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Development of solid-phase microextraction methodology for analysis of headspace volatile compounds in simulated beef flavour

Soo-Yeun Moon, Eunice C.Y. Li-Chan *

Faculty of Agricultural Sciences, The University of British Columbia, Food Science Building, 6650 North West Marine Drive, Vancouver, BC, Canada V6T 1Z4

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Abstract

The conditions for headspace solid phase microextraction (HS-SPME) analysis of volatile compounds in simulated beef flavour were determined by fractional factorial experimental design based on Taguchi's orthogonal array. Adsorption temperature (30–60 °C), adsorption time (20–60 min), and the type of SPME phase (65 μm PDMS/DVB, 65 μm CW/DVB, 75 μm CAR/PDMS and 50/30 μm DVB/CAR/PDMS) were significant factors affecting total peak area and the number of peaks in the gas chromatogram, while added salt concentration (0–6%) as well as the 2-factor interactions of adsorption temperature with time, salt concentration or SPME phase, did not significantly affect the gas chromatogram. A sensitive and reproducible HS-SPME method was achieved using the following conditions: adsorption at 60 °C for 60 min on 50/30 μm DVB/CAR/PDMS, followed by desorption of extracted volatiles at 250 °C for 3 min and gas chromatographic separation on a DB-5 analytical column.

Keywords: Headspace analysis; Solid phase microextraction; Simulated beef flavour; Fractional factorial experimental design

1. Introduction

A characteristic beef odour is of prime importance to the quality of processed beef products as well as beef analogue products such as vegetable protein based meat substitutes for vegetarian consumers. Among the vegetable protein materials, soy protein has become especially popular as meat substitutes over the past few years due to the reported hypocholesterolemic effects of soy protein resulting in reducing risks of cardiovascular disease (Anderson, Johnstone, & Cook-Newell, 1995). However, flavour problems have been a major technical impediment in the increased usage of soy proteins in human foods (Maheshwari, Ooi, & Nikolov, 1995). Many studies have been reported on flavour of soy protein products with respect to the "beany" odour (Boatright & Lei, 1999; Lei & Boatright, 2001; Wolf, 1975), and on reducing the off-flavour (Inouye, Shiihara, Uno, & Takita, 2002; Maheshwari et al., 1995; McDaniel & Chan, 1988). In addition to indigenous undesirable soy aroma components that are difficult to remove, soy proteins may interact with desirable components of added flavour formulations such as simulated beef flavour. The presence of soy protein in aqueous systems has been reported to increase the retention of volatile components in samples (Gremli, 1974), and suppression of chicken flavour in a formulated soup at high levels of soy protein has also been reported (Malcolmson & McDaniel, 1987). Moreover, a significant reduction in perceived flavour intensity was observed when simulated beef flavours were added at the supplier's recommended dosage to formulate soy protein based beef substitutes (Yves Veggie Cuisine, 2002, personal communication). However, there is little information on the specific underlying mechanism for these observations, which could provide potential solutions for the problem. To elucidate the interaction

* Corresponding author. Tel.: +1-604-822-6182; fax: +1-604-822-3959.
E-mail address: ecyl@interchange.ubc.ca (E.C.Y. Li-Chan).

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between soy protein and simulated beef flavours, it is crucial to use a sensitive and reproducible but convenient method for the beef flavour analysis.

