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Physicochemical Properties of Wheat Gluten Hydrolysates Produced by Different Proteases

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ABSTRACT

Physicochemical Properties of Wheat Gluten Hydrolysates Produced by Different Proteases

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Hydrolysis of proteins has been widely used to produce ingredients with functional characteristics (foaming, solubility, emulsifying, etc.) as well as various physiological activities (antioxidant, antimicrobial, anti-inflammatory, anti-hypertensive activities, etc.). Enzymatic hydrolysis has been applied to various foods of animal and plant sources. Wheat gluten, which is produced while separating starch from wheat, is relatively inexpensive. The objective of the study was to evaluate the changes in chemical characteristics and antioxidant properties of wheat gluten hydrolysates (WGH) and utilize the WGH as an ingredient in a beverage system.

Single (2, 6, 12 and 24 h) and sequential enzymatic hydrolyses (8, 10 and 12 h) were conducted to produce WGH using commercial proteases (Alcalase, Flavourzyme, Protamex and Neutrase). Wheat gluten was suspended in distilled water (20%, w/w) and hydrolyzed with the enzyme to wheat gluten ratio of 1:100 (w/w) at 50˚C in a water bath.

Yields of all the produced WGH were over 50%. Degree of hydrolysis (DH) of the WGH significantly increased with hydrolysis time ($p<0.05$). DH of the WGH produced by Flavourzyme was the highest, independent of hydrolysis time, while that of the WGH by Neutrase was the lowest. Sequential enzymatic hydrolysis remarkably decreased the fraction with more than 10 kDa and increased the fraction with less than 500 Da compared with the single enzymatic hydrolysis. Contents and compositions of free amino acids in the WGH were significantly affected by the enzyme types and hydrolysis conditions including the number of treated enzymes and sequences of the treated enzymes. DPPH radical scavenging activity of the WGH significantly increased with hydrolysis time ($p<0.05$). On the other hand, ABTS radical scavenging activity of the WGH was rarely affected by the enzyme types and hydrolysis conditions. The WGH produced by Protamex showed lower turbidity, better thermal stability and higher solubility, suggesting they may be suitable for beverage development.

Keywords: Wheat gluten; Protein hydrolysate; Sequential enzymatic hydrolysis; Beverage

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INTRODUCTION

Hydrolysis of proteins has been widely used to produce ingredients with functional characteristics (foaming, solubility, emulsifying, etc.) (Neklyudov et al., 2000) and various bioactivities (antioxidant, antimicrobial, anti-inflammatory, anti-hypertensive activities, etc.) (Castro and Sato, 2015). Chemicals, including acidic/alkaline compounds, and enzymes have been used for hydrolysis. Chemical treatments are difficult to control specific cleavages in amino acid sequences of proteins and can destroy essential amino acids (Finley et al., 1982). During neutralization of acidic/alkaline conditions, undesirable salts are readily formed. On the other hand, proteases have certain specificities for substrates, hydrolyzing proteins into peptides in a mild condition. The specificity and reaction conditions (pH, temperature and time) during enzymatic hydrolysis can affect characteristics of protein hydrolysates; peptide size, amino acid sequences and amount of free amino acids (Sarmadi and Ismail, 2010). Enzymatic hydrolysis has been utilized in a wide range of food materials including animal (milk, egg, beef, pork, chicken, etc.) and plant sources (rice, corn, soy, wheat, etc.) (Agyei and Danquah, 2012). Wheat is one of the cereal crops widely consumed in the world. Wheat is available at a low cost because it is harvested over 700 million tons annually with technological advances. Wheat gluten, a by-product of the wheat starch industry, is rarely water-soluble due to gluten forming a continuous network between gliadin and glutenin proteins (Shewry, 2009). Not only different functional characteristics (Kong et al., 2007; Wang et al., 2006) but also biological activities such as antioxidant (Qiu et al., 2013) and anti-hypertensive activities (Cian et al., 2015)

have been reported on wheat gluten hydrolysates (WGH) produced by enzymatic hydrolysis.

Single enzymatic hydrolysis of wheat gluten has been investigated to determine the effect of enzyme types and hydrolysis time on the characteristics of hydrolysates (Kong et al., 2007). Liu et al. (2016) reported the effect of sequential enzymatic hydrolysis using endo- and exo-peptidases to reduce bitterness. Besides, sequential enzyme treatment was attempted to enhance the efficiency of hydrolysis in food proteins such as Nile tilapia proteins (*Oreochromis niloticus*) (Yarnpakdee et al., 2015), duck egg white proteins (Ren et al., 2014) and muscle of brown stripe red snapper (Khantaphant et al., 2011). However, little information on the changes in characteristics of WGH was available in terms of comparison between single and sequential enzymatic hydrolyses. Moreover, it is important to understand sensory properties of WGH because individual peptides have unique taste properties like sweetness, sourness, umami and bitterness (Shahidi, 2012). However, the evaluation of physicochemical and sensory properties of WGH in a beverage system has not been studied.

The objective of this study was to evaluate the changes in chemical characteristics and antioxidant properties of the WGH produced by single and sequential enzymatic hydrolyses using commercial proteases (Alcalase, Flavourzyme, Protamex, and Neutrase) and also to determine turbidity, thermal stability, pH solubility and sensory characteristics of a beverage system made from the WGH.

MATERIALS AND METHODS

1. Materials

Wheat gluten (78.9±0.58% crude protein (N X 5.7), 4.83±0.15% water, 4.36±0.20% crude fat and 0.60±0.02% crude ash) was obtained from Anhui Ruifuxiang Company (Anhui, China). Alcalase (EC 3.4.21.62, from *Bacillus licheniformis,* 2.4 AU/g), Flavourzyme 1000 L (EC 3.4.11.1, from *Aspergillus oryzae*, 1000 AU/g), Protamex (EC 3.4.24.28, from *Bacillus subtilis*, 1.5 AU/g) and Neutrase (EC 3.4.24.28, from *Bacillus amyloliquefaciens,* 0.8 AU/g) were purchased from Novozymes (Bagsvaerd, Denmark). o-Phthaldialdehyde (OPA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), N, N-dimethyl- 2-mercaptoethylammonium chloride (DMMAC), cytochrome C from equine heart, aprotinin from bovine lung, bacitracin, Gly-Gly-Tyr-Arg, Gly-Gly-Gly and potassium persulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium tetraborate decahydrate was from Junsei Chemical Co., Ltd. (Tokyo, Japan). 9-Fluorenylmethyl chloroformate (FMOC) was from Agilent Technologies (Palo Alto, CA, USA). Mite hot chocolate powder was purchased from Dongsuh Food Co., Ltd. (Seoul, Korea). The other chemicals used in the present study were of analytical grade and purchased from Samchun Chemical Co. (Seoul, Korea).

