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# Evaluation of the fermentation properties of different soybean (*Glycine max* L.) cultivars

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## ABSTRACT

The enzymatic activity (EA) and amino-type nitrogen (ATN), viscous substance (VS), organic acid (OA), free amino acid (FAA) and volatile compound (VC) content in fermented soybeans of six cultivars (*Glycine max* L. 'Taekwangkong' [TKK], 'Daechankong' [DCK], 'Misokong' [MSK], 'Seonpungkong' [SPK], 'Uramkong' [URK] and 'Chamolkong' [COK]) were compared. Fermentation increased the quality indices such as EA, ATN and VS in soybean and their highest content were observed in DCK. The content of major OAs decreased in the following order: lactic acid > acetic acid > fumaric acid. The essential amino acid (EAA) content was increased by fermentation and FAA production had a positive effect on the abundance of the fermentation metabolites γ-amino-n-butyric acid and ornithine. The content of the VS pyrazine differed with cultivar. Overall, soybean cultivars showed differences in the composition and fermentation properties, with raw DCK soybeans being particularly suitable for fermentation.

**Key words:** Cultivars, Fermentation property, *Glycine max* L., Soybean.

## INTRODUCTION

Fermented soybean foods are rich in nutrients (e.g., protein, fat, essential amino acids, and fatty acids) and have a unique flavor. They are also known to possess antioxidants (Eom, *et al*. 2009) and are effective against chronic diseases and geriatric disease (Gholamhoseini *et al*. 2018). Various food products prepared from fermented soybean (e.g., Korean cheonggukjang and doenjang, Japanese miso and natto, and Chinese shuidouchi) have been used as nutritious food for more than thousands of years in East Asia (Lee *et al*. 2017; Naoko *et al*. 2018; Sunantha and Yukiharu, 2019). Their consumption has been increasing because of not only their unique flavors, but also their nutritional (high protein) and functional effects (Luo *et al*. 2014; Lee *et al*., 2018).

The fermentation process increases the nutritional value of soybeans (e.g., vitamin and mineral content). New functional bioactive substances (such as isoflavone, polyglutamate, polypeptide, peptone and amide) are generated or their levels are increased as microorganism (e.g., *Bacillus*, *Lactobacillus*, *Aspergillus,* and yeasts) enzymes decompose proteins and carbohydrates during the fermentation process (Eom *et al*. 2009). These substances are converted to bioavailable form (Fabricio *et al*. 2018). Additionally, fermentation enhances the taste and smell of soybeans by altering the composition of volatile compounds.

Microorganisms suitable for fermentation (e.g., *Lactobacillus*) have been investigated in relation to the antioxidant properties of fermented soybean products (Sasithorn *et al*. 2017), physiological benefits of fermented soybean (Cao *et al*. 2019), manufacturing process (Ghosh *et al*. 2018) and changes in volatile compound (e.g., butanoic acid, and 2,3-dimethylpyrazine) profiles with fermentation time and conditions (Park *et al*. 2017). In addition, there is little information on how chemical composition affects the quality of soybean and the suitability of a cultivar for fermentation.

To address this issue, in the present study, we compared the fermentation characteristics of six soybean cultivars including enzymatic activity (EA) and amino-type nitrogen (ATN), viscous substance (VS), organic acid (OA), free amino acid (FAA) and volatile compound (VC) content.

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## MATERIALS AND METHODS

### Sample preparation

Six Korean soybean cultivars (*Glycine max* L. 'Taekwangkong' [TKK], 'Daechankong' [DCK], 'Misokong' [MSK], 'Seonpungkong' [SPK], 'Uramkong' [URK] and 'Chamolkong' [COK]) were grown in the National Institute of Crop Science, Rural Development Administration (Miryang, South Korea) during the 2017 growing season and were stored at 4°C. Bacterial strains isolated from traditional Korean

soybean paste were used as the starter culture.

**Soybean fermentation**

Soybeans were soaked for 15 h at room temperature; after draining the water, the soybeans were sterilized by autoclaving at 12°C for 30 min. Sterile soybeans were inoculated with a suspension of *Bacillus amyloliquefaciens* at a concentration of $10^6$ CFU/g and approximately 200 g (wet weight) was placed in a sterile styrofoam cup that was incubated at 40°C for 24 h. The fermented soybeans were crushed or freeze dried and maintained at -70°C until use.

