Chemical and Biochemical Characterization of *Doenjang* (Korean Soy Paste) Supplemented with Glasswort (*Salicornia herbacea* L.) and Rice (*Oryza sativa* L.)

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Abstract: Four types of *Doenjang* were prepared from 100% (w/w) defatted soybean (DFS), a mixture of 80% (w/w) defatted soybean and 20% (w/w) glasswort (DFS-G), a mixture of 80% (w/w) defatted soybean and 20% (w/w) rice (DFS-R), or a mixture of 60% (w/w) defatted soybean, 20% (w/w) glasswort and 20% (w/w) rice (DFS-GR). Maturity of the DFS-G *doenjang* was the highest, which was proportional to total amino acid content. Antioxidative compounds and total amino acids were relatively higher in the *doenjang* with glasswort (DFS-G, DFS-GR) than those in the other preparations (DFS, DFS-R). Succinic and pyroglutamic acid were relatively higher but levulinic acid was relatively lower in the *doenjang* with glasswort compared to those in the others. Mn, Fe, Zn, and Al were relatively higher in the *doenjang* with glasswort compared to those in the others. Volatile organic compounds were differently produced depending on *doenjang* type and influenced definitely by addition of glasswort and rice. Some bacterial communities responsible for *meju* fermentation were changed during ripening for *doenjang* whereas others were conserved.

Key words: *Doenjang* (Korean soy paste), *meju*, glasswort, rice, bacterial community.

1. Introduction

Inorganic and organic compounds contained in glasswort, which is a land plant growing in salty soil around the seashore, are similar to those of seaweeds and edible vegetables, respectively [1, 2]. The vegetable- and seaweed-like compounds of glasswort may be useful as nutritional supplements for fermenting food. The concentration of NaCl contained in the cellular fluid of glasswort is similar to seawater, which can be concentrated to < 15% in dried glasswort [3-5]. NaCl in the dried glasswort is an inhibitory factor against growth of microorganisms that are responsible for *nuruk* and *meju* fermentation. NaCl contained in the dried glasswort is effectively removed by washing with water, because the highly concentrated NaCl must be crystallized on the

glasswort surface after drying. Other minerals contained in dried glasswort except NaCl are preserved mostly after the NaCl is washed out [3, 6]. In previous research, dried glasswort flake has been used as a subsidiary material to prepare *nuruk* and *meju*, whereas *makgeolli* and vinegar are prepared with *nuruk* by fermentation, and *kanjang* has been extracted from *meju* by long-term ripening [7, 8].

Soybean is composed of nutritionally rich proteins and physiologically functional compounds such as isoflavones, vitamin E, saponin, and anthocyanin [9-12]. In particular, the physiologically active compounds function as anticancer agents [13, 14]. *Meju* has been prepared with only cooked soybean by natural fermentation and *doenjang* has been prepared with *meju* itself by long-term ripening under high salt conditions of about 16%-20% NaCl [15]. In various *in vitro* tests, *doenjang* extract functions as an anticancer, antipostmenopausal, and antiosteoporosis agent

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[16-20]. The pharmacological, physiological, and antioxidative effects of glasswort are useful materials for cosmetics and foods [21-23]. Accordingly, the physiological functions of *doenjang* could be improved by adding glasswort during the *meju*-preparing process. Inorganic and organic compounds contained in glasswort may not only be a substrate but act as growth factors for microorganisms during *meju* fermentation and may be converted to *doenjang* nutritional ingredients during ripening.

Glasswort is generally dried to effectively preserve it, because freshly edible glasswort is produced in limited amounts during the spring and is easily spoiled even in the refrigerator. The inorganic and organic compounds in glasswort are extracted naturally during ripening and fermentation and during preparation of *kanjang* and *makgeolli* [7, 8]. However, the process of extracting physiologically useful compounds from glasswort contained in *meju* cannot be conducted while preparing *doenjang* because *meju* itself becomes *doenjang* by ripening. Mixing fine glasswort powder with soybean during cooking and fermentation may be the best way to preserve all glasswort chemical compounds in *doenjang* without loss.

In this study, *doenjang* was prepared with *meju* made from soybean only, a mixture of soybean and glasswort, a mixture of soybean and rice, or a mixture of soybean, glasswort, and rice to reinforce the nutrition in *doenjang*. The nutritional ingredients of four different *doenjang* preparations were analyzed and compared, by which a new recipe for preparing nutritionally improved *doenjang* is expected to be developed.