2. Preparation of wheat gluten hydrolysates

Single enzymatic hydrolysis of wheat gluten using the four commercial proteases (Alcalase, Flavourzyme, Protamex and Neutrase) was carried out under the conditions as follows: Wheat gluten was suspended in distilled water (20%,

w/w). The suspension was continuously stirred using an overhead stirrer (Wisestir HS-30D, Daihan Scientific Co., Seoul, Korea) at 200 rpm, and temperature was kept at 50±1˚C in a water bath. Wheat gluten was hydrolyzed with enzyme to wheat gluten ratio of 1:100 (w/w) for 2, 6, 12 and 24 h. After hydrolyzed, the mixture was heated for 10 min at 95˚C to inactivate the enzyme before centrifuging at 8,000×g for 10 min at 20˚C. Alcalase and Protamex were selected to conduct sequential enzymatic hydrolysis due to their high efficiency compared to the other two enzymes. Hydrolysis using the first enzyme was conducted for 6 h under the same hydrolysis conditions to the single enzymatic hydrolysis. After the hydrolysis, the mixture was heated at 95˚C to inactivate the first enzyme and cooled down to 50˚C slowly. The second enzyme was added with enzyme to wheat gluten ratio of 1:100 (w/w), and hydrolysis was additionally conducted for 2, 4 and 6 h. After hydrolysis, the mixture was heated at 95˚C for 10 min to inactivate the enzyme before centrifuging at 8,000×g for 10 min at 20˚C. WGH powder obtained after lyophilization of the supernatant was stored at -20 ˚C until further analysis.

3. Yield of the WGH

Yield of WGH was calculated using the following equation:

$$\text{Yield (\%)} = (W_1/W_0) \times 100,$$

where W_0 is weight of wheat gluten used (g, dry basis) and W_1 is weight of freeze-dried WGH (g).

4. Degree of hydrolysis (DH)

DH was determined by OPA method described by Wang et al. (2006) and

Frisher et al. (1988). Each sample of the WGH was dissolved at 1.25 mg/mL in 12.5 mM sodium borate buffer (pH 8.5) containing 2% (w/v) SDS. Fifty μL of this solution was added to 1 mL of a reagent composed of 50 mL 0.1 M sodium borate buffer (pH 9.3), 1.25 mL 20% (w/v) SDS solution, 100 mg DMMAC and 40 mg OPA dissolved in 1 mL methanol before 2 min incubation at room temperature. Absorbance of the mixture was measured at 340 nm. The number of amino groups was determined with reference to an L-leucine standard curve (between 0.5 and 5 mM). DH was calculated using the following equation:

$DH\ (\%) = [(\alpha - n_i)/(n_T - n_i)] \times 100,$

where n_T is the total number of amino groups in the totally hydrolyzed gluten treated with 6 M HCl at 110˚C for 24 h, n_i is the number of amino groups in native gluten and α is the number of free amino groups of WGH.

5. Molecular weight (MW) distribution

5.1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted according to Laemmli (1970) using 15% acrylamide separating gel and 5% acrylamide stacking gel. Each sample of the WGH was prepared in 50 mM Tris-HCl buffer (pH 6.8) containing 0.1% bromophenol blue, 10% glycerol, 2.5% SDS and 0.1 M 1, 4-dithiothreitol, and loaded onto gel. After electrophoresis, gels were stained with Coomassie brilliant blue R-250 to detect proteins.

5.2. Size exclusion chromatography (SEC)

MW distribution of WGH was determined by SEC using an HPLC system

consisting of Waters 2695 and Waters 2996 PDA detector from Waters (Milford, MA, USA). TSK gel 2000 SWXL column (300 X 7.8 mm) from Tosoh (Tokyo, Japan) was used with 70:30 (v/v %) acetonitrile/water containing 0.1% trifluoroacetic acid (TFA) as mobile phase. Injection volume was 20 μL. Flow rate was 0.5 mL/min. Detection wavelength was 214 nm. Data processing was performed using Empower software version 2 from Waters (Milford, MA, USA). A MW calibration curve was prepared by following standards: cytochrome C from equine heart (12,500 Da), aprotinin from bovine lung (6,500 Da), bacitracin (1,450 Da), Gly-Gly-Tyr-Arg (451 Da) and Gly-Gly-Gly (189 Da). A relationship between the retention times and the log of MW of the proteins used as standards was established. Samples were divided into the following classes: 0–500 Da, 500–1,000 Da, 1,000-3,000 Da, 3,000–5,000 Da, 5,000–10,000 Da and above 10,000 Da. The relative area of each fraction was given in percentage of the total area.

6. Free amino acids

Free amino acids in the WGH were determined by an HPLC system consisting of Dionex Ultimate 3000 and FL detector (Dionex, Idstein, Germany). VDSspher 100 C18 column (150 X 4.6 mm) from VDS Optilab (Berlin, Germany) was used with mobile phase A (40 mM sodium phosphate dibasic buffer, pH 7) and mobile phase B (water/acetonitrile/methanol, 10:45:45, v/v %). The gradient elution was as follows: 95% A and 5% B for 3 min, in 24 min to 45% A and 55% B, in 31 min to 20% A and 80% B and 95% A and 5% B in 35 min. Flow rate was 1.5 mL/min, and injection volume was 0.5 μL. Immediately after injection, an auto-sampler was used for the inline-derivatization by FMOC/OPA post column derivatization. OPA-derived amino acids were monitored at emission 450 nm and excitation 340 nm

and FMOC-derived amino acids were monitored at emission 305 nm and excitation 266 nm. Data processing was performed using Chromeleon software 6.8 version from Dionex (Idstein, Germany). Individual free amino acids were expressed as mg/g of the lyophilized WGH.

7. Antioxidant properties

7.1. DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method described by Kong et al. (2008) with some modification. Each sample of the WGH was dissolved in distilled water at 5 mg/mL. Two hundred μL of the solution was mixed with 200 μL 0.2 mM DPPH dissolved in 95% (v/v) ethanol. The mixture was shaken and then incubated in the dark for 20 min. The mixture was centrifuged at 8,000×g for 10 min after incubation. Absorbance of the supernatant was measured at 517 nm. DPPH radical scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (%) = $[(A_0 – A_s)/A_0] \times 100$,

where A_0 is absorbance of blank mixture (200 μL distilled water mixed with 200 μL DPPH solution) and A_s is absorbance of the WGH.

7.2. ABTS radical scavenging activity

ABTS radical scavenging activity was determined as described by Re et al. (1999). ABTS radical solution was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate at a ratio of 1:1 (v/v). The mixture was allowed to stand in the dark for 12-16 h before use. The ABTS radical solution was diluted with distilled water to an absorbance of 0.7±0.05 at 734 nm. Then 50 μL of WGH was

added to 950 μL diluted ABTS radical solution. The mixture was shaken and then incubated in the dark for 10 min. The mixture was centrifuged at 8,000×g for 10 min. Absorbance of the supernatant was measured at 734 nm. ABTS radical scavenging activity was calculated using the following equation:

ABTS radical scavenging activity (%) = $[(A_0 – A_s)/A_0] \times 100$,

where A_0 is absorbance of blank mixture (50 μL distilled water mixed with 950 μL ABTS solution) and A_s is absorbance of the WGH.

8. Physicochemical properties of WGH solution

8.1. Turbidity and thermal stability

WGH were dissolved in distilled water at 50 mg/mL. Turbidity of the samples was determined by measuring the optical density (OD) at 600 nm using a spectrophotometer (Optizen 2120UV; Mecasys, Daejeon, Korea). To determine thermal stability, the samples were heated at 90˚C for 10 min and cooled down to room temperature. The turbidity of the samples was determined as mentioned above. Distilled water was used as blank.

