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A comparison of solid-phase microextraction (SPME) with simultaneous distillation-extraction (SDE) for the analysis of volatile compounds in heated beef and sheep fat.

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Abstract

A comparison has been made on the application of SPME and SDE for the extraction of volatile compounds from heated beef and sheep fat with separation and measurement by gas chromatography-mass spectrometry. **As far as we know, this report represents the first time that such a comparison has been made for the measurement of volatile compounds in heated sheep fat.** Approximately 100 compounds (in relatively high abundance) were characterised in the volatile profiles of heated beef and sheep fat using both techniques. Differences were observed in the volatile profiles obtained from each technique, independent of compound class. Rather than rate one technique as superior to another, the techniques can be regarded as complementary to each other.

Keywords SPME, SDE, solid-phase microextraction, simultaneous distillation and extraction, sheep fat, beef fat.

1 Introduction

Flavour is an important component of the eating quality of meat, and can be regarded as a combination of taste, the sensation perceived by the taste buds, and odour, the sensation perceived by the olfactory organ (Maarse, 1991). In its fresh uncooked state, meat has very little flavour and it is only as a result of cooking that meat develops a flavour, often characteristic of the product. During cooking, a complex series of thermally induced reactions occur between the non-volatile components of lean and fat tissues which generates a large number of products (Mottram, 1998). While some compounds contribute to the meat' s taste, it is mostly the volatile compounds formed from cooking that are responsible for the aroma and which typify the specific flavour associated with the meat. The major precursors of meat flavour are either lipids or water-soluble components that, during cooking, are subject to two sets of reactions: Maillard reactions between amino acids and reducing sugars, and thermal degradation of the lipid content. Mottram (1998) also notes that the lipid-derived volatiles are the compounds primarily responsible for explaining the differences between the volatile profiles of meat species, and are the main contributors to the species-specific flavour.

For sheep, two aromas are associated with the cooked meat of the animal. The first, 'mutton' flavour, is related to an animal's age while the second aroma, known as 'pastoral' flavour, is related to an animal's diet. Mutton flavour, regarded as the characteristic flavour associated with the cooked meat of older animals, becomes more pronounced as the meat is being cooked (Young and Braggins 1998). A range of fatty acids in cooked mutton fat were reported to be responsible for this aroma (Wong, Nixon & Johnson, 1975), with focus been given to branched chain fatty acids (BCFAs) as the main contributors to the aroma (Young and Braggins 1998). The presence of this particular note has been cited as one of the reasons historically that sheepmeat consumption has been low in some markets (Sink and Caporaso 1977). 'Pastoral' flavour can be present in the cooked meat of pasture fed ruminants (Berry, Maga, Calkins, Wells, Carpenter & Cross, 1980) and, for sheep meat, is linked to the presence of 3-methylindole and, to a lesser extent, *p*-cresol (4-methylphenol,

Young, Lane, Priolo & Fraser, 2003). The presence of a 'pastoral' flavour in sheepmeat may not be consequential to Australian consumers, who are unable to distinguish between grilled lamb from animals finished on either pasture or concentrate-based feeding systems (Pethick *et al.* 2005). However, the presence of this flavour note could cause the product to be less palatable to other lamb consumers, more accustomed to the meat from grain fed sheep (Prescott, Young & O'Neill, 2001).

In order to characterise 'pastoral' flavour in sheep meat, simultaneous distillation and extraction (SDE) has been the principal technique for the extraction of 3-methylindole and *p*-cresol from sheep fat (Ha and Lindsay 1990; Ha and Lindsay 1991; Lane and Fraser 1999; Osorio, Zumalacárregui, Cabeza, Figueira & Mateo, 2008; Schreurs *et al.* 2007; Young *et al.* 2003) as it is a one-step isolation-concentration process using steam distillation to extract the analytes from the sample (Chaintreau 2001). While it is a relatively simple extraction technique, it has also been regarded as lengthy and laborious (Young and Braggins 1998; Prescott *et al.* 2001).

Recently, solid-phase microextraction (SPME) has become the method of choice for aroma analysis since it offers solvent-free, rapid sampling with low-cost, ease of operation and sensitivity (Sides, Robards & Helliwell, 2000). SPME integrates several steps of the analytical process, and allows sample extraction and introduction to be performed as a simple process (Stashenko and Martinez 2004). Due to its simplicity and ease of use, SPME has been widely applied to the measurement of aroma profiles of, and monitoring lipid oxidation in, meat and related products (e.g. ham (Garcia-Esteban, Ansorena, Astiasarán, Martín, & Ruiz, 2004), beef (Giuffrida, Golay, Destaillats, Hug & Dionisi, 2005; Machiels & Istasse 2003; Moon & Li-Chan 2004; Moon, Cliff & Li-Chan, 2006; Watanabe, Ueda, Higuchi, & Shiba, 2008, Song *et al*, 2011) and goat (Madruga, Elmore, Dodson, & Mottram, 2009). **SPME has also been used to monitor the volatile profile of cooked lamb (Vasta *et al*, 2010; Nieto, Bañón & Garrido, 2011, Nieto *et al*, 2011) and lamb fat (Vasta *et al,* 2011) as well.** The aim of this work was to evaluate the

performance of SPME for measuring the volatile profile of heated sheep fat in comparison to that found with SDE. **For comparison, we included beef fat in this study, reflecting the interest in the literature in SPME's application to the measurement of volatile compounds in beef and related products.**

2 Materials and methods

2.1 Materials

Divinylbenzene /Carboxen®/polydimethylsilicone **(50 / 30 µm DVB/Car/PDMS)** SPME fibres **(Cat. no. 57329-U)** were purchased from Supelco, Inc. (Sydney, Australia). The SPME fibre was pre-conditioned at 300 °C for 1 hr as per the manufacturer's recommendation.

2.2 Fat samples

A commercial beef fat ("Allowrie Prime Beef Dripping") was purchased from a local retail store. **Subcutaneous fat samples, taken from forty 22-month old sheep, were combined to form an aggregate sample, representative of sheep fat**. These samples were taken from carcasses from Resource Flock 1 of Australian Sheep Industry Co-operative Research Centre (Hopkins *et al.* 2007).