Headspace solid phase microextraction (HS-SPME) is a sample preparation technique that has been gaining popularity in recent years. Volatiles are captured by SPME based on the theory of equilibrium partitioning of the analytes between the solid phase of SPME, liquid or solid sample matrix, and headspace above the matrix (Zhang & Pawliszyn, 1993), and can be analyzed by gas chromatography (GC). Traditional methods for the extraction and concentration of volatile compounds for analysis by GC such as liquid–liquid extraction, solid phase extraction, supercritical fluid extraction, static headspace sampling or dynamic headspace (purge-and-trap) methods have one or more drawbacks such as high cost, multi-step preparation, low sensitivity, prolonged extraction time, artifact formation or solvent contamination (Braggins, Grimm, & Visser, 1999). Compared to the previous methods, the SPME technique has been regarded as a simple, rapid, and economical method requiring no solvent (Yang & Peppard, 1994). Originally, the SPME technique was developed for analysis of pollutants in environmental water samples, by immersing the SPME fiber in an aqueous sample (Arthur et al., 1992; Arthur & Pawliszyn, 1990). It has also now been applied to flavour analysis, by employing headspace SPME sampling in foods such as cheese (Lecanu, Ducruet, Jouquand, Gratadoux, & Feigenbaum, 2002), edible oils (Steenson, Lee, & Min, 2002), coffee (Akiyama et al., 2003), ginger (Shao, Marriott, Shellie, & Hugel, 2003), kimchi (Lee, Kang, & Min, 2003b), and sweet wines (Rodriguez-Bencomo, Conde, Garcia-Montelongo, & Perez-Trujillo, 2003).

However, it has also been reported that the SPME analysis is drastically affected by several factors such as the nature of the SPME solid phase, adsorption time, adsorption temperature, salt addition, stirring condition, and sample size (Lee et al., 2003b; Rodriguez-Bencomo, Conde, Rodriguez-Delgado, Garcia-Montelongo, & Perez-Trujillo, 2002; Steenson et al., 2002). Generally 'one factor at a time' experiments have been conducted in most of the previous studies to determine the analysis conditions with SPME, but ''one factor at a time' designs often overlook interactions among the factors. In contrast, Liu and Yang (2002) used response surface methodology (RSM) coupled with a two-factor central composite rotatable design to study the interaction between adsorption time and adsorption temperature. However, RSM is not the best choice when dealing with multiple factors due to the large number of experiments required. In order to investigate the effects of multiple factors as well as potential interactions between these factors in a time and cost effective manner, fractional factorial design based on Taguchi's orthogonal array can be considered (Arteaga, Li-Chan, Vazquez-Arteaga, & Nakai, 1994). To date, there has been no report on important factors for optimum adsorption condition to analyze headspace volatile compounds in simulated beef flavour. Therefore, Taguchi's fractional factorial experimental design was proposed in this study to screen significant factors, which would have a great impact on adsorption condition for headspace analysis of simulated beef flavour.

Therefore, the objectives of this study were to investigate the conditions that may influence HS-SPME of volatile compounds from simulated beef flavour by means of the Taguchi's method, and to investigate the effects of the factors along with potential interactions between these factors. The achievement of a sensitive and reproducible analytical method that is able to monitor changes in the headspace composition of the flavour sample would be the basis of future research to elucidate the mechanism of soy protein–flavour interactions that may be responsible for suppression of perceived intensity of beef flavour in soy protein products.

2. Experimental methods

2.1. Materials

Commercially produced simulated beef flavour for beef substitutes was a gift from Yves Veggie Cuisine, a division of The Hain Celestial Group (Delta, BC, Canada). It is a blended flavour based on vegetable proteins with other vegetable origin materials and the beef character is derived from Maillard reaction during roasting of the protein fractions. The solid phase assembly holder and four commercially available fibers, 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65 μm carbowax/divinylbenzene (CW/DVB), 75 μm carboxen/polydimethylsiloxane (CAR/PDMS), and 50/30 μm stableflex divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) were purchased from Supelco (Sigma–Aldrich, Canada, Oakville, ON). Before use, each fiber was exposed to a splitless/split injection port under helium flow and conditioned for the recommended time at recommended temperatures according to the manufacturer's instruction. GC sample vials with 15 ml capacity and polypropylene hole cap with PTFE/silicone septa were purchased from Supelco.

