Journal of Food Technology 3 (3): 435-439, 2005

Fidentification of Major Volatile Components in Chinese Traditional Smoke-cured Beef

[1]Ai-Nong Yu and [2]Bao-Guo Sun
[1] Department of Chemical Engineering, Hubei Institute for Nationalities, Enshi 445000, P.R. China
[2]The College of Chemistry and Environment Engineering,
Beijing Technology and Business University, Beijing 100037, P R. China

Abstract: Volatile components of smoke-cured beef, which is traditional food of southwest China, were trapped by condensing and dissolving in organic solvent (ether and n-pentane), using the nitrogen purge-and-steam distillation (NPSD) method. Quantitative analysis of the components obtained was carried out by gas chromatography(GC) with 1,2-dichlorobenzene, which was used as the internal standards. Qualitative analysis of the components obtained was carried out by gas chromatography-mass spectrometry (GC-MS). 38 compounds were identified. 11 Compounds were the first report in Chinese traditional smoke-cured meat. These compounds included phenol acetate, 3-ethylphenol, 1,2-epoxyhexadecane, 3,4-dimethyl-3-cyclohexen-1-carboxaldehyde, 5-ethyl-2-methylpyridine, 4-isopropenyl-5-methyl-4-hexen-1-al, 4-(2,5-dihydro-3-methoxyphenyl)butylamine, octadecanal, 4-propylguaiacol, 3-(2,2-dimethylpropylidene)bicyclo[3.3.1]nonane-2,4-dione and 4,7-dimethyl-3,8-phenanthroline. 24 Odor-active compounds are significant for the characteristic Chinese traditional smoke-cured beef aroma. Among them, the phenolic derivatived volatiles is important odor-active compounds for smoke-cured beef aroma.

Key words: Smoke-cured beef, aroma compounds, meat, flavor, odor, volatile, GC-MS

INTRODUCTION

In continuation of our attempts to identify the key components that are responsible for the Chinese traditional smoke-cured meat aroma[1,2] we report here the results of our investigation of Chinese traditional smoke-cured beef. Smoke-cured beef is one of another Chinese traditional smoke-cured meat. In our previous work, we had provided qualitative and quantitative information on flavor substances present in Chinese traditional smoke-cured bacon[1,2]. We isolate the volatiles from Chinese traditional smoke-cured beef, using the nitrogen purge-and-steam distillation (NPSD) method. The results of which are discussed in this paper.

A great many of efforts have been made in the past years to determine the chemical composition of fresh beef or fresh beef product and a number of outstanding reviews on the progress of fresh beef or fresh beef product flavor chemistry have been published[3-10]. The reports have been published on the chemical aspects of meat smoking[11] and the chemical composition of meat smoke[12]. In recent years, the volatile substances of smoked food have been reported[11,13-20]. To our knowledge, almost no published report on the volatiles of Chinese traditional smoke-cured beef is available except our previous report on Chinese traditional smoke-cured bacon[1,2]. We expect this work may help stimulate interest in studies on Chinese traditional smoke-cured beef products.

EXPERIMENTAL

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

Corresponding Author: Ai-Nong Yu, Department of Chemical Engineering, Hubei Institute for Nationalities, Enshi 445000, P.R. China

chromatographic standards 1,2-dichlorobenzene was purchased from Shanghai No.1 Chemical Reagent Factory. Anhydrous sodium sulfate (analytical grade) were purchased from Tianjin Chemical Reagent CO., Inc.

Cooking meat: Ground smoke-cured beef 200.0 g was placed in a 1000 mL flask. 400 mL distilled water was added and the contents were refluxed for 40 min. in an oil bath to produce volatile components. The cooked smoke-cured beef samples were cooled to room temperature.

Isolation of volatile components: The method of trapping volatile substances was adopted the nitrogen purge-and-steam distillation (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 beef 200.0 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, condense water and water-soluble components by condenser pipe into 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 distillating period. At the end of the experiment, the condensate of the first cold trap(1st Trap) was got 150 mL and extracted with 50 mL ether twice and 50 mL n-pentane twice. The extracted solution was 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.

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 x 0.25 mm(i.d) x 0.25 μm] and flame ionization detector (FID) were 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 the smoke-cured beef volatile concentrate was carried out with 1,2-dichlorobenzene (4.9 mg mL^{-1} in n-pentane) as the internal standard[21]. From the peak areas of different known concentration of 1,2-dichlorobenzene (RT about 6.000 min), the amount of individual constituents present in smoke-cured beef was calculated and expressed in terms of milligrams per kilogram of smoke-cured beef.

