# Volatile reduced sulphur compounds in butter by solid phase microextraction

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Summary. The reduced sulphur volatiles, methanethiol and dimethyldisulphide (but not dimethylsulphide), have been detected in the headspace of butter samples. Their concentrations in the butter itself were calculated by determination of the distribution coefficient ($K$) through spiking butter samples with known quantities of methanethiol and dimethyldisulphide. The $K$ values obtained suggest that the dimethyldisulphide had a greater affinity than methanethiol for the lipid phase of the butter. Solid phase microextraction used in conjunction with gas chromatography –mass spectrometry has been shown to be an effective method for headspace analysis and was used for quantifying the butter headspace concentrations of the reduced sulphur compounds. Seasonal variation of both methanethiol and dimethyldisulphide were found in a series of butter samples, the highest concentration being in spring with an overall decrease through the New Zealand summer. Grass type and condition appeared to influence the concentrations of these compounds in the butter. Storage of the series of butter samples at 4 °C for 5 weeks resulted in a significant decrease in the concentrations of both methanethiol and dimethyldisulphide. The dimethylsulphide concentration in all butter samples was below the detection limit of the analytical system used.

Volatile sulphur compounds are shown to influence the flavour of a variety of foods and beverages (Mussinan & Keelan, 1994). While various compounds, particularly those not containing sulphur, have been found to influence the flavour and aroma of butter (Abdel-Mageed & Fadel, 1995), relatively little is known about the occurrence and chemistry of the volatile sulphur compounds in butter. Since milk is a butter precursor, volatile sulphur compounds in milk would be expected to be important in the determination of butter odour and flavour characteristics (Forss, 1993; Mariaca *et al.* 1997).

Sulphur compounds are important in determining the flavour and aroma of milk and treated milks (Reddy *et al.* 1967; Christensen & Reineccius, 1992; Bosset *et al.* 1994; Imhof & Bosset, 1994), and recently Calvo & de la Hoz (1992) reviewed the influence of a variety of compounds on the flavour of heated milk. A variety of reduced sulphur compounds have been found in raw and treated milks, including hydrogen sulphide ($H_2S$), methanethiol (MeSH), dimethylsulphide (DMS) and dimethyldisulphide (DMDS) (Jaddou *et al.* 1978; Badings & Neeter, 1980). Although

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the details of the formation of these compounds are not yet known, they could include oxidative, thermal, microbial and enzymic reactions. Also possible are disproportionation, methylation and photochemical transformations.

Few of the sulphur-containing compounds found in milk have been identified in fresh butter. While Patton *et al.* (1956) were the first to suggest that DMS is present in milk, Day *et al.* (1964) were the first to report its presence in butter. However, sulphur compounds have been detected in a number of specific situations related to butter. For example, Kinsella (1975) suggested that thiophenes and other compounds may be produced by the heat treatment of butter and butter cultures and they could contribute to the flavour of butter. Moreover, butter tainted by the cruciferous weed *Coronopus didymus* in feedstock is reported to contain the higher molecular mass sulphur compounds, benzylmethylsulphide and benzylthiol (Forss, 1971). The low molecular mass sulphur-containing compounds, $H_2S$ and MeSH, while not previously identified in butter, are reported to enhance the flavour of butter cultures at 1–5 $\mu$g/kg (Lindsay *et al.* 1967).

While it is apparent that sulphur compounds have a role in determining butter flavour, a variety of non-sulphur-containing compounds have also been found to be important, examples being indole, diacetyl, skatole and 4-cis-heptenal together with various acids, carbonyl compounds and lactones (Ramshaw, 1974; Badings & Neeter, 1980).

Various methods have been used to extract volatile compounds from milk and milk products, many based on low temperature vacuum distillation, degassing, headspace analysis, freeze concentration and absorption (Badings & Neeter, 1980). Chromatography has always been popular for separation and identification, while more recently capillary gas chromatography–mass spectrometry (GC–MS) has been used. Solid phase microextraction (SPME) is now being applied for a variety of analytical purposes, particularly headspace analysis. SPME combines sampling and preconcentration into one step and makes it possible to transfer analytes directly into a gas chromatograph. We report here the use of a polyacrylate SPME fibre for the extraction of several reduced sulphur compounds from the headspace of butter samples, and their quantitation using GC–MS. One distinct advantage of using SPME fibres is that drastic extraction procedures such as steam vacuum distillation are avoided, thus minimizing the possibility of modifying (oxidizing) any reduced sulphur compounds present.

