Physicochemical properties of enzyme-treated waxy rice flour and expansion properties of *Gangjung* (a traditional Korean oil-puffed rice snack)

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Abstract Long steeping process of waxy rice involved in *Gangjung* production is essential for obtaining good quality *Gangjung*, but it causes many related problems. The objective was to examine physicochemical properties of waxy rice flour and expansion properties of *Gangjung* manufactured using waxy rice flour treated with protease (P-flour), α -amylase (A-flour), or in combination of the two (PA-flour) over different durations. While crude protein and reducing sugar contents of P-flour and A-flour differed depending on enzyme treatment time, it was not observed for PA-flour. *Gangjung* made with P-flour or A-flour could show similar expansion rate to that of *Gangjung* made with waxy rice flour steeped under the optimal condition, however, *Gangjung* made with PA-flour showed lower expansion rate than of the optimal one. These findings indicate that treatment of waxy rice flour with either protease or α -amylase, but not both, could reduce the steeping time involved in *Gangjung* production.

Keywords: *Gangjung*, waxy rice, enzyme, protease, α -Amylase

Introduction

Enzymes play an important role in food processing, storage, and cooking thereby influencing the quality of food (1). Starch-hydrolyzing enzymes are used for modifying starch to increase its functionality (2,3). Starch-hydrolyzing enzymes used in the processing of starchy foods include α - and β -amylase, amyloglucosidase, and isoamylase (4). Among these, α -amylase is widely distributed in plants, animals, and microorganisms. This enzyme produces dextrin by acting on starch and randomly hydrolyzing the linkage of amylose molecule; dextrin is then broken down into maltose, maltotriose, maltotetraose, etc (5). α -Amylase has been used to promote generation of fermentable sugar in baking, and to improve starch quality, as well as liquefaction and saccharification of starch (4).

Protease has been used in snack foods such as bread and cracker to improve their quality by hydrolyzing part of the protein in the system. However, protease has not been applied to rice or processed rice foods because the contribution of protein to the quality is insignificant due to its low concentration (10%) in rice (6). Nevertheless, protease treatment has been shown to improve the texture quality of cooked rice (6-11), suggesting that protease does affect rice and processed rice food products.

Gangjung (a type of *Yukwa*) is a traditional Korean oil-puffed snack using waxy rice. The production process has not been completely automated thus making large-scale and standardized production difficult. Therefore several steps of the process are still performed manually (12,13). The steeping process, a crucial step in the preparation of *Gangjung*, which takes the longest time in the manufacturing process, involves partial hydrolysis of starch and protein and reduction of mineral contents from enzymatic reactions. This step is closely related to the quality improvement of *Gangjung*, which has been identified in previous studies (14). However, the steeping process causes time and environmental issues, hence the increasing cost of production of *Gangjung* (15). Although some researchers have attempted to shorten steeping process in *Gangjung* production by applying microorganisms, enzymes, or both (16,17), there is a lack of research on the changes in the quality of *Gangjung* depending on the treatment conditions such as types of enzymes, amount of added enzyme, and processing time.

Therefore, the aim of the present study was to examine the possibility of shortening the lengthy steeping process involved in *Gangjung* production by preparing enzyme-treated waxy rice flour (referred to as "En-flour"). To this end, the utility of enzyme-treated waxy rice flour was investigated by comparing the physicochemical

characteristics of En-flours and the expansion properties of *Gangjung* made using these flours.

Materials and Methods

Materials Waxy rice (Bakunchalbyeo, Korea) was purchased in Iksan, Cheonrabuk-do, Korea and stored at 4°C before *Gangjung* production. Frying oil (Baeksul corn oil, CJ, Incheon, Korea), rice wine (Subok Gold, Doosan, Kunsan, Korea), and sugar (Baeksul sugar, CJ) were purchased from local stores. Protease (EC 3. 4. 21. 4., Cat. No. P5380) from *Bacillus licheniformis* and α -amylase (EC 3. 2. 1. 1., Cat. No. A6380) from *Bacillus amyloliquefaciens* were purchased from Sigma-Aldrich (St. Louis, MO, USA). The enzyme activities of protease and α -amylase were 11.6 and 1870 units/mg solid, respectively.

Experimental design The enzyme concentration used in the study was selected based on the preliminary experiment, considering the expansion ratio of *Gangjung*. The optimal time was investigated under different duration: 5, 60, 120, and 180 min. The condition (i.e., pH and treatment time) of each enzyme were selected based on previous literatures (18,19) and pilot experiment on enzyme activities and the activity on grain product recommended by manufacturers, and accordingly, the corresponding amount of enzyme per 100 g of waxy rice flour was used for the treatment condition.

Preparation of waxy rice flour Three kilograms of waxy rice were steeped in 7.5 L of distilled water in a covered plastic vessel (20 L size) for 2 h at room temperature. The steeped waxy rice were then washed and drained before milling. The prepared waxy rice were milled twice using a roll mill (DK104; Donggang, Daegu, Korea). During the 1st milling, there was no gap between the rollers, and during the 2nd milling the gap was 2 mm. The milled waxy rice flour was then passed through a 20-mesh screen and stored at 20°C until enzyme treatment.

