Characterization and Assessment of Flavor Compounds and Some Allergens in Three Iranian Rice Cultivars during Gelatinization Process by HS-SPME/GC-MS

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Abstract: A combined gas chromatography mass spectrometry with headspace solid-phase microextraction method has been utilized for the analysis of the flavor volatiles of three different rice cultivars including two modified Iranian rice cultivars and Hashemi rice cultivar during gelatinization. As a result, while gelatinization would progress, the amount of the volatile compounds would be also increased. Altogether, 74, 55 and 66 components were identified for Hashemi, HD5 and HD6 rice samples, respectively, which 56 unique compounds were not identified, previously. Subsequently, seven fragrance chemicals have been detected, which were most frequently reported as contact allergens in the European Union.

Keywords: Rice (*Oryza sativa* L.), Flavor Volatiles Components, Solid-Phase Microextraction (SPME), Gas Chromatography Mass Spectrometry (GC/MS), Contact Allergens.

Introduction

Rice is the major product of people's diet in many countries and also one of the most momentous factors in market business is the aroma of 'fragrant rices'. This is a feature which discriminates it from regular rice^{1,2}.

There are numerous volatile components in cooked rice grains, as well as those which are due to biochemically distinct pathways, and are very rich sources of hydrocarbons, organic acids, alcohols, aldehydes, ketones, esters and phenols. On the other hand, there are some other compounds which are extracted as a result of chemical breakdowns, which fatty acids are good sample among them³.

Aromatic can have both positive and negative influence on people's tastes. The former can be flavor and fragrance components such as 2-acetyl-1-pyrroline (2AP) and the aromatic alcohols and the latter can be off-flavors of hexanal and 2-pentylfuran⁴. 2AP is one of the

aromatic compounds, which recognized as the most imperative key flavor component of rice aroma⁵⁻⁸.

In order to afford sample preparation methods and analytical techniques, Solid-phase microextraction (SPME) is introduced as a rapid, sensitive and consistent technique for the extraction and concentration of volatile compounds from different sample matrices. This technique has many advantages as well as less time for extraction, effortlessness, preventing the loss of analytes and field sampling with portable field sampler^{9,10}.

This technique has a enormous sensitivity mostly in the study of identifying flavor indicators of rice, Since the extracted fraction on the fiber is statistically introduced into the gas chromatography (GC) by thermal desorption^{11,12}. In present study, our main intention is to use HS-SPME-GC-MS technique as a reliable method for effective trapping and screening of a broad range volatile flavor compounds in the headspace of Iranian rice samples during the gelatinization process.

Experimental

Three Iranian fragrant rice samples were employed in this study, including two new modified varieties (HD5, HD6) and one Hashemi variety (HD1), which are consumed in Iran, predominantly. These samples were collected from Rice Research Institute. All samples were harvested in August 2009 and contained 22% moisture. Following 24 h from harvesting, the samples were sun-dried to about 12-13% moisture content, after that they were dehulled at the growing area and were transferred to the laboratory and stored in nylon bags and located in the refrigerator at 4 °C until the experiments were accomplished.

Gelatinization process

Firstly, some glass balls were poured into a glass of 275 mL water and were placed under primary heating till they reach to the boiling point. Afterward, 50 g of one of the tested samples was added into the boiling water and stirred. After 7 min, 10 rice grains were chosen randomly and placed on a glass plate with equal distance. Subsequently, the samples were covered with another plate, slip it till gelatinized grains observed and then the number of gelatinized rice grains were counted, accurately. Following 8 min, the experiment were carried out every one minute to complete the gelatinization all of the grains. This method was used for all samples in this study, which was performed for the first time.

Rice cooking

The conventional Iranian rice cooking was utilized, by which a mixture of 150 g of white rice and 400 mL of distilled water were added. The whole process was divided into 4 steps, including all the stages from the beginning till the end of gelatinization process. The cooking time of this process was determined by the temperature at which melting of crystalline structure took place. Rice with high GT requires more time to cook, while rice with low GT, requires less time; usually up to 4 min. The earlier has unacceptable texture².

