Chapter 12

Off-Flavors in Milk

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Chapter Outline

I. INTRODUCTION

The nutritional benefits of cow's milk, described in Chapter 4, make it an important source of nutrients for infants and young adults in their daily diets. Besides milk fulfilling nutritional requirements, consumers enjoy the delicate flavor of milk and other milk-derived dairy products. The flavor of milk becomes a key parameter of product quality as acceptance is largely dependent on flavor (Drake *et al.*, 2006). Fresh milk is a rather bland product: it has a pleasant, slightly sweet aroma and flavor, and a pleasant mouth-feel and aftertaste. Since fresh milk has a very delicate flavor, any off-balance of the flavor profile can emerge into 'off-flavor' which can be easily detected by the consumer. The flavor of milk is influenced by a variety of factors involved in milk production, including the genetics of the cow, the physical and physiological condition of the cow, the type of feed consumed by the cow, the environment around the cow and the milking area, and biological, chemical and enzymatic changes in milk during production and distribution (Franklin, 1951). The flavor composition of milk is complex; at least 400 volatile compounds have been reported in milk, covering a wide range of chemical classes including lactones, acids, esters, ketones, aldehydes, alcohols, furans, carbonyls, pyrazines, sulfur compounds, and aliphatic and aromatic hydrocarbons (Moio *et al.*, 1994). The off-balance of these volatile compounds in milk, as well as the generation of some new off-flavor compounds, can cause off-flavor in milk. According to the Committee on Flavor Nomenclature and Reference Standards of the American Dairy Science Association, off-flavors in milk can be categorized into heated, light-induced, lipolyzed, microbial, oxidized, transmitted, and miscellaneous, as shown in Table 12.1 (Shipe *et al.*, 1978). The most common off-flavor issues in dairy industry, and the possible methods to minimize or eliminate these off-flavors, will be addressed in this chapter.

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TABLE 12.1 Categories of Off-Flavors in Milk

Cause	Descriptive or Associated Terms
Heated	Cooked, caramelized, scorched
Light-induced	Light, sunlight, activated
Lipolyzed	Rancid, butyric, bitter, goaty[a]
Microbial	Acid, bitter, fruity, malty, putrid, unclean
Oxidized	Papery, cardboard, metallic, oily, fishy
Transmitted	Feed, weed, cowy, barny
Miscellaneous	Flat, chemical, foreign, lacks freshness, salty

[a]*Bitter flavor may arise from a number of different causes. If a specific cause is unknown it should be classified under miscellaneous.*
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II. OFF-FLAVORS IN MILK

A. Lipolyzed Flavors

Lipolyzed flavors, one of the most common types of off-flavor in milk and dairy products, are produced by the enzymatic hydrolysis of milk fat triglycerides. This results in the accumulation of free fatty acids (FFAs) as major degradation products, mono glycerides and diglycerides, and possibly glycerol (Figure 12.1). At one time this type of flavor defect was described as 'rancid', which caused considerable confusion because of the term's association with lipid oxidation. This was eventually resolved by differentiating hydrolytic rancidity from oxidative rancidity, which more closely described 'oxidized flavors'. Heat-resistant lipases from psychrotrophic bacteria, predominantly *Pseudomonas* species, have been associated with lipolyzed flavors (Saxby, 1992).

FFAs can be derived from lipolysis, proteolysis, and lactose fermentation during cheese ripening. Both esterase and lipase have lipolytic activities, and can hydrolyze milk lipids to FFAs. Most of the FFAs with carbon chain

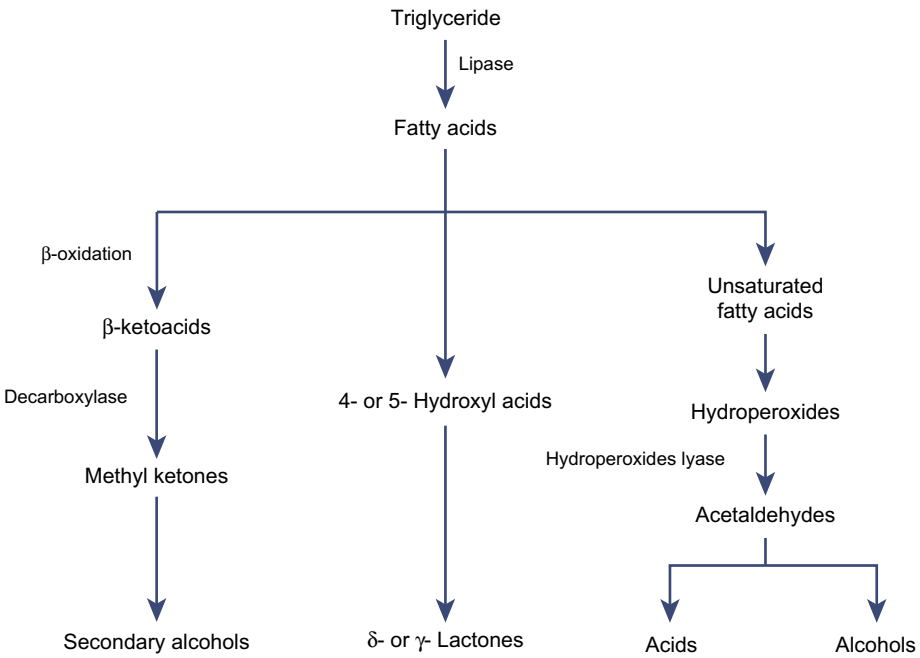

FIGURE 12.1 General pathways for the metabolism of milk triglycerides and fatty acids. *(From Singh* et al.*, 2003. With kind permission from Springer Science and Business Media.)*

lengths from C4 to C20 come from triglyceride hydrolysis by milk and microbial lipases during cheese aging. A lower proportion of FFAs with carbon chain length from C2 to C6 can come from lactose fermentation and amino acid degradation. Lactose fermentation produces acetic acid, propionic acid, and butanoic acid. Amino acid degradation, mainly catalytic deamination, can generate branched chain fatty acids such as isobutanoic and isovaleric acid (Kuzdzal-Savoie, 1980). A small amount of FFAs can also be generated from the oxidation of alcohols, aldehydes, ketones, and esters.

1. Methods for Determination of Free Fatty Acids

The determination of lipolysis is very important for evaluating milk quality. The degree of lipolysis can be measured by several methods. The acid degree value (ADV) has been widely used to monitor FFA liberation in milk, cream, and cheese (Richardson, 1985; Lin and Jeon, 1987; Ikins *et al.*, 1988). However, the method measures only the total liberated FFA by titration. It does not provide information about the concentration of individual FFAs. The ability of ADV to accurately predict rancidity in milk was questioned by a number of researchers (Duncan *et al.*, 1990, 1991) as milk with high ADV values was not always found to be rancid. This discrepancy was attributed to the difference in solubility of the fatty acids in the milk (Duncan *et al.*, 1990). ADV methods only partially recover medium-chain fat-soluble fatty acids (C10−C16) as the shorter chain fatty acids implicated in rancid flavor (C4−C12) are more hydrophilic and remain in the aqueous phase of the milk. Thus, the determination of individual fatty acid concentrations is important, particularly short-chain FFAs (between C4:0 and C12:0), as they have strong, often undesirable flavors, and are the major contributors to lipolyzed flavor in milk.

Individual FFAs can be accurately determined by gas chromatography (GC) with or without derivatization. The determination of individual FFAs by GC consists of lipid extraction, FFA isolation, and quantification (Deeth *et al.*, 1983). High extraction efficiency was achieved with acidified diethyl ether when the sample pH was brought to 1.5 with sulfuric acid (Needs *et al.*, 1983). Before the individual FFAs can be analyzed by GC, the extracted FFAs need to be separated out from fat. The separation technique needs to provide quantitative separation of FFAs from fat and have no contamination from the fat.

Among many methods used to isolate FFAs, the alkaline arrest silicic acid column method was widely used in early work (Woo and Lindsay, 1982). In this method, both FFAs and lipids were dissolved in petroleum ether−diethyl ether (80:20), and the triglycerides were passed through the KOH−silicic acid column. The FFAs were trapped by the column as the potassium salts, and eluted from the column with 2% formic acid in diethyl ether. However, not only is the preparation of KOH−silicic acid column tedious, but also the column performance lacks reproducibility. The potential for channeling in the column can greatly reduce the separation efficiency. In addition, the prolonged fat contact with KOH column can greatly increase fat hydrolysis and cause variability in the analysis.

An alumina column has been used to separate FFA from triglyceride (Deeth *et al.*, 1983; De Jong and Badings, 1990). The neutral lipids were eluted from a deactivated alumina column with diethyl ether−heptane (1:1), while FFAs were extracted with diethyl ether containing 3% formic acid. The recovery of FFA in cheese samples was high (De Jong and Badings, 1990).

Strong anion-exchange resins have also been used to isolate FFAs in milk (Needs *et al.*, 1983; Spangelo *et al.*, 1986). After the triglycerides were eluted out, the absorbed FFAs were methylated by mixing the dried resin with HCl−methanol. The methyl esters of fatty acids were extracted with diethyl ether and analyzed by GC. However, the prolonged contact with the strong basic resin can cause triglyceride hydrolysis (Needs *et al.*, 1983). The isolated FFAs are typically methylated for GC analysis. The methylation of FFAs is catalyzed by acid or base.

