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Headspace Volatiles from Heated Pork Fat

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ABSTRACT

Volatile chemicals formed in the headspace of heated pork fat were purged into dichloromethane, and the chemicals trapped were analyzed by gas chromatography and gas chromatography/mass spectrometry. Volatile compounds identified in the headspace samples were nine n-alkanes, 19 n-alkenes, eight cyclic alkanes, three cyclic alkenes, five aromatic hydrocarbons and three ketones. Fifty-three peaks out of 124 peaks on the gas chromatogram of six replicated headspace samples were used for the statistical treatments.

INTRODUCTION

The study of volatile chemicals formed in cooked foods has been one of the most active areas of flavor chemistry. In the 1970s, volatile compounds formed in cooked meat received much attention and numerous flavor chemicals were isolated and identified from various cooked meats (Persson & Von Sydow, 1973; Wilson *et al.*, 1973; Mussinan & Walradt, 1974). Most important flavor chemicals found in cooked meats are heterocyclic compounds including pyrazines, thiazoles, thiophenes, and pyridines (Shibamoto, 1980), which are known to form from carbonyl and amine compounds via the Maillard reaction (Bailey, 1983). Recently, lipids have

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come to be considered precursors of carbonyl compounds in the Maillard reaction (Ohnishi & Shibamoto, 1984). In fact, it has long been known that lipids play an important role in the formation of flavor chemicals in cooked meat (Yamamoto *et al.*, 1970). Thus the identification of volatiles formed from lipids is a necessary step to better understand the complex processes involved in flavor changes of food during cooking.

The isolation of volatile chemicals from lipids or lipid-rich samples is one of the most difficult analytical procedures. The most common method is to perform organic solvent extraction followed by steam distillation. Headspace sampling is also widely used to isolate volatiles from a lipid sample. The headspace of a heated sample is purged by either a nitrogen stream or an air stream, and volatiles are entrained onto porous polymers such as Tenax or Porapak Q (Uchman & Jennings, 1977; Mottram *et al.*, 1982, 1984; Mottram, 1985). In the present study, qualitative and quantitative analysis of headspace volatiles obtained from heated pork fat were conducted by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

MATERIALS AND METHODS

Materials

Pork fat was purchased from a local market. Fat tissue was ground with small amounts of dry ice in a blender. The pulverized, solidified fat was placed in a glass container and maintained in a water bath at 70–80°C. Nonfat materials such as blood, muscle, and connective tissue were denatured by the heat and removed from the melted fat by filtration. The purified pork fat was weighed (1260 g) and stored at −5°C for later use. Authentic reference compounds were obtained from reliable commercial sources.

Sample preparations

Headspace samples were collected using the apparatus shown in Fig. 1. Two traps were filled with 40 ml each of dichloromethane and chilled in an ice bath during sample collection. The purified pork fat (150 g) was placed in a two-necked flask and heated at 300°C with a mantle heater. The headspace volatiles formed from pork fat were purged with purified air into the dichloromethane traps for 6 h. The dichloromethane from the two traps was combined even though the gas chromatogram of the second trap showed no peaks. After the combined solution was dried over anhydrous sodium sulfate for 12 h, it was concentrated by distillation. The experiment was