Headspace solid-phase microextraction sampling

Process of gelatinization was accomplished Using SPME, a sampling device was designed in order to collect the sample of flavor volatiles. The flavor volatiles of rice during gelatinization were released out throughout the first side arm, while SPME fiber was located in the second side of sampling device with a flexible septum. In all stages of gelatinization, the fiber was located in the manually operated SPME holder and the septum was covered with a Teflon-coated silicone in order to prevent volatile components release in septum. The fiber was lowered in the sampling port to adsorb flavor volatile components of rice and desorbed them thermally in the injection port of GC-MS instrument for 10 min at 250 °C.

Afterwards, the fiber was exposed to the SPME fiber conditioner at 250 °C for 1hr for reconditioning before being subjected to the next volatile samples. SPME fibers with PDMS (100 µm, non-bonded), CAR/PDMS (75 µm, bonded) and DVB/CAR/PDMS (65 µm, bonded) coating, provided by Supelco (Bellefonte, PA, USA) were applied as commercial fibers and were preconditioned in an SPME fiber conditioner (GL Sciences) at 250 °C for 1 h before the first measurement.

Gas chromatography-mass spectrometry

GC separation was performed on an HP-6890 GC system (HEWLETT PACKED, USA), coupled to a mass detector (HP-5973, USA). A column, HP-5MS (5% Phenyl dimethyl siloxan) was applied using a 30 m x 0.25 mm x 0.32 µm film thickness. Analytes extracted onto the commercial fiber, were injected using 9.5mm Thermogreen LB-2 septa (Supelco). The injector's temperature was 250°C in this case. Helium purified at 99.99% was selected as a carrier gas at a flow rate of 1 mL/min. The temperature program was performed at 60°C for 3 min, then increased at 220°C at rate of 5°C/min and maintained at 220 °C. The mass selective detector was applied in an electron impact ionization mode at 70 ev.

An alkane mixture with C8-C20 alkane and concentration of 40 mg/mL in hexane was purchased from Fluka. The mixture was used to approximate retention indices (RI), which was injected into the fiber by 5min headspace extraction from a 10 mL SPME vial, including 1 mL HPLC-grade water spiked with 10 µL of the mixture. The volatile components were positively identified by matching their mass spectra, with the spectra of reference compounds in Adams mass spectra library (9th edition) and verified on the basis of mass spectra and RI values reported in the literature^{13,14}.

Results and Discussion

Cooking time

The cooking time of rice is determined by the temperature at which the crystalline structure of the starch begins to melt. This is called gelatinization temperature (GT). In rice samples, GT ranges from 55-85 °C. Hashemi rice sample with high GT requires more time to cook. Lowering the GT of the modified (HD5, HD6) rice grains could decrease average cooking time.

In Figures 1, 2 and 3, gelatinization time for 0%, 50%, 90% and 100% of the whole grains is recognized and during this time, SPME fiber is injected. The whole process was separated into four stages: I, 17':00; II, 21':00; III, 24':90"; IV, 27':30 were assigned to Hashemi rice sample leading to gelatinization 0, 50, 90, and 100 percent of rice grains. For HD5 variety at cooking stages: I, 15':00; II, 18':42"; III, 20':69"; IV, 22':27" leading to gelatinization 0, 50, 90 and 100 percent of rice grains. In HD6 variety at cooking stages: I, 15':00; II, 18':38"; III, 21':67"; IV, 24':30 leading to gelatinization 0, 50, 90 and 100 percent of rice grains.

Figure 1. Gelatinization profile of Hashemi rice samples.

Figure 2. Gelatinization profile of HD5 rice samples.

Figure 3. Gelatinization profile of HD6 rice samples.

Flavor volatiles in the three Iranian rice cultivars

Using headspace SPME method, flavor volatiles in the three Iranian rices were extracted during gelatinization and analyzed by GC-MS. These compounds were determined by comparing their mass spectra and RI values with authentic compound, whereas others were identified by their corresponding mass spectra (Adams mass libraries) and RI values, when RI values on the HP-5MS capillary column were available in the literature^{13,14}. A whole range of 74, 55 and 66 components were identified for Hashemi, HD5 and HD6, respectively. For HD5, 23, 11, 23 and 36 specific compounds were identified at stages I, II, III and IV, respectively. And regarding HD6, 14, 19, 47 and 32 specific compounds were identified at the same stages as HD5 and 14, 42, 37 and 43 particular compounds for Hashemi.

