7

Flavour generation in dairy products

A. E. M. Boelrijk, C. de Jong and G. Smit, NIZO Food Research, The Netherlands

7.1 Introduction

In this chapter, the focus is primarily on the chemical aspects of flavour generation in dairy products as opposed to biological flavour formation. However, a strict separation between chemical and biological aspects of flavour generation is rather artificial (whether you use a proton or an enzyme for a catalytic transformation, in the end it is all chemistry!). In order to prevent overlap with other chapters in this book which focus on the role of commercial cultures and enzymes in flavour production as much as possible, we decided to focus primarily on the flavour and off-flavour generation in several dairy products caused by processing steps (such as heating and ageing). In this review we deal with dairy products from bovine milk. Although the chapter aims to cover the literature in this field as much as possible, the main focus is on the literature that has been published since 1990. For the literature before 1990 we refer to excellent reviews by Badings (1991) and Adda (1986) on the flavours in milk.

7.1.1 Chemical pathways that are important for the generation of dairy flavours

All dairy products start from raw milk that, apart from minor ingredients such as salts and vitamins, consists mainly of milk fat, proteins and lactose. These three main groups can be degraded to building blocks that have a flavour of their own, or derivatives from each group can react with each other to form new products that have a flavour (Adda, 1986; Dumont and Adda, 1979). The possible degradation reactions for the three main groups are summarised in Fig. 7.1(a)

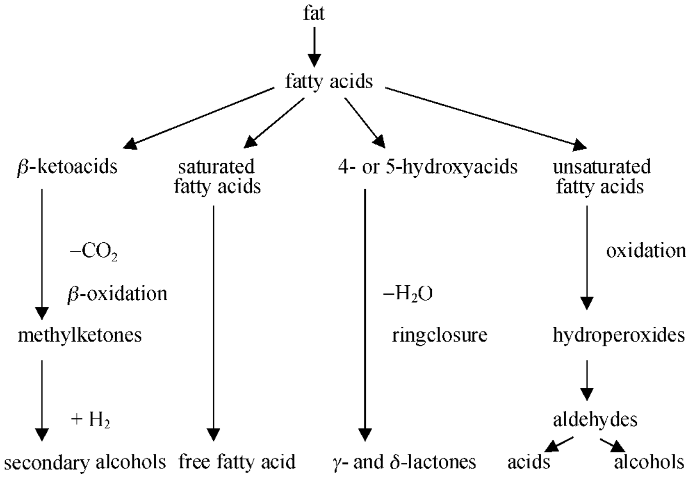

Fig. 7.1(a) Degradation of milk fat.

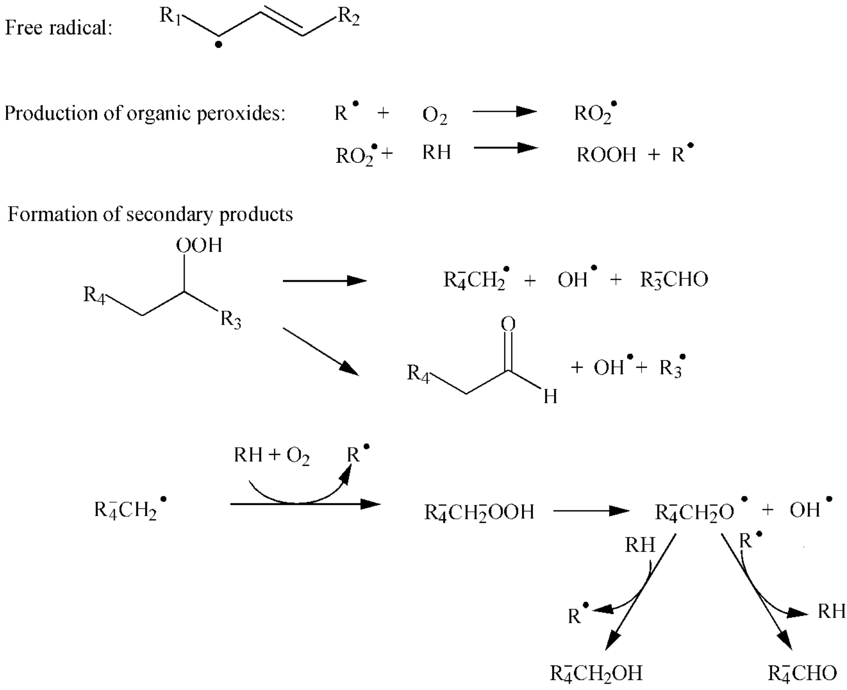

Fig. 7.1(b) Auto-oxidation of unsaturated fatty acids.

(degradation of milk fat) and Fig. 7.1(b) (auto-oxidation of unsaturated fatty acids), and later in the chapter in Fig. 7.2 (degradation of proteins) and Fig. 7.3 (degradation of lactose). We start this chapter by discussing these conversion pathways in more detail.

7.1.2 Degradation of milk fat

The degradation of milk fat results in a large number of different volatile flavours (Adda, 1986). Lipases (either bacterial or milk-own) degrade milk fat in C_4–C_{10} free fatty acids (Driessen, 1981). β-Keto acids are formed from saturated fatty acids and in turn result in the formation of methyl ketones after proton- or enzyme-catalysed decarboxylation (van Duin, 1965). The methyl ketones can also be formed by β-oxidation of saturated fatty acids. Hydrogenation of the methyl ketones leads to secondary alcohols. By a cyclisation step (β-oxidation followed by splitting off a water molecule), γ- and δ-lactones are formed from γ- or δ-hydroxy fatty acids. The γ- or δ-hydroxy fatty acids are present in milk fat in small quantities from the start and can also be formed from unsaturated free fatty acids by a hydration reaction (Mick *et al.*, 1982). The hydroxy acids can also lead to C_3–C_{15} esters by intramolecular esterification reactions. An important route for the formation of aldehydes and ketones from unsaturated fatty acids is auto-oxidation. An example of a general auto-oxidation scheme is given in Fig. 7.1(b). The auto-oxidation reaction cascade starts with radical formation. The resulting organic peroxide degrades in secondary products such as ketones, aldehydes and alcohols. The origin of the starting radical can be manyfold and will be discussed in more detail in section 7.2.3 on the generation of off-flavours in milk. Aldehydes are important starting products for the formation of acids (by oxidation) or for the formation of Maillard products after their reaction with amino acids. The Maillard reaction will be discussed in detail in section 7.2.2 on heated milk.

