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Instrumental measurement of milk flavour and colour

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Abstract: Flavour and colour are among the main determinants of milk product quality. The understanding of how certain variables, such as production, processing and storage, impact the flavour and colour of fluid and dried milk products is of great interest to dairy scientists. For this reason instrumental methods have been developed for the measurement of the flavour and colour properties of milk products. Colour measurement is usually made using mature technologies based on colorimeters or spectrophotometers. On the other hand, the analysis of the flavour of milk and milk products presents a unique challenge and improved analytical methods are in constant development. This chapter presents a detailed discussion of modern instrumental methods used for the analysis of the flavour components and colour attributes of fluid and dried milk products.

Key words: flavour, odour, colour, milk, dried milk, dairy, gas chromatography, electronic nose, colorimeter.

7.1 Introduction

In the past two decades considerable progress has been made towards our understanding of the flavour chemistry/biochemistry of dairy products, but defining milk flavour continues to challenge researchers working in the sensory and flavour science fields. Fresh milk (raw, pasteurized and/or homogenized) of good quality has a subtle, yet distinctive clean and fresh dairy flavour. According to Badings (1991) there are three basic elements responsible for the sensory properties of milk, including: (1) pleasant mouth-feel due to presence of

macromolecules, such as colloidal proteins and fat globules, (2) sweet and salty taste due to lactose and milk salts, respectively, and (3) a weak and delicate aroma profile due to the proper balance of numerous volatile constituents found in low abundance. These elements, and to some extent the colour and appearance attributes, are the main determinants of the sensory quality of milk. Production, processing and storage practices can profoundly affect milk quality. For this reason the dairy industry has been actively engaged in the development of methods for the measurement of milk flavour and colour.

This chapter presents an overview of the instrumental methods used for the analysis of the flavour components and colour of fluid and dried milk products.

7.2 Flavour and colour of milk

Over the past five decades numerous researchers have demonstrated the involvement of carbonyls, esters, alcohols, short-chain free fatty acids, aromatics, sulphur-containing compounds and other miscellaneous compounds in the flavour of milk and milk products. Characteristic aroma components of fluid and dried milks that have been identified using gas chromatography–olfactometry methods are listed in Tables 7.1 and 7.2. The flavour components of milk are derived from two main sources: (1) feed constituents and rumen and animal metabolism during milk formation, and (2) reaction of milk protein, fat or lactose during processing and storage of milk (Forss, 1979; Badings, 1991). Before, during and after processing, the flavour of milk can be negatively affected by numerous chemical or biochemical reactions and processes (Forss, 1979). These include feed source, oxidative and hydrolytic rancidity, thermal degradation, packaging interactions and microbial contamination (Table 7.3). Several excellent reviews have been published on the volatile flavour components of milk and milk products (Forss, 1979; Forss and Sugisawa, 1981; Adda, 1986; Badings, 1991; Nursten 1997; Singh *et al.*, 2007; Cadwallader and Singh, 2009). In particular, the review by Cadwallader and Singh (2009) presents a detailed discussion of the occurrence and formation of flavour and off-flavour compounds in milk and dairy products.

In contrast to flavour, very little attention has been paid to the appearance characteristics, and, in particular, the colour of milk and dried milk. As pointed out by Burton (1956) the reason for this might be because the colour of milk is generally taken for granted and doesn't become a quality issue until it is altered from its normal or expected state. Fluid milk varies in colour from opaque white to yellowish-white or even to a blueish tinted white (Doan, 1924). The observed colour of milk is attributed to the light reflectance properties of the milk components such as fat globules, colloidal substances (e.g., proteins), β -carotene and riboflavin (Solah *et al.*, 2007). From a quality standpoint it has mainly been the discolouration of milk caused by Maillard (nonenzymatic) browning that has concerned the dairy industry. Colour changes in pasteurized fluid milk are generally minimal and are usually not of much concern. On the other hand,

Table 7.1 Potent odourants identified by gas chromatography–olfactometry in raw, pasteurized and UHT fluid milks (compounds listed in decreasing order of relative odour potency)

<i>Raw milk (whole)^{a,b}</i>	<i>Pasteurized milk (whole)^b</i>	<i>Pasteurized milk (whole/skim blend)^c</i>	<i>UHT milk (glass bottles)^f</i>
ethyl hexanoate	dimethyl sulphone	1-octen-3-one	δ -decalactone
ethyl butanoate	hexanal	dimethyl sulphide	vanillin
dimethyl sulphone	nonanal	hexanoic acid	6-(<i>Z</i>)-dodecenyl- γ -lactone
nonanal	1-octen-3-ol	2-methylthiophene	γ -dodecalactone
1-octen-3-one	indole	acetic acid	<i>trans</i> -4,5-epoxy-(<i>E</i>)-2-decenal
indole	benzothiazole	phenylacetaldehyde	δ -octalactone
heptanal	δ -decalactone	1-nonen-3-one	2-acetyl-2-thiazoline
ethyl octanoate	2-tridecanone	dimethyl trisulphide	3-(methylthio)propanal
octanal		2,3-butanedione	(methional)
	<i>Pasteurized milk (skim, 2% and whole)^d</i>	3-methyl-2-butenal	2-acetyl-1-pyrroline
<i>Raw milk (whole)^c</i>	2-acetyl-1-pyrroline	benzothiazole	<i>UHT milk (PE bottles)^f</i>
3-(methylthio)propanal	dimethylsulphide	2-methoxyphenol (guaiacol)	δ -decalactone
(methional)	methylpropanal		γ -dodecalactone
δ -decalactone	3-methylbutanal	<i>UHT milk^b</i>	3-methoxy-4-
2-acetyl-1-pyrroline	1-octen-3-one	2-heptanone	hydroxybenzaldehyde (vanillin)
2-acetyl-2-thiazoline	octanal	2-nonanone	(<i>Z</i>)-4-heptenal
6-(<i>Z</i>)-dodecenyl- α -lactone	1-hexen-3-one	2-undecanone	3-(methylthio)propanal
4-hydroxy-2,5-dimethyl-3(2 <i>H</i>)-	methanethiol	δ -decalactone	(methional)
furanone (furanol)	hexanal	2-tridecanone	hexanoic acid
2-isopropyl-3-methoxypyrazine	3-methylindole (skatole)	dimethyl sulphone	δ -octalactone
3-methylindole (skatole)	1-nonen-3-one	benzothiazole	<i>trans</i> -4,5-epoxy-(<i>E</i>)-2-decenal
(<i>E,Z</i>)-2,6-nonadienal		hexanal	γ -nonalactone
δ -octalactone		indole	δ -nonalactone
decanoic acid			
(<i>E</i>)-2-nonenal			
(<i>E,E</i>)-2,4-nonadienal			
γ -decalactone			
1-octen-3-one			