FFAs can be methylated and analyzed without being separated from triglycerides. Dowex strong anion-exchange resin has been used as a heterogeneous catalyst to methylate FFAs directly in milk extract (Spangelo *et al.*, 1986). The methylation was carried out in dimethylformamide, CH_3I, and pyridine at 40°C. The methyl esters were then extracted with hexane and analyzed by GC. The catalyst allows complete methylation of FFAs without potential hydrolysis of triglycerides and transesterification with other lipids. However, the reproducibility of butyric acid is very poor, owing to potential evaporation loss of methyl butyrate during methylation steps.

Tetramethylammonium hydroxide (TMAH) has been directly added to a lipid extract to convert FFAs to the tetramethylammonium soaps, the latter being transformed to methyl esters in the GC injector and analyzed by GC (Metcalfe and Wang, 1981; Martinez-Castro *et al.*, 1986; Martin-Hernandez *et al.*, 1988; Chavarri *et al.*, 1997). The methyl esters from transesterification of triglycerides were in the top ether layer, while the FFA ammonium soaps stayed in the methanol layer. After pH adjustment, the methanol layer was injected into the GC and the pyrolysis methylation was carried out in the injection port of a gas chromatograph. The method does not require FFA

isolation from triglyceride. However, the short-chain fatty acid esters from glycerol can be dissolved in the lower metholic phase and gave higher results (Chavarri *et al.*, 1997). In addition, the pyrolysis process generates excess large amounts of trimethylamine, which flashes off short-chain fatty acid esters from the splitter and alters the results (Martin-Hernandez *et al.*, 1988).

An aminopropyl weak anion-exchange column has been successfully used to isolate FFAs from lipid extract (De Jong and Badings, 1990; Chavarri *et al.*, 1997). The neutral lipids were rinsed from the column with chloroform−isopropanol (2:1), and then the FFAs were eluted with diethyl ether containing 2% formic acid. The FFAs were directly injected onto a gas chromatograph and separated by an FFAP column. This method is simple and quick, and nearly 100% recoveries were achieved with most FFAs. Hydrolysis of triglycerides and lactic acid contamination were not observed with this method. All fatty acids can be analyzed, with good repeatability. The method has also been used to analyze FFAs in cheeses (Qian and Reineccius, 2002)

Liquid chromatographic methods were developed from a protocol utilizing FFA derivatization (Garcia *et al.*, 1990). Recently, several new approaches have been reported that allow rapid quantitative analysis of short-chain FFAs. Capillary electrophoresis and indirect ultraviolet (UV) absorption (Vallejo-Cordoba *et al.*, 1998) were used for the quantification of FFAs in lipolyzed cream. Solid-phase microextraction (SPME) and GC were used to quantitatively determine short-chain FFAs in milk (Gonzalez-Cordova and Vallejo-Cordoba, 2001). In this method, FFA extraction consisted of placing 40 ml of milk containing 28% NaCl at pH 1.5 in a sealed vial and equilibrating for 30 minutes at 70°C. The FFAs were then extracted with an SPME fiber and thermally desorbed for GC analysis. Using multiple regression analysis, Gonzalez-Cordova and Vallejo-Cordoba (2003) reported a highly significant ($p < 0.001$) correlation coefficient (R^2) of 0.84 between their SPME and gas chromatographic method for detecting short-chain FFAs and rancidity scores determined by sensory evaluation in 19 commercial milks. Using this method they could detect and predict hydrolytic rancidity in milk based on the formation of short-chain fatty acids.

2. Sensory Properties of Free Fatty Acids

The sensory detection thresholds of FFAs have been investigated in both water and oil by several authors (Patton, 1964; Siek *et al.*, 1969; Urbach *et al.*, 1972; Brennand, 1989), and the reported values vary widely owing to different methods used in the studies (Table 12.2). Short-chain FFAs have higher sensory threshold values in water than in oil, possibly due to their higher solubility (and thus lower vapor pressure) in water. Milk is an emulsion or a colloid of butterfat globules, which can be considered a water-based fluid. Butanoic (C4), hexanoic (C6), and octanoic (C8) acids are often described as rancid, sweaty (body odor), goat-like, and generally unpleasant, while decanoic (C10) and dodecanoic (C12) acids are described as having soapy and waxy aromas. From butanoic acid (C4:0) to octanoic acid (C8:0), sensory thresholds in water generally increase with increasing chain length. In ordinary fresh milk, the concentrations of butanoic (C4), hexanoic (C6), octanoic (C8), decanoic (C10), dodecanoic (C12) acids are typically below their sensory thresholds (De Jong and Badings, 1990; Gonzalez-Cordova and Vallejo-Cordoba, 2001). Thus, these compounds may not contribute significantly to the overall aroma of ordinary fresh milk. However, high lipolytic activity in milk can increase the short-chain fatty acid concentration to extents that exceed their sensory thresholds, and cause lipolyzed off-flavor in milk.

B. Lipases in Milk

Bovine milk contains very high lipolytic activity, predominantly from β-type esterases. These include glycerol tricarboxyl esterases, aliphatic esterases, diesterases, and lipases (EC 3.1.1.3), with a pH optimum of 8−9. The majority of lipase activity is associated with casein, of which 70% is bound to micellar casein (Downey and Andrews 1966). This association is largely electrostatic as the enzyme can be released from the micelle by sodium chloride or heparin. The remainder of lipase is present as a soluble casein−enzyme complex in the milk serum (Hoynes and Downey, 1973; Anderson, 1982).

One particular lipase in milk is lipoprotein lipase (LPL) (EC 3.1.1.34), a glycoprotein with two N-linked oligosaccharides, which appear to be necessary for its activity (Egelrud and Olivecrona, 1972). It accounts for most, if not all, of the lipolytic activity in bovine milk. It exists as a homodimer with a molecular mass of approximately 100 kDa (Kinnunen *et al.*, 1976). LPL plays an important role in removing lipids from the blood to the mammary gland and its presence may be due to leakage from the tissue (Shirley *et al.*, 1973; Mendelson *et al.*, 1977). However, bovine milk LPL does not serve any known biological purpose in milk. LPL is relatively unstable to heat and acid, and would normally be deactivated in the stomach. High-temperature, short-time (HTST) pasteurization

TABLE 12.2 Aroma Attributes and Sensory Thresholds of the Volatile Free Fatty Acids

Compound	Aroma Attributes	Threshold (ppm)	Medium	References
Acetic acid	Vinegar, sour, pungent	22–100	Water	Patton (1964), Siek *et al.* (1971), Manning and Robinson (1973)
		0.12–7	Oil	Patton (1964), Siek *et al.* (1971), Urbach *et al.* (1972), Reiners and Grosch (1998)
Propanoic acid	Sour, pungent	20–40	Water	Amoore *et al.* (1968), Salo (1970)
Butyric acid	Rancid, cheesy, sharp	0.3–6.8	Water	Patton (1964), Amoore *et al.* (1968), Siek *et al.* (1971), Baldwin *et al.* (1973)
		0.14–3	Oil	Patton (1964), Siek *et al.* (1971), Urbach *et al.* (1972), Schieberle *et al.* (1993)
2-Methylpropanoic acid	Cheesy, rancid, caramel	0.05–8.1	Water	Salo (1970), Brennand (1989), Larsen and Poll (1992)
Pentanoic acid	Cheesy, sour, meaty, sweaty	1.1–6.5	Water	Amoore *et al.* (1968), Brennand (1989)
2-Methylbutanoic acid	Cheesy, sour, rancid, sweaty	0.07	Water	Brennand (1989)
		0.02	Oil	Reiners and Grosch (1998)
Hexanoic acid	Cheesy, goaty, sharp	0.29–27	Water	Amoore *et al.* (1968), Siek *et al.* (1971), Baldwin *et al.* (1973), Buttery (1993)
		2.5–10	Oil	Patton (1964), Siek *et al.* (1971), Urbach *et al.* (1972), Schieberle *et al.* (1993)
Heptanoic acid	Cheesy, goaty, rancid	0.28–10.4	Water	Amoore *et al.* (1968), Brennand (1989)
Octanoic acid	Cheesy, sweaty	3–19	Water	Patton (1964), Amoore *et al.* (1968), Baldwin *et al.* (1973), Buttery (1993)
		10–350	Oil	Patton (1964), Urbach *et al.* (1972)
Nonanoic acid	Fatty, green	2.4–8.8	Water	Amoore *et al.* (1968), Brennand (1989)
Decanoic acid	Soapy, waxy	1.4–10	Water	Patton (1964), Amoore *et al.* (1968), Baldwin *et al.* (1973), Buttery (1993)
		5–200	Oil	Patton (1964), Urbach *et al.* (1972)
Dodecanoic acid	Soapy, metallic	2.2–16	Water	Brennand (1989)
		700	Oil	Patton (1964), Siek *et al.* (1971), Urbach *et al.* (1972)

(72°C held for 15 seconds) can deactivate most of the enzymes in milk, hydrolyzing long- and short-chain triacylglycerols, partial glycerides, and phospholipids (Egelrud and Olivecrona, 1973; Scow and Egelrud, 1976).

