spray-dried powder, the fat globules are distributed throughout the powder particles. The amount of free fat depends on the total fat content, and may be about 25% of total fat. Homogenization pre-drying reduces the level of free fat formed.

Further liberation of 'free fat' may occur under adverse storage conditions. If powder absorbs water it becomes 'clammy' and lactose crystallizes, resulting in the expulsion of other milk components from the lactose crystals into the spaces between the crystals. De-emulsification of the fat may occur due to the mechanical action of sharp edges of lactose crystals on the fat globule membrane. If the fat is liquid at the time of membrane rupture, or if it becomes liquid during storage, it will adsorb on to the powder particles, forming a water-repellant film around the particles.

The state of fat in powder has a major influence on wettability, i.e. the ease with which the powder particles make contact with water. Adequate wettability is a prerequisite for good dispersibility. Free fat has a water-repelling effect on the particles during dissolution, making the powder difficult to reconstitute. Clumps of fat and oily patches appear on the surface of the reconstituted powder, as well as greasy films on the walls of containers. The presence of 'free fat' on the surface of the particles tends to increase the susceptibility of fat to oxidation. A scum of fat–protein complexes may appear on the surface of reconstituted milk; the propensity to scum formation is increased by high storage temperatures.

3.15 Lipid oxidation

Lipid oxidation, leading to oxidative rancidity, is a major cause of deterioration in milk and dairy products. The subject has been reviewed by Richardson and Korycka-Dahl (1983) and O'Connor and O'Brien (1995).

Lipid oxidation is an autocatalysed free-radical chain reaction which is normally divided into three phases: initiation, propagation and termination (Figure 3.33).

The initial step involves abstracting a hydrogen atom from a fatty acid, forming a fatty acid (FA) free radical, e.g.

$$CH_3\text{----}CH_2\text{—}CH{=}CH\text{—}\dot{C}H\text{—}CH{=}CH\text{—}CH_2\text{-----}COOH$$

Although saturated fatty acids may lose a $H^{\cdot}$ and undergo oxidation, the reaction principally involves unsaturated fatty acids, especially polyunsaturated fatty acids (PUFA), the methylene, $—CH_2—$, group between double bonds being particularly sensitive:

