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Development of *Kanjang* (Traditional Korean Soy Sauce) Supplemented with Glasswort (*Salicornia herbacea* L.)

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Abstract

Five types of *meju* were prepared from 100% defatted soybean (DFSG0), a mixture of 90% DFS and 10% glasswort (DFSG1), a mixture of 80% DFS and 20% glasswort (DFSG2), a mixture of 70% DFS and 30% glasswort (DFSG3), and a mixture of 60% DFS and 40% glasswort (DFSG4). Five types of *kanjang* were separately prepared from the 5 types of *meju* by ripening in brine for 6 months. The contents of certain minerals (Mg, Ca, Fe, Mn, and Zn), organic acids (citric acid, malic acid) and the antioxidative effects in the *kanjang* were increased in proportion to the glasswort content in the *meju*. However, the free amino acid contents in the *kanjang* were reduced in proportion to the glasswort content in the *meju*. DFSG1- and DFSG2-*kanjang* did not show distinct differences from DFSG0-kanjang based on aroma, flavor, and taste that were compared simply by panel tests. The bacterial and fungal community in the fermented *meju* and *kanjang* was not affected by the addition of glasswort to the *meju*-making process. Bacteria belonging to the *Lactobacillus* and *Bacillus* genera and the *Lactobacillus* family predominated, and yeasts belonging to the *Saccharomyces* genus and fungi belonging to the *Aspergillus* genus predominated in the fermented *meju* and *kanjang*. In conclusion, the glasswort was a supplement that nutritionally improved the *kanjang* (except for free amino acid contents) but didn't influence the growth of microorganisms that are responsible for the fermentation of *meju* and *kanjang*.

Key words: glasswort, *meju*, *kanjang*, organic acids, amino acids, microbial community

INTRODUCTION

Glasswort, which is a halophyte, has been studied as a potential component of cosmetics and food additives due to its pharmacological and antioxidative effects (1-4), however, its utility for the production of foods or cosmetics is limited, due to its relatively high NaCl contents. The nutritional ingredients of the glasswort do not differ markedly from those of edible vegetables, but its mineral contents are quite similar to seaweeds－most notably, brown seaweed and tangle (5,6). Generally, dried glasswort is used in food processing applications, as fresh glasswort, like other vegetables, can be easily spoiled. The direct application of the dried glasswort to a fermentation process may be one factor that inhibits or limits the growth of bacteria and fungi (yeast). To resolve the limitations induced by NaCl, a desalting technique for dried glasswort was employed to the processes of *makgeolli*- and vinegar-making (7,8). The seaweed-like nutrients and vegetable-like growth of glasswort may prove advantageous for its use as an additive or a raw material for food processing, due to the ease with which it can be cultivated and harvested.

Meju has traditionally been prepared from cooked and crushed soybeans, which are fermented naturally for 20～30 days in a natural environment; *kanjang* is made by the fermentation and ripening of *meju* in brine for more than 6 months (9,10). The long-term fermentation of *meju* and ripening of *kanjang* may involve selective enrichments of the microbial community. Generally, the quality of *meju* is determined by its microbial community, incubation time, and environmental conditions, since the raw material for *meju* has been traditionally limited to the soybean (11). The nutritional constituents of the soybean are sufficient for the growth of bacteria, fungi, and yeast, which are responsible for fermentation in the *meju*-making process (12), nevertheless, roasted wheat powder is mixed with defatted soybeans in the *koji*-making process (13). The differences in principal characteristics between *kanjang* and commercial soy sauce may be caused by the differences in raw materials and fermentation techniques for the production of *meju* and *koji*.

In this study, a mixture of glasswort and defatted soybean (DFS) was fermented to prepare *meju* via the traditional technique. The *kanjang* prepared from the *meju* was analyzed and the nutrients compared to estimate the effects of glasswort on the quality of *kanjang*. The microbial community was analyzed in the fermented *meju*

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and *kanjang* in order to estimate the effects of glasswort on microbial growth. The principal objective of this study was to develop a new recipe for Korean traditional *kanjang* supplemented with glasswort.

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from the Korean branch of Sigma-Aldrich (St. Louis, MO, USA), except those used for amino acid analysis.

Desalting and grinding of glasswort

Dried glasswort purchased from Buan Hamcho (Buan, Jeonnam, Korea) was soaked for 10～15 min in running tap water to wash and remove the salts that crystallized with dust outside of the glasswort body during drying. The desalted glasswort was then completely dried under sunlight and ground to a particle size of less than 50-mesh using a ceramic ball mill (SW-BM117, 11.5 L volume, SW Engineering, Seoul, Korea).

