

Flavours and Off-Flavours in Milk and Dairy Products

K.R. Cadwallader and T.K. Singh

14.1. Introduction

Milk and milk products are an important part of daily nutrition in many regions of the world. Besides fulfilling nutritional requirements, the flavour of milk and milk products is a key parameter for consumer acceptance and marketing (Drake *et al.*, 2007a). The market for dairy products in more traditional dairying countries has been growing steadily; most of this growth can be attributed directly to the introduction of novel product options and increasing application of milk constituents in other food formulations. Due to the importance of dairy products in daily life, especially for consumers in traditional dairying countries, they are being used increasingly as delivery systems for biologically active/nutraceutical preparations. Even higher growth in the consumption of milk and milk products is now coming from countries which did not have any tradition of dairying; such countries offer tremendous opportunity for further enhanced sales. At the same time this increased consumption also challenges researchers and manufacturers to create new product solutions to better suit the palette of consumers recently introduced to dairy products.

During the last two decades, considerable progress has been made towards understanding the flavour chemistry/biochemistry of dairy products, but defining fresh dairy or dairy flavour continues to challenge researchers working in sensory/flavour area. Fresh milk (raw or pasteurized) of overall

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good flavour quality has a bland but characteristic flavour (Badings, 1991). Products made from this basic raw material vary in flavour from mild/weak (e.g., yoghurt, Mozzarella cheese) to intensely flavoured (e.g., Blue cheeses, hard Italian cheeses). For dairy products, three elements comprise the overall sensory experience:

- (1) The **mouthfeel** from the constituents of the milk, especially milk fat and proteins which are essential for viscosity and/or texture
- (2) **Taste** components, e.g., slight sweet/salty taste from lactose, milk salts and added salt (NaCl) to bitterness in cheese caused by peptides
- (3) **Aroma** caused by a proper balance of numerous volatile organic compounds

In the past, the stability of milk and milk products was the primary consideration but is no longer the principal objective due to the evolution of modern sanitary practices, as well as pasteurization. Today, the manufacture of dairy products of consistently good flavour and texture is crucial. In early flavour studies, researchers identified hundreds of volatile compounds, with little or no attention paid to their sensory contribution to the overall flavour of the products. The availability of powerful chromatographic separation techniques, like high-resolution capillary gas chromatography in combination with mass spectrometry and olfactory detection techniques (use of olfactory detection ports), has revolutionized work on the characterization of flavour compounds. Advances in instrumental/chemical analysis have paralleled the developments in sensory methods for the analysis of flavour compounds. Recently, published reviews by Parliment and McGorin (2000), McGorin (2001), Singh *et al.* (2003a, 2007), Cadwallader (2007) and Drake *et al.* (2007a) described various sensory-directed analytical flavour techniques used in the evaluation of key aroma compounds in milk and dairy products. A recently published text, '*Sensory-Directed Flavour Analysis*' edited by Marsili (2007a), is highly recommended to readers who may be interested in more details on flavour analysis techniques.

This chapter presents a discussion on the aroma/taste compounds in various dairy products and reactions involved with their production. Mechanisms involved in the production of odorants responsible for the specific flavour notes and off-flavours in dairy products are also discussed.

14.2. Reactions Involved in the Formation of Flavour Compounds in Milk and Milk Products

The volatile flavour and taste compounds in milk products originate from the degradation of the major milk constituents, namely lactose, citrate, milk lipids and milk proteins (particularly the caseins). The physico-chemical

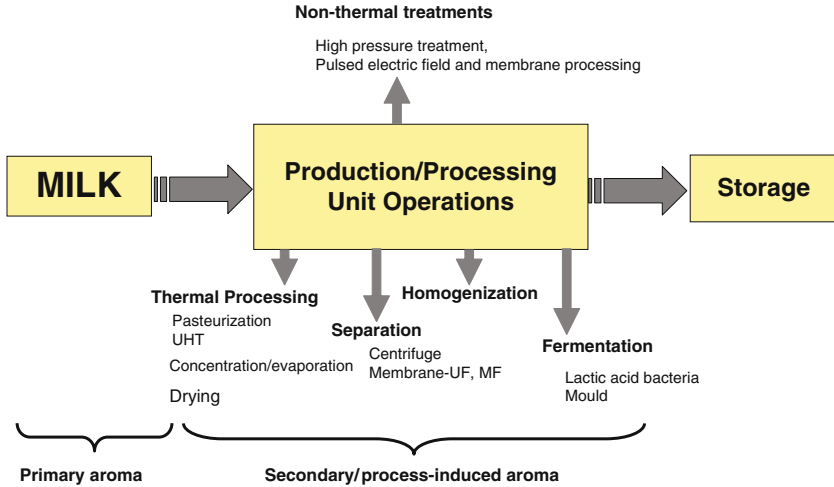


Figure 14.1. Main sources of aroma components in dairy products.

parameters, i.e., heat treatment, pH, water activity, salt concentration and ripening temperature, necessary for the right balance of biochemical changes are set during manufacturing (Figure 14.1). In case of deviation of any of these parameters, cheeses or other dairy products could potentially develop texture and/or flavour inconsistencies or defects. The degradation of milk constituents during the manufacture of dairy products involves a concerted series of chemical and biochemical reactions. Reactions and/or pathways involved in the production milk and milk products can be sub-divided into three major categories:

- (1) Lipid oxidation
- (2) Process-induced changes
 - a. Heat-induced changes
 - b. Changes induced by non-thermal processing technologies
- (3) Fermentation by lactic acid bacteria and other cultures.

14.2.1. Lipid Oxidation

Auto-oxidation of fat in milk and milk products occurs initially in the polyunsaturated phospholipids fraction of the milk fat globule membrane, followed by the main triacylglycerol fraction. It has been reported that different milks vary widely in susceptibility to oxidation. The oxidative

deterioration of milks is classified empirically as (a) spontaneous (developing oxidized flavours within 2 days without added metal catalyst), (b) susceptible (developing oxidized flavours within 2 days after the addition of copper ions) and (c) resistant or non-susceptible (not developing oxidized flavours after 2 days even after addition of copper or iron ions) (Frankel, 1980). Unlike fluid milk, in dry milk products it is the triacylglycerol fraction which is more susceptible to oxidation and the phospholipids act as antioxidants (Frankel, 1980). From this it appears that the susceptibility of milk phospholipids to oxidation depends on whether they are suspended in water or a continuous fat phase. This difference in oxidative stability influences the development of different flavour or off-flavour compounds in various dairy products.

A simplified scheme of lipid oxidation involving three distinct steps is shown in Figure 14.2 (for further details see Frankel, 1980; Walstra *et al.*, 1999). Some of these oxidation products can be perceived at exceptionally low concentrations and thereby cause off-flavours, described as fatty, fried, plastic, tallowy, fishy, metallic or cardboard-like. Off-flavour development can cause problems in fluid milk, sour-cream butter milk, cream, butter, butter oil, a variety of milk powders, cheeses and casein and whey protein products.

Some volatile compounds produced by the auto-oxidation of specific unsaturated fatty acids are listed below:

- **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*

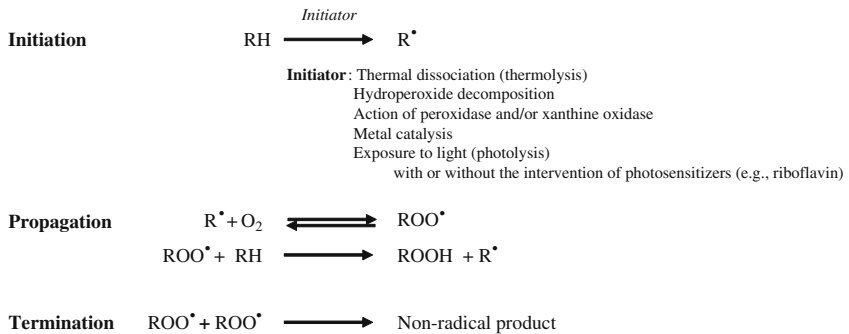


Figure 14.2. General pathway way for the autoxidation of unsaturated lipid (modified from Singh *et al.*, 2007).

14.2.2. Processing-Induced Changes

14.2.2.1. Heat-Induced Changes

Thermal treatment of fluid milk, such as pasteurization or the production of various concentrated/dried milk products, leads to a whole series of desirable, and undesirable, chemical changes which can have major consequence for the texture, taste and flavour. Selected changes, which directly influence the flavour/off-flavour of milk and milk products, are

- Thermal degradation of lipids (e.g., accelerated degradation of hydroperoxides resulting in the formation of 2-alkanones). (For more information on the thermal degradation of lipids, see Nawar, 1989; Yoo *et al.*, 1989).
- Thermal reactions involving amino acid side chains (e.g., generation H₂S and other S-compounds).
- Maillard reactions: e.g., reaction of lactose, a reducing sugar, with ϵ -amino groups on the side chain of lysine residues in milk proteins (e.g., resulting in the formation of a wide variety of odorants, namely furfurals, 3-/4-furanones, Strecker aldehydes, pyrazines, pyrroles, thiazoles maltol, furaneol, etc., see Chapter 7), (Figure 14.3).
- Limited information is available on the interaction of lipid oxidation and Maillard reaction products on the flavour and off-flavour chemistry of milk and milk products. These reactions also contribute to non-enzymatic browning in food systems, which may or may not be desirable (see details in review by Zamora and Hidalgo, 2005; see also Chapter 7).

This is not a complete list of thermally induced reactions in milk and milk products. Other reactions such as hydrolysis of peptide bonds, dephosphorylation of proteins, etc., also occur but are not important from a flavour point of view.

14.2.2.2. Changes Induced by Non-Thermal Processing Technologies

New technologies are needed to process milk without compromising its flavour. Several non-thermal processing technologies have been explored to achieve microbial safety and minimize the formation of off-flavour. Some promising technologies are

- Membrane filtration
- High pressure processing
- Pulsed electric field treatment

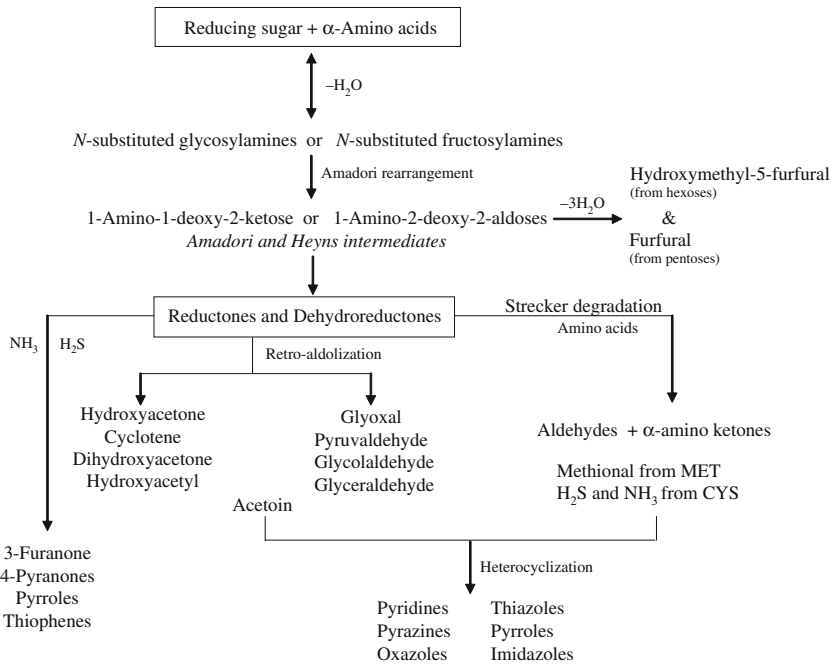


Figure 14.3. Formation of aroma compounds *via* Maillard reaction/non-enzymatic browning (modified from Singh *et al.*, 2007).

Microfiltration (MF) using cross-flow membrane separation has shown promising results in eliminating bacteria from milk and increasing shelf-life without the development of off-flavours (Elwell and Barbano, 2006; Rysstar and Kolstad, 2006).

High hydrostatic pressure processing (HPP), a new technology to the food industry (Torres and Velazquez, 2004), can destroy microorganisms by high hydrostatic pressure without heat (Berlin *et al.*, 1999; Velazquez *et al.*, 2002). This technology has been gaining commercial acceptance in the manufacture of food products with 'fresh' flavour that are not possible with other preservation technologies. To retain the 'fresh' milk flavour, HPP has been studied as a potential alternative to the pasteurization of milk. A microbiological reduction and significant extension in the shelf-life of milk compared to that of pasteurized milk has been achieved using pressure treatments. HPP can reduce the size of casein micelles in milk at pressures above 230 MPa, resulting in a decrease in whiteness and turbidity and an increase in the viscosity of milk (Buchheim and El-Nour, 1996). High pressure can also affect the crystallization properties of milk fat. It is generally assumed that

HPP at low temperature will retain the flavour of the product; however, Hofmann *et al.* (2005) reported that HPP could potentially change the formation of Maillard-derived compounds at high temperature.

Recent work by Vazquez-Landaverde *et al.* (2006a) showed that HPP at a low temperature causes minimum change of the volatile composition of milk. However, under extreme pressure and temperature conditions, volatile compound formation is different from that under atmospheric pressure conditions. Heat treatment at high temperature promotes the formation of both aldehydes and methyl ketones, whereas high pressure at high temperature favours the formation of aldehydes. The formation of sulphur compounds was also different under high pressure.

Further work is required to understand the mechanism/kinetics of volatile formation under high hydrostatic pressure, pulsed electric field and/or their combination with heat treatment.

14.2.3. Fermentation by Lactic Acid Bacteria

Considerable knowledge on the principal changes and pathways involved in manufacture of fermented milk and cheese ripening has been accumulated over the last several decades. The three primary biochemical processes are

- Metabolism of lactose, lactate and citrate ('Glycolysis')
- Lipolysis
- Proteolysis

The relative importance of each of these processes depends on the type of fermented dairy product. These primary changes are followed and overlapped by many secondary catabolic changes, including deamination, transamination, decarboxylation and desulfurylation of amino acids, β -oxidation of fatty acids and even purely synthetic chemical changes, e.g., formation of thioesters. The above-mentioned primary reactions are mainly responsible for the basic textural changes and are also largely responsible for the basic flavour of fermented dairy products. However, secondary transformations are mainly responsible for the finer aspects of cheese flavour and for modification of cheese texture. Glycolysis, lipolysis, proteolysis and related reactions are further discussed in the next few sections.

