Volatile sulfur compounds in pasteurised and UHT milk during storage

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ORIGINAL PAPER

Volatile sulfur compounds in pasteurised and UHT milk during storage

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Abstract Cooked or sulfurous off-flavour caused by volatile sulfur compounds (VSCs) limits acceptance of ultra-high temperature (UHT) milk in some parts of the world. Therefore, the concentrations of VSCs in UHT milk over 16 weeks of storage were studied and compared with those in pasteurised milk. The major VSCs contributing to the cooked flavour were identified using solid-phase microextraction and gas chromatography with pulsed flame photometric detection. Nine VSCs were detected in commercial indirectly processed UHT skim and whole milk. These were hydrogen sulfide, carbonyl sulfide, methanethiol, dimethyl sulfide, carbon disulfide, dimethyl disulfide, dimethyl sulfoxide, dimethyl sulfone and dimethyl trisulfide. An additional VSC was detected but not identified. The concentrations of hydrogen sulfide, methanthiol, dimethyl sulfide and dimethyl trisulfide were initially higher than their reported threshold values indicating their importance in milk flavour, especially cooked flavour. However, they decreased slowly during storage to levels below their threshold values. This decrease corresponded to a decrease in dissolved oxygen level. Four VSCs, carbon disulfide, dimethyl sulfide, dimethyl sulfoxide and dimethyl disulfide, were detected in pasteurised milk; however, their concentrations were lower than their reported threshold values. This paper puts into perspective the significance of VSCs in the flavour of UHT and pasteurised milk, both initially and during storage, and indicates the period of storage for minimisation of cooked flavour in UHT milk.

Keywords Milk · Solid-phase microextraction · Pulsed flame photometric detector · Volatile sulfur compounds

1 Introduction

Heating milk at ultra-high temperature (UHT) is necessary to produce a stable, safe and long shelf life product. However, this causes changes in flavour which is a major

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consumer concern for UHT milk (Colahan-Sederstrom and Peterson 2005; Perkins and Deeth 2001). A common flavour defect in UHT milk is cooked flavour which is a strong barrier to UHT milk acceptance (Lewis and Heppell 2001). The flavour is intense directly after processing and gradually disappears during storage. This off-flavour is caused by volatile sulfur compounds (VSCs) formed during denaturation of whey proteins, especially β-lactoglobulin. Maillard reactions and fat globule membrane proteins are other precursors of VSCs. The presence of VSCs and their possible sources in UHT milk was critically reviewed by Al-Attabi et al. (2009) and Zabbia et al. (2012).

VSCs including hydrogen sulfide (H_2S), carbonyl sulfide (COS), methanethiol (MeSH), dimethyl sulfide (DMS), carbon disulfide (CS_2), dimethyl disulfide (DMDS), dimethyl sulfoxide (DMSO), dimethyl sulfone (Me_2SO_2) and dimethyl trisulfide (DMTS) have been detected in several types of milk, including raw milk, pasteurised milk, UHT milk and sterilised milk. Differences in sample preparation and gas chromatography (GC) detectors, which have different sensitivities to sulfur compounds, have led to a lack of agreement on which VSCs contribute to the cooked/sulfurous flavour of UHT milk. Also, differences in the concentrations of these volatiles in different milk samples could be attributed to the different heat treatments and different origins of the milk samples.

Working with VSCs is a challenge; they are highly volatile and reactive, easily oxidised and sensitive to heat, which make them difficult to quantify accurately. Therefore, reliable quantitative data of the majority of sulfur compounds in UHT milk such as MeSH, DMSO, Me_2SO_2, CS_2 and COS are scant. Recently, VSCs were quantified in different commercial milk samples with different fat percentages (Vazquez-Landaverde et al. 2006) and their kinetics of formation in skim milk were elucidated (De Wit and Nieuwenhuijse 2008). The latter authors described the reactions as complex and dependent on the sample matrix (e.g. fat content).

Solid-phase microextraction (SPME), carboxen (CAR)/polydimethylsiloxane fibre (PDMS) shows high sensitivity to VSCs when combined with gas chromatography and pulsed flame photometric detection (PFPD) (Vazquez-Landaverde et al. 2006). The CAR/PDMS fibre is the most widely used fibre for sulfur extraction (Mestres et al. 2000; Burbank and Qian 2005; Vazquez-Landaverde et al. 2006). In the work described in this paper, the concentrations of the different VSCs in raw, pasteurised and UHT-processed milk (skim and whole) were determined using SPME/GC/PFPD.

The major objective of the work was to assess the contributions of the various VSCs to the cooked flavour of UHT milk. This was achieved through monitoring the concentrations of the VSCs during storage of the processed milk and relating them to their reported flavour thresholds.

