Change in profiles of volatile compounds from two types of *Fagopyrum esculentum* (buckwheat) *soksungjang* during fermentation

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Abstract *Fagopyrum esculentum* (buckwheat) *soksungjang* is one of the traditional soybean pastes in Korea. This study profiled and compared volatile compounds between traditionally manufactured (TBS) and commercially modified buckwheat *soksungjang* (CBS) according to their fermentation periods. More volatile compounds were generated and non-uniform increases or decreases in volatiles were more common during TBS fermentation. In addition, the changes in and differences between the volatiles from TBS and CBS during the fermentation process (after 0, 1, 2, and 5 weeks) were investigated in partial least squares-discriminant analysis models. The changes were accelerated during CBS fermentation in comparison with TBS fermentation. Several major volatile compounds, such as methyl decanoate, 3-hydroxy-2,6-dimethylpyran-4-one, and methyl heptanoate were found in the final stage of fermentation in TBS, in contrary, tridecane, (Z)-hex-3-en-1-ol, furan-2-carbaldehyde, and ethyl tetradecanoate were contributed to the latest of fermentation in CBS.

Keywords Buckwheat *soksungjang* · Volatile profile · Fermentation · Novel starter · Partial least squares-discriminant analysis

Introduction

Various fermented soybean products (e.g., Korean *doenjang* and *gochujang*, Japanese *miso* and *natto*, and Chinese *douchi*) have been enjoyed for more than thousands of years in Eastern Asia. Their consumption has recently been increasing due not only to their own characteristic flavors but also their various nutritional (high protein) and functional benefits, such as anti-oxidant [1, 2], fibrinolytic [3, 4], and anti-cancer [5, 6] effects. In particular, *doenjang* is the oldest and the most popular Korean fermented soybean product. Traditional *doenjang* is made with *meju*, which is prepared by soaking, streaming, and crushing soybeans, forming them into a rectangular block, and then hanging this up with rice straw for 1–3 months to facilitate the growth of wild microorganisms (e.g., *Bacillus*, *Lactobacillus*, *Aspergillus* species, and yeasts) [7, 8]. *Meju* is then brined and ripened for another 2–3 months. After fermentation, it is separated into two parts (e.g., liquid and solid residue) and the solids are additionally ripened for more than 2-month (*doenjang*).

Soksungjang (literally, ‘*soksung*’ of *soksungjang* in Korean means ‘quick’ in English) is similar to *doenjang*, except that it is made by mixing, soaking, steaming, molding, and fermenting soybeans together with grains (e.g., barley, rice, wheat, and buckwheat). It requires a shorter (e.g., 5–6 weeks) fermentation period than most fermented soybean products due to this, which generally require 6–12 months of fermentation. Furthermore, brining and ripening processes are applied, but not the separating process. In this study, *Fagopyrum esculentum* (buckwheat) *soksungjang* (BS) was prepared. Buckwheat is a rich source of starch, protein, dietary fiber, vitamin B, and minerals [9, 10]. In particular, its beneficial effects are mainly due to its abundance of flavonoids (especially,

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rutin) [11]. Rutin is the glycoside between the flavonol quercetin and the disaccharide rutinose (α -L-rhamnopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranose) that exhibits anti-oxidative, anti-inflammatory, and anti-carcinogenic effects, and can also reduce the fragility of blood vessels related to hemorrhagic disease and hypertension in humans [12].

In general, fermented soybean products have strong and characteristic flavors which can be caused by generating specific volatile compounds, such as butanoic acid (sweaty), 3-methylbutanoic acid (rancid, sweaty), 2-pentylfuran (beany), methyl 2-methylbutanoate (fruity), 2- and 3-methylbutanal (malty), and 2,3-dimethylpyrazine (nutty). Therefore, it is important to profile volatile compounds of fermented soybean products in order to predict their qualities [13, 14]. In addition, during fermentation, the volatile compounds were changed according to a starter which is a predominant microorganism due to its type and activity of enzymes. In this study, there are two types of BSs: (1) traditionally manufactured buckwheat *soksungjang* (TBS) is non-uniformly fermented by natural microorganisms, and thus unequal qualities can be produced during fermentation, while (2) commercial modified buckwheat *soksungjang* (CBS) is generally made by inoculating certain microorganisms (e.g., *Bacillus*, *Rhizopus*, *Mucor*, and *Aspergillus* species) that known to be the dominant microflora of fermented soybean products [15, 16]. Microorganisms in *soksungjang* degrade macromolecules (e.g., proteins, sugars, and fatty acids) in soybeans with various enzymes and provide a substrate pool for further biological and chemical reactions, which can produce the characteristic tastes and odors of fermented foods [17]. *Aspergillus oryzae* (PS03) and *Bacillus amyloliquefaciens* (RD7-7) previously isolated from *meju* were applied for CBS as the novel multi-starter in this study.

The aims of this study were (1) to profile and compare volatile compounds between two BSs, since the microorganisms involved in fermentation could contribute to the formation of unique flavor characteristics, and (2) to observe their changes and differences according to fermentation periods by multivariate statistical analysis.

Materials and methods

Sample preparation

BS samples were prepared by two different types of method, traditional and commercial modified methods. The procedures for manufacturing BS are as follows; soybeans were soaked in water and steamed at 121 °C for 40 min and mixed with buckwheat at 7:3 ratio (w/w). In CBS, the novel multi-starter (*Aspergillus oryzae* PS03 : *Bacillus amyloliquefaciens* RD7-7 = 1:1) was inoculated, and then

mixtures were formed in block shape (500 g). On the other hands, traditional BS (TBS) was only exposed to the natural strains. Their surfaces were dried at 25 ± 5 °C for 2 h using a drying oven (DS-80-2; Dasol Scientific Co., Ltd., Hwaseong-si, Korea), then put them at 28–30 °C for 2 weeks. The fermented soybeans were called, BS *meju*. Finally, BS samples were prepared by crushing dried BS *meju* submerged in 15.5% sea salt solution (1.0 kg dry BS *meju* plus 1.4 kg sea salt solution) at 20–25 °C during 5 weeks. Samples were collected at the 0, 1, 2, and 5 weeks of fermentation to analyze their volatile compounds.

Extraction of volatile compounds in BS

Solid phase micro-extraction (SPME) with carboxen/polydimethylsiloxane fiber (CAR/PDMS, 75 μ m, Supelco, Bellefonte, PA, USA) was used to extract volatile compounds in BS. BS sample (5.0 g) was added into a 20 mL vial (headspace screw top clear vial, Agilent technologies, Santa Clara, CA, USA) and sealed with a cap (Ultraclean 18 mm screw-cap w/septa, Agilent Technologies). The vial was kept at 60 °C with agitating at 250 rpm for 30 min to obtain an equilibrium state. SPME fiber (22 mm) was then exposed into the headspace of the vial to adsorb volatile compounds for 30 min. Then it was desorbed in the GC injection port (230 °C, splitless mode) for 5 min. All experiments were conducted in triplicate.

Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS analysis was performed using a 7890B series gas chromatograph connected to a 5977A mass selective detector (MSD) (Agilent Technologies) and multi-purpose sampler MPS 2 (Gerstel, Mülheim an der Ruhr, Germany) were used in the present study. 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) was equipped and the oven temperature was initially held at 40 °C (6 min) and followed by ramping to 200 °C (10 min) at a rate of 3 °C/min. The other GC–MS conditions were as follows: Both front inlet and detector transfer line temperatures were 230 °C. Helium, as a carrier gas, was used at a constant flow rate of 0.8 mL/min. Mass scanning range were 35–550 m/z and mass spectra were obtained at 70 eV in the electron ionization (EI) mode.