In general, the volatile compounds in the three Iranian rice samples during gelatinization, belong to the chemical classes of aldehydes, ketones, hydrocarbons, esters and phenols, *etc.* these chemical classes corresponding with those previously mentioned in non- Iranian rice using various methods of extraction.

Table 1, 2 and 3 show all compounds detected in the collected rice samples. Those compounds which are listed in Table 1, 2 and 3 were mentioned before in rice. However, 56 new unique compounds were detected (Docosane, 2-Methyl-undecanal, Phthalic acid, Tetracosane, 1,3-dimethoxy-Benzene, Anizole, Pentacosane, Heneicosane, Tricosane, Octane, Octyl formate, 2,6- Dimethoxy-phenol, Neryl acetone, Tetradecanal, 1-Methoxynaphthalene, 2-acetylnaphthalene, Naphthalene, 1-Hexadecene, ar-Turmerone, 2-Ethylhexyl-salicylate, 1,3-Dimethoxy-benzene, Biphenyl, Isopropyl tetradecanoate, <2Z,6E>Dodecadiene-1-al, alpha-Pinene, Camphene, <6-isopropyl>Quinoline, <2-acetyl>Furan, 1,2-Benzenedicarboxylic acid, n- Octyl- 1- iodo- Octane, 2- butyl-2- Octenal, 2, 6, 10, 14- tetramethyl- Hexadecane, 2-hydroxy, 2-methyl- Benzoic acid, beta.Bisabolene, 2-Methyl-naphthalene, Cyclosativene, 1,4-Dimethylnaphthalene, Methyl isoeugenol, Diethylphthalate, Isobutyl salicylate, Turmerone, <6-methyl->Heptan-2-ol, 1,3-Diethyl-benzene, 1,2,4-Trimethyl benzene, lilia, 2,3,6-Trimethyl-naphthalene, 1- ethyl- Naphthalene, 3-methyl-Tetradecane, 3-methyl-Pentadecane, 2,6,10,14-tetramethyl-Pentadecane, 1,3- dimethyl- Naphthalene, 2- phenyl- 2- methyl-Aziridine, 2- methyl- Tridecane, 4-ethyl- 3,4- dimethyl- Cyclohexanone, n- Octyl-Cyclohexane, 9- methyl- Nonadecane), which were not reported in the previous studies^{15,16}.

Table 1. Flavor volatiles identified in headspace of Hashemi rice cultivar.

NO	Compound	Odor Description	KI in experiment	KI in literature	I, %	II, %	III, %	IV, %
1	N-Hexanal	Green, grass-like	805	802	31.2	3.8	7.4	12.4
2	2-Butylfuran		881	890	4.3			
3	2- Phenyl- 2-methyl- Aziridine		888					0.6
4	2-Acetyl-1-pyrroline		915	920			3.7	5.8
5	Benzaldehyde	Nutty, bitter	960	960		2.1		1.3
6	<6-Methyl->Heptan-2-ol		960	965		1.5		

Condt...

