid oxidation, with cysteine, tryptophan and lysine showing the most pronounced effects. Comparison of the effects of amino acids with structurally similar analogues, in which the amino group was absent or blocked, indicated that the primary amino group plays a major role in the inhibitory activity of amino acids. An amino group on the side chain of an amino acid also exerted an antioxidant effect, although to a lesser degree than that of α-amino groups. The indolyl group of L-tryptophan also exerted a strong antioxidant effect.

16.5.6. Products of Browning Reactions

Carbonyl-amine reactions, such as those between lactose and milk proteins, have been reported to produce potent antioxidants (Dugan, 1980; Eichner, 1980; Ericksson, 1982). Browning reaction products can stabilize milk fat considerably (Wyatt and Day, 1965). However, it is important to note that browning reaction products may exert adverse nutritional and toxicological effects (O'Brien and Morrissey, 1989).

The effect of browning compounds on the auto-oxidative stability of ghee has been reported by Nath and Murthy (1988). Browning compounds were prepared by heating leucine and dicarbonyls (either dihydroxyacetone, methyl glyoxal or glyoxal). All three types of browning oil, when added, afforded protection against autoxidation of ghee heated to 120°C for 5 min. However, BHT (0.02%) was a more powerful antioxidant than the most effective dicarbonyl/amino acid combination (dihydroxyacetone/leucine).

Lingnert *et al*. (1983) reported that addition of 0.3% of the Maillard products of histidine and glucose to milk powder and storage under nitrogen gave the reconstituted milk both a low initial intensity of "cardboard" flavor and almost total inhibition of its further development after reconstitution.

Calligaris *et al*. (2004) studied changes in antioxidant and pro-oxidant activity in milk subjected to different heat treatments. Their results indicated that short heat treatments can be potentially responsible for a depletion in the overall antioxidant properties of milk. Only the application of severe heat treatments, associated with the formation of brown melanoidins, allowed a recovery and even a possible increase in the antioxidant properties of milk.

16.6. Milk Fat Globule Membrane (MFGM)

Fat in milk exists primarily in the form of globules surrounded by a complex membrane, which contains a mixture of unsaturated phospholipids, proteins, glycoproteins and other minor components (Mulder and Walstra, 1974; Keenan *et al*., 1983; see Chapter 4; Keenan and Dylewski, 1995). The proximity of unsaturated phospholipids to various pro-oxidants in the lipoprotein matrix makes the MFGM a focal point for the oxidation of milk lipids (Mulder and Walstra, 1974; Bouzas *et al*., 1985). O'Mahony and Shipe (1970) found that the concentration of phosphatidylethanolamine (PE) was lowest in milk classified as least susceptible to copper-induced oxidation. PE is known to bind Cu^{2+} strongly (Morita and Fujimaki, 1972). Reconstituted milk was reported to be more stable to oxidation than regular milk due to removal of membrane material (Krukovsky, 1952). The rate of copper-catalyzed oxidation of cream containing MFGM was faster than when the membrane was removed. Ascorbic acid (10 mg/kg) accelerated the oxidation of cream under these conditions (Chen and Nawar, 1991a). A decrease in the relative concentration of membrane phospholipids and copper was proposed as the mechanism by which the development of oxidized and "tallowy" flavor in homogenized milk was inhibited (Tarassuk and Koops, 1960). Rapid oxidation of isolated MFGM was demonstrated by King (1962, 1963). Oxidation in isolated MFGM was influenced by Cu and ascorbic acid concentration. Once oxidation is initiated in the MFGM, diffusion of the propagating chain reaction radicals into the more saturated fat globule core from the fat-plasma interface results in generalized oxidation of milk fat triglycerides.

About one-third of the phospholipids in freshly drawn milk are located in the milk serum as small lipoprotein particles, sometimes referred to as "milk microsomes." Their proportion in milk serum can be increased in processed milk as a result of disruption of the MFGM and release of membrane phospholipids into the aqueous phase (Mulder and Walstra, 1974; McPherson and Kitchen, 1983). Modification of the MFGM by processing treatments that may alter the distribution of pro-oxidants and antioxidants can markedly affect the stability of milk (McPherson and Kitchen, 1983).

Xanthine oxidoreductase, a metalloprotein abundant in the MFGM, may also be partially responsible for the susceptibility of the membrane to lipid oxidation (Allen and Humphries, 1977; Aurand *et al*., 1977; Bruder *et al*., 1982; Bouzas *et al*., 1985). Allen and Humphries (1977) prepared two protein fractions from MFGM and found that oxidative activity resided almost entirely in the first fraction, devoid of phospholipids, but richer in Xanthine oxidoreductase. They proposed that the metalloprotein, and not

phospholipids, was probably responsible for the inherent oxidative capability of the membrane material.

The mechanism(s) by which Xanthine oxidoreductase exerts its pro-oxidant effect(s) is not fully understood. Hydrogen peroxide, resulting from oxidation of a suitable substrate by Xanthine oxidoreductase, could oxidize milk lipids. However, normal milk contains little or no substrate for the enzyme. A possible mechanism involving interaction between native and denatured Xanthine oxidoreductase in MFGM and lactoperoxidase or copper in milk serum has been proposed (Hill, 1979; Allen and Wreiden, 1982b).

Hill (1979) used milk containing up to 35% (w/w) linoleic acid in the milk fat to investigate the mechanism of action of Xanthine oxidoreductase. He proposed a coupled enzyme system in which lactoperoxidase catalyses lipid oxidation, generating aldehyde substrates for Xanthine oxidoreductase that in turn generates H_2O_2 for use by lactoperoxidase as an oxidizing agent. Hill (1979) reported that milk rich in linoleic acid was much more oxidatively stable when pasteurized at 80°C/15 s than after pasteurization at 72°C/15 s. The increased stability was attributed to thermal inactivation of the oxidative enzymes at the higher temperature since after pasteurization of the milk at 80°C/15 s, rapid development of oxidized flavor occurred if Xanthine oxidoreductase or lactoperoxidase was added. When substrate for Xanthine oxidoreductase was added also, oxidative processes were accelerated. Addition of small amounts (1 mg/l) of superoxidase dismutase and catalase improved the oxidative stability of this milk, indicating that $O_2^{\bullet}$ is involved in the oxidative process. However, oxidation of milk lipids after addition of 0.1 mg/kg Cu^{2+} was not inhibited by superoxide dismutase and catalase. When formate (an $^{\bullet}OH$ scavenger) was added to the milk, lipid oxidation was inhibited, suggesting that $^{\bullet}OH$ was the active pro-oxidant. Hill (1979) postulated that two major systems in milk catalyze lipid oxidation:

1. Generation of $^{\bullet}OH$ by copper-ascorbic acid;
2. Generation of $O_2^{\bullet}$ and $^1O_2$ by Xanthine oxidoreductase-lactoperoxidase.

Allen and Wrieden (1982b) used a trilinolein model system to confirm the strong pro-oxidant role of lactoperoxidase which was retarded by heating at 80°C/20 s. They proposed that, in addition to its enzymatic effects, lactoperoxidase was pro-oxidant by virtue of generalized haem catalysis. In the presence of 10 μM added Cu^{2+}, Xanthine oxidoreductase rapidly oxidized trilinolein in the absence of a substrate for Xanthine oxidoreductase (Allen and Wrieden, 1982b). They suggested that the added Cu^{2+} inactivated the Xanthine oxidoreductase and that any $O_2^{\bullet}$ produced could be converted to very strong oxidizing species by the Cu^{2+} bound to the inactive enzyme. The FAD associated with Xanthine oxidoreductase may also act as a photosensitizer in the production of $O_2^{\bullet}$ (Korycka-Dahl and Richardson, 1978). The potentially longer lifetime of

$O_2^{\bullet}$ in the membrane lipids in association with bound Cu^{2+} could make these reactions of some significance in lipid oxidation.

Several workers have reported low concentrations of cytochromes in the MFGM (Bailie and Morton, 1958a,b; Plantz *et al.*, 1973; Gregory *et al.*, 1976; Bernstein, 1977; Jarasch *et al.*, 1977). Ferri-porphyrin proteins have been shown to be powerful pro-oxidants (Kendrick and Watts, 1969). Furthermore, their proximity to unsaturated phospholipids in the MFGM and the longer life-times of active oxygen species in a nonpolar environment, suggest a role for cytochromes in the oxidation of MFGM lipids. Bernstein (1977) reported a b_5-type cytochrome and a carbon monoxide-binding cytochrome in MFGM. The CO-binding cytochrome promoted lipid oxidation to the same extent as hemoglobin, whereas the native b_5-type cytochrome was inactive. Thermal processing of milk may expose or mask ferri-porphyrin groups in MFGM and, hence, may influence lipid oxidation.

16.7. Storage Temperature

The effect of storage temperature on the oxidative stability of milk and milk products is unclear. Storage, in air, at 2°C inhibited the development of oxidized flavor in dry whole milk when compared with control samples held at 38°C (Pyenson and Tracy, 1946). Oxidative deterioration of UHT cream occurred two to three times more rapidly at 18°C than at 10°C, while little or no oxidation occurred at 4°C (Downey, 1969). The oxidation–reduction potential of butter and the rate of flavor deterioration have been reported to increase as the storage temperature increased (Weihrauch, 1988).

In a study on butteroil held at a temperature ranging from −10 to +50°C, oxidation rate increased with increasing temperature but the same flavor was formed on storage and the reaction sequence for flavor formation was similar at all temperatures (Hamm *et al.*, 1968). Dunkley and Franke (1967) reported a decrease in flavor intensity and thiobarbituric acid (TBA) values in liquid milk as storage temperature was increased from 0 to 4 to 8°C. Schwartz and Parks (1974) reported that condensed milk stored at −17°C was more susceptible to oxidized flavor development than at −7°C.

Kristensen (2001) reported that increasing the storage temperature from 5 to 37°C for processed cheese resulted in significantly enhanced oxidation which was apparent after a few days of light-exposed storage.

