Characterization of Pyrazines in Some Chinese Liquors and Their Approximate Concentrations

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Pyrazines are very important impact aroma compounds in Chinese liquors. The identification of pyrazine derivatives was carried out by liquid–liquid extraction (LLE). The liquor sample was adjusted to the H^+ concentration of 1 N with 12 N HCl and then concentrated by rotatory evaporator under vacuum condition. The concentrated liquor was extracted by diethyl ether, and the residual aqueous phase was adjusted to pH 10. The basic compounds were detected and identified by gas chromatography (GC)–mass spectrometry (MS). A total of 27 pyrazines were identified in Chinese liquors, mainly alkyl- and acetylpyrazines. A method for determining pyrazines in Chinese liquors was developed. It involves extraction by headspace (HS) solid phase microextraction (SPME) and determination using GC–flame thermionic detector (FTD). The optimum method was that the sample alcohol concentration was diluted to 12% vol by freshly redistilled–deionized water, and the diluted samples were saturated with NaCl and equilibrated at 50 °C for 15 min and extracted for 30 min at the same temperature. The developed method enabled detection limits of <200 ng/L. Linearity (R^2 > 0.99) and recovery rate were satisfied in all cases. Pyrazines of 12 commercial typical Chinese liquors were quantified by HS-SPME followed by GC-FTD and had a wide range of concentration.

KEYWORDS: Headspace solid phase microextraction (HS-SPME); pyrazines; Chinese liquor; gas chromatography–mass spectrometry (GC-MS); flame thermionic detector (FTD)

INTRODUCTION

Chinese liquor is one of the oldest distillates in the world and is typically made from sorghum or a mixture of wheat, barley, corn, rice, and sorghum. Because of different Daqu qualities, liquor-making processes, and fermentations, Chinese liquors are obviously different in sensory quality. According to aroma characteristics, Chinese liquors can be classified into five types on the basis of the sensory evaluation: strong aroma style, light aroma style, soy sauce aroma style, sweet honey style, and miscellaneous style liquors (*1, 2*). The strong aroma style liquor has strong fruity, pineapple-, banana-, and apple-like aromas. The sensory quality of light aroma style liquor is similar to that of the strong aroma style, but the aroma intensity is weaker. The soy sauce aroma style liquor has soy sauce-like and roasted aromas, whereas the miscellaneous aroma style liquor is between the say sauce aroma style and the strong aroma style liquors by sensory assessment. The complex aroma style liquor is one of the most important styles in the miscellaneous liquor, and it has become very popular in China recently.

Chinese liquor mainly consists of ethanol (40–55% vol) and water. However, its aroma is mainly contributed by micro aroma compounds, such as alcohols, esters, acids, acetals, ketones, aldehydes, sulfur-containing compounds, lactones, and heterocyclic compounds (*1, 3, 4*). A few studies (*1, 3, 4*) have reported that ethyl esters and some fatty acids were the most important aroma compounds in the strong aroma style liquors, whereas no studies of aromas relating to the light, soy sauce, and complex aroma style liquors have been published.

Pyrazine is one of the most important classes of aroma compounds in raw and processed food and alcoholic beverages, for example, baked potato (*5*), cheese (*6*), fermented soybean (*7, 8*), wine (*9–11*), and liquor (*1, 3, 12*). Pyrazines are usually formed by Maillard reaction (*13*). Recently, researchers have reported that *Bacillus subtilis* can produce pyrazines in the solid-state fermentation on soybean (*7, 14*). Therefore, some researchers presumed that pyrazines could be the most important aroma compounds of the soy sauce aroma style liquor (*15–17*).

In fact, several searchers have reported that pyrazines were detected in distillates. Benn and Peppard (*12*) analyzed the volatile compounds of tequila, a kind of liquor made in Mexico, using liquid–liquid extraction (LLE) followed by gas chromatography (GC)–mass spectrometry (MS). They identified five pyrazines, including 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3-methylpyrazine, and 2,3,5,6-tetramethylpyrazine, and their relative levels were also given. Moreover, nitrogen-containing heterocyclic compounds

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were also reported in whiskey (*18, 19*). Fan and Qian (*1, 3*) reported that several pyrazines have been detected by GC–olfactometry (GC-O) and GC-MS in Chinese liquors, including 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2,3,5-trimethylpyrazine, 2,6-diethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 2,3-dimethyl-5-ethylpyrazine, 2,3,5,6-tetramethylpyrazine, 2,3,5-trimethyl-6-ethylpyrazine, 2-butyl-3,5-dimethylpyrazine, and 3,5-dimethyl-2-pentylpyrazine. These pyrazine derivative compounds contributed to baked, nutty, and roasted notes, and they were very important to Chinese liquors on the basis of FD values. However, no quantification data were done in those papers. On the other hand, Yu and co-workers (*20*) employed a method using a rotary vacuum evaporator to eliminate most of the water, alcohol, and other volatile compounds; 29 pyrazines in Moutai (MT) liquor were identified by GC-MS. Among these, four pyrazines, including 2-methylpyrazine, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine, and 2,3,5,6-tetramethylpyrazine, were quantified, and the others were semiquantified. Moreover, identifications of some unknown pyrazines were suspected.