2.3 Headspace solid-phase micro-extraction (SPME).

Samples were stored at −80 °C for 12 months and then removed and allowed to reach room temperature prior to analysis. **The fats were heated using a bench-top heater until it became molten.** Aliquots (5.00 **± 0.01 g, *n* = 10**) of molten fat were transferred to 20 mL headspace vials and sealed with polytetrafluoroethylene (PTFE, Teflon®)/silicone septa and steel caps. The vials and their contents were pre-heated at 100 °C for 5 min **in a CombiPAL autosample**r (CTC, Switzerland) prior to the insertion of the DVB/Car/PDMS SPME fibre into the headspace where it was held for 60 min. The fibre was then withdrawn and inserted into the GC injector to allow the adsorbed compounds to be transferred to the analytical column. The fibre was held in the injector for 7 min.

2.4 Simultaneous distillation-extraction (SDE).

Aliquots (5.00 **± 0.01 g, *n* = 6**) of molten fat were transferred to 100 mL flasks containing 30 mL of saturated brine (i.e. NaCl) solution. The flask was attached to a modified Likens-Nickerson apparatus (Chrompack, Netherlands) with a second flask containing 2 mL dichloromethane attached to the apparatus. Dichloromethane (4 mL), followed by saturated brine (2 mL), was added to the apparatus solvent return loop and both the solvent and sample mixture were heated to their respective boiling temperatures and maintained at these temperatures for 60 min. The condenser was cooled to a temperature of -5 °C. The organic extract (2 mL) was cooled to ambient temperature and then dried over anhydrous Na_2SO_4 prior to analysis.

2.5 Analysis by gas chromatography-mass spectrometry (GC-MS)

The volatile compounds were separated using a DB5-MS fused silica capillary column (J&W, 30m x 0.25 mm i.d. x 250 μm film thickness) in an Agilent GC-MS system (Palo Alto, CA, USA) comprising a Model 6890 gas chromatograph and Model 5973 mass selective detector with a CombiPAL autosampler (CTC, Switzerland). The GC oven temperature was initially held at 40 °C for 2 min, increased at a rate of 6 °C min^{-1} to 260 °C where it was held for a further 6.33 min. For SPME, the injector, heated at 260 °C, was held in the splitless mode for the first 2 min of the analysis and then in the split mode (20:1) for the remainder of the analysis. **The SPME fibre remained in the injector for 7 min to clean the fibre.** For SDE, the extract (1 μL) was injected under the same conditions with a solvent delay time of 3.5 min. Helium was used as the carrier gas with a constant flowrate of 2.0 ml min^{-1}.

A series of *n*-alkanes (C_8 to C_{24}) were analysed under the same chromatographic conditions in order to calculate the van den Dool and Kratz (1963) retention indices, RIs, which were calculated using:

$$RI = 100.n + (100.z).\frac{t_r\,(\text{compound}) - t_r(n)}{t_r(N) - t_r(n)}$$

where t_r is the retention time, n and N are respectively the number of carbon atoms in the alkanes eluting before and after the compound, and z is the difference between the number of carbon atoms between the smaller and larger alkane.

The MS was operated in electron ionisation mode (70 eV) and data was acquired in full scan mode for range of 40 to 360 Da. The temperature of the source and the detector were 150 and 230 °C, respectively, while the MS transfer line was 280 °C. Compounds were tentatively identified by comparing the mass spectra to those found in the NIST 05 mass spectral library and comparison of van den Dool and Kratz indices to those reported in the literature. Peak areas for each compound were calculated using the total ion chromatogram, assuming a relative response factor of one for each compound. The results of the volatile analysis were reported as percentages, representing the proportion of each identified peak to the total area of identified peaks in each chromatogram.

2.6 Statistical analysis

The data was analysed using analysis of variance ("aov" command) using R (R Development Team 2008).

3 Results and discussion

3.1 Comparison of samples

A total of **100** compounds were detected in the commercially available rendered beef fat sample using both SPME and SDE with GC-MS (Table 1) while, for the sheep fat, a total of **97** compounds was detected using both techniques (Table 2). For the beef fat, 89 compounds were extracted with SPME while 55 compounds were extracted using SDE with 44 compounds common to both techniques. For the sheep fat, 74 and 67 compounds were extracted by SPME and SDE, respectively, with 44 compounds common to both techniques. It was not possible though to identify every compound since, in some cases, no conclusive match could be made between the mass spectra of these compounds and the reference spectra in the mass spectral library. In these instances, the compounds were

deemed as unknown and, for beef fat, there were 35 and 15 unknowns for SPME and SDE respectively while, for the sheep fat, these were 20 and 22 respectively.

There were four main classes of compounds which were identified; these were aldehydes, hydrocarbons, acids and ketones/lactones (Table 3) and account for most of the identified compounds in beef and sheep fat for both sampling techniques. For beef fat using SPME, the most abundant compound class was the hydrocarbons (46.3 %), followed by the acids (15.7 %), aldehydes (10.6 %) and the ketones/lactones (4.2 %) as the least abundant. A similar trend was observed with SDE but the order for the acids and aldehydes was reversed; alkanes (53.9 %), aldehydes (17.2 %), acids (14.4 %) and ketones/lactones (1.6 %). For the sheep fat, the alkanes were the most abundant compound class (42.0 and 38.0 % for SPME and SDE, respectively), followed by acids (16.8 and 21.7 % for SPME and SDE), with ketones/lactones (11.3 %) then aldehydes (8.3 %) for SPME while, for SDE, the order was aldehydes (12.6 %) then ketones/lactones (6.0 %). This is contrast to the comparative study of the volatile compounds from dry-cured ham where higher proportions of aldehydes and aliphatic hydrocarbons were extracted and identified using SDE, compared to SPME, while SPME showed a higher number of ketones, acids and alcohols (Garcia-Esteban *et al,* 2004).

It was evident that differences existed between the proportions of the extracted compounds that were common to both techniques**. For beef fat, SPME extracted lower proportions of four aldehydes (heptanal, octenal, nonanal, 2,4-undecadienal), 2,3-octanedione, tetradecanoic acid, a phyt-1-ene isomer (RI = 1787), neophytadiene and five unknown compounds whereas SDE extracted lower proportions of the following compounds, 2,4-heptadienal (both isomers), *E*,*E*-2,4-nonadienal, 3,5-octane-2-dione, 2-tridecanone, naphthalene, butyrated hydroxytoluene (BHT) and three unknown compounds. No differences between each technique were observed for five aldehydes (*E*-2-nonenal, *E*-2-decenal, dodecanal, tridecanal and tetradecanal), six alkanes**

(pentadecane, octadecane, phytane, phyt-1-ene (RI = 1812), and phyt-2-ene (RI = 1830 and 1844)), diethyl phthalate, 2-heptadecanone and three unknown compounds.