Another lipase source in milk is from psychrotrophic bacteria such as *Pseudomonas*, which have a major effect on the lipolysis of milk and dairy products (Sørhaug and Stepaniak, 1997). These bacterial lipases have different characteristics from LPL. The major difference is their ability to pass through the milk fat globule membrane (MFGM) into the intact fat globules (Fitz-Gerald and Deeth, 1983). Another significant difference is that bacterial lipases are stable to HTST, and even to ultra-high-temperature (UHT) treatment (~140°C for 4 seconds) (Christen *et al.*, 1986).

1. Lipolysis of Milk

Intrinsic milk enzymes are present in sufficient amounts to cause extensive fat hydrolysis with concomitant flavor impairment (Herrington, 1954). The level of enzymes is not the critical factor in determining the susceptibility of milk

to lipolysis, but rather the MFGM, which protects the micelle triacylglycerols from lipolytic attack. In freshly secreted milk, this biological membrane is intact and forms an effective barrier around the fat. However, this protection is reduced or completely eliminated in certain situations, such as physical damage to the membrane in raw milk. Lipolysis in milk can be broadly categorized into two types: spontaneous and induced. Spontaneous lipolysis is initiated by the simple act of cooling raw milk below 10°C soon after secretion. By contrast, induced lipolysis is initiated by physical damage to the MFGM, which allows lipase access to the fat substrate (Deeth, 2006). Both spontaneous and induced lipolysis progress during storage, mostly on the first day of refrigeration (Ouattara *et al.*, 2004).

2. Microbial Generated Off-Flavors

Milk is an ideal medium for microbial growth. Thus, it is particularly important to utilize the most thorough sanitization procedures and proper cooling and holding temperatures to optimize raw milk quality on the farm. Off-flavors generally develop in processed milk when the bacterial population rises to $\geq 10^7$ cfu/ml (Schroder *et al.*, 1982). Off-flavors develop in three stages: loss of freshness; increased perception of staleness; and generation of rancid, fruity, and bitter flavors. Bitter flavors usually accompany protein degradation. Soapy and rancid flavors are generally a result of lipid breakdown (Cousin, 1982). Most microbial spoilage and associated off-flavors are due to postpasteurization contamination, generally involving psychrotrophic bacteria. Psychrotrophs are bacteria capable of growth at temperatures at ≤ 7°C (44.6°F). Rapid cooling and refrigerated storage of raw milk have favored the growth of psychrotrophic bacteria. During cold storage these bacteria dominate the flora and produce extracellular enzymes (mainly proteases and lipases), which are the major contributors to dairy spoilage (Sørhaug and Stepaniak, 1997). For example, digestion of casein by proteases can cause a bitter flavor and the gelation of milk. Lipases hydrolyze milk fat to produce FFAs, which cause milk to taste rancid, bitter, unclean, and soapy. Lecithinase degrades MFGMs and increases the susceptibility of milk fat to the action of lipases. A better understanding of bacterial-induced spoilage is needed.

Psychrotrophic bacteria from numerous genera have been isolated from milk, both Gram-negative (e.g. *Pseudomonas*, *Aeromonas*, *Serratia*, *Acinetobacter*, *Alcaligenes*, *Achromobacter*, *Enterobacter*, and *Flavobacterium* spp.) and Gram-positive (e.g. *Bacillus* (Meer *et al.*, 1991), *Clostridium*, *Corynebacterium*, *Microbacterium*, *Micrococcus*, *Streptococcus*, *Staphylococcus*, and *Lactobacillus* spp.) (Champagne *et al.*, 1994; Shah, 1994). Of these, *Pseudomonas* is the most frequently reported psychrotroph in raw milk. The growth of *Pseudomonas* strains and their production of proteases were reported as the cause of the release of plasmin and plasminogen from the casein micelle into the whey fraction (Fajardo-Lira and Nielsen, 1998; Nielsen, 2002). Eight unique milk spoilage aromas were used to differentiate milk spoiled by Pseudomonas strains: rotten; barn; shrimpy; medicinal; fruity; cheesy; cooked; and overall spoilage aroma. *Ps. fragi* was confirmed to produce fruity aromas in milk, while *Ps. putida* produced fruity, cheesy, rotten, and barn aromas. Spoilage aroma characteristics were found to be influenced not only by species, but also by fat level and time (Hayes *et al.* 2002). This suggested that the extracellular enzyme activity patterns among *Pseudomonas* isolates appeared to be associated with ribotypes (Dogan and Boor, 2003). A potential early detection system of microbial spoilage, which utilizes an electronic nose unit and 14 conducting polymer sensors, has been studied with bacteria (*Pseudomonas aureofaciens*, *P. fluorescens*, *Bacillus cereus*) and yeasts (*Candida pseudotropicalis*, *Kluyveromyces lactis*) (Magan *et al.*, 2001).

3. Proteolyzed Off-Flavors

Proteolytic enzymes degrade proteins and release a range of nitrogenous compounds. Those proteases, which attack either casein or whey proteins, result in the coagulation of milk and production of bitter flavors. While bacteria may be killed by heat treatment, certain heat-resistant enzymes produced in raw milk by psychrotrophic bacteria may cause proteolytic and lipolytic action. Proteolysis can be measured as the increase in trichloroacetic-acid-soluble free amino groups, and subsequently determined by trinitrobenzene sulfonic acid (TNBS) via colorimetric analysis (Cogan, 1977). The relationship between proteolysis measured with TNBS and off-flavor development was investigated by McKellar (1981). The study showed that this method may be used as an indicator of shelf-life as proteolysis can be detected prior to off-flavor development. Proteolysis can also be measured by the determination of tyrosine value, which has been reported to be associated with high somatic cell counts in milk (Senyk *et al.*, 1985). The reported proteolytic-derived aromatic products include *p*-cresol, methional, phenethanol, phenylacetaldehyde, 3/2-methylbutanal, and 2-methylpropanal (Dunn and Lindsay, 1985). Off-flavor associated with proteolysis can be affected by proteolytic activity, via species differences (Stead, 1986) or environmental conditions (Matselis and Roussis, 1998).

C. Oxidized Off-Flavors

Lipid oxidation influences the quality of food products through flavor and taste deterioration, as well as reduction in nutritive value. The flavors produced by oxidation of dairy products have been described as oxidized, cardboard-like, beany, green, metallic, oily, fishy, bitter, fruity, soapy, painty, rancid, grassy, buttery, and tallow-like. 'Oxidized flavor' was recommended as the generic term to describe all of these flavors (Shipe *et al.*, 1978). Lipid oxidation typically involves the reaction of molecular oxygen with unsaturated fatty acids via a free radical mechanism or light-induced oxidation, influenced by factors such as degree of unsaturation of fatty acids, presence of transition metal ions, and antioxidant tocopherols and carotenoids. The initial products of lipid oxidation, lipid hydroperoxides, are quite unstable; they can break down rapidly to produce short-chain volatile compounds such as hydrocarbons, acids, alcohols, aldehydes, and ketones, which elicit undesirable flavors.

Sensitivity to oxidation can be monitored also by measuring the antioxidative capacity, because oxidation can only occur in the case of imbalance between reactive oxidants and the antioxidant defense (Halliwell, 1996). A review by Antolovich *et al.* (2002) gave a comprehensive summary on the method for measuring the oxidative stability on antioxidative capacity, such as peroxide value, diene conjugation, thiobarbituric acid reactive substances, hexanal formation, total radical trapping antioxidant parameter, and electron spin resonance (ESR) spin-trap test. Recently, the ferric reducing antioxidant power (FRAP) and diphenyl picryl hydrazyl (DPPH) methods have been used to measure antioxidative capacity in order to monitor oxidation sensitivity in milk (Smet *et al.*, 2008). The methods showed higher sensitivity to oxidation than the more conventional peroxide value method during the first hours and days of storage.

Polyunsaturated acids, including oleic, linoleic, linolenic, and arachidonic acid are major precursors for the formation of aldehyde compounds owing to their prevalence in milk products. Table 12.3 lists the possible origins of aldehydes produced by autoxidation. In the autoxidation process, oxygen reacts with a methylene group adjacent to a double bond under catalysis of trace metals (such as copper) and enzymes (Forss, 1979), leading to the formation of hydroperoxides. These further decompose to straight-chain aldehydes.

Removal of oxygen can effectively prevent autoxidation. Flushing the sample headspace with nitrogen reduces lipid oxidation. Oxygen-scavenging packaging can lower the dissolved oxygen content in UHT milk, and thus reduce the formation of stale flavor volatiles, including methyl ketones and aldehydes, compared with milk packaged without oxygen-scavenging film (Perkins *et al.*, 2007).

Autoxidation also generates methyl ketones and hydrocarbons, although the majority of methylketones are generated through thermal breakdown of fatty acids. Methyl ketones with an odd number of carbon atoms (C7, C9, C11, C12), such as 2-pentanone, 2-heptanone, 2-nonanone, and 2-undecanone, are major ketones found in milk. Such are formed during heat treatment from the oxidation of FFAs into β-ketoacids, and the subsequent decarboxylation of these into methyl ketones (Moio *et al.*, 1993). A proposed pathway for the formation of ketones is presented in Figure 12.1, with their thresholds listed in Table 12.4.