Many extraction techniques had been used in the quantification of pyrazines, such as LLE (*21*), vacuum distillation followed by LLE (*22*), selective purge-and-trap method (*23*), solid phase extraction (*9, 24*), ion-exchange resin extraction (*25*), supercritical carbon dioxide extraction (*26*), and simultaneous distillation–extraction (SDE) (*27*). However, these methods are laborious, tedious, and time-consuming. In addition, artifacts in SDE would be possibly formed. More recently, several researchers employed a simple and rapid method for the quantification of pyrazines, which is headspace (HS) solid phase microextraction (SPME) coupled with nitrogen–phosphorus detection (NPD) (*10, 11, 28*) or coupled with GC–atomic emission detection (ACD) (*29*). Hartmann and co-workers (*28*) studied 3-alkyl-2-methoxypyrazines in spiked model wines by HS-SPME. The divinylbenzene/carboxen/poly(dimethylsiloxane) (DVB/CAR/ PDMS) coated fibers resulted in the highest analyte recoveries at optimum extraction conditions. Sala and co-workers (*10, 11*) developed the HS-SPME method for the determination of 3-alkyl-2-methoxypyrazines in musts and wines. They all reported the measurement of alkylmethoxypyrazines at the nanograms per liter level in grape musts and wines and concluded that the HS-SPME method could be successfully applied to the experimental musts and red wine; thus, the evolution of their pyrazine contents during the ripening or the winemaking process could be monitored.

The aim of our study was to identify pyrazines in Chinese liquors, to set up a method for determining pyrazines by means of HS-SPME followed by GC-FTD, and to determine the profile of pyrazines in the difference aroma-type Chinese liquors.

MATERIALS AND METHODS

Chemicals. Pyrazine (98.0%), 2-methylpyrazine (98.0%), 2,3-dimethylpyrazine (98.0%), 2,5-dimethylpyrazine (98.0%), 2,6-dimethylpyrazine (98.0%), 2-ethylpyrazine (98.0%), 2,3,5-trimethylpyrazine (98.0%), 2,3,5,6-tetramethylpyrazine (98.0%), and 2-acetylpyridine (99.0%) were purchased from Sigma-Aldrich (Shanghai, China).

Sodium chloride, anhydrous sodium sulfate, hydrochloric acid, sodium hydroxide, diethyl ether, hydrochloric acid, and absolute ethanol were purchased from China National Pharmaceutical Group Corporation (Shanghai, China).

Liquor Samples. All Chinese liquor samples were purchased from local stores, including Moutai liquor (MT, 500 mL, 53% vol), Moutaiyingbin liquor (MTYB, 500 mL, 53% vol), Langjiu liquor (LJ, 500 mL, 53% vol), Wuliangye liquor (WLY, 500 mL, 52% vol), Yanghelansejindian liquor (YHLS, 500 mL, 46% vol), Jinshiyun liquor (JSY, 500 mL, 42% vol), Gujinggong liquor (GJG, 500 mL, 55% vol),

Xifengjiu liquor (XFJ, 500 mL, 45% vol), Dongjiu liquor (DJ, 500 mL, 54% vol), Shite liquor (ST, 500 mL, 52% vol), Jiannanchuan liquor (JNC, 500 mL, 52% vol), and Fenjiu liquor (FJ, 500 mL, 45% vol). All of these Chinese liquors were bottled in 2005, and all samples were stored at 4 °C until analysis.

LLE for Identification of Pyrazines. A total of 1000 mL of Chinese liquor was adjusted H^+ to a final concentration of 1 N with 12 N HCl and then was concentrated to about 20 mL using the Rotavapor (Büchi, Postfach, Switzerland) under vacuum at 50 °C. The concentrate was transferred to a separatory funnel and extracted three times with 20 mL of freshly redistilled diethyl ether each. The residual aqueous phase was adjusted to pH 7 with 12 N NaOH and then to pH 10 with 1 N NaOH. Then, it was saturated with NaCl and extracted three times with 20 mL of freshly redistilled diethyl ether each. The basic extracts were combined and dried over 5 g of anhydrous sodium sulfate. The dried solution was filtered and then slowly concentrated to a final volume of 0.5 mL under a gentle stream of nitrogen. The concentrate was stored at −20 °C for further GC-MS analysis.

