Comparison of volatile profiles in *Fagopyrum esculentum* (buckwheat) *soksungjang* prepared with different starter cultures during fermentation

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Abstract This study investigated the differences and changes in the volatile profiles of buckwheat *soksungjang* (BS) inoculated with multiple microbial starters (*Lactobacillus brevis* + *Aspergillus oryzae*, BS-LA vs. *Lactobacillus brevis* + *Bacillus amyloliquefaciens*, BS-LB) during fermentation using SPME coupled with GC–MS and partial least square-discriminant analysis. BS samples fermented for 5 weeks could be differentiated from other BS samples with shorter fermentation periods, and the BS-LA and BS-LB samples fermented for 5 weeks were separated. Acids, benzenes, and esters were main volatile compounds in both BS samples, however, their differences and changes were varied. The increase of 3-methylbutanoic acid was bigger in BS-LB than BS-LA, while the contents of 2- and 3-methylbutanal were relatively higher in BS-LA

than BS-LB. Furthermore, the contents of esters of BS-LA significantly increased during fermentation. These results indicate that the volatile profiles of BS samples depend on the fermentation periods and the combination of microbial starters.

Keywords *Soksungjang* · Soybean *kaji* · Volatile profiles · Fermentation · Partial least squares-discriminant analysis

Introduction

Fermented soybean products have been consumed in most parts of the world due to their characteristic flavors as well as nutritional and functional benefits (Choi et al., 2009; Kwak et al., 2007; Wang et al., 2008). The formation of these flavors is generally determined by the predominant microorganisms present during fermentation. These microorganisms degrade macromolecules (e.g., proteins, carbohydrates, and lipids) in the main ingredients via various enzymes and cause further biological and chemical reactions, which can produce and enhance the characteristic tastes and aromas (Park et al., 2010). Various microorganisms, such as fungi, bacteria, yeast, and lactic acid bacteria, were involved in fermented soybeans products. In particular, *Aspergillus oryzae*, *Bacillus amyloliquefaciens*, and *Lactobacillus* spp. were reported as important starters for soybean fermentation (Kim et al., 2009a; 2009b; Lee et al., 2014; Lee et al., 2017). However, their combinational effects on the formation of volatile compounds in fermented soybean products have not been clearly determined. Thus, a synergistic combination of lactic acid bacteria with bacteria or fungi was applied to *soksungjang* in this study.

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Soksungjang (also called ‘*beolmijang*’) is one of the traditional fermented soybean pastes consumed in Korea. It is very similar to *doenjang* (the most popular fermented soybean product). However, it has a shorter fermentation period (e.g., 5–6 weeks) than that of *doenjang* and is made by mixing, soaking, steaming, molding, and fermenting soybeans together with grains (Choi et al., 2011; Park et al., 2017). In this study, buckwheat was considered as one of grain source due to absence of gluten and various health benefits (Kreft et al., 1999; Park et al., 2000).

Compositions and contents of volatile compounds in fermented soybean products has steadily studied and, in particular, butanoic acid, 3-methylbutanoic acid, 2-pentylfuran, 2- and 3-methylbutanal, methyl 2-methylbutanoate, 4-methylpentanoate, and some pyrazines were regarded as main volatile compounds having unique flavor characteristics in fermented soybean pastes (Cho et al., 2017; Yoo et al., 1999). On the other hand, some volatile compounds, such as 2- and 3-methylbutanal, phenylacetaldehyde, furfural, hexanal, 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone, 2,4-decadienal, and phenol compounds, were reported as main volatile compounds in various buckwheat-based products (Starowicz et al., 2018). However, there is a lack of research on volatile compounds in buckwheat *soksungjang* (BS). Our previous study profiled and compared the volatile compounds present during fermentation between BS samples manufactured traditionally and commercially (Park et al., 2017). It was demonstrated that there are differences in the volatile profiles between *soksungjang* fermented with starters (*A. oryzae* + *B. amyloliquefaciens*, in the commercial method) and without starters (in the traditional method). In particular, the inoculation of starter(s) at the initial stage of fermentation could accelerate the biochemical reactions (especially the more effective breakdown of soybean proteins) and the production of secondary metabolites.