## Materials and methods

Our analytical method involved placing butter samples in a septum-capped silanized glass vial (44·3 ml), exposing an SPME fibre for 10 min to the vial headspace and desorbing the adsorbed volatiles in the injection port of a GC–MS. The MS scan mode was used for retention time determination of selected sulphur compounds and the single ion (SI) mode for their quantitation. We focused on the reduced sulphur compounds of low molecular mass, MeSH, DMS and DMDS.

### *Solid phase microextraction*

Polyacrylate SPME fibres (85 $\mu$m; Supelco, Bellefonte, PA 16823-0048, USA) were used to adsorb the polar sulphur compounds in the butter headspace. All fibres were conditioned in the injection port of the gas chromatograph at 300 °C for 2 h before use and desorption from the fibre was achieved by exposure at 200 °C for 1 min. An injection port liner was used to minimize the injection port volume and to

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Table 1. *Butter samples analysed and pasture conditions at the time of milk production*

| Sample† | Date packed | Pasture conditions during milk production |
|---|---|---|
| BF | 6 Sep 95 (Very early season) | Wet conditions, soft ground, animal footprints visible |
| CF | 23 Oct 95 (Early season) | Wet conditions, soft ground, animal footprints visible, supplementary feeding |
| DF | 25 Nov 95 (Early mid season) | Lush grass |
| GG | 1 Feb 96 (Mid season) | Standard summer conditions, summer type pasture |
| HG | 1 Mar 96 (Late season) | Summer conditions |

† Samples were provided by the New Zealand Dairy Research Institute, Palmerston North, New Zealand.

improve the peak height to width ratio, thus avoiding the need for cryogenic focusing.

*Butter samples*

Samples of supermarket butter were used for method development and preliminary investigations prior to quantitation of reduced sulphur compounds in five samples of butter provided by the New Zealand Dairy Research Institute (Palmerston North, New Zealand). These samples, in the form of cubes 100 mm on a side, were prepared during the period September 1995–March 1996, spanning one New Zealand dairy production season (Table 1). Once prepared, the butter samples were stored at between −9 and −10 °C until overnight transport to Auckland on 25–26 March 1996, where they were held at 5 °C until analysis. Samples were obtained from 25 mm inside each block immediately prior to analysis.

*Headspace analysis*

The fibres were exposed to both butter headspace and standard gases in vials with a hole cap and a Teflon-faced silicone rubber septum. Samples (10·0 g) of butter were placed in the vials and warmed to 30–35 °C while being magnetically stirred using a 25 mm bar. After 30 min stirring a fibre was exposed to the vial headspace for 10 min before immediate desorption in the GC–MS injection port.

*Gas chromatography–mass spectrometry calibration*

Liquid DMS and DMDS were supplied by Merck (D-64293 Darmstadt 1, Germany; 98% purity). Methanethiol gas was obtained from a small (0·1 $m^3$) gas cylinder (0·1 MPa pressure) supplied by Matheson (East Rutherford, NJ 07073, USA). Gaseous standards of MeSH in the mg/kg range were prepared by successive dilution of the pure gas using gas tight syringes and the vials. DMDS standards (1 g/kg) were prepared by injection of 1·2 $\mu$l DMDS liquid into a 308·7 ml conical flask with a septum. After allowing the liquid to evaporate, standards at the $\mu$g/ml level were prepared by dilution into the vials. DMS standards (1 g/kg) were prepared from 1·0 $\mu$l of liquid in a similar manner. Adequate stability of the sulphur compounds was achieved by silanization of the vials using a 30 g/l solution of dimethyldichlorosilane in toluene. Calibration curves were obtained using freshly prepared standards and an SPME fibre.

*Instrumentation*

A Hewlett Packard G1800A GCD system (gas chromatograph and electron ionization detector (GC–MS)) with a split–splitless injector used in splitless mode and fitted with an SPME injection port liner (i.d. 0·75 mm; Supelco) was used for all

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headspace analyses. A Hewlett Packard 30 m HP1 capillary column (i.e. 0·32 mm, film thickness 4 $\mu$m) with He carrier gas and an effluent splitter kit was also used for all analyses. Temperature programming was utilized to maximize separation of the sulphur compounds: 70 °C for 2 min followed by a 10 deg C/min increase to 150 °C. The injector and detector temperatures were set at 200 and 275 °C respectively, and the column flow rate was 1·6 ml/min.