Identification and quantification

Volatile compounds were identified by comparing their mass spectral data with those of the National Institute of Standards and Technology (NIST) version 08 mass spectral

Table 1 The volatile compounds identified in TBS according to fermentation periods

No.	Possible compound	RI ¹	Relative Peak Area ²				ID ³
			0w	1w	2w	5w	
Acids							
v1	Acetic acid	1455	1.519 ± 0.347a ⁴	2.028 ± 0.340a	1.247 ± 0.180a	4.091 ± 0.892	B
v2	3-Methylbutanoic acid	1672	0.153 ± 0.018b	0.332 ± 0.088c	ND ⁵ a	NDa	B
v3	Hexanoic acid	1851	0.028 ± 0.003b	NDa	NDa	NDa	B
Alcohols							
v4	Ethanol	935	NDa	5.650 ± 0.741a	21.437 ± 5.471b	149.245 ± 13.491c	B
v5	4-Methoxybutan-1-ol	936	1.400 ± 0.076b	NDa	NDa	1.415 ± 0.312b	B
v6	2-Methylpropan-1-ol	1095	0.151 ± 0.030b	0.561 ± 0.110c	0.841 ± 0.096d	NDa	B
v7	Butan-1-ol	1102	NDa	NDa	NDa	0.480 ± 0.130b	B
v8	3-Methylbutan-1-ol	1206	3.589 ± 0.654a	8.273 ± 2.247a	8.027 ± 1.040a	22.876 ± 7.077b	B
v9	3-Methylbut-3-en-1-ol	1248	0.136 ± 0.014b	0.316 ± 0.092c	0.091 ± 0.027a	NDa	B
v10	2-Methylbut-2-en-1-ol	1321	0.263 ± 0.056b	0.842 ± 0.198c	0.328 ± 0.056b	NDa	B
v11	Hexan-1-ol	1354	0.073 ± 0.015a	0.300 ± 0.050b	0.244 ± 0.045b	0.262 ± 0.074b	B
v13	Oct-1-en-3-ol	1450	0.647 ± 0.171a	8.956 ± 1.412d	4.719 ± 0.722b	7.284 ± 0.554c	B
v15	Butane-2,3-diol	1542	1.529 ± 0.291a	1.232 ± 0.284a	1.571 ± 0.236a	2.690 ± 0.212b	B
v16	Butane-1,3-diol	1580	1.729 ± 0.196ab	2.498 ± 0.521b	1.143 ± 0.157a	2.070 ± 0.267ab	B
v17	4-Methyl-1-propan-2-ylcyclohex-3-en-1-ol	1604	0.063 ± 0.012a	0.709 ± 0.141c	0.450 ± 0.112b	NDa	B
v18	(4-Terpineol)						
	3-Hydroxy-2,6-dimethylpyran-4-one (Methyl maltol)	1970	0.071 ± 0.012a	0.846 ± 0.074c	0.591 ± 0.177b	0.672 ± 0.090b	B
v19	3-Hydroxy-2-methylpyran-4-one (Maltol)	1976	0.045 ± 0.010a	NDa	0.308 ± 0.087b	0.725 ± 0.218c	B
Benzens and benzene derivatives							
v20	1,3-Xylene	1104	NDa	NDa	NDa	0.414 ± 0.085b	B
v22	Benzaldehyde	1532	0.976 ± 0.127a	3.242 ± 0.406c	1.915 ± 0.153b	7.564 ± 0.725d	B
v24	Methyl benzoate	1630	1.285 ± 0.361a	11.987 ± 1.590b	8.945 ± 2.180b	16.327 ± 2.351c	B
v25	2-Phenylacetaldehyde	1654	0.855 ± 0.222a	9.236 ± 1.689c	6.464 ± 0.805b	13.536 ± 2.081d	B
v26	2-Hydroxy-benzaldehyde	1688	0.089 ± 0.011a	0.842 ± 0.088b	0.606 ± 0.133b	1.340 ± 0.197c	B
v27	1,2-Dmethoxybenzene	1734	0.037 ± 0.009a	0.465 ± 0.082bc	0.310 ± 0.043b	0.562 ± 0.144c	B
28	Methyl 2-phenylacetate	1766	0.107 ± 0.031a	2.377 ± 0.563b	1.737 ± 0.348b	3.411 ± 0.861c	B
v29	2-Phenylprop-2-enal	1810	0.172 ± 0.040a	3.655 ± 0.726bc	2.648 ± 0.659b	4.063 ± 0.764c	B
v30	Methyl 3-phenylpropanoate	1854	0.046 ± 0.010b	NDa	NDa	NDa	B
v31	Phenylmethanol	1884	0.197 ± 0.054a	2.517 ± 0.366c	1.707 ± 0.166b	2.912 ± 0.084d	B
v32	2-Phenylethanol	1915	0.366 ± 0.097a	7.096 ± 1.460b	8.040 ± 0.989b	25.367 ± 3.971c	B
v34	2-Phenylbut-2-enal	1939	NDa	0.102 ± 0.011a	0.452 ± 0.080a	1.894 ± 0.476b	B
v35	Benzoic acid	>2000	0.059 ± 0.011a	9.868 ± 0.211c	0.126 ± 0.024a	1.087 ± 0.778b	A
Carbonyls							
v37	2-Methylbutanal	915	0.523 ± 0.038b	1.452 ± 0.302c	0.587 ± 0.096b	NDa	B
v38	3-Methylbutanal	919	4.244 ± 0.419b	4.992 ± 1.296b	1.543 ± 0.297a	3.604 ± 1.165b	B
v39	Octan-3-one	1252	NDa	0.668 ± 0.147c	0.200 ± 0.028b	NDa	B
v40	3-Hydroxybutan-2-one	1288	0.115 ± 0.013b	NDa	NDa	NDa	B
v41	6-Methylhept-5-en-2-one	1337	NDa	0.419 ± 0.022c	0.279 ± 0.103b	NDa	B
Esters							
v42	Methyl acetate	<900	8.202 ± 1.219a	17.967 ± 3.742c	8.083 ± 1.418a	13.084 ± 2.259b	A
v43	Ethyl acetate	<900	NDa	NDa	0.878 ± 0.090a	4.905 ± 1.764b	A
v44	Methyl 2-methylpropanoate	922	NDa	NDa	0.532 ± 0.071b	NDa	B
v45	Methyl butanoate	989	0.243 ± 0.028b	0.655 ± 0.169d	0.463 ± 0.058c	NDa	B

