Study on volatile compounds in rice by HS-SPME and GC-MS

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Abstract

Headspace solid phase micro-extraction (HS-SPME), together with gas chromatography and mass spectrometry, were used to investigate volatile compounds in rice. Experimental conditions including extraction time, temperature, the amount of sample and equilibrium time, were optimized. Optimal conditions were that a 20 g sample was heated for 30 min at 80°C prior to headspace absorption, and then extracted for 30 min with HS-SPME. The volatile compounds in indica and japonica rice were alcohols, aldehydes, ketones, esters, hydrocarbons, organic acids, as well as heterocyclic compounds. Aldehydes were the most abundant making up around 30% of volatiles by weight.

Keywords: Headspace solid phase micro-extraction, Rice, Gas chromatographic and mass spectrum, Volatile substances

1. Introduction

Rice is the main grain staple for about half of the world's population and China is one of world's three main rice producers. Paddy or rough rice is the main security reserve crop in China (LONG Qilin, 2004), so that it may be held in storage for considerable periods. As paddy respires actively its quality may decline during storage. Therefore, research on volatile components of rice and exploring new method of rapidly evaluating the quality of rice in storage has important practical significance for the guidance of scientific storage of paddy.

The solid-phase micro-extraction (SPME) method was developed by Arthur and Paw (1990) in the last century. SPME is a technology of sample pre-treatment, which combines sampling, extracting, concentrating and injection. Combined with gas or liquid chromatography, it can be effectively used to analyze trace organic compounds in samples. It has good repeatability and a detection limit up to µg/L (MA Jipin et al., 2002). SPME with the characteristics of simple preparation, rapid analysis, low cost, and without organic solvents, is widely used in the food, environmental, chemical, and pharmaceutical industry.

The changes during storage of volatile components in rice have been little studied and reported (Maga, 1984; Ling ,1988; Zhou et al., 2005). A total of 12 group components in rice were separated by gas chromatography by Zhou et al. (2005), but no volatile components were identified. The volatile carbonyl compound (VCC) composition and content were analysed using column chromatography by Ling (1988). In this paper, we identify and classify the volatile components of rice, and compare indica and japonica varieties, using solid-phase micro-extraction and GC-MS.

2. Materials and methods

2.1. Experimental material

Paddy samples: Two indica rice samples were collected from Hubei and Guangdong provinces and two japonica samples collected from Heilongjiang Province and Liaoning provinces in China.

2.2. Main instruments

Solid-phase micro-extraction device and extraction head (CAD-PDMS 75µm, PDMS 100µm), the United States Supelco, Inc.; Gas-MS analyzer, Thermo Electron Corporation; Laboratory test Huller, type JLGJ4.5, Zhejiang Taizhou Food Instrument Factory; Water bath, Shanghai Shenxin Experimental Instruments Co. Ltd; Extraction bottles with Cypriot.

2.3. Sample preparation

Brown rice sample preparation: The paddy was cleaned of impurities then husked. The resultant brown rice was placed in a ziplock bag and mixed before use.

Headspace solid-phase extraction: 20g brown rice was placed in 100mL glass bottle, and water added in a ratio of 1:2 was added. The glass bottle was put in heated water bath until there was an equilibrium between the gas in the top space and the liquid in the lower part. The head of the solid-phase micro-extraction fiber was inserted into the glass bottle through a hole in the top. After the extraction period was complete, the fiber was removed and inserted quickly into the injection hole of a gas chromatogram, and desorbed at 250°C for 5min in splitless mode.

2.4. Gas chromatographic conditions

Column is DB5-MS capillary column (30m × 0.25mm × 0.25μm); Carrier gas helium with flow rate 1.0mL/min; Initial column temperature 50°C, and heated to 165°C with 10°C/ min, keeping 5min, and then to 250°C with 15°C/min, keeping 3min; Non-split injection.

2.5. MS conditions

Interface temperature is 280°C; Ion source is the EI.Ion at 230°C; Electron energy is 70ev; Mass scan range(M/Z) is 35-350amu.