14.2.3.1. Glycolysis and Related Reactions

During the manufacture of fermented milks and cheese, starter lactic acid bacteria ferment lactose to (mainly L) lactic acid (Figure 14.4). In the case of Cheddar-type cheeses, most of the lactic acid is produced in the vat before

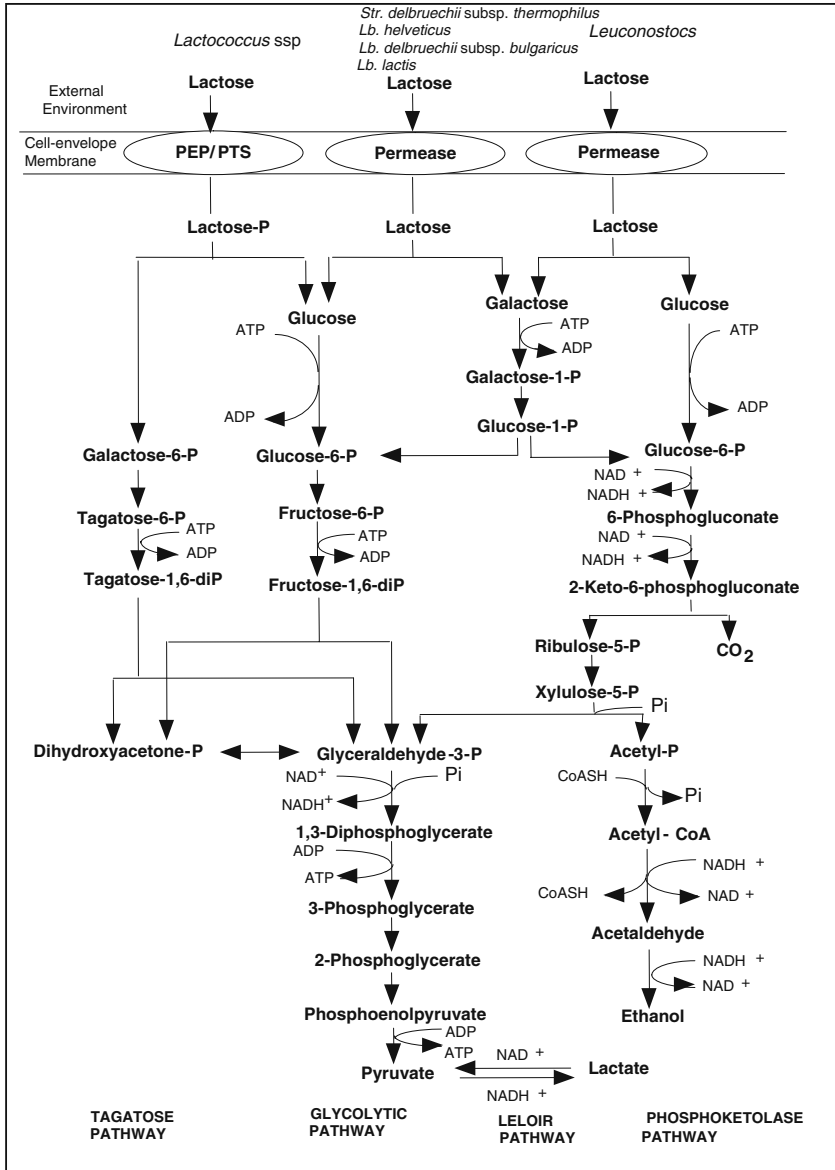


Figure 14.4. Pathways for the metabolism of lactose by mesophilic and thermophilic lactic acid bacteria (reproduced with permission from Singh *et al.*, 2003; © Blackwell Publishing, Inc.).

salting and moulding. During manufacture or shortly thereafter, curd pH reaches ~ 5.0 , but the rate is characteristic of the cheese variety (6–24 h). Even after losing $\sim 98\%$ of the total milk lactose in the whey as lactose or lactate, the cheese curd still contains 0.8–1.5% lactose at the end of manufacture (Huffman and Kristoffersen, 1984).

The pH at whey drainage largely determines the mineral content of a cheese. The loss of Ca^{2+} and phosphate from casein micelles determines the extent to which the micelles are disrupted and this largely determines the basic structure and texture of a cheese (Lawrence *et al.*, 1983). In general, curds with a low pH at drainage have a crumbly texture, e.g., Cheshire, while high pH curds tend to be more elastic, e.g., Emmental.

The racemization of L-lactate is probably not significant from a flavour viewpoint, but D-lactate may have undesirable nutritional consequences in infants. Calcium-D-lactate is believed to be less soluble than calcium-L-lactate and may crystallize in cheese, especially on cut surfaces (Dybing *et al.*, 1988). Consumers may mistake the crystals as spoilage, and crystal formation is generally considered negative.

Lactic acid is metabolized by propionic acid bacteria, e.g., in the production of Swiss-type cheeses, to propanoic and acetic acids, H_2O and CO_2 . The production of CO_2 is responsible for the eye formation which is a characteristic of Swiss-type cheeses (Fox *et al.*, 1995). Oxidation of lactate may also occur in cheese to a very limited extent. During this process, lactate is converted to acetate and CO_2 . Acetate is present at fairly high concentrations in Cheddar and is considered to contribute to cheese flavour, although a high concentration may cause off-flavour (Aston and Dulley, 1982).

14.2.3.2. Citrate Metabolism

Bovine milk contains relatively low levels of citrate (~ 8 mM). Approximately 90% of the citrate in milk is soluble and most is lost in the whey; however, the concentration of citrate in the aqueous phase of cheese is ~ 3 times that in whey (Fryer *et al.*, 1970), presumably reflecting the concentration of colloidal citrate. Cheddar cheese contains 0.2–0.5 % (w/w) citrate, which is not metabolized by *L. lactis* ssp. *lactis* or *L. lactis* ssp. *cremoris*, but is metabolized by citrate-positive lactococci and *Leuconostoc* sp. with the production of diacetyl and CO_2 . Due to CO_2 production, citrate metabolism is responsible for the characteristic eyes in Dutch-type cheeses. Diacetyl and acetate produced from citrate contribute to the flavour of Dutch-type and Cheddar cheeses (Aston and Dulley, 1982). Several species of mesophilic lactobacilli metabolize citrate with the production of diacetyl and formate (Fryer, 1970); the presence of lactose influences the amount of formate formed.

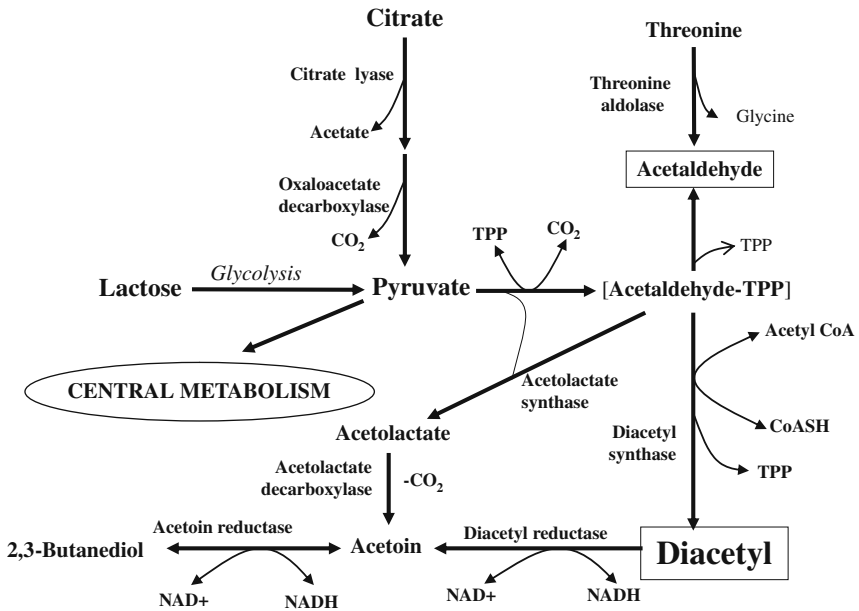


Figure 14.5. Metabolism of citrate by lactic acid bacteria (modified from Singh *et al.*, 2003).

The principal flavour compounds produced from the metabolism of citrate are acetate, diacetyl (2,3-butanedione), acetoin (3-hydroxy-2-butanone) and 2,3-butanediol (Cogan, 1995) (Figure 14.5). Diacetyl is usually produced in small amounts but acetoin is generally produced at much higher concentration (10–50 fold higher than diacetyl).

14.2.3.3. Lipolysis and Related Reactions

Like all types of food with a high fat content, lipolytic (enzymatic hydrolysis by lipases and esterases) and oxidative (chemical) changes are likely to occur in dairy products. Lipases and esterases in cheese originate from milk, starter, secondary starter and non-starter bacteria. A number of psychrotrophic organisms, which can dominate the microflora of refrigerated milk, produce heat-stable lipases. The hydrolysis of triglycerides, which constitute more than 98% of milk fat, is the principal biochemical transformation of fat, which leads to the production of free fatty acids (FFAs), di- and mono-glycerides and possibly glycerol (Figure 14.6). FFAs contribute to the aroma of cheese. Individual FFAs, particularly

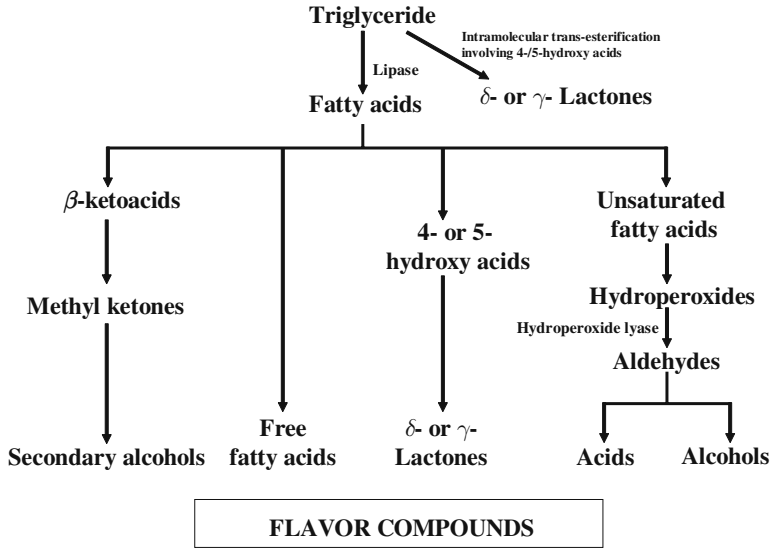


Figure 14.6. General pathways for the metabolism of milk triglycerides and fatty acids (reproduced with permission from Singh *et al.*, 2003; © Blackwell Publishing, Inc.).

acids between C_{4:0} and C_{12:0}, have specific flavours (rancid, sharp, goaty, soapy, coconut-like). The flavour intensity of FFAs depends not only on the concentration but also on the distribution between aqueous and fat phases, the pH of the medium, the presence of certain cations (e.g., Na⁺, Ca²⁺) and protein degradation products (Adda *et al.*, 1982). The pH has a major influence on the flavour impact of FFAs. At the pH of Cheddar cheese (pH ~5.2), a considerable portion of the FFAs are present as salts, which are non-volatile, thus reducing their flavour impact. In most cheese varieties, relatively little lipolysis occurs during ripening and too much is considered undesirable. Most consumers would consider Cheddar, Dutch and Swiss-type cheeses containing even moderate levels of free fatty acids to be rancid. Even lesser amounts of FFAs would make fermented milk such as yogurt rancid. However, extensive lipolysis is desirable as part of overall flavour development in certain cheeses, such as hard Italian cheeses (Romano, Provolone), Blue and Feta.

The fat fraction of dairy products is also important for the development of typical flavour. Cheddar cheese made from non-fat milk does not develop full aroma, even after 12 months (Ohern and Tuckey, 1969). Foda *et al.* (1974) suggested that the fatty acid composition and natural emulsion of milk fat are important for flavour development.

14.2.3.4. Fatty Acid Metabolism

FFAs are involved in several types of reactions which vary in importance with the type of dairy product involved (Figure 14.6). Methyl ketones are produced from fatty acids by oxidative degradation. The production of methyl ketones involves oxidation of fatty acids (mainly from C_{6:0} to C_{12:0}) to β -ketoacids, which are then decarboxylated to corresponding methyl ketones with one carbon atom less (Hawke, 1966). Methyl ketones are responsible for the characteristic aroma of blue-veined cheeses (Gripou *et al.*, 1991). However, they play a limited role in Cheddar cheese flavour. Ultimately, methyl ketones can be reduced to secondary alcohols, which do not contribute to cheese aroma. Another reaction in which polyunsaturated and, perhaps, monounsaturated fatty acids can be involved, is oxidation. The extent of oxidation in cheese is, however, rather limited, possibly due to a low redox potential. This, together with the presence of natural antioxidants, could prevent the initiation of oxidation mechanisms, or create conditions under which the primary oxidation products are reduced (Adda *et al.*, 1982).

Aliphatic and aromatic esters play an important part in the flavour and, sometimes, the off-flavour of cheese. This synthesis mainly concerns the above-mentioned short- or medium-chain fatty acids and an aliphatic (ethanol) or aromatic (phenylethanol) alcohol or thiol (methanethiol) may be involved. Esters generally contribute a fruity flavour to dairy products which is desirable and characteristic in many cheeses (Parmesan, Parrano) but undesirable in others (Cheddar).