16.8. Water Activity

Labuza (1971) has described the complex relationship between water activity (a_w) and lipid oxidation, with a minimum observed at intermediate a_w (~0.4)

levels and an increased rate of oxidation at either very low or high a_w. At an a_w values >0.8, the rate of lipid oxidation decreases. Labuza (1971), Karel (1980) and Schaich (1980) explained these complex relationships, suggesting that at very low a_w, lipid oxidation is favored because a water monolayer is not available to mask pro-oxidants or to retard the decomposition of hydroperoxides by hydrogen bonding. As the amount of water is increased to form a monolayer, pro-oxidants may be masked *via* hydration and the monolayer serves as a barrier to oxidation. Higher a_w promotes lipid oxidation by mobilizing pro-oxidants and facilitating their diffusion through the food. However, very high a_w values retard oxidation by diluting the reactants.

In contrast to the hypothesis of Labuza (1971), Loncin *et al.* (1968) reported that autoxidation of milk powder was stimulated by water activity below 0.11 and unaffected by water activities between this value and 0.75. Stapelfeldt *et al.* (1977a) investigated the oxidative stability of whole milk powder at water activities of 0.11, 0.23 and 0.33 at 25°C and 0.11, 0.17 and 0.31 at 45°C for 2 months of storage. As one would expect, lipid oxidation was affected greatly by higher storage temperature. In contrast to the generalization of Labuza (1971), least oxidation was observed at a water activity between 0.11 and 0.23 and most oxidation at higher water activities (0.31 at 45°C).

16.9. Measurement of Lipid Oxidation

Routine procedures to assay the extent of oxidation in lipids and lipid-containing foods should be simple, reliable and sensitive. Results from routine procedures should ideally correlate well with results obtained from sensory taste panels. St. Angelo (1996) has described volatile compound profiles formed during lipid oxidation in different groups of food products. However, because of the complexity of lipid oxidation, no single test can be equally useful at all stages of the oxidative process. The methods should be capable of detecting autoxidation before the onset of off-flavor. This is particularly true in the case of milk products where a low level of oxidation can lead to off-flavor.

Measurement of hydroperoxides is the classical method for quantifying lipid oxidation and a variety of assay procedures are available. The oxidation of ferrous to ferric iron by hydroperoxides in the presence of ammonium thiocyanate to produce ferric thiocyanate, which can be quantified spectrophotometrically at 505 nm, has been used extensively to study lipid oxidation in milk (Loftus-Hills and Thiel, 1946). Newstead and Headifen (1981) recommend that extraction of fat from whole milk powder be carried out in the dark when using this procedure to avoid artefactually high

peroxide values. A second assay procedure for hydroperoxides is based on the reaction of oxidized fat with 1,5-diphenyl-carbohydrazide to yield a red-colored product (Hamm *et al.*, 1965). A third procedure is based upon the liberation of iodine from potassium iodide by hydroperoxides (AOCS, 1971). A standardized protocol exists for peroxide value determination in butterfat (IDF, 1991). A major caveat with procedures based on the direct or indirect determination of hydroperoxides is that they may not correlate well with the level of off-flavor in the product, particularly when the oxidative process is at an advanced stage (Kliman *et al.*, 1962). During the course of oxidation, peroxide values reach a peak and then decline. However, the early stage of lipid oxidation in dairy products can be followed by HPLC analysis of hydroperoxides or the peroxide value (Emmons *et al.*, 1986).

Procedures involving thiobarbituric acid (TBA) as an analytical reagent have also been used widely to follow the progress of autoxidation in dairy products (Dunkley and Jennings, 1951; King, 1962). Both procedures are based on the condensation of two molecules of TBA with one molecule of the oxidation end-product, malonaldehyde, resulting in the formation of a red-colored complex which can be determined spectrophotometrically at 532 nm. However, compounds other than malonaldehyde may react with this reagent to give artefactually high results (Slater, 1984). The appropriateness of the TBA assay for milk has been questioned (Ward, 1985). High-performance liquid chromatographic procedures are now available to determine malonaldehyde directly (Madere and Behrens, 1992). A gas chromatographic procedure is also available (Frankel and Neff, 1983).

King (1962) showed that the TBA method correlates well with the intensity of oxidized flavor in liquid milk. Downey (1969) suggested that the TBA procedure of Dunkley and Jennings (1951) is more applicable than that of King (1962) for determining the extent of the off-flavor.

Other traditional methods available for monitoring the extent of lipid oxidation include the Anisidine value, the Kreis test (Mehlenbacher, 1960), methods based on the carbonyl content of oxidized fats (Henick *et al.*, 1954; Lillard and Day, 1961), and measurement of oxygen uptake either by manometry or polarography (Tappel, 1955; Hamilton and Tappel, 1963).

In recent years, modern instrumental methods have been developed to monitor lipid oxidation in biological samples, including dairy products. These include use of electron spin resonance (ESR) spectrometry, direct measurement of secondary oxidative products such as malonaldehyde, static and dynamic GC/MS methods. ESR spectrometry permits detection of free radicals formed in the very early stages of oxidation prior to the formation of peroxides. The method has been applied successfully to dairy products such as milk powders and processed cheese (Nielsen *et al.*, 1997; Stapelfeldt

et al., 1997a,b; Kristensen and Skibsted, 1999). Data indicate that the method correlates well with the TBA method and with sensory evaluation of oxidation in these dairy products. The method may have potential for application in quality control and accelerated testing of dairy products for oxidative stability.

The traditional TBA and anisidine value tests can be replaced by a qualitative or quantitative analysis of oxidation-derived volatiles using headspace GC/MS methodology or the so-called "electronic nose" apparatus. These techniques have been applied successfully to a variety of dairy products (Lee *et al*., 1991; Park and Goins, 1992; Ulberth and Roubicek, 1995; Christensen and Holmer, 1996; Kim and Morr, 1996). For example, Kim and Morr (1996) reported that exposure of milk to fluorescent light for 48 h and subjected to dynamic headspace analysis resulted in the identification of five major volatile compounds: 2-butanone, 2-propanol, pentanal, dimethyl disulfide and hexanal. The classes of volatiles recovered were remarkably similar to those reported by Dimick (1982). Results of dynamic headspace analysis correlated well with sensory panel results. Frankel (1993) has stated that headspace analysis of volatile oxidation products by gas chromatography gives better information about the origin of flavor and odor volatiles than traditional chemical analyses and is the best method for comparison with sensory panel results.

Bibliography

Adhikari, A.K., Singhal, O.P. 1992. Effect of dissolved oxygen content on the flavor profile of UHT milk during storage. *Aust. J. Dairy Technol*. **47**, 1–6.

Allen, C., Parks, O.W. 1975. Evidence for methional in skim milk exposed to sunlight. *J. Dairy Sci*. **58**, 1609–1611.

Allen, J.C., Humphries, C. 1977. The oxidation of lipids by components of bovine milk-fat globule membrane. *J. Dairy Res*. **44**, 495–507.

Allen, J.C., Wrieden, W.L. 1982a. Influence of milk proteins on lipid oxidation in aqueous emulsion. I. Casein, whey protein and α-lactalbumin. *J. Dairy Res*. **42**, 239–248.

Allen, J.C., Wrieden, W.L. 1982b. Influence of milk proteins on lipid oxidation in aqueous emulsion. II. Lactoperoxidase, lactoferrin, superoxide dismutase and Xanthine oxidoreductase. *J. Dairy Res*. **42**, 249–263.

Amr, A.S. 1991. Effectiveness of synthetic and potential natural antioxidants in improving the stability of sheep's anhydrous butterfat during long-term storage. *J. Sci. Food Agric*. **55**, 75–85.

Andersson, I., Oste, R. 1994. Nutritional quality of pasteurized milk. Vitamin B12, folacin and ascorbic acid content during storage. *Int. Dairy J*. **4**, 161–172.

Andersson, K., Lingnert, H. 1998. Influence of oxygen concentration and light on the oxidative stability of cream powder. *Lebensm. Wiss. Technol*. **31**, 169–176.

AOCS. 1971. *Official and Tentative Methods. American Oil Chemists' Society*, Chicago, IL.

Arrington, L.R., Krienke, W.A. 1954. Inhibition of the oxidized flavor of milk with chelating compounds. *J. Dairy Sci*. **37**, 819–824.

Asada, K. 1976. Occurrence of superoxide dismutase in bovine milk. *Agric. Biol. Chem.* **40**, 1659–1660.

Astrup, H.N. 1963. Oxidized flavor in milk and the Xanthine oxidoreductase inhibitor. *J. Dairy Sci.* **46**, 1425.

Aulakh, J.S., Stine, C.M. 1971. Binding of copper by certain milk proteins as measured by equilibrium dialysis. *J. Dairy Sci.* **54**, 1605–1608.

Aurand, L.W., Woods, A.E. 1959. Role of Xanthine oxidoreductase in the development of spontaneously oxidized flavor in milk. *J. Dairy Sci.* **42**, 1111–1118.

Aurand, L.W., Boone, N.H., Giddings, G.G. 1977. Superoxide and singlet oxygen in milk lipid peroxidation. *J. Dairy Sci.* **60**, 363–369.

Aurand, L.W., Chu, T.M., Singleton, J.A., Shen, R. 1967. Xanthine oxidoreductase activity and development of spontaneously oxidized flavor in milk. *J. Dairy Sci.* **50**, 465–471.

Badings, H.T. 1960. Principles of auto-oxidation processes in lipids with special regard to the development of auto-oxidation off-flavor. *Neth. Milk Dairy J.* **14**, 215–242.

Badings, H.T., Neeter, R. 1980. Recent advances in the study of aroma compounds of milk and dairy products. *Neth. Milk Dairy J.* **34**, 9–30.

Bailie, M.J., Morton, R.K. 1958a. Comparative properties of microsomes from cow's milk and from mammary gland. I. Enzymic activities. *Biochem. J.* **69**, 35–44.

Bailie, M.J., Morton, R.K. 1958b. Comparative properties of microsomes from cow's milk and from mammary gland. II. Chemical composition. *Biochem. J.* **69**, 44–51.

Baldwin, A.J., Ackland, J.D. 1991. Effect of preheat treatment and storage on the properties of whole milk powder. *Neth. Milk Dairy J.* **45**, 169–181.

Barrefors, P., Granelli, K., Appelqvist, L.A., Bjoerck, L. 1995. Chemical characterization of raw milk samples with and without oxidative off-flavor. *J. Dairy Sci.* **78**, 2691–2699.