7.1.3 Degradation of proteins

A large diversity of volatile and non-volatile dairy flavours can be formed from proteins (Adda, 1986; Calvo and de la Hoz, 1992; Contarini *et al.*, 1997). Figure 7.2 summarises the different pathways. Proteinases and peptidases (bacterial or milk-own) degrade the proteins to peptides and free amino acids. Several (non-volatile) peptides have a bitter taste (Driessen, 1981) and will be discussed in detail in Chapter 22. Amino acids can be degraded to aldehydes by Strecker degradation (Nursten, 1986; Ho, 1996), which in turn can take part in the Maillard reaction or can be oxidised to form acids. Sulphur-containing amino acids, i.e. cysteine and methionine, are important sources for the generation of volatile sulphur containing compounds which have a distinct flavour. Protonation of activated sulphydryl groups leads to liberation of H_2S, while it is thought that protonation of methionine residues leads to liberation of methional (Nursten, 1986; Ho, 1996). Nitrogen-containing amino acids

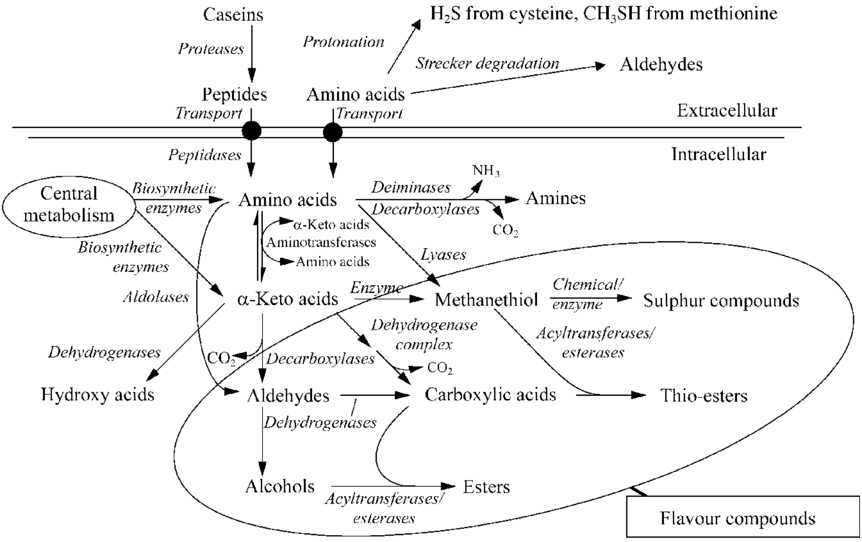

Fig. 7.2 Degradation of proteins.

eventually lead to volatile nitrogen-containing flavours such as pyrazines, pyrroles and so on. Photochemical degradation of thiamine (vitamin B1) followed by a reaction with H_2S has been reported to produce a strong aromatic volatile with a rubber taste (Buttery *et al.*, 1981), whose molecular identity has not been established yet.

7.1.4 Degradation of lactose

For the preparation of yoghurt the enzymatic degradation of lactose into lactate, acetaldehyde and diacetyl is essential. However, lactose can also react non-enzymatically with amino acids via the Maillard reaction which eventually leads to a number of volatile flavour compounds depending on the amino acids that are available and the reaction conditions that are used (van Boekel, 1998). The degradation will be discussed in more detail in the paragraph dealing with the Maillard reaction in milk in section 7.2.2.

The mixture of flavours that is eventually produced from the different milk ingredient groups (fat, protein and lactose) depends on the processing conditions and micro-organisms that are used for preparation of the dairy product. Usually a complex interaction between biological and chemical degradation pathways leads to the desired or characteristic flavour. In the next sections the pathways leading to the characteristic flavours will be discussed for different dairy products: raw milk, heated milk and fermented liquid dairy products (yoghurt and buttermilk).

7.2 Raw and heat-treated milk

7.2.1 Raw milk

Fresh, raw milk has a characteristic but very bland taste (Adda, 1986; Badings, 1991; Nursten, 1997). The sensory perception of fresh milk is mainly determined by a pleasant mouthfeel from the physical constitution of milk (Walstra and Jenness, 1988), a slight sweet/salty taste that is caused by lactose and milk salts, and a delicate flavour caused by many odorous compounds. Although at least 400 volatile compounds (carbonyl compounds, alkanols, free fatty acids, sulphur compounds, etc.) have been detected in raw milk (Badings, 1991), most of them are present in very low concentrations. In total, all aroma compounds together comprise no more than 1–100 mg per kg milk. Adda, Badings and their colleagues have extensively analysed the volatile flavours of fresh milk with GC-olfactometry and GC-MS (Adda, 1986; Badings, 1991). Their conclusions were that, although the concentrations of many of these volatiles were of a sub-threshold level, the delicate, weak flavour of milk is the result of a specific ratio between these volatiles. An interesting question is whether some of those 400 volatiles found in fresh milk are more important for sensory perception than others. Moio *et al.* (1993) tried to answer this question by doing a CHARM (Combined Hedonic Aroma Response Measurement) analysis. A CHARM analysis combines olfactometry measurements (olfactometry = using a human nose as a detector behind a gas chromatograph) with time intensity measurements and therefore gives an estimation of the flavour impact of the different volatiles (Acree *et al.*, 1984; Friedrich and Acree, 1998). Based on the CHARM analysis of fresh milk, Moio *et al.* concluded that ethylbutanoate, ethylhexanoate and dimethylsulphone have a relatively large impact on the total milk flavour.

The origin of flavours in normal fresh milk is thought to be in the metabolism of the cow. However, transfer of volatiles from the forage of the milk may also take place. A combination of certain forage and the metabolism of the cow may lead to increased concentration of certain volatiles in the raw milk such as indole, skatole, sulphides, mercaptans, nitriles, thiocyanates, etc., which lead to off-flavours in the milk (Badings, 1991; Bendall, 2001). This topic is discussed in more detail in section 7.2.3. Recently, the effects of storage conditions on lypolysis, proteolysis and sensory attributes in raw milk have been studied. Overall results showed that most of the changes in raw milk occur within the first 24 hours (Wiking *et al.*, 2002).

7.2.2 The effect of different heat treatments on the flavour profile of milk

The main reason for giving milk a heat treatment is to prolong its shelf-life. Heat treatments have a major effect on the flavour of milk. Different heat treatments lead to different flavour profiles and therefore result in different types of milk. The most widely used heat treatments and their resulting flavour profiles are discussed below.

