

# Changes in Volatile Compounds of Chinese Rice Wine Wheat Qu During Fermentation and Storage

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## ABSTRACT

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In order to understand the optimum time of using wheat Qu for Chinese rice wine brewing on the basis of the contents of volatile compounds, changes in volatile compounds of Chinese rice wine wheat Qu during fermentation and storage were studied by headspace solid phase microextraction (HS-SPME) combined with gas chromatography–mass spectrum (GC-MS). It was shown that the concentrations of most groups of volatiles, including alcohols, aldehydes, aromatic compounds, phenols, sulphides, furans, and nitrogen-containing compounds, increased progressively from the first day to the fourth day of fermentation, and then decreased gradually. A minority group of volatile compounds such as acids and ketones peaked after one day of fermentation, esters increased slowly from the first day to the eighth day of fermentation. Lactones and esters rose slowly from the first day to the fifteenth day of fermentation respectively, and decreased afterwards, whereas the evolution of terpenes was somewhat erratic. These results suggested that changes in the concentrations of most groups of volatile compounds during fermentation and storage were uniform. This made it possible to determine the optimum level of fermentation and storage for Chinese rice wine wheat Qu on the basis of the contents of volatile compounds.

**Key words:** Chinese rice wine, fermentation, storage, volatile compounds, wheat Qu.

## INTRODUCTION

Chinese rice wine is a traditional alcoholic beverage, which is consumed widely in south China and has a unique aroma, subtle flavor and low alcoholicity<sup>32</sup>. Chinese rice wine is typically fermented from glutinous rice or rice with Chinese rice wine qu and yeast. The saccharifying cultures used for Chinese rice wine are wheat Qu, Xiaoqu, or other enzyme preparations. Wheat Qu is the most widely used culture and is made from wheat through a spontaneous inoculation of molds, bacteria and yeasts in the solid state. In Chinese rice wine brewing, wheat Qu is a source of saccharification enzymes, and provides nutri-

tion for yeast multiplication and flavor and aroma substances for Chinese rice wine. Its role in Chinese rice wine production is similar to the koji of Japanese sake, but is somewhat wider in scope, since it may include many microorganisms and produce a variety of enzymes. During the process of wheat Qu production, the wheat is typically extruded into pieces, mixed with water, and pressed into a brick-shaped block. Wheat Qu is then incubated under spontaneous conditions for at least 15 days and stored for several months before being used to make Chinese rice wine. As a result of fermentation, wheat Qu is rich in various microorganisms such as *Aspidia corymbifera*, *Rhizopus oryzae*, *Rhizomucor pusillus*, *Aspergillus oryzae*, *Emericella nidulans*, *Clavispora lusitaniae*, *Aspergillus fumigatus*, *Aspergillus niger*, *Rhizopus microsporus*, *Saccharomyces cerevisiae*, *Candida tropicalis*, *Pichia guilliermondii* and *Pichia anomala*<sup>30</sup>. A wide variety of enzymes including amylases, glucoamylase, proteases, phytase and phosphatase also accumulate in the wheat Qu during fermentation.

In general, the quality of Chinese rice wines is directly related to Chinese rice wine wheat Qu, which determines the organoleptic characteristics of the finished Chinese rice wine<sup>28</sup>. Research has generally focused on the enzymes and microorganisms of wheat Qu<sup>24,30</sup>, and the flavor of wheat Qu is judged by its simple sensory description with the term “normal or no odor of wheat Qu”. It is the volatile compounds of wheat Qu however, that are the most important factors which influence the flavor of Chinese rice wine<sup>23</sup>, and for this reason it was important to analyze and characterize the volatile compounds from wheat Qu.

Wheat Qu is usually fermented for 15 days and stored for at least 2 months. During the fermentation and storage period, changes in enzymes, microorganisms, and substances occur and these changes depend on many factors such as temperature, humidity, and time of fermentation and storage<sup>28</sup>. These differences in time of fermentation and storage alter the composition of the enzymes and microorganisms of wheat Qu resulting in subtle changes in the composition of the volatile compounds.

The primary aim of this research effort was to determine the volatiles in Chinese rice wine wheat Qu produced during fermentation and storage, using headspace solid phase microextraction (SPME). SPME is a solvent-free extraction technique and has been used to extract a wide range of volatile and semi-volatile aroma compounds in solid food samples such as mushroom<sup>11</sup>, beef<sup>20</sup>,

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breakfast cereal<sup>15</sup>, olive oil<sup>16</sup> and alcoholic beverages<sup>7,29,31</sup>. The volatiles were analyzed and the concentration changes in the volatile compounds during fermentation and storage were documented. These findings could assist in optimizing the brewing time required for wheat Qu in the production of Chinese rice wine.

## MATERIALS AND METHODS

### Chemicals and reagents

Ethanol (special grade reagent) was obtained from Hanbon Sci. & Tech. Co., Ltd (Jiangsu, China) and analytical-grade anhydrous calcium chloride and sodium chloride were obtained from China National Pharmaceutical Group Corporation (Shanghai, China). Ultrapure water was produced using a Milli-Q purification system (Millipore, Bedford, MA, USA). The 2-octanol ( $\geq 96.0\%$ ) was obtained from Sigma-Aldrich (Shanghai, China) and used as the internal standard. Other reagents were purchased from Sigma-Aldrich China Co. (Shanghai, China).