7	1-Octen-3-ol	Raw mushroom	982	979				0.9
8	2-Pentylfuran	Nutty, bean	998	990		1.2	1.6	3.2
9	1,2,4-Trimethyl benzene		999	994		0.8		0.3
10	Octanal	Green, citrus-like	1007	999	2.1	0.9	1.6	3.5
11	D- Limonene		1042	1029				1.3
12	1-Nitro-hexane		1051			2.0	2.3	2.4
13	1,3-Diethyl-benzene		1065	1059		1.2		
14	N-Octanol		1072	1068				1.8
15	N-Nonanal	Soapy, citrus-like	1110	1101	5.9	6.0	7.7	10.4
16	(E)-2-Nonenal	Fatty, tallowy	1166	1162			1.2	0.6
17	Naphthalene		1173	1181		2.2		
18	2,6-Dimethylaniline		1179	1168		0.5		
19	Dodecane		1201	1200			1.4	1.6
20	Decanal	Green, soapy	1207	1207			2.7	2.4
21	(E,E)-2,4-Nonadienal	Fatty, waxy	1207	1212		1.8		
22	(E,E)-2,4-Octadienal		1223				0.8	1.5
23	(E)-2-Decenal	waxy	1270	1264		5.9		2.0
24	(E,Z)-2,4-Decadienal	Fatty, green	1283	1293		6.8		
25	Indole	Sweet, burnt, floral	1289	1291		0.4		
26	<cis-2-tert-butyl- >Cyclohexanol acetate		1302	1293		1.2	1.4	0.6
27	Tridecane		1303	1300	1.6		3.3	3.4
28	(E,E)-2,4-Decadienal	Fatty, waxy	1310	1317		3.0	3.3	
29	2-Methyl-naphthalene		1324			1.2	1.3	0.3
30	1-Methoxy-naphthalene		1343	1346		1.1		0.7
31	2,6-Dimethoxy-phenol		1355	1349		3.3		
32	2- methyl- Tridecane		1366					0.4
33	Cyclosativene		1371	1383				0.4
34	Undecanol		1372	1370			0.6	1.9
35	2,6,10,14-tetramethyl- Hexadecane		1379				2.5	3.1
36	Tetradecane		1402	1400	3.7	4.8	7.8	6.9
37	Cyclohexanone		1405					0.4
38	(E)-2-Octenal	Green, fatty	1420	1425		1.7	1.5	1.3
39	2,6-Dimethylnaphthalene		1427	1431		2.6	1.0	1.4
40	Isobutyl salicylate		1429	1425	3.1	2.1		
41	1,3- dimethyl- Naphthalene		1434			1.3		
42	2-Pentadecanone		1441		9.2			
43	1,4-Dimethylnaphthalene		1450			4.2	5.1	0.8
44	n- Octyl- Cyclohexane		1459					1.0
45	Isoeugenol		1461	1451				0.5

Condt...

5	2- Acetyl- 1-pyrroline		915	920	1.4			
6	Anizole		917	918	1.8	3.3	3.5	3.9
7	Ethanol	sweet	920	925	0.9			
8	Benzaldehyde	Nutty, bitter	974	960	0.9			
9	Hepten-2-ol<6-methyl-5->		982	992	1.0			
10	2-Pentylfuran	Nutty, bean	985	990	4.0	11.2	3.3	4.3
11	1-Octen-3-ol	Raw mushroom	989	979				2.4
12	1,3,5-trimethyl-Benzene		990	996	0.8	4.8		
13	<i>n</i> -Octanal	Green, citrus-like	993	999	2.6		2.7	4.4
14	<i>n</i> -Decane		1009	1000			1.6	1.7
15	<i>D</i> - Limonene		1022	1029			6.2	
16	Undecane		1091	1100	0.6			
17	<i>n</i> -Nonanal	Soapy, citrus-like	1100	1101	6.1	7.6	8.8	11.7
18	Octyl formate		1140	1131				1.9
19	(<i>E</i>)-2-Nonenal	Fatty, tallowy	1157	1162				1.4
20	1,3-dimethoxy-Benzene		1178	1169	1.2			
21	Dodecane		1200	1200	2.5	1.6	0.5	1.1
22	Tridecane		1297	1300	0.4	3.2	1.7	2.4
23	<i>E</i> -2-Heptenal	Herbaceous	1320	1318				1.4
24	Hexyl furan		1322	1329				2.1
25	2,6-Dimethoxy-phenol		1340	1349				3.5
26	2- Methyl- undecanal		1366	1368				0.9
27	<i>n</i> - Undecanol		1372	1370				0.8
28	N.D.		1379				0.9	
29	N.D.		1379					0.7
31	Tetradecane		1391	1400		4.0	4.1	3.0
32	(<i>E</i>)-2-Octenal	Green,fatty	1432	1425				2.6
33	2,6-Dimethyl-naphthalene		1442	1431				1.3
34	Geranyl acetone	Magnolia, green	1463	1455				1.6
35	1-Heptanol	Green,fatty	1466	1457			1.6	
36	N.D.		1469					1.7
37	N.D.		1491		0.4			
38	Pentadecane		1500	1500		3.6	6.3	3.6
39	Butylated hydroxytoluene		1524	1516				1.5
40	1-Octanol	Fruity, floral	1560	1565	0.6		1.0	
41	Hexadecane		1600	1600		2.2	4.8	2.1
42	1-Nonanol	Floral, citrus-like	1670	1671				1.1
43	<i>n</i> -Tetradecanol		1670	1673				1.0
44	Heptadecane		1700	1700			0.6	1.0
45	Phthalic acid		1703		3.4			
46	Farnesol		1710	1718	2.2			
47	1-Octadecene		1799	1790				0.6
48	<i>n</i> -Nonadecane		1905	1900				0.7

Condt...

