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Headspace Solid Phase Microextraction and Gas Chromatography−Olfactometry Dilution Analysis of Young and Aged Chinese "Yanghe Daqu" Liquors

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The aroma compounds of young and aged Chinese "Yanghe Daqu" liquor samples were extracted by solid phase microextraction (SPME) and analyzed by gas chromatography (GC)−olfactometry dilution analysis. The original liquor samples were diluted with deionized water to give a final alcohol content of 14% (v/v). The samples were stepwise diluted (1:1) with 14% (by volume) ethanol−water solution and then extracted by headspace SPME. The samples were preequilibrated at 50 °C for 15 min and extracted with stirring at the same temperature for 30 min prior to injection into GC. The aroma compounds were identified by both GC-MS and GC−olfactometry using DB-Wax and DB-5 columns. The results suggested that esters were the major contributors to Yanghe Daqu liquor aroma. Ethyl hexanoate, ethyl butanoate, and ethyl pentanoate had very high flavor dilution values in both young and aged liquors (FD > 8192). Methyl hexanoate, ethyl heptanoate, ethyl benzoate, and butyl hexanoate could also be very important because of their high flavor dilution values (FD ≥ 256). Moreover, two acetals, 1,1-diethoxyethane and 1,1-diethoxy-3-methylbutane, also were shown high flavor dilution values in Yanghe Daqu liquors (FD ≥ 256). Other aroma compounds having moderate flavor dilution values included acetaldehyde, 3-methylbutanol, and 2-pentanol (FD ≥ 32). Comparing young and aged liquors, the aroma profiles were similar, but the aroma compounds in the aged sample had higher flavor dilution values than in the young ones.

KEYWORDS: HS-SPME; gas chromatography−olfactometry; young and aged liquor; Chinese liquor; Yanghe Daqu; aroma

INTRODUCTION

Chinese liquor is a popular alcoholic beverage with an annual consumption of 4 million kiloliters. Like other distilled beverages such as brandy, whiskey, rum, and vodka, its aroma can be developed during fermentation, distillation, and the aging process (*1*, *2*).

"Yanghe Daqu" is a famous Chinese liquor with an output of 30000 metric tons annually. It is fermented from grains (rice, sticky rice, sorghum, wheat, and corn) with Daqu powder made from wheat or a mixture of wheat, barley, and pea, as a starter. Fresh distillate has undesirable characteristics often described as "harsh", "green", and "raw". These characteristics are often associated with young liquor and generally decrease or disappear upon aging. At the same time, a well-balanced, "matured" liquor aroma is developed during this process.

"Yanghe Daqu" liquor is usually aged in a sealed pottery jar for at least 3 years. Many chemical reactions such as oxidation, esterification, hydrolysis, and rearrangement can occur during this aging process (*2*). In addition, water and ethanol can slowly permeate through the jar causing a reduction in volume with storage time. It is possible that some aroma compounds could escape from the jar and get lost, while other aroma compounds, being larger and less polar than water and ethanol, are more difficult to migrate through the ceramic jar and thus are concentrated during the storage. Recently, of particular interest was an attempt to accelerate the aging process (*3*), but the aroma compositions of young and aged Yanghe Daqu liquors have not yet been determined.

Solid phase microextraction (SPME) is a solvent-free and low-cost sampling method. This technique has been applied to the volatile and aroma analysis of wine (*4*, *5*), whiskey (*6*, *7*), brandy (*8*, *9*), and spirits (*10*, *11*). Ebeler and co-workers *(9)* reported that liquid−liquid extraction (LLE) using Freon 11 was more efficient for extracting the higher alcohols in brandy than the PDMS [poly(dimethylsiloxane)]-SPME technique, while SPME was more selective for esters than LLE. Castro et al. (*5*) compared volatile compounds of "fino" sherry wine using rotatory and continuous LLE with those extracted by SPME method using a Carboxen-PDMS fiber. They found that the compounds extracted by SPME were similar to those obtained from rotary and continuous LLE and the SPME showed higher sensitivities than LLE for several compounds.

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The SPME extraction technique has also been coupled with gas chromatography−olfactometry (GC/O) to study aroma compounds. Bazemore and co-workers (*12*) studied aroma active compounds in orange juice with headspace (HS)-SPME-Osme. Kim and co-workers (*13*) extracted aroma compounds from Yuzu (*Citrus junos* Tanaka) with HS-SPME and achieved GC/O dilution analysis by adjusting the GC injector split ratio. Martí and co-workers (*14*) studied wine aroma using SPME and GC/O analysis of successively diluted samples. They concluded that HS-SPME [divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS fiber)] was a good technique to study wine aroma, because a wide range of odorants can be extracted and the approach could be suitable for GC/O analysis. Yanghe Daqu is a major liquor in China; yet, its aroma has not been fully understood. The objective of this study is to employ the HS-SPME GC/O dilution analysis technique to analyze the aroma compounds in young and aged Yanghe Daqu liquors.

MATERIALS AND METHODS

Chemicals. Methyl hexanoate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, propyl butanoate, 1-pentanal, heptanoic acid, octanoic acid, and 1-octanol were from Eastman (Rochester, NY). Ethyl 2-methylpropanoate, propyl hexanoate, pentyl butanoate, pentyl hexanoate, hexyl hexanoate, 2-methylpropyl acetate, 2-methylpropyl hexanoate, and 3-methylbutyl 3-methylbutanoate were obtained from K & K Laboratories (Plainview, NY). 2-Pentanol, 1-heptanol, and ethyl 2-hydroxypropanoate were from Matheson Coleman & Bell (East Rutherford, NJ). Ethyl benzoate was obtained from EKC Inc. (Rosemont, IL). Acetic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, pentanoic acid, hexanoic acid, acetaldehyde, 2-methylbutanal, 3-methylbutanal, 2-methylpropanol, 1-butanol, 2-butanol, 3-methylbutanol, 1-hexanol, 2-heptanol, benzeneethanol, benzeneacetaldehyde, furfural (2-furancarboxaldehyde), 2-pentanone, ethyl acetate, ethyl propanoate, ethyl butanoate, ethyl pentanoate, ethyl hexanoate, ethyl heptanoate, ethyl 3-methylbutanoate, ethyl benzeneacetate, ethyl 3-phenylpropanoate, 3-methylbutyl hexanoate, butyl acetate, butyl hexanoate, pentyl acetate, hexyl acetate, hexyl butanoate, hexyl octanoate, diethyl butanedioate, 2-phenylethyl acetate, 3-methylbutyl acetate, dimethyl disulfide, dimethyl trisulfide, 4-ethylguaiacol, and 1,1-diethoxyethane were from Sigma-Aldrich Co. (St. Louis, MO). Ethanol, absolute-200 proof, was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY). Sodium chloride was obtained from Sigma-Aldrich, Inc.