Manufacturing process of En-flour Enzyme treated waxy rice flour (referred to as En-flour) was manufactured as follow. Firstly, in order to increase the effect of enzyme treatment in the preparation of En-flour, wet-processed waxy rice flour, steeped at room temperature for 2 h, was used as control group I (referred to as "CO-I"). In addition, waxy rice flour steeped under the optimal conditions (31.5°C, 9 days), as used in our previous study, was used as control group II (referred to as "CO-II") (20). P-flour and A-flour is defined as waxy rice flour treated with 20 units of protease or 25 units of α -amylase, respectively, for 5, 60, 120, and 180 min. PA-flour is waxy rice flour treated firstly with 20 units of protease for 5 min, followed by 25 units of α -amylase for 5, 60, 120, and 180 min.

P-flour was prepared according to the method of Lumdubwong and Seib (21), with slight modification. Waxy rice flour suspension (20% w/v, dry basis) was adjusted to pH 10.0 using 1 M NaOH at

50°C, and protease dissolved in 1 mL distilled water (units/100 g rice flour, dry basis) was added. Subsequently, NaOH (1 M) was added to the suspension to maintain pH at 10.0. The enzyme activity was disrupted by adding 1 M HCl, and the pH was neutralized to 7.0 with 1 M NaOH. The reaction mixture was then cooled in ice and was centrifuged at 1,000×*g* for 30 min. The suspension was washed three times with distilled water and freeze-dried (Programmable freeze dryer, Ilshin Lab Co. Seoul, Korea). The waxy rice flour was then passed through a 20-mesh screen and stored at 20°C before being used for *Gangjung* preparation.

A-flour was prepared according to the method of Lee *et al.* (22) with some modification. Waxy rice flour suspension (20% w/v, dry basis) was adjusted to pH 6.0 with 1 M HCl at 37°C and α -amylase dissolved in 1 mL distilled water was added. Subsequently, 1 M HCl was added to the suspension to maintain pH of 6.0. A-flour was then prepared using the same process as which described for the protease-treated waxy rice flour.

For preparation of PA-flour, the amount of enzyme used and the application process of protease and α -amylase was the same to the process of making P-flour and A-flour, respectively. Because the surface and inner part of rice is mainly composed of protein and starch, respectively, waxy rice flour was treated with protease first and then with α -amylase, according to the methods described by Kim *et al.* (23) and Park (1). The yields of flours obtained using the traditional steeping method was approximately 50%, whereas the yields of P-flour, A-flour, and PA-flour were approximately 50–60% in yields.

Preparation of *Gangjung* *Gangjung* was prepared according to the method described by Kim *et al.* (20). Steeped waxy rice flour, rice wine, and sugar were mixed, and water was added until the moisture content of the mixture was 48%. The mixture was then steamed at 100°C for 20 min, kneaded for 10 min using a screw kneader (Speed No. 2, Model K5-A; Kitchen Aid, Dayton, OH, USA), pressed into a 0.5 cm-thick sheet, and then dried at room temperature for 1 h. The dried sheet was cut into small pieces (3×1×0.5 cm) and was dried again (40°C for 10 h) using a hot-air dryer (DF-360 DI; DURi Science, Seoul, Korea). The small dried pieces were then fried twice in electric deep-fat fryers (HD6155; Philips Electronics, Echarr-aranaz, Spain). The first and second frying conditions were 1 min at 120°C and 0.5 min at 160°C, respectively.

Crude protein and reducing sugar contents Crude protein content of P-flour was estimated by the Kjeldahl method, and reducing sugar content of A-flour was estimated using the DNS method (24). For waxy rice flour treated with combination of protease and α -amylase, both reducing sugar and crude protein contents were analyzed.

Mineral contents Mineral contents of En-flour were estimated according to AOAC methods (25), using inductively coupled-plasma-atomic emission spectrophotometer (ICP-AES; z6100; Hitachi, Tokyo, Japan).

Pasting properties Pasting properties of steeped waxy rice flour were analyzed by using a Rapid Visco Analyzer (RVA) (Newport Scientific Pty, Ltd, Warriewood, Australia) according to AACC 08-01 method (26). The moisture content of the En-flours was adjusted to meet 14% to follow the method of AACC 08-01. Steeped waxy rice flour (3.5 g, db) was suspended in distilled water (25 mL), and the suspension was stirred in the RVA at 960 rpm for 10 s, and then at 160 rpm for the remainder of the test. The samples were held at 50°C for 1 min, and then heated up to 95°C for 3.5 min and held at 50°C for 2.5 min, cooled down to 50°C for 4 min, and maintained at 50°C for 2 min. The parameters recorded were initial pasting temperature (P_{temp}), peak viscosity (PV), hold or hot paste viscosity (HPV), final or cool paste viscosity (CPV), breakdown (PV HPV), and setback (CPV PV).