2 Materials and methods

2.1 Chemical standards

Dimethyl disulfide ($(CH_3)_2S_2$), dimethyl sulfide ($(CH_3)_2S$), dimethyl trisulfide ($(CH_3)_2S_3$), CS_2, dimethyl sulfoxide (CH_3SOCH_3), dimethyl sulfone ($CH_3SO_2CH_3$), ethyl methyl sulfide ($CH_3CH_2SCH_3$) and isopropyl disulfide ($C_6H_{14}S_2$) were purchased from Sigma-Aldrich (Sydney, NSW, Australia). Methanethiol (CH_3SH)

(1 mL, 1,000 mg.L^{-1} in methanol) and COS (1 mL, 2,000 mg.L^{-1} in toluene) were purchased from Novachem Pty Ltd (Collingwood, VIC, Australia). H_2S was generated from sodium sulfide ($Na_2S{\cdot}9H_2O$) (Ajax Finechem, Taren Point, NSW, Australia) according to the method of Vazquez-Landaverde et al. (2006) by dissolving it in 50 mmol.L^{-1} phosphate buffer (pH 8.5).

2.2 Commercial milk samples

Three batches (1 L packages) each of full-cream pasteurised (less than 4% fat), UHT skim and UHT whole milk samples were collected from a local dairy processor directly after processing. A total of 99 samples were collected to enable samples to be withdrawn for analysis during storage. Raw milk samples were collected with each batch and immediately analysed for sulfur volatiles. The UHT milk was indirectly processed using a tubular UHT plant and aseptically packaged into Tetra Brick paperboard cartons with no or very little headspace, while the pasteurised milk was packaged in high-density polyethylene bottles. The pasteurised milk was stored at 5 °C for 14 days, while the UHT milk samples were stored at 22–23 °C for up to 16 weeks (113 days). One package of each batch was opened for duplicate VSC analysis. The pasteurised milk samples were analysed on days 1, 3, 6, 9, 12 and 14, while the UHT milk samples were analysed on days 1, 3, 6, 9, 12 and 15 and then weekly up to 113 days. This experiment was run using a split plot design.

2.3 Calibration procedure

Calibration curves of VSCs were constructed using a spike addition method. The standards were prepared in raw whole milk. Immediately after receipt of the milk, sodium azide (0.5 g.L^{-1}) was added and the milk was stored at −18 °C till used (within 3 days). Stock solutions of most of the sulfur compounds were prepared in methanol. The stock solution of Me_2SO_2 was prepared in distilled water (Vazquez-Landaverde et al. 2006). The raw whole milk sample was spiked with the solutions of the standard sulfur compounds to give the required concentrations. All analyses were performed in triplicate and coefficients of determination (R^2) were calculated.

2.4 Calibration curves

The calibration curve equations of the VSCs are given in Table 1. The standard curves for H_2S and MeSH were difficult to construct due to the high volatility and susceptibility to oxidation of these compounds. In addition, H_2S is highly reactive and binds in significant amounts to milk components (Thomas et al. 1976). The concentration of H_2S and MeSH can be measured from the DMS calibration curve when sulfur-selective PFPD is used as this detector has an equimolar response (Amirav et al. 2008). This means that compounds with the same number of sulfur atoms per molecule will produce signals of equal size. Consequently, when the PFPD is used in the linear mode, a calibration based on one sulfur-containing compound can be used for all compounds with an equal number of sulfur atoms. The analysis of H_2S and MeSH assumed that their partitioning between the liquid and gas phases is the same as for DMS.

Table 1 The calibration curve concentrations, calibration curve equations and correlation coefficients for standards of volatile sulfur compounds

Compounds	Calibration concentrations	Calibration curve equation	R^2
COS ($mg.L^{-1}$)	0.2, 0.5, 1, 2.5, 5, 8	$y=10.285x$	0.98
DMS ($\mu g.L^{-1}$)	4, 6, 10, 15, 34, 50	$y=1.1795x$	0.98
	94, 200, 400, 650, 800	$y=0.0832x+50.247$	0.96
CS_2 ($\mu g.L^{-1}$)	0.2, 2, 4, 6, 8, 12	$y=1.5149x$	0.996
DMDS ($\mu g.L^{-1}$)	0.09, 0.8, 1.5, 2, 2.8, 3.6	$y=4.6716x$	0.97
DMSO ($mg.L^{-1}$)	0.8, 5, 50, 100, 200, 350, 450	$y=0.0401x$	0.98
Me_2SO_2 ($mg.L^{-1}$)	3, 5, 6.5, 8, 8.5, 10.5	$y=0.0602x+0.2345$	0.98
DMTS ($\mu g.L^{-1}$)	1, 2, 3, 6, 9, 12	$y=0.3651x$	0.99

Gaafar (1987) calculated the H_2S concentration from a DMS calibration curve using a flame photometric detector (FPD). He assumed that the response of the FPD was the same for all compounds with one sulfur compound. A correction equation was used for the molecular weight (MW) for both DMS and H_2S. In the present work, the H_2S and MeSH concentrations were calculated in a similar manner based on the DMS calibration curve using the following equation:

$$Y = 1.1795x$$

where Y is the square root area of H_2S or MeSH in UHT milk and x is the H_2S or MeSH concentration in micrograms per litre.