Bartholomew, B.P., Ogden, L.V. 1990. Effect of emulsifiers and fortification methods on light stability of vitamin A in milk. *J. Dairy Sci.* **73**, 1485–1488.

Bassett, R., Keeney, M. 1960. Identification of some volatile carbonyl compounds from nonfat dry milk. *J. Dairy Sci.* **43**, 1744–1750.

Bassett, R., Fung, D.Y.C., Mantha, V.R. 1986. Off-flavors in milk. *CRC Crit. Rev. Food Sci. Nutr.* **24**, 1–52.

Bateman, L., Hughes, H., Morris, A.L. 1953. Hydroperoxide decomposition in relation to the initiation of radical chain reactions. *Disc. Faraday Soc.* **14**, 190–199.

Begeman, P.H., Koster, J.C. 1964. Components of butterfat, 4-*cis*-hepenal: a cream-flavored component of butter. *Nature* **202**, 552–553.

Bakbolet, M. 1990. Light effects on food. *J. Food Prot.* **53**, 430–440.

Belitz, H.D., Grosch, W. 1999. *Food Chemistry*, 2nd edn, Springer, Berlin.

Bell, R.W., Anderson, H.A., Tittsler, R.P. 1962. Effect of L-ascorbic acid on the flavor stability of concentrated sweetened cream. *J. Dairy Sci.* **45**, 1019–1020.

Bergman, T., Beetelsen, E., Berglof, A., Larsson, S. 1962. The occurrence of flavor defects in milk exposed to cold storage prior to pasteurization. *Proc. Int. Dairy Congr.* (Copenhagen) **A**, 579–588.

Berliner, L.J., Ogata, T. 1997. Riboflavin-sensitized singlet oxygen formation in milk. In: *Food and Free Radicals* (M. Hiramatsu, T. Yoshikawa, M. Inoue, eds.), pp. 119–122, Plenum Press, New York.

Bernstein, B.A. 1977. *Cytochromes in the Fat Globule Membrane of Bovine Milk and in the Microsomes of the Lactating Bovine Mammary Gland*. PhD thesis, University of Wisconsin, Madison.

Bihel, S., Birlouez-Aragon, I. 1998. Inhibition of tryptophan oxidation in the presence of iron-vitamin C by bovine lactoferrin. *Int. Dairy J.* **8**, 637–641.

Bolland, J.L., Gee, G. 1946. Kinetic studies in the chemistry of rubber and related materials. *Trans. Faraday Soc*. **42**, 236–252.

Borle, F., Sieber, R., Bosset, J.O. 2001. Photo-oxidation and photoprotection of foods, with particular reference to dairy products. *Sci. de Aliment*. **21**, 571–590.

Bosset, J.O., Gallmann, P.U., Sieber, R. 1994. Influence of light transmittance of packaging materials on the shelf-life of milk and dairy products—a review. In: *Food Packaging and Preservation* (M. Mathlouthi, ed.), pp. 222–268, Blackie Academic and Professional, Glasgow.

Bosset, J.O., Sieber, R., Gallmann, P.U. 1995. Light transmittance: Influence on the shelf life of milk and milk products. In: *Technical Guide for the Packaging of Milk and Milk Products*, 3rd edn, *Bulletin* 300, pp. 19–39, International Dairy Federation, Brussels.

Bouzas, J., Kamarei, A.R., Karel, M. 1985. Storage stability of the milk fat globule membrane. *J. Food Proc. Preserv*. **9**, 11–24.

Bradley, D.G., Min, D.B. 1992. Singlet oxygen oxidation of foods. *CRC Crit. Rev. Food Sci. Nutr*. **31**, 211–236.

Bradley, R.L. Jr. 1980. Effect of light on alteration of nutritional value and flavor of milk: a review. *J. Food Prot*. **43**, 314–320.

Bray, S.L., Duthie, A.H., Rogers, R.P. 1977. Consumers can detect light induced flavor in milk. *J. Food Prot*. **40**, 586–587.

Bruder, G., Heid, H.W., Jarasch, E.D., Keenan, T.W., Mather, I.H. 1982. Characteristics of membrane bound and soluble forms of Xanthine oxidoreductase from milk and endothelial cells of capillaries. *Biochim. Biophys. Acta*. **701**, 357–369.

Bruhn, J.C., Franke, A.A. 1971. Influence of copper and tocopherol on the susceptibility of herd milk to spontaneous oxidized flavor. *J. Dairy Sci*. **54**, 761–762.

Bruhn, J.C., Franke, A.A., Goble, G.S. 1976. Factors relating to development of spontaneous oxidized flavor in raw milk. *J. Dairy Sci*. **59**, 828–833.

Brunner, J.R. 1974. Physical equilibria in milk: the lipid phase. In: *Fundamentals of Dairy Chemistry* (B.H. Webb, A.H. Johnson, J.A. Alford, eds.), pp. 474–602, AVI Publishing, Westport, CT.

Bugaud, C., Buchin, S., Coulon, J.B., Hauwuy, A., Dupont, D. 2001. Influence of the nature of alpine pastures on plasmin activity, fatty acid and volatile compound composition of milk. *Le Lait*. **81**, 401–414.

Bull, M. 1992. Packaging requirements for cream. *Bulletin* **271**, pp. 40–44, International. Dairy Federation, Brussels.

Burton, G.W., Ingold, K.U. 1981. Auto-oxidation of biological molecules. I. The antioxidant activity of vitamin E and related chain breaking phenolic antioxidants *in vitro*. *J. Am. Chem. Soc*. **103**, 6472–6477.

Calligaris, S., Manzocco, L., Anese, M., Nicoli, M.C. 2004. Effect of heat-treatment on the antioxidant and pro-oxidant activity of milk. *Int. Dairy J*. **14**, 421–427.

Carlsson, D.J., Suprunchuk, T., Wiles, D.W. 1976. Photooxidation of unsaturated oils: effects of singlet oxygen quenchers. *J. Am. Oil Chem. Soc*. **53**, 656–660.

Chan, H.W.S. 1987. The mechanism of auto-oxidation. In: *Auto-oxidation of Unsaturated Lipids* (H.W.C. Chan, ed.), pp. 1–16, Academic Press, London.

Charmley, E., Nicholson, J.W.G. 1995. Influence of dietary fat source on oxidative stability and fatty acid composition of milk from cows receiving a low or high level of dietary vitamin E. *Can. J. Anim. Sci*. **74**, 657–664.

Charmley, E., Nicholson, J.W.G., Butler, G. 1991. Effect of intramuscular injection of α-tocopherol on its concentration in blood plasma and milk. *J. Dairy Sci*. **74** (Suppl 1), 274 (abstr.).

Chen, Z.Y., Nawar, W.W. 1991a. Role of milk fat globule in the auto-oxidation of milk fat. *J. Food Sci*. **56**, 398–401, 426.

Chen, Z.Y., Nawar, W.W. 1991b. The role of amino acids in the auto-oxidation of milk fat. *J. Am. Oil Chem. Soc*. **68**, 47–50.

Chilson, W.H. 1935. What causes most common off-flavor of market milk? *Milk Plant Monthly* **24**, 24–28.

Christensen, T.C., Holmer, G. 1996. GC/MS analysis of volatile aroma components in butter during storage in different catering packaging. *Milchwissenschaft*. **51**, 134–139.

Cladman, W., Scheffer, S., Goodrich, N., Griffiths, M.W. 1998. Shelf life of milk packaged in plastic containers with and without treatment to reduce light transmission. *Int. Dairy J*. **8**, 629–636.

Corbett, W.J., Tracy, P.H. 1943. The incidence of oxidized flavor in the milk of individual cows within one herd. *J. Dairy Sci*. **43**, 585–588.

Cotton, F.A., Wilkinson, G. 1972. *Advanced Inorganic Chemistry*, 4th edn, John Wiley, New York.

Czulak, J., Hammond, L.A., Horwood, J.F. 1974. Cheese and cultured dairy products from milk with high linoleic acid content. *Aust. J. Dairy Technol*. **29**, 124–128.

Day, E.A., Lillard, D.A., Montgomery, M.W. 1963. Auto-oxidation of milk lipids. III. Effect of flavor of the additive interactions of carbonyl compounds at sub-threshold concentrations. *J. Dairy Sci*. **46**, 291–294.

Deger, D., Ashoor, S.H. 1987. Light induced changes in taste, appearance, odor and riboflavin content of cheese. *J. Dairy Sci*. **70**, 1371–1376.

Dimick, P.S. 1976. Effect of fluorescent light on amino acid composition of serum proteins from homogenized milk. *J. Dairy Sci*. **59**, 305–308.

Dimick, P.S. 1982. Photochemical effects on flavor and nutrients of fluid milk. *Can. Inst. Food Sci. Technol. J*. **15**, 247–256.

Downey, W.K. 1969. Lipid oxidation as a source of off-flavor development during the storage of dairy products. *J. Soc. Dairy Technol*. **22**, 154–162.

Dugan, L.R. 1980. Natural antioxidants. In: *Auto-oxidation in Food and Biological Systems* (M.G. Simic, M. Karel, eds.), pp. 261–282, Plenum Press, New York.

Dunkley, W.L., Franke, A.A. 1967. Evaluating susceptibility of milk to oxidized flavor. *J. Dairy Sci*. **50**, 1–9.

Dunkley, W.L., Jennings, W.G. 1951. A procedure for application of the thiobarbituric acid test to milk. *J. Dairy Sci*. **34**, 1064–1069.

Dunkley, W.L., Franke, A.A., Robb J. 1968b. Tocopherol concentration and oxidative stability of milk from cows fed supplements of D- or DL-α-tocopheryl acetate. *J. Dairy Sci*. **51**, 531–534.

Dunkley, W.L., Franke, A.A., Robb, J., Ronning, M.J. 1968a. Influence of dietary copper and ethylenediaminetetraacetate on copper concentration and oxidative stability of milk. *J. Dairy Sci*. **51**, 863–866.

Dunkley, W.L., Ronning, M., Smith, L.M. 1966. Influence of supplemental tocopherol and carotene on oxidative stability of milk and milk fat. *Proc. 17th Int. Dairy Congr* (Munich) **A**, 223–227.

