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Analysis of volatile components in a Chinese fish sauce, Fuzhou Yulu, by gas chromatography-mass spectrometry*

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Abstract: Volatile components of Fuzhou Yulu, a Chinese fish sauce, were analyzed by gas chromatography-mass spectrometry (GC-MS), and two pretreatment methods, i.e., purge and trap (P&T) GC-MS and ethyl acetate extraction followed by GC-MS, were compared. P&T-GC-MS method determined 12 components, including sulfur-containing constituents (such as dimethyl disulfide), nitrogen-containing constituents (such as pyrazine derivatives), aldehydes and ketones. Ethyl acetate extraction followed by GC-MS method detected 10 components, which were mainly volatile organic acids (such as benzenepropanoic acid) and esters. Neither of the two methods detected alcohols or trimethylamine. This study offers an important reference to determine volatile flavor components of traditional fish sauce through modern analysis methods.

Key words: Fish sauce, Fuzhou Yulu, Volatile component, Purge and trap, Gas chromatography-mass spectrometry (GC-MS)
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INTRODUCTION

Yulu, also known as a fish sauce, is a clear brown liquid, produced by fermenting small fish and shrimp or byproducts from aquatic products processing, which have been preconditioned with salt (Sanceda *et al*., 2003a; 2003b). Fermentation results from the action of endogenous enzyme or additional microbial enzymes (Fukami *et al*., 2004a; 2004b; Siringan *et al*., 2006; Dissaraphong *et al*., 2006). The regions of fish sauce producing and consumption are comparatively scattered mainly in the southeast of Asia, east coast of China, Japan, and the north of Philippines. As one of the most famous brands of fish sauces in China, Fuzhou Yulu has a maximal yield and has been exported to 16 nations or regions in the world.

Because of its unique, traditional flavor, the fish sauce has been widely consumed as one of the condiments for cooking, although the use of amount is considerably tiny. While there have been many reports focusing on the nutrient compositions of fish sauces (Ijong and Ohta, 1995; Brillantes and Samosorn, 2001; Park *et al*., 2001; 2002a; 2002b; Sanceda *et al*., 2003a; 2003b), limited reports about their volatile flavor components are available.

In the present study, we analyzed the volatile components in Fuzhou Yulu, a typical and representative Chinese fish sauce by using gas chromatography-mass spectrometry (GC-MS), and compared two pretreatment methods, purge and trap (P&T) GC-MS and ethyl acetate extraction followed by GC-MS. The component fingerprint we described here can be used as a control for the comparison between different brands of fish sauce. In addition, the flavor component analysis method we used here can also be applied to other traditional food products.

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MATERIALS AND METHODS

Materials

During the production of Fuzhou Yulu, all raw materials were fermented with fish enzymes and salt-resistant microorganisms at natural temperature for 10~12 months, and high concentration of salt was used to prevent spoilage. Generally, the fermented mash was extracted and passed through the filters three times to produce the first, second, and third extracts, respectively. In this study, we only chose the first extract for analysis, as the first extraction sauce represents the most unique flavors of different brands of fish sauces. All samples were collected from 8 factories in Fuzhou, Fujian Province, China. All these producers use traditional brewing techniques without adding any food additives. All chemicals used were of analytical grade and obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

Preparation of samples

1. Ethyl acetate extraction

The 50 ml sample was mixed with 20 ml ethyl acetate and loaded into a 250-ml separatory funnel; after shaking vigorously, the funnel was laid still for 20 min; the upper organic phase was collected. Then repeated this process three times. The final extract was stored at 4 °C before GC-MS analysis.

2. Purge and trap (P&T)

The 5 ml sample was introduced into a glass vessel at 60 °C and purged with 60 ml/min of N_2 for 11 min. Stainless steel cartridges packed with 100 mg Tenax TA (previously deactivated under N_2 at 250 °C for 2 h) were used as traps. After that, the cartridges were capped at 210 °C for 4 min, with a split ratio of 2:1.