**Measurement of EA and ATN and VS content**

The protease activity assay was carried out as previously described (Anson, 1938), with some modification. The α-amylase activity was measured using the dextrinogenic unit of nagase method (Lee *et al*. 2017). The ATN content was measured using the Formol method (Lee *et al*. 2017). Viscous substances were weighed by evaporating a certain amount of the sample at 105°C and were marked as dry substances (%).

**Measurement of OA content**

One gram of freeze-dried sample was added to 80% MeOH and extracted using a sonicator (BKUP-600N; Biokonvision, Gyeonggi, South Korea) for 1 h. The extract was passed through a 0.2-μm membrane filter and the filtrate was analyzed by high-performance liquid chromatography (HPLC) (Ultimate 3000 with pump and autosampler; Dionex, Sunnyvale, CA, USA) using an HLB Sep-pak C18 cartridge (Waters, Milford, MA, USA) and Aminex 87H column (300 mm × 6.5 mm; Waters), with 0.01 N sulfuric acid (99%; Fluka, Ronkonkoma, NY, USA) as the mobile phase.

**Measurement of FAA content**

The FAA content was determined using a post reaction-type AA analyzer (L-8900; Hitachi, Tokyo, Japan). Freeze-dried powdered sample (1 g) was extracted in 50 mL of distilled water at 80°C for 15 min. After centrifugation (Ultra-4.0; Hanil, Gimpo, South Korea) of 1 mL of the extract in 5% (w/v) trichloroacetic acid at 10 000 rpm for 10 min, the supernatant was passed through a 0.2-μm membrane filter and analyzed as previously described (Yoo and Chang, 1999).

**Measurement of VC content**

VCs were extracted from fermented soybeans using the solid-phase microextraction (SPME) method with 75 mL of carboxen/polydimethylsiloxane fiber (Supelco, Bellefonte, PA, USA). The extract was concentrated by rotary evaporation (N-1000; Eyela, Tokyo, Japan), transferred to a vial, and concentrated to 100 μL under nitrogen gas. VCs were analyzed by gas chromatography (GC) (TRACE1310; Thermo Fisher Scientific, Waltham, MA, USA)/mass spectrometry (MS) (TSQ8000; Thermo Fisher Scientific using a DB-WAX column (60 m × 0.25 mm, 0.5-μm film thickness; Agilent Technologies, Santa Clara, CA, USA) (Park *et al*. 2017).

**Statistical analysis**

Experiments were carried out with triplicate samples and all data are expressed as mean ± standard deviation (SD). Significant differences among treatment means was determined by one-way analysis of variance. Data were analyzed using SPSS v.12.0 software (SPSS Inc., Chicago, IL, USA). Differences between groups were evaluated by Duncan's multiple range test, and the significance level was set at 0.05.

## RESULTS AND DISCUSSION

**EA and ATN and VS content in fermented soybeans**

The EA of the extracts of six soybean cultivars was measured after 24 h of fermentation (Table 1). The protease activity was the highest in DCK (664.07 U/g), followed by SPK (650.14 U/g). α-Amylase is an important enzyme in fermented soybeans as it hydrolyzes carbohydrates to confer a sweet taste. The α-amylase activity was the highest in TKK and DCK at 21.56 and 16.57 U/g, respectively. These results demonstrate that there was a significant difference in the EA of fermented soybeans of different cultivars. The activity of protease from microorganisms involved in fermentation affects the taste of a product (Gil *et al*. 2016). Although the biochemical mechanisms of fermentation were similar among soybean cultivars, differences in nutritional value and quality exist due to variable activities of hydrolytic enzymes, indigenous microflora and fermentation