2.6. Data Processing

The volatile components of unknown samples were identified by computer through searching and matching NIST and Weily MS libraries. The volatile components were quantified using peak area normalization.

3. Results and discussion

3.1. Optimization of extraction method

There are many factors that can have a direct impact during the headspace-solid phase micro-extraction process these include the equilibrium time, extraction time and temperature.

3.2. Selection of extraction temperature

Solid-phase micro-extraction and the enrichment of the sample is a dynamic equilibrium process. The temperature is the most important parameters affecting the distribution coefficient, which is a thermodynamic constant. Raising the temperature can concentrate the gas phase of gas-liquid equilibrium, increasing the volatile compounds that reach the headspace and the fiber surface, but the adsorption process of SPME is generally an exothermic reaction, so increasing temperature will reduce the absorption capacity of the fiber coating. It is therefore best to keep the extraction medium at a higher temperature, while the extraction fiber surface should maintain at a lower temperature. The different nature of the compounds is also an important consideration for the extraction process. When the conditions for the equilibrium temperature, equilibrium time, extraction time, and sample weight are set at 80°C, 30 min, 30 min, and 20 g respectively, extraction temperatures of 50, 60, 70, 80, and 90°C were selected to explore the impact of extraction temperature. The results showed that the optimal extraction temperature is 80°C (Fig. 1).
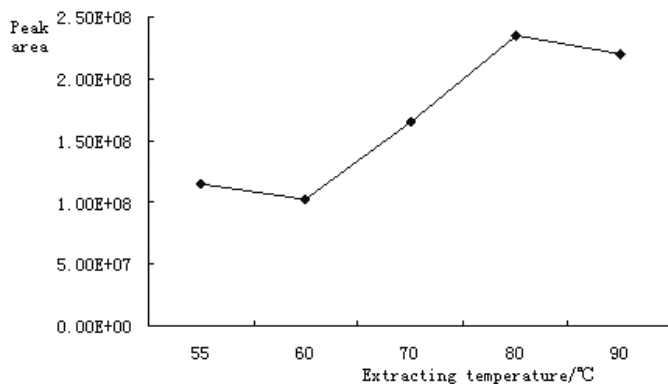
Figure 1   Effect of different extraction temperatures on total peak area.

3.3. Selection of equilibrium time

The gas phase partial pressure of each component is not only affected by temperature, but also has a close relationship to the equilibrium time. When the conditions for equilibrium temperature, extraction time, extraction temperature and sample weight were set at 80°C, 30 min, 80°C, and 20 g respectively, the equilibrium times of 10, 25, 40, 60, and 75 min were selected for testing. The results showed that 60 min was the optimal equilibrium time (Fig. 2).
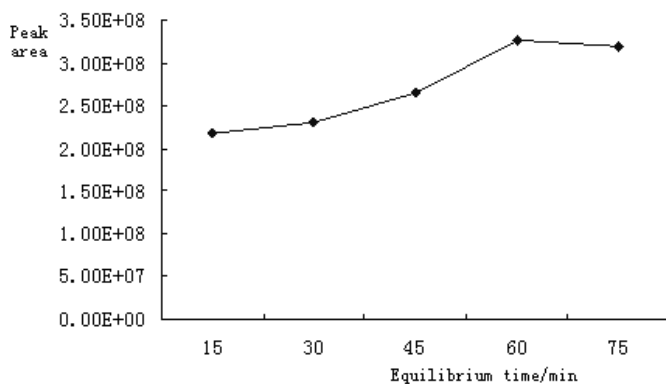
Figure 2   Effect of different equilibrium times on the peak area.