3. Determination by GC-MS

The GC-MS system used for analysis was Agilent Technologies 6890N with Agilent 5975 inert XL Mass selective Detector (Agilent Technologies Co., Ltd., Palo Alto, USA) operating at 70 eV in EI (electron ionization) mode. Data acquisition and analysis were performed by using Enhanced Data Analysis software, which is supplied by the manufacturer. Substances were separated on a fused silica capillary column, HP-5MS flexible glass capillary gas chromatography column (30 m×0.25 mm×0.25 μm, Agilent Technologies Co., Ltd., Palo Alto, USA). The column was kept at 35 °C for 4 min, heated from 35 to l00 °C at a programmed rate of 30 °C/min and held at l00 °C for 2 min, and then heated from 100 to 300 °C at a rate of 15 °C/min and held at 300 °C for 10 min. The temperatures for the injection port, ion source, quadrupole, and interface were set at 250, 230, 150, 280 °C, respectively. Helium with a flow rate of 0.5 ml/min was used as carrier gas, and split ratio was 2:1. The parameter was monitored in full scan mode with a scan range of m/z 40~400.

RESULTS

Analytical results of purge and trap

The GC-MS with P&T chromatographic profile of the sample is shown in Fig.1, and the list of the principal volatile compounds in Fuzhou Yulu is given in Table 1. A total of 12 peaks were identified, and 10 matched to the known compounds: dimethyl disulfide, 2-heptanone, 2,6-dimethyl pyrazine, benzaldehyde, dimethyl trisulfide, 2-ethyl-6-methyl pyrazine, 2-methyl-5-(1-methylethyl) pyrazine, acetophenone, 3-ethyl-2,5-dimethyl pyrazine, dimethyl tetrasulfide, respectively (Table 1). The match degrees of these 10 peaks were all above 78%. The other two peaks failed to match to any known compounds due to their low match degrees (Table 1).

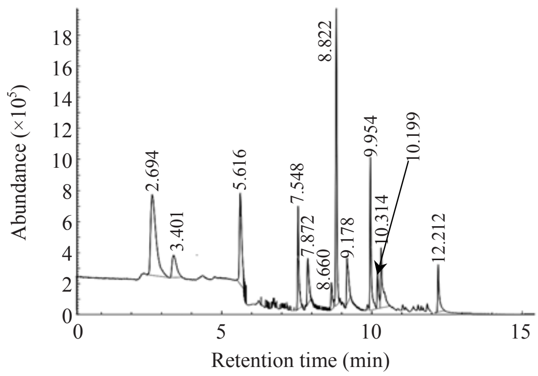

Fig.1 GC-MS with P&T chromatogram of Fuzhou Yulu

Analytical results of extracts by ethyl acetate

The GC-MS chromatographic profiles of the solvent (ethyl acetate) and the ethyl acetate extracted sample are shown in Figs.2a and 2b, respectively. The comparison of principal volatile compounds between solvent and the extracted sample is given in Table 2.

Table 1 Volatile components identified in Fuzhou Yulu by GC-MS with P&T

Peak No.	*RT* (min)	Component name	Match (%)
1	2.694	Acetone	9
2	3.401	Formic acid ethenyl ester	9
3	5.616	Dimethyl disulfide	96
4	7.548	2-Heptanone	91
5	7.872	2,6-Dimethyl pyrazine	72
6	8.660	Benzaldehyde	95
7	8.822	Dimethyl trisulfide	95
8	9.178	2-Ethyl-6-methyl pyrazine	90
9	9.954	2-Methyl-5-(1-methylethyl) pyrazine	87
10	10.199	Acetophenone	87
11	10.314	3-Ethyl-2,5-dimethyl pyrazine	91
12	12.212	Dimethyl tetrasulfide	78

RT: retention time

Table 2 Volatile components identified in extracted Fuzhou Yulu by GC-MS

Peak No.	*RT* (min)	Component name	Match (%)
Ethyl acetate			
1~14	3.490~4.386	Ethyl acetate	90~72
15	5.240	Propanoic acid ethyl ester	72
16	5.976	Toluene	95
17	6.589	Acetic acid, butyl ester	90
Fuzhou Yulu			
1~27	3.401~4.386	Ethyl acetate	90~72
28	5.240	Propanoic acid ethyl ester	64
29	5.976	Toluene	91
30	6.323	2-Methyl propanoic acid	64
31	6.593	Acetic acid, butyl ester	90
32	6.876	Butanoic acid	91
33	7.350	3-Methyl butanoic acid	81
34	7.583	2-Methyl hexanoic acid	78
35	12.429	Benzeneacetic acid	90
36	13.376	Benzenepropanoic acid	96
37	17.520	Diethyldithiophosphinic acid	43
38	17.791	Phthalic acid isobutyl octyl ester	90
39	18.192	2,3,4-Trihydroxybenzaldehyde	47
40	18.298	2,3,4-Trihydroxybenzaldehyde	43
41	18.429	1,2-Benzenedicarboxylic acid butyl 2-methylpropyl ester	93

