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Analytical Methods

Analysis of volatile compounds in traditional smoke-cured bacon (CSCB) with different fiber coatings using SPME

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Abstract

The volatile compounds of Chinese traditional smoke-cured bacon (CSCB) were studied using SPMS-GC/MS. There were 48 volatile compounds identified and quantified, which belonged to several classes of chemical: 1 alkane, 16 aldehydes, 5 ketones, 9 alcohols, 4 thioethers and thiols, 3 furans and 10 phenols compounds. All the volatile compounds except for alkane was responsible for CSCB characteristic flavor. The major volatile compounds of CSCB came from smoking, oxidation and Maillard reaction, etc. Many volatile compounds were not reported in previous paper isolated by steam distillation method or nitrogen purge-and-steam distillation method on CSCB. It should be because of different method of isolating volatile substances from CSCB. Among the fibers tested, CAR/PDMS (carboxen/polydimethylsiloxane) fiber coating showed the highest area counts for most volatile compounds. CAR/PDMS coating extracted better those compounds whose linear retention indices (LRI) was lower than 926 (on average) and DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) those with higher LRI.

Keywords: Smoke-cured bacon; GC–MS; Meat; Volatile compounds; Solid-phase microextraction

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1. Introduction

Chinese traditional smoke-cured bacon (CSCB) is a traditional meat product in China. It is made by curing and smoking and has a distinctive flavor considered as tangy, delicate, meaty and salty (Yu & Sun, 2005). By far, the reports about volatile compounds of CSCB separated by steam distillation method or nitrogen purge-and-steam distillation method have only two papers and conclude that the phenolic derivatives are responsible for the typical flavor (Yu & Sun, 2005; Yu & Wu, 2003).

Solid-phase microextraction (SPME) is a versatile sample preparation technique (Arthurand & Pawliszyn, 1990) that has been used, among other applications, to describe the volatile flavor profile of foodstuffs (Yang & Peppard, 1994). In comparison to traditional techniques for analyzing volatile constituents of foodstuffs, SPME is inexpensive, solvent free, easy to handle, sensitive, and selective (Zhang & Pawliszyn, 1993). In addition, SPME allows using mild sampling conditions, such as an extraction temperature below 65 °C, avoiding artifacts formation during sample analysis (Nigel, Brunton, Cronin, & Monahan, 2000).

The key component of an SPME device is a fused-silica fiber that is coated on the outside with an appropriate stationary phase. Analytes from the sample are directly extracted and concentrated onto the fiber coating. The choice of the fiber coating is a key factor, since the type and amount of compounds that are extracted from the sample depends on the physico-chemical characteristics of the fiber stationary phase and on the film thickness. The affinity of the fiber for an analyte relies on the principle

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of ‘like dissolves like’, and coating fibers having different properties or thickness are selected in accordance with target compounds (Kataoka, Lord, & Pawliszyn, 2000). Non-polar polydimethylsiloxane (PDMS) fiber is preferred for the extraction of non-polar analytes, whereas the more polar polyacrilate (PA) fiber is more appropriate for the extraction of polar analytes. Mixed coating fibers, containing divinylbenzene (DVB) copolymers, templated resin (TPR) or carboxen (CAR) increase retention capacity. PDMS/DVB and CAR/DVB can be used for the extraction of low molecular weight volatile and polar analytes (Kataoka et al., 2000).

The objective of the present study is to analyze the profile of the volatile compounds of CSCB by SPME to increase the knowledge of the substances responsible for its characteristic flavor, and to study the effect of fiber coating on the volatile profile of CSCB analyzed by SPME, in order to determine the most suitable fiber for further analysis.

2. Materials and methods

2.1. Material

CSCB was prepared by traditional method of southwest China (Yu & Sun, 2005). Ground CSCB 5.0 g was placed in a 50 mL flask. Twenty-five milliliters of distilled water was added, and the ground CSCB were refluxed for 60 min in an oil bath at 100 °C. The cooked smoke-cured bacon samples were cooled to room temperature.