Table 1 continued

No.	Possible compound	RI ¹	Relative Peak Area ²				ID ³
			0w	1w	2w	5w	
v46	Dimethyl carbonate	999	0.144 ± 0.011b	NDa	NDa	NDa	B
v47	Methyl 2-methylbutanoate	1009	NDa	NDa	0.938 ± 0.150b	NDa	B
v50	Methyl hexanoate	1186	0.284 ± 0.061a	3.467 ± 0.160d	1.619 ± 0.216b	2.920 ± 0.229c	B
v53	Ethyl hexanoate	1286	NDa	NDa	NDa	0.974 ± 0.137b	B
v54	Methyl heptanoate	1390	NDa	0.845 ± 0.151c	0.407 ± 0.044b	0.468 ± 0.114b	B
v55	Methyl octanoate	1431	0.257 ± 0.050a	3.468 ± 0.425d	2.016 ± 0.324b	2.719 ± 0.422c	B
v56	Methyl oct-4-enoate	1434	NDa	0.768 ± 0.124c	0.353 ± 0.048b	NDa	B
v57	Ethyl octanoate	1492	NDa	NDa	0.190 ± 0.046ab	0.643 ± 0.186b	B
v58	Methyl nonanoate	1533	0.098 ± 0.011a	1.146 ± 0.181c	0.481 ± 0.086b	NDa	B
v59	Methyl decanoate	1630	0.063 ± 0.015a	1.620 ± 0.177c	1.095 ± 0.256b	0.870 ± 0.120b	B
v60	Methyl pyridine-3-carboxylate	1767	NDa	NDa	NDa	0.153 ± 0.033b	B
v61	Ethyl 2-phenylacetate	1804	NDa	NDa	0.270 ± 0.058b	0.843 ± 0.202c	B
v62	Methyl dodecanoate	1811	0.093 ± 0.021a	1.279 ± 0.196b	1.188 ± 0.377b	NDa	B
v63	Ethyl dodecanoate	>2000	NDa	NDa	NDa	0.375 ± 0.049b	A
v64	Methyl tetradecanoate	>2000	0.386 ± 0.098a	3.960 ± 0.804b	3.780 ± 1.663b	3.614 ± 1.165b	A
v65	Ethyl tetradecanoate	>2000	NDa	NDa	0.152 ± 0.077a	1.135 ± 0.422b	A
v66	Methyl 12-methyltetradecanoate	>2000	0.075 ± 0.021a	0.434 ± 0.078b	0.210 ± 0.100a	0.435 ± 0.105b	A
v67	Methyl pentadecanoate	>2000	0.129 ± 0.038a	1.358 ± 0.124b	1.176 ± 0.578b	NDa	A
v68	Methyl 14-methylpentadecanoate	>2000	0.015 ± 0.013b	NDa	NDa	NDa	A
v69	Methyl hexadecanoate	>2000	6.453 ± 1.844a	80.933 ± 12.665c	65.240 ± 6.335b	NDa	A
v70	Methyl hexadec-7-enoate	>2000	0.089 ± 0.023a	1.356 ± 0.407a	2.044 ± 0.350a	19.767 ± 5.816b	A
v71	Methyl (Z)-hexadec-9-enoate	>2000	0.093 ± 0.018a	0.692 ± 0.169b	1.199 ± 0.246c	0.418 ± 0.075b	A
v72	Ethyl hexadecanoate	>2000	0.048 ± 0.012a	0.576 ± 0.175b	2.874 ± 0.480c	NDa	A
v73	Methyl (7E,10E)-hexadeca-7,10-dienoate	>2000	NDa	NDa	0.361 ± 0.082b	NDa	A
v75	Methyl octadecanoate	>2000	0.156 ± 0.053a	3.418 ± 0.900b	3.450 ± 0.926b	1.240 ± 0.190a	A
v76	Methyl octadec-9-enoate	>2000	1.846 ± 0.505a	21.875 ± 6.452b	19.762 ± 4.565b	0.497 ± 0.089a	A
v77	Methyl (9Z,12Z)-octadeca-9,12-dienoate	>2000	2.040 ± 0.638a	18.006 ± 4.342b	1.986 ± 0.201a	3.235 ± 0.275a	A
v79	Ethyl octadec-9-enoate	>2000	NDa	NDa	0.456 ± 0.170b	0.166 ± 0.057a	A
<i>Furans and furan derivatives</i>							
v80	Furan-2-carbaldehyde	1472	NDa	NDa	0.092 ± 0.008a	0.342 ± 0.099b	B
v81	Oxolan-2-one	1641	0.045 ± 0.007b	NDa	NDa	NDa	B
v82	Furan-2-ylmethanol	1667	0.032 ± 0.009b	0.189 ± 0.026c	0.222 ± 0.018d	NDa	B
v83	4-Methyl-2H-furan-5-one	1722	NDa	NDa	NDa	0.748 ± 0.108b	B
<i>Hydrocarbons</i>							
v85	2-Methylnonane	956	NDa	0.817 ± 0.055b	NDa	NDa	B
v87	Decane	1000	0.522 ± 0.099a	6.982 ± 0.528b	4.072 ± 0.864c	NDa	B
v88	Dodecane	1200	NDa	NDa	0.184 ± 0.035b	NDa	B
v89	Tridecane	1300	NDa	NDa	0.428 ± 0.083a	21.686 ± 4.288b	B
v90	Tetradecane	1400	NDa	NDa	0.172 ± 0.046b	NDa	B
v92	Heptadecane	1700	0.039 ± 0.008b	NDa	NDa	NDa	B
<i>Phenols</i>							
v93	2-Methoxyphenol	1868	0.028 ± 0.005a	0.502 ± 0.086b	0.524 ± 0.026b	0.464 ± 0.026b	B
v94	4-Ethyl-2-methoxyphenol	>2000	NDa	NDa	NDa	0.568 ± 0.196b	A
v95	2-Methoxy-4-prop-2-enylphenol	>2000	NDa	0.123 ± 0.019c	0.089 ± 0.020b	NDa	A
v96	3-Ethylphenol	>2000	NDa	0.077 ± 0.015a	0.046 ± 0.004a	58.997 ± 3.688b	A
v97	4-Ethenyl-2-methoxyphenol	>2000	0.053 ± 0.007a	1.135 ± 0.099b	1.535 ± 0.100c	2.505 ± 0.246d	A

Table 1 continued

No.	Possible compound	RI ¹	Relative Peak Area ²				ID ³
			0w	1w	2w	5w	
v98	4-Ethenylphenol	>2000	0.052 ± 0.007a	0.497 ± 0.078a	0.386 ± 0.039a	16.190 ± 7.193b	A
	Pyrazines						
v99	2,6-Dimethylpyrazine	1329	NDa	0.477 ± 0.127b	0.339 ± 0.094b	NDa	B
v101	2,3,5,6-Tetramethylpyrazine	1475	0.113 ± 0.015a	0.267 ± 0.014b	0.133 ± 0.015a	0.375 ± 0.054c	B
	<i>Sulfur-containing compounds</i>						
v102	(Methyltrisulfanyl)methane (Dimethyl trisulfide)	1380	0.031 ± 0.011a	NDa	0.108 ± 0.041b	NDa	B
v103	3-Methylsulfanyl-propan-1-ol (Methionol)	1722	NDa	NDa	0.164 ± 0.020a	1.890 ± 0.245b	B
	<i>Miscellaneous compounds</i>						
v104	Styrene	1208	NDa	NDa	NDa	4.448 ± 1.060b	B
v106	Alpha-cubebene	1435	NDa	NDa	NDa	0.267 ± 0.047b	B
v107	Alpha-murolene	1723	0.110 ± 0.022a	1.765 ± 0.220c	0.112 ± 0.025a	0.388 ± 0.045b	B
v108	Delta-cadinene	1755	0.153 ± 0.031a	2.194 ± 0.189b	1.537 ± 0.402b	1.806 ± 0.592b	B

¹ Retention indices were determined using n-paraffins C7–C22 as external standards

² Average of contents compared to that of the internal standard ± standard deviation

³ Tentative identification was performed as follow: A, mass spectrum was consistent with that of Wiley mass spectrum database; B, mass spectrum and retention index were consistent with those of the mass spectrum database and literatures

⁴ There are significant differences ($p < 0.05$) among samples collected according to fermentation period by using Duncan's multiple comparison test between the samples having different letter in low

⁵ Not detected

database. Also retention index (RI) of each compound were determined with *n*-paraffins from C₇ to C₂₂ as external standards. The relative level of each volatile compound was performed by comparing their peak areas to that of the internal standard compound (300 µl of 200 ppm methyl cinnamate in methanol) on the GC–MS total ion chromatogram (TIC).