^a Moio *et al.* (1993). ^b Moio *et al.* (1994). ^c Colahan-Sederstrom and Peterson (2005). ^d Cadwallader and Howard (1998). ^e Ott *et al.* (1997).^f UHT milks stored in either glass or polyethylene (PE) bottles (Czerny and Schieberle, 2007).

Table 7.2 Potent odourants identified by gas chromatography–olfactometry in nonfat and wholefat dried milks (data for products having normal flavour quality; compounds listed in decreasing order of relative odour potency)

<i>Nonfat dry milk (low heat)^a</i>	<i>Nonfat dry milk (medium heat)^a</i>	<i>Nonfat dry milk (high heat)^a</i>
4-hydroxy-2,5-dimethyl-3(2 <i>H</i>)-furanone (furanol)	δ-decalactone	4-hydroxy-2,5-dimethyl-3(2 <i>H</i>)-furanone (furanol)
butanoic acid	4-hydroxy-2,5-dimethyl-3(2 <i>H</i>)-furanone (Furanol)	butanoic acid
3-(methylthio)propanal (methional)	butanoic acid	3-(methylthio)propanal (methional)
<i>o</i> -aminoacetophenone	3-(methylthio)propanal (methional)	<i>o</i> -aminoacetophenone
δ-decalactone	<i>o</i> -aminoacetophenone	<i>trans</i> -4,5-Epoxy-(<i>E</i>)-2-decenal
octanoic acid	<i>trans</i> -4,5-epoxy-(<i>E</i>)-2-decenal	δ-decalactone
pentanoic acid	3-methoxy-4-hydroxybenzaldehyde (vanillin)	pentanoic acid
3-methoxy-4-hydroxybenzaldehyde (vanillin)	2-acetyl-1-pyrroline	3-hydroxy-4,5-dimethyl-2(5 <i>H</i>)-furanone (sotolon)
2-acetyl-1-pyrroline	2-acetyl-2-thiazoline	3-methoxy-4-hydroxybenzaldehyde (vanillin)
hexanoic acid	3-hydroxy-4,5-hydroxy-2(5 <i>H</i>)-furanone (sotolon)	phenylacetic acid
nonanal	hexanoic acid	nonanal
2-acetyl-2-thiazoline	phenylacetic acid	1-octen-3-one
γ-dodecalactone	γ-dodecalactone	2-acetyl-1-pyrroline
(<i>E</i>)-2-nonenal	(<i>E</i>)-2-undecenal	hexanoic acid
(<i>E</i>)-2-undecenal	(<i>E,E</i>)-2,4-decadienal	octanoic acid
<i>trans</i> -4,5-epoxy-(<i>E</i>)-2-decenal	3-phenylpropanoic acid	γ-dodecalactone
3-hydroxy-4,5-hydroxy-2(5 <i>H</i>)-furanone (sotolon)		(<i>E</i>)-2-nonenal
phenyl acetic acid		3-phenylpropanoic acid
1-octen-3-one		3-hydroxy-2-methyl-4 <i>H</i> -pyran-4-one (maltol)
(<i>E,E</i>)-2,4-decadienal		
3-phenylpropanoic acid		
3-hydroxy-2-methyl-4 <i>H</i> -pyran-4-one (maltol)		
2-methylpropanoic acid		

Nonfat dry milk^b

2-acetyl-1-pyrroline
 hexanoic acid
 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone
 (Furaneol)
 δ -octalactone
o-aminoacetophenone
 3-(methylthio)propanal
 γ -dodecalactone
 γ -octalactone
 4-hydroxy-2-ethyl-5-methyl-3(2*H*)-furanone
 (homofuraneol)
 1-octen-3-one
 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone
 (sotolon)
 6-(*Z*)-dodecenyl- γ -lactone
 butanoic acid
 octanoic acid
 2/3-methylbutanoic acid
 phenylacetaldehyde
 octanoic acid
 2/3-methylbutanoic acid

phenylacetaldehyde
 acetic acid
 nonanal
 (*E,Z*)-2,6-nonadienal
 propanoic acid
 pentanoic acid
 2-acetylthiazole
 2-acetyl-2-thiazoline
 3-methylindole