D. Fishy Off-Flavors

The development of a fishy off-flavor, reminiscent of rotting fish, was reported in bulk milk from Red and White dairy breeds in Sweden (Lunden *et al.*, 2002a). This phenomenon, often confused with oxidized flavor, and was incorrectly thought to be associated with the formation of trimethylamine (TMA) oxide. The connection between fish odor and TMA is well established, with the olfactory threshold for detection being around 1−2 ppm (Mehta *et al.*, 1974;

TABLE 12.3 Possible Origins of Aldehyde Obtained from Specific Unsaturated Fatty Acids

Unsaturated Fatty Acid	Aldehyde Obtained
Oleic acid	Octanal, nonanal, decanal, 2-decenal, 2-undecenal
Linoleic acid	Hexanal, 2-octenal, 3-nonenal, 2,4-decadienal
Linolenic acid	Propanal, 3-hexenal, 2,4-heptadienal, 3,6-nonadienal, 2,4,7-decatrienal
Arachidonic acid	Hexanal, 2-octenal, 3-nonenal, 2,4-decadienal, 2,5-undecadienal, 2,5,8-tridecatrienal

TABLE 12.4 Aroma Attributes and Sensory Thresholds of Some Volatile Ketones

Compound	Aroma Attributes	Threshold (ppm)	Medium	References
Acetone	Acetone-like, pungent	500	Water	Manning and Robinson (1973)
		125	Oil	Siek *et al.* (1969)
2-Butanone	Acetone-like	50	Water	Wick (1966)
		30	Oil	Siek *et al.* (1969)
2-Pentanone	Floral, fruity, wine, acetone-like	2.3	Water	Siek *et al.* (1971)
		61	Butter	Siek *et al.* (1969)
2-Hexanone	Floral, fruity	0.93	Water	Siek *et al.* (1971)
2-Heptanone	Blue cheese, fruity, sweet	0.14	Water	Buttery *et al.* (1988)
		1.5–15	Butter	Siek *et al.* (1969), Preininger and Grosch (1994)
2-Octanone	Fruity, musty, unripe apple, green	2.5–3.4	Butter	Siek *et al.* (1969)
2-Nonanone	Fruity, musty, rose, tea-like	0.2	Water	Buttery *et al.* (1988)
		7.7	Cheese	Siek *et al.* (1969)
2-Decanone	Fruity, musty	0.19	Water	Siek *et al.* (1971)
2-Undecanone	Floral, herbaceous, fruity	0.007–5.4	Water	Karahadian *et al.* (1985), Buttery *et al.* (1988)
		3.4	Oil	Kubicková and Grosch (1998)

von Gunten *et al.*, 1976). TMA is oxidized by the liver enzyme, flavin-containing monooxygenase (FMO), to TMA oxide, however, it is tasteless and colorless (Hlavica and Kehl, 1977). It is the impaired oxidation of TMA, however, that results in the fishy odor phenomenon (Pearson *et al.*, 1979; Spellacy *et al.*, 1979). Feeding wheat pasture was particularly associated with the development of this fish odor/flavor problem in milk (Mehta *et al.*, 1974; von Gunten *et al.*, 1976; Kim *et al.*, 1980). Using dynamic headspace GC, Lunden *et al.* (2002a) showed that milk samples with a fish taint had $>$ 1 mg TMA/kg of milk compared to normal milk in which TMA was not detected (Figure 12.2). There appeared to be a dose-dependent relationship between TMA levels and the development of a fishy off-flavor score.

Fishy odor or trimethylaminuria is an autosomal recessive inborn error of metabolism in humans in which there is an abnormal secretion of TMA in breath, urine, sweat, saliva, and vaginal secretions. This phenomenon appears to be due to impaired oxidation of TMA resulting from loss-of-function mutations in the *FMO3* gene encoding the isoform of flavin-containing monooxygenase (Dolphin *et al.*, 1997; Treacy *et al.*, 1998; Ackerman *et al.*, 1999; Basarab *et al.*, 1999; Forrest *et al.*, 2001). Lunden *et al.* (2002b) showed that this phenomenon in cow's milk was due to the nonsense mutation (R238X) in bovine *FM03* gene ortholog. The R238X substitution was not found in Swedish Holstein, Polled, or Jersey cows, but was surprisingly common in the Swedish Red and White breeds.

E. Light-Induced Off-Flavors

The primary factor responsible for the development of light-induced–oxidized flavor in milk was shown by Aurand *et al.* (1966) to be riboflavin, with ascorbic acid playing a secondary role. Riboflavin acts as a photosensitizer in milk by accelerating the oxidation of amino acids, DNAs, and unsaturated fatty acids (Choe *et al.*, 2005). The use of fluorescent lights to illuminate dairy display cases is responsible for the flavor deterioration and loss of nutrient quality in milk (Bradley, 1980; Dimick, 1982; Hoskin and Dimick, 1979; Sattar and deMan, 1975). In particular, the effects of 'white' fluorescent light, in widespread use in supermarkets, with a spectral output of 350–750 nm and peaks at 470 and 600 nm, are shown in Figure 12.3. The radiant energy emitted by the fluorescent light is absorbed by and interacts with milk components such as riboflavin (Dunkley *et al.*, 1962). When exposed to light, riboflavin forms singlet oxygen and superoxide anions from triplet oxygen (Jernigan, 1985; Bradley and Min, 1992;

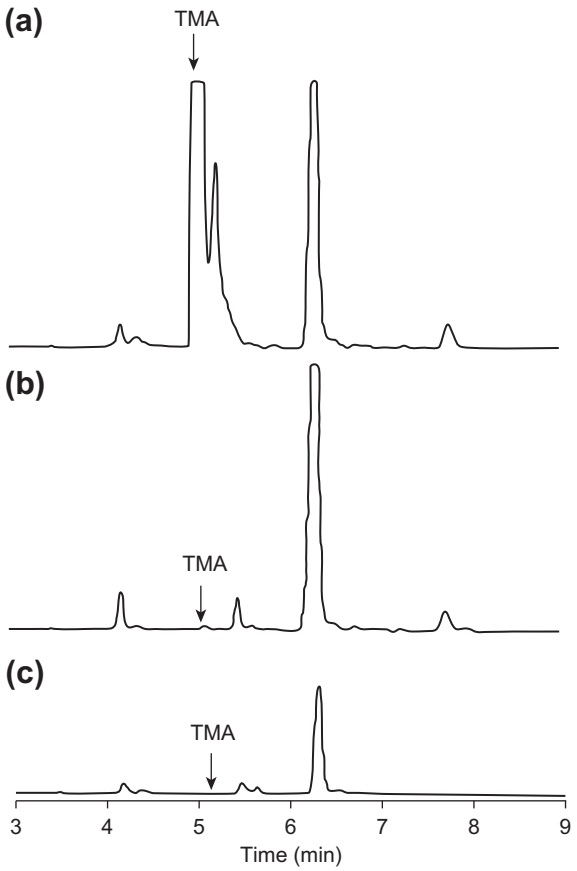

FIGURE 12.2 Gas chromatography–flame ionization detection chromatogram of: (a) a milk sample with strong fishy off-flavor; (b) normal Swedish milk; and (c) Swiss UHT reference milk. Sample treatment using a purge-and-trap system (dynamic headspace analysis) and gas chromatography with flame ionization detection. TMA: trimethylamine *(Lunden* et al.*, 2002a).*

Naseem *et al.*, 1993). The mechanism of activation has been extensively studied and involves several mechanisms, referred to as type I and type II (de la Rochette *et al.*, 2003). Excitation by light leads to the formation of a riboflavin triplet active state, a diradical (Choe *et al.*, 2005). The type I mechanism involves the formation of free radicals by hydrogen or electron transfer between the riboflavin triplet activated state (^{1}RF*) and substrates (Edwards and

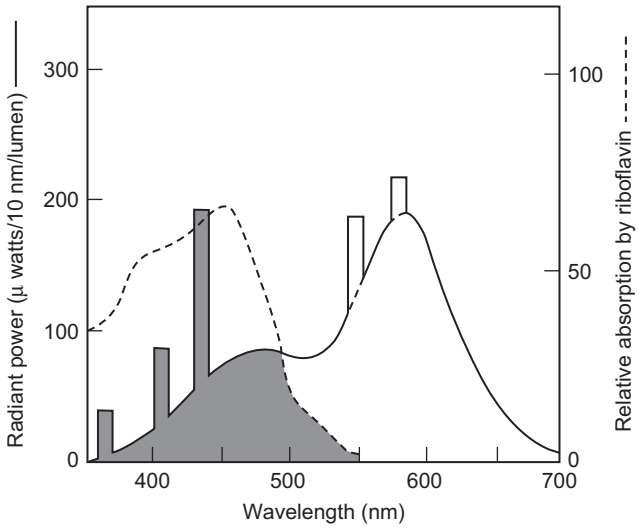

FIGURE 12.3 Emission spectra of a cool white fluorescent lamp compared with absorption of riboflavin *(Dunkley* et al.*, 1962).*

Silva, 2001). Type II involves the formation of singlet oxygen ($^1O_2$*) by energy transfer from ^{1}RF* to molecular oxygen (Boff and Min, 2002). Both of these mechanisms are outlined in Figure 12.4 (Choe *et al.*, 2005).