Then, the x value is substituted in the following correction equation to calculate the H_2S/MeSH concentrations.

$$\mathrm{H_2S/MeSH}\left(\mu g.L^{-1}\right) = \frac{x \times \text{density of DMS} \times \text{MW of DMS}}{\text{MW of } H_2S/\text{MW of MeSH}}$$

where density of DMS=0.8483, MW of DMS=62.13, MW of H_2S=34.08 and MW of MeSH=48.11.

2.5 SPME/gas chromatography analysis

Five millilitres of milk was placed in a 10-mL screw-top vial, fitted with a PTFE-faced silicone septum. The extraction was performed using a CAR-PDMS, 85 μm (Supelco, Australia) SPME fibre at 30 °C for 15 min (Vazquez-Landaverde et al. 2006).

The analyses were performed on a Varian CP-3800 gas chromatograph equipped with a pulsed flame photometric detector (GC/PFPD). The analytes were separated on a CP-SIL 5 CB column (fused silica, 30 m×0.32 mm id, 4 μm film thickness; Varian, Brisbane, QLD, Australia). The SPME fibre was thermally desorbed in the GC-PFPD injector at 250 °C for 7 min operating in the splitless mode. The injector was fitted with a narrow bore (0.75 mm ID) inlet liner (Supelco, Australia). The oven temperature was programmed as follows: 35 °C for 2 min then increased at 15 °C/min to 150 °C and held for 1 min, increased at 20 °C/min to 250 °C and then held for 2 min. The total run time was 16.2 min. The detector parameters were temperature, 300 °C; photomultiplier

tube voltage, 550 V; trigger level, 200 mV; sampling delay, 6 ms; sampling width, 20 ms; and gain factor, 2.

2.6 Dissolved oxygen analysis

Dissolved oxygen was measured in parts per million (milligrams per litre) immediately after the package was opened using a bench-top dissolved oxygen meter, Smart CHEM-Lab, fitted with an O_2 probe with ED1 sensor (TPS, Australia). Probe calibration was performed before each measurement according to the manufacturer's manual.

3 Results

3.1 VSCs in commercial milk

Four VSCs, CS_2, DMS, DMSO and DMDS, were detected in pasteurised milk; all except Me_2SO_2 are also present in raw milk. Nine VSCs that were previously reported in UHT milk, namely H_2S, COS, MeSH, DMS, CS_2, DMDS, DMSO, Me_2SO_2 and DMTS, plus the unknown compound with a retention time of around 7.8 min (Fig. 1) were detected.

Comparative chromatograms between the whole UHT milk on day 1 and week 12 (85 days) are shown in Fig. 1. The VSCs in pasteurised milk and whole and skim UHT milk are summarised in Figs. 2 and 3. The reported flavour threshold values of VSCs in milk and water are summarised in Table 2.

There was an overall decrease in the concentrations of VSCs during storage of the pasteurised and UHT milk samples. H_2S was not detected in the raw and pasteurised samples analysed in this work. However, a very high concentration was detected in UHT whole (50.7 $\mu g.L^{-1}$) and skim milk (12.3 $\mu g.L^{-1}$). A marked reduction in H_2S concentration was observed in whole milk from day 1 to day 3, and then, a gradual reduction occurred until week 3 (day 22) when it could not be detected. Its concentration rapidly decreased to below the threshold value of 10 $\mu g.L^{-1}$ by day 6 (7.4 $\mu g.L^{-1}$) and day 3 (5.13 $\mu g.L^{-1}$) for whole and skim milk, respectively. It became undetectable after 12 days of storage in UHT skim milk. On the other hand, COS disappeared quickly from UHT skim milk after 6 days of storage and after 15 days from UHT whole milk.

MeSH was detected in UHT milk, but not in raw and pasteurised milk. However, its initial concentration in UHT whole milk (9.8 $\mu g.L^{-1}$) was more than double that in skim milk (4 $\mu g.L^{-1}$). Up to day 6, the concentration was higher than its flavour threshold value in water of 0.02–2.1 $\mu g.L^{-1}$ (Table 2), indicating its possible contribution to cooked flavour at that time. Moreover, its concentration was still above the threshold value in UHT whole and skim milk until after 3 weeks (22 days) and 4 weeks (29 days) of storage, respectively, when it was undetectable.