### Manufacture of wheat Qu

The wheat Qu was produced by a Chinese rice wine manufacturer. The process used to make the wheat Qu was as follows. Wheat (500 kg) was broken into pieces and mixed well with water (100–110 kg, 25–30°C). The mixture was pressed into a model to shape bricks 24 cm long, 15.7 cm wide and 7 cm high. The bricks were moved into a room for fermentation under suitable conditions of temperature, moisture, carbon dioxide and oxygen levels. During the process of fermentation, the initial temperature of the wheat Qu was generally kept at 25–30°C. The temperature rose slowly as fermentation progressed during the first 3 days, due to microbial growth. When it reached 55°C, the temperature of the wheat Qu was controlled within a range of 45–55°C for 2 days by ventilating the room. The wheat Qu was cooled slowly to room temperature and fermented for about 10 days. The wheat Qu bricks were then conveyed to another room and stored for at least 2 months before use.

### Wheat Qu samples

A series of wheat Qu samples were obtained from a Chinese rice wine manufacturer in Shanghai City, China. These included wheat Qu samples with 0 (Wheat not fermented), 1, 2, 3, 4, 6, 8, 10, 15 days of fermentation, and 30 days of fermentation and storage.

### Sample preparation

Wheat Qu (~500 g) was ground and stored at 4°C before analysis. The ground wheat Qu (10 g) was weighed into a 50 mL centrifuge tube with a PTFE-lined screw cap. Each sample was suspended in 20 mL of ultrapure water, and 0.1 g anhydrous calcium chloride was added. The mixture was homogenized using a glass stick. The centrifuge tube was sealed with a cap and placed into a 25  $\pm$  3°C ultrasound cleaning bath (AS2060B, 60w, China) and sonicated at 60 w for 30 min. After sonication, the mixture was centrifuged at 7,690 g for 10 min at 4°C. The supernatant was filtered and collected in a glass jar. The extraction was repeated twice and the filtrates combined and stored at –20°C.

### Headspace solid phase microextraction (HS-SPME) and gas chromatography–mass spectrum (GC-MS) analysis

Analysis of the volatile compounds was performed using HS-SPME coupled with a GC-MS according to the procedure described by Luo et al.<sup>18</sup> with modifications. The volatile compounds of wheat Qu were extracted using a 50/30  $\mu\text{m}$  divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Inc., Bellefonte, PA). Each wheat Qu sample (8 mL) was placed in a 25 mL SPME glass vial together with 2.5 g of sodium chloride and 5  $\mu\text{L}$  of the internal standard 2-octanol (64.55 mg/L in absolute ethanol). The vial was tightly capped, stirred and left to equilibrate for 15 min and then incubated for 40 min at 50°C. After extraction, the fiber was introduced into the injection port of the GC-MS system (at 250°C for 5 min) and the analytes extracted from the fiber were thermally desorbed. All samples were analysed in triplicate.

GC-MS analysis was carried out using an Agilent 6890 GC-5975 mass selective detector (MSD). The column was a CP-Wax (60 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness, Varian Chrompack, Middelburg, The Netherlands). The oven and injector temperatures were 250°C. Helium at a constant flow rate of 2 mL/min was used as the column carrier gas. The oven temperature was held at 50°C for 2 min, and then raised to 230°C at a rate of 6°C/min and held at 230°C for 15 min. An Agilent 5975 MSD was used for the identification of the unknown compounds. The electron impact (EI) energy was 70 eV. The ion source and transfer line temperatures were set at 230°C and 280°C, respectively. EI mass spectra ranged from 30 to 550 amu.

### Retention indices (RI) calculations and identification of unknowns

Unknown compounds were identified by comparison with those in the NIST05a.L Database (Agilent Technologies Inc.). RIs of unknown compounds were calculated in accordance with a modified Kovats method<sup>2</sup>. A standard mixture of C5–C30 was injected into the GC-MS and the retention times were used to calculate the RIs of the unknown compounds. The sample and some standard mixtures were co-injected into the GC-MS. Positive identification was achieved by comparing the sample mass spectra and RIs with those of standards or those published in the literature under the same conditions. Tentative identification was achieved by comparing mass spectra only.

### Analysis of semi-quantification

Semi-quantification of the volatile compounds was performed using 2-octanol as the internal standard. Data were collected in total ion mode for all mixed standards and samples. Semi-quantitative data of the volatile compounds were acquired according to the method described by Luo et al. with the following formula<sup>18</sup>:

$$C(\mu\text{g/L}) = \frac{A_c}{A_{is}} C_{is}(\mu\text{g/L})$$

C: the relative concentration of analyte (expressed as  $\mu\text{g/L}$ );  $C_{is}$ : the final concentration of internal standard (IS)

in sample;  $A_c$ : peak area of analyte;  $A_{is}$ : peak area of internal standard.

The results were expressed as the mean value of three replicates of wheat Qu samples. The standard deviations (RSDs) of the analyses were less than 10%.