Synthesis of Esters. 2-Methylpropyl butanoate, 3-methylbutyl butanoate, 3-methylbutyl octanoate, propyl octanoate, butyl pentanoate, heptyl hexanoate, 2-phenylethyl butanoate, and 2-phenylethyl hexanoate were synthesized by mixing butanoic acid with 2-methylpropanol, butanoic acid with 3-methylbutanol, octanoic acid with 3-methylbutanol, octanoic acid with propanol, pentanoic acid with butanol, hexanoic acid with heptanol, butanoic with 2-phenylethanol, and hexanoic acid with 2-phenylethanol, respectively. Six hundred microliters of acid was mixed with 2 mL of alcohol in a 20 mL vial. The reactions were catalyzed by acid (1 N H_2SO_4, 500 μL) at 100 °C for 1 h. After the reaction, the mixture was cooled and 5 mL of saturated NaCl solution was added, and the esters were extracted with 5 mL of Freon 11 in a separatory funnel. One microliter of extract was injected into GC-MS (split ratio 100:1) for identification.

Synthesis of Acetals. 1,1-Diethoxy-2-methylpropane, 1,1-diethoxy-2-methylbutane, 1,1-diethoxy-3-methylbutane, 1,1-diethoxynonane, and 1,1-diethoxy-2-phenylethane were synthesized by reacting 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, nonanal and phenylacetaldehyde, and alcohols in acidic conditions (*15*). Six hundred microliters of aldehydes was mixed with 20 mL of ethanol. Two milliliters of H_2SO_4 (1 N) was added. The mixture was stirred at 58 °C for 1 h. After cooling, 50 mL of saturated NaCl solution was added to the reaction mixture and the product was extracted with 10 mL of Freon 11 in a separatory funnel. One microliter of acetal solution was injected to GC-MS (split ratio 100:1) for identification.

Chinese Yanghe Daqu Liquors. Young and aged Yanghe Daqu liquors were produced at Yanghe Distillery Co. Ltd., Jiangsu province of China, as described previously (*16*). These samples were made from a mixture of sorghum, wheat, corn, rice, sticky rice, and rice hull using Daqu powder, which was fermented with barley, wheat, and peas as the starter. The raw materials of making liquor were milled, added with water, cooked, and then fermented for 60 days at 28−32 °C under anaerobic conditions in a solid state. The fermentor (3.4 m length, 1.8 m width, and 2.0 m height) was built with clay, and the inside was coated with a layer of fermentation mud made of clay, spent grain, bean cake powder, and fermentation bacteria (*Clostridium* sp.). After fermentation, the liquor was distilled out with steam. The fresh distillate was aged in a sealed china jar (1000 L) at 15−25 °C.

One sample (young Yanghe Daqu liquor) was produced in May 2004 and stored in a china jar for 4 months. Another sample (aged Yanghe Daqu liquor) was made in May 1999 and aged for more than 5 years. Five hundred milliliters of these two liquors was transferred from china jars into a glass bottle in September 2004 and then stored at −15 °C. The samples were analyzed within 3 months.

HS-SPME. A 50/30 μm DVB/CAR/PDMS fiber (Supelco, Inc., Bellefonte, PA) was used for aroma extraction. Each liquor sample (2 mL) was diluted with deionized water to a final concentration of 14% (v/v) ethanol, and the total 10 mL solution volume was put into a 20 mL vial (item #: S126-0020, I-CHEM, NC). The diluted sample was saturated with sodium chloride. Then, the vial was tightly capped with a silicon septum. This sample was equilibrated at 50 °C in a thermostatic bath for 15 min and extracted for 30 min at the same temperature under stirring. After extraction, the fiber was inserted into the injection port of GC (250 °C) to desorb the analytes.

Linearity of HS-SPME. To check the linearity of HS-SPME for Yanghe Daqu liquor analysis, one of the liquor samples (young) was sequentially diluted with 14% aqueous ethanol at a 1:1 ratio. An automatic headspace sampling system (MultiPurposeSample MPS 2 with a SPME adapter, from GERSTEL Inc., Baltimore, MD) with a 50/30 μm DVB/CAR/PDMS fiber was used for extraction of volatile compounds. GC-MS was carried out using an Agilent GC 6890-5973 mass selective detector. Samples were analyzed on an HP-5 column (30 m length, 0.32 mm i.d., 0.25 μm film thickness, Agilent Technology). The samples were equilibrated at 50 °C for 15 min and extracted for 30 min at the same temperature under stirring (250 rpm, on for 20 s, off for 0 s). After extraction, the fiber was inserted into the injection port of GC (250 °C) to desorb the analytes for 5 min. The oven and injector temperatures were identical to GC/O analysis described below on a DB-5 column. Each dilution was analyzed twice, and the first dilution was assigned a concentration number of ten.