2. Materials and Methods

2.1 Desalting and Grinding of the Glasswort

Dried glasswort was purchased from Buan Hamcho (Buan, Cheonranam-do, Korea) and was soaked for 15 min in running tap water for washing and desalting. The desalted glasswort was subsequently dried under sunlight for 24 h and ground to a particle size of < 300

mesh using a ceramic ball mill (SW-BM117, 11.5 L volume, SW Engineering, Seoul, Korea).

2.2 Meju Fermentation

Four types of *meju*-making dough were prepared with the following ingredients (mixed ratio) based on dry weight: 100% (w/w) defatted soybean (DFS), a mixture of 80% (w/w) defatted soybean and 20% (w/w) glasswort (DFS-G), a mixture of 80% (w/w) defatted soybean and 20% (w/w) rice (DFS-R), and a mixture of 60% (w/w) defatted soybean, 20% (w/w) glasswort, and 20% (w/w) rice (DFS-GR). The fine glasswort powder was mixed thoroughly with roughly ground (< 50 mesh) DFS and rice, then moistened with drinking water. The moistened *meju* ingredients were steamed for 90 min and then cooled at room temperature. The cooked *meju* ingredients were cast into round molds (25 cm diameter × 10 cm height) to prepare the *meju*-making dough. The prepared *meju*-making dough was incubated on rice straw at 20-25 °C. The *meju*-making dough was fermented into *meju* and completely dried during incubation for 10 weeks.

2.3 Preparation of Doenjang

The fermented and dried *meju* was roughly ground with a stone mortar and then put in a glass jar with a ventilation-type cap to which 20% (w/v) brine prepared with sun-dried salt was added. NaCl content in the brine was determined quantitatively by titration with AgNO₃ based on a standard NaCl solution (0.1 M). The *meju* was completely wetted by adding brine, and the ratio of brine to *meju* was 2.2-2.4 based on weight. The wetted *meju* with brine was ripened for 10 months at 20-25 °C. The glass jar cap was periodically opened and exposed to sunlight for more than 6 h per day at intervals of 2-3 days until the surface of the *doenjang* was completely dried. The film-like dried surface protected against mold contamination.

2.4 Analysis of Minerals

Mineral content was analyzed using inductively

coupled plasma (ICP) optic emission spectrometry (SPECTRO Analytical Instruments, Kleve, Germany). The properly diluted *doenjang* extract was directly injected into an ICP injector under specific wavelengths for Mg (279.553 nm), Na (589.592 nm), K (766.491 nm), Ca (396.847 nm), Mn (293.306 nm), Zn (213.856 nm), Al (167.08 nm), and Fe (259.940 nm). The mineral concentrations were calculated based on the absorbance obtained with standard materials (AccuTrace Reference Standard, AccuStandard, New Haven, CT, USA) and dilution rates.

2.5 Analysis of Organic Acids

Organic acid content was analyzed by high performance liquid chromatography (HPLC) (Beckman, Coulter System Gold, Brea, CA, USA) using an ion exclusion column (Shodex, Rspak KC-811, Showa Denko, Tokyo, Japan) and a refractive index detector (Shodex, RI-101). Free amino acids were analyzed based on the general technique employed for food analyses [24].

2.6 Analysis of Amino Acids

Free amino acids were determined on an ammonia filtration column (LCA, k04/Na, 4.6×100 mm, Sykam, GmbH, Eresing, Germany) equipped with an S433 automatic amino acid analyzer (Sykam).

2.7 Analysis of Volatile Compounds

Eleven volatile organic compounds, including 3-methylbutanol, propionic acid, furfuryl alcohol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 4-hydroxy-2-ethyl-5-methyl furan-3-one, methionol, 2-phenylethanol, 2-methyl-propanol, 1-butanol, 4-ethylguaiacol, and 4-hydroxy-2-methylene-5-methyl-3(2H)-furanone were analyzed using gas chromatography (GC) (Varian CP-3800, Palo Alto, CA, USA) and GC/mass spectrometry (MS) (Varian Saturn 2100D) equipped with an HP-INNOWAX capillary column (Agilent Technologies, Santa Clara, CA, USA) and an FI detector (Varian). Injector and

detector temperatures were adjusted to 230 °C and 260 °C, respectively. Initial oven temperature was maintained at 50 °C for 5 min and then gradually increased to 230 °C at a rate of 10 °C per min. Samples were directly extracted from *doenjang* (100 g) with methyl acetate (25 mL), and the extraction process was repeated three times for single *doenjang* samples. Extracts obtained via triple extraction were mixed together and adjusted to a final volume of 100 mL by adding fresh methyl acetate. 2 µL of solvent extract was subsequently injected into the GC injector in splitless mode.