Low-pasteurised milk

This is a very mild heat treatment (e.g. 15 s, 72ºC) which eliminates pathogenic micro-organisms and gives only a modest prolongation of shelf-life. Because the effect of this treatment on the milk flavour is minimal, the milk keeps its basic taste. Only a very weak cooked flavour may be detected, which is caused by traces of H_2S. Other flavour components of LP milk were summarised by Badings (1991). Recently, hept-*cis*-4-enal was determined to make an important contribution towards the flavour of fresh homogenised/pasteurised milk at concentrations in the low to medium pg/g range (Bendall and Olney, 2001).

Medium-pasteurised milk

This heat treatment (e.g. 20 s, 75ºC) is slightly more intense than the LP treatment. The result is a milk flavour that is very similar to that of LP milk but with a slightly more pronounced cooked flavour.

High-pasteurised milk

Again the heat treatment is more intense than the preceding ones (e.g. 20 s, 85ºC). This milk has a distinct cooked flavour, a slight UHT/ketone flavour and sometimes a trace of caramel flavour.

Sterilised milk

When a very long shelf-life is desired, sterilisation procedures are used, either batchwise, continuously or by combining the two methods (e.g. 30 s, 145ºC continuous, followed by 20 min, 115ºC batchwise in a bottle). This extensive heat treatment strongly affects the flavour and colour of the product. After the heat treatment the colour turns from off-white to yellow-brownish. The sensory profile of the product is determined mainly by a strong cooked flavour, UHT/ketone type and caramelisation/sterilisation flavours. The key aroma compounds of sterilised milk have been determined based on AEDA (aroma extract dilution analysis) (Iwatsuki *et al.*, 1999) and CHARM analysis (Moio *et al.*, 1994). Key aroma compounds for sterilised milk based on AEDA have been reported to be respectively 2-pentanone, 2-heptanone, 2-nonanone, 2-undecanone, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2-ethyl-3-methylpyrazine, methional, pentanoic acid, benzothiazole and vanillin (Iwatsuki *et al.*, 1999). Key aroma compounds reported for sterilised milk determined by CHARM analysis are respectively 2-nonanone, hexanal, benzothiazole and δ-decalactone (Moio *et al.*, 1994). Sulphur-containing volatiles such as H_2S, methanethiol, dimethylsulphide and carboxylsulphide, which are associated mainly with the cooked flavour, are produced due to the heat-induced denaturation of serum proteins (Adda, 1986; Badings, 1991; Nursten, 1997) – in particular, β-lactoglobulin starts denaturation already at 60ºC – and their production is accelerated at high temperatures.

Another pathway that operates under these conditions and produces sulphur-containing and other volatiles that have a high impact on the flavour is the Maillard reaction. For the flavour of heated milk, the Maillard reaction is important because it results in the production of volatile flavours such as aldehydes, ketones, maltol, isomaltol, pyrazines, furanones and methanethiol and H_2S (when methionine and cysteine take part as the amino acids respectively). Furthermore, the Maillard reaction gives sterilised milk its characteristic yellow-brown colour and depletes the milk of some of its nutrients (vitamin C and amino acids). The extent of the Maillard reactions is proportional to the strength of the heat treatment.

The Maillard reaction is an important reaction leading to key flavours in generally all dairy products that have undergone heat treatment and/or have aged for a certain period (Ho, 1996). Therefore we discuss it in more detail. The Maillard reaction can be divided into different sub-reactions (see Fig. 7.3(a) and (b)) (van Boekel, 1998). The first step is the production of an *N*-substituted glycosylamine from a reducing sugar (in milk primarily lactose) and an amino acid (in milk primarily protein-bound lysine). This first step is a reversible reaction that is followed by an Amadori or Heyns rearrangement, which is irreversible and produces 1-amino-1-deoxy-2-ketoses or 1-amino-2-deoxy-2-aldoses (in milk primarily 1-amino-1-deoxylactulosyllysine). At this stage the 1-amino-1-deoxylactulosyllysine is rather stable as long as the heating conditions are not too drastic (Mauron, 1981).

From 1-amino-1-deoxy-2-ketoses and 1-amino-2-deoxy-2-aldoses, different pathways are possible. Under acidic conditions, dehydration pathways will lead to furfurals (furfural or hydroxymethyl-5-furfural). Since this route needs acidic conditions, it is not so important in milk. Products such as hydroxymethyl-furfural (HMF), furfural, furfurylalcohol and lysylpyrraline are formed in milk but indeed in very small amounts (Berg, 1993; Morales and van Boekel, 1996). Under neutral (milk) conditions the 1-deoxyosone-pathway is the most important pathway leading to β-pyranone, 3-furanone, cyclopentenone, galactosylisomaltol and (after fragmentation) to reductons and dehydroreductons. β-Pyranone is not very stable and is isomerised into cyclopentenone and subsequently converted to galactosylisomaltol (Pischetsrieder and Severin, 1996). In the presence of amino groups, β-pyranone, cyclopentenone and galactosylisomaltol are converted to nitrogen-containing products such as acetylpyrrole, pyridinium betaine and furanone-amine. The reductons and dehydroreductons can be converted to aldehydes and α-aminoketones by Strecker degradation pathways or by retro-aldolisation pathways that lead to compounds such as acetyl, diacetyl, pyruvaldehyde and other aldehydes. The reactive α-dicarbonyl compounds such as pyruvaldehyde and diacetyl react further with other intermediates to form many different potent odorants such as pyridines, pyrazines, oxazoles, thiazoles, pyrroles and imidazoles (Nursten, 1986).

In the final stage of the Maillard reaction a polymerisation takes place of the different reaction intermediates that results in different polymers called melanoidins, which are characterised by a brownish colour. This final stage is not very well characterised from a chemical point of view (van Boekel, 1998). The exact type of Maillard flavours that are formed in each product depends on the ingredients and reaction conditions. Since the Maillard reaction is very

