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Flavour substances of Chinese traditional smoke-cured bacon

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Abstract

Flavour components from Chinese traditional smoke-cured bacon were trapped by condensing and dissolving in organic solvent (ether and *n*-pentane), using the nitrogen purge-and-steam distillation (NPSD) method. Qualitative and quantitative characterizations of the extract were performed by means of gas chromatography-mass spectrometry and gas chromatography with a flame ionization detector, respectively. Using Chinese traditional smoke-cured bacon, under the condition of stewing, the identification of 27 constituents which were not reported in a previous paper was achieved by the steam distillation method on Chinese traditional smoke-cured bacon and 4 phenols was not previously reported. The use of NPSD technique not only serves as a carrier of aroma constituents but also provided a medium which prevented the oxidation of the cellular components. This study indicates that must of the volatiles from Chinese traditional smoke-cured bacon are phenolic-derivatived. The volatiles in Chinese traditional smoke-cured bacon, however, are very different from fresh pork and Jinhua ham. The phenolic derivatives volatiles in Chinese traditional smoke-cured bacon should be related to smoking during manufacturing.

Keywords: Smoke-cured bacon; GC-MS; Aroma compounds; Meat

1. Introduction

Smoke-cured bacon, a traditional food of the Chinese, has a long history. It is the main meat food in Hubei, Sichuan, Hunan, Guizhou, Yunnan of China. Chinese traditional smoke-cured bacon is made by salting, smoking and dry-curing. The smoke-cured bacon has a distinctive flavour considered as tangy, delicate, meaty and salty. It has a different aroma from fresh pork, especially in stewing. The flavour composition of smoke-cured bacon is fairly complex, with many flavour compounds. A great many of efforts have been made in past years to determine the chemical composition of fresh pork and a number of outstanding reviews on the progress of fresh pork flavour chemistry have been published (Chang & Peterson, 1977; Ramarathnam, Rubin, & Diosady, 1991a, 1993; Shahidi, Rubin, & D'Souza, 1986). Reports have been published on chemical aspects of meat smoking (Tóth & Potthast, 1984) and the chemical composition of meat smoke (Christopher, James, & Glen, 1999). The volatile substances of Chinese traditional Jinhua ham and Cantonese sausage have also been published (Du & Ahn, 2001). In recent years, the volatile substances of smoked food have been reported roughly (Hanson, 2000; Kjällstrand & Petersson, 2001). But, as far as we know, reports on volatile substances of Chinese traditional smoke-cured bacon have not been found except for previous report (Yu & Wu, 2003).

The objective of the present study is to provide qualitative and quantitative information on flavour substances present in Chinese traditional smoke-cured bacon. Isolating volatile substances from meat, the usual methods are conventional steam distillation and continuous steam distillation-extraction (SDE) (Ramarathnam et al., 1991a; Wettasinghe, Vasanthan, & Temelli, 2001), headspace solid-phase microextraction (SPME) (Du & Ahn, 2001; Elmore, Mottram, & Hierro, 2000; Gorraiz, Beriain, & Chasco, 2002; Insausti, Beriain, & Gorraiz, 2002; Ruiz, Cava, & Ventanas, 1998), gas purge-and-trap

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technique (Ramarathnam et al., 1993) and supercritical CO_2 extraction (Thongwong, Fernando, & Grün, 1999). In our previous attempts, we provided quantitative information on flavour substances present in the aroma concentrate of Chinese traditional smoke-cured bacon isolated by the steam distillation method (Yu & Wu, 2003). In this paper, we adopt nitrogen purge-and-steam distillation (NPSD) methods, which can thoroughly extract volatile substances of meat and can extract meat flavour volatiles similar to those in stewed meat.

2. Materials and methods

2.1. Materials and reagents

Chinese traditional smoke-cured bacon was prepared by a traditional method of southwest China. The fresh pork was salted for 3 days, and then was smoked by raw firewood about for one month until the meat was dried. After careful removal of the dust and the excess depot fat, the smoke-cured bacon sample was deboned manually, cut into small pieces, and then ground until transformed into meat slurry.