Riboflavin radicals are very strong oxidizing species, so that light-induced off-flavor (LIOF) development in milk is dependent on the availability of oxygen and ultraviolet light. LIOFs are created through photosensitization of lipids and amino acid sulfur groups, dependent on the wavelength and intensity of the light, exposure time, product temperature, and light-transmission properties of the container. The two major distinctive off-flavors in milk induced by irradiation of light energy are sunlight and cardboard flavor. Sunlight flavor refers to a burnt and oxidized odor in milk after light exposure for more than 2 days. Methionine was first implicated in the formation of LIOF by Patton and Josephson (1953) by the formation of dimethyl disulfide (DMDS) and methional. The mechanism involved in the formation of LIOF is somewhat controversial. Jung *et al.* (1998) reported that singlet oxygen, formed from triplet oxygen under sunlight in the presence of riboflavin in milk, reacts with methional, forming the hydroperoxide. Figure 12.5 shows that the hydroperoxide decomposes to form methional and thiomethyl radicals, with the latter producing dimethyl sulfide (DMS) (Choe *et al.*, 2005). The formation of DMDS was highly correlated with the LIOF sensory scores (Jung *et al.*, 1998). Because ascorbic acid is a good quencher of singlet oxygen, its presence reduces the formation of DMDS. Cardboard-like or metallic flavors, which develop in milk through prolonged light exposure, are caused by secondary lipid oxidation products including aldehydes, ketones, alcohols, and hydrocarbons

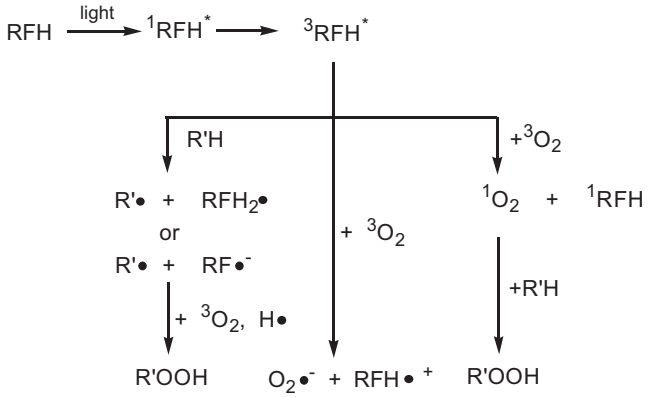

FIGURE 12.4 Photosensitization of riboflavin and type I and type II mechanisms *(Choe* et al., *2005).*

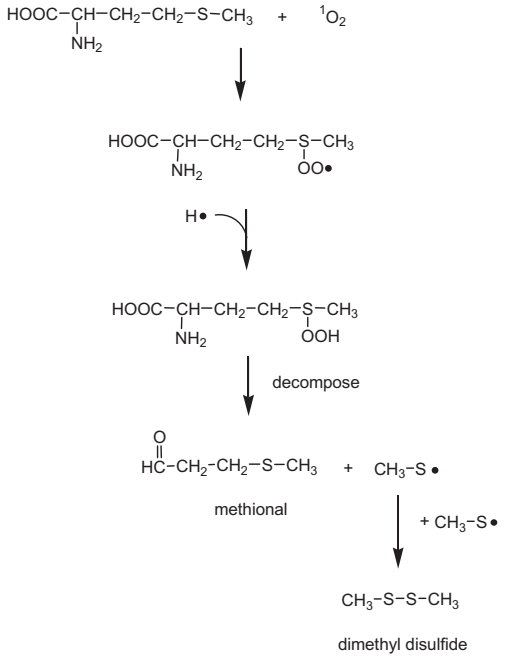

FIGURE 12.5 Oxidation of methionine by singlet oxygen *(Choe* et al., *2005).*

(Gaafar and Gaber 1992). Dynamic headspace analysis has been used to evaluate the volatile compounds hexanal, pentanal, DMDS, 2-butanone, and 2-propanol (Kim and Morr, 1996).

Packaging can directly prevent the development of LIOF by protecting the product from both light and oxygen. All plastic containers, such as polyethylene terephthalate (PET) and high-density polyethylene (HDPE), can provide very good convenience through easy opening and reclosing, minimizing recontamination. Pigmented PET bottles have excellent mechanical properties and offer good oxygen and light protection. Pigmented HDPE bottles, both monolayer and multilayer, with a greater thickness than current PET, are more suitable for the fresh milk packaging market (Cladman *et al.*, 1998). Chemical and sensorial aspects were studied in low-fat pasteurized milk bottled by various packaging materials (Moyssiadi *et al.* 2004). Multilayer TiO_2-pigmented HDPE, monolayer HDPE, clear PET, and pigmented PET were compared with paperboard cartons for a period of 7 days. The result showed that all packaging materials with regard to microbiological and chemical parameters provided good protection of milk flavor over the test period. The multilayer provided the best overall protection for the product, followed by the monolayer HDPE bottle. The degradation of volatiles by chemical reactions could explain these results, such as oxidation caused by oxygen diffusion and light transmission into the packaging. In the authors' unpublished work, samples of 2% pasteurized milk were packaged in three different treated bottles: translucent HDPE bottles stored in the dark, translucent 7HDPE bottles stored under fluorescent light, and HDPE bottles coated with light-blocking pigment stored under fluorescent light, in order to determine the cause of oxidation. Dynamic headspace analysis clearly demonstrated that translucent HDPE bottled milk stored under fluorescent light had much higher concentrations of hexanal, heptanal, and octanal than the milk stored under dark or in UV-blocking bottles (authors' unpublished data). The hexanal level in translucent HDPE bottled milk was five times higher than in those stored in dark or light-blocking bottles (Figure 12.6). This trend was also observed in cream cheese. The surface of cream cheese had much higher aldehyde formation than the center of the cream cheese (Figure 12.7) owing to poor packaging protection (authors' unpublished data). A similar study was conducted to assess the sensory differences in milk packaged with different materials (Boccacci Mariani *et al.*, 2006). No off-flavor was found in milk packaged in the paperboard during the storage period studied; however, a taint off-flavor was found in the PET-bottled milk (due to light-induced oxidative changes) after 1−2 days of storage (as assessed by the trained panel) and 2−3 days of storage (as assessed

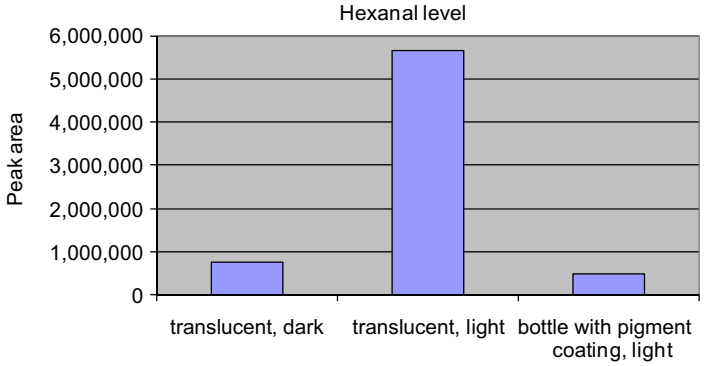

FIGURE 12.6 Hexanal level in milk stored in different bottles *(Qian* et al.*, unpublished data).*

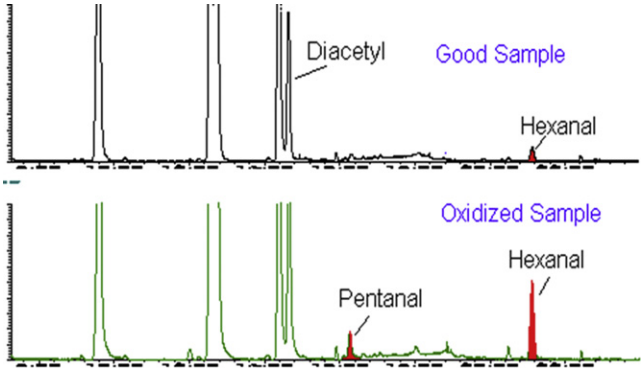

FIGURE 12.7 Gas chromatogram showing the oxidation of cream cheese *(Qian* et al.*, unpublished data).*

by consumers). In addition to offering microbial and light protection, the packaging material can absorb aroma or off-aroma from the food matrix; this scalping property may help to maintain product quality, depending on the product. Polyethylene packaging has much stronger adsorption to lactones, aldehydes, and FFAs in UHT milk compared with glass bottles (Czerny and Schieberle, 2007).

Many consumers, however, prefer packaging material that allows them to see the food they are purchasing (Sattar *et al.*, 1976; Rosenthal, 1992; Cladman *et al.*, 1998; Doyle, 2004). Therefore, milk is still packaged in HDPE or PET containers which transmit between 62% and 85% of light with wavelengths between 300 and 700 nm. UV absorbers, such as iridescent films, can be added to polymer packaging materials to block UV wavelengths without affecting the clarity of the packaging material. Webster *et al.* (2009) demonstrated the ability of these films to protect against the development of LIOF in 2% milk exposed to visible excitation wavelengths of riboflavin (400, 446, and 570 nm). It was evident from their research, however, that blocking the transmission of all riboflavin excitation wavelengths was insufficient to completely protect against the development of LIOF. This suggested the presence of other components in milk, such as chlorins and porphyrins, identified previously by Wold *et al.* (2005) in the photooxidation of cheese.