2.8 Determination of Physiologically Active Compounds

Doenjang was suspended in double distilled water, incubated in a refrigerator for 24 h, and then diluted from 10 to 100 times based on dry weight. The diluted *doenjang* extract was used as a sample for analysis of physiologically active compounds. Polyphenol content in the *doenjang* extract was assessed using the Prussian blue spectrophotometric method [25, 26]. Total phenolic contents in *doenjang* extract were determined via the Folin-Ciocalteu colorimetric method [27]. Antioxidative activity of *doenjang* extract was determined using a modified version of the method developed by Brand-Williams et al. [28] for DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity assays [29]. The concentrations of physiologically active compounds were determined based on the absorbance obtained with standard materials and the dilution ratio.

2.9 Temperature Gradient Gel Electrophoresis (TGGE)

Chromosomal DNA of microorganisms grown in *meju* and *doenjang* was directly extracted from the fermented *meju* and the ripened *doenjang* using a Power Soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA) and a bead beater (FastPrep-24, Irvine, CA, USA). The 16S-rDNA was amplified

using the chromosomal DNA extracted from *meju* and *doenjang*. A variable region of 16S-rDNA was amplified with the forward primer (eubacteria, V3 region) 341f 5'-CCTACGGGAGGCAGCAG-3' and the reverse primer (universal, V3 region) 518r 5'-ATTACCGCGGCTGCTGG-3'. A GC clamp (5'-CGCCCCGCCGCGCGCGGGCGGGGCGGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3') was attached to the 5'-end of the GC341f primer [30].

2.10 Identification of TGGE Bands

DNA was extracted from the TGGE band and purified with a DNA Gel Purification kit (Accuprep, Bioneer, Daejeon, Korea). The purified DNA was subsequently amplified with the same primers and procedures employed for TGGE sample preparation, except the GC clamp was not attached to the forward primer. The species-specific identities of the amplified 16S-rDNA variable region were determined based on sequence homology using the GenBank database system.

3. Results and Discussion

3.1 General Characterization of *Doenjang*

Moisture and salinity of *doenjang* were not significantly different among the four types of *doenjang*, mineral content and maturity was relatively higher in the *doenjang* containing glasswort, and pH was relatively lower in the *doenjang* containing rice (Table 1). Maturity is generally proportional to concentration of soluble peptides or amino acids produced from soy protein by catalysis of microbial enzymes. The production efficiency of soluble peptides and amino acids may be dependent upon

physiological activity of microorganisms and environmental conditions [31]. However, if the substrate (soybean protein) is not limited by microbial growth, production of soluble peptides and amino acids may be more influenced by microbial activity than substrate concentration. Theoretically, organic substrates in *meju* composed of soybean cannot be deficient in microbial growth but inorganic substrates may be. Glasswort contains higher Mg, Fe, Zn, Mn, and Ca contents than those of soybean and rice [32]. Trace minerals are absolutely required as a prosthetic group for enzymes catalyzing bacterial energy metabolism. Accordingly, glasswort may be a useful mineral source to activate *meju* fermentation and improve the nutrition of *doenjang*; however rice is exceptionable based on maturity and mineral content.

3.2 Minerals in *Doenjang*

As previously stated, glasswort is seaweed-like plant that contains relatively higher mineral content than that of soybean and rice [5]. Fine glasswort powder was used as the raw material in the *meju*-making process, which became a *meju* ingredient during fermentation. The minerals contained in the glasswort may be substrates for microorganisms responsible for *meju* fermentation and become ingredients of *doenjang* during ripening. Accordingly, the absolute-value of the minerals contained in the *doenjang* with glasswort such as Mg, Ca, Mn, Fe, Zn, and Al was higher than that of *doenjang* without glasswort (Table 2). The mineral content of *doenjang* was not affected by adding rice, which was expected due to the mineral content of the rice [5]. Mineral content of *doenjang* may not be a

Table 1 General characteristics of *doenjang* made of DFS, DFS-G, DFS-R and DFS-GR *doenjang*.