**Table 1:** Enzymatic activity, amino type nitrogen and viscous substance of fermented soybean according to cultivars.

| Cultivars[1)] | Enzymatic activity | | Amino type nitrogen | Viscous substance |
|---|---|---|---|---|
| | Protease(unit/g) | α-Amylase(unit/g) | (mg%) | (%) |
| TKK | 564.86±5.67[2)c3)] | 21.56±0.83[a] | 239.53±1.05[b] | 6.50±0.08[c] |
| DCK | 664.07±5.54[a] | 16.57±0.71[b] | 241.35±2.16[a] | 8.00±0.06[a] |
| MSK | 384.43±3.28[f] | 6.63±0.64[f] | 188.11±5.24[f] | 6.03±0.09[e] |
| SPK | 650.14±3.72[b] | 9.93±0.56[e] | 217.36±1.92[c] | 7.88±0.12[b] |
| URK | 496.29±5.64[e] | 11.36±0.12[d] | 207.98±2.81[d] | 6.27±0.31[d] |
| COK | 526.29±7.72[d] | 12.98±0.92[c] | 196.99±2.8[2] | 5.99±0.19[e] |

[1)]TKK: *Glycine max* L. Taekwangkong, DCK: L. Daechankong, MSK: L. Misokong, SPK: L. Seonpungkong, URK: L. Uramkong, COK: L. Chamolkong.

[2)]Each value is the mean ± SD (n=3).

[3)]Values with different superscripts are significantly different at $p < 0.05$ according to Duncan's multiple range tests.

conditions (Chukeatirote, 2015).

The protease activity during fermentation leads to the deamination of proteins and their breakdown into AAs; it serves as a quality indicator of soybean fermentation (Ghosh *et al*. 2018). The ATN content was the highest in DCK (Table 1), reflecting a high enzymatic activity (Eom *et al*. 2009). It has been reported that the amounts of sugar and AAs produced by microorganisms during soybean fermentation are highly correlated (Gil *et al*. 2016). VSs produced by fermentation are a polyglutamate mixture of levan and protein decomposed from fructan in soybeans (Bang *et al*. 2011). The VS content was higher in DCK than in other cultivars. These results suggest the difference in the composition of species due to genetic diversity depending on the growth environment conditions of soybean (Jain *et al*. 2018).

**OA content in fermented soybeans**

The OA content in fermented soybean varied among cultivars, as determined by HPLC (Table 2). The major OAs were lactic acid > acetic acid > fumaric acid. Citric acid was not detected in any of the samples. DCK had the highest OA content at 7034.58 mg/kg. Fig 1 shows the percentage of OAs according to cultivar; MSK had the highest lactic acid content (86.58%), whereas the acetic acid content was the highest in URK (34.30%).

A previous study reported differences in the OA content in eight soybean cultivars (Yoo and Chang, 1999). This is in agreement with our findings, which also showed that lactic acid and acetic acid were present when *B. subtilis* was used for fermentation. On the contrary, another study showed that the main OAs in soybeans fermented using *Lactobacillus* were lactic acid and oxalic acid (Lee *et al*. 2017). These differences may be due to the soybean cultivars and starter strains that were used. It is expected that the OA composition affects the antimicrobial activity and flavor of the products.

**FAA content in fermented soybeans**

FAAs in soybeans have a high nutritional value and have several health benefits (Gibbs *et al*. 2004). The FAA content varied with the soybean cultivar. The EAA contents tended to increase after fermentation in all of the tested cultivars, but were the highest in DCK and lowest in MSK (Fig 2). Notably, in DCK, the EAA content after fermentation was about seven times higher than that before fermentation. The most abundant EAA was Val, followed by Leu, Lys, Thr, Try, Phe, Ile and Met. Glu was the most abundant FAA in all the cultivars, with levels 2–10 times higher in fermented soybean than in raw soybean (Fig 3). DCK had the highest FAA content following fermentation, and also showed the greatest increase. In an earlier study, the Glu concentration showed the greatest increase among FAAs following fermentation (Jung *et al*. 2009). Although it depends on the cultivar, changes in the FAA content caused by fermentation generally increase the palatability, bitterness, and sweetness of soybean (Gil *et al*. 2016). This is because the AAs resulting from the breakdown of proteins during the fermentation process combine to produce a unique flavor (Katekan, *et al*. 2011). The increase in FAAs after fermentation of soybeans results in functional benefits to the health of humans (Luo *et al*. 2014; Shekar and Pushpendra, 2017).