Oxygen-free nitrogen gas was purchased from Hubei Yichang Lantian Gases Co., Ltd. (99.999%). Ether (spectral grade) and *n*-pentane (spectral grade) were purchased from Tianjin Kermel Chemical Reagent Development Center; ether was dried over K_2CO_3 and distilled. Gas chromatographic standard 1,2-dichlorobenzene was purchased from Shanghai No. 1 Chemical Reagent Factory. Anhydrous sodium sulfate (analytical grade) was purchased from Tianjin Chemical Reagent Co., Inc.

2.2. Cooking

Ground smoke-cured bacon (200 g) placed in a 1000 ml flask. 400 ml distilled water were added, and the contents were refluxed for 40 min in an oil bath. The cooked smoke-cured bacon samples were cooled to room temperature.

2.3. Nitrogen purge-and-steam distillation

The method for trapping flavour substances was the NPSD. The apparatus comprised a 1000 ml two-neck flask. One of the necks served as the inlet for the purging gas, oxygen-free nitrogen. The second neck was equipped with an ordinary distiller condenser. The cooked smoke-cured bacon (200 g) was placed in the two-neck flask, where it was constantly maintained at 102 ± 5 °C with the oil bath. A slow stream of oxygen-free nitrogen gas was passed through the meat slurry so as to purge the volatiles from the headspace. The effluent stream was made to condense in a condenser pipe, which was connected to a cold trap (1st Trap) maintained at 2–4 °C with crushed ice, next through the second cold trap (2nd Trap), containing 50 ml of ether maintained at -20 ± 5 °C with the help of an ice–HCl mixture, and finally through the third cold trap (3rd Trap), containing 50 ml of *n*-pentane maintained at -20 ± 5 °C with the help of an ice–HCl mixture too. This mode of fractionation was designed so as to help some of the less volatile components, condensed water and water-soluble components, by condenser pipe, into the first cold trap. Some volatile components and oil-soluble components were absorbed in ether in the second cold trap and in *n*-pentane in the third cold trap.

The volatiles were collected over a 3 h purging and distilling period. At the end of the experiment, the condensate of the first cold trap (1st Trap) was 150 ml and was extracted with 50 ml ether, twice, and 50 ml *n*-pentane, twice. The extracted solutions were combined, dried over anhydrous sodium sulfate, and concentrated by passing a slow stream of oxygen-free nitrogen gas to a final volume of around 0.5 ml. The second cold trap (2nd Trap) and the third cold trap (3rd Trap) were dried and concentrated as described above.

2.4. Quantitation of the individual components (GC-FID)

Quantitative characterizations of the extract were performed by means of gas chromatograph with flame ionization detector. A gas chromatograph (HP-6890; Hewlett–Packard Co. Wilmington, Del., USA), equipped with a DB-5 capillary column [30 m × 0.25 mm(i.d) × 0.25 μm] and flame ionization detector (FID) was used. Analysis was carried out by using helium as the carrier gas, with the column temperature maintained initially at 60 °C for 2 min and then programmed from 60 to 260 °C at a rate of 10 °C/min, where it was held for 8 min. Quantitation of the individual constituents identified in the smoke-cured bacon aroma concentrate was carried out with 1,2-dichlorobenzene (4.9 mg/ml in *n*-pentane) as the internal standard (Elmore et al., 2000). From the peak areas of different known concentration of 1,2-dichlorobenzene, the amount of individual constituents present in smoke-cured bacon was calculated and expressed in terms of milligrammes per kilogramme of smoke-cured bacon.

2.5. Gas chromatography-mass spectrometry (GC-MS)

Qualitative characterizations of the extract were performed by means gas chromatography-mass spectrometry. A Finnigan TRACE GC-MS (Thermo Quest Finnigan Co., USA) equipped with a DB-5 capillary column [30 m × 0.25 mm(i.d) × 0.25 μm] was used. Analysis was carried out by using helium as the carrier gas, with the column temperature maintained initially at 60 °C for 2 min and then programmed from 60 to 260 °C at a rate of 10 °C/min, where it was held for 8 min. The source and analyzer temperatures were 200 and 250 °C, respectively. The ionization voltage applied was 70 eV.

Mass spectra obtained were compared with those of known compounds in the Mainlib, Replib, Wiley, Nist library by using a computer.