Dunkley, W.L., Ronning, M., Franke, A.A., Robb, J. 1967. Supplementing rations with tocopherol and ethoxyquin to increase oxidative stability of milk. *J. Dairy Sci*. **50**, 492–499.

Edmondson, L.F., Douglas, F.W., Avants, J.K. 1971. Enrichment of pasteurized whole milk with iron. *J. Dairy Sci*. **54**, 1422–1426.

Eichner, K. 1980. Anti-oxidative effect of Maillard reaction intermediates. In: *Auto-oxidation in Food and Biological Systems* (M.G. Simic, M. Karel, eds.), pp. 267–285, Plenum Press, New York.

El-Negoumy, A.M. 1965. Relation of composition of the aqueous phase to oxidized flavor development by dialysed globular milk fat. *J. Dairy Sci.* **48**, 1406–1412.

Emmons, D.B., Paquette, G.J., Froehlich, D.A. 1986. Oxidation of butter by low intensities of fluorescent light in relation to retail stores. *J. Dairy Sci.* **69**, 2437–2450.

Erickson, D.R., Dunkley, W.L. 1964. Spectrophotometric determination of tocopherol in milk and milk lipids. *Anal. Chem.* **36**, 1055–1058.

Erickson, D.R., Dunkley, W.L., Ronning, M. 1963. Effect of intravenously injected tocopherol on oxidized flavor in milk. *J. Dairy Sci.* **46**, 911–915.

Erickson, M. 1997. Chemical and microbial stability of fluid milk in response to packaging and dispensing. *Int. J. Dairy Technol.* **50**, 107–111.

Eriksson, C.C. 1987. Oxidation of lipids in food systems. In: *Auto-oxidation of Unsaturated Lipids* (H.W.S. Chan, ed.), pp. 207–231, Academic Press, London.

Eriksson, C.E. 1982. Lipid oxidation catalysts and inhibitors in raw materials and processed foods. *Food Chem.* **9**, 3–19.

Fakourelis, N., Lee, E.D., Min, D.B. 1987. Effects of chlorophyll and β-carotene on the oxidation stability of olive oil. *J. Food Sci.* **52**, 234–235.

Farag, R.S., Osman, S.A., Hallabo, S.A.S., Nasr, A.A. 1978. Linoleic acid oxidation catalysed by various amino acids and cupric ions in freeze-dried model systems. *J. Am. Oil Chem. Soc.* **55**, 703–710.

Farmer, E.H., Bloomfield, G.F., Sundralingam, A., Sutton, D.A. 1942. The course and mechanism of auto-oxidation reactions in olefinic and polyolefinic substances, including rubber. *Trans. Faraday Soc.* **38**, 348–356.

Finley, J.W. 1968. *A Study of Chemical and Physical Factors Affecting the Development of Light-Induced Off-Flavor in Milk*. PhD Thesis, Cornell University.

Finley, J.W., Shipe, W.F. 1971. Isolation of a flavor producing fraction from light exposed milk. *J. Dairy Sci.* **54**, 15–20.

Focant, M., Mignolet, E., Marique, M., Clabots, F., Breyne, T., Dalemans, D., Larondelle, Y. 1998. The effect of vitamin E supplementation of cow diets containing rapeseed and linseed on the prevention of milk fat oxidation. *J. Dairy Sci.* **81**, 1095–1101.

Foote, C.S. 1976. Photosensitized oxidation and singlet oxygen: consequences in biological systems. In: *Free Radicals in Biology*, Volume 2 (W.A. Pryor, ed.), pp. 85–133, Academic Press, New York.

Foote, C.S., Denny, R.W. 1968. Chemistry of singlet oxygen. VII. Quenching by β-carotene. *J. Am. Chem. Soc.* **90**, 6233–6235.

Foote, C.S., Ching, T., Geller, G.G. 1974. Chemistry of singlet oxygen. XVIII. Rates of reaction and quenching of α-tocopherol and singlet oxygen. *Photochem. Photobiol.* **20**, 511–513.

Forss, D.A., Angelini, P., Bazinet, M.L., Merritt, C. 1967. Volatile compounds produced by copper-catalysed oxidation of butterfat. *J. Am. Oil. Chem. Soc.* **44**, 141–143.

Forss, D.A., Dunstone, E.A., Stark, W. 1960a. Fishy flavor in dairy products. II. The volatile compounds associated with fishy flavor in butterfat. *J. Dairy Res.* **27**, 211–219.

Forss, D.A., Dunstone, E.A., Stark, W. 1960b. Fishy flavor in dairy products. III. The volatile compounds associated with fishy flavor in washed cream. *J. Dairy Res.* **27**, 373–380.

Forss, D.A., Dunstone, E.A., Stark, W. 1960c. The volatile compounds associated with tallowy and painty flavor in butterfat. *J. Dairy Res.* **27**, 381–387.

Forss, D.A., Pont, E.G., Stark, W. 1955a. The volatile compounds associated with oxidized flavor in skim milk. *J. Dairy Res.* **22**, 91–102.

Forss, D.A., Pont, E.G., Stark, W. 1955b. Further observations on the volatile compounds associated with oxidized flavor in skim milk. *J. Dairy Res.* **22**, 345–348.

Fox, P.F., Morrissey, P.A. 1981. Indigenous enzymes of bovine milk. In: *Enzymes and Food Processing* (G.G. Birch, N. Blakebrough, K.J. Parker, eds.), pp. 213–238, Applied Science Publishers, London.

Frankel, E.N. 1980. Lipid oxidation. *Prog. Lipid Res.* **19**, 1–22.

Frankel, E.N. 1982. Volatile lipid oxidation products. *Prog. Lipid Res.* **22**, 1–33.

Frankel, E.N. 1985. Chemistry of free radical and singlet oxidation of lipids. *Prog. Lipid Res.* **23**, 197–221.

Frankel, E.N. 1988. Hydroperoxidation of unsaturated fatty esters. In: *Oxygen Radicals in Biology and Medicine* (M. Simic, K.A. Taylor, J.F. Ward, C. von Sonntag, eds.), pp. 265–282, Plenum Press, New York.

Frankel, E.N. 1991. Recent advances in lipid oxidation. *J. Sci. Food Agric.* **54**, 495–511.

Frankel, E.N. 1993. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends Food Sci. Technol.* **4**, 220–223.

Frankel, E.N. 1998. *Lipid Oxidation*. The Oily Press, Dundee, UK.

Frankel, E.N., Neff, W.E. 1983. Formation of malonaldehyde from lipid oxidation products. *Biochim. Biophys. Acta*. **754**, 264–270.

Frankel, E.N., Hu, M.L., Tappel, A.L. 1989. Rapid headspace gas chromatography of hexanal as a measure of lipid peroxidation in biological samples. *Lipids*. **24**, 976–981.

Frankel, E.N., Nowakowska, J., Evans, C.D. 1961. Formation of methyl azalaldlehydate on auto-oxidation of lipids. *J. Am. Oil. Chem. Soc.* **38**, 161–168.

Fridovich, I. 1976. Oxygen radicals, hydrogen peroxide and oxygen toxicity. In: *Free Radicals in Biology*, Vol. 1 (W.A. Pryor, ed.), pp. 239–277, Academic Press, New York.

Fridovich, I. 1977. Oxygen is toxic. *Bioscience*. **27**, 462–466.

Gardner, H.W. 1989. Oxygen radical chemistry of polyunsaturated fatty acids. *Free Rad. Biol. Med.* **7**, 65–86.

Goering, H.K., Gordon, C.H., Wrenn, T.R., Bitman, J, King, R.L., Douglas, F.W.. 1976. Effect of feeding protected safflower oil on yield, composition, flavor and oxidative stability of milk. *J. Dairy Sci.* **59**, 416–425.

Granelli, K., Barrefors, P., Bjorck, L., Appelqvist, L.A. 1998. Further studies on lipid composition of bovine milk in relation to spontaneous oxidized flavor. *J. Sci. Food Agric.* **77**, 161–171.

Greenbank, G.R., Wright, P.A., Deysher, E.F., Holm, G.E. 1946. The keeping quality of samples of commercially dried milk packed in air and in inert gas. *J. Dairy Sci.* **29**, 55–61.

Gregory, J.F., Shipe, W.F. 1975. Oxidative stability of milk. I. The anti-oxidative effect of trypsin treatment and aging. *J. Dairy Sci.* **58**, 1263–1271.

Gregory, J.F., Babish, J.G., Shipe, W.F. 1976. Role of heme proteins in peroxidation of milk lipids. *J. Dairy Sci.* **59**, 364–368.

Grosch, W. 1987. Reactions of hydroperoxides – products of low molecular weight. In: *Auto-oxidation of Unsaturated Lipids* (H.W.S. Chan, ed.), pp. 95–139, Academic Press, London.

Gutteridge, J.M.C., Patterson, S.K., Segal, A.W., Halliwell, B. 1981. Inhibition of lipid peroxidation by the iron-binding protein lactoferrin. *Biochem. J.* **199**, 259–261.

Haase, G., Dunkley, W.L. 1969a. Ascorbic acid and copper in linoleate oxidation. I. Measurement of oxidation by ultraviolet spectrophotometry and the thiobarbituric acid test. *J. Lipid Res.* **10**, 555–560.

Haase, G., Dunkley, W.L. 1969b. Ascorbic acid and copper in linoleate oxidation. II. Ascorbic acid and copper as oxidation catalysts. *J. Lipid Res.* **10**, 561–567.

Haase, G., Dunkley, W.L. 1969c. Ascorbic acid and copper in linoleate oxidation. III. Ascorbic acid and copper as oxidation catalysts. *J. Lipid Res.* **10**, 568–576.

Haase, G., Dunkley, W.L. 1970. Copper in milk and its role in catalyzing the development of oxidized flavors. *Milchwissenschaft*. **25**, 656–661.

Haisman, D.R., Groenendijk, C.B.M., O'Sullivan, G.J. 1992. The effect of light on flavor and nutritional quality of milk. Paperboard vs plastic containers. *Food Technol.* New Zealand **27** (2), 16–20.

Hall, G., Lingnert, H. 1986. Analysis of prediction of lipid oxidation in foods. *Dev. Food Sci.* **12**, 735–743.