Preparation of *meju*

The ground DFS was moistened with drinking water, steamed for 90 min, then cooled at room temperature. Non-cooked glasswort powder was thoroughly mixed with the steamed DFS to make the *meju*-making dough, which was then cast in round-shaped molds (diameter 20 cm × height 10 cm). Five types of the *meju*-making dough were prepared using the following ingredients: DFSG0 (100% DFS), DFSG1 (mixture of 90% DFS and 10% glasswort), DFSG2 (mixture of 80% DFS and 20% glasswort), DFSG3 (mixture of 70% DFS and 30% glasswort), and DFSG4 (mixture of 60% DFS and 40% glasswort). The prepared *meju*-making dough was incubated on rice straw at 20～25°C. The meju-making dough was fermented into *meju* and naturally dried during 9 weeks of incubation.

Making of *kanjang*

The fermented and dried *meju* was put in a glass jar with a cap, to which 18% (NaCl, w/v) of brine prepared with sun-dried salt was added. NaCl content in the brine was quantitatively determined by titration with $AgNO_3$ on the basis of standard NaCl solution (0.1 M). The ratio of *meju* to brine was adjusted to 1 to 3 based on weight. The *meju* steeped in the brine was ripened for 6 months at 20～25°C. Five types of *kanjang*－DFSG0-, DFSG1-, DFSG2-, DFSG3-, and DFSG4-*kanjang*－were obtained from the five types of *meju* via the filtration and removal of micro-particles.

Analysis of minerals

The minerals were analyzed using an inductively-coupled plasma optic emission (ICPOE) spectrometer (SPECTRO Analytical Instrument, Kleve, Germany). The *kanjang* was filtered through a 0.45 μm membrane filter (0.45 μm filter, Satorius, GmbH, Göttingen, Germany) and diluted 20-fold with double-distilled water. The diluted filtrate was then directly injected into the ICPOE injector under specific wavelengths for Mg (279.553 nm), Na (589.592 nm), K (766.491 nm), Ca (396.847 nm), Mn (257.61 nm), Zn (213.856 nm) and Fe (259.940 nm). The concentrations of minerals were calculated based on the absorbance obtained using standard materials (AccuTrace™ Reference Standard, AccuStandard, New Haven, CT, USA) and dilution rates.

Analysis of organic acids

The organic acid contents of the *kanjang* were analyzed via HPLC (Beckman, Coulter System Gold, Brea, CA, USA) with an ion-exclusion column (Shodex, Rspak KC-811, Showa Denko, Tokyo, Japan) and a refractive index detector (Shodex, RI-101). The column and detector were adjusted to a temperature of 40°C. The mobile phase was $HClO_4$ (6 mM) and the flow rate was adjusted to 0.8 mL/min. The samples prepared via 30 min of centrifugation at 12,000 × *g* and 4°C were filtered through a 0.45 μm membrane filter (Satorius, GmbH) and subsequently desalted by passing through an ion-exchange column (Amberlite IR120, Rohm and Haas, PA, USA). Thirty μL of filtrated and desalted sample was then injected into the HPLC injector. The organic acid concentrations were calculated based on the peak area in the chromatograms generated using standard materials.

Analysis of total nitrogen

Total nitrogen contents of *kanjang* were analyzed via the micro-Kjeldahl method. The *kanjang* samples were diluted properly to fit the range for the chemical oxidation reaction of samples.

Free amino acids

Free amino acids of *kanjang* were analyzed on the basis of the general technique employed for food analysis (14). *Kanjang* was diluted 20-fold with sodium citrate buffer and filtered through a 0.45 μm filter (Satorius, GmbH), which was used for amino acid analysis. Free amino acids were determined with an Ammonia Filtration Column (LCA, k04/Na, 4.6 × 100 mm, Sykam GmbH, Eresing, Germany) equipped with an S433 automatic amino acid analyzer (Sykam GmbH). All of the buffers used for amino acid analysis by the hydrolysate program were purchased from Sykam: buffer A-1, buffer B-1, regeneration solution, and dilution buffer.

Polyphenol content determination

Polyphenol contents were determined via the Prussian

blue spectrophotometric method (15,16). 3.0 mL of 0.1 M $FeCl_3$ in 0.1 M HCl were added to 1 mL of the 1,000× diluted *kanjang* or 10% of glasswort extract, followed immediately by the timed addition of 3.0 mL of freshly prepared 0.008 M $K_3Fe(CN)_6$. The absorbance was measured via spectrophotometry (UV-1601, Shimadzu, Tokyo, Japan) at 720 nm, 10 min after the introduction of the reagents. A standard curve was prepared to express the results as tannic acid equivalents, i.e. the quantity of tannic acid (mg/L) required to achieve a color intensity equivalent to that of the polyphenols after blank correction. Five identical tests were carried out at the same time and under the same conditions.

