Available online at www.sciencedirect.com
SCIENCE DIRECT
Food Chemistry 99 (2006) 759–766
Food Chemistry
www.elsevier.com/locate/foodchem

Identification of volatile compounds in soymilk using solid-phase microextraction-gas chromatography

Allaoua Achouri, Joyce Irene Boye *, Youness Zamani

Agriculture and Agri-Food Canada, Food Research and Development Centre, 3600 Casavant Boulevard West, St. Hyacinthe, Que., Canada J2S 8E3

Received 30 June 2005; accepted 12 September 2005

Abstract

Headspace solid-phase microextraction (HS-SPME) gas chromatography was used to analyze volatile compounds in soymilk. The effect of incubation temperature (30–70 °C) and time (5–60 min), sample volume (0.5–5 ml), and type of SPME fiber (65 μm CWAX–DVB, 70 μm PDMS–DVB and 85 μm CAR–PDMS) were studied. All the factors markedly affected sensitivity and selectivity. Among the three fibers tested, the CAR–PDMS fiber had greater sensitivity to a more diverse range of volatile compounds, followed by PDMS–DVB and CWAX–DVB fibers using both soymilk and water with added volatiles as a matrix. SPME optimization conducted using a water matrix with added known soy volatiles, showed the following conditions to be optimal for selectivity and sensitivity: incubation temperature of 40 °C, incubation time of 20 min, and sample volume of 5 ml (for volatile compound concentration of ~25 ppm). The selected conditions were used for the analysis of volatiles in six commercial soymilk samples. A total of 30 volatile compounds were identified. The results showed significant differences in the total volatiles of the soymilk products. The repeatability of measurements of total volatiles compounds of soymilk was ~5.4% for four replicate analyses. Similar volatile compounds were present in all the samples analyzed but at different concentrations. The method proposed is simple and can be used to measure both hexanal and/or total volatiles in soymilk samples.

Keywords: Solid-phase microextraction; Flavour; Volatile compounds; Soymilk; Gas chromatography

1. Introduction

Solid-phase microextraction (SPME)-gas chromatography is increasingly been used for the analysis of volatile, semi-volatile, polar and non-polar compounds in various matrices. Briefly, the technique involves the adsorption of volatile compounds present in a system (e.g. food matrix) onto an adsorbent fiber. Adsorption is based on the equilibrium partitioning of the analytes between the solid-phase of the SPME fiber, liquid or solid sample matrix, and headspace above the matrix. Adsorbed analytes are later desorbed by heat onto a column and analyzed by gas chromatography (GC) (Eisert & Levsen, 1996; Pawliszyn, 1995; Prosen & Zupancic-Kralj, 1999).

Since its development (Arthur & Pawliszyn, 1990; Zhang & Pawliszyn, 1993), headspace solid-phase microextraction (HS-SPME), has found application in several research studies, particularly in the area of food quality and the environment. More recently, SPME has gained popularity as the instrumental technique of choice for the analysis of food products such as, honey (Pérez, Brunete, Calvo, & Tadeo, 2002), cheese (Lecanu, Ducruet, Jouquand, Gratadoux, & Feigenbaum, 2002), olive, soy and corn oils (Cavalli, Fernandez, Lizzani-Cuvelier, & Loiseau, 2004; Steenson, Lee, & Min, 2002), fruit juices and nectars (Lambropoulou & Albanis, 2002; Liu & Yang, 2002; Riu-Aumatell, Castellari, Lopez-Tamames, Galassi, & Buxaderas, 2004), meats (Brunton, Cronin, & Monahan, 2001) and spices (Jirovetz, Buchbauer, Ngassoum, & Geissier, 2002).

Interest in the application of SPME to characterize soy volatiles is growing. Flavour problems have been a major

* Corresponding author. Tel.: +1 450 773 1105; fax: +1 450 773 8461.
E-mail address: boyej@agr.gc.ca (J.I. Boye).

0308-8146/$ - see front matter.
doi:10.1016/j.foodchem.2005.09.001

technical impediment to the increased use of soy products in food formulation. Several investigators have reported the volatile flavour compounds present in soy foods to include pentanol, hexanol, heptanol, hexanal and ethyl vinyl ketone. Lipoxygenase (LOP), an enzyme naturally present in soybeans, mediates the conversion of polyunsaturated fatty acids to hydroperoxides; subsequent degradation products are responsible for the off-flavours generated. The primary off-flavour precursors in soybean are $C_{18:2}$ linoleate which produces hexanal, and $C_{18:3}$ linolenate which produces 2-hexenal and 3-hexenol via hexenal (Kobayashi, Tsuda, Hirata, Kubota, & Kitamura, 1995). More recently, Min, Yu, Yoo, and St.Martin (2005) using dynamic headspace and capillary gas chromatography have reported that factors such as soybean variety and growing location can have significant effects on the volatile compounds in soymilk. Continued growth in the development and use of soymilk derived products requires in-depth understanding and characterization of the flavour profiles generated as a result of different processing procedures. This information continues to be lacking in the literature.