Expansion properties The longitudinal-cut area (LA) and cross-sectional area (CA) of the *Gangjung* were measured using a modified method of Chun *et al.* (27), by using a ruler instead of an image analyzer. The volume of *Gangjung* was calculated by multiplying the values of *Gangjung* base length (D) and CA. Density of each sample was represented by the value of weight divided by volume. The expansion ratio (ER), the ratio of *Gangjung* volume expanded from the volume of *bandegi*, was calculated as follow:

$$\text{Expansion ratio (\%)} = \frac{(CA \cdot D) - V}{V} \cdot 100$$

CA: Cross-section area of *Gangjung* (cm²), D: Length of *Gangjung* base (cm), V: Volume of *bandegi* (cm³)

Statistical analysis The physicochemical analysis was repeated three times and each replicate consisted of three measurements. When examining the expansion properties of *Gangjung*, the frying process was repeated three times, because the variation in expansion was very large. Nine samples were measured in every repetition. Analysis of variance (ANOVA) was performed to test the treatment effect on each measurement variable. When significant treatment effect was found, Duncan's multiple range test was performed to determine significant difference among the sample means. PCA was employed in order to summarize the interrelationship among physicochemical properties and the samples. All statistical analyses were conducted using SAS (version 9.1) software (SAS Institute Inc., Cary, N.C., U.S.A) and XLSTAT for Windows 14.0 (Addinsoft, Paris, France).

Results and Discussion

Effect of enzyme treatment on crude protein and reducing-sugar contents of waxy rice flour According to the previous studies, the expansion of *Gangjung* is highly correlated with the protein content of waxy rice flour (20). As shown in Table 1, when the change in

Table 1. Crude protein¹⁾ of waxy rice flour treated with protease under different conditions

Flours ²⁾	Enzyme treatment time (min)	Crude Protein (%)
CO-I		8.90±0.04 ^a
CO-II		4.74±0.03 ^e
P-flour	5	8.85±0.13 ^a
	60	8.62±0.03 ^b
	120	5.93±0.04 ^c
	180	5.29±0.07 ^d
CO-I		8.90±0.04 ^c
CO-II		4.74±0.03 ^d
PA-flour	5	9.83±0.68 ^a
	60	9.45±0.16 ^{ab}
	120	9.76±0.13 ^a
	180	9.03±0.07 ^{bc}

¹⁾Means of three replicates. Values within a column followed by a different letter are significantly different ($p < 0.05$), Duncan's multiple range test.

²⁾CO-I, Waxy rice steeped for 2 h; CO-II, Waxy rice steeped under optimum conditions (31.5°C, 9 days); P-flour, Waxy rice flour treated with 20 units of protease with varying treatment time; PA-flour, Waxy rice flour treated with 20 units of protease for 5 min followed by 25 units of α -amylase with varying treatment time.

protein content of P-flour was first examined, the crude protein content decreased with longer duration of protease treatment. The protein content in the P-flour steeped for 120 min at 20 units was 5.93%, which is slightly higher than 4.74% in CO-II. A previous study showed that non-starch components, including protein and minerals, are widely distributed around the exterior part of the grain; with 30% milling ratio, protein decreased by 27.9%, suggesting that as the milling ratio increases, the ER of *Gangjung* increases (17). This indicates that protein is one of the key attributes that interfere with the ER of *Gangjung*.

The extent of hydrolysis of starch is determined by measuring the reducing sugar content in the supernatant when starch is decomposed by α - and β -amylases (28). Reducing sugar contents of CO-I and CO-II supernatants treated by α -amylase were 27.40 and 4.57 mg/mL, respectively (Table 2). The reducing sugar content of supernatant treated with α -amylase showed a time-dependent increase, which indicates that hydrolysis of starch increases proportionally with the treatment duration. In contrast, as the steeping duration increased, the reducing sugar content of A-flour decreased, indicating that as the duration of α -amylase treatment increased, the reducing sugar generated was eluted as supernatant. Furthermore, in all the samples except one treated with 25 units of α -amylase for 180 min, the reducing sugar content of waxy rice flour was higher than that of CO-II.

The protein content of waxy rice subjected to combined treatment was expected to be decreased by protease treatment, however, there was no significant difference even with longer treatment duration, and also it showed higher protein contents than control groups (Table 1). Reducing-sugar content of supernatant treated with

Table 2. Reducing sugar¹⁾ of waxy rice flour treated with α -amylase under different conditions

Flours ²⁾	Enzyme treatment time (min)	Reducing Sugar	
		Supernatant (mg/mL)	Waxy Rice Flour (%)
CO-I		27.40±0.27 ^d	15.59±1.16 ^c
CO-II		4.57±0.22 ^e	18.33±0.23 ^c
A-flour	5	29.61±1.12 ^d	36.24±0.47 ^a
	60	32.51±0.54 ^c	26.63±4.08 ^b
	120	42.76±0.49 ^b	27.99±6.63 ^b
	180	79.75±0.73 ^a	17.10±6.19 ^c
CO-I		27.40±0.27 ^b	15.59±1.16 ^b
CO-II		4.57±0.22 ^c	18.33±0.23 ^a
PA-flour	5	29.14±1.34 ^a	9.32±0.12 ^d
	60	29.21±0.38 ^a	13.84±0.35 ^c
	120	29.23±0.96 ^a	12.80±0.28 ^c
	180	30.30±0.17 ^a	12.80±1.51 ^c

¹⁾Means of three replicates. Values within a column followed by a different letter are significantly different ($p < 0.05$), Duncan's multiple range test.

