Quality Characteristics of Low-Salt Gochujang Added with Glycyrrhiza uralensis and Brassica juncea

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Abstract

The effects of Glycyrrhiza uralensis and Brassica juncea on the quality and palatability of low-salt gochujang were investigated in terms of the microbial characteristics, enzyme activities, pH, acidity, amino nitrogen and sensory evaluation during 40 days of fermentation. The proliferation of fungi in low-salt gochujang with added G. uralensis and B. juncea were inhibited, while the numbers of total viable bacteria and lactic acid bacteria were not affected. In terms of α -amylase and β -amylase activity, no significant difference was observed by the salt concentration or additives. However, lowering the salt concentration increased protease activity. The amount of amino-nitrogen in low-salt gochujang at 20 days was similar to that in the control gochujang at 40 days. In the sensory test, low-salt gochujang was preferred compared to control gochujang (8.5% salt). Particularly, the 4.3% salt gochujang with additives was the most preferred.

Key words: Glycyorrhiza uralensis, Brassica juncea, low-salt, gochujang

INTRODUCTION

Gochujang (red pepper paste), Korea's starch-based fermented food made of glutinous rice and red pepper powder mixed with Koji and salt, has a unique taste and palatability attributed to the organic acid, alcohol, sugars, amino acids, and other byproducts created from the metabolism and fermentation of microbes. About 10% (w/ w) salt is added to gochujang to give the food a salty taste and enhance storability by controlling the activities of microbes during fermentation. While salt plays a key role in maintaining the human body's homeostasis, excessive salt or sodium intake is known to cause or worsen high blood pressure and vascular, cardiac, or renal diseases (1-3). According to the Korea National Health and Nutrition Examination Survey, the average amount of sodium intake per person was 4,388 mg in 2007 and 4,553 mg in 2008, more than twice the 2,000 mg recommended amount of sodium intake by the World Health Organization (WHO) (4). To solve this problem, the Korea Food & Drug Administration (KFDA) established the Sodium Reduction Commission in 2010, which promotes the sodium reduction policy that seeks to reduce the average sodium intake per person by at least 10%. To achieve such a goal, it is important to examine the sodium intake pathway and promote sodium reduction by priority among main foods containing high sodium levels. Therefore, sodium reduction in paste foods, such as gochujang or doenjang (soy bean paste), which account for about 73% of Koreans' sodium intake, is an important measure (5). Additionally, *gochujang* has recently been registered as a codex food and discovered to function against obesity (6) and diabetes (7). To further promote such interest and develop *gochujang* as a global sauce, sodium reduction is essential. However, reducing the salt concentration in *gochujang* can lead to gas formation and deteriorated sensory quality during aging and storage. To solve such problems in manufacturing low-salt *gochujang*, many studies on quality and fermentation properties of *gochujang* with added alcohol and lactic acid (8), sake cake (9), horseradish (10), food preservatives (11), and gamma irradiation (12) have been attempted. However; the results of these studies have not been commercialized.

Glycyrrhiza uralensis, a plant with rich ethnobotanical history, is used as folk medicine both in European and Eastern countries. Glycyrrhiza uralensis ethanol extract has an antibacterial activity against Staphylococcus aureus, Listeria monocytogenes, Streptococcus mutans and Bacillus subtilis (13), while isolated liquiritigenin and liquiritin are also known to have anti-microbial functions (14). Allyl isothiocyanate, a component of Burassica juncea, has strong anti-fungal activity against yeast (15) and inhibits the growth of Candida boidinii, the cause of pellicle formation on the surface of gochujang (16). In a recent study, the shelf life of low-salt soybean paste was extended by adding B. juncea, G. uralensis and chitosan (17). Therefore, the purpose of this study was to examine

the possibility of manufacturing low-salt *gochujang* by adding *B. juncea* powder and *G. uralensis* ethanol extract.

MATERIALS AND METHODS

Material and reagents

G. uralensis, meju, starch syrup, malt, red pepper powder, glutinous rice flour, and salt were purchased from Ilsin (Seoul, Korea) and whole *B. juncea* powder was ground from oriental *B. juncea* seeds #202 (G.S DUNNCO LTD., Ontario, Canada). All of the reagents were purchased from Junsei (Tokyo, Japan) and media were purchased from Difico (Detroit, MI, USA).

Extraction of Glycyrrhiza uralensis

Washed and added 1 kg of *G. uralensis* to 4 L of 85% ethanol for extraction using an extractor (Hanbak Scientific Co., Bucheon, Korea) at 40°C for 3 hr. The extract was filtered using Whatman No. 2 and was concentrated down to 3 L. This mixture was prepared for low-salt *gochujang*.

Low salt gochujang preparation

The mixing ratio for preparing *gochujang* according to the traditional methods of Moonokraega is given in Table 1. *Gochujangs* were divided into seven groups depending on the addition of salt, *G. uralensis* and *B. juncea*. Control was made with 8.5% salt, and low-salt *gochujangs* were added with 5.9%, 4.3% or 2.0% salt, and low salt *gochujangs* with additives were added with 0.8% of *G. uralensis* ethanol extract and 0.7% of *B. juncea* powder. The amount of added *G. uralensis* ethanol extract and *B. juncea* powder were determined in a pretest. At this concentration, *G. uralensis* ethanol extract and *B. juncea* powder did not affect the taste and flavor of *gochujang*. *Gochujangs* were fermented at 28°C for 50 days, and analyzed every 10 days.

Microbiological analysis

To enumerate the microorganism populations in *go-chujang* samples, 25 g of the samples were aseptically transferred to sterile bags containing 225 mL of 0.85% NaCl and pummeled in stomacher (Tekmar Co., Los Angeles, CA, USA) for 2 min. The homogenates were serially diluted with 0.85% NaCl. The 1 mL of diluent was plated on petri dishes, with 15 mL of plate count agar (Difco Co.) for total bacteria, MRS containing 1% bromophenol for lactic acid bacteria, or potato dextrose agar for fungi poured over the diluent. After the media solidified, the plates were incubated at 37°C for 24 hr (for total bacterial and lactic acid bacteria) or at 30°C for 48 hr (for fungi) and the colonies were manually counted.