2.2. Fractional factorial design

A Taguchi's $L_{27}(3^{13})$ orthogonal array was used to evaluate potentially significant factors affecting the adsorption of simulated beef flavour compounds onto SPME fibers (Table 1). The main effects of four factors were investigated: adsorption temperature (A), adsorption time (B), salt concentration (C), and SPME phase

Table 1
Column assignment for the four factors and three interactions in this study to Taguchi's $L_{27}(3^{13})$ orthogonal array

Exp. no.	Exp. order	Column number												
		1 A	2 B	3 A × B	4 A × B	5 C	6 A × C	7 A × C	8 D	9 A × D	10 A × D	11 e	12 e	13 e
1	17	0	0	0	0	0	0	0	0	0	0	0	0	0
2	19	0	0	0	0	1	1	1	1	1	1	1	1	1
3	24	0	0	0	0	2	2	2	2	2	2	2	2	2
4	23	0	1	1	1	0	0	0	1	1	1	2	2	2
5	2	0	1	1	1	1	1	1	2	2	2	0	0	0
6	27	0	1	1	1	2	2	2	0	0	0	1	1	1
7	18	0	2	2	2	0	0	0	2	2	2	1	1	1
8	8	0	2	2	2	1	1	1	0	0	0	2	2	2
9	7	0	2	2	2	2	2	2	1	1	1	0	0	0
10	1	1	0	1	2	0	1	2	0	1	2	0	1	2
11	20	1	0	1	2	1	2	0	1	2	0	1	2	0
12	22	1	0	1	2	2	0	1	2	0	1	2	0	1
13	25	1	1	2	0	0	1	2	1	2	0	2	0	1
14	5	1	1	2	0	1	2	0	2	0	1	0	1	2
15	6	1	1	2	0	2	0	1	0	1	2	1	2	0
16	13	1	2	0	1	0	1	2	2	0	1	1	2	0
17	12	1	2	0	1	1	2	0	0	1	2	2	0	1
18	26	1	2	0	1	2	0	1	1	2	0	0	1	2
19	9	2	0	2	1	0	2	1	0	2	1	0	2	1
20	3	2	0	2	1	1	0	2	1	0	2	1	0	2
21	14	2	0	2	1	2	1	0	2	1	0	2	1	0
22	15	2	1	0	2	0	2	1	1	0	2	2	1	0
23	21	2	1	0	2	1	0	2	2	1	0	0	2	1
24	11	2	1	0	2	2	1	0	0	2	1	1	0	2
25	16	2	2	1	0	0	2	1	2	1	0	1	0	2
26	4	2	2	1	0	1	0	2	0	2	1	2	1	0
27	10	2	2	1	0	2	1	0	1	0	2	0	2	1

A: adsorption temperature (30, 45, and 60 °C as levels 0, 1, and 2).
B: adsorption time (20, 40, and 60 min as levels 0, 1, and 2).
C: salt concentration (0%, 3%, and 6% as levels 0, 1, and 2).
D: SPME phase (50/30 μm DVB/CAR/PDMS, 65 μm PDMS/DVB, and 65 μm CW/DVB as levels 0, 1, and 2).
e: error term.

(D). The three levels selected for each factor were 30, 45, and 60 °C for adsorption temperature, 20, 40, and 60 min for adsorption time, 0%, 3%, and 6% of added sodium chloride concentration, and 65 μm PDMS/DVB, 65 μm CW/DVB, and 50/30 μm DVB/CAR/PDMS for SPME phase.

In addition to the four main factors, three interactions were also investigated: adsorption temperature × adsorption time (A × B), adsorption temperature × salt concentration (A × C), and adsorption temperature × SPME phase (A × D). The linear graph used for this experimental design is illustrated in Fig. 1 and the column assignment and the levels of each factor are shown in Table 2. In this design, columns 1, 2, 5, and 8 were assigned to the main factors (A, B, C, and D). Columns 3 + 4, 6 + 7, and 9 + 10 were used to investigate three of the possible interactions between these factors, while columns 11, 12, and 13 were employed for the estimation of the error term. The levels of each factor were selected within an applicable range of the processing for commercial meat substitutes according to the recommendation by Ross (1996).