2.2. Headspace solid-phase microextraction–gas chromatography–mass spectrometry (headspace-SPME–GC–MS)

2.2.1. SPME sampling

The assayed fibers were carboxen/polydimethylsiloxane (CAR/PDMS) (75 μm thickness), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm thickness) and polydimethylsiloxane/divinylbenzene (PDMS/DVB) (65 μm thickness) (Supelco, Bellefonte, PA, USA). Before the SPME fiber was inserted into the vial, the sample was equilibrated for 15 min at 60 °C. The extraction time is 30 min at 60 °C.

Prior to analysis the SPME fiber was preconditioned in the injection port of the gas chromatograph at the temperature and for the time suggested by the manufacturers (CAR/PDMS is 270 °C, DVB/CAR/PDMS and PDMS/DVB are 250 °C for 2 h.). This temperature was the same as that set for the injection port of the gas chromatograph during desorption. Temperatures set for desorption were 280 °C for CAR/PDMS, 260 °C for DVB/CAR/PDMS and PDMS/DVB. Analysis of each tested condition was repeated twice.

2.2.2. Gas chromatography–mass spectrometry

Analyzes were performed using a Agilent 6890N gas chromatograph coupled to a Agilent 5975i mass selective detector (Agilent Technologies, Inc., USA). Volatiles were separated using a DB-5 capillary column [30 m × 0.25 mm(i.d) × 0.25 μm]. The SPME fiber was desorbed and maintained in the injection port at the oven temperature (280 °C for CAR/PDMS, 260 °C for DVB/CAR/PDMS and PDMS/DVB) and for the time (5.0 min) suggested by manufacturers. The injection port was in splitless mode. The temperature program was isothermal for 10 min at 40 °C, raised to 200 °C at a rate of 5 °C min^{-1} and then raised to 250 °C at a rate of 20 °C min^{-1} and held for 5 min. C_5–C_{22} *n*-alkanes (Pure Chemical Analysis Co., Ltd.) were run under the same chromatographic conditions as the samples to calculate the linear retention indices (LRI) of detected compounds. The transfer line to the mass spectrometer was maintained at 280 °C. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1753 V, and collecting data at a rate of 1 scan s^{-1} over the *m*/*z* range of 30–550 u.m.a. Compounds were tentatively identified by comparing their mass spectra with those contained in the Nist05 and Wiley275 libraries and by comparison of their LRI with those reported in the literature. Area counts of volatiles were provided by integrating in initial threshold 18.0 using Agilent chemstation.

3. Results and discussion

3.1. Volatile Compounds of CSCB

We identified 48 volatile compounds in CSCB samples, which included 1 alkane, 16 aldehydes, 5 ketones, 9 alcohols, 4 thioethers and thiols, 3 furans and 10 phenols compounds. Total volatile compounds isolated are presented in Table 1. The predominant volatile compound (peak area $>10^8$) in CSCB was hexanal, followed by pentanal, nonanal, octanal, heptanal, benzaldehyde, 2-methoxyphenol and 3-methylbutanal.

It is important to identity the origin of the volatile compounds to understand the formation of favor in CSCB. As reported in the same type of meat product such as the smoked sausages, it suggests the volatile compounds might be due to smoking, seasoning, and the reactions between carbonhydrates, lipids and protein by microbial or endogenous enzymes (Ansorena, Gimeno, Astiasarán, & Bello, 2001; Johansson, Berdague, Larson, Tran, & Borch, 1994; Mateo & Zumalacárregui, 1996).