Total phenolic content (TPC) determination

TPC was determined via the Folin-Ciocalteu colorimetric method (17). In a 10 mL conical tube, 6.4 mL of distilled water, 0.1 mL of 100× diluted kanjang or 10% (w/v) of glasswort extract and 0.5 mL of Folin-Ciocalteu reagent (1:1 with water) were mixed together. After exactly 1 min, 3.0 mL of Na_2CO_3 (10 g/100 mL) was added, and the mixture was allowed to stand for 2 hr at room temperature in darkness. The absorbance was read at 765 nm, and the total polyphenol concentration was calculated from a calibration curve developed using 10～100 mg/L of gallic acid. Five identical tests were carried out at the same time and under the same conditions.

Antioxidation activity

Antioxidants were assessed using a modified method of Brand-Williams et al. (18) for an assay of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity (19). One hundred μm of DPPH was dissolved in 80% aqueous methanol to make the stock solution, which has to be prepared fresh daily. 0.1 mL of 1,000× diluted *kanjang* and 10% (w/v) of glasswort extract were added to 2.9 mL of the methanolic DPPH solution. The mixture was vigorously shaken and permitted to stand at 23°C in darkness for 30 min. The decrease in absorbance of the resulting solution was monitored at 517 nm at 30 min. The controls consisted of 0.1 mL of 50% aqueous methanol and 2.9 mL of DPPH solution. The DPPH radical scavenging activity of *kanjang* was expressed as the mg/kg standard compound of vitamin C equivalent antioxidant capacity in 30 min of reaction time.

Temperature gradient gel electrophoresis

The 16S-rDNA amplified from chromosomal DNA extracted directly from the *meju* was employed as a template for the preparation of the TGGE sample (16S-rDNA variable region). A variable region of 16S-rDNA was amplified with forward primer (eubacteria, V3 region) 341f 5'-CCTACGGGAGGCAGCAG-3' and reverse primer (universal, V3 region) 518r 5'-ATTACCG-CGGCTGCTGG-3'. A GC clamp (5′-CGCCCGCCGCG-CGCGGCGGGCGGGGCGGGGGCACGGGGGGCC-TACGGGAGGCAGCAG-3') was attached to the 5'-end of the GC341f primer (20). The procedures for PCR and DNA amplification were identical to the 16S-rDNA amplification conditions, with the exception of the annealing temperature. The 18S-rDNA amplified from chromosomal DNA was utilized as a template for the preparation of the TGGE sample (18S-rDNA variable region). A variable region of 18S-rDNA was amplified with forward primer (wide range of fungal taxa) EF3 5'-TCCTCTAAATGACCAAGTTTG-3' and reverse primer (wide range of fungal taxa) EF4 5'-GGAAGGGRTGTA-TTTATTAG-3'. A GC clamp (5′-CGCCCGCCGCGCG-CGGCGGGCGGGGCGGGGGCACGGGGGGG-3') was attached to the 5'-end of NS-3 (5'-GCAAGTCTGGTGC-CAGCAGCC-3') (21,22). The procedures for PCR and DNA amplification were identical to the 18S- rDNA amplification conditions, with the exception of the annealing temperature. The TGGE system (Bio-Rad, Dcode™, Universal Mutation Detection System, Hercules, CA, USA) was operated in accordance with the manufacturer's specifications. Aliquots (45 mL) of the PCR products were electrophoresed in gels containing 8% acrylamide, 8 M urea, and 20% formamide with a 1.5 ×TAE buffer system at a constant voltage of 100 V for 12.5 hr and then at 40 V for 0.5 hr, applying a temperature gradient of 39 to 52°C. Prior to electrophoresis, the gel was equilibrated to the temperature gradient for 30 to 45 min.

Amplification and identification of TGGE bands

DNA was extracted from the TGGE band and purified with a DNA gel purification kit (Accuprep, Bioneer, Seoul, Korea). The purified DNA was then amplified with the same primers and procedures employed for TGGE sample preparation, in which the GC clamp was not attached to the forward primer. The species-specific identities of the amplified variable 16S-rDNAs or 18S-rDNAs were determined on the basis of sequence homology, according to the information in the GenBank database system.