In this study, we focused on the differences and changes in volatile profiles between two different BS samples prepared with *L. brevis* and *A. oryzae* or *B. amyloliquefaciens* during fermentation in order to investigate combinational effects of lactic acid bacteria with bacteria or fungi. Moreover, partial least squares-discriminant analysis (PLS-DA) was applied to elucidate the main contributors to sample discrimination.

Materials and methods

Materials and chemicals

Soybeans (Korean *Bactae*, *Glycine max* L.) were obtained from local market. *L. brevis* JSB22, *A. oryzae* PS03, and *B. amyloliquefaciens* were previously isolated from Korean

traditional fermented products. The SPME fiber (carboxen/polydimethylsiloxane fiber, 75 μ m) and automatic SPME holder were purchased from Supelco (Bellefonte, PA, USA). Methyl cinnamate, an internal standard, and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sample preparation

BS samples was produced using a previously described method (Park et al., 2017). In brief, steamed soybeans were inoculated by two different starters (*L. brevis* JSB22 + *A. oryzae* PS03, BS-LA or *L. brevis* JSB22 + *B. amyloliquefaciens* RD7-7, BS-LB). Then, they were mixed with buckwheat at 7:3 ratio (w/w) and ripened at 20–25 °C for 5 weeks. BS-LA and BS-LB samples were collected after 0, 1, 2, and 5 weeks of fermentation, and their volatiles were analyzed.

Analysis of volatile compounds in BS samples

Volatile compounds in BS samples were extracted by solid phase micro-extraction (SPME) coated with carboxen/polydimethylsiloxane fiber. Then GC–MS analysis was conducted using a 7890B series gas chromatograph connected to a 5977A mass selective detector (MSD) (Agilent Technologies) equipped with DB-FFAP capillary column (30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness, J&W Scientific, Folsom, CA, USA) and multi-purpose sampler MPS 2 (Gerstel, Mülheim an der Ruhr, Germany). Volatile compounds were identified and quantified according to the method of Park et al. (2017). The identification was accomplished with comparing of their mass spectral database (NIST 08 and Wiley 9n.1) and retention index (RI). Quantitative data was calculated by comparing their peak areas to that of the internal standard compound.

Statistical analysis

PLS-DA was applied to the raw values ($n = 3$) of the relative peak areas obtained by GC–MS using SIMCA-P software (version 11.0, Umetrics, Umeå, Sweden). Analysis of variance and the *t* test were implemented using SPSS statistical package (version 12.0, Chicago, IL, USA). The criterion for significant difference was $p < 0.05$.

Results and discussion

Comparison of volatile profiles between BS-LA and BS-LB

PLS-DA was applied to investigate the differences and changes in volatile compounds (based on Supplementary Table 1) among BS samples inoculated with different

multiple microbial starters during fermentation. PLS-DA is commonly performed to identify the separation between groups of observations and to understand which variables carry the class-separating information (Ledauphin et al., 2010; Zhang et al., 2013). Figure 1 shows the distribution of all BS samples for the first and second components of the PLS-DA score plot. The values of the model parameters ($R^2Y = 0.98$ and $Q^2 = 0.91$) indicated high degree of explained variance (R^2Y) and cross-validated predictive capability (Q^2). The BS-LA and BS-LB samples during the initial and intermediate stages of fermentation (below 2 weeks) were located on the positive $t[1]$ axis, and those fermented for 5 weeks (later stage of fermentation) were separated on the negative $t[2]$ axis. These observations could be explained by the change in volatile profiles among BS samples inoculated with those starters in the later stage of fermentation (from 2 to 5 weeks) being accelerated relative to those in the initial and middle stage of fermentations (from 0 to 2 weeks).