## RESULTS AND DISCUSSION

### Analysis of changes of volatile compounds in wheat Qu during fermentation and storage

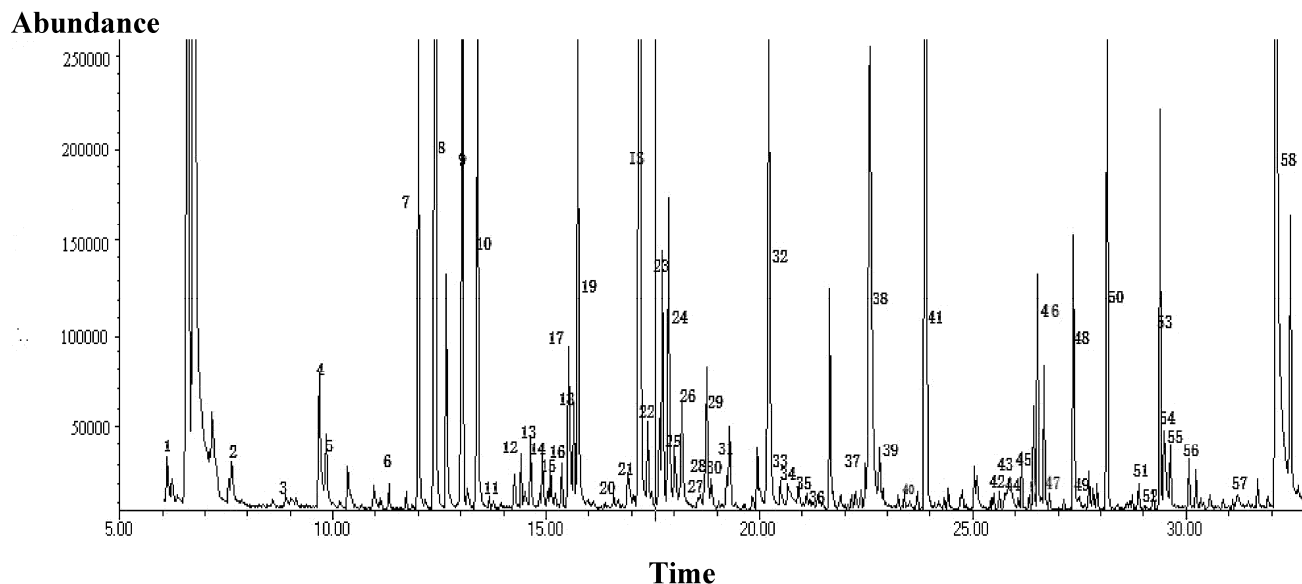
Total ion chromatograms of the wheat Qu sample on the fourth day of fermentation using the HS-SPME method are shown in Fig. 1. The volatile compounds identified are shown in Table I, along with the relative concentrations in the extract. A total of 58 volatile compounds were identified and measured in the wheat Qu samples including 11 alcohols, 6 acids, 3 esters, 7 ketones, 5 aldehydes, 9 aromatics, 1 lactone, 4 phenols, 2 sulphides, 3 furans, 4 nitrogen-containing compounds, and 3 terpenes. Most of these compounds have been previously detected in Chinese rice wine<sup>18</sup>.

The evolution of each group of volatile compounds during fermentation and storage of wheat Qu is illustrated in Fig. 2. The different groups of compounds generally behaved as expected, with all of the volatile compounds, except menthol, increasing from the first day of fermentation (Table I).

The alcohols identified in Chinese rice wine wheat Qu during fermentation and storage were 2-methylpropanol,

1-penten-3-ol, 3-methylbutanol, 1-pentanol, 2-heptanol, 1-hexanol, 1-octen-3-ol, 1-heptanol, 2-ethyl-1-hexanol, 1-octanol, and 1-nonanol (Table I). There was a clear trend in concentration changes in these compounds in the Chinese rice wine wheat Qu during fermentation and storage. The levels of alcohols rose from the first day to the fourth day of fermentation, decreased gradually, and then tended to stabilize. The concentrations of 3-methylbutanol, 1-pentanol, and 1-hexanol, increased sharply from the first day to the fourth day of fermentation, decreased from the sixth day to the fifteenth day of fermentation, and then tended to stabilize gradually. While 2-methylpropanol, 1-penten-3-ol, 2-heptanol, 1-octen-3-ol, 1-heptanol, 2-ethyl-1-hexanol, 1-octanol, and 1-nonanol were present in very small amounts in Chinese rice wine wheat Qu during fermentation and storage, their concentrations gradually rose, decreased, and then tended to stabilize. These alcohols have been described with fruity, floral and alcohol-like aromas.