GC/O Dilution Analysis. GC/O analysis was performed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactometer. The column carrier gas was nitrogen at a constant pressure (15 psi, 2 mL/min column flow measured at 25 °C). Half of the column flow was directed to the FID, while the other half was directed to a heated sniffing port with a fused silica outlet splitter (Alltech Associates, Inc., Deerfield, IL). Samples were analyzed on a DB-Wax column (30 m length, 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA) and a DB-5 column (30 m length, 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific). The oven temperature was held at 40 °C for 2 min, and then increased to 230 °C at a rate of 4 °C/min, and held at 230 °C for 5 min on a DB-Wax column, while the final temperature was 250 °C on a DB-5 column. Injector and detector temperatures were 250 °C.

The samples were stepwise diluted with 14% (by volume) aqueous ethanol solution using a 1:1 dilution before being extracted with HS-SPME and analyzed by GC/O dilution analysis technique (*14*). The flavor dilution (FD) factors were determined for the odor active compounds in each sample (*17*).

Two panelists (one male and one female) were selected for the GC/O study. Both panelists were familiar with GC/O technique and had more than 100 h of training. The panelists responded to and recorded the retention time and descriptor of the aroma compounds. Each sample was sniffed in replicate by each panelist with the same fiber. When a volatile compound was detected at least twice, this analyte was determined to be a declared aroma compound.

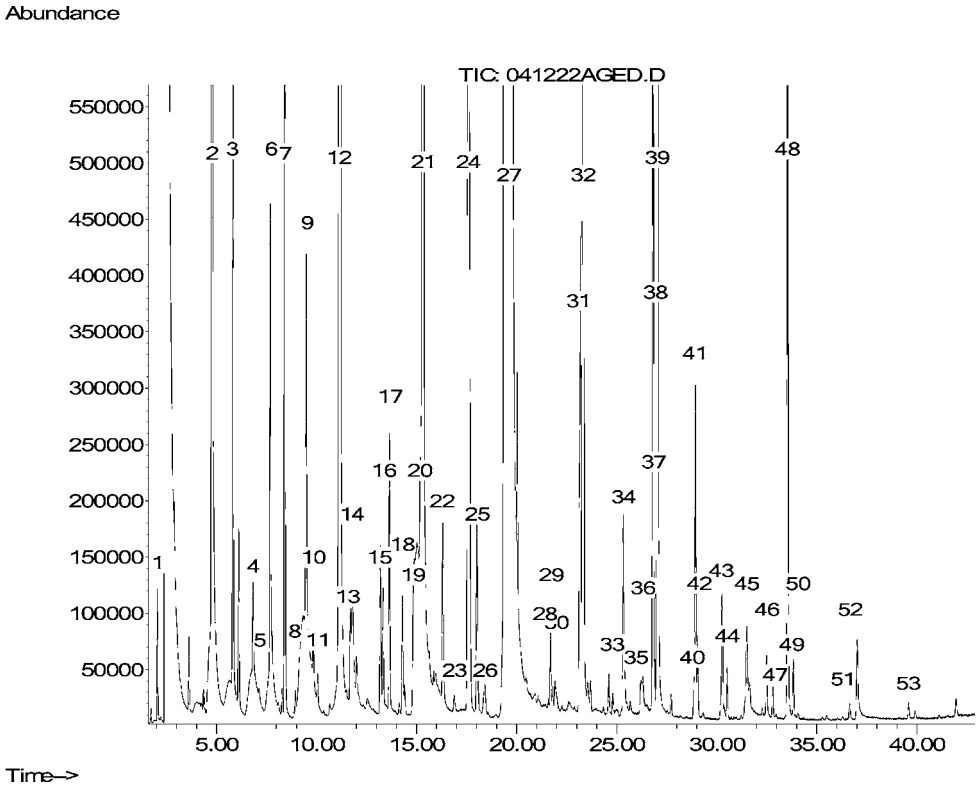

Figure 1. Chromatogram of aged Yanghe Daqu liquor detected on DB-5 column. Key: 1, acetaldehyde; 2, ethyl acetate; 3, 3-methylbutanal; 4, 2-pentanone; 5, 2-pentanol; 6, ethyl propanoate; 7, 1,1-diethoxyethane; 8, dimethyl disulfide; 9, ethyl 2-methylpropanoate; 10, 3-methylbutanol; 11, butanoic acid; 12, ethyl butanoate; 13, butyl acetate; 14, ethyl 2-hydropropanoate; 15, ethyl 2-methylbutanoate; 16, ethyl 3-methylbutanoate; 17, 1,1-diethoxy-2-methylpropane; 18, 3-methylbutyl acetate; 19, 1-hexanol; 20, propyl butanoate; 21, ethyl pentanoate; 22, methyl hexanoate; 23, 2-methylpropyl butanoate; 24, 1,1-diethoxy-3-methylbutanoate; 25, ethyl 4-methylpentanoate; 26, dimethyl trisulfide; 27, ethyl hexanoate; 28, 3-methylbutyl butanoate; 29, pentyl butanoate; 30, ethyl 2-hydroxyhexanoate; 31, propyl hexanoate; 32, ethyl heptanoate; 33, ethyl cyclohexanecarboxylate; 34, 2-methylpropyl hexanoate; 35, ethyl benzoate; 36, diethyl butanedioate; 37, butyl hexanoate; 38, hexyl butanoate; 39, ethyl octanoate; 40, ethyl benzeneacetate; 41, 3-methylbutyl hexanoate; 42, 2-phenylethyl acetate; 43, pentyl hexanoate; 44, ethyl nonanoate; 45, 1,1-diethoxy-2-phenylethane; 46, ethyl 3-phenylpropanoate; 47, 3,5-dimethyl-2-pentylpyrazine; 48, hexyl hexanoate; 49, furfuryl hexanoate; 50, ethyl decanoate; 51, 2-phenylethyl butanoate; 52, ethyl 2-hydroxy-3-phenylpropanoate; and 53, hexyl octanoate.

Retention Indices (RI). RIs were calculated in accordance with a modified Kovats method (*18*). A standard mixture of paraffin homologues C5−C25 was prepared. The sample and the hydrocarbon standard mixture were coinjected into the GC, and the retention times were used to calculate RI.