Wholefat dry milk^b

hexanoic acid
 3-methylindole (skatole)
 2-acetyl-1-pyrroline
o-aminoacetophenone
 γ -octalactone
 γ -dodecalactone
 butanoic acid
 δ -octalactone
 acetic acid
 octanoic acid

δ -dodecalactone
 3-hydroxy-2-methyl-4*H*-pyran-4-one (maltol)
 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone
 (sotolon)
 δ -decalactone
 2/3-methylbutanoic acid
 2-methylthiophene
 4-hydroxy-2-ethyl-5-methyl-3(2*H*)-furanone
 (homofuraneol)
 (*E*)-2-decenal
 2-acetylthiazole
 (*E*)-2-nonenal
 2-acetyl-2-thiazoline
 propanoic acid
 pentanoic acid
 1-octen-3-one
 2-methoxyphenol
 2,3-butanedione
 3-(methylthio)propanal (methional)
 nonanal
 (*E,E*)-2,4-nonadienal
 phenylacetaldehyde

^a Karagül-Yüceer *et al.* (2001, 2003).

^b Carunchia Whetstone and Drake (2007).

Table 7.3 Off-flavours and flavour defects in fluid and dried milks

Flavour defect	Chemicals	Origin	References
Feed	Dimethyl sulphide, acetone, butanone, isopropanol, ethanol, propanal	Metabolites of feed and silage	Badings (1991)
	(<i>E</i>)-2- and (<i>Z</i>)-2-nonenal	Fresh cut alfalfa	Marsili (2003)
	Dimethyl sulphide, 2-butanone, hexanal	Grass silage	Mounchili <i>et al.</i> (2005)
	Terpenes	Grass (pasture)	Schlichtherle-Cerny <i>et al.</i> (2007)
Fishy	Trimethylamine	Microbial	Cornfield (1955); Humphriss (1953); Lunden <i>et al.</i> (2002); Ampuero <i>et al.</i> (2002)
Fruity	Ethyl esters (e.g., ethyl butanoate, ethyl hexanoate)	Microbial, enzymatic	Wellnitz-Ruen <i>et al.</i> (1982); Whitfield <i>et al.</i> (2000); Marsili (2003)
Heat abuse, cooked	Maltol, furans, pyrazines and other Maillard reaction products	Thermally induced	Badings (1991)
	H ₂ S, methanethiol, dimethylsulphide and other sulphides, 2-alkanones, methylpropanal, 3-methylbutanal, <i>n</i> -aldehydes	Thermally induced	Contarini <i>et al.</i> (1997); Boelrijk and de Jong (2003); Marsili and Miller (1998); Vazquez-Landaverde <i>et al.</i> (2005, 2006)
Light-abuse	Dimethyl sulphide, dimethyl disulphide, <i>n</i> -aldehydes, 2-alkanones, vinyl ketones	Light-induced oxidation	Mehta and Bassette (1979); Jeng <i>et al.</i> (1988); Cadwallader and Howard (1998); Jung <i>et al.</i> (1998), Kim and Morr (1996); Marsili and Miller (1998); van Aardt <i>et al.</i> (2005)

Oxidized	<i>n</i> -Aldehydes, 2/3-alkenals, 2,4-alkadienals, 2,6-nonadienal, 2,4,7-decatrienal, ketones, vinyl ketones	Lipid oxidation (e.g., copper-induced)	Forss <i>et al.</i> (1955a,b); Day <i>et al.</i> (1963), Hammond and Seals (1972); Badings (1991); Barrefors <i>et al.</i> (1995); Granelli <i>et al.</i> (1998); Marsili and Miller (1998)
	(<i>E</i>)-6-Nonenal	Ozonolysis	Parks <i>et al.</i> (1969)
Phenolic	<i>p</i> -Cresol	Enzymatic, enzymatic	Badings and Neeter (1980)
Rancid	Volatile short-chain fatty acids (C4–C10)	Microbial, enzymatic, sanitizer	Azzara and Campbell (1992); Marsili (2000); Whitfield <i>et al.</i> (2000)
Stale, storage	2-Alkanones, <i>o</i> -aminoacetophenone, Strecker aldehydes (e.g., 3-methylbutanal, methional), <i>n</i> -aldehydes, 2-alkenals, 2,4-alkadienals, vinyl ketones	Storage (lipid-oxidation, Maillard reaction)	Arnold <i>et al.</i> (1966); Parks <i>et al.</i> (1964); Anderson and Lingnert (1998); Preininger and Ullrich (2001); Valero <i>et al.</i> (2001); Karagül-Yüceer <i>et al.</i> (2002); Perkins <i>et al.</i> (2005); Carunchia Whetstine and Drake (2007)
Taints, chemical	Propylacetate	Solvent residue from packaging	Marsili (2003)
Taints, weed	Indole, skatole, mercaptans, sulphides, nitriles, thiocyanates	Metabolites of weeds	Badings (1991)
	Skatole, benzylthiol, benzyl methyl sulphide	Metabolites of weeds	Park (1969); Park <i>et al.</i> (1969)

browning reactions in UHT, condensed and dried milks can be considerable and may affect product quality (Patton, 1955; Popov-Raljić *et al.*, 2008). Several factors are known to promote browning reactions in milk products, including use of excessive processing temperatures and adverse storage conditions. Heating of fluid milk has two main effects on its colour: at moderate temperatures (65°C) or immediately after a UHT process an initial 'whitening' may occur, while at high temperatures (>90°C) browning readily occurs due to the Maillard reaction (Burton, 1955, 1956; Kessler and Fink, 1986; Rhim *et al.*, 1988; Singh and Creamer, 1992; Browning *et al.*, 2001; Popov-Raljić *et al.*, 2008). The increased rate of the Maillard reaction in concentrated and dried milks is due to the lower water activities and increased concentrations of reactant molecules in these products (van Boekel, 1998).