Characteristics	DFS (1)	DFS-G	DFS-R	DFS-G-R
Moisture (%)	45.7 ± 1.2	44.6 ± 0.9	45.0 ± 1.1	44.3 ± 1.1
Salinity (%)	17.9 ± 0.2	18.3 ± 0.2	18.1 ± 0.3	18.0 ± 0.2
Minerals (mg/kg)	5,653 ± 110	6,121 ± 95	4,744 ± 88	6,027 ± 94
pH	6.37 ± 0.2	6.20 ± 0.2	5.84 ± 0.2	5.98 ± 0.1
Maturity (mg%)	520 ± 26	680 ± 18	460 ± 23	558 ± 11
Ethanol (%)	0.23 ± 0.03	0.15 ± 0.02	0.15 ± 0.02	0.64 ± 0.03

Table 2 Mineral contents in *doenjang* made of DFS, DFS-G, DFS-R, and DFS-GR *doenjang*.

Minerals (mg/kg)	DFS (1)	DFS-G	DFS-R	DFS-G-R
Na	147,921 ± 941	146,959 ± 1,091	148,400 ± 1,083	146,542 ± 1,118
Mg	3,501 ± 16	3,710 ± 19	3,108 ± 15	3,645 ± 15
K	11,874 ± 32	10,979 ± 42	7,208 ± 36	10,249 ± 33
Ca	1,903 ± 82	2,002 ± 66	1,332 ± 65	1,998 ± 71
Mn	8.50 ± 0.23	11.55 ± 0.25	3.89 ± 0.31	9.61 ± 0.18
Fe	130.8 ± 6.1	178.2 ± 6.1	126.9 ± 4.9	171.7 ± 5.8
Zn	16.86 ± 0.13	19.14 ± 0.11	11.26 ± 0.27	18.24 ± 0.19
Al	92.9 ± 3.8	200.6 ± 2.1	162.4 ± 2.7	185.1 ± 3.2
Ni, Cd, Cr, Cu, La, Pb, Sb, Se, Sn, Ti	N.D	N.D	N.D	N.D

Table 3 Free amino acid contents in *doenjang* made of DFS, DFS-G, DFS-R, and DFS-GR *doenjang*.

Taste	Amino acids (%)	DFS (1)	DFS-G	DFS-R	DFS-G-R
Sweet	Thr	0.16 ± 0.01	0.19 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
	Ser	0.18 ± 0.01	0.21 ± 0.01	0.16 ± 0.01	0.18 ± 0.01
	Gly	0.13 ± 0.01	0.14 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
	Ala	0.21 ± 0.01	0.29 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
	Lys	0.20 ± 0.01	0.24 ± 0.01	0.15 ± 0.01	0.16 ± 0.01
	Subtotal	0.65 ± 0.5	1.07 ± 0.05	0.77 ± 0.05	0.82 ± 0.05
Savory	Asp	0.36 ± 0.02	0.43 ± 0.02	0.34 ± 0.03	0.32 ± 0.02
	Glu	0.67 ± 0.02	0.80 ± 0.03	0.49 ± 0.02	0.55 ± 0.02
	Cys	0.06 ± 0.001	0.07 ± 0.001	0.05 ± 0.001	0.06 ± 0.001
	Subtotal	1.09 ± 0.041	1.30 ± 0.051	0.88 ± 0.051	0.93 ± 0.041
Bitter	Met	0.04 ± 0.001	0.04 ± 0.001	0.04 ± 0.001	0.04 ± 0.001
	Ile	0.18 ± 0.01	0.20 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
	Leu	0.28 ± 0.01	0.30 ± 0.01	0.23 ± 0.01	0.22 ± 0.01
	Subsotal	0.50 ± 0.021	0.54 ± 0.021	0.41 ± 0.021	0.40 ± 0.021
Others	Pro	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.17 ± 0.01
	Val	0.23 ± 0.03	0.27 ± 0.03	0.19 ± 0.03	0.19 ± 0.02
	Tyr	0.14 ± 0.01	0.13 ± 0.01	0.08 ± 0.01	0.10 ± 0.01
	Phe	0.16 ± 0.01	0.18 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
	His	0.07 ± 0.001	0.08 ± 0.001	0.00 ± 0	0.00 ± 0
	Arg	0.22 ± 0.01	0.26 ± 0.01	0.18 ± 0.01	0.22 ± 0.01
	Subtotal	0.98 ± 0.071	1.09 ± 0.071	0.73 ± 0.07	0.80 ± 0.06
Total		3.22 ± 0.267	3.99 ± 0.227	2.79 ± 0.268	2.95 ± 0.198