49	n-Nonadecane	1907	1900			4.1	
50	n-Eicosane	1995	2000	0.3		2.0	
51	n-Heneicosane	2101	2100		1.9	0.7	1.2
52	Docosane	2209	2200	0.5		0.7	3.5
53	n-Tricosane	2291	2300			2.0	
54	Tetracosane	2411	2400	0.6		2.2	0.6
55	Pentacosane	2510	2500			1.8	1.3
Total				97.0	100.0	99.9	99.3

N.D. = not detected.

Table 3. Flavor volatiles identified in the headspace of HD6 rice cultivar.

NO	Compound	Odor Description	KI in experiment	KI in literature	I, %	II, %	III, %	IV, %
1	Pentanal	Woody, fruity	710	706			1.5	
2	Octane		801	800			1.4	
3	N-Hexanal	Green, grass-like	802	802	49.0	42.2	14.2	36.3
4	N-Heptanal	Fruity, fatty	900	902	3.0	2.2		2.2
5	<2-Acetyl>Furan		911	913				1.8
6	Anizole		919	918	5.3	7.1	5.2	2.5
7	Alpha-Pinene		935	939				1.5
8	Camphene		951	954				1.1
9	Benzaldehyde	Nutty, bitter	960	960			2.0	1.2
10	2-Pentylfuran	Nutty, bean	981	990	6.6	4.4	3.8	5.3
11	1-Octen-3-ol	Raw mushroom	982	979	1.9	2.0	2.2	1.3
12	Octanal	Green, citrus-like	990	999		4.7	2.3	
13	1,3,5-trimethyl-Benzene		992	996	4.0			2.8
14	D- Limonene		1020	1029				2.6
15	Undecane		1091	1100			2.7	
16	N-Nonanal	Soapy, citrus-like	1095	1101	10.9	17.4	10.0	6.1
17	Octyl formate		1139	1131		1.7		0.9
18	(E)-2-Nonenal	Fatty, tallowy	1152	1162		0.9		0.5
19	1,3-Dimethoxy-benzene		1175	1169			0.4	1.1
20	Naphthalene		1187	1181			1.0	
21	Dodecane		1200	1200			0.3	
22	n- Octyl- 1- iodo-Octane		1250			2.6		
23	(E)-2-Decenal	waxy	1260	1264			1.2	
24	(E,Z)-2,4-Decadienal	Fatty, green	1299	1293			1.3	
25	Tridecane		1300	1300	1.4	1.1	1.4	0.9
26	Undecanal	Fresh, lemon-like	1312	1307		0.6	0.3	
27	(E)-2-Heptenal	Herbaceous	1316	1318			0.8	0.7
28	2,6-Dimethoxyphenol		1357	1349	0.9	1.8	4.6	
29	2- Butyl-2- Octenal		1383			0.7	1.0	
30	Biphenyl		1385	1377			0.6	
31	Tetradecane		1400	1400	2.2	2.3	2.1	1.6
32	2, 6, 10, 14-tetramethyl-Hexadecane		1421					3.0
33	(E)-2-Octenal	Green, fatty	1433	1425		1.4	1.7	1.7

Condt...