7.3 Flavour measurement

The availability of powerful chromatographic separation techniques, such as high-resolution capillary gas chromatography–mass spectrometry (GC-MS) and GC–olfactometry (GCO), has allowed for the detailed identification and characterization of critical flavour components of milk. In addition, the development of various types of electronic noses has provided the dairy industry with a rapid means to assess milk flavour quality. Reviews by Mairaca and Bosset (1997), Parliment and McGorin (2000), McGorin (2007), Singh *et al.* (2003), Cadwallader (2007) and Drake *et al.* (2007) describe some of the analytical techniques used in the evaluation of key aroma compounds in milk and dairy products. The book *Sensory-directed Flavor Analysis* edited by Marsili (2007a) is highly recommended to those readers interested in greater detail about the general analytical techniques used in flavour research. The review by Ampuero and Bosset (2003) provides important insights into the application of electronic nose technology for the analysis of dairy products.

The initial and often the most critical step in aroma analysis is the isolation of the volatile compounds from the nonvolatile matrix components. Following isolation, instrumental methods of analysis, such as GC-MS, are employed to separate, identify and quantify the various volatile components of the isolate. In some cases, combined sensory–instrumental methods, such as GC–olfactometry (GCO), can be applied to indicate important contributors to the characteristic aroma of the product. This section focuses on modern procedures used for the isolation and analysis of volatile flavour compounds of fluid and dried milk products.

7.3.1 Flavour compound isolation/extraction

The analysis of the volatile components of milk is a difficult process due to the presence of only minute amounts of volatile solutes in a highly complex nonvolatile matrix. Furthermore, milk volatile compounds comprise many

different chemical classes, such as acids, ketones, aldehydes, alcohols, etc., and are subject to further chemical breakdown due to oxidation and thermal decomposition. For this reason the volatile isolation procedure is usually the most critical step in the analysis. The isolation of volatile constituents may be accomplished by considering the differences in chemical properties between the volatile compounds and nonvolatile milk matrix components. Generally, this means taking advantage of the higher volatility and/or the relative nonpolar nature of the volatile components. Methods that depend on the volatile nature of the aroma components include headspace and distillation techniques. On the other hand, solvent extraction and adsorption methods rely on the relative nonpolar nature of the volatile compounds to isolate them from the matrix. A good approach is to use a method that takes advantage of both volatility and polarity differences in the isolation step, such as combined headspace–adsorption and extraction–distillation methods.

There is no single ‘perfect’ volatile isolation procedure and all volatile isolation techniques will impart some degree of sampling bias (Etiévant, 1996; Reineccius, 2006). For greater accuracy the method chosen should maintain sample integrity, minimize loss of labile (sensitive) aroma compounds, and isolate all volatile compounds equally. To help assure accuracy, it is common practice to use two or more complementary isolation methods that are based on different separation criteria. In this way, the sampling bias of each method can be accounted for in the final results. Methods most commonly employed for the isolation of volatile constituents of milk products are discussed below.

Headspace methods

All aroma compounds possess some degree of volatility. Headspace isolation techniques take advantage of this property by monitoring the gaseous headspace above a liquid or solid material in a sealed container. Headspace methodologies, including static headspace, dynamic headspace and purge-and-trap, have been reviewed (Hinshaw, 1990; Cole and Woolfenden, 1992; Wampler, 2002). Some unique advantages of headspace methods are that they provide some indication of the composition of the volatiles above the food (headspace aroma composition), they are nondestructive (mild conditions), and minimal sample preparation is required.

Static headspace analysis

In principle, static headspace analysis (SHA) is the simplest among the headspace analysis techniques. In SHA the food product (liquid or solid) is contained in a closed vessel (typically a vial is used), and the volatile components are allowed to come into equilibrium between the sample matrix and the surrounding headspace. For analysis, an aliquot of the headspace is then withdrawn and injected into a GC. SHA is influenced by numerous parameters, such as temperature, sample and container size, ratio of sample to headspace volume, nature of the sample matrix, addition of matrix modifiers (salting out) and whether or not the sample is stirred or agitated during equilibration. Advantages

of SHA include quick and simple sample preparation, elimination of reagents (no solvent peak during GC analysis) and low risk of artifacts. The main disadvantage of SHA is that it is limited to products that contain appreciable levels of highly volatile 'headspace' components. SHA may be a good choice for targeted analyses of some highly volatile components of milk, such as acetaldehyde, hydrogen sulphide, diacetyl, methanethiol, dimethylsulphide and low molecular weight Strecker aldehydes (e.g. methylpropanal, 2-methylbutanal and 3-methylbutanal). SHA has been successfully used for the analysis of highly volatile sulphur compounds in heated milk (Christensen and Reineccius, 1992) and for general volatile profiling of fluid milk (Toso *et al.*, 2002).