²⁾CO-I, Waxy rice steeped for 2 h; CO-II, Waxy rice steeped under optimum conditions (31.5°C, 9 days); A-flour, Waxy rice flour treated with 25 units of α -amylase with varying treatment time; PA-flour, Waxy rice flour treated with 20 units of protease for 5 min followed by 25 units of α -amylase with varying treatment time.

combined treatment was higher compared to the supernatant of the control groups while lower compared to α -amylase treatment alone (Table 2). Further, the reducing sugar content of all the PA-flour was

lower than that of control groups, which was not significantly affected by time-dependent manner. We hypothesized that, after removing protein, α -amylase would break down starch more easily thereby reducing sugar content would increase, but the results contrasted with the hypothesis. Whitehurst and Law (29) suggested that the presence of other enzymes in food played a role to lower the stability of added enzymes, which corroborates our finding that the presence of the protease was assumed to lower the stability of α -amylase.

Effect of enzyme treatment on mineral content of waxy rice flour

Previous studies (30,31) have shown a relationship between mineral release in steeped waxy rice and expansion properties of puffed products. Moreover, in our previous study (20), phosphorous was found to be one of the most important minerals that influenced the ER of *Gangjung*.

As shown in Table 3, mineral content, including that of P, K, Ca, Mg, and Zn, of P-flour decreased with increasing duration of treatment. The P and Zn contents of P-flour were lower compared to the CO-I, but higher compared to the CO-II. Notably, the K, Ca, and Mg contents of P-flour were similar to those of CO-II, except the case of 20 unit and 5 min.

The mineral contents of A-flour were lower than those of CO-I but higher than those of CO-II, which was not significantly affected by the treatment period. The mineral content of waxy rice flour subjected to combined treatment did not show significant change in proportion

Table 3. Mineral contents¹⁾ of waxy rice flour treated with enzymes under different conditions

Flours ²⁾	Enzyme treatment time (min)	P (mg%)	K (mg%)	Ca (mg%)	Mg (mg%)	Zn (mg%)
CO-I		79.52±3.18 ^a	51.42±2.74 ^a	8.00±1.79 ^b	20.00±0.33 ^a	1.17±0.07 ^a
CO-II		14.40±0.15 ^e	7.80±0.05 ^c	1.88±0.13 ^c	4.10±0.56 ^c	0.51±0.04 ^e
P-flour	5	40.00±0.20 ^b	27.48±2.42 ^b	14.85±2.86 ^a	11.01±1.24 ^b	0.81±0.04 ^b
	60	26.70±1.67 ^c	6.57±2.65 ^{cd}	1.05±0.70 ^c	4.23±0.32 ^c	0.74±0.12 ^{bc}
	120	24.81±0.34 ^c	1.90±0.76 ^d	1.45±0.70 ^c	5.03±0.09 ^c	0.75±0.03 ^{bc}
	180	20.83±1.00 ^d	4.96±5.56 ^{cd}	0.86±0.39 ^c	4.37±0.26 ^c	0.61±0.11 ^{cd}
CO-I		79.52±3.18 ^a	51.42±2.74 ^a	8.00±1.79 ^b	20.00±0.33 ^a	1.17±0.07 ^b
CO-II		14.40±0.15 ^d	7.80±0.05 ^d	1.88±0.13 ^c	4.10±0.56 ^e	0.51±0.04 ^d
A-flour	5	37.90±0.03 ^c	19.30±0.14 ^b	2.10±0.04 ^c	15.00±0.33 ^b	1.90±0.01 ^a
	60	45.72±4.29 ^b	16.91±0.71 ^b	2.96±0.82 ^c	15.36±0.17 ^b	0.74±0.03 ^c
	120	44.13±6.21 ^b	19.48±0.34 ^b	7.78±1.11 ^b	9.35±0.09 ^d	0.82±0.02 ^c
	180	37.31±0.89 ^c	18.08±2.76 ^b	10.32±6.90 ^a	13.83±1.14 ^c	0.86±0.00 ^c
CO-I		79.52±3.18 ^a	51.42±2.74 ^a	8.00±1.79 ^a	20.00±0.33 ^a	1.17±0.07 ^c
CO-II		14.40±0.15 ^d	7.80±0.05 ^b	1.88±0.13 ^{bc}	4.10±0.56 ^c	0.51±0.04 ^d
PA-flour	5	24.7±0.26 ^{dc}	19.10±0.79 ^b	2.90±0.05 ^b	1.70±0.01 ^d	2.10±0.03 ^b
	60	33.50±0.17 ^b	20.30±1.30 ^b	1.00±0.02 ^c	5.87±0.23 ^b	0.30±0.01 ^e
	120	33.80±0.12 ^b	19.30±0.24 ^b	2.50±0.06 ^b	5.61±0.04 ^b	0.30±0.01 ^e
	180	24.40±0.17 ^c	21.60±1.72 ^b	2.80±0.12 ^b	3.95±0.35 ^c	2.40±0.02 ^a

¹⁾Data dry base content (%). Means of three replicates. For P-flour, A-flour, and PA-flour compared to CO-I and CO-II, respectively, values within a column followed by a different letter are significantly different ($p < 0.05$), Duncan's multiple range test.