$$C_{18:3} \gg C_{18:2} \gg C_{18:1} > C_{18:0}.$$

The polar lipids in milk fat are richer in PUFA than neutral lipids and are

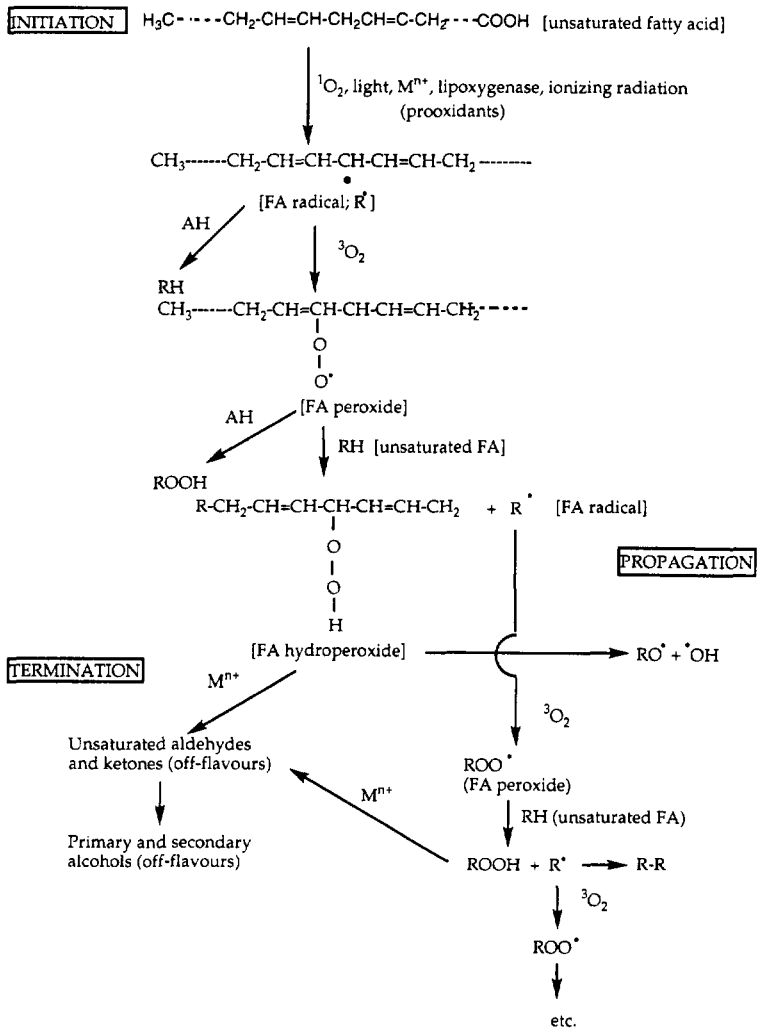


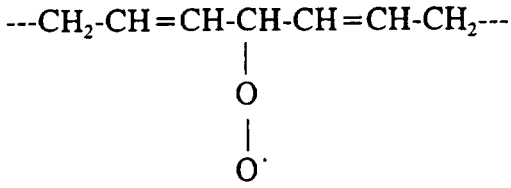
Figure 3.33 Autooxidation of fatty acids. AH, antioxidant; M^{n+}, polyvalent metal (e.g. Fe^{2+}, Cu^{2+}).

concentrated in the fat globule membrane in juxtaposition with several pro-oxidants and are, therefore, particularly sensitive to oxidation.

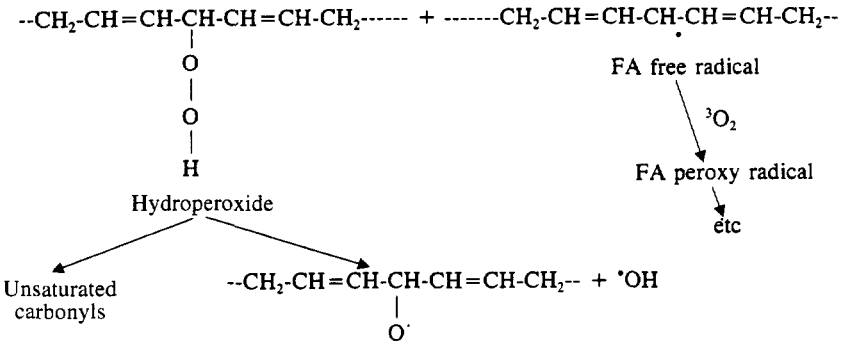
The initiation reaction is catalysed by singlet oxygen ($^1O_2$, produced by ionizing radiation and other factors), polyvalent metal ions that can undergo a monovalent oxidation/reduction reaction ($M^{n+1} \rightarrow M^n$), especially copper (the metal may be free or organically bound, for example, xanthine oxidase, peroxidase, catalase or cytochromes), or light, especially in the

presence of a photosensitizer, e.g. riboflavin (in the case of vegetable products, lipoxygenase is a major pro-oxidant but this enzyme is not present in milk or dairy products).

The FA free radical may abstract a H from a hydrogen donor, e.g. an antioxidant (AH), terminating the reaction, or may react with molecular triplet oxygen, $^3O_2$, forming an unstable peroxy radical:



In turn, the peroxy radical may obtain a H from an antioxidant, terminating the reaction, or from another fatty acid, forming a hydroperoxide and another FA free radical, which continues the reaction.



Two free radicals, each of which can initiate a new oxidation cycle

The intermediate products of lipid oxidation are themselves free radicals, and more than one may be formed during each cycle; hence the reaction is autolcatalytic, i.e. the rate of oxidation increases with time, as shown schematically in Figure 3.34. Thus, the formation of only very few (theoretically only one) free radicals by an exogenous agent is necessary to initiate the reaction. The reaction shows an induction period, the length of which depends on the presence of pro-oxidants and antioxidants.