GC-MS Analysis. GC-MS was carried out using an Agilent GC 6890-5973 mass selective detector (MSD). Samples were analyzed on a DB-Wax column (30 m length, 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific) and a DB-5 column (30 m length, 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific). The oven and injector temperatures were identical to GC/O analysis described previously. The column carrier gas was helium at a constant flow rate of 2 mL/min. An Agilent 5973 MSD was used for identification. 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.). Positive identification was achieved by comparing mass spectrum, aroma, and RI of the standards. Tentative identification was achieved by comparing aroma or mass spectrum only.

RESULTS AND DISCUSSION

HS-SPME Parameters. Four fibers, coated with PA (polyacrylate), PDMS, CAR/PDMS, and DVB/CAR/PDMS, were evaluated for the extraction of the aroma compounds in Yanghe Daqu liquor. The DVB/CAR/PDMS fiber extracted more compounds with higher intensities while the PA fiber extracted the fewest compounds. This observation was consistent with previous reports (*6*, *14*). Addition of salts typically improves the extraction efficiency, but this was not the case for Yanghe Daqu liquor. It was observed that the addition of salt improved the extraction efficiency of highly volatile compounds such as ethyl acetate and ethyl butanoate but decreased the extraction efficiency for less volatile compounds such as ethyl heptanoate and ethyl octanoate. This phenomenon is probably due to the competitive absorption of the SPME fiber (*19*). The extraction time is also very important for extraction efficiency. It has been reported that the concentration of short chain fatty acid ester decreases while the long chain fatty acid ester increases with extraction time (*19*). The Yanghe Daqu liquor sample was extracted for 20, 30, 40, and 60 min. It was observed that extraction efficiency for long chain esters such as ethyl heptanoate, butyl hexanoate, and ethyl octanoate all improved with extraction time while there was not much change for short chain esters. It is well-known that the extraction is strongly influenced by temperature in the HS-SPME analysis. The extraction temperatures (30, 40, 50, and 60 °C) were evaluated in the HS-SPME parameter screening experiment. The results showed that the volatile compounds increased with extraction temperature up to 50 °C. When the extraction temperature was raised to 60 °C, the extraction efficiencies for all esters were dramatically decreased. For alcoholic beverages like Yanghe Daqu liquor, ethanol concentration is a very important factor for volatile extraction (*9*). Three alcohol levels, 7, 14, and 35% (by volume), were tested. The 14% ethanol yielded the highest

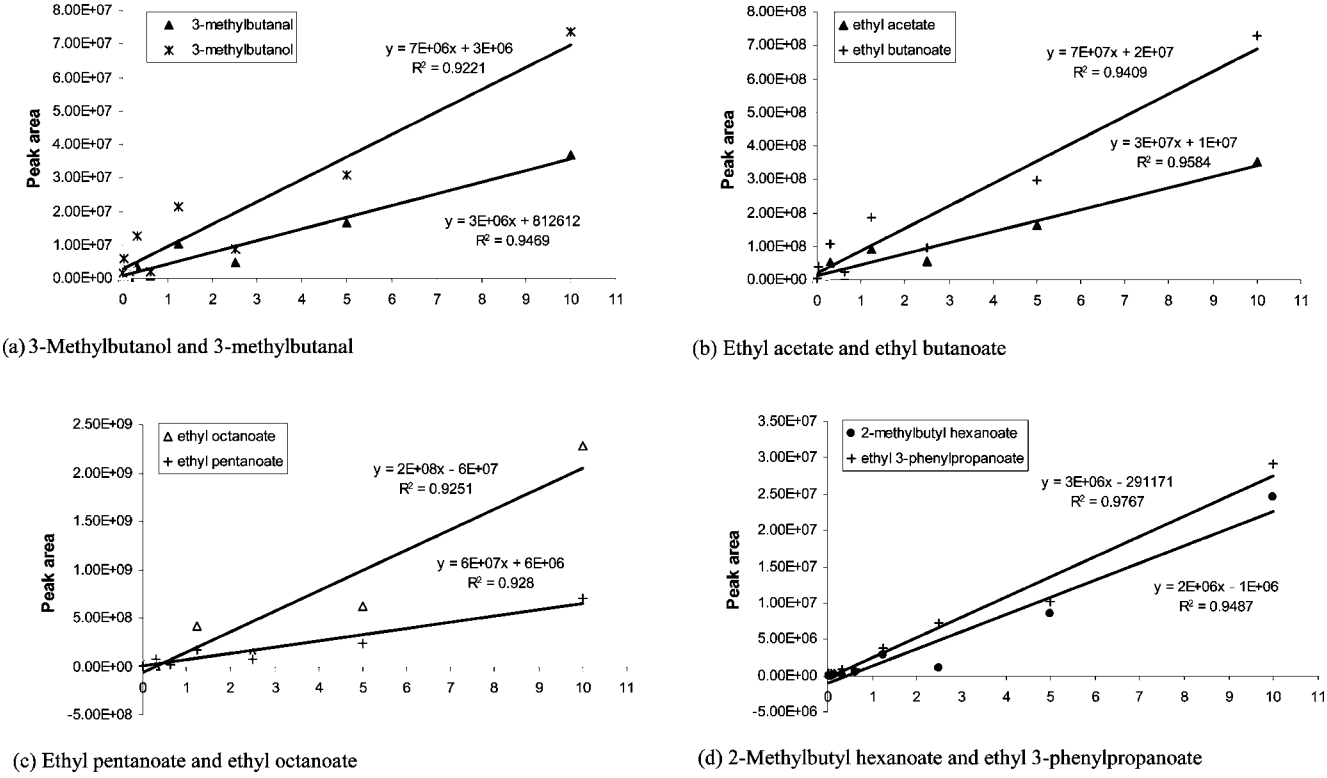

Figure 2. Linearity of aldehydes, alcohols, and esters from young Yanghe Daqu liquor extracted by HS-SPME (DVB/CAR/PDMS fiber) and detected on a DB-5 column using a series of 1:1 dilutions; the first dilution was assigned a number of 10. (**a**) 3-Methylbutanol and 3-methylbutanal, (**b**) ethyl acetate and ethyl butanoate, (**c**) ethyl pentanoate and ethyl octanoate, and (**d**) 2-methylbutyl hexanoate and ethyl 3-phenylpropanoate.

sensitivity for HS-SPME extraction of the volatile compounds. On the basis of these observations, the volatiles were extracted at 50 °C for 30 min and the alcohol content was adjusted to 14% for the GC/O analysis.

