Exploring *Nuruk* Aroma; Identification of Volatile Compounds in Commercial Fermentation Starters

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Abstract Volatile compounds of 7 commercial fermentation starters, including 4 traditional *nuruk*, 2 *ipguk*, and 1 crude amylolytic enzyme, were extracted using solvent-assisted flavor evaporation (SAFE), and extracts were analyzed using GC-MS. A total of 70 volatile compounds, including 4 esters, 10 acids, 16 alcohols, 16 hydrocarbons, 9 ketones, 5 aldehydes, 3 volatiles phenols, and 7 miscellaneous components were identified. Concentrations of volatile compounds, including 4,6-dimethylundecane, methyl 2-hydroxybenzoate, 1-hexanol, 2-phenylethanol, 3-methylbutanoic acid, and hexanoic acid were higher in 4 traditional *nuruk* starters than in others. The major volatile components in 2 *ipguk* starters were the hydrocarbons dodecane, heptadecane, octadecane, and nonadecane. Concentrations of 2-methoxy-4-vinylphenol and 3-hydroxy-2-butanone were highest in the crude amylolytic enzyme starter. Compositions and amounts of volatile compounds were different among the 7 commercial fermentation starters.

Keywords: fermentation starter, volatile, solvent assisted flavor evaporation, *nuruk*, *ipguk*

Introduction

A fermentation starter as a saccharification agent that converts starch into sugars in alcohol fermentation has been used in brewing Korean traditional alcoholic beverages. Traditional *nuruk*, *ipguk* (Japanese style *koji* used since the Japanese colonial era), and a crude amylolytic enzyme that was developed and supplied in the 1960s, have been used in Korea as fermentation starters (1). Traditional *nuruk*, which is usually made from wheat or grits, allows growth of *Rhizopus* sp., *Aspergillus* sp., *Saccharomyces* sp., *Hansenula* sp., and *Lactobacillus* sp. (2), production of which enhances the flavor of *makeolli* and *yakju* (3). *Ipguk* originates from the Japanese brewing method in which microorganisms are intensively incubated and nurtured via artificial vaccination of sterilized media with a specific fungus (4). The crude amylolytic enzyme has a heightened saccharogenic power via artificial incubation of *Rhizopus* sp. and *Aspergillus* sp. for diastatic enzyme production as the main bacteria of traditional *nuruk* for promotion of both the complicated flavor of traditional *nuruk* and the saccharogenic power (5). Growth and development of microorganisms, including activation of enzymes, alcohol fermentability, and organic acid production, differ according to types of fermentation starters, affecting volatile component profiles, tastes, and color of *makeolli* or *yakju* (6).

Studies on brewing of traditional alcoholic beverages have been weighted towards isolation and identification of microorganisms

related to alcoholic fermentation, and enhancement of the fermentation process by changing brewing conditions using different fermentation starters, raw materials, and physical settings. Effective yeasts (7-8), fungi, and lactic acid bacteria (9-10) for alcoholic fermentation were selected from different types of *nuruk*.

Sensory studies of *yakju* and *makeolli* have been recently reported (11-15). Sensory characteristics of *nuruk* aroma as yeasty, moldy, or fermented aromas, were reported as main sensory characters. Those sensory characteristics were mainly considered as related to the fermentation starters *nuruk*, *ipguk*, or the crude amylolytic enzyme. Aroma terms, also known as lexicons, were defined as *nuruk* smells associated with activated yeast and mold growth (mildew) with reference compounds. However, these definitions and reference compounds could lead to confusion for consumers. Generally, *nuruk*, which includes traditional *nuruk*, improved *nuruk* (crude amylolytic enzyme), and Japanese style *koji* (*ipguk*), exhibits different aroma characteristics. Therefore, more detailed definitions and reference compounds for *nuruk* related sensory terms were required for clear communications with consumers and industry practitioners.

Reference compounds for sensory characteristics have been defined with exact chemical compounds for wine, beer, and Sake (16-19). For example, a wine aroma wheel was developed in 1984 to promote better understanding of sensory characteristics of wines and to facilitate communication among members of the wine industry (16). Over 90 aroma characteristics were determined and reference

compounds were suggested. For determination of more detailed reference compounds for *nuruk* related aroma characteristics, the first step would be to identify volatile compounds in different types of *nuruk*. However, volatile compositions of different types of *nuruk* fermentation starters have not been thoroughly studied.