Some methylketones and linear aliphatic aldehydes isolated from CSCB might formed by β-oxidation of fatty acids, which generated a large proportion of the total volatiles. Hexanal was the main linear aliphatic aldehydes. A cyclopentenone (2,3-dimethyl-2-cyclopenten-1-one) was observed in CSCB, which are typical volatiles of wood smoke (Maga, 1987). Some branched short-chain aldehydes

Table 1
Total area counts of identified CSCB volatile compounds extracted by SPME with either a CAR/PDMS, a DVB/CAR/PMS or a PDMS/DVB coated fiber

Volatiles	LRI	Identification	CAR/PDMS ($\times 10^6$)	DVB/CAR/PDMS ($\times 10^6$)	PDMS/DVB ($\times 10^6$)	Total ($\times 10^6$)
Alkanes						
Butane	<500	MS[a], LRI[b]	24.76	ND[c]	ND	24.76
		Total	24.76	0	0	24.76
Aldehydes						
2-Methylpropanal	550	MS, LRI	11.92	ND	ND	11.92
3-Methylbutanal	643	MS, LRI	108.81	ND	TR[d]	108.81
2-Methybutanal	653	MS, LRI	45.33	ND	ND	45.33
Pentanal	695	MS, LRI	213.12	39.80	7.14	260.06
3-Methyl-2-butenal	779	MS, LRI	TR	ND	ND	TR
Hexanal	799	MS, LRI	584.76	191.62	43.54	819.92
2-Hexenal	848	MS, LRI	TR	TR	ND	TR
Heptanal	896	MS, LRI	78.40	70.40	16.66	165.46
Benzaldehyde	952	MS, LRI	81.70	51.06	ND	132.76
Octanal	993	MS, LRI	48.49	85.15	33.64	167.28
5-Ethylcyclopent-1-enecarboxaldehyde	1018	MS	4.75	8.91	ND	13.66
2-Octenal	1046	MS, LRI	8.38	20.91	ND	29.29
Nonanal	1088	MS, LRI	52.67	127.33	37.12	217.12
2-Nonenal	1142	MS, LRI	6.70	30.40	ND	37.10
2-Decenal	1242	MS, LRI	4.67	42.61	ND	47.28
2-Undecenal	1340	MS	ND	17.48	ND	17.48
		Total	1249.71	685.66	138.10	2073.47
Ketones						
2-Butanone	596	MS, LRI	47.39	ND	ND	47.39
2-Heptanone	888	MS, LRI	7.38	5.72	4.97	18.07
1-Octen-3-one	972	MS, LRI	TR	ND	ND	TR
2,3-Octanedione	978	MS, LRI	30.90	8.48	ND	39.38
2,3-Dimethyl-2-cyclopenten-1-one	1027	MS, LRI	TR	ND	ND	TR
		Total	85.68	14.20	4.97	104.85
Alcohols						
1-Penten-3-ol	675	MS, LRI	16.89	ND	ND	16.89
3-Methyl-1-butanol	726	MS, LRI	27.82	ND	ND	27.82
1-Pentanol	759	MS, LRI	48.84	9.18	ND	58.02
1-Hexanol	868	MS, LRI	10.33	TR	ND	10.33
1-Heptanol	967	MS, LRI	7.17	18.14	ND	25.31
1-Octen-3-ol	974	MS, LRI	8.54	32.14	ND	40.68
4-Ethylcyclohexanol	999	MS, LRI	TR	ND	ND	TR
1-Octanol	1060	MS, LRI	4.61	18.52	ND	23.13
Phenylethyl alcohol	1097	MS, LRI	8.02	ND	ND	8.02
		Total	132.22	77.97	0	210.19
Thioethers and thiols						
Methanethiol	<500	MS, LRI	9.58	ND	ND	9.58
Dimethyl disulfide	732	MS, LRI	24.48	ND	ND	24.48
3-(Methylthio)propanal	901	MS, LRI	23.00	9.50	ND	32.50
Dimethyl trisulfide	958	MS, LRI	14.62	ND	ND	14.62
		Total	71.68	9.50	0	81.18
Furans						
2-Furfural	827	MS, LRI	TR	ND	ND	TR
2-Furanmethanol	858	MS, LRI	TR	TR	ND	TR
2-Pentylfuran	983	MS, LRI	16.32	17.44	18.05	51.81
		Total	16.32	17.44	18.05	51.81
Phenols						
2-Methylphenol	1049	MS, LRI	29.78	ND	ND	29.78
3-Methylphenol	1069	MS, LRI	21.13	ND	ND	21.13
4-Methylphenol	1070	MS, LRI	TR	ND	ND	TR