In the scan mode of the electron ionization detector, the mass range scanned was 30–200 amu. In the SI mode the instrument software made it possible to analyse only two compounds concurrently; consequently, MeSH (ions 47, 48) and DMDS (ions 79, 94) were analysed in one injection using the same SI method, with the injection being repeated for DMS (ions 47, 62). The appropriate ions for each compound were chosen by the software using a scan mode spectrum obtained by injection of a standard. When using the SI mode, ions appearing at the appropriate compound retention time were taken as an indication of the presence of the compound. The retention time of each compound was determined using standards. Instrument calibration and component quantitation were conducted using the SI mode; in this mode the instrument sensitivity was enhanced by a factor of approximately ten.

## RESULTS

### *Method development*

Using the polyacrylate fibre and GC–MS, we focused on the identification and detection of MeSH, DMS and DMDS in the headspace of New Zealand butter samples. A blank (unexposed) polyacrylate fibre was investigated to determine the retention time at which the minor decomposition or bleed products often seen from fibres would occur. The only observable peaks in the scan mode were at 1·96 and 2·08 min. The SI mode using the MeSH–DMDS method and a blank fibre gave peaks at 1·90 and 2·20 and 9·50 min, while the SI mode method for DMS gave no observable peaks. Hence blank fibre injections did not result in peaks that interfered with those resulting from fibre injections of any of the three sulphur compounds.

The time required for the fibre to equilibrate with the butter headspace gases was determined by a series of exposures ranging from 2 to 15 min in length. As shown in Fig. 1 the absorption equilibrium was essentially complete after 10 min fibre exposure.

The reproducibility of the fibre method was determined by five repeat fibre injections using the SI mode and the prepared MeSH and DMDS standards. The CV for MeSH and DMDS were 3 and 6 % respectively. We noted that the reproducibility of DMDS was poor when the same standard was used for repeat fibre injections and the vial containing the standard was unsilanized. This was caused by a decrease over several hours in the DMDS in the vial, but this loss was minimized by silanization and frequent replacement of the septum.

After heating the butter in a vial to 30–35 °C the time for equilibrium to be established between the volatile butter components and the vial headspace was determined by varying the stirring time prior to the 10 min fibre extraction. After $\sim$ 30 min stirring no further increase in the amounts absorbed by the fibre was noted, so this stirring time was adopted.

Calibration of the GC–MS was achieved using known concentrations of gas phase MeSH, DMS and DMDS. The three calibration plots were linear over the concentration range investigated. Relevant gas chromatographic information is given in Table 2.

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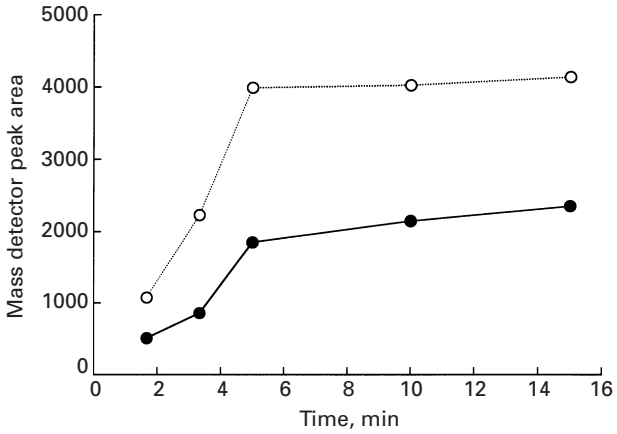



Fig. 1. Increase in mass detector response (measured as peak area) for ○, dimethyldisulphide and ●, methanethiol with increasing exposure time of a polyacrylate fibre in the headspace above a butter sample.

Table 2. *Gas chromatography retention times, calibration and detection limits for the reduced sulphur gases analysed in this study*

| Compound | Retention time, min | Concentration range, μl/l | No. of samples | $r^2$ | Detection limit μl/l† |
|---|---|---|---|---|---|
| Methanethiol | 3·1 | 0–2·0 | 5 | 1·00 | 0·01 |
| Dimethylsulphide | 4·5 | 0–10·0 | 5 | 0·99 | 0·1 |
| Dimethyldisulphide | 8·9 | 0–10·0 | 4 | 1·00 | 0·01 |

† Estimated from chromatograms obtained by progressive dilution of standards.

