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Research Article

Effects of enzyme combinations on physicochemical characteristics and antioxidant activities of domestic soybean protein hydrolysates

Hae Jin Kim[1†], Eun Ji Lee[1†], Do Wan Kim[2*], Kyung Young Yoon[1*]

[1]*Department of Food and Nutrition, Yeungnam University, Gyeongsan 38541, Korea*
[2]*Department of Food and Pharmaceuticals, Jungwon University, Goesan 28024, Korea*

Abstract This study evaluated the effects of different enzyme combinations on protein hydrolysis, solubility, functional properties, and antioxidant activity of protein hydrolysates prepared from domestically cultivated soybean (*Glycine max*) varieties, including *Daewon*, *Seoritae*, and *Seomoktae*. Soybean proteins were hydrolyzed using two enzyme systems: Protamex-Flavourzyme (A) and Alcalase-Flavourzyme (B). Enzymatic hydrolysis increased soluble protein content, nitrogen solubility, and degree of hydrolysis, with higher values observed for enzyme B treatment. In contrast, hydrolyzed samples showed reduced water- and oil-holding capacities (3.12-3.52 g/g and 1.84-1.95 g/g, respectively), foaming ability (2.00-3.00%), emulsifying activity (43.79-65.22%), apparent turbidity, and viscosity (0.81-1.31 cP) compared with non-hydrolyzed samples, indicating improved dispersibility and suitability for liquid food applications. Furthermore, enzymatic treatment enhanced total polyphenol content and DPPH radical scavenging activity, with the highest values observed in enzyme B-treated *Seomoktae* (124.29 mg GAE/100 g) and enzyme A-treated *Daewon* (92.69%), respectively. These results demonstrate that enzyme selection plays a critical role in modulating the functional and antioxidant properties of soybean protein hydrolysates. The findings suggest that hydrolyzed soybean proteins may serve as promising functional ingredients for liquid food systems, while further studies are needed to evaluate their applicability in specialized nutrition products and plant-based protein formulations.

Keywords soybean protein hydrolysate, enzymatic hydrolysis, physicochemical properties, antioxidant activity, Korean soybean cultivars

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†These authors contributed equally to this study.
*These authors contributed equally to this study.

***Corresponding author**
Do Wan Kim
Tel: +82-43-830-8612
E-mail: dwkim1126@juw.ac.kr

Kyung Young Yoon
Tel: +82-53-810-2878
E-mail: Yoonky2441@ynu.ac.kr

1. Introduction

Soybean (*Glycine max*) is an important plant-based food resource with a high protein content of approximately 35-40%, making it an important dietary protein source, particularly in cereal-based diets where protein intake is often insufficient. Soybeans also contain approximately 20% lipids, of which more than 80% are unsaturated fatty acids, which contribute to reducing cholesterol level and preventing cardiovascular diseases (Kim, 2018). In addition, soybeans contain a large amount of bioactive compounds such as polyphenol, isoflavones, and saponins, which have been reported to exhibit antioxidant, anti-inflammatory, and metabolic regulatory effects, thereby increasing their value as functional food ingredients (Messina et al., 2022).

Despite these nutritional advantages, the utilization of soybean protein is limited by several factors. Native soybean proteins possess compact tertiary structures and high molecular weights, which reduce their digestibility and absorption efficiency. Furthermore, antinutritional factors such as trypsin inhibitors in soybeans can further impair protein utilization (Gilani et al., 2019; Rackis,

1981). In addition, inadequate processing conditions may induce undesirable beany flavors, negatively affecting sensory quality and consumer acceptance (Yang et al., 2023). Consequently, various processing strategies have been explored to overcome these limitations, among which enzymatic hydrolysis has gained considerable attention.

Enzymatic hydrolysis converts large molecules into smaller ones, i.e. protein into peptides and free amino acids, thereby improving protein digestibility and bioavailability. This structural modification also enhances protein solubility and processing functionality, while potentially reducing allergenicity through the degradation of allergenic epitopes (Tavano, 2013). Importantly, the type of protease and its substrate specificity strongly influence peptide size distribution and amino acid composition, which in turn affect the physicochemical characteristics, functional properties, and bioactivities of protein hydrolysates (Ashaolu, 2020). Soybean protein hydrolysates have been widely applied in protein supplements and liquid protein beverages due to their superior solubility, dispersibility, and ease of digestion. In addition, their reported bioactivities, such as antioxidant and antihypertensive effects, have promoted their use as functional food ingredients (Park et al., 2010). Owing to their low viscosity and reduced allergenic potential, soybean protein hydrolysates are also suitable for specialized nutritional products, including infant formulas, medical foods, and elderly-friendly care foods that require minimal swallowing effort. Previous studies have demonstrated that different proteolytic enzymes exert distinct effects on the functional properties and antioxidant activities of soybean protein hydrolysates (Li et al., 2025; Song et al., 2021).