No overall trends between extraction technique and compound class or type trends were found in this study. It would reasonable to assume that such trends might have been observed in this study (e.g. higher amounts of chemically similar compounds extracted by one technique in comparison to the other) but this was not the case. For example, nonanal and *t*-2-nonenal are chemically similar compounds yet higher amounts of nonanal were extracted from beef fat using SPME compared to that obtained from SDE yet no difference was observed for *t*-2-nonenal using either technique. In fact, in the general case, no distinct trend was observed for aldehydes for each extraction techniques. This is in contrast to other comparative studies in sampling the volatile compounds of meat products where relationships have been reported to exist between chemical class and extraction technique. Garcia-Esteban *et al*. (2004) found that, for dry-cured ham, SPME was more efficient in extracting low molecular weight compounds of high volatility, while SDE was more suitable in extracting compounds of low volatility that could not be extracted by SPME. **These workers used Carboxen®/PDMS SPME fibres that are more suited for the analysis of low molecular weight volatile compounds and could explain the observed higher efficiency for SPME in the study of Garcia-Esteban and co-workers.** Other workers have reported differences in the amount and type of compounds extracted using these techniques. For the volatile profile of a meat product derived from mini-pigs, similar volatile profiles were found with both SDE and SPME but SDE was the preferred technique as it allowed the generation of semi-quantitated data (Xie, Sun, Zheng, & Wang, 2008). After evaluating three extraction techniques (SPME, SDE and purge-and-trap (P&T)) for the measurement of the volatile profile of goat meat, the extraction profile was found to vary with the extraction technique (Madruga *et al.* 2009). These workers found that better extraction of volatiles of low molecular weight was afforded with SPME and

P&T while SDE extracted more high boiling volatile compounds. This concurs with the view expressed by Garcia-Esteban *et al*. (2004) in measuring the volatile profile of dry-cured ham. Madruga *et al*. (2009) also suggested that both SPME and SDE could be regarded as techniques that provide complementary information rather than rate one technique as more superior to another in performance.

3.2 Beef fat

For beef fat, some of the compounds identified in this work have been reported elsewhere. For example, diterpenoids (e.g. phyt-1-ene, phyt-2-ene and neophytadiene) were measured by SPME/GC-MS and found in higher levels in fat originating from Australian animals compared to Wagyu beef (Watanabe *et al*, 2008). The diterpenoids were present in significant amounts in the commercial beef fat (~ 20 to 25 %) and were also the main contributors to the hydrocarbon class for this sample. These compounds were also present in the sheep fat but not in the same abundance. The diterpenoids originate from chlorophyll and their presence implies that the animals fed on green grass (Watanabe *et al.* 2008). Other volatile compounds (2,3-octanedione, 3,5-octadien-2-one (Sivadier, Ratel & Engel, 2009) and phytol (Dawson and Hemington 1974)) are also indicators of a pasture diet and were present as volatiles in the beef fat. Sulphur compounds, furans and pyrazines have been detected by SPME for cooked beef meat (Machiels and Istasse 2003) but these were not identified in the fat sample used in this present study. This would imply that the meat used in Machiels and Istasse (2003) was the source of these compounds rather than the fat. High amounts of lactones (γ-dodecalactone, δ-decalactone, δ-dodecalactone, δ-tetradecalactone and δ-hexadecalactone) have been reported in Australian beef fat by Watanable *et al*. (2008). In this study, lactones were found in both beef and sheep fat. The mass spectra of two unknown compounds in the beef fat (RI = 2021 and 2129) were indicative of δ-lactones but their identity was not fully established.

3.3 Sheep fat

As for beef fat, differences existed between the volatile compounds extracted in sheep fat by both techniques. For SDE, higher proportions, compared to those found with SPME, were found for the following compounds: *t*-2-octenal, nonanal, *t*-2-decenal, tetradecanal, 2,3-octanedione, aromadendrene, 1-pentadecene, 2-tridecanone, tetradecanoic acid, phyt-2-ene (RI = 1831) and four unknown compounds. In contrast, higher proportions of the following compounds were extracted by SPME compared to SDE; 2,4-heptadienal (both isomers), tridecanal, hexadecane, phyt-1-ene (RI = 1785), octadecane, phytane and two unknown compounds. Compared to beef fat, a larger number of volatile compounds were found in the sheep fat where no statistically significant difference existed between the extracted proportions obtained from either technique ($P > 0.05$, Table 2). These were four aldehydes (heptanal, 2-heptenal, *E*-2-nonenal and *E*,*E*-2,4-decadienal), two methyl ketones (2-undecanone and 2-heptadecanone), three fatty acids (hexadecanoic, oleic and octadecanoic acids), six alkanes (tetradecane, pentadecane, heptadecane, neophytadiene, phyt-2-ene (RI = 1844) and heneicosane), diethyl phthalate, 2,6-diisopropylnaphthalene and three unknown compounds. As for beef fat, there does not appear to be general trends which exist between the extraction method and the chemical class of the compound.

Using P&T, recent work has described the presence of over 200 compounds in sheep fat (Engel & Ratel, 2007). While the compound classes described by these workers were similar to those shown in Table 2, there were some notable differences. Engel and Ratel (2007) found additional esters, aromatic hydrocarbons, a furan, sulphur containing compounds and terpenes compared to this study. In the current work, attention was only given to reasonably abundant peaks in the chromatogram in order to increase the likelihood that identification could be made of the compound responsible for the peak. This would mean that other minor components in the chromatograms would not have been identified. **Engel and Ratel also used P&T, a dynamic headspace**

technique, that is more sensitive for the measurement of volatile compounds in comparison to SPME, which can be regarded as a static headspace technique. Thus, there exists the possibility that some minor components may not have been detected. Alternatively, some compounds may have co-eluted and thus would not be observed in the chromatogram, meaning that they would not have been detected using the approach used in this study. They would be hidden in the chromatogram and would not readily be identified unless a specific search was made for a particular compound. An example of this is 3-methylindole ("skatole"), a compound responsible for 'pastoral' flavour in sheepmeat. The characteristic ions in the mass spectra of 3-methylindole are *m*/*z* = 130 and 131 (Powers, 1968), and a search for these ions in the chromatogram indicated that the compound was present but in very low abundance in comparison to the other compounds (Figure 1). **It was not detected when the initial characterisation of the compounds had been performed, and was only found within the chromatogram when a specific search was made for this compound. This could well be the case for other compounds which may be present in very low abundance within the sample and so would not detectable unless a specific search is made for these compounds.**