It is well known that there are many metabolic pathways are involved in the biosynthesis of alcohols in cheese<sup>5</sup>. These metabolic pathways include amino acid metabolism, lactose metabolism, methyl ketone reduction as well as degradation of linoleic and linolenic acids. Chinese rice wine wheat Qu, made from wheat through spontaneous fermentation, was rich in various microorganisms and complex enzyme systems. Cramer et al.<sup>4</sup> reported that the lipid content was about 1.70% and the protein content ranged from 15.5 to 16.8% in the wheat varieties. Alcohols in wheat Qu could be produced through lipid oxida-



**Fig. 1.** Total ion chromatogram of volatile compounds in a wheat Qu on the fourth day of fermentation extracted by HS-SPME. Key: 1, ethyl acetate; 2, 2,3-butanedione; 3, 1-penten-3-one; 4, hexanal; 5, 2-methylpropanol; 6, 1-penten-3-ol; 7, ethyl hexanoate; 8, 3-methylbutanol; 9, 2-pentylfuran; 10, 1-pentanol; 11, 3-octanone; 12, 2-octanone; 13, 3-hydroxy-2-butanone; 14, 2-heptanol; 15, (E)-2-heptenal; 16, 2,5-dimethylpyrazine; 17, 6-methyl-5-hepten-2-one; 18, 2,6-dimethylpyrazine; 19, 1-hexanol; 20, 2-nonanone; 21, nonanal; 22, ethyl octanoate; 23, 2,3,5-trimethylpyrazine; 24, 1-octen-3-ol; 25, 1-heptanol; 26, acetic acid; 27, 3-(methylthio)-propanal; 28, furural; 29, 2-ethyl-1-hexanol; 30, 2,3,5,6-tetramethylpyrazine; 31, decanal; 32, benzaldehyde; 33, 1-octanol; 34, 3-methylbutanoic acid; 35, menthol; 36, 1-nonanol; 37, 2-furanmethanol; 38, phenylacetaldehyde; 39, acetophenone; 40, ethyl benzoate; 41, 1,2-dimethoxybenzene; 42, (E,E)-2,4-decadienal; 43, hexanoic acid; 44, geosmin; 45, geranylacetone; 46, guaiacol; 47, benzyl alcohol; 48, 2-phenylethanol; 49, heptanoic acid; 50, 1,2,3-dimethoxybenzene; 51, phenol; 52, benzothiazole; 53, 4-ethenyl-1,2-methoxybenzene; 54,  $\gamma$ -nonalactone; 55, 4-ethylguaiacol; 56, octanoic acid; 57, nonanoic acid; 58, 4-vinylguaiacol.

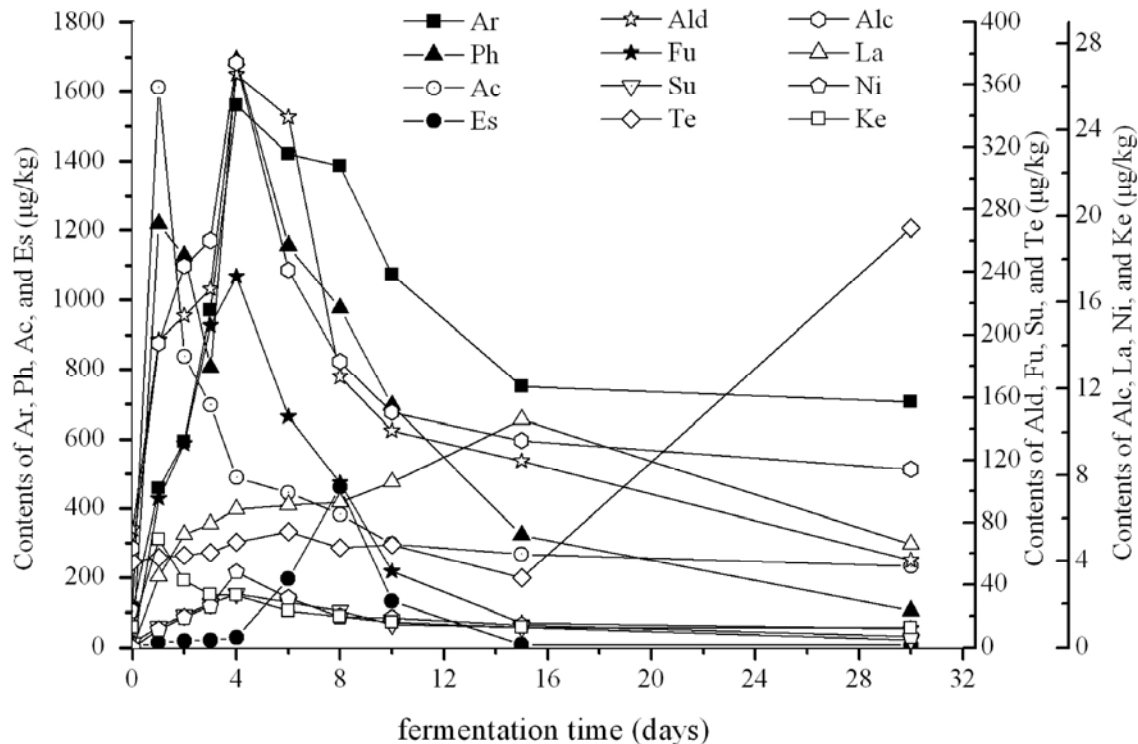
tion catalyzed by lipoxygenase originating from the microbiota present in the medium or from microbial amino acid catabolism, as well as methyl ketone reduction.