²⁾CO-I, Waxy rice steeped for 2 h; CO-II, Waxy rice steeped at optimum condition (31.5°C, 9 days); P-flour, Waxy rice flour treated with 20 units of protease with varying treatment time; A-flour, Waxy rice flour treated with 25 units of α -amylase with varying treatment time; PA-flour, Waxy rice flour treated with 20 units of protease for 5 min followed by 25 units of α -amylase with varying treatment time.

Table 4. RVA properties¹⁾ of waxy rice flour treated with enzymes under different conditions

Flours ²⁾	Enzyme treatment time (min)	Initial Pasting Temp (°C)	Viscosity			Breakdown (P-H)	Setback (C-P)
			Peak (P)	Hold (H)	Final (C)		
CO-I		64.01±0.30 ^b	175.33±4.51 ^e	74.33±3.51 ^d	105.33±3.51 ^d	101.00±1.00 ^d	-70.00±1.00 ^a
CO-II		65.78±0.50 ^a	218.67±4.51 ^d	55.00±3.46 ^e	77.33±4.04 ^e	163.67±2.52 ^b	-141.33±2.52 ^d
P-flour	5	64.70±0.42 ^{ab}	264.50±2.12 ^b	94.50±0.71 ^c	143.00±1.41 ^c	170.00±1.41 ^a	-121.67±0.58 ^c
	60	64.00±1.26 ^b	272.33±4.73 ^a	105.67±2.31 ^a	152.33±2.08 ^a	166.67±2.52 ^b	-120.00±2.65 ^c
	120	64.32±0.96 ^{ab}	261.33±4.62 ^{bc}	102.33±4.62 ^{ab}	149.00±1.73 ^b	159.00±0.00 ^c	-112.33±2.89 ^b
	180	64.67±1.01 ^{ab}	254.67±2.89 ^c	97.33±4.93 ^{bc}	140.00±4.36 ^c	157.33±2.08 ^c	-114.67±1.53 ^b
CO-I		64.01±0.30 ^b	175.33±4.51 ^b	74.33±3.51 ^a	105.33±3.51 ^a	101.00±1.00 ^d	-70.00±1.00 ^a
CO-II		65.78±0.50 ^a	218.67±4.51 ^a	55.00±3.46 ^b	77.33±4.04 ^b	163.67±2.52 ^a	-141.33±2.52 ^d
A-flour	5	65.81±0.35 ^a	119.00±8.54 ^f	20.00±3.00 ^d	40.33±3.51 ^c	99.00±5.57 ^d	-78.67±5.13 ^a
	60	65.87±0.35 ^a	140.50±2.12 ^e	48.50±0.71 ^d	48.50±0.71 ^c	115.50±2.12 ^c	-91.33±2.31 ^b
	120	66.63±0.32 ^a	149.33±3.51 ^d	27.00±0.00 ^d	50.33±2.08 ^c	122.33±3.51 ^b	-99.00±1.73 ^{bc}
	180	66.00±0.21 ^a	160.33±3.21 ^c	35.33±0.58 ^c	49.67±11.85 ^c	125.00±2.65 ^b	-110.67±15.01 ^c
CO-I		64.01±0.30 ^c	175.33±4.51 ^b	74.33±3.51 ^a	105.33±3.51 ^a	101.00±1.00 ^c	-70.00±1.00 ^a
CO-II		65.78±0.50 ^b	218.67±4.51 ^a	55.00±3.46 ^c	77.33±4.04 ^c	163.67±2.52 ^a	-141.33±2.52 ^d
PA-flour	5	66.00±0.43 ^a	168.67±14.57 ^{bc}	41.00±3.61 ^d	71.33±5.13 ^c	127.67±11.02 ^b	-97.33±9.50 ^b
	60	66.52±0.10 ^{bc}	222.33±4.93 ^a	62.00±3.00 ^b	94.67±3.51 ^b	160.33±2.52 ^a	-127.67±2.52 ^c
	120	66.67±0.37 ^a	223.67±5.51 ^a	63.00±4.35 ^b	94.67±3.52 ^b	160.67±3.52 ^a	-129.00±4.00 ^c
	180	66.28±0.82 ^a	157.00±1.73 ^d	23.00±1.00 ^e	54.33±4.94 ^d	134.00±1.00 ^b	-102.67±6.66 ^b

¹⁾Means of three replicates. For P-flour, A-flour, and PA-flour compared to CO-I and CO-II, respectively, values within a column followed by a different letter are significantly different ($p < 0.05$), Duncan's multiple range test.