In this study, volatile components of 4 different traditional varieties of *nuruk*, 2 different varieties of *ipguk* (Japanese style *koji*), and an improved *nuruk* with a crude amyolytic enzyme, were investigated using solvent-assisted flavor evaporation with GC-MS. Different volatile compositions of each starter sample were compared using a principal components analysis (PCA).

Materials and Methods

Materials Seven commercial fermentation starters were purchased from different manufacturing companies in June of 2013 (Table 1). Four of the starters (T1, T2, T3, and T4) were collected from local companies producing Korean traditional *nuruk*. Two additional starters (IP1 and IP2) were acquired from Joen grain, Co., Ltd. (Hwaseong, Korea) and Seoul Jangsu Co., Ltd. (Seoul, Korea) producing *ipguk* using rice inoculated with *Aspergillus kawachii*. In addition, a remaining starter (CE) was purchased from Korea enzyme Co., Ltd. (Hwaseong, Korea). *n*-Alkane standards (C6-C25) were purchased from Aldrich Chemical Company Inc. (Milwaukee, WI, USA). Sodium sulfate, 4,6-dimethyl-undecane, heptadecane, octadecane, 1-methoxy-2-propanol, 4-methyl benzaldehyde, 2-cyclohexen-1-one, and 2-methyl-1-pentanol (internal standard) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dichloro-methane was purchased from J.T Baker (Philipsburg, NJ, USA).

Extraction and isolation of volatile compounds using a solvent assisted flavor evaporation (SAFE) apparatus A 10 g sample of each fermentation starter was subjected to extraction using 90 mL of dichloro-methane for 12 h at 20°C. A 0.5 mL internal standard (IS) of 2-methyl-1-pentanol at 50 µg/mL in methanol was added to starter samples before extraction. A SAFE apparatus was used for isolation of volatile compounds from fermentation starter samples. The SAFE procedure was adapted from Engel *et al.* (20) and Lee and Lee (21). Dichloromethane extracts were separated using centrifugation (Combi 514R; Hanil science industrial, Incheon, Korea) at 5,000*g* for 10 min and the supernatant was collected and used for analysis. The filtered extract supernatant was added to the SAFE dropping funnel (Ace glassware, Vineland, NJ, USA), and the SAFE apparatus was heated to 50°C using a circulating water bath. A 500 mL distillation flask was heated to 50°C in a water bath. The receiving flask for distillation and the safety-cooling trap were cooled to −196°C using liquid nitrogen. The SAFE apparatus was connected to a high-vacuum pump, then the extract in the dropping funnel was added in small aliquots to the distillation flask over a period of 25 min. The resulting collected and frozen distillate was thawed at 20°C. Thawed extracts

Table 1. Materials and ingredients

Code	Production area	Manufacturing process	Raw materials
T1	Gyeonggi-do	Traditional	Wheat
T2	Gyeongsangbuk-do	Traditional	Wheat
T3	Jeollanam-do	Traditional	Wheat
T4	Gyeongsangnam-do	Traditional	Wheat
CE	Gyeonggi-do	Improved	Wheat bran
IP1	Gyeonggi-do	Improved	Rice
IP2	Gyeonggi-do	Improved	Rice

were dried over anhydrous sodium sulfate and concentrated to approximately 1 mL using a Vigreux column in a water bath at 45°C. The collected solvent and volatile compounds from the 90 min SAFE process were concentrated to 0.5 mL using liquid nitrogen. Concentrated extracts were stored at −20°C in the deep freezer (Model JP/CLN-40U; Towa scientific Co., Tokyo, Japan) prior to analysis (22-23).