Hamilton, J.W., Tappel, A.L. 1963. Evaluation of antioxidants by a rapid polarographic method. *J. Am. Oil Chem. Soc.* **40**, 52–54.

Hamm, D.L., Hammond, E.G., Hotchkiss, D.K. 1968. Effect of temperature on rate of auto-oxidation of milk fat. *J. Dairy Sci.* **51**, 483–491.

Hamm, D.L., Hammond, E.G., Parvanah, V., Snyder, H.E. 1965. The determination of peroxides by the Stamm method. *J. Am. Oil Chem. Soc.* **42**, 920–922.

Hammond, E.G., 1970. Stabilizing milk fat with antioxidants. *Am. Dairy Rev.* **32** (6), 40–41, 43, 76–77.

Hamzawi, L.F. 1990. Role of phospholipids and alpha-tocopherol as natural antioxidants in buffalo butterfat. *Milchwissenschaft.* **45**, 95–97.

Hannan, R.S., Boag, J.W. 1952. Effects of electronic irradiation on fats. *Nature.* **169**, 152–153.

Hannan, R.S., Shepherd, H.J. 1952. An after-effect in butter-fat irradiated with high-energy electrons. *Nature.* **170**, 1021–1022.

Hansen, E., Skibsted, L.H. 2000. Light-induced oxidative changes in a model dairy spread. Wavelength dependence of quantum yields. *J. Agric. Food Chem.* **48**, 3090–3094.

Hanzlik, R.P. 1976. *Inorganic Aspects of Biological and Organic Chemistry.* Academic Press, New York.

Havemose, M.S., Weisbjerg, M.R., Wender, W.L.P., Bredie, L.P., Nielsen, J.H. 2004. Influence of feeding different types of roughage on the oxidative stability of milk. *Int. Dairy J.* **14**, 563–570.

Hedrick, T.I., Glass, L. 1975. Chemical changes in milk during exposure to fluorescent light. *J. Milk Food Technol.* **38**, 129–131.

Hegenauer, J., Saltman, P., Ludwig, D., Ripley, L., Bajo, P. 1979a. Effects of supplemental iron and copper on lipid oxidation in milk. I. Comparison of metal complexes in emulsified and homogenized milk. *J. Agric. Food Chem.* **27**, 860–867.

Hegenauer, J., Saltman, P., Ludwig, D., Ripley, L., Ley, A. 1979b. Iron-supplemented cow milk. Identification and spectral properties of iron bound to casein micelles. *J. Agric. Food Chem.* **27**, 1294–1301.

Helal, F.R., El-Bagoury, K., Rifaat, I.D., Hofi, A.A., El-Sokkary, A. 1976. The effect of some antioxidants and emulsifiers on the keeping quality of buffalo whole milk powder during storage. *Egypt J. Dairy Sci.* **4**, 115–119.

Henick, A.S., Benca, M.F., Mitchell, J.H. 1954. Estimating carbonyl compounds in rancid fats and foods. *J. Am. Oil Chem. Soc.* **31**, 88–91.

Hermansen, J.E. 1995. Prediction of milk fatty acid profile in dairy cows fed dietary fat differing in fatty acid composition. *J. Dairy Sci.* **78**, 872–879.

Hill, R.D. 1975. Superoxide dismutase activity in bovine milk. *Aust. J. Dairy Technol.* **30**, 26–28.

Hill, R.D. 1979. Oxidative enzymes and oxidative processes in milk. *CSIRO Food Res. Q.* **39**, 33–37.

Hill, R.D., van Leeuwen, V., Wilkinson, R.A. 1977. Some factors influencing the auto-oxidation of milks rich in linoleic acid. *N.Z. J. Dairy Sci. Technol.* **12**, 69–77.

Hoffman, G. 1962. 1-Octen-3-ol and its relation to other oxidative cleavage products from esters of linoleic acid. *J. Am. Oil Chem. Soc.* **39**, 439–444.

Holbrook, J., Hicks, C.L. 1978. Variation of superoxide dismutase in bovine milk. *J. Dairy Sci.* **61**, 1072–1077.

Hong, C.M., Wendorff, W.L., Bradley, R.L. Jr. 1995. Factors affecting light-induced pink discoloration of annatto-colored cheese. *J. Food Sci*. **60**, 94–97.

Horvat, R.J., McFadden, W.H., Hawkins, N.G., Black, D.R., Lane, W.G., Teeter, R.M. 1965. Volatile products from mild oxidation of methyl linoleate. Analysis by combined mass spectrometry-gas chromatography. *J. Am. Oil Chem. Soc*. **42**, 1112–1115.

IDF. 1982. Outer Packaging of Milk and Milk Products, *Bulletin* 143, International Dairy Federation, Brussels.

IDF 1991. Anhydrous milk fat—determination of the peroxide value. Standard 74A, International Dairy Federation, Brussels.

Jackson, W.P., Loo, C.C. 1959. A solid in-package oxygen absorbent and its use in dry milk products. *J. Dairy Sci*. **42**, 912 (abstr.).

Jarasch, E.D., Bruder, G., Keenan, T.W., Franke, W. 1977. Redox constituents in milk fat globule membranes and rough endoplasmic reticulum from lactating mammary gland. *J. Cell Biol*. **73**, 223–241.

Jarrett, W.D. 1979. A review of the important trace elements in dairy products. *Aust. J. Dairy Technol*. **34**, 28–34.

Jenq, W., Bassett, R., Crang, R.E. 1988. Effects of light and copper on volatile aldehydes of milk and milk fractions. *J. Dairy Sci*. **71**, 2366–2372.

Jensen, R.G. 1995. Fat-soluble vitamins in bovine milk. In: *Handbook of Milk Composition* (R.G. Jensen, ed.), pp. 718–725, Academic Press, San Diego.

Jeon, I.J., Thomas, E.L., Reineccius, G.A. 1978. Production of volatile flavor comounds in ultra high temperature processed milk during aseptic storage. *J. Agric. Food Chem*. **26**, 1183–1188.

Johnson, A.H. 1974. The composition of milk. In: *Fundamentals of Dairy Chemistry* (B.H. Webb, A.H. Johnson, J.A. Alford, eds.), pp. 1–57, AVI Publishing, Westport, CT.

Josephson, D.V., Doan, F.J. 1939. Observations on cooked flavor in milk—its source and significance. *Milk Dealer* **29** (2), 35–54.

Jung, M.Y., Min, D.B. 1991. Effects of quenching mechanisms of carotenoids on the photosensitized oxidation of soyabean oil. *J. Am. Oil Chem. Soc*. **68**, 653–656.

Kamaleldin, A., Appelqvist, L.A. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* **31**, 671–701.

Kanner, J., Kinsella, J.E. 1983. Lipid deterioration initiated by phagocytic cells in muscle foods: β-carotene destruction by a myeloperoxidase-hydrogen peroxide-halide system. *J. Agric. Food Chem*. **31**, 370–376.

Kanno, C., Yamauchi, K., Tsugo, T. 1968. Occurrence of α-tocopherol and variation of α- and β-tocopherol in bovine milk fat. *J. Dairy Sci*. **51**, 1713–1719.

Karel, M. 1980. Lipid oxidation, secondary reactions, and water activity of foods. In: *Autooxidation in Foods and Biological Systems* (M. Simic, M. Karel, eds.), pp. 191–206, Plenum Press, New York.

Keenan, T.W., Dylewski, D.P. 1995. Intracellular origin of milk lipid globules and the nature and structure of the milk lipid globule membrane. In: *Advanced Dairy Chemistry: Lipids*, 2nd edn (P.F. Fox, ed.), pp. 89–130, Chapman and Hall, London.

Keenan, T.W., Moon, T.O., Dylewski, D.P. 1983. Lipid globules retain globule material after homogenization. *J. Dairy Sci*. **66**, 196–203.

Kendrick, J., Watts, B.M. 1969. Acceleration and inhibition of lipid oxidation by heme compounds. *Lipids* **4**, 454–458.

Kim, Y.D., Morr, C.V. 1996. Dynamic headspace analysis of light activated flavor in milk. *Int. Dairy J*. **6**, 185–193.

King, R.L. 1958. *Variation and Distribution of Copper in Milk in Relation to Oxidized Form*. PhD Thesis, University of California, Davis.