factor in the taste and flavor of the final product but must be considered for nutritional quality.

3.3 Free Amino Acids in *Doenjang*

Some specific bacterial strains can produce amino acids from sugars by fermentation only under specific growth conditions [33, 34]. However, it is certainly possible that free amino acids in *meju* and *doenjang* are generated only by enzymatic hydrolysis of proteins contained in the DFS, glasswort, or rice. The

protein content in glasswort and rice is not higher than 2.0% (w/w) and 8.0% (w/w), respectively, whereas the protein content of DFS is > 40% (w/w) [5]. Accordingly, the protein content of the *meju*-making materials was not a limiting factor for microbial growth, and amino acid production may be proportional to the bacterial activity for protein enzymatic hydrolysis. The free amino acid content in *doenjang* was not correlated with the percentage balance of DFS but was correlated with glasswort (Table 3). Adding glasswort to

doenjang significantly increased free amino acid content when compared based on the ratio of free amino acid content to DFS content. The species and contents of free amino acids may be one of the factors that determine the taste of *doenjang*. The content of sweet-tasting and savory-tasting free amino acids was distinctly higher but the bitter-tasting amino acids were slightly higher in *doenjang* with glasswort than those in the others. These results demonstrate that adding glasswort to *meju* activated microbial growth and amino acid production but rice did not alter amino acid composition or content in *doenjang*.

3.4 Organic Acids in *Doenjang*

Non-volatile organic acids produced by microorganisms responsible for *meju* fermentation may have accumulated in the *doenjang* during ripening. Citric and lactic acids effectively improve the taste of soy sauce [35], by which *doenjang* taste may also be improved. Organic acids are theoretically produced from sugars by fermentative metabolism of specific microorganisms and may be produced in proportion to the balance of carbohydrate contained in *meju*-making dough. The balance of carbohydrate in DFS-R and DFS-GR *meju* corresponded to 16% (w/w), considering a 20% (w/w) rice balance in *meju* and an 80% (w/w) starch balance in rice. Citric and acetic

acid production was influenced by adding rice but malic, succinic and lactic acid production was not observed (Table 4). Pyroglutamic acid was about 50% higher in the *doenjang* with glasswort than in samples without. These results suggest that rice or glasswort is not the factor that activates specific microorganisms to induce organic acid production but is nutritional sources supplementing carbohydrate and inorganic compound for *meju*-making. It is possible that the conditions and microorganisms employed in the *meju*-making process may be slightly influenced but may not be greatly changed by adding glasswort or rice.

3.5 Antioxidant Content in *Doenjang*

Dried glasswort composed of leaves and stems contains various pigments including chlorophylls and carotenoids for photosynthesis. Phenolic compounds are also ubiquitous chemicals found in most plants but those contents differ according to plant species. Polyphenols are high-molecular weight polymers, and phenolic compounds contain a simple phenolic ring bearing one or more hydroxyl groups. In particular, phenolic compounds are of considerable interest due to their antioxidant properties [36]. Polyphenol content in *doenjang* did not increase but total phenolic compounds increased about 50% after adding glasswort (Table 5). The activity of *doenjang* for

Table 4 Organic acid contents in *doenjang* made of DFS, DFS-G, DFS-R, and DFS-GR *doenjang*.