Changes of volatile profiles in two different BS samples according to fermentation periods

The changes in the volatile profiles of each BS samples according to fermentation periods (0, 1, 2, and 5 weeks) were also observed in the PLS-DA models, respectively (Fig. 2A, B). In both score plots, BS samples fermented for below 2 weeks and for 5 weeks could be discriminated by $t[1]$. Tables 1 and 2 show significant variables contributing to the $t[1]$ dimension (p value > 0.07) based on a threshold of 0.7 on the validity importance in projection (VIP) test. VIP values from PLS-DA that describes a quantitative estimation of the discriminatory power of each individual

feature. It is used to separate samples and identify important individual features that maximize an ability of classification (Cho et al., 2008). The main variables that contribute to the positive $t[1]$ dimension among BS-LA samples were 2-phenylethanol and 2-ethylhexan-1-ol (Table 1), whereas, dimethyl carbonate, pentanoic acid, and 2-(2-ethoxyethoxy)ethanol were major compounds related to the positive $t[1]$ dimension among BS-LB samples during fermentation (Table 2).

In addition, more variables contributed to the negative $t[1]$ dimension (in the later period of fermentation) in both BS samples, which means that the generation of volatile compounds could enhance and alter the increase in microbial activities during fermentation. The important contributors to the later period of fermentation (5 weeks) in the BS-LA sample were methyl esters of fatty acids (e.g., methyl heptanoate, methyl octanoate, and methyl butanoate), which would be mainly generated via the β -oxidation pathway of fatty acids (Etschmann et al., 2002). Moreover, esters (e.g., 3-methylbutyl acetate, methyl acetate, hexyl acetate, hexyl acetate, ethyl and methyl 2-phenylacetate), fusel aldehydes, alcohols, and acids (e.g., 3-methylbutanal, benzaldehyde, 2-methylbutanal, and 3-methylbutanoic acid), which were produced via *ehrich* pathway (Smit et al., 2009), were also represented as main volatile compounds at a later part of fermentation.

On the other hand, acetic acid, which are typical compounds via the metabolism of LAB in fermented products (Coulibaly et al., 2017), were predominantly found in the later period of fermentation in BS-LB. During fermentation, *L. brevis*, a heterofermentative LAB, can convert lactic acid to acetic acid in order to providing a stable pH in the presence of oxygen (Xu et al., 2017). It might be explained that the activity of *L. brevis* was higher when be in BS-LB samples than BS-LA samples. On the other hand, butane-2,3-diol was shown as an important variables at BS-LB samples. *B. amyloliquefaciens* is regarded as safe and efficient butane-2,3-diol producer as well as *L. brevis* (Song et al., 2018).

Differences of volatile compositions between two BS samples

As shown in Fig. 1, BS samples fermented for 5 weeks were noticeably distinguishable from BS samples fermented for shorter periods (less than 2 weeks). At this work, volatile compositions and contents of two different samples fermented for 5 weeks were compared in order to elucidate the differences between BS-LA and BS-LB samples. Table 3 lists the profiles of the volatile compounds (a total of 83) identified in BS-LA and BS-LB fermented for 5 weeks, their retention index (RI), and relative peak areas on DB-FFAP columns. The volatile

Fig. 1 PLS-DA score plot of BS samples prepared by different multiple starters during fermentation (filled triangle, BS-LA and open triangle, BS-LB)

Fig. 2 PLS-DA score plots of BS-LA (A) and BS-LB (B) samples according to fermentation periods (filled triangle, BS-LA and open triangle, BS-LB)

Table 1 The contribution of volatile variances with p value > 0.7 in BS-LA samples according to fermentation periods

