Undesirable Sulphur and Carbonyl Flavour Compounds in UHT Milk: A Review

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ABSTRACT

UHT processing, leads to the formation of “cooked” and “flat” flavours in milk. These undesirable notes occur due to the volatile formation of a variety of sulphur containing compounds, methyl ketones and aliphatic aldehydes derived from the constituents of the milks matrix during thermal processing and storage. The “cooked” flavour of UHT milk is associated with the presence of a variety of sulphur containing compounds while the “stale” flavour is characterised by the dissipation of these sulphur volatiles and an increase of the formation and presence of both methyl ketones and aliphatic aldehydes over time. The extent that the individual volatiles contribute to the overall flavour of UHT milk is not clear. The proposed formation of these volatiles; methods to control the intensity of “cooked” and “stale” flavours associated with UHT milk and extraction techniques for the isolation of these volatiles from milk have been reviewed.

Keywords: UHT milk, cooked flavour, stale flavour
INTRODUCTION

Ultra High Temperature (UHT) milk is a shelf-stable dairy product which is produced by holding milk at a high temperature (138-145°C) for a short time interval (1.5-9.0 seconds) (Valero et al., 2001) and packaged under aseptic conditions. This treatment provides an advantageous shelf-life of 6-9 months at room temperature when compared to approximately 14 day’s refrigerated shelf-life of pasteurised milk (Perkins et al., 2005). However, according to Clare et al. (2005) UHT milk has poor consumer acceptability. This can be attributed to the chemical formation of “cooked” and “flat” flavours (Vazquez-Landaaverda et al., 2005; Vazquez-Landaaverde et al., 2006) which are derived from the constituents of the milks matrix during processing and storage. These chemical changes lead to an increase in the concentration of various flavour compounds such as sulphur containing compounds, methyl ketones and aliphatic aldehydes which contribute to the overall flavour of the milk (Jeon et al., 1978; Shibamoto et al., 1980; Contarini et al., 1997; Vazquez-Landaaverda et al., 2005 and Vazquez-Landaaverde et al., 2006). In addition to the “cooked” and “stale” character, heated milk may also be perceived as having a “heated” or “rich” flavour (Shipe et al., 1978). This characteristic flavour is believed to be imparted via products of the Maillard reaction during thermal processing (Rerkrai et al., 1987). These Maillard compounds include various furans, diketones, cyclic ketones, pyrazines and other sulphur and nitrogen containing compounds (Shibamoto et al., 1980). Initially UHT milk is described as having a “cooked” flavour which is attributed to the increase in the formation of sulphur containing compounds in the milk due to thermal denaturation of milk serum proteins during processing (Patrick and Swaisgood, 1976 and Vazquez-Landaaverde et al., 2006). This “cooked” flavour however dissipates after several weeks and is replaced by an off flavour note characterised as being “stale” (Valero et al., 2001 and Vazquez-Landaaverde et al., 2005). This is due to the formation of methyl ketones via the thermal decarboxylation of β-keto acids as well as the initiation of lipid oxidation in the milk leading to an increase in the formation of aliphatic aldehydes during storage (Shibamoto et al., 1980 and Moio et al., 1994). To consumers, flavour is one of the most significant quality attributes owing to the overall acceptability of a food product (Guichard, 2002) and due to the formation of these compounds in milk during processing and storage some consumers find the flavour of UHT milk to be undesirable (Clare et al., 2005).
Thus if the concentration of these “cooked”, “stale” and “heated” off-flavour producing compounds in UHT milk can be significantly reduced the overall consumer acceptance of the product may improve. The formation of off-flavour volatiles in UHT milk will be reviewed. Furthermore methods to reduce the concentration of these volatiles; the interactions of these volatiles with milk packaging materials and analytical methods used to study these volatiles in milk are discussed.