SPME gas chromatography, although simple, is greatly affected by factors such as the nature of the SPME solid-phase, temperature of incubation, incubation time, and sample volume (Lee, Kang, & Min, 2003; Rodriguez-Bencomo, Conde, Rodriguez-Delgado, Garcia-Montelongo, & Perez-Trujillo, 2002; Steenson et al., 2002). The goals of this study, therefore, were two-fold: (1) to develop and optimize a headspace SPME method to study the flavour profile of soymilk, (a) by comparing the absorption efficiency of three different SPME fibers (carbowax–divinyl benzene (CWAX–DVB-65 μm), polydimethylsiloxane–divinyl benzene (PDMS–DVB-70 μm) and carboxen–polydimethylsiloxane (CAR–PDMS-85 μm)) on the recovery of selected volatile standards, using water as a matrix; (b) by studying the effect of incubation temperature, incubation time as well as sample volume on volatile recovery and (2) to use the optimized method developed to characterize volatile flavour compounds present in six commercial soymilk samples available on the Canadian market.

2. Materials and methods

2.1. Materials

Twenty one volatile standards from different chemical classes including aldehydes (hexanal, heptanal, pentanal, *trans-trans* 2,4 decadienal, *trans*-2-heptenal), alcohols (hexanol, 2-hexanol, 1-heptanol, 1-pentanol, 2-pentanol, 1-octen-3-ol, decanol), ketones (2-heptanone, 2,3-pentanedione, 3-hydroxy-2-butanone), aromatic compounds (benzaldehyde, *n*-butyl benzene), esters (ethyl heptanoate, ethyl hexanoate, ethyl butyrate) and furans (2-pentyl furan) were purchased from Sigma–Aldrich Chimica . These standards were selected based on their reported presence in soy-derived products. Six commercial soymilk products were purchased from a Canadian supermarket and analyzed. For confidentiality purposes the samples will be referred to as products A–F.

2.2. Fiber selection

Three fibers, 65 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB), 70 μm carbowax–divinylbenzene (CWAX–DVB) and 85 μm carboxen–polydimethylsiloxane (CAR–PDMS) were purchased from Supelco Company (Bellefonte, PA, USA). The fibers were provided attached to a stainless steel plunger sheathed by a protective needle. During analysis the needle passes through the septum of the sample container and is depressed to expose the fiber to the headspace for analyte adsorption. After a specified period of incubation, the fiber is retracted into the needle and then ejected from the sample vial prior to being inserted into the GC injector port for analytes desorption onto the column. All fibers were preconditioned in the injection port of the gas chromatograph according to the instructions provided by the supplier, prior to analysis.

2.3. Sample preparation

Stock solutions were prepared by dissolving the individual volatile standards in Millipore water at concentrations of 0.1 μl/ml. A gas tight syringe was used for sample preparation to minimise loss. Aliquots (5 ml) of the stock solutions were pipetted into 10 ml glass vials, closed using a Teflon/Silicone (TEF/SIL) septum and analyzed by SPME–GC–MS.

2.4. SPME analysis

A Saturn 2000 gas chromatograph–mass spectrometer (GC–MS) (Varian Analytical Systems, San Fernando, CA) was used for the analysis. The GC was equipped with a split/splitless model CP-3800 injector. The MS detector was used in the electron impact (EI) mode, with a mass range of 40–300 m/z. The adsorbed volatiles were desorbed in the injector port in splitless mode at 300 °C for 3 min. Volatiles were eluted with helium gas in a wall-coated open tubular (WCOT) fused silica 30 m × 0.25 mm column coated with 0.25 μm of chemically bonded polysiloxane low bleed phase (CP-SIL 8 CB Low Bleed/MS). The temperature was programmed as follows: initial temperature was kept at 35 °C for 3 min, and then increased to 210 °C at 6 °C/min, and held for 10 min. Compounds were identified based on National Institute of Standards and Technology (NIST) database through Saturn mass spectra library search. The identities of compounds were also confirmed by comparing their mass spectra and retention times with those obtained for the respective standards.

2.5. Optimization of SPME procedure

The best performing fiber based on sensitivity and selectivity was retained for the optimization studies. The effects of incubation time (5–60 min), incubation temperature (30–70 °C) and sample volume (0.5–5 ml) were studied. A stock solution containing 2.5 μl (25 ppm) of each of the following

standards (1-pentanol, hexanol, heptanol, 1-octen-3-ol, hexanal, heptanal, pentanal, 2-heptanone, benzaldehyde, 2-pentyl furan and ethyl hexanoate) in 100 ml of water was prepared. The solution was well vortexed to ensure complete mixing. Samples (5 ml) of the stock solution were placed into 10 ml GC vials and used for the optimization studies (the GC response obtained for each standard was, therefore, equivalent to a volume of 0.125 μl).

2.6. Statistical analysis

Each peak measurement (integrator counts) represents the mean of triplicate headspace analyses, and error bars indicate the standard deviation. Statistical significance of differences between the total volatiles of the six commercial soymilks was evaluated by the Duncan's test at the 5% level of probability.