Meanwhile, Korea relies heavily on imported soybeans to meet domestic demand, and recent global supply chain instability has highlighted the need to increase the utilization of domestically produced agricultural resources (Park et al., 2025). Korean soybean cultivars exhibit considerable variation in protein content, amino acid composition, and functional components, which can significantly influence processing characteristics and final product quality (Kim et al., 2022; Song et al., 2021). Nevertheless, systematic studies evaluating the functional properties and antioxidant activity of protein hydrolysates derived from Korean soybean cultivars, particularly with respect to enzyme-specific effects, remain limited.

Therefore, the purpose of this study was to enhance the food industry applicability of Korean soybeans by producing soybean protein hydrolysates using different enzyme systems and comparing their protein hydrolysis, solubility, functional properties, and antioxidant activities. Through this approach, the present study aims to provide scientific evidence supporting the value-added utilization of domestic soybean cultivars as functional ingredients for protein supplements and functional food applications.

2. Materials and methods

2.1. Materials

Soybean cultivars used in this study-*Daewon*, *Seoritae*, and *Seomoktae*-were cultivated in Goesan, Chungbuk Province, Korea, and purchased from an agricultural corporation (Goesan Japgok, Goesan, Chungbuk, Korea). The soybeans were washed and steamed at 121℃ for 20 min using a high-pressure steamer (DY-AS2, Daeyoung, Gunpo, Korea). The steamed samples were subsequently dried at 60℃ for 10 h in a hot-air dryer (DY-HD1, Daeyoung), ground using a food mixer (FM-681C, Hanil, Incheon, Korea), and passed through a 100-mesh sieve. The resulting soybean powder was stored in a deep freezer at -40℃ until further use.

2.2. Preparation of soybean protein hydrolysates

For the preparation of soybean protein hydrolysates, Alcalase 2.4 L, Flavourzyme 500 MG, and Protamex 1.5 MG (Novozymes, Bagsværd, Denmark) were used. Two enzyme mixtures were selected based on previously reported studies on soybean protein hydrolysis under similar reaction conditions (Anh et al., 2020; Islam et al., 2022; Yang et al., 2023). Enzyme A consisted of Protamex and Flavourzyme mixed at a ratio of 2:1, whereas enzyme B consisted of Alcalase and Flavourzyme mixed at a ratio of 1:1. Each enzyme mixture was dissolved in 0.1 M sodium phosphate buffer (pH 7.0) and used as the enzyme solution.

Briefly, 100 g of soybean powder was dispersed in 900 mL of distilled water (DW), and the enzyme solution was added at 2% (v/v) of the sample volume. The mixture was hydrolyzed in a shaking water bath (BS-11, JeioTech, Seoul, Korea) at 50℃ for 3 h with constant shaking at 100 rpm. These hydrolysis conditions were selected based on previous optimization studies reporting effective soybean protein hydrolysis at approximately pH 7.0, 50-55℃, and 2-3 h reaction time (Anh et al., 2020; Hoa and Dao, 2017). After

enzymatic hydrolysis, the reaction was terminated by heating at 98℃ for 10 min to inactivate the enzymes. The resulting hydrolysates were then dried at 55℃ for 24 h, ground into powder, and used for subsequent analyses.

For the non-treated control, soybean powder was processed under the same conditions, including dispersion, incubation at 50℃ for 3 h, heat treatment, drying, and grinding, but without enzyme addition. Therefore, the reported DH values represent the contribution of enzymatic hydrolysis, while the independent effect of steaming on protein structural changes was not separately evaluated.

2.3. Measurement of protein hydrolysis and solubility

2.3.1. Soluble protein content

Soluble protein content was determined according to the Lowry method (Lowry et al., 1951). Briefly, sample solution (0.5 mL) was mixed with 2.5 mL of Lowry reagent and allowed to react at room temperature for 10 min. Subsequently, 2.5 mL of Folin-Ciocalteu reagent diluted with distilled water at a ratio of 1:1 (v/v) was added, and the mixture was placed at room temperature for 30 min. Absorbance was measured at 750 nm using a spectrophotometer. Protein concentration was calculated from a standard curve prepared using bovine serum albumin (BSA; Sigma-Aldrich Co., St. Louis, MO, USA).

2.3.2. Nitrogen solubility

Nitrogen solubility (NS) was measured according to the method described by Kim et al. (2013). Briefly, 1 g of sample was dispersed in 100 mL of DW and stirred for 1 h at room temperature. The dispersion was then centrifuged at 3,000 rpm for 20 min at 4℃, and the supernatant was collected. The nitrogen content of the supernatant was measured using the Lowry method (Lowry et al., 1951). Total nitrogen content was determined by the Kjeldahl method using a micro-Kjeldahl apparatus (BUCHI Distillation Unit K-350). The NS was calculated using the following equation:

$$\text{Nitrogen solubility} = \frac{\text{Water} - \text{soluble nitrogen}}{\text{Total nitrogen}} \times 100$$

2.3.3. Degree of hydrolysis

The degree of hydrolysis (DH) was measured using a method of Park and Yoon (2018). The sample solution was mixed with an equal volume of 20% (w/v) trichloroacetic acid (TCA) and centrifuged at 1,600 rpm for 30 min. An aliquot of the supernatant was collected and quantified for 10% TCA-soluble protein content using the Lowry method (Lowry et al., 1951). Total protein content was measured by the Kjeldahl method using a micro-Kjeldahl apparatus (BUCHI Distillation Unit K-350, Flawil, Switzerland). The DH was calculated on a protein basis as the percentage of TCA-soluble protein relative to the total protein content using the following equation:

$$\text{Degree of hydrolysis (\%)} = \frac{\text{10\% TCA} - \text{soluble protein}}{\text{Total protein}} \times 100$$

The DH of untreated samples was set to 0.00% as a baseline for comparison, representing the extent of hydrolysis induced by enzymatic treatment (Adler-Nissen, 1986).