The mechanism of synthesis of esters in cheese is still largely unknown (Liu *et al.*, 2004b). It is generally accepted that the enzymes of the cheese microflora are involved in the formation of cheese esters (Hosono *et al.*, 1974; Harper *et al.*, 1980; Cristiani and Monnet, 2001; Liu *et al.*, 2004b), but some authors also suggest that cheese esters are not of enzymatic origin (Forss, 1972; Adda *et al.*, 1982). Most *S*-methyl thioesters can be formed spontaneously in cheese from the reaction of acylCoA with methanethiol (Helinck *et al.*, 2000). Two ester-producing reactions could be involved in ester formation in cheese, esterification and alcoholysis. Until recently, ester synthesis in cheese was regarded as resulting from the esterification of an alcohol and an acid. The acid or acyl CoA moieties of esters are formed from the action of the cheese microflora and their enzymes on lactose, lactate, lipids and proteins of cheese curd (Urbach, 1997a). It has been shown recently that cheese esters could also be synthesized directly from glycerides and alcohols *via* an alcoholysis reaction. Esterases of lactic acid bacteria can catalyse this reaction, involving the transfer of a fatty acyl group from triglycerides (and, preferably, mono- and di-glycerides) to an alcohol, without the direct involvement of water (Liu *et al.*, 2003, 2004a). Alcoholysis could be a more common route for

esters synthesis in aqueous environments than the esterification reaction, which is favoured under low water activity conditions (Liu *et al.*, 2004b). The rate-limiting factors of ester synthesis in cheese are unknown. Substrates, enzymes and the environment may all determine the rate of ester formation. In Cheddar and Swiss cheeses, however, ethanol is regarded as the limiting factor for ester synthesis (Liu *et al.*, 2004b; Thierry *et al.*, 2006). The 'fruitiness' defect of Cheddar cheese, which results from the formation of ethyl esters (Bills *et al.*, 1965), was found significantly correlated to the concentration of ethanol (Manning, 1979). It was reported that the addition of ethanol to Cheddar curd produced increased levels of ethyl esters (Urbach, 1993). Ethanol is the main alcohol detected in Cheddar cheese (McGugan *et al.*, 1975). Its formation in Cheddar results mainly from the activity of heterofermentative lactobacilli and/or yeasts. A recent study by Thierry *et al.* (2006) demonstrated that ethanol is the limiting factor for ethyl ester synthesis in Swiss cheese.

Lactones (γ - and δ -) are potent fat-derived flavour compounds that play an important role in the overall cheese flavour profile, particularly in Cheddar (Wong *et al.*, 1973; Drake *et al.*, 2001). Data from a recently published work (Alewijn *et al.*, 2006) showed that lactic acid bacterial enzymatic activities played no role in the formation of lactones from milk triglycerides or free fatty acids. The same study also demonstrated that the mechanism of lactone formation in cheese is a one-step, non-enzymatic reaction, where a hydroxyl fatty acid esterified in a triglyceride undergoes trans-esterification to release the lactone directly.

14.2.3.5. Proteolysis and Related Reactions

Proteolysis is the most widely studied biochemical change in dairy products. During the manufacture of fermented milks and cheese and during the ripening of cheese, a gradual decomposition of caseins occurs due to the combined action of various proteolytic enzymes. These generally include enzymes from the following sources:

- Coagulant
 - a) Chymosin (genetically engineered-MaxirenTM, ChymaxTM)
 - b) Chymosin/pepsin (from calf stomach)
 - c) Rennet substitutes microbial (enzymes from *Rhizomucor pusillus*, *R. miehei*, *Cryphonectria parasitica*, *Aspergillus oryzae* and *Irpex lactis*), and
 - d) Plant rennet (e.g., *Cynara cardunculus*)
- Indigenous milk enzymes (e.g., plasmin, cathepsins)

- Starter and non-starter bacterial enzymes
 - a) Cell envelope-associated proteinases (lactocepins)
 - b) Peptidases (including endopeptidases, aminopeptidases, di-/tripeptidases and proline-specific peptidases)
- Secondary starter enzymes
- Exogenous enzymes used as ripening aids

Enzymes from the first three sources are active in most ripened cheeses. The secondary starters (microorganisms added to cheese milk or curd for purposes other than acidification, e.g., surface smear organisms, blue/white moulds) exert considerable influence on the maturation of cheese varieties in which they are used. Exogenous enzymes, when present can be very influential.

The correct pattern of proteolysis is generally considered to be a prerequisite for the development of the correct flavour of cheese. Products of proteolysis *per se* (i.e., peptides and free amino acids) probably are significant in cheese taste, at least to 'background' flavour, and some off-flavours, e.g., bitterness, but are unlikely to contribute aroma. Compounds arising from the catabolism of amino acids contribute directly to cheese taste and aroma.

14.2.3.5.1. Hydrolysis of α_{s1} -Casein

In the cheese environment, with a high ionic strength and a low a_w , rennet-induced breakdown of α_{s1} -casein proceeds much faster than that of β -casein (α_{s2} - and *para*- κ -caseins are quite resistant to hydrolysis by the enzymes of rennet) (Visser, 1993). The residual chymosin rapidly hydrolyses α_{s1} -casein at the Phe₂₃–Phe₂₄ bond during the initial stages of ripening (Creamer and Richardson, 1974). Hydrolysis of this single bond of α_{s1} -casein causes a rapid change in the rubbery texture of young Cheddar curd into a smoother, more homogeneous product (Lawrence *et al.*, 1987). However, more recent work of O'Mahony *et al.* (2005) showed that the initial softening of texture is more highly correlated with the level of insoluble calcium than with the level of intact α_{s1} -casein in cheeses early in ripening. The authors of this study concluded that the hydrolysis of α_{s1} -casein at Phe₂₃–Phe₂₄ is not a prerequisite for softening which is due principally to the partial solubilization of colloidal calcium phosphate associated with the *para*- κ -casein matrix of Cheddar cheese during the early stages of ripening. The peptide α_{s1} -casein f1-23, produced by chymosin action on the bond Phe₂₃–Phe₂₄ of α_{s1} -casein, is further hydrolyzed in Cheddar cheese (Singh *et al.*, 1994) by the CEP from starter *L. lactis* ssp. *cremoris*, resulting in the production of a range of small molecular weight peptides, representing N-terminal (α_{s1} -casein f1–7, 1–9,

1–13 and 1–14) and C-terminal (α_{s1} -casein f14–17, 17–21) sequences, which were found to be bitter in taste (Richardson and Creamer, 1973; Lee *et al.*, 1996). The large peptide produced by chymosin, α_{s1} -casein f24–199, is further hydrolyzed by chymosin and CEP (for further details see McSweeney *et al.*, 1994; Singh *et al.*, 1995, 1997).

14.2.3.5.2. Hydrolysis of β -Casein

Chymosin has limited action on β -casein in Cheddar, although some activity is indicated by the presence of the peptide β -casein f1–192 (McSweeney *et al.*, 1994). Hydrolysis of the bond Leu₁₉₂–Tyr₁₉₃ of β -casein by chymosin releases a small C-terminal fragment, β -casein f193–209, which is extremely bitter (Singh *et al.*, 2004b). Nearly half of the β -casein in Cheddar cheese is hydrolyzed during ripening by plasmin, an indigenous milk proteinase. Plasmin hydrolysis of β -casein results in the formation of three γ -caseins [γ_1 - (β -casein f29–209), γ_2 - (β -casein f106–209) and γ_3 - (β -casein f108–209)], representing the C-terminal region, and five proteose-peptones (β -casein f1–28, β -casein 1–105/107 and β -casein f29–105/107), representing the corresponding N-terminal region. The γ -caseins seem to accumulate in Cheddar over the ripening period. The proteose-peptones are extensively hydrolyzed by the starter bacterial CEP and peptidases to produce small peptides and free amino acids (Singh *et al.*, 1995, 1997).

Proteolysis in cheese seems to be a sequential process involving enzymes from rennet, milk proteinases (particularly plasmin), the starter culture, secondary microorganisms and non-starter lactic acid bacteria (NSLAB):

- The hydrolysis of casein to high molecular weight peptides is thought to be primarily the result of chymosin and plasmin.
- The subsequent hydrolysis of high molecular weight peptides into small peptides and free amino acids is primarily the result of proteolytic enzymes from lactic acid bacteria (Singh *et al.*, 2003a).

Proteolytic degradation of caseins into peptides in Cheddar (Singh *et al.*, 1994, 1995, 1997; Fernandez *et al.*, 1998), Emmental (Gagnaire *et al.*, 2001) and Parmigiano-Reggiano (Addeo *et al.*, 1992, 1994) cheeses has been characterized in detail.

14.2.3.6. Catabolism of Amino Acids

In lactococci, the first step in the degradation of amino acids is transamination (Figure 14.7; Gao *et al.*, 1997), leading to the formation of α -keto acids (α -KAs). Aromatic aminotransferases have been characterized from *L. lactis* ssp *cremoris* (Yvon *et al.*, 1997; Rijnen *et al.*, 1999a) and *L. lactis* ssp

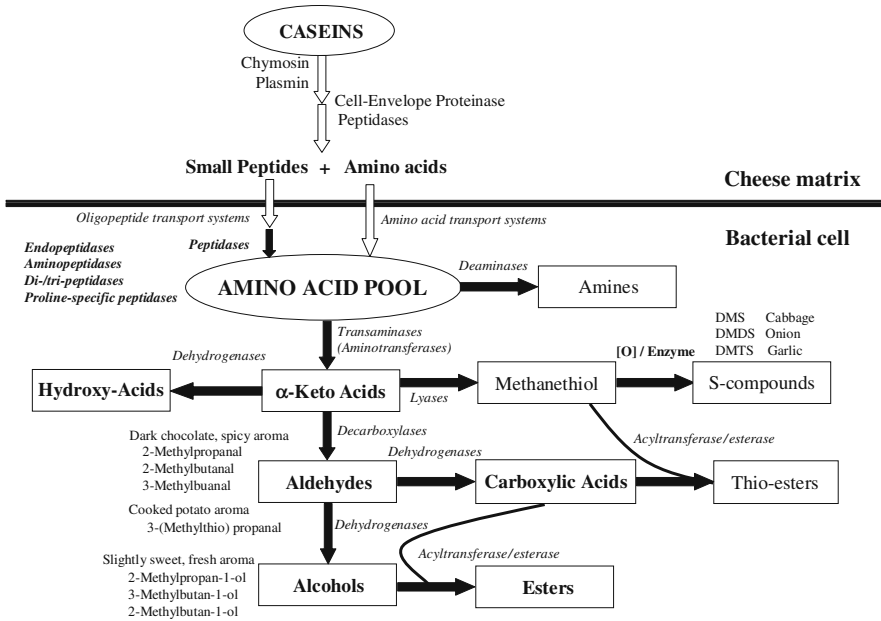


Figure 14.7. Generation of flavour compounds from milk protein degradation. DMS, dimethyl sulphide; DMDS, dimethyl disulphide; DMTS, dimethyl trisulphide (modified from Singh *et al.*, 2003).

lactis (Gao and Steele, 1998). These enzymes initiate the degradation of Val, Leu, Ile, Phe, Tyr, Trp and Met, all of which are known precursors of cheese flavour compounds. Inactivation of aminotransferases involved in the breakdown of amino acids by lactococci has been shown to reduce aroma formation during cheese ripening (Rijnen *et al.*, 1999b).

α -KAs are central intermediates and can be converted to hydroxyl acids, aldehydes and CoA-esters (Figure 14.7). These reactions are mostly enzymatic, but some chemical conversion steps have also been described, like the formation of benzaldehyde from phenylpyruvic acid (Smit *et al.*, 2004). The aldehydes formed can generally be dehydrogenated or hydrogenated to their corresponding alcohols or organic acids, which are, in their turn, substrates for esterases and acyltransferases leading to (thio)esters (Figure 14.7).

Other enzymatic routes for the conversion of amino acids involve lyases (e.g., cystathionine β -lyase, threonine aldolase) and deamination/decarboxylation (resulting in the formation of amines). For further details see Smit *et al.* (2005).

The volatile fraction of cheese contains several sulfur-containing compounds such as methanethiol, methional, dimethyl sulphide, dimethyldisulphide, dimethyltrisulphide, dimethyltetrasulphide, carbonyl sulphide and hydrogen

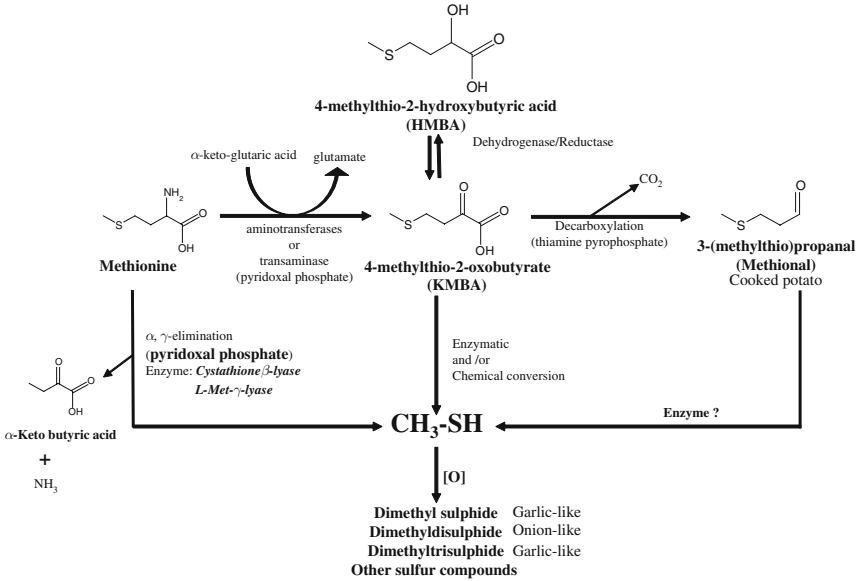


Figure 14.8. Degradation of methionine to potent sulphur-containing odorants (reproduced with permission from Singh *et al.*, 2003; © Blackwell Publishing, Inc.).

sulphide (Lindsay and Rippe, 1986; Urbach, 1995; Weimer *et al.*, 1999) which contribute to the aroma of cheese (Milo and Reineccius, 1997). These compounds are known to originate from Met (Figure 14.8). Methanethiol has been associated with desirable Cheddar-type sulfur notes in good-quality Cheddar cheese (Manning and Price, 1977; Manning and Moore 1979; Price and Manning 1983). However, alone or in excess, methanethiol does not produce typical Cheddar cheese flavour (Weimer *et al.*, 1999).

Methanethiol is readily oxidized to dimethyl disulphide and dimethyl trisulphide (Parliment *et al.*, 1982; Chin and Lindsay, 1994). The occurrence of these compounds is a direct result of the methanethiol content and is modulated by the low redox potential of cheese. Methanethiol can potentially oxidize during analysis to form these compounds, and this may account for some reports of dimethyl disulphide and dimethyl trisulphide in cheese. Dimethyl sulphide (Milo and Reineccius, 1997) and dimethyl trisulphide were noted recently as important odorants in aged Cheddar cheese (Milo and Reineccius, 1997; Suriyaphan *et al.*, 2001b; Zehentbauer and Reineccius, 2002). Further work is needed to define the mechanism and cheese conditions needed for the production of these compounds.