3.4. Selection of extracting time

When conditions of equilibrium temperature, equilibrium time, extraction time, extracting temperature, and sample weight were 80°C, 30 min, 80°C, and 20 g respectively, extraction times of 10, 25, 40, 55, and 70 min were selected to test the effect of extraction time on the total peak area. The total peak area grows as extraction time increases (Fig. 3) and is maximal at 55 min. It then tends to balance and the impurity peaks increase rapidly after 55 min. In order to avoid impurity peaks, 50 min was adopted as the optimal extraction time.
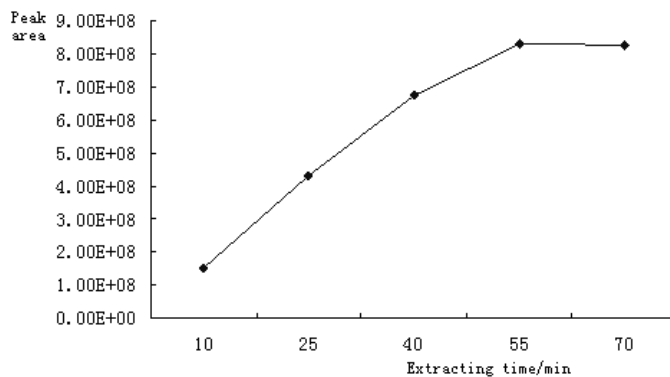
Figure 3   Effect of different extraction times on total peak area.

3.5. Choice of sample amount

When conditions for equilibrium temperature, equilibrium time, extraction time, extraction temperature were set at 80°C, 30 min, 30 min, and 80°C respectively, the sample weights of 10, 15, 20, 25, and 30 g were selected to test how sample weight affected total peak area. A sample weight of 20 g gave the greatest peak area (Fig. 4) and so was adopted as the optimal sample size.
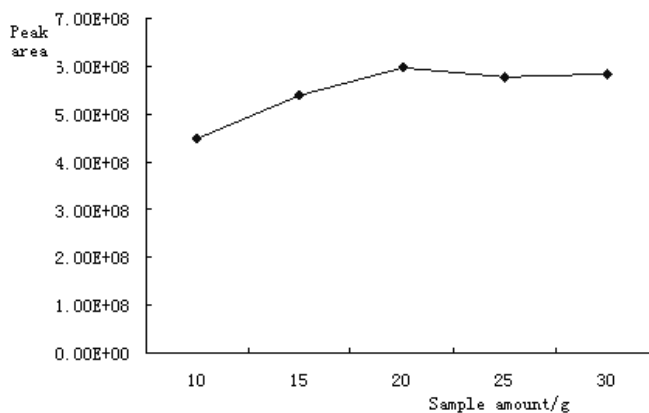
Figure 4   Effect of different sample amounts on total peak area.

3.6. Precision test

Using the optimum conditions for solid-phase micro-extraction, the same sample was analysed five times. The relative standard deviation of the total peak height was 2.54%, and the relative standard deviation of the total peak area was 4.31% (Tab. 1). This shows that the method has good precision.

Table 1   Reproducible experimental analysis results of volatile components in rice.

Test times	The total peak height	The total peak area
1	236714734	711688705
2	232831897	711763421
3	229953884	689801699
4	242762815	745467910
5	243723859	663439100
mean	237197438	704432167
SD	6027595	30351355
RSD (%)	2.54	4.31

3.7. The GC-MS total ion peaks map of volatile components in rice

Solid-phase micro-extraction can fully extract the volatile components in rice which can then be well separated (Fig. 5 and 6). This shows that solid-phase micro-extraction is a feasible pre-processing technology for the volatile components in rice.
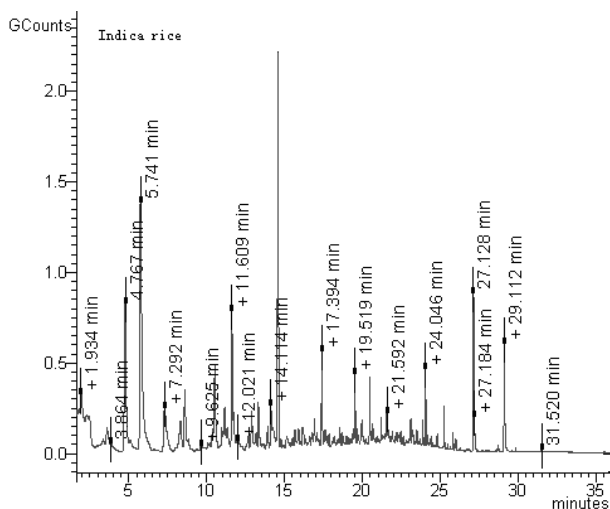
Figure 5   The GC-MS total ion peaks map of volatile components in indica rice.
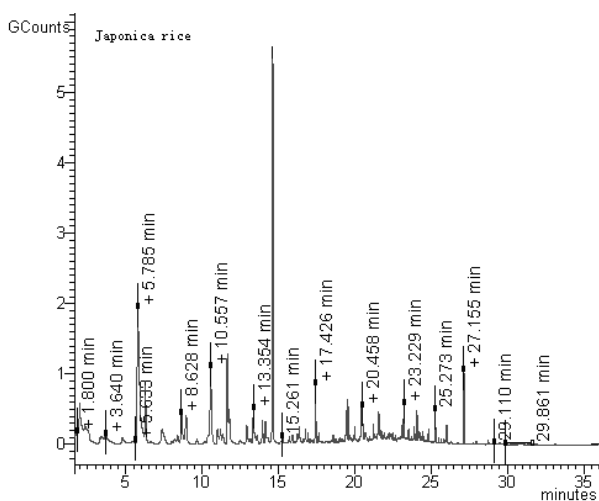
Figure 6   The GC-MS total ion peaks map of volatile components in japonica rice.