F. Heat-Induced Off-Flavors

Thermal treatment can destroy spoilage bacteria and deactivate enzymes, increasing the shelf-life of milk. The most popular thermal treatments, pasteurization and UHT, develop new volatile compounds which bring desirable and undesirable flavors, affecting taste and texture. The main volatile compounds contributing to heated flavor are Maillard reaction products. Thermal degradation of lipids generates stale or oxidized flavor during storage. Thermal reactions involving amino acid side-chains generate sulfur compounds which are responsible for off-flavor in UHT milk. Other thermal-induced reactions such as hydrolysis of peptide bonds, dephosphorylation of proteins, and the interaction of lipid oxidation and Maillard reaction, may also affect the flavor to some extent. Heat treatments, particularly UHT processing, can promote the development of thermally derived off-flavor compounds such as aldehydes, methyl ketones, and various sulfur compounds (Scanlan *et al.*, 1968; Jeon *et al.*, 1978; Moio *et al.*, 1994; Contarini *et al.*, 1997; Contarini and Povolo 2002).

Contarini and Povolo (2002) studied the effect of heat treatments on volatile compounds in commercially processed milk samples using headspace SPME and GC. They identified 11 compounds, five of which (2-pentanone, 2-heptanone, 2-nonanone, benzaldehyde, and 2-undecanone) exhibited a correlation with the severity of the heat treatment. Vazquez-Landaverde *et al.* (2005) quantified some volatile flavor compounds in milk subjected to different heat treatments. Concentrations of ketones were not different in raw and pasteurized milk samples; however, their concentrations were markedly higher in UHT milk. At the same fat level, UHT milk contained approximately 12 times the amount present in raw and pasteurized milk. The major contributors were 2-heptanone and 2-nonanone, followed by 2,3-butanedione, 2-pentanone, and 2-undecanone. The concentrations of 2-heptanone and 2-nonanone in UHT milk were 34 and 52 times higher, respectively, than in raw and pasteurized samples. The odor activity values (OAVs; ratio of concentration to sensory threshold) for 2-heptanone and 2-nonanone were less than 1 in raw and pasteurized milk, indicating that they were not important aroma contributors. However, the OAVs in UHT milk were in the range of 4−10, suggesting that these compounds could be very important contributors to the aroma of heated milk. Contarini *et al.* (1997) and Contarini and Povolo (2002) reported that the concentrations of 2-pentanone, 2-hexanone, 2-heptanone, 2-nonanone, and 2-undecanone increased in direct proportion to the severity of the heat treatment and were associated with the development of stale-heated flavor in UHT milk. Moio *et al.* (1994) identified 2-heptanone and 2-nonanone as the most abundant volatile flavor compounds in UHT milk.

Although methyl ketones are naturally present in raw milk, they can be formed during heat treatment by β-oxidation of saturated fatty acids followed by decarboxylation (Nawar, 1996). Milk fat contains 10% (w/w) of C6, C8, C10, and C12 fatty acids, which are precursors for odd-carbon-numbered C5, C7, C9, and C11 methyl ketones produced during heat treatment. Fat content seems to have an impact on the concentration of methyl ketones in UHT milk, where their concentration in 3% fat milk was found to be almost double that found in 1% fat milk (Vazquez-Landaverde *et al.*, 2005). Methyl ketones can also be formed through direct decarboxylation of β-ketoacids present in raw milk. Milk fat contains approximately 1% lipids in which oxo fatty acids of various chain lengths are esterified to glycerol. These oxo fatty acids can be liberated as β-ketoacids and decarboxylated to C6−C16 methyl ketones when the fat is heated in the presence of water (Grosch, 1982; Jensen *et al.*, 1995).

Vazquez-Landaverde *et al.* (2005) found that the concentration of 2,3-butanedione in UHT milk was higher than in raw milk, while its concentration varied widely in pasteurized milk. The OAV was higher than 1 for UHT and

pasteurized milk, suggesting that 2,3-butanedione is contributing to the aroma of heated milk. 2,3-Butanedione has been reported as a very important flavorant contributing to the rich 'heated' note in UHT milk, giving a buttery, pastry-like aroma (Scanlan *et al.*, 1968). Although its formation has been suggested to be heat induced (Scanlan *et al.*, 1968), it is also attributed to microbial activity in milk (Badis *et al.*, 2004), therefore being an ambiguous indicator for the heat treatment.

Aldehyde concentrations are also affected by heat treatment. The aroma contributors and sensory thresholds are summarized in Table 12.5. All these volatiles are associated with green, grass-like odors; the shorter chain aldehydes are also considered to be pungent and malt-like, and the longer chain aldehydes have more fatty notes. According to Vazquez-Landaverde *et al.* (2005), UHT milk had higher concentrations of total aldehydes than raw and pasteurized milk. Hexanal, octanal, and nonanal showed a higher concentration in 3% fat UHT milk while 2-methylpropanal, 3-methylbutanal, 2-furaldehyde, heptanal, and decanal concentrations were higher for both 1% and 3% fat UHT milk. The total aldehyde concentration was not different between raw and pasteurized milk. Based on

TABLE 12.5 Aroma Attributes and Sensory Thresholds of Some Volatile Aldehydes

Compound	Aroma Attributes	Threshold (ppm)	Medium	References
Acetaldehyde	Pungent, fruity penetrating	0.025	Water	Guth and Grosch (1994)
		0.0002	Oil	Buttery *et al.* (1995)
Propanal	Pungent, acrid, solvent	0.037	Water	Boelens and Van Gemert (1987)
		0.009	Oil	Reiners and Grosch (1998)
2-Methylpropanal	Malty, cocoa, green, pungent	0.002	Water	Amoore *et al.* (1976)
2-Methylbutanal	Cocoa, coffee, almond, malty	0.002–0.14	Oil	Guadagni *et al.* (1972), Reiners and Grosch (1998)
3-Methylbutanal	Malty, cocoa	0.013	Oil	Guadagni *et al.* (1972), Kubicková and Grosch (1998)
Butanal	Pungent, malty, green	0.018	Water	Boelens and Van Gemert (1987)
Pentanal	Malty, apple, green	0.012–0.07	Water	Siek *et al.* (1971), Buttery *et al.* (1988)
Hexanal	Grassy, green, tallow	0.009–0.05	Water	Ahmed *et al.* (1978), Larsen and Poll (1992)
		0.19–0.3	Oil	Siek *et al.* (1971), Guth and Grosch (1990)
(E)-2-Hexenal	Green, sweet, leafy, apple	0.017–0.05	Water	Ahmed *et al.* (1978), Buttery *et al.* (1988), Larsen and Poll (1992)
		0.42	Oil	Reiners and Grosch (1998)
Heptanal	Fatty, green, woody, fruity	0.031–0.25	Oil	Siek *et al.* (1971), Guadagni *et al.* (1972)
Octanal	Fatty, citrus	0.001	Water	Ahmed *et al.* (1978), Buttery *et al.* (1988)
		0.056	Oil	Reiners and Grosch (1998)
Nonanal	Citrus, green, fatty, floral	0.002	Water	Ahmed *et al.* (1978)
		1	Oil	Siek *et al.* (1969)
Decanal	Waxy, floral, citrus	0.002	Water	Ahmed *et al.* (1978), Boelens and Van Gemert (1987)
Dodecanal	Citrus, powerful	0.0005–0.002	Water	Ahmed *et al.* (1978), Boelens and Van Gemert (1987)
Furfural	Sweet, almond, penetrating	3	Water	Guadagni *et al.* (1972)
Phenyl acetaldehyde	Floral, hyacinth, green	0.002	Water	Whetstine *et al.* (2005)

their OAVs, nonanal and decanal appeared to be important contributors to the aroma of raw, pasteurized and UHT milk, while octanal, hexanal, 2-methylbutanal, 3-methylbutanal, and 2-methylpropanal were important only for UHT milk aroma. Contarini and Povolo (2002) found that 3-methylbutanal increased with the heat treatment severity, while hexanal and heptanal did not. The presence of 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal in heated milk is due to the Strecker degradation of amino acids during Maillard reactions (Damodaran, 1996). Hexanal, heptanal, octanal, nonanal, and decanal result from the autoxidation of unsaturated fatty acids (C18:1 and C18:2) and also the spontaneous decomposition of hydroperoxides promoted by heat (Grosch, 1982). Hexanal can also be transferred to milk from cow's feed (Scanlan *et al.*, 1968) or originate from light-induced lipid oxidation (Marsili, 1999). Rerkrai *et al.* (1987) stated that the increase in C2 and C7−9 saturated aldehyde concentration is the main cause of the stale flavor in UHT milk, due to their low flavor thresholds. 2-Furaldehyde has been found in UHT milk (Vazquez-Landaverde *et al.*, 2005), but the OAV was too low to be considered an important contributor to milk aroma. However, it is considered a good indicator of the heat treatment because it is the precursor of melanoidins in Maillard reactions between sugars and the free amino group of milk proteins or amino acids (BeMiller and Whistler 1996).

Ethyl acetate has been found to increase in concentration up to 10 times in UHT milk compared to raw milk (Vazquez-Landaverde *et al.*, 2005). It has been reported that ethyl acetate is formed by esterification of ethanol and acetic acid via the Fischer reaction, catalyzed by heat (Hart, 1991). However, its very low OAV in the samples analyzed suggest that this compound is not an important contributor to the aroma of milk.