GC-MS Analysis. Identification was carried out using an Agilent 6890N GC coupled to an Agilent 5975 mass selective detector (MSD). The concentrate (1 μL) was analyzed on a DB-Wax column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific) and on a HP-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent). The injector temperature was 250 °C, and the splitless mode was used. The oven temperature was held at 50 °C for 2 min, then raised to 230 °C at a rate of 4 °C/min, and held at 230 °C for 15 min on the DB-Wax column. For the HP-5 column, the oven temperature was held at 50 °C for 2 min, increased to 140 °C at a rate of 2 °C/min, then held for 0 min, raised to 280 °C at a rate of 10 °C/min, and held at 280 °C for 10 min The column carrier gas was helium at a constant flow rate of 2 mL/min. The electron impact energy was 70 eV, and the ion source temperature was set at 230 °C. Mass spectra of unknown compounds were compared with those in the Wiley 275.L database (Agilent Technologies, Inc.), NIST05a.L, and AMDIS. Retention indices (RIs) of unknown compounds were calculated in accordance with a modified Kovats method (*30*). Identification of unknown pyrazines was achieved by comparing mass spectra and RIs of the standards or RIs from the literature (RILs).

HS-SPME Parameter. The SPME holder and a 50/30 μm DVB/CAR/ PDMS fiber (2 cm, Supelco, Inc., Bellefonte, PA) were used for pyrazine extraction. Before analysis, the fiber was conditioned by inserting it into the GC injector at the 250 °C for 2 h to prevent contamination. Each liquor sample was diluted with freshly redistilled–deionized water to a final concentration of 12% vol ethanol. A total of 5 mL of diluted sample was put into a 15 mL vial and spiked with 50 μL of 2-acetylpyridine (internal standard, IS), 10 mg/L in ethanol. The diluted solution was saturated with NaCl, and a small magnetic stirrer was added. The vial was tightly capped with a Teflon-faced silicone septum. This sample was equilibrated at 50 °C in an isothermal bath for 15 min and extracted for 30 min at the same temperature under continuous stirring. After extraction, the fiber was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC for thermal desorption at 250 °C for 5 min. All analyses were made in triplicate.

GC-Flame Thermionic Detector (FTD) Analysis. Chromatographic analyses were made on s GC 2010 coupled with s FTD (Shimadzu, Kyoto, Japan). The injection was made in the split mode (split ratio 1:1) at 250 °C. Each sample was analyzed on a DB-Wax (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific) fused-silica capillary column. The column carrier gas was high-purity helium at a constant flow of 3 mL/min. The makeup gas to the detector was nitrogen at 27.5 mL/min. The H_2 flow was1.5 mL/min and the air flow 144.9 mL/min. The temperature of the detector was held at 250 °C. The oven temperature was held at 50 °C for 2 min, and then it was raised at a rate of 2 °C/min to 85 °C and held for 0.1 min. Finally, the oven temperature was increased at a rate of 5 °C/min to 210 °C and held at 210 °C for 2 min.

Quantitation Method. *Calibration of Standard Curves and Quantification of Samples.* The HS-SPME and GC-FTD conditions were set as described previously. The standard curves for individual pyrazines were built up by plotting the response ratio of target compound and IS

Table 1. Calibration Data of Pyrazines Standard and Their Recovery in Chinese Liquors

pyrazine	slope	intercept	R^2	n	linear range (μg/L)	DL (ng/L)	synthetic liquor recovery (%)	RSD (%)	MT recovery (%)	RSD (%)	GJG recovery (%)	RSD (%)
pyrazine	0.6463	0.0451	0.9954	6	1.25–320	156.25	99.39	5.70	99.59	8.93	ND[a]	ND
2-methylpyrazine	2.2330	−0.0978	0.9915	7	0.78–400	97.66	119.10	4.72	95.47	5.23	93.53	6.81
2,5-dimethylpyrazine	2.3034	0.1408	0.9954	10	0.63–320	125.00	93.74	4.70	84.54	9.41	91.58	5.42
2,6-dimethylpyrazine	2.5908	−0.0574	0.9948	7	0.78–400	65.10	103.38	4.09	89.43	5.76	108.02	5.91
2-ethylpyrazine	4.0155	−0.1282	0.9927	7	0.78–200	78.12	103.26	5.13	89.68	10.86	88.17	10.29
2,3-dimethylpyrazine	1.9084	0.0811	0.9955	9	0.47–240	117.19	89.66	6.17	95.67	9.64	117.16	9.72
2,3,5-trimethylpyrazine	3.0638	0.0555	0.9999	7	1.56–400	195.53	101.46	6.83	92.58	9.68	102.53	8.49
2,3,5,6-tetramethylpyrazine	2.7129	0.1411	0.9996	5	1.56–400	156.25	83.20	11.52	91.27	6.97	109.81	4.04

[a] ND, not detected in this liquor.