No. ^a	Compound	p Value ^b	No.	Compound	p Value
v77	2-Phenylethanol	0.10	V36	Haxan-1-ol	– 0.07
v48	2-Ethylhexan-1-ol	0.07	V62	3-Methylbutanoic acid	– 0.09
			v6	3-Methylbutanal	– 0.09
			v12	Decane	– 0.10
			v34	Methyl 2-hydroxypropanoate	– 0.10
			v15	Hexanal	– 0.10
			v74	Hexanoic acid	– 0.11
			v20	1,3-Xylene	– 0.12
			v3	Ethyl acetate	– 0.12
			v18	3-Methylbutyl acetate	– 0.12
			v40	Octan-3-ol	– 0.12
			v2	Methyl acetate	– 0.12
			v29	Hexyl acetate	– 0.12
			v10	Methyl butanoate	– 0.13
			v5	2-Methylbutanal	– 0.13
			v75	2-Methoxyphenol	– 0.13
			v39	Methyl octanoate	– 0.13
			v30	Methyl heptanoate	– 0.13
			v70	Ethyl 2-phenylacetate	– 0.13
			v82	5-Pentyloxolan-2-one	– 0.13
			v76	Phenylmethanol	– 0.13
			v54	Methyl decanoate	– 0.13
			v69	Methyl 2-phenylacetate	– 0.13
			v57	Methyl benzoate	– 0.13
			v86	4-Ethenyl-2-methoxyphenol	– 0.13
			v50	Benzaldehyde	– 0.13
			v73	Calamenene	– 0.13

^aNumbered as in the order of retention indices (RI)

^b p Value is the probability that the results from the sample data occurred by chance

Table 2 The contribution of volatile variances with p value > 0.7 in BS-LB samples according to fermentation periods

No. ^a	Compound	p Value ^b	No.	Compound	p Value
v11	Dimethyl carbonate	0.11	v43	Acetic acid	– 0.12
v67	Pentanoic acid	0.09	v10	Methyl butanoate	– 0.12
v56	2-(2-Ethoxyethoxy)ethanol	0.07	v18	3-Methylbutyl acetate	– 0.12
			V33	Oct-1-en-3-one	– 0.12
			V35	Ethyl 2-hydroxypropanoate	– 0.11
			340	Octan-3-ol	– 0.11
			V15	Hexanal	– 0.11
			V51	Butane-2,3-diol	– 0.10
			V37	(Methyltrisulfanyl)methane	– 0.10

^aNumbered as in the order of retention indices (RI)^b p Value is the probability that the results from the sample data occurred by chance

compounds in BS-LA comprised 3 acids, 4 aldehydes, 8 alcohols, 2 aliphatic hydrocarbons, 13 benzenes, 25 esters, 4 furans, 2 lactones, 2 phenols, 1 sulfur-containing compound, 3 terpenes, and 1 other compound. On the other hand, the volatile compounds in BS-LB comprised of 5 acids, 4 aldehydes, 10 alcohols, 4 aliphatic hydrocarbons, 14 benzenes, 15 esters, 3 furans, 1 ketone, 2 lactones, 2 pyrazines, 4 phenols, 1 sulfur-containing compound, 3 terpenes, and 1 other compound.

Figure 3 shows the quantitative composition of identified volatile compounds in both BS samples according to chemical functional groups. In particular, esters, benzenes, and acids predominated in both BS-LA and BS-LB fermented for 5 weeks: esters > benzenes > acids in BS-LA vs acids > benzenes > esters in BS-LB. In general, volatile compounds in fermented soybeans can be produced by various complex metabolism processes, such as glycolysis, lipolysis, and pyruvate and proteolytic metabolism processes, with microorganisms being involved as the fermentation process progresses. In particular, mutual exchanges between two or more microbial starters also occur in different ways during the fermentation process, which increases the complexity of generating volatile compounds as secondary metabolites (Singh and Lee, 2018). Table 3 also shows the differences of two BS samples comparing relative contents of volatile compounds in detail.