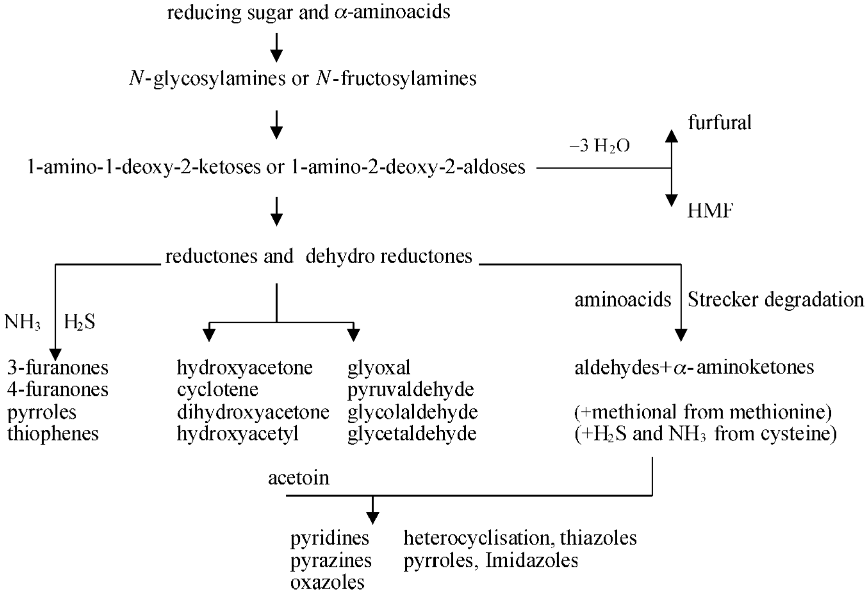

Fig. 7.3(a) General overview of the Maillard reaction.

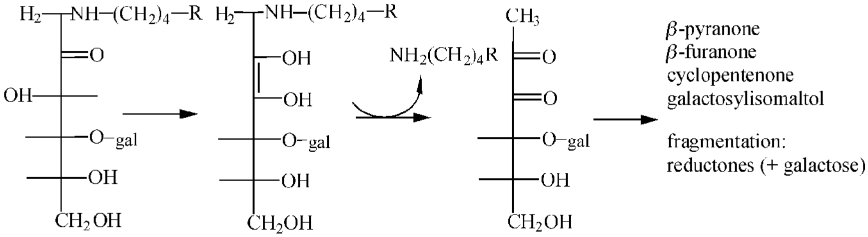

Fig. 7.3(b) Breakdown of the Amadori product under neutral conditions in milk in the second stage of the Maillard reaction (from Vermin and Parkanyi, 1982).

complex, it is hard to obtain a kinetic model for the reaction in milk. An important complication is that lactose is not only a reactant in the Maillard reaction but also subject to isomerisation/degradation reactions (Berg, 1993; Berg and van Boekel, 1994). Van Boekel and co-workers (van Boekel, 1996, 2001) have proposed a simplified kinetic model to describe the Maillard reaction in milk.

UHT (Ultra High Temperature) milk

Over the last decade a lot of effort has been devoted to the issue of extending the shelf-life of milk as much as possible with a minimal effect on flavour. This is because in a large number of countries consumers see the flavour of pasteurised milk as the standard flavour. The process that has been most successful from a commercial and qualitative point of view is the UHT process. In this process the milk is heated for a short time (3–15 s) at a high temperature (140–150ºC). Two types of continuous flow UHT processes are common (Hinrichs and Kessler, 1995): ‘direct heating’ in which the milk is heated directly by a steam injection, and ‘indirect heating’ in which the milk is heated indirectly by tubular or plate-heat exchangers. Depending on the dispersion in residence time in the heating system and the heating temperature, UHT milk can be produced by relatively mild treatment (e.g. 4 s, 142ºC, directly) or by a relatively harsh treatment (e.g. 15 s, 150ºC, indirectly). As a consequence of these different heat treatments, UHT milk can be produced with flavour profiles that vary between wide limits, i.e. close to pasteurised milk with a mild treatment or close to sterilised milk with a harsh treatment. In this chapter we focus on the UHT milk with a mild heat treatment.

Three important attributes dominate the sensory perception of UHT milk (Badings, 1991): ‘cooked’ (sometimes also called ‘sulphur’ or ‘cabbage’), ‘sterile’ (sometimes also called ‘Maillard’, ‘caramel’ or even ‘gluey’) and ‘oxidation’ (sometimes also called ‘stale’ or ‘ketone’) flavour. A fourth attribute that is sometimes mentioned is the intrinsic UHT flavour that seems to be a combination of ‘ketone’, ‘oxidation’ and ‘rich’ attributes.

Table 7.1 summarises the chemical volatiles that are held responsible for the different sensory attributes in UHT milk and the processes that affect the strength of the attributes (Anderson and Oste, 1995). Badings and co-workers have developed a synthetic flavour mix that can give a ‘UHT taste’ to pasteurised milk (Badings *et al.*, 1981). The synthetic mix consists of a mixture of methylketones, diacetyl, H_2S, methanethiol, methylisothiocyanate, ethylisothiocyanate, benzathiazole, several lactones, maltol, isobutylmercaptan and dimethylsulphide.

Table 7.1 Aromatic volatiles that are responsible for the main attributes of UHT milk and the factors that govern them

Sensory attribute	Aromatic volatile	Factors of influence
Cooked flavour	Free SH groups Volatile sulphides H_2S Methanethiol Dimethylsulphide Carboxylsulphide	Amount of vitamin C Sulphydryl oxidase O_2 permeability of carton Volume of headspace in carton Concentration of O_2 in milk Ageing conditions
Sterile flavour	2-Alkanones Lactones Maltol Furanones	Extent of Maillard reaction Ageing conditions
Oxidation flavour (also called ‘stale’)	Aldehydes (C_2, C_3, C_5, C_6) Acetone Ketones (C_5, C_7, C_8, C_9)	Extent of auto-oxidation Extent of Maillard reaction Ageing conditions

The cooked flavour in UHT milk is caused by sulphur-containing flavours (e.g. H_2S, methanethiol, dimethylsulphide and carboxylsulphide) that are produced due to the denaturation of serum proteins (Calvo and de la Hoz, 1992; Anderson and Oste, 1995) or the Maillard reaction, as has been discussed above. The generation of dimethylsulphide from S-methylmethionine sulphonium salt (sometimes present in the milk due to specific forage) has also been reported (Keenan and Lindsay, 1969). In general, this cooked flavour attribute increases with increasing process temperatures. However, exemptions are reported in which an increase of heat treatment leads to a lower cooked flavour. This is thought to be due to oxidation of the free sulphur groups into disulphides or a participation of the free sulphur groups in the Maillard reaction. The threshold value for the perception of cooked flavour in milk has been reported to be a concentration of free SH groups of 20 μmol/l (Thomas *et al.*, 1975). During the ageing process of UHT milk, the cooked flavour decreases (Mehta and Bassette, 1978; Fink and Kessler, 1986a, 1986b; Calvo and de la Hoz, 1992; Anderson and Oste, 1992, 1995). This is due to the oxidation of the free SH groups by the dioxygen that is present in milk (equation 7.1):

$$2\text{R–SH} + \text{O}_2 \rightarrow \text{R–SS–R} + \text{H}_2\text{O}_2 \qquad 7.1$$