Enzyme activity

The extracts of gochujang (10 g each) were prepared by shaking in 100 mL distilled water for 30 min at room temperature. After centrifugation (Beckman Instruments, Inc., Palo Alto, CA, USA) at 3000 rpm for 10 min, the supernatant was filtered using Whatman No. 2 filter paper. Filtrate was then gathered for determination of amylase and protease activity. To measure α-amylase activity, Fuwa's colorimeric method (18) of iodine-starch color reaction was performed. 0.1 mL of filtrate was added to a mixture of 0.5 mL of 0.5% soluble starch and 4 mL of 0.04 M potassium phosphate buffer (pH 5.9). After the mixture was reacted at 25°C for 10 min, 0.1 mL of 0.1 N HCl was added to stop the reaction. A 0.1 mL aliquot of the reaction mixture was then mixed with 1 mL of 1/3,000 N iodine reagent. After incubating at room temperature for 20 min, the absorbance was measured at 660 nm using a UV-spectrophotometer (V560, Jasco, Tokyo, Japan). One unit of amylase activity was defined as the amount of enzyme that decreased the absorbance at 660 nm by 10% per 1 min. A β-amylase activity was determined by the dinitrosalicylic acid method (19), where a mixture of 0.1 mL of 1% soluble starch

Table 1. The mixing ratio of ingredients for the preparation of <i>gochujang</i> (unit	t:	٤	g)	
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	8.5%	5.9%	5.9%+LM ¹⁾	4.3%	4.3%+LM	2.0%	2.0%+LM
Meju	210	216	195	219	198	225	204
Water	1,248	1,284	1,260	1,305	1,281	1,338	1,314
Starch syrup	105	108	108	111	111	111	111
Malt	132	135	135	138	138	141	141
Red pepper powder	420	432	432	438	438	450	450
Glutinous rice flour	630	648	648	660	660	675	675
Salt	255	177	177	129	129	60	60
	(8.5%)	(5.9%)	(5.9%)	(4.3%)	(4.3%)	(2%)	(2%)
B. juncea	` — ´	` — <i>`</i>	21	` — ´	21	` — <i>`</i>	21
G. uralensis extract	_	_	24	_	24	_	24
Total	3,000	3,000	3,000	3,000	3,000	3,000	3,000

¹⁾LM: G. uralensis 0.8% and B. juncea 0.7%.

and 0.1 mL of 0.016 M sodium acetate buffer (pH 6.8) was added to 0.1 mL of the supernatant and warmed for 10 min at 30°C. Zero point three mL of dinitrosalicylic acid was added to the reaction mixture, and the activity level was determined as the absorbance at 550 nm using the UV-spectrophotometer. Using maltose as the standard, a unit of enzyme activity was expressed as the liberation of 1 mg of glucose per 1 mL of the supernatant. Protease activity was determined by modifying the Anson method (20). Zero point 5 mL of filtrate was added to 2 mL of 0.6% casein (pH 7.0), and reacted at 30°C for 30 min. The protease activity was then measured as the absorbance at 660 nm by a UV-spectrophotometer, and the units were expressed as the liberation of 1 μg of tyrosine per 1 mL of filtrate.

pH, acidity and amino nitrogen levels

Five g of *gochujang* was well mixed with 250 mL of distilled water for 1 hr at room temperature, and filtered using Whatman No. 2 filter paper. This filtrate was used for measurement of pH, acidity and amino nitrogen. The pH of test solution (50 mL) was measured using the pH meter (Orion 3 star, Thermo Electron Co., Beverly, MA, USA). Titratable acidity of test solution (50 mL) was determined as the titration volume in mL of 0.1 N NaOH needed to bring the pH to 8.4. For the amino nitrogen test, the pH of filtrate (50 mL) was adjusted to 8.4 using

0.1 N NaOH, and followed by the addition of 30 mL of 50% formalin. The pH was again adjusted to 8.4 with 0.01 N NaOH. The final titrated volume was used to calculate the amino nitrogen content. The distilled water was used as the blank test.

Color

Color of *gochujang* samples was determined using a colorimeter (CR-300, KONICA MINOLTA, Osaka, Japan) calibrated with a calibration plate (L^* =99.48, a^* =-0.07, b^* =-0.6).

Sensory evaluation

The low salt *gochujangs* were evaluated by ten panelists at 40 days. Seven samples were presented in random order. The samples were evaluated using a 9 point scale for color, flavor (savory, salt, unpleasant, sour), taste (savory, sweet, salt, sour), and overall palatability. The color, flavor, taste and overall palatability were evaluated as follows: very poor (1 point), moderate (5 point), very good (9 point). The savory, salty, unpleasant, and sour flavor and taste were evaluated as follows: very low (1 point), medium (5 point), very strong (9 point).

Statistical analysis

Statistical analysis was performed with SAS (Statistical Analysis System). Mean and standard deviations were calculated, and Tukey's multiple range tests were

Table 2. Changes in microbial population of low-salt *gochujang* added with *G. uralensis* and *B. juncea* during fermentation at 28°C (Unit: Log CFU/g)

