Headspace Volatile Compounds of Steamed *Liriopsis* Tuber Tea Affected by Steaming Frequency

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ABSTRACT: Flavor quality of *Liriopsis* tuber tea that was made using a steaming process was studied by measuring changes in headspace volatile compounds. Headspace volatile compounds of the prepared samples were isolated, separated and identified by the combined system of purge & trap, automatic thermal desorber, gas chromatography, and mass selective detector. As steaming frequencies were increased, the area percent of aldehydes decreased from 32.01% to 3.39% at 1 and 9 steaming frequency times, respectively. However, furans and ketones increased from 18.67% to 33.86% and from 9.60% to 17.40% at 1 and 9 times, respectively. The savory flavor of *Liriopsis* tuber tea was due to a decrease in aldehydes contributing a fresh flavor at the 1st steaming process and newly generated furans from nonenzymatic browning with repeated steaming frequencies. These results will provide basic information for quality control of the newly developed *Liriopsis* tuber tea.

Keywords: *Liriopsis* tuber tea, headspace volatile compound, steaming frequency

INTRODUCTION

Liriopsis tuber is a perennial plant of Liliaceae, which is similar to barley in roots and to glutinous millet in leaves. It is used as ornamental plants due to its beautiful flowers and as medicinal resources for roots. Traditional uses of *Liriopsis* tuber include calming cough, removing phlegm, bringing stamina, improving diuresis, stopping thirst and others in oriental medicine (1). Based on these traditional uses, it has been studied and reported as blood sugar depressing, antidiabetes, antiinflammation, immunodulatory, liver protection, anticancer, and brain cell protection/enhancement (1,2). However, there are few studies about use of *Liriopsis* tuber in food like tea.

Tea originated from China and later distributed to Korea and Japan. Nowadays, it is used worldwide. Tea is sometimes made using a steaming process. The steaming process prevents destruction of active components and makes new compounds by changing components via the indirect thermal treatment (3). Particularly, “Gujeung-Gupo” is the traditional steaming process that passes through a steaming and drying process 9 times. This is usually used for the processing of medicinal herbs such as red ginseng (4,5) and *Rehmannia glutinosa* (6). The

reason for short steaming hours and fewer steaming times is to minimize loss of effective components that occur during long time steaming. Also, another result of steaming time and frequency is to increase Maillard reaction products by the repetitive process of steaming and drying (4). However, there are few papers on flavor quality of *Liriopsis* tuber tea according to steaming process.

Flavor quality of tea is different depending on aromatic compounds in its final product. Aromatic compounds of green tea are numerous and volatile compounds of a complex flavor such as tea are affected by the process method (7). Comparison of aromatic compounds between green tea and roasted green tea showed 10 species of volatile compounds such as leaf alcohol, and leaf aldehyde are decreased, but species of ester, ketone, and nitrogenous compounds are increased. Delicate flavor compounds were identified from Maillard reaction products that are caused by heat treatment (7). Also, *Liriopsis* tuber composition is reported by several studies to change in general components and physicochemical characters after heat treatment methods like steaming or roasting (1,8,9). However, reports lack analysis data of volatile aromatic compounds. Therefore, changes of volatile compounds of *Liriopsis* tuber according to steaming frequency were investigated.

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MATERIALS AND METHODS

Preparation of steamed *Liriopsis* tuber powder

Liriopsis tuber harvested in the early spring of 2010 was purchased in Miryang, Korea. Tuber was kept at -40°C before sample treatment. *Liriopsis* tuber sample was made using different steaming conditions. Dried *Liriopsis* tuber (200 g) was steamed with 150 mL distilled water at 99°C for 3 h and dried at 70°C for 21 h using hot air. This process was repeated 1, 3, 5, 7, and 9 times. Then, samples were dried at 70°C for 51 h using hot air. Samples were then freeze-dried for 48 h. The dried samples were crushed and filtered using a 50 mesh sieve.

Adsorption and desorption of headspace volatile compounds

The 5 g of five *Liriopsis* tuber samples of 1, 3, 5, 7, and 9 times steaming frequencies were put into each 250 mL dark brown bottle (Supelco Inc., Bellefonte, PA, USA) and sealed. The sealed serum bottles were put in a dry oven at 60°C for 30 min. After they were cooled at room temperature for 30 min. A syringe was connected with a thin tube placed in a silicon septum and other side of the tube was connected to an adsorption pipe. Headspace volatile compounds created in the dark brown bottle were adsorbed in the stainless steel adsorption pipe (Agilent Technologies Inc., Santa Clara, CA, USA) of 90 cm length packed with Tenax-TA for 5 min using a VPC-10 vacuum pump (Shimadzu Corporation, Kyoto, Japan) and mass flow controller (Fig. 1). The stainless steel adsorption pipe was installed in an automated thermal desorber (ATD 400, PerkinElmer, Inc., Waltham, MA, USA) and desorbed in the opposite direction of adsorption direction. The desorption temperature conditions had the first desorption at 350°C for 4 min. Then, the second desorption took place at 350°C for 1 min. Desorb flow was maintained at 50.2 mL/min.