3.8. The overall composition of volatile components in rice

Rice contains alcohols, aldehydes, ketones, esters, hydrocarbons, acids, and heterocyclic volatile compounds (Fig. 7). Hydrocarbons compounds were the most numerous, followed by aldehydes, alcohols and ketones, organic acids, esters and heterocyclic components. Aldehydes and hydrocarbons make up the highest proportion of volatiles compounds by weight in rice followed by alcohols, ketones and heterocyclic (Fig. 8). In indica and japonica rice the total aldehyde content was very similar, 32.22% and 33.47% respectively, with hydrocarbon content only a little less so at 24.34% and 29.15%. In the case of total alcohol indica rice at 9.1% was almost double japonica at 5.6%.
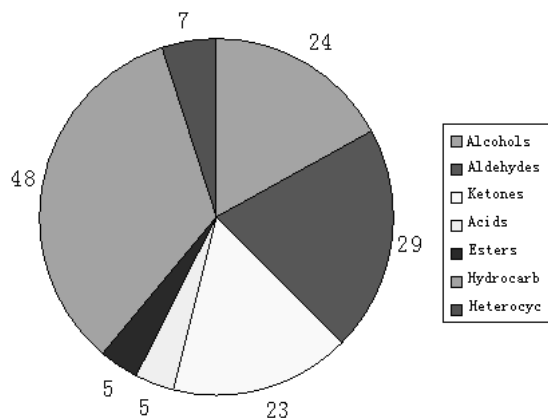
Figure 7   The numbers of various volatile compounds in rice.
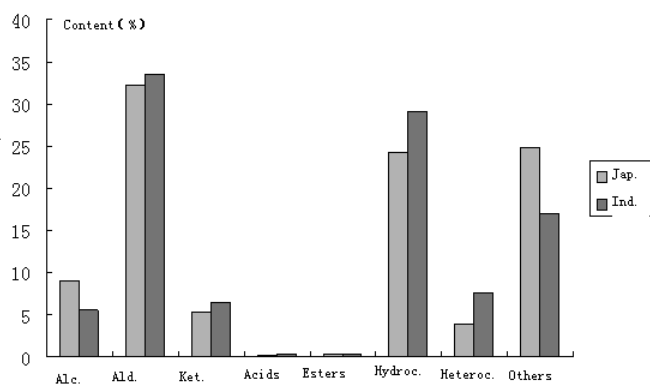
Figure 8   The proportion of various volatile components in rice.

3.9. Volatile alcohols

There were a total of 22 different volatile alcoholic compounds detected in the rice, of which there were 19 in indica and 13 in japonica (Tab. 2). The most abundant volatile alcohols in indica rice were n-hexyl alcohol, n-octanol and 2-hexyl-1-octanol, while in japonica they were n-octanol, 2- hexyl-1-octanol, and 3,7,11-trimethyl-1-12 alcohol.