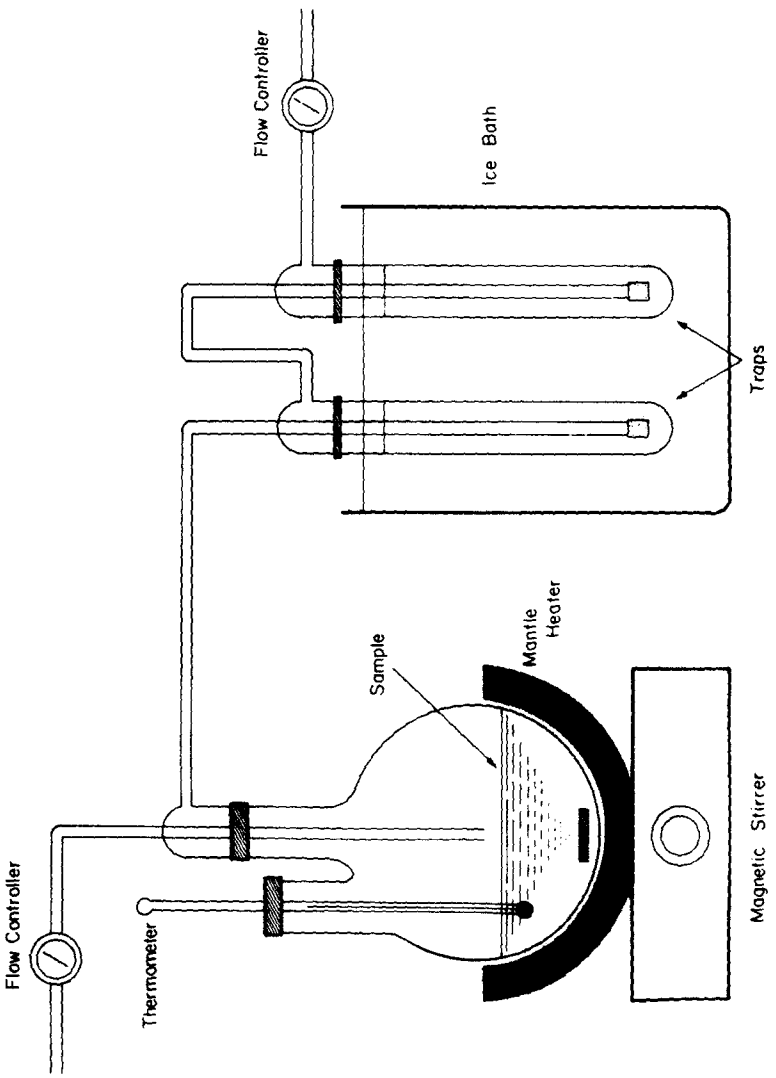

Fig. 1. Apparatus used for headspace sampling.

TABLE 1
Volume of Air Purged for Each Sample

Sample No.	*Volume (ml)*	*Average flow rate (ml/min)*
1	5082	14·1
2	4271	11·9
3	6636	18·4
4	6866	19·1
5	7570	21·0
6	6520	18·1

replicated six times using samples of the same purified pork fat. The calculated air volumes purged into the traps for each experiment are shown in Table 1.

When the air stream (7–10 ml/min) was passed through two blank dichloromethane traps cooled at 0°C for 7 h, the amount of dichloromethane in the first and second traps reduced from 40 ml to 38·3 ml and 39·0 ml, respectively, indicating that the amount of volatiles escaping from the traps during collection was negligible.

Qualitative and quantitative analysis of compounds in the headspace samples

Qualitative analysis was conducted using Kovats GC retention index (Kovats, 1965) and GC/MS techniques. The Kovats index (I) and MS fragmentation pattern of each component were compared with those of the authentic chemicals to identify the constituents of the samples. Quantitative analysis was conducted by GC using a standard curve of *n*-undecane (Ettre, 1967).

Statistical treatment of data

Among the nearly 200 peaks observed on the gas chromatogram, 124 peaks were used for the statistical treatment with the following equation (Yasuhara *et al.*, 1987):

$$S(A, B) = \sum(A_i B_i)/[\sqrt{\sum(A_i^2)} \times \sqrt{\sum(B_i^2)}]$$

where S is a factor of similarity between samples A and B, and A_i and B_i are the relative concentrations of component i in samples A and B, respectively. Interpretations of X values are as follows: $S \geq 0{\cdot}9$, almost identical; $0{\cdot}9 > S \geq 0{\cdot}8$, very similar; $0{\cdot}8 > S \geq 0{\cdot}7$, fairly similar; $0{\cdot}7 > S \geq 0{\cdot}5$, slightly similar; $S < 0{\cdot}5$, not similar.

Instruments

A Hewlett-Packard Model 5890 GC equipped with a 30 m × 0·25 mm i.d. DB-1 fused silica capillary column and a flame ionization detector was used for the qualitative and quantitative analyses of the samples. The oven temperature was held at 40°C for 5 min and then programmed to 250°C at 4°C/min. The injector temperature was 250°C and the detector temperature was 280°C. The injector split ratio was 1:45. The GC peak areas were integrated with an HP 3390-A reporting integrator. A Hewlett-Packard Model 5890 GC interfaced to a VG Trio II mass spectrometer with VG 11-250 computer data system was used for MS identification of the GC components at MS ionization voltage 70 eV. The column and oven conditions for GC/MS were as described for the HP 5890 GC.

RESULTS AND DISCUSSION

The compounds identified and their calculated concentrations in the headspace from heated pork fat are shown in Table 2.