Chemical ingredients of glasswort

The practical balance of glasswort in the DFSG1-, DFSG2-, DFSG3-, and DFSG4-*meju* was 10, 20, 30, and 40% (w/w), respectively, from which *kanjang* was extracted directly. On the basis of this balance, 10% (w/v) of glasswort (GE10) was prepared via direct extraction of the desalted glasswort powder with double-distilled water at 60°C for 180 min. Total nitrogen (TN), minerals, organic acids, free amino acids, and antioxidative com-

pounds contained in the GE10 were analyzed for direct comparison with *kanjang*.

Panel test

After ripening for more than 6 months, the aroma, flavor and taste of the DFSG0-, DFSG1-, DFSG2-, DFSG3-, and DFSG4-*kanjang* were sensuously compared by 20 panelists (10 females and 10 males), who are junior and senior students. The aroma was compared using the only olfactory sense by inhalation of air around mouth of test tubes (*kanjang* volume, 10 mL; tube volume, 15 mL) containing the undiluted soy sauces. Distance between nose and test tube mouth was adjusted horizontally to 15 cm. The flavor was compared using the tongue sense without swallowing by dropping the undiluted *kanjang* (100 μL) on center of upper part of tongue. The taste was compared using the tongue sense by keeping 25 mL of a 10 times-diluted *kanjang* in mouth for 10 sec without swallowing. For the blind test, the DFSG0-*kanjang* was double used as a reference and a test group. The similarity between the reference and the test groups was determined based on the following five criteria: completely same (5 points), almost same (4 points), a little different (3 points), different (2 points) and completely different (1 point).

RESULTS AND DISCUSSION

Effect of glasswort on general character of *kanjang*

The protein content in glasswort is substantially lower than that in DFS or soybean (23). TN may be a major factor in evaluating *kanjang* quality, which must be proportional to the protein content of raw materials employed for *meju*-making. Practically, the TN content in *kanjang* was correlated with the percentage balance of DFS as shown in Table 1. TN content in the DFSG0-kanjang was 1.37%, which is within the range of 0.66～2.75% of the *kanjang* made of traditional *meju* (14) and 1.25～1.40% of the commercial soy sauce made of *koji* (9). Meanwhile, TN content in the DFSG1-, DFSG2-, DFSG3-, and DFSG4-*kanjang* was 1.16～1.09, which also is within the range of 0.66～2.75% of the traditional *kanjang,* but lower than the 1.25～1.40% range of commercial soy sauce. Accordingly, the reduction in TN resulting from the addition of glasswort does not result in a falling-off in quality based on the TN content of the traditional *kanjang*.

Effect of glasswort on mineral content in *kanjang*

The minerals in traditional *kanjang* are derived from soybean and brine; meanwhile, those in the *kanjang* prepared herein may have originated from DFS, glasswort, and brine. The content of total minerals in the *kanjang* was not proportional to the glasswort balance; however, the content of Mg, Ca, Fe, Mn, and Zn was generally proportional to the glasswort balance, as shown in Table 2. The majority of Mg and Mn in glasswort were extracted as *kanjang* minerals; however, part of the Ca, Fe, and Zn in glasswort was extracted into the *kanjang*. These results may be caused by the relatively low solubility of Ca, Fe, and Zn in the *kanjang* containing high salt and mineral contents. The minerals of *kanjang* may not be a determining factor in taste and flavor, but has to be considered for nutritional quality.

Effect of glasswort on organic acid content in kanjang

Non-volatile organic acids are known to influence the taste of *kanjang*; in particular, citric acid and lactic acid

Table 1. General characters of *kanjang* made of 100% defatted soybean (DFSG0), a mixture of 90% DFS and 10% glasswort (DFSG1), a mixture of 80% DFS and 20% glasswort (DFSG2), a mixture of 70% DFS and 30% glasswort (DFSG3), a mixture of 60% DFS and 40% glasswort (DFSG4)-*meju*, and 10% (w/v) of glasswort extract (GE10)

Characters	DFSG0	DFSG1	DFSG2	DFSG3	DFSG4	GE10
TN (%)	1.37±0.04	1.16±0.05	1.12±0.03	1.12±0.03	1.09±0.02	0.04
pH	5.62±0.1	5.49±0.2	5.45±0.1	5.37±0.1	5.32±0.1	6.20
Salts (%)	20.56±0.5	20.62±0.5	20.91±0.2	20.56±0.6	20.85±0.3	2.69
Na (%)	8.14±0.4	8.36±0.2	8.12±0.6	7.73±0.2	7.76±0.4	1.91

Table 2. Content of minerals in *kanjang* made of 100% defatted soybean (DFSG0), a mixture of 90% DFS and 10% glasswort (DFSG1), a mixture of 80% DFS and 20% glasswort (DFSG2), a mixture of 70% DFS and 30% glasswort (DFSG3), a mixture of 60% DFS and 40% glasswort (DFSG4)-*meju*, and 10% (w/v) of glasswort extract (GE10)