The volatile sulphur compounds from Met can also be produced non-enzymatically. Wolle *et al.* (2006) reported that incubation of Met in the

presence of pyridoxal-5'-phosphate (PLP) under cheese-like conditions (pH 5.2 + 4% salt at 7°C) resulted in the production of methanethiol, dimethyl disulphide and dimethyl trisulphide. A 1000-fold increase in the production of volatile sulphur compounds was observed when the reaction temperature was raised from 7 to 37°C. This reaction in conjunction with an adjunct culture with higher peptidolytic activity may offer an improved method to control and enhance the production of volatile sulphur compounds in cheeses or enzyme-modified cheeses.

Amino acid degradation plays a vital role in flavour development in cheese. A number of researchers have attempted to enhance the free amino acid content of Cheddar cheese by direct addition of amino acids (Wallace and Fox, 1997) or genetic modification of lactococci with increased aminopeptidase N activity (McGarry *et al.*, 1994; Christensen *et al.*, 1995). However, an increased amino acid content in Cheddar did not affect flavour development, which led Yvon *et al.* (1998) to hypothesize that the rate-limiting factor in flavour biogenesis was not the release of amino acids but their subsequent conversion to aroma compounds. Yvon *et al.* (1998) identified the transaminase acceptor, α -ketoglutarate, as the first limiting factor in the degradation of amino acids. Addition of α -ketoglutarate to Cheddar curd resulted in increased volatile components originating from branched-chain and aromatic amino acids (Banks *et al.*, 2001). Results of a recent study showed that Cheddar cheese made using an adjunct starter *Lactobacillus casei* (genetically modified to enhance the expression of hydroxyl acid dehydrogenase, HADH) retarded flavour development (Broadbent *et al.*, 2004). HADH catalyses the conversion of α -KAs to α -hydroxy acids, which have little or no importance from a flavour point of view. It may still be possible to selectively suppress aromatic amino acid (Phe, Tyr, Trp)-derived off-flavour compounds by overexpression of an alternative HADH with more narrow specificity for aromatic amino acid-derived α -KAs.

The effect of membrane permeabilisation, by treatment with the bacteriocin lactacin 3147, on the branched-chain amino acid transamination by *L. lactis* IFPL359 was investigated by Martinez-Cuesta *et al.* (2002). Membrane permeabilisation of the cells made them non-viable, but they remained metabolically active and facilitated free amino acids diffusion into the cell. These changes made intracellular enzymes more accessible to their substrates and hence increased branched-chain amino acid transamination.

The detailed understanding of the mechanistic pathways involved in the degradation of amino acids to cheese flavour compounds and the capacity of starter/non-starter bacteria to generate volatiles will not only result in enhanced control/acceleration of cheese flavour development but also in minimizing the occurrence of off-flavours.

14.3. Characteristic Flavours of Milk and Milk Products

According to the 'Component Balance Theory', the flavour of cheese is produced by the correct balance and concentrations of a wide range of sapid and aromatic compounds (Mulder, 1952; Kosikowski and Mocquot, 1958). If a proper balance of components is not achieved, then undesirable or defective flavour occurs. Decades of flavour research on dairy products have confirmed the accuracy of this concept not only for cheese but also for various other milk and milk products. See Tables 14.1, 14.2, 14.3, 14.4, 14.5, 14.6, 14.7, 14.8 and 14.9 for a list of odorants found in milk and milk products.

14.3.1. Aroma of Fluid Milk

The delicate but weak flavour of milk is caused by very low concentrations of numerous odorants (Badings, 1991; Bendall, 2001). Thermal processing is a commonly employed method to achieve microbial safety and shelf-life stability of milk, e.g., high-temperature short-time pasteurization (typically at 72°C for 15 s; the shelf-life is only 20 days at refrigeration temperatures) and ultrahigh-temperature (UHT) processing (135–150°C for 3–5 s; UHT-treated milk is stable at room temperature for up to 6 months). When milk is severely heated, both flavour and colour are affected. Varying levels of heat treatment during processing of raw milk, e.g., pasteurization, UHT-treatment or sterilization, have been shown to increase lipid oxidation products (Table 14.1). The UHT process can induce a chalky taste and strong 'cooked' off-aroma notes in milk (Shipe, 1980). Numerous studies have linked the 'cooked' off-aroma defect to the production of volatile sulphur compounds, aldehydes and methyl ketones (Scanlan *et al.*, 1968; Jeon *et al.*, 1978; Moio *et al.*, 1994; Contarini *et al.*, 1997). Moio *et al.* (1994) compared the aroma-impact compounds in raw milk to those in pasteurized and UHT milks using gas chromatography–olfactometry–mass spectrometry (GCO–MS) and aroma extract dilution analysis (AEDA) (Table 14.1). Recently published work by Czerny and Schieberle (2007) demonstrates a remarkably different set of odorants in UHT milk (see Table 14.1 for details). Such differences may be attributed to different breeds of cow, the cow feeding or milk processing, although 2-alkanones have rather high odour thresholds and may, thus, not contribute much to the overall milk aroma. Czerny and Schieberle (2007) also reported the influence of the packaging material on the odorant profiles of UHT milk (Table 14.1).

Volatile compounds that contribute particularly to sterilized milk are lipid oxidation products (mainly 2-alkanones: C_{5, 7, 9, 11}) and several heterocyclic compounds such as pyrazines, furans, lactones and other products of

Table 14.1. Aroma compounds identified in fluid milk

Milk (Fresh) ^a	Milk (Pasteurized) ^a	Milk (UHT) ^a	Milk (UHT) ^b Glass bottles	Milk (UHT) ^b PE bottles
Ethyl hexanoate	Dimethyl sulfone	2-Heptanone	δ -Decalactone (1024)	δ -Decalactone (>4096)
Ethyl butyrate	Hexanal	2-Nonanone	Vanillin (128)	γ -Dodecalactone (>4096)
Dimethyl sulfone	Nonanal	2-Undecanone	γ -(Z)-6-dodecenolactone (128)	Vanillin (256)
1-Octen-3-ol	1-Octen-3-ol	δ -Decalactone	γ -Dodecalactone (128)	(Z)-4-Heptenal (64)
Indole	Indole	2-Tridecanone	<i>trans</i> -4,5-epoxy- <i>E</i> -2-decenal (128)	3-(Methylthio) propanal (64)
	Benzothiazole	Dimethyl sulfone	δ -Octalactone (128)	Hexanoic acid (64)
	δ -Decalactone	Benzothiazole	2-Acetyl-2-thiazoline (64)	δ -Octalactone (64)
	2-Tridecanone	Hexanal	3-(Methylthio) propanal (64)	<i>trans</i> -4,5-epoxy- <i>E</i> -2-decenal (64)
		Indole	2-Acetyl-1-pyrroline (64)	γ -Nonalactone (64)
				δ -Nonalactone (64)
				γ -(Z)-6-Dodecenolactone (64)

^aRaw, pasteurized and ultra-high temperature (UHT) treated milks analysed by gas chromatography-olfactometry/aroma extract dilution analysis/GC-mass spectrometry (GCO/AEDA/GC-MS) (Moto *et al.*, 1994).

^bUHT treated milks stored in glass and polyethylene (PE) bottles analysed by GCO/AEDA/GC-MS (Czerny and Schieberle, 2007). Values in parenthesis represent flavour dilution factors

Table 14.2. Aroma compounds identified in cream

Cream ^a	Pasteurized cream (15% Fat) ^b	Sterilized cream (25% Fat) ^c
γ -Decalactone	2,3-Butanedione (diacetyl)	Dimethyl trisulphide
δ -Decalactone	3-Hydroxy-2-butanone (acetoin)	2-Alkanones (C ₅ , C ₇ and C ₉)
δ -Dodecalactone	Dimethyl trisulphide	2-Furfural
(<i>Z</i>)-4-Heptenal	2-Nonanone	2-Furanmethanol
(<i>E,E</i>)-2,4-Nonadienal	Butanoic acid	
	Acetic acid	
	Dimethyl sulphide	
	2-Butanone	

^aMcGorin (2001).

^{b,c}Pionnier and Hugelshofer (2006).

non-enzymatic browning/Maillard reactions [e.g., maltol, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (HDMF), 2,5-dimethylpyrazine and *o*-aminoacetophenone] (Badings, 1991). Sweetened condensed milk has a similar volatile flavour profile (Shimoda *et al.*, 2001).

14.3.2. Aroma of Fat-Enriched Milk Products

The flavour of cream is due mainly to the contributions from the aqueous phase of milk and from the fat globule membrane (Bading and Neeter, 1980), while butter aroma is derived primarily from the volatile compounds present in the fat fraction (Mallia *et al.*, 2008). Whipping of cream may increase slightly the concentrations of oxidation products and, provided that it is not overdone, the flavour is improved. Begemann and Koster (1964) found (*Z*)-4-heptenal to be important in cream flavour. Pionnier and Hugelshofer (2006) analysed cream that had been subjected to different processes (pasteurisation, sterilization, UHT) and having different fat levels by GCO (Table 14.2). They identified 32 aroma compounds, which included ketones, acids, lactones and sulphur compounds. The aroma profile of sterilized cream was dominated by compounds such sulphides, ketones and Maillard products.

The key aroma compounds in sweet-cream butter were studied using AEDA by Budin *et al.* (2001) and Peterson and Reineccius (2003a). In the first study, lactones, ketones and aldehydes were found to have high aroma dilution factors (Table 14.3). Interestingly, skatole was also found as a key odorant of sweet-cream butter, showing an aroma dilution factor of 128. Peterson and Reineccius (2003a) identified additional aroma compounds in the headspace of sweet-cream butter, namely hydrogen sulphide, acetaldehyde, dimethyl sulphide, diacetyl, hexanal, 2-methylbutanal,

Table 14.3. Aroma compounds identified in butter and butter oil

Sweet cream butter ^a	Sweet cream butter ^b	Heated butter ^a	Sour cream butter ^c	Butter oil ^d
δ -Decalactone	Dimethyl trisulphide	1-Hexen-3-one	δ -Decalactone	1-Octen-3-one
δ -Dodecalactone	1-Acetyl-2-pyrroline	1-Octen-3-one	(Z)-6-Dodecen- γ -lactone	(Z)-3-Hexenal
(Z)-6-Dodecen- γ -lactone	2,3-Butanedione	(E)-2-Nonenal	Skatole	(Z)-2-Nonenal
1-Hexen-3-one	1-Octen-3-one	(Z)-2-Nonenal	Butanoic acid	(E)-2-Nonenal
1-Octen-3-one	δ -Decalactone	(E,E)-2,4-Decadienal	2,3-Butanedione	(E,E)-2,4-Decadienal
(E)-2-Nonenal	γ -Nonalactone	<i>trans</i> -4,5-Epoxy-(E)-2-decenal	(Z)-2-Nonenal	
(E,E)-2,4-Decadienal	Butanoic acid	2,5-Dimethyl-4-hydroxy-3(2H)-furanone (HDMF)	Acetic acid	
<i>trans</i> -4,5-Epoxy-(E)-2-decenal	Ethyl butanoate	Methional	(E)-2-Nonenal	
(Z)-2-Nonenal		δ -Octalactone	(Z,Z)-3,6-Nonadienal	
Skatole		δ -Decalactone	γ -Octalactone	
		δ -Dodecalactone	Hexanoic acid	
		Skatole	1-Penten-3-one	
			Unknown compound (fatty/nutty odour)	
			(E,E)-2,4-Decadienal	
			<i>trans</i> -4,5-Epoxy-(E)-2-decenal	
			(E,E)-2,4-Nonadienal	
			Hexanal	
			1-Octen-3-one	

^aBudin *et al.* (2001).^bLozano *et al.* (2007)-Commercial samples of fresh sweet-cream butter obtained within the 24 h of production.^cSchieberle *et al.* (1993).^dWidder *et al.* (1991).

3-methylbutanal, butanoic acid, dimethyl trisulphide, hexanoic acid, δ -hexalactone, nonanal, δ -octalactone and γ -dodecalactone. Aroma recombination studies, followed by sensory analysis, indicated that the synthetic aroma model mixture was significantly different from the reference (direct from manufacturing plant) but ranked the same (similarity index) as the aroma of a commercial margarine or an unsalted fresh butter. According to Peterson and Reineccius (2003a), sweet-cream butter was characterised by δ -octalactone, δ -hexalactone and γ -dodecalactone. In particular, δ -hexalactone and γ -dodecalactone had creamy and peach-like odours, respectively, but were identified in sweet-cream butter only by Peterson and Reineccius (2003a). Day *et al.* (1964) identified DMS in butter and considered it to be a desirable component, which smooths the strong flavour of diacetyl. In a study by Schieberle *et al.* (1993), the overall odour impression of sweet-cream butter was evaluated by a trained sensory panel and compared with the odorants of different types of sour-cream butter. The results showed that the concentration of diacetyl is lower without a fermentation process as in sweet-cream butter, resulting in an overall mild and sweet odour impression.

Schieberle *et al.* (1993) studied different kinds of butter: sour-cream butter, Irish sour-cream butter, German farm sour-cream butter and cultured butter and compared them with sweet-cream butter. AEDA on Irish sour-cream butter, showing the most intense odour during a preliminary sensory analysis, revealed 18 odour-active compounds (Table 14.3). Sunflower oil was spiked with diacetyl, δ -decalactone and butanoic acid at the same concentrations occurring in cultured butter that was chosen as standard for the most typical butter odour. The results indicated that sunflower oil containing the three odorants exhibited an aroma note, which in quality and intensity was very similar to the odour of the cultured butter.