Table 2. Identification of Pyrazines in Chinese Liquors by LLE-GC-MS and HS-SPME Coupled with FTD on both DB-Wax and DB-5 Columns

peak	RI_{DB-Wax}	RIL_{polar}	RI_{DB-5}	$RIL_{nonpolar}$	pyrazine	identification[a]
1	1201	1207 (45)	730	734 (13)	pyrazine	MS, RI
2	1265	1262 (45)	832	820 (34)	2-methylpyrazine	MS, RI
3	1321	1320 (45)	917	909 (34)	2,5-dimethylpyrazine	MS, RI
4	1330	1326 (45)	920	907 (34)	2,6-dimethylpyrazine	MS, RI
5	1334	1357 (46)	931	931 (47)	2-ethylpyrazine	MS, RI
6	1342	1309 (33)	922	916 (34)	2,3-dimethylpyrazine	MS, RI
7	1375	1380 (45)	999	977 (33)	2-ethyl-6-methylpyrazine	MS, RIL
8	1387	1385 (45)	1002	980 (33)	2-ethyl-5-methylpyrazine	MS, RIL
9	1397	1367 (33)	1001	984 (33)	2-ethyl-3-methylpyrazine	MS, RIL
10	1402	1404 (45)	990	1000 (34)	2,3,5-trimethylpyrazine	MS, RI
11	1415	1415 (1)	1078	1088 (34)	2,6-diethylpyrazine	MS, RIL
12	1430	1435 (6)	1083	1078 (34)	2,5-dimethyl-3-ethylpyrazine	MS, RIL
13	1445	1460 (48)	1076	1084 (34)	2,3-dimethyl-5-ethylpyrazine	MS, RIL
14	1447	1455 (45)	1077	1083 (34)	3,5-dimethyl-2-ethylpyrazine	MS, RIL
15	1460	1460 (1)	1089	1090 (34)	2,3,5,6-tetramethylpyrazine	MS, RI
16	1469		1159	1160 (49)	3,5-diethyl-2-methylpyrazine	MS, RIL
17	1491	1491 (1)	1163	1163 (1)	2,3,5-trimethyl-6-ethylpyrazine	MS, RIL
18	1506		1185	1184 (50)	2,5-dimethyl-3-isobutylpyrazine	MS, RIL
19	1522	1521 (31)	1016	1031 (47)	2-methyl-6-vinylpyrazine	MS, RIL
21	1567	1567 (33)	1069	1061 (33)	2-acetyl-3-methylpyrazine	MS, RIL
22	1583	1583 (1)	1254	1268 (34)	2-butyl-3,5-dimethylpyrazine	MS, RIL
23	1618	1618 (33)	1088	1088 (33)	2-acetyl-6-methylpyrazine	MS, RIL
24	1628		1156		2-methyl-6-(Z-1-propenyl)pyrazine[b]	MS
25	1654		1174		2-acetyl-3,5-dimethylpyrazine[b]	MS
26	1680		1357		2,5-dimethyl-3-pentylpyrazine[b]	MS
27	1702		1247	1217 (34)	2,3-dimethyl-5-(Z-1-propenyl)-pyrazine	MS, RIL

[a] MS, compounds were identified by MS spectra with a Qual ≥94%. RI, compounds were identified by comparison to the authentic standard. RIL, compounds were identified by comparison with the RI from the literature. [b] Tentatively identified.

against the concentration ratio. The ranges of concentrations of the calibration curves are list in **Table 1**.

When the concentrations of pyrazines in liquor samples exceeded the upper linear range, the liquor samples would be diluted with a 12% vol ethanol/water solution until the levels of pyrazines were within the linear ranges.

Calculation of Recovery. Known amounts of pyrazine standards were evaluated in synthetic liquor, as well as in the MT and GJG liquors. The concentrations of pyrazines in these liquors before and after the addition of pyrazines were quantified according to the procedure described previously. The recovery rates of pyrazines in these liquors were calculated as recovery = [(detected amount after addition − detected amount before addition) × 100%]/added amount.

RESULTS AND DISCUSSION

Extraction and Identification of Unknown Pyrazines. Pyrazines have basic characteristics resulting from the presence of two nitrogen atoms. For the identification of unknown pyrazines, liquor samples could be adjusted to pH 1–2 with HCl or H_2SO_4 according to a traditional method (1, 6). However, some literature data indicate that the pK_a value of pyrazine is 0.65, whereas the pK_a of 2,5-dimethylpyrazine is 1.85 (27). Hérent and co-workers (22) studied the relationship of pyrazine

extraction with pH. Initial samples were adjusted to pH 1, 0.7, 0.5, 0.3, and 0.1. The results showed that the recovery of all pyrazines increased with decreasing pH value. Alkyl-substituted pyrazines are more basic, which leads to better recovery rate in the basic phase, whereas low basicity compounds, such as acetyl- and methoxypyrazine derivatives, stay in the acidic-neutral phase. Yu and co-workers (20) reported that about 90% of pyrazine would react with acid when the H^+ concentration of Chinese liquor was adjusted to 1 N. Therefore, prior to extraction by rotary evaporator in this study, Chinese liquor samples were adjusted to an H^+ concentration of 1 N with concentrated HCl.