Minerals (mg/kg)	DFSG0	DFSG1	DFSG2	DFSG3	DFSG4	GE10
Mg	3,118±59	3,238±144	3,377±96	3,336±128	3,411±105	440
K	12,560±392	12,420±255	11,660±219	11,340±415	11,240±388	1,127
Ca	225±9	237±14	267±11	269±5	278±16	170
Fe	6.65±0.6	6.90±0.2	7.15±0.2	7.50±0.5	7.75±0.3	37.8
Mn	0.03>	0.10±0	1.09±0.02	1.67±0.02	2.05±0.01	2.1
Zn	7.10±0.3	7.18±0.5	7.25±0.5	8.35±0.4	8.85±0.5	13.4

Table 3. Content of organic acids in *kanjang* made of 100% defatted soybean (DFSG0), a mixture of 90% DFS and 10% glasswort (DFSG1), a mixture of 80% DFS and 20% glasswort (DFSG2), a mixture of 70% DFS and 30% glasswort (DFSG3), a mixture of 60% DFS and 40% glasswort (DFSG4)-*meju*, and 10% (w/v) of glasswort extract (GE10)

Organic acids (mg/kg)	DFSG0	DFSG1	DFSG2	DFSG3	DFSG4	GE10
Citric acid	3,922±184	4,364±156	4,517±174	4,718±206	4,880±195	598
Malic acid	5,635±135	6,024±211	6,100±249	6,539±240	6,609±158	158
Succinic acid	1,488±43	1,343±48	1,291±26	1,347±53	1,294±33	0
Lactic acid	996±38	948±42	935±33	969±54	942±27	0
Acetic acid	821±37	800±18	792±55	813±36	782±57	217
Pyroglutamic	179±7	162±7	165±4	168±8	162±3	0

are effective in improving the taste of *kanjang* (24). Lactic acid was not increased in proportion to the glasswort balance; however, citric acid and malic acid were increased, as is shown in Table 3. The increments of citric acid and malic acid in the *kanjang* were higher than the citric acid and malic acid contents in the GE10. Accordingly, glasswort may be not only a supplementary source for citric acid and malic acid, but also a physiological factor in the activation of fermentation metabolism for the production of organic acids in the *meju*- and *kanjang*-making process.

Effect of glasswort on free amino acid content in *kanjang*

Free amino acids may be generated via enzymatic hydrolysis of protein or bacterial metabolism for amino acid fermentation. Amino acids cannot be produced by bacterial fermentation in the *meju*- and *kanjang*-making process, because specific bacterial strains can generate amino acid via fermentation under specific growth conditions (17,25). Accordingly, it remains quite possible that the sole source of free amino acid production in *meju* and *kanjang* may be proteins contained in the DFS or glasswort. The protein content in glasswort is not higher than 2.0%, and that in DFS is more than 40% (21,26). The free amino acid contents in the *kanjang* were correlated with the percentage balance of DFS used for the *meju*-making dough, as shown in Table 4. This result shows the same tendency as TN content in the *kanjang*. Contents of free amino acids in the DFSG1-, DFSG2-, DFSG3-, and DFSG4-*kanjang* were about 79 ~83% of those in the DFS *kanjang*, however, which is significantly higher than those in the *kanjang* manufactured in other studies (14,24). The species and contents of free amino acids may be one of the determining factors in the taste of *kanjang*. Contents of the sweet-

Table 4. Free amino acids content in *kanjang* made of 100% defatted soybean (DFSG0), a mixture of 90% DFS and 10% glasswort (DFSG1), a mixture of 80% DFS and 20% glasswort (DFSG2), a mixture of 70% DFS and 30% glasswort (DFSG3), a mixture of 60% DFS and 40% glasswort (DFSG4)-*meju*, and 10% (w/v) of glasswort extract (GE10)

