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A Headspace Solid Phase Microextraction Method for Using to Monitor Hexanal and Heptanal Content in Food Samples

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ABSTRACT

Headspace solid phase microextraction (HS-SPME) technique coupled with Gas Chromatography (GC) was developed to measure hexanal and heptanal (off-flavour compounds, occurred from lipid oxidation) in some food samples. The HS-SPME and GC parameters were studied. Six food samples; infant cereal, instant noodle, potato French fried, deep fried peanut, fried rice (Khawtan) and fried pork skin (Capmoo) were analyzed. Hexanal and heptanal were found in the range of 0.15-10.40 μg/g and 0.14-1.65 μg/g, respectively. Hexanal content in Capmoo increased significantly during storage. It was found that increase of lipid content in food samples can reduce the analysis recoveries.

Key words: hexanal, heptanal, headspace solid-phase microextraction

INTRODUCTION

Oxidative degradation due to autooxidation of polyunsaturated fatty acids, present in variable amounts in most foods, is one of the well recognized factors that may limit the shelf-life of lipid containing foods. The loss of food quality through flavour deterioration, decrease in nutritional value, and the generation of potentially toxic substances can have a significant impact on food safety (Fenaille *et al.,* 2003). The primary oxidation products for unsaturated fatty acids are hydroperoxides, highly reactive compounds that decompose rapidly, yielding a complex mixture of non-volatile compounds and volatile compounds such as hydrocarbons (ethane and pentane), aldehydes (pentanal, hexanal, hexenal, 2-octenal and 2-nonenal) and ketones, which affect the overall quality of the product. Aldehydes are particularly important in relation to flavour alteration and a toxicological perspective [Frankel, 1993]. The major product of fat oxidation that increases in content during storage is hexanal. This has become a known indicator of fat oxidation (Brunton et al., 2000). Hexanal content is directly related to oxidative off-flavours, and the compound is easily detected because of its low odour threshold (5 ppb) (Buttery *et al.,* 1988). Different methods have been reported for determining volatile compounds originated during lipid oxidation, different sampling techniques have been described to isolate and concentrate volatile compounds prior to GC injection including liquid extraction, solid phase extraction, headspace and headspace solid-phase microextraction. The present study optimizes and validates a headspace solid phase microextraction (HS-SPME) for determining hexanal and heptanal in some Thai foods that is useful in monitoring the quality of the products during storage.

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MATERIALS AND METHODS

Samples

All the products: infant cereal, instant noodle, potato French fried, deep fried peanut, baked Thai rice (Khawtan) and fried pork skin (Capmoo) were kept in their original sealed containers at room temperature.

Reagents and materials

The reagard and materials need in this study were: Hexanal and heptanal (Fluka); methanol (Fisher Scientific); sodium chloride and sodium hydroxide (Ajax Fine- chem); hydrochloric acid (J. T. Baker); manual type SPME holder and SPME fibers: CAR/PDMS, CW/PDMS, PDMS/DVB DVB/CAR/PDMS and PDMS (Supelco). All solvents and reagents used in this study were of analytical grade purity.

Preparation of standard solutions

The 1000 ppm stock solution of mixed hexanal and heptanal in methanol was prepared and kept in a refrigerator. The working standard solutions and aqueous synthetic samples were prepared daily by appropriate dilution of stock solution.

HS-SPME procedure

10.0 mL of standard solution or sample solution (1.00 g of powered sample in 10.00 mL solution) were prepared in a 20-mL headspace vial that was immediately crimped with an aluminium seal containing a rubber septum. The vials were shaken and stayed at room temperature (25°C), a conditioned (1 h at 200°C) SPME fiber was exposed to the headspace of the sample. SPME holder assembly was adjusted to 3.0 scale units to ensure that the fiber was identically positioned from run to run. Following adsorption process, the fiber was immediately inserted in the GC injection port.

GC-FID analysis

Analysis of hexanal and heptanal was carried out using a Varian Gas Chromatograph model CP 3800 equipped with a Flame Ionization Detector (FID).

Method optimization

The analytical conditions including GC conditions; oven temperature and carrier gas flow rate and HS-SPME conditions; type of SPME fiber, desorption temperature, desorption time, adsorption temperature, adsorption time, pH of solution and sodium chloride concentration for hexanal and heptanal determination were optimized and the detail was shown in Table 1. In the optimization of the method, synthetic aqueous sample (10.0 ppm of hexanal and heptanal in water) was used.