8.2. pH solubility

The WGH were suspended at 50 mg/mL in a buffer solution using 0.2 M sodium phosphate and 0.1 M citric acid at pH 3, 5 and 7. The mixture was centrifuged at 15,000×g for 10 min. The supernatant was discarded, and the pellet was oven-dried at 105˚C for 1 h. The solubility (%) was calculated using the following equation:

Solubility (%) = $(W_0\text{-}W_1/W_0) \times 100$,

where W_0 is weight of dry wheat gluten used (g) and W_1 is weight of dried WGH

(g).

8.3. Color

WGH were dissolved in distilled water at 50 mg/mL. Color of WGH solution was measured using a colorimeter (CM-5, Konica Minolta Co., Tokyo, Japan). Results are given as lightness (L^*), redness (a^*) and yellowness (b^*).

9. Sensory evaluation

Sensory evaluation was conducted two times. Firstly, the WGH produced by the single and sequential enzymatic hydrolyses for 12 h were selected for sensory evaluation. The WGH suspended at 1% concentration in water were presented for evaluation at room temperature in a randomized order. Thirty six participants scored all the samples for flavor, appearance, taste, bitterness and overall acceptability. Flavor, appearance, taste and overall acceptability were scored on 15 cm line scales with anchors labeled ‘dislike very much’ (0) and ‘like very much’ (15). Bitterness was scored on a 15 cm line scale with anchors labeled ‘very weak’ (0) and ‘very strong’ (15). Secondly, based on the results of the first sensory evaluation, the WGH produced by 12 h hydrolyses using Alcalase and Protamex were selected as ingredients of a chocolate beverage because of their higher overall acceptability than the others. The chocolate beverage was prepared using Mite hot chocolate powder at 18% (w/w) concentration in hot water. The WGH were added at two different concentrations of 2.5 and 5% (w/w). The beverages were served at room temperature in a randomized order and evaluated under the same procedure of the first sensory evaluation.

10. Statistical analysis

All the data were obtained by the tests conducted in triplicate. Results were subject to one-way analysis of variance (ANOVA) and Duncan's new multiple range test at significance level of $p<0.05$ using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

1. Yield and DH

Yields of the WGH hydrolyzed by all the tested proteases were over 50% at any hydrolysis conditions (Table 1). Hydrolysis time little affected the yield of the WGH produced by Alcalase. On the other hand, the yield of the WGH by 2 h hydrolysis using Flavourzyme increased from 59.2% to 67.4% by 24 h hydrolysis. Yields of the WGH produced by Protamex and Neutrase tended to decrease during the enzymatic hydrolysis. These results indicate that Alcalase and Flavourzyme are more efficient to produce water-soluble hydrolysates than Protamex and Neutrase with hydrolysis time. The sequential enzymatic hydrolysis using Alcalase after 6 h hydrolysis with Protamex produced more water-soluble hydrolysates than the hydrolysis using only Protamex, implying that the sequential enzymatic hydrolysis may overcome low yield of single enzymatic hydrolysis using Protamex.

As shown in Table 1, DH of the WGH was up to 52.9% depending on the enzyme types and hydrolysis conditions including hydrolysis time and the number of treated enzymes. The hydrolysis of wheat gluten with the proteases seemed to slow down after 6 h hydrolysis. DH of the WGH produced by Flavourzyme was the highest, indicating that Flavourzyme is the most efficient for wheat gluten hydrolysis. After 24 h hydrolysis, DH of the WGH produced by Alcalase was similar to that of the WGH produced by Protamex, while that of the WGH produced by Neutrase was the lowest. Kechaou et al. (2009) reported that Alcalase was the best to hydrolyze cuttlefish viscera (*Sepia officinalis*) and sardine viscera (*Sardina pilchardus*) among commercial proteases (Alcalase, Flavourzyme and Protamex).

The sequential enzymatic hydrolysis using Alcalase after 6 h hydrolysis with Protamex showed higher efficiency than the single enzymatic hydrolysis with Protamex. On the other hand, the sequential enzymatic hydrolysis using Protamex after 6 h hydrolysis with Alcalase showed lower efficiency than the single enzymatic hydrolysis with Alcalase. The sequential enzymatic hydrolysis seemed to be more efficient on the production of WGH than the single enzymatic hydrolysis. However, MW distribution determined by SEC should be considered to evaluate the hydrolysis efficiency because DH was only calculated by the number of amino groups.

Table 1. Yield and degree of hydrolysis (DH) of wheat gluten hydrolysates produced by single and sequential enzymatic hydrolyses

	Enzyme	Hydrolysis time (h)	Yield (%)	DH (%)
Single	Alcalase	2	64.2±0.39^{e}	8.74±1.21^{e}
		6	64.9±0.06^{d}	13.5±0.79^{d}
		8	66.4±0.06^{c}	16.2±2.05^{cd}
		10	66.9±0.11^{b}	18.4±2.31^{bc}
		12	67.5±0.08^{a}	22.5±1.96^{ab}
		24	66.8±0.11^{b}	26.3±4.18^{a}
	Flavourzyme	2	59.2±0.70^{c}	13.4±0.77^{d}
		6	63.1±0.71^{b}	33.3±0.30^{c}
		12	66.9±0.46^{a}	38.7±2.03^{b}
		24	67.4±0.42^{a}	52.9±2.76^{a}
	Protamex	2	66.4±0.59^{a}	7.15±1.26^{e}
		6	63.7±2.20^{b}	14.0±1.78^{d}
		8	62.8±0.18^{b}	14.2±0.87^{cd}
		10	60.0±0.13^{c}	16.2±0.63^{c}
		12	59.4±0.25^{c}	18.8±1.01^{b}
		24	55.8±1.46^{d}	25.4±0.71^{a}
	Neutrase	2	64.4±0.07^{a}	5.65±0.60^{d}
		6	66.4±1.03^{a}	8.83±0.37^{c}
		12	60.9±0.05^{b}	13.0±0.53^{b}
		24	54.6±0.77^{c}	22.1±1.29^{a}
Sequential	Alcalase→ Protamex	6→2	66.7±0.07^{a}	13.9±1.59^{b}
		6→4	66.9±0.46^{a}	18.7±0.53^{a}
		6→6	65.4±0.50^{b}	20.1±1.46^{a}
	Protamex→ Alcalase	6→2	62.6±0.17	17.3±2.96
		6→4	63.1±0.13	17.6±2.08
		6→6	63.0±0.37	21.3±2.11

All data represent the mean and standard deviation (n=3).

Different small letters in the same columns indicate significant differences among wheat gluten hydrolysates produced by same enzyme ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

2. MW distribution of the WGH

MW of all the WGH in the SDS-PAGE patterns dramatically decreased to below 15 kDa (data not shown), compared with raw wheat gluten, which consisted of high MW subunit of glutenin (67-88 kDa), low MW subunits of glutenin (32-35 kDa) and gliadin (28-55 kDa) (Wieser, 2007).

As shown in Table 2, the SEC revealed that the fraction of the hydrolysates with more than 10 kDa decreased during hydrolysis, while the fraction with less than 1 kDa increased on all the WGH. After 2 h hydrolysis, Alcalase was the best to hydrolyze wheat gluten into peptides among the treated enzymes. The WGH produced by Alcalase contained the smallest amount of the fraction with more than 10 kDa and the largest amount of the fraction with less than 500 Da. The WGH produced after 6 h hydrolysis by Flavourzyme contained smaller amount of the fraction with more than 10 kDa and larger amount of the fraction with less than 500 Da than those produced by the other enzymes. However, it is hard to evaluate whether Flavourzyme is more efficient for the production of WGH with lower MW because it has both endo- and exo-protease activities. Therefore, the amount of free amino acids should be considered. On the other hand, Neutrase showed the lowest efficiency to hydrolyze wheat gluten into peptides for all the hydrolysis times. The WGH produced by Neutrase contained the largest amount of the fraction with more than 10 kDa and the smallest amount of the fraction with less than 500 Da at any hydrolysis times. Therefore, Neutrase may not be a suitable enzyme to hydrolyze wheat gluten effectively.