3.4 Origins of compounds

Nearly all of the compounds found in the beef and sheep fats originate either from lipid oxidation or are related to a pasture diet (Mottram 1998). Given that a high temperature (100 °C) was used for extraction of the volatiles using SPME and SDE, it is not surprising to see the presence of compounds, such as aldehydes, ketones and hydrocarbons, which are produced from the oxidation and degradation of the fatty acid components of lipids (Mottram 1998; Liu, Xu & Zhou,. 2007). The characteristic flavour of the different meat species is generally believed to be derived from lipid sources (Mottram 1998). In the case of sheepmeat, there are two aroma notes that are commonly associated with the cooked product from this animal, 'mutton' and 'pastoral' aroma. Branched chain fatty acids (BCFAs), the main contributors to 'mutton' aroma, were not detected in either set of the TICs resulting from sampling sheep fat with SPME and SDE. Other

fatty acids (e.g. hexadecanoic, octadecenoic and octadecanoic) in high abundance in sheep fat were only present at low levels, and given that the BCFAs levels in sheep fat range fat from 0.0 to 1.4 mg kg^{-1} (Watkins *et al,* 2010), it is likely these compounds were not detected by these techniques, under these experimental conditions.

Some novel compounds were also identified in the volatile composition of both beef and sheep fat. Aromadendrene, a sesquiterpene, was extracted using SPME in the commercial beef fat sample and has been reported as an odour-active compound present in simulated beef flavour (Moon, Cliff & Li-Chan, 2006). This compound was also found in sheep fat with both SPME and SDE and, as far as we are aware, has not been previously reported for sheep meat or fat. Butyrated hydroxytoluene (BHT), an anti-oxidant, and diethyl phthalate, used as a plasticiser, were also found in the commercial beef fat. **The presence of BHT was not unexpected since it is a lipophilic compound used as a food antioxidant and would be added to the commercial fat sample as a supplement in order to reduce the risk of fat oxidation, particularly for storage in retail stores.** Diethyl phthalate is a phthalate ester and, as a class, these compounds have been in worldwide production as plasticisers and, with their frequent use and application, have become ubiquitous in the environment (Xu, Liang & Zhang, 2007). One can only assume this compound was introduced to the fat as part of the commercial preparation of this product. *N*-cyclohexylcyclohexanamine and *N*-ethyl-2-methylbenzenesulfonamide were also identified by the mass spectral library search but it is unclear what the source of these compounds could be. Of course, the assignment of these compounds need to be regarded as tentative and would need additional confirmation such as authentic standards.

For the sheep fat, two compounds, 2,6-diisopropylnaphthalene and 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, were detected and, as far as this author is aware, have not been previously reported in sheep fat. 2,6-Diisopropylnaphthalene has been observed as the main compound in the boiling of seed coats of legumes (Mucuna beans) and used as plant

growth regulator and as a solvent for manufacturing of printing materials (Mwatseteza & Torto 2010). The source of this compound was not clear. The other compound, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, found in waste landfill leachates (Badoil & Benanou 2009), is an oxidation product of 2,6-di-*t*-butylphenol. This latter compound is used as a UV stabiliser and an antioxidant for hydrocarbon based materials, which was also detected in the TIC (*m*/*z* = 191 and 206, RI = 1513). The most likely source of the compound would be the plastic tubes used for storage of the sheep fat. As for beef fat, these assignments need to regarded as tentative and would need further confirmation.

3.5 Repeatability

The repeatability of each technique was tested by performing replicate extractions and measurements (*n* = 10 for SPME and *n* = 6 for SDE) on the same day. The mean value and associated standard deviation for each analyte are shown in Table 1 for beef fat and in Table 2 for sheep fat. In nearly all cases, the RSD associated with SPME measurements was lower compared to the SDE results. For example, the RSD's for the SPME measurement of phyt-1-ene and phyt-2-ene in beef fat were 1.5 and 6.1 %, which are considerably lower than those found with the SDE results (23.2 and 24.4 %, respectively). One reason for the large variation in the SDE results could be due to the number of the preparative steps associated with this technique (Liu *et al.* 2007). Additionally, three sets of SDE apparatus were used for extracting the volatiles from each fat, with two replicates extracted on one day with each apparatus. Given that some variation will exist between each apparatus, it is likely that this will also contribute to the differences between results. For example, the SDE result for octadecanoic acid in beef fat (4.56 ± 4.15) shows that a large variation exists for this compound. Inspection of the original chromatograms revealed that differences existed between the absolute amounts for this compound between replicates, despite the same conditions being employed for each replicate. A similar trend was also apparent for some aldehydes (e.g. nonanal, decanal, and *E*-2-decenal) but this was not observed for all aldehydes (e.g. heptanal, RSD = 6.1 %). This suggests that the differences between the repeatability for some

compounds with the two techniques cannot be generalised to a compound's class. The reason for the differences between these techniques remains unclear.

For this comparative study, the results were expressed as percentages of the combined areas for every identified peak. This is quite suitable for the purposes of this study but does not reflect the abundance of material extracted by the techniques. Higher abundances of volatile compounds were extracted by SPME from both fats in comparison to SDE (Figure 2). This is most likely related to the SPME sampling mode where the volatile compounds in the headspace would be at a higher concentration, compared to the semi-volatile compounds, due to the higher vapour pressure of the volatile compounds. Thus, the volatile compounds would be more readily adsorbed onto the SPME fibre and as a result higher amounts of these compounds would be detected. The selection of a suitable internal standard would allow semi-quantitative analyses to be performed and thus direct comparisons across samples could then be made. For this work, the use of proportions meant that comparison of the two techniques could be made for each sample but not between samples. This could be done by quantifying each analyte by preparing suitable calibration curves of analyte response from standard solutions of known concentrations, and a suitable selection of compounds similar to the analytes could be used as internal standards for the analysis.

4 Conclusions

A comparison has been made between SPME and SDE for extracting volatile compounds from heated beef and sheep fat. **As far as we are aware, this represents the first time that such a comparison of these two techniques has been made for measuring the volatile profile of sheep fat.** Around 100 compounds (in relatively high abundance) were characterised in the volatile profiles using SDE and SPME. It was not possible to identify every compound by comparison to a commercial mass spectral library. Differences were observed in the volatile profiles obtained by each extraction technique, making it difficult

to rate one as superior to the other. Rather, it would be more appropriate to regard the techniques as complementary to each other.