Statistical analysis

Analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS, version 12.0, Chicago, IL, USA) program was conducted to evaluate statistical change/or difference of the volatile compounds in each BSs during fermentation, respectively. The result of Duncan's multi-range test presented the significant different level at $p < 0.05$. The values of volatile compounds were presented by average ± standard deviation of the three repetitive results. Partial least squares-discriminant analysis (PLS-DA) was conducted to the raw values ($n = 3$) of the relative peak areas obtained by GC–MS using SIMCA-P (version 11.0, Umetrics, Umeå, Sweden).

Results and Discussion

Volatile profiles from two BSs during the fermentation

The volatiles in the two BSs (TBS and CBS) were extracted using SPME and then analyzed by GC–MS. The volatile compounds identified in TBS and CBS according to their fermentation periods, their relative peak areas, and RIs on the DB-FFAP column were listed in Tables 1 and 2. A total of 91 and 80 volatile compounds were identified in TBS and CBS during fermentation, respectively. The volatile compounds of TBS encompassed 3 acids, 14 alcohols, 13 benzenes, 5 carbonyls, 32 esters, 4 furans, 6 hydrocarbons, 6 phenols, 2 pyrazines, 2 sulfur-containing compounds, and 4 miscellaneous compounds. On the other hand, volatiles of CBS were composed of 3 acids, 10 alcohols, 13 benzenes, 4 carbonyls, 28 esters, 3 furans, 6 hydrocarbons, 4 phenols, 3 pyrazines, 1 sulfur-containing compound, and 4 miscellaneous compounds. During the fermentation process of soybean products, various enzymes (predominantly protease, lipase, and amylase) can generate free amino acids, organic acids, mono- or di-saccharides, and fatty acids, which can then produce characteristic

Table 2 The volatile compounds identified in CBS according to fermentation periods

No.	Possible compound	RI ¹	Relative Peak Area ²				ID ³
			0w	1w	2w	5w	
<i>Acids</i>							
v1	Acetic acid	1454	1.467 ± 0.735a ⁴	1.609 ± 0.484a	1.737 ± 0.156a	1.269 ± 0.063a	
v2	3-Methylbutanoic acid	1672	0.133 ± 0.026a	0.193 ± 0.024a	0.730 ± 0.096c	0.380 ± 0.023b	
v3	Hexanoic acid	1849	ND ⁵ a	NDa	0.073 ± 0.015b	NDa	
<i>Alcohols</i>							
v5	4-Methoxybutan-1-ol	937	3.438 ± 0.445b	NDa	NDa	NDa	
v8	3-Methylbutan-1-ol	1207	1.966 ± 0.568b	0.472 ± 0.169a	2.526 ± 0.390b	0.716 ± 0.077a	
v11	Hexan-1-ol	1353	NDa	0.046 ± 0.007b	0.184 ± 0.037c	0.072 ± 0.019b	
v12	(Z)-Hex-3-en-1-ol	1383	NDa	NDa	NDa	0.067 ± 0.007b	
v13	Oct-1-en-3-ol	1451	0.106 ± 0.002a	0.067 ± 0.016a	1.873 ± 0.277c	1.000 ± 0.131b	
v14	2-Ethylhexan-1-ol	1492	0.018 ± 0.003a	0.230 ± 0.037b	NDa	NDa	
v15	Butane-2,3-diol	1544	4.424 ± 1.169c	0.111 ± 0.019a	2.805 ± 0.728b	0.614 ± 0.069a	
v16	Butane-1,3-diol	1581	0.736 ± 0.174a	3.158 ± 0.720b	0.839 ± 0.209a	0.149 ± 0.011a	
v17	4-Methyl-1-propan-2-ylcyclohex-3-en-1-ol (4-Terpineol)	1603	NDa	NDa	0.230 ± 0.012c	0.099 ± 0.001b	
v19	3-Hydroxy-2-methylpyran-4-one (Maltol)	1972	0.034 ± 0.003a	NDa	0.439 ± 0.182b	0.167 ± 0.008a	
<i>Benzens and benzene derivatives</i>							
v21	1,4-Xylene	1138	NDa	1.138 ± 0.527b	0.180 ± 0.030a	NDa	
v22	Benzaldehyde	1532	0.706 ± 0.095a	2.565 ± 0.363b	3.532 ± 0.340c	2.569 ± 0.348b	
v23	Benzonitrile	1613	NDa	NDa	0.075 ± 0.017c	0.054 ± 0.009b	
v24	Methyl benzoate	1629	0.289 ± 0.023a	0.029 ± 0.026a	7.844 ± 2.147c	3.229 ± 0.338b	
v25	2-Phenylacetaldehyde	1653	0.330 ± 0.041a	NDa	7.435 ± 1.010c	5.072 ± 0.574b	
v26	2-Hydroxybenzaldehyde	1679	0.067 ± 0.005a	NDa	1.062 ± 0.253c	0.672 ± 0.013b	
v27	1,2-Dimethoxybenzene	1757	NDa	0.065 ± 0.008c	NDa	0.055 ± 0.001b	
v28	Methyl 2-phenylacetate	1768	0.038 ± 0.001a	0.065 ± 0.006a	1.550 ± 0.349c	0.456 ± 0.035b	
v29	2-Phenylprop-2-enal	1812	0.045 ± 0.004a	0.028 ± 0.006a	1.194 ± 0.246c	0.860 ± 0.059b	
v30	Methyl 3-phenylpropanoate	1853	0.008 ± 0.013a	0.625 ± 0.153b	0.176 ± 0.032c	0.061 ± 0.011ab	
v31	Phenylmethanol	1886	0.025 ± 0.001a	0.116 ± 0.003a	0.550 ± 0.140b	0.293 ± 0.017b	
v32	2-Phenylethanol	1920	0.106 ± 0.009a	NDa	1.769 ± 0.312c	1.356 ± 0.065b	
v35	Benzoic acid	>2000	0.040 ± 0.008b	NDa	0.068 ± 0.060c	0.113 ± 0.005c	
<i>Carbonyls</i>							
v36	Butanal	<900	NDa	0.075 ± 0.032b	0.159 ± 0.033c	NDa	
v37	2-Methylbutanal	919	0.399 ± 0.078ab	0.559 ± 0.128b	1.037 ± 0.231c	0.179 ± 0.006a	
v38	3-Methylbutanal	921	3.475 ± 0.597c	1.674 ± 0.690ab	2.557 ± 0.248b	0.920 ± 0.061a	
v40	3-Hydroxybutan-2-one	1288	0.171 ± 0.042c	0.077 ± 0.009b	NDa	0.088 ± 0.005b	
<i>Esters</i>							
v42	Methyl acetate	<900	4.944 ± 0.506a	7.113 ± 0.886a	16.428 ± 2.521b	6.374 ± 0.043a	
v45	Methyl butanoate	988	NDa	0.138 ± 0.032b	0.294 ± 0.013c	0.146 ± 0.008b	
v46	Dimethyl carbonate	999	0.180 ± 0.048b	NDa	NDa	NDa	
v47	Methyl 2-methylbutanoate	1010	NDa	0.707 ± 0.105b	1.547 ± 0.254c	NDa	
v48	Methyl pentanoate	1019	NDa	NDa	0.286 ± 0.069b	NDa	
v49	Methyl 4-methylpentanoate	1145	NDa	0.049 ± 0.015a	2.453 ± 0.337c	0.836 ± 0.080b	
v50	Methyl hexanoate	1194	NDa	0.583 ± 0.099b	2.161 ± 0.302c	1.041 ± 0.071b	
v51	Methyl 4-methylhexanoate	1255	NDa	0.218 ± 0.036a	1.993 ± 0.400c	0.624 ± 0.029b	
v52	Methyl 4-methylpent-3-enoate	1277	NDa	0.209 ± 0.063b	0.182 ± 0.048b	0.068 ± 0.006a	
v55	Methyl octanoate	1391	0.081 ± 0.010a	0.521 ± 0.077b	1.787 ± 0.319c	0.705 ± 0.042b	
v56	Methyl oct-4-enoate	1432	NDa	0.272 ± 0.054c	0.476 ± 0.054d	0.122 ± 0.006b	
v58	Methyl nonanoate	1494	0.068 ± 0.014a	NDa	0.713 ± 0.143c	0.534 ± 0.098b	
v59	Methyl decanoate	1598	0.060 ± 0.017a	0.337 ± 0.042b	0.753 ± 0.139c	0.365 ± 0.062b	
v62	Methyl dodecanoate	1805	NDa	0.020 ± 0.004a	0.609 ± 0.128b	NDa	
v64	Methyl tetradecanoate	>2000	0.126 ± 0.023a	0.437 ± 0.019a	2.647 ± 0.360c	2.106 ± 0.163b	