FLAVOUR COMPOUNDS IN UHT MILK

According to Jaddou et al. (1978); Jeon et al. (1978); Contarini and Povolo (2002) and Vazquez-Landa verda et al. (2005) the flavour of milk is liable to change when milk is subjected to a thermal treatment and storage. Heat induced flavours in milk can be classified into four distinct groups: cooked or sulphurous; heated or rich, caramelised and scorched (Shipe et al., 1978). According to Clare et al. (2005) UHT milk has poor consumer acceptability, when compared to pasteurized milk, due to the formation of “cooked” and “flat” flavours. Several compounds which give milk a “heated” or “rich” character also appear to contribute to the overall “stale” (Scanlan et al., 1968; Jeon et al., 1978; Shibamoto et al., 1980; Rerkrai et al., 1987; Vazquez-Landaverde et al., 2005) and “cooked” (Colahan-Sederstrom and Peterson, 2005) flavour of UHT milk. Furthermore, Ferretti and Flanagan (1972) proposed that the “stale” flavour associated with dairy products is possibly due to both lipid oxidation and the Maillard reaction. The initial “cooked” flavour of UHT milk dissipates several days after UHT milk production (Patrick and Swaisgood, 1976). The milk is then rather described as “stale” (Thomas et al., 1975). These flavours occur due to the increase in the formation of various carbonyl and sulphur containing compounds in UHT milk (Jeon et al., 1978; Shibamoto et al., 1980; Contarini et al., 1997; Vazquez-Landa verda et al., 2005 and Vazquez-Landaverde et al., 2006). The concentrations of these flavour compounds are lower in raw and pasteurized milk when compared to the flavour profile of UHT milk (Table 1). The mentioned flavour compounds in UHT milk are derived via thermal denaturation of milk proteins; lipid oxidation or non enzymic reactions of the constituents of the milk’s matrix i.e. milk proteins, carbohydrates, lipids and other milk constituents (Calvo and de la Hoz, 1992).
Table 1. The Concentration (μg/kg) and Odour Thresholds of Some Flavour Compounds in Raw, Pasteurized and UHT Milk (3% milk fat). Adapted from Rychlik et al. (1998), Vazquez-Landaverde et al. (2005) and Vazquez-Landaverde et al. (2006)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Raw milk</th>
<th>Pasteurised milk</th>
<th>UHT milk</th>
<th>Odour Threshold (μg/kg)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ketones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diacetyl</td>
<td>0.48</td>
<td>1.71</td>
<td>7.39</td>
<td>5</td>
</tr>
<tr>
<td>2-pentanone</td>
<td>0.28</td>
<td>0.14</td>
<td>9.53</td>
<td>n.d.</td>
</tr>
<tr>
<td>2-hexanone</td>
<td>0.37</td>
<td>0.17</td>
<td>1.18</td>
<td>n.d.</td>
</tr>
<tr>
<td>2-heptanone</td>
<td>0.95</td>
<td>0.55</td>
<td>34.46</td>
<td>5</td>
</tr>
<tr>
<td>2-octanone</td>
<td>3.82</td>
<td>2.15</td>
<td>4.51</td>
<td>n.d.</td>
</tr>
<tr>
<td>2-nonanone</td>
<td>0.24</td>
<td>0.33</td>
<td>52.64</td>
<td>5</td>
</tr>
<tr>
<td>2-decanone</td>
<td>n.d.</td>
<td>n.d.</td>
<td>1.33</td>
<td>n.d.</td>
</tr>
<tr>
<td>2-undecanone</td>
<td>4.58</td>
<td>0.7</td>
<td>9.7</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Aldehydes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-methylpropanal</td>
<td>0.40</td>
<td>0.48</td>
<td>2.52</td>
<td>0.7</td>
</tr>
<tr>
<td>3-methylbutanal</td>
<td>n.d.</td>
<td>0.17</td>
<td>1.14</td>
<td>0.04</td>
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<tr>
<td>2-methylbutanal</td>
<td>0.90</td>
<td>0.11</td>
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<td>0.9</td>
</tr>
<tr>
<td>Hexanal</td>
<td>2.68</td>
<td>0.82</td>
<td>12.97</td>
<td>4.5</td>
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<td>2-furaldehyde</td>
<td>0.20</td>
<td>0.13</td>
<td>0.38</td>
<td>3000</td>
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<tr>
<td>Heptanal</td>
<td>0.20</td>
<td>0.08</td>
<td>1.68</td>
<td>3</td>
</tr>
<tr>
<td>Octanal</td>
<td>0.52</td>
<td>0.09</td>
<td>0.95</td>
<td>0.7</td>
</tr>
<tr>
<td>Nonanal</td>
<td>1.36</td>
<td>0.28</td>
<td>3.92</td>
<td>1</td>
</tr>
<tr>
<td>Decanal</td>
<td>2.72</td>
<td>1.26</td>
<td>6.68</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Sulphur compounds</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>hydrogen sulphide</td>
<td>1.21</td>
<td>0.5</td>
<td>12.0</td>
<td>10</td>
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<tr>
<td>Methanethiol</td>
<td>4.80</td>
<td>0.4</td>
<td>23.9</td>
<td>5</td>
</tr>
<tr>
<td>carbon disulphide</td>
<td>0.0221</td>
<td>0.0313</td>
<td>0.0589</td>
<td>n.d.</td>
</tr>
<tr>
<td>Compound</td>
<td>Raw milk</td>
<td>Pasteurised milk</td>
<td>UHT milk</td>
<td>Odour Threshold (µg/kg)</td>
</tr>
<tr>
<td>------------------------</td>
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<td>------------------</td>
<td>----------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>dimethyl sulphide</td>
<td>8.16</td>
<td>14.2</td>
<td>21.41</td>
<td>2</td>
</tr>
<tr>
<td>dimethyl disulphide</td>
<td>0.0334</td>
<td>0.0101</td>
<td>0.0328</td>
<td>30</td>
</tr>
<tr>
<td>dimethyl trisulphide</td>
<td>0.0367</td>
<td>0.0157</td>
<td>0.0473</td>
<td>0.008</td>
</tr>
<tr>
<td>dimethyl sulfoxide</td>
<td>1260</td>
<td>820</td>
<td>1460</td>
<td>n.d.</td>
</tr>
<tr>
<td>dimethyl sulfone</td>
<td>2640</td>
<td>4720</td>
<td>1260</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