Analysis of samples

The method was applied to six food samples. Two of the samples: Capmoo (high content of lipid) and Khawtan (low content of lipid) were analyzed after 1, 2, 5, 7, 10, and 14 storage weeks at the same conditions.

RESULTS AND DISCUSSION

Optimization

It was found that the oven temperature of 60 °C and carrier gas flow rate of 2.0 mL/min was the most suitable condition because hexanal and heptanal can be separated completely in the short time. The retention times of hexanal and heptanal were 2. 21 and 3.32 min, respectively as shown in Figure 1.

The result of fiber types was shown in Figure 2. It was found that 3 types of fiber: CAR/PDMS or DVB/CAR/PDMS or PDMS/DVB were suitable to be use in this study because hexanal and heptanal were detected with greater peak areas than CW/DVB and PDMS, respectively. PDMS/DVB fiber was selected to be used in this study

Table 1 GC and HS-SPME conditions.

GC CONDITIONS:	
GC column	30 m × 0.53 mm × 5 μm film thickness an Equity 5™ (5% diphenyl/ 95% dimethyl-siloxane capillary column)
Oven temperature	50 °C, 60 °C and 70 °C
Carrier gas (He) flow rate	1.0 mL/min and 2.0 mL/min
HS-SPME CONDITIONS:	
SPME fiber type	PDMS, CAR/PDMS, DVB/CAR/PDMS, CW/DVB and PDMS/DVB
Desorption temperature	100 °C, 150 °C and 200 °C
Desorption time	0.5, 1, 2, 3 and 5 min
Adsorption temperature	25 °C (room temp), 60 °C, 70 °C, 80 °C and 90 °C
Adsorption time	2, 5, 7, 10, 15, 20 and 30 min
pH	3.0 and 10.0
Sodium chloride concentration	0, 0.5, 1.0, 2.0 and 3.0* g/10 mL(*saturated solution)

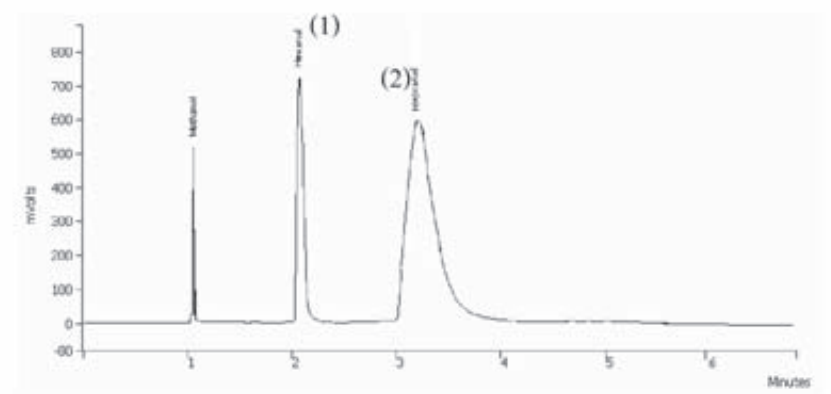

Figure 1 GC chromatogram of headspace gas of synthetic aqueous sample peak (1) is hexanal and peak (2) is heptanal.

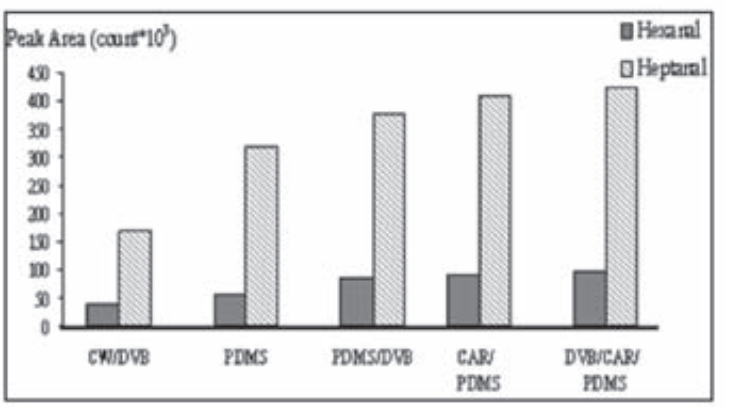

Figure 2 Influence of fiber types on peak area of hexanal and heptanal.

with the reason of flexibility and availability.