A total of 27 pyrazines were detected and identified in Chinese liquors by LLE-GC-MS in this work (**Table 2**). All pyrazines identified in this study were alkyl- and acetylpyrazine derivatives, and no alkoxypyrazines were detected. Of these pyrazines, 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2,3,5-trimethylpyrazine, 2,6-diethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 2,3-dimethyl-5-ethylpyrazine, 2,3,5,6-tetramethylpyrazine, 2,3,5-trimethyl-6-ethylpyrazine, and 2-butyl-3,5-dimethylpyrazine had been identified by GC-MS and GC-O in Chinese liquors (1, 3). Several pyrazines were also detected by Yu

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and co-workers (*20*), including pyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3-methylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 3,5-diethyl-2-methylpyrazine, and 2,5-dimethyl-3-isobutylpyrazine. There were seven pyrazines that were first identified in Chinese liquors on both the DB-Wax and DB-5 columns, including 2-methyl-6-vinylpyrazine, 2-acetyl-3-methylpyrazine, 2-acetyl-6-methylpyrazine, 2-methyl-6-(*Z*-1-propenyl)pyrazine (tentatively identified), 2-acetyl-3,5-dimethylpyrazine [1-(3,5-dimethyl-2-pyrazinyl)-1-ethanone, tentatively identified], 2,5-dimethyl-3-pentylpyrazine (tentatively identified), and 2,3-dimethyl-5-(*Z*-1-propenyl)pyrazine. Their RIs (polar and nonpolar columns) and RILs from the literature are given in **Table 2**. 2-Methyl-6-vinylpyrazine had been detected by static headspace GC-MS in espresso coffee (*31*). 2-Acetyl-3-methylpyrazine was found by Coleman and Ho in the baked potato (*32*). 2-Acetyl-6-methylpyrazine (*33*) and 2,3-dimethyl-5-(*Z*-1-propenyl)pyrazine (*34*) had also been reported in the literature. No RILs of 2-methyl-6-propenylpyrazine, 2-acetyl-3,5-dimethylpyrazine, and 2,5-dimethyl-3-pentylpyrazine were found in the literature.

Optimization of HS-SPME Parameters. Because the HS-SPME method is solvent-free and simple and requires only a small amount of sample, it could be used as a quick method to screen the potentially important aroma compounds. FTD, also named NPD, is a nitrogen-selective detector, and it can greatly simplify the task of detecting nitrogen-containing heterocyclic compounds in the complex flavor samples. HS-SPME coupled with NPD had been applied to detect alkylmethoxypyrazines in musts and wines (*10, 11*). In this study, HS-SPME combined with FTD was employed to detect pyrazines in Chinese liquors. All 27 pyrazines identified by LLE-GC-MS were detected using HS-SPME coupled with FTD.

Different Fiber Type Effects. Four fibers, coating polyacrylate (PA), poly(dimethylsiloxane) (PDMS), carboxen/PDMS (CAR/DMS), and DVB/CAR/PDMS, were used to compare their extraction of the aroma compounds in Chinese liquors. The results showed that the CAR/PDMS and PDMS fibers were more sensitive than the PA fiber for pyrazines, and the DVB/CAR/PDMS fiber had the best enrichment capacity when compared to PA, PDMS, and CAR/PDMS. These results differed from Sala's conclusion (*10*), but were consistent with the paper of Hartmann and co-workers (*28*).

Salt Concentration Effect. Some researchers reported that high ionic strength increased the extraction efficiency in headspace analysis (*35, 36*). The liquor samples saturated with NaCl or without salt were studied. All pyrazines found in this study were more sensitive in the sample saturated with NaCl than the sample without salt. The total peak areas of pyrazines increased >50% in the sample saturated with salt, compared to the sample without salt. Therefore, taking into account both the single pyrazine concentration and total peak areas, the sample saturated with salt was chosen for all investigated pyrazines.

Extraction Time Effect. To obtain the best extraction, 20, 30, 40, and 50 min sampling times were tested. The results showed that the concentrations of pyrazines absorbed to the fiber reached a maximum value at 30 min. More extraction time did not increase the total amount and number of pyrazines. An equilibration time of 15 min and extraction for 30 min were considered to be adequate.

Extraction Temperature Effect. It is well-known that the extraction rate is strongly influenced by temperature in HS-SPME analysis. Extraction temperatures of 30, 40, 50, and 60 °C were tested, respectively. The results showed that the concentrations of pyrazines increased with extraction temperature up to 50 °C. When the adsorption temperature was at 60 °C, pyrazine levels were less than those at 50 °C. The temperature of 50 °C was the most sensitive for pyrazines detection.

Ethanol Concentration Effect. The matrix or ethanol concentration is a very important factor for volatile extraction. Three matrix ethanol levels, 6, 12, and 18% vol, were tested. The results showed that the sample with 12% ethanol was the most sensitive for HS-SPME extraction of pyrazines.

On the basis of these observations, in our study, the sample alcohol concentration was diluted to 12% vol by freshly redistilled–deionized water, and the diluted samples were saturated with NaCl and equilibrated at 50 °C for 15 min and extracted for 30 min at the same temperature.