Numerous studies focused on *Bacillus* and *Aspergillus* species as the main microorganisms in fermented soybean products and found volatile organic acids, alcohols, and esters were predominant and their levels varied with the associated microorganisms during fermentation process (Kum et al., 2015; Leejeerajumnean et al., 2001; Seo et al., 2018). Recently, Seo et al. (2018) reported the differences of volatile profiles between *koji* fermented by *B. amyloliquefaciens* and *A. oryzae*. 2-Methoxyphenol, 2-methylpropanoic acid, 3-methylbutanoic acid, and

4-methylpentanoic acid, exhibiting cheesy and rancid odor notes, were more found in *B. koji*, in contrast, the contents of some volatiles related to malty odor note (e.g., 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal) were relatively higher in *A. koji* compared to those of *B. koji* (Seo et al., 2018).

In this study, (1) higher levels of acids were found in a BS-LB sample, except 3-methylbutanoic acid having low threshold and cheesy and rancid odor notes (Park et al., 2007). However, the level of 3-methylbutanoic acid has shown a remarkable increase according to fermentation periods in BS-LB compared to in BS-LA (Supplementary Table 1). Acetic acid could be generated by catalytic oxidation from aldehydes, which could be produced by the catalytic hydration of acetylene or by the catalytic dehydrogenation of ethanol, and its level was twofold higher in BS-LB than in BS-LA. Heptanoic acid and octanoic acid, which could be produced by lipolysis metabolism, were detected only in BS-LB. (2) Some aldehydes, such as 2- and 3-methylbutanal, showed higher contents in BS-LA compared to BS-LB, corresponding to previous study (Seo et al., 2018). Furthermore, 6-methylhept-5-en-2-ol and 2-ethylhexan-1-ol were only detected in BS-LB. (3) Benzenes were also predominant volatile compounds in both BS-LA and BS-LB in this study. Most of the benzenes identified usually originate from phenylalanine catabolism, which could vary with the type and level of activity of enzymes. This study found that higher levels of styrene, benzaldehydes, 2-phenyl acetaldehyde, and phenylmethanol (which come from the catabolism of cinnamate) in BS-LB, while the level of 2-phenylethanol was higher in BS-LA (Etschmann et al., 2002). It appears that phenylalanine catabolism is relatively accelerated in BS-LB fermentation. (4) Esters reportedly contribute to positive aroma properties (described as fruity or flowery odor notes) of food (Reineccius, 2006), and they could be formed by the esterification of alcohols with fatty acids (Zhao et al.,

Table 3 Comparison of the volatile compounds between BS samples fermented for 5 weeks