superscript a odour thresholds determined in water. n.d. = not determined

**Sulphur containing compounds**

The initial “cooked” flavour is the first of two problematic flavours occurring in UHT milk (Mehta, 1980). As raw milk is heated during UHT processing, serum proteins denature leading to the formation of free “reactive” sulphydryl groups which correlate to the liberation of volatile sulphur compounds and the “cooked” flavour (Blankenagel and Humbert, 1963; Patrick and Swaisgood, 1976). However according to Jaddou et al. (1978) the “cooked/cabbagy” flavour of UHT milk may not only be due to the collective liberation of sulphur containing compounds (hydrogen sulphide, carbonyl sulphide, methanethiol, dimethyl sulphide and carbon disulphide) but perhaps a result of these compounds interacting with unidentified compounds from the carbonyl fraction of milk.

Initially hydrogen sulphide was considered the major sulphur containing volatile contributing to the “cooked” flavour of heated milk (Thomas et al., 1976). However more recently, Vazquez-Landaverde et al. (2006) suggested that hydrogen sulphide does not contribute as extensively to the aroma of UHT milk as previously indicated. Furthermore Vazquez-Landaverde et al. (2005) identified that dimethyl sulphide is an important aroma contributor and Vazquez-Landaverde et al. (2006) showed that methanethiol is possibly the most potent sulphur containing aroma compound in UHT milk. Both these compounds are present in UHT milk at concentrations well above their odour thresholds (Table 1) (Rychlik et al., 1998). Hydrogen sulphide is formed due to the thermal denaturation of β-lactoglobulin in particular as well as other whey proteins and from denatured protein containing material, associated with the milk fat globule membranes.
(Townley and Gould, 1943; Hutton and Patton, 1952; Badings and van der Pol, 1973). These protein molecules contain the sulphur containing amino acids cysteine and methionine (Aboshama and Hansen, 1977). The now exposed sulphydryl groups of the denatured polypeptides may be oxidised leading to the release of hydrogen sulphide (Townly and Gould, 1943; Hutton and Patton, 1952). Alternatively, Strecker degradation of cysteine residues with a diketone may take place leading to the formation of hydrogen sulphide (Schutte and Teranishi 1974). Although the exact formation of dimethyl sulphide is unclear there are two proposed reaction mechanisms. Keenan and Lindsay (1968) showed that dimethyl sulphide and homoserine concentrations increased when s-methylmethionine sulphonium salts, occurring in milk from various plant food sources, decreased due to thermal desiccation during processing. However Ballance (1961) believes that dimethyl sulphide is formed from the sulphur containing amino acid methionine. During the heating of milk, methional is formed due to the Strecker degradation of methionine and is further converted to methanethiol (Schutte and Koenders 1972). Methanethiol is then oxidised to form dimethyl disulphide (Ballance, 1961) which can be further converted to dimethyl trisulphide and dimethyl sulphide (Fig. 1). According to Thomas et al. (1975) the loss of the “cooked” flavour of heated milk during storage is due to the oxidation of reactive sulphydryl groups with molecular oxygen over time. However dimethyl sulphide is known to increase in concentration during storage (Jaddou et al., 1978) possibly because dimethyl sulphide is a product of the oxidation of methanethiol (Ballance, 1961).