Table 2 continued

No.	Possible compound	RI ¹	Relative Peak Area ²				ID ³
			0w	1w	2w	5w	
v65	Ethyl tetradecanoate	>2000	NDa	NDa	NDa	0.057 ± 0.005b	
v66	Methyl 12-methyltetradecanoate	>2000	0.070 ± 0.017ab	0.079 ± 0.068b	1.068 ± 0.108c	NDa	
v67	Methyl pentadecanoate	>2000	0.024 ± 0.002a	0.129 ± 0.012b	0.567 ± 0.058d	0.405 ± 0.041c	
v68	Methyl 14-methylpentadecanoate	>2000	NDa	0.052 ± 0.002b	0.237 ± 0.012c	0.317 ± 0.031d	
v69	Methyl hexadecanoate	>2000	1.109 ± 0.059a	0.033 ± 0.008a	29.559 ± 2.841c	16.946 ± 0.849b	
v70	Methyl hexadec-7-enoate	>2000	NDa	0.287 ± 0.003b	0.315 ± 0.062b	0.273 ± 0.026b	
v71	Methyl (Z)-hexadec-9-enoate	>2000	NDa	2.742 ± 0.293c	0.345 ± 0.044b	0.365 ± 0.028b	
v72	Ethyl hexadecanoate	>2000	NDa	0.269 ± 0.063c	0.151 ± 0.017b	1.504 ± 0.092d	
v74	Methyl heptadecanoate	>2000	NDa	1.971 ± 0.424b	0.049 ± 0.004a	0.048 ± 0.002a	
v75	Methyl octadecanoate	>2000	NDa	NDa	1.266 ± 0.342c	0.345 ± 0.036b	
v76	Methyl octadec-9-enoate	>2000	0.199 ± 0.030a	NDa	7.653 ± 1.647c	3.363 ± 0.147b	
v77	Methyl (9Z,12Z)-octadeca-9,12-dienoate	>2000	0.212 ± 0.024a	NDa	5.061 ± 0.347c	3.661 ± 0.189b	
v78	Methyl octadeca-9,12,15-trienoate	>2000	NDa	NDa	0.365 ± 0.039c	0.281 ± 0.030b	
<i>Furans and furan derivatives</i>							
v80	Furan-2-carbaldehyde	1460	NDa	NDa	NDa	0.107 ± 0.011b	
v81	Oxolan-2-one	1639	0.075 ± 0.020b	0.193 ± 0.033c	0.072 ± 0.016b	NDa	
v82	Furan-2-ylmethanol	1667	NDa	0.023 ± 0.020b	0.203 ± 0.010d	0.147 ± 0.007c	
<i>Hydrocarbons</i>							
v84	Hexane	<900	1.016 ± 0.256b	2.694 ± 0.322c	0.768 ± 0.014b	NDa	
v85	2-Methylnonane	956	NDa	0.199 ± 0.040b	0.465 ± 0.041d	0.313 ± 0.006c	
v86	3-Methylnonane	966	NDa	0.153 ± 0.043b	0.261 ± 0.039c	0.189 ± 0.007b	
v87	Decane	1000	0.405 ± 0.082a	1.669 ± 0.037b	4.194 ± 0.132c	4.480 ± 0.576c	
v89	Tridecane	1300	nda	NDa	NDa	0.148 ± 0.006b	
v91	Hexadecane	1600	0.050 ± 0.006b	0.151 ± 0.043c	NDa	NDa	
<i>Phenols</i>							
v93	2-Methoxyphenol	1870	0.040 ± 0.002a	0.092 ± 0.010a	4.879 ± 0.919c	3.183 ± 0.335b	
v95	2-Methoxy-3-prop-2-enylphenol	>2000	NDa	NDa	0.053 ± 0.012b	NDa	
v97	4-Ethenyl-2-methoxyphenol	>2000	0.023 ± 0.000a	0.051 ± 0.014a	0.599 ± 0.015c	0.468 ± 0.058b	
v98	4-Ethenylphenol	>2000	0.050 ± 0.007a	71.482 ± 6.445b	NDa	0.276 ± 0.028a	
<i>Pyrazines</i>							
v99	2,6-Dimethylpyrazine	1330	NDa	0.027 ± 0.000a	0.181 ± 0.041b	0.150 ± 0.010b	
v100	2,3,5-Trimethylpyrazine	1406	NDa	0.084 ± 0.021b	0.218 ± 0.049c	0.189 ± 0.009c	
v101	2,3,5,6-Tetramethylpyrazine	1477	0.091 ± 0.017b	0.039 ± 0.017a	0.162 ± 0.009d	0.116 ± 0.001c	
<i>Sulfur-containing compounds</i>							
v102	(Methyltrisulfanyl)methane	1380	NDa	0.046 ± 0.011b	0.157 ± 0.007d	0.084 ± 0.014c	
<i>Miscellaneous compounds</i>							
v105	1-(1H-Pyrrol-2-yl)ethanone (2-Acetyl-1-pyrroline)	1338	NDa	0.224 ± 0.037b	0.222 ± 0.012b	NDa	
v106	Alpha-cubebene	1434	NDa	NDa	NDa	0.073 ± 0.008b	
v107	Alpha-murolene	1725	0.031 ± 0.006a	0.307 ± 0.054b	0.702 ± 0.079c	0.000 ± 0.000a	
v108	Delta-cadinene	1758	0.038 ± 0.009a	0.035 ± 0.007a	0.829 ± 0.099c	0.293 ± 0.023b	

¹ Retention indices were determined using n-paraffins C7–C22 as external standards² Average of contents compared to that of the internal standard ± standard deviation³ Tentative identification was performed as follow: A, mass spectrum was consistent with that of Wiley mass spectrum database; B, mass spectrum and retention index were consistent with those of the mass spectrum database and literatures⁴ There are significant differences ($p < 0.05$) among samples collected according to fermentation period by using Duncan's multiple comparison test between the samples having different letter in low⁵ Not detected

flavor compounds [18]. In particular, volatile organic acids, alcohols, and esters have been considered important contributors to the unique flavor properties of fermented soybean products [19]. As indicated in Tables 1 and 2, these were also the predominant volatile compounds in the BSs in this study.