Dynamic headspace analysis

The sensitivity of headspace analysis can be greatly improved by use of an intermediate trapping or adsorption step to enrich the volatile constituents prior to GC analysis. This technique is commonly referred to as dynamic headspace analysis (DHA) or purge-and-trap analysis and is useful for the analysis of trace-level components of high and intermediate volatility. In DHA an inert gas (nitrogen or helium) is swept over or through a thermostatted sample (contained in a closed vessel) for a period of time sufficiently long enough to 'extract' the volatile constituents. During this process the volatiles are enriched by an intermediate trapping step facilitated by use of adsorbent materials (porous polymers or charcoal) or by cryogenic focusing. Adsorbent trapping is most commonly used since it avoids the trapping of water vapour, which can adversely affect the cryogenic injector or GC column performance. Immediately after collection, trapped volatiles are transferred, most commonly by thermal desorption, to the GC for subsequent analysis. In order to improve GC performance, it is common practice to cryofocus the thermally desorbed volatiles in the GC inlet prior to analysis. Applications of adsorbent trapping–thermal desorption techniques are discussed in greater detail elsewhere (Hartman *et al.*, 1993; Butrym, 1999; Wampler, 2002).

DHA has similar advantages to SHA in that there is only minimal sample preparation required, no reagents are used, which means there is no solvent peak during GC analysis, and there is a low risk of artifact formation due to sample decomposition. A major limitation is that DHA is not an efficient method for analysis of semi-volatile compounds. Due to its ease of use, high sample throughput and relatively low cost per unit analysis, DHA has been the most extensively used method for the analysis of volatile components of fluid and dried milk products (Wellnitz-Ruen *et al.*, 1982; Park and Goins, 1992; Vallejo-Cordoba and Nakai, 1993; Imhof and Bosset, 1994; Barrefors *et al.*, 1995; Kim and Morr, 1996; Señorans *et al.*, 1996; Anderson and Lingnert, 1998; Cadwallader and Howard, 1998; Granelli *et al.*, 1998; Rysstad *et al.*, 1998; Valero *et al.*, 1999, 2001; Contarini and Povolito, 2002; Toso *et al.*, 2002; Fernandez *et al.*, 2003; Solano-Lopez *et al.*, 2005; Schlichtherle-Cerny *et al.*, 2007).

Static headspace–solid phase microextraction

In the past decade static headspace–solid phase micro extraction (HS-SPME) has emerged as one of the most commonly used methods for the isolation of volatile compounds from foods. HS-SPME is a rapid, solventless technique and is based on the partitioning of the volatile components between the sample headspace and a polymer-coated fibre. For analysis, the adsorbed volatiles are thermally desorbed in the heated inlet of the GC. The application of HS-SPME in the analysis of volatiles in foods has been reviewed and critically evaluated (Roberts *et al.*, 2000; Harmon, 2002; Marsili, 2002b; Werkhoff *et al.*, 2002; Vas and Vékey 2004).

Several adsorbent phases and film thicknesses are available for SPME. All the parameters of SHA should be considered in HS-SPME, as well as the nature of the fibre coating and fibre exposure (extraction) time. It is very important that the chosen sampling temperature does not result in any sample decomposition. Roberts *et al.* (2000) recommended the use of short extraction times (1–5 min) for highly volatile compounds and longer extraction times (5–30 min) for semi-volatile compounds. Transfer of the volatiles from the fibre to the GC is generally accomplished using hot, splitless injection, which may result in loss of some thermally labile volatile compounds.

Contarini and Povo (2002) reported that DHA and HS-SPME provided similar precision, but differed in their recovery factors, for the analysis of volatiles in fluid milks subjected to different heat treatments. HS-SPME has also been applied for the study of milk with off-flavours (Whitfield *et al.*, 2000; Mounchili *et al.*, 2005; Hedegaard *et al.*, 2006) and thermally derived off-flavours (Vazquez-Landaverde *et al.*, 2005), and to measure volatile profiles of high pressure processed milks (Vazquez-Landaverde *et al.*, 2006, 2007).

7.3.2 Solvent extraction–distillation methods

The combination of distillation with solvent extraction has been extensively used for the isolation of volatile compounds from foods (Chaintreau, 2001; Parliment, 2002). The traditional method of simultaneous distillation–solvent extraction (SDE) has, in recent years, been replaced by milder methods, e.g. direct solvent extraction–high vacuum distillation, that avoid the formation of thermally generated artifacts due to sample (thermal) decomposition and that minimize the loss of thermally labile volatile components.

Direct solvent extraction

Most volatile organic compounds in foods are considerably less polar than the bulk, mostly aqueous food matrix and, therefore, can be readily isolated by direct solvent extraction (DSE). The main concern with this approach for the analysis of milk volatiles is that the resulting solvent extract will contain appreciable amounts of nonpolar and nonvolatile lipids and minor amounts of other nonvolatile material. Therefore, these extracts should not be injected directly into a GC without first taking some precautionary steps. One such

approach has been to perform an additional cleanup step, such as high vacuum distillation, to isolate the volatile components of the extract from the nonvolatile material prior to GC analysis.

Direct solvent extraction is an effective means of isolating a broad range of volatile constituents from milk. It is especially suitable for extracting semi-volatile constituents (e.g. lactones, free fatty acids, phenolics, etc.) that cannot be effectively analysed using headspace methods. Repeated extractions are generally necessary to minimize some of the bias caused by the inherent selectivity of the solvent chosen for the extraction. A good general solvent is diethyl ether for several reasons: it has good selectivity toward most aroma compounds, it has a relatively low density, which enables ease of recovery, and it has a low boiling point so that it can be evaporated without significant losses of the extracted volatile substances. An important consideration in solvent extraction is solvent purity. This includes water if it is used in the analysis or in the preparation of other reagents. It is prudent to run a blank to account for any impurities or artifacts introduced by the solvent or other reagents used in the analysis.