MW distributions of the WGH produced by the single and sequential enzymatic hydrolyses are shown in Fig. 1. The sequential enzymatic hydrolysis decreased the fraction with more than 10 kDa and increased the fraction with less than 500 Da

compared to the single enzymatic hydrolysis during the same hydrolysis time. The fraction with more than 10 kDa remarkably was less in the WGH produced by 2 h hydrolysis using Protamex after 6 h hydrolysis using Alcalase (3.0%) than by 8 h hydrolysis using Alcalase (7.8%). Moreover, the fraction with less than 500 Da was much more in the WGH produced by 2 h hydrolysis using Protamex after 6 h hydrolysis using Alcalase (45.5%) than by 8 h hydrolysis using Alcalase (37.8%). The fraction with more than 10 kDa in the WGH produced by Alcalase after 6 h hydrolysis using Protamex (2.3%) was also less than by 8 h hydrolysis using Protamex (10.4 %). Therefore, adding the second enzyme effectively hydrolyzed wheat gluten into smaller peptides.

Table 2. Molecular weight distribution of wheat gluten hydrolysates produced by single enzymatic hydrolysis

Enzyme	Hydrolysis time (h)	>10 kDa	5-10 kDa	3-5 kDa	1-3 kDa	0.5-1 kDa	<500 Da
Alcalase	2	18.2 ± 1.40^{aD}	7.75 ± 0.72^{aC}	7.79 ± 0.68^{a}	24.1 ± 0.71^{aA}	15.9 ± 0.73^{cA}	26.4 ± 0.67^{dA}
	6	9.97 ± 1.51^{bB}	6.09 ± 0.38^{bB}	6.75 ± 0.48^{bB}	24.7 ± 0.32^{aB}	18.1 ± 0.72^{bB}	34.4 ± 1.67^{cA}
	12	3.02 ± 0.47^{cB}	3.54 ± 0.38^{cC}	5.40 ± 0.51^{cB}	22.4 ± 0.69^{bC}	20.1 ± 0.44^{aA}	45.5 ± 1.91^{bA}
	24	1.57 ± 0.25^{cB}	2.50 ± 0.25^{dC}	4.41 ± 0.44^{cB}	19.7 ± 0.34^{cC}	19.5 ± 0.68^{aB}	52.3 ± 1.06^{aB}
Flavourzyme	2	31.1 ± 1.28^{aB}	10.1 ± 0.10^{aAB}	8.44 ± 1.01^{a}	22.5 ± 0.84^{cB}	10.6 ± 0.53^{cC}	17.4 ± 0.56^{dB}
	6	6.97 ± 1.02^{bC}	6.76 ± 0.37^{bB}	6.88 ± 0.20^{bAB}	26.5 ± 0.34^{aA}	15.9 ± 0.34^{bC}	37.0 ± 0.74^{cA}
	12	4.04 ± 0.35^{cB}	5.18 ± 0.27^{cB}	5.79 ± 0.09^{cB}	24.0 ± 0.23^{bB}	16.9 ± 0.10^{aC}	44.1 ± 0.83^{bA}
	24	2.02 ± 0.39^{dB}	1.86 ± 0.11^{dC}	2.78 ± 0.15^{dC}	14.4 ± 0.36^{dD}	16.1 ± 0.25^{bC}	62.9 ± 1.08^{aA}
Protamex	2	24.4 ± 1.41^{aC}	9.65 ± 0.25^{aB}	7.73 ± 0.64^{a}	23.7 ± 0.28^{AB}	16.1 ± 0.50^{bA}	18.4 ± 1.57^{cB}
	6	10.6 ± 2.22^{bB}	9.31 ± 1.64^{aA}	6.55 ± 0.34^{aB}	25.1 ± 0.42^{B}	20.3 ± 0.55^{aA}	28.2 ± 3.43^{bB}
	12	3.20 ± 1.12^{cB}	5.30 ± 1.29^{bB}	5.26 ± 0.65^{bB}	23.7 ± 1.11^{B}	21.0 ± 0.38^{aA}	41.5 ± 4.36^{aA}
	24	1.76 ± 0.16^{cB}	3.94 ± 0.97^{bB}	4.74 ± 0.84^{bB}	23.7 ± 2.31^{B}	20.8 ± 0.17^{aA}	45.1 ± 3.73^{aC}
Neutrase	2	34.8 ± 1.38^{aA}	10.7 ± 0.41^{aA}	8.26 ± 0.20^{a}	21.0 ± 0.68^{cC}	12.2 ± 0.33^{cB}	13.1 ± 0.49^{dC}
	6	22.3 ± 1.15^{bA}	10.7 ± 0.39^{aA}	7.55 ± 0.40^{bA}	24.8 ± 0.44^{bB}	16.1 ± 0.52^{bC}	18.6 ± 0.02^{cC}
	12	13.5 ± 1.94^{cA}	10.3 ± 0.65^{aA}	7.76 ± 0.14^{bA}	26.3 ± 0.42^{aA}	18.3 ± 0.82^{aB}	23.9 ± 0.82^{bB}
	24	8.29 ± 1.49^{dA}	8.98 ± 0.54^{bA}	7.56 ± 0.10^{bA}	26.6 ± 0.85^{aA}	19.3 ± 0.86^{aB}	29.2 ± 0.46^{aD}

All data represent the mean and standard deviation (n=3).

Different small letters in the same columns indicate significant differences among the wheat gluten hydrolysates produced by the same enzymes

($p<0.05$; one-way ANOVA and Duncan's multiple range test).

Different large letters in the same columns indicate significant differences among the wheat gluten hydrolysates produced during the same hydrolysis times ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