Taste	Amino acids (%)	DFSG0	DFSG1	DFSG2	DFSG3	DFSG4	GE10
Sweet	Thr	0.12±0.004	0.10±0.002	0.10±0.002	0.09±0.004	0.09±0.003	0.00
	Ser	0.18±0.01	0.15±0.005	0.15±0.005	0.14±0.006	0.14±0.008	0.00
	Gly	0.08±0.005	0.07±0.003	0.06±0.001	0.07±0.003	0.07±0.002	0.00
	Ala	0.26±0.01	0.21±0.009	0.20±0.006	0.20±0.001	0.19±0.006	0.00
	Lys	0.08±0.01	0.07±0.002	0.06±0.001	0.07±0.001	0.07±0.001	0.00
	Subtotal	0.72±0.039	0.6±0.021	0.57±0.013	0.57±0.015	0.56±0.021	0
Savory	Asp	0.16±0.003	0.13±0.008	0.13±0.003	0.12±0.002	0.12±0.004	0.00
	Glu	0.51±0.006	0.43±0.005	0.42±0.01	0.41±0.003	0.40±0.003	0.00
	Cys	0.04±0.001	0.04±0.005	0.04±0.001	0.04±0.003	0.04±0.002	0.01
	Subtotal	0.71±0.01	0.6±0.018	0.6±0.014	0.57±0.008	0.56±0.009	0.01
Bitter	Met	0.05±0.001	0.04±0.001	0.04±0.001	0.04±0.001	0.04±0.001	0.00
	Ile	0.15±0.001	0.12±0.005	0.11±0.003	0.11±0.005	0.11±0.004	0.00
	Leu	0.26±0.007	0.21±0.004	0.20±0.003	0.20±0.004	0.19±0.004	0.00
	Subtotal	0.46±0.009	0.37±0.01	0.35±0.007	0.35±0.01	0.34±0.009	0
Others	Pro	0.06±0.002	0.06±0.001	0.06±0.001	0.06±0.002	0.07±0.002	0.00
	Val	0.19±0.006	0.15±0.008	0.14±0.001	0.14±0.006	0.13±0.008	0.00
	Tyr	0.08±0.002	0.08±0.002	0.08±0.003	0.08±0.003	0.08±0.003	0.00
	Phe	0.15±0.004	0.12±0.004	0.12±0.002	0.12±0.003	0.11±0.003	0.00
	His	0.06±0.001	0.05±0.002	0.05±0.001	0.05±0.001	0.05±0.003	0.02
	Arg	0.14±0.005	0.13±0.003	0.12±0.004	0.13±0.006	0.12±0.006	0.02
	Subtotal	0.68±0.018	0.59±0.02	0.56±0.012	0.58±0.021	0.56±0.022	0.04
Total		2.64±0.076	2.22±0.069	2.15±0.046	2.14±0.054	2.10±0.061	0.05

tasting and savory-tasting free amino acids in all of the *kanjang* did not differ greatly from one another and are commonly significantly higher than those of the bitter-tasting amino acids. These results demonstrated that glasswort is neither the source of free amino acids nor the cause of altered amino acid composition in the *kanjang*.

Effect of glasswort on antioxidant contents in *kanjang*

Contents of total phenolic compounds, polyphenols, and antioxidants in the DFSG0-*kanjang* were 2,613, 267, and 3,647 mg/kg, respectively, and may have been produced in the *meju*- and *kanjang*-fermentation processes. Increments of total phenolic compounds, polyphenols, and antioxidants in the DFSG1-, DFSG2-, DFSG3-, and DFSG4-*kanjang* were substantially lower than those in the GE10, as shown in Table 5. According to these results, it is possible that some of the physiological active compounds contained in the glasswort may remain in the final DFSG1～DFSG4-*kanjang*, but most of them lost their functions in tandem with the scavenging reaction of the unnecessary oxidants during the fermentation of *meju* and the ripening of *kanjang* (27-29). Polyphenol, which is found in a variety of vegetables, fruits, and teas, is a bioactive compound capable of defending against deleterious oxidative damage (30). Phenolic compounds can delay *in vitro* and *in vivo* oxidation processes and scavenge reactive oxygen species (31). Accordingly, the antioxidant in the *kanjang* may be effectively increased by the addition of glasswort to the *meju*-making dough.

Effect of glasswort on savor of kanjang

The glasswort may influence the aroma, flavor and taste of *kanjang*. The panelists distinguish distinct differences of aroma, flavor, and taste of DFSG3- and DFSG4-*kanjang* from DFSG0-*kanjang* as shown Table 6. The relatively higher concentration of glasswort contained in the DFSG3- and DFSG4-*kanjang* may cause aroma, flavor, and taste to be differentiated from the DFSG0-, DFSG1-, and DFSG2-*kanjang*. Practically, the *kanjang*-specific compounds influencing aroma, flavor, and taste that are organic acids, volatile compounds, minerals, and amino acids were changed significantly by addition of glasswort. Accordingly, the proper content of glasswort in the *kanjang* is thought to be 10～20% (w/v) based on the contents of specific minerals (Mg, Ca, Fe, Mn) and physiologically active compounds (total phenolic compounds, polyphenol, antioxidant), and similar choice of DFSG1- and DFSG2-*kanjang* with DFSG0-*kanjang* by panelists.