Gas chromatograph-mass spectrometric (GC-MS): Qualitative characterizations of the extract were performed by means of gas chromatograph-mass spectrometry. A Finnigan TRACE GC-MS (Thermo Quest Finnigan Co., USA) equipped with a DB-5 capillary column [30 m x 0.25 mm(i.d) x 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°Cnothing to 260°C at a rate of 10°C/ min, where it was held for 8 min. The source and analyzer temperatures were 200°C 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 computer.

RESULTS AND DISCUSSION

The components identified in the three volatile concentrates of smoke-cured beef prepared according to the NPSD method, as described above, are listed in Table 1. In all, 38 components were identified in the different fractions of the beef volatile concentrates. Of the total number of compounds identified, 6 are hydrocarbons, 12 phenols or phenolic derivatives, 6 carbonyls, 1 alcohol, 1 amide, 3 esters, 2 amines, 3 carboxylic acides and 4 heterocyclics. The component of the compounds is different from the components identified in the fresh beef or fresh beef product[2-9]

The first cold trap, maintained at 2-4°C with crushed ice, is mainly used with the intention of condensing water vapor and the water-soluble components. This fraction showes the presence of 5 hydrocarbons, 12 phenols or phenolic derivatives, 3 carbonyls, 1 ester, 2 amines, 3 carboxylic acides and 3 heterocyclics. 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 beef.

Table 1. Compounds identified in the volatile concentrates of smoke-cured beef by the NPSD method

RT (min)	Volatiles	Area	1st Trap10^{-3} mg kg^{-1}	2nd Trap10^{-3} mg kg^{-1}	3rd Trap10^{-3} mg kg^{-1}
5.147	phenol	3420.42	5.23	ND[a]	ND
5.470	phenol acetate	174.54	0.27	ND	ND
6.159	o-cresol	160.57	0.25	ND	ND
6.409	N-isopropylmethanesulfonamido-2-cyclohexene-2-one	1209.10	1.85	ND	ND
6.841	p-cresol	5454.19	8.33	ND	ND
7.203	2-nonen-1-ol	7.17	ND	0.030	ND
8.057	2,4-xylenol	939.54	1.44	ND	ND
8.397	3-ethylphenol	1288.06	1.97	ND	ND
8.697	3,4-dimethyl-3-cyclohexen-1-carboxaldehyde	TR[b]	ND	TR	ND
8.716	5-ethyl-2-methylpyridine	1.91	ND	ND	0.0071
8.718	n-dodecane	3146.29	4.81	ND	ND
9.358	2,4,5-trimethylphenol	431.20	0.66	ND	ND
10.041	4-ethylguaiacol	1597.52	2.44	ND	ND
10.156	4-(2,5-dihydro-3-methoxyphenyl)butylamine	TR	TR	ND	ND
10.608	4-isopropenyl-5-methyl-4-hexen-1-al	374.16	0.57	ND	ND
11.225	4-propenylguaiacol (isoeugenol)	413.15	0.63	ND	ND
11.363	4-propylguaiacol	355.23	0.54	ND	ND
11.984	tetradecane	131.54	0.20	ND	ND
12.053	cedrene	1.17	ND	ND	0.0044
12.608	butyl hydroxy toluene	337.18	0.52	ND	ND
13.254	3-(2,2-dimethylpropylidene)bicyclo[3.3.1] nonane-2,4-dione	10.75	ND	0.023	0.019
14.096	2,3,5-trimethoxytoluene	173.50	0.27	ND	ND
14.460	hexadecane	118.68	0.18	ND	ND
14.951±0.025	diphenylamine	3054.98	4.33	0.91	0.021
15.700	heptadecane	73.00	0.11	ND	ND
16.574	octadecane	886.47	1.36	ND	ND
16.877	myristic acid	161.60	0.25	ND	ND
17.118±0.008	hexadecanal	443.19	0.67	0.017	ND
18.103	octadecanal	TR	ND	ND	TR
18.676	isobutyl phthalate	4.36	ND	0.018	ND
18.706	dibutyl phthalate	1100.11	1.68	ND	ND
18.804	hexadecanoic acid	1104.05	1.69	ND	ND
19.333	oleic acid	86.74	0.13	ND	ND
20.622	1,2-epoxyhexadecane	1358.86	2.08	ND	ND
22.682	9-octadecenamide	7.22	ND	0.031	ND
22.696	4,7-dimethyl-3,8-phenanthroline	97.12	0.15	ND	ND
24.519	diisooctyl phthalate	20.85	ND	ND	0.077
24.559	apparicine	628.21	0.96	ND	ND