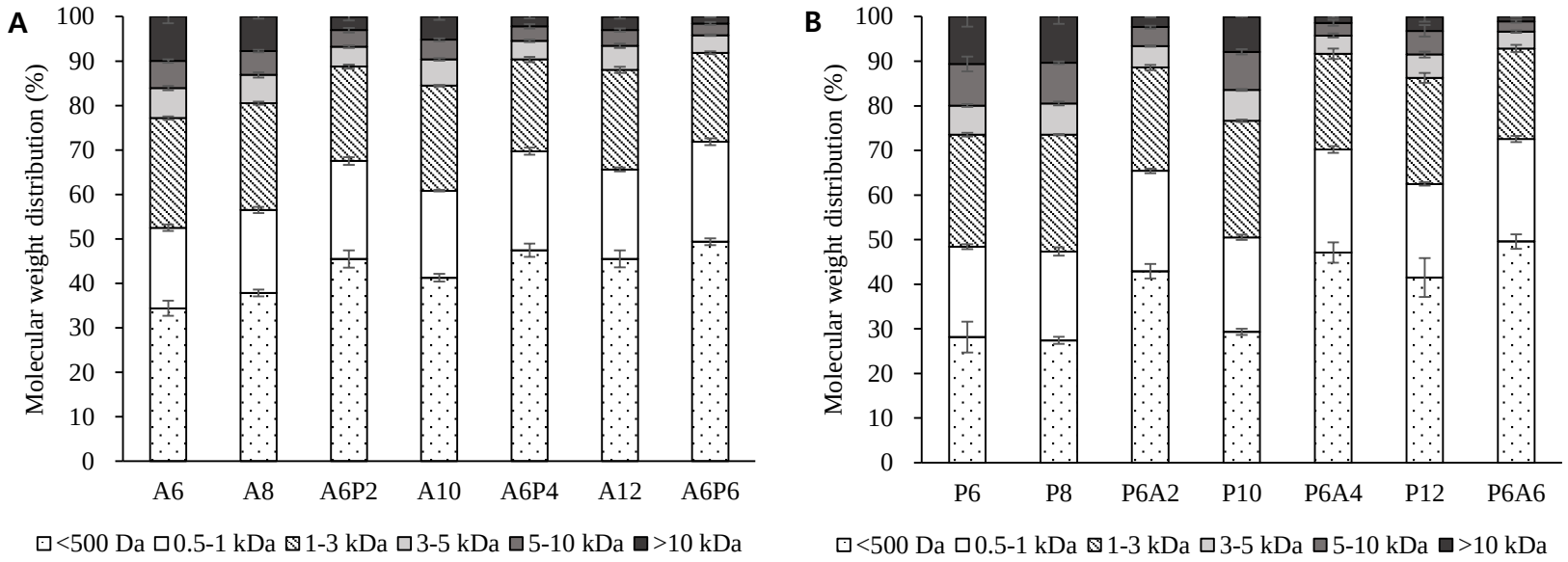

Figure 1. Molecular weight distribution of wheat gluten hydrolysates determined by size exclusion chromatography.

(A) Single enzymatic hydrolysis using Alcalase and sequential enzymatic hydrolysis using Protamex and Alcalase. (B) Single enzymatic hydrolysis with Protamex and sequential enzymatic hydrolysis using Alcalase and Protamex. Alcalase, A; Protamex, P; and numbers after A and P are hydrolysis times (h). All data represent the means and standard deviations (n=3).

3. Free amino acids of the WGH

The enzyme types and the number of the treated enzymes significantly affected the amounts of free amino acids and compositions of the WGH (Table 3). Flavourzyme produced more free amino acids than the others. Although the amounts of the fractions with less than 500 Da in the WGH produced by 12 h hydrolyses using Alcalase, Flavourzyme and Protamex were not significantly different ($p>0.05$), the amount of free amino acids in the WGH produced by Flavourzyme (24.5 %) was much higher than in the WGH produced by Alcalase and Protamex, indicating that the WGH produced by 12 h hydrolysis using Flavourzyme contained less amount of peptides with less than 500 Da than the others at the same hydrolysis time. Free amino acids except proline in the WGH produced by Flavourzyme were the largest. Protamex produced significantly more free amino acids than Alcalase ($p<0.05$). Free amino acids in the WGH produced by 12 h hydrolysis using Protamex, except glutamic acid and threonine, were significantly more than in the WGH produced by 12 h hydrolysis using Alcalase. The WGH produced by 12 h hydrolysis using Alcalase had significantly more glutamic acid than by 12 h hydrolysis using Protamex ($p<0.05$), while the amount of threonine was not significantly different ($p>0.05$). Although total free amino acids were not significantly different between the WGH produced by Alcalase and by Neutrase, the compositions of free amino acids were remarkably different. Aaslyng et al. (1988) reported that free amino acids contribute to the taste of hydrolyzed soy proteins, and especially the content of glutamic acids is important for umami taste.

Although the enzyme and time for the hydrolysis were the same, sequence of enzyme treatments significantly affected the compositions and total amounts of

free amino acids of the hydrolysates (Table 3). Total free amino acids in the WGH produced by 6 h hydrolysis using Alcalase after 6 h hydrolysis using Protamex (32.3 mg/g) were significantly higher than by 6 h hydrolysis using Protamex after 6 h hydrolysis using Alcalase (22.9 mg/g) ($p<0.05$). Most of the free amino acids in the WGH produced by 6 h hydrolysis using Alcalase after 6 h hydrolysis using Protamex were significantly more than by 6 h hydrolysis using Protamex after 6 h hydrolysis using Alcalase ($p<0.05$). This might result from the changes in the cleavage sites available for the second enzymes due to the changes in the amino acid sequences of the WGH by the first enzymes.

Table 3. Free amino acids in wheat gluten hydrolysates produced by single and sequential enzymatic hydrolyses for 12 h (mg/g, dry basis)

Amino acid	A12	F12	P12	N12	A6P6	P6A6
Aspartic acid	0.25±0.01^{c}	2.52±0.06^{a}	0.30±0.02^{b}	0.19±0.01^{d}	0.18±0.00^{d}	0.18±0.01^{d}
Glutamic acid	3.57±0.14^{b}	5.42±0.16^{a}	1.75±0.15^{c}	0.69±0.04^{e}	1.21±0.09^{d}	1.33±0.17^{d}
Asparagine	0.42±0.01^{c}	5.28±0.22^{a}	0.68±0.04^{b}	0.37±0.04^{c}	0.30±0.01^{c}	0.38±0.03^{c}
Serine	1.94±0.05^{c}	14.1±0.27^{a}	3.11±0.09^{b}	1.97±0.12^{c}	1.22±0.05^{d}	1.84±0.09^{c}
Glutamine	5.82±0.15^{c}	81.9±1.09^{a}	8.31±0.68^{b}	4.24±0.03^{d}	3.83±0.10^{d}	4.15±0.35^{d}
Histidine	0.55±0.01^{d}	7.66±0.21^{a}	1.41±0.09^{b}	0.71±0.02^{cd}	0.33±0.04^{e}	0.74±0.07^{c}
Glycine	0.23±0.00^{e}	2.69±0.07^{a}	0.95±0.06^{b}	0.80±0.06^{c}	0.21±0.01^{e}	0.71±0.03^{d}
Threonine	1.19±0.04^{b}	7.21±0.17^{a}	1.32±0.04^{b}	0.83±0.04^{c}	0.89±0.05^{c}	0.83±0.05^{c}
Arginine	3.11±0.10^{c}	12.0±0.35^{a}	5.17±0.22^{b}	3.09±0.19^{c}	2.34±0.13^{d}	3.24±0.16^{c}
Alanine	1.51±0.04^{d}	7.25±0.15^{a}	2.70±0.09^{b}	1.75±0.07^{c}	1.00±0.02^{e}	1.58±0.11^{d}
Tyrosine	1.34±0.03^{c}	9.22±0.17^{a}	3.40±0.05^{b}	1.60±0.69^{c}	0.77±0.04^{d}	1.53±0.08^{c}
Valine	1.51±0.02^{c}	15.3±0.29^{a}	2.27±0.07^{b}	1.42±0.02^{c}	0.90±0.02^{d}	1.05±0.08^{d}
Methionine	1.68±0.01^{c}	5.76±0.09^{a}	1.94±0.12^{b}	1.12±0.01^{d}	0.93±0.01^{e}	1.16±0.09^{d}
Phenylalanine	2.32±0.05^{e}	13.3±0.21^{a}	5.28±0.13^{b}	3.67±0.03^{c}	1.43±0.05^{f}	2.53±0.12^{d}

Table 3. (continued)