GC/O Dilution Analysis. The GC/O dilution analysis was performed by successively diluting the liquor samples with 14% (by volume) ethanol solution according to Martí et al (*14*). The FD factors were determined for the odor active compounds in each sample as described by Grosch (*17*). **Figure 1** was the chromatograph of aged Yanghe Daqu liquor detected on a DB-5 column.

To ensure reliable GC/O dilution analysis, the concentrations of extracted compounds by SPME fiber must have a linear relationship with the dilutions. Yanghe Daqu liquor was sequentially diluted at a 1:1 ratio with 14% ethanol, and the responses (total ion abundance) for an alcohol, an aldehyde, and several esters with varying chain lengths were evaluated. The results showed that 3-methylbutanal, 3-methylbutanol, ethyl acetate, ethyl butanoate, ethyl pentanoate, ethyl octanoate, 2-methylbutyl hexanoate, and ethyl 3-phenylpropanoate from Yanghe Daqu liquor had adequate linearity for GC/O dilution analysis because their linear correlation coefficients (R^2) were all greater than 0.92 (**Figure 2**). It is expected that the linear response is even better for SPME fiber at lower concentration (*14*).

On the basis of the FD values detected on a DB-Wax column (**Table 1**), the potentially important alcohols were 2-butanol, 3-methylbutanol, 2-pentanol, and 1-hexanol (FD ≥ 32). These alcohols impart fruity, floral, green, and alcoholic odors. These alcohols had high FD values in aged liquor and low FD values in young liquor. Among them, 3-methylbutanol and 2-pentanol had FD values more than 256 in aged liquor and less than 4 in young liquors. In addition, 2-methylpropanol, 1-butanol, 1-heptanol, 2-heptanol, and 1-octanol were detected in both Yanghe Daqu liquors. These alcohols had fruity and green aromas. Benzeneethanol, which gave rosy and honey aromas, was also identified in both liquors, but it had extremely low FD values (FD ≤ 2) in this study. Long-chained alcohols were probably formed by yeast through Ehrlich pathway in the fermentation of liquor (*20*), whereas benzeneethanol could be produced by *Saccharomyces cerevisiae* (*21*).

Esters seemed to be the most important aroma compounds in Yanghe Daqu liquors, especially ethyl esters (**Table 1**). Ethyl hexanoate, ethyl butanoate, and ethyl pentanoate had extremely high FD values (FD > 8192) in both young and aged liquors. Ethyl heptanoate, butyl hexanoate, and methyl hexanoate also had high FD values (FD ≥ 256) in both liquors. Ethyl acetate, ethyl 3-methylbutanoate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, pentyl hexanoate, hexyl butanoate, hexyl hexanoate, 3-methylbutyl butanoate, and 2-methylpropyl hexanoate could also contribute to Yanghe Daqu aroma (FD ≥ 32). These esters contributed to fruity, floral, sweet, pineapple, and apple aromas.

Several aromatic esters were identified in both liquors in this study, including ethyl benzoate, ethyl benzeneacetate, ethyl 3-phenylpropanoate, 2-phenylethyl acetate, 2-phenylethyl butanoate, and 2-phenylethyl hexanoate. Among them, ethyl benzoate (FD ≥ 256), ethyl benzeneacetate (FD = 128), and ethyl 3-phenylpropanoate (FD ≥ 64) could be very important to Yanghe Daqu aroma. Ethyl benzeneacetate contributed to rosy and honey aromas, while ethyl benzoate and ethyl 3-phenylpropanoate impacted floral and fruity notes. Several hydroxyl fatty acid esters were identified in this study. Ethyl 2-hydroxypropanoate, ethyl 2-hydroxyhexanoate, and ethyl 2-hydroxy-3-methylbutanoate had floral, jasmine, and fruity aromas. These compounds had been identified in freshly distilled Calvados and Cognac (*15*). Diethyl butanedioate was also identified, and it contributed to fruity and sweet aromas. According to our knowledge, ethyl cyclohexanecarboxylate was

Table 1. Potent Aroma Compounds Detected by HS-SPME and GC/O Dilution Analysis on a DB-Wax Column

