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Characteristics of Red Pepper Paste by Using Germinated Barley with Increased γ-Amino Butyric Acid

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Abstract

Germinated barley, instead of glutinous rice, was used to make health-enhancing fermented red pepper paste. The proximate components of commercial glutinous rice red pepper paste (CGRPP) and germinated barley red pepper paste (GBRPP) were analyzed during fermentation. The sensory characteristics and γ-amino butyric acid (GABA) contents of CGRPP and GBRPP were evaluated. The contents of β-glucan and GABA showed the highest value after 48 hrs of germination. During the fermentation, the contents of GABA in GBRPP increased up to 28 days and then decreased. During sensory evaluation, the consumer liked the GBRPP more than CGRPP. The GABA contents were increased during fermentation and GABA contents of GBRPP were twice as much as that of CGRPP. These results suggest that the GBRPP can have consumer acceptance for its health benefits and taste and can therefore become commercialized.

Key words: red pepper paste, γ-amino butyric acid (GABA), germinated barley, fermentation, β-glucan

INTRODUCTION

The beneficial effects of healthy diets on the quality of life involve the identification of new sources of nutraceuticals. Red pepper paste is an example of such a nutraceutical source and could be a basis for functional food products. Red pepper paste is made by mixing red hot pepper, glutinous rice powder, fermented soybean powder, and salt. It is a dark and reddish paste with a piquant flavor. Red pepper paste's primary ingredient is red hot pepper. In red hot pepper, capsaicinoids are predominant, and this causes the hot-tasting sensation. Hot pepper also possesses biologically beneficial properties that may affect human health (1). For example, red pepper paste has become popular because of its anti-obesity effects (2). It also reduces high blood pressure (3). Furthermore, red pepper paste has a high amount of poly-γ-glutamate (γ-PGA), which contains highly absorbable hydrogels and has great potential as a biodegradable polymer in a broad range of food industries (4).

To broaden the application of healthy functional red pepper paste, barley was used as the starch source and was germinated to increase its health-enhancing components. Specifically, changes in β-glucan and γ-amino butyric acid (GABA), which are known to be effective in reducing hypertension and controlling diabetes, were selected to be analyzed during germination. Barley, which is composed mainly of β-glucans (5), is an important source of dietary fiber. We confirmed that barley plant seed tissues accumulate β-glucans under environmental stress conditions, such as germination. Barley is one of the most commonly used germinated plants in health foods, including one food called "BarleyGreen". Brown rice germination also results in increased accumulation of a beneficial product, which is GABA. GABA is known to be an effective blood pressure regulator and to participate in the recovery of alcohol-related symptoms (6). We wanted to see if GABA levels also increased during barley germination.

The germinated barley and glutinous rice were used for making the pre-mixture, which contained the inoculated *Aspergillus oryzae* on steamed soybean (*meju*) and salt. The proximate component changes of the pre-mixtures were analyzed during fermentation. When making red pepper paste, the red hot pepper was mixed with each pre-mixture and post-fermented for 10 days. Each post-fermented red pepper paste was regarded as the final product. The sensory characteristics and GABA contents of each final product were analyzed.

MATERIALS AND METHODS

Materials

Pentafluorobenzylbromide and GABA were purchased from Sigma Aldrich (St. Louis, MO, USA). The ingredients for making red pepper paste, such as de-hulled barley, red pepper powder, salt, soybean and glutinous rice powder, were purchased from a local market place.

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Measurement of β-glucan

The β-glucan content of germinated barley was determined with the method of McCleary and Codd (7), using the mixed-linkage β-glucan assay kit (Megazyme Ltd., Wicklow, Ireland). Analyses were replicated four times and represented as mean values.