GC-MS analysis

A Tenax adsorption column was used with an automatic thermal desorber (ATD 400, PerkinElmer, Inc.) and the headspace volatile compounds were automatically injected into the GC-2010 Gas Chromatograph (Shimadzu Corporation). Volatiles were separated with a GC-column and identified through Shimadzu QP-2010 Plus mass selective detector (Shimadzu Corporation). The

GC column used was an AT-1 ($60\text{ m}\times 0.32\text{ mm}\times 1.0\text{ }\mu\text{m}$, Alltech, Nicholasville, KY, USA). The temperature conditions of the GC oven were divided into 4 steps. First, the temperature was kept at 35°C for 10 min and in the second step, it was increased up to 120°C at the rate of $8^{\circ}\text{C}/\text{min}$ and then maintained for 10 min. In the third one, it was increased up to 180 at the rate of $12^{\circ}\text{C}/\text{min}$ and kept for 7 min. Finally, it was increased until 230°C at the rate of $15^{\circ}\text{C}/\text{min}$ and then maintained for 10 min. The temperature of GC interface was kept at 230°C . Carrier gas was helium gas of purity 99.9999 percent.

The temperature of MSD was 250°C , mass range was set to 20~350 m/z, ionization voltage was 70 eV. The identification of each peak in the total ion chromatogram was carried out by comparing its mass spectrum with Wiley 211 or Nist 107 mass spectral databases (John Wiley & Sons, Inc., Hoboken, NJ, USA). Then, it was positively identified by comparing with Kovats retention index. The quantitative analysis of identified volatile compounds was shown in relative area percent with the total peak area equaling 100 percent.

RESULTS AND DISCUSSION

Total ion chromatograms of headspace volatile compounds isolated from *Liriopsis* tuber according to steaming frequency are shown in Fig. 2. Their identified volatile compounds are represented in Table 1.

The volatile compounds in 5, 8, 11, 16, 20, 22, and 24 retention time (min) were decreased with the increased steaming frequencies. These volatile compounds were 2-methyl propanal, 3-methyl butanal, 3-hydroxy-2-butanone, *n*-hexanal, 2,6-dimethyl pyrazine, 2-pentyl furan, and D-limonene. These were thought not to relate to savory flavors of *Liriopsis* tuber. Pyrazine is a typical roasted flavor (10). 2,6-Dimethyl pyrazine was thought not to relate with savory flavor of *Liriopsis* tuber.

In Table 1, the total numbers of identified volatile compounds were 74, 49, 48, 41, and 51 from 1, 3, 5, 7, and 9 frequencies, respectively. As the steaming frequencies increased, the volatile compound species of the samples decreased, except for the 9th steaming sample. Five aldehydes, 2 alcohols, 10 furans, 3 aromatic compounds, 3 acids, 3 esters, 2 ketones, and 2 aliphatic

Fig. 1. Schematic diagram of adsorption stage of sample.

Fig. 2. Total ion chromatograms of headspace volatile compounds isolated from steamed *Liriopsis* tuber powders for different steaming frequencies of 1, 3, 5, 7, and 9 times.

compounds were identified from all the samples of 1 to 9 frequencies.

The changes of area percentages of volatile compound classes are shown Fig. 3. As steaming frequency increased, the area percentage of aldehydes, aliphatic compounds, sulfur-containing compounds, terpenes, and pyrazines were decreased, but furans, acids, ketones, esters, and aromatic compounds were increased.

Aldehyde, the most abundant class among total volatile compounds of *Liriopsis* tuber, respectively was 32.01%, and 33.41% at steaming 1st and 3rd times. Then, they decreased to 9.96%, 4.12%, and 3.39% in 5, 7, and 9 frequencies, respectively. Because aldehydes such as 2-methyl propanal, 3-methyl butanal, and 2-methyl butanal had low boiling points, they disappeared as the steaming process was repeated (11). 3-Methyl butanal and 2-methyl butanal were identified compounds from tea such as green tea, and semi-fermented tea, and are known to contribute to their sweet flavor (10,12). C₆-aldehydes like hexanal, and 2-hexenal contribute to a fresh flavor of tea. Hexanal was identified in Chinese-fermented tea like Liu Bao tea. 2-Hexenal was found from black tea (10), and is

known to be created by reaction of unsaturated fatty acids such as linoleic acid (C_{18:2}) and linolenic acid (C_{18:3}), and enzymes such as lipoxygenase, hydroperoxylyase and isomerase (7). Nonanal, with a strong sweet and flower aroma and benzaldehyde with a roasted almond aroma decreased with the increased steaming frequencies (14).