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Table 1 Volatile composition (as proportion of total of identified peaks) for the headspace measurement of beef fat using solid-phase microextraction (SPME) and simultaneous distillation and extraction (SDE) with GC-MS

RI[A]	Compound	SPME[B,D]	SDE[C,D]	*P*	ID[E]	Mass spectra[F]
< 800	Acetic acid	1.16 ± 0.27	-		5	
< 800	Pentenal	1.00 ± 0.17	-		5	
802	Hexanal	0.30 ± 0.06	-		3	
828	2,5,5-Trimethyl-2-hexene	-	0.16 ± 0.08		6	
833	Unknown	-	0.20 ± 0.18			83,55,68,41
840	Unknown	-	1.62 ± 0.46			43,59,101,83
852	Unknown	0.06 ± 0.01	-			43,98,83,55,69,106
874	2,4,6-Trimethyl-3-heptene	-	0.11 ± 0.11		6	
883	Pentanoic acid	0.21 ± 0.03	-		5	
892	Heptanal	0.58 ± 0.05	1.43 ± 0.09	< 0.05	1	
898	Unknown	0.05 ± 0.02	-			43,55,70,87
908	1,2,3,4,5-Pentamethylcyclopentane	-	0.32 ± 0.29		6	
917	Unknown	0.07 ± 0.03				57,43,59,85
936	Unknown	0.37 ± 0.03				81,79,124,41,53,95,109
937	Unknown	-	1.01 ± 0.81			70,71,43,55,140
939	Unknown	0.55 ± 0.06	-			81,79,124,41,53,95,109
948	Z-2-Heptenal	0.20 ± 0.05	-		1	
954	Unknown	0.09 ± 0.01	-			83,55,112,152
980	2,3-Octanedione	0.18 ± 0.03	2.79 ± 1.09	< 0.001	1,2	
992	2,4-Heptadienal	0.70 ± 0.11	0.57 ± 0.21	NS	5	
1001	Octanal	-	0.93 ± 1.14		1,2,4	
1006	2,4-Heptadienal	1.13 ± 0.21	0.79 ± 0.44	NS	5	
1020	Unknown	0.40 ± 0.13	-			81,67,41,55,95,89,108
1035	Unknown	0.11 ± 0.03	-			110,81,109,58

RI[A]	Compound	SPME[B,D]	SDE[C,D]	*P*	ID[E]	Mass spectra[F]
1055	E-2-Octenal	0.17 ± 0.03	0.48 ± 0.42	< 0.05	1,2	
1059	Unknown	0.42 ± 0.04	-			57,85,43
1068	3,5-Octadien-2-one	0.58 ± 0.05	-		5	
1070	1-Octanol	-	0.38 ± 0.22		2	
1076	*p*-Cresol	0.29 ± 0.03	-		4	
1084	Heptanoic acid	0.42 ± 0.07	-		5	
1090	3,5-Octadien-2-one	0.62 ± 0.08	0.40 ± 0.20	< 0.05	3	
1103	Nonanal	0.81 ± 0.15	2.67 ± 2.93		2,3	
1158	E-2-Nonenal	1.25 ± 0.22	1.31 ± 0.96	NS	3	
1176	Napthalene	0.80 ± 0.08	0.31 ± 0.14	< 0.001	3	
1204	Unknown	0.11 ± 0.02	-			118,133,55,41,83,69
1206	Decanal	-	0.27 ± 0.38		2	
1212	E,E-2,4-Nonadienal	0.30 ± 0.02	0.12 ± 0.05	< 0.001	1	
1220	Unknown	0.17 ± 0.02	-			88,43,99,71,144,55
1245	Unknown	0.31 ± 0.05	0.29 ± 0.28	NS		81,55,125,166,98,41
1260	E-2-Decenal	0.99 ± 0.35	2.34 ± 2.62	NS	2	
1265	Unknown	0.21 ± 0.02	-			59,44,102,83
1287	1-Methylnaphthalene	0.59 ± 0.05	-		5	
1289	2-Undecanone	-	0.53 ± 0.17		7	
1293	Unknown	0.54 ± 0.17				112,82,96,152,71
1294	E,Z-2,4-Decadienal	-	0.61 ± 0.24		3	
1306	Undecanal	-	0.33 ± 0.28		1	
1316	2,4-Undecadienal	0.40 ± 0.15	1.71 ± 0.68	< 0.001	5	
1334	Unknown	0.56 ± 0.06	-			86,57,41,70,69
1351	Unknown	0.32 ± 0.05	-			57,43,86,99,71,109,127
1362	2-Undecanal	1.42 ± 0.58	2.52 ± 2.60	NS	1	
1372	*n*-Decanoic acid	1.14 ± 0.08	-		2	
1399	Tetradecane	-	0.27 ± 0.09		2	
1405	Unknown	-	0.05 ± 0.02			69,81,95,41,58,163

RI[A]	Compound	SPME[B,D]	SDE[C,D]	*P*	ID[E]	Mass spectra[F]
1407	Dodecanal	0.20 ± 0.03	0.25 ± 0.24	NS	2	
1412	N-cyclohexyl-cyclohexanamine	0.15 ± 0.06	-		5	
1417	2,4-Dodecadienal	0.18 ± 0.06	-		5	
1438	Aromadendrene	0.22 ± 0.08	-		5	
1462	Unknown	0.29 ± 0.05	0.66 ± 0.22	< 0.001		71,57,53,85,141,113,99,183
1496	2-Tridecanone	0.78 ± 0.06	0.57 ± 0.16	< 0.01	5	
1500	Pentadecane	0.35 ± 0.04	0.42 ± 0.12	NS	5	
1510	Tridecanal	0.24 ± 0.02	0.21 ± 0.14	NS	3	
1516	BHT	0.31 ± 0.10	0.08 ± 0.01	< 0.001	5	
1524	Unknown	1.97 ± 0.09	0.40 ± 0.09	< 0.001		124,137,55,180
1532	Unknown	0.05 ± 0.03	-			137,194,109,165
1565	Dodecanoic acid	0.64 ± 0.04	-		5	
1573	Unknown	3.53 ± 0.28	0.80 ± 0.22	< 0.001		57,82,43,69,95,109
1592	Diethyl phthalate	0.88 ± 0.21	1.03 ± 0.30	NS	5	
1595	Unknown	0.18 ± 0.04	-			71,43,159,111,243
1610	Tetradecanal	0.69 ± 0.06	0.67 ± 0.16	NS	5	
1641	Unknown	0.11 ± 0.01	-			43,57,97.71,213,111,84,151,126
1649	N-Ethyl-2-methylbenzenesulfonamide	0.22 ± 0.03	-		5	
1676	Unknown	0.82 ± 0.23	-			85,57,43,69,86,109,123,137,180
1683	Unknown	0.86 ± 0.08	0.86 ± 0.19	NS		57,41,70,95,82,109,123,197
1700	Heptadecane	1.72 ± 0.10	1.68 ± 0.32	NS	5	
1706	δ–Dodecalactone	0.60 ± 0.04	-		5	
1713	Unknown	0.46 ± 0.06	0.35 ± 0.07	< 0.01		57,82,43,96,68,109,123,182
1729	Unknown	0.23 ± 0.02	0.23 ± 0.05	NS		57,69,111,43,126,97,155,197,212
1751	Myristoleic acid	1.35 ± 0.16	-		5	
1767	Tetradecanoic acid	1.84 ± 1.09	5.50 ± 0.48	< 0.001	5	
1787	Phyt-1-ene (isomer)	19.93 ± 0.30	24.76 ± 5.74	< 0.01	2	
1796	Octadecane	1.94 ± 0.12	1.74 ± 0.41	NS	2	
1806	Phytane	1.43 ± 0.10	1.37 ± 0.31	NS	5	