Measurement of GABA

The GABA contents were analyzed by modifying the Struy method (8). One gram of each sample was acidified by adding 400 μL of 6 N HCl and heated to 110°C for 4 hr to accomplish hydrolysis of the GABA. After cooling to room temperature, the solution was neutralized by the addition of 200 μL of 12 N NaOH. Then 800 μL of phosphate buffer (1 M, pH 11.5) and 50 μL of methylchloroformate was added to form N-methylcarbonyl derivatives for 10 min at room temperature while being constantly shaken. The mixture was acidified using 150 μL of 5 N HCl and any derivatives that formed were then extracted into 4 mL of ethyl acetate by manual shaking for 1 min. After 2 min of centrifugation, the supernatant was transferred to another test tube and blown to dryness with nitrogen at 40°C. Pentafluoro-benzyl (PFB) derivatives were formed by treating the residue with 10 μL of tri-ethyl amine and 100 μL of 7% pentafluorobenzyl bromide in acetonitrile (v/v) for 15 min at room temperature. After adding 200 μL of 0.5 N HCl, the formed derivatives were extracted with 1 mL hexane. The hexane fraction was blown to dryness and resolved in 50 μL of hexane. From this final solution 1 μL was used for the GC analysis. The separation of GABA was performed by using the gas chromatography (M600D system, Young-Lin Co., Gyeonggido, Korea). The GC operation conditions were as followed; the column was fused silica capillary column (Supelco-Wax™-10, 30 m × 0.53 mm × 0.5 μm film thickness), injection temperature was 200°C, oven temperature was 180°C, and detector temperature was 250°C. The peak of GABA was confirmed with matching the same retention time with a reference. Calibration curves were established by carrying various amounts of GABA throughout the entire procedure. The observed values were used for linear regression analysis. Analyses were replicated four times and represented as mean values.

Preparation of germinated barley

De-hulled barley (*Hordeum vulgare*) was soaked in tap water at 30°C for 24 hr, and the extra water was discarded. The soaked barley was placed on a small porous plate covered with gauze. Then the plate was put into a sterilized incubator and was adjusted at 30°C for 96 hr. Water was sprayed on the plate every 4 hours. Samples were taken after 0 hr (de-hulled barley before soaking), 24 hr (soaked de-hulled barley), 48 hr, 72 hr and 96 hr. The samples were immediately freeze dried after being picked and stored at -18°C until used.

Preparation of germinated barley powder for making red pepper paste

The barley germinated for 48 hr was shown to have the highest value of the contents of β-glucan and GABA; therefore, this sample was used to make the red pepper paste. The 48-hr germinated barley was dried with a vacuum dryer (VO-10X Vacuum Oven, Jeio Tech., Gyeonggido, Korea) until the moisture content reached 15%. The vacuum-dried germinated barley was milled using a pulverizer (Cyclotec 1093 Sample mill, Tecator Co., Höganäs, Sweden), and then screened using 100 mesh sieve. The powder passed through a 100 mesh sieve that was used for making red pepper paste.

Preparation of red pepper paste

Starter soybean was soaked overnight, steamed for 15 min at 121°C and cooled to 30°C. Then *Aspergillus oryzae* (Chungmu fermentation Co. Ltd., Ulsan, Korea) was inoculated and incubated at 40°C for 24 hr. This is referred to as *meju*. After incubation, the *meju* was dried and made into powder.

Preparation of pre-mixture: For making the commercial glutinous rice red pepper paste (CGRPP), *meju* (25%), glutinous rice powder (30%), salt (10%) and water (35%) were blended. For making the germinated barley powder red pepper paste (GBRPP), germinated barley powder was used instead of glutinous rice, while all the other ingredients remained the same as for CGRPP. Both pre-mixtures were fermented for 28 days at 30°C.

Preparation of red pepper paste: Each pre-mixture (63%), red pepper powder (20%) and corn starch syrup (17%) were blended and chopped. Each pre-mixture of chopped red pepper paste was post-fermented at room temperature for 10 days. Each post-fermented red pepper paste was regarded as the final product and was used for sensory evaluation and GABA analysis.

Proximate analysis of red pepper paste during fermentation

Analysis of amino nitrogen (AN): Five grams of each pre-mixture was put into the 250 mL Erlenmeyer flask and 100 mL of water was added. The pre-mixture and water were mixed well and filtered with filter paper (Whatman No 2.). Fifteen milliliters of each filtrate was poured into a 100 mL Erlenmeyer flask and titrated with 0.1 N-NaOH until the pH value reached 8.4. The AN was calculated with the following equation.

$$AN\ (mg\%) = \{(\text{Consumed 0.1 N NaOH mL} \times \text{0.1 N NaOH factor} \times 1000 \times 0.0014)/\text{Total sample amount (g)}\} \times 100$$

pH: For measuring pH, 5 g of each sample and 45 g of distilled water was homogenized well. The value of pH was measured using a pH meter (model 720, Thermo orion, Beverly, MA, USA).