		Fermentation time (day)					
		0	10	20	30	40	50
	8.5%	8.43 ± 0.10^{AB}	$8.44 \pm 0.18^{\text{bAB}}$	8.49 ± 0.10^{A}	8.35 ± 0.07^{AB}	8.31 ± 0.15^{aB}	8.04 ± 0.13^{dC}
	5.9%	8.45 ± 0.12^{A}	$8.26\pm0.08^{\rm cBC}$	8.31 ± 0.11^{B}	8.19 ± 0.11^{CD}	8.12 ± 0.08^{bD}	$8.07 \pm 0.09^{\text{cdD}}$
Total viable	$5.9\% + LM^{1)}$	8.29 ± 0.06	8.26 ± 0.15^{c}	8.29 ± 0.14	8.19 ± 0.10	$8.28 \pm 0.02^{\mathrm{ab}}$	8.22 ± 0.06^{ab}
cell	4.3%	8.35 ± 0.20	8.26 ± 0.14^{c}	8.32 ± 0.09	8.30 ± 0.16	8.32 ± 0.18^{a}	8.20 ± 0.11^{bc}
Cell	4.3% + LM	8.30 ± 0.07	$8.25 \pm 0.15^{\circ}$	8.27 ± 0.22	8.26 ± 0.13	8.27 ± 0.16^{ab}	8.07 ± 0.12^{cd}
	2.0%	8.43 ± 0.21^{AB}	8.53 ± 0.05^{abA}	8.42 ± 0.05^{AB}	8.34 ± 0.18^{B}	$8.30 \pm 0.03^{\mathrm{aB}}$	$8.27 \pm 0.07^{\text{abB}}$
	2.0% + LM	$8.33\pm0.08^{\mathrm{B}}$	8.67 ± 0.10^{aA}	$8.40 \pm 0.25^{\mathrm{B}}$	$8.28 \pm 0.12^{\mathrm{B}}$	8.42 ± 0.18^{aB}	8.34 ± 0.13^{aB}
	8.5%	4.42 ± 0.17^{bcA}	$3.67 \pm 0.10^{\text{cB}}$	3.45 ± 0.20^{bC}	$2.97 \pm 0.13^{\text{bD}}$	2.79 ± 0.08^{bE}	2.70 ± 0.13^{bE}
	5.9%	$4.43 \pm 0.27^{\text{bcA}}$	$3.44 \pm 0.08^{\mathrm{dB}}$	$2.85 \pm 0.11^{\text{cC}}$	2.95 ± 0.03^{bC}	$2.54 \pm 0.03^{\text{cD}}$	ND^{cE}
	5.9% + LM	4.60 ± 0.17^{abA}	$\mathrm{ND}^{\mathrm{fB}}$	$\mathrm{ND}^{\mathrm{eB}}$	$\mathrm{ND}^{\mathrm{dB}}$	$\mathrm{ND}^{\mathrm{eB}}$	$\mathrm{ND}^{\mathrm{cB}}$
Fungi	4.3%	4.69 ± 0.03^{aA}	$3.31 \pm 0.10^{\text{eB}}$	2.26 ± 0.24^{dD}	$2.65 \pm 0.03^{\text{cC}}$	1.65 ± 0.03^{dE}	$\mathrm{ND}^{\mathrm{cF}}$
-	4.3% + LM	4.36 ± 0.21^{cA}	$\mathrm{ND}^{\mathrm{tB}}$	ND^{eB}	ND^{dB}	$\mathrm{ND}^{\mathrm{eB}}$	ND^{cB}
	2.0%	4.69 ± 0.10^{aB}	4.49 ± 0.10^{bC}	$4.56 \pm 0.00^{\mathrm{aB}}$	4.11 ± 0.13^{aD}	$6.32 \pm 0.00^{\mathrm{aA}}$	6.14 ± 0.03^{aA}
	2.0% + LM	4.55 ± 0.16^{abcA}	4.04 ± 0.01^{aA}	$\mathrm{ND}^{\mathrm{eB}}$	$\mathrm{ND}^{\mathrm{dB}}$	$\mathrm{ND}^{\mathrm{eB}}$	$\mathrm{ND}^{\mathrm{cB}}$
	8.5%	$7.58 \pm 0.09^{\text{abAB}}$	$7.34 \pm 0.30^{\text{eC}}$	7.72 ± 0.15^{cA}	$7.46 \pm 0.09^{\text{cdBC}}$	$7.58 \pm 0.15^{\text{cAB}}$	7.28 ± 0.06^{bC}
	5.9%	7.41 ± 0.33^{bcC}	7.93 ± 0.17^{bcA}	$7.55 \pm 0.13^{\text{dBC}}$	7.64 ± 0.18^{abcBC}	$7.72 \pm 0.04^{\text{abcAB}}$	$7.57 \pm 0.20^{\mathrm{aBC}}$
Lastic said	5.9% + LM	$7.34 \pm 0.02^{\text{cBC}}$	7.65 ± 0.16^{dA}	$7.65 \pm 0.16^{\text{cdA}}$	$7.40 \pm 0.21^{\mathrm{dB}}$	$7.60 \pm 0.08^{\text{bcA}}$	7.21 ± 0.14^{bC}
Lactic acid bacteria	4.3%	7.39 ± 0.06^{bcC}	$7.80 \pm 0.18^{\text{cdA}}$	$7.61 \pm 0.08^{\text{cdB}}$	$7.70 \pm 0.08^{\text{abAB}}$	7.63 ± 0.18^{bcAB}	7.57 ± 0.20^{aB}
	4.3% + LM	7.55 ± 0.06^{abcB}	$7.79 \pm 0.05^{\text{cdA}}$	$7.58 \pm 0.14^{\text{cdB}}$	$7.49 \pm 0.02^{\text{bcdB}}$	7.79 ± 0.06^{aA}	7.33 ± 0.08^{bC}
	2.0%	7.51 ± 0.28^{abcE}	8.04 ± 0.11^{bA}	$7.92 \pm 0.05^{\text{bAB}}$	$7.84 \pm 0.08^{\mathrm{aBC}}$	$7.72 \pm 0.09^{\text{abcCD}}$	7.58 ± 0.12^{aDE}
	2.0% + LM	7.67 ± 0.06^{aC}	$8.58 \pm 0.03^{\mathrm{aA}}$	8.25 ± 0.13^{aB}	$7.62 \pm 0.33^{\text{bcdC}}$	7.74 ± 0.14^{abC}	$7.31 \pm 0.17^{\text{bD}}$

 $^{^{1)}}$ LM: G. uralensis 0.8% and B. juncea 0.7%. $^{2)}$ ND: Not detected. Values are mean \pm SD (n=3).

Means with different superscripts in the same column (a-f) and row (A-E) are significantly different (p<0.05).

applied. A probability level of less than 0.05 (p<0.05) was indicated as statistical significance.