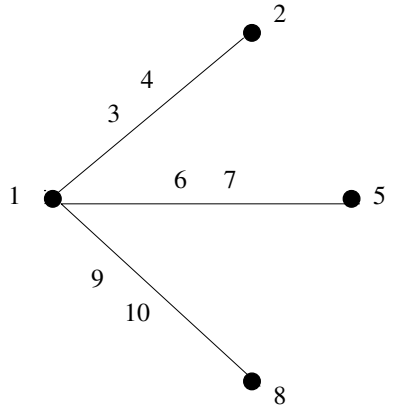

Fig. 1. The standard linear graph of orthogonal array $L_{27}(3^{13})$ used in this study.

2.3. Solid-phase microextraction procedure

Flavour stock solution consisting of 10 g of flavour and 40 g of 30 mM Tris–HCl buffer (pH 6.0) was prepared fresh daily. In a 15 ml capacity GC sampling vial

Table 2
Experimental design based on Taguchi's $L_{27}(3^{13})$ orthogonal array and the measured responses of total area count and the number of peaks in the gas chromatogram for each experimental run

Exp. no.	Factors[a]				Responses[b]	
	A	B	C	D	Total area	No. of peaks
1	30	20	0	DVB/CAR/PDMS	228801	9
2	30	20	3	PDMS/DVB	36325	1
3	30	20	6	CW/DVB	30013	1
4	30	40	0	PDMS/DVB	68431	1
5	30	40	3	CW/DVB	63933	2
6	30	40	6	DVB/CAR/PDMS	369733	15
7	30	60	0	CW/DVB	43944	1
8	30	60	3	DVB/CAR/PDMS	383206	14
9	30	60	6	PDMS/DVB	143972	5
10	45	20	0	DVB/CAR/PDMS	236792	11
11	45	20	3	PDMS/DVB	47968	1
12	45	20	6	CW/DVB	37258	2
13	45	40	0	PDMS/DVB	87752	2
14	45	40	3	CW/DVB	27000	1
15	45	40	6	DVB/CAR/PDMS	432501	19
16	45	60	0	CW/DVB	24796	1
17	45	60	3	DVB/CAR/PDMS	524510	22
18	45	60	6	PDMS/DVB	154745	6
19	60	20	0	DVB/CAR/PDMS	287959	13
20	60	20	3	PDMS/DVB	78910	3
21	60	20	6	CW/DVB	33728	2
22	60	40	0	PDMS/DVB	156345	8
23	60	40	3	CW/DVB	60726	3
24	60	40	6	DVB/CAR/PDMS	777677	35
25	60	60	0	CW/DVB	79574	5
26	60	60	3	DVB/CAR/PDMS	1089718	45
27	60	60	6	PDMS/DVB	271640	13

[a] Factors were assigned to columns as shown in Table 1, where A: adsorption temperature (°C), B: adsorption time (min), C: salt concentration (%), and D: SPME phase.
[b] Area reject: 10,000, initial threshold: 1 and peak width: 0.04.

with a magnetic stirring bar, 2.5 g of flavour stock solution and 3 g of buffer solution were mixed, to provide a flavour concentration similar to that of commercial beef flavour products. To examine the salt effect, 0, 0.165, or 0.330 g NaCl was added to give 0%, 3.0%, and 6% (w/w) added salt concentration, respectively. Since the simulated beef flavour originally had 20% salt, this resulted in 1.8%, 4.8% and 7.8% final salt concentration in the samples. These specific salt concentrations were selected within the range of salt levels of beef substitute products. The vial was tightly capped with a polypropylene hole cap with a PTFE/silicone septum. The SPME fiber (either 65 μm PDMS/DVB, 65 μm CW/DVB, or 50/30 μm DVB/CAR/PDMS) was exposed to the headspace above the sample solution for 20, 40, or 60 min at 30, 45, or 60 °C according to the experimental design shown in Table 2. Stirring with a magnetic stirring bar was consistently applied for all samples.

2.4. Optimization of adsorption time

2.5 g of previously prepared flavour stock solution and 3 g of 30 mM Tris–HCl buffer (pH 6.0) buffer solution were added in a 15 ml GC sampling vial with a magnetic stirring bar. The vial was tightly capped as described above. To study the effect of adsorption time at 60 °C on SPME by 50/30 μm DVB/CAR/PDMS fiber, samples were maintained for 20, 40, 60, 80, 100 or 120 min with stirring. All treatments were made in triplicate and average and standard deviation values were calculated.