Butter generates potent odorants during heating (Grosch, 1986). Budin *et al.* (2001) studied odorants in heated sweet-cream butter using AEDA. The volatile fraction of butter, heated to 105–110°C for 15 min, was isolated by high-vacuum distillation. The odorants with the highest aroma dilution factors are listed in Table 14.3. The key aroma compounds of heated butter were compared with those of fresh butter: δ -decalactone, skatole, 1-octen-3-one, (*E*)- and (*Z*)-2-nonenal, (*E,E*)-2,4-decadienal and *trans*-4,5-epoxy-(*E*)-2-decenal had higher aroma dilution values in heated butter. Schieberle *et al.* (1993) determined the sensory threshold of δ -decalactone as 120 $\mu\text{g}/\text{kg}$ sunflower oil. It is present in heated butter approximately 50 times above its threshold, which suggests that it is the most important odorant in heated butter (Budin *et al.*, 2001). Due to their high odour activity values (OAVs), 1-octen-3-one, methional, HDMF and *trans*-

4,5-epoxy-(*E*)-2-decenal were found to contribute to heated butter aroma. Peterson and Reineccius (2003b) studied the key aroma compounds of heated butter, using static headspace analysis, and confirmed methional, (*E*)-2-nonenal, 1-hexen-3-one, 1-octen-3-one, δ -octalactone, δ -decalactone, HDMF and skatole as potent odorants. In addition, they found hydrogen sulphide, methanethiol, acetaldehyde, diacetyl, 2-heptanone, dimethyl trisulphide, nonanal, butanoic acid, 3-methylbutanoic acid, δ -hexalactone and hexanoic acid. According to these authors, 3-methylbutanoic acid (cheese-like odour), methional (potato-like), HDMF (caramel-like) and 2-heptanone (blue cheese-like) characterize the odour of heated butter. These compounds were not detected in fresh sweet-cream butter. On the other hand, odorants such as 2- and 3-methylbutanal, hexanal, γ -dodecalactone and dimethyl sulphide, found in fresh sweet-cream butter, were not detected in heated butter. Concentrations of ketones and especially lactones increased significantly in heated butter.

Widder *et al.* (1991) investigated the key odorants of butter oil, using vacuum distillation for isolation and GCO-MS for the identification of the volatile compounds. Sixteen potent odorants were identified by AEDA; the most potent odorants are listed in Table 14.3. Vanillin was reported by the authors in butter oil for the first time. The most important aroma compounds with the highest flavour dilution (FD) factors are listed in Table 14.3 (Widder *et al.*, 1991). In the same study, the odour-active compounds in fresh butter oil were compared with those in butter oil which had been stored for 42 days at room temperature. The FD factors of the carbonyl compounds formed by lipid peroxidation increased.

Volatile compounds in traditional sour-cream butter (Wadodkar *et al.*, 2002) and in particular of Smen, a fermented butter produced in Morocco and in other Arab countries, were studied by GCO (Triqui and Guth, 2001). The results of an AEDA indicate butanoic and hexanoic acids as potent odorants. The primary mechanism of aroma development in this product is lipolysis.

14.3.3. Aroma of Dried Milk Products

Milk powder is used widely as a raw material in food formulation. Therefore, flavours and off-flavours originating from milk powders could appear in the final products (Shiratsuchi *et al.*, 1994a). Consequently, flavour monitoring is a part of the research, development, production and quality control of milk powders. The volatile flavour compounds of commercial skim milk powder were studied by extraction of volatiles using simultaneous steam distillation–extraction under reduced pressure (SDE) followed by analysis of the extracts by GCMS. The major compounds were

hydrocarbons, aldehydes, ketones, alcohols, fatty acids, esters, furans, phenolic compounds, lactones and nitrogenous compounds, which constituted over 99.5% of the total volatiles recovered. Results obtained showed that the levels of flavour compounds in the skim milk powder were very low and that their compositions were extremely complicated. Among them, free fatty acids and lactones, which were present at relatively high levels, were considered to be fundamental contributors to the flavour of skim milk. Moreover, aldehydes, aromatic hydrocarbons and some heterocyclic compounds, such as indoles or thiazole, seem to participate indirectly in the flavour of skim milk.

The aroma of dried milk products, such as non-fat dry milk, with varying heat treatment, involves very similar compounds as shown in Table 14.4, but the high heat-treated non-fat dried milk particularly has a strong aroma intensity caused by HDMF, butanoic acid, methional, *o*-aminoacetophenone, *trans*-4,5-epoxy-(*E*)-2-decenal, sotolon and vanillin (Karagul-Yuceer *et al.*, 2001, 2003a). A stored sample of non-fat dried milk had particularly high odour intensities for methional and *o*-aminoacetophenone (Table 14.4; Karagul-Yuceer *et al.*, 2002). The odorant profile of whole milk powder showed a significant effect of the season, as monitored by the levels of compounds such as dimethyl sulphide, pentanal, hexanal and butanoic acid (Biolatto *et al.*, 2007). Whole milk powder manufactured in summer had significantly higher levels of hexanal, pentanal and dimethyl sulphide as compared to autumn and winter samples. On the other hand, butanoic acid showed significant differences between autumn and spring.

Other dried products such as rennet casein, an important food ingredient, particularly suffer from off-flavour. Results of AEDA indicated *o*-aminoacetophenone to be a potent odorant; however, descriptive sensory analysis of model aroma systems revealed that the typical odour of rennet casein was caused principally by hexanoic acid, indole, guaiacol and *p*-cresol (Karagul-Yuceer *et al.*, 2003c). It is essential for rennet casein to be bland and free of any off-flavour for its use as a food ingredient.

Dried whey and dried whey products are important ingredients in the food industry. Liquid whey is further processed into dried whey powder, whey protein concentrates (WPC; 35–80% protein) and whey protein isolates (WPI; >90% protein). Dried whey proteins are commonly used as ingredients due to their exceptional functional characteristics (Morr and Foegeding, 1990) and nutritional value (Quach *et al.*, 1999). From the literature, a somewhat vague description of dried and liquid whey flavour can be obtained. Based on US standards for dry whey (USDA, 2000), reconstituted whey should have a normal liquid whey flavour, free from undesirable flavours. Characteristically, cheese whey has a slightly

Table 14.4. Aroma compounds identified in non-fat dried milk manufactured with varying heat treatments and storage

Non-fat dry milk (Low heat) ^a	Non-fat dry milk (Medium heat) ^a	Non-fat dry milk (High heat) ^a	Non-fat dry milk (stored) ^b
2,5-Dimethyl-4-hydroxy-3(2 <i>H</i>)-furanone (HDMF)	δ -Decalactone	HDMF	3-(Methylthio) propanal
Butanoic acid	HDMF	Butanoic acid	<i>o</i> -Aminoacetophenone
3-(Methylthio) propanal	Butanoic acid	3-(Methylthio) propanal	HDMF
<i>o</i> -Aminoacetophenone	3-(Methylthio) propanal	<i>o</i> -Aminoacetophenone	2-Methyl-3-hydroxy-4 <i>H</i> -pyran-4-one (Maltol)
δ -Decalactone	<i>o</i> -Aminoacetophenone	(<i>E</i>)-4,5-Epoxy-(<i>E</i>)-2-decal	Butanoic acid
(<i>E</i>)-4,5-Epoxy-(<i>E</i>)-2-decal	(<i>E</i>)-4,5-Epoxy-(<i>E</i>)-2-decal	δ -Decalactone	Pentanoic acid
Pentanoic acid	Vanillin	Pentanoic acid	Acetic acid
4,5-Dimethyl-3-hydroxy-2(5 <i>H</i>)-furanone (Sotolon)	2-Acetyl-1-pyrroline	Sotolon	Hexanoic acid
3-Methoxy-4-hydroxybenzaldehyde (Vanillin)	2-Acetyl-2-thiazoline	Vanillin	Octanoic acid
2-Acetyl-1-pyrroline	Sotolon	Phenyl acetic acid	Decanoic acid
2-Acetyl-2-thiazoline	Hexanoic acid	Nonanal	Dodecanoic acid
Hexanoic acid	Phenyl acetic acid	1-Octen-3-one	<i>p</i> -Cresol
Phenyl acetic acid	γ -Dodecalactone	2-Acetyl-1-pyrroline	Skatole
Octanoic acid	(<i>E</i>)-2-Undecenal	Hexanoic acid	Dimethyl trisulphide
Nonanal	(<i>E</i>)-2,4-Decadienal	Octanoic acid	(<i>E</i>)-2,4-Decadienal
1-Octen-3-one		(<i>E</i>)-2-Nonenal	Furfuryl alcohol
			Phenyl acetic acid
			1-Octen-3-one

^aNon-fat dried milks analyzed by gas chromatography-olfactometry/aroma extract dilution analysis/GC-mass spectrometry (GCO/AEDA/GC-MS)(Karagul-Yuceer *et al.*, 2001, 2003a).

^bStored nonfat dried milks analyzed by GCO/AEDA/GC-MS (Karagul-Yuceer *et al.*, 2002).

dirty-sweet/acidic taste and odour (Bodyfelt *et al.*, 1988). The flavour quality of whey depends on

- The quality of the milk from which the cheese was made
- The type of cheese manufactured
- The method of whey handling immediately after curd draining
- The elapsed time between whey draining and pasteurization (Bodyfelt *et al.*, 1988)

The presence of strong flavours and flavour variability can limit dried whey ingredient applications. Flavour variability may be inherent in the liquid whey itself, or may be an outcome of downstream processing techniques. A better understanding of flavour variability in liquid whey may lead to methods to minimize flavour variability in dried whey ingredients. Volatiles from fresh Cheddar cheese whey batches from different processing plants/starter culture rotations were extracted with diethyl ether followed by isolation of volatiles by high-vacuum distillation (Karagul-Yuceer *et al.*, 2003b). GCO-AEDA analysis showed that 2,3-butanedione (buttery), hexanal (green), 2-acetyl-1-pyrroline (popcorn), methional (potato), (*E,E*)-2,4-decadienal (frying oil) and (*E,E*)-2,4-nonadienal (frying oil) were potent neutral/basic aroma-active compounds identified in all whey samples. Odour intensities of hexanal, (*E,E*)-2,4-nonadienal, 2,3-butanedione and (*E,E*)-2,4-decadienal were variable. Short-chain volatile acids were predominant in acidic fractions and their intensities differed among the whey samples. Results obtained by GCO agreed with quantitation results. Liquid whey aroma components are influenced by starter culture rotation (Karagul-Yuceer *et al.*, 2003b).

Few studies have specifically addressed the flavour of dried whey or whey protein products (Stevenson and Chen, 1996; Quach *et al.*, 1999; Mahajan *et al.*, 2004). Recently, aroma-impact compounds in WPC and WPI were studied using descriptive sensory analysis in conjunction with instrumental volatile aroma compound analysis studies (Carunchia-Whetstine *et al.*, 2005b). Sensory analysis of whey protein preparations showed that the samples exhibited sweet aromatic, cardboard/wet paper, animal/wet dog, soapy, brothy, cucumber and cooked/milky flavours, along with a bitter taste and an astringent mouthfeel. Key volatile flavour compounds in WPC (80% protein) and WPI are listed in Table 14.5. According to authors, these baseline data on flavour chemicals in whey proteins will help to identify the most appropriate whey ingredients to use to control or minimize flavour variability in whey-enhanced products.

Table 14.5. Aroma compounds identified in dairy ingredients

Liquid cheese whey ^a	Whey protein concentrate/ isolate ^b	Rennet casein ^c
2,3-Butanedione	Butanoic acid	<i>o</i> -Aminoacetophenone
Hexanal	2-Acetyl-1-pyrroline	2-Methoxyphenol
2-Acetyl-1-pyrroline	2-Methyl-3-furanthiol	Hexanoic acid
3-(Methylthio) propanal	2,5-Dimethyl-4-hydroxy- 3-(2 <i>H</i>)-furanone (HDMF)	Maltol
(<i>E,E</i>)-2,4-Decadienal	2-Nonenal	HDMF
(<i>E,E</i>)-2,4-Nonadienal	(<i>E,Z</i>)-2,6-Nonadienal	Sotolon
Short chain acids	(<i>E,Z</i>)-2,4-Decadienal	Decanoic acid
		γ -(<i>Z</i>)-6-Dodecenolactone
		Skatole
		Dodecanoic acid
		Vanillin

^aLiquid Cheddar whey analysed by dynamic headspace/gas chromatography-mass spectrometry (DHS/GC-MS) (Karagul-Yuceer *et al.*, 2003b; Carunchia-Whetstine *et al.*, 2003a).

^bWhey protein concentrate (80% protein) and isolates were analyzed by gas chromatography-olfactometry/aroma extract dilution analysis/GC-mass spectrometry (GCO/AEDA/GC-MS)(Carunchia-Whetstine *et al.*, 2005b).

^cRennet casein analysed by GCO/AEDA/GC-MS (Karagul-Yuceer *et al.*, 2003c).

14.3.4. Aroma of Fermented Milk Products

Diacetyl and acetaldehyde are important odorants in yoghurt and are produced by lactic acid bacteria used as starter cultures (Table 14.6). Numerous other fermented milks are produced around the world involving lactic acid bacteria and/or in some cases fairly complicated sets of microflora in which a host of volatile compounds play a crucial role in flavour. Fat content has a profound effect on flavour release, and the extent of this effect is determined to some extent by the physical chemistry of the compound concerned. Low-fat yogurts (0.2%) were found to release volatiles more quickly and at a higher intensity but with less persistence than yogurts containing fat at 3.5 or 10% (Brauss *et al.*, 1999). Sensory assessment of the yogurts showed significant differences in intensity and speed of onset of flavour, but not the overall duration of perception. Because different compounds are affected differently, the physical chemistry of flavour molecules should be considered when formulations designed to accommodate changes in fat content are created.

There has been extensive research on the flavour of cheeses, but despite this effort, only limited information is available on the chemistry of the flavour of most cheese varieties and the flavour of none is characterized

Table 14.6. Aroma compounds identified in yoghurt and Mozzarella cheese

Yoghurt ^a	Mozzarella (Bovine milk) ^b	Mozzarella (Water buffalo milk) ^b
2,3-Butanedione	Ethyl-3-methyl butanoate	1-Octen-3-ol
Acetaldehyde	Ethyl isobutanoate	Nonanal
Dimethyl sulphide	2- and 3-Methyl-1-butanol	Indole
Benzaldehyde	Phenyl acetaldehyde	3-Hydroxy-2-butanone
2,3-Pentanedione	Ethyl hexanoate	3-Methyl-2-buten-1-ol
	Ethyl butanoate	2-Octanone
	Nonanal	2-Hydroxy-3-pentanone
	1-Octen-3-ol	Heptanal

^aYoghurt data adapted from McGorin (2001).