Thermal processing can generate a cooked, sulfurous, cabbage-like off-flavor in milk (Shipe, 1980). Researchers have identified that hydrogen sulfide (H_2S), methanethiol (MeSH), carbon disulfide (CS_2), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) are related to this cooked off-flavor defect (Shipe, 1980; Christensen and Reineccius, 1992; Simon and Hansen, 2001; Datta *et al.*, 2002). Several other sulfur compounds, including benzothiazole, dimethyl sulfoxide (DMSO), dimethyl sulfone (Me_2SO_2), carbonyl sulfide (COS), sulfur dioxide, butanethiol, and thiophene, have been found in heated milk, but their roles in milk flavor have not been well elucidated (Scanlan *et al.*, 1968; Shibamoto *et al.*, 1980; Shipe, 1980; Moio *et al.*, 1994).

The concentration of sulfur compounds in milk is related to the severity of heat treatment of the milk. Vazquez-Landaverde *et al.* (2006a) found that UHT milk contained significantly higher concentrations of H_2S, MeSH, CS_2, DMTS, and DMSO than raw and pasteurized milk. H_2S was the sulfur compound with the highest increase in concentration, up to seven times. MeSH increased about five times, and the DMSO concentration increased almost three times more in UHT than in raw milk (Vazquez-Landaverde *et al.*, 2005).

Sulfur compounds have a very low sensory threshold. Calculated OAVs suggest that many of the sulfur compounds are very important contributors to the flavor of both heated and fresh milk. OAV values for MeSH and DMTS in UHT milk samples were much greater than 1, thus contributing to the aroma. According to the magnitude of its OAVs, MeSH could be the most important sulfur-containing contributor to the aroma of UHT milk, in which its concentration was 80−119 times higher than its reported threshold. It has a strong and unpleasant cabbage, sulfur-like aroma (Rychlik *et al.*, 1998). A correlation has been found between the increase in concentration of this compound and the increase in 'cooked' flavor defect due to the heat treatment of milk (Badings *et al.*, 1981; Christensen and Reineccius, 1992; Simon and Hansen, 2001). MeSH is thought to be liberated during heat treatment from methionine, by breakdown of the sulfur-bearing side-chain (Damodaran, 1996), but the actual pathway has not been well elucidated. Despite the importance of MeSH to the flavor of milk and dairy products, its study has been limited owing to its high reactivity and volatility. Only a few researchers have reported reliable quantification techniques for this compound (Burbank and Qian, 2005; Fang and Qian, 2005).

The concentration of H_2S in milk increases linearly with the intensity of heating (Hutton and Patton, 1952; Christensen and Reineccius, 1992). In addition, the log of the concentration of H_2S has a strong linear relationship with the heated flavor intensity of milk (Badings, 1978). This compound is also indirectly responsible for the formation of other sulfur compounds (Zheng and Ho, 1994). H_2S is produced mainly from temperature-activated sulfhydryl groups of sulfur-containing amino acids (cysteine) in β-lactoglobulin (Badings *et al.*, 1981; Damodaran, 1996), which are then oxidized, forming H_2S. It has been suggested that H_2S could be the most important contributor to the 'cooked' flavor of milk because it is the major sulfur compound formed in heated milks (Badings *et al.*, 1978; Jaddou *et al.*, 1978; Badings *et al.*, 1981; Rerkrai *et al.*, 1987; Christensen and Reineccius, 1992) and it also has a characteristic sulfur-like aroma (Rychlik *et al.*, 1998). However, Vazquez-Landaverde *et al.* (2006a) showed that H_2S concentration in UHT milk was only slightly higher than its reported threshold, and calculated OAV values indicate that H_2S could be less important to the aroma of heated milk than previously thought.

Although DMS is present naturally in raw milk (Toso *et al.*, 2002), it can also be formed from the sulfhydryl group of milk proteins subjected to thermal denaturation (Datta *et al.*, 2002). The formation of DMDS is probably due to the oxidation of MeSH (Ferreti, 1973; Chin and Lindsay, 1994). Jaddou *et al.* (1978) reported that DMDS concentration increased in UHT milk, but decreased in sterilized samples. DMDS has a sulfur- and cabbage-like aroma (Rychlik *et al.*, 1998), with low threshold values. DMTS has a low concentration in milk, but it also has a very low sensory threshold, indicating that this compound could also be a contributor to the sulfurous aroma in heated milk, although it may not be as important as MeSH.

Although its origin has not been well elucidated, carbon disulfide (CS_2) has been identified as a product of the breakdown of other sulfur compounds (Urbach, 1993). Since there is correlation between CS_2 and heat treatments (Vazquez-Landaverde *et al.*, 2006a), this compound could be a good indicator of the heat treatment. It has a sweet, ethereal, slightly green, sulfur-like aroma (Rychlik *et al.*, 1998). Because of its high sensory threshold, it probably will not contribute to milk flavor.

It has been proposed that dimethyl sulfone ($DMSO_2$) is produced in milk by the heat-induced oxidation of DMS via DMSO as the intermediate (Shibamoto *et al.*, 1980). Under oxidant conditions, methionine is easily oxidized to methionine sulfoxide and methionine sulfone, which eventually breakdown to yield DMSO and $DMSO_2$, respectively (Damodaran, 1996). Shibamoto *et al.* (1980) found that $DMSO_2$ concentration decreases when milk is subjected to treatments between 60 and 90°C, but it starts to increase considerably at temperatures above 90°C. Moio *et al.* (1994) found that $DMSO_2$ concentration was lower for UHT milk than that for raw and pasteurized samples. $DMSO_2$ has an aroma defined as being like hot milk, leather, and bovine sweat (Rychlik *et al.*, 1998).

A general trend has been observed that the concentration of H_2S, MeSH, and DMTS in heated milk increases with the fat content in milk (Vazquez-Landaverde *et al.*, 2006a), suggesting that the heat-induced formation of sulfur compounds in milk is affected by fat level, but the mechanism is not clear. It was proposed by de Koning *et al.* (1990) that the membrane proteins of the milk fat globules contributed to the formation of sulfides.

Lactones could be important contributors to the flavor of heated milk. Lactones are cyclic esters that usually have pronounced fruity aromas associated with peaches, apricots, and coconut (Table 12.6). From γ-hexalactone to γ-dodecalactone, or δ-hexalactone to δ-dodecalactone, their sensory thresholds in water generally decrease with increasing chain length. Lactones can be formed in the ruminant mammary gland from the hydrolysis of saturated fatty acids and subsequent cyclization of free hydroxyacids (Dumont and Adda, 1978), and are therefore present in very small amounts in fresh, unheated milk. These compounds can be formed during heat treatments from the thermal breakdown of γ- and δ-hydroxyacids through intramolecular esterification of hydroxyacids, where the loss of water results in ring formation (Fox *et al.*, 2000). Recent work by the authors of this chapter demonstrated that lactone concentrations are much higher in UHT milk than in pasteurized milk at the same fat content (Figure 12.8).

G. Non-Thermal Processing and Off-Flavor Formation

New processing technology is needed to increase the shelf-life of milk without compromising its natural flavor. Thermal processing is the prevailing method to achieve microbial safety and shelf-life stability of milk. Although HTST pasteurization of milk (typically at 72°C for 15 seconds) is acceptable to most consumers, the process does impart a slight cooked, sulfurous note, and the final product shelf-life is only 20 days at refrigeration temperatures. UHT processing (135−150°C for 3−5 seconds) produces a product that is stable at room temperature for up to 6 months; however, this process can induce strong 'cooked' off-aroma notes in milk (Shipe, 1980), thus limiting its marketing in the USA and many other countries (Steely, 1994).

Promising non-thermal methods including membrane filtration, high-pressure processing (HPP), and pulsed electric field treatment are used to achieve a microbial shelf-life similar to that of UHT milk while minimizing the generation of off-flavor compounds. To retain the 'fresh' milk flavor, HPP has been studied as an alternative to the pasteurization of milk. A similar microbiological reduction to that of pasteurized milk has been achieved using pressure treatments of 400 MPa for 15 minutes or 500 MPa for 3 minutes at room temperature (Rademacher and Kessler, 1996). At moderate temperature (55°C), HPP (586 MPa for 5 minutes) can significantly extend the shelf-life of milk to 45 days, which is beyond that of pasteurized milk (Tovar-Hernandez *et al.*, 2005). Although it is generally assumed that HPP at low temperature will not change the aroma or flavor of the product (Cheftel, 1995; Berlin *et al.*, 1999; Velazquez *et al.*, 2002), HPP under certain conditions has been reported to change the concentration of some important flavor compounds. Hofmann *et al.* (2005) reported that HPP could influence the formation of Maillard-derived compounds in a sugar−amino acid model solution. In another study using milk, Vazquez-Landaverde *et al.*