²⁾CO-I, Waxy rice steeped for 2 h; CO-II, Waxy rice steeped at optimum condition (31.5°C, 9 days); P-flour, Waxy rice flour treated with 20 units of protease with varying treatment time; A-flour, Waxy rice flour treated with 25 units of α -amylase with varying treatment time; PA-flour, Waxy rice flour treated with 20 units of protease for 5 min followed by 25 units of α -amylase with varying treatment time.

to the duration of treatment. The mineral content of combined treatment were also lower than that of CO-I, higher than that of CO-II, showing a similar result to that of protease or α -amylase treatment alone, which indicates that combined treatment does not have an influence on the mineral content. Park (1) reported that, Zn, Ca, and Mg content of waxy rice treated with a combination of α -amylase and glucoamylase was lower than that of waxy rice steeped for 2 h. Further, compared to waxy rice steeped for 28 h, Ca and Mg contents were higher but Zn content was lower in the enzyme-treated group which was inconsistent with the result of present study, suggesting that the change of mineral contents by enzyme treatment could be different by conditions and duration of enzyme treatment.

Effect of enzyme treatment on pasting properties of waxy rice flour by the RVA The pasting properties of En-flour investigated with using the RVA are shown in Table 4. The initial pasting temperature of CO-I and CO-II were 64.01, and 65.78°C, respectively. P-flour did not show significant change in initial pasting temperature with the duration of treatment and the values were similar to those of CO-I and CO-II.

The peak viscosity of amylogram depends on resistance to heat and shear of expanded starch, presence of soluble starch eluted from granules during heating, and interaction between expanded granules (32). The peak viscosities of the control groups CO-I and CO-II were

175.33 and 218.67 RVU, respectively, showing a significant difference. All peak viscosity of P-flour, which was 250–270 RVU, was higher than CO-II, while peak viscosity of A-flour was lower than CO-II. This result suggests that the peak viscosity of P-flour increased due to exposure of the starch because of proteins between starch granules are reduced by protease treatment, resulting in higher peak viscosity of P-flour than that of CO-II in general. For A-flour, α -amylase treatment resulted in decreased viscosity due to starch hydrolysis. On the other hand, the peak viscosity of A-flour slightly increased as the enzyme treatment time prolonged, while a decrease was expected as a result of starch hydrolysis. Similarly, in the study of Kim *et al.* (20), when kernels for making *Gangjung* were steeped in an environment where both protease and α -amylase was available to act, an increased peak viscosity was observed as the steeping time prolonged. In this environment, protease was expected to act first to break down the proteins surrounding the kernel, which then helps α -amylase to act in starch molecules in the inner part of the kernel. Thus, as the steeping period prolongs, a decrease in peak viscosity was expected, while an increase was observed. There seems to be other factors involved other than starch hydrolysis, and explanation of the phenomenon remains to be studied. The peak viscosity of PA-flour was not consistent regardless of enzyme treatment time.

Breakdown, which the value of subtracting hold viscosity from peak viscosity, is a stability index of the flour products, whereas setback, which the value of subtracting peak viscosity from final

Table 5. Expansion properties¹⁾ of *Gangjung* made with enzyme treated waxy rice flour under different conditions