**Ketones**

The “stale” note is the second of the two problematic flavours of UHT milk (Mehta, 1980). It appears in the milk during storage as the “cooked” flavour begins to decrease (Thomas et al., 1975). A number of different ketones contribute to the overall flavour of UHT milk. Methyl ketones for instance contribute to the perceived “stale” flavour (Contarini and Povolo, 2002) while diketones and cyclic ketones add “heated” flavour notes to the milk (Shibamoto et al., 1980). Methyl ketones are present in raw and pasteurised milk however their total concentration
Figure 1  Formation of dimethyl sulphide via Strecker degradation of methionine and the oxidation of methanethiol. Adapted from Ballance (1961) and Schutte and Koenders (1972).
methyl ketones increase during storage (Valero et al., 2001) and the rate of their formation is dependent on the milk's storage temperature (Jeon et al., 1978). Contarini et al. (1997) and Vazquez-Landaverde et al. (2005) identified that the overall concentration of methyl ketones is proportional to the extent of the thermal processing treatment and the development of a “stale” flavour in UHT milk. Methyl ketones, particularly ones with odd numbered carbon atoms, are produced via the hydrolysis and decarboxylation of β-ketoalkanoic acid esters in milk fat during heating (Parks et al., 1964) (Fig. 2). Methyl ketone formation requires low activation energy (Schwartz et al., 1966) and so formation continues readily throughout the storage period (Kochhar, 1996) of the milk.
Although the overall concentration of methyl ketones are relatively high in UHT milk, they occur at levels far below their odour threshold values and contribute little to the overall “stale” flavour when compared with aliphatic aldehydes (Table 1) (Jeon et al., 1978). However, Contarini and Povolo (2002) showed that an increase in the perceived “stale” flavour of UHT milk correlated with an increase in the concentration of thermally derived methyl ketones in the product. It has been suggested by Langler and Day (1964) that a mixture of methyl ketones may act synergistically together to contribute to a perceivable flavour when the compounds are all present below their threshold values. Moio et al. (1994) suggested that 2-heptanone and 2-nonanone in particular are the two most potent aroma compounds in UHT milk. Recently, based on odour active values (OAV) Vazquez-Landaverde et al. (2005) identified 2-heptanone and 2-nonanone as important odourants in the overall “stale” aroma of UHT milk. Using an olfactory evaluation of captured UHT milk volatiles Naudé et al. (2009) identified that 2-heptanone had a “soapy” aroma where as 2-nonanone was perceived as more “fruity”. However the synergistic effect of 2-nonanone and 2-heptanone produced a pungent cheese, sour milk-like aroma in UHT milk.

Aldehydes

According to Vazquez-Landaverde et al. (2005) aldehydes may strongly contribute to the flavour of UHT milk albeit that they occur at very low concentrations. The rate at which aldehydes form in milk are influenced by two factors, namely 1) dissolved oxygen content and 2) temperature during storage (Jeon et al., 1978). Aliphatic aldehydes are produced in UHT milk, during storage, by the oxidation of casein bound unsaturated fatty acids (Ramshaw and Dunstone, 1969) as well as via the degradation of various amino acids in the milk’s matrix. For example 3-Methylbutanal or 2-methylbutanal are synthesised via the degradation of the amino acid leucine in the Maillard reaction (Morgan et al., 1957) which occurs during the heating of milk. Nonanal however, is formed as a secondary oxidation product of a C10 hydroxide derived from the autoxidation of oleic acid (Day and Lillard, 1960) (Fig. 3).

Aliphatic aldehydes are mostly present at concentrations near to or above their odour threshold values in UHT milk (Jeon et al., 1978; Rerkrai et al., 1987; Rychlik et al., 1998 and Vazquez-
Figure 3  Formation of octanal, decanal, nonanal and undecanal via the oxidative degradation of oleic acid. Adapted from Day and Lillard (1960).
Landaverde et al., 2005). Thus, Jeon et al. (1978) concluded that even though the concentrations of methyl ketones are higher, aliphatic aldehydes in particular contribute more extensively to the overall “stale” flavour of UHT milk. Based on OAV’s octanal, nonanal, decanal, 2-methylbutanal, 2-methylpropanal and methylpropanal are major contributors to the “stale” flavour of UHT milk (Vazquez-Landaverde et al., 2005). Furthermore, Day et al. (1963) suggested that a mixture of aliphatic aldehydes could act in a synergistic manner and give rise to an oxidised flavour in milk fat samples when all the aldehydes were present below their threshold values. This indicates that aldehydes may considerably contribute to the “stale” flavour of UHT milk all be it that their concentration in the milk is relatively low (Vazquez-Landaverde et al. 2005).

**Compounds associated with the Maillard reaction**

Nursten (1981) proposed that volatile compounds derived from the nonenzymic pathways of the Maillard reaction can be classified into three groups (Table 2). During milk processing, lactose and its derivatives, glucose and galactose, may either thermally degrade or interact with amino acids and other nitrogenous compounds to form a variety of flavour compounds via the Maillard reaction (Calvo and de la Hoz, 1992). Many of these compounds are believed to contribute to the “heated” or “rich” flavour of heated milks (Shipe et al., 1978 and Rerkrai et al., 1987). Maillard derived compounds such as benzaldehyde (Joen et al., 1978), acrolein (Mehta and Bassette, 1978), maltol, diacetyl (Scanlan et al., 1968), acetaldehyde, isobutanal (Rerkari et al., 1987), 1-butanol (Jaddou et al., 1978) 2-methylbutanal and 3-methylbutanal (Vazquez-Landaverde et al., 2005) have been identified in UHT milk. Diacetyl in particular, has been suggested to contribute excessively towards the “heated” flavour of UHT milk (Scanlan et al., 1968). According to Jeon et al. (1978) diacetyl occurs above its odour threshold in UHT milk and thus contributes to the flavour of the final product. The concentration of diacetyl in milk increases proportionally to the increasing severity of the applied heat treatment (Scanlan et al., 1968).