γ-Amino-n-butyric acid (GABA) and ornithine (Orn), which are AA metabolites produced by soybean fermentation, were the most abundant in TKK and DCK

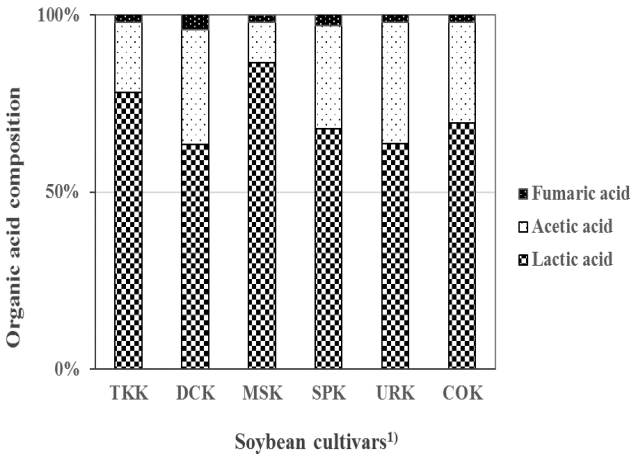



**Fig 1:** Organic acid composition ratio of fermented soybean of fermented soybean according to cultivars. [1)] TKK: *Glycine max* L. Taekwangkong, DCK: L. Daechankong, MSK: L. Misokong, SPK: L. Seonpungkong, URK: L. Uramkong, COK: L. Chamolkong.

**Table 2:** Organic acid composition of fermented soybean of fermented soybean according to cultivars.

| | Cultivars[1)] | | | | | |
|---|---|---|---|---|---|---|
| Organic acid | TKK | DCK | MSK | SPK | URK | COK |
| Acetic acid | 1,497.38 | 2,418.80 | 881.24 | 1,752.44. | 2,531.84 | 2,174.09 |
| Citric acid | ND[2)] | ND | ND | ND | ND | ND |
| Fumaric acid | 2.32 | 4.55 | 2.20 | 3.59 | 2.52 | 2.42 |
| Lactic acid | 5,389.63 | 4,611.23 | 5,709.53 | 3,714.24 | 4,441.33 | 4,790.00 |
| Oxalic acid | ND | ND | ND | ND | ND | ND |
| Succinic acid | ND | ND | ND | ND | ND | ND |
| Total | 6,889.33 | 7,034.58 | 6,592.97 | 5,470.27 | 6,975.69 | 6,966.51 |

[1)]TKK: *Glycine max* L. Taekwangkong, DCK: L. Daechankong, MSK: L. Misokong, SPK: L. Seonpungkong, URK: L. Uramkong, COK: L. Chamolkong.

[2)]ND: Not detected.