Flours ²⁾	Enzyme treatment time (min)	Cross-sectional Area (A. cm ²)	Longitudinal-cut Area (B. cm ²)	Length/Height	Density (g/cm ³)	Expansion Ratio (%)
CO-I		1.74±0.33 ^c	4.10±0.68 ^c	2.37±0.23 ^a	0.51±0.07 ^a	970.0±255.9 ^d
CO-II		3.11±0.55 ^b	7.27±1.04 ^b	2.36±0.19 ^a	0.30±0.06 ^b	1941.7±344.5 ^{b,c}
P-flour	5	3.25±0.47 ^b	7.44±1.13 ^b	2.30±0.22 ^a	0.29±0.08 ^{b,c}	1916.2±410.7 ^{b,c}
	60	1.93±0.29 ^c	4.51±0.49 ^c	1.67±0.14 ^b	0.20±0.04 ^c	1608.8±306.0 ^c
	120	3.58±0.87 ^{ab}	8.06±1.01 ^b	2.30±0.24 ^a	0.25±0.06 ^{b,c}	2256.2±571.6 ^{ab}
	180	3.88±0.75 ^a	9.58±1.32 ^a	2.50±0.29 ^a	0.26±0.06 ^{b,c}	2575.9±828.6 ^a
CO-I		1.74±0.33 ^b	4.10±0.68 ^b	2.37±0.23 ^a	0.51±0.07 ^b	970.0±255.9 ^b
CO-II		3.11±0.55 ^a	7.27±1.04 ^a	2.36±0.19 ^a	0.30±0.06 ^d	1941.7±344.5 ^a
A-flour	5	1.96±0.20 ^b	4.63±0.34 ^b	2.37±0.21 ^a	0.47±0.05 ^{b,c}	936.1±128.1 ^b
	60	1.76±0.16 ^b	4.32±0.26 ^b	1.76±0.14 ^b	0.22±0.03 ^d	1194.0±229.2 ^b
	120	3.07±0.49 ^a	6.99±0.87 ^a	2.29±0.18 ^a	0.34±0.03 ^{cd}	2079.5±439.0 ^a
	180	1.11±0.19 ^c	2.72±0.45 ^c	2.47±0.14 ^a	1.04±0.20 ^a	387.1±161.9 ^c
CO-I		1.74±0.33 ^{cd}	4.10±0.68 ^{cd}	2.37±0.23 ^{ab}	0.51±0.07 ^a	970.0±255.9 ^{cd}
CO-II		3.11±0.55 ^a	7.27±1.04 ^a	2.36±0.19 ^{ab}	0.30±0.06 ^c	1941.7±344.5 ^a
PA-flour	5	2.21±0.32 ^b	4.93±0.40 ^b	2.25±0.25 ^b	0.40±0.07 ^b	1041.9±266.7 ^c
	60	1.56±0.27 ^d	4.08±0.67 ^{cd}	1.88±0.11 ^c	0.28±0.06 ^c	1339.8±354.3 ^b
	120	1.90±0.20 ^c	4.65±0.28 ^{bc}	1.76±0.15 ^d	0.20±0.02 ^d	1489.1±308.7 ^b
	180	1.58±0.27 ^{cd}	3.88±0.49 ^d	2.48±0.33 ^a	0.51±0.07 ^a	719.5±160.5 ^d

¹⁾Means of three replicates. For P-flour, A-flour, and PA-flour compared to CO-I and CO-II, respectively, values within a column followed by a different letter are significantly different ($p<0.05$), Duncan's multiple range test.

²⁾CO-I, Waxy rice steeped for 2 h; CO-II, Waxy rice steeped at optimum condition (31.5°C, 9 days); P-flour, Waxy rice flour treated with 20 units of protease with varying treatment time; A-flour, Waxy rice flour treated with 25 units of α -amylase with varying treatment time; PA-flour, Waxy rice flour treated with 20 units of protease for 5 min followed by 25 units of α -amylase with varying treatment time.

viscosity, is an index of amylose retrogradation (32). The previous study showed that breakdown is one of the important variables in expansion (20). The breakdown of the P-flour was 157.33–170.00, and it decreased with increasing duration of enzyme treatment. These values were higher than that of CO-I. These findings indicate that the peak viscosity and breakdown values increase as protein content decreases because of protease treatment. For *Mikwa*, a traditional Japanese rice snack that is manufactured by a similar process to *Gangjung*, it was reported that as the breakdown value is higher, the puffing increases (33). The manufacturing process of *Mikwa* includes cooling, slicing, drying, reheating, and puffing processing after pregelatinizing rice. Hence, this indicates that the breakdown value is related to the ER. Furthermore, Chen *et al.* (34) indicated that physical and chemical changes in expansion attribute to starch gelatinization, starch breakdown, and protein denaturation, and as the changes have an influence on appearance, flavor, smell, and texture, the protein and pasting properties are clearly related to the expansion. It is assumed that the change in breakdown values caused by protease treatment in our study contributes to the expansion of *Gangjung*. The setback was 121.67–112.33, which was lower than that of the CO-I waxy rice flour but higher than that of the CO-II waxy rice flour.

The initial pasting temperature of CO-II and A-flour was slightly higher than that of CO-I. The peak viscosity of A-flour for 5 min was lower than that of CO-I and CO-II. Lee *et al.* (35) found that the

viscosity of rice flour extrudates treated with amylase was much lower than that of non-enzyme-treated ones, probably because of the degradation of starch by enzyme hydrolysis. Furthermore, the breakdown value of A-flour alone was 99.00–125.00, higher than that of CO-I and lower than that of CO-II, whereas the setback was –110.67~–78.67, which was lower than that of CO-I and higher than that of CO-II.

As for the pasting properties, the initial pasting temperature of PA-flour was higher than that of CO-I and similar to that of CO-II. The peak viscosity of PA-flour was higher than that of A-flour, but lower than that of P-flour. While the breakdown and setback of single En-flour showed consistent change (decrease and increase, respectively), depending on the duration of the treatment, those of PA-flour did not show the same tendency. PA-flour was expected to have lower viscosity and breakdown values than those of P-flour or A-flour, because the combined treatment would break down both proteins and starch granules. These results suggest that, as indicated above, the two enzymes play as an inactivator in interaction.