Quantification Method of Pyrazines. For most pyrazines, the linear responses were obtained with R^2 ranging from 0.9915 (2-methylpyrazine) to 0.9999 (2,3,5-trimethylpyrazine), and the detection limits (DL) were all below 200 ng/L; the detection limit of 2,6-dimethylpyrazine was particularly low at 65.10 ng/L. The recoveries of all pyrazines in the synthetic liquor ranged from 83.2 to 119.10%, and similar results were obtained in both the MT and GJG liquors (**Table 1**). Except for eight pyrazines (**Table 1**), the other target compounds were only semiquantified. Because none of these pyrazine standards were available, and the number of C atoms of these pyrazines is greater than that of 2,3,5,6-tetramethylpyrazines, their slopes and intercepts were appointed as 2.7129 and 0, respectively; these pyrazines included 2,6-diethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 2,3-dimethyl-5-ethylpyrazine, 3,5-dimethyl-2-ethylpyrazine, 3,5-diethyl-2-methylpyrazine, 2,3,5-trimethyl-6-ethylpyrazine, 2,5-dimethyl-3-isobutylpyrazine, 2-methyl-6-vinylpyrazine, 2-acetyl-3-methylpyrazine, 2-butyl-3,5-dimethylpyrazine, 2-acetyl-6-methylpyrazine, 2-methyl-6-(*Z*-1-propenyl)pyrazine, 2-acetyl-3,5-dimethylpyrazine, 2,5-dimethyl-3-pentylpyrazine, and 2,3-dimethyl-5-(*Z*-1-propenyl)pyrazine.

Pyrazines in Chinese Liquors. Twelve different typical commercial liquor samples obtained from a supermarket were analyzed by HS-SPME coupled with GC-FTD (**Table 3**). Twenty-two pyrazines were detected in the MT liquor, 21 in the MTYB liquor, 17 in the LJ liquor, and 15 in both the WLY and DJ liquors. Only two pyrazines, 2,6-dimethylpyrazine and 3,5-diethyl-2-methylpyrazine, were detected in the FJ liquor, whereas only one pyrazine, 2,6-dimethylpyrazine, was detected in the ST liquor. 2,6-Dimethylpyrazine was detected in all samples and ranged from 20.34 to 1057.46 μg/L. Several pyrazines, including 2-methylpyrazine (39.19–1011.55 μg/L), 2,5-dimethylpyrazine (0.63–182.15 μg/L), 2-ethylpyrazine (19.52–101.7 μg/L), 2-ethyl-3-methylpyrazine (47.21–897.49 μg/L), 2,3,5-trimethylpyrazine (0.41–2327.95 μg/L), 2,6-diethylpyrazine (84.05–1621.30 μg/L), 3,5-diethyl-2-methylpyrazine (41.12–545.58 μg/L), 2-methyl-6-propenylpyrazine (41.86–450.76 μg/L), and 2-acetyl-3,5-dimethylpyrazine (38.56–337.90 μg/L), were detected and determined in most of the Chinese liquors.

The different aroma style liquors had different numbers and amounts of pyrazines. According to aroma classification, both the MT and LJ liquors are the soy sauce aroma style liquor, and the JSY liquor belongs to the complex aroma style liquor. The MTYB, GJG, YHLS, WLY, JNC, and ST liquors are strong aroma style liquors by sensory assessment, whereas the FJ liquor is a light aroma style liquor, and both the DJ and SF liquors belong to miscellaneous styles. **Table 3** showed that the MT liquor had higher concentrations of 2-acetyl-6-methylpyrazine, 2-ethyl-6-methylpyrazine, and 3,5-dimethyl-2-ethylpyrazine. The

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Table 3. Concentrations (Micrograms per Liter) of Pyrazines in Chinese Liquors Detected by GC-FTD ($n = 3$)