No. ^a	Compound	RI ^b	Relative peak area ^c		<i>t</i> Value ^d	<i>p</i> Value ^e
			BS-LA-5w ^f	BS-LB-5w ^g		
<i>Acids</i>						
v43	Acetic acid	1445	29.428 ± 1.888	40.662 ± 6.126	3.035	0.039*
v62	3-Methylbutanoic acid	1673	0.711 ± 0.164	0.495 ± 0.065	− 2.117	0.102
v74	Hexanoic acid	1850	0.607 ± 0.143	0.951 ± 0.257	2.025	0.113
v78	Heptanoic acid	1942	ND ^h	0.094 ± 0.030	5.410	0.033*
v83	Octanoic acid	> 2000	ND	0.168 ± 0.026	11.002	0.000*
<i>Aldehydes</i>						
v5	2-Methylbutanal	916	0.242 ± 0.039	0.102 ± 0.010	− 6.069	0.004*
v6	3-Methylbutanal	918	1.777 ± 0.170	0.960 ± 0.089	− 7.373	0.002*
v15	Hexanal	1080	0.153 ± 0.030	0.228 ± 0.040	2.624	0.059
v22	3-Methylbut-2-enal	1203	0.577 ± 0.088	0.131 ± 0.017	− 8.663	0.001*
<i>Alcohols</i>						
v7	Ethanol	934	1.172 ± 0.244	1.307 ± 0.328	0.572	0.598
v23	3-Methylbutan-1-ol	1210	0.406 ± 0.098	0.577 ± 0.037	2.817	0.480
v36	Hexan-1-ol	1352	0.389 ± 0.112	0.538 ± 0.186	1.194	0.298
v38	(Z)-Hex-3-en-1-ol	1384	0.127 ± 0.037	0.174 ± 0.064	1.116	0.327
v40	Octan-3-ol	1392	0.351 ± 0.081	0.239 ± 0.059	− 1.943	0.124
v44	Oct-1-en-3-ol	1450	4.064 ± 0.897	3.964 ± 0.982	− 0.131	0.902
v45	6-Methylhept-5-en-2-ol	1459	ND	0.573 ± 0.138	7.196	0.019*
v48	2-Ethylhexan-1-ol	1491	ND	0.264 ± 0.070	6.522	0.003*
v51	Butane-2,3-diol	1544	0.744 ± 0.074	0.560 ± 0.180	− 1.643	0.176
v55	4-Methyl-1-propan-2-ylcyclohex-3-en-1-ol	1603	0.810 ± 0.175	0.553 ± 0.100	− 2.210	0.092
<i>Aliphatic hydrocarbons</i>						
v8	2-Methylnonane	956	ND	0.600 ± 0.129	8.038	0.001*
v9	3-Methylnonane	965	ND	0.364 ± 0.021	29.931	0.001*
v12	Decane	1002	0.555 ± 0.078	2.982 ± 0.909	4.607	0.010*
v32	Tridecane	1300	0.234 ± 0.058	0.059 ± 0.015	− 5.057	0.007*
<i>Benzenes and benzene derivatives</i>						
v13	Toluene	1038	0.879 ± 0.140	0.914 ± 0.143	0.304	0.776
v17	Ethylbenzene	1098	0.143 ± 0.032	0.167 ± 0.055	0.679	0.535
v19	1,4-Xylene	1136	0.799 ± 0.197	0.682 ± 0.013	− 1.021	0.365
v20	1,3-Xylene	1180	0.406 ± 0.047	0.443 ± 0.049	0.948	0.397
v27	Styrene	1246	0.672 ± 0.050	0.226 ± 0.049	− 11.029	0.000*
v50	Benzaldehyde	1532	8.670 ± 0.640	3.202 ± 0.553	− 11.199	0.000*
v57	Methyl benzoate	1629	5.532 ± 0.165	0.351 ± 0.152	− 39.999	0.000*
v60	2-Phenylacetaldehyde	1653	14.037 ± 1.464	5.452 ± 0.852	− 8.779	0.002*
v63	2-Hydroxybenzaldehyde	1689	0.695 ± 0.147	0.238 ± 0.055	− 5.053	0.007*
v69	Methyl 2-phenylacetate	1767	0.302 ± 0.012	0.080 ± 0.013	− 21.018	0.000*
v70	Ethyl 2-phenylacetate	1793	0.241 ± 0.033	0.189 ± 0.040	− 1.718	0.161
v76	Phenylmethanol	1885	0.948 ± 0.048	0.850 ± 0.121	− 1.306	0.262
v77	2-Phenylethanol	1918	ND	0.676 ± 0.091	12.881	0.006*
v93	Benzoic acid	> 2000	1.117 ± 0.226	0.311 ± 0.023	− 6.154	0.024*
<i>Esters</i>						
v2	Methyl acetate	835	10.574 ± 0.469	2.560 ± 0.579	− 18.627	0.000*
v3	Ethyl acetate	891	2.843 ± 0.190	1.195 ± 0.324	− 7.594	0.004*
v10	Methyl butanoate	986	0.139 ± 0.020	0.076 ± 0.007	− 5.132	0.007*
v18	3-Methylbutyl acetate	1120	0.341 ± 0.097	0.368 ± 0.097	0.338	0.753
v21	Methyl hexanoate	1185	8.307 ± 1.327	0.374 ± 0.082	− 10.338	0.009*
v25	Ethyl hexanoate	1230	ND	0.076 ± 0.020	6.607	0.003*
v28	Methyl (E)-hex-3-enoate	1260	0.462 ± 0.140	ND	− 5.701	0.029*
v29	Hexyl acetate	1271	0.225 ± 0.045	0.197 ± 0.020	− 0.974	0.385
v30	Methyl heptanoate	1288	0.895 ± 0.025	ND	− 62.606	0.000*
v34	Methyl 2-hydroxypropanoate	1320	2.637 ± 0.574	0.477 ± 0.049	− 6.493	0.022*