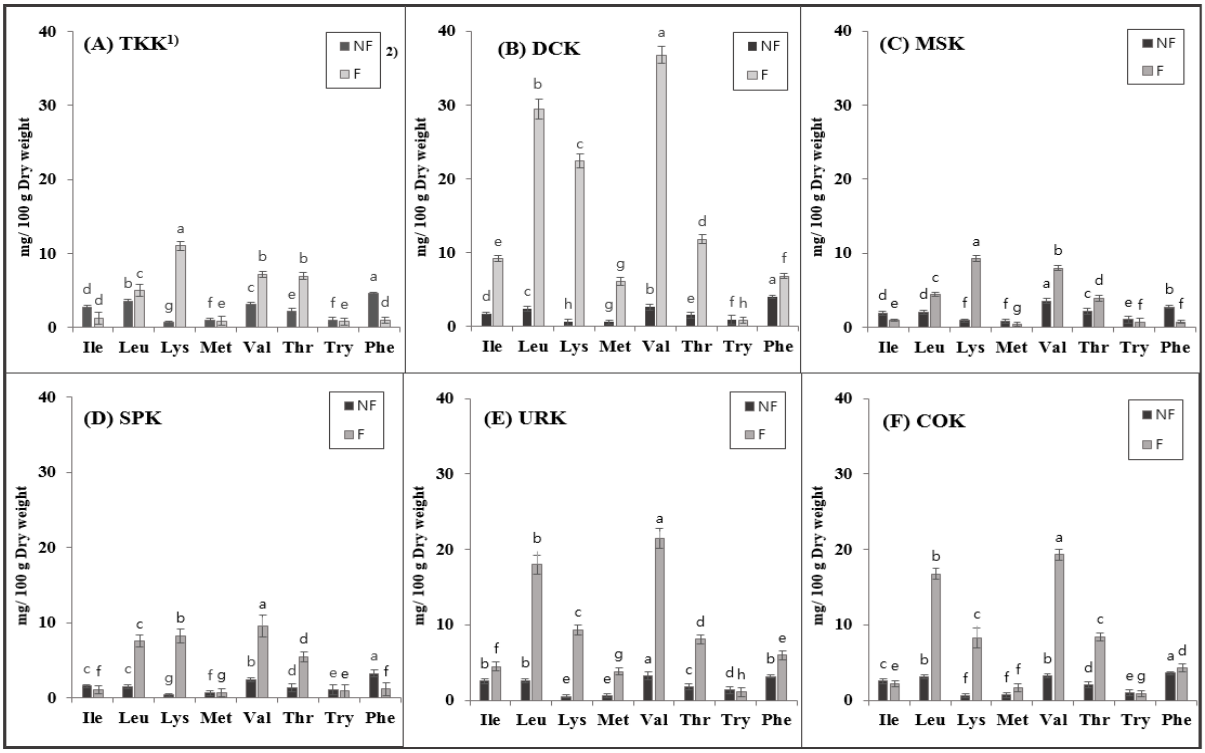



**Fig 2:** Essential amino acids contents of raw soybean and fermented soybean according to cultivars. Values with different superscripts are significantly different at P < 0.05 according to Duncan's multiple range tests. [1)](A) TKK: *Glycine max* L. Taekwangkong, (B) DCK: L. Daechankong, (C) MSK: L. Misokong, (D) SPK: L. Seonpungkong, (E) URK: L. Uramkong, (F) COK: L. Chamolkong. [2)] NF: Non-fermentation and F: Fermentation.