King, R.L. 1962. Oxidation of milk fat globule membrane. I. Thiobarbituric acid reaction as a measure of oxidized flavor in milk and model systems. *J. Dairy Sci.* **45**, 1165–1171.
King, R.L. 1963. Oxidation of milk fat globule membrane material. II. Relation of ascorbic acid and membrane concentration. *J. Dairy Sci.* **46**, 267–274.
King, R.L. 1968. Direct addition of tocopherol to milk for control of oxidized flavor. *J. Dairy Sci.* **51**, 1705–1707.
King, R.L., Dunkley, W.L. 1959a. Role of chelating compound in the inhibition of oxidized flavor. *J. Dairy Sci.* **42**, 897 (abstr.).
King, R.L., Dunkley, W.L. 1959b. Relation of natural copper in milk to incidence of spontaneous oxidized flavor. *J. Dairy Sci.* **42**, 420–427.
King, R.L., Burrows, F.A., Hemken, R.W., Bashore, D.L. 1967. Control of oxidized flavor by managed intake of vitamin E from selected forages. *J. Dairy Sci.* **50**, 943–944.
King, R.L., Luick, J.R., Litman, I.I., Jennings, W.G., Dunkley, W.L. 1959. Distribution of natural and added copper and iron in milk. *J. Dairy Sci.* **42**, 780–790.
King, R.L., Tikriti, H.H., Oskarsson, M. 1966. Natural and supplemented tocopherol in the dairy ration and oxidized flavor. *J. Dairy Sci.* **49**, 1574 (abstr.).
Kliman, P.G., Tamsma, A., Pallansch, M.J. 1962. Peroxide value – flavor score relationships in stored foam-dried whole milk. *J. Agric. Food Chem.* **10**, 496–498.
Kochhar, S.P. 1996. Oxidation pathways to the formation of off-flavor. In: *Food Taints and Off-flavor*, 2nd edn (M.J. Saxby, ed.), pp. 168–225, Blackie Academic and Professional, London.
Kochi, J. 1973. *Free Radicals*, John Wiley, New York.
Kolakowska, A. 2003. Lipid oxidation in food systems. In: *Chemical and Functional Properties of Food Lipids* (Z.E. Sikorski, A. Kolakowska, eds.), pp. 133–166, CRC Press, Boca Raton, FL.
Kooops, J. 1964. Antioxidant activity of ascorbyl plamitate in cold stored butter. *Neth. Milk Dairy J.* **18**, 38–51.
Koops, J. 1969. The effect of pH on the partition of natural and added copper in milk and cream. *Neth. Milk Dairy J.* **23**, 200–213.
Korhonen, H., Korpela, R. 1994. The effects of dairy processes on the components and nutritional value of milk. *Scand. J. Nutr.* **38**, 166–172.
Korycka-Dahl, M., Richardson, T. 1978. Photogeneration of superoxide anion in serum of bovine milk and in model systems containaing riboflavin and amino acids. *J. Dairy Sci.* **61**, 400–407.
Korycka-Dahl, M., Richardson, T. 1979. Photogeneration of superoxide anion upon illumination of bovine milk serum proteins with fluorescent light in the presence of riboflavin. *J. Dairy Sci.* **62**, 183–188.
Korycka-Dahl, M., Richardson, T. 1980. Initiation of oxidative changes in foods. *J. Dairy Sci.* **63**, 1181–1198.
Korycka-Dahl, M., Richardson, T., Hicks, C.L. 1979. Superoxide dismutase activity in bovine milk serum. *J. Food Prot.* **42**, 867–871.
Korycka-Dahl, M., Vassal, L., Ribadeau Dumas, B., Mocquot, G. 1983. Studies on lipid oxidation during ripening of Camembert cheese and its impact on cheese flavor. *Sci. Alim.* **3**, 79–90.
Krinsky, N.I. 1979. Carotenoid protection against oxidation. *Pure Appl. Chem.* **51**, 649–660.
Kristensen, D., Skibsted, L.H. 1999. Comparison of three methods based on electron spin resonance spectrometry for evaluation of oxidative stability of processed cheese. *J. Agric. Food Chem.* **47**, 3099–3104.
Kristensen, D., Hansen, E., Arndal, A., Trinderup, R.A., Skibsted, L.H. 2001. Influence of light and temperature on the colour and oxidative stability of processed cheese. *Int. Dairy J.* **11**, 837–843.

Kristensen, D., Hedegaard, R.V., Nielsen, J.H., Skibsted, L.H. 2004. Oxidative stability of buttermilk as influenced by the fatty acid composition of cow's milk manipulated by diet. *J. Dairy Res.* **71**, 46–50.

Krukovsky, V.N. 1952. The origin of oxidized flavors and factors responsible for their development in milk and milk products. *J. Dairy Sci.* **35**, 21–29.

Krukovsky, V.N. 1955. Organoleptic study of oxygenated and copper treated milk prior to pasteurization. *J. Dairy Sci.* **38**, 595 (abstr.).

Krukovsky, V.N. 1961. Review of biochemical properties of milk and lipid deterioration in milk and milk products as influenced by natural varietal factors. *J. Agric. Food Chem.* **9**, 439–447.

Krukovsky, V.N., Guthrie, E.S. 1945. Ascorbic acid oxidation, a key factor in the inhibition or promotion of the tallowy flavor in milk. *J. Dairy Sci.* **28**, 565–579.

Krukovsky, V.N., Whiting, F., Loosli, J.K. 1950. Tocopherol, carotenoid and vitamin A content of the milk fat and the resistance of milk to the development of oxidized flavors as influenced by breed and season. *J. Dairy Sci.* **33**, 791–796.

Kurtz, F.E., Tamsma, A., Pallansch, J. 1973. Effect of fortification with iron on susceptibility of skim milk and nonfat dry milk to oxidation. *J. Dairy Sci.* **56**, 1139–1143.

Labuza, T.P. 1971. Kinetics of lipid oxidation. *CRC Crit. Rev. Food Technol.* **2**, 355–404.

Larsson, B.L., Jenness, R. 1950. The reducing capacity of milk as measured by an iodimetric titration. *J. Dairy Sci.* **33**, 896–903.

Lee, S.R., Macku, C., Shibamoto, T. 1991. Isolation and identification of headspace volatiles formed in heated butter. *J. Agric. Food Chem.* **39**, 1972–1975.

Lillard, D.A., Day, E.A. 1961. Auto-oxidation of milk lipids. II. The relationship of sensory to chemical methods for measuring the oxidized flavor of milk fats. *J. Dairy Sci.* **44**, 623–632.

Lindmark-Mansson, H., Akesson, B. 2000. Antioxidative factors in milk. *Br. J. Nutr.* **84** (Suppl. 1), S103–S110.

Lingnert, H., Eriksson, C., Waller, G.R. 1983. Characterization of anti-oxidative Maillard reaction products from histidine and glucose. In: *The Maillard Reaction in Food and Nutrition* (G.R. Waller, M.S. Feather, eds.), pp. 335–345, ACS Symposium Series 215, American Chemical Society, Washington, DC.

Loftus-Hills, G., Thiel, C.C. 1946. The ferric thiocyanate method of estimating peroxides in fat or butter, milk and dried milk. *J. Dairy Res.* **14**, 340–353.

Lundberg, W.O. 1962. Mechanisms. In: *Lipids and their Oxidation* (H.W. Schultz, E.A. Day, R.O. Sinnhuber, eds.), pp. 31–50, AVI Publishing, Westport, CT.

Madere, R., Behrens, W.A. 1992. Malonaldehyde determination in foods by ion-pairing high-performance liquid chromatography. *Food Res. Int.* **25**, 37–40.

Manson, W., Cannon, J. 1978. The reaction of α_{s1}- and β-casein with ferrous ions in the presence of oxygen. *J. Dairy Res.* **45**, 59–67.

Matsushita, S., Terao, J. 1980. Singlet oxygen-initiated photooxidation of unsaturated fatty acids and esters and inhibitory effect of tocopherols and β-carotene. In: *Auto-oxidation in Food and Biological Systems* (M.G. Simic, M. Karel, eds.), pp. 27–44, Plenum Press, New York.

McCormick, A.M., Napoli, J.L., Schnoes, H.K., DeLuca, H.F. 1978. Isolation and identification of 5,6-epoxyretinoic acid: a biologically active metabolite of retinoic acid. *Biochemistry* **17**, 4085–4090.

McPherson, A.V., Kitchen, B.J. 1983. Reviews of the progress of Dairy Science: the bovine milk fat globule membrane – its formation, composition, structure and behaviour in milk and dairy products. *J. Dairy Res.* **50**, 107–133.

Mehlenbacher, V.C. 1960. *The Analysis of Fats and Oils*, The Garrand Press, Champagne, IL.

Meijboom, P.W. 1964. Relationship between molecular structure and flavor perceptibility of aliphatic aldehydes. *J. Am. Oil Chem. Soc*. **41**, 326–328.

Meyer, R.I., Jokay, L. 1960. The effect of an oxygen scavenger packet, dessicant in-package system on the stability of dry whole milk and dry ice cream mix. *J. Dairy Sci*. **43**, 844 (abstr.).

Min, D.B., Lee, H.O. 1996. Chemistry of lipid oxidation. In: *Food Lipids and Health* (R.E. McDonald, D.B. Min, eds.), pp. 241–268, Marcel Dekker, Anaheim, CA.

Misra, H.P. 1974. Generation of superoxide free radical during the auto-oxidation of thiols. *J. Biol. Chem*. **249**, 2151–2155.

Morales, M.S., Palmquist, D.L., Weiss, W.P. 2000. Milk fat composition of Holstein and Jersey cows with control or depleted copper status and fed whole soybeans or tallow. *J. Dairy Sci*. **83**, 2112–2119.

Morita, M., Fujimaki, M. 1972. Characterization of the copper-binding of phosphatidyl ethanolamine emulsion in its rapid auto-oxidation. *Agric. Biol. Chem*. **36**, 1751–1754.

Mortensen, A., Skibsted, L.H., Truscott, T.G. 2001. The interaction of dietary carotenoids with radical species. *Arch. Biochem. Biophys*. **385**, 13–19.

Mortensen, G., Bertelsen, G., Mortensen, B.K., Stapelfeldt, H. 2004. Light-induced changes in packaged cheeses – a review. *Int. Dairy J*. **14**, 85–102.

Mortensen, G., Sorensen, J., Stapelfeldt, H. 2002. Effect of light and oxygen transmission characteristics of packaging materials on photooxidative quality changes in Havarti cheeses. *Packaging Technol. Sci*. **15**, 121–127.

Mortensen, G., Sorensen, J., Stapelfeldt, H. 2003. Response surface models for prediction of photooxidative quality changes in Havarti cheese. *Euro. Food Res. Technol*. **216**, 93–98.

Mulder, H., Walstra, P. 1974. *The Milk Fat Globule: Emulsion Science as Applied to Food Products and Comparable Foods*, Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.

Mulder, H., Menger, J.W., Meijers, P. 1964. The copper content of cow's milk. *Neth. Milk Dairy J*. **18**, 52–65.

Murty, G.K., Rhea, U.S., Peeler, J.T. 1972. Copper, iron, manganese, strontium and zinc content of market milk. *J. Dairy Sci*. **55**, 1666–1674.

Nath, B.S., Murthy, M.K.R. 1988. Effect of browning compounds on the auto-oxidative stability of ghee. *Indian J. Dairy Sci*. **41**, 116–119.

Nawar, W.W. 1985. Lipids. In: *Food Chemistry* (O.R. Fennema, ed.), pp. 139–244, Marcel Dekker, New York.

Nelson, K.H., Cathcart, W.M. 1984. Transmission of light through pigmented polyethylene milk bottles. *J. Food Prot*. **47**, 346–348.

Newstead, D.F., Headifen, J.M. 1982. A reappraisal of the method for estimation of the peroxide value in fat in whole milk powder. *N.Z. J. Dairy Sci. Technol*. **15**, 13–18.

Nielsen, B.R., Stapelfeldt, H., Skibsted, L.H. 1997. Early prediction of the shelf-life of medium-heat whole milk powders using stepwise multiple regression and principal component analysis. *Int. Dairy J*. **7**, 341–348.

Niki, E. 1991. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *Am. J. Clin. Nutr*. **54**, 1119S–1124S.

