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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 fats

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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 fats 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 fats 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.

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 occurs between the non-volatile components of lean and fat tissues which generate 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 & 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 & 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 & Caporaso, 1977). 'Pastoral' flavour can be present in the cooked meat of pasture fed ruminants (Berry et al., 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 & Lindsay, 1990, 1991; Lane & Fraser, 1999; Osorio, Zumalacárregui, Cabeza, Figueira, & Mateo, 2008; Schreurs et al., 2007; Young et al., 2003) as it

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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 (Prescott et al., 2001; Young & Braggins, 1998).

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 & 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, Cliff, & Li-Chan, 2006; Moon & Li-Chan, 2004; Song et al., 2011, Watanabe, Ueda, Higuchi, & Shiba, 2008) and goat (Madruga, Elmore, Dodson, & Mottram, 2009)). SPME has also been used to monitor the volatile profile of cooked lamb (Nieto, Bañón, & Garrido, 2011; Nieto, Estrada, Jordán, Garrido, & Bañón, 2011, Vasta et al., 2010) 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 h 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, Stanley, Martin, & Gilmour, 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 autosampler (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, 30 m × 0.25 mm i.d. × 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}) was analysed under the same chromatographic conditions in order to calculate the van den Dool and Kratz (1963) retention indices, RIs, which were calculated using:

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

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 Core 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,