The relative levels of acetic acid and 3-methylbutanoic acid in CBS increased until 2 weeks of fermentation and then decreased after 5 weeks of fermentation. In contrast, the amount of acetic acid in TBS increased until 1 week of fermentation, decreased after 2-weeks of fermentation, and then again increased from 5 weeks of fermentation. Notably, 3-methylbutanoic acid was not detected in TBS from 2 weeks of fermentation. Previous studies reported that the content of volatile organic acids varied according to the predominant microorganism in fermented products, with *Bacillus* species being mainly associated with the production of these compounds [20, 21]. The irregular aspect of 3-methylbutanoic acid not being detected in TBS could have been due to the unequal progression of fermentation with natural microflora, in contrast to CBS being fermented with a single predominant microorganism (*Bacillus amyloliquefaciens* RD7-7).

3-Methylbutan-1-ol, butane-2,3-diol, butane-1,3-diol, and 4-methoxybutan-1-ol were dominant alcohols in both two BSs during the initial stage of fermentation, and their contents or aspects tended to vary with the progression of fermentation. In additional, ethanol was the most abundant compound in the later state of fermentation in TBS, while this was not found in CBS. A previous study [22, 23] found that ethanol could be generated in soybean by the metabolism of yeast. In the present study, the higher amount of ethanol in TBS could also have resulted from the higher activities of yeast and/or bacteria during TBS fermentation.

Esters contribute to positive aroma properties of fermented products [24, 25], and they can be produced by the esterification of alcohols with fatty acids [26]. In particular, esters with low molecular weights have floral, sweet, and fruity aroma properties and help to reduce the sharpness of fatty acids and the bitterness of amines [25]. In contrast, esters with high molecular weights commonly exhibit fatty and oily aroma notes [25]. In the present study, the contents of esters with low molecular weights ($RI < 1600$) was higher in TBS, which could have been due to the higher amount of ethanol in TBS facilitating the generation of esters.

Changes in volatile compounds in two BSs according to fermentation periods by PLS-DA

The PLS-DA is one of efficient statistical techniques to clarify the separation between samples, to build their classification models, and to see how the underlying

variables affect the separation between samples. Recently, the models have been successfully applied to discriminate grape musts based on their volatile compositions during fermentation [27]; water-soluble metabolites of *meju* according to fermentation periods [28]; olive oil samples from the Catalonia region with the presence of a variety of defects [29], and so on. In this study, the discriminations of BSs according to the fermentation periods were accomplished by applying their volatile profiles to PLS-DA, to ensure the objective interpretation of the results in Tables 1 and 2.

In a PLS-DA model, the X variables (the predictors) are reduced to scores of component (estimates of the latent variables or their rotation), which are denoted by t_a ($a = 1, 2, 3, \dots$). In other words, the variables are correlated and summarized by new variables t_a scores whose values explain the variation represented by each principal component. The score t_1 (first component) explains the largest

Fig. 1 PLS-DA score plot of TBS samples during fermentation

Fig. 2 PLS-DA score plot of CBS samples during fermentation

variation of the X space, followed by t_2 , and so on [30]. The PLS-DA models revealed clear clustering among each BS groups based on the volatile compounds of BSs during fermentation (0, 1, 2, and 5 weeks) in this study (Figs. 1, 2). Their internal cross-validations on PLS-DA were performed and component 4 and parameters, such as $R^2X = 0.931$, $R^2Y = 0.982$, and $Q^2Y = 0.957$ in TBS and $R^2X = 0.969$, $R^2Y = 0.981$, and $Q^2Y = 0.964$ in CBS, respectively. The score plot in Fig. 1 indicates that the TBS samples were located on the positive $t[1]$ axis during the initial and middle stages of fermentation (up to 2 weeks), while these samples were on the negative $t[2]$ axis during the latest stage of fermentation (5 weeks). On the other hand, the score plot for CBS in Fig. 2 shows that CBS samples during the initial and first middle stages of fermentation (up to 1 week) and during the second middle and latest stage of fermentation

(2–5 weeks) could be clearly separated by $t[1]$. Meanwhile, the discrimination between 0- and 1-week CBS samples and the separation between 2- and 5-week CSB samples could be done by $t[2]$: the sample at the initial stage of fermentation sample (0 week) was located on the positive $t[1]$ and $t[2]$ axes, that at the first middle stage of fermentation (1 week) was on the positive $t[1]$ and negative $t[2]$ axes, that at the second middle stage of fermentation (2 weeks) being on the negative $t[1]$ and $t[2]$, and the latest stage of fermentation sample (5 weeks) was on the negative $t[1]$ and positive $t[2]$ axes. It seems that the change in the volatiles of CBS was accelerated relative to TBS because CBS had initially been fermented with a novel multi-starter. A starter culture could induce a high biosynthetic capacity of fermented soybean products and uniformly control them to produce flavor compounds [31, 32].

Table 3 The contribution of volatile variances in TBS according to fermentation period

No.	p[1]	p[2]	No.	p[1]	p[2]	No.	p[1]	p[2]
v1	-0.143	0.020	v38	0.005	0.001	v73	0.047	0.038
v2	0.085	0.051	v39	0.076	0.139	v75	0.050	0.148
v3	0.053	-0.156	v40	0.053	-0.156	v76	0.094	0.128
v4	-0.159	0.012	v41	0.089	0.140	v77	0.046	0.118
v5	-0.092	-0.132	v42	-0.019	0.116	v79	0.005	0.039
v6	0.105	0.095	v43	-0.149	0.006	v80	-0.149	0.010
v7	-0.153	-0.001	v44	0.048	0.037	v81	0.053	-0.155
v8	-0.141	0.040	v45	0.126	0.100	v82	0.105	0.117
v9	0.108	0.073	v46	0.053	-0.156	v83	-0.161	0.003
v10	0.109	0.101	v47	0.048	0.036	v85	0.059	0.120
v11	-0.038	0.150	v50	-0.059	0.151	v87	0.096	0.139
v13	-0.052	0.160	v53	-0.158	0.001	v88	0.047	0.038
v15	-0.134	-0.015	v54	-0.007	0.166	v89	-0.160	0.004
v16	-0.021	0.045	v55	-0.043	0.164	v90	0.047	0.038
v17	0.097	0.137	v56	0.084	0.145	v92	0.053	-0.154
v18	-0.039	0.168	v57	-0.111	0.018	v93	-0.039	0.160
v19	-0.143	0.010	v58	0.091	0.135	v94	-0.156	0.004
v20	-0.156	0.000	v59	0.005	0.175	v95	0.090	0.143
v22	-0.151	0.056	v60	-0.159	0.003	v96	-0.154	0.004
v24	-0.110	0.126	v61	-0.152	0.017	v97	-0.128	0.093
v25	-0.118	0.117	v62	0.096	0.131	v98	-0.151	0.008
v26	-0.126	0.109	v63	-0.161	0.002	v99	0.088	0.140
v27	-0.097	0.134	v64	-0.039	0.148	v101	-0.127	0.078
v28	-0.110	0.121	v65	-0.153	0.010	v102	0.063	-0.013
v29	-0.084	0.145	v66	-0.081	0.131	v103	-0.161	0.006
v30	0.053	-0.154	v67	0.095	0.129	v104	-0.158	0.002
v31	-0.094	0.141	v68	0.042	-0.120	v106	-0.160	0.003
v32	-0.152	0.055	v69	0.100	0.133	v107	0.033	0.127
v34	-0.156	0.020	v70	-0.157	0.015	v108	-0.012	0.164
v35	0.043	0.124	v71	0.037	0.119			
v37	0.115	0.094	v72	0.063	0.060			

Table 4 The contribution of volatile variances in CBS according to fermentation period