The magnitude that all Maillard reaction compounds contribute to the overall “heated” flavour of the product is still not fully understood. However, it appears that several compounds contributing to the “heated” or “rich” character may also contribute to the overall “stale” and
“cooked” flavour of UHT (Scanlan et al., 1968; Jeon et al., 1978; Shibamoto et al., 1980; Rerkrai et al., 1987; Colahan-Sederstrom and Peterson, 2005; Vazquez-Landaaverde et al., 2005).

A number of different studies have been carried out in order to limits or decrease off “cooked” and “stale” flavour production in milk. According to Boyd and Gould (1957) the addition of copper sulphate, calcium chloride or disodium phosphate to milk before heating, reduces the total concentration of sulphhydryl groups (-SH) and hydrogen sulphide in milk. The addition of 0.5ppm copper, prior to and after heating, led to the reduction of hydrogen sulphide where as

Table 2. Classification of Volatile Products Derived During The Maillard Reaction (Nursten, 1981)

<table>
<thead>
<tr>
<th>Volatile Classification</th>
<th>Volatile Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Dehydration and fragmentation products from &quot;simple&quot; sugars</td>
<td>Furans</td>
</tr>
<tr>
<td></td>
<td>Pyrones</td>
</tr>
<tr>
<td></td>
<td>Cyclopentenes</td>
</tr>
<tr>
<td></td>
<td>Carbyonyls</td>
</tr>
<tr>
<td></td>
<td>Acids</td>
</tr>
<tr>
<td>2- Degradation products from &quot;simple&quot; amino acids</td>
<td>Aldehydes</td>
</tr>
<tr>
<td></td>
<td>Sulphur compounds</td>
</tr>
<tr>
<td>3- Volatiles formed by further interactions</td>
<td>Pyrroles</td>
</tr>
<tr>
<td></td>
<td>Pyridines</td>
</tr>
<tr>
<td></td>
<td>Imidazoles</td>
</tr>
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<td></td>
<td>Pyrazines</td>
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<tr>
<td></td>
<td>Oxazoles</td>
</tr>
<tr>
<td></td>
<td>Thiazoles</td>
</tr>
</tbody>
</table>
Inhibition of “cooked” and “flat” flavours in milk

2.0ppm led to no hydrogen sulphide being produced when compared to a control. However, Marsili (2000) indicated that when copper was added to milk in excess it led to the formation of an adverse “oxidised” flavour in the milk. Samuelsson and Borgström (1973) used sodium iodate or sodium bromate or potassium iodate or potassium bromate, which are oxidising agents, to reduce the presence of sulphydryl groups and decrease the “cooked” flavour in sterilised milk without increasing the production of an “oxidised” flavour. Ferretti (1973) and Ferretti et al. (1974) showed that the “cooked” flavour of UHT milk can be controlled by the addition of organic thiolsulphonates and organic thiosulphates to the milk before heating. However, apart from two of these compounds adding other undesirable flavours to the milk (Ferretti, 1973) neither are permitted to be used as food additives (Ferretti et al., 1974) and may not be added to milk. The “cooked” flavour of UHT milk may be removed by treating the milk with a native milk enzyme, sulphydryl oxidase. The enzyme initiates the conversion of –SH groups in heated milk to form disulphides but, it cannot be added to milk prior to heating because high temperatures lead to the inactivation of the enzymic activity of sulphydryl oxidase (Swaisgood, 1977). Renner and Berlage-Weining (1983) added L-cysteine to heated milk in order to reduce the concentration of hydrogen sulphide and the overall intensity of the “cooked” flavour in heated milk where cysteine is formed due to the reaction of L-cysteine with hydrogen sulphide.

According to Colahan-Sederstrom and Peterson (2005) sulphur-containing methional showed a significant reduction along with other Maillard reaction-derived compounds when the polyphenol, epicatchin (0.2%), was added to milk prior to UHT processing. Although the addition of epicatchin lead to a decrease in the “cooked” flavour, at the effective level of 0.2% it also imparts a bitter note to milk (Colahan-Sederstrom and Peterson, 2005). Al-Attabi et al. (2009) have recently and extensively similarly summarised a number of the attempted methods used to reduce the “cooked” flavour of heated milk.