RI	aroma compounds	descriptor	basis of identification[a]	FD factors	
				aged	young
713	acetaldehyde	green, malt	MS, aroma, RI	256	32
889	1,1-diethoxyethane	fruity	MS, aroma, RI	2048	256
889	ethyl acetate	pineapple	MS, aroma, RI	2048	256
913	3-methylbutanal	green, malt, mint	MS, aroma, RI	16	8
917	2-methylbutanal	malt, green,	MS, aroma, RI	2	2
929	1-pentanal	malt, green	MS, aroma, RI	1	16
931	ethyl propanoate	banana, fruity	MS, aroma, RI	8	2
962	ethyl 2-methylpropanoate	fruity, sweet	MS, aroma, RI	8	2
976	1,1-diethoxy-2-methylpropane	fruity	MS, aroma, RI	ND	2
1005	2-methylpropyl acetate	floral, fruity	MS, aroma, RI	2	2
1022	2-butanol	fruity	MS, aroma, RI	32	2
1030	ethyl butanoate	pineapple	MS, aroma, RI	>8192	>8192
1042	ethyl 3-methylbutanoate	apple	MS, aroma, RI	128	64
1066	dimethyl disulfide	onion, cabbage	MS, aroma, RI	8	2
1067	1,1-diethoxy-2-methylbutane	fruity	MS, aroma, RI	8	4
1073	1,1-diethoxy-3-methylbutane	fruity	MS, aroma, RI	2048	128
1113	2-methylpropanol	wine, solvent	MS, aroma, RI	ND	2
1128	3-methylbutyl acetate	banana, fruity	MS, aroma, RI	ND	2
1130	2-pentanol	fruity, alcoholic	MS, aroma, RI	256	2
1145	ethyl pentanoate	apple	MS, aroma, RI	>8192	>8192
1156	1-butanol	pungent, alcoholic	MS, aroma, RI	8	ND
1185	pentyl acetate	fruity	MS, aroma, RI	64	16
1193	methyl hexanoate	floral, fruity	MS, aroma, RI	256	128
1210	3-methylbutanol	rancid, nail polish	MS, aroma, RI	256	4
1234	ethyl hexanoate	fruity, floral, sweet	MS, aroma, RI	>8192	>8192
1263	hexyl acetate	fruity, floral	MS, aroma, RI	8	2
1266	3-methylbutyl butanoate	floral, fruity	MS, aroma, RI	32	2
1290	1,1,3-triethoxypropane	mushroom	MS, aroma, RIL	16	64
1296	butyl pentanoate	fruity	MS, aroma, RI	4	2
1300	propyl hexanoate	pineapple, sweet	MS, aroma, RI	2	2
1310	2-heptanol	fruity	MS, aroma, RI	2	1
1315	ethyl heptanoate	fruity	MS, aroma, RI	256	64
1328	2-methylpropyl hexanoate	sweet, apple	MS, aroma, RI	64	64
1349	ethyl 2-hydroxypropanoate	fruity	MS, aroma, RI	ND	4
1362	1-hexanol	floral, green	MS, aroma, RI	64	4
1363	3-methylbutyl 3-methylbutanoate	fruity	MS, aroma, RI	16	16
1368	dimethyl trisulfide	sulfur, rotten cabbage	MS, aroma, RI	32	2
1385	butyl hexanoate	pineapple, fruity	MS, aroma, RI	1024	128
1388	hexyl butanoate	fruity, floral	MS, aroma, RI	8	32
1397	ethyl 2-hydroxy-3-methylbutanoate	floral	MS, aroma, RIL	8	64
1407	ethyl cyclohexanecarboxylate	fruity, floral	MS, aroma, RIL	16	32
1411	ethyl octanoate	fruity	MS, aroma, RI	128	32
1430	3-methylbutyl hexanoate	fruity, apple, green	MS, aroma, RI	2	8
1457	1-heptanol	green, fruity	MS, aroma, RI	8	8
1459	2,3,5,6-tetramethylpyrazine[b]	roasted, baked	aroma, RIL	ND	4
1467	2-furancarboxaldehyde	sweet, almond	MS, aroma, RI	32	16
1473	acetic acid	acidic, vinegar	MS, aroma, RI	4	8
1485	pentyl hexanoate	fruity	MS, aroma, RI	64	64
1487	hexyl pentanoate	floral, fruity	MS, aroma, RI	4	4
1504	propyl octanoate	fruity	MS, aroma, RI	32	ND
1511	1,1-diethoxynonane	fruity, floral	MS, aroma, RI	64	64
1521	ethyl nonanoate	fruity, rose-like	MS, aroma, RI	32	64
1540	ethyl 2-hydroxyhexanoate	floral, jasmine	MS, aroma, RIL	32	16
1557	1-octanol	green	MS, aroma, RI	8	4
1572	2-methylpropanoic acid	acid, rancid	MS, aroma, RI	8	ND
1577	unknown	woody, fruity		4	8
1603	hexyl hexanoate	apple, peach	MS, aroma, RI	64	16
1625	ethyl decanoate	fruity, grape	MS, aroma, RI	64	16
1641	butanoic acid	rancid, cheesy	MS, aroma, RI	64	16
1649	ethyl benzoate	herbal, fruity	MS, aroma, RI	32	256
1654	3-methylbutyl octanoate	fruity, pineapple	MS, aroma, RI	8	4
1668	diethyl butanedioate	fruity, sweet	MS, aroma, RI	16	16
1679	3-methylbutanoic acid	rancid, acidic	MS, aroma, RI	16	16
1706	heptyl hexanoate	fruity	MS, aroma, RI	4	16
1717	1,1-diethoxy-2-phenylethane	floral, fruity	MS, aroma, RIL	32	64
1751	pentanoic acid	sweaty, rancid	MS, aroma, RI	32	4
1775	ethyl benzeneacetate	rosy, honey	MS, aroma, RI	128	128
1807	2-phenylethyl acetate	rosy, floral	MS, aroma, RI	64	16
1857	hexanoic acid	sweaty, cheesy	MS, aroma, RI	64	16
1878	ethyl 3-phenylpropanoate	fruity, floral	MS, aroma, RI	32	64
1936	benzeneethanol	rosy, honey	MS, aroma, RI	2	2
1962	heptanoic acid	unpleasant	MS, aroma, RI	64	16
2026	4-ethylguaiacol	clove, spicy	MS, aroma, RI	64	32
2090	4-methylphenol	animal, phenol	MS, aroma, RIL	2	4
2165	2-phenylethyl hexanoate	fruity	MS, aroma, RI	4	16
2186	4-ethylphenol	smoky	MS, aroma, RIL	64	16
2265	unknown	goaty, smoky		ND	16

[a] MS: Compounds were identified by MS spectra. Aroma: Compounds were identified by the aroma descriptors. RI: Compounds were identified by comparison to pure standard. RIL: Compounds were identified by comparison with RI from the literature. [b] Tentatively identified. ND, not detected by GC/O.