3. Results and discussion

3.1. Gas chromatography-mass spectrometric (GC-MS) analysis

The components identified in the three aroma concentrates of smoke-cured bacon, prepared by the NPSD method, as described above, are listed in Table 1. In all, 40 compounds were detected in the different fractions of the aroma concentrates, 27 of which was not reported in our previous paper where isolated by the steam distillation method (Yu & Wu, 2003). Of the total number of components identified, 9 are hydrocarbons, 12 phenols, 2 carbonyls, 1 alcohol, 2 amides, 7 esters, 1 amine, 5 carboxylic acids, 1 heterocyclic. The compounds are different from the components identified in fresh pork (Chang & Peterson, 1977; Ramarathnam et al., 1991a, 1993; Shahidi et al., 1986).

Table 1
Compounds identified in the aroma concentrates of smoke cured bacon by the NPSD method

RT (min)	Volatiles	Area	1st Trap (10^{-3} mg/kg)	2nd Trap (10^{-3} mg/kg)	3rd Trap (10^{-3} mg/kg)
5.133	Phenol	2288.7913	8.23	ND[a]	ND
6.795	*N*-isopropylmethanesulfonamido-2-cyclohexene-2-one	900.7397	3.24	ND	ND
6.934	*p*-Cresol	628.7829	2.26	ND	ND
7.200	2-Nonen-1-ol	D[b]	ND	D	ND
8.359	3,4-dimethylphenol	686.6793	2.47	ND	ND
8.621	*p*-Ethylphenol	D	ND	D	ND
8.663	2-(2-Isopropenyl-5-methyl-cyclopentyl)-acetamide	579.8613	2.08	ND	ND
8.719	*n*-Dodecane	1.7230	ND	ND	0.007605
8.815	4-Hydroxy-non-2-ynoic acid,ethyl ester	D	ND	D	ND
9.330	10-Undecenoic acid,octyl ester	D	ND	D	ND
9.335	2,4,5-Trimethylphenol	365.6969	1.31	ND	ND
10.019	4-Ethylguaiacol	260.9545	0.938	ND	ND
10.180	*o*-Tertbutylphenol	78.4462	0.282	ND	ND
10.610	2,6-Dimethoxyphenol	194.0267	0.697	ND	ND
10.750	Geranyl isovalerate	D	ND	D	ND
11.415	Tetradecane	149.9264	0.539	ND	ND
12.091	Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	D	ND	ND	D
12.105	Cedrene	D	ND	D	ND
12.676	Isoeugenol	356.9945	1.283	ND	ND
13.258	Butyl hydroxy toluene	209.0300	ND	0.8125	ND
13.260	Acenaphthalene	87.5175	0.315	ND	ND
13.599	2,3,5-Trimethoxytoluene	51.5165	0.185	ND	ND
14.941 ± 0.01	Diphenylamine	1577.4963	4.34	1.4241	0.01500
15.695	Heptadecane	27.7111	0.0996	ND	ND
16.526	Octadecane	222.2542	0.799	ND	ND
16.956	Myristic acid	106.9297	0.384	ND	ND
17.115 ± 0.004	Hexadecanal	592.5866	2.00	0.1460	ND
18.563	Isobutyl phthalate	327.5808	1.18	ND	ND
18.677	Dibutyl phthalate	8.4421	ND	ND	0.03726
18.810	10-Methoxy-nb-α-methylcorynantheol	1333.7488	4.79	ND	ND
18.875 ± 0.2	Hexadecanoic acid	92.1231	0.293	0.04082	ND
20.069	Oleic acid	7.9624	ND	0.03095	ND
20.618	9-Octadecenoic acid	1048.6995	3.77	ND	ND
20.762	Octadecanoic acid	121.1014	0.435	ND	ND
21.143	Octadecyl acetate	10.5811	ND	0.04113	ND
22.376 ± 0.3	9-Octadecenamide	14.5875	D	0.05670	ND
22.684	2,2′-Methylenebis[6-(1,1-dimethylethyl)-4-methyl]-phenol	52.4592	0.189	ND	ND
23.193	1-Docosene	D	ND	D	ND
24.060	Heptacosane	D	D	ND	ND
24.527 ± 0.007	Diisooctyl phthalate	249.5711	0.826	0.07715	ND

[a] ND = not detected.
[b] D = detected.