The results of varying desorption temperature, desorption time, adsorption temperature, adsorption time, pH of solution and sodium chloride concentration were shown in Figure 3

From the experiment, the fiber type DVB/CAR/PDMS was found to be the most appropriate type for detecting of hexanal and heptanal, due to the result of high peak area, and the less efficiency types are CAR/PDMS, PDMS/DVB, CW/DVB, and PDMS respectively. However, PDMS/DVB type was selected for this experiment because the reason of flexibility and availability. The desorption at temperature of 150 °C was selected from the result of the highest peak area within 3 minutes of desorption time. There was no significant difference desorption between 3 minutes and 5 minutes desorption time, and the result of adsorption temperature shown peak area decreased while the temperature increased. Therefore, the test performed at temperature of 25°C which was general controlled room temperature, and adsorption time was 10

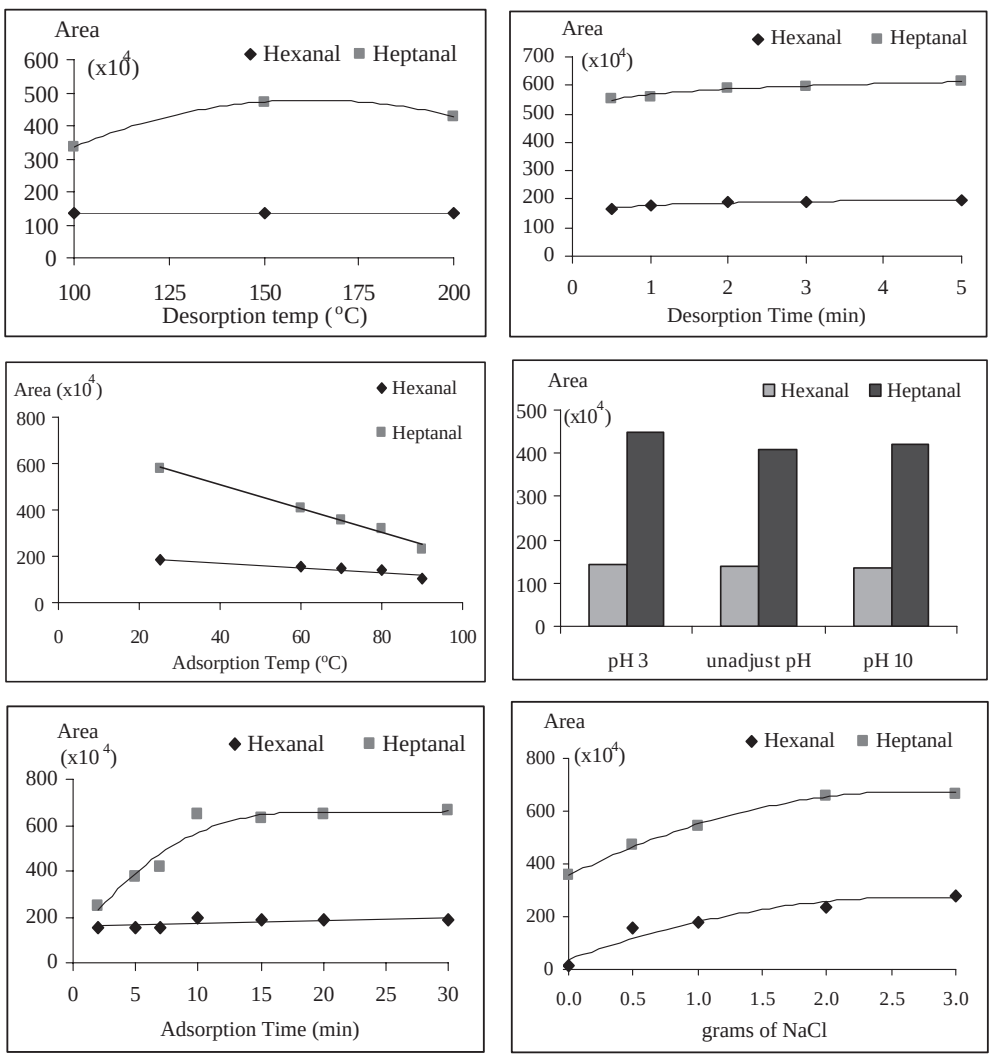

Figure 3 Results of HS-SPME conditions on peak areas of hexanal and heptanal.