GC-MS One µL of each concentrated extract was analyzed in duplicate on a Hewlett-Packard (HP) GC model 7890A (Palo Alto, CA, USA) coupled to a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and equipped with a Stabilwax®-DA bonded fused capillary column (30 m×0.25 mm i.d., film thickness=0.25 µm) (Restek, Bellefonte, PA, USA). Analysis was performed with an inlet temperature of 220°C in splitless mode for 1 min with a purge flow rate to the split vent of 50 mL/min for 1 min, a column head pressure of 14.14 psi, a helium carrier gas flow rate of 1.3 mL/min, and an average helium gas velocity of 30 cm/s. The oven temperature was held at 40°C for 4 min with a programmed increase at 5°C/min until 185°C, followed by isothermal holding for 20 min. Mass spectra were generated in electron impact mode (EI) at 70 eV at an ion source temperature of 230°C. Spectra were obtained over an *m/z* range of 40-350. Total ion chromatograms (TIC) acquired via GC-MS were used for peak area integration. HP-MSD Chemstation software G1701BA ver.D.02.00 was used for data acquisition.

Compound identification and quantification Volatile compounds were positively identified based on comparison with Kovats retention indices (KI) (13) and MS fragmentation patterns were compared with reference compounds, or with mass spectra in the Wiley 275 mass spectral database (Hewlett-Packard). KI values of unknown compounds were determined via injection with a homologous series of alkanes (C6-C25). GC-MS conditions were the same as described above. For quantification of volatiles, starter samples were run in duplicate, and integrated areas based on the TIC were normalized to IS average values. Relative volatile concentrations of 7 starter samples were determined based on comparison with IS concentrations, assuming a response factor of 1.

Statistical analysis of GC-MS data All statistical analyses were performed using SAS version 6.12 (SAS Institute, Cary, NC, USA) or

XLSTAT ver.2007.1 (Addinsoft, New York, NY, USA). For evaluation of significant differences between volatile components of fermentation starter samples, an analysis of variance (ANOVA) was performed following the general line model (GLM) procedure. PCA was performed using mean values of relative volatile concentrations with a correlation matrix and no rotation. PCA was applied to clarify relationships among fermentation starters prepared using different manufacturing processes and volatile components present. The GC data set included 71 identified volatile compounds.

Results and Discussion

Compositions of volatile compounds in commercial fermentation starters Volatiles in 7 commercial fermentation starters isolated using a SAFE apparatus and analyzed using GC-MS are shown in Table 2, including identified volatile components, chemical classes, relative concentrations, and KI values. A total of 70 volatile compounds were identified, including 4 esters, 16 hydrocarbons, 16 alcohols, 10 acids, 5 aldehydes, 9 ketones, 3 volatile phenols, and 7 miscellaneous compounds. Among identified volatile compounds, 22 were present in all 7 starters. Volatiles were grouped by chemical class. Alcohols, acids, and hydrocarbons were the largest groups with abundant amounts of 2,3-butanediol, 2-cyclohexen-1-ol, phenylethyl-alcohol, benzyl alcohol, 3-methyl-butanoic acid, and dodecane.

Among 16 hydrocarbons identified, 5-ethyl-5-methyldecane, 4-methylundecane, dodecane, and 4,6-dimethylundecane were the major compounds. The overall hydrocarbon levels in starter samples T2, T4, and IP2 were higher than in other starter samples. Overall concentrations of hydrocarbons did not exhibit differences based on use of fermentation starters. The alkanes dodecane, pentadecane, hexadecane, heptadecane, and nonadecane were also previously reported as major volatile compounds in traditional *nuruk* (24). Dodecane is a known volatile component of soy sauce, soybean paste (25), and unrefined rice wine (24). Alkanes were formed due to decarboxylation of fatty acids. However, because of large threshold values, many straight hydrocarbons do not have a direct impact on smell (26).

Esters are major volatiles in many alcoholic beverages, including *yakju*, *makgeolli*, and others (11,12,15). These ester compounds are mainly produced by yeasts during alcoholic fermentation. However, only 4 esters were identified in this study. Contrary to ethyl esters that are commonly identified in alcoholic beverages, methyl 2-hydroxybenzoate (methyl salicylate) was the major ester in fermentation starters in this study, which widely present in plants, particularly in blueberries, as a naturally produced ester (27).