Effect of enzyme treatment on the expansion properties of *Gangjung* Expansion ratio (ER) has been reported to be one of the most important indexes to show the quality of *Gangjung* (20). Hence, ER is one of the most important factors to be considered while manufacturing high-quality *Gangjung*. As shown in Table 5, *Gangjung* prepared from waxy rice flour treated with 20 units of

protease for 5 min showed a similar ER to that of CO-II. A sample treated with 20 units protease for 180 min showed highest increase in 2575.9% in the ER. When waxy rice flour was treated with protease at 20 units for 5 and 60 min, protein content was lower by 0.1–0.3% compared to that of CO-I, but the ER of *Gangjung* made from P-flour was significantly higher than that of *Gangjung* made from CO-I. These findings indicate that the small amount of waxy rice protein degraded by protease can have a significant influence on the expansion size.

The expansion properties of *Gangjung* made from waxy rice flour treated with 25 units α -amylase for 5 to 180 min showed that the ER of *Gangjung* increased with increasing duration of enzyme treatment from 5 to 120 min, but the ER of *Gangjung* at 25 units for 180 min was lower than that of CO-I. Therefore, appropriate treatment duration for 25 units α -amylase to meet similar ER to that of CO-II was 120 min. These findings indicate that starch hydrolysis by α -amylase has to be controlled to the appropriate levels. Park (1) reported that the level of starch hydrolysis suitable for *yukwa* (popped rice snack) processing is 39–58%, and a high percentage of starch hydrolysis makes it difficult to form structure of *yukwa*, which supports the results of the present study.

The ER of *Gangjung* made from PA-flour was 719.5–1489.1%, which is similar to or higher than that of CO-I, but lower than that of CO-II. The ER of samples treated with 25 units α -amylase for 60 and 120 min following protease treatment was the highest among PA-flour samples. These results indicate that the ER may be related to the breakdown, because these values were higher at 60 and 120 min. Nonetheless, the ER under this condition was significantly lower compared to that of CO-II, indicating that combined treatment is less effective than treatment with either enzyme alone. These findings provide evidence that the two enzymes act as an inactivator on another, rather than showing synergistic effect to each other, as shown in the results of the physicochemical analysis of waxy rice flour.

The correlation among mineral content and RVA properties of the waxy rice flour treated with different enzymes, and expansion ratio of *Gangjung* To summarize and explain the correlation between the physicochemical properties of waxy rice flour treated with enzyme and the expansion properties of *Gangjung*, the PCA analysis was carried out. The first two components explained 46.05 and 18.60% of the total variation (64.65%), respectively. As shown in Fig. 1, viscosity, breakdown, and ER of the *Gangjung* were loaded in the positive direction of PC1; whereas minerals and density were loaded in the negative direction. Concerning the PCA plots of the En-flour samples based on PC1, CO-II and the P-flour were loaded in the positive direction; whereas CO-I and the A-flour were loaded in the negative direction. The PA-flour was located in the middle. In other words, *Gangjung* treated with protease loaded in positive direction was found to have similar physicochemical and expansion characteristics to CO-II, concerning higher levels of break down, ER, and so one. On

Fig. 1. Principal components analysis (PCA) of the physicochemical properties of En-flour and expansion properties of *Gangjung* based on first (x) and second (y) PCs. Sample code: CO-I, waxy rice steeped for 2 h; CO-II, waxy rice steeped at optimum condition (31.5°C, 9 days); a-5, -60, -120, and -180, waxy rice flour treated with 25 units of α -amylase for 5, 60, 120 and 180 min; P-5, -60, -120, and -180, waxy rice flour treated with 20 units of protease for 5, 60, 120, and 180 min; PA-5, -60, -120, and -180, waxy rice flour treated with 20 units of protease treatment for 5 min followed by 25 units of α -amylase for 5, 60, 120, and 180 min.

the other hand, *Gangjung* treated with amylase loaded in the negative direction, and had similar characteristics to CO-I, with higher levels of mineral contents and setback.

Based on PC2, initial pasting temperature was loaded in the positive direction, while P content was loaded in the negative direction. In *Gangjung* sample, CO-I was strongly loaded in the negative direction of PC2, and the rest of the enzyme-treated samples and CO-II were located in middle. CO-I, the wet-processed waxy rice flour steeped for two hours, was the only sample irrespective of enzyme treatment. On the other hand, CO-II was not treated with the enzyme directly, however, it could be considered as the sample affected by the enzyme since it generated the enzymes during the long steeping processing. Thus, the difference between samples in PC2 was considered to be from the enzyme effect.

Overall, considering the physicochemical properties of En-flours and expansion properties of *Gangjung* made using these flours, it was found that modifying waxy rice flours using protease and α -amylase, but not both, could reduce the steeping time involved in *Gangjung* making without sacrificing the quality of *Gangjung*. That is, waxy rice flours treated with 25 units α -amylase for 120 min and with 20 units protease for 5–180 min were found to produce *Gangjung* similar to the one made from waxy rice flour steeped for optimal condition from a physicochemical point of view. However, none of the *Gangjung* made using waxy rice flours treated with protease and α -amylase was able to provide similar expansion rates to that of optimal one. Further studies are required to examine sensory

characteristics and molecular level properties of starch and protein of *Gangjung* made using En-flour, in order to confirm the usefulness of using enzymes for reducing steeping time involved in *Gangjung* making.

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