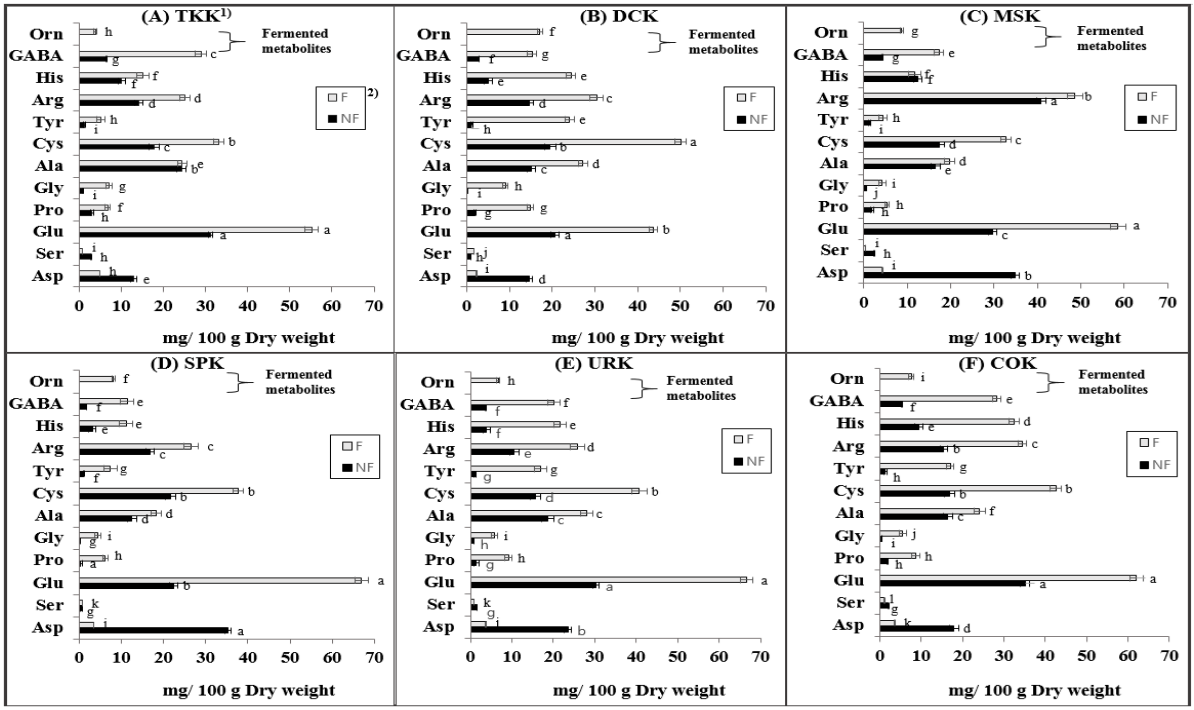



**Fig 3:** Non-essential amino acids contents of raw soybean and fermented soybean according to cultivars. Values with different superscripts are significantly different at P < 0.05 according to Duncan's multiple range tests. [1)](A) TKK: *Glycine max* L. Taekwangkong, (B) DCK: L. Daechankong, (C) MSK: L. Misokong, (D) SPK: L. Seonpungkong, (E) URK: L. Uramkong, (F) COK: L. Chamolkong. [2)]NF: Non-fermentation and F: Fermentation.

cultivars, respectively (Fig 3). GABA is an inhibitory neurotransmitter that is produced from the FAA Glu, which is important for brain function (Marseglia *et al*. 2014).

Intermediate products of soybean fermentation such as GABA and Orn may be important for evaluating the properties of the fermentation products. The observed differences in FAA composition in soybean cultivars are likely due to variations in the synthesis or decomposition of their chemical constituents. The findings of the present study suggested that fermented DCK was superior in terms of taste and quality (e.g., EAA content) to those of the other cultivars.

**VC content in fermented soybeans**

The VC content in fermented soybeans was analyzed by SPME GC/MS (Table 3). Thirty-four VCs were present in the six cultivars; the most abundant was acetoin, followed by styrene and ethylester-butanoic acids. Acetoin is primarily detected in processed products (e.g., fermented milk) and is a precursor of diacetyl ($CH_3COCOCH_3$), a fermentation product generated by microorganisms. VCs are produced during soybean fermentation by various enzymes (e.g., protease and amylase) and substrates (e.g., aminoic acid, organic acid and saccharide) (Park *et al*. 2017).

Pyrazine compounds, which are major VCs in fermented soybeans, were present at the highest and lowest content in DCK and MSK, respectively (Table 3). Trimethylpyrazine was the most abundant compound followed by