Amino acid	A12	F12	P12	N12	A6P6	P6A6
Isoleucine	1.12±0.03^{d}	15.1±0.27^{a}	2.80±0.10^{b}	1.61±0.04^{c}	0.66±0.01^{e}	1.09±0.08^{d}
Leucine	7.78±0.19^{c}	34.6±0.71^{a}	11.1±0.65^{b}	6.90±0.02^{d}	5.51±0.07^{e}	7.03±0.27^{d}
Lysine	0.75±0.02^{d}	2.39±0.12^{a}	1.52±0.05^{b}	0.98±0.03^{c}	0.68±0.04^{d}	1.00±0.06^{c}
Proline	0.74±0.04^{e}	2.89±0.07^{b}	3.77±0.10^{a}	2.76±0.05^{c}	0.50±0.07^{f}	1.90±0.04^{d}
Total	35.9±0.88^{c}	244.7±3.38^{a}	57.8±2.30^{b}	34.7±1.12^{c}	22.9±0.53^{d}	32.3±1.87^{c}

Alcalase, A; Flavouzyme, F; Protamex, P; Neutrase, N; and numbers after the letters are hydrolysis times (h).

All data represent the means and standard deviations (n=3).

Different small letters in the same rows indicate significant differences ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

4. Antioxidant properties of the WGH

DPPH radical scavenging activity of the WGH increased up to 68.1% depending on the types of enzymes and hydrolysis time (Table 4). DPPH radical scavenging activity of the WGH generally increased with hydrolysis time. DPPH radical scavenging activity of the WGH produced by 24 h hydrolysis using Alcalase was the highest. The WGH produced by Alcalase and Protamex showed higher DPPH radical scavenging activity than those by Flavourzyme. These results revealed that protein hydrolysates with higher contents of smaller peptides might have higher antioxidant properties than those with lower contents of smaller peptides. Alashi et al. (2014) also reported similar results that the fraction with less than 1 kDa exhibited high antioxidant properties in Australian canola meal protein hydrolysates.

ABTS radical scavenging activity was over 60% regardless of enzyme types and hydrolysis time (Table 4). This result seems to be different from the result of DPPH radical scavenging activity, which was influenced by the enzyme types and correlated with hydrolysis time. ABTS radical scavenging activity of the WGH was little changed by the enzyme types and hydrolysis time. Khantaphant et al. (2012) reported the same results that DPPH radical scavenging activity in protein hydrolysates from the muscle of brown stripe red snapper significantly increased with increasing DH, but ABTS radical scavenging activity was little changed with increasing DH.

Table 4. DPPH and ABTS radical scavenging activities of wheat gluten hydrolysates produced by single and sequential enzymatic hydrolyses

	Enzyme	Hydrolysis time (h)	DPPH (%)	ABTS (%)
Single	Alcalase	2	9.01±2.46^{e}	67.2±1.60^{a}
		6	28.3±4.97^{d}	64.4±0.53^{bc}
		8	29.8±1.17^{d}	66.3±0.61^{ab}
		10	40.3±3.23^{c}	67.1±0.90^{a}
		12	51.7±3.32^{b}	64.2±0.81^{c}
		24	68.1±2.40^{a}	64.7±1.43^{bc}
	Flavourzyme	2	N.D.	64.8±0.80^{ab}
		6	N.D.	63.3±0.46^{b}
		12	10.7±1.41	63.3±0.73^{b}
		24	20.1±1.46	66.7±1.88^{a}
	Protamex	2	14.7±3.72^{c}	67.9±1.05
		6	47.1±2.14^{b}	67.3±0.62
		8	48.8±3.51^{b}	68.9±2.37
		10	52.2±2.61^{b}	66.8±4.92
		12	60.4±2.76^{a}	67.6±1.07
		24	62.5±3.41^{a}	73.0±1.20
	Neutrase	2	N.D.	65.2±1.77
		6	23.7±4.47	64.4±0.68
		12	26.9±3.17	66.1±0.66
		24	32.9±3.57	66.0±0.46
Sequential	Alcalase→ Protamex	6→2	47.0±0.30^{c}	65.2±4.38
		6→4	53.0±2.33^{b}	67.6±0.22
		6→6	61.9±1.46^{a}	66.2±1.11
	Protamex→ Alcalase	6→2	46.0±3.42^{c}	65.4±2.36
		6→4	53.8±2.43^{b}	65.3±2.95
		6→6	62.3±3.61^{a}	65.7±2.08

N.D.: not determined

All data represent the means and standard deviations (n=3).

Different small letters in the same columns indicate significant differences among wheat gluten hydrolysates produced by the same enzymes ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

5. Physicochemical properties of WGH solution

The treated enzymes, except Protamex, and hydrolysis conditions including hydrolysis time and the number of treated enzymes significantly affected turbidity of WGH solution (Fig. 2). All the WGH solution had low turbidity after 12 h hydrolysis. The WGH solution prepared by Protamex had lower turbidity regardless of hydrolysis time than the others. Turbidity of the WGH solution prepared by Alcalase decreased after 10 h hydrolysis. Adding Alcalase after 6 h hydrolysis with Protamex was better to prepare a WGH solution with lower turbidity than adding Protamex after 6 h hydrolysis with Alcalase. Thermal processing little influenced turbidity of the WGH regardless of the enzyme types and hydrolysis conditions including hydrolysis time and the number of treated enzymes (Fig. 2). This result is attributed to the fact that unpredictable aggregation is rarely formed during thermal processing in WGH with shorter peptides due to the lack of secondary structures.

Most of the beverage industry considers food processing to avoid undesirable turbidity and precipitation in the final products (Pinelo et al., 2010). Moreover, a maintenance of clarity has been a concern in beverage products, and thermal treatments are required for safety and shelf stability in the beverage industry (Beecher et al., 2008; LaClair and Etzel, 2010). As a result, WGH with shorter peptides, having lower turbidity and higher thermal stability, are suitable to be ingredients for the beverages. Enzymatic hydrolysis could be also utilized to improve functional properties such as turbidity and thermal stability of proteins in food manufacturing.

Solubility of wheat gluten was less than 10% at pH 3, 5 and 7, while most of the WGH had high solubility over 96% at pH 3, 5 and 7 except the WGH produced by

2 and 6 h hydrolyses using Alcalase (data not shown). This result might be attributed to the fact that smaller and more hydrophilic peptides are produced via enzymatic hydrolysis. Kong et al. (2007) also reported that solubility of WGH increased over 60 % by enzymatic hydrolysis using commercial proteases.

*L**, *a** and *b** values of the WGH solution were shown in Table 5. The *L** values of the WGH solution prepared with single enzymatic hydrolysates decreased with hydrolysis time, while the *b** values of the WGH solution increased with hydrolysis time. On the other hand, sequential enzymatic hydrolysis little affected the *L**, *a** and *b** values of the WGH solution. Dong et al. (2008) also reported that the *L** values decreased with hydrolysis time, while the *b** values increased with hydrolysis time in the Silver carp (*Hypophthalmichthys molitrix*) protein hydrolysates produced by Alcalase and Flavourzyme.