Preliminary results suggested that DMS may not actually have been present in significant quantities in the butter samples used in this study. In the follow up investigation an attempt was made to quantify the DMS concentrations in several additional butter samples which included one described as 'cowy'. It has been suggested (Patton *et al.* 1956) that a 'cowy' off flavour in milk may be ascribed to high levels of DMS. The concentrations of DMS in these butter samples were also below our detection limit, therefore it was not investigated further.

*Concentrations of methanethiol and dimethyldisulphide in the headspace of selected butter samples*

Detectable quantities of MeSH and DMDS were found using the fibre method in the headspace of all five of the DRI butter samples. These samples (BF, CF, DF, GG and HG) formed a series, with sample BF representing butter manufactured early in the New Zealand dairy milking season (September–April) and sample HG being manufactured from milk produced late in the season.

To determine the distribution coefficients of MeSH and DMDS between butter and its headspace, known quantities of each compound were added separately to the headspace of a sealed vial containing 10 g butter. After equilibration of the gas between the butter and the vial headspace, the headspace concentration was determined and the equilibrium constants $K_{\mathrm{MeSH}}$ and $K_{\mathrm{DMDS}}$ were calculated from the difference between the quantities added to the vial and the amounts measured in the headspace after equilibrium. Fig. 2 shows the linear relationship between

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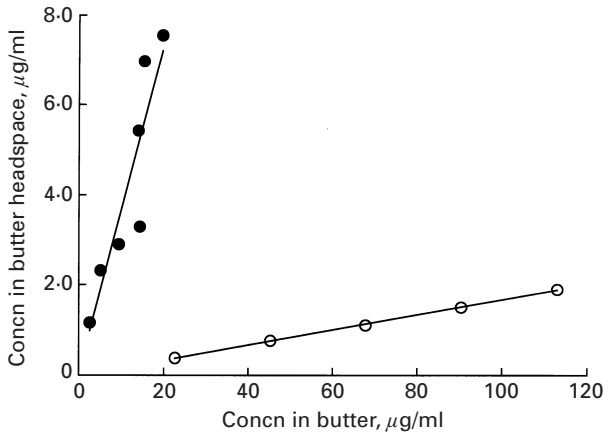



Fig. 2. Dependence of the concentrations of ○, dimethyldisulphide and ●, methanethiol in the headspace above a butter sample on the concentrations in the butter itself.

Table 3. *Concentrations of dimethyldisulphide and methanethiol in the headspace of closed vials containing butter, and the associated butter concentrations† calculated from headspace concentrations using the measured dimensionless distribution coefficients*

(Values are μg/ml for headspace, μg/g for butter; means for $n = 2$, CV = 20%)

| | Dimethyldisulphide concentration | | | | Methanethiol concentration | | | |
|---|---|---|---|---|---|---|---|---|
| Sample‡ | Headspace 27 Mar 96 | Butter 27 Mar 96 | Butter 11 Apr 96 | Butter 2 May 96 | Headspace 27 Mar 96 | Butter 27 Mar 96 | Butter 11 Apr 96 | Butter 2 May 96 |
| BF | 0·10 | 6·3 | 2·5 | 2·5 | 0·20 | 1·2 | 0·39 | 0·39 |
| CF | 0·08 | 4·7 | 2·5 | 2·5 | 0·18 | 1·1 | 0·35 | 0·35 |
| DF | 1·20 | 7·4 | 2·5 | 4·7 | 0·22 | 1·3 | 0·39 | 0·35 |
| GG | 0·04 | 2·4 | 0·27 | 1·2 | 0·14 | 0·86 | 0·35 | 0·27 |
| HG | 0·04 | 2·4 | 0·35 | 1·2 | 0·10 | 0·63 | 0·35 | 0·35 |

† A butter density of 0·912 g/ml was assumed.
‡ Butter samples were provided by the New Zealand Dairy Research Institute, Palmerston North, New Zealand.

MeSH and DMDS concentrations in the headspace and their concentrations in the butter. The dimensionless distribution coefficients ($K$) were defined as

$$K = \frac{\text{concentration in headspace}}{\text{concentration in butter}}.$$

Values of 0·38 for $K_{\text{MeSH}}$ and 0·017 for $K_{\text{DMDS}}$ at 30 °C were obtained from the results shown in Fig. 2.

The headspace concentrations of MeSH and DMDS for the five butter samples analysed as soon as possible after receipt are given in Table 3. The concentrations of MeSH and DMDS in these butter samples are also shown, and were calculated from the butter headspace concentrations using the distribution coefficients $K_{\text{MeSH}}$ and $K_{\text{DMDS}}$.