2.5. Gas chromatography

A Hewlett–Packard 5890 gas chromatograph with flame ionization detector (FID) and a DB-5 analytical fused silica capillary column (30 m × 0.32 mm × 0.25 μm film thickness from J&W Scientific, Folsom, CA) were used for analysis of the volatile compounds. The injection was conducted in a splitless mode for 3 min at 250 °C. The oven temperature was held at 40 °C for 3 min, ramped to 180 °C at the rate of 3 °C/min and then to 260 °C at 10 °C/min, and maintained at 260 °C for 2 min. Helium was used as a carrier gas at a column-head pressure of 12 p.s.i. (1 p.s.i. = 6894.76 Pa). The temperature of the FID detector was 280 °C and it was supplied with 35 ml/min of hydrogen, 350 ml/min of air

and 30 ml/min of helium as a make-up gas. To increase reliability in terms of number of peaks and peak area, several parameters in the chromatogram were adjusted. Peak width was set to 0.04 and initial threshold was set to 1. The peaks with peak area under 10,000 were not regarded as reliable peaks.

A 0.75 mm I.D. inlet liner was employed to minimize broadening effect, and resulted in decreased peak width compared to a 2.0 mm injection glass liner. We also tested the type of injection mode (splitless/split), desorption time (0.5–3 min) and desorption temperature (220–260 °C) along with oven temperature programs to improve peak shape and sensitivity while reducing carry-over from the previous analysis. The detection of the analytes was improved using splitless mode, 3 min as desorption time, and 250 °C as desorption temperature (data not shown).

2.6. Statistical analysis

General linear model of analysis of variance (ANOVA) was performed by using Minitab (version 13.30, Minitab inc., PA, USA) to analyze the significance of the four main factors, namely, adsorption temperature (A), adsorption time (B), salt concentration (C), and SPME phase (D), and three interactions between the factors ($A \times B$, $A \times C$, and $A \times D$). Tukey's multiple-range test was conducted for comparison of mean values of obtained data at the 95% confidence level.

3. Results and discussion

3.1. Screening of significant factors on the headspace analysis of the beef flavour

The FID responses in terms of total area and number of peaks for the chromatograms of each of the 27 experimental runs are shown in Table 2. Typical total ion chromatograms (TICs) are illustrated in Fig. 2, which demonstrates the variation in both total peak area and number of peaks in the TICs of the headspace volatile compounds in simulated beef flavour under three different adsorption conditions corresponding to experiment numbers 25, 26, and 27 (Table 2).

Table 3 shows the results of ANOVA for significance of the main factors and the selected interactions between the factors, on the total peak area and the number of peaks of headspace volatile compounds in simulated beef flavour. For both total peak area and number of peaks, adsorption temperature and adsorption time were significant factors ($P \leqslant 0.05$) whereas SPME phase was highly significant ($P < 0.001$). Salt concentration did not have a significant effect ($P > 0.05$) within the studied range.

The number of interaction effects that can be examined is limited by the number of columns in a design, and