Table 2. Potent Aroma Compounds Detected by HS-SPME and GC/O Dilution Analysis on a DB-5 Column

RI	aroma compounds	descriptor	basis of identification[a]	FD factors	
				aged	young
<500	acetaldehyde	green, malt	MS, aroma, RI	128	32
534	2-methylpropanal	green	MS, aroma, RI	ND	64
584	ethyl acetate	fruity, ester	MS, aroma, RI	256	64
618	2-methylpropanol	wine	MS, aroma, RI	ND	4
629	3-methylbutanal	green, malt	MS, aroma, RI	16	8
671	2-pentanone	fruity	MS, aroma, RI	4	8
703	2-pentanol	alcoholic, fruity	MS, aroma, RI	32	8
705	ethyl propanoate	sweet, fruity	MS, aroma, RI	2	32
726	1,1-diethoxyethane	fruity	MS, aroma, RI	256	64
740	dimethyl disulfide	cooked onion	MS, aroma, RI	2	16
754	ethyl 2-methylpropanoate	fruity, sweet	MS, aroma, RI	16	4
783	3-methylbutanol	nail polish, rancid	MS, aroma, RI	64	4
795	butanoic acid	rancid, butter, cheese	MS, aroma, RI	64	8
800	ethyl butanoate	sweet, fruity	MS, aroma, RI	>8192	>8192
812	butyl acetate	fruity	MS, aroma, RI	1	4
815	ethyl 2-hydroxypropanoate	fruity	MS, aroma, RI	4	8
849	ethyl 2-methylbutanoate	apple, fruity	MS, aroma, RI	256	128
852	ethyl 3-methylbutanoate	apple	MS, aroma, RI	512	256
859	1,1-diethoxy-2-methylpropane	fruity	MS, aroma, RI	2	8
875	3-methylbutyl acetate	fruity	MS, aroma, RI	8	8
888	1-hexanol	floral, green	MS, aroma, RI	64	4
896	propyl butanoate	fruity, floral	MS, aroma, RI	4	16
900	ethyl pentanoate	fruity	MS, aroma, RI	>8192	>8192
915	2-heptanol	fruity	MS, aroma, RI	ND	4
924	methyl hexanoate	green, fruity	MS, aroma, RI	256	128
938	2-methylpropyl butanoate	fruity, floral	MS, aroma, RI	8	64
955	1,1-diethoxy-3-methylbutane	fruity	MS, aroma, RI	4096	1024
963	ethyl 4-methylpentanoate	fruity	MS, aroma, RI	128	256
976	dimethyl trisulfide	rotten cabbage, vegetative	MS, aroma, RI	128	64
1010	ethyl hexanoate	ester, fruity	MS, aroma, RI	>8192	>8192
1056	3-methylbutyl butanoate	fruity	MS, aroma, RI	128	16
1059	pentyl butanoate	fruity, floral	MS, aroma, RI	2	2
1062	ethyl 2-hydroxyhexanoate	fruity, jasmine	MS, aroma, RIL	2	8
1078	heptanoic acid	acidic, unpleasant	MS, aroma, RI	ND	2
1093	propyl hexanoate	floral, fruity	MS, aroma, RI	32	16
1097	ethyl heptanoate	fruity	MS, aroma, RI	256	128
1136	ethyl cyclohexanecarboxylate	fruity	MS, aroma, RIL	32	64
1150	2-methylpropyl hexanoate	woody, fruity	MS, aroma, RI	1	8
1163	2,3,5-trimethyl-6-ethylpyrazine[b]	baked, nut	MS, aroma	8	4
1171	octanoic acid	acid, sour	MS, aroma, RI	ND	8
1175	ethyl benzoate	floral	MS, aroma, RI	64	32
1176	diethyl butanedioate	fruity, wine	MS, aroma, RI	4	32
1183	unknown	nut, roasted		1	8
1189	butyl hexanoate	fruity	MS, aroma, RI	1024	256
1191	hexyl butanoate	floral, fruity	MS, aroma, RI	8	32
1196	ethyl octanoate	fruity	MS, aroma, RI	256	64
1247	ethyl benzeneacetate	rosy, honey	MS, aroma, RI	64	64
1249	3-methylbutyl hexanoate	fruity, apple, green	MS, aroma, RI	32	8
1260	2-phenylethyl acetate	rosy, honey	MS, aroma, RI	32	16
1287	pentyl hexanoate	fruity	MS, aroma, RI	64	8
1294	ethyl nonanoate	fruity	MS, aroma, RI	32	16
1328	1,1-diethoxy-2-phenylethane	fruity	MS, aroma, RI	32	64
1353	ethyl 3-phenylpropanoate	fruity	MS, aroma, RI	2	8
1357	3,5-dimethyl-2-pentylpyrazine	baked, roasted	MS, aroma, RIL	2	8
1368	furfuryl hexanoate	fruity	MS, aroma, RI	32	16
1385	hexyl hexanoate	apple, peach	MS, aroma, RI	64	16
1394	ethyl decanoate	green, fruity	MS, aroma, RI	32	16
1401	unknown	roasted		32	64
1447	2-phenylethyl butanoate	fruity	MS, aroma, RI	8	8
1456	ethyl 2-hydroxy-3-phenylpropanoate	goaty, smoky	MS, aroma, RIL	32	8
1495	unknown	fruity		1	64
1556	unknown	roasted		16	8
1579	hexyl octanoate	green, fruity	MS, aroma, RI	32	8

[a] MS: Compounds were identified by MS spectra. Aroma: Compounds were identified by the aroma descriptors. RI: Compounds were identified by comparison to pure standard. RIL: Compounds were identified by comparison with RI from the literature. [b] Tentatively identified. ND, not detected by GC/O.

first detected in Chinese liquor. It gave fruity and floral odors and had a FD ≥ 32. Esters were mostly formed through esterification of alcohols with fatty acids during the fermentation and aging processes. Fan et al. (*22*) reported that Daqu had both high hydrolase and esterase activities. The esterases could be very active during the fermentation process and catalyze ester synthesis.