*Effect of storage on concentrations of MeSH and DMDS in butter*

In addition to the seasonal variations in MeSH and DMDS in the butter samples, the levels of both compounds were found to decrease with storage. The butter samples were kept at 5 °C after receipt on 25 March 1996 until the final analysis on 2 May

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1996, 5 weeks later. During this period there were significant decreases in the concentrations of both MeSH and DMDS (Table 3), with the decrease for both compounds being similar at $\sim 60\,\%$, most of which occurred in the first 2 weeks.

## DISCUSSION

Day *et al.* (1964) claimed to have identified DMS in a variety of butter samples by GC with u.v. absorption spectra being used for confirmation. It was noted that, although sensory evaluation indicated that the concentration of DMS fell during storage of the butter, the chromatographic peak believed to represent DMS did not decrease during storage. Although the concentrations of DMS in butter were not quantified, there seems to have been an assumption, based on sensory evaluation, that the concentration was $\sim 30\,\mu g/kg$.

Publications over the last 30 years have made numerous references to the paper by Day *et al.* (1964). However our results suggest that the small peak observed by these authors may have arisen from co-elution of two or more reduced sulphur compounds, perhaps with a common $CH_3$-S component, for example MeSH, DMS and/or DMDS. Our detection of both MeSH and DMDS (but not DMS) suggests that they may have been misidentified in the past. The lack of other published results on either compound prevents any useful comparisons with the concentrations we found.

It has been suggested that the Strecker degradation, in which sulphur-containing amino acids are deaminated and decarboxylated to aldehydes by $\alpha$-dicarbonyl compounds such as reducing sugars, may provide the mechanism for the formation of sulphur compounds in dairy products (Forss, 1979). For example, methionine can yield methional by this mechanism. In addition, a mechanism for forming DMDS in milk has been suggested by Joblin (1992) who, in discussing rumen microorganisms and their influence on milk flavour, states that S-methylcysteine sulphoxide, a free amino acid found in *Brassica* forage crops, is degraded via hydrolytic and reductive reactions in the rumen to DMDS, which is in turn transferred to milk fat. Chin & Lindsay (1994) have suggested that in disrupted cabbage tissue DMDS is formed by chemical disproportionation of methanethiosulphinate, while MeSH and dimethyl-trisulphide formation involves $H_2S$ reacting with thiosulphinates and thio-sulphanates following the action of cysteine sulphoxide lyases.

Dimensionless distribution coefficients of organosulphur compounds in aqueous solution at a variety of temperatures and ionic strengths have been measured (Przyjazny *et al.* 1983), with values comparable to ours being 0·101 for MeSH and 0·045 for DMDS (at low ionic strength and 25 °C). These indicate an enhanced solubility of DMDS in butter compared with water, but a lower MeSH solubility in butter. This is in keeping with MeSH having a greater solubility than DMDS in water (Lide & Frederikse, 1994). For the purpose of calculating the concentrations of MeSH and DMDS we assumed that our $K$ values were applicable to all the butter samples analysed in this study.

Both MeSH and DMDS concentrations in the five butter samples can be rationalized in terms of the season and type of feed that was available at the time, with the high levels being found in the vigorous grass growing part of the season, (see Table 1). A clear relationship between feed type and milk flavour has been shown (Urbach, 1990).

The observed loss of both MeSH and DMDS from all the stored butter samples may have been the result of their diffusion through the butter and subsequent escape from the surface of the sample. However one might expect a difference in the

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diffusion rates of these two compounds resulting in an observable difference in their loss rates and percentage decreases. Alternatively the loss may have been due to microbiological activity or to a reaction such as the partial oxidation of MeSH to produce DMDS, dimethyltrisulphide and other polysulphides. It has been suggested that aqueous formation of a compound such as dimethyltrisulphide and interconversion of sulphur compounds may involve microbiological mechanisms (Wajon & Heitz, 1995).

While the effect of storage on the volatiles of fresh cows' butter has been investigated by Abdel-Mageed & Fadel (1995), sulphur compounds do not appear in the list of the compounds identified. The use of vacuum stream extraction and choice of GC technique may have prevented this. However, oxidation processes occurring in the stored butter were evident, again suggesting that oxidation may have been involved in the observed decrease in the concentrations of MeSH and DMDS in our stored butter samples.

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