3.2. Analysis of the first cold trap fraction

The gas chromatogram (GC) of the separated constituents trapped in the first cold trap fraction is illustrated in Fig. 1. The first cold trap, maintained at 2–4 °C with crushed ice, is mainly used with the intention of condensing water vapour and the water-soluble components. This fraction shows the presence of 10 phenols, 2 carbonyls, 2 amides, 5 hydrocarbons, 1 amine, 4 carboxylic acids, 2 esters and 1 heterocyclic. These identified components constitute 67.5% of all identified volatiles compounds in the smoke-cured bacon. Organoleptic evaluation of the contents of the first cold trap strongly indicates the presence of the components responsible for the desirable meaty aroma of stewing smoke-cured bacon. The concentration of phenol (RT 5.133 min) is the highest in the smoke-cured bacon (8.23×10^{-3} mg/kg). The odour threshold of phenol is 40 ppb (150 μg/m^3) (Amoore & Hautala, 1983). Other compounds are also in the smoke-cured bacon. These compounds include 10-methoxy-nb-α-methylcorynantheol (RT 18.810 min), diphenylamine (RT 14.951 min), 9-octadecenoic acid (RT 20.618 min), *N*-isopropylmethanesulfonamido-2-cyclohexene-2-one (RT 6.795 min), 3,4-dimethylphenol (RT 8.359 min), *p*-cresol (RT 6.934 min), 2-(2-isopropenyl-5-methyl-cyclopentyl)-acetamide (RT 8.663 min), hexadecanal (RT 17.119 min), 2,4,5-trimethylphenol (RT 9.335 min), isoeugenol (RT 12.676 min), isobutyl phthalate (RT 18.563 min), 4-ethyl-2-methoxyphenol (RT 10.019 min), diisooctyl phthalate (RT 24.534 min), octadecane (RT 16.526 min), 2,6-dimethoxyphenol (RT 10.610 min) and tetradecane (RT 11.415 min).

3.3. Analysis of the second cold trap fraction

The second cold trap, containing 50 ml of ether maintained at -20 ± 5 °C with ice–HCl mixture, is mainly used to trap the more volatile compounds that are not condensed earlier in the condenser pipe. The gas chromatogram (GC) of aroma concentrates of smoke-cured bacon prepared from the second cold trap is shown in Fig. 2. In all, 15 compounds were identified in this fraction; of these, 1 is an alcohol, 2 are phenols, 5 are esters, 2 are hydrocarbons, 1 is an amine, 1 is an aldehyde, 2 are carboxylic acids and 1 is an amide. Among them, 10 compounds were identified only in this fraction. These compounds include 2-nonen-1-ol (RT 7.200 min), *p*-ethylphenol (RT 8.621 min), 4-hydroxy-non-2-ynoic acid,ethyl ester (RT 8.815 min), 10-undecenoic acid, octyl ester (RT 9.330 min), geranyl isovalerate (RT 10.750 min), cedrene (RT 12.105 min), butyl hydroxy toluene (RT 13.258 min), oleic acid (RT 20.069 min), octadecyl acetate (RT 21.143 min) and 1-docoscene (RT 23.193 min). The concentrations of identified compounds in this fraction were generally lower than in the first cold trap. Comparatively speaking, the concentrations of diphenylamine (1.42×10^{-3} mg/kg), butyl hydroxy toluene (0.813×10^{-3} mg/kg) and hexadecanal (0.146×10^{-3} mg/kg) were higher than the others in this fraction.

3.4. Analysis of the third cold trap fraction

The third cold trap, containing 50 ml of *n*-pentane maintained at -20 ± 5 °C with ice–HCl mixture, is mainly used to trap the compounds that escape absorption components in the first cold trap and second cold trap, and oil-soluble components. The gas chromatogram (GC) of aroma concentrates of smoke-cured bacon prepared from the third cold trap is shown in Fig. 3. In all, only four compounds were identified in this fraction. These compounds included *n*-dodecane, decahydro-4, 8,8-trimethyl-9-methylene-1,4-methanoazulene, diphenylamine and dibutyl phthalate. *n*-Dodecane, decahydro-