Gamma amino butyric acid (GABA): GABA was analyzed with the same method as the germinated barley powder with exception of adding one gram of each pre-mixture and red pepper paste instead of germinated barley.

Sensory evaluation of red pepper paste

Panel selection and training for descriptive analysis: Nine panelists (5 females; 4 males; ages ranging from 21 to 28) were selected by the scores obtained from 6 triangle tests containing 4 products of red pepper paste. Attributed descriptors for evaluating the red pepper paste were developed by panelists using the quantitative descriptive analysis (QDA^{TM}) methodology (9). Ballot developments were accomplished during 4 sessions, and the references were determined by panel consensus. The descriptors and references are listed in Table 1.

After developing the ballot, the panelists were trained to evaluate the sensory characteristics of the red pepper paste. During the first period, training was performed using red pepper paste with 4 different commercial products. Also, the panelists were asked to quantify differences among the samples in descriptive terms on a scale of 0 to 15. The references were served along with the red pepper paste during training. This procedure was repeated during 4 working sessions which took an hour and a half per session. After the first period, three tests were performed. Then the weak point of each panelist was analyzed and training was repeated as in the first period. For the final training period, the test procedure was introduced to ensure panelist familiarity with the scorecard.

Sample presentation for descriptive analysis: Twenty grams of each sample was placed on a white plate 15 cm in diameter with blind three-digit codes. At each testing period, sample containers were mixed by inversion under fluorescence lighting at each booth. These samples were presented to panel members seated in individual booths when the samples reached 20°C (about room temperature). Panelists evaluated the sensory characteristics of red pepper paste on the 15 cm line scale labeled in a range from "not detectable" to "intense". The results were statistically analyzed at $p<0.05$ level.

Consumer acceptance analysis: Fifty-six panelists (36 females; 20 males; ages ranging from 21 to 45) were selected for the consumer acceptance test. The tests were performed in individual booths and the respondents were asked to rate four terms. The evaluator marked each term on a 9 point hedonic scale. Sensory scores were 9, very good; 7, good; 5, fair; 3, poor; 1, very poor (10). Ten grams of CGRPP and GBRPP were placed on a flat white plate, 10 cm in diameter. First, the panelists were asked "How much do you like the appearance?" Second, they were asked "How much do you like the flavor?" Third, they ate a spoonful, which contained 0.5 g of each sample, and then were asked "How much do you like the taste?" Finally, they were asked to rate their overall acceptance. The results were statistically analyzed at $p<0.05$ level.

Table 1. Definitions and references of the descriptive terms for sensory descriptive analysis

Sensory attribute	Terms	Definitions	References
Appearance	Glossy	Shiny and gleaming surface	Sugar glazed apple
	Stickiness	The properties related with glue	10% wheat flour glue
	Redness	Degree of red color	Munsell color (5R 4/10)
Aroma	Soy sauce	The odor related with soy sauce	Soy sauce (Haechandle Co. Ltd., Chungnam, Korea)
	Chungkookjang	The odor related with fermented *Bacillus subtillis* on steamed soybean	Chungkookjang (Honghoa Co., Gyeongbuk, Korea)
	Pungent	The odor related with hot pepper	Hot pepper powder (Haechandle Co. Ltd.)
	Sweet	The odor related with corn syrup	Corn syrup (Daesang Co., Gyeonggi, Korea)
	Sulfury	The odor related with boiled egg	Boiled egg
Taste	Salty	The taste sensation of NaCl	NaCl
	Pungent	The taste sensation of capsaicin	Capsaicin
	Bitterness	The taste sensation of caffeine	Caffeine
	Nutty	The taste sensation related with roasted peanut	Roasted peanut
	Umami	The taste sensation related with MSG	MSG
Texture	Mouth coat	The sensation related with covering a thin layer on the surface of tongue	Butter
	Roughness	The sensation related with coarse grainy	Brown rice

Statistical analysis

The data was presented as a mean±standard deviation (SD) in quadruple, except the sensory evaluation, which was performed once. Statistical evaluation of the results was performed by analysis of the variance (ANOVA) followed by the Duncan multiple comparison. Statistical significance was defined as $p<0.05$. The data was analyzed using the Minitab 13 statistical software package (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

β-Glucan and GABA analysis results during germination of barley

Barley was germinated to increase the health-enhancing materials, such as β-glucan and GABA. The changes in β-glucan and GABA contents after germination are shown in Table 2. After 48 hr of germination, β-glucan levels increased up to 3.3 times starting levels, then remained at that high for 72 hr before decreasing. Also after 48 hr of germination, the length of the sprout was about 0.35±0.15 cm and the GABA content was increased up to 2.5 times, before then decreasing. For these reasons, the 48-hour germinated barley was selected for making GBRPP.