^bMozzarella cheese analyzed by gas chromatography-olfactometry/aroma extract dilution analysis/GC-mass spectrometry (GCO/AEDA/GC-MS) (Moio *et al.*, 1993).

sufficiently to permit its reproduction by mixtures of pure compounds in a cheese model (Fox *et al.*, 1995; McGorin, 2001; Parliment and McGorin, 2000; Singh *et al.*, 2003a). In many previous studies, cheeses were simply analyzed for flavour by cheese graders. Such qualitative sensory data have limited use. More defined and analytical information using descriptive sensory and instrumental analysis is required. In the last couple of decades, a number of published works attempted to characterize the mechanism/enzymology of various reactions involved in the generation of volatiles in cheese. Only recent work in the last decade has attempted to study cheese flavour in detail.

In recent years, key odorants in a number of cheese varieties have been characterized by GCO and AEDA. Mozzarella cheeses produced using buffalo or cow milk were found to have very different volatiles compounds in their aroma profiles (Table 14.6).

Odorants in surface mould-ripened cheeses, like Camembert, were studied in detail by Kubickova and Grosch (1997, 1998a,b) (Table 14.7). Aroma compounds were analyzed by GCO using both AEDA and aroma extract concentration analysis (AECA). Compounds like 1-octen-3-ol and the corresponding ketone were found to be responsible for the mushroom/musty aroma note of Camembert. Kubickova and Grosch (1998a) incorporated key odorants identified in Camembert in a model cheese, which was found to be close to the genuine Camembert. The origins and properties of compounds involved in the flavour of surface-ripened cheeses were reviewed by Molimard and Spinnler (1996). Gorgonzola is an Italian soft blue-veined cheese made from whole cows milk inoculated with spores of *Penicillium roqueforti* var. *weidmanni*. There are two commercial types of Gorgonzola cheese: a traditional variety, also termed 'natural', and a creamy type also called 'sweet',

with a more delicate taste and less pungent flavour. 1-Octen-3-ol, ethyl hexanoate, 2-nonanone, 2-heptanone, 2-heptanol, ethyl butanoate, 2-nonanol and 4-methylanisole were the key odorants of the natural cheese, whereas 2-heptanone, 2-heptanol, ethyl butanoate, 3-(methylthio)propanal and an unidentified constituent with a fruity odour were characteristic of the creamy Gorgonzola cheese (Moio *et al.*, 2000) (Table 14.7). On the basis of high odour activity values, 2-nonanone, 1-octen-3-ol, 2-heptanol, ethyl hexanoate, methylanisole and 2-heptanone were the most important odorants in natural and creamy Gorgonzola cheese aroma (Moio *et al.*, 2000).

Key odorants in the aroma profile of Swiss Emmental were methional, HDMF, ethyl furaneol, diacetyl, 3-methyl butanal and esters (Preininger and Grosch, 1994). Cheese models composed of methional, HDMF, ethyl furaneol, acetic acid, propanoic acid, lactic acid, succinic acid, glutamic acid, sodium, potassium, calcium, magnesium, ammonium, phosphate and chloride were judged to match the flavour of Swiss Emmental cheese very well (Preininger *et al.*, 1996). The flavour of typical Swiss Gruyère cheese and a Gruyère sample exhibiting a potato-like off-flavour were characterized by Rychlik and Bosset (2001a,b). Odorants like methional, 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine were the probable source of potato-like off-flavour in Gruyère.

Münster cheese made in the United States, using *Streptococcus thermophilus* as culture and no surface smear, was described by cooked/milky, whey, milk fat/lactone, sour and salty notes using descriptive sensory analysis (Singh *et al.*, 2003b). The use of dynamic headspace dilution analysis (DHDA) methodology, previously described by Cadwallader and Baek (1998), showed that the most aromatic compounds in the headspace of Münster were 2,3-butanedione, dimethyl sulphide, dimethyl disulphide, 2/3-methylbutanal and 2-acetyl-2-thiazoline (Singh *et al.*, 2003b).

Numerous (>80) odour-active compounds were identified in fresh Chèvre-style goat cheese and assessed by sensory analysis of model cheeses for their specific role in the overall aroma. Overall, the flavour was found to be dominated by 2,3-butanedione, 1-octen-3-one, *o*-aminoacetophenone, lactones and octanoic acid. In addition, 4-methyl octanoic and 4-ethyl octanoic acids were found to impart waxy/crayon odour to fresh goat cheese (Carunchia-Whetstine *et al.*, 2003b).

The flavour of Cheddar cheese is by far the most widely studied. It is generally accepted that the flavour quality of Cheddar cheese in the marketplace today differs considerably from that manufactured before the widespread use of pasteurization, microbial rennets and other modern manufacturing practices (Dunn and Lindsay, 1985). Much of the differences between traditional and contemporary Cheddar flavours probably should be attributed to current

Table 14.7. Aroma compounds identified in mould ripened and Swiss Gruyère cheeses

Blue-type ^a	Gorgonzola (Normal) ^b	Gorgonzola (Sweet or creamy) ^b	Camembert ^c	Swiss Gruyère ^d
2,3-Butanedione	1-Octen-3-ol	2-Heptanone	2,3-Butanedione	2- and 3-Methyl butanal
2-Methyl propanal	Ethyl hexanoate	2-Heptanol	3-Methyl butanal	3-(Methylthio)propanal
3-Methyl butanal	2-Nonanone	Ethyl butanoate	3-(Methylthio)propanal	Dimethyl trisulphide
Ethyl butanoate	2-Heptanone	3-(Methylthio)propanal	1-Octen-3-ol	Phenyl acetaldehyde
Ethyl hexanoate	2-Heptanol	Unknown (fruity odour)	1-Octen-3-one	2-Ethyl-3,5-dimethylpyrazine
3-(Methylthio) propanal	Ethyl butanoate		Phenyl ethyl acetate	2,3-diethyl-5-methylpyrazine
Dimethyl trisulphide	2-Nonanol		2-Undecanone	Methanethiol
2-Heptanone	4-Methylanisole		δ-Decalactone	Acetic acid
2-Nonanone			Methanethiol	Propanoic acid
			Dimethyl sulphide	Butanoic acid
			Acetaldehyde	3-Methyl butanoic acid
			Hexanal	Phenyl acetic acid
			Dimethyl trisulphide	
			Butanoic acid	
			Isovaleric acid	

^aBlue cheese analyzed by gas chromatography-olfactometry/aroma extract dilution analysis/GC-mass spectrometry (GCO/AEDA/GC-MS) (Qian *et al.*, 2002).

^bGorgonzola cheese analyzed by GCO/AEDA/GC-MS (Moto *et al.*, 2000).

^cCamembert cheese analyzed by GCO/AEDA/aroma concentration analysis (AECA)/GC-MS and GCO of decreasing headspace samples (GCO-H) (Kubicova and Grosch, 1997).

^dSwiss Gruyère cheese analyzed by GCO/AEDA/GC-MS and dynamic headspace (DH)-GC-MS (Rychlik and Bosset, 2001a,b).

marketing of bland-flavoured young cheeses. However, even longer-aged cheeses are frequently criticized for a lack of adequate Cheddar-type flavour.

The significance of sulfur compounds, such as H₂S, methanethiol and dimethyl sulphide, in Cheddar was shown by Manning and Robinson (1973). The compounds with low vapor pressure/high boiling points in the distillate, such as 2,3-butanedione, methyl ketones and volatile fatty acids were also considered to play an important role in Cheddar flavour. Analysis of Cheddar headspace volatiles also reconfirmed the important role played by H₂S, methanethiol and dimethyl sulphide in flavour (Manning and Price 1977; Manning and Moore 1979; Price and Manning 1983).

In order to evaluate important odorants, GCO/AEDA was first applied to Cheddar cheese by Christensen and Reineccius (1995). The components found to have the highest potency (dilution factor) in a 3-year-old Cheddar cheese were ethyl acetate, 2-methylbutanal, 3-methylbutanal, 2,3-butanedione, α -pinene, ethyl butyrate, ethyl caproate, 1-octen-3-one, acetic acid, methional, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, decanoic acid and dodecanoic acid. The authors pointed out that the technique did not allow the determination of the most volatile odour fraction, which included hydrogen sulphide, acetaldehyde and methanethiol. Descriptive sensory analysis was not conducted on the cheese used in the study, which limited conclusions about the role of individual compounds on specific cheese flavours. Based on these results, a subsequent sensory study using a concept matching technique was conducted. Dacremont and Vickers (1994) found that a recognizable Cheddar aroma was produced by a mixture of 2,3-butanedione, methional and butanoic acid. However, the authors also indicated a possible contribution of other aroma compounds that were not commercially available at that time.

Milo and Reineccius (1997) applied both traditional high-vacuum isolation-aroma extract dilution analysis (AEDA) and GCO of decreasing headspace samples (GCO-H) to further study the aroma of a regular and a low-fat Cheddar cheese (Table 14.8). After quantification and calculation of the respective odour activity values, based on sensory thresholds in oil and water, they suggested that acetic acid, butyric acid, methional, 2,3-butanedione and homofuraneol are the primary odorants responsible for the pleasant mild aroma of Cheddar cheese. In addition to these compounds, the contribution of highly volatile sulfur compounds such as methanethiol and dimethyl sulphide to nasal perception of Cheddar cheese was quite obvious on the basis of GCO analysis of static headspace samples. The authors further hypothesized that the meaty-brothy odour characteristic of low-fat Cheddar was caused by high concentrations of methional, HDMF and especially homofuraneol. The HDMF-type odorants are known to be produced by certain strains of lactobacilli (Preininger, 1995). While the mixture of these

volatile compounds in a model cheese matrix had Cheddar aroma, attribute profiling described it as lacking in sour, mouldy and sulfurous notes relative to the real cheese. Also, the overall odour was described as weak. This discrepancy in sensory character between the aromatized model and the real cheese was caused partially by aroma–matrix interactions, which resulted in quantitative errors (Wang and Reineccius, 1998).

A comparison of the volatile components of full- and reduced-fat Cheddar showed that the level of methanethiol in the cheese is highly correlated with the flavour grade. This observation may indicate that the lack of aroma in reduced-fat Cheddar is due to the lack of methanethiol. However, a combination of methanethiol and decanoic acid or butanoic acid in all cheeses gave a better correlation with Cheddar flavour than methanethiol alone (Dimos *et al.*, 1996). Addition of methanethiol to a bland slurry of reduced-fat Cheddar produced a strong Cheddar aroma (Urbach, 1997b).

The use of dynamic headspace dilution analysis (DHDA) methodology has suggested additional volatiles as being important to Cheddar cheese aroma as compared to the aforementioned results from GCO-H and solvent extraction/AEDA (Zehentbauer and Reineccius, 2002) (Table 14.8). Results of DHDA showed that in addition to the odorants previously identified by AEDA and GCO-H, (*Z*)-4-heptenal, 2-acetyl-1-pyrroline, dimethyl trisulphide, 1-octen-3-one, (*Z*)-1,5-octadiene-3-one and (*E*)/(*Z*)-2-nonenal, which have been underestimated or not even perceived during AEDA, may also contribute to the overall aroma of Cheddar cheese.

The volatile aroma components of two sharp Cheddar cheeses of British Farmhouse origin, made using raw milk and ripened for at least 1 year, were analyzed by AEDA (Suriyaphan *et al.*, 2001b, Table 14.8). Descriptive sensory analysis of these cheeses was also conducted. Key flavours in sharp Cheddar cheeses were barnyard and earthy. Following instrumental analysis, model system addition was used to confirm compounds responsible for specific flavour notes. *p*-Cresol was mainly responsible for a ‘cowy-barny’ note, whereas an intense ‘soil-like’ note was due to 2-isopropyl-3-methoxy-pyrazine. At much lower odour intensity, 2-isobutyl-3-methoxypyrazine contributed a ‘bell pepper-like’ note. Direct addition of *p*-cresol (>100 µg/kg) or 2-isopropyl-3-methoxypyrazine (>3 µg/kg) to a mild domestic Cheddar cheese resulted in increased intensities of cowy/phenolic and earthy/bell pepper aroma notes. Additionally, within the same wedge of cheese, the concentrations of *p*-cresol and 2-isopropyl-3-methoxypyrazine were lower at the center than at the rind.

Avsar *et al.* (2004) determined that the aldehydes, 2- and 3-methyl butanal and methyl propanal, which are derived from leucine, isoleucine and valine, respectively, have central roles in the nutty flavour of Cheddar

Table 14.8. Aroma compounds identified in Cheddar and Parmigiano-Reggiano cheeses

Mild Cheddar ^a	Sharp Cheddar (British Farmhouse) ^b	Parmigiano Reggiano ^c
2,5-Dimethyl-4-hydroxy-3-(2H)furanone (HDMF)	2-Isopropyl-3-methoxypyrazine	2-Methylpropanal
(E)-2-Nonenal	3-(Methylthio) propanal	2-Methylbutanal
2,3-Butanedione	<i>p</i> -Cresol	3-Methylbutanal
(Z)-4-Heptenal	δ -Dodecalactone	Dimethyl trisulphide
3-(Methylthio) propanal	Butanoic acid	2,3-Butanedione
1-Octen-3-one	3-methyl butanoic acid	3-(Methylthio) propanal
2-Acetyl-2-thiazoline	2-Phenylethanol	Phenyl acetaldehyde
Dimethyl trisulphide	Ethyl octanoate	Ethyl butanoate
(Z)-1,5-Octadien-3-one	Acetic acid	Ethyl hexanoate
(Z)-2-Nonenal	β -Damascenone	Ethyl octanoate
Ethyl butanoate	Octanoic acid	Acetic acid
Hexanal	Sotolon	Butanoic acid
2-Isobutyl-3-methoxypyrazine	Phenyl acetic acid	Hexanoic acid
<i>trans</i> -4,5-Epoxy-2-(E)-decenal	Ethyl butanoate	Octanoic acid
2-Nonanone	Ethyl hexanoate	
2-Isopropyl-3-methoxypyrazine	Dimethyl trisulphide	
Decanal	Phenyl acetaldehyde	
2/3-Methyl butanal	Pentanoic acid	
Ethyl octanoate	Guaiacol	
1-Hexen-3-one	γ -Decalactone	
Methyl propanal	δ -Decalactone	
Ethyl hexanoate	1-Octen-3-one	
Homofuraneol	2-Acetylpyrazine	
Butanoic acid	2-Isobutyl-3-methoxypyrazine	
	Linalool	
	(E,Z)-2,6-Nonadienal	
	Geosmin	
	HDMF	

^aMild Cheddar cheese analyzed by gas chromatography-olfactometry/aroma extract dilution analysis/GC-mass spectrometry (GCO/AEDA/GC-MS) and GCO/dynamic headspace dilution analysis (DHDA)/GC-MS (Zehentbauer and Reineccius, 2002).