Alcohols, which were the second in area percentage among the identified total volatile compounds of *Liriopsis* tuber, were 28.16% at steaming 1 time. The area percentage of alcohols increased to 38.30% at the 5th steaming time, to 42.54% at the 7th steaming time, but the alcohol percentage of the final product decreased to 29.75%. This was largely on account of changes of ethanol which had the highest area percentage among alcohols. However, it has been reported that alcohols don't greatly affect food flavor unless high concentrations of alcohol were present because alcohols have high odor detection threshold values (15).

Furan contents were 18.67%, 26.03%, 29.71%, 27.75%, and 33.86% from 1, 3, 5, 7, and 9 frequencies, respectively. As the frequencies increased, furan amounts were

Table 1. Volatile compounds in steamed *Liriopsis* tuber powder according to different steaming frequencies (times)

Compounds	RT ¹⁾	RI ²⁾	Area (%)					RI ³⁾
			1	3	5	7	9	
Aldehyde (14)								
Formaldehyde ^{T4)}	2.92	<500	0.07	0.45	0.17	0.11	0.39	
Acetaldehyde	3.20	<500	2.68	4.00	2.23	2.02	1.46	c
2-Methyl propanal	5.05	544	8.77	9.62	5.23	1.30	0.67	c
3-Methyl butanal	8.32	643	6.08	4.26	1.55	0.22	0.31	b, c
2-Methyl butanal ^T	8.84	653	12.51	14.00	—	—	—	
<i>n</i> -Hexanal	15.95	791	1.57	0.88	0.57	0.48	0.48	b, c
2-Hexenal	17.96	846	0.01	—	—	—	—	b
2,4-Dimethyl pentanal ^T	19.33	885	—	0.09	—	—	—	
Heptanal	19.57	891	—	—	0.11	—	—	c
2-Ethyl hexanal ^T	21.25	939	—	—	0.09	—	—	
Benzaldehyde	21.60	949	0.13	—	—	—	—	a, c
2,4-Undecadienal ^T	22.68	979	—	—	—	—	0.08	
<i>n</i> -Nonanal	26.68	1095	0.09	0.11	0.01	—	—	b, c
2-Butyl-2-octenal ^T	37.50	1379	0.11	—	—	—	—	
Subtotal			32.01	33.41	9.96	4.12	3.39	
Alcohol (14)								
Methanol	3.32	<500	1.79	1.76	0.84	—	0.82	c
Ethanol	3.74	<500	20.24	15.92	37.00	38.83	28.77	c
2-Propanol	4.25	504	—	—	—	0.24	—	c
2-Methyl-2-propanol ^T	4.65	525	3.44	4.47	—	2.04	—	
1-Propanol ^T	5.50	564	0.25	—	—	—	0.01	
2-Methyl-1-propanol ^T	7.97	636	0.37	—	—	—	—	
<i>n</i> -Butanol	10.25	678	0.31	0.14	0.11	1.36	0.15	c
3-Methyl-3-butenol ^T	13.77	748	0.04	0.05	—	—	—	
3-Methyl-1-butanol ^T	14.68	767	—	—	0.23	—	—	
1-Pentanol ^T	15.40	781	0.42	—	—	—	—	
1,2-Butanediol ^T	16.13	794	0.17	—	—	—	—	
<i>n</i> -Hexanol	19.03	876	0.91	0.20	0.12	—	—	a
2-Methyl-pentanol ^T	20.78	926	0.02	—	—	—	—	
2,7-Dimethyl-1-octanol ^T	26.40	1088	0.19	—	—	0.07	—	
Subtotal			28.16	22.54	38.30	42.54	29.75	
Furan (17)								
Furan	4.18	501	0.41	0.32	0.32	0.40	0.67	c
2,5-Dihydrofuran ^T	5.37	558	—	—	—	—	0.01	
2-Methyl furan	6.52	602	0.30	0.67	1.21	1.77	2.44	c
2-Ethyl furan	11.63	699	0.10	—	—	—	—	c
2,5-Dimethyl furan	12.00	708	0.19	0.27	0.46	0.74	0.82	b, c
2-Methyl dihydro-3(2H)-furanone	16.20	796	6.30	4.83	5.78	6.34	6.53	b
Furfural	17.36	828	4.32	12.67	16.36	14.37	18.80	c
5-Methyl-2(3H)-furanone ^T	18.48	861	0.36	0.21	0.20	0.22	0.19	
2-Furanmethanol ^T	18.61	865	0.82	1.71	1.24	0.39	0.40	
2- <i>n</i> -Butyl furan ^T	19.60	892	0.42	—	—	—	—	
Dihydro-2(3H)-furanone ^T	19.64	893	—	0.50	0.17	0.10	0.12	
Furfuryl formate ^T	19.89	899	—	0.10	0.10	0.09	0.08	
2-Acetyl furan ^T	20.08	905	1.69	3.33	2.39	1.78	1.71	
5-Methyl-2(5H)-furanone	20.62	921	0.01	0.01	0.10	0.07	0.11	b
5-Methyl-2-furfural	21.59	949	0.56	1.00	1.23	1.49	1.98	a
2-Furan methanol, acetate ^T	22.55	975	—	—	0.01	—	—	
2-Pentyl furan	22.67	979	3.20	0.41	0.15	—	—	a, b
Subtotal			18.67	26.03	29.71	27.75	33.86	
Aromatic compound (6)								
Benzene	9.11	658	0.58	0.94	1.28	0.78	2.53	b
Methyl benzene	14.90	771	0.27	0.30	0.34	0.21	0.35	b
Ethyl benzene	18.60	864	0.07	—	—	—	—	b
1,2-Dimethyl benzene	18.86	872	0.07	0.24	0.18	0.11	0.17	b
Styrene	19.55	890	—	—	—	—	0.23	a
(1-Methylethenyl) benzene	22.41	972	—	—	—	—	0.04	b
Subtotal			0.99	1.47	1.81	1.10	3.32	