3. Results and discussion

3.1. Comparative response of the three SPME fibers

Table 1 shows comparative responses of the three SPME fibers (CWAX–DVB-65 μm, PDMS–DVB-70 μm and CAR–PDMS-85 μm) for the selected volatile compounds in a water matrix under the same running conditions. Of the three fibers, our results showed the CAR–PDMS fiber to be the most selective and the most sensitive. Close examination of the results revealed that the recoveries for the different volatiles classes were higher for the PDMS–DVB fiber compared to the CWAX–DVB. The relative increases observed were as follows: aldehydes (24–38-fold), alcohols (6–58-fold), ketones (20-fold), aromatic compounds (6–10-fold), esters (10–21-fold) and furan (10-fold). In addition, volatiles with low molecular weights (80–100 Da) such as 2-pentanol (alcohol), pentanal (aldehyde), 3-hydroxy-2-butanone, 2,3-pentanedione (ketones) and having less than five carbon atoms were not detected by the CWAX–DVB fiber. The CAR–PDMS fiber, gave a much greater response in adsorptivity (sensitivity and selectivity) when compared to the other two fibers. The relative increase in recovery observed for the CAR–PDMS fiber when compared to the PDMS–DVB fiber were as follows: aldehydes (32–122-fold), alcohols (9–238-fold), ketones (56-fold), aromatic compounds (13–44-fold), esters (29–36-fold) and furan (20-fold) (Table 1). The stronger response of the CAR–PDMS fiber is likely related to the properties of the adsorbent Carboxen 1006, a porous carbon with a very high surface area (1000 m^2/g), which is mixed with PDMS to make the fiber coating. This type of matrix, which is rich

Table 1
MS detector response (peak area) of selected volatile compounds in a water matrix analyzed by SPME–MS using three different fibers

Compound	MS detector response (area)				
	CWAX–DVB fiber (70 μm)	PDMS–DVB fiber (65 μm)		CAR–PDMS fiber (85 μm)	
	Peak area ($\times 10^4$)	Peak area ($\times 10^4$)	Fold increase[a] (2/1)	Peak area($\times 10^4$)	Fold increase[a] (3/2)
Aldehydes					
Hexanal	3.1 ± 0.05	83.1 ± 0.4	26.8	377.6 ± 0.4	4.5
Heptanal	2.9 ± 0.04	70.4 ± 0.2	24.15	241.6 ± 1.9	3.4
Pentanal	ND	8.6 ± 0.09		61.2 ± 0.2	7.1
t−*t* 2,4 Decadienal	28.8 ± 0.2	907.8 ± 1	31.5	939 ± 1.5	1.0
trans-2 Heptenal	4.4 ± 0.07	167.3 ± 0.8	38.1	536.5 ± 0.9	3.2
Alcohols					
Hexanol	1.7 ± 0.01	79.7 ± 0.0	46.6	407.4 ± 0.6	5.1
2-Hexanol	2.4 ± 0.05	63.7 ± 0.3	26.3	277.6 ± 1.8	4.4
1-Heptanol	8.0 ± 0.02	285.2 ± 1.6	35.6	753.6 ± 2.9	2.6
1-Pentanol	1.3 ± 0.0	21.5 ± 0.4	16.7	180 ± 2.4	8.4
2-Pentanol	ND	1.3 ± 0.03		15.5±	12.1
1-Octen-3-ol	3.5 ± 0.09	204 ± 1.0	58.0	439 ± 0.4	2.2
Decanol	234.9 ± 1.9	1430 ± 17.4	6.1	2161 ± 0.2	1.5
Ketones					
2-Heptanone	10.2 ± 0.1	204.6 ± 0.46	20.0	572 ± 0.7	2.8
3-Hydroxy 2butanone	ND	1.6 ± 0.05		9.7 ± 0.1	6.1
2,3-Pentanedione	ND	ND		12.7 ± 0.07	
Aromatic compounds					
Benzaldehyde	7.8 ± 0.2	47.1 ± 0.4	6.0	345 ± 0.3	7.3
n-Butyl benzene	62.6 ± 0.5	604.7 ± 5.9	9.7	837 ± 0.8	1.4
Esters					
Ethyl heptanoate	11.9 ± 0.1	253.5 ± 0.8	21.3	427 ± 0.2	1.7
Ethyl hexanoate	13.0 ± 0.2	183.6 ± 0.4	14.1	395 ± 0.9	2.1
Ethyl butyrate	4.7 ± 0.03	46.7 ± 0.3	9.9	137 ± 0.4	2.9
Furan					
2-Pentyl furan	36.0 ± 0.8	357 ± 1.3	9.9	771 ± 2.5	2.2

ND: not detected. Fold increase[a]: (2/1) = fiber 2 versus fiber 1; (3/2) = fiber 3 versus fiber 2.

in micropores is efficient at adsorbing gases, trace-level volatiles and low molecular weight compounds (MW 30–225) (Shirey & Mindrup, 1999). The divinylbenzene (DVB) polymer, although rich in macropores, is better suited for retaining semi-volatile analytes. Vas and Vékey (2004) earlier reported that the type and thickness of the fiber coating material is the most important feature determining the analytical performance of SPME. Overall, the CAR–PDMS fiber gave the best performance, showing higher sensitivity and greater selectivity to a wider range of volatile compounds with varying polarities and molecular weights and was selected for subsequent work.

The repeatability (within-day variability) of measurements (precision) expressed by the relative standard deviation (R.S.D.) was found to be dependent on the type of compounds analyzed. The % R.S.D. values for aldehydes, alcohols, ketones, aromatic compounds, esters and furans using the CAR–PDMS fiber, ranged from 1.6 to 10.7%, 0.6 to 5.3%, 0.6 to 1.3%, 9.7%, 2.3 to 5.6% and 3.3%, respectively. R.S.D. values were in the satisfactory range (~5%) with automatic sampling for most of the samples analyzed. The exception was for volatiles having high adsorptivities, such as hexanal (10.7% R.S.D.) and benzaldehyde (9.7% R.S.D.). Matisovà, Medved'ovà, Vraniakova, and Simon (2002), Zhang, Yang, and Pawliszyn (1994) earlier found that the precision of HS-SPME measurements also depends on the analyte concentration, sample volume, matrix, and number of measurements.