The evolution of aromatic compounds was similar to those of alcohols. The sum of the aromatic compounds showed a sharp increase between the first day and the fourth day of fermentation, and then decreased gradually. An exception was the concentrations of 1,2,3-trimethoxybenzene and 2-phenylethanol in this group, which increased from the first day to the eighth day of fermentation, and then decreased gradually from the ninth day of fermentation. Among all of the aromatic compounds examined, 1,2,3-trimethoxybenzene and 4-ethenyl-1,2-methoxybenzene were identified only in wheat Qu and not in wheat. Benzaldehyde, phenylacetaldehyde, benzyl alcohol, ethyl benzoate, acetophenone, 1,2-dimethoxybenzene, and 2-phenylethanol, which were detected in the wheat Qu throughout all fermentations and storage stages studied, were also detected in Chinese rice wine<sup>18</sup>. The compound 1,2,3-trimethoxybenzene, which is associated with smoke and musty odors, presented a higher concentration on the fourth day of fermentation. The compound 2-phenylethanol, which contributes to rosy and honey aromas and is important in wheat Qu, also had the highest concentration in the Chinese rice wine<sup>18</sup>.

The volatile phenolic compounds identified in Chinese rice wine wheat Qu during fermentation and storage, include phenol, guaiacol, 4-ethylguaiacol, and 4-vinylguaiacol, can be formed from lignin degradation of raw materials in wheat<sup>19</sup>. Different studies have been performed to determine the capacity of certain microorganism to pro-

duce volatile phenols<sup>3,27</sup>. These volatile phenolic compounds, except for guaiacol, have been reported in Chinese rice wine<sup>18</sup>. The production of volatile phenols was somewhat more variable than the other compounds, but the tendency was for an increase in the concentration until the fourth day of fermentation, from which time the concentration started to fall (Table I). The levels of these compounds increased sharply on the first day of fermentation, and then decreased slowly from the second day to the third day of fermentation, but tended to increase from the third day to the fourth day of fermentation, and then tended to fall. The concentration of 4-vinylguaiacol increased markedly from the first day to the fourth day of fermentation, gradually decreased, and then remained stable. The concentration of 4-ethylguaiacol achieved a peak after two days of fermentation and then steadily decreased

Aldehydes and nitrogen-containing compounds were present in small amounts, and sulphides and furans were found in trace amounts in the studied samples. Small fluctuations of these groups of volatile compounds were observed in Chinese rice wine wheat Qu during fermentation and storage, (Table I). The tendency was for a slight increase from the first day to the fourth day of fermentation, and then for a slight decrease. An exception was the concentration of (E,E)-2,4-decadienal and (E)-2-heptenal of aldehydes, which achieved a peak on the second day and the third day of fermentation respectively, and then steadily decreased. Aldehydes, such as hexanal and (E,E)-2,4-decadienal, are the expected oxidation products of linoleic acid<sup>26</sup>. A total of four nitrogen-containing com-



**Fig. 2.** Changes in the volatile compounds in the Chinese rice wheat Qu during fermentation and 30 day storage period. Abbreviations: Ar, aromatic compounds; Ph, phenols; Ac, acids; Es, esters; Ald, aldehydes; Fu, furans; Su, sulphides; Te, terpenes; Alc, alcohols; La, lactones; Ni, nitrogen-containing compounds; Ke, ketones.

**Table I.** Volatile compounds identified in the Chinese rice wine wheat Qu during fermentation and 30 day storage period.