No.	p[1]	p[2]	No.	p[1]	p[2]	No.	p[1]	p[2]
v1	-0.024	-0.106	v37	-0.088	-0.157	v75	-0.139	-0.030
v2	-0.143	-0.024	v38	0.023	-0.034	v76	-0.143	0.003
v3	-0.126	-0.075	v40	0.117	0.099	v77	-0.139	0.057
v5	0.083	0.076	v42	-0.127	-0.096	v78	-0.139	0.058
v8	-0.069	-0.002	v45	-0.129	-0.089	v80	-0.024	0.175
v11	-0.139	-0.059	v46	0.081	0.075	v81	0.057	-0.216
v12	-0.024	0.175	v47	-0.101	-0.165	v82	-0.139	0.038
v13	-0.143	0.018	v48	-0.124	-0.074	v84	0.071	-0.204
v14	0.072	-0.179	v49	-0.142	-0.020	v85	-0.134	-0.036
v15	-0.001	0.050	v50	-0.141	-0.046	v86	-0.123	-0.060
v16	0.056	-0.208	v51	-0.140	-0.045	v87	-0.122	0.053
v17	-0.142	0.002	v52	-0.055	-0.199	v89	-0.024	0.176
v19	-0.137	-0.007	v55	-0.138	-0.068	v91	0.095	-0.166
v21	0.043	-0.195	v56	-0.109	-0.155	v93	-0.142	0.036
v22	-0.116	-0.086	v58	-0.138	0.063	v95	-0.126	-0.075
v23	-0.140	0.048	v59	-0.133	-0.082	v97	-0.137	0.053
v24	-0.142	-0.003	v62	-0.125	-0.082	v98	0.065	-0.182
v25	-0.140	0.049	v64	-0.139	0.041	v99	-0.137	0.042
v26	-0.141	0.040	v65	-0.024	0.175	v100	-0.131	0.004
v27	0.041	-0.030	v66	-0.119	-0.094	v101	-0.122	0.075
v28	-0.139	-0.033	v67	-0.141	0.016	v102	-0.139	-0.039
v29	-0.140	0.049	v68	-0.113	0.096	v105	-0.050	-0.216
v30	0.030	-0.205	v69	-0.143	0.030	v106	-0.024	0.175
v31	-0.142	-0.021	v70	-0.094	-0.092	v107	-0.100	-0.163
v32	-0.139	0.062	v71	0.049	-0.183	v108	-0.141	-0.019
v35	-0.113	0.144	v72	-0.028	0.146			
v36	-0.098	-0.169	v74	0.061	-0.183			

Loading plots were produced to classify which variables contribute most to the discrimination ability. Tables 3 and 4 show their contribution on variables with loadings in a loadings plot, denoted by “p_a”, contribute heavily to observations whose scores are found in a similar position in a scores plot [30]. The major volatile compounds that contribute to the t[1] dimension during TBS fermentation in the following order in Table 3: ethyl dodecanoate (v63) > 4-methyl-2H-furan-5-one (v83) > 3-methylsulfanylpropan-1-ol (v103) > tridecane (v89) > α -cubebene (v106) > ethanol (v4) > methyl pyridine-3-carboxylate (v60) > ethyl hexanoate (v53) > styrene (v104) > methyl hexadec-7-enoate (v70) > 1,3-xylene (v20) > 2-phenylbut-2-enal (v34) > 4-ethyl-2-methoxyphenol (v94). In particular, since these compounds were on the negative t[1] axis, they were strongly associated with the latest stage of TBS fermentation. This also means that their amounts were increased after 5 weeks of TBS fermentation. On the other hand, the following compounds were important contributors to the PC 2 dimension (also in decreasing

order): methyl decanoate (v59) > 3-hydroxy-2,6-dimethylpyran-4-one (v18) > methyl heptanoate (v54) > delta-cadinene (v108) > oct-1-en-3-ol (v13) > 2-methoxyphenol (v93) > methyl 3-phenylpropanoate (v30) > heptadecane (v92) > oxolan-2-one (v81) > 3-hydroxybutan-2-one (v40) > hexanoic acid (v3) > dimethyl carbonate (v46). Methyl 3-phenylpropanoate (v30), heptadecane (v92), oxolan-2-one (v81), 3-hydroxybutan-2-one (v40), hexanoic acid (v3), and dimethyl carbonate (v46) were located on the positive t[1] and negative t[2] axes and were mainly associated with the initial stage of fermentation in TBS samples, whereas methyl decanoate (v59) on the positive t[1] and t[2] axes, was the dominant compound during the middle stage of TBS fermentation.

In addition, the main volatiles on the t[1] dimension contributing to discrimination among CBS samples were methyl hexadecanoate (v69), methyl octadec-9-enoate (v76), 3-methylbutanoic acid (v2), oct-1-en-3-ol (v13), methyl benzoate (v24), methyl 4-methylpentanoate (v49), 2-methoxyphenol (v93), phenylmethanol (v31), and

4-methyl-1-propan-2-ylcyclohex-3-en-1-ol (v17) (Table 4). In particular, these compounds were located on the negative t[1] axis, suggesting that they were predominantly produced during the second middle stage of CBS fermentation process (first middle stage → second middle stage). On the other hand, oxolan-2-one (v81), 1-(1H-pyrrol-2-yl) ethanone (v105), butane-1,3-diol (v16), methyl 3-phenylpropanoate (v30), hexane (v84), methyl 4-methylpent-3-enoate (v52), 1,4-xylene (v21), methyl (Z)-hexadec-9-enoate (v71), methyl heptadecanoate (v74), 4-ethenylphenol (v98), 2-ethylhexan-1-ol (v14), tridecane (v89), (Z)-hex-3-en-1-ol (v12), furan-2-carbaldehyde (v80), ethyl tetradecanoate (v65), and α -cubebene (v106) were important volatile variables on the t[2] dimension. In particular, 3-hydroxybutan-2-one (v40), located on the positive t[1] and t[2] axes, was related to the initial stage of CBS fermentation, and oxolan-2-one (v81), butane-1,3-diol (v16), methyl 3-phenylpropanoate (v30), hexane (v84), 1,4-xylene (v21), methyl (Z)-hexadec-9-enoate (v71), methyl heptadecanoate (v74), 4-ethenylphenol (v98), and 2-ethylhexan-1-ol (v14), being on the positive t[1] and negative t[2] axes, were strongly associated with the first middle stage of CBS fermentation. In addition, 1-(1H-pyrrol-2-yl) ethanone (v105) and methyl 4-methylpent-3-enoate (v52), being on the negative t[1] and t[2] axes, were especially produced between the first (1 week) and second (2 weeks) middle stages of CBS fermentation. Lastly, tridecane (v89), (Z)-hex-3-en-1-ol (v12), furan-2-carbaldehyde (v80), ethyl tetradecanoate (v65), and α -cubebene (v106) which were located on the negative t[1] and positive t[2] axes were associated with the latest stage of CBS fermentation.