The efficiency of the CAR–PDMS fiber for the selected volatiles was confirmed using soymilk as a matrix instead of water. Fig. 1 shows the mass spectrometer detector response (peak area) of a commercial soymilk sample, analyzed using the three different SPME fibers. The results clearly demonstrate the higher adsorptivity of the CAR–PDMS fiber with soymilk as sample matrix. The repeatability (within-day variability) of measurements of total volatiles compounds (sum of all volatiles observed) was 5.4% for four replicate analyses done using the soymilk matrix.

3.2. Optimization of solid-phase microextraction

The CAR–PDMS fiber was retained and used for further optimization studies (i.e. effect of incubation time, temperature, and sample volume) because of its greater sensitivity and selectivity.

3.2.1. Effect of incubation time

Fig. 2A shows the effect of incubation time on the adsorptivity of the selected volatiles. For the purposes of this work, adsorptivity is defined as the ability of the volatile standards to adhere to the SPME fiber under the conditions studied and was measured by the MS detector response peak area. An increase in total volatile recovery (sum of all the volatile peaks) was observed with increasing incubation time from 5 min to a maximum after 40 min. As the incubation time was increased further (50–60 min), total volatiles recovery decreased. This decrease may be attributed to reverse diffusion of analytes from fiber to sample in an attempt to maintain the partition equilibrium, as previously reported by Prosen and Zupancic-Kralj (1999). The time required to saturate the fiber, however, varied for each standard, and was highly dependent on the chemical nature of the compounds being analyzed. For most of the individual standards tested (aldehyde, alcohol, ketone, aromatic compound, ester, and furan) the maximum fiber saturation was reached within the range of 30–50 min of incubation (data not shown). Our results further showed that for most of these standards, 90% of their maximum adsorption capacity was reached after 20 min. For practical applications, a 20 min incubation period might, therefore, be appropriate for analysis (optimal volatile recovery).

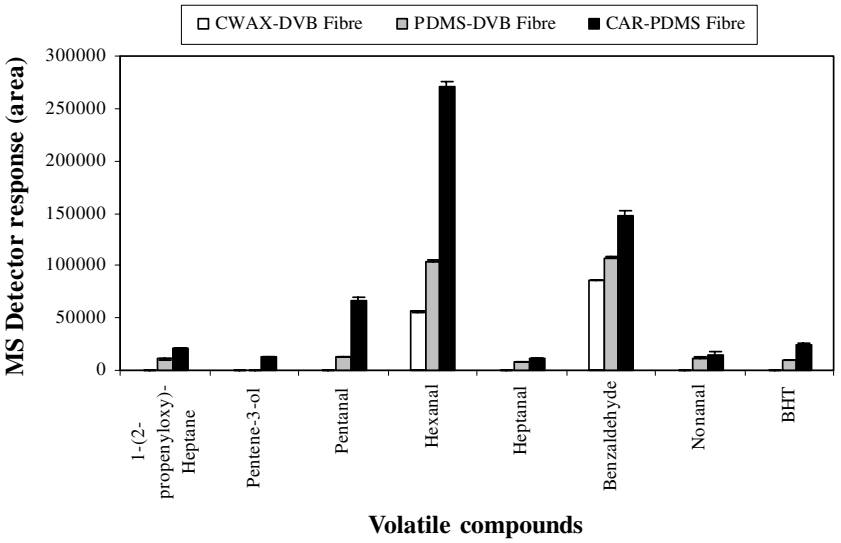

Fig. 1. Mass spectrometer detector response (peak area) of a commercial soymilk sample using the three different SPME fibers: (□) CWAX–DVB, (▩) PDMS–DVB, (■) CAR–PDMS.