		MT[a]		LJ		MTYB		GJG		SF		YHLS		FJ		ST		WLY		DJ		JNC		JSY	
peak	pyrazine	concn	SD	concn	SD	concn	SD	concn	SD	concn	SD	concn	SD	concn	SD	concn	SD	concn	SD	concn	SD	concn	SD	concn	SD
1	pyrazine	34.57	5.67	ND[b]		365.88	66.19	ND		ND		ND		ND		ND		ND		ND		ND		ND	
2	2-methylpyrazine	125.05	4.53	122.36	4.98	150.50	23.26	39.19	2.25	ND		1011.55	34.85	ND		ND		247.64	8.92	ND		179.66	9.33	182.15	12.34
3	2,5-dimethylpyrazine	56.61	0.13	67.72	1.44	53.10	3.69	70.74	4.07	21.77	1.07	67.33	1.57	20.34	0.91	47.53	0.14	<0.63		<0.63		<0.63		1057.46	46.77
4	2,6-dimethylpyrazine	395.11	8.03	414.72	8.42	1012.94	26.23	23.94	1.33	ND		ND		ND		ND		143.48	4.07	80.62	2.13	179.12	8.98	ND	
5	2-ethylpyrazine	60.31	4.71	40.25	2.50	101.70	7.42	<0.63		ND		ND		ND		ND		20.56	0.15	19.52	0.08	20.58	0.09	ND	
6	2,3-dimethylpyrazine	79.47		108.27		116.78	10.18	<0.47		ND		ND		ND		ND		79.05	5.40	ND		<0.47		220.19	10.33
7	2-ethyl-6-methylpyrazine[c]	639.86	56.04	ND		51.94	1.03	ND		ND		ND		ND		ND		ND		ND		ND		ND	
8	2-ethyl-5-methylpyrazine[c]	87.25	3.65	225.02	12.35	1836.52	38.91	54.53	1.88	6.12	0.83	907.97	71.30	ND		ND		143.36	19.92	ND		ND		ND	
9	2-ethyl-3-methylpyrazine[c]	47.21	3.03	96.92	10.38	180.17	7.42	0.41	0.01	ND		ND		ND		ND		15.31	3.25	ND		ND		897.49	22.11
10	2,3,5-trimethylpyrazine	474.95	20.55	538.96	37.50	34.53	0.19	84.05	6.39	ND		ND		ND		ND		274.06	27.71	1178.95	39.32	286.68	50.61	2327.95	110.23
11	2,6-diethylpyrazine[c]	ND		ND		1621.30	16.12	ND		ND		ND		ND		ND		ND		20.76	0.41	ND		ND	
12	2,5-dimethyl-3-ethylpyrazine[c]	171.66	7.59	ND		516.45	9.90	ND		ND		114.72	11.18	ND		ND		38.80	1.54	ND		ND		ND	
13	2,3-dimethyl-5-ethylpyrazine[c]	12.68	0.54	ND		37.44	0.08	ND		ND		ND		ND		ND		80.89	7.89	ND		ND		ND	
14	3,5-dimethyl-2-ethylpyrazine[c]	545.58	34.84	41.12	1.79	167.38	2.09	ND		ND		114.81	7.82	10.49	0.47	ND		ND		<1.56		ND		ND	
15	2,3,5,6-tetramethylpyrazine	440.01	11.70	178.40	8.26	1657.84	45.28	96.17	7.69	69.80	7.03	ND		ND		ND		41.00	2.47	9.84	0.53	ND		247.61	11.87
16	3,5-diethyl-2-methylpyrazine[c]	46.15	1.19	279.96	24.19	420.37	13.77	24.86	1.58	ND		ND		ND		ND		29.86	4.11	16.30	1.27	ND		ND	ND
17	2,3,5-trimethyl-6-ethylpyrazine[c]	50.92	2.81	67.49	4.76	35.82	0.04	8.50	1.40	ND		92.03	8.74	ND		ND		ND		49.71	8.24	ND		ND	
18	2,5-dimethyl-3-isobutylpyrazine[c]	ND		ND		92.94	2.08	ND		ND		137.76	9.04	ND		ND		ND		172.25	6.21	ND		ND	
19	2-methyl-6-vinylpyrazine[c]	127.31	1.19	93.59	1.95	ND		15.42	0.50	ND		ND		ND		ND		ND		ND		ND		ND	
20	2-acetyl-3-methylpyrazine[c]	66.56	2.82	53.92	1.04	75.78	3.23	ND		27.41	0.33	ND		ND		ND		118.57	4.05	101.91	10.88	46.80	0.10	ND	
21	2-butyl-3,5-dimethylpyrazine[c]	906.82	70.12	450.76	75.14	ND		190.69	12.67	ND		57.04	1.72	ND		ND		ND		134.07	9.11	ND		ND	
22	2-acetyl-6-methylpyrazine[c]	41.86	0.83	291.21	13.91	349.52	27.62	ND		ND		ND		ND		ND		38.56	4.60	62.52	4.23	ND		ND	
23	2-methyl-6-propenylpyrazine[c]	337.90	6.72	60.45	3.60	149.88	4.74	ND		ND		ND		ND		ND		ND		75.70	4.81	ND		ND	
24	2-acetyl-3,5-dimethylpyrazine[c]	61.97	1.34	15.23	0.83	ND		ND		ND		ND		ND		ND		ND		ND		213.30	25.24	136.2	8.28
25	2,5-dimethyl-3-pentylpyrazine[c]	217.78	0.65	ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND	
26	2,3-dimethyl-5-propenylpyrazine[c]	ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND		ND	
	total	5027.60		3146.35		9028.80		608.51		125.11		2503.20		30.83		47.53		1271.14		1922.15		926.14		5069.05	

[a] Cocn, mean concentration; SD, standard deviation. [b] ND, not detected by GC-FTD. [c] Slopes and intercepts of these pyrazines were appointed 2.7129 and 0, respectively.

MTYB liquor had high concentrations of 2-ethyl-5-methylpyrazine, 2,3,5,6-tetramethylpyrazine, 2,6-diethylpyrazine, and 2,6-dimethylpyrazine, whereas the main pyrazines in the JSY liquor were 2,3,5-trimethylpyrazine and 2,6-dimethylpyrazine.