RI	Basis of identification <sup>a</sup>	Compounds	Fermentation time (days) and content ( $\mu\text{g}/\text{kg}$ -dried wheat Qu)									
			0	1	2	3	4	6	8	10	15	30
<b>Alcohols</b>												
1106	MS, RI	2-methylpropanol	0.57	2.42	6.95	8.72	11.43	2.35	1.21	0.86	0.54	0.51
1165	MS	1-penten-3-ol <sup>b</sup>	0.66	3.53	3.89	4.61	5.22	3.41	2.42	2.38	1.24	0.97
1229	MS, RI	3-methylbutanol	3.01	33.04	78.19	78.58	97.84	20.62	19.57	14.63	13.83	8.37
1255	MS, RI	1-pentanol	3.01	18.13	19.37	29.69	39.71	36.29	30.56	23.83	12.27	11.92
1312	MS, RI	2-heptanol	4.47	4.53	8.67	9.43	12.82	9.17	8.67	7.72	6.95	3.92
1345	MS, RI	1-hexanol	23.36	56.71	61.15	74.21	161.49	129.43	86.53	71.33	70.98	56.83
1437	MS, RI	1-octen-3-ol	21.04	67.72	56.98	44.33	34.86	28.65	22.02	18.98	17.56	24.18
1440	MS, RI	1-heptanol	2.68	3.36	3.39	3.41	4.06	4.86	5.28	4.69	3.73	3.13
1462	MS, RI	2-ethyl-1-hexanol	2.62	2.87	3.19	4.99	3.56	3.31	3.28	2.87	2.83	2.09
1548	MS, RI	1-octanol	1.62	1.68	1.69	1.73	2.57	2.39	2.37	2.32	1.67	1.42
1645	MS, RI	1-nonanol	0.37	0.51	0.55	0.58	0.74	0.82	0.96	0.89	0.85	0.69
		$\Sigma$	63.41	194.5	244.02	260.28	374.3	241.3	182.87	150.5	132.45	114.03
<b>Acids</b>												
1445	MS, RI	acetic acid	29.62	821.78	421.29	371.06	207.23	194.86	146.49	120.15	101.35	88.69
1630	MS, RI	3-methylbutanoic acid	354.32	188.59	116.01	99.81	95.35	84.36	68.22	62.81	44.77	354.32
1820	MS, RI	hexanoic acid	27.48	385.39	197.48	187.61	162.97	139.71	138.09	95.35	93.82	92.78
1962	MS, RI	heptanoic acid	0.32	3.21	2.34	2.12	1.75	1.73	1.63	1.33	1.26	1.08
2050	MS, RI	octanoic acid	8.17	46.45	26.14	20.07	17.02	12.19	10.78	10.62	7.67	4.62
2178	MS, RI	nonanoic acid	0.68	1.25	1.51	1.57	1.82	1.62	1.39	1.26	1.14	0.64
		$\Sigma$	102.64	1612.43	837.35	698.44	490.6	445.46	382.74	296.93	268.05	232.58
<b>Esters</b>												
885	MS, RI	ethyl acetate	0.52	9.21	11.63	14.13	17.57	187.37	456.62	128.21	5.88	4.66
1205	MS, RI	ethyl hexanoate	0.39	2.27	2.29	2.32	2.83	2.87	2.95	2.63	2.07	2.06
1405	MS, RI	ethyl octanoate	0.82	3.09	4.62	4.87	9.1	5.72	1.85	1.23	0.96	0.61
		$\Sigma$	1.73	14.57	18.54	21.32	29.5	195.96	461.42	132.07	8.91	7.33
<b>Ketones</b>												
991	MS, RI	2,3-butanedione	n.d. <sup>c</sup>	21.89	11.04	8.13	7.84	4.44	3.38	1.71	0.88	0.69
1036	MS	1-penten-3-one <sup>b</sup>	0.47	2.39	1.68	1.48	1.44	1.36	1.32	1.26	1.23	1.19
1262	MS, RI	3-octanone	n.d.	2.91	1.55	1.39	1.02	0.81	0.65	0.65	0.64	0.61
1275	MS, RI	2-octanone	8.79	8.11	7.78	7.98	7.35	8.11	7.48	7.41	7.11	7.04
1366	MS, RI	2-nonanone	n.d.	1.59	2.17	2.23	2.39	2.56	1.68	1.54	1.42	1.39
1294	MS, RI	3-hydroxy-2-butanone	n.d.	28.49	13.65	6.95	6.44	1.32	0.84	0.45	n.d.	n.d.
1340	MS, RIL	6-methyl-5-hepten-2-one	3.64	3.77	4.79	5.38	6.66	4.52	4.26	3.21	1.77	1.46
		$\Sigma$	12.9	69.15	42.66	33.54	33.14	23.12	19.61	16.23	13.05	12.38
<b>Aldehydes</b>												
1093	MS, RI	hexanal	2.29	6.07	8.98	9.53	16.71	9.26	6.85	5.54	5.88	2.26
1332	MS, RIL	(E)-2-heptenal	0.39	1.73	1.24	2.08	1.33	0.67	1.01	0.98	0.87	0.45
1369	MS, RI	nonanal	1.42	1.51	1.62	1.71	3.34	3.01	2.66	2.16	0.88	0.61
1518	MS, RI	decanal	1.21	1.68	1.78	1.84	1.92	1.74	1.59	0.97	0.24	0.47
1812	MS, RIL	(E,E)-2,4-decadienal	0.18	3.25	1.75	1.45	1.27	0.64	0.45	0.42	0.79	0.24
		$\Sigma$	5.49	14.24	15.37	16.61	24.57	15.32	12.56	10.07	8.66	4.03

(continued on next page)

<sup>a</sup> MS - compounds were identified by MS spectra. RI - compounds were identified by comparison to a pure standard. RIL - compounds were identified by comparison with RI from the literature<sup>12,17,26</sup>.

<sup>b</sup> Not detected.

<sup>c</sup> Tentatively identified.

pounds were detected only in the Chinese rice wine wheat Qu and not in wheat. Wheat Qu was generally exposed to 45–55°C for at least 2 days, a high fermentation temperature. Nitrogen-containing compounds could be produced through both non-enzymatic pathways such as the Maillard reaction and enzymatic pathways produced by *Bacillus subtilis*<sup>8</sup>. In wheat Qu, during fermentation the high temperature would benefit the Maillard reaction. Similar to nitrogen-containing compounds, a high temperature would also facilitate furan formation through the thermal degradation of carbohydrate followed by cyclation in Maillard-type systems<sup>1</sup>. Two sulphides were present in wheat Qu at low concentrations. Sulfur-containing compounds often have very low sensory thresholds, and these compounds can arise from the degradation of sulfur-containing amino acids<sup>9</sup>.