Researchers have employed direct solvent extraction for recovery of milk volatiles (Cadwallader and Howard, 1998; Polineni and Peterson, 2005). Once a solvent extract is prepared, it is usually subjected to a high vacuum distillation cleanup step, separated into neutral/basic and acidic fractions and then concentrated by removing the bulk of the extraction solvent prior to GC analysis. These steps are discussed below.

High vacuum distillation

High vacuum distillation is the method of choice for 'cleaning up' volatile extracts prepared by direct solvent extraction. This step is especially important if the aroma extract is to be analysed by cool, on-column GC or other injection technique where the nonvolatile material will either interfere with the injection or lead to formation of thermally generated artifacts if a heated GC inlet is used. A careful and highly efficient solvent assisted flavour evaporation (SAFE) distillation system was developed for high vacuum distillation of either liquid products or solvent extracts (Engel *et al.*, 1999). SAFE has been used, often in combination with DSE, for the preparation of aroma extracts from milk products (Carunchia Whetstone *et al.*, 2005, 2006, 2007; Colahan-Sederstrom and Peterson, 2005). SAFE has been shown to be effective for the extraction of most volatiles from solvent extracts of cheese, including difficult polar and semi-volatile constituents (Werkhoff *et al.*, 2002).

Class fractionation and concentration of extracts

Aroma extracts prepared by direct solvent extraction–high vacuum (SAFE) distillation often contain hundreds of volatile constituents of varying polarities and containing different functional groups. Various methods, such as adsorption column chromatography and preparative GC, can be used to simplify the analysis by fractionation of the extract prior to GC analysis. One of the simplest

and most common methods is the use of acid/base chemistry to fractionate the extract into its acidic, basic and neutral components. The advantage of performing this fractionation step is that the GC chromatograms are less crowded with peaks. Furthermore, one does not need to compromise as much on GC column selection, since the optimum column may be used for the different fractions. For milk products, it is generally not beneficial to fractionate the basic compounds from the neutral ones, since milk contains only a low abundance of basic volatile compounds (e.g. 2-acetyl-1-pyrroline, *o*-aminoacetophenone, indole and skatole).

The general approach for fractionation of aroma extracts has been to back-extract the acidic components from the solvent extract using aqueous bicarbonate or other suitable aqueous base (Carunchia Whetstone *et al.*, 2005). The neutral/basic components are retained in the organic solvent. The aqueous base is then acidified and then subjected to solvent extraction to yield the acidic fraction (contained in the organic solvent).

Prior to GC analysis, it is usually necessary to enrich the concentration of the volatile analytes in the aroma extract. This is most often accomplished by careful distillation (e.g., use of Kuderna–Danish concentration apparatus) or by slow evaporation of the solvent under a gentle stream of inert gas (e.g. nitrogen). A major drawback to this concentration step is that some volatile constituents are lost (evaporate) along with the extraction solvent. It is for this reason that direct solvent extraction is generally not suitable for the analysis of highly volatile components.

7.3.3 Other isolation/extraction methods

In addition to the methods described above, there are some new and emerging methods that have not yet been widely applied to the study of milk flavour. These include stir bar sorptive extraction (SBSE) (Baltussen *et al.*, 1999), solid-phase dynamic extraction (SPDE) (Bicchi *et al.*, 2004; Christ *et al.*, 2007) and single droplet microextraction (SDME) (Wood *et al.*, 2004). The main attraction of these methods is that they are suitable for use with multifunctional GC autosamplers.

7.3.4 Instrumental methods of analysis

Gas chromatography–mass spectrometry

Tandem gas chromatography–mass spectrometry (GC-MS) is the method of choice for the analysis of volatile food components. The pre-eminence of GC-MS is due to the fact that high resolution GC provides the highest overall efficiency and performance of all separation methods and is readily operated in tandem with MS.

Present day GC involves the use of high resolution open tubular columns with bonded phases that are capable of separating hundreds of volatile constituents in a single run. GC is a mature methodology and the theory and general application

will not be discussed here. Two critical parameters that can profoundly influence the results of GC, injection technique and analytical column stationary phase, are discussed below.

The injection inlet is used to introduce the volatile compounds, typically 1 to 3 μL of an aroma extract or 1 to 25 mL of headspace vapour, into the GC column. Programmable temperature vaporizer (PTV) and on-column injectors represent the best options among the many available GC injectors. The PTV inlet has particular appeal since it allows for cryogenic focusing and ramped heated (programmable) injections in either split or splitless modes among other possible configurations. Cool on-column injection has been the method of choice for analysis of aroma extracts prepared by direct solvent extraction–high vacuum distillation since it avoids thermal degradation and all of the components of the extract are completely introduced into the GC column (i.e. there is no injector discrimination).

In general, only two types of GC phases are required for the identification and quantitation of the volatile constituents of milk. Polarity of the stationary phase is the most important parameter and should be matched as closely as possible with the polarity of the analytes. For identification purposes it is best to determine retention indices for each analyte on two columns of differing polarity. Polyethylene glycol phases, such as a Carbowax (e.g. DBWAX) or free fatty acid phase (e.g. FFAP), are suitable for the analysis of most polar compounds. A 95% dimethyl–5% diphenylpolysiloxane phase (e.g. DB5) is commonly used for the analysis of nonpolar compounds. Some compounds are incompatible with certain phases, e.g. short-chain free fatty acids do not separate well on nonpolar phases. In such cases it may be necessary to use an intermediate polarity phase, e.g., 86% dimethyl–14% cyanopropylphenylpolysiloxane (DB-1701). Blank (2002) presented a detailed discussion of the importance of GC stationary phase in aroma research.