Table 2   The different volatile alcohol compounds in rice and the relative content (%).

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
1	n-hexanol	3.35	0.17	—	—
2	n-heptanol	0.26	0.15	0.12	0.34
3	n-octanol	1.24	0.40	0.30	1.53
4	2-hexyl-1-octanol	1.14	0.67	0.09	1.01
5	1-octene-3-ol	0.58	0.32	0.17	—
6	2-ethyl-1-decanol	—	1.43	2.35	—
7	2-butyl-1-octanol	0.78	—	—	—
8	n-nonanol	0.46	—	—	—
9	2-ethyl-1-decanol	0.59	—	—	—
10	2-methyl- Undecanol	0.74	—	—	—
11	n-Dodecanol	0.20	0.15	—	0.33
12	3,7,11-trimethyl-1- Dodecanol	0.01	1.65	2.09	0.07
13	tetradecanol	—	—	—	0.04

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
14	6,10,13-trimethyl-tetradecanol	0.29	—	—	—
15	10-pentadecene-1-ol	—	—	0.268	0.48
16	n-hexadecane	0.76	0.69		0.12
17	3,7,11,15 -tetramethyl-2-hexadecimalen-1-ol	—	1.23	0.79	—
18	n-heptadecanol	0.13	—	—	—
19	10-heptadec en-1-ol	—	0.11	—	—
20	n-eicosanol	0.21	—	—	—
21	pentacosene	—	—	1.12	—
22	phytol	0.50	—	—	—

3.10. Volatile aldehydes

A total of 28 different volatile aldehydes were detected of which there were 18 in indica and 23 in japonica (Tab. 3). Both indica and japonica contained pentanal, hexanal, heptanal, 2-heptene aldehyde, octanal, nonanal, decyl aldehyde and benzene formaldehyde. The most abundant aldehyde was hexanal, which on average accounted for 13.31% of the aldehydes (averaging 14.69% for indica and 1.93% for japonica), followed by nonanal which accounted for an average of 7.93%. Pentanal, hexanal, heptanal, octanal, nonanal, decyl aldehyde, and benzene formaldehyde were present at relatively high levels.

Table 3   The different volatile aldehyde compounds in rice and the relative content (%).

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
1	3-methyl-2- butyraldehyde	—	—	—	0.11
2	pentanal	0.33	0.29	0.32	1.45
3	2,4-pentadiene aldehyde	0.02	—	—	—
4	hexanal	13.5	15.9	9.27	14.59
5	2-heptene aldehyde	0.095	—	0.07	0.16
6	2,4-hexadiene aldehyde	—	—	—	0.204
7	2-ethyl -2-hexenal	—	0.44	—	—
8	heptanal	3.12	0.79	0.93	2.33
9	2-heptenal	0.65	0.48	0.42	0.92
10	octanal	4.0	1.13	1.18	5.09
11	2-octenal	0.63	0.51	0.49	—
12	2-butyl-2-Octenal	—	1.48	1.03	—
13	2,3,7-trimethyl-Octanal	—	0.53	—	—
14	nonanal	10.0	2.53	2.96	16.25
15	nonnenal	0.52	—	—	0.51
16	2,4-nonadienal	—	—	—	0.17
17	decyl aldehyde	2.46	0.53	0.47	2.18
18	2-decenal	—	—	—	1.07
19	undecane	0.46		0.11	0.47
20	citral	—	—	—	0.05
21	dodecanal	—	—	—	0.19
22	2,4-diene dodecanal	—	—	0.11	0.13
23	tridecanal	0.26	—	—	—
24	tetradecanal	0.26	—	—	0.23
25	hexadecanal	0.16		0.12	0.52
26	benzene formaldehyde	1.49	1.0	1.41	1.24
27	4-methyl benzene formaldehyde	—	—	0.04	0.07
28	benzene acetaldehyde	—	—	0.09	—

3.11. Volatile ketones

A total of 23 different volatile ketones were detected of which there were 19 in indica and 13 in japonica rice (Tab. 4). Even within the two rice varieties, there was considerable variation in the types of ketone detected. The ketones content was much lower than the aldehyde content.