The hydroperoxides are unstable and may break down to various products, including unsaturated carbonyls, which are mainly responsible for the off-flavours of oxidized lipids (the FA free radicals, peroxy radicals and

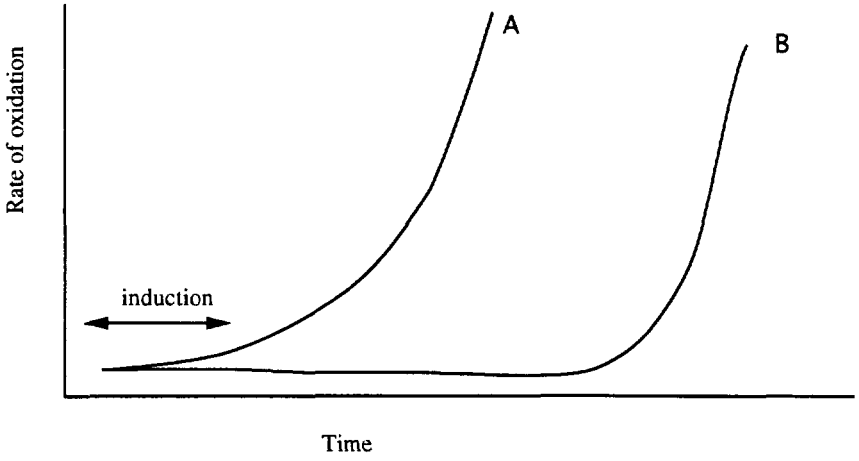


Figure 3.34 Rate of oxidation in the absence (A) or presence (B) of an antioxidant.

Table 3.14 Compounds contributing to typical oxidized flavour

Compounds	Flavours
Alkanals C_6–C_{11}	Green tallowy
2-Alkenals C_6–C_{10}	Green fatty
2,4-Alkadienals C_7–C_{10}	Oily deep-fried
3-*cis*-Hexenal	Green
4-*cis*-Heptenal	Cream/putty
2,6- and 3,6-Nonadienal	Cucumber
2,4,7-Decatrienal	Fishy, sliced beans
1-Octen-3-one	Metallic
1,5-*cis*-Octadien-3-one	Metallic
1-Octen-3-ol	Mushroom

From Richardson and Korycka-Dahl (1983).

hydroperoxides are flavourless). Different carbonyls vary with respect to flavour impact and since the carbonyls produced depend on the fatty acid being oxidized, the flavour characteristics of oxidized dairy products vary (Table 3.14).

The principal factors affecting lipid oxidation in milk and milk products are summarized in Table 3.15.

3.15.1 Pro-oxidants in milk and milk products

Probably the principal pro-oxidants in milk and dairy products are metals, Cu and to a lesser extent Fe, and light. The metals may be indigenous, e.g.

Table 3.15 Major factors affecting the oxidation of lipids in milk and dairy products[a]

A. Potential pro-oxidants
1. Oxygen and activated oxygen species
   Active oxygen system of somatic cells?
2. Riboflavin and light
3. Metals (e.g. copper and iron) associated with various ligands
   Metallo-proteins
   Salts of fatty acids
4. Metallo-enzymes (denatured?)
   Xanthine oxidase
   Lactoperoxidase, catalase (denatured)
   Cytochrome P420
   Cytochrome b_5
   Sulphydryl oxidase?
5. Ascorbate (?) and thiols (?) (via reductive activation of metals?)