TABLE 2
Compounds Identified in Headspace Samples of Heated Pork Fat

Compound	$I_{DB\text{-}1}$[a]	*Concentration in headspace (μg/l)*[b]
Alkanes		
n-hexane	600	14·8 ± 5·16
n-heptane	700	233 ± 88·6
n-octane	800	522 ± 177
n-nonane	900	673 ± 327
n-decane	1 000	135 ± 81·2
n-undecane	1 100	274 ± 26·9
n-dodecane	1 200	102 ± 98·2
n-tridecane	1 300	23·5 ± 9·27
n-tetradecane	1 400	12·4 ± 12·8
Alkenes		
C_6H_{12}	643	21·2 ± 14·4
C_6H_{10}	669	71·7 ± 35·9
(Z)-2-heptene	704	26·8 ± 4·70
(E)-2-heptene	711	32·4 ± 5·26
3-heptene	717	47·1 ± 32·9
3-ethyl-2-pentene	728	42·2 ± 68·2
C_7H_{12}	775	55·1 ± 20·7
1-octene	794	19·2 ± 3·70

(*continued*)

TABLE 2—*contd.*

Compound	$I_{DB\text{-}1}$[a]	*Concentration in headspace (μg/l)*[b]
C_8H_{16}	797	37·5[c]
(Z)-2-octene	804	152 ± 94·0
(E)-2-octene	910	72·0 ± 68·4
1-nonene	895	45·1 ± 10·0
(Z)-2-nonene	902	73·2[c]
(E)-2-nonene	910	39·4 ± 5·78
1-decene	993	20·3 ± 2·36
(Z)-2-decene	1 003	51·7 ± 24·2
(E)-2-decene	1 009	29·5 ± 8·60
1-undecene	1 093	20·8 ± 5·56
(Z)-2-undecene	1 106	8·94 ± 5·43
(E)-2-undecene	1 110	137 ± 31·6
$C_{11}H_{20}$	1 121	54·6 ± 54·1
$C_{11}H_{20}$	1 191	42·8 ± 27·5
1-dodecene	1 191	28·2 ± 11·0
(Z)-2-dodecene	1 205	3·21[c]
(E)-2-dodecene	1 210	16·4 ± 9·76
Cyclic alkanes		
cyclopentane	685	305 ± 49·2
methylcyclohexane	767	6·15 ± 1·82
cyclooctane	787	407 ± 75·0
ethylcyclohexane	883	14·2 ± 7·96
propylcyclohexane	986	129 ± 27·7
butylcyclohexane	1 082	14·2 ± 7·96
pentylcyclohexane	1 184	95·0 ± 27·9
hexylcyclohexane	1 287	9·04 ± 7·31
Cyclic alkenes		
ethylcyclopentene	714	3·30[c]
1-methylcyclohexane	730	18·3 ± 20·0
4-methylcyclohexane	740	1·76 ± 1·83
Aromatic hydrocarbons		
toluene	750	17·3 ± 2·33
ethylbenzene	847	31·3 ± 26·1
propylbenzene	930	18·9 ± 13·3
butylbenzene	1 042	56·3 ± 19·7
pentylbenzene	1 144	20·4 ± 15·9
Ketones		
2-butanone	572	22·1 ± 8·97
cyclopentanone	755	58·7 ± 21·8
cyclohexanone	860	70·9 ± 30·4

[a] Kovat Index on DB-1.
[b] Values are mean ± standard deviation of three replicated samples.
[c] Values in two samples are less than 0·01.

TABLE 3
The Pattern Similarity Factors (S) for the Six Replicate Samples

Sample No.	*No. 1*	*No. 2*	*No. 3*	*No. 4*	*No. 5*	*No. 6*
1	—	0·644	0·667	0·589	0·671	0·562
2			0·800	0·859	0·772	0·834
3				0·858	0·527	0·808
4					0·768	0·980
5						0·715
6						—

Even though heterocyclic compounds such as pyrazines and thiazoles are major volatiles found in cooked pork (Mussinan & Walradt, 1974), no heterocyclic compounds were identified due to lack of nitrogen and sulfur sources in the present study (see Table 2). Mottram (1985) found that headspace volatiles of pork were dominated by aldehydes and alcohols originating from the thermal oxidation of lipids. However, saturated and unsaturated hydrocarbons were the major constituents of the headspace volatiles of pork fat in the present study. Identification of those hydrocarbons, including *n*-alkanes, *n*-alkenes, and alkylcyclohexanes, is consistent with our previous works (Ohnishi & Shibamoto, 1984; Umano & Shibamoto, 1987).

The GC of each replicated sample showed some differences in volatile composition even though the collection conditions were held as equal as possible. The S values of six replicated samples are shown in Table 3. Samples 3, 4 and 6 were chosen to report concentration of each component in the headspace because the S values for their interactions (3, 4; 3, 6; and 4, 6) were greater than 0·8 (very similar). The variation in composition of the replicated samples was rather large. This may be due to the variations in the amount of air purged. In addition to the flow rate fluctuation, many factors may be involved in the formation of volatile chemicals from the thermal degradation of lipids, including variations in temperature, oxygen content, and the presence of formation of catalysts. Once degradation occurs, reactions may not progress in the same pattern for each fat sample, and subsequently, variation in the composition of headspace will occur.

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