Among 16 alcohols identified, the main components were 2,3-butanediol, 2-cyclohexen-1-ol, 2-phenylethanol, and benzyl alcohol. Overall total concentrations of quantified alcohols were higher in the 4 traditional *nuruk* varieties T1, T2, T3, and T4, and the crude amylolytic enzyme variety (CE), than in *ipguk* varieties IP1 and IP2.

2,3-Butanediol comprised approximately 47% of the total of quantified alcoholic compounds. However, 2,3-butanediol was not detected in *ipguk* samples IP1 or IP2. Occurrence of 2,3-butanediol is mediated by the metabolic fate of the microorganisms *Aerobacter aerogenes*, *Klebsiella pneumonia*, and *Bacillus polymyxa* (28). Since 2,3-butanediol was detected in traditional *nuruk* and co-enzyme starter samples, different microorganisms were involved in production of 2,3-butanediol during the saccharification process. Moreover, 2,3-butanediol does not occur in *ipguk* that is inoculated with a single microorganism. 2-phenylethanol, which has a mild, warm-rose, honey-like odor, was a significant component that affected the flavor of *makgeolli* (15), and soybean paste (29). In this study, 2-phenylethanol was detected in 6 starter samples, with *ipguk* (IP1) as the only exception. The highest concentration of 2-phenylethanol was observed in traditional *nuruk* (T1).

Among 10 acid compounds identified, 3-methylbutanoic acid, hexanoic acid, and acetic acid were major volatiles comprising approximately 75% of the total of quantified acids. Starter sample T1 contained the highest concentration of acids at 33.77 mg/L, while in starter samples T2, T3, T4, CE, and IP2, acid concentrations ranged from 5.99-8.44 mg/L. A minimal acid level was observed in starter sample IP1. Acids were present in red pepper paste (30) and *takju* (24). Acids with sharp, rancid, or sweaty notes were considered to be important contributors to flavor.

The main aldehyde components were 4-methylbenzaldehyde, 2-methylbenzaldehyde, and hexanal with the highest level in starter sample IP2. Different types of aldehydes were developed when starches in wheat or rice were decomposed using enzyme reactions of different microorganisms (31).

Among identified volatile phenol compounds, 2-methoxy-4-vinylphenol is a compound with a flavor of buckwheat and smoke. Ferulic acid, abundant in wheat, can be converted into 2-methoxy-4-vinylphenol by *Saccharomyces cerevisiae* and *Pseudomonas fluorescens* (32). 2-methoxy-4-vinylphenol was present in both traditional *nuruk* and the variety with a co-enzyme, which used wheat as raw material in this study. Also, 2,4-bis(1,1-dimethylethyl)-phenol was reported as a volatile compound of mulberries (33) and extracts of Nelumbinis semen (34).

Comparison of volatile compositions using PCA PCA was performed to achieve an overall picture of the volatile distribution with separation of starter samples (Fig. 1). The first principal component (PC1) explained 28.37% of variation across starter samples, while the second PC2 explained 23.09% of the variance. Butanoic acid (ac2), heptanoic acid (ac9), 2,3-dihydrothiophene (ms7), pentadecane (hc13), 2-cyclohexen-1-ol (al9), benzyl alcohols (al15), and 5-pentyl-2(5H)-furanone (ke8) were located on the positive side of PC1 (Fig. 1A). In traditional *nuruk* samples T1 and T2, levels of the above compounds (ac2, ac9, ms7, hc13, al9, al15, and ke8) were significantly higher ($p < 0.05$), compared with respective levels in *ipguk* samples IP1 and IP2. 4-methyldecane (hc1), nonadecane (hc8), octadecane

(hc16), ethyl hexadecanoate (es4), benzaldehyde (ad2), 2-octanone (ke5), and 2-thiophenecarbaldehyde (ms5) were located on the strong negative side of PC1.