Table 4 The different volatile ketone compounds in rice and the relative content (%).

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
1	acetone	0.97	—	—	—
2	4-hydroxy-4-methyl-2-pentanone	0.01	—	—	—
3	2-heptanone	—	0.95	—	—
4	6-methyl-2-heptanone	0.16	0.48	—	—
5	heptene-dione	—	0.286	—	—
6	6-methyl-5-ene-2-heptanone	1.28	—	—	—
7	3,5-heptadiene-2-one	0.21	0.15	—	—
8	2-octanone	—	0.35	0.91	0.71
9	2,3- octandione	—	—	0.32	0.88
10	3-octene-2-one	—	0.46	0.45	—
11	3-octdiene-2-one	—	—	—	0.06
12	6-methyl-5-heptanone	—	—	0.691	1.83
13	5-ethyl-6-methyl-2- heptanone	—	—	0.08	0.39
14	6-methyl-3,5-heptadiene-2-one	—	—	0.39	0.76
15	2-nonanone	—	0.169	—	—
16	3-nonene-2-one	—	—	0.25	—
17	2-undecanone	—	0.32	0.24	—
18	6,10 -dimethyl-2-undecanone	0.67	—	0.51	0.72
19	6,10-dimethyl-5,9-undecandione	0.291	—	—	—
20	2-dodecanone	—	0.56	0.55	0.12
21	2-tridecanone	—	—	0.17	0.19
22	2-pentadecanone	0.26	—	—	0.11
23	6,10,14-trimethyl-2-pentadecanone	2.60	0.41	0.79	1.99

3.12. Volatile hydrocarbons

A total of 48 volatile hydrocarbons volatile were detected in the rice (Tab. 5). Both indica and japonica contained 2,6,10-trimethyl-12 alkane, pentadecane, 2,6,10-trimethyl-15alkane, hexadecane, heptadecane, 19 and 20 alkyl alkanes all of which were relatively abundant, averaging 1.39%, 1.75%, 3.14%, 2.60%, 2.52%, 1.62% and 3.52%, respectively.

Table 5 The different volatile hydrocarbon compounds in rice and the relative content (%).

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
1	propane	—	—	—	0.02
2	3,5-dimethyl-octane	0.01	—	—	—
3	3-ethyl-2-methyl-heptane	1.12	—	—	—
4	nonane	—	—	—	0.25
5	decane	—	—	—	0.48
6	2,6-dimethyl-decane	0.70	—	—	—
7	2,4,6-trimethyl-decane	—	—	0.17	—
8	undecane	1.70	—	0.04	0.06
9	2,5-dimethyl-undecane	0.17	0.05	0.05	—
10	dodecane	—	0.53	0.18	0.11
11	2-methyl-dodecane	0.15	—	—	—
12	2,6,10-trimethyl- dodecane	0.42	3.45	1.45	0.25
13	tridecane	0.67	—	—	1.59
14	5-methyl-tridecane	0.22	0.22	0.18	—
15	tetradecane	—	1.544	—	—
16	2,6,10-trimethyl-tetradecane	—	—	0.56	—
17	pentadecane	1.72	0.06	2.96	2.28
18	5-methyl-pentadecane	—	—	0.591	—
19	2,6,10-trimethyl-pentadecane	1.48	3.03	7.82	0.25
20	hexadecane	1.07	3.43	4.24	1.69
21	3-methyl-hexadecane	—	—	0.90	—
22	2,6,10,14-tetramethyl-hexadecane	—	—	1.72	—
23	heptadecane	3.17	0.92	5.72	0.26
24	2,6-dimethyl-heptadecane	0.19	1.22	0.28	—
25	2,6,10,14-tetramethyl-heptadecane	—	1.47	1.55	—