RESULTS AND DISCUSSION

Change in the number of bacteria in *gochujang* depending on the fermentation period

The initial number of bacteria in all the test groups was 8.30 to 8.45 log CFU/g. There was no significant change in the number of bacteria during fermentation, and the approximate level of 8 log CFU/g was maintained until 50 days. This result is similar to that of previous researches in which the total number of bacteria in normal gochujang was 7~8 log CFU/g (21,22) and the total number of bacteria was not affected by controlling the amount of salt or adding food additives such as B. juncea or G. uralensis (23,24). This result is related to the spectrum of anti-bacterial substances added to gochujang to control the growth of microbes. We proposed that although B. juncea and G. uralensis used in this study have excellent anti-bacterial activity against yeast, Bacillus spp., and some food poisoning bacteria (25,26), they cannot fully control the growth of all bacteria in gochujang. The number of lactic acid bacteria detected in the early storage period was between 7.34 and 7.67 log CFU/g in all the test groups, and the changes in the numbers of lactic acid bacteria in the test groups except 2.0% salt gochujangs were negligible. In the 2.0% salt gochujang and 2.0% salt gochujang with additives groups, however, the lactic acid bacteria increased by $0.5 \sim 1.0$ log CFU/g after storage for 10 days. The number of fungi in the early fermentation was $4.41 \sim 4.70 \log \text{CFU/g}$, lower than that detected in general gochujang (about 5~ 6 log CFU/g). The number of fungi in 8.5, 5.9, and 4.3% salt gochujang gradually decreased to 2.7~3.5 log CFU/g during the fermentation, but no fungi were detected in these groups with additives after 10 days. The number of fungi in 2.0% salt gochujang rapidly increased up to 6 log CFU/g during fermentation; however, no fungi were detected in the 2.0% salt gochujang with additives after 30 days. The fungi in the gochujang with B. juncea and horseradish were not detected after 60 days of storage, according to Shin et al. (10). Compared to a previous result, adding B. juncea and G. uralensis is more effective in controlling the proliferation of fungi, including yeast.

Although yeast plays a positive role in the flavor of *gochujang* (27), it can also produce gas causing the package to expand (28) or the pellicle on the surface (16). Restraining the production of gas by controlling yeast and continuously increasing amino acid nitrogen via enzymes and bacteria can result in better-sensory quality

of *gochujang* (29). In this study, the addition of additives to *gochujang* also drastically decreased the number of fungi, but did not significantly affect the number of other bacteria. Therefore, the addition of *B. juncea* and *G. uralensis* may positively influence the amount of amino acid nitrogen or the sensory test.

Change in the activity of enzymes

The α-amylase in gochujang liquefies its starchy ingredients. The optimal pH for the activity of α -amylase is around 6.0. Thus, the activity of α -amylase generally increases in the early aging process and decreases with fermentation time (30,31), as the pH decreases for the fermentation. In this study, the α -amylase activity also increased significantly until 20 days and gradually decreased in all the test groups. The β-amylase, a diastatic enzyme that decomposes starch and produces reducing sugar during fermentation, increased up to 5.4~6.6 units at first, then decreased to 3.5~3.9 at 10 days. After 10 days, the activity of β-amylase was maintained until 50 days. These results show that lowering salt concentration or adding B. juncea and G. uralensis to gochujang has little effect on α and β -amylase activity. Such results differ from when antibacterial ingredients were added to the gochujang, the activity of the enzymes decreased more compared to control (23). In producing gochujang, the degradation of the diastatic activity makes the gochujang rough and reduces its flavor. Thus, it is recommended that the amylase activity be adequately maintained. Therefore, the amounts of B. juncea and G. uralensis added to gochujang in this study were appropriate to maintain the activity of amylase.

The amount of free amino acids, related to the savory taste of gochujang, was affected by the protease activity. Protease activity, therefore, is an important index of the taste of gochujang, along with the amount of aminonitrogen. The activity of protease significantly increased in all the test groups during fermentation, and was higher in the low-salt gochujang than in the high-salt; the activity of the enzymes in 8.5% salt gochujang increased by about 3 units, $4 \sim 5$ units in the 5.9 and 4.3% salt; and about 7 units in the 2.0% salt gochujang. These results suggest the protease is sensitive to the salt concentration. Protease activity by the addition of B. juncea powder and G. uralensis extract showed some significant differences in 5.9% and 4.3% salt gochujang in the middle of the aging process (at $10 \sim 30$ days). But, considering that such a difference was negligible and that no regular pattern was observed with the addition of such ingredients, it is considered that the addition of B. juncea and G. uralensis did not greatly affect the activity of protease.

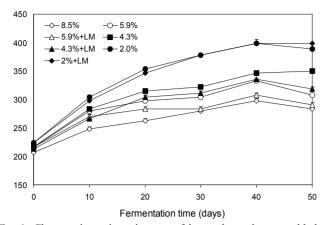


Fig. 1. Changes in amino nitrogen of low salt *gochujang* added with *G. uralensis* and *B. juncea* during fermentation at 28°C. LM: *G. uralensis* 0.8% and *B. juncea* 0.7%. Values are mean \pm SD (n=3).

Amino-nitrogen content

Amino-nitrogen allows the observation of how much protein was decomposed into free amino acids, making it an important index in determining the quality of *go-chujang*. Fig. 1 shows the change in the amino-nitrogen content of *gochujang*. The amino-nitrogen content was initially between 206.50 and 224.00 mg% in all test groups, and consistently increased until 40 days, identical to reports of Park et al. (32) and Shin et al. (33). The effect of salt concentration on amino nitrogen con-

tent was similar to the results reported by Lim and Song (24) and Oh et al. (34) whereby amino nitrogen content of gochujang rapidly increased with the reduction of its salt (24,34). At 20 days, the amount of amino-nitrogen in the 5.9, 4.3, and 2.0% salt gochujang was 297.70, 315.00, and 352.50 mg%, respectively, and 283.50, 303.00, and 386.50 mg% in the same groups with additives, respectively. Therefore, the amount of amino-nitrogen in all the test groups was either similar to or higher than 297.70 mg%, which is the amount of amino-nitrogen in the 8.9% salt gochujang (control) at 40 days. These results indicate that if the salt concentration is lowered to or below 5.9%, the fermentation period in the preparation of gochujang can be shortened by about 20 days. This may be due to the greater activity of the enzymes that decompose protein in low-salt gochujang, paralleling the result in this study, suggesting that the lower the salt concentration, the protease activity increases (Table 3). No significant difference in the amount of amino-nitrogen was observed in the 2.0% salt gochujang with and without additives. In the 5.9% and 4.3% salt gochujang, however, mixing additives resulted in a significantly lower amount of amino-nitrogen than in the gochujang without additives. However, considering that the amount of amino-nitrogen in the low-salt gochujang with additives was either higher or about the same as