Table 3 continued

No. ^a	Compound	RI ^b	Relative peak area ^c		<i>t</i> Value ^d	<i>p</i> Value ^e
			BS-LA-5w ^f	BS-LB-5w ^g		
v35	Ethyl 2-hydroxypropanoate	1347	0.564 ± 0.177	0.506 ± 0.023	− 0.563	0.629
v39	Methyl octanoate	1390	1.449 ± 0.187	ND	− 13.390	0.006*
v49	Methyl nonanoate	1493	0.739 ± 0.242	ND	− 5.280	0.034*
v54	Methyl decanoate	1596	0.267 ± 0.029	ND	− 15.835	0.000*
v66	Benzyl acetate	1735	0.159 ± 0.022	0.167 ± 0.019	0.470	0.663
v71	Methyl dodecanoate	1804	0.468 ± 0.052	ND	− 15.468	0.000*
v80	Methyl tetradecanoate	> 2000	1.507 ± 0.304	ND	− 8.575	0.001*
v84	Methyl pentadecanoate	> 2000	0.484 ± 0.168	ND	− 4.986	0.008*
v87	Methyl hexadecanoate	> 2000	43.250 ± 9.761	3.554 ± 0.395	− 7.038	0.002*
v88	Methyl (Z)-hexadec-9-enoate	> 2000	0.554 ± 0.110	ND	− 8.718	0.001*
v89	Ethyl hexadecanoate	> 2000	2.363 ± 0.381	0.384 ± 0.041	− 8.938	0.011*
v90	Methyl heptadecanoate	> 2000	0.107 ± 0.031	ND	− 5.981	0.004*
v92	Methyl octadecanoate	> 2000	1.206 ± 0.325	0.083 ± 0.018	− 5.978	0.004*
v94	Methyl octadec-9-enoate	> 2000	11.592 ± 3.026	0.827 ± 0.116	− 6.157	0.004*
v95	Methyl (9Z,12Z)-octadeca-9,12-dienoate	> 2000	10.774 ± 2.210	0.524 ± 0.143	− 8.018	0.015*
v96	Methyl octadeca-9,12,15-trienoate	> 2000	0.879 ± 0.181	ND	− 8.420	0.014*
<i>Furans</i>						
v24	2-Pentylfuran	1229	0.463 ± 0.379	0.161 ± 0.023	− 1.380	0.301
v26	2-(Methoxymethyl)Furan	1234	0.088 ± 0.014	ND	− 10.704	0.009*
v46	Furan-2-carbaldehyde	1475	9.378 ± 1.554	2.349 ± 0.867	− 6.842	0.002*
v61	Furan-2-ylmethanol	1668	2.769 ± 0.440	1.217 ± 0.317	− 4.959	0.008*
<i>Ketones</i>						
v33	Oct-1-en-3-one	1311	ND	0.147 ± 0.045	5.658	0.030*
<i>Lactones</i>						
v64	5-Ethylloxolan-2-one	1711	0.575 ± 0.063	0.201 ± 0.036	− 8.979	0.001*
v82	5-Pentylloxolan-2-one	> 2000	0.170 ± 0.014	0.076 ± 0.013	− 8.397	0.001*
<i>Pyrazines</i>						
v42	2,3,5-Trimethylpyrazine	1409	ND	0.081 ± 0.026	5.442	0.006*
v47	2,3,5,6-Tetramethylpyrazine	1476	ND	0.567 ± 0.125	7.882	0.001*
<i>Phenols</i>						
v75	2-Methoxyphenol	1868	0.078 ± 0.014	0.188 ± 0.023	7.034	0.002*
v81	Phenol	> 2000	ND	0.155 ± 0.022	12.221	0.007*
v86	4-Ethenyl-2-methoxyphenol	> 2000	0.107 ± 0.002	0.127 ± 0.016	2.152	0.159
v91	4-Ethenylphenol	> 2000	ND	0.117 ± 0.013	15.314	0.004*
<i>Sulfur-containing compounds</i>						
v37	(Methyltrisulfanyl)methane	1380	0.461 ± 0.127	0.071 ± 0.013	− 5.298	0.032*
<i>Terpenes</i>						
v65	Alpha-Murolene	1724	0.523 ± 0.138	0.219 ± 0.067	− 3.435	0.026*
v68	Delta.-Cadinene	1758	1.286 ± 0.904	0.337 ± 0.041	− 1.816	0.211
v73	Calamenene	1834	0.960 ± 0.023	0.401 ± 0.098	− 9.574	0.008*
<i>Miscellaneous compounds</i>						
v79	3-Hydroxy-2-methylpyran-4-one	1979	7.767 ± 1.983	4.246 ± 0.607	− 2.941	0.042*