The rate of decrease of free SH groups is affected by the dioxygen concentration in the milk during processing, and the dioxygen concentration during ageing and the ageing temperature. The dioxygen concentration during ageing is dependent on the initial dioxygen concentration in the milk and the headspace volume above the milk in the container and dioxygen permeability of the container. In general a higher dioxygen concentration and a higher temperature will result in a faster decline of the cooked flavour (Fink and Kessler, 1986a, 1986b; Adhikari and Singhal, 1992). Fink and Kessler (1986a, 1986b) studied the concentrations of free SH groups, vitamin C and dioxygen in UHT milk that was aged under different conditions. Based on these results they were able to construct a kinetic model which enabled them to predict the second-order reaction that describes the decline of the concentration of free SH groups and vitamin C. A dioxygen concentration between 3 and 5 ppm has been reported to be optimal. A higher dioxygen concentration will result in negative side-effects such as oxidation of nutrients (vitamin C) and oxidation reactions with other milk ingredients leading to off-flavours. Alternatively, the free SH groups can be transformed by other reagents than dioxygen. Examples of reported methods are:

- Reaction of free SH groups with organic thiosulphonates and thiosulphates (the so-called sulphydryl blocking agents) (Ferretti, 1973; Ferretti *et al.*, 1974)
- Addition of L-cystine to the milk before processing (Badings *et al.*, 1978)
- Use of immobilised sulphydryl oxidase to decrease the free SH groups (Swaisgood *et al.*, 1980)
- Addition of cumin seeds before processing (Josephson, 1989).

However, to our knowledge none of the methods have been commercialised so far.

Once the cooked flavour has decreased to a certain extent during the ageing of UHT milk, the 'oxidation' taste will start to increase. In a way the free SH groups in UHT milk protect the milk against formation of volatiles that are responsible for the oxidation flavour. The same phenomenon is observed in another dairy product, whole milk powder (Rotkiewicz *et al.*, 1979; Baldwin *et al.*, 1991; Stapelfeldt *et al.*, 1997). In that case the high pre-heat treatment of the milk prior to the manufacture of milk powder is the major factor controlling the oxidative stability of the product. The increased anti-oxidative capacity of milk powder has been ascribed to protein unfolding which exposes thiol groups (Walstra and Jenness, 1984). The thiols can, due to the rather low binding energy of the S–H bond, act as hydrogen atom donors and quench reactive oxygen species. The resulting thiyl radicals yield inactive disulphides in chain terminating reactions.

In the literature 'oxidation' is sometimes described as 'stale'. It has been proposed that the difference between these descriptions is due to a difference in aroma concentration (the attribute 'stale' would be caused by a lower concentration of aroma than the attribute 'oxidation'), but this has not been proven. It has also been suggested that the stale attribute is mainly due to aldehydes, while the oxidation attribute is caused by ketones. Again, this hypothesis has not been proven by measurements. In this chapter we use the term oxidation. There is no clear consent in the literature about the identity of the volatiles that are responsible for the oxidation attribute. However, it is clear that aldehydes and ketones play a crucial role. Both aldehydes and ketones are produced from milk fat, but can also be derived from the Maillard reaction (see above). The C_3, C_5 and C_6 aldehydes (Mehta and Bassette, 1978), 2-heptanone and 2-nonanone (Badings *et al.*, 1981; Baldings, 1991), acetaldehyde, acetone, 2-pentanone and 2-heptanone (Nursten, 1997), acetaldehyde, propanal, pentanal, hexanal, 2-pentanone and 2-heptanone (Gaafer, 1991) and 2-heptanone and 2-octanone (Moio *et al.*, 1994) have been reported to be key flavours for the oxidation attribute. The differences between the reports might be caused by differences between the UHT processing of the analysed samples and/or the conditions (time, temperature) at which the samples are aged. However, all reports agree that the oxidation flavour in UHT milk is already present at low ageing temperatures and increases as the ageing temperature increases. Unfortunately, up to now no kinetic model has been developed to predict the level of oxidation flavour under different ageing conditions as was done before for the cooked flavour (as described above).

The 'sterile' attribute in UHT milk is caused by flavour compounds that are formed in the Maillard reaction, which has been discussed in detail above. The extent of the sterile attribute in UHT milk is dependent on the UHT processing parameters and the ageing parameters. A higher ageing temperature and time increase the sterile attribute.

Recently, mathematical models have been developed that can predict the taste of UHT milk based only on the chemical analyses of key aroma components (Boelrijk and de Jong, 2002). In order to obtain a predictive model, both the analytical and sensory data were analysed and linked using multivariate statistics. Linear discriminant analysis (LDA) proved to be most successful since it provided ‘correct predicted classifications’ higher than 90%.

7.2.3 Off-flavours in milk and their chemical or biological origin

Off-flavours induced by light and/or metal ions

Independent of heating processes, each oxidation process in milk has to start with a reaction of dioxygen with one of the milk ingredients. However, to be able to react with organic compounds a catalyst should activate dioxygen (e.g. change the electronic state of dioxygen from a triplet into a singlet state). A notorious oxidation off-flavour is formed in many dairy products by a combination of light, riboflavin (a photosensitiser) and dioxygen or metal ions (i.e. primarily catalytic amounts of Cu^{2+} ions) and dioxygen (Wishner, 1964; Schröder, 1983; Rysstad *et al.*, 1998). Both systems are able to generate activated dioxygen that is reactive enough to break down serum proteins and produce volatile thiols (Jung *et al.*, 1998), sulphides and disulphides or to form organic peroxides from fatty acids. The organic peroxides are able to start a cascade of reactions leading to aldehydes, ketones and acids (see Fig. 7.1(b)). Although the copper-catalysed oxidation off-flavour is well controlled these days, the light-induced oxidation off-flavour remains a topic of further investigation (Whited *et al.*, 2002). Schröder has shown that the extent of light-induced oxidation flavour is dependent on the volume of headspace above the milk in the container (Schröder, 1983). A solution for this type of off-flavour should be found in the development of improved packaging materials (Rysstad *et al.*, 1998; Simon and Hanson, 2001a, 2001b).