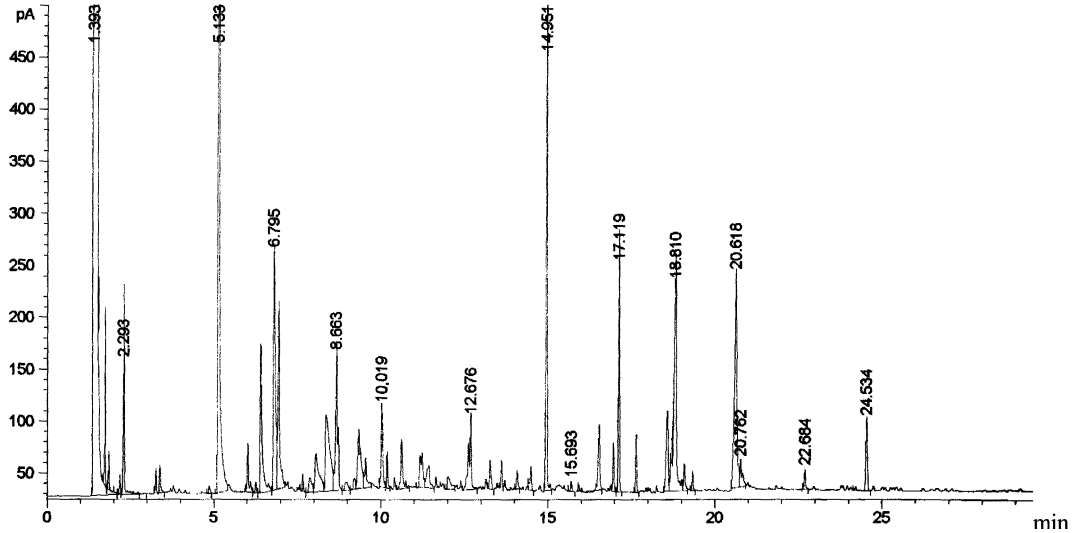

Fig. 1. Gas chromatogram (GC) of Chinese traditional smoke cured bacon flavour concentrate isolated by the NPSD method (first cold trap).

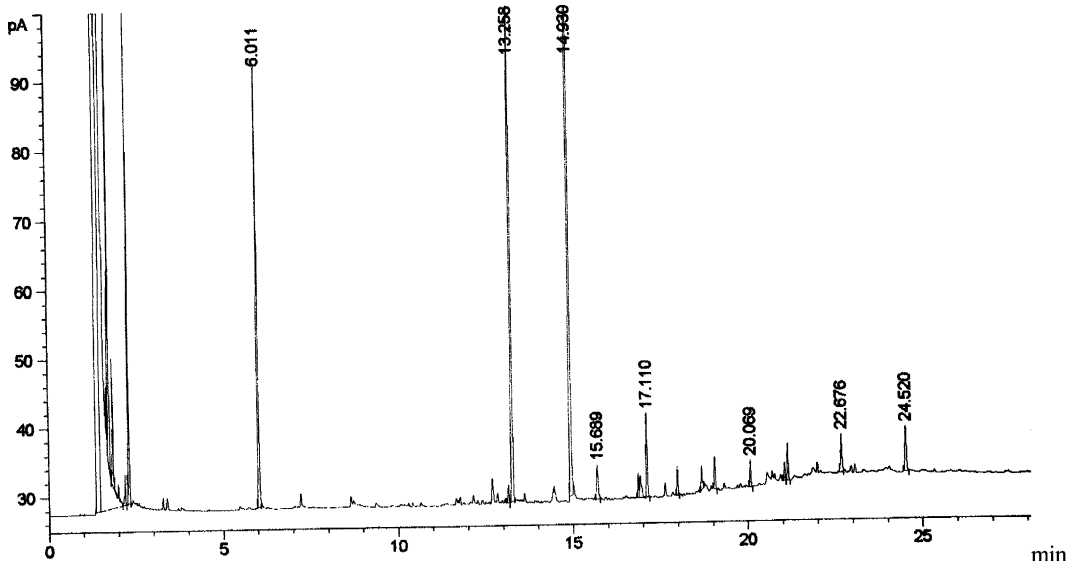

Fig. 2. Gas chromatogram (GC) of Chinese traditional smoke cured bacon flavour concentrate isolated by the NPSD method (second cold trap).