RT: retention time

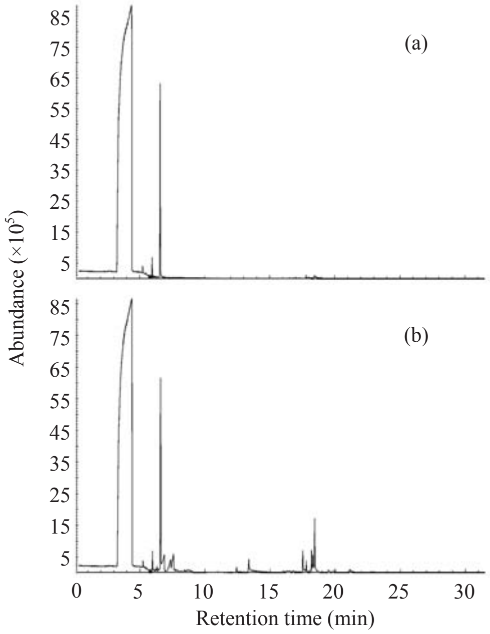

Fig.2 GC-MS chromatogram of (a) ethyl acetate and (b) Fuzhou Yulu extracted by ethyl acetate

As shown in Fig.2a, all peaks of ethyl acetate have flown out within 7 min. Therefore, the peaks after 7 min in Fig.2b belong to the extracted sample. Except for ethyl acetate, propanoic acid ethyl ester, toluene, acetic acid and butyl ester which exist in extractant, 2-methyl propanoic acid, butanoic acid, 3-methyl butanoic acid, 3-methyl pentanoic acid, 2-methyl butanoic acid, 2-methyl hexanoic acid, benzeneacetic acid, benzenepropanoic acid, phthalic acid isobutyl octyl ester, and 1,2-benzenedicarboxylic acid butyl 2-methylpropyl ester were also determined in ethyl acetate extracted samples. In addition, diethyldithiophosphinic acid and 2,3,4-trihydroxybenzaldehyde could not be confirmed due to their low match degrees.

DISCUSSION

Effect of different method on analytical results

In previous studies (Shimoda *et al*., 1996; Michihata *et al*., 2002), a group of volatile compounds in fish sauce were identified by using headspace gas analysis, which includes alcohols, nitrogen-containing constituents, sulfur-containing constituents and aldehydes. However, none of fatty acid or long-chain alcohols was detected by this method. Considering the powerful analytical ability of GC-MS in determining volatile flavor components, we successfully applied GC-MS analysis on fish sauce. The results reveal comprehensive constitution of volatile components in fish sauce, with some components detected for the first time, such as acetophenone.

We also compared the two different pretreatment methods in preparing the samples, i.e., P&T and ethyl acetate extractions. We found considerable differences between these two methods. When the fish sauce sample was pretreated with P&T method, GC-MS could detect sulfur-containing constituents and nitrogen-containing constituents, i.e., aldehydes and ketones. When the fish sauce sample was treated by ethyl acetate extraction, GC-MS could detect organic acids and esters. These data indicate that different sampling injection and different pretreated method were able to detect different components. Therefore, in order to determine a more comprehensive fingerprint of the volatile components of fish sauce, we need to collect different samples widely and to combine different analytical methods as well.

Characteristics of volatile components of Fuzhou Yulu

A total of 22 volatile components of fish sauce were detected in this study, including sulfur-containing constituents, nitrogen-containing constituents, aldehydes, ketones, organic acids, and esters. While the results show a great similarity between Fuzhou Yulu and other brands of fish sauces, the characteristics of Fuzhou Yulu were also detected. Fuzhou Yulu and other brands of fish sauces were all rich in dimethyl sulfides, pyrazine derivatives and volatile organic acids, but the constitution of organic acids in Fuzhou Yulu was slightly different to that in others. In this study, only phthalic acid isobutyl octyl ester and 1,2-benzenedicarboxylic acid butyl 2-methylpropyl ester were detected in Fuzhou Yulu, but others reported that fish sauce has many different types of esters (Shimoda *et al*., 1996). There were a few kinds of aldehydes and ketones in Fuzhou Yulu, especially simple aldehydes and ketones. In addition, lower alcohols and trimethylamine were not detected in Fuzhou Yulu.