DMS concentrations in raw and pasteurised milk were 13.4 and 12.9 $\mu g.L^{-1}$ respectively. A very high concentration of DMS (327 $\mu g.L^{-1}$) was generated in UHT whole milk, which was 4.6 times higher than in UHT skim milk (70.8 $\mu g.L^{-1}$). A slight increase in the DMS concentration in skim milk on day 3 was observed before it gradually decreased. The decrease in the concentration of DMS in UHT whole milk

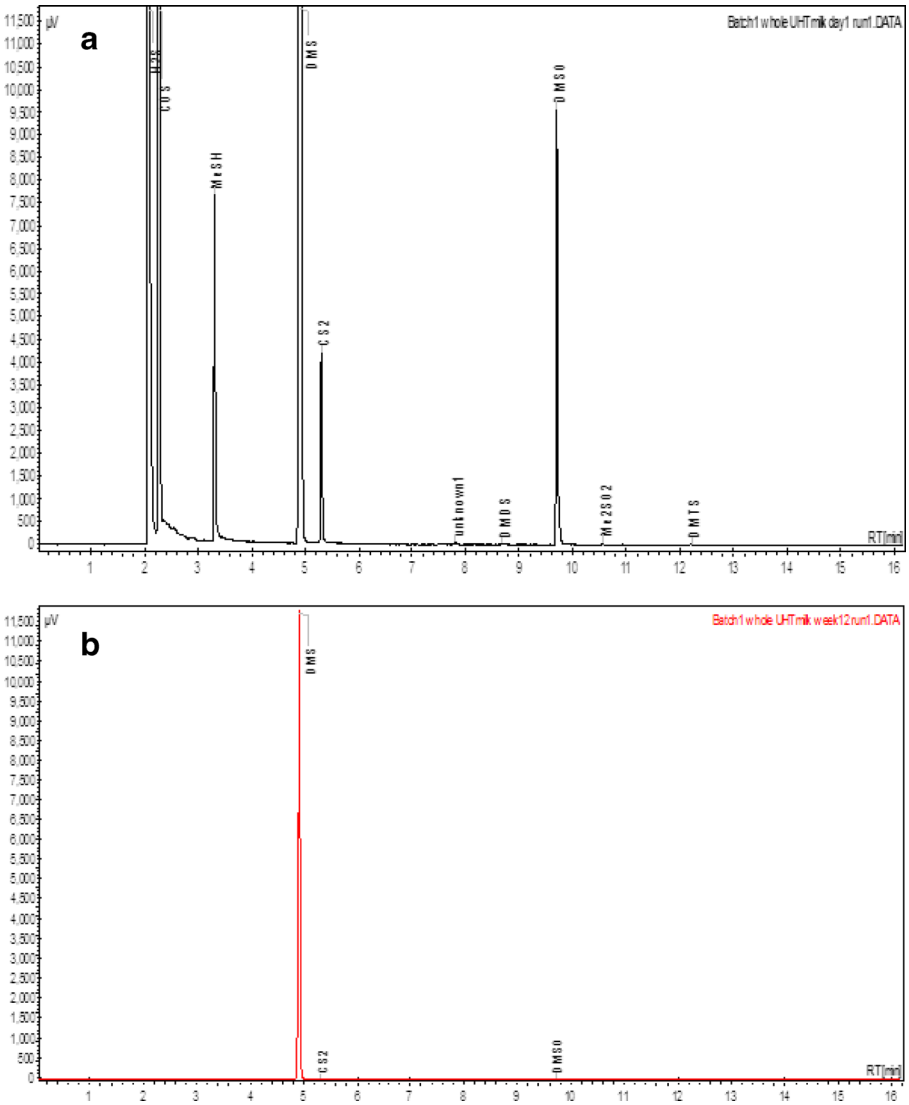

Fig. 1 Chromatograms of volatile sulfur compounds SPME extracted from whole UHT milk at **a** day 1 and **b** week 12 (85 days)

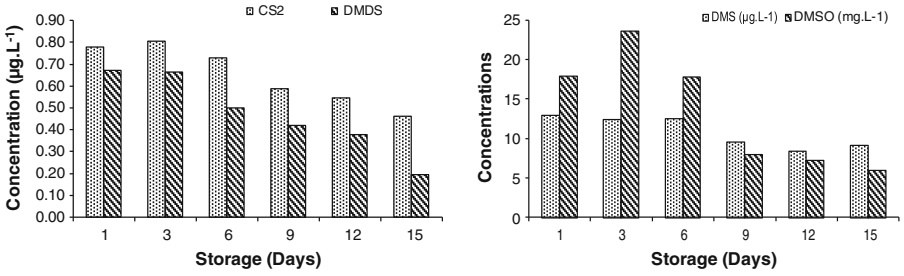

Fig. 2 Concentration of VSCs in commercial whole pasteurised milk during storage at 5 °C for 14 days