Table 3. Changes in enzyme activity of low salt *gochujang* added with *G. uralensis* and *B. juncea* during fermentation at 28°C (unit/g)

20 C							(umu 5)
		Fermentation time (day)					
		0	10	20	30	40	50
	8.5%	1.5 ± 0.20^{bC}	$2.5 \pm 0.34^{\mathrm{B}}_{-}$	5.14 ± 0.07^{A}	4.53 ± 0.16^{A}	$1.33 \pm 0.28^{\text{C}}$	1.82 ± 0.03^{C}
	5.9%	1.2 ± 0.41^{bC}	$2.35 \pm 0.20^{\mathrm{B}}$	$5.29 \pm 0.23^{\mathrm{A}}$	5.0 ± 0.21^{A}	$1.32 \pm 0.16^{\text{C}}$	$2.00 \pm 0.10^{\mathrm{B}}$
	$5.9\% + LM^{1)}$	$1.2 \pm 0.03^{\mathrm{bD}}$	$2.3 \pm 0.16^{\text{C}}$	5.3 ± 0.09^{A}	$4.41 \pm 0.27^{\mathrm{B}}$	1.05 ± 0.11^{D}	2.37 ± 0.18^{C}
α-	4.3%	2.5 ± 0.50^{aC}	$2.37 \pm 0.23^{\text{CD}}$	5.51 ± 0.03^{A}	4.51 ± 0.09^{B}	1.40 ± 0.32^{D}	1.78 ± 0.05^{CD}
amylase	4.3% + LM	2.4 ± 0.25^{aCB}	2.64 ± 0.10^{B}	5.12 ± 0.37^{A}	4.60 ± 0.10^{A}	$1.14 \pm 0.07^{\mathrm{D}}$	$1.80 \pm 0.29^{\text{CD}}$
	2.0%	2.4 ± 0.05^{aC}	2.41 ± 0.23^{C}	5.39 ± 0.40^{A}	4.54 ± 0.26^{B}	1.22 ± 0.06^{D}	1.99 ± 0.11^{DC}
	2.0% + LM	2.3 ± 0.30^{aCB}	$2.37 \pm 0.05^{\mathrm{B}}$	4.98 ± 0.34^{A}	4.55 ± 0.07^{A}	1.54 ± 0.19^{C}	1.56 ± 0.22^{C}
	8.5%	6.1 ± 0.20^{abA}	$3.6 \pm 0.21^{\text{B}}$	4.1 ± 0.05^{aB}	3.7 ± 0.23^{aB}	$4.1 \pm 0.13^{\text{B}}_{-}$	$3.9 \pm 0.11^{B}_{-}$
	5.9%	$6.6 \pm 0.50^{\mathrm{aA}}$	3.6 ± 0.05^{B}	$4.0\pm0.26^{ m abB}$	3.6 ± 0.19^{aB}	4.2 ± 0.10^{B}	3.7 ± 0.45^{B}
0	5.9% + LM	5.4 ± 0.22^{bA}	$3.5 \pm 0.26^{\mathrm{B}}$	$4.2 \pm 0.11^{\mathrm{aB}}$	3.8 ± 0.18^{aB}	$4.2 \pm 0.26^{\mathrm{B}}$	3.7 ± 0.16^{B}
β-	4.3%	$6.2 \pm 0.01^{\mathrm{abA}}$	$3.9 \pm 0.22^{\mathrm{B}}$	$4.0\pm0.27^{\rm abC}$	3.4 ± 0.14^{bC}	4.0 ± 0.11^{C}	3.8 ± 0.16^{C}
amylase	4.3% + LM	5.9 ± 0.20^{abA}	3.7 ± 0.15^{B}	$4.1 \pm 0.25^{\mathrm{aB}}$	$3.8 \pm 0.05^{\mathrm{aB}}$	4.3 ± 0.24^{B}	$4.0 \pm 0.24^{\mathrm{B}}$
	2.0%	6.5 ± 0.17^{aA}	$3.5 \pm 0.05^{\mathrm{B}}$	3.8 ± 0.09^{abB}	$3.2 \pm 0.01^{\rm cB}$	$3.6 \pm 0.40^{\mathrm{B}}$	$3.2 \pm 0.45^{\mathrm{B}}$
	2.0% + LM	6.2 ± 0.17^{abA}	$3.8 \pm 0.16^{\mathrm{B}}$	$3.4 \pm 0.16^{\mathrm{bB}}$	$3.2 \pm 0.00^{\text{cC}}$	$4.2 \pm 0.08^{\rm C}$	$3.3 \pm 0.01^{\text{C}}$
	8.5%	6.67 ± 0.35^{aD}	$7.48 \pm 0.17^{\text{cDC}}$	7.48 ± 0.40^{bC}	$8.65 \pm 0.07^{\mathrm{dB}}$	$9.98 \pm 0.30^{\mathrm{bA}}$	9.78 ± 0.22^{bA}
Protease	5.9%	$6.72 \pm 0.27^{\mathrm{aD}}$	7.27 ± 0.15^{bcD}	8.30 ± 0.27^{abC}	$9.22 \pm 0.18^{\text{cdB}}$	$9.75 \pm 0.17^{\mathrm{bB}}$	11.08 ± 0.28^{abA}
	5.9% + LM	$6.67 \pm 0.08^{\mathrm{aD}}$	7.88 ± 0.20^{abC}	$8.72 \pm 0.35^{\mathrm{aBC}}$	9.65 ± 0.12^{cB}	$9.80 \pm 0.30^{\mathrm{bA}}$	$10.27 \pm 0.33^{\mathrm{bA}}$
	4.3%	6.77 ± 0.17^{aD}	$8.03 \pm 0.37^{\mathrm{aC}}$	8.83 ± 0.18^{aC}	9.87 ± 0.10^{bcB}	$10.45 \pm 0.25^{\text{bB}}$	12.02 ± 0.30^{aA}
	4.3% + LM	$6.53 \pm 0.20^{\mathrm{aD}}$	$5.45 \pm 0.28^{\rm cD}$	8.30 ± 0.35^{abC}	$9.67 \pm 0.25^{\text{cB}}$	10.17 ± 0.18^{bB}	11.43 ± 0.10^{aA}
	2.0%	5.47 ± 0.38^{bD}	$8.32 \pm 0.00^{\mathrm{aC}}$	9.37 ± 0.30^{aC}	10.60 ± 0.40^{abB}	11.35 ± 0.42^{aB}	12.48 ± 0.23^{aA}
	2.0% + LM	6.12 ± 0.22^{abD}	8.25 ± 0.08^{aC}	8.73 ± 0.28^{aC}	10.97 ± 0.27^{aB}	11.62 ± 0.12^{aB}	13.00 ± 0.42^{aA}

 $[\]overline{^{1)}}$ LM: G. uralensis 0.8% and B. juncea 0.7%.