Although copper has been used to decrease the “cooked” flavour of UHT milk (Boyd and Gould, 1957; Marsili, 2000) the presence of copper increased the formation of acetal, propanal, n-pentanal and n-hexanal in pasteurised milk because copper ions may initiate lipid oxidation.
leading to the production of “stale” flavours (Jenq et al., 1988). To control the “flat” flavour of UHT milk research has focused on active packaging to selectively adsorb aldehydes and ketones. Brodie III and Visioli (1994) decreased the concentration of hexanal from a “hexanal environment” by exposing the environment to a polyethylene imine and polyolefin polymer which can absorb volatile aldehydes. Suloff et al. (2003) lowered the concentration of acetaldehyde, propionaldehyde, butyraldehyde, valeraldehyde and caproaldehyde from an acidified aqueous model solution by active packaging incorporated with aldehyde-scavenging agent’s nylon MXD6, D-sorbitol and \( \alpha \)-cyclodextrin. This film showed selective binding for lower molecular weight aldehydes (Suloff et al., 2003). There is no indication that this film can bind methyl ketones, which are key component of the “stale” flavour of UHT milk (Shibamoto et al., 1980 and Contarini and Povolo, 2002) and the analysis was also not carried out in a more complex matrix such as milk. Colahan-Sederstrom and Peterson (2005) added epicatchin (0.2%) to milk prior to UHT processing. Even though epicatchin is a powerful oxidising agent it had a limited affect on the total production of nonanal a compound formed during lipid oxidation (Colahan-Sederstrom and Peterson, 2005). Recently Perkins et al. (2006) incorporated an oxygen scavenging film into a UHT milk package which lowered the amount of dissolved
oxygen in UHT milk, leading to the reduction of 2-hexanone, hexanal, 2-heptanone, 2-nonanone and nonanal by 23-41% after 14 weeks of storage when compared to a control. However based on odour rather than flavour a sensory panel found no significant difference between the treated and untreated samples and the oxygen scavenger used in the trials was not an approved food additive (Perkins et al., 2006).

PACKAGING OF UHT MILK

In the food packaging industry, low density polyethylene (LDPE) is the most used plastic film (Robertson, 2006) because it is a good moisture barrier; it is stable at high processing temperature; it is inert to most food products; it is resistant to chemical degradation (Sajilata et al., 2007) and it is the most cost effective plastic film available on a per unit mass basis (Selke, 2003). UHT milk is aseptically packaged into a polyethylene (LDPE)-lined paper-board pack containing a layer of aluminium foil (Robertson, 2006). This package is known as a Tetra Brick ® (Tetra Pak, 2008). The three materials used in the multilayered brick are allocated into six individual layers, where each layer fulfils a desired function (Fig. 4 and Table 3).

Table 3 The Function of LDPE, Paper-Board and Aluminium Foil Layers in The Multilayered Tetra Brick®. Adapted from Tetra Pak (2008)

<table>
<thead>
<tr>
<th>Layer</th>
<th>Material</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LDPE</td>
<td>Interior moisture barrier, preventing the gain of moisture to the milk</td>
</tr>
<tr>
<td>2</td>
<td>LDPE</td>
<td>Binding surface for layer 1 and 3</td>
</tr>
<tr>
<td>3</td>
<td>Aluminium Foil</td>
<td>Prevents the penetration of UV light and O₂ into the milk (prevents oxidation⁴)</td>
</tr>
<tr>
<td>4</td>
<td>LDPE</td>
<td>Binding surface for layer 3 and 5</td>
</tr>
<tr>
<td>5</td>
<td>Paper-Board</td>
<td>Provides mechanical rigidity to the package</td>
</tr>
<tr>
<td>6</td>
<td>LDPE</td>
<td>Exterior moisture barrier, preventing the loss of moisture from the milk</td>
</tr>
</tbody>
</table>

LDPE = low density polyethylene. ⁴ Lipid and nutrient oxidation
Interactions between flavour compounds and UHT packaging material

Interactions between flavour compounds inherent in milk and the UHT packaging material itself have been studied (van Willige et al., 2000b, Simon et al., 2001 and Czerny and Schieberle, 2007). According to Hansen et al. (1974) after 16 days of storage, flavour sorption of volatiles to the UHT packaging material takes place in milk packaged inside polyethylene and cardboard boxes. Flavour volatiles in UHT milk, including 2-nonanal, may move into and through polyethylene packaging material during storage (Czerny and Schieberle, 2007). Furthermore the scalping of these compounds by LDPE is influenced by the volatiles molecular size as well as the polarity and solubility characteristics of the volatile and the LDPE itself (Nielsen et al., 1992). Arora et al. (1991) showed that the more hydrophobic flavour compounds with longer carbon chains have a greater affinity for non polar LDPE films while Linssen et al. (1991) stated that volatiles in drinking yoghurt with shorter carbon chains have a lower affinity to polyethylene bottles.

According to Arora et al. (1991) LDPE has an affinity for a range of hydrophobic compounds adsorbing aldehydes to a greater degree than methyl ketones in aqueous flavour solutions. LDPE also has an affinity for branched sulphur containing compound (Arora et al., 1991). Most studies on the interaction of flavours with packaging materials are completed in simple aqueous flavour solutions and not with actual food products with complex matrixes.

In model food systems the concentration of flavour compounds adsorbed by linear low density polyethylene (LLDPE) was influenced by the presence of oil or fat, polysaccharides and proteins where these constituents decreased the amount of flavour adsorbed by the packaging material (van Willige et al., 2000a and van Willige et al., 2000b).