TABLE 12.6 Aroma Attributes and Sensory Thresholds of Some Major Lactones

Compound	Aroma Attributes	Threshold (ppm)	Medium	References
γ-Hexalactone	Coconut, fruity, sweet	1.6–13	Water	Siek *et al.* (1971), Engel *et al.* (1988)
		8	Oil	Siek *et al.* (1971)
γ-Heptalactone	Coconut, fruity, nutty	0.52	Water	Siek *et al.* (1971)
		3.4	Oil	Siek *et al.* (1971)
δ-Octalactone	Coconut, animal	0.4–0.57	Water	Siek *et al.* (1971), Engel *et al.* (1988)
		0.1–3	Oil	Siek *et al.* (1971), Urbach *et al.* (1972)
γ-Octalactone	Coconut, fruity	0.095	Water	Siek *et al.* (1971)
		3.5	Oil	Siek *et al.* (1971)
γ-Nonalactone	Coconut, peach	0.065	Water	Siek *et al.* (1971)
		2.4	Oil	Siek *et al.* (1971)
δ-Decalactone	Coconut, apricot	0.1–0.16	Water	Siek *et al.* (1971), Urbach *et al.* (1972), Engel *et al.* (1988)
		0.4–1.4	Oil	Siek *et al.* (1971), Preininger *et al.* (1994)
γ-Decalatone	Coconut, apricot, fatty	0.005–0.09	Water	Siek *et al.* (1971), Engel *et al.* (1988), Larsen *et al.* (1992)
		1	Oil	Siek *et al.* (1971)
δ-Dodecalactone	Fresh fruit, peach	0.1–1	Water	Siek *et al.* (1971)
		0.12–10	Oil	Siek *et al.* (1971), Schieberle *et al.* (1993)
γ-Dodecalactone	Peach, butter, sweet, floral	0.007	Water	Engel *et al.* (1988)
		1	Oil	Urbach *et al.* (1972)

(2006b) found that pressure, temperature, and time, as well as their interactions, all had significant effects ($p < 0.001$) on off-flavor generation in milk. Pressure and time effects were greatest at 60°C, while their effects were almost negligible at 25°C. It was observed that the off-flavor generation of pressure-heated samples at 60°C was different from that of heated-alone samples. Heat treatment at 60°C tended to promote mostly the formation of methanethiol, H_2S, and methyl ketones, while high-pressure treatment at the same temperature mostly formed H_2S and aldehydes such as hexanal and octanal. The results demonstrated that the off-flavor generation at high pressure and moderate temperature was different from that under atmospheric pressure conditions.

Although the actual formation mechanisms under high pressure are not known, oxygen becomes more soluble under high pressure, therefore potentially increasing the formation of hydroperoxides and leading to more aldehyde generation. It is also possible that high pressure affects the kinetics of volatile formation. According to Le Chatelier's principle (Galazka and Ledward, 1996), if the formation of hydroperoxides from oxygen and fatty acids involves equilibrium reactions with a volume reduction, high pressure will favor this reaction and thus lead to more aldehyde generation. Another highly likely possibility is that the hydrostatic pressure affects the rate of formation according to its reaction activation volume (ΔV^*) defined as the difference between the partial molar volume of the transition or activated state and that of the reactant at the same temperature and pressure (McNaught and Wilkinson, 1997). When pressure is applied, $\Delta V^* < 0$ leads to an increase in reaction rate, whereas $\Delta V > 0$ has the opposite effect. The sensitivity of a chemical reaction to pressure will increase with the absolute value of ΔV^* (Mussa and Ramaswamy, 1997). The formation of H_2S seems to be affected by both pressure and holding time (Vazquez-Landaverde *et al.*, 2006b). A dramatic increase in H_2S was observed under high-pressure treatments even at 25°C. The concentration of MeSH also increased at 25°C under high pressure. However, when the pressure increased to 620 MPa, the concentration of MeSH decreased (Vazquez-Landaverde *et al.*, 2006b). Although

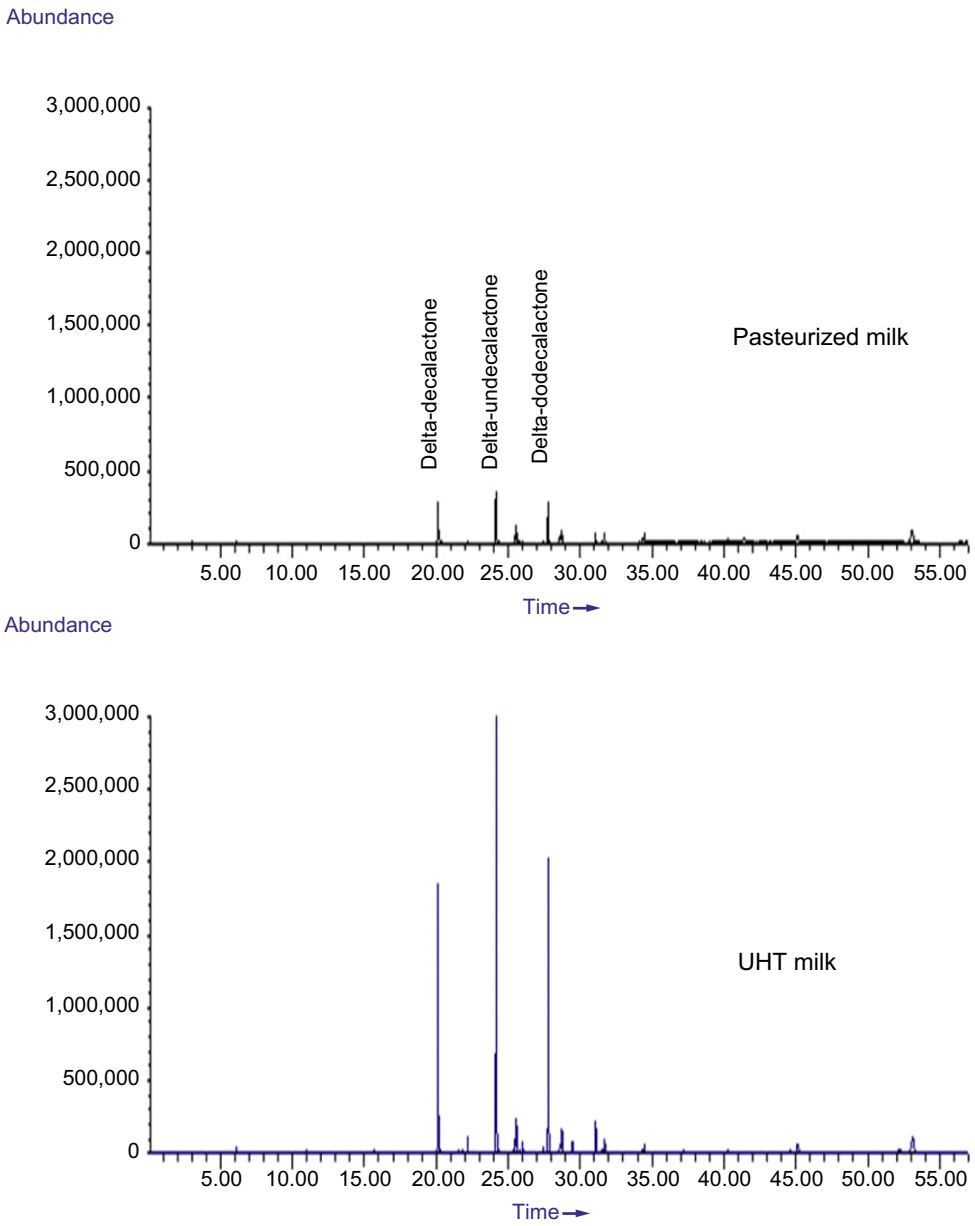

FIGURE 12.8 Gas chromatography–mass spectrometry chromatograms (selected ion monitoring, 99 *m/z*) of UHT milk and pasteurized milk by stir bar sorptive extraction *(Qian* et al.*, unpublished data).*

methanethiol formation appeared to be inhibited under pressure, it is also possible that it was converted to other compounds. In addition, the formation and conversion of methanethiol could be pH dependent, due to pressure-induced pH shifts.

A kinetic study conducted by Vazquez-Landaverde *et al.* (2007) provided an improved understanding of the formation mechanisms of volatile compounds in milk subjected to high hydrostatic pressure. Hexanal, heptanal, octanal, nonanal, and decanal formation followed first order kinetics with rate constants increasing with pressure and temperature. Activation energies for these five straight-chain aldehydes decreased with pressure, suggesting that pressure has a catalytic effect on their formation reactions in milk. Formation of 2-methylpropanal, 2,3-butanedione, and H_2S followed zero-order kinetics with rate constants increasing with temperature but with an unclear pressure effect. Activation energies for 2-methylpropanal and 2,3-butanedione increased with pressure, whereas the values for H_2S remained constant in the pressure range studied. The concentrations of other off-flavor compounds studied, including the powerful off-flavor compound methanethiol, remained unchanged in all

pressure-treated samples. In the case of the methyl ketones 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone, 2-decanone, and 2-undecanone, their concentration did not depend on time and pressure. The results supported a previous suggestion (Vazquez-Landaverde *et al.*, 2007) that high hydrostatic pressure affects the formation kinetics of off-flavor compounds in milk differently, inhibiting some and promoting others. Conventional heat treatment of food products will produce different responses depending on the free energy, ΔG, or activation free energy, ΔG^*, of the various reactions. But the reactions that are commonly observed during heat treatment will not be observed during HPP unless they have an optional pathway of reaction that involves the application of a mechanical reduction of the volume, which has been defined as the main difference between high pressure and thermal processing (Galazka and Ledward, 1996). Although temperature has to be controlled when applying HPP treatments so as not to change the flavor of milk, Vazquez-Landaverde and Qian (2007) suggested that the combination of high pressure, heat, and antioxidants could be used to develop a commercial product that is much more shelf stable while possibly reducing or completely eliminating cooked off-flavor.

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