In the beer industry, β-glucan, a water soluble dietary fiber (11), is produced during barley steeping and creates unfavorable effects during fermentation, such as increased viscosity (12). Even though β-glucan produces undesired effects in beer industry, the high viscosity β-glucan promotes a beneficial dietary effect by slowing down the digestibility in animal intestines and reducing the nutritive value (13). In red paper paste, the viscosity-forming properties of β-glucans make potential alternatives as thickening agents (14). The β-glucans in barley are linear homopolysaccharides of glucose with approximately 70% (1→4)-linkages and 30% (1→3) linkage. The β-glucans in barley have been reported to lower the total serum- and LDL-cholesterol in humans and animals (15,16). McIntosh et al. (17) demonstrated that plasma LDL-cholesterol concentrations were lowered by 7% in mildly hypercholesterolacemic men, who consumed 8 g/day of barley β-glucan for 4 weeks. Also, HlotekjØlen et al. (5) mentioned that hull-less varieties of barley (*Hordeum vulgare*) seem suitable for human consumption, with high levels of soluble fiber and nutritive contents such as β-glucans and soluble non-starch polysaccharides. β-Glucan in barley could also be suitable for functional food products aimed at enhancing health and preventing cancer. Therefore, red pepper paste made from germinated barley powder should give health benefits as a functional food product.

The content of GABA was increased up to 2.5 times during germination (Table 2). These results were the same as the sprouted cereal containing a relatively higher concentration of GABA than normal cereal (7). GABA is a ubiquitous non-protein amino acid produced primarily by the α-decarboxylation of L-glutamic acid, which is catalyzed by the enzyme glutamate decarboxylase. Brown and Shelp (18) reported that GABA accumulated rapidly and largely in a variety of plant tissues under conditions of environmental stress. Oh et al. (6) also mentioned that brown rice germinated in glutamic acid enhanced the level of GABA. Recently, there has been an increased interest in the utilization of plant-derived GABA for its bioactivity. GABA plays a number of beneficial roles, such as functioning as a major inhibitory neurotransmitter (19), a regulator of cardiovascular functions (20), a controller of pain sensation and anxiety (21), and the recovery helper of alcohol-related symptoms (22). Therefore, we also assume that GBRPP can increase the bioactivity of red pepper paste.

Proximate analysis of pre-mixture during fermentation

When evaluating the adjustable fermentation periods, the proximate analysis of each pre-mixture for making red pepper paste was measured in terms of the contents of amino nitrogen and GABA and pH values. During fermentation, β-glucan could not be analyzed because the pre-mixture had salt in it, and the enzymatic analysis method did not working in this condition.

Amino nitrogen: The protein was hydrolyzed to peptides and amino acids. These peptides and amino acids contribute to the umami taste in red pepper paste (23). Therefore, amino nitrogen during fermentation can act as a kind of indicator for detecting how much fermentation has occurred (24). In this study, the amino nitrogen was dramatically increased up to 28 days of fermentation

Table 2. Changes of sprout length and contents of β-glucan and GABA during germination of barley

Germination time (hr)	0	24	48	72	96
Length of sprout (cm)	−	$0.05\pm0.02^{1)d2)}$	0.35 ± 0.15^{c}	0.63 ± 0.21^{b}	0.97 ± 0.32^{a}
β-Glucan (w/w %)	0.82 ± 0.21^{c}	1.0 ± 0.32^{c}	2.7 ± 0.67^{a}	2.8 ± 0.56^{a}	1.6 ± 0.56^{b}
GABA (mg/100 g)	19.21 ± 3.43^{c}	33.0 ± 10.2^{b}	47.8 ± 3.20^{a}	36.4 ± 19.3^{b}	31.3 ± 4.96^{b}

[1)]Values are expressed as mean±standard deviation (n=4).
[2)]Mean values with different letters are significantly different within the same row, $p<0.05$.