Table 1. Continued

Compounds	RT ¹⁾	RI ²⁾	Area (%)					RI ³⁾
			1	3	5	7	9	
Acid (6)								
Acetic acid ^T	8.10	639	3.64	4.37	9.48	6.79	7.82	b
Propanoic acid ^T	12.93	730	0.20	0.24	0.44	0.44	0.44	
2-Propenoic acid	13.29	738	—	—	—	0.01	0.03	
2-Methyl propanoic acid ^T	15.60	785	0.14	0.12	0.06	0.03	0.05	
2-Methyl butanoic acid ^T	18.80	870	0.29	0.25	—	—	—	
Benzoic acid ^T	31.81	1193	—	—	—	—	0.47	
Subtotal			4.26	4.98	9.98	7.26	8.81	
Ester (11)								
Formic acid, methyl ester ^T	3.46	<500	0.79	0.78	0.81	2.31	2.06	a, b c
Formic acid, ethyl ester	4.43	514	0.39	2.02	0.10	0.12	0.11	
Acetic acid, methyl ester	4.67	526	0.41	—	0.34	—	0.37	
Sulfurous acid, dimethyl ester ^T	5.24	553	—	—	—	—	0.07	a, b
Acetic acid, ethyl ester	7.01	614	0.38	0.17	0.20	0.14	0.23	
2-Hydroxy-propanoic acid, methyl ester ^T	13.97	752	—	—	—	0.03	0.04	
1-Methoxy-2-propyl acetate ^T	18.83	871	—	0.45	0.02	0.24	0.27	b a
Pentanoic acid, ethyl ester	19.70	894	0.28	—	—	—	—	
Hexanoic acid, methyl ester	20.47	917	0.02	—	—	—	—	
Oxalic acid, 2-ethylhexyl hexyl ester ^T	23.42	998	0.18	0.12	0.09	—	—	a
Octanoic acid, ethyl ester	31.85	1193	0.07	—	—	—	—	
Subtotal			2.51	3.54	1.56	2.84	3.15	
Ketone (13)								
2-Propanone	4.01	<500	5.53	4.93	3.08	4.45	4.34	c
2,3-Butanedione	5.74	574	0.62	—	2.83	5.21	7.39	c
2-Butanone	6.14	589	0.60	0.25	0.14	0.26	—	c
2-Pentanone ^T	10.60	684	0.05	—	—	—	—	a
2,3-Pentanedione	10.73	686	0.76	1.24	2.06	4.18	5.53	
3-Hydroxy-2-butanone	11.74	702	1.44	0.53	0.21	—	—	
4-Methyl-2-pentanone ^T	13.64	745	—	—	—	0.03	0.03	a
1-Hydroxy-2-butanone ^T	14.72	768	—	—	—	—	0.08	
2-Hexanone	15.75	787	0.06	—	—	—	—	
2-Heptanone	19.32	884	0.44	—	—	—	0.04	c
3-Octanone	20.80	927	0.02	—	—	—	—	c
2,6-Dimethyl-3-heptanone ^T	20.98	932	0.02	0.01	—	—	—	a
2-Octanone	22.21	966	0.07	—	—	—	—	a
Subtotal			9.60	6.96	8.31	14.13	17.40	
Aliphatic compound (9)								
<i>n</i> -Pentane	4.17	500	1.10	—	—	—	—	a
<i>n</i> -Hexane	6.43	600	0.15	—	—	—	—	a
2,3,4-Trimethyl pentane ^T	14.46	762	0.11	0.14	0.05	0.10	0.03	b
2,3,3-Trimethyl pentane ^T	14.66	766	0.16	—	—	—	—	
2,2,3,3-Tetramethyl pentane ^T	14.73	768	—	—	—	—	0.09	
2-Octene	16.48	801	0.11	—	—	—	—	b
3,5-Dimethyl heptane	17.89	844	0.02	—	—	—	—	b
<i>n</i> -Nonane	19.91	900	0.07	—	—	—	—	a
Dodecane	32.25	1200	0.08	0.20	0.21	0.13	0.17	a
Subtotal			1.81	0.33	0.26	0.23	0.29	
Others (5)								
Diacetyl sulfide ^T	12.61	723	—	—	—	—	0.01	c
Dimethyl disulfide	13.53	743	0.14	0.11	—	0.03	0.02	
2,6-Dimethyl pyrazine	20.29	911	0.77	0.47	0.02	—	—	
α -Pinene	21.20	938	0.11	—	—	—	—	a
D-Limonene	24.25	1024	0.97	0.16	0.09	—	—	a
Subtotal			1.99	0.74	0.11	0.03	0.03	
Total			100.00	100.00	100.00	100.00	100.00	