Acids and ketones peaked after one day of fermentation, decreased gradually from the second day of fermentation, and then stabilized. The concentrations of acids increased sharply on the first day of fermentation, and then decreased gradually. Acids were found at a high concentration in wheat Qu at all stages. Acids can be produced either by the yeasts during alcoholic fermentation<sup>25</sup> or by lactic acid bacteria (LAB) genera during fermentation of cheese<sup>10</sup>. Many LAB isolated from cheese are known to contain lipases that can hydrolyze lipids, resulting in the formation of volatile fatty acids. Fatty acids are not only aroma compounds by themselves, but also serve as precursors of alcohols, esters, lactones and methyl ketones. Most ketones are produced by lipid oxidation and the  $\beta$ -oxidation of free fatty acids by microbial fermentation in fermented meat products, and the ketones may be reduced

**Table I.** Volatile compounds identified in the Chinese rice wine wheat Qu during fermentation and 30 day storage period. (continued from previous page)

RI	Basis of identification <sup>a</sup>	Compounds	Fermentation time (days) and content (µg/kg-dried wheat Qu)									
			0	1	2	3	4	6	8	10	15	30
<b>Aromatic compounds</b>												
1533	MS, RI	benzaldehyde	0.34	16.23	27.05	38.67	64.42	28.81	22.19	11.46	10.01	7.32
1656	MS, RI	phenylacetaldehyde	0.91	4.54	4.69	5.93	6.33	6.29	6.28	6.25	3.46	2.58
1660	MS, RI	acetophenone	1.53	38.27	105.98	110.88	195.26	145.98	74.41	59.35	15.71	2.14
1678	MS, RI	ethyl benzoate	0.59	0.67	0.44	0.48	0.72	0.83	1.09	0.73	0.74	1.36
1731	MS, RI	1,2-dimethoxybenzene	1.99	6.86	10.22	105.33	370.98	212.72	145.51	119.11	39.92	43.44
1891	MS, RI	benzyl alcohol	0.06	0.36	0.44	0.58	1.19	0.52	0.37	0.28	0.27	0.27
1932	MS, RI	2-phenylethanol	105.63	355.51	374.26	375.41	393.53	409.59	447.57	263.06	208.62	264.84
1987	MS	1,2,3-trimethoxybenzene <sup>b</sup>	n.d.	20.82	44.36	223.33	350.96	479.22	570.73	540.45	407.16	326.04
1998	MS	4-ethenyl-1,2-methoxybenzene <sup>b</sup>	n.d.	14.77	25.61	109.63	176.94	137.36	118.95	72.44	66.16	59.35
		Σ	111.05	458.03	593.05	970.24	1560.33	1421.32	1387.11	1073.33	752.05	707.34
<b>Lactones</b>												
2003	MS, RI	γ-nonalactone	n.d.	45.22	72.01	78.85	88.58	90.99	92.93	105.99	145.94	65.95
		Σ		45.22	72.01	78.85	88.58	90.99	92.93	105.99	145.94	65.95
<b>Phenols</b>												
1989	MS, RI	phenol	7.69	22.64	23.77	27.22	40.23	41.55	43.06	160.763	41.15	36.228
1876	MS, RI	guaiacol	n.d.	47.75	152.32	154.61	169.29	170.13	185.73	190.31	223.69	28.23
2010	MS, RI	4-ethylguaiacol	n.d.	884.32	609.27	130.57	125.44	71.62	22.41	8.09	7.42	n.d.
2188	MS, RI	4-vinylguaiacol	2.86	262.82	341.96	494.39	1357.21	872.21	724.26	338.57	50.71	41.01
		Σ	10.56	1217.53	1127.32	806.79	1692.17	1155.11	975.46	697.73	322.97	105.47
<b>Sulphides</b>												
1449	MS, RIL	3-(methylthio)propanal	n.d.	0.36	0.89	1.23	1.64	1.51	1.18	0.79	0.56	0.22
1996	MS, RI	benzothiazole	0.23	0.61	0.61	0.79	0.83	0.58	0.51	0.29	0.36	0.14
		Σ	0.23	0.97	1.50	2.02	2.47	2.09	1.69	1.08	0.92	0.36
<b>Furans</b>												
1647	MS, RI	2-furanmethanol	n.d.	3.83	5.47	3.07	2.64	1.15	1.11	n.d.	n.d.	n.d.
1240	MS, RIL	2-pentylfuran	n.d.	0.38	0.56	0.85	1.57	1.49	1.45	1.16	0.84	0.75
1452	MS, RI	fural	n.d.	2.69	3.43	10.99	12.99	8.07	5.09	2.36	0.29	0.11
		Σ	n.d.	6.9	9.46	14.91	17.2	10.71	7.65	3.52	1.13	0.86
<b>Nitrogen-containing compounds</b>												
1474	MS, RI	2,3,5,6-tetramethylpyrazine	n.d.	0.29	0.32	1.23	2.62	2.81	1.32	0.51	0.55	0.26
1337	MS, RI	2,5-dimethyl-pyrazine	n.d.	4.39	9.74	12.25	23.81	13.52	5.82	5.09	1.58	0.91
1342	MS, RI	2,6-dimethyl pyrazine	n.d.	2.31	3.15	5.01	6.52	4.94	3.37	2.19	1.76	1.18
1422	MS, RI	2,3,5-trimethylpyrazine	n.d.	0.96	1.71	2.23	8.55	3.23	1.49	1.45	0.72	0.44
		Σ	n.d.	11.56	19.06	26.31	48.07	31.48	19.47	18.90	14.2	6.96
<b>Terpenes</b>												
1639	MS <sup>b</sup>	menthol <sup>b</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.41	0.66	0.64	1.35
1861	MS, RI	geosmin	n.d.	0.19	0.27	0.31	0.31	0.34	0.37	0.54	1.01	16.67
1868	MS, RI	geranylacetone	3.96	3.97	3.99	4.08	4.57	5.01	3.84	3.54	1.58	1.41
		Σ	3.96	4.16	4.26	4.39	4.88	5.35	4.62	4.74	3.23	19.43