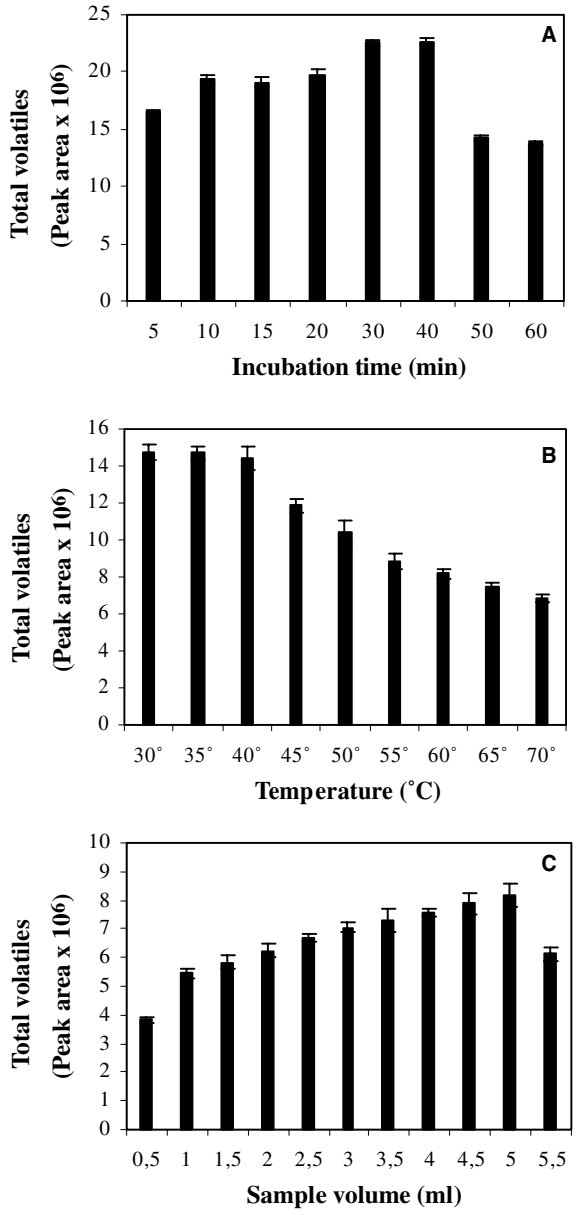

Fig. 2. Optimization of headspace solid-phase microextraction, using CAR–PDMS fiber. Effect of (A) incubation time; (B) incubation temperature; (C) sample volume on total volatiles recovery (MS response-peak areas).

3.2.2. Effect of incubation temperature

The effect of incubation temperature on adsorptivity over the range of 30–70 °C is shown in Fig. 2B. Maximum adsorptivity was observed between 30 and 40 °C. Increasing sample temperature above 40 °C resulted in a consistent decrease in total volatile recovery (total peak area). This general trend was observed for all the six volatile classes (aldehydes, alcohols, ketones, aromatic compounds, esters, and furan) individually. Desorption of volatiles from the fiber with increasing temperature during HS-SPME analysis has already been reported and been interpreted to result from a competition on the fiber between the different

Table 2
Identification of volatile compounds in six commercial soymilks using SPME–GC–MS

Volatiles	MS identification[a]
Product A	
Pentanal	Standard
Hexanal	Standard
2-Hydroxycyclopenten-1-one	Library
2-Heptanone	Standard
Heptanal	Standard
2-Butyl octanol	Library
Benzaldehyde	Standard
2-Pentylfuran	Standard
3,5-Octadiene-2-one	Library
Benzoic acid ester	Library
Hexanoic acid ester	Library
Butylated hydroxytoluene (BHT)	Standard
Product B	
1-(2-propenyloxy)-Heptane	Library
1-Penten-3-ol	Library
Pentanal	Standard
Hexanal	Standard
Heptanal	Standard
Benzaldehyde	Standard
Nonanal	Standard
Benzoic acid ester	Library
Butylated hydroxytoluene (BHT)	Standard
Product C	
1-Penten-3-ol	Standard
Pentanal	Standard
3-Hydroxy-2-butanone	Standard
Dimethyl disulfide	Library
Hexanal	Standard
2-Heptanone	Standard
Heptanal	Standard
Benzaldehyde	Standard
3-Octen-2-one	Library
Ethyl heptanoate	Library
Ethyl hexanoate	Library
Decanol	Standard
Butanoic acid-ester	Library
Product D	
Pentanal	Standard
3-Hydroxy 2 butanone	Standard
Hexanal	Standard
2-Butanone (MEK)	Standard
3-Metylacetylacetone	Library
Benzaldehyde	Standard
2-Pentylfuran	Standard
Ethyl heptanoate	Standard
Octanoic acid	Library
Ethyl octanoate	Standard
Butanoic acid, 1,1-dimethylpropyl ester	Library
Butylated hydroxytoluene (BHT)	Standard
Product E	
1-Penten-3-ol	Standard
Pentanal	Standard
2,4,4-Trimethyl-1-hexene	Library
3-Methyl hexane	Library
Hexanal	Standard
Isoamylacetate	Standard
2-Heptanone	Standard
Heptanal	Standard

(*continued on next page*)

Table 2 (*continued*)

Volatiles	MS identification[a]
Benzaldehyde	Standard
2-Pentyl furan	Standard
2-2-Dimethyl-octane	Library
2-Butyl-1-octanol	Library
3-Methylnonane	Library
Ethyl heptanoate	Standard
Ethyl octanoate	Standard
Butanoic acid, 1,1-dimethylpropyl ester	Library
Product F	
1-(2-Propenyloxy)-Heptane	Library
1-Pentene-3-ol	Standard
Pentanal	Standard
Hexanal	Standard
Benzaldehyde	Standard
Ethyl heptanoate	Standard
Ethyl octanoate	Standard
Butanoic acid, 1,1-dimethyl propyl ester	Library

[a] The volatiles were either positively identified using MS of pure standards or identified using the GC–MS spectra library.

compounds released in the headspace (Lambropoulou & Albanis, 2002; Lecanu et al., 2002; Liu & Yang, 2002). An incubation temperature of not greater than 40 °C, therefore, appears optimal for the recovery of the volatile compounds studied independent of their chemical nature.