Off-flavours transferred from cow to milk

During lactation, the digestive tract, blood circulation and respiratory system of the cow are important organs for determining the sensory and nutritional quality of the raw milk (Fearon *et al.*, 1998; Bassette *et al.*, 1986). Compounds which have been reported to be responsible for feed-related off-flavours are dimethyl sulphide, acetone, butanone, isopropanol, ethanol and propanol (Badings, 1991; Bendall, 2001). Indole, skatole, mercaptans, sulphides, nitriles and thiocyanates have been reported to be involved in off-flavours produced by weed taints that are digested in the cow (Forss, 1979).

Off-flavours in milk caused by micro-organisms or enzymatic reactions

Lipolytic rancidity in milk is caused by the liberation of C_4–C_{12} fatty acids from milk fat by milk lipase or bacterial lipases (Badings, 1991). Terms to describe this off-flavour are soapy, rancid and butyric acid. Sometimes lipolytic rancidity is accompanied by bitterness as a result of the formation of certain mono- and diglycerides. An unclean flavour can be due to an increase of dimethylsulphide above the threshold of 14 μg/kg, which is caused by psychotrophic bacteria.

Some of these bacteria can also produce ethyl esters of butyric, isovaleric and caproic acids that will lead to a fruity off-flavour. Milk contaminated with *Streptococcus lactis* var. *maltigenes* may develop a malty flavour as a result of the formation of 3-methylbutanal, 2-methylbutanal and 2-methylpropanal. Sterile milk produced by mild UHT heating may develop a bitter off-flavour on ageing as a result of the remaining activity of thermostable bacterial proteinases, which break down milk proteins to bitter peptides.

7.3 Yoghurt and buttermilk

7.3.1 Aspects of yoghurt (off)-flavour

Yoghurt is prepared by fermentation of milk by usually two types of bacteria, namely *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Marshall, 1993; Driessen, 1988). The flavour of yoghurt is determined by a unique combination of volatile organic compounds that are mainly formed in the first couple of hours of the fermentation process. Quite a number of studies dealing with qualitative data on aromatic volatiles that are present in yoghurt have been published. More than 90 different volatiles have been identified in yoghurt including carbohydrates, alcohols, aldehydes, ketones, acids, esters, lactones, sulphur-containing compounds, pyrazines and furan derivatives (Viani and Horman, 1973; Marshall, 1982; Ott *et al.*, 1997). However, in studies where quantitative results are reported usually only a few of these volatiles are followed, primarily a combination of ethanol, acetaldehyde, diacetyl, acetone, acetoin, butanone, formic acid, acetic acid and dimethylsulphide (Botazzi and Dellaglio, 1967; Hamden *et al.*, 1971; Dutta *et al.*, 1973; Marshall and Cole, 1983; Thornhill and Cogan, 1984; Scolari *et al.*, 1985; Yuguchi *et al.*, 1989; Hegazi and Abo-Elgena, 1990; Perez *et al.*, 1991; Zouari and Desmazeaud, 1991; Zouari *et al.*, 1991). From these compounds, acetaldehyde is usually thought to have the largest impact on the total yoghurt flavour. Diacetyl is thought to be important for the 'roundness' of the yoghurt flavour (Groux, 1973). A popular point of discussion in the literature is the preferred ratio between acetaldehyde and diacetyl. Too much acetaldehyde compared to diacetyl would lead to a 'green' off-flavour. A ratio between acetaldehyde and diacetyl of 1:1 would give a preferred typical taste of yoghurt (Zouari and Desmazeaud, 1991). Also the ratio of acetaldehyde to acetone (2.8:1) has been mentioned to be important for an optimal yoghurt taste. There is much discussion about the concentrations of these volatiles that are needed in order to produce an optimal yoghurt flavour. However, so far no agreement is found in the literature.

Ott and co-workers (Ott *et al.*, 1997) have done a type of AEDA (aroma extract dilution analysis) study to determine the key aroma components of two-week-old yoghurt. AEDA is a GC-olfactometry technique, which delivers comparable information to a CHARM analysis (see section 7.2.1). The results for yoghurt are compared with an AEDA study of pasteurised milk. Some volatiles appear to be important key aroma components in both products while others appear to be specific for yoghurt. Based on this study, the key aroma components for yoghurt are acetaldehyde, diacetyl, 2,3-pentanedione, methional, 2-methyltetrahydrothiophene-3-one, (2E)-nonanal, 3-methylbutyric acid, guiacol, benzothiazole and two unidentified compounds. Imhof and co-workers (Imhof *et al.*, 1994, 1995), choose another approach to determine the key aroma components in yoghurt and use the odour activity value (OAV) concept. OAV is defined as the ratio between the concentration of the volatile in the product and its threshold concentration. The higher the OAV value, the more important the volatile is for the total flavour. Using this method, Imhof *et al.* identified six volatiles that should have a high impact (OAV > 2) on the yoghurt flavour: acetaldehyde, dimethylsulphide, diacetyl, 2,3-pentanedione, L-limonene and undecanal. It is mentioned that undecanal is a component already available in milk and is not produced by the yoghurt cultures. A critical remark concerning the OAV method is that the threshold values for the volatiles that are used in this method are usually determined in water and not in dairy products. However, it is known that the main ingredients (fat, proteins and carbohydrates) in dairy products have a major effect on the release of volatiles and subsequently on the threshold values (Fares *et al.*, 1998; Roberts and Pollien, 2000; Hansen and Heines, 1991). Therefore, the OAV data obtained by Imhof and co-workers, for which they used threshold values in water, should be interpreted with caution.

Importance of acidity

An important factor in the sensory evaluation of yoghurt is the acidity of the product. In recent years, consumers have shown a preference for milder, less acidic yoghurts (Eberhard *et al.*, 1995). However, such products were rated as less flavourful (Kneifel, 1992; Kneifel *et al.*, 1992). It appears that important differences are found in the concentrations of flavour impact compounds such as acetaldehyde, diacetyl and 2,3-pentanedione between the mild and the more acidic yoghurts (Ott *et al.*, 1999). Studies have found a decrease in acetaldehyde concentration and an increase in diacetyl and 2,3-pentanedione concentrations in mild yoghurts compared with more acidic products. Recently, Ott and co-workers (Ott *et al.*, 2000a) reported results that show a mutual influence between the key aroma compounds and the acidity of the yoghurt. In their study they characterised the sensory properties of traditional acidic and mild, less acidic yoghurts by a trained panel using descriptive analysis. Their conclusion was that there are important flavour (sensory) differences between the two classes of yoghurts but that they are mainly due to the differences in acidity and not due to different concentrations of the three flavour impact compounds (acetaldehyde, diacetyl and 2,3-pentanedione) that were analysed. Deodorisation and impact aroma compound addition had much less influence on yoghurt flavour than did pH variations. This study clearly emphasises the importance of acidity in the perception of yoghurt flavour. The same can be said for the yoghurt texture which is vital for the perception. Less viscous yoghurts are usually perceived as more acidic. However, texture–taste interactions will not be discussed here.