^{1,2)}RT and RI stand for retention time and Kovat retention index, respectively.

³⁾Rf is references. a is Flavornet.org, b is Pherobase.com, c is Makkhen's thesis

⁴⁾The superscript of "T" of the compounds means tentatively identified compound by matching mass spectrum data of sample with reference one. The other compounds are positively identified by using MS and RI.

Fig. 3. The changes of ratios of volatile compounds according to different steaming frequencies.

found with the increased area percentage. Furans such as furfural, 2-methyl furan and 5-methyl-2-furfural increased. Furan has been known to contribute to savory smell of food and is produced by nonenzymatic browning reactions such as amino-carbonyl reactions (14). Furfural has an almond and sweet flavor. Furfural is known to be generated from pentose and 5-methyl-2-furfural. 5-Methyl-2-furfural has caramel and burnt sugar flavor. 2-Acetyl furan has a sweet cigarette flavor. 2-Acetyl furan is known to be made from hexose by non-enzymatic browning reactions (16). The savory smell increased with the increased steaming frequencies (10,14). When furan compounds exist in moderate concentrations they have a savory and sweet smell. When they exist excessively, it is burnt (14).

Acid area percents were 4.26%, 4.98%, 9.98%, 7.26%, and 8.81% from 1, 3, 5, 7, and 9 frequencies, respectively. They were increased until 5 frequencies and then

their amounts fluctuated. The reason was thought that acetic acid was the most abundant acid was the main factor of these changes.

Ketone area percents were 9.60%, 6.96%, 8.31%, 14.13%, and 17.40% from 1, 3, 5, 7, and 9 frequencies, respectively. In the third frequency, they were a little decreased, but after the third one, they were consistently increased in Fig. 3. The amounts of 2,3-butanedione and 2,3-pentanedione were increased according to the increased steaming frequencies. Sour taste was evaluated to be high among other tastes in sensory evaluation results of hot water extract from *Liriopsis* tuber (9). Also, 2,3-pentanedione was identified from buckwheat-green tea (10).

Sulfur-containing compounds, terpenes, and pyrazines were identified in low amounts. Dimethyl disulfide is known to have an onion, cabbage, and putrid flavor (18). But as frequency was increased, these compounds dis-

appeared. Terpenes are known to have forest or green aromas (14). These compounds disappeared with increased steaming frequencies.

Esters, other aliphatic compounds, and other aromatic compounds were found in small area percentages among total volatile compounds. Moreover, most of the aliphatic compounds disappeared after the first steaming frequency.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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