(*continued on next page*)

Table 1 (*continued*)

Volatiles	LRI	Identification	CAR/PDMS ($\times 10^6$)	DVB/CAR/PDMS ($\times 10^6$)	PDMS/DVB ($\times 10^6$)	Total ($\times 10^6$)
2-Methoxyphenol	1075	MS, LRI	55.27	40.78	20.72	116.77
2,5-Dimethylphenol	1135	MS, LRI	TR	ND	ND	TR
2,3-Dimethylphenol	1136	MS, LRI	TR	ND	ND	TR
3-Ethylphenol	1156	MS, LRI	TR	ND	ND	TR
p-Methylguaiacol	1174	MS, LRI	25.97	29.90	26.17	82.04
3,4-Dimethoxytoluene	1219	MS	4.66	ND	ND	4.66
p-Ethylguaiacol	1261	MS, LRI	6.85	10.57	13.48	30.90
		Total	143.65	81.26	60.38	285.28
		All total	1724.02	886.02	221.49	2831.53

[a] MS: mass spectrum tentatively identified using Nist05 and Wiley275 libraries.

[b] LRI: LRI calculated for a DB-5 capillary column and LRI in agreement with literature (Ansorena et al., 2001; Boulanger, Chassagne, & Crouzet, 1999; Boulanger & Crouzet, 2000,2001; da Silva, Borba, Semir, & Marsaioli, 1999; Donnelly, Abdel-Hamid, Jeter, & Gurka, 1993; Engel, Baty, le Corre, Souchon, & Martin, 2002; García, Martín, Timón, & Córdoba, 2000; Joffraud, Leroi, Roy, & Berdagué, 2001; Kovacevic, Pavlovic, Menkovic, Tzakou, & Couladis, 2002; Lee, Suriyaphan, & Cadwallader, 2001; Macku & Shibamoto, 1991; Meynier, Novelli, Chissolinim, Zanardi, & Gandemer, 1999; Pino, Marbot, & Fuentes, 2003; Pitarokili, Tzakou, Loukis, & Harvala, 2003; Rostad & Pereira, 1986; von Kováts, 1958; Zehentbauer & Reineccius, 2002).

[c] ND: not detected.

[d] TR: trace amount (area counts $< 0.20 \times 10^6$).

and alcohols were detected in CSCB. The origin of branched aldehydes and their corresponding alcohols in CSCB is in Strecker degradation reactions of amino acids (Ventanas et al., 1992), which is an important pathway associated to Maillard reaction. In Strecker reaction, amino acids are decarboxylated and deaminated forming aldehydes, while dicarbonyls formed in Maillard reaction are converted to aminoketones or aminoalcohols, which can also react with themselves or with other compounds providing a wide variety of aromatic compounds (Mottram, 1998). 3-Methylbutanal produces bacon-like flavor via reactions with sulphur-containing compounds (Shu, Mookherjee, Bondarovich, & Hagedom, 1985). 2-Methylbutanal, 3-methylbutanal and 1-octen-3-ol have been described as odor active compounds in Iberian hams (Carrapiso, Jurado, Timón, & García, 2002).

4 Thioethers and thiol compounds were detected in CSCB. These compounds include dimethyl disulfide, 3-(methylthio)propanal, dimethyl trisulfide and methanethiol. They are considered to be particularly important aroma compounds. During cooking, a major route to these compounds is the Maillard reaction between reducing sugars and the amino acid cysteine (Mottram & Nobrega, 2002). The desulfuration of cysteine and methionine can result in the formation of sulfide and thiol volatiles (Pianotti, Lachette, & Dills, 1986).