^aNumbered as in the order of retention indices (RI)^bRetention indices were determined using *n*-paraffins C₇–C₂₂ as external standards^cMean value of relative peak areas to that of an internal standard ± standard deviation^d*t* Value is the ratio of the departure of the estimated value of a parameter from its hypothesized value to its standard error (*Significant difference in *p* < 0.05)^e*p* Value is the probability that the results from the sample data occurred by chance^fBuckwheat *soksungjang* inoculated by *L. brevis* and *A. oryzae* and fermented for 5 week^gBuckwheat *soksungjang* inoculated by *L. brevis* and *B. amyloliquefaciens* and fermented for 5 week^hNot detected

Fig. 3 Composition of volatile compounds in BS samples prepared by different multiple starters during fermentation (100% stacked bar chart)

2009). Relatively high levels of esters were found in BS-LA in the present study, with the amounts of ethyl esters of fatty acids being higher in BS-LA compared to BS-LB. It is usually more difficult to form ethyl esters than methyl esters due to the different activities of alcohols with catalysts required to produce the alkoxide ion during biological and/or chemical reactions during fermentation (Meher et al., 2006). Thus, the esterification reactions were probably more active in BS-LA. (5) On the other hand, pyrazines in fermented soybean paste could be generated by the Maillard reaction and the activity of *B. species* (Lee and Ahn, 2009), and two pyrazines (2,3,5-trimethyl- and 2,3,5,6-tetramethylpyrazines) were found only in BS-LB in this study. In summary, these observations indicate that the diversity of volatile compounds produced by microbial starters in BS samples can vary with the fermentation period and multiple combinations of microorganisms, which might be due to changes in their own activities or their potential to act enzymatically.

This study found that the profiles of volatile compounds in BS samples varied with the fermentation period and the type of microbial starters. In general, volatile compounds

in fermented soybeans can be generated by various complex metabolism processes with microorganisms being involved as the fermentation process progresses. In this study, fusel acids and aldehydes, ethyl ester of fatty acids were relatively superior in BS-LA, while, catabolism of cinnamate and pyrazines were highly promoted in BS-LB. This study elucidated the impact of multiple starters, especially lactic acid bacteria and other microorganisms, on the formation of volatile compounds and could provide fundamental information of optimizing fermentation conditions of buckwheat *soksungjang*.

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