3.2.3. Effect of sample volume

The effect of sample volume on adsorptivity response is given in Fig. 2C. An increase in total volatile recovery of 113.7% was observed as sample volume was increased from 0.5 to 5 ml. Increasing the volume further to 5.5 ml resulted in a decrease (−25%) in recovery compared to that obtained for the 5 ml sample. Reverse diffusion of analytes from fiber to sample as a result of overload has been reported by Prosen and Zupancic-Kralj (1999). The amount of adsorbed analyte is generally proportional to sample volume. The larger the sample size, the greater the quantity of analytes that will be adsorbed onto the fiber, and the shorter the time for an equilibrium to be

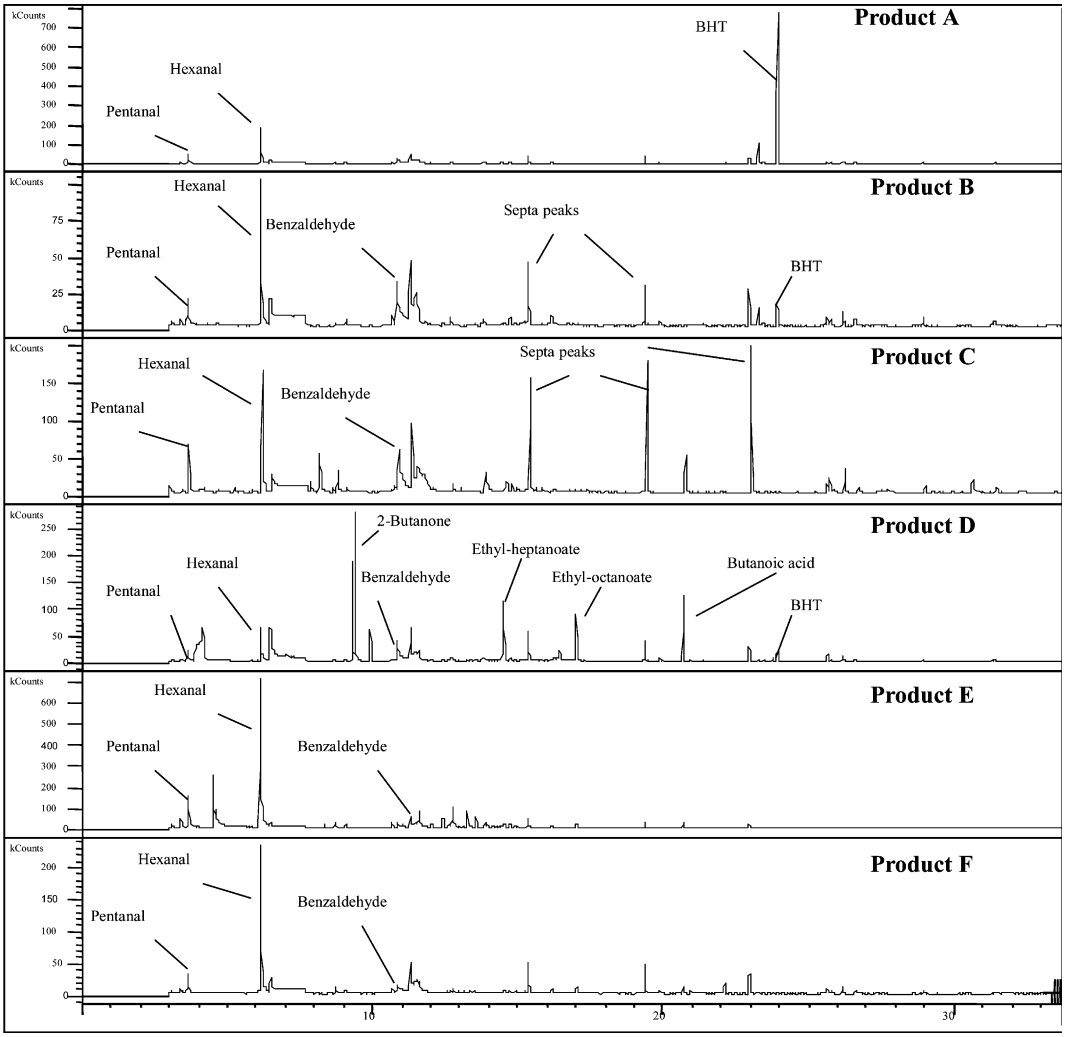

Fig. 3. Gas chromatograms of volatile flavours in six commercial soymilk products.

reached. With increasing incubation time, the partition coefficient (concentration of analyte in the fiber versus the concentration of analyte in the sample) decreases. If the sample volume is large and the incubation time lengthened, a desorption phenomenon can be expected to occur. For the standard mixture studied (i.e. containing 25 ppm of each standard) a saturation capacity of the fiber appeared to have been reached with the 5 ml sample volume.

Interestingly, below 2.5 ml sample size, some volatiles from the alcohol class such as pentanol and hexanol were not detected (data not shown). This may suggest that for such low volatility fractions, a larger sample volume may be required for better detection and quantification. Repeatability of measurements, as expressed by the relative standard deviation, was also found to be sample volume dependent. The best repeatability was observed for the lowest volumes (0.5–2.5 ml) with average R.S.D. values in the range of 1.92–3.7%. Using higher sample volumes (3–5.5 ml) increased the R.S.D. values to 3.0–8.7%. Similar findings have been reported by Matisovà et al. (2002).

3.3. SPME analysis of commercial soymilk volatiles

Based on the results obtained, the following conditions were selected for the analysis of volatiles in commercial soymilk samples: incubation temperature of 40 °C, incubation time of 20 min and a sample volume of up to 5 ml. Preliminary studies were conducted using different volumes of the soymilk samples to determine if a 5 ml sample volume fell within the range identified above. A sample volume of 5 ml was retained.