**Table 3:** Volatile compound of fermented soybean according to cultivars.

| Possible compound | | RT[1] | Relative Peak Area (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | (min) | TKK[2] | DCK | MSK | SPK | URK | COK |
| Alcohol | 2,3-Butanediol-(S-(R*,R*)) | 52.28 | 1.37 | 1.72 | 1.21 | 2.40 | 3.70 | 0.43 |
| | 2,3-Butanediol-(R-(R*,R*)) | 52.30 | ND[3] | ND | 23.26 | ND | 30.64 | 13.08 |
| | 3-Octanol | 46.77 | 0.09 | 0.41 | 0.06 | 0.29 | 0.08 | 0.15 |
| | Benzeneethanol | 12.95 | 0.61 | 0.56 | 0.37 | 0.53 | 0.39 | 0.56 |
| | 2-Furanmethanol | 59.46 | 1.44 | 0.33 | 0.19 | 0.25 | 0.57 | 0.37 |
| | Benzyl alcohol | 51.73 | 0.23 | 0.41 | 0.29 | 0.46 | 0.26 | 0.24 |
| | Geraniol | 74.19 | 0.13 | 0.29 | 0.16 | 0.25 | 0.25 | 0.11 |
| | Oxirane | 38.02 | 0.43 | 0.50 | 0.20 | 0.47 | 0.41 | 0.40 |
| Acid | Acetic acid | 41.81 | 1.17 | 3.99 | 1.05 | 1.63 | 1.52 | 0.92 |
| | Butanoic acid | 60.02 | 0.80 | 1.68 | 0.64 | 1.11 | 0.16 | 0.56 |
| | Propanoic acid | 53.78 | 0.30 | 0.36 | 0.12 | 0.29 | 0.67 | 0.14 |
| | Dimethyl-propanedioic acid | 53.79 | 1.28 | 0.83 | 0.56 | 1.09 | 1.66 | 0.40 |
| | 2-Mehtylbutanoic acid | 60.10 | 1.42 | 1.64 | 1.84 | 3.61 | 1.93 | 0.69 |
| | 3-Mehtylbutanoic acid | 60.02 | 1.54 | 1.88 | 1.75 | 3.37 | 1.89 | 1.07 |
| | 2-Ethyl-butanoic acid | 67.33 | 1.53 | 2.00 | 0.59 | 2.39 | 2.99 | 0.24 |
| Ketone | Acetoin | 35.68 | 34.68 | 40.54 | 40.54 | 41.70 | 10.01 | 47.85 |
| | 3-(Acetyloxy)-2-butanone | 35.71 | 0.14 | 0.32 | 0.15 | 0.22 | 0.04 | 0.13 |
| | 2-Nonanone | 26.60 | 0.25 | 0.28 | 0.17 | 0.30 | 0.30 | 0.19 |
| | 2-Tridecanone | 32.62 | 0.07 | 0.05 | 0.06 | 0.07 | 0.16 | 0.08 |
| | 2-Tetradecanone | 48.79 | ND | 0.04 | 0.03 | 0.04 | 0.07 | 0.03 |
| | 3-Hydroxy-2-methylpyran-4-one | 79.46 | 17.87 | 10.82 | 8.54 | 13.93 | 11.14 | 6.89 |
| | 1-Phenylethanone | 59.66 | 0.72 | 0.87 | 1.05 | 1.03 | 0.21 | 0.28 |
| Phenol | Phenol | 77.55 | 0.60 | 0.43 | 0.39 | 0.49 | 0.54 | 0.46 |
| | 2-Methoxy-phenol | 74.19 | 8.65 | 2.23 | 2.92 | 3.94 | 4.91 | 6.41 |
| | 2-Methoxy-4-vinylphenol | 88.68 | 4.93 | 1.92 | 1.42 | 1.87 | 3.28 | 3.31 |
| | 2,6-Di-tert-butyl-4-methylphenol | 90.45 | 6.93 | 5.45 | 2.04 | 0.98 | 0.04 | 0.23 |
| Ester | 2-Mehtylpropanoic acid-ethyl ester | 15.08 | 0.12 | 0.27 | 0.14 | 0.21 | 0.24 | 0.06 |
| | Ethylester-butanoic acid | 41.81 | 4.42 | 5.56 | 1.69 | 7.44 | 8.21 | 1.07 |
| Furan | 2,3-Dihydro-benzofuran | 93.60 | 2.37 | 1.12 | 1.41 | 1.54 | 1.46 | 0.89 |
| Pyrazine | 2,5-Dimethyl-pyrazine | 38.70 | 0.72 | 1.79 | 0.52 | 0.88 | 1.09 | 0.41 |
| | 2,6-Dimethyl-pyrazine | 38.50 | 0.14 | 0.25 | 0.10 | 0.18 | 0.20 | 0.11 |
| | Tetramethyl-pyrazine | 49.14 | 0.38 | 3.46 | 0.79 | 1.15 | 0.56 | 1.03 |
| | Trimethyl-pyrazine | 44.40 | 1.17 | 3.99 | 1.05 | 1.63 | 1.52 | 0.92 |
| Miscellaneous | Styrene | 34.03 | 3.50 | 3.99 | 4.70 | 4.27 | 8.91 | 10.27 |

[1]Retention time

[2]TKK: *Glycine max* L. Taekwangkong, DCK: L. Daechankong, MSK: L. Misokong, SPK: L. Seonpungkong, URK: L. Uramkong, COK: L. Chamolkong.

[3]ND: Not detected.

tetramethylpyrazine. An earlier study has showed that pyrazine compounds were present in soybean samples fermented using *Bacillus* but were absent in those fermented using *Lactobacillus* (Lee *et al*. 2017), which is in disagreement with the results of the present study.

The main compound responsible for the unpleasant odor that is sometimes associated with fermented soybean is tetramethylpyrazine generated by fermentation starter strains although a small amount of this VC can positively affect the taste. The amount of tetramethylpyrazine produced varies depending on fermentation conditions (e.g., microbial strain, water, salinity and temperature) and the type and amount of other compounds. Therefore, maintaining an appropriate VC content is important for increasing consumer preference.

## CONCLUSION

DCK soybean cultivar showed excellent fermentation characteristics including the highest OA, FAA and Orn content. Furthermore, the VC content, which can confer an unpleasant odor when present in excess amount, was also higher in DCK than in MSK. Therefore, although DCK has suitable characteristics to produce fermented foods, the fermentation process may require optimization in order to achieve a VC content that is acceptable by consumers.

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