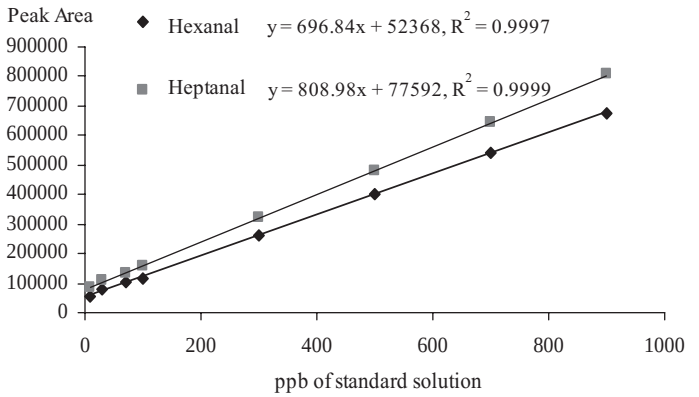

Figure 4 Calibration curve of hexanal and heptanal standard solution (range 1 ppb-900 ppb).

minutes. The result of pH adjustment affected the peak area of heptanal, but rarely not affect that of hexanal. Then the test was set to perform without adjustment of pH, and add some NaCl for increase amount of salt to increase peak area result.

Linearity of Calibration curve

A plot of the peak areas against the concentrations of hexanal and heptanal was obtained as shown in Figure 4. It showed a good linearity with a correlation coefficient (R^2) > 0.999.

Detection and quantification limit

The detection limits (LOD) of hexanal and heptanal, estimated with defined as signal 3 times the height of the noise level were below the ppb level. The quantification limits (LOQ) obtained for hexanal and heptanal were 10 ppb which correspond to 100 ng/g in sample.

Hexanal and heptanal content in food samples

Six food samples; infant cereal, instant noodle, potato French fried, deep fried peanut, fried rice (Khawtan) and fried pork skin (Capmoo) were analyzed at the optimum conditions. Hexanal and heptanal contents in the analyzed foods were shown in Table 2.

Effect of storage time

The hexanal and heptanal contents of stored samples were determined and the results were shown in Table 3 and Figure 6. It was found that hexanal content in Capmoo increased significantly from 0.95 to 14.23 μg/g during increase of the storage time due to lipid oxidation resulting in deterioration of food quality. Hexanal

Table 2 Hexanal and heptanal content in food samples.

Sample	Average content (mg/g)	
	Hexanal	Heptanal
Potato chip	0.15	0.20
Infant cereal	0.31	0.17
Instant noodle	0.34	0.22
Fried rice (Khawtan)	0.25	0.14
Fried peanut	5.23	0.23
Fried pork skill (Capmoo) sample I	0.37	0.15
Fried pork skill (Capmoo) sample II	10.40	1.65

is an off-flavour and it was used as a good indicator of rancidity. The onset of rancid odours was found to occur when the hexanal content in Capmoo increased to 10 μg/g. During this time, hexanal content in Khawtan, a low fat snake, heptanal content in both Khawtan and Capmoo not significantly increased.

Effect, of oil content in sample

Palm oil was added into Khawtan prior

Table 3 Hexanal and heptanal contents in samples at various storage times.

Storage time (Weeks)	μg/g of hexanal and heptanal in samples			
	Capmoo		Khawtan	
	Hexanal	Heptanal	Hexanal	Heptanal
1	0.95	0.19	0.38	0.17
2	2.32	0.22	0.42	0.18
5	9.60	0.48	0.47	0.20
7	11.60	0.53	0.51	0.21
10	13.27	0.72	0.53	0.26
14	14.23	0.69	0.57	0.28

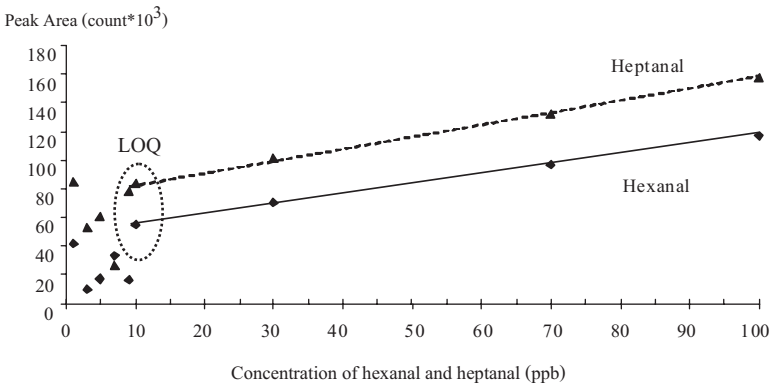

Figure 5 LOQ of hexanal and heptanal determination was found at 10 ppb.