^bBritish farmhouse Cheddar cheese analyzed by GCO/AEDA/GC-MS (Suriyaphan *et al.*, 2001b).

^cParmigiano Reggiano cheese analyzed by dynamic headspace analysis-GC-MS (Qian and Reineccius, 2002).

cheese. Nutty flavours are desirable, but they are generally found only in very mature Cheddar cheeses (>9 mo; Avsar *et al.*, 2004). After the identification of volatiles responsible for the nutty flavour in Cheddar, Carunchia-Whets-tine *et al.* (2006) attempted to create this specific aged cheese flavour note by employing specific adjunct cultures in pilot-scale cheese-making trials. Adding *L. lactis* ATCC 29146 at the level of 10⁴ or 10⁵ cfu/mL of milk as an

adjunct culture during manufacture of Cheddar cheese resulted in an increase in nutty flavour perception in the cheese. Cheeses ripened at 13°C developed aged flavours (including nutty) more rapidly than cheeses ripened at 5°C. Panellists described the 1-week old cheeses as nutty/malty, whereas the 4- and 8-mo cheeses were described only as nutty. The concentrations of 2- and 3-methyl butanal and methyl propanal increased during aging and were higher in the cheeses with the adjunct culture added and in the cheeses ripened at 13°C. This study demonstrates the advantages of linking descriptive sensory analysis, flavour chemistry and starter culture biochemistry to control cheese flavour. These results allow cheese manufacturers the opportunity to optimize the cheese-making procedure to produce a consistent nutty-flavoured Cheddar cheese.

It is important to note that in each of the studies mentioned previously, different Cheddar cheeses of different ages, microflora, and biochemistry were studied. Cheddar cheese encompasses a wide category and there are numerous potential flavour profiles. Thus, to elucidate Cheddar cheese flavour is a large task and descriptive sensory analysis should be conducted in conjunction with any instrumental study to provide clarification.

14.4. Specific Flavours or Off-Flavours in Milk and Milk Products

In addition to the characteristic desirable flavours, dairy products frequently suffer from specific flavour defects. While desirable flavour has been difficult to define in chemical and sensory terms since consumers vary in preference and definition of dairy product flavour, the specific cause(s) of many of these specific flavour or off-flavour notes have been established more or less definitively. This section presents an overview on chemicals responsible for the specific flavour/off-flavour notes in dairy products.

It is important to note that at the beginning of the development of flavour defects, or before the aroma detection threshold of certain off-flavours is reached, the fluid milk may show a poor/flat flavour that lacks freshness (Badings, 1991). GCO analysis performed on five milk samples (one of good flavour and four samples tainted with off-flavour characterized as 'feed') revealed approximately 75 aroma-active compounds (Mouchili *et al.*, 2005). Nearly all those odorants were common to all the milk extracts analysed (both good-quality and off-flavoured samples), suggesting that off-flavour originated from the concentration differences of a common set of compounds rather than from the absence or presence of specific compounds. Off-flavours in milk may develop *via* the different

mechanisms outlined below (see Table 14.9 for possible causative chemicals/mechanisms involved):

- Off-flavour transferred to milk during lactation
 - Feed off-flavours (e.g., from green forages, silage, etc., consumed by the animal a few hour before milking)
 - Weed off-flavour (e.g., volatiles formed from certain weeds during digestion)
- Cowy flavours (related to ketosis and acetonemia in cattle resulting in an increased concentration of acetone)
- Stable off-flavours (e.g., transferred via the respiratory system from stable/feed to cow)
- Lipid oxidation—most widely implicated in the defects in milk and milk products
- Microbial action
- Contamination (e.g., compounds from sanitizers)
- Thermal abuse
- Adsorption/permeation of aroma compounds with/through packaging material

Some of the mechanisms of off-flavour development, in particular lipid oxidation and microbial metabolites, have also been responsible for the development of a whole variety of off-flavours in milk and milk products (Table 14.9). Specific flavours like nutty or brothy flavours were also characterized in Cheddar cheese (Table 14.9), which may or may not be considered as an off-flavour depending on the consumer preference.

14.5. Taste Compounds in Milk and Milk Products

Research on taste compounds in dairy products is fairly limited but there seems to be an increasing interest in this area in recent years. In food systems such as milk and milk products, study of taste compounds in isolation or in matrices devoid of contribution from volatile aroma compounds is difficult due to complex nature, in terms of both number of food constituents and their competing/synergistic effects on taste and/or aroma. Compounds which contribute to the taste of milk and milk products can originate from three possible sources:

- (1) Naturally found in milk, e.g., lactose
- (2) Added/produced during the manufacturing process (e.g., NaCl, lactic acid)
- (3) Produced by many biochemical reactions occurring during fermentation.

Table 14.9. Specific flavour and off-flavour compounds in cheeses (modified from Singh *et al.*, 2007)

Defect	Chemicals	Mechanism of Formation	Reference
Processed milk			
Oxidized	Heptanal, octanal, nonanal, 2-octenal, 2-nonenal	Lipid oxidation, light abuse, Cu oxidation	Forss <i>et al.</i> (1955a, b)
Malty	2- and 3-methylbutanal	Microbial, enzymatic, Strecker degradation	Morgan (1970a,b)
Metallic Fishy	1-Octen-3-one Trimethylamine	Lipid oxidation Microbial	Day <i>et al.</i> (1963) Corfield (1955), Humphriss (1953)
Phenolic Fruity	<i>p</i> -Cresol Ethyl butanoate, ethyl hexanoate	Enzymatic, microbial Microbial, enzymatic	Lunden <i>et al.</i> (2002) Badings and Neeter (1980) Wellnitz-Ruen <i>et al.</i> (1982) Whitfield <i>et al.</i> (2000)
Rancid	FFAs (C4-10)	Microbial, enzymatic, sanitizer	Marsili (2003) Azzara and Campbell (1992), Marsili (2000)
Oxidized	Hexanal, 1-octen-3-one	Lipid oxidation, light abuse, Cu oxidation	Cadwallader and Howard (1998) Marsili and Miller (1998)
Oxidized (cardboard)	1-Octen-3-one, octanal	Lipid oxidation	Hammond and Seals (1972)
Oxidized (general)	Aldehydes and ketones	Lipid oxidation	Badings (1991)
Oxidized (fatty/fried)	2-Alkenals (C7-C10), 2,4-alkadienals (C7, C10)	Lipid oxidation	Badings (1991)
Oxidized (green/cucumber)	(Z)-3-Hexenal, (E,Z)-2,6-nonadienal	Lipid oxidation	Badings (1991)
Oxidized (tallowy)	2-Alkenals (C7-C10), alkanals	Lipid oxidation	Badings (1991)
Oxidized (fishy)	2,4,7-Decatrienal	Lipid oxidation	Badings (1991)

(Continued)

Table 14.9. (Continued)

Defect	Chemicals	Mechanism of Formation	Reference
Cooked flavour	H ₂ S, methanethiol, dimethylsulphide and other sulphides	Thermally induced	Boelrijk and de Jong (2003)
Weed taints	Maltol, furans, pyrazines and other Maillard reaction products	Thermally induced	Badings (1991)
	Indole, skatole, mercaptans, sulphides, nitriles, Thiocyanates	Metabolites of weed	Badings (1991)
Feed flavours	Skatole (fecal odour)	Metabolites of weed	Park (1969)
	Benzylthiol, benzyl methyl sulphide (burnt odour)	Metabolites of weed	Park <i>et al.</i> (1969)
Feed flavours	Dimethyl sulphide, acetone, butanone, isopropanol, ethanol, propanal	Metabolites of feed and silage	Badings (1991)
	(<i>E</i>)-2- and (<i>Z</i>)-3-hexenal	Freshly cut alfalfa	Marsili (2003)
Feed flavours Light induced	Dimethyl sulphide, 2-butanone, hexanal	Grass silage	Mouchili <i>et al.</i> (2005)
	Dimethyl disulphide	Light-induced degradation (singlet oxygen oxidation) of methionine	Jung <i>et al.</i> (1998)
Heat abuse, cooked	2-Pentanone, 2-heptanone, 2-nonanone	Thermally induced, microbial	Marsili and Miller (2003)
	Methanethiol, dimethyl sulphide, dimethyl trisulphide, hydrogen sulphide	Thermally induced	Vazquez-Landaverde <i>et al.</i> (2005, 2006b)
Stale Cooked/stale/sulphury	C3-11, 13 2-alkanones / C4-10 saturated aldehydes	Thermally induced	Perkins <i>et al.</i> (2005)
	2,3-Butanedione, 2-alkanones, 2-methylpropanal, 3-methylbutanal, nonanal, decanal, dimethyl sulphide	Thermally induced	Vazquez-Landaverde <i>et al.</i> (2005)

(Continued)

Table 14.9. (Continued)

Defect	Chemicals	Mechanism of Formation	Reference
Chemical, solvent-like	Propyl/acetate	Solvent residue from packaging material	Marsili (2003)
Butter			
Green or yogurt-like	Acetaldehyde	Microbial, enzymatic	Lindsay <i>et al.</i> (1965)
Metallic	1,5-Octadiene-2-one	Lipid oxidation	Swodoba and Peers (1977)
Stale	Styrene	Residue from packaging material	Lozano <i>et al.</i> (2007)
Burnt/rubbery/sulphur	Benzyl methyl sulphide	Unknown	Drake <i>et al.</i> (2007b)
Butter oil			
Cardboard	(E)-/(Z)-2-Nonenal	Lipid oxidation	Grosch <i>et al.</i> (1994) Widder and Grosch (1997)
Sterilized concentrated milk			
Stale	<i>o</i> -Aminoacetophenone	–	Arnold <i>et al.</i> (1966)
Ice cream			
Putrid flavour	Dimethyl disulphide, hexanal	Light-induced degradation (singlet oxygen oxidation) of methionine / lipid oxidation	Marsili (2003)
Sour cream buttermilk			
Metallic	(E,Z)-2,6-Nonadien-1-ol	Lipid oxidation, microbial, enzymatic	Helier and Schieberle (1997a, b)
Yogurt			
Smoky or phenolic aroma	Guaiaicol (defect found in vanilla flavoured products)	Microbial	Whitfield (1998)
Cheese-like off-note	Short-chain fatty acids	Microbial, enzymatic	Rychlik <i>et al.</i> (2006)

(Continued)

Table 14.9. (Continued)

Defect	Chemicals	Mechanism of Formation	Reference
Rancid, unclean Swiss Gruyère Potato-like aroma	Short-chain fatty acids Methional	Enzymatic, microbial Microbial, enzymatic	Cadwallader (unpublished) Rychlik and Bosset (2001a,b)
Gruyère de Comté Potato-like aroma	3-Methoxy-2-propylpyridine	Microbial	Dumont <i>et al.</i> (1975)
Smear-coated cheese Potato-like aroma	2-Methoxy-3-isopropylpyrazine	Microbial	Dumont <i>et al.</i> (1983)
Feta Kerosene-like	<i>trans</i> -1,3-Pentadiene	Microbial, enzymatic	Horwood <i>et al.</i> (1981)
Goat cheese Waxy/crayon	4-Methyl octanoic acid, 4-ethyl octanoic acid	Milk, enzymatic	Carunchia-Whetstine <i>et al.</i> (2003b)
Oxidized	1-Heptanol, heptanal, nonanal, 2-decenal	Lipid oxidation, light-induced	Kim <i>et al.</i> (2003)
Brie / Camembert Musty/earthy	Methylisoborneol	Microbial	Karahadian <i>et al.</i> (1985)
Cheddar cheese Unclean/off-flavour Catty flavour	2-Methyl propanoic acid, 3-methyl butanoic acid 2-mercapto-2-methylpentan-4-one and sulphide	Microbial, enzymatic Reaction of mesityl oxide (contaminant)	Nakae and Elliot (1965a,b) Badings (1967), Spencer (1969a,b)
Fruitiness	Ethyl butanoate, ethyl hexanoate, ethyl octanoate	Microbial, enzymatic	Drake <i>et al.</i> (2001, 2002) Bills <i>et al.</i> (1965), Morgan (1970b)

(Continued)

Table 14.9. (Continued)

Defect	Chemicals	Mechanism of Formation	Reference
Floral/rose-like	Phenyl ethanol, Phenyl acetaldehyde	Microbial, enzymatic	Dunn and Lindsay (1985)
Unclean, utensil-like	<i>p</i> -Cresol (Off-flavour enhanced by FFAs)	Microbial, enzymatic	Dunn and Lindsay (1985)
Unclean (dull harsh)	2- and 3-Methylbutanal, 2-methylpropanal	Microbial, enzymatic	Dunn and Lindsay (1985)
Rosy/floral	Phenylacetaldehyde, phenylacetic acid	Microbial, enzymatic	Carunchia-Whetstone <i>et al.</i> (2005a)
Yeast flavour	Ethanol, ethyl acetate, ethyl butanoate	Microbial, enzymatic	Horwood <i>et al.</i> (1987)
Phenolic	<i>p</i> -Cresol	Microbial, enzymatic	Ramshaw <i>et al.</i> (1990)
Mayonnaise/bread-like	(<i>E,E</i>)/(<i>E,Z</i>)-2,4-Decadienal	Microbial, lipid oxidation, enzymatic	Suriyaphan <i>et al.</i> (1999, 2001a)
Cow / barny-flavours	<i>p</i> -Cresol	Microbial, enzymatic	Suriyaphan <i>et al.</i> (2001b)
Earthy / bell pepper	2-isopropyl-3-methoxypyrazine	Microbial, Maillard reaction	Suriyaphan <i>et al.</i> (2001b)
Brothy flavour	3-(Methylthio) propanal, 2,5-Dimethyl-4-hydroxy-3(2 <i>H</i>)-furanone, Ethyl furaneol, 2-Methyl-3-furanthiol (and its dimerized form)	Microbial, enzymatic, Maillard reaction	Singh <i>et al.</i> (2004a), Cadwallader <i>et al.</i> (2006)
Nutty flavour	2-Methylpropanal, 2- and 3-methylbutanal	Microbial, enzymatic	Avsar <i>et al.</i> (2004) Carunchia-Whetstone <i>et al.</i> (2006)
Mothball/grassy	Acetic acid, 2-methyl butanoic acid, skatole	Microbial, nzymatic	Drake <i>et al.</i> (2007b)
Italian cheeses (Provolone, Pecorino, Romano and Parmesan)			
Medicinal/cow	Phenol, <i>m</i> - and <i>p</i> -cresol, ethyl phenol, 3,4-dimethyl phenol, 2-isopropyl phenol, thymol and carvacrol	Microbial, enzymatic, feed	Ha and Lindsay (1991), Ney (1973)