Origin of the key aroma components of yoghurt

The key aroma components of yoghurt are produced during fermentation. Therefore, the production routes towards these components are mainly catalysed by enzymes. In this chapter we summarise the steps for the formation of acetaldehyde in Fig. 7.4 (Ott *et al.*, 2000c) and the possible formation routes towards fatty acids in Fig. 7.5.

There are basically two routes towards the formation of acetaldehyde, one based on lactose and the other based on milk protein. Since this chapter focuses mainly on the chemical aspects of flavour generation, we refer to Chapter 6 for more details on the enzymatic steps. In the present chapter, however, we would like to mention a recent publication by Ott *et al.* (2000b) reporting the results of a study that aims to elucidate the mechanism of diacetyl and 2,3-pentanedione formation in yoghurt. Their results suggest that the addition of branched-chain amino acids (BCAA) or an inhibitor of the BCAA biochemical pathways during fermentation of milk with a lac^{-} mutant of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* strongly influence the formation of diacetyl and 2,3-pentanedione as well as their precursors 2-acetolactate and 2-acetohydroxybutyrate. Based on ^{13}C incorporation studies they conclude that glucose is the major precursor via pyruvate and activated acetaldehyde for diacetyl, and L-threonine is the precursor via 2-ketobutyrate for 2,3-pentanedione. However, glucose can also be the precursor for 2,3-pentanedione via activated acetaldehyde. They also suggest an alternative route towards 2,3-pentanedione via 3-methylaspartate, an intermediate in the glutamate synthesis.

Ageing of the fresh yoghurt changes the sensory profile. However,

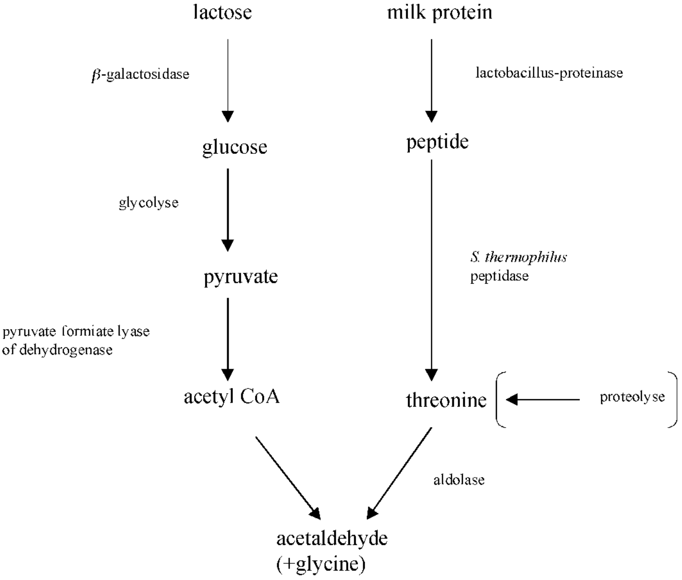

Fig. 7.4 Metabolic routes leading to acetaldehyde in yoghurt.

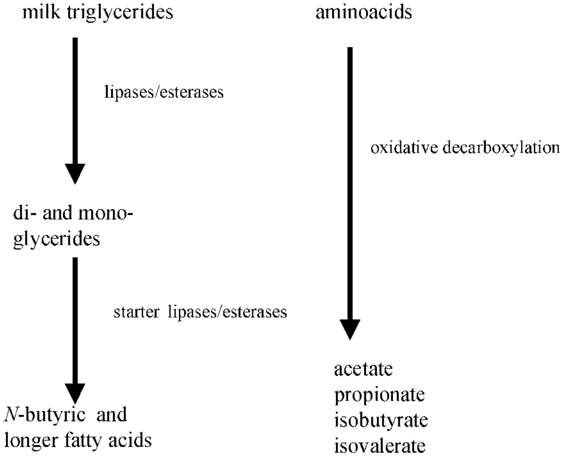

Fig. 7.5 Metabolic routes leading to volatile fatty acids.

contradictory information is published about the qualitative or quantitative change in flavour components over time (Laye *et al.*, 1993; Imhof *et al.*, 1994; McGregor and White, 1987; Kang *et al.*, 1988). Some authors find a decrease of acetaldehyde and diacetyl, while others do not detect any change or even detect an increase of certain flavour components during ageing. It is clear that the type of yoghurt (i.e. the strains that are used for fermentation), packaging material, sunlight and dioxygen levels play a role in these studies and their results.

7.3.2 Chemical aspects of buttermilk (off)-flavour

Buttermilk is obtained as a by-product of the process of making butter (Frank, 1983). There are two types of buttermilk: sweet cream buttermilk which is produced by treating the pasteurised cream with butter starter cultures after separation of the butterfat to yield so-called ‘fermented buttermilk’, and sour cream buttermilk which is prepared by a procedure in which the fermentation is done before separation of the butterfat. We would like to focus in this paragraph on the chemical origin of one of the main off-flavours encountered in sour cream buttermilk, namely the metallic off-flavour that becomes important during ageing of fresh buttermilk. Heiler and Schieberle (1996, 1997a, 1997b) have recently performed a detailed study to elucidate the compound responsible for this off-flavour and its metabolic and chemical origin. By using comparative AEDA of buttermilk products with and without metallic off-flavour they determined that (E,Z)-2,6-nonadienol is the key odorant responsible for the metallic off-flavour that develops during storage of sour cream buttermilk. They also showed that the flavour-quality contribution of (E,Z)-2,6-nonadienol is critically dependent on its concentration. For example, in concentrations ranging from 0.3 to 0.6 μg/l, (E,Z)-2,6-nonadienol *enhances* the buttermilk flavour, while a final concentration of 1.3 μg/l (after addition of synthetic (E,Z)-2,6-