RI[A]	Compound	SPME[B,D]	SDE[C,D]	*P*	ID[E]	Mass spectra[F]
1812	Phyt-1-ene (isomer)	1.06 ± 0.15	1.57 ± 0.42	< 0.01	2	
1822	3,7,11,15-Tetramethyl-2-hexadecene	0.39 ± 0.02	-		5	
1830	Phyt-2-ene (isomer)	0.90 ± 0.34	1.22 ± 0.48	NS	2	
1836	Neophytadiene	6.25 ± 0.40	7.87 ± 2.18	< 0.05	2	
1844	Phyt-2-ene (isomer)	8.64 ± 0.53	9.75 ± 2.38	NS	2	
1860	Unknown	0.84 ± 0.13	0.54 ± 0.16	< 0.001		81,95,68,123,57,53,278
1878	Unknown	0.99 ± 0.07	0.55 ± 0.17	< 0.001		82,81,95,123,68,57,43,109,278
1897	2-Heptadecanone	1.03 ± 0.04	0.90 ± 0.27	NS	5	
1915	δ-Tetradecalactone	1.83 ± 0.22	-		2	
1920	Unknown	0.67 ± 0.23	1.00 ± 0.32	< 0.001		55,69,83,41,97,111,236
1964	*n*-Hexadecanoic acid	7.07 ± 1.72	5.21 ± 6.27	NS	5	
2013	Unknown	0.12 ± 0.02	-			71,57,82,43,,96,123,109,166,137,151
2019	16-Octadecenal	-	0.18 ± 0.13		6	
2021	Unknown	0.11 ± 0.01	-			99,71,114,192,236
2035	Unknown	0.06 ± 0.02	-			55,69,41,97,83,110,250,185,221
2058	Unknown	0.12 ± 0.02	-			98,43,111,55,74,83,129,227,140,270
2075	Phytol	0.52 ± 0.10	-		5	
2097	Heneicosane	0.19 ± 0.01	-		5	
2128	Unknown	0.75 ± 0.04	0.44 ± 0.13	< 0.001		99,71,55,83,114,192,236
2135	Oleic acid	1.31 ± 0.72	3.16 ± 4.83	NS	5	
2158	Octadecanoic acid	0.56 ± 0.33	4.56 ± 4.15	< 0.01	5	

[A]RI = van den Dool and Kratz retention index [B]*n* = 10 [C]*n* = 6 [D]Mean ± standard deviation [E]1 - Liu *et al.* 2007 2 - Watanabe *et al.* 2008 3 - Xie *et al.* 2008 4 Madruga *et al.* 5 - RI value found with NIST MS Search 2.0 6 - RI estimate from NIST MS Search 2.0 7 - Acree and Arn [F]Ions in order of decreasing abundance

Table 2 Volatile composition (as proportion of total of identified peaks) for the headspace measurement of sheep fat using solid-phase microextraction (SPME) and simultaneous distillation and extraction (SDE) with GC-MS

RI[A]	Compound	SPME[B,D]	SDE[C,D]	*P*	ID[E]	Mass spectra[F]
<800	Acetone	1.60 ± 0.61	-		5	
<800	Acetic acid	2.34 ± 0.33	-		5	
<800	Pentanal	0.45 ± 0.09	-		5	
839	4-Hydroxy-4-methyl-2-pentanone	2.92 ± 2.60	-		2	
892	Heptanal	0.34 ± 0.02	0.72 ± 0.67	NS	1	
900	2,5-Dimethylpyrazine	0.31 ± 0.02	-		5	
908	Unknown	-	0.44 ± 0.25			57,69,83,55,41,140,125,11
936	Unknown	-	0.80 ± 0.52			71,70,43,140,111,83
944	Unknown	-	0.23 ± 0.14			71,70,43,140,111,83
948	2-Heptenal	0.27 ± 0.01	0.30 ± 0.13	NS	1	
980	2,3-Octanedione	1.14 ± 0.10	5.60 ± 2.11	< 0.001	1,2	
991	2,4-Heptadienal	0.60 ± 0.09	0.17 ± 0.10	< 0.001	5	
1000	Octanal	0.79 ± 0.78	-		1,2,4	
1006	2,4-Heptadienal	1.12 ± 0.06	0.32 ± 0.14	< 0.001	5	
1021	Unknown	0.33 ± 0.02	0.39 ± 0.25	NS		81,55,51,67,95,109
1024	Limonene	-	0.28 ± 0.17		5	
1035	Unknown	0.16 ± 0.01	-			110,81,109,58