[a]ND = not detected [b]TR = trace

The concentration of p-cresol (RT 6.841 min) is the highest in the smoke-cured beef (8.3340×10^{-3} mg kg^{-1}). Other compounds are also higher in the smoke-cured beef than the others. These compounds include phenol (RT 5.147 min, 5.2264×10^{-3} mg kg^{-1}), n-dodecane (RT 8.718 min, 4.8075×10^{-3} mg kg^{-1}), diphenylamine (RT 14.951±0.025 min, 4.3314×10^{-3} mg kg^{-1}), 4-ethylguaiacol (RT 10.041 min, 2.4410×10^{-3} mg kg^{-1}), 1,2-epoxyhexadecanel (RT 20.622 min, 2.0763×10^{-3} mg kg^{-1}) and 3-ethylphenol (RT 8.397 min, 1.9682×10^{-3} mg kg^{-1}). Among them, p-cresol, phenol, n-dodecane, 4-ethylguaiacol, 1,2-epoxyhexadecanel and 3-ethylphenol are identified only in this fraction. Another 22 compounds are identified only in this fraction too.

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. In all, 7 compounds of smoke-cured beef volatile concentrates of this fraction are identified. Of these, 1 is an alcohol, 3 are carbonyls, 1 is an amine, 1 is an ester and 1 is an amide. Among them, 4 compounds are identified only in this fraction. These compounds include 2-nonen-1-ol (RT 7.203 min), isobutyl phthalate (RT 18.676 min), 3, 4-dimethyl-3-cyclohexen-1-carboxaldehyde (RT 8.697 min) and 9-octadecenamide (RT 22.682 min). The concentration of identified compounds in this fraction is generally lower than the first cold trap. Comparatively speaking, the concentration of diphenylamine (0.9067×10^{-3} mg kg^{-1}) is higher than the others in this 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 first cold trap and second cold trap and oil-soluble components. In all, 6 compounds are identified in this fraction. 4 Compounds are identified only in this fraction. These compounds include 5-ethyl-2-methylpyridine (RT 8.716 min), cedrene (RT 12.053

min), octadecanal (RT 18.103 min) and diisooctyl phthalate (RT 24.519 min).

Among all detected compounds in three fractions, n-dodecane, tetradecane, cedrene, hexadecane, heptadecane, octadecane, myristic acid, isobutyl phthalate, dibutyl phthalate, hexadecanoic acid, oleic acid, 9-octadecenamide and diisooctyl phthalate generally are considered to smell faint[22]. 12 phenols or phenolic derivatives are responsible for tangy flavour[13-15,17,23] Phenols or phenolic derivatives have aromas judged as pungent, cresolic, burnt and smoky[14,23]. We should pay most attention to p-cresol, phenol, 4-ethylguaiacol and 3-ethylphenol, which are the highest in the smoke-cured beef identified in first fraction. The odor threshold of phenol is 40 ppb (150 $\mu g\ m^{-3}$)[24]. 4-Ethylguaiacol (RT 10.019 min) has smoky roasted flavor and burnt taste and 3-ethylphenol has burnt taste[25]. Phenol acetate (RT 5.470 min) is newly identified which is not seen previously reported on smoked products[11,13-20]. Otherwise, among all detected compounds in three fractions, 2-nonen-1-ol, hexadecanal and octadecanal are in some degree meaty. 5-Ethyl-2-methylpyridine affords fatty and green odours[25] 1,2-Epoxyhexadecane, diphenylamine, 4-(2,5-dihydro-3-methoxyphenyl)butylamine, 3,4-dimethyl-3-cyclohexen-1-carboxaldehyde, 4,7-dimethyl-3,8-phenanthroline, 3-(2,2-dimethylpropylidene)bicyclo[3.3.1]nonane-2,4-dione, N-isopropyl-methanesulfonamido-2-cyclohexene-2-one and 4-isopropenyl-5-methyl-4-hexen-1-al are vague in organoleptic character, but should make contributions to the aroma of Chinese traditional smoke-cured beef on the basis of their structural formulae. Apparicine is a plant alkaloid, which was perhaps taken into it in smoked. So, phenolic derivatives, diphenylamine, 2-nonen-1-ol, 3,4-dimethyl-3-cyclohexen-1-carboxaldehyde, 4,7-dimethyl-3,8-phenanthroline, 5-ethyl-2-methylpyridine, 1,2-epoxyhexadecane, 4-(2,5-dihydro-3-methoxyphenyl)-butylamine, N-isopropylmethanesulfonamido-2-cyclohexene-2-one, 4-isopropenyl-5-methyl-4-hexen-1-al, 3-(2,2-dimethylpropylidene)bicyclo[3.3.1]nonane-2,4-dione, hexadecanal and octadecanal are responsible for the Chinese traditional smoke-cured beef aroma. The volatiles in Chinese traditional smoke-cured beef, however, are very different from fresh beef. The phenolic derivatived volatiles in Chinese traditional smoke-cured beef should be related to smoking during manufacturing.