(Continued)

Table 14.9. (Continued)

Defect	Chemicals	Mechanism of Formation	Reference
Club cheese Musty/dirty	2-Alkanone (C7,9,11), 8-nonen-2-one	Mould growth/ metabolites	Marsili (2007b)
Skim milk powder Stale Stale	<i>o</i> -Aminoacetophenone 2-Furaldehyde, 2-furfuryl butyrate, alkylpyrazine, <i>N</i> -ethyl-2-formylpyrrole	– Non-enzymatic browning	Parks <i>et al.</i> (1964) Ferrenti and Flanagan (1972)
Cow house-like Papery/cardboard-like	β -Ionone, benzothiazole, tetradecanal (<i>E, E</i>)-2,4-Nonadienal, (<i>E, E</i>)-2,4-decadienal	– Lipid oxidation	Shiratsuchi <i>et al.</i> (1994b) Karagul-Yuceer <i>et al.</i> (2003a)
Infant formula Rancid	Propanal, pentanal, hexanal	Lipid oxidation	Romeu-Nadal <i>et al.</i> (2007)
Whey protein concentrates/isolates Cabbage off-flavour	Dimethyl trisulphide	Degradation of Met	Wright <i>et al.</i> (2006)
Sour cream powder Melon/ripened kiwi-like	2,4,5-Trimethyloxazole	Reaction of diacetyl and Arg/Cys	Marsili (2007c)

Table 14.10. Taste compounds in milk and milk products (modified from Singh *et al.*, 2007)

Compounds	Taste/other complex sensation	Products
Lactose	Sweet	Milk, concentrated / evaporated milks, dried milk powders
Sucrose	Sweet	Sweetened yogurt, ice creams, sweetened condensed/evaporated milks
Lactic acid	Sour	Fermented milks / cream, cheeses
Acetic/propanoic acid	Sour	Fermented milks, cheeses
Propionic acid	Sour, umami	Umami taste in Swiss cheese
Succinic acid	Umami	Umami taste in Swiss cheese
Ca/Mg salt of propanoic acid	Sweet	Cheeses
Peptides	Mostly bland, some can be bitter, sour or umami	Milk, Fermented milks, cheeses
γ -Glutamyl dipeptides	Sour, salty, brothy metallic	Comté Cheese
Amino acids		
Gly, Ala, Ser, Thr	Sweet	Cheeses
Glu, Asp, Gln, Asn	Sour, umami	Cheeses
His	Sour (?)	Cheeses
Pro, Lys	Sweet, bitter	
Leu, Val, Ile, Arg, Phe, Tyr	Bitter	Cheeses
Trp		
NaCl	Salty	Cheeses
Ethanol	Slightly sweet, cooling/drying sensation	Fermented milks (e.g., Kefir, Koumiss)
CO ₂	Sour, fresh, cooling sensation	Fermented milks (e.g., Kefir, Koumiss)

Table 14.10 lists a number of compounds, and their taste contribution, present in dairy products. Important details available in the literature on taste compounds are summarized below:

- The main taste compounds in milk are lactose (approximately 0.3 times as sweet as sucrose) and the dissolved salts, which cause a sweet and salty taste, respectively. The sweet taste dominates, whereas salty taste is prevalent if the Na/lactose ratio is high, as in the case of mastitic milk. The casein reportedly somewhat masks the sweet taste of lactose in milk (Walstra *et al.*, 1999). Lactose-hydrolyzed milk and whey have a sweeter taste than regular pasteurized milk.

- A chalky taste is noticed in high heat-treated or UHT-treated milks. This may be the result of precipitation of colloidal calcium phosphate.
- Sodium chloride is an important contributor to the taste of cheeses. The apparent saltiness of cheese increases with maturity, increased NaCl concentration and decreasing pH (McSweeney, 1997).
- The principal acid in fermented milk and cheese is lactic acid. The concentration of lactic acid, and also the pH, varies considerably with:
 - The type of fermented dairy product
 - Initial production by the starter culture
 - Extent of loss in whey
 - Its metabolism by the non-starter microflora

Several other acids, e.g., acetic, propanoic, and C₄₋₁₀, also contribute to sour/soapy taste but they contribute mostly towards the aroma. Some of the characteristic taste (sour, sweet, salty) compounds of Emmental (Swiss) cheese are acetic acid, propanoic acid, lactic acid, succinic acid glutamic acid, each in free form and/or as ammonium, sodium, potassium, magnesium and calcium salts, as well as corresponding chlorides and phosphates (Warmke *et al.*, 1996). Magnesium and calcium propionate mainly cause the sweetish note in the taste profile of Emmental cheese.

- Casein is hydrolyzed to varying degrees depending on the type of fermented milk and cheese, resulting in the production of peptides and free amino acids. The precise role of the intermediate to small molecular weight peptides is not clear, but they are generally accepted to play an important role in the background taste of cheese (Fox *et al.*, 1994). Several peptides have been identified in different types of cheeses as bitter (see Table 14.11 for a list of bitter peptides). An interesting relationship was established by Ney (1981) between the average hydrophobicity (Q) of a peptide, as measured by the hydrophobicity of amino acid side chains determined by Tanford (1962), and bitterness. Peptides with a Q value >1400 cal mol⁻¹ residue⁻¹ and a molecular weight up to 6000 Da (molecules >6000 Da are likely to be too large to interact with the taste receptors) taste bitter, and no bitterness occurs when Q is <1300 cal mol⁻¹ residue⁻¹. Peptide β -CN f193–209, identified in both Cheddar and Gouda, was shown to be bitter by detailed sensory analysis (Singh *et al.*, 2004b). This peptide with a Q value of 1762 cal mol⁻¹ residue⁻¹ and molecular weight of 1882.51 Da was

Table 14.11. Bitter peptides identified in cheeses

Peptide	Sequence	Hydrophobicity cal mol ⁻¹ residue ⁻¹	Type of cheese and Reference
α_1 -CN f1-7	H.Arg.Pro.Lys.His.Pro.Ile.Lys.OH	1771.0	Cheddar ; Lee <i>et al.</i> (1996)
α_6 -CN f1-13	H.Arg.Pro.Lys.His.Pro.Ile.Lys. His.Gln.Gly.Leu.Pro.Gln.OH	1363.0	Cheddar ; Lee <i>et al.</i> (1996)
α_1 -CN f11-14	H.Leu.Pro.Gln.Glu.OH	1367.0	Cheddar ; Lee <i>et al.</i> (1996)
α_6 -CN f14-17	H.Glu.Val.Leu.Asn.OH	1162.5	Cheddar ; Hodges <i>et al.</i> (1972), Richardson and Creamer (1973), Hamilton <i>et al.</i> (1974)
α_1 -CN f17-21	H.Asn.Glu.Asn.Leu.Leu.OH	1074.0	Cheddar ; Hodges <i>et al.</i> (1972), Richardson and Creamer (1973), Hamilton <i>et al.</i> (1974)
α_1 -CN f26-32	H.Ala.Pro.Phe.Pro.Glu.Val.Phe.OH	1930.0	Cheddar ; Richardson and Creamer (1973)
α_1 -CN f26-33	H.Ala.Pro.Phe.Pro.Glu.Val. Phe.Gly.OH	1688.8	Cheddar ; Hodges <i>et al.</i> (1972), Hamilton <i>et al.</i> (1974)
α_1 -CN f198-199	H.Leu.Trp.OH	2710.0	Alpkäse ; Guigoz and Solms (1974)
α_2 -CN f191-197	H.Lys.Pro.Trp.Ile.Gln.Pro.Lys.OH	2010.0	Cheddar ; Lee <i>et al.</i> (1996)
β -CN f8-16	H.Val.Pro.Gly.Glu.Ile.Val. Glu.Ser(P).Leu.OH	1390.0	Cheddar ; Lee <i>et al.</i> (1996)
β -CN f 46-67	H.Gln.Asp.Lys.Ile.His.Pro.Phe. Ala.Gln.Thr.Gln.Ser.Leu. Val.Tyr.Pro.Phe.Pro.Gly.Pro.Ile. (Pro/His).OH	1580.5	Cheddar ; Richardson and Creamer (1973), Hamilton <i>et al.</i> (1974)
β -CN f 61-69	H.Pro.Phe.Pro.Gly.Pro.Ile. Pro.Asn.Ser.OH	1792.2	Butterkäse ; Huber and Klostermeyer (1974)
β -CN f46-84	H.Gln.Asp.Lys.Ile.His.Pro.Phe.Ala. Gln.Thr.Gln.Ser.Leu.Val.Tyr.Pro. Phe.Pro.Gly.Pro.Ile.(Pro/ His).Asn.Ser.Leu.Pro.Gln.Asn.Ile. Pro.Pro.Leu.Thr.Gln.Thr.Pro.Val. Val.Val.OH	1508.5	Cheddar ; Hamilton <i>et al.</i> (1974)

(Continued)

Table 14.11. (Continued)

Peptide	Sequence	Hydrophobicity cal mol ⁻¹ residue ⁻¹	Type of cheese and Reference
β -CN f84-89	H.Val.Pro.Pro.Phe.Leu.Gln.OH	1983.3	Gouda ; Visser <i>et al.</i> (1983)
β -CN f193-209	H.Tyr.Gln.Glu.Pro.Val.Leu.Gly.Pro. Val.Arg.Gly.Pro.Phe.Pro.Ile.Ile. Val.OH	1762.4	Cheddar ; Kelly (1993), Broadbent <i>et al.</i> (1998), Soeryapranata <i>et al.</i> (2002a, b), Singh <i>et al.</i> (2004b, 2005) Gouda ; Visser <i>et al.</i> (1983)
β -CN f193-208	H.Tyr.Gln.Glu.Pro.Val.Leu.Gly. Pro.Val.Arg.Gly.Pro.Phe.Pro.Ile. Ile.OH	1766.9	Gouda ; Visser <i>et al.</i> (1983)
β -CN f193-207	H.Tyr.Gln.Glu.Pro.Val.Leu.Gly. Pro.Val.Arg.Gly.Pro.Phe. Pro.Ile.OH	1686.7	Gouda ; Visser <i>et al.</i> (1983)

also classified as potentially bitter in the Q value model proposed by Ney (1981). Amino acids are also known to elicit different taste (see Table 14.10).

- Several small peptides were identified in Comté cheese. Cyclic dipeptides were described as bitter (Roudot-Algaron *et al.*, 1993) and dipeptides with a gamma-glutamyl residue were found to be sour (e.g., γ -Glu-Tyr), apart from γ -Glu-Phe described to have a complex taste, which was brothy and slightly sour, salty and metallic (Roudot-Algaron *et al.*, 1994). However, because of their low concentration compared to their taste threshold value, they could not be directly responsible of cheese taste.

A number of workers have studied taste-active compounds in Camembert (Engel *et al.*, 2001a,b,c), Cheddar (Yang and Vickers, 2004), Comté (Salles *et al.*, 1995), Goat (Engel *et al.*, 2000a,b; Engel *et al.*, 2002; Salles *et al.*, 2002) and Emmentaler cheeses (Warmke *et al.*, 1996). Drake *et al.* (2007c) studied compounds responsible for the umami taste in Cheddar and Swiss cheeses. Low and high intensity umami-tasting cheeses were selected using a trained sensory panel. Some compounds, namely monosodium glutamate (MSG), disodium-5'-inosine monophosphate (IMP), disodium-5'-guanosine monophosphate (GMP), sodium chloride, lactic acid, propionic acid and succinic acid, were quantified in both types of cheese with and without umami taste. Comparison of analytical data and sensory thresholds indicated that IMP and GMP thresholds were 100-fold higher than their concentrations in cheese. All other compounds contributed some umami taste within their concentration range in umami cheeses. Sensory analysis of model cheeses clearly demonstrated that Glu played a major role in the umami taste of both Cheddar and Swiss cheese, while succinic and propionic acids contributed in Swiss cheese. The knowledge of umami-tasting components of cheeses will be useful in developing technologies to control and regulate the level of this specific taste attribute in cheeses.

14.6. Conclusions

A concerted series of chemical and biochemical reactions are involved in the formation of dairy flavour and off-flavour compounds. The general chemical/biochemical pathways, i.e., (1) heat-induced changes, (2) lipid oxidation, (3) glycolysis, (4) lipolysis and (5) proteolysis, involved in the degradation of milk constituents are now fairly well characterized. Recent works on the enzymology and genetic manipulation of the starter and non-starter lactic acid bacteria have helped in the better understanding of further catabolic modification of the products of primary degradation pathways. This has lead

to immense progress in understanding the flavour chemistry of fermented dairy products. So far, a large number of volatile compounds have been identified from various types of cheese but still it is not possible to duplicate cheese flavour by pure chemicals in model systems. However, there is now a good understanding of the causes of bitterness and specific flavours/off-flavours in dairy products.

Growing consumer awareness and demand for minimally processed fresh food products has led to the application of non-thermal processing technologies in the processing of milk and milk products. The influence of new processing technologies like high pressure and pulsed electric field on sensory and flavour chemistry will need to be studied further. In addition, there is a demand for rapid and high-throughput processes that create dairy foods with traits (in particular, flavour and texture) identical to those of traditional dairy products that may take months or even years to develop.

Recent developments in sensory and instrumental methodologies in flavour analysis have been of immense help in furthering our understanding of the flavour chemistry of dairy products. Further work on the characterization of flavour (both aroma- and taste-active) compounds, flavour-matrix interaction mechanisms and flavour release mechanisms are needed to elucidate fully the complex nature of dairy flavours. The better understanding of flavour chemistry will be useful in the development of new technologies/mechanisms for the effective control and acceleration of the ripening process in cheese.

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