Table 3. Results of proximate analysis between pre-mixture of the commercial marketed glutinous red pepper paste (CGRPP) and the germinated barley red pepper paste (GBRPP) during fermentation

		0 day	7 days	14 days	21 days	28 days	35 days
Amino type nitrogen (mg%)	CGRPP	134.2±11.2[1)aD]	184.4±9.7[aC]	242.8±16.9[aB]	268.4±14.7[aB]	325.6±18.9[aA]	348.1±17.8[aA]
	GBRPP	142.2±9.8[aC]	190.4±6.8[aB]	246.2±7.8[aAB]	271.1±20.1[aB]	331.5±17.3[aA]	345.9±28.6[aA]
pH	CGRPP	5.13±0.02[aAB]	4.95±0.03[aB]	4.88±0.08[aB]	4.89±0.03[aB]	5.11±0.09[aAB]	5.17±0.12[aA]
	GBRPP	5.17±0.05[aA]	5.03±0.09[aB]	4.91±0.11[aC]	4.95±0.08[aBC]	5.09±0.13[aAB]	5.18±0.07[aA]
GABA (mg/100 g)	CGRPP	6.5±0.91[aC]	6.7±1.01[aC]	7.6±0.89[aB]	8.2±1.22[aAB]	8.6±0.87[aA]	7.9±0.96[aAB]
	GBRPP	12.1±1.21[bD]	13.9±1.11[bC]	15.2±0.67[bB]	16.4±1.19[bA]	16.2±0.97[bA]	15.8±0.81[bAB]

[1)]Values are expressed as mean±standard deviation (n=4).
[a,b]Mean values with different letters are significantly different at same column for each analysis item, $p<0.05$.
[A-D]Mean values with different letters are significantly different within the same row, $p<0.05$.

and then leveled out. This result suggests that the protease derived from *Asp. oryzae* was most active in this fermentation period. The amino nitrogen increased during the fermentation period, but there was no difference in levels between GBRPP and CGRPP (Table 3). This result suggests that the use of germinated barley instead of glutinous rice should not affect the fermentation; therefore, we assume that the germinated barley can be used for making red pepper paste.

pH: The pH values were reduced up to 21 days and then increased (Table 3). The pH value increase is caused by the production of acids during fermentation, when sugars and amino acids are decomposed to acids by the microbial activation (24). In this study, there was no significant difference in the pH value between GBRPP and CGRPP pre-mixtures ($p>0.05$).

GABA: The changes in GABA during fermentation are shown in Table 3. The GABA contents increased during fermentation up to 28 days. After that time, they decreased. Therefore, the pre-mixture after 28 days of fermentation was chosen for making the final red pepper paste product. The GABA contents between GBRPP and CGRPP pre-mixture were shown to be significantly different ($p>0.05$). The GABA content in the GBRPP was twice as high as that of CGRPP. This result suggested that the germinated barley can be used as a health-enhancing food product because of its increased GABA content.

Comparison of sensory evaluation results between GBRPP and CGRPP

The results of the sensory descriptive analysis are shown in Table 4. For the appearance of the pastes, the stickiness and redness of the product did not result in a statistical difference between GBRPP and CGRPP ($p>0.05$). However, the glossy nature of the product resulted in a significant statistical difference between GBRPP and CGRPP ($p<0.05$). The glutinous rice starch in CGRPP was changed to maltodextrin and/or glucose during fermentation. These decomposed carbohydrates have shiny and sticky properties. GBRPP was evaluated less glossy than CGRPP because barley has less starch than glutinous rice. All aroma attributes, such as soy sauce, chungkookjang, pungent, sweet and sulfury, and salty and pungent taste attributes did not show a statistical difference between GBRPP and CGRPP ($p>0.05$). In terms of the bitterness and the umami, the sensory intensity of CGRPP was significantly higher than that of GBRPP. In terms of a nutty taste, the sensory intensity of GBRPP was significantly higher than that of CGRPP. In texture attributes, the term of mouth coat, which had a negative effect in overall consumer acceptance, had a significantly lower sensory strength in GBRPP. However, the sensory strength of roughness did not show statistical difference ($p>0.05$).