Microbial community variations in *meju* and *kanjang*

The TGGE band patterns of the 16S- and 18S-rDNA variable region obtained from five types of *meju* and *kanjang* were very similar to one another, as shown in Fig. 1 and 2, respectively. According to these results, it can be presumed that glasswort is a general nutritional factor for the microbial community, which is responsible for the fermentation of *meju* and *kanjang*; however, it may not be a growth factor for any specific microorganism. The bacteria and fungi homologous for the variable region of 16S- and 18S-rDNA extracted from the TGGE gel were identified on the basis of the data in the GenBank database, as shown in Table 7. Bacteria belonging to the *Lactobacillus* and *Bacillus* genera and the *Lactobacillus* family predominated, and yeasts belonging to *Saccharomyces* genus and fungi belonging to the *Aspergillus* genus predominated. *Bacillus subtilis* and

Table 5. Content of total phenolic compounds, polyphenol, and antioxidant in *kanjang* made of 100% defatted soybean (DFSG0), a mixture of 90% DFS and 10% glasswort (DFSG1), a mixture of 80% DFS and 20% glasswort (DFSG2), a mixture of 70% DFS and 30% glasswort (DFSG3), a mixture of 60% DFS and 40% glasswort (DFSG4)-*meju*, and 10% (w/v) of glasswort extract (GE10)

Contents (mg/kg)	DFSG0	DFSG1	DFSG2	DFSG3	DFSG4	GE10
Total phenolic contents	2,613±98	2,705±121	2,935±105	2,997±158	3,191±85	2,467
Polyphenol	267±54	275±32	276±46	288±38	289±43	190
Antioxidant	3,647±119	5,738±184	6,267±249	7,655±232	8,978±411	6,362

Table 6. Comparison of the aroma, flavor, and taste among reference *kanjang* (DFSG0) and the test *kanjang* (DFSG0～DFSG4)

Test items	Similarities of test groups with the reference (DFSG0-*kanjang*)				
	DFSG0	DFSG1	DFSG2	DFSG3	DFSG4
Aroma	4.80±0.41	4.75±0.64	4.75±0.55	4.60±0.59	4.45±0.76
Flavor	4.75±0.44	4.70±0.57	4.70±0.66	4.45±0.56	4.35±0.81
Taste	4.85±0.49	4.80±0.52	4.70±0.66	4.30±0.80	4.20±0.52

The *kanjang* was made of 100% defatted soybean (DFSG0), a mixture of 90% DFS and 10% glasswort (DFSG1), a mixture of 80% DFS and 20% glasswort (DFSG2), a mixture of 70% DFS and 30% glasswort (DFSG3), and a mixture of 60% DFS and 40% glasswort (DFSG4)-*meju*.

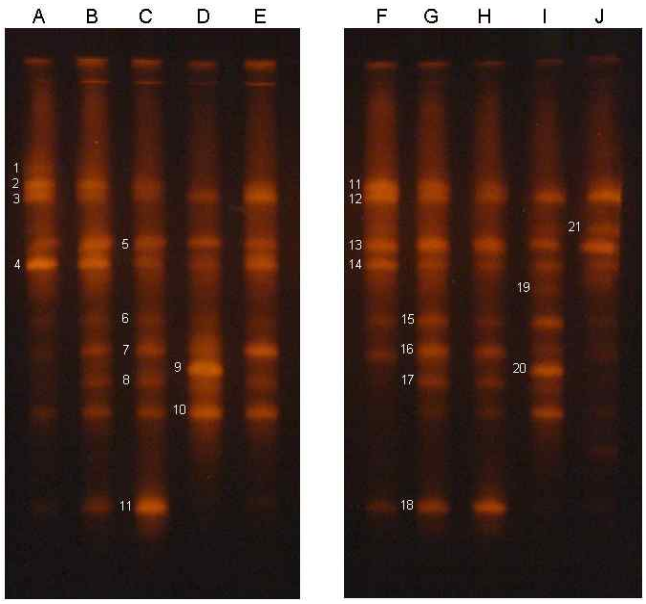

Fig. 1. TGGE pattern of 16S-rDNA variable region amplified with genomic DNA extracted from DFSG0- (lane A), DFSG1- (lane B), DFSG2- (lane C), DFSG3- (lane D), and DFSG4-*meju* (lane E); and DFSG0- (lane F), DFSG1- (lane G), DFSG2- (lane H), DFSG3- (lane I), and DFSG4-*kanjang* (lane J).