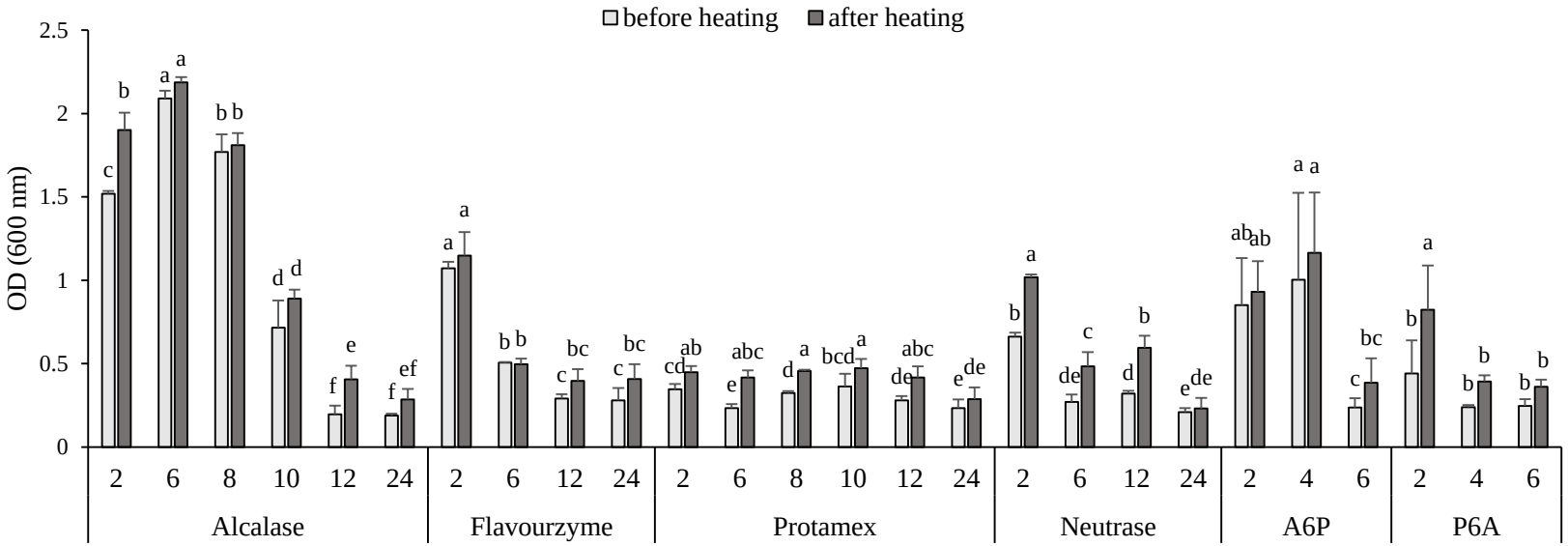

Figure 2. Turbidity and thermal stability of wheat gluten hydrolysates produced by single and sequential enzymatic hydrolyses using Alcalase, Flavourzyme, Protamex and Neutrase.

A6P, wheat gluten hydrolysates produced by Protamex after 6 h hydrolysis with Alcalase; and P6A, wheat gluten hydrolysates produced by Alcalase after 6 h hydrolysis with Protamex. All data represent the means and standard deviations (n=3). Different small letters indicate significant differences among wheat gluten hydrolysates produced by the same enzymes ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

Table 5. *L**, *a** and *b** values of wheat gluten hydrolysates produced by single and sequential enzymatic hydrolyses

	Enzyme	Hydrolysis time (h)	*L**	*a**	*b**
Single	Alcalase	2	80.6±5.26^{bc}	1.05±0.58^{a}	12.4±0.51^{c}
		6	77.6±0.74^{c}	-0.01±0.05^{b}	12.5±0.08^{c}
		8	77.2±0.95^{c}	-0.06±0.08^{b}	13.6±0.22^{b}
		10	85.8±1.05^{a}	-1.15±0.31^{c}	13.8±0.62^{b}
		12	86.8±0.12^{a}	-2.26±0.11^{d}	13.4±0.13^{b}
		24	83.9±0.30^{ab}	-2.64±0.11^{d}	17.5±0.02^{a}
	Flavourzyme	2	81.0±1.39^{c}	0.44±0.12^{a}	11.7±0.09^{c}
		6	85.9±0.38^{a}	-0.70±0.26^{c}	11.9±0.59^{c}
		12	82.9±0.78^{b}	-0.48±0.13^{bc}	14.6±0.31^{b}
		24	80.9±0.44^{c}	-0.33±0.12^{b}	15.7±0.72^{a}
	Protamex	2	92.3±0.06^{a}	-0.80±0.11^{a}	8.37±0.31^{c}
		6	88.0±0.78^{b}	-1.37±0.40^{b}	12.7±1.02^{b}
		8	87.0±0.42^{b}	-1.65±0.04^{bc}	13.4±0.21^{a}
		10	86.0±1.97^{b}	-1.49±0.47^{b}	14.1±0.78^{ab}
		12	86.4±0.74^{b}	-2.11±0.12^{c}	13.1±0.26^{ab}
		24	87.3±2.95^{b}	-1.63±0.27^{bc}	13.9±0.98^{ab}

Table 5. (continued)

	Enzyme	Hydrolysis time (h)	*L**	*a**	*b**
	Neutrase	2	90.9±1.65^{a}	0.05±0.54^{a}	10.0±1.12^{c}
		6	89.0±0.85^{ab}	-1.20±0.22^{b}	13.0±1.23^{b}
		12	85.7±0.78^{b}	-1.42±0.08^{bc}	15.2±1.21^{ab}
		24	87.7±2.95^{ab}	-1.87±0.18^{c}	14.6±0.63^{a}
Sequential	Alcalase→ Protamex	6→2	83.6±2.79	-0.49±0.21^{a}	14.1±0.06^{a}
		6→4	86.3±2.08	-1.23±0.24^{b}	13.9±0.11^{a}
		6→6	87.9±0.09	-2.21±0.05^{c}	13.5±0.23^{b}
	Protamex→ Alcalase	6→2	86.4±1.15	-1.22±0.65^{a}	14.3±1.22
		6→4	87.7±0.26	-2.03±0.07^{b}	13.3±0.11
		6→6	86.6±0.05	-2.17±0.08^{b}	14.2±0.14

All data represent the mean and standard deviation (n=3).

Different small letters in the same columns indicate significant differences among wheat gluten hydrolysates produced by same enzyme ($p<0.05$; one-way ANOVA and Duncan's multiple range test).

6. Sensory characteristics of WGH beverage

Sensory profiles of WGH solutions and chocolate beverages with WGH are shown in Fig. 3. The WGH solutions produced by single and sequential enzymatic hydrolyses for 12 h differed mainly in flavor, taste, bitterness and overall acceptability (Fig. 3A). The WGH produced by 12 h hydrolyses using Alcalase and Protamex had higher overall acceptability. On the other hand, overall acceptability of the WGH produced by Neutrase was the lowest. The WGH produced by the sequential enzymatic hydrolysis had stronger bitterness than by the single enzymatic hydrolysis. Bitterness of the WGH produced by 12 h hydrolysis using Alcalase was the lowest among the tested six hydrolysates. The WGH with lower bitterness tasted better as expected.