PC2 was mainly defined by a contrast between starter samples T1 and T2 and volatile compounds of these 2 starter samples were located along PC2. Concentrations of 5-ethyl-5-methyldecane (hc2), 7-methyl-2-decene (hc9), hexadecane (hc14), 1-methoxy-2-propanol (al2), 2-methylbenzaldehyde (ad5), 4-isopropyl-1,3-cyclohexadione (ke3), styrene (ms1), and 1-methyl-4-isopropylbenzene (ms2) were

higher in traditional *nuruk* T2 than in other starter samples. Volatiles positioned on the negative side of PC2 were 2-butyl-1-octanol (al8), 2-methyl-3-buten-2-ol (al14), 2-phenoxyethanol (al16), 3-methylbutanoic acid (ac3), pentanoic acid (ac4), 3-hydro-2-methylpropanoic acid (ac8), and octanoic acid (ac10), which were all dominant in traditional *nuruk* sample T1.

Based on inspection of the distribution of starter samples (Fig. 1B), traditional *nuruk* samples T1 and T2 were positioned on the positive side on PC1, and *ipguk* samples IP1 and IP2 were located on the

Table 2. Volatile flavor compounds (mg/L¹⁾) in 7 commercial fermentation starters

Code	Kl ²⁾	Volatile compound ³⁾	Starter							Id ⁴⁾	Ref ⁵⁾
			T1	T2	T3	T4	CE	IP1	IP2		
Hydrocarbones											
hc1	1007	4-methyl-decane	0.65	-	-	-	-	0.74	1.84	B	22, 23
hc2	1010	5-ethyl-5-methyl-decane	-	2.83	-	3.74	-	-	-	B	
hc3	1110	4-methyl-2-undecane	0.59	2.00	0.72	3.05	1.34	1.05	3.58	B	
hc4	1196	dodecane	1.83	2.32	1.13	2.60	1.02	0.83	2.48	A	
hc5	1213	4,6-dimethyl-Dodecane	0.22	0.87	0.49	1.34	0.30	0.23	1.17	B	
hc6	1219	4,6-dimethyl-Undecane	2.22	1.25	1.69	0.57	0.37	0.50	0.79	A	
hc7	1238	2,4,6-trimethyl-decane	-	-	-	0.52	0.36	-	0.70	B	22
hc8	1238	nonadecane	-	-	-	-	-	0.16	0.33	A	
hc9	1310	7-methyl-2-decene	-	1.69	-	2.20	-	0.83	-	B	
hc10	1320	1-methoxy-2-butene	-	2.61	-	-	-	-	-	B	
hc11	1320	4-ethyl-2-octene	-	-	-	2.61	-	-	-	B	22, 24
hc12	1418	2-methyl-decane	0.42	0.95	1.01	0.64	0.60	0.12	0.60	A	
hc13	1489	pentadecane	0.38	0.68	0.28	0.28	0.27	0.34	0.18	A	
hc14	1589	Hexadecane	0.43	1.13	0.03	0.37	0.18	0.31	0.85	A	
hc15	1691	heptadecane	0.22	-	-	-	-	0.02	0.38	A	
hc16	1760	octadecane	-	-	-	-	-	0.19	0.21	A	
Total hydrocarbones			6.96	16.33	5.35	17.92	4.44	5.32	13.11		
Esters											
es1	1759	butyl 2-methylpentanoate	0.92	0.47	0.52	0.38	0.78	0.06	0.37	B	25, 28, 28
es2	1782	methyl 2-hydroxybenzoate	2.56	1.59	0.55	2.64	0.96	0.61	2.13	B	
es3	1875	butyl butanoate	0.95	0.31	2.71	-	-	-	-	A	
es4	2252	ethyl hexadecanoate	-	-	-	-	-	0.02	0.17	A	
Total esters			4.43	2.37	3.78	3.03	1.74	0.69	2.67		
Alcohols											
al1	1095	1,8-nonadien-3-ol	0.13	0.45	0.16	0.31	0.42	0.11	0.13	B	6, 22, 23, 28, 30
al2	1132	1-methoxy-2-propanol	-	0.88	-	-	-	0.30	-	A	
al3	1161	1-methyl-2-cyclopentenol	-	-	-	0.56	-	0.50	-	B	
al4	1205	3-methyl-1-butanol	-	1.54	-	-	-	-	-	A	
al5	1221	2-ethoxy ethanol	-	0.51	0.05	0.72	-	0.84	-	B	
al6	1350	1-hexanol	2.77	1.07	0.94	0.67	0.31	-	-	A	
al7	1397	2-butoxy ethanol	-	-	-	0.80	-	-	0.37	A	6, 32, 24
al8	1465	2-butyl-1-octanol	2.43	-	-	0.30	-	-	0.26	B	
al9	1466	2-cyclohexen-1-ol	2.56	4.41	2.17	3.29	2.18	1.19	0.80	A	6, 27, 7, 23
al10	1569	2,3-butanediol	2.84	32.15	4.99	3.15	26.18	-	-	A	
al11	1584	1,2-propanediol	-	1.98	-	-	0.55	-	-	B	
al12	1614	2-(2-ethoxyethoxy)-ethanol	0.09	0.11	0.16	0.20	0.19	-	0.47	A	
al13	1807	2-phenylethanol	7.73	3.80	1.80	0.28	0.20	-	0.47	A	
al14	1870	2-methyl-3-buten-2-ol	1.52	-	-	-	-	-	-	B	
al15	1871	benzyl alcohol	6.57	2.86	1.78	0.30	0.67	-	0.39	A	
al16	2142	2-phenoxy-ethanol	1.92	0.19	0.10	-	-	0.08	-	A	
Alcohol total			34.89	53.63	12.15	10.81	30.94	3.02	2.89		