Table 2 lists the volatile compounds identified in the six commercial soymilks. A total of 30 volatile compounds were identified. The main constituents were hexanal, pentanal, benzaldehyde, 3-hydroxy-2-butanone, 2-pentylfuran, 2-butanone, heptanal, nonanal, penten-3-ol, 2-heptanone, ethyl heptanoate, and ethyl octanoate. Other compounds present but at lower concentrations (below the instrument threshold) were also identified but not quantified (summed) into the total volatiles.

Significant ($p < 0.05$) differences in the total volatiles were observed between the analyzed soymilk products, except between products C and F. The product with the lowest level of total volatiles was product B, followed by products F, C, A, D and E (Fig. 4A). For all the soymilk products, the highest responses observed were for hexanal and pentanal (Fig. 3). Products E and D had higher contents of hexanal, pentanal, trimethyl-1-hexene, and 3-hydroxy-2-butanone, 2-butanone, ethyl heptanoate, ethyl octanoate, and butanoic acid-ester. Sunflower oil and barley flour were used as ingredients in the processing of these two soymilk products, which may explain the high levels of these compounds. Products C and B had high levels of benzaldehyde. Wilkens and Lin (1970) previously reported that this flavour compound may not necessarily contribute to the beany flavour of soymilk, but might have a masking effect due to its cherry or almond like aroma. With the exception of the BHT peak, the product A flavour profile scored the lowest compared to all the other soymilk samples (Fig. 3). This product was processed from defatted soy protein isolate and, therefore, contained less quantities of the polyunsaturated fatty acids which act as precursors to off-flavour compounds. Its medium ranking position among the other soymilk products as shown in Fig. 4A is due to the presence of the strong BHT peak, which represented 65% of the total volatiles. BHT is usually added to limit lipid oxidation in soymilk during storage.

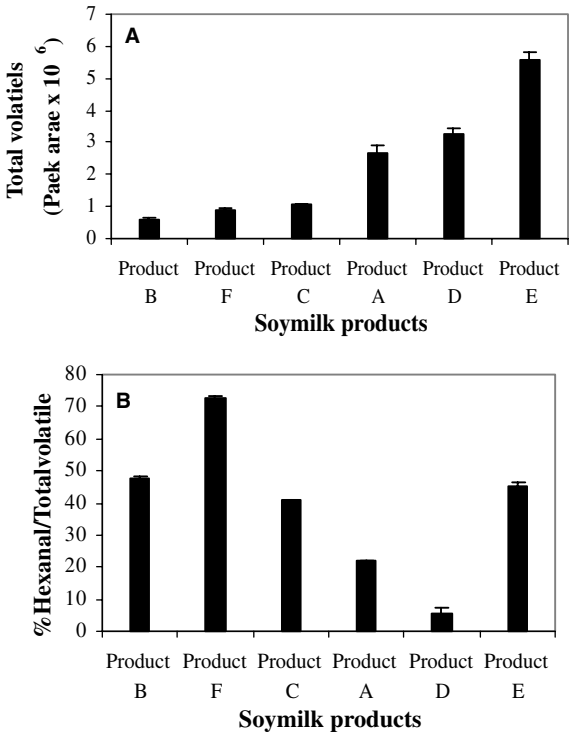

Fig. 4. (A) Total volatiles recovery (MS response-peak areas) and (B) percent ratio of hexanal to total volatiles recovery of six commercial soymilk products.

It is widely accepted that the principal contributors to off-flavours in soymilk are the volatile carbonyl compounds, particularly hexanal. Fig. 4B shows the percent of hexanal relative to the total volatiles in the different soymilk products. Product D had the lowest hexanal followed by products A, C, E, B and F. In contrast to product D, which had low concentration of hexanal but high total volatiles, product F had a high concentration of hexanal, although its total volatiles level was one of the lowest. This finding shows the importance of analyzing for individual volatiles, particularly hexanal. It also demonstrates the complexity of quality evaluation of soymilk products based only on instrumental flavour characterization. Unless tightly coupled with sensory evaluation to establish the correlation between specific flavour components and acceptability, results based on total volatiles alone could be misleading.

4. Conclusion

Evaluated for its performance in the recovery of volatile compounds, the adsorptivity of the CAR–PDMS fiber was found to be superior to those of porous polymer materials such as PDMS–DVB and CWAX–DVB. Its high sample capacity, sensitivity, and wider selectivity in respect to the chemical nature of the compounds being analyzed made it much more suitable than the other fibers, for the analysis of volatile flavours in soymilk. Selection of an appropriate SPME fiber depends on the targeted compounds and, therefore, on the particular food under study. Our results have demonstrated that for soymilk, a good compromise is to perform the SPME analysis using an incubation time of 20 min, incubation temperature of 40 °C, and a 5 ml sample volume. Significant ($p < 0.05$) differences in total volatiles were observed between the six commercial soymilk products analyzed; most of the volatiles compounds identified were present in all the samples but in different amounts. Our results further suggest that a decision on soymilk flavour/acceptability should not be based on total volatiles alone but on the relative concentration of specific volatile compounds (e.g. hexanal); whenever possible these results should be supported by sensory analysis tests.