The chocolate beverage without the WGH had the highest overall acceptability, taste, appearance and the lowest bitterness (Fig. 3B). Bitterness of the chocolate beverage prepared with the WGH produced by 12 h hydrolyses using Alcalase and Protamex significantly increased depending on the WGH concentration. Chocolate beverage containing 5% (w/w) WGH prepared with the WGH produced by 12 h hydrolysis using Protamex had lower bitterness, better taste and higher overall acceptability than that produced by 12 h hydrolysis using Alcalase. Although there was little difference in MW distribution of the WGH produced by 12 h hydrolyses using Alcalase and Protamex, the differences of peptide sequence and the content of free amino acids in the WGH might contribute to sensory properties. Liu et al. (2012) reported that smaller peptides with larger amounts of hydrophobic amino acids had stronger bitterness in soy protein hydrolysates and high content of free amino acids, especially glutamic acid, had umami taste. Moreover, the bitterness of protein hydrolysates has been concerned for application in food systems (Yang et

al., 2012). Therefore, agents inhibiting bitter taste in WGH need to be studied for developing a better beverage.

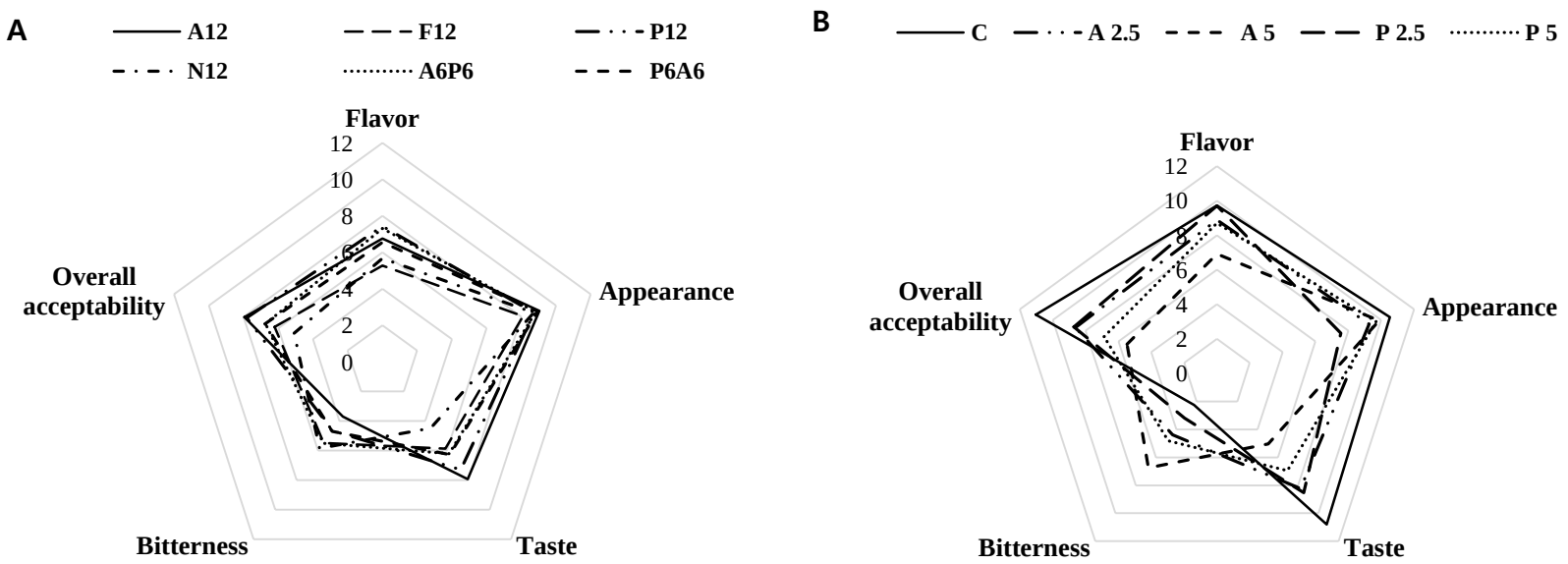

Figure 3. Sensory profile of wheat gluten hydrolysates.

(A) Wheat gluten hydrolysates suspended in water (1%, w/w) and (B) chocolate beverages prepared with wheat gluten hydrolysates (2.5 and 5%, w/w) produced by 12 h hydrolyses with Alcalase and Protamex. Each value represents the mean scored on a 15 cm line scale by 36 panelists. Alcalase, A; Flavourzyme, F; Protamex, P; Neutrase, N; Control, C; numbers after the letters are hydrolysis times (h) and numbers before the letters are concentrations of wheat gluten hydrolysates.

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국문초록

밀 글루텐 효소가수분해물의 이화학적 특성

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단백질 가수분해는 용해도, 거품 형성능, 유화능과 같은 물리적 특성이나 항산화, 항염증, 항균, 항고혈압과 같은 생리활성을 가지는 단백질 가수분해물을 생산하는데 사용되어 왔다. 단백질 효소 가수분해는 우유, 달걀, 돼지, 소, 닭과 같은 동물성 원료와 쌀, 옥수수, 밀, 콩과 같은 식물성 원료를 포함한 다양한 식품을 활용하고 있다. 그 중 밀 글루텐은 밀에서부터 전분을 생산하는 공정 중에 생산되기 때문에 가격이 비교적 저렴하다. 본 연구는 상업적으로 이용되는 단백질 가수분해효소를 이용하여 생산한 밀 글루텐 가수분해물의 화학 특성과 항산화능의 변화를 평가하고 이를 음료의 원료로 활용하기 위하여 수행하였다.

밀 단백질을 단백질 가수분해효소 4 종류(Alcalase, Flavouryzme, Protamex, Nerutrase)를 이용하여 단일(2, 6, 12, 24시간) 또는 순차적 조합 효소 가수분해(8, 10, 12시간)를 수행했다. 밀 글루텐을 증류수에 20%(w/w)로 넣고 효소 대 밀 글루텐의 비를 1:100(w/w)으로 첨가하

여 50℃에서 가수분해했다.

생산된 모든 밀 글루텐 가수분해물의 수율은 50% 이상이었다. 밀 글루텐 가수분해물의 가수분해도는 가수분해 시간에 따라 유의적으로 증가했다($p<0.05$). Flavourzyme을 이용하여 생산한 밀 글루텐 가수분해물의 가수분해도는 가수분해 시간에 상관없이 가장 높았으나, Neutrase를 이용하여 생산한 밀 글루텐 가수분해물의 가수분해도는 가장 낮았다. 순차적 조합 효소 가수분해는 단일 효소 가수분해에 비해서 분자량이 10 kDa 이상인 분획을 현저하게 감소시켰고 500 Da 이하인 분획을 증가시켰다. 밀 글루텐 가수분해물의 유리 아미노산의 함량과 조성은 처리한 단백질 가수분해 효소의 수와 처리한 단백질 가수분해 효소의 순서와 같은 가수분해 조건과 효소 유형에 의해 영향을 받았다. 특히, Flavourzyme을 12시간 처리한 밀 글루텐 가수분해물의 24%는 유리 아미노산이었다. 밀 글루텐 가수분해물의 DPPH 라디칼 소거능은 가수분해 시간에 따라 유의적으로 증가했다($p<0.05$). 반면, 밀 글루텐 가수분해물의 ABTS 라디칼 소거능은 가수분해 처리 조건과 효소 유형에 큰 영향을 받지 않았다. Protamex를 이용하여 생산한 밀 글루텐 가수분해물을 원료로 사용하여 제조한 음료가 탁도가 비교적 낮고, 열 안정성이 우수하고, 용해도가 높아 이를 음료 개발에 활용하기에 적합하다고 판단했다.

주요어: 밀 글루텐; 단백질 가수분해물; 효소 가수분해; 음료

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