Organic acids (mg/kg)	DFS (1)	DFS-G	DFS-R	DFS-G-R
Citric acid	10,634 ± 195	11,262 ± 166	13,134 ± 184	13,706 ± 212
Malic acid	13,581 ± 53	13,535 ± 83	11,341 ± 88	11,335 ± 65
Succinic acid	815 ± 18	1,201 ± 21	999 ± 12	655 ± 11
Lactic acid	1,404 ± 68	1,564 ± 77	1,526 ± 55	1,446 ± 32
Acetic acid	777 ± 16	721 ± 24	957 ± 21	1,000 ± 29
Levulinic acid	1,102 ± 45	584 ± 12	592 ± 9	809 ± 18
Pyroglutamic acid	1,106 ± 55	1,517 ± 62	1,035 ± 49	1,585 ± 68
Total	29,419 ± 450	30,383 ± 445	30,586 ± 418	30,538 ± 435

Table 5 Antioxidative compounds in *doenjang* made of DFS, DFS-G, DFS-R, and DFS-GR *doenjang*.

Antioxidative compounds (mg/kg)	DFS (1)	DFS-G	DFS-R	DFS-G-R
Polyphenol	7,540 ± 388	7,645 ± 268	7,632 ± 293	6,983 ± 255
Total phenolic contents	7,750 ± 316	11,189 ± 495	8,696 ± 382	12,607 ± 388
DPPH scavenging activity	1,652 ± 125	2,189 ± 107	1,948 ± 169	2,625 ± 151
Reduction activity of Fe ³⁺ to Fe ²⁺	1,827 ± 83	2,162 ± 58	1,809 ± 79	2,404 ± 61

DPPH scavenging and reducing Fe^{3+} also increased about 30% by adding glasswort. These results show that adding glasswort to *meju* may cause phenolic compounds to increase in *doenjang* but may not be a factor increasing polyphenol content in *doenjang* [21]. The antioxidants contained in *doenjang* may function as agents to protect against deteriorating *doenjang* quality due to oxidation reactions, which increased effectively by adding glasswort to the *meju*-making dough.

3.6 Volatile Organic Compounds in Doenjang

Certain volatile compounds that cause fragrant flavors and off-flavors may be generated by biochemical conversion of amino acids and metabolic fermentation of sugars during *meju* fermentation and *doenjang* ripening. Eleven volatile compounds were quantitatively analyzed, and seven were identified in the *doenjang* after ripening; however, both the type and concentration of volatile organic compounds were definitively reduced in the *doenjang* made with *meju* containing glasswort and rice (Table 6). All kinds of volatile organic compounds decreased significantly or were not produced in *doenjang* made of *meju* with glasswort and rice. In particular, off-flavor compounds such as 2-methyl-1-butanol and propionic acid are not produced or are greatly reduced in the *doenjang* with glasswort or rice [37-39]. Volatile organic compounds may be better at lowering off-flavor or unwanted flavors in *doenjang* (peppermint, caramel, bacon, spice, and smoky), which are not detected or detected at trace levels [40]. It is possible that glasswort or rice added to fermenting *meju* or ripening *doenjang* may

influence microorganisms not to produce or to produce limited amounts of volatile organic compounds.

3.7 Bacterial Community Variation in Doenjang

DNA was directly extracted from *meju* after fermentation and after sufficient ripening of *doenjang*, which was used as template to amplify 16S-rDNA. The TGGE pattern of the 16S-rDNA variable region (16S-rDNA-VR) obtained from DFS, DFS-G, DFS-R, and DFS-GR *meju* was slightly different depending on source materials, whereas most of the bands were maintained in the *doenjang* TGGE gel (Fig. 1). Thus, the bacterial populations responsible for *meju* fermentation were mostly maintained but others may have been degraded under the high salinity conditions or contaminated during the *doenjang*-making process. Endospores may be more stably maintained than bacterial vegetable cells during the *doenjang*-making process. The homologous bacterial species with the 16S-rDNA-VR extracted from the TGGE gel mostly belonged to *Bacillus* except the uncultured bacterium (Table 7). In particular, *B. licheniformis* and *B. subtilis* are typical bacterial species that have been isolated from commercial and traditional *meju* [35, 41, 42]. *B. amyloliquefaciens* has been identified as the predominant bacterial species occupying the bacterial communities isolated from *kochujang* [43]. In our findings, the community diversity of the bacteria detected in the *meju* was slightly altered due to adding glasswort and rice, and the bacterial species detected in the *meju* were mostly homologous with those detected in the *doenjang*.

Table 6 Volatile organic compounds in *doenjang* made of DFS, DFS-G, DFS-R, and DFS-GR *doenjang*.