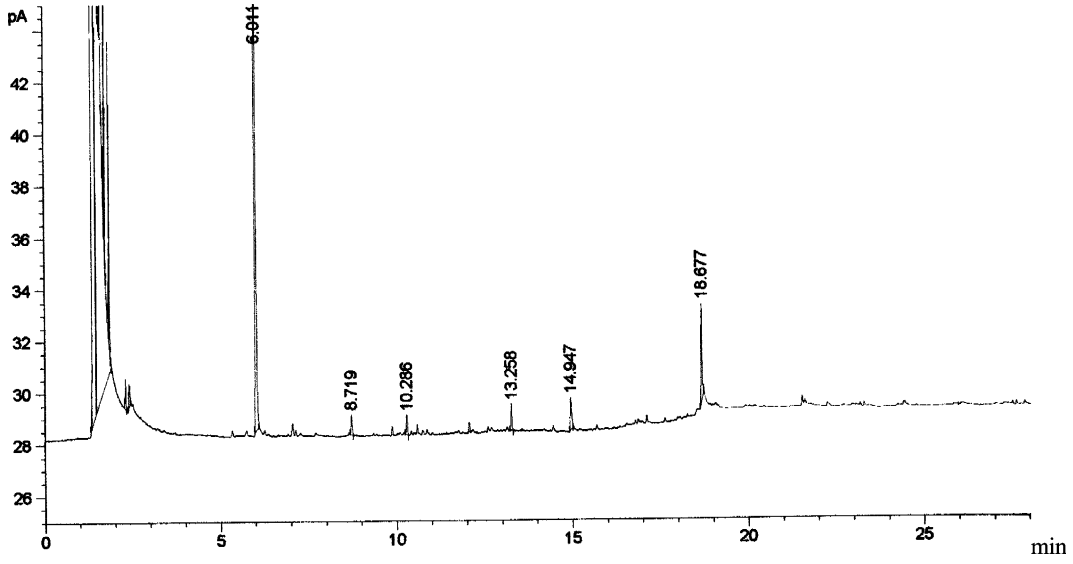

Fig. 3. Gas chromatogram (GC) of Chinese traditional smoke cured bacon flavour concentrate isolated by the NPSD method (third cold trap).

4,8,8-trimethyl-9-methylene-1,4-methanoazulene and dibutyl phthalate were identified only in this fraction.

3.5. Analysis of the flavour substances of Chinese traditional smoke-cured bacon

Among all detected compounds in the three fractions, we should pay most attention to the 12 phenols, which are responsible for the tangy flavour. The phenols constitute 30% of the identified components in our test. 4-Methoxyphenols were detected in our test, and reflect the structure of the corresponding units in the plant lignin (Kjällstrand & Petersson, 2001). Eight phenols and alkylphenols were detected, such as phenol, *p*-cresol, 3,4-dimethylphenol, *p*-ethylphenol, 2,4,5-trimethylphenol, *o*-tertbutylphenol, butylhydroxytoluene and 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl]-phenol. Some of the above compounds have been detected in liquid smoke preparations (Tóth & Potthast, 1984). But Tóth and Potthast (1984) do not report 3,4-dimethylphenol, *o*-tertbutylphenol, butyl hydroxy toluene, 2,3,5-trimethoxytoluene or 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl]-phenol. Smoked meat flavour and aroma are mainly due to the phenols present in wood smoke (Hanson, 2000). But Hanson does not report specific phenols. Guillén and Manzanos (1999) report that the aqueous smoke flavouring is mainly phenolic derivatives. The flavour of liquid smoke is basically due to phenol derivatives, which have aromas judged as pungent, cresolic, burnt, and smoky (Fujimaki, Kim, &