Noll, C.I., Supplee, G.C. 1951. Factors affecting the gas content of milk. *J. Dairy Sci*. **24**, 993–1013.

O'Brien, J., Morrissey, P.A. 1989. Nutritional and toxicological aspects of the Maillard browning reaction in foods. *CRC Crit. Rev. Food Sci. Nutr*. **28**, 211–248.

O'Mahony, J.P., Shipe, W.F. 1970. Effect of variations in phospholipid composition of fat globule membrane fractions on the oxidative stability of milk. *J. Dairy Sci*. **53**, 636 (abstr.).

Olson, F.C., Brown, W.C. 1942. Oxidized flavor in milk. XI. Ascorbic acid, glutathione, and hydrogen peroxide as mechanisms for the production of oxidized flavor. *J. Dairy Sci.* **25**, 1027–1039.

Park, P.S.W., Goins, R.E. 1992. Determination of volatile lipid oxidation products by dynamic headspace-capillary gas chromatographic analysis with application to milk-based nutritional products. *J. Agric. Food Chem.* **40**, 1581–1585.

Parks, O.W., Allen, C. 1977. Photodegradation of riboflavin to lumichrome in milk exposed to sunlight. *J. Dairy Sci.* **60**, 1038–1041.

Parks, O.W., Patton, S. 1961. Volatile carbonyl compounds in stored dry whole milk. *J. Dairy Sci.* **44**, 1–9.

Parks, O.W., Keeney, M., Schwartz, D.P. 1963. Carbonyl compounds associated with the off-flavor in spontaneously oxidized milk. *J. Dairy Sci.* **46**, 295–301.

Parks, O.W., Wong, N.P., Allen, C.A., Schwartz, D.P. 1969. 6-*trans*-Nonenal: an off-flavor component of foam spray-dried milks. *J. Dairy Sci.* **52**, 953–956.

Parmer, S., Sharma, R. 1986. Use of mango seeds in enhancing the oxidative stability of ghee. *Asian J. Dairy Res.* **5**, 91–99.

Patton, S. 1954. The mechanism of sunlight flavor formation in milk with special reference to methionine and riboflavin. *J. Dairy Sci.* **37**, 446–452.

Patton, S., Barnes, I.J., Evans, L.E. 1959. *n*-Deca-2,4-dienal, its origin from linoleate and flavor significance in fats. *J. Am. Oil Chem. Soc.* **36**, 280–283.

Peters, I.I., Trout, G.M. 1945a. The attraction between the fat globules and the leucocytes of milk. *J. Dairy Sci.* **28**, 277–281.

Peters, I.I., Trout, G.M. 1945b. The influence of pH on the attraction between the fat globules and leucocytes of milks. *J. Dairy Sci.* **28**, 283–289.

Plantz, P.E., Patton, S., Keenan, T.W. 1973. Further evidence of plasma membrane material in skim milk. *J. Dairy Sci.* **56**, 978–983.

Pokorny, J. 1987. Major factors affecting the auto-oxidation of lipids. In: *Auto-oxidation of Unsaturated Lipids* (H.W.S. Chan, ed.), pp. 141–206, Academic Press, London.

Pont, E.G. 1952. Studies on the origin of oxidized flavor in whole milk. *J. Dairy Res.* **19**, 316–327.

Porter, N.A. 1986. Mechanisms for auto-oxidation of polyunsaturated lipids. *Acta Chem. Res.* **19**, 262–268.

Pryor, W.A., Fuller, D.L., Stanley, J.P. 1972. Reactivity patterns of the methyl radical. *J. Am. Oil Chem. Soc.* **94**, 1632–1638.

Pyenson, H., Tracy, P.H. 1946. A spectrophotometric study of the changes in peroxide value of spray-dried whole milk powder during storage. *J. Dairy Sci.* **29**, 1–12.

Rajan, T.S., Richardson, G.A., Stein, R.W. 1962. Xanthine oxidoreductase activity of milks in relation to stage of lactation, feed and incidence of spontaneous oxidation. *J. Dairy Sci.* **45**, 933–934.

Ramaswamy, R., Baer, R.J., Schingoethe, D.J., Hippen, A.R., Kasperson, K.M., Whitlock, L.A. 2001. Composition and flavor of milk and butter from cows fed fish oil, extruded soybeans, or their combinations. *J. Dairy Sci.* **84**, 2144–2151.

Ranby, B., Rabek, J.F. 1978. *Singlet Oxygen: Reactions with Organic Compounds and Polymers*, John Wiley, New York.

Rao, V.D., Murthy, M.K.R. 1987. Influence of the metal catalysts on the pattern of carbonyl production during the auto-oxidation of cow milk fat. *Indian J. Anim. Sci.* **57**, 475–478.

Richardson, T., Korycka-Dahl, M. 1983. Lipid oxidation. In: *Developments in Dairy Chemistry: Lipids*, Vol. 2 (P.F. Fox, ed.), pp. 241–363, Elsevier Applied Science Publishers, London.

Riest, U., Ronning, M., Dunkley, W.L., Franke, A.A. 1967. Oxidative stability of milk as influenced by dietary copper, molybdenum and sulfate. *Milchwissenschaft*. **22**, 551–554.

Riisom, T., Sims, R.J., Fioriti, J.A. 1980. Effect of amino acids on the auto-oxidation of safflower oil in emulsions. *J. Am. Oil Chem. Soc*. **57**, 354–359.

Robertson, G.L. 1993. Food Packaging—Principles and Practice. Marcel Dekker, New York.

Roh, J.K., Bradley, R.L., Richardson, T., Weckel, K.G. 1976. Removal of copper from milk. *J. Dairy Sci*. **59**, 382–385.

Rysstad, G., Ebbesen, A., Eggested, J. 1998. Sensory and chemical quality of UHT milk stored in paperboard cartons with different oxygen and light barriers. *Food Add. Contam*. **15**, 112–122.

Saidi, B., Warthesen, J.J. 1995. Effect of heat and homogenization on riboflavin photolysis in milk. *Int. Dairy J*. **5**, 635–645.

Salin, M., McCord, J.M. 1977. Free radicals in leukocyte metabolism and inflammation. In: *Superoxide and Superoxide Dismutases* (A.M. Michelson, J.M. McCord, I. Fridovich, eds.), pp. 257–270, Academic Press, New York.

Samuelsson, E.G. 1966. The copper content in milk and the distribution of copper to various phases of milk. *Milchwissenschaft*. **21**, 335–341.

Sargent, J.S.E., Stine, C.M. 1964. Effects of heat on the distribution of residual and added copper in whole fluid milk. *J. Dairy Sci*. **47**, 662–663.

Sattar, A., de Man, J.M. 1975. Photooxidation of milk and milk products—a review. *CRC Crit. Rev. Food Sci. Nutr*. **7**, 13–37.

Sattar, A., de Man, J.M., Alexander, J.C. 1977a. Wavelength effects on light-induced decomposition of vitamin A and β-carotene in solutions and milk fat. *Can. Inst. Food Sci. Technol. J*. **10**, 56–64.

Sattar, A., de Man, J.M., Alexander, J.C. 1977b. Light-induced degradation of vitamins. II. Kinetic studies on ascorbic acid decomposition in solution. *Can. Inst. Food Sci. Technol. J*. **10**, 65–68.

Schaffer, P.S., Greenbank, G.R., Holm, G.E. 1946. The rate of auto-oxidation of milk fat in atmospheres of different oxygen concentration. *J. Dairy Sci*. **29**, 145–150.

Schaich, K.M. 1980. Free radical initiation in proteins and amino acids by ionizing and ultraviolet radiations and lipid oxidation . Part III. Free radical transfer from oxidizing lipids. *CRC Crit. Rev. Food Sci. Nutr*. **13**, 189–244.

Schingoethe, D.J., Parsons, J.G., Ludens, F.C., Schaffer, L.V., Shave, H.J. (1979). Response of lactating cows to 300 mg of supplemental vitamin E daily. *J. Dairy Sci*. **62**, 333–338.

Schroder, M.J.A. 1982. Effect of oxygen on the keeping quality of milk. I. Oxidized flavor development and oxygen uptake in milk in relation to oxygen availability. *J. Dairy Res*. **49**, 407–424.

Schwartz, D.P., Parks, O.W. 1974. The lipids of milk: deterioration. In: *Fundamentals of Dairy Chemistry* (B.H. Webb, A.H. Johnson, J.A. Alford, eds.), pp. 220–272, AVI Publishing, Westport, CT.

Scott, G. 1965. *Atmospheric Oxidation and Antioxidants*, Elsevier Publishing, New York.

Seely, G.R., Meyer, T.H. 1971. The photosensitized oxidation of β-carotene. *Photochem. Photobiol*. **13**, 27–32.

Seerless, S.K., Armstrong, J.G. 1970. Vitamin E, vitamin A and carotene contents of Alberta butter. *J. Dairy Sci*. **53**, 150–154.

Sharma, S. 1981. Ghee: a resume of recent research. *J. Food Sci. Technol*. (India) **18**, 70–77.

Sharp, P.F., Guthrie, E.S., Hand, D.B. 1941. A new method of retarding oxidized flavor and preserving vitamin C. *Int. Assoc. Milk Dealers Bull*. **20**, 523–545.

Shipe, W.F. 1964. Oxidations in the dark. *J. Dairy Sci*. **47**, 221–230.

Shipe, W.F., Bassette, R., Deane, D.D., Dunkley, W.L., Hammond, E.G., Harper, W.J., Kleyn, D.H., Morgan, M.E., Nelson, J.H., Scanlan, R.A. 1978. Off-flavor in milk. *J. Dairy Sci*. **61**, 855–869.

Shipe, W.F., Senyk, G.F., Weetall, H.H. 1972. Inhibition of oxidized flavor development in milk by immobilized trypsin. *J. Dairy Sci.* **55**, 647–648.

Sidhu, G.S., Brown, M.A., Johnson, A.R. 1975. Auto-oxidation in milk rich in linoleic acid. I. An objective method for measuring auto-oxidation and evaluating auto-oxidations. *J. Dairy Res.* **42**, 185–195.