34	2,6-Dimethylnaphthalene		1437	1431		1.2		
35	1-Methoxy-naphthalene		1440	1446		1.2		
36	Neryl acetone		1441	1436	2.1	1.0	1.0	
37	2-hydroxy, 2-methyl- Benzoic acid		1449					2.3
38	<2Z,6E> Dodecadiene-1-al		1455	1447		0.5		
39	2-Pentadecanone		1459	1451		7.5		
40	Geranyl acetone	Magnolia, green	1460	1455	1.4	2.2		
41	1,2- Benzenedi-carboxylic acid		1469		2.4			
42	Pentadecane		1500	1500	2.1	2.6	2.7	1.3
43	<6-isopropyl> Quinoline		1519	1511				0.9
44	N.D.		1539			0.6		
45	N.D.		1553					2.7
46	1-Octanol	Fruity, floral	1570	1565		1.3		
47	N.D.		1573			0.6		
48	N.D.		1595					2.0
49	1-Hexadecene		1599	1590		0.7		
50	Hexadecane		1600	1600	2.1	2.4	4.4	4.0
51	2-acetylnaphthalene		1602	1609		1.6		
52	N.D.		1612			0.8		
53	Tetradecanal		1617	1613		0.6		
54	N.D.		1658			0.7		
55	N.D.		1673			0.6		
56	ar-Turmerone		1675	1669		1.7		
57	1-Nonanol	Floral, citrus-like	1681	1671		0.7		
58	Heptadecane		1700	1700				0.9
59	(E)-2-Undecenal	Fatty, sweet	1759	1750		0.9		
60	Octadecane		1809	1800				3.1
61	2-Ethylhexyl-salicylate		1811	1807		1.8		
62	Tridecanal		1830	1821				0.7
63	Isopropyl tetradecanoate		1839	1830		1.3	2.1	
64	Hexadecanoic acid		2006	1995				2.7
65	2-Methoxy-4-vinylphenol	Spicy, clove-like	2191	2180		1.0		
66	Docosane		2209	2200		1.0		
Total					93	100	92.3	98.7

N.D. = not detected.

Variation in flavor volatiles of rice during four different cooking stages

Significant differences were investigated in the volatiles of rice during the four different gelatinization stages. Two major compounds were detected at stage I for Hashemi, HD5 and HD6 rice cultivars known as nonanal and hexanal. The latter is known as an important lipid oxidation product in rice. However, there are other components which have been identified only at stage I for HD5, such as ethanol, which were apparently lost by steam vaporization at later cooking stages during the cooking process. In contrast, hexadecanoic acid which was

identified as a predominant compound at stage IV for HD6, as well as pentacosane were detected at stage III & IV for HD5.

Primary heating of rice at gelatinization stages I and II resulted in the evaporation of aldehydes in rice, and fatty acids in steam distillation in all cultivars, respectively. But, excess steam and heat have debilitative influence on the extraction of low-boiling-point volatiles. Thus, further heating at stages III and IV, increased the rate of evaporation of a broad range of the flavor volatiles of rice. As anticipated, the amount of key odorant compounds for instance (*E*)-2 nonenal (HD5, HD6 and Hashemi), (*E,Z*)-2, 4-decadienal (HD6 and Hashemi), 2-methoxy-4-vinylphenol (HD6 and Hashemi) and indole (Hashemi) commonly increased, while those of low-boiling point volatiles decreased upon cooking in all cultivars.

Similarities and differences among the three different Iranian rice cultivars

By comparing the volatile components in Table 1, 2 and 3 it might infer the similarities and differences in the three Iranian rice samples. These samples were examined on the basis of the same condition.

There were no significant differences in the profiles of flavor volatiles; nevertheless, less volatile compounds were recognized in two modified rice samples (HD5 & HD6), comparing Hashemi. For instance, some components such as Phthalic acid, Farnesol, Ethanol, alpha-Pinene and Camphene were detected in two modified samples, whereas Hashemi's major compounds were beta.Bisabolene, Cyclosativene, Methyl isoeugenol, Isobutyl salicylate, Turmerone, lilia, <cis-2-tert-butyl->Cyclohexanol acetate, 2-phenyl- 2-methyl- Aziridine, 4-ethyl- 3,4- dimethyl- Cyclohexanone, *n*- Octyl- Cyclohexane, 9-methyl- Nonadecane, 2- methyl- Tridecane, (*E,E*)-2,4-Decadienal and Indole.

A broad range of the flavor volatiles of rice during gelatinization could be extracted and identified in a single headspace SPME/GC-MS, of which Naphthalene compounds and Benzene compounds liberated widely and predominantly from different rice samples, which these components have not been previously reported in rice.