B. Potential antioxidants
1. Tocopherols
2. Milk proteins
3. Carotenoids (β-carotene; bixin in anatto)
4. Certain ligands for metal pro-oxidants
5. Ascorbate and thiols
6. Maillard browning reaction products
7. Antioxidant enzymes (superoxide dismutase, sulphydryl oxidase)

C. Environmental and physical factors
1. Inert gas or vacuum packing
2. Gas permeability and opacity of packaging materials
3. Light
4. Temperature
5. pH
6. Water activity
7. Reduction potential
8. Surface area

D. Processing and storage
1. Homogenization
2. Thermal treatments
3. Fermentation
4. Proteolysis

[a]Many of these factors are interrelated and may even present paradoxical effects (e.g. ascorbate and thiols) on lipid oxidation.
Modified from Richardson and Korycka-Dahl (1983).

as part of xanthine oxidase, lactoperoxidase, catalase or cytochromes, or may arise through contamination from equipment, water, soil, etc. Contamination with such metals can be reduced through the use of stainless-steel equipment.

Metal-containing enzymes, e.g. lactoperoxidase and catalase, and cytochromes, can act as pro-oxidants owing to the metals they contain rather than enzymatically; the pro-oxidant effect of these enzymes is increased by heating (although there are conflicting reports). Xanthine oxidase, which

contains Fe and Mo, can act enzymatically and as a source of pro-oxidant metals.

Riboflavin is a potent photosensitizer and catalyses a number of oxidative reactions in milk, e.g. fatty acids, proteins (with the formation of 3-methyl thiopropanal from methionine which is responsible for light-induced off-flavour) and ascorbic acid. Milk and dairy products should be protected from light by suitable packaging and exposure to UV light should be minimized.

Ascorbic acid is a very effective anti-oxidant but combinations of ascorbate and copper can be pro-oxidant depending on their relative concentrations. Apparently, ascorbate reduces Cu^{2+} to Cu^{+}.

3.15.2 Antioxidants in milk

Antioxidants are molecules with an easily detachable H atom which they donate to fatty acid free radicals or fatty acid peroxy radicals, which would otherwise abstract a H from another fatty acid, forming another free radical. The residual antioxidant molecule (less its donatable H) is stable and antioxidants thus break the autocatalytic chain reaction.

Milk and dairy products contain several antioxidants, of which the following are probably the most important:

- Tocopherols (vitamin E), which are discussed more fully in Chapter 6. The principal function of tocopherols *in vivo* is probably to serve as antioxidants. The concentration of tocopherols in milk and meat products can be increased by supplementing the animal's diet.
- Ascorbic acid (vitamin C): at low concentrations, as in milk, ascorbic acid is an effective antioxidant, but acts as a pro-oxidant at higher concentrations.
- Superoxidase dismutase (SOD). This enzyme, which occurs in various body tissues and fluids, including milk, scavenges superoxide radicals (O_2^-) which are powerful pro-oxidants. SOD is discussed more fully in Chapter 8.
- Carotenoids can act as scavengers of free radicals but whether or not they act as antioxidants in milk is controversial.
- The thiol groups of β-lactoglobulin and proteins of the fat globule membrane are activated by heating. Most evidence indicates that thiol groups have antioxidant properties but they may also produce active oxygen species which could act as pro-oxidants under certain circumstances. The caseins are also effective antioxidants, possibly via chelation of Cu.
- Some products of the Maillard reaction are effective antioxidants.

The addition of synthetic antioxidants, e.g. β-hydroxyanisole or butylated hydroxytoluene, to dairy products is prohibited in most countries.

3.15.3 Spontaneous oxidation

Between 10 and 20% of raw individual-cow milk samples undergo oxidation rapidly while others are more stable. Milks have been classified into three categories, based on their propensity to lipid oxidation:

- Spontaneous: milks which are labile to oxidation without added Cu or Fe.
- Susceptible: milks which are susceptible to oxidation on addition of Cu or Fe but not without.
- Non-susceptible milks that do not become oxidized even in the presence of added Cu or Fe.

It has been proposed that spontaneous milks have a high content (10 times normal) of xanthine oxidase (XO). Although addition of exogenous XO to non-susceptible milk induces oxidative rancidity, no correlation has been found between the level of indigenous XO and susceptibility to oxidative rancidity. The Cu–ascorbate system appears to be the principal pro-oxidant in susceptible milk. A balance between the principal antioxidant in milk, α-tocopherol (Chapter 6), and XO may determine the oxidative stability of milk. The level of superoxide dismutase (SOD) in milk might also be a factor but there is no correlation between the level of SOD and the propensity to oxidative rancidity.