References

Arthur, C. L., & Pawliszyn, J. (1990). Solid-phase microextraction with thermal desorption using silica optical fibers. *Analytical Chemistry, 62*, 2145–2148.

Brunton, N. P., Cronin, D. A., & Monahan, F. J. (2001). The effects of temperature and pressure on the performance of Carboxen/PDMS fibres during solid-phase microextraction (SPME) of headspace volatiles from cooked and raw turkey breast. *Flavour Fragrance Journal, 16*, 294–302.

Cavalli, J. F., Fernandez, X., Lizzani-Cuvelier, L., & Loiseau, A. M. (2004). Characterization of volatile compounds of French and Spanish virgin olive oils by HS-SPME: Identification of quality-freshness markers. *Food Chemistry, 88*, 151–157.

Eisert, R., & Levsen, K. (1996). Soild-phase microextraction coupled to gas chromatography: A new method for the analysis of organics in water. *Journal of Chromatography A, 733*, 143–157.

Jirovetz, L., Buchbauer, G., Ngassoum, M. B., & Geissier, M. (2002). Analysis of the headspace aroma compounds of the seeds of the Cameroonian 'garlic plant' Hua gabonii using SPME/GC/FID, SPME/GC/MS and olfactometry. *European Food Resource and Technology, 214*, 212–215.

Kobayashi, A., Tsuda, Y., Hirata, N., Kubota, K., & Kitamura, K. (1995). Aroma constituents of soybean [Glycine max (L.) Merrill.] milk lacking lipoxygenase isozymes. *Journal of Agricultural and Food Chemistry, 43*, 2449–2452.

Lambropoulou, D. A., & Albanis, T. A. (2002). Headspace solid-phase microextraction applied to the analysis of organophosphorus insecticides in strawberry and cherry juices. *Journal of Agricultural and Food Chemistry, 50*, 3359–3365.

Lecanu, L., Ducruet, V., Jouquand, C., Gratadoux, J. J., & Feigenbaum, A. (2002). Optimization of headspace solid-phase microextraction (SPME) for the odor analysis of surface-ripened cheese. *Journal of Agricultural and Food Chemistry, 50*, 3810–3817.

Lee, J. H., Kang, J. H., & Min, D. B. (2003). Optimization of solid-phase microextraction analysis of headspace volatile compounds in kimchi, a traditional Korean fermented vegetable product. *Journal of Food Science, 68*, 844–848.

Liu, T., & Yang, T. S. (2002). Optimization of solid-phase microextraction analysis for studying change of headspace flavor compounds of banana during ripening. *Journal of Agricultural and Food Chemistry, 50*, 653–657.

Matisovà, E., Medved'ovà, M., Vraniakova, J., & Simon, P. (2002). Optimization of solid-phase microextraction of volatiles. *Journal of Chromatography A, 960*, 159–164.

Min, S., Yu, Y., Yoo, S., & St.Martin, S. (2005). Effect of soybean varieties and growing locations on the flavor of soymilk. *Journal of Food Science, 70*, C1–C7.

Pawliszyn, J. (1995). New directions in sample preparation for analysis of organic compounds. *Trends in Analytical Chemistry, 14*(3), 113–122.

Pérez, R. A., Brunete, C. S., Calvo, R. M., & Tadeo, J. L. (2002). Analysis of volatiles from spanish honeys by solid-phase microextraction and GC–MS. *Journal of Agricultural and Food Chemistry, 50*, 2633–2637.

Prosen, H., & Zupancic-Kralj, L. (1999). Solid-phase microextraction. *Trends in Analytical Chemistry, 18*(4), 272–282.

Riu-Aumatell, M., Castellari, M., Lopez-Tamames, E., Galassi, S., & Buxaderas, S. (2004). Characterization of volatile compounds of fruit juices and nectars by HS-SPME and GC–MS. *Food Chemistry, 87*, 627–637.

Rodriguez-Bencomo, J. J., Conde, J. E., Rodriguez-Delgado, M. A., Garcia-Montelongo, F., & Perez-Trujillo, J. P. (2002). Determination of esters in dry and sweet white wines by headspace solid-phase microextraction and gas chromatography. *Journal of Chromatography A, 963*, 213–223.

Shirey, R. E., & Mindrup, R. F., (1999). A systematic approach for selecting the appropriate SPME fiber. Supelco Park, Bellefonte, PA 16823. Available from: http://www.sigmaaldrich.com/Brands/Supelco_Home/Datanodes.html.

Steenson, D. F., Lee, J. H., & Min, D. B. (2002). Solid-phase microextraction of volatile soybean oil and corn oil compounds. *Journal of Food Science, 67*, 71–76.

Vas, G., & Vékey, K. (2004). Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. *Journal of Mass Spectrometry, 39*, 233–254.

Wilkens, W. F., & Lin, F. M. (1970). Gas chromatographic and mass spectral analyses of soybean milk volatiles. *Journal of Agricultural and Food Chemistry, 18*, 333–336.

Zhang, Z., & Pawliszyn, J. (1993). Headspace solid-phase microextraction. *Analytical Chemistry, 65*, 1843–1852.

Zhang, Z., Yang, M. J., & Pawliszyn, J. (1994). Solid-phase microextraction. A solvent free alternative for sample preparation. *Analytical Chemistry, 66*, 844A–853A.