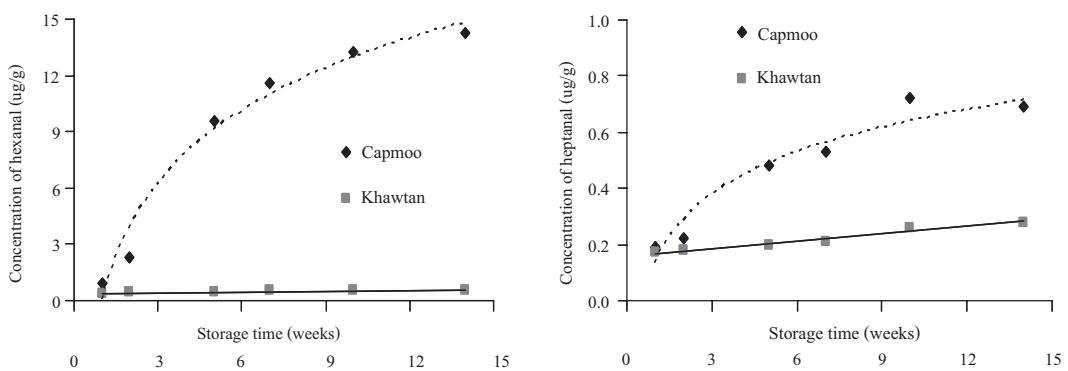

Figure 6 Effects of storage time on hexanal and heptanal contents in Capmoo and Khawtan.

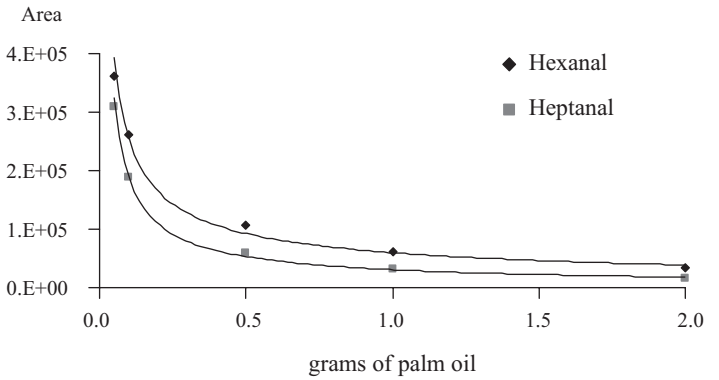

Figure 7 The effects of oil contents on the peak areas of hexanal and heptanal in Khawtan.

to analysis with different weights and the results was shown in Figure 7. It was found that the oil content in sample could be reduce the GC response due to decrease of volatility of analyses.

Precision and accuracy

The reproducibility of the method was determined by the measurements of four samples which had good precision with relative standard deviation values of 0.9-4.0 % and 0.5-2.3 % (n=3) for hexanal and heptanal, respectively. Accuracy was estimated by recovery assays. The recovery percentages of hexanal and heptanal were obtained by spiking aliquots of the sample with an amount of standard. Recovery percentages of 77.3 % and 80.9 % were obtained for hexanal and heptanal, respectively.

CONCLUSION

HS-SPME/GC-FID is useful for determining hexanal and heptanal contents in foods. The optimum condition for extraction was investigated. 1.00 g sample in 10 mL saturated NaCl solution was prepared in 20 mL headspace vial, 3 types of fiber: CAR/PDMS or DVB/CAR/PDMS or PDMS/DVB could be be used at an adsorption time of 10 min and desorption in GC injector at 150 ^{o}C for 3 min. Linearity is verified over a wide range (10 ppb-900 ppb with R^2 = 0.999). This method shows good precision with RSD values of 0.5-4.0 %, the recovery percentages range from 77.3 % to 80.9 % and the detection limit below the ng/g level.

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