Table 2. Continued

Code	KI ²⁾	Volatile compound ³⁾	Starter							Id ⁴⁾	Ref ⁵⁾
			T1	T2	T3	T4	CE	IP1	IP2		
Acids											
ac1	1451	acetic acid	0.13	1.80	-	0.34	4.42	0.43	4.65	A	4,6,7,22, 24, 30
ac2	1623	butanoic acid	0.43	0.49	0.28	0.18	0.20	0.05	-	A	28
ac3	1662	3-methyl butanoic acid	16.25	1.14	6.83	0.59	-	-	-	A	20, 28
ac4	1731	Pentanoic acid	3.02	0.29	-	0.61	2.04	0.04	0.25	A	23, 32
ac5	1761	2-methyl hexanoic acid	0.64	0.91	0.28	0.51	0.18	0.39	0.52	B	
ac6	1791	3-methyl-2-butenic acid	-	0.73	-	-	-	-	-	A	28
ac7	1836	hexanoic acid	10.61	1.99	1.08	4.35	1.73	-	-	A	6, 22, 23, 30, 32
ac8	1861	3-hydro-2-methyl-Propanoic acid	1.98	0.68	0.44	0.26	-	0.05	0.57	B	
ac9	1947	heptanoic acid	0.34	0.41	0.07	0.07	0.23	-	-	A	23, 24
ac10	2051	octanoic Acid	0.39	-	0.08	0.13	0.36	-	-	A	6, 23, 32
		Total acids	33.79	8.44	9.06	7.04	9.16	0.96	5.99		
Aldehydes											
ad1	1089	hexanal	0.15	0.40	0.62	0.93	0.77	-	-	A	23, 32
ad2	1529	benzaldehyde	-	-	-	0.26	-	-	1.34	A	23,24,28, 31
ad3	1648	Benzene acetaldehyde	0.18	-	0.01	0.14	0.46	-	-	A	28
ad4	1655	4-methyl benzaldehyde	1.68	0.93	0.72	1.12	0.53	0.26	1.34	A	
ad5	1656	2-methyl benzaldehyde	0.43	1.40	0.29	0.88	0.68	0.45	0.71	A	
		Total aldehydes	2.44	2.73	1.64	3.33	2.44	0.71	3.39		
Volatile phenols											
ph1	2103	2,6-bis(1-methylpropyl)-phenol	0.04	0.27	0.27	0.49	0.13	0.02	0.18	B	
ph2	2210	2-methoxy-4-vinylphenol	0.14	0.18	0.29	0.03	2.15	-	-	A	28, 31
ph3	2303	2,4-bis(1,1-dimethylethyl)-phenol	2.61	0.49	0.21	0.57	0.39	0.36	3.22	A	32, 33
		Total volatile phenols	2.79	0.94	0.77	1.09	2.67	0.38	3.40		
Ketones											
ke1	1209	4-methyl-2-heptanone	-	-	1.00	0.14	0.11	0.09	0.24	B	
ke2	1281	3-hydroxy-2-butanone	0.22	0.67	-	-	2.55	-	-	A	28
ke3	1328	4-isopropylcyclohexane-1,3-dione	0.86	1.85	0.82	1.47	0.77	0.47	1.73	B	
ke4	1437	2-cyclohexen-1-one	1.55	2.19	0.98	1.83	1.27	1.20	1.33	A	
ke5	1617	2-octanone	-	-	-	-	-	0.10	0.24	A	28
ke6	1633	butyrolactone	0.24	-	0.22	0.30	0.22	-	-	B	
ke7	1706	5-ethyldihydro-2(3 <i>H</i>)-furanone	1.54	0.59	0.17	0.20	0.32	0.08	-	A	
ke8	2079	5-pentyl-2(5 <i>H</i>)-furanone	0.12	0.27	0.04	0.06	0.25	-	-	B	
ke9	2330	1,1'-(1,4-phenylene)bis-ethanone	3.08	1.09	0.49	1.81	0.77	0.49	4.33	B	
		Total ketones	7.61	6.66	3.72	5.81	6.26	2.43	7.87		
Miscellaneous											
ms1	1253	Styrene (ethenyl-benzene)	0.03	0.58	0.21	0.63	-	-	-	A	28
ms2	1364	1-methyl-4-(1-methylethyl) benzene	0.06	0.69	0.48	0.17	0.39	0.06	0.25	B	
ms3	1425	1,3-bis(1,1-dimethylethyl)-benzene	6.19	7.96	4.61	11.62	5.00	3.95	12.41	B	
ms4	1448	1,2-dimethyl-hydrazine	-	1.12	0.29	-	-	-	-	B	
ms5	1555	2-thiophenecarbaldehyde	-	-	-	-	-	0.13	0.17	B	
ms6	1898	dimethyl sulfone	0.30	0.16	0.14	0.51	1.19	-	-	A	
ms7	2372	2,3-dihydro-thiophene	0.21	0.25	0.04	0.05	0.13	0.06	0.09	B	
		Total miscellaneous	6.79	10.76	5.77	12.98	6.71	4.20	12.92		