11 Compounds are newly identified in Chinese traditional smoke-cured meat.These compounds include phenol acetate, 3-ethylphenol, 3,4-dimethyl-3-cyclohexen-1-carboxaldehyde, 1,2-epoxyhexadecane, 5-ethyl-2-methylpyridine, 4-(2,5-dihydro-3-methoxyphenyl)butylamine, 4-propylguaiacol, 4,7-dimethyl-3,8-phenanthroline, 4-isopropenyl-5-methyl-4-hexen-1-al, 3-(2,2-dimethylpropylidene) bicyclo[3.3.1]nonane-2,4-dione and octadecanal. These compounds were not identified in our previously report on Chinese traditional smoke-cured bacon[1,2].

ACKNOWLEDGEMENTS

The authers thank the significant fund of the education department of Hubei province (2001Z08002) and national natural science foundation of China (20376003) for the financial support of this investigation.

REFERENCES

1. Yu Ai-Nong and Sun Bao-Guo, 2005. Food Chemistry, 89: 227-233.
2. Yu Ai-Nong and Wu Shao-Yan, 2003. Food Science(China), 24: 135-138.
3. Gorraiz, C., M.J. Beriain and J. Chasco, 2002. J. Food Sci., 67: 916-922.
4. Insausti, K., M.J. Beriain and C. Gorraiz, 2002. J. Food Sci., 67: 1580-1589.
5. Machiels, D. and L. Istasse, 2003. Talanta, 61: 529-537.
6. Ramarathnam, N., L.J. Rubin and L.L. Diosady, 1991. J. Agric. Food Chem., 39: 1839-1847.
7. Ramarathnam, N., L.J. Rubin and L.L. Diosady, 1993. J. Agric. Food Chem., 41: 939-945.
8. Thongwong, A., L.N. Fernando and I.U. Grün, 1999. J. Food Sci., 64: 387-389.
9. Vercellotti, J.R., J.C.W. Kuan, R.H. Liu, M.G. Legendre, A.J.St. Angelo and H.P. Dupuy, 1987. J. Agric. Food Chem., 35: 1030-1035.
10. Wettasinghe, M., T. Vasanthan and F. Temelli, 2001. Food Res. Intl.,, 34: 149-158.
11. Toth, L. and K. Potthast, 1984. Advances in Food Res., 29: 87-158.
12. Christopher, G.N., J.S. James and R.C. Glen, 1999. Environ. Sci. Technol., 33: 3313-3316.
13. Daun, J.H., 1979. Food Technol., 33: 66-71, 83.
14. Fujimaki, M., K. Kim and T. Kurata, 1974. Agric. Biol. Chem., 38: 45-52.
15. Guillen, M.D. and M.J. Manzanos, 1999. J. Sci. Food Agric., 79: 1267-1274.
16. Guillen, M.D. and M.C. Errecalde, 2002. J. Sci. Food Agric., 82: 945-952.
17. Hanson, R., 2000. Meat Intl., 10: 18-20.
18. Hierro, E., L. Hoz and J.A. Ordonez, 2004. Food Chem., 85: 649-657.
19. Kjällstrand, J. and G. Petersson, 2001. Food Chem., 74: 85-89.

20. Polignne, A. Collignan and G. Trystram, 2002. J. Food Sci., 67: 2976-2986.
21. Elmore, J.S., D.S. Mottram and E. Hierro, 2000. J. Chromatography, 905: 233-240.
22. Huang, Z.X., Q.Z. Jin, S.G. Luo and L.H. Chen, 1991. Flavor Chemistry and Technology. Beijing: Chinese Light Industry Press, pp: 207-214.
23. Baltes, W. and I. Söchtig, 1979. Zeitschrift fur Lebensmittel-Untersuchung und-Forschung, 169: 9-16.
24. Amoore, J.E. and E. Hautala, 1983. J. Applied Toxicol., 3: 272-290.
25. Winter, M., I.M. Goldman and F. Gautschi, 1976. Flavoring agent. United States Patent, 3: 943,260.