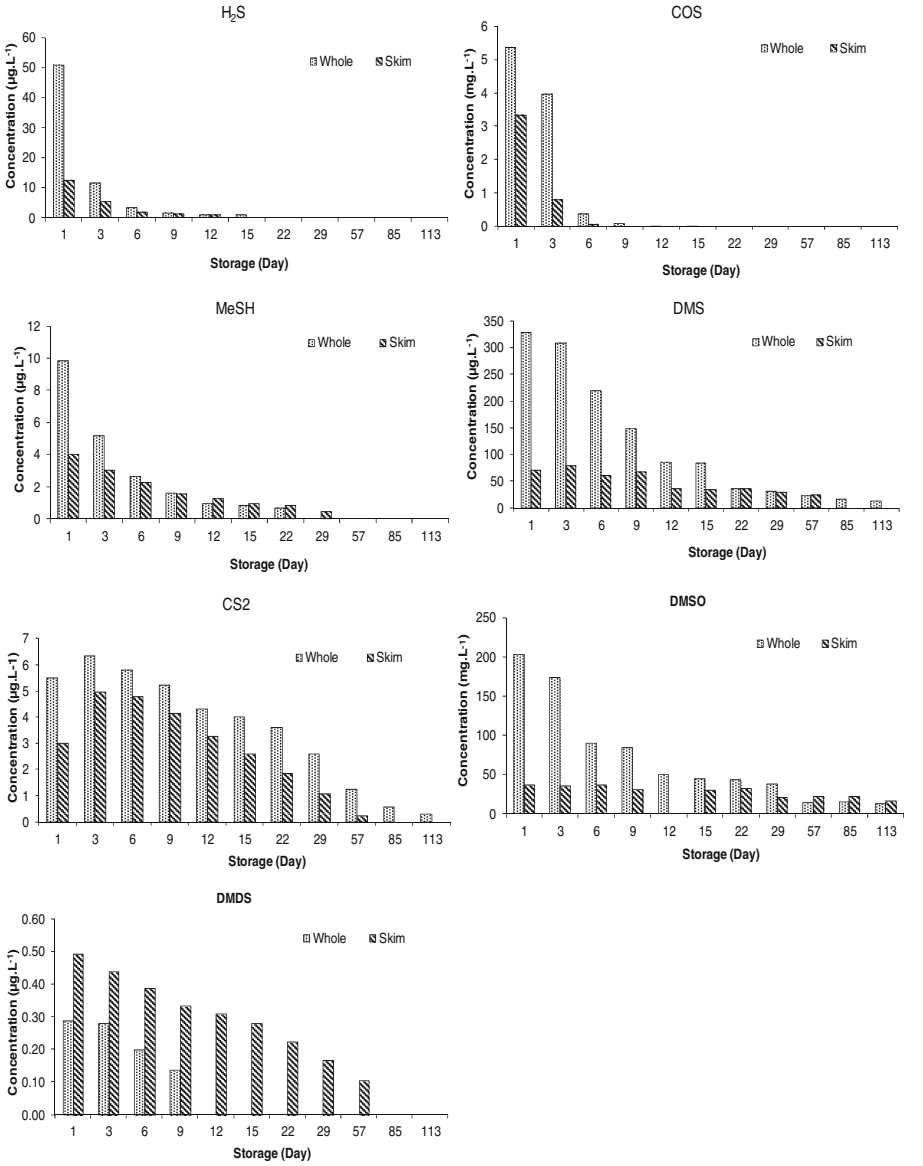

Fig. 3 Concentrations of VSCs in whole and skim UHT milk during storage at 22–23 °C (Note: *Y*-axis for COS and DMSO is in milligrams per litre, all others are in micrograms per litre)

was very rapid during storage until week 3 (22 days), whereafter the concentration in both whole and skim gradually decreased at the same rate. However, its concentration remained higher than its flavour threshold value in milk (20 $\mu g.L^{-1}$) until week 8 (57 days) in whole milk (21.9 $\mu g.L^{-1}$) and skim milk (23.2 $\mu g.L^{-1}$). The concentrations for week 12 (85 days) and week 16 (113 days) for skim milk were not recorded due to technical problems encountered with the GC/PFPD.