Furans are produced because of smoke and Maillard reaction. 2-Furfural, 2-furanmethanol and 2-pentylfuran were detected in CSCB. Furaldehydes are less polar and more carbohydrate degradation products. 2-Furanmethanol has been found in various sugar/amino acid Maillard reactions (Ames, Guy, & Kipping, 2001). Furan derivatives possess caramel, sweet, butterscotch, brandy, burnt and sugar notes (Viani & Horman, 1974).

As was expected from a smoked sausage, typical wood smoke compounds were found in CSCB. Phenols and methoxyphenols are the most classical smoke components, which accorded with our results. As seen from Table 1, there were 4 methoxyphenols and 6 phenols found in CSCB. 2-Methoxyphenol is a predominant smoke component of methoxyphenols in CSCB. Smoke from softwood biomass contains 2-methoxyphenols (Kjällstrand, Ramnäs, & Petersson, 2000). The concentrations of phenols such as dimethylphenols and ethylphenols were relatively lower compared to methoxyphenols. Methoxyphenols and phenols are powerful aromatic compounds with pungent, cresolic, heavy, burnt, and smoky notes (Guillén & Manzanos, 2002), together with antioxidant and antimicrobial activity, and their odor threshold values are between 0.1 and 1 ng l^{-1} (Hierro, Hoz, & Ordóñez, 2004). The sensory threshold values of those compounds make them important contributors to the flavor of CSCB.

Many volatile compounds were not reported in our previous paper isolated by steam distillation method or nitrogen purge-and-steam distillation method on CSCB (Yu & Sun, 2005; Yu & Wu, 2003). It should be because of different method of isolating volatile substances from CSCB.

3.2. SPME fiber coating

Total area counts obtained for each coated fibers are shown in Table 1. Total ion chromatograms of CSCB samples extracted with CAR/PDMS, DVB/CAR/PDMS and PDMS/DVB fibers are shown in Fig. 1. CAR/PDMS coated fibers extracted the highest total amount of volatile compounds, followed by DVB/CAR/PDMS and PDMS/DVB. The total amount of compounds extracted with PDMS/DVB was much lower. Therefore, PDMS/DVB fibers may be discarded for further detailed consideration about individual compounds due to the low total area counts and the smaller number of compounds extracted. The wider range of compounds and higher total area extracted with the bipolar porous coatings CAR/PDMS and DVB/CAR/PDMS than with PDMS/DVB fibers have been previously shown by other authors in several food-