Many detectors are available for GC. The flame ionization detector (FID) is most commonly used for routine GC; however, for the analysis of highly complex volatile mixtures a mass spectrometric (MS) detector is preferred since it provides both qualitative and quantitative information. The main advantage of GC-MS is that it allows for mass spectral library matching for identification of unknown chromatographic peaks. The total ion chromatogram generated by full scan GC-MS can also be used for routine peak quantitation. For analysis of trace constituents, selected ion monitoring (SIM) mass spectrometry and ‘mass chromatography’ are often used. In SIM only selected ions representative of a specific compound or class of compounds are recorded during GC-MS analysis. The technique provides high sensitivity for analysis of known constituents but does not provide any useful information for the identification of unknown compounds, since full mass spectra are not recorded. Mass chromatography can be considered as retrospective SIM. With this method complete mass spectra are recorded throughout the GC-MS run rather than just selected ions. The data analysis software can then be used to re-plot only specific ions from full spectra data, with the aim of resolving co-eluted peaks. Mass chromatography has the

advantage over SIM in that full spectra are available for detailed interpretation; however, the method is somewhat less sensitive than SIM.

Gas chromatography–olfactometry

As mentioned earlier, high resolution GC is capable of separating and detecting hundreds of volatile compounds in a single run. However, it is likely that only a small number of these compounds actually contribute to the aroma of the product. Various approaches have been taken to identify these key odourants. These include the calculation of odour activity values (OAVs), use of GC–olfactometry (GCO) and sensory analysis of aroma models (Grosch, 1993, 1994, 2001). In GCO, the analytes are first separated by the GC and then delivered, generally in parallel with an electronic detector, to an olfactometer (sniff port) where they are mixed with humidified air. Human panelists continuously evaluate (nasally) the air emitted from the olfactometer and record their perceptions, such as odour intensity and odour description of the detected compounds. GCO has been reviewed (Acree, 1993; Blank, 2002). Common methods based on GCO include aroma extract dilution analysis (AEDA) (Grosch, 1993, 1994), CharmAnalysis (Acree, 1993), Osme (McDaniel *et al.*, 1990), nasal impact frequency (NIF, Pollien *et al.*, 1997; SNIF, Chaintreau, 2002) and post-peak intensity scaling (Avsar *et al.*, 2004). These methods differ mainly in how the GCO data are recorded and analysed. All GCO methods should be considered as screening methods since they do not provide an absolute measure of odour potency. Instead, GCO data should be used to indicate odour-active volatiles for subsequent quantitative analysis and sensory studies. Among the various GCO methods available, the dilution techniques have been most often used in the analysis of milk flavour.

GCO has been applied for aroma characterization of fresh (Moio *et al.*, 1993; Iwatsuki *et al.*, 1999; Bendall, 2001), pasteurized and UHT milks (Moio *et al.*, 1994; Iwatsuki *et al.*, 1999), milk with light-induced flavour (Cadwallader and Howard, 1998) and nonfat dry milk (Karagül-Yüceer *et al.*, 2001; Carunchia Whetstine and Drake, 2007). Tables 7.1 and 7.2 provide listings of characteristic aroma components identified in fluid and dried milk products by GCO. Two reviews of the application of GCO for the analysis of dairy products have also been published (Friedrich and Acree, 1998; Parliment and McGorin, 2000).

Quantitative analysis

The ideal quantitative analysis procedure should have high precision and accuracy. Internal standard methodology has been the preferred method for routine analysis of volatile compounds in cheese products. In the internal standard method, known amounts of surrogate internal standards that mimic the analytes of interest as much as possible are added to the sample matrix prior to performing the volatile isolation procedure. The internal standards compensate for variations in extraction procedures, injection volume and detector drift. Internal standards should possess chemical, spectral and chromatographic properties that are similar to the compounds being analysed. They should be

readily available, stable, inexpensive, of high (or known) purity, and must not interfere with the analytes during GC. For highest accuracy it is important to determine the relative recoveries and GC response factors for the internal standards relative to the target analytes. These recovery factors may be based on GC-MS (full scan, SIM, or mass chromatography) or use of a selective detector (e.g. flame photometric detector for selective analysis of sulphur-containing compounds).

The perfect internal standard is an isotopic analogue of the analyte of interest. This method is called stable isotope dilution analysis (SIDA) and involves the use of stable isotopes (deuterium or carbon-13 labelled) as internal standards. GC-MS analysis (by either electron-impact-MS or chemical-ionization-MS) is required, since the isotopic internal standard co-elutes with the analyte but is resolvable by MS. The method is self-correcting for recovery losses during sample work-up and chromatography, since the labelled and unlabelled compounds have essentially the same physical and chemical properties. The main disadvantage of SIDA is the cost associated with the synthesis of the isotopic analogues. SIDA has been used in the analysis of milk aroma constituents (Bendall and Olney, 2001; Preininger and Ullrich, 2001; Czerny and Schieberle, 2007).