3.15.4 Other factors that affect lipid oxidation in milk and dairy products

Like many other reactions, lipid oxidation is influenced by the water activity (a_w) of the system. Minimal oxidation occurs at $a_w \sim 0.3$. Low values of a_w (< 0.3) are considered to promote oxidation because low amounts of water are unable to 'mask' pro-oxidants as happens at monolayer a_w values ($a_w \sim 0.3$). Higher values of a_w facilitate the mobility of pro-oxidants while very high values of a_w may have a diluent effect.

Oxygen is essential for lipid oxidation. At oxygen pressures below 10 kPa (≈ 0.1 atm; oxygen content $\sim 10\,mg\,kg^{-1}$ fat), lipid oxidation is proportional to O_2 content. Low concentrations of oxygen can be achieved by flushing with inert gas, e.g. N_2, the use of glucose oxidase (Chapter 8) or by fermentation.

Lipid oxidation is increased by decreasing pH (optimum ~pH 3.8), perhaps due to competition between H^+ and metal ions (M^{n+}) for ligands, causing the release of M^{n+}. The principal cause may be a shift of the Cu distribution, e.g. at pH 4.6, 30–40% of the Cu accompanies the fat globules.

Homogenization markedly reduces the propensity to oxidative rancidity, perhaps due to redistribution of the susceptible lipids and pro-oxidants of the MFGM (however, the propensity to hydrolytic rancidity and sunlight oxidized flavour (due to the production of methional from methionine in protein) is increased).

NaCl reduces the rate of auto-oxidation in sweet-cream butter but increases it in ripened cream butter (*c.* pH 5); the mechanism in unknown.

In addition to influencing the rate of lipid oxidation via activation of thiol groups and metallo-enzymes, heating milk may also affect oxidation via redistribution of Cu (which migrates to the FGM on heating) and possibly by the formation of Maillard browning products, some of which have metal chelating and antioxidant properties.

The rate of auto-oxidation increases with increasing temperature ($Q_{10} \sim 2$) but oxidation in raw and HTST-pasteurized milk is promoted by low temperatures whereas the reverse is true for UHT-sterilized products (i.e. the effect of temperature is normal). The reason(s) for this anomalous behaviour is unknown.

3.15.5 Measurement of lipid oxidation

In addition to organoleptic assessment, several chemical/physical methods have been developed to measure lipid oxidation. These include: peroxide value, thiobarbituric acid (TBA) value, ultraviolet absorption (at 233 nm), ferric thiocyanate, Kreis test, chemiluminescence, oxygen uptake and analysis of carbonyls by HPLC (see Rossell, 1986).

3.16 Rheology of milk fat

The rheological properties of many dairy products are strongly influenced by the amount and melting point of the fat present. The sensory properties of cheese are strongly influenced by fat content but the effect is even greater in butter in which hardness/spreadability is of major concern. The hardness of fats is determined by the ratio of solid to liquid fat which is influenced by: fatty acid profile, fatty acid distribution and processing treatments.

3.16.1 Fatty acid profile and distribution

The fatty acid profile of ruminant fats (milk and adipose tissue) is relatively constant due to the 'buffering' action of the rumen microflora that modify ingested lipids. However, the proportions of various fatty acids in milk lipids show seasonal/nutritional/lactational variations (Figure 3.5) which are reflected in seasonal variations in the hardness of milk fat (Figure 3.7).

The fatty acid profile can be modified substantially by feeding encapsulated (protected) polyunsaturated oils to cows. The oil is encapsulated in a film of polymerized protein or in crushed oil-rich seeds. The encapsulating protein is digested in the abomasum, resulting in the release of the unsaturated lipid, a high proportion of the fatty acids of which are then incorporated into the milk (and adipose tissue) lipids. The technical