Values are mean \pm SD (n=3).

Means with different superscripts in the same column (a-d) and row (A-D) are significantly different (p<0.05).

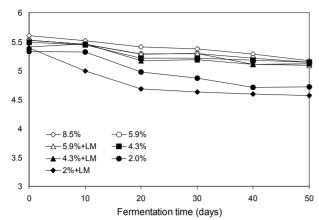


Fig. 2. Changes in pH of low salt *gochujang* added with *G. uralensis* and *B. juncea* during fermentation at 28° C. LM: *G. uralensis* 0.8% and *B. juncea* 0.7%. Values are mean \pm SD (n=3).

that in the control, it is expected that adding 0.7% of *B. juncea* and 0.8% of *G. uralensis* does not affect the shortening fermentation period at a low salt concentration.

pH and acidity

Fig. 2 shows that the pH of gochujang decreased from $5.39 \sim 5.60$ in the early fermentation stage to $4.57 \sim 5.17$ as time passed, which is a typical pH change pattern in gochujang (35). The lower the salt concentration, the lower the pH became. Regardless of the additives, the pH in the 5.9% and 4.3% salt gochujang were between 5.0 and 5.5, indicating a slight difference with the pH of the control group. The pH of the 2.0% salt gochujang tended to decrease to 4.5~4.7. The acidity in all the test groups increased rapidly in the early fermentation process, and tended to slow down as fermentation continued (Fig. 3), suggesting that the organic acids turned into either alcohol or ester in the later fermentation process (36). The change in the acidity, depending on the salt concentration, corresponded to the pH change. As the salt concentration decreased, the higher the acidity became. The acidity of the 2.0% salt gochujang with and without B. juncea and G. uralensis were particularly high. In the 5.9% and 4.3% salt gochujang, the acidity did not significantly differ with the use of additives. While such a result is similar to that of the previous test where lowering the salt concentration increased the acidity (35), the acidity of the gochujang added with B. juncea and secondary ingredients was lower than that of the control group (37). Various bacteria create succinic acid (38), citric acid (39), and pyroglutamic acid (40), among others, in gochujang during fermentation. Such organic acids change the acidity and pH, altering the taste of gochujang; the acidity is closely related to the sourness of gochujang. This study showed that lowering the salt con-

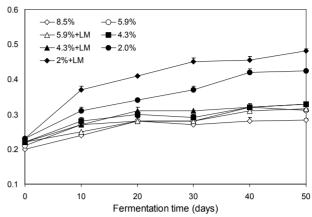


Fig. 3. Changes in acidity of low salt *gochujang* added with *G. uralensis* and *B. juncea* during fermentation at 28° C. LM: *G. uralensis* 0.8% and *B. juncea* 0.7%. Values are mean \pm SD (n=3).

centration of *gochujang* to 2.0% or lower will degrade the quality and sensory palatability of *gochujang* due to sourness resulting from low acidity.

Color

In this study, the change in color during fermentation, an important factor in consumer evaluation of gochujang quality, was measured using a color difference meter (Table 4). Until 40 days of storage, there was no significant change due to the salt concentration, additives, or duration of the storage, with $31 \sim 33$ for L*, $17 \sim 21$ for a*, and 10~13 for b*. Such a result is similar to that of a previous study where L^* was $29 \sim 32$, a^* was $16 \sim 18$, and b* was 14~18 during the fermentation process in traditional gochujang (41). At 50 days, however, L* rapidly decreased to 18~20, and a* and b* greatly increased to 32~24. Moon and Kim (42) reported that the dominant color wavelength of gochujang is between 594 and 597 nm. As gochujang ages, its color turns to brown, becoming darker and deeper due to the Maillard reaction. L* rapidly decreased and a* and b* greatly increased at 50 days in this study because as the browning rapidly progressed, the color of the gochujang became darker and thicker. While the color of the gochujang changed during the aging process in this study, it was not affected by the difference in salt concentration or additives, compared to other studies. Oh et al. (34) and Lim and Song (24) reported that the lower the salt concentration was, the greater the values of L*, a*, and b* increased, while Park and Kim (23) reported that adding anti-bacterial ingredients such as chitosan or B. juncea restrained the browning of gochujang. The reason for the difference in results under similar process conditions is that fermenting gochujang shows a wide variety of color change patterns, depending on the granularity of the red pepper powder, the storage conditions, the use of heat treatment,

Table 4. Changes in color of low salt gochujang added with G. uralensis and B. juncea during fermentation at 28°C