Volatile compounds (mg/kg)	DFS (1)	DFS-G	DFS-R	DFS-G-R	General properties
2-methyl-1-butanol	0.99 ± 0.01	0.0	0.0	0.0	Organic solvent, odor
3-methylbutanol	3.67 ± 0.2	0.6 ± 0.01	0.29 ± 0.01	0.68 ± 0.02	Strong fragrant of peppermint or camphor
Propionic acid	12.16 ± 0.2	5.46 ± 0.3	3.47 ± 0.2	5.42 ± 0.2	Off-flavor (odor) for wine
Furfuryl alcohol	92.10 ± 1.4	67.47 ± 2.1	13.23 ± 1.6	15.97 ± 1.3	Caramel, sweet, woody flavor
HDMF	0.61 ± 0.1	0.37 ± 0.02	0.08 ± 0.01	0.0	Caramel-like smelling
HEMF	1.32 ± 0.02	0.27 ± 0.01	0.71 ± 0.01	0.05 ± 0.001	Caramel-like smelling
4-EG	1.06 ± 0.01	0.0	0.0	0.18 ± 0.01	Bacon, spice, clove, smoky aroma

Fig. 1 TGGE pattern of 16S-rDNA variable region amplified with genomic DNA extracted from DFS (lane 1), DFS-G (lane 2), DFS-R (lane 3), and DFS-GR (lane 4) *meju* and *doenjang*.

Table 7 The homologous bacteria with DNAs extracted from the numbered bands in TGGE gel were arranged in the order of the band numbers in Fig. 1.

Band No.	Lanes in TGGE of DNA obtained from <i>meju</i>			
	1 (DFS)	2 (DFS-G)	3 (DFS-R)	4 (DFS-GR)
1	Uncultured bacterium	Uncultured bacterium	<i>Bacillus</i> sp.	<i>Bacillus cereus</i>
2	Uncultured bacterium	<i>Bacillus</i> sp.	Uncultured <i>Bacillus</i> sp.	Uncultured bacterium
3	<i>Bacillus</i> sp.	<i>Bacillus licheniformis</i>	Uncultured bacterium	<i>Bacillus</i> sp.
4	<i>Bacillus licheniformis</i>	Uncultured bacterium	<i>Bacillus</i> sp.	Uncultured bacterium s
5	Uncultured bacterium	Uncultured <i>Bacillus</i> sp.	Uncultured <i>Bacillus</i> sp.	Uncultured <i>Bacillus</i> sp.
6	Uncultured <i>Bacillus</i> sp.			Uncultured bacterium
7				<i>Bacillus subtilis</i>
8				Uncultured <i>Bacillus</i> sp.
Band No.	Lanes in TGGE of DNA obtained from <i>Doenjang</i>			
	1 (DFS)	2 (DFS-G)	3 (DFS-R)	4 (DFS-GR)
1	<i>Bacillus</i> sp.	Uncultured bacterium	Uncultured bacterium	<i>Bacillus</i> sp.
2	Uncultured bacterium	Uncultured bacterium	<i>Bacillus</i> sp.	<i>Bacillus licheniformis</i>
3	<i>Bacillus licheniformis</i>	<i>Bacillus</i> sp.	Uncultured bacterium	<i>Bacillus amyloliquefaciens</i>
4	<i>Bacillus sonorensis</i>	Uncultured bacterium	Uncultured bacterium	Uncultured bacterium
5	<i>Bacillus subtilis</i>	<i>Bacillus licheniformis</i>	<i>Bacillus amyloliquefaciens</i>	
6		<i>Bacillus amyloliquefaciens</i>	<i>Bacillus sonorensis</i>	
7		<i>Bacillus sonorensis</i>		

4. Conclusion

The glasswort may be useful as a supplement during the *meju*-making process based on nutrition, free amino acid production, and antioxidant activity. However, rice may not be functionally based on *doenjang* quality depending on the nutritional supplement, free amino acid content, and antioxidant activity. Various physiologically active compounds of glasswort besides minerals and antioxidants may also play roles in the final composition of *doenjang*. Glasswort is largely utilized as a food additive or nutritional supplement. The use of glasswort in the *meju*-making process may be helpful to improve *doenjang* quality, because the fermentation and ripening process may help to chemically and nutritionally balance and stabilize the DFS and glasswort ingredients.

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