From more than 300 rice volatiles identified by many research groups, 2-acetyl-1-pyrroline (2AP) as a principal aroma compound. We were able to detect 2-acetyl-1-pyrroline in the Hashemi rice sample at gelatinization stage III and IV and HD5 rice sample at gelatinization stage I, although we were unable to detect any 2-acetyl-1-pyrroline in the HD6 modified rice sample.

Aromatic can be directly linked to consumer preference in both positive terms (such as 2AP) and negative terms of which Hexanal and 2-Pentylfuran were detected in the headspace of different rice sample.

In Hashemi cultivar the amount of Hexanal decreased significantly at gelatinization stage II and increased gradually at gelatinization stage III and IV. In contrast, the amount of 2-Pentylfuran increased at gelatinization stage II, III and IV.

In HD5 cultivar the amount of Hexanal decreased with increasing gelatinization. The amount of 2-Pentylfuran increased significantly at gelatinization stage II and decreased at stage III, and then increased slightly at stage IV.

In HD6 cultivar the amount of Hexanal decreased with increasing gelatinization stage I, II, III and then increased significantly at gelatinization stage IV. Similar results were observed for the 2-Pentylfuran.

Optimization of different experimental parameters

In order to optimize the suitable conditions of flavor compounds, hexanal target were investigate. Initially, three types of commercial fibers (PDMS, CAR/PDMS and DVB/CAR/PDMS) were selected in order to extract volatiles from the headspace above the rice samples

at the beginning of the experiment. The latter is known as the most effective compound in extraction of flavor volatiles. The DVB/CAR/PDMS fiber was used in all applications (Figure 4).

In order to estimate the effect of water content of rice samples on the SPME of target analytes, different volumes of water were added to 150 g rice sample and SPME experiments were performed. The results in Figure 1 indicate that increasing water content up to 400 mL was correspondent with the extraction efficiency increase of hexanal. However, addition of >400 mL water showed a great decrease in extraction efficiency, since this would result in producing an adhesive mixture that was not easily agitated and made the diffusion of analytes more difficult. On the other hand, addition of <400 mL water was not enough for the cooking of rice. Therefore, 400 mL water was selected as a suitable amount of water for the rest of experiment (Figure 5).

Figure 4. Comparison of the extraction efficiencies of the PDMS, CAR/PDMS and DVB/CAR/PDMS fibers for volatile compounds in the headspace of rice sample.

Figure 5. Comparison of the extraction efficiencies of the various additions of water.

For the investigation of the effect of equilibrium time on the target analytes, the rice samples were extracted 5-30 min. As Figure 6 shows, with the increase in equilibrium time, the extracted amounts of hexanal increased in to a maximum threshold after 30 min of extraction. As a whole, the optimum equilibrium time was considered as 30 min to reach the equilibrium for hexanal (Figure 6).

Figure 6. Equilibrium time profile.

Identification of contact allergens in rice cultivars

Rice flavor consists of a wide range of volatile compounds. Along with, in this study seven fragrance chemicals were detected, which were most frequently reported as contact allergens. Their names are listed as follows: Lilial (Lilial is a synthetic compound listed as a fragrance allergen), Cyclohexanone, Diethylphthalate, Farnesol (Farnesol is one of the most frequent contact allergens), Isoeugenol (common allergen), Limonene, Alpha-pinene.

Conclusion

This study has demonstrated the application of HS-SPME/GC-MS as a powerful combination to perform a rapid analytical method for the direct profiling of the flavor volatiles in three different rice cultivars including two modified Iranian rice cultivars and Hashemi rice cultivar during gelatinization process. Therefore, a broad range of the flavor volatiles of rice could be extracted, concentrated and identified in the headspace of different rice samples using HS-SPME/GC-MS.

All of free flavor volatiles, the bound flavor components and the compounds formed by the thermal decomposition of the non-volatile constituents existing in rice should be liberated during the gelatinization process.

This is a first study which was accomplished regarding the contact allergens in rice during gelatinization process, which was not previously reported. Further studies should be accomplished to consider future separation and identification on these compounds, as well as comparing these compounds among Iranian, non- Iranian samples and modified samples.

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