CS_2 remained at approximately the same concentration in pasteurised milk as in raw milk up to the third day of storage (0.81 $\mu g.L^{-1}$ compared with 0.74 $\mu g.L^{-1}$) and then

Table 2 Reported flavour threshold values of volatile sulfur compounds

Compounds	Threshold (t) ($\mu g.L^{-1}$)	Threshold in	Reference
H_2S	10	Water	Jaddou et al. (1978), Rychlik et al. (1998)
MeSH	0.02–2.1; 0.2	Water	Jaddou et al. (1978), Rychlik et al. (1998)
CS_2	1,000>t>100	Milk	Jaddou et al. (1978)
DMS	20	Milk	Jaddou et al. (1978)
DMDS	21; 19	Milk	Jaddou et al. (1978), Rychlik et al. (1998)
DMTS	0.008	Water	Rychlik et al. (1998)

gradually decreased over the remainder of the storage period. Its concentration was higher in UHT milk than in pasteurised milk. However, its concentration increased in both skim (to 2.98 $\mu g.L^{-1}$) and whole milk (to 5.5 $\mu g.L^{-1}$) until day 3 and then gradually decreased.

DMDS was detected in raw and pasteurised milk at a concentration of 0.73 and 0.67 $\mu g.L^{-1}$, respectively. The DMDS concentration in UHT whole milk (0.29 $\mu g.L^{-1}$) was less than in the UHT skim milk (0.49 $\mu g.L^{-1}$) immediately after processing. However, it disappeared faster in whole UHT milk (after 9 days) than in skim UHT milk (after 57 days).

In pasteurised milk, DMSO increased from 26.8 $mg.L^{-1}$ on day 1 to 35.4 $mg.L^{-1}$ on day 3 and then gradually decreased. A very high concentration (203 $mg.L^{-1}$) was detected directly after processing in UHT whole milk compared with 36.3 $mg.L^{-1}$ in skim milk.

Me_2SO_2 was detected in raw milk (5.9 $mg.L^{-1}$) and whole UHT milk (5.2 $mg.L^{-1}$), but was not detected in the pasteurised and skim UHT milk. On the other hand, DMTS (1.9 $\mu g.L^{-1}$) was only detected in UHT whole milk. By day 6, both Me_2SO_2 and DMTS had disappeared.

3.2 Dissolved oxygen in UHT milk

Dissolved oxygen in commercial UHT milk (Fig. 4) was measured during 16 weeks (113 days) of storage at 22–23 °C. The collected samples were processed indirectly and packaged in Tetra Brik® cartons with no headspace.

The dissolved oxygen concentration in the commercial UHT milk, directly after processing, was 2.37 $mg.L^{-1}$. The initial drop in the dissolved oxygen level during 9 days of storage is largely attributable to the oxidation of sulfur volatiles. Thereafter, the concentration gradually decreased with storage time until it reached 0.28 $mg.L^{-1}$ on week 16 (113 days).

4 Discussion

4.1 VSCs in commercial milk

Several VSCs were previously detected in pasteurised milk including H_2S, MeSH, CS_2, DMS, DMDS, DMTS, DMSO and Me_2SO_2. However, most of them are at sub-flavour threshold concentrations, so they do not contribute to the flavour of pasteurised milk

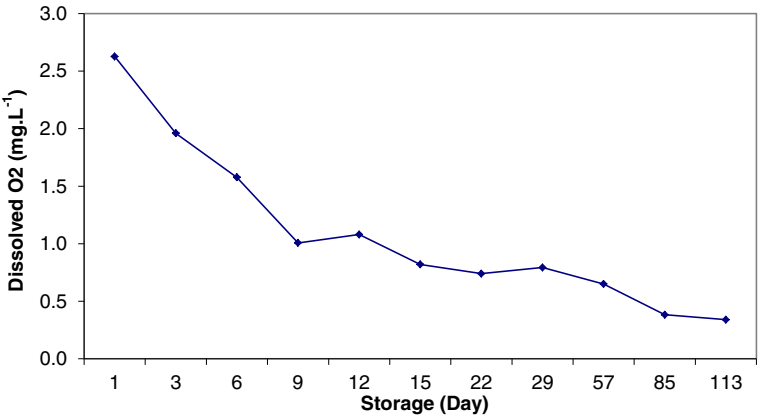

Fig. 4 Dissolved oxygen concentrations in commercial UHT milk during storage at 22–23 °C for 16 weeks

(Al-Attabi et al. 2009). In the current research, the concentrations of the four sulfur compounds detected in pasteurised milk were lower than their threshold values.

The higher heat severity of UHT processing compared to pasteurisation causes a marked difference in the concentration of VSCs between UHT and pasteurised milk. A major reason for this is the much greater extent of β-lactoglobulin denaturation in UHT milk. Other milk components such as thiamine may also be sources of VSCs under UHT conditions (Dwivedi and Arnold 1973). Thus, the concentrations of DMS and CS_2 were much higher in UHT milk than in pasteurised milk. Three other VSCs, H_2S, COS and MeSH, which were not present in pasteurised milk were detected in UHT milk. Furthermore, the concentration of VSCs in whole UHT milk was higher than in skim milk with two extra compounds being detected, namely Me_2SO_2 and DMTS. This difference is attributed to the significant role of milk fat globule membrane protein in VSC development (Gaafar 1987). Generally, as the fat percentage increases, the concentration of VSCs increases (Gaafar 1987; Vazquez-Landaverde et al. 2006).