Application of multivariate analysis techniques and electronic noses

Chemometrics/multivariate analysis (MVA) techniques can be applied for the interpretation of complex instrumental data for three main purposes: (1) to explore patterns of association in datasets, (2) to continuously monitor properties of materials, and (3) to classify materials (Marsili, 2007b). While researchers have applied MVA techniques to understand the effect of production, processing and storage on milk flavour volatiles (Contarini *et al.*, 1997; Contarini and Povoio, 2002; Fernandez *et al.*, 2003; Vazquez-Landaverde *et al.*, 2006), this approach is most commonly used for the analysis of data from electronic nose (e-nose) instruments which utilize sensory arrays to obtain multiple responses for vapour (e.g., headspace) samples. Some commercial electronic nose devices make use of conducting polymer or metal oxide sensor arrays (Harper, 2001; Pearce *et al.*, 2003). These instruments have experienced limited success due to sensor drift, need for frequent calibration and other factors (Marsili, 2002a). Nowadays, the mass spectrometric detector (MSD)-based e-noses are more popular since they overcome many of the problems of the sensor array-based instruments (Marsili, 2002a).

Electronic noses are effective for discriminating different kinds of milk (Visser and Taylor, 1998; Brudzewski *et al.*, 2004; Yu *et al.*, 2007) and can be used to predict shelf-life (Labreche *et al.*, 2005). Korel and Balaban (2002) were able to correlate sensory data for milk with microbial loads using an electron nose equipped with 12 conducting polymer sensors. Meanwhile, Capone *et al.* (2001) demonstrated that an electronic nose based on semiconductor thin films could distinguish between UHT and pasteurized milk.

MS-based electronic noses have been successfully used to detect specific flavour defects in milk, such as trimethylamine (Ampuero *et al.*, 2002), for

differentiation of normal-tasting milk from those containing off-flavours from light, heat, copper abuse or from microbial contamination (Marsili, 1999), and for shelf-life prediction (Marsili, 2000).

7.4 Measurement of colour

Realizing the importance of colour, dairy researchers early on developed a number of methodologies to measure the colour attributes of milk products. The first attempts at measuring the colour of fluid milk involved the use of crude colorimeters (Doan, 1924). The Munsell colour system was later used to measure degree of browning by the visual comparison of solutions containing extracted brown pigments to colour references (Webb and Holm, 1930). Degree of browning was also estimated using spectrophotometric methods based on the reflectance properties of the unaltered product (Kass and Palmer, 1940; Nelson, 1948; Burton, 1954), based on the transmittance properties of solutions containing extracted pigments (Choi *et al.*, 1949; Tinkler *et al.*, 1955), and by absorption of 2-thiobarbituric acid reactive products (of 5-hydroxyfurfural) (Keeney and Bassette, 1959). Browning has also been estimated by absorption at 420 nm (Olano and Martínez-Castro, 1996).

Today, the measurement of colour can be considered a mature science (MacDougall, 2002; Francis, 2003). This is due to the development and commercialization of dedicated colour measurement devices, such as trichromatic (or tristimulus) colorimeters in the mid-1950s (Hunter, 1958) and, more recently, spectrophotometer-based systems (Francis, 2003). These systems provide a rapid and convenient means for the routine analysis of food colour (MacDougall, 2002; Francis, 2003). Since 1960, nearly all of the studies conducted on milk colour have made use of tristimulus colorimeters or spectrophotometers (Pagliarini *et al.*, 1990; Hardy and Fanni, 1981; Rampilli and Andreini, 1992; Popov-Raljić *et al.*, 2008). Hunter (CIE) $L^*a^*b^*$ and CIELAB $L^*a^*b^*$ are two commonly used systems for the analysis of colorimeter data, where L = lightness or darkness, $+a$ = redness, $-a$ = greenness, $+b$ = yellowness and $-b$ = blueness (Francis, 2003). The $L^*a^*b^*$ values are often transformed into colour difference values (ΔE) for comparison between any two samples using the following equation:

$$\Delta E = [\Delta L^2 + \Delta a^2 + \Delta b^2]^{0.5}$$

Since the whiteness of milk products is often the most critical colour attribute, some researchers have also used the variation in lightness value (ΔL) as a measure of degree of difference in whiteness compared to a white standard (Hardy and Fanni, 1981). Some instruments are capable of transforming the data into whiteness values (WT) (Gervilla *et al.*, 2001). Yellow index (YI) was used by Pagliarini *et al.* (1990) to monitor the colour changes in heat-treated milk. In addition to the above-mentioned tristimulus systems, spectral reflectance measurements (400 to 700 nm) are also a suitable estimate of degree of

whiteness in milk (Nelson, 1948; Quiñones *et al.*, 1997; Solah *et al.*, 2007). Some recent applications of colorimetric and spectrophotometric systems for measurement of colour in milk products can be found in the published literature (Nielsen *et al.*, 1997; Browning *et al.*, 2001; Gervilla *et al.*, 2001; Nozière *et al.*, 2006; Solah *et al.*, 2007; Popov-Raljić *et al.*, 2008).

7.5 Future trends

The understanding of how certain variables, such as production, processing and storage, impact the flavour and colour of milk will continue to interest dairy scientists. Measurement of milk colour using colorimeters and spectrophotometers is a mature science and little development in this field is expected to occur in the near future. On the other hand, techniques used for flavour measurement of milk products are constantly evolving. Such methods include the difficult and labour-intensive GCO and GC-MS methods, which allow for detailed aroma characterization. In addition, relatively simple and easy to use methods, including SHA, DHA and HS-SPME and electronic nose technology, provide useful, albeit somewhat limited, information about how the volatile constituents of milk are impacted by new and emerging dairy processing techniques, as well as other factors such as packaging and storage.

7.6 Sources of further information and advice

Texts that provide more detailed information about new and emerging methods for measuring volatile compounds in foods are highly recommended (Reneiccius, 2006; Marsili, 2007a). For the latest developments in flavour research the reader should consult scientific journals, proceedings (symposia) of scientific meetings and technical papers presented at scientific meetings.

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