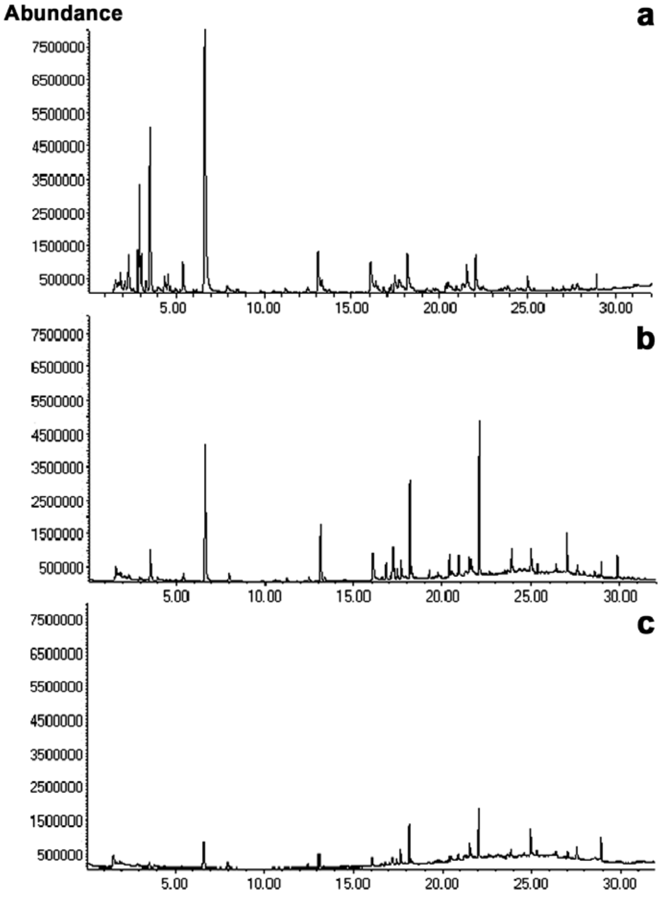

Fig. 1. Total ion chromatogram of CSCB volatiles extracted for 30 min with CAR/PDMS (a) DVB/CAR/PDMS, (b) PDMS/DVB and (c) SPME fibers, respectively.

stuffs (Dufour, Delbecq, & Perez, 2001; Gianelli, Flores, & Toldrá, 2002).

Table 1 shows the area counts of the different chemical groups extracted by CAR/PDMS and DVB/CAR/PDMS fibers in CSCB samples. DVB/CAR/PDMS fiber favored the extraction of long-chain aldehydes, long-chain alcohols, furans and *p*-methyl (ethyl) guaiacol. In CSCB samples extracted with CAR/PDMS fiber the major group was that of the short-chain alcohols followed by sulfur compounds, ketones and short-chain aldehydes. These results concerning the type of compounds adsorbed by each kind of fiber were in agreement with previous works (Elmore, Mottram, & Hierro, 2000; Gianelli et al., 2002). For all families of compounds in CSCB samples CAR/PDMS extracted higher amounts of compounds with lower LRI, whereas the amount of compounds with higher LRI was greater using the DVB/CAR/PDMS fiber. This agrees with previous available information, in which the CAR/PDMS coating is generally recommended for extracting small analytes (Elmore et al., 2000) whereas the DVB/CAR/PDMS is recommended for larger analytes. Carboxen is a porous carbon with a high surface area (around 1200 $m^2 g^{-1}$) which has been described as adequate for extraction of low boiling point compounds, although it allows the adsorption of a wide range of compounds due to the presence of different types of pores (micro-, meso-, and macropores). On the other hand, the layer of PDMS-DVB in the DVB/CAR/PDMS fiber provides this type of fibers the ability to extract high boiling point compounds. These characteristics explain the different amounts of compounds with low and high boiling point extracted with each type of fiber observed in this study.

Although CAR/PDMS extracted a higher number of compounds than DVB/CAR/PDMS in CSCB, the compounds with higher LRI was greater using the DVB/CAR/PDMS fiber such as 2-undecenal, 2-decenal, 2-nonenal, nonanal, 1-octanol and *p*-ethylguaiacol, etc. Thus, it seems that both porous fibers tested, CAR/PDMS and DVB/CAR/PDMS, provides the volatile compounds profile for CSCB, and that both satisfactorily extracts most compounds previously highlighted as odor active compounds in CSCB.

4. Conclusions

The objective of the present investigation was to analyze the profile of the volatile compounds responsible for its characteristic flavor of CSCB by SPME and to study the effect of fiber coating on the volatile profile of CSCB analyzed by SPME. This result shows that (1) 1 Alkane, 16 aldehydes, 5 ketones, 9 alcohols, 4 thioethers and thiols, 3 furans and 10 phenols compounds were identified. Among them, aldehydes, ketones, alcohols, thioethers and thiols and furans are also responsible for CSCB characteristic flavor besides phenols compounds. The major volatile compounds of CSCB came from smoking, oxidation and Maillard reaction, etc. Many volatile compounds were not reported in our previous paper isolated by steam distillation method or nitrogen purge-and-steam distillation method on CSCB (Yu & Sun, 2005; Yu & Wu, 2003). It should be because of different method of isolating volatile substances from CSCB. (2) CAR/PDMS and DVB/CAR/PDMS satisfactorily extract most odor active compounds in CSCB. CAR/PDMS coating extracted better those compounds whose linear retention indices (LRI) was lower than 926 (on average) and DVB/CAR/PDMS those with higher LRI.

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