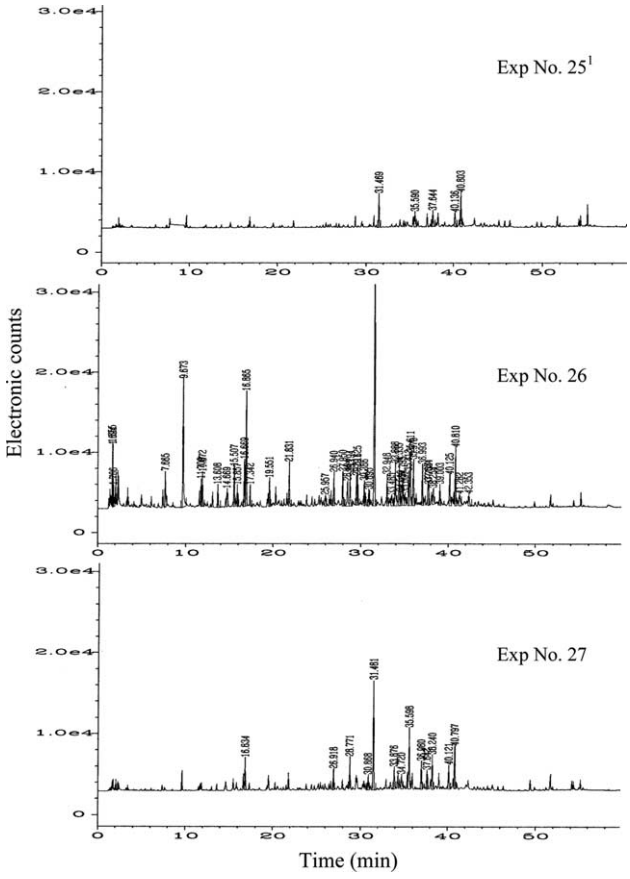

Fig. 2. Total ion chromatograms (TICs) of headspace volatile compounds in simulated beef flavour using the SPME adsorption conditions described in Table 2 for experiment number 25, 26, and 27. [1]Experiment numbers correspond to those of Table 2.

interactions that are higher than second order are assumed not to be important. In this experiment, in addition to the four main factors, three selected interactions were investigated, which were adsorption temperature × adsorption time, adsorption temperature × salt concentration, and adsorption temperature × SPME phase. These interactions were selected based on previous research results demonstrating that adsorption temperature was one of the most important factors affecting adsorption efficiency by SPME (del Castillo & Dobson, 2002; Diaz, Ventura, & Galceran, 2002; Liu & Yang, 2002; Steenson et al., 2002). None of the selected interactions was statistically significant at the 5% significance level.

3.2. Effects of SPME fibers

Based on the response of the total peak area obtained in preliminary experiments (data not shown), three types of SPME fibers (i.e. 65 μm PDMS/DVB, 65 μm CW/DVB, and 50/30 μm DVB/CAR/PDMS) were chosen from the four fibers mentioned above to conduct the fractional factorial experiment. Among the three SPME fibers

Table 3
Analysis of variance of the main factors and the selected interactions between the factors on the total peak area and the number of peaks of headspace volatile compounds in simulated beef flavour

Source	Degree of freedom	Total peak area		No. of peaks	
		F-value	Probability	*F*-value	Probability
A	2	5.14	0.050*	9.13	0.015*
B	2	5.95	0.038*	6.53	0.031*
C	2	3.10	0.119	3.52	0.098
D	2	36.13	0.000***	43.15	0.000***
A × B	4	1.05	0.455	1.53	0.304
A × C	4	0.56	0.702	0.43	0.780
A × D	4	2.46	0.156	2.82	0.124
Error	6	–	–	–	–

*, **, and *** significant at $p \leqslant 0.05$, $p \leqslant 0.01$, and $p \leqslant 0.001$, respectively.
A: adsorption temperature, B: adsorption time, C: salt concentration, and D: SPME phase.

tested, 50/30 μm DVB/CAR/PDMS fiber coating showed remarkably higher signal response ($P < 0.001$) than the other fibers for both total area and number of peaks (Fig. 3). Fig. 2 also illustrates the larger total peak area and number of peaks obtained using the 50/30 μm DVB/CAR/PDMS fiber. This fiber has improved stability of coating materials compared to other commercially available fibers (Supelco catalog 2003/2004). A coefficient of variation of 2.9% was observed for five replicate analyses of the total area counts of the headspace volatiles in beef flavour, using the 50/30 μm DVB/CAR/PDMS fiber for 60 min at 60 °C. In addition, no considerable carry-over from the previous extraction was observed after 3 min desorption in the injector. Therefore, 50/30 μm DVB/CAR/PDMS fiber was selected as the most suitable fiber due to the high sensitivity with good reproducibility as well as durable property of the fiber.