RI[A]	Compound	SPME[B,D]	SDE[C,D]	*P*	ID[E]	Mass spectra[F]
1039	Benzeneacetaldehyde	0.10 ± 0.01	-		5	
1051	Unknown	0.12 ± 0.04	-			110,81,109,58
1055	E-2-Octenal	0.16 ± 0.01	0.38 ± 0.19	< 0.01	1,2	
1071	1-Octanol	-	0.16 ± 0.18		2	
1076	*p*-Cresol	0.32 ± 0.03	-		4	
1085	Unknown	-	0.16 ± 0.10			43,87,142,99,71,57,113
1094	4-Nonen-4-ol	-	0.63 ± 0.28		5	
1103	Nonanal	0.60 ± 0.46	1.70 ± 1.43	< 0.05	2,3	
1158	E-2-Nonenal	0.86 ± 0.09	0.72 ± 0.37	NS	3	
1183	Octanoic acid	0.67 ± 0.38	-		2	
1192	2-Decanone	-	0.09 ± 0.07		5	
1199	Dodecane	-	0.08 ± 0.03		5	
1205	Decanal	-	0.38 ± 0.45		2	
1220	Unknown	0.41 ± 0.06	0.22 ± 0.11	< 0.001		88,43,99,71,87,144
1242	Unknown	0.10 ± 0.01	-			43,99,71,72
1248	Unknown	-	0.25 ± 0.15			83,70,55,41,110,97
1260	E-2-Decenal	1.25 ± 0.08	3.13 ± 1.24	< 0.001	2	
1282	Nonanoic acid	2.20 ± 0.48	-		3	
1292	2-Undecanone	0.80 ± 0.07	0.74 ± 0.28	NS	7	

RI[A]	Compound	SPME[B,D]	SDE[C,D]	*P*	ID[E]	Mass spectra[F]
1299	Tridecane	-	0.23 ± 0.11		5	
1307	Unknown	-	0.31 ± 0.40			57,43,82,71,96,126,109
1314	E,E-2,4-Decadienal	0.64 ± 0.03	0.61 ± 0.24	NS	3	
1324	Methyl decanoate	0.21 ± 0.02	-		5	
1349	Unknown	-	0.10 ± 0.10			70,83,41,55,124
1362	2-Undecanal	0.65 ± 0.04	1.55 ± 1.25		1	
1377	Unknown	-	0.43 ± 0.14			83,55,182,98,125,139,111
1379	n-Decanoic acid	4.89 ± 0.26	-		2	
1380	Unknown	1.44 ± 0.08	1.86 ± 0.63	< 0.05		124,137,55,189,152
1389	Unknown	0.20 ± 0.01	-			123,110,55,166,68,96,92
1398	Tetradecane	0.92 ± 0.59	0.35 ± 0.16	NS	5	
1401	Nictonamide	0.42 ± 0.48	-		5	
1404	Unknown	-	0.19 ± 0.08			69,81,95,41,55,163,123
1407	Dodecanal	0.19 ± 0.02	0.41 ± 0.32		2	
1417	Unknown	0.24 ± 0.12	-			151,109,43,81
1426	Unknown	0.28 ± 0.03	-			71,73,57,129,127,85,41
1436	Aromadendrene	0.17 ± 0.02	0.31 ± 0.14	< 0.01	5	
1462	Unknown	1.31 ± 0.17	1.46 ± 0.73	NS		71,57,43,85,151,113,99,183
1486	Unknown	1.13 ± 0.10	0.83 ± 0.42	< 0.05		69,83,55,97,43,210,111,125

RI[A]	Compound	SPME[B,D]	SDE[C,D]	*P*	ID[E]	Mass spectra[F]
1489	Unknown	0.36 ± 0.03	-			55,69,83,97,41,210,111
1492	1-Pentadecene	2.01 ± 0.14	1.44 ± 0.65	< 0.05	5	
1496	2-Tridecanone	2.45 ± 0.12	1.72 ± 0.70	< 0.01	4	
1500	Pentadecane	1.11 ± 0.09	0.87 ± 0.44	NS	5	
1505	Unknown	0.54 ± 0.52	0.69 ± 0.33	NS		55,97,83,69,41,210,281,110,125
1516	Tridecanal	0.23 ± 0.02	0.16 ± 0.08	< 0.05	3	
1525	Unknown	3.96 ± 0.15	2.65 ± 0.76	< 0.001		124,137,55,180
1533	Unknown	-	0.15 ± 0.03			137,194,109,79,125,165,151,179
1566	Dodecanoic acid	0.92 ± 0.08	-		5	
1573	Unknown	3.58 ± 0.21	0.42 ± 0.08	< 0.001		127,43,55,82,99
1592	Diethyl phthalate	1.83 ± 0.55	1.46 ± 0.89	NS	5	
1597	Hexadecane	1.31 ± 0.05	0.92 ± 0.39	< 0.01		
1610	Tetradecanal	0.17 ± 0.03	0.40 ± 0.15	< 0.001	5	
1642	Unknown	0.42 ± 0.45	-			97,57,43,69,83,111,213,126,151
1677	Unknown	0.63 ± 0.04	0.34 ± 0.11	< 0.001		57,70,82,95,41,109,123
1684	γ-Dodecalactone	-	0.19 ± 0.06		7	
1698	Heptadecane	4.46 ± 0.14	3.93 ± 0.83	NS	5	
1705	δ-Dodecalactone	0.23 ± 0.03	-		5	

RI[A]	Compound	SPME[B,D]	SDE[C,D]	*P*	ID[E]	Mass spectra[F]
1715	Unknown	-	0.24 ± 0.06			57,82,96,41,68,111,123,138,154
1729	2,6-Diisopropylnaphthalene	0.25 ± 0.01	0.21 ± 0.06	NS	5	
1762	Tetradecanoic acid	0.48 ± 0.31	2.21 ± 0.27	< 0.001	5	
1781	E-3-Octadecene	0.87 ± 0.05	-		2,5	
1785	Phyt-1-ene	4.78 ± 0.15	3.65 ± 1.19	< 0.01	2	
1796	Octadecane	5.47 ± 0.22	4.46 ± 1.38	< 0.05	2	
1806	Phytane	1.35 ± 0.04	1.00 ± 0.33	< 0.01	2	
1814	Hexadecanal	-	0.86 ± 0.17		5	
1823	Unknown	0.40 ± 0.03	-			82,95,123,68,57,43,137,128
1831	Phyt-2-ene	0.55 ± 0.03	0.66 ± 0.13	< 0.05	2	
1836	Neophytadiene	7.51 ± 0.35	7.12 ± 1.65	NS	2	
1844	Phyt-2-ene	11.11 ± 0.42	9.74 ± 2.94	NS	2	
1861	Unknown	1.05 ± 0.12	0.72 ± 0.20	NS		81,57,43,96,68,110,137,124,250
1878	Unknown	0.69 ± 0.05	-			82,95,123,68,57,43,137,278
1898	2-Heptadecanone	2.19 ± 0.13	2.39 ± 0.55	NS	5	
1915	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	2.26 ± 0.59	-		5	
1960	n-Hexadecanoic acid	3.95 ± 1.09	6.41 ± 4.89	NS	5	
1990	Ethyl hexadecanoate	0.15 ± 0.05	-		5	
2017	Unknown	-	0.63 ±			82,57,43,96,68,110,137,125,250