In conclusion, the volatile compounds in BSs changed or differed according to the fermentation type (TBS or CBS) and the fermentation period (0, 1, 2, and 5 weeks). In particular, the PLS-DA models identified different trends between TBS and CBS fermentation. During TBS fermentation, 0-, 1-, and 2-week samples and 5-week samples were preferentially separated, whereas 0- and 1-week samples and 2- and 5-week samples were discriminated during CBS fermentation. It seems that the generation of flavors in BS could be closely related to the microorganisms involved in fermentation. The use of a starter culture at the beginning of fermentation could facilitate the biosynthetic and chemical reactions in fermented products and subsequently increase the production of secondary metabolites, including flavor compounds.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Kwak CS, Lee MS, Park SC. Higher antioxidant properties of *Chungkookjang*, a fermented soybean paste, may be due to increased aglycone and malonylglycoside isoflavone during fermentation. *Nutr. Res.* 27: 719–727 (2009)
- Wang D, Wang LJ, Zhu FX, Zhu JY, Chen XD, Zou L, Saito M, Li LT. *In vitro* and *in vivo* studies on the antioxidant activities of the aqueous extracts of *Douchi* (a traditional Chinese salt-fermented soybean food). *Food Chem.* 107: 1421–1428 (2008)
- Fujita M, Nomura K, Hong K, Ito Y, Asada A, Nishimuro S. Purification and characterization of a strong fibrinolytic enzyme (Nattokinase) in the vegetable cheese *natto*, a popular soybean fermented food in Japan. *Biochem. Biophys. Res. Com.* 197: 1340–1347 (1993)
- Choi NS, Chung DM, Han YJ, Kim SH, Song JJ. Purification and characterization of a subtilisin D5, a fibrinolytic enzyme of *Bacillus amyloliquefaciens* DJ-5 isolated from *Doenjang*. *Food Sci. Biotechnol.* 18: 500–505 (2009)
- Jung KO, Park SY, Park KY. Longer aging time increases the anticancer and antimetastatic properties of *doenjang*. *Nutrition* 22: 539–545 (2006)
- Kerwin SM. Soy Saponins and the anticancer effects of soybeans and soy-based foods. *Curr. Med. Chem. Anti-Cancer Agents* 4: 263–272 (2004)
- Kim YS, Kim MC, Kwon SW, Kim SJ, Park IC, Ka JO, Weon HY. Analyses of bacterial communities in *meju*, a Korean traditional fermented soybean bricks, by cultivation-based and pyrosequencing methods. *J. Microbiol.* 49: 340–348 (2011)
- Lee JH, Kim TW, Chang HC, Kim HY. Determination of microbial diversity in *meju*, fermented cooked soya beans, using nested PCR-denaturing gradient gel electrophoresis. *Lett. Appl. Microbiol.* 51: 388–394 (2010)
- Park CH, Kim YB, Choi YS, Heo K, Kim SL, Lee KC. Rutin content in food products processed from groats, leaves and flowers of buckwheat. *Fagopyrum*, 17: 63–66 (2000)
- Kreft S, Knapp M, Kreft I. Extraction of rutin from buckwheat (*Fagopyrum esculentum* Moench) seeds and determination by capillary electrophoresis. *J. Agr. Food Chem.* 47: 4649–4652 (1999)
- Sun T, Ho CT. Antioxidant activities of buckwheat extracts. *Food Chem.* 90: 743–749 (2005)
- Oomah BD, Mazza G. Flavonoids and antioxidative activities in buckwheat. *J. Agr. Food Chem.* 44: 1746–1750 (1996)
- Jo YJ, Cho IH, Song CK, Shin HW, Kim YS. Comparison of fermented soybean paste (*Doenjang*) prepared by different methods based on profiling of volatile compounds. *J. Food Sci.* 76: 368–379 (2011)
- Cho KM, Lim HJ, Kim MS, Hwang CE, Nam SH, Joo OS, Lee BW, Kim JK, Shin EC. Time course effects of fermentation on fatty acid and volatile compound profiles of *Cheonggukjang* using new soybean cultivars. *J. Food Drug Anal. In press* (2016)
- Yoo SK, Cho WH, Kang SM, Lee SH. Isolation and identification of microorganisms in Korean traditional soybean paste and soybean sauce. *Med. Microbiol. Lett.* 27: 113–117 (1999)
- Kim TW, Lee JW, Kim SE, Park MH, Chang HC, Kim HY. Analysis of microbial communities in *doenjang*, a Korean fermented soybean paste, using nested PCR-denaturing gradient gel electrophoresis. *Int. J. Food Microbiol.* 131: 265–271 (2009)
- Park MK, Cho IH, Lee SR, Choi HK, Kwon DY, Kim YS. Metabolite profiling of *Cheonggukjang*, a fermented soybean paste, during fermentation by gas chromatography–mass spectrometry and principal component analysis. *Food Chem.* 122: 1313–1319 (2010)

18. Kim GE, Kim MH, Choi BD, Kim TS, Lee JH. Flavor compounds of domestic meju and Doenjang. *J. Korean Soc. Food Nutr.* 21:557–565 (1992)
19. Tavaría FK, Ferreira SF, Malcata X. Volatile Free Fatty Acids as Ripening Indicators for Serra da Estrela Cheese. *J. Dairy Sci.* 87: 4064–4072 (2004)
20. Ji WD, Yang SH, Choi MR, Kim JK. Volatile components of Korean soybean paste produced by *B. subtilis* PM3. *J. Microbiol. Biotechnol.* 5: 143–148 (1995)
21. Park HK, Kim JK. Optimal manufacturing conditions for Korean soybean paste and soy sauce, using *Aspergillus oryzae* AJ 100 as a flavor improver. *Food Sci. Biotechnol.* 17: 208–211 (2008)
22. Fleet GH, Lafon-lafourcade S., Ribereau-Gayon P. Evolution of yeasts and bacteria during fermentation and storage of bordeaux wines. *Appl. Environ. Microbiol.* 48: 1034–1038 (1984)
23. Bontinis TG, Mallatou H, Pappa EC, Massouaas T, Alichanidis E. Study of proteolysis, lipolysis and volatile profile of a traditional Greek goat cheese (Xinotyri) during ripening. *Small Rumin Res.* 105: 193–201 (2012)
24. Francis IL, Newton JL. Determining wine aroma from compositional data. *Aust. J. Grape Wine Res.* 11: 114–126 (2005)
25. Reineccius G. *Flavor Chemistry and Technology*. 2nd ed. Taylor & Francis, New York, USA. pp.73–98 (2006)
26. Zhao Y, Xu Y, Li J, Fan W, Jiang W. Profile of volatile compounds in 11 brandies by headspace solid-phase microextraction followed by gas chromatography–mass spectrometry. *J. Food Sci.* 74: 90–99 (2009)
27. Malherbe S, Watts VH, Nieuwoudt H, F. Bauer F, Du Toit M. Analysis of volatile profiles of fermenting grape must by head-space solid-phase dynamic extraction coupled with gas chromatography–mass spectrometry (HS-SPDE GC–MS): Novel application to investigate problem fermentations. *J. Agr. Food Chem.* 57: 5161–5166 (2009)
28. Kang HJ, Yang HJ, Kim MJ, Han ES, Kim HJ, Kwon DY. Metabolomic analysis of *meju* during fermentation by ultra performance liquid chromatography–quadrupole-time of flight mass spectrometry (UPLC–Q–TOF MS). *Food Chem.* 127: 1056–1064 (2011)
29. Borràs E, Ferré J, Boqué R, Mestres M, Aceña L, Calvo A, Busto O. Olive oil sensory defects classification with data fusion of instrumental techniques and multivariate analysis (PLS-DA). *Food Chem.* 203: 314–322 (2016)
30. Szymańska E, Saccenti E, Smilde AK, Westerhuis JA. Double-check: validation of diagnostic statistics for PLS-DA models in metabolomics studies. *Metabolomics* 8: 3–16 (2012)
31. Guldeldt LU, Sørensen KI, Strøman P, Behrndt H, Williams D, Johansen E. Effect of starter cultures with a genetically modified peptidolytic or lytic system on Cheddar cheese ripening. *Int. Dairy J.* 11: 373–382 (2001)
32. Kum SJ, Yang SO, Lee SM, Chang PS, Choi YH, Lee JJ, Hurh BS, Kim YS. Effects of *Aspergillus* Species Inoculation and Their Enzymatic Activities on the Formation of Volatile Components in Fermented Soybean Paste (*doenjang*). *J. Agr. Food Chem.* 63: 1401–1418 (2015)