No information could be found regarding the interaction of flavour compounds with the paper-board of the brick type aseptic packages. However, packaging material consisting of polyethylene (PE)/paper-board/PE/aluminium/PE retains sulphur containing hydrogen sulphide and methanethiol for longer than packages without the aluminium barrier because the barrier reduces the overall transfer of volatiles in the system (Simon et al., 2001).
EXTRACTION TECHNIQUES FOR THE ISOLATION OF VOLATILES FROM FOOD MATRICES

In general gas chromatography (GC) involves the headspace extraction and analysis of volatile compounds in the gaseous phase above a non-volatile liquid or solid phase (Snow and Slack, 2002 and Wampler, 2002). Due to the fact that flavour or aroma compounds are volatile in nature, techniques used to sample or extract the volatiles from the headspace above milk include static headspace extraction, dynamic headspace (purge-and-trap) extraction, headspace solid-phase microextraction (HS-SPME) and stir bar sorptive extraction (SBSE) (Christensen and Reineccius, 1992; Marsili, 1999; Naudé et al., 2009; Valero et al., 2001 and van Aardt et al., 2009). These extraction techniques are then usually coupled to analytical methods such as GC-MS and GC-flame ionisation detection (FID) to identify and quantify the extracted volatiles (Vazquez-Landaverde et al., 2005 and Naudé et al., 2009). Headspace sampling/extraction techniques allow for solvent free extraction with the extracted volatiles being more representative of the food flavour profile being analysed when compared to solvent extraction techniques (Naudé et al., 2009). This review will further cover the basic principles pertaining to static headspace extraction, dynamic headspace (purge-and-trap) extraction, HS-SPME and SBSE.

Static headspace extraction

Static headspace extraction involves the sampling of the headspace above a food matrix sample in a sealed vial under predetermined equilibrium time/temperature conditions to reach equilibrium (Snow and Slack, 2002 and Wampler, 2002). Once at equilibrium the volatiles in the headspace are sampled using a gas-tight syringe and injected directly into a GC-MS or incorporated into the GC-MS via a pressurised heated transfer line (Friedrich and Acree, 1998 and Snow and Slack, 2002). According to Wampler (2002) static headspace extraction can easily be set up for automatic sampling. Furthermore is has low analysis costs and uncomplicated sample preparation. However, a drawback of this technique is its poor sensitivity for less volatile
compounds, when compared to dynamic headspace (purge-and-trap) extraction or SPME, and is mainly used to sample volatiles at higher concentrations (Snow and Slack, 2002).

**Dynamic headspace (purge-and-tap) extraction**

This sampling technique involves the bubbling or purging of an inert carrier gas into an aqueous sample in a closed purging vessel (Snow and Slack, 2002). The gas carrying the purged volatiles passes through a trap made up of a sorbent material, with a large surface area, which adsorbs and concentrates the volatiles (Wampler, 2002 and Friedrich and Acree, 1998). The trapped volatiles are then thermally desorbed from the trap and analysed using GC-MS or GC-FID (Contarini et al., 1997; Contarini and Povolo, 2002 and Naudé et al., 2009). According to Imhof and Bosset (1994) artefact formation during dynamic headspace extraction is subsequently reduced due to the single extraction and concentration step at relatively low extraction temperatures. Furthermore the variety of different sorbent traps available, such as Tenax, Carbosieve, Carboxen and Ambersorb allow for efficient concentration and selective extraction of specific volatiles during headspace analysis (Wampler, 2002). However, due to the fact that these traps are so tightly packed with sorbent material some have been noted to cause problematic desorption because of high inlet pressure and gas flow shutdown (Naudé et al., 2009). Polydimethylsiloxane (PDMS) multi channel traps (MCT) traps have been used to sample the headspace of different food matrices including UHT milk (Sivakumar et al., 2008 and Naudé et al., 2009). It has been noted by Naudé et al. (2009) that gas flow and inlet pressure problems were not experienced during the thermal desorption of PDMS MCT’s due to the open tubular structure of the MCT. A major advantage of dynamic headspace extraction is the techniques sensitivity allowing analysis of volatiles at ppb concentration ranges (Snow and Slack, 2002 and Wampler, 2002). However, at high analyte concentrations volatile carry-over can occur between consecutive GC sequences leading to incorrect volatile quantification (Marsili, 1999). Furthermore analyte breakthrough and collection of moisture on the traps can lead to volatile losses and decreased retention time repeatability of early eluting compounds (Naudé et al., 2009).
**Headspace solid-phase microextraction (HS-SPME)**

HS-SPME involves the direct extraction and concentration of volatiles in the headspace above a sample onto a sorbent phase coated onto the outside of a fused silica fibre (Snow and Slack, 2002). The trapped analytes are then thermally desorbed from the fibre and analysed using a GC-MS or in the case of solvent desorption, injected into a high performance liquid chromatograph (HPLC) (Kataoka et al., 2000 and Povolo and Contarini, 2003). In-tube-SPME or solid-phase dynamic extraction (SPDE) involves the coating of a sorbent phase onto the inside surface of a fused silica fibre or the inside walls of the needle of a gas tight syringe (Zhang et al., 1994 and Lord and Pawliszyn, 2000). This coating arrangement allows for larger extraction volumes (up to six times) when compared with a 100 µl SPME fibre (Musshoff et al., 2002). According to Jochmann et al. (2006) SPDE has comparable or improved sensitivity for a number of volatile compounds, from water samples, when compared with other enrichment techniques including SPME. SPME techniques can be executed manually or by using an autosampler (Zhang et al., 1994).