3.3. Effects of salt concentration

The addition of salt at concentrations up to 6% (which is equivalent to 7.8% of total salt concentration considering the indigenous salt content of the flavour) did not significantly affect either total peak area or the number of peaks of the beef flavour samples as shown in Table 3. Generally, the presence of salt has been reported to stimulate adsorption of the volatile components from samples by changing the phase border properties and decreasing the solubility of hydrophobic components in the aqueous phase, the so called "salting out" effect (Yang & Peppard, 1994). However, a salt concentration of 20–30% (w/v) was suggested to be required to yield an adsorption effect for most flavour compounds (Lee, Diono, Kim, & Min, 2003a). Liu and Yang (2002) reported that low concentrations of salt in the range of 0–10% did not affect adsorption efficiency of volatiles such as ethyl isovalerate and isoamyl acetate while adsorption of both compounds significantly increased at 20% salt. In our study, an upper level of up to 6% of sodium chloride was added since higher salt concentration would not be relevant in the context of the type of food systems under consideration, i.e. beef flavoured vegetable products. High concentration of salt is also known to stimulate denaturation of proteins (Cheftel, Cuq, & Lorient, 1985).

3.4. Effects of adsorption temperature

As adsorption temperature increased from 45 to 60 °C, both total peak area ($p < 0.1$) as well as the number of total peaks ($p < 0.05$) in the GC chromatograms of the samples increased while there was no significant difference between 30 and 45 °C (Fig. 3). The increases of volatile compounds can be explained by the fact that higher temperature tends to drive the volatiles from the liquid phase to the gas phase. Diaz et al. (2002) also demonstrated that the adsorption efficiency of brominated analogs in water increased by increasing the temperature up to 50 °C and Steenson et al. (2002) found an increase of volatile compounds in vegetable oils with higher extraction temperature. In this study, 60 °C was proposed as the adsorption temperature for the beef flavour due to the high sensitivity of both total area counts and number of peaks. Furthermore, beef analogue products such as vegetarian hot dogs are frequently served at temperatures close to 60 °C (Yves Veggie Cuisine, 2002, personal communication).

3.5. Effects of adsorption time

Table 3 shows adsorption time was also one of the significant factors in the analysis of headspace volatile compounds of the beef flavour. The effect of adsorption time on the analysis of headspace volatile compounds is illustrated in Fig. 3. Even though there were no statistically significant differences between the responses at 20 and 40 min or at 40 and 60 min, significant difference was found between 20 and 60 min ($P < 0.05$) in total peak area in addition to the number of total peaks. The increase of volatile compounds with adsorption time was also reported by other research groups (Steenson et al., 2002; Tombesi & Freije, 2002). In contrast, Lee