RI[A]	Compound	SPME[B,D]	SDE[C,D]	*P*	ID[E]	Mass spectra[F]
2097	Heneicosane	0.47 ± 0.04	0.23 0.59 ± 0.22	NS	5	
2133	Oleic acid	0.58 ± 0.31	7.52 ± 5.94	NS	5	
2157	Octadecanoic acid	0.72 ± 0.38	7.76 ± 7.31	NS	5	

[A]RI = van den Dool and Kratz retention index [B]*n* = 10 [C]*n* = 6 [D]Mean ± standard deviation [E]See Table 1, note E

[F]See Table 1, Note F

Table 3 Numbers of volatiles in beef and sheep fat extracted by SPME and SDE.

Chemical class	Beef fat		Sheep fat	
	SPME	SDE	SPME	SDE
Aldehydes	17	18	15	15
Hydrocarbons	16	11	17	19
Acids	9	4	9	4
Ketones/lactones	8	5	6	6
Others	4	2	7	1
Unknown	35	15	20	22
Total	89	55	74	67

Figure 1. Partial total ion chromatogram indicating elution order of 3-methylindole, sampled by solid-phase microextraction. The inset shows the mass spectra of 3-methylindole measured at this retention time. The abundant compound is the unknown at KI = 1524.

Figure 2 Overlay of total ion chromatograms of volatile compounds sampled in (a) beef and (b) sheep fat using solid-phase microextraction (SPME, shown in blue) and simultaneous distillation and extraction (SDE, shown in red).

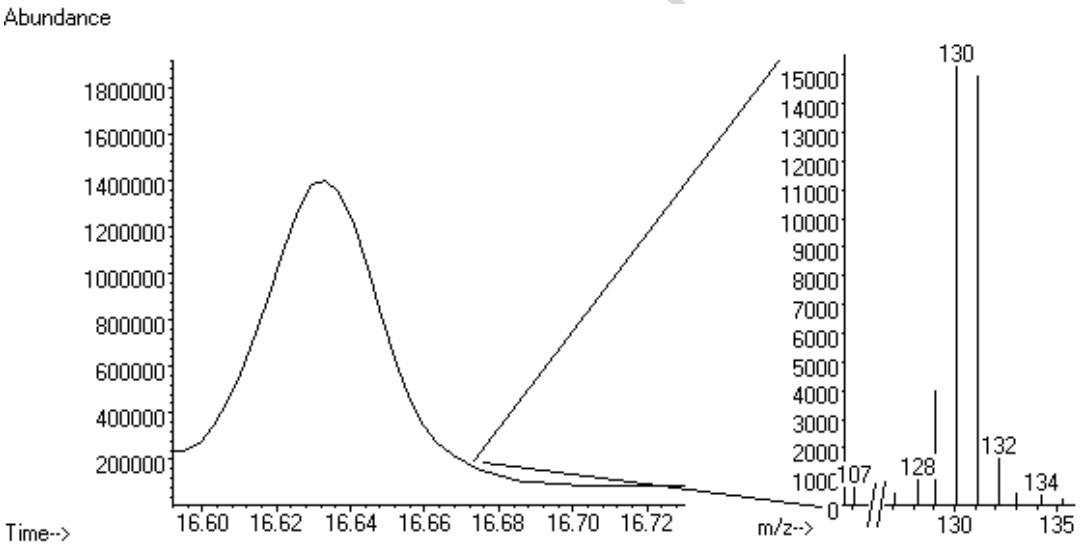

Figure 1

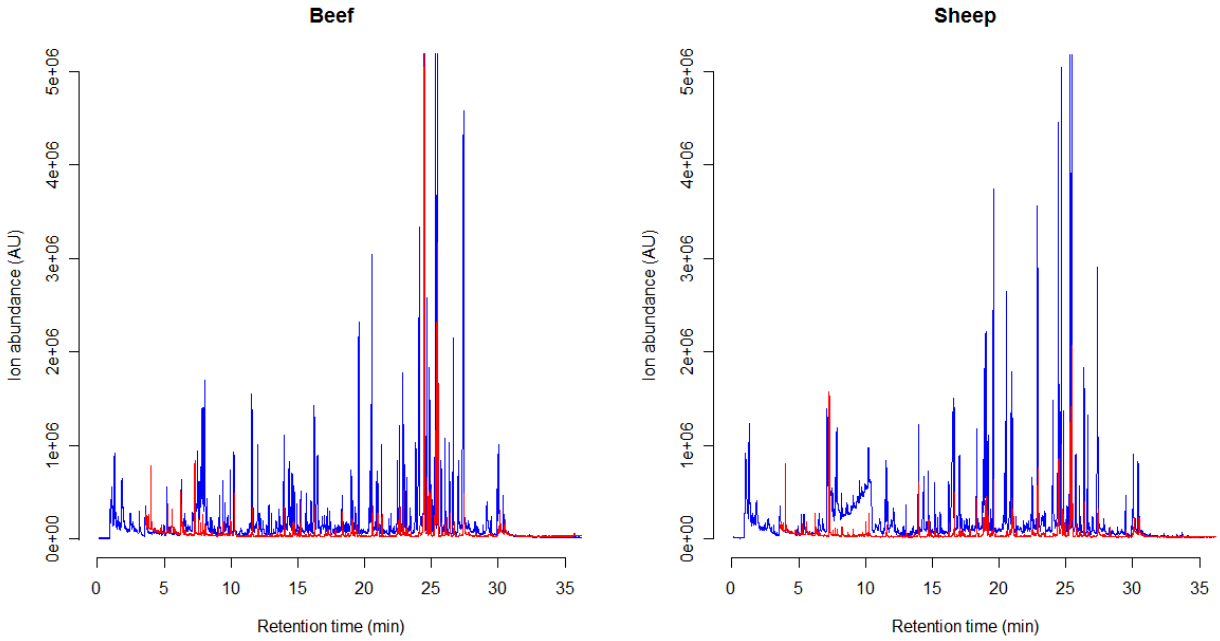

Figure 2