Kurata, 1974; Baltes & Söchtig, 1979). Fujimaki et al. (1974) report 13 phenols. They are mainly (unsubstituted, 4-methyl, 4-ethyl, 4-allyl) guaiacol, phenol, *o*-, *m*- and *p*-cresols, 2,4-, 2,6-, 3,4-, 3,5-xylenol with some 2,6-dimethoxyphenol homologues. In our investigation, phenol, *p*-cresol, 3,4-dimethylphenol, 4-ethylguaiacol and 2,6-dimethoxyphenol were detected similar to Fujimaki et al. (1974) but did not report *p*-ethylphenol, 2,4,5-trimethylphenol, *o*-tertbutylphenol, isoeugenol, butyl hydroxy toluene, 2,3,5-trimethoxytoluene or 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl]–phenol. Daun (1979) reports that the key aroma compounds of the smoked food are primarily due to phenols, but Daun only reported 3 phenols which are guaiacol, 4-ethylguaiacol and 2,6-dimethoxyphenol. Among them, 4-ethylguaiacol (RT 10.019 min) and 2,6-dimethoxyphenol (RT 10.610 min) are detected in our test. Unfortunately did not we detect guaiacol. But guaiacol gives a smoky taste, whereas 2,6-dimethoxyphenol gives a smoky odour (Daun, 1979). Otherwise, 3,4-dimethylphenol (RT 8.359 min) has a slightly burnt taste, 2,4,5-trimethylphenol (RT 9.335 min) has a phenolic note, 4-ethylguaiacol (RT 10.019 min) has a smoky roasted flavour and burnt taste (Winter, Goldman, & Gautschi, 1976) and 2,6-dimethoxyphenol (RT 10.610 min) has a woody note and herby flavour (Zhu, Xue, & Li, 1993).

So, *o*-tertbutylphenol, butyl hydroxy toluene, 2,3,5-trimethoxytoluene and 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl]-phenol are not seen previously reported on smoked products. The phenolic derivative volatiles in Chinese traditional smoke-cured bacon should be related to smoking during manufacturing.

Otherwise, among all detected compounds in the three fractions, *n*-dodecane, tetradecane, heptadecane, octadecane, myristic acid, isobutyl phthalate, dibutyl phthalate, hexadecanoic acid, oleic acid, 9-octadecenoic acid, octadecanoic acid, 9-octadecenamide, 1-docosene, heptacosane and diisooctyl phthalate generally are considered to smell faint(Huang, Jin, Luo, & Chen, 1991). 2-Nonen-1-ol and hexadecanal are in some degree meaty. *N*-isopropylmethanesulfonamido-2-cyclohexene-2-one,2-(2-isopropenyl-5-methyl-cyclopentyl)-acetamide, decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene, cedrene, 10-methoxy-nb-α-methylcorynantheol, acenaphthalene and diphenylamine, are vague in organoleptic character, but should make contributions to the aroma of Chinese traditional smoke-cured bacon on the basis of their structural formulae. 10-Undecenoic acid octyl ester, geranyl isovalerate, octadecyl acetate and 4-hydroxy-non-2-ynoic acid ethyl ester afford fruity and fatty odours (Huang et al., 1991).

So, the phenolic derivatives, 2-nonen-1-ol, hexadecanal, 10-undecenoic acid octyl ester, geranyl isovalerate, octadecyl acetate, 4-hydroxy-non-2-ynoic acid ethyl ester, *N*-isopropylmethane-sulfonamido-2-cyclohexene-2-one, 2-(2-isopropenyl-5-methyl-cyclopentyl)-acetamide, decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene, cedrene, 10-methoxy-nb-α-methylcorynantheol, acenaphthalene, diphenylamine are responsible for the Chinese traditional smoke-cured bacon aroma. Among them phenolic derivatives should be key flavour substances and are responsible for the tangy flavour.

4. Conclusions

The objective of the present investigation was to isolate the volatiles from Chinese traditional smoke-cured bacon in three different fractions, depending on their volatility and solubility. It is believed that this mode of collection would facilitate the identification of the key compounds responsible for the Chinese traditional smoke-cured bacon aroma, with little or no interference from constituents that are usually present in high concentration but makes, little or no contribution to the flavour notes mentioned above. Using Chinese traditional smoke-cured bacon, under the condition of stewing, 27 constituents were not reported in our previous paper isolated by the steam distillation method on Chinese traditional smoke-cured bacon (Yu & Wu, 2003) and 4 phenols were not previously reported in smoked product. Nitrogen in the purge-and-steam distillation technique not only serves as a carrier of aroma constituents but also provided an inert medium which prevented oxidation of the cellular components. This study indicates most of the volatiles from Chinese traditional smoke-cured bacon are phenolic derivatives. The volatiles in Chinese traditional smoke-cured bacon, however, are very different from fresh pork and Jinhua ham. The phenolic derivatived volatiles in Chinese traditional smoke-cured bacon should be related to smoking during manufacturing.

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