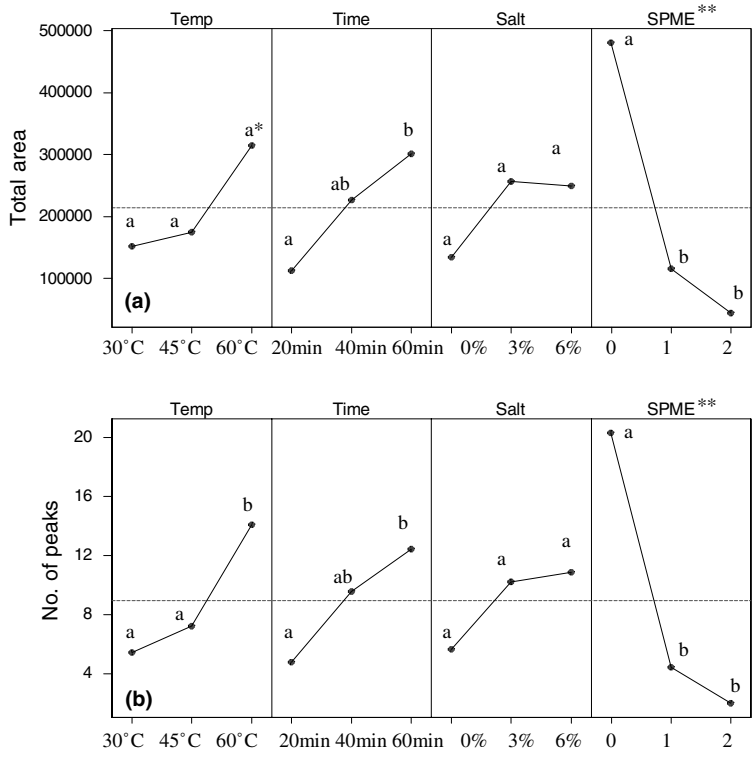

Fig. 3. Effects of adsorption temperature, adsorption time, salt concentration, and SPME phase on (a) means of total area counts and (b) means of the number of peaks obtained in the analysis of headspace volatile compounds in simulated beef flavour Different letters (a and b) within each plot indicate significant difference ($P < 0.05$). *Different at 10% significance level ($P = 0.0571$). **Levels 0, 1, and 2 of the SPME Phase refer to 50/30 μm DVB/CAR/PDMS, 65 μm PDMS/DVB, and 65 μm CW/DVB, respectively.

et al. (2003b) reported that the isolation of volatile compounds in kimchi adsorbed onto SPME increased with longer time up to 30 min, while it tended to decrease at times longer than 30 min depending on the adsorption temperature.

Since there was no interaction between factors, subsequent experiments were conducted using 50/30 μm DVB/CAR/PDMS fiber at 60 °C to investigate the adsorption time required to reach maximum total area responses of the samples. Fig. 4 shows the effect of adsorption time at 60 °C by 50/30 μm DVB/CAR/PDMS fiber on the headspace volatile compounds of the beef flavour. The total area of volatile compounds was increased with longer adsorption times up to 80 min ($p < 0.05$) when equilibrium between the headspace of the beef flavour sample and the SPME fiber was reached. However, it was suggested that the SPME sampling time should be no longer than the total GC run time for maximum productivity, because good precision can be accomplished without achieving equilibrium as long as the adsorption time is controlled accurately (Penton, 1999). Therefore, the adsorption time of 60 min was selected, considering a good reflection of transferring of volatile compounds from sample solution to headspace, a suitable analysis time for the routine analysis, and a low coefficient of variation (2.9%).

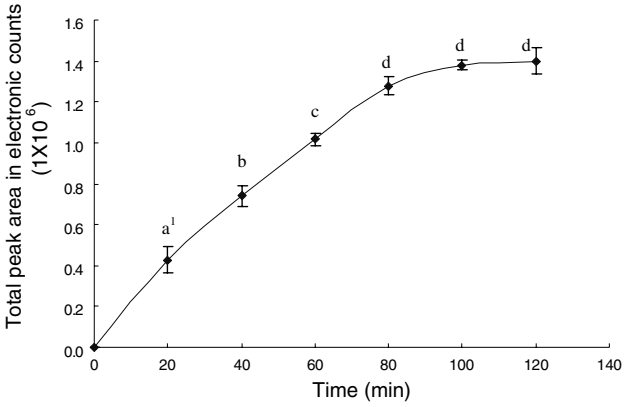

Fig. 4. Effect of adsorption time on the headspace volatile analysis of simulated beef flavour by 50/30 μm DVB/CAR/PDMS SPME at 60 °C. Points are averages from triplicate analyses and error bars are ±standard deviation. [1]Different letters indicate significant difference ($P < 0.05$).

4. Conclusion

This study has demonstrated the application of fractional factorial experimental design based on Taguchi's orthogonal array for screening the significant factors with SPME for analysis of the headspace volatile compounds in simulated beef flavour. Out of four main

factors and three potential interactions researched, adsorption temperature, adsorption time, and SPME phase were the factors that significantly affect the headspace analysis of the simulated beef flavour in terms of the total peak area and the number of peaks. The selected adsorption conditions for SPME were adsorption by 50/30 μm DVB/CAR/PDMS fiber for 60 min at 60 °C. The establishment of this reproducible and representative analytical method by HS-SPME paves the way for ongoing research to monitor changes in the headspace composition of the simulated beef flavour, to elucidate the basis for the interactions between flavour compounds and soy protein in vegetarian food products.

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