to alcohols<sup>21</sup>. Thus, most of acids and ketones in wheat Qu were speculated to be derived from lipid oxidation by microbial fermentation. These two groups of volatile compounds are intermediate compounds, which may be transformed into the other volatile compounds. Therefore, the levels of these volatile compounds in wheat Qu increased sharply on day 1 of fermentation and then decreased.

Only three esters were detected in wheat Qu. Esters tended to increase slowly from the first day to a peak on the eighth day of fermentation, decreased gradually, and then stabilized. Esters, which impart fruity and floral odors to Chinese rice wine wheat Qu, could be formed either via the esterification of alcohols with fatty acids or through the synthesis in the microorganism's cells by alcohol acetyltransferase using acetyl-CoA and higher alcohols as substrates during fermentation. Generally speaking, the latter plays a more important role in the formation of esters during the fermentation<sup>6</sup>. Therefore, ester formation can be influenced by the concentration of

the two substrates (acetyl-CoA and higher alcohols) and the activity of alcohol acetyltransferase. All factors that influence substrate concentrations or enzyme activity will affect ester production. Many factors such as fermentation temperature, fatty acid, nitrogen and oxygen levels can affect ester production. In the fermentation processes of the Chinese rice wine wheat Qu, suitable conditions such as temperature and carbon dioxide and oxygen levels were maintained, thus favoring the formation of esters.

Lactones tended to increase slowly from the first day to a peak on the fifteenth day of fermentation, and then decreased. In cheese, lactones are generated by the hydrolysis of hydroxy-fatty acid triglycerides, followed by lactonisation<sup>5</sup>. Only one lactone, namely γ-nonalactone was detected in wheat Qu in this study. This compound, which probably came from a bacterial fermentation<sup>22</sup>, may be formed by other volatiles such as acids, that is, it is a secondary metabolite. The concentration of γ-nonalactone increased slowly up to the fifteenth day of fermentation

and  $\gamma$ -nonalactone has also been reported in Chinese rice wine<sup>18</sup>.

The evolution of terpenes was somewhat erratic because the concentrations of trans-1,10-dimethyl-trans-9-decalinol (geosmin) tended to increase slowly during the fermentation stage, and then increased rapidly during the storage period studied. Terpenes usually exist in the free and glycosylated form in grapes. During alcoholic fermentation, the content of free terpenes often increases due to the  $\beta$ -glucosidase activity of yeasts<sup>13</sup>. For this reason, the increase of some terpenes observed in wheat Qu may be attributed to the  $\beta$ -glucosidase activity originating from microbiota present in the medium. Geosmin is produced by a number of microorganisms, including most *Streptomyces* and several species of myxobacteria, cyanobacteria and fungi<sup>14</sup>. Terpenes, which were present in small amounts in wheat Qu, were not detected in the Chinese rice wine<sup>18</sup>.

As noted above, most groups of volatile compounds including alcohols, aldehydes, aromatic compounds, phenols, sulphides, furans, and nitrogen-containing compounds are primary metabolites. The levels of these groups of volatile compounds peaked on the fourth day of fermentation, and then tended to decrease. Acids and ketones are not only primary metabolites, but also the precursors of other aromatic compounds. The levels of these volatile compounds in wheat Qu increased sharply on the first day of fermentation and then decreased. A minority group of volatile compounds such as esters and lactones, which were secondary metabolites, could be produced via other volatiles such as alcohols, fatty acids or other substances. These volatile compounds attained a peak on the eighth day and the fifteenth day, respectively, and afterwards tended to decrease. Although the levels of these volatiles concentrations were low, they may be important in Chinese rice wine. Terpenes increased slowly during the fermentation stage, and then increased rapidly during storage. These volatiles were also present in small amounts in wheat Qu, but not in Chinese rice wine. According to the analysis above, during fermentation there was a marked change in all groups of volatiles except for the terpenes, however, no significant change was shown during storage. The results suggest that wheat Qu incubated for 4 days could be used for brewing of Chinese rice wine, in terms of the general evolution of volatile compounds during fermentation and storage, and provide a theoretic reference for shortening the wheat Qu-making period of Chinese rice wine production.

## CONCLUSIONS

A total of 58 volatile compounds were identified and semi-quantified by HS-SPME-GC-MS in wheat Qu samples within 30 days of fermentation and storage. These compounds included alcohols, acids, esters, ketones, aldehydes, aromatic compounds, lactones, phenols, sulphides, furans, nitrogen-containing compounds, and terpenes. The concentrations of most groups of volatile compounds attained a peak on the fourth day of fermentation and then gradually decreased, suggesting that shortening the wheat Qu making period to 4 days may provide an optimal level of fermentation.

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