¹⁾Average value (mg/L) (w/v) (n=2)

$$\text{mg/L} = \frac{\text{Area of each compound} \times \text{Amount of internal standard}}{\text{Area of internal standard} \times \text{Amount of sample}/10^6}$$

²⁾Kovats indices of unknown compounds on a DA-WAX column³⁾Compounds by order of Kovats indices in a chemical class⁴⁾Volatiles were identified based on the following criteria: A, mass spectrum and retention index consistent with previous reports, or an authentic standard; B, mass spectrum consistent with the Wiley 275 mass spectrum database⁵⁾Reference

Fig. 1. Principal component analysis loadings for 71 volatile compounds (A) and scores for the 7 commercial fermentation starters (B). Starter samples and volatile compound codes are defined in Table 2.

negative side of PC1. Starter sample CE was located on the slightly positive side of PC1. On PC2, the contrast between traditional *nuruk* samples T2 and T1 was large. Overall starter sample clustering based on the manufacturing process of traditional vs. improved-Japanese style starters was not observed. However, *ipguk* samples IP1 and IP2 showed a similar PCA grouping. Different microorganisms were involved in each traditional *nuruk* sample and volatile compositions of each *nuruk* sample were diverse.

In conclusion, more volatile components were identified in traditional *nuruk* at higher levels than in *ipguk* manufactured following the Japanese style. Starters made using natural microflora showed large differences in volatile profiles, perhaps due to differences in microorganism actions in the natural environment. In contrast, *ipguk*, prepared using only *Aspergillus oryzae*, contained fewer volatiles than other starters. For identification of more sensory relevance of volatiles present in different types of *nuruk*, GC-olfactometry should be applied in future studies. Monitoring of changes in volatile compounds during and after alcoholic fermentation using different types of fermentation starters would be valuable.

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