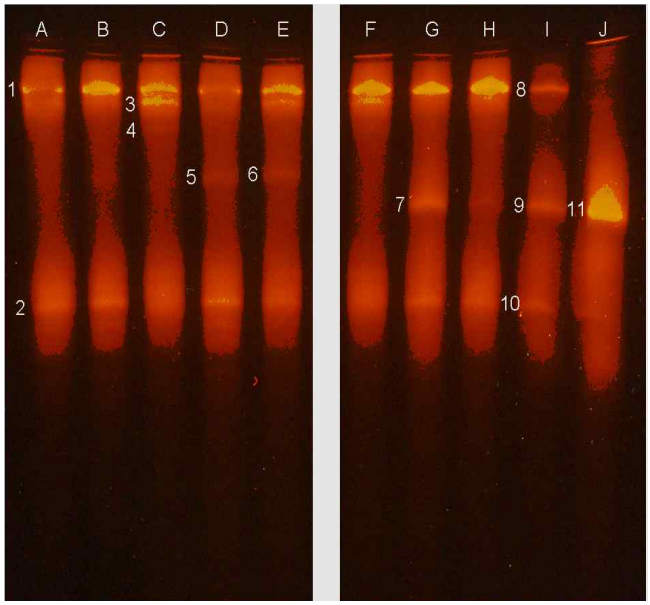

Fig. 2. TGGE pattern of 18S-rDNA variable region amplified with genomic DNA extracted from DFSG0- (lane A), DFSG1- (lane B), DFSG2- (lane C), DFSG3- (lane D), and DFSG4-*meju* (lane E); and DFSG0- (lane F), DFSG1- (lane G), DFSG2- (lane H), DFSG3- (lane I), and DFSG4-*kanjang* (lane J).

Aspergillus sp. are typical microorganisms used in *meju* fermentation (32), and were isolated from fermented *meju* (24). Lactic acid bacteria belonging to the genera *Lactobacillus* and *Pediococcus*, and yeast including *Saccharomyces* spp., *Endomyces* spp., and *Ogataea* spp. are typical microorganisms that grow in fermented foods. However, other microorganisms including *Olivibacter* sp., uncultured bacteria, *Monascus* sp., and *Eladia* sp. may be contaminants without any special functions or significant impacts on *meju* and *kanjang* fermentation (33).

In conclusion, some of the chemical components of glasswort were turned into the nutritional ingredients of

Table 7. The homologous microorganisms with DNAs extracted from the numbered bands in TGGE gel were arranged in the order of the band numbers in Fig. 2 and 3

Band No	16S-rDNA		18S-rDNA	
	Genus or species (Accession number)	Homology (%)	Genus or species (Accession number)	Homology (%)
1	*Lactobacillus plantarum* (HM058985)	98	*Saccharomyces fibuligera* (EU057520)	97
2	Uncultured bacterium (HM073691)	97	*Endomyces* sp. (D86913)	98
3	*Lactobacillus pentosus* (HM067026)	98	*Ogataea polymorpha* (FJ914908)	98
4	*Olivibacter sitiensis* (DQ421387)	99	*Saccharomyces* sp. (DQ345288)	99
5	*Bacillus* sp. (FJ763943)	98	*Aspergillus candidus* (EU883597)	96
6	Uncultured bacterium (HM263074)	99	*Monascus fuliginosus* (HM188433)	97
7	Uncultured bacterium (EU464747)	99	*Aspergillus* sp. (EU365862)	99
8	Uncultured bacterium (FJ032513)	97	Uncultured *Eurotiomycetes* family (FN598407)	99
9	*Bacillus substilis* (AB210949)	99	*Aspergillus* sp. (DQ810192)	99
10	Uncultured *Lactobacillaceae* (HM076834)	98	*Saccharomyces malanga* (EU057521)	97
11	*Bacillus* sp. (EU162022)	99	*Eladia saccula* (AJ748275)	99
12	*Lactobacillus pentosus* (HM067026)	99		
13	*Bacillus* sp. (FJ763943)	99		
14	*Olivibacter sitiensis* (DQ421387)	97		
15	Uncultured bacterium (HM263074)	97		
16	Uncultured bacterium (DQ824670)	99		
17	Uncultured bacterium (FJ032513)	99		
18	*Bacillus* sp. (EU162022)	99		
19	*Pediococcus* sp. (AF349938)	98		
20	*Bacillus substilis* (AB210949)	99		
21	Uncultured bacterium (HM265895)	99		

kanjang, they activated fermentation metabolism for citric and malic acid production, and they may have functioned as oxidant scavengers. Glasswort is largely utilized as a food additive or nutritional supplement without biochemical or biological processes. The use of glasswort in the *meju*-making process may be helpful in improving the quality of *kanjang*, because the fermentation and ripening process may help to chemically and nutritionally balance and stabilize ingredients of DFS and glasswort.

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