Sidhu, G.S., Brown, M.A., Johnson, A.R. 1976. Auto-oxidation in milk rich in linoleic acid. II. Modification of the initial system and control of oxidation. *J. Dairy Res.* **43**, 239–250.

Singleton, J.A., Aurand, L.W., Lancaster, F.W. 1963. Sunlight flavor in milk. I. A study of components involved in the flavor development. *J. Dairy Sci.* **46**, 1050–1053.

Skibsted, L.H. 2000. Light-induced changes in dairy products. *Bulletin* 346, pp. 4–9, International Dairy Federation, Brussels.

Slater, T.F. 1984. Overview of methods used for detecting lipids peroxidation. In: *Methods in Enzymology* (L. Packer, ed.), pp 283–293, Academic Press, New York.

Smith, G.J., Dunkley, W.L. 1960. Xanthine oxidoreductase and incidence of spontaneous oxidized flavor in milk. *J. Dairy Sci.* **43**, 278–280.

Smith, G.J., Dunkley, W.L. 1962a. Copper binding in relation to inhibition of oxidized flavor by heat treatment and homogenization. *Proc. Int. Dairy Congr.* (Copenhagen) **A**, 625–632.

Smith, G.J., Dunkley, W.L. 1962b. Pro-oxidants in spontaneous development of oxidized flavor in milk. *J. Dairy Sci.* **45**, 170–181.

Smith, G.J., Dunkley, W.L. 1962c. Ascorbic acid oxidation and lipid peroxidation in milk. *J. Food Sci.* **27**, 127–134.

Spikes, J.D., Livingstone, R. 1969. The molecular biology of photodynamic action: sensitized photooxidations in biological systems. *Adv. Radiat. Biol.* **3**, 28–121.

St. Angelo, A.J. 1996. Lipid oxidation in foods. *CRC Crit. Rev. Food Sci. Nutr.* **36**, 175–224.

St. Laurent, A.M., Hidiroglou, M., Snoddon, M., Nicholson, J.W.G. 1990. Effect of α-tocopherol supplementation to dairy cows on milk and plasma α-tocopherol concentrations and on spontaneous oxidized flavor in milk. *Can. J. Anim. Sci.* **70**, 561–570.

Stapelfeldt, H., Nielsen, B.R., Skibsted, L.H. 1997a. Effect of heat treatment, water activity and storage temperature on the oxidative stability of whole milk powder. *Int. Dairy J.* **7**, 331–339.

Stapelfeldt, H., Nielsen, B.R., Skibsted, L.H. 1997b. Towards use of electron spin resonance spectrometry in quality control of milk powder. Correlation between sensory score of instant whole milk powders and concentration of free radicals and 2-thiobarbituric acid reactive substances. *Milchwissenschaft*. **52**, 682–685.

Stapelfeldt, H., Nielsen, K.N., Jensen, S.K., Skibsted, L.H. 1999. Free radical formation in freeze-dried raw milk in relation to its α-tocopherol level. *J. Dairy Res.* **66**, 461–466.

Stark, W., Forss, D.A. 1962. A compound responsible for metallic flavor in dairy products. I. Isolation and identification. *J. Dairy Res.* **29**, 173–180.

Stark, W., Forss, D.A. 1966. N-Alkan-1-ols in oxidized butter. *J. Dairy Res.* **33**, 31–36.

Suttles, M.L., Marshall, R.T. 1993. Interactions of packages and fluorescent light with the flavor of ice cream. *J. Food Protect.* **56**, 622–624.

Swaisgood, H.E., Abraham, P. 1980. Oxygen activation by sulfhydryl oxidase and the enzyme's interaction with peroxidase. *J. Dairy Sci.* **63**, 1205–1210.

Tada, M., Kobayashi, N., Kobayashi, S. 1971. Studies on the photosensitized degradation of food constituents. II. Photosensitized degradation of methionine by riboflavin. *J. Agric. Chem. Soc. Japan* **45**, 471–476.

Tamsma, A., Kurtz, F.E., Pallansch, M.J. 1967. Effect of oxygen removal technique on flavor stability of low-heat foam spray dried whole milk. *J. Dairy Sci.* **50**, 1562–1565.

Tamsma, A., Mucha, T.J., Pallansch, M.J. 1962. Factors relating to flavor stability of foam-dried milk. II. Effect of heating milk prior to dairying. *J. Dairy Sci.* **45**, 1435–1439.

Tamsma, A., Pallansch, M.J., Mucha, T.J., Patterson, W.I. 1961. Factors related to the flavor stability of foam-dried whole milk. I. Effect of oxygen level. *J. Dairy Sci.* **44**, 1644–1649.

Tappel, A.L. 1955. Catalysis of linoleate oxidation by copper proteins. *J. Am. Oil Chem. Soc.* **32**, 252–254.

Tarassuk, N.P., Koops, J. 1960. Inhibition of oxidized flavor in homogenized milk as related to the concentration of copper and phospholipids per unit of fat globule surface. *J. Dairy Sci.* **43**, 93–94.

Taylor, M.J., Richardson, T. 1980a. Antioxidant activity of cysteine and protein sulfhydryls in a linoleate emulsion oxidized by hemoglobin. *J. Food Sci.* **45**, 1223–1227, 1230.

Taylor, M.J., Richardson, T. 1980b. Antioxidant activity of skim milk: effect of heat and resultant sulfhydryl groups. *J. Dairy Sci.* **63**, 1783–1795.

Teixeira Neto, R.O., Karel, M., Saguy, I., Mizraki, S. 1981. Oxygen uptake and β-carotene decoloration in a dehydrated food model. *J. Food Sci.* **46**, 665–669, 676.

Terao, J., Yamauchi, R., Murakauri, H., Matsushita, S. 1980. Inhibitory effects of tocopherols and β-carotene on singlet oxygen-initiated photooxidation of methyl linoleate and soybean oil. *J. Food Proc. Preserv.* **4**, 79–93.

Timmons, J.S., Weiss, W.P., Palmquist, D.L., Harper, W.J. 2001. Relationships among dietary roasted soybeans, milk components and spontaneous oxidized flavor of milk. *J. Dairy Sci.* **84**, 2440–2449.

Timms, R.E., Roupas, P., Rogers, W.P. 1982. The content of dissolved oxygen in air-saturated liquid and crystallized anhydrous milk fat. *Aust. J. Dairy Technol.* **37**, 39–40.

Tong, L.M., Sasaki, S., McClements, D.J., Decker, E.A. 2000. Mechanism of antioxidant activity of a high molecular weight fraction of whey. *J. Agric. Food Sci.* **48**, 1473–1478.

Toyoda, M., Ito, Y.U., Iwaida, M., Itsugi, Y., Ohashi, M., Fuju, T. 1982. Simple and rapid determination of hydrogen peroxide contained in milk by use of an oxygen electrode. *N.Z. J. Dairy Sci. Technol.* **17**, 41–46.

Ulberth, F., Roubicek, D. 1995. Monitoring of oxidative deterioration of milk powder by headspace gas chromatography. *Int. Dairy J.* **5**, 523–531.

Urbach, G. 1990. Effect of feed on flavor in dairy foods. *J. Dairy Sci.* **73**, 3639–3650.

Urbach, G.E. 1989. Effect of feed on flavor in dairy products. *J. Dairy Sci.* **72** (Suppl. 1), 108 (abstr.).

Wade, V., Al-Tahiri, R., Crawford, R. 1986. The auto-oxidative stability of anhydrous milk fat with and without antioxidants. *Milchwisssenschaft*. **41**, 479–482.

Ward, D.D. 1985. The TBA assay and lipid oxidation: a overview of the relevant literature. *Milchwissenschaft*. **40**, 583–588.

Warner, K., Frankel, E.N. 1987. Effects on β-carotene on light stability of soybean oil. *J. Am. Oil. Chem. Soc.* **64**, 213–218.

Waters, W.A. 1971. The kinetics and mechanism of metal-catalysed auto-oxidation. *J. Am. Oil Chem. Soc.* **48**, 427–433.

Weihrauch, J.L. 1988. Lipids of milk: deterioration. In: *Fundamentals of Dairy Chemistry*, 3rd edition (N.P. Wong, ed.), pp. 215–278, van Nostrand Reinhold, New York.

Wilson, H.K., Herreid, E.O. 1969. Controlling oxidized flavors in high-fat sterilized cream. *J. Dairy Sci.* **52**, 1229–1232.

Wishner, L.A., Keeney, M. 1963. Carbonyl pattern of sunlight-exposed milk. *J. Dairy Sci.* **46**, 785–788.

Wold, J.P., Jorgensen, K., Lundby, F. 2002. Non-destructive measurement of light-induced oxidation in dairy products by fluorescence spectroscopy and imaging. *J. Dairy Sci.* **85**, 1693–1704.

Wold, J.P., Veberg, A., Nilsen, A., Iani, V., Juzenas, P., Moan, J. 2005. The role of naturally occurring chlorophyll and porphyrins in light-induced oxidation of dairy products. A study based on fluorescence spectroscopy and sensory analysis. *Int. Dairy J.* **14**, 343–353.

Wong, N.P. Walter, H.E., Vestal, J.H., Lacroix, D.E., Alford, J.A. 1973. Cheddar cheese with increased polyunsaturated fatty acids. *J. Dairy Sci.* **56**, 1271–1275.

Wyatt, C.J., Day, E.A. 1965. Evaluation of antioxidants in deodorized and non-deodorized butteroil stored at 30°C. *J. Dairy Sci.* **48**, 682–686.

Yamauchi, R., Matsushita, S. 1977. Quenching effect of tocopherols on methyl linoleate photooxidation and their oxidation products. *Agric. Biol. Chem.* **41**, 1425–1430.

Yee, J.J., Shipe, W.F. 1982. Effects of sulfhydryl compounds on lipid oxidations catalysed by copper and heme. *J. Dairy Sci.* **65**, 1414–1420.

Zweig, A., Henderson, W.A. 1975. Singlet oxygen and polymer photooxidations. I. Sensitizers, quenchers and reactants. *J. Polym. Sci.* Part A **1**, 717–736.

Zygoura, P., Moyssiadi, T., Badeka, A., Kondyli, E., Savvaidis, I., Kontominas, M.G. 2004. Shelf life of whole pasteurized milk in Greece: effect of packaging material. *Food Chem.* **87**, 1–9.