Among Chinese liquors, the MTYB liquor had the highest concentration of pyrazines, which is 9028.80 µg/L, whereas the lowest concentration of pyrazines was detected in the FJ liquor, only 30.83 µg/L. The MT, LJ, and JSY liquors also had higher total concentrations of pyrazines, which were 5027.60, 3146.35, and 5069.05 µg/L, respectively (**Table 3**). These results indicated that the soy sauce and complex aroma style liquors had higher pyrazines concentrations, followed by the strong aroma style liquor, whereas the light aroma style liquor had low levels of pyrazines. However, the MTYB liquor, which belongs to the strong aroma style, differed from the other strong aroma style liquors.

It is obvious that not all of the volatile compounds occurring in a food contribute to its aroma (37); only those with odor activity values (OAVs) of 1 or more can contribute to the aroma. The concentrations of pyrazine, 2-methylpyrazine, 2-ethylpyrazine, and 2,3-dimethylpyrazine in Chinese liquor are lower than their thresholds, 300000, 30000, 4000, and 400 µg/L in water (38, 39), respectively. This means that these four pyrazines cannot contribute to aromas. The threshold of 2,5-dimethylpyrazine was 80 µg/L in water (33), whereas the mean concentration in all Chinese liquors studied in this paper was 99.14 µg/L. Therefore, this pyrazine might contribute to aromas of Chinese liquors. Although Chinese liquors had high concentrations of 2,6-dimethylpyrazine, of which the average concentration was more than 320 µg/L, the mean OAV is still less than 1, because its threshold was 400 µg/L (33). 2,3,5-Trimethylpyrazine and 2,3,5,6-tetramethylpyrazine had much greater concentration in several Chinese liquors, for example, JSY, MT, LJ, and MTYB liquors. The thresholds of 2,3,5-trimethylpyrazine and 2,3,5,6-tetramethylpyrazine were 400 µg/L (40) and 1000 µg/L in water (34), respectively. These two pyrazines could contribute to aromas in some Chinese liquors.

Pyrazines could be formed either via the Maillard reaction between saccharide and amino residues or through the ambient temperature reaction of microbial metabolites in Chinese liquor. Daqu is fermented from wheat or a mixture of wheat, barley, and pea (4). Daqu is not only the starter but also the fermentation material because it accounts for 25–30% of the total of grains used in the fermentation. Daqu used for the MT, JSY, and LJ liquors was exposed to 60–65 °C for 10–12 days, that used for both the WLY and JNC liquors was exposed to 58–60 °C for 10–12 days, and that used for FJ liquor was exposed to 40–45 °C for around 10 days (1, 2). **Table 3** reveals that the total of pyrazines was more than 3000 µg/L in the MT, JSY, and LJ liquors, 900–1300 µg/L in the WLY and JNC liquors, and around 30 µg/L in the FJ liquor. We presumed that high temperature in the Daqu process would benefit the formation of pyrazines. On the other hand, Fan and Qian (1) reported that the concentrations of pyrazines in the WLY and JNC liquors were more than those in the YHDQ liquor. However, the YHLS liquor studied in this paper was another brand made at the Yanghe Distillery. YHLS liquor was produced by blending a strong aroma style liquor with a soy sauce aroma style liquor; thus, it is reasonable that the concentrations of pyrazines in the YHLS liquor were greater than those of pyrazines in the WLY and JNC liquors.

The metabolic activities of microorganisms in the solid-state fermentation of Chinese liquor would generate a variety of precursors, such as α-acetolactate, 3-hydroxy-2-butanone (ac-

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etoin), free amino acids, and ammonia; these compounds could convert to pyrazines by non-enzyme-catalyzed reactions (*41, 42*). Rizzi found that acetoin combined with ammonia to form 2,3,5,6-tetramethylpyrazine at 22 °C (*41*), whereas acetoin had been detected in Chinese liquors (*1*). Tai and Ho (*43*) reported that 1-hydroxy-2-propanone was the precursor of 2,5-dimethylpyrazine and 2,3-dimethylpyrazine, and the ratio of 1-hydroxy-2-propanone/acetoin in the reaction system had a significant effect on their total sensory quality. In recent reports, Larroche and co-workers (*7, 14*) thought that L-threonine was the precursor of 2,5-dimethylpyrazine for *Bacillus subtilis* in the solid-state fermentation on soybean, and acetoin could stimulate 2,3,5,6-tetramethylpyrazine synthesis. Similar to the formation of alkylpyrazines, the formation of acetylpyrazines involves the addition of ammonia to a dicarbonyl compound. The resulting product reacts with *C*-methyltriose reductone from a sugar retro-aldol reaction to form an intermediate that loses two water molecules to form an acetylpyrazine (*44*).

In summary, 27 pyrazines were identified and detected by LLE-GC-MS in Chinese liquors, mainly alkyl- and acetylpyrazines, and no alkoxypyrazines were detected. A HS-SPME coupled with FTD method could detect all 27 pyrazines. The developed method had a good recovery in the linear range. Pyrazines in Chinese liquors had a broad range of concentration, and MTYB liquor had the highest concentration of total pyrazines among all Chinese liquor samples, whereas FJ liquor had the lowest.

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