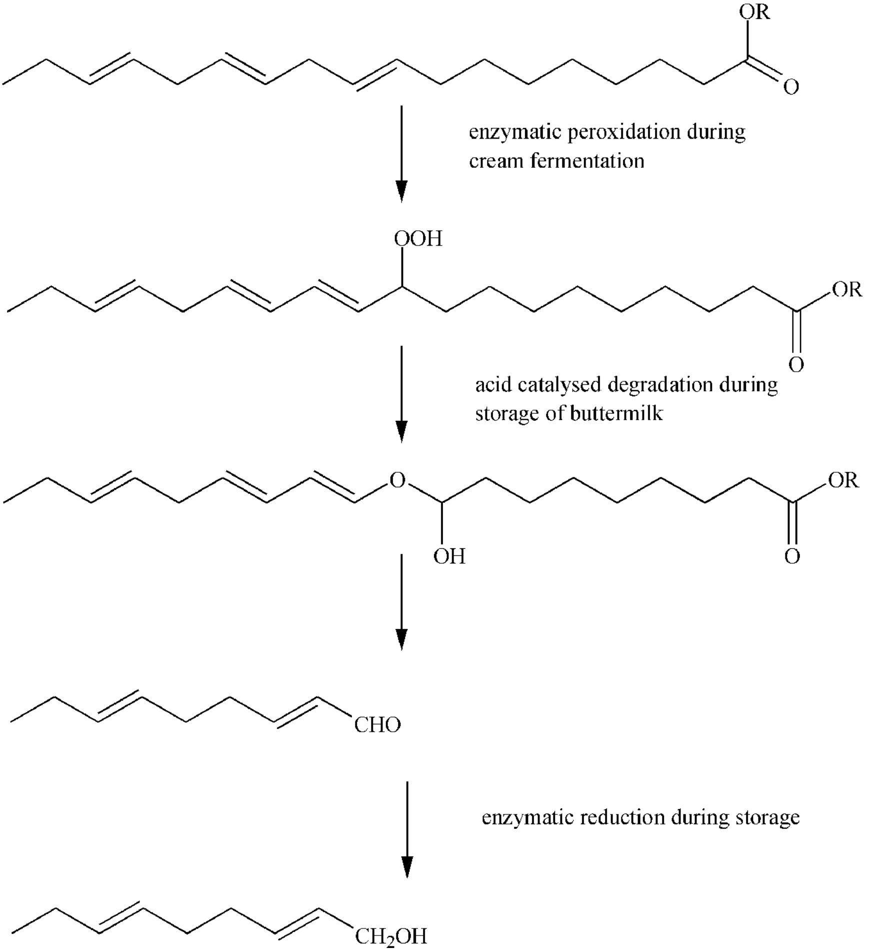

Fig. 7.6 Reaction scheme explaining the formation of (E,Z)-2,6-nonadienol in sour cream buttermilk (reproduced from Heiler and Schieberle, 1997b).

nonadienol) *decreases* the quality of the buttermilk flavour and causes the metallic off-flavour (Heiler and Schieberle, 1996, 1997a, 1997b). A detailed model study (Heiler and Schieberle, 1997b) revealed that (E,Z)-2,6-nonadienol is produced via a cascade of reactions from α-linolenic acid, as summarised in Fig. 7.6. In the first step α-linolenic acid is peroxidised by oxygenases from the starter culture, yielding glycerol-bound 9-hydroperoxy-10,12,15-octadecatrienoate. During the preparation of butter, this polar hydroperoxide is transferred into the buttermilk and degraded into (E,Z)-2,6-nonadienal via two acid-catalysed steps. During storage of the sour cream buttermilk, the dienal is reduced to (E,Z)-2,6-nonadienol, which is the cause of the metallic off-flavour. To prevent the metallic off-flavour, the authors recommend the use of starter cultures that are low in oxygenases or creams that are low in α-linolenic acid.

Since the concentration of α-linolenic acid is usually higher in high-fat creams, the formation of (E,Z)-2,6-nonadienol is generally higher in sour cream buttermilk, which allows the starter cultures to act on cream that contains a much larger percentage of fat than in fermented sweet cream buttermilk.

7.4 Conclusion and future trends

This chapter has given a general overview of the chemical and some biochemical aspects of flavour generation in dairy products. It can be concluded that many researchers have focused their studies on determining the key flavour components that are responsible for the sensory perception of a variety of dairy products. These key flavour compounds and their origin have been reviewed for different dairy products. It is obvious that the so-called flavour balance is of great importance for sensory properties of dairy products and of milk with its bland flavour, in particular. The key flavour components that are found in dairy products originate from milk and the processing conditions (fermentation, different heat treatments and ageing conditions) that are used to produce the specific products. A disturbance of the flavour balance or the introduction of unwanted compounds may cause off-flavours.

In general, heat treatment of dairy products leads to protein and fat degradation and the Maillard reaction, leading to flavour compounds that have a large impact on sensory perception. Although already a vast amount of results on these processes have been generated in the past, more work needs to be done to be able to predict, control and optimise the processing conditions better in order to produce higher quality products for the consumer. Especially the complex Maillard reaction (important for so many dairy products) remains a subject for further study. We expect that the generation of kinetic models that are better able to predict flavour in dairy products under different processing and ageing conditions will be a major topic for future studies.

To understand the formation of flavours in dairy products, one should study the metabolic as well as the chemical pathways during production and storage. One sees in general that a certain combination of metabolic and processing steps leads to the formation of the specific flavours. The specific flavour can be a desired attribute (for example, in the formation of yoghurt flavour) or an undesired attribute or off-flavour (as is the case for the metallic off-flavour in buttermilk). In order to study the metabolic and chemical pathways in a most efficient way, it is important to have a clear view of the chemical structure of the key aroma compounds that are responsible for sensory perception. Since a large number of flavour compounds are usually produced in dairy products, it is important to separate the less important ones from the important (key) flavour components. Such methods as (comparative) AEDA, CHARM and OAV analyses have proved to be essential for this matter. Also, new techniques such as MS-Nose (Brauss *et al.*, 1999; Weel *et al.*, 2002) that enable the study of flavour release from the dairy product directly *in vivo* in the human nose while the product is being eaten, will become more important. With this technique it is possible to quantify the effect of fat, proteins and carbohydrates in the dairy matrix on the release of key aroma compounds from the dairy product into the human nose, where the flavours are perceived.

These methods, together with the development of analytical in-line control methods that enable the control of process variables earlier in the process, will result in the efficient development of high quality products that are preferred by consumers (Boelrijk and De Jong, 2002). Knowledge of the possible causes of off-flavours and the ability to control the production processes via in-line analytical measurements will also lead to a more constant quality of the end product.

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7.6 References

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