		Fermentation time (day)						
		0	10	20	30	40	50	
	8.5%	33.24 ± 0.07^{A}	31.91 ± 0.10^{A}	31.80 ± 0.47^{A}	32.36 ± 0.21^{A}	32.36 ± 0.53^{A}	19.18 ± 0.58^{B}	
	5.9%	32.84 ± 0.10^{A}	31.73 ± 0.57^{A}	31.80 ± 0.51^{A}	32.59 ± 1.55^{A}	32.48 ± 0.21^{A}	18.93 ± 0.54^{B}	
_ *	5.9%+LM ¹⁾	33.11 ± 1.15^{A}	31.91 ± 0.12^{A}	32.08 ± 0.35^{A}	32.24 ± 0.21^{A}	32.61 ± 0.54^{A}	18.77 ± 0.32^{B}	
L^*	4.3%	$33.88 \pm 0.23^{\text{A}}$	33.29 ± 1.15^{A}	33.12 ± 0.51^{A}	$33.62 \pm 2.35^{\text{A}}$	33.54 ± 1.78^{A}	$19.78 \pm 0.10^{\mathrm{B}}_{\mathrm{p}}$	
	4.3%+LM	33.35 ± 0.49^{A}	33.91 ± 1.23^{A}	33.88 ± 0.69^{A}	33.10 ± 1.63^{A}	33.51 ± 3.42^{A}	$20.63 \pm 1.48^{\mathrm{B}}_{\mathrm{p}}$	
	2.0%	32.85 ± 1.11^{A}	32.04 ± 0.21^{A}	$32.47 \pm 0.25^{\text{A}}$	$32.72 \pm 0.43^{\text{A}}$	32.61 ± 0.24^{A}	$19.23 \pm 0.52^{\mathrm{B}}_{\mathrm{p}}$	
	2.0%+LM	32.97 ± 0.68^{A}	32.79 ± 0.66^{A}	32.59 ± 0.96^{A}	32.90 ± 0.12^{A}	32.74 ± 1.51^{A}	$19.84 \pm 5.13^{\mathrm{B}}$	
	8.5%	$19.52 \pm 0.48^{\mathrm{bB}}$	$17.85 \pm 0.16^{\mathrm{bB}}$	$18.15 \pm 1.23^{\mathrm{bB}}$	$19.26 \pm 0.73^{\mathrm{B}}$	$18.9 \pm 0.67^{\mathrm{B}}$	33.40 ± 0.57^{A}	
	5.9%	19.78 ± 0.31^{abB}	$17.49 \pm 0.33^{\mathrm{bB}}$	$18.50 \pm 2.30^{\mathrm{bB}}$	$19.35 \pm 0.23^{\mathrm{B}}$	$18.61 \pm 1.20^{\mathrm{B}}$	33.22 ± 0.53^{A}	
*	5.9%+LM	$19.89 \pm 0.21^{\mathrm{abB}}$	$18.16 \pm 0.36^{\mathrm{bB}}$	$18.33 \pm 0.10^{\mathrm{bB}}$	18.58 ± 0.71^{B}	$18.97 \pm 2.13^{\mathrm{B}}$	32.59 ± 0.48^{A}	
a	4.3%	$20.04 \pm 1.06^{\mathrm{abB}}$	$20.17 \pm 1.24^{\mathrm{abB}}$	$20.19 \pm 1.00^{\mathrm{abB}}$	$20.79 \pm 2.34^{\mathrm{B}}$	$20.86 \pm 0.65^{\mathrm{B}}$	33.50 ± 1.24^{A}	
	4.3%+LM	21.94 ± 1.23^{aB}	21.66 ± 0.40^{aB}	21.18 ± 1.59^{aB}	21.44 ± 1.38^{B}	21.90 ± 3.48^{B}	33.62 ± 0.99^{A}	
	2.0%	$19.75 \pm 0.23^{\mathrm{abB}}$	18.92 ± 0.68^{abB}	$19.14 \pm 1.63^{\mathrm{abB}}$	$19.70 \pm 0.44^{\mathrm{B}}$	$19.42 \pm 0.82^{\mathrm{B}}$	$33.73 \pm 0.83^{\text{A}}$	
	2.0%+LM	$19.68 \pm 0.12^{\text{abB}}$	$20.07 \pm 1.36^{\text{abB}}$	20.11 ± 0.88^{abB}	$20.64 \pm 0.63^{\mathrm{B}}$	$20.00 \pm 0.13^{\mathrm{B}}$	33.84 ± 0.64^{A}	
	8.5%	$12.44 \pm 0.54^{\mathrm{B}}_{-}$	$10.89 \pm 0.37^{\mathrm{B}}_{-}$	$11.02 \pm 0.46^{\mathrm{B}}_{-}$	$12.19 \pm 1.20^{\mathrm{B}}$	$12.00 \pm 0.88^{\mathrm{B}}_{-}$	33.91 ± 1.20^{A}	
	5.9%	$12.73 \pm 0.33^{\mathrm{B}}_{-}$	$10.53 \pm 0.20^{\mathrm{B}}$	$11.27 \pm 0.56^{\mathrm{B}}_{-}$	$12.33 \pm 1.45^{\mathrm{B}}_{-}$	$11.78 \pm 0.12^{\mathrm{B}}$	32.46 ± 0.24^{A}	
b*	5.9%+LM	$12.85 \pm 0.21^{\mathrm{B}}$	$11.37 \pm 0.10^{\mathrm{B}}$	11.47 ± 0.43^{B}	$11.79 \pm 1.22^{\mathrm{B}}$	12.36 ± 0.43^{B}	32.18 ± 0.72^{A}	
D	4.3%	$12.04 \pm 0.54^{\mathrm{B}}$	$12.95 \pm 1.00^{\mathrm{B}}$	$11.95 \pm 1.23^{\mathrm{B}}$	$12.91 \pm 2.43^{\mathrm{B}}$	$12.94 \pm 0.82^{\mathrm{B}}$	33.23 ± 0.81^{A}	
	4.3%+LM	$12.39 \pm 0.34^{\mathrm{B}}_{-}$	$13.11 \pm 1.50^{\mathrm{B}}$	$12.60 \pm 2.34^{\mathrm{B}}_{-}$	$13.44 \pm 1.54^{\mathrm{B}}$	$13.54 \pm 0.59^{\mathrm{B}}$	34.44 ± 0.33^{A}	
	2.0%	$12.63 \pm 0.49^{\mathrm{B}}$	11.63 ± 0.39^{B}	$11.89 \pm 1.20^{\mathrm{B}}$	$12.51 \pm 0.44^{\mathrm{B}}$	12.35 ± 0.41^{B}	32.99 ± 0.56^{A}	
	2.0%+LM	$12.66 \pm 0.34^{\mathrm{B}}$	$13.00 \pm 0.45^{\mathrm{B}}$	$12.59 \pm 0.75^{\mathrm{B}}$	$13.41 \pm 1.43^{\mathrm{B}}$	$12.83 \pm 1.17^{\mathrm{B}}$	34.03 ± 0.24^{A}	

 $[\]overline{^{1)}}$ LM: G. uralensis 0.8% and B. juncea 0.7%.

Values are mean \pm SD (n=3).

Means with different superscripts in the same column (a,b) and row (A,B) are significantly different (p<0.05).

or the activity of the enzymes.