CONCLUSION

In summary, we analyzed the volatile components in Fuzhou Yulu by GC-MS, and compared two pretreatment methods, P&T-GC-MS and ethyl acetate extraction followed by GC-MS. We found that the main volatile components of Fuzhou Yulu are similar to those of other brands of fish sauces, but it also has its unique characteristics, which is probably due to producers' proprietary usage of raw materials and the influence of local climate. The component fingerprint we described here can be used as a control for the comparison between different brands of fish sauces. In addition, the flavor components analysis method proposed in this study can also be applied to the analysis of other traditional food products.

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References

Brillantes, S., Samosorn, W., 2001. Determination of histamine in fish sauce from Thailand using a solid phase extraction and high-performance liquid chromatography. *Fisheries Science*, **67**(6):1163-1168. [doi:10.1046/j.1444-2906.2001.00375.x]

Dissaraphong, S., Benjakul, S., Visessanguan, W., Kishimura, H., 2006. The influence of storage conditions of tuna viscera before fermentation on the chemical, physical and microbiological changes in fish sauce during fermentation. *Bioresource Technology*, **97**(16):2032-2040. [doi:10.1016/j.biortech.2005.10.007]

Fukami, K., Satomi, M., Funatsu, Y., Kawasaki, K.I., Watabe, S., 2004a. Characterization and distribution of *Staphylococcus* sp. implicated for improvement of fish sauce odor. *Fisheries Science*, **70**(5):916-923. [doi:10.1111/j.1444-2906.2004.00887.x]

Fukami, K., Funatsu, Y., Kawasaki, K., Watabe, S., 2004b. Improvement of fish-sauce odor by treatment with bacteria isolated from the fish-sauce mush (moromi) made from frigate mackerel. *Food Microbiology and Safety*, **69**(2):45-49.

Ijong, F.G., Ohta, Y., 1995. Amino acid compositions of bakasang, a traditional fermented fish sauce from Indonesia. *Lebensmittel Wissenschaft Technologie*, **28**(2): 236-237.

Michihata, T., Yano, T., Enomoto, T., 2002. Volatile compounds of headspace gas in the Japanese fish ishiru. *Bioscience Biotechnology and Biochemistry*, **66**(10): 2251-2255. [doi:10.1271/bbb.66.2251]

Park, J.N., Fukumoto, Y., Fujita, E., Tanaka, T., Washio, T., Otsuka, S., Shimizu, T., Watanabe, K., Abe, H., 2001. Chemical composition of fish sauces produced in southeast and east Asian countries. *Journal of Food Composition and Analysis*, **14**(2):113-125. [doi:10.1006/jfca.2000.0963]

Park, J.N., Ishida, K., Watanabe, T., Endoh, K.I., Watanabe, K., Murakami, M., Abe, H., 2002a. Taste effects of oligopeptides in a Vietnamese fish sauce. *Fisheries Science*, **68**(4):921-928. [doi:10.1046/j.1444-2906.2002.00511.x]

Park, J.N., Watanabe, T., Endoh, K.I., Watanabe, K., Abe, H., 2002b. Taste-active components in a Vietnamese fish sauce. *Fisheries Science*, **68**(4):913-920. [doi:10.1046/j.1444-2906.2002.00510.x]

Sanceda, N.G., Suzuki, E., Kurata, T., 2003a. Branched chain amino acids as source of specific branched chain volatile fatty acids during the fermentation process of fish sauce. *Amino Acids*, **24**(1):81-87.

Sanceda, N., Suzuki, E., Kurata, T., 2003b. Quality and sensory acceptance of fish sauce partially substituting sodium chloride or natural salt with potassium chloride during the fermentation process. *International Journal of Food Science and Technology*, **38**(4):435-443. [doi:10.1046/j.1365-2621.2003.00710.x]

Shimoda, M., Peralta, R.R., Osajima, Y., 1996. Headspace gas analysis of fish sauce. *Journal of Agricultural and Food Chemistry*, **44**(11):3601-3605. [doi:10.1021/jf960345u]

Siringan, P., Raksakulthai, N., Yongsawatdigul, J., 2006. Source and changes of proteinase activities of Indian anchovy (*Stolephorus* spp.) during fish sauce fermentation. *Journal of the Science of Food and Agriculture*, **86**(12):1970-1976. [doi:10.1002/jsfa.2581]