Several acetals were detected in both liquors on the DB-Wax column (**Table 1**). Among them, 1,1-diethoxy-3-methylbutane gave fruity aroma and had an extremely high FD value (FD ≥ 2048). This acetal could be very important to Yanghe Daqu aroma. 1,1-Diethoxyethane could also be an important aroma compound, which contributed a fruity note. It coeluted with ethyl acetate on a DB-Wax column, but was separated on a DB-5 column (FD ≥ 256). 1,1-Diethoxynonane and 1,1,3-triethoxypropane could be important to Yanghe Daqu liquors aroma. These two compounds gave fruity and floral aromas and were detected in both liquors with relative high FD values (FD ≥

64). 1,1,3-Triethoxypropane was identified in Calvados as a defect (*21*), and it was produced from acetalization of acrolein in the presence of excess of ethanol. 1,1-Diethoxy-2-methylpropane, 1,1-diethoxy-2-methylbutane, and 1,1-diethoxy-2-phenylethane were also identified with low FD values in this study. They contributed a fruity aroma. Acetals have been found as important aroma components in freshly distilled Calvados (*23*), Cognac (*24*), and white wine (*25*), and they were likely formed from reaction of alcohols and aldehydes in the presence of excess alcohols.

Aldehydes detected in Yanghe Daqu liquors included acetaldehyde, 2-methylbutanal, 3-methylbutanal, 1-pentanal, and 2-furancarboxaldehyde (furfural) (**Table 1**). Among them, acetaldehyde could be very important to the liquor aroma based on its FD values (FD ≥ 256), which gave green and malt aromas. The SPME fiber has low affinity for acetaldehyde, so its aroma contribution could have been even greater. 2-Furancarboxaldehyde, which imparted sweet and almond notes, could be important because its FD value was more than 32. Aldehydes were probably formed by yeast (*26*).

Among all the fatty acids detected on a DB-Wax column (**Table 1**), butanoic, hexanoic, heptanoic, and pentanoic acids had relative high FD values (FD ≥ 32) and could be important aroma compounds in Yanghe Daqu liquors. They contributed rancid, sweaty, and cheesy notes. Acetic, 2-methylpropanoic acid, 3-methylbutanoic, and octanoic acids were found in both the young and the aged liquors in this study, but they had low FD values (FD ≤ 16). Most of the fatty acids in the Yanghe Daqu liquors were produced by microbial fermentation (*27*, *28*).

Two sulfur compounds, dimethyl disulfide and dimethyl trisulfide, were detected on the DB-Wax column in young and aged liquors with low FD values. Dimethyl disulfide gave onion and cabbage odors. Dimethyl trisulfide contributed to sulfur and rotten cabbage aromas. Sulfur-containing compounds probably came from the degradation of sulfur-containing amino acids (*29*).

4-Ethylguaiacol and 4-ethylphenol could be important aroma compounds to Yanghe Daqu liquors based on the FD values (FD ≥ 64) detected on the DB-Wax column (**Table 1**). 4-Ethylguaiacol gave clove and spicy notes, while 4-ethylphenol contributed a smoky aroma. 4-Methylphenol was also found in the liquor samples (FD ≤ 4). It had animal, phenol, and smoky aromas. Phenolic compounds were probably derived from lignin degradation of raw materials (*30*).

Several pyrazines, including 2,3,5,6-tetramethylpyrazine, 2,3,5-trimethyl-6-ethylpyrazine (tentatively identified), and 3,5-dimethyl-2-pentylpyrazine, were identified in both liquors, although they had relatively low FD values (FD ≤ 16). These pyrazines imparted baked, roasted, and nut notes and can be formed though both nonenzymatic and enzymatic pathways (*31*, *32*).

Very similar results were observed on the DB-5 column (**Table 2**). The potentially important aroma compounds identified on the nonpolar column were ethyl acetate, ethyl butanoate, ethyl pentanoate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl 3-methylbutanoate, methyl hexanoate, butyl hexanoate, 1,1-diethoxyethane, 1,1-diethoxy-3-methylbutane, and acetaldehyde. Their proposed importance was based on their high FD values (FD ≥ 256). Several esters were detected on a DB-5 column but not detected on a DB-Wax column, including butyl acetate, ethyl 2-methylbutanoate, propyl butanoate, 2-methylpropyl butanoate, ethyl 4-methylpentanoate, pentyl butanoate, 2-phenylethyl butanoate, ethyl 2-hydroxy-3-phenylpropanoate, and hexyl octanoate. These esters contributed to apple, fruity, and floral aromas except for ethyl 2-hydroxy-3-phenylpropanoate, which impart goaty and smoky notes. Of these, ethyl 2-methylbutanoate and ethyl 4-methylpentanoate were very important to Yanghe Daqu liquor aroma because of their high FD values (FD ≥ 256). 2-Methylpropyl butanoate, ethyl 2-hydroxy-3-phenylpropanoate, and hexyl octanoate could be important to young and aged liquors because their FD values were more than 32. 2-Methylpropanal was only detected on the DB-5 column with a high FD value (FD ≥ 64), and it gave green odor. Only one ketone, 2-pentanone, was identified on a DB-5 column in this study. It had a low FD value in both the young and the aged liquors (FD ≤ 8). It contributed a fruity aroma.

In summary, HS-SPME using DVB/CAR/PDMS fiber is a good technique for detecting aroma compounds in Yanghe Daqu liquors. The potentially important aroma compounds can be evaluated by HS-SPME GC/O dilution analysis. In this study, esters were identified as the major aroma compounds in Yanghe Daqu liquors. On the basis of the FD values, ethyl hexanoate, ethyl butanoate, and ethyl pentanoate were probably the most important aroma compounds. In addition, acids, aldehydes, alcohols, acetals, sulfide compounds, phenols, and pyrazines could significantly contribute toward the aroma of young and aged liquors. The aroma profile was similar between young and aged liquors, but the aroma compounds in aged liquor had higher FD values than in young ones. However, further quantitative analysis and sensory work are needed to identify the difference in young and aged liquors and to clarify the mechanisms involved in the aging process.

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