Sensory evaluation

In terms of overall palatability, 5.9% and 4.3% salt gochujang with and without additives, were significantly higher than control gochujang. The high palatability of other low salt gochujangs, except the 2.0% salt, is related to taste than flavor. Flavor palatability of gochujangs did not result in a significant difference among test groups, and in terms of savory, unpleasant, and salty flavor, sensory intensity did not manifest a significant difference among test groups. In terms of taste, a significant difference was observed in taste palatability; 2.0% salt gochujang with and without additives had significantly lower scores than other groups, and 5.9% and 4.3% salt gochujang with and without additives had higher scores than the control gochujang. In terms of savory and sweet taste, low salt gochujangs scored higher than the control and were significantly sweeter and more savory in taste than the other test groups (Table 4). The savory taste was higher in the low salt gochujang than in the control group because the lower salt concentration contained more amino-nitrogen. One exception was that while it was expected that the 2.0% salt gochujang would be more preferred by the panel due to the protease activity and the high amino-nitrogen content, it scored lower than other low-salt gochujang groups in overall preference because of a sourer taste and a peculiar smell due to low

pH and high acidity. In conclusion, even when the salt concentration of *gochujang* is reduced up to 4.3%, adding *G. uralensis* and *B. juncea* can inhibit the proliferation of filamentous fungi, which causes quality degradation in low salt *gochujang*, and allows production of high-quality *gochujang* with excellent sensory palatability.

CONCLUSION

When G. uralensis extract and B. juncea powder, containing an anti-bacterial substance, were added to lowsalt gochujang, the proliferation of fungi such as yeast was inhibited. The α-amylase activity increased and then subsequently decreased during the aging process, while β-amylase activity tended to decrease during the process. The protease activity tended to increase in correlation with lowering the salt concentration, although the effects of the additives were negligible. The amount of amino-nitrogen in the low-salt gochujangs at 20 days was similar to that in the control gochujang at 40 days. The result of the sensory evaluation that was conducted on 40 days showed that the low-salt gochujangs were more preferred than the control gochujang. In particular, the 4.3% low-salt gochujang with additives were the most preferred, followed by the 5.9% low-salt gochujang with additives. In conclusion, a low-salt gochujang adding G. uralensis and B. juncea is also expected to reduce the

 5.80 ± 1.17^{ab}

 4.90 ± 1.51^{ab}

8.5% 5.9% 5.9% + LM2% + LM4.3% 4.3% + LM2.0% 4.90 ± 1.58^{abc} 5.20 ± 1.33^{abc} 4.60 ± 0.80^{abc} 5.70 ± 1.27^{ab} $4.10 \pm 0.83^{\circ}$ 4.20 ± 0.83^{bc} $6.00 \pm 0.89^{\circ}$ Color Flavor 4.00 ± 0.77 5.00 ± 1.18 5.10 ± 1.45 5.30 ± 1.49 5.20 ± 0.98 4.80 ± 1.33 4.60 ± 1.50 4.70 ± 0.78 3.80 ± 0.60 4.30 ± 1.19 5.00 ± 1.26 5.10 ± 0.70 5.10 ± 0.70 4.20 ± 1.25 Savory Unpleasant 4.10 ± 1.14 4.20 ± 1.17 4.60 ± 0.66 4.20 ± 0.98 4.10 ± 0.70 4.80 ± 0.87 4.30 ± 0.64 5.10 ± 0.94 4.70 ± 1.19 4.90 ± 0.83 4.80 ± 0.87 5.20 ± 1.25 5.10 ± 1.14 5.00 ± 0.63 Salt 4.20 ± 0.98^{ab} 3.90 ± 0.94^{ab} 4.11 ± 0.99^{ab} 3.60 ± 0.80^{ab} 5.50 ± 0.92^{ab} 3.60 ± 0.80^{ab} 4.60 ± 0.80^{ab} Sour 4.10 ± 0.94^{ab} 5.20 ± 1.54^{ab} 5.50 ± 1.02^{ab} 3.70 ± 1.42^{b} Taste 5.70 ± 1.49^{a} 5.70 ± 1.42^{a} 3.70 ± 1.35^{b} 4.60 ± 1.02^{ab} 3.90 ± 0.70^{b} 4.20 ± 0.87^{t} 5.90 ± 0.70^{a} 5.80 ± 0.98^{a} 5.90 ± 1.04^{a} Savory $6.00 \pm 1.00^{\rm a}$ 5.30 ± 1.10^{ab} 5.20 ± 1.08^{ab} 5.30 ± 1.35^{ab} 4.10 ± 0.70^{b} 5.60 ± 0.92^{a} 5.80 ± 0.60^{a} 5.30 ± 1.10^{t} Sweet 6.00 ± 0.77^{ab} 5.70 ± 1.10^{ab} 5.70 ± 1.35^{ab} 5.80 ± 0.75^{ab} 5.80 ± 1.17^{ab} Salt $6.20 \pm 0.98^{\circ}$ 4.70 ± 0.90^{t}

Table 5. Sensory evaluation of low salt *gochujang* added with *G. uralensis* and *B. juncea* during fermentation at 28°C for 40 days

 $\overline{^{1)}}$ LM: G. uralensis 0.8% and B. juncea 0.7%.

Sour

Overall acceptability

Color, flavor, taste, overall acceptability were evaluated as follows: very poor (1 point), moderate (5 points), very good (9 points), and savory, unpleasant, sweet, salt, sour were evaluated as follows: very low (1 point), medium (5 points), very strong (9 points). Values are mean \pm SD (n=10). ^{a-c}Means with different superscripts in the same row are significantly different (p<0.05).

 5.20 ± 0.87^{bc}

 6.20 ± 1.60^a

 5.00 ± 0.45^{bc}

 6.40 ± 0.92^a

 4.90 ± 0.70^{bc}

 6.40 ± 0.80^{a}

duration of the fermentation and improve sensory palatability as well as to prevent quality degradation caused by yeast, which can be observed in low-salt *gochujang*.

 $4.30 \pm 0.64^{\circ}$

 5.00 ± 0.89^{ab}

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 6.60 ± 0.66^{a}

 3.90 ± 1.22^{b}

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 5.40 ± 0.80^{bc}

 6.50 ± 1.12^a

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