A number of articles have been published comparing dynamic headspace (purge-and-trap) techniques to HS-SPME to analyse the flavour profile of different food stuffs (Contarini and Povolo, 2002; Elmore et al., 1997; Marsili, 1999 and Povolo and Contarini, 2003).

In the volatile analysis of two different cola drinks Elmore et al. (1997) indicated that there was no difference in the reproducibility between the two techniques and that for trace analysis dynamic headspace extraction is most suitable where for analysis of major volatile components HS-SPME is a better option. Similarly, comparable reproducibility for the two different methods was reported by Contarini and Povolo (2002) and Povolo and Contarini (2003) for the volatile analysis of milk and butter respectively. Furthermore greater amounts of volatiles were extracted by dynamic headspace extraction however; HS-SPME had superior extraction efficiency for higher molecular weight volatiles (Povolo and Contarini, 2003). In a study for the analysis of light induced lipid oxidation products in milk Marsili (1999) indicated that HS-SPME, when compared to dynamic headspace (purge-and-trap) techniques, is less expensive; did not lead to carryover or artefact formation and showed superior precision and accuracy. Furthermore the
author indicated that the sensitivity of HS-SPME is equal to that of dynamic headspace techniques. In general HS-SPME is a rapid, sensitive, uncomplicated, inexpensive and versatile technique (Kataoka et al., 2000; Roberts et al., 2000, Snow and Slack, 2002 and Vazquez-Landaverde et al., 2005). However limitations such as competition of volatiles for the sorbent phase of the fibre which may lead to erroneous results (Grote and Pawliszyn, 1997 and Roberts et al., 2000). It has been noted that the risk of artefact formation can increase at higher extraction temperatures (Perkins et al., 2005). Furthermore the sorbent phase coated onto the SPME fibre has a relatively small sorbent volume of approximately 0.9 mm$^3$ (Schulz et al., 2007) when compared to a volume of approximately 635 mm$^3$ of the PDMS MCT, used in dynamic headspace analysis, providing a much larger sample enrichment (Naudé et al., 2009).

**Stir Bar Sorptive Extraction (SBSE)**

SBSE, a SPME variant and relatively new extraction technique, involves the coating of glass covered magnetic stir bars with a thick layer of PDMS (Baltussen et al., 1999 and Snow and Slack, 2002). By introducing the phase coated stir bar directly into an aqueous sample, extraction can take place during stirring (Baltussen et al., 1999). After efficient stirring the stir bar can be inserted into a glass desorption tube and thermally desorbed into a GC inlet for analysis (Snow and Slack, 2002). Blasco et al. (2004) reported that SBSE has a greater volatile concentration capacity, accuracy and sensitivity for several different pesticides in honey when compared to SPME at similar experimental conditions. Similarly van Aardt et al. (2009) indicated that SBSE proved to be more sensitive than SPME for the quantitative analysis of volatile compounds in UHT milk. These advantages are achieved because the coated stir bars have a larger sorbent volume of 25-200 µl PDMS (Bicchi et al., 2004) compared to the relatively lower sorbent volume of 0.6-0.9 µl associated with the SPME fibre coating (Bicchi et al., 2000; Bicchi et al., 2004 and Schulz et al., 2007). However, SBSE cannot be fully automated which is a clear disadvantage of the procedure (Popp et al., 2003).
CONCLUSION

UHT processing leads to the formation of a variety of volatile compounds derived from the milks chemical constituents. Some of these volatiles have been identified to contribute to specific undesirable flavour notes in UHT milk. The “cooked” flavour of UHT milk is associated with the presence of a variety of sulphur containing compounds while the “stale” flavour is characterised by the dissipation of these sulphur volatiles and an increase of the formation and presence of both methyl ketones and aliphatic aldehydes over time. However, the extent that the individual volatiles contribute to the overall flavour of UHT milk is still unclear. A number of different methods, including the use of drop in additives as well as active packaging, have been identified as potential means to remove or decrease the occurrence of these undesirable flavours associated with UHT milk and ultimately improve the consumer acceptability of the product. Continuous improvements in gas chromatography and the development of new analytical techniques, may offer a better understanding as to how the volatile components of UHT milk interact with the milks constituents, packaging material and storage environment. Furthermore, this may improve the understanding of which odorants contribute most to the undesirable flavour of UHT milk, allowing for further research to focus on selective reduction of the these flavour volatiles from the milk.

REFERENCES