No.	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
26	octadecane	—	3.99	—	0.58
27	nondecane	0.45	3.74	1.83	0.46
28	eicosane	0.27	3.31	10.0	0.47
29	docosane	0.19	—	—	—
30	tricosane	—	0.97	—	—
31	tetracosane	—	3.22	2.13	—
32	3,5,23-trimethyl-tetracosane	—	—	2.09	—
33	hexacosane	—	0.46	—	0.25
34	heptacosane	—	—	0.59	—
35	octacosane	0.29	—	—	—
36	triacontane	0.41	0.36	0.61	
37	dotriacontane	0.15	0.20	—	—
38	17-hexadecyl-tetratriacontane	—	—	0.75	—
39	hexatriacontane	0.22	0.59		
40	1,1-dimethyl-2-octyl-cyclobutane	0.12	—	—	—
41	alkyl-cyclopentane	—	—	—	0.09
42	nonyl-cyclohexane	—	0.34	—	0.17
43	undecyl-cyclohexane	—	—	1.21	—
44	pentadecyl-cyclohexane	0.13	1.49	—	—
45	heptadecyl-cyclohexane	—	—	0.46	—
46	toluene	—	—	—	0.68
47	1,2,3-trimethyl-benzene	—	—	0.02	—
48	1-ethyl-2-methyl-benzene	0.16	—	—	0.26

3.13. Other volatile compounds

Five organic acids, five esters, and seven heterocyclic compounds were detected (Tab. 6). There were the big differences in the organic acids and esters of the two rice varieties. The heterocyclic components in both indica and japonica contain 2-pentyl-furan and methyl-naphthalene, with average contents of 3.37% and 0.80% respectively.

Table 6 The other volatile components in rice and the relative content (%).

N	Compounds	Indica rice 1	Indica rice 2	Japonica rice 1	Japonica rice 2
	Organic acids				
1	acetic acid	—	0.02	0.19	0.14
2	propyl acid	0.13	—	—	—
3	2,3-dihydroxy-succinic acid	—	—	0.04	—
4	3,7-dimethyl-hexanoic acid	—	—	—	0.084
5	palmitate	0.09		0.09	0.12
	Esters				
1	formic acid hexate	—	—	0.35	—
2	hexanoic acid dodecate	—	—	0.24	—
3	phenyl acetic acid-4-tridecate	—	0.05	—	—
4	acetic acid tetradecate	—	—	—	0.19
5	oxalic acid-cyclohexyl methyl nonate	—	0.56	—	—
	Heterocyclic Compounds				
1	2-methyl-furan	—	—	—	0.74
2	2-pentyl - furan	3.31	2.63	2.15	5.41
3	2-methyl-5-isopropyl-furan	0.24	—	0.10	0.28
4	2,3-dihydrobenzofuran	—	—	—	1.53
5	methyl-naphthalene	0.30	1.22	1.40	0.28
6	methoxy-phenyl-oxime	—	—	0.94	2.38
7	indole	—	—	—	0.06

4. Conclusions

Headspace solid phase micro-extraction, together with gas chromatography and mass spectrography, were used to study for volatile compounds in rice. The optimal conditions for extraction were a 20 g sample heated for 30 min at 80°C prior to headspace absorption, and then extracted for 30 min with HS-SPME.

The volatile compounds in rice are alcohols, aldehydes, ketones, esters, hydrocarbons, organic acids, as well as heterocyclic compounds. Aldehydes were the main volatile compounds. Hexanal is the most abundant, averaging 13.31%; followed by nonanal, averaging 7.93%.

References

Arthur, C. L., Paw, L., 1990. Solid phase with thermal desorption using fused silica optical fibers. Analytical Chemistry 62, 2145-2148.

Ling, J., 1988. Volatile carbonyl compounds (VCC) of grain, analysis and determination. Journal of Grain Storage 1, 20-25.

Long, Q., 2004. Rice knowledge and overview. Journal of Chinese Rice 3, 44-47.

Ma, J., Wang, H., Guan, Y., 2002. New technology of solid-phase micro-extraction. Journal of Chromatography 1, 16-20.

Maga, J.A., 1984. Rice product volatiles: A Review. Journal of Agriculture and Food Chemistry 32, 964-970.

Zhou X., Zhang Y., Zhao Q., Zhou Z., Bian K., Zhong L., 2005. Changes of volatile substances in rice during storage and its relationship with the fresh degree. Journal of the Food and Feed Industry 2, 1-3.