H_2S was not detected in the raw and pasteurised samples analysed in this work. Until recently, it had not been detected in raw milk as it was only known to be a heat-generated compound. However, Vazquez-Landaverde et al. (2006) detected H_2S in raw whole milk and pasteurised milk at concentrations lower than its threshold value. In this study, the initial H_2S concentrations in both whole and skim UHT milk were higher than the reported threshold value in water (10 $\mu g.L^{-1}$) indicating its contribution to cooked flavour. Similar results were found for UHT whole milk by Badings and de Jong (1984) and Badings et al. (1981), 50 $\mu g.kg^{-1}$ and 47.2 $\mu g.L^{-1}$, respectively. Higher concentrations (88 $\mu g.L^{-1}$) of H_2S were found by Gaafar (1987), while even higher concentrations of >250 $\mu g.L^{-1}$ were reported by Dumont and Adda (1978). A lower concentration (~20 $\mu g.L^{-1}$) was detected by Jaddou et al. (1978). Recently, Vazquez-Landaverde et al. (2006) reported H_2S in concentrations of 12 $\mu g.kg^{-1}$ in 3.25% fat UHT milk.

There are two possible mechanisms of H_2S formation: (a) Strecker degradation of cysteine in the presence of a diketone and (b) thermal degradation of thiamine (Al-Attabi et al. 2009). De Wit and Nieuwenhuijse (2008) suggested that H_2S, together with DMS, is produced from MeSH oxidation. Even though H_2S has been reported to react with other milk components (Badings et al. 1981) or be oxidised to other products

(Christensen and Reineccius 1992), the mechanism of its disappearance from milk has not been elucidated. One possibility is that it reacts with carbon dioxide to form COS, which is further converted to other compounds yet to be identified (De Wit and Nieuwenhuijse 2008). COS is odourless; therefore, less attention has been given to this VSC. However, it may play a role in flavour through its conversion to other volatiles.

The initial concentrations of MeSH in UHT whole milk and skim milk are within the concentrations previously reported. These concentrations are higher than its reported threshold value of 0.02–2.1 $\mu g.L^{-1}$ (Table 2). A similar concentration of MeSH in UHT skim milk has been detected in raw milk with 3.25% fat (4.8 $\mu g.kg^{-1}$) and in pasteurised milk with 0% fat (5.97 $\mu g.kg^{-1}$) (Vazquez-Landaverde et al. 2006). The same authors detected a higher concentration in 3.25% fat UHT milk (23.9 $\mu g.kg^{-1}$) than in 1% fat UHT milk (16.1 $\mu g.kg^{-1}$). They indicated the importance of the MeSH to the flavour of UHT milk at concentrations 80 to 119 times higher than its threshold value (0.2 $\mu g.kg^{-1}$). It has also been detected in sterilised milk at a concentration of 5 $\mu g.kg^{-1}$ (Badings and de Jong 1984). De Wit and Nieuwenhuijse (2008) correlated cooked flavour with MeSH production. The high volatility and reactivity of this VSC have limited its quantification in milk (Vazquez-Landaverde et al. 2006), and hence, the data for it in the literature may not be reliable. MeSH is formed by the Strecker degradation of methionine and also from riboflavin.

The concentrations of DMS in raw and pasteurised milk are less than the threshold value in milk (20 $\mu g.L^{-1}$). However, their concentrations are markedly higher in UHT milk especially whole milk indicating that this compound increases upon heating and contributes to sulfurous flavour at the concentrations detected in this work.

The increases in the DMS concentration in UHT skim milk on day 3 could be a result of continuing decomposition of the DMS precursor, *S*-methyl methionine sulfonium salt. Slinkard (1976) found an increase in DMS in the first 15 days of storage. This was related to slow decomposition of the DMS precursor. The previous highest concentrations of DMS were reported by Bosset et al. (1996) in UHT milk (70–140 $\mu g.kg^{-1}$) and retort sterilised milk (180 $\mu g.kg^{-1}$). The current concentration and the above reported high concentrations of DMS strongly indicate its possible contribution to UHT milk flavour. Other authors have supported this conclusion (Steely 1994; Vazquez-Landaverde et al. 2005). Its formation is a result of Strecker degradation of methionine. In skim milk, DMS has been suggested to be oxidised to an unknown compound, since the known oxidation products, DMSO and Me_2SO_2, could not be detected (De Wit and Nieuwenhuijse 2008).