Consumer acceptance: In terms of texture, consumer acceptance did not show a statistical difference between GBRPP and CGRPP (Table 5). The glossy appearance

Table 4. Comparison of sensory descriptive analysis results between the germinated barley red pepper paste (GBRPP) and the commercial marketed glutinous red pepper paste (CGRPP)

Sensory attribute	Terms	GBRPP	CGRPP
Appearance	Glossy	6.25±1.39[1)b2)]	10.13±1.25[a]
	Stickiness	7.13±3.18[a]	8.75±1.91[a]
	Redness	10.75±1.98[a]	9.63±2.20[a]
Aroma	Soy sauce	8.63±2.33[a]	8.38±2.07[a]
	Chungkookjang	8.50±2.51[a]	9.63±2.77[a]
	Pungent	6.88±2.70[a]	8.13±2.17[a]
	Sweet	4.00±1.31[a]	4.63±1.51[a]
	Sulfury	7.00±1.60[a]	8.25±1.98[a]
Taste	Salty	7.75±1.67[a]	9.13±2.30[a]
	Pungent	8.88±1.64[a]	10.63±1.92[a]
	Bitterness	6.00±1.07[b]	8.13±1.96[a]
	Nutty	7.50±1.60[a]	5.75±1.16[b]
	Umami	5.50±1.77[b]	7.25±2.19[a]
Texture	Mouth coat	6.25±1.16[b]	8.13±1.36[a]
	Roughness	5.63±1.19[a]	5.38±0.74[a]

[1)]Values are expressed as mean±standard deviation (n=8).
[2)]Mean values with different letters are significantly different, $p<0.05$.

Table 5. Comparison of consumer acceptance between the commercial marketed glutinous red pepper paste (CGRPP) and the germinated barley red pepper paste (GBRPP)

	CGRPP	GBRPP
Overall acceptance	4.38±1.97[1)b2)]	4.92±1.67[a]
Appearance	3.75±1.45[b]	5.42±1.59[a]
Odor & taste	4.50±1.59[a]	4.21±1.32[b]
Texture	5.04±1.43[a]	4.88±1.62[a]

[1)]Values are expressed as mean±standard deviation (n=56).
[2)]Mean values with different letters within the same row are significantly different, $p<0.05$.

Table 6. Comparison of GABA contents between the commercial marketed glutinous red pepper paste (CGRPP) and the germinated barley red pepper paste (GBRPP)

Varieties of red pepper paste	GBRPP	CGRPP
Contents (mg/100 g)	14.9±0.83[1)a2)]	7.17±0.69[b]

[1)]Values are expressed as mean±standard deviation (n=4).
[2)]Mean values with different letters are significantly different, $p<0.05$.

of red pepper paste makes the product attractive. The glossy appearance of germinated barley red pepper paste (GBRPP) was evaluated to have less intensity than commercial glutinous rice red pepper paste (CGRPP). However, in terms of having a nutty taste, the sensory intensity of GBRPP was significantly higher than that of CGRPP. Regarding appearance, consumers showed a low acceptance for GBRPP. In odor and taste, consumer accepted GBRPP over CGRPP. In overall acceptance, the consumer liked GBRPP more than CGRPP.

Comparison of GABA contents between GBRPP and CGRPP

The amount of GABA in the GBRPP product was twice as high as the CGRPP (Table 6). A large amount of GABA already exists in the raw material for making GBRPP before fermentation, but that level is increased during fermentation. Wang et al. (25) mentioned that GABA content was increased during fermentation. Their results are similar to the ones reported here. Based on the results, the germinated barley red pepper paste can become commercialized by increasing the GABA contents.

CONCLUSION

For the development of healthy functional red pepper paste, barley was used and germinated to increase health effective components. After 48 hours of germination, the contents of β-glucan and γ-amino butyric acid (GABA) were increased up to 3.3 and 2.5 times, respectively. In overall acceptance, the consumer likes GBRPP more than CGRPP. Also, GABA was increased during fermentation and the GABA contents of GBRPP were twice as much as the CGRPP. These results suggest that the germinated barley red pepper paste (GBRPP) can have consumer acceptance for its health benefits and taste and can therefore become commercialized.

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