The concentrations of CS_2 in raw, pasteurised and UHT milk found in this work are less than its threshold value (t) in milk of $1{,}000 > t > 100$ $\mu g.L^{-1}$, indicating an insignificant contribution to the flavour of milk. The reason for increases in the concentration up to the third day is not clear. However, it is clear that the concentrations increased upon heating, with a higher concentration in whole milk than in skim milk. Therefore, Vazquez-Landaverde et al. (2006) suggested that CS_2 could be a good indicator of heat treatment. These authors detected CS_2 at parts per trillion concentrations in UHT milk. This indicates the high sensitivity of the pulsed flame photometric detector along with the use of SPME. The origin of this compound in milk is not clear.

The current concentrations of DMDS in UHT milk are less than those previously detected in indirectly processed UHT milk, which were in the range of 2–5 $\mu g.kg^{-1}$ (Bosset et al. 1996), although recently it was detected at 30.3–32.8 $ng.kg^{-1}$ in UHT milk (Vazquez-Landaverde et al. 2006). The DMDS concentrations were the lowest among the VSCs detected. There was a decrease in the DMDS concentration in whole UHT milk, during the first few days of storage, resulting in a low concentration (15 $\mu g.L^{-1}$); however, the concentration in skim milk was higher at manufacture and took a much longer time to decrease to this level (Fig. 3). The contribution of DMDS to milk flavour is insignificant, as its detected concentrations were considerably less than its reported threshold value in milk (Table 2). Trace amounts of DMDS are formed as a result of the Strecker degradation of methionine.

The concentrations of DMSO in raw, pasteurised and UHT milk are higher than previously reported (Al-Attabi et al. 2009). It could still be detected at week 16 (113 days). The reason for the increase in DMSO on day 3 in pasteurised milk is unclear. Me_2SO_2 is reported to form as a result of DMS oxidation, where DMSO is formed as an intermediate compound (Shibamoto and Mihara 1980). However, Me_2SO_2 was not detected in the current analyses of raw and pasteurised milk. The contribution of DMSO to cooked flavour is unknown as its threshold value has not been reported in either milk or water. The presence of Me_2SO_2 in raw milk is influenced by feed composition (Toso et al. 2002). Since Me_2SO_2 is described as flavourless, it is unlikely to make a contribution to the flavour of milk.

DMTS, which was detected at a concentration higher than its threshold value in water (0.008 $\mu g.L^{-1}$) (Table 2), was previously described as an odour impact compound in milk (Bendall 2001) and has recently been indicated as a major contributor to milk flavour (Vazquez-Landaverde et al. 2006), especially the sulfurous flavour of UHT milk. DMTS is generated from the Strecker degradation of methionine, where MeSH is produced and further oxidised to DMDS and DMTS (Bendall 2001).

4.2 Dissolved oxygen in UHT milk

UHT milk with low oxygen concentration and little or no headspace can have a poor flavour compared with milk with a moderate headspace and hence some dissolved oxygen (Zadow and Birtwistle 1973). There was no difference in the oxidation of free –SH groups in indirectly processed UHT milk samples with and without headspace, when the initial concentration of dissolved oxygen was higher than 5 $mg.L^{-1}$. Therefore, the disappearance rate of the cooked flavour is the same for both samples (Fink and Kessler 1986b). However, at low dissolved oxygen, as in directly processed UHT milk (0.35 $mg.L^{-1}$), the headspace was shown to be important in the rate of cooked flavour disappearance during storage (Fink and Kessler 1986a). The reported concentrations of oxygen in indirectly UHT milk are between 4 and 9 $mg.L^{-1}$ (Al-Attabi et al. 2009). In this study, the dissolved oxygen concentration in the UHT milk was lower than that. The low initial concentration and little or no headspace could result in less oxidation of VSCs, especially those related to cooked flavour, and hence delay their disappearance. This is true for the current commercial UHT milk where the concentrations of VSCs were high and took a long time to decrease to levels below their threshold values; DMS is a good example of that. Sufficient oxygen is required in

UHT milk to oxidise VSCs to a level below their threshold value, but which retains their antioxidant properties to prevent the appearance of stale or oxidised flavour.

5 Conclusion

Extraction using SPME coupled with GC analysis using PFPD enabled the quantification of a range of volatile sulfur compounds in raw, pasteurised and UHT milk. The concentrations of the VSCs in UHT milk during storage showed a rapid decrease, particularly in H_2S and MeSH, during the days immediately after manufacture, which corresponded to a significant decrease in dissolved oxygen. Since this also corresponds to the disappearance of the initial strong sulfurous flavour, it is suggested that oxidation of the VSCs by the dissolved oxygen is responsible for the flavour change. Of the VSCs detected in UHT milk, only H_2S, MeSH, DMS and DMTS were present initially in concentrations higher than their reported threshold values, indicating that they contribute to milk flavour. However, during storage, their concentrations were reduced to lower than or close to the reported threshold values by days 6, 29, 57 and 6, respectively.

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