2.4. Measurement of functional properties

2.4.1. Water-holding capacity

Water-holding capacity (WHC) was determined using a modified method of Park and Yoon (2019). Briefly, 1 g of sample was mixed with 10 mL of DW and vortexed thoroughly, followed by incubation at room temperature for 1 h. The mixture was then centrifuged at 1,600 ×*g* for 30 min at 25℃. After removing the supernatant, the weight of the remaining precipitate was measured. WHC was calculated using the following equation:

$$\text{WHC (g/g)} = \frac{\text{Weight of sample after removing supernatant}}{\text{Weight of dry sample}}$$

2.4.2. Oil-holding capacity

Oil-holding capacity (OHC) was measured according to a modified method of Nguyen et al. (2015). Briefly, 1 g of sample was mixed with 20 mL of soybean oil and vortexed, followed by incubation at room temperature for 1 h. The mixture was centrifuged at 1,600 ×*g* for 30 min, after which the supernatant was discarded. The tube was then tilted at a 45° angle and allowed to stand for an additional 10 min to remove residual free oil. The weight of the remaining

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precipitate was measured, and OHC was calculated using the following equation:

$$\text{OHC (g/g)} = \frac{\text{Weight of oil} - \text{bound sample}}{\text{Weight of dry sample}}$$

2.4.3. Foaming ability and foam stability

Foaming ability (FA) and foam stability (FS) were determined using a modified method of Park and Yoon (2019). Briefly, 2 g of sample was dispersed in 100 mL of DW and homogenized using a homogenizer (AM-1, Nissei, Japan) at 8,000 rpm for 1 min to generate foam. The total volume of the mixture after homogenization was immediately recorded, and FA was calculated using the following equation:

$$\text{Foaming ability (\%)} = \frac{\text{Volume after whipping} - \text{Initial volume}}{\text{Initial volume}} \times 100$$

The FS was evaluated by measuring the remaining foam volume after standing at room temperature for predetermined time intervals (e.g., 10, 30, and 60 min). FS was expressed as the percentage of foam volume retained relative to the initial foam volume using the following equation:

$$\text{Foaming stability (\%)} = \frac{\text{Foam volume at time t}}{\text{Initial foam volume}} \times 100$$

2.4.4. Emulsifying activity and emulsion stability

Emulsifying activity (EA) and emulsion stability (ES) were determined using a method of Park and Yoon (2019). Briefly, 100 mL of sample solution prepared at a concentration of 3% (w/v) was mixed with 100 mL of soybean oil and homogenized at 8,000 rpm for 5 min to form an emulsion. The resulting emulsion was centrifuged at 1,600 ×*g* for 10 min, and EA was calculated based on the height of the emulsified layer relative to the total height of the mixture using the following equation:

$$\text{Emulsifying activity (\%)} = \frac{\text{Height of emulsified layer}}{\text{Total height of mixture}} \times 100$$

The ES was evaluated using the same procedure as described for EA to prepare the emulsion. The freshly prepared emulsion was then heated in a water bath at 80℃ for 30 min, followed by cooling to 25℃. After heat treatment, the emulsion was centrifuged at 1,000 rpm for 10 min, and ES was calculated using the following equation:

$$\text{Emulsion stability (\%)} = \frac{\text{Height of emulsified layer after heating}}{\text{Total height of mixture}} \times 100$$

2.4.5. Turbidity

Turbidity was measured according to the method of Chamba et al. (2014). The sample was dispersed in DW to prepare solutions with various concentrations of 10, 20, 30, 40, and 50 mg/mL. The dispersions were stirred at room temperature for 1 h to ensure uniform suspension. Apparent absorbance at 600 nm (A_{600}) was then measured and used as a relative indicator of turbidity and dispersion behavior rather than as an absolute quantitative measure, due to potential light scattering effects at high sample concentrations.

2.4.6. Viscosity

The viscosity of sample solution was measured using a viscometer (DV2T, Brookfield, Middleboro, MA, USA) according to the method described by Chel-Guerrero et al. (2002). Briefly, 1 g of sample was mixed with 10 mL of DW and stirred thoroughly. The pH of the dispersion was adjusted to 7.0 using 1 N NaOH or 1 N HCl. An aliquot (1 mL) of the dispersion was transferred to the sample container, and viscosity was measured using a CP-42 spindle at a rotational speed of 100 rpm for 1 min. The viscometer was calibrated using a viscosity standard (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) prior to measurement.

2.5. Determination of antioxidant activity

2.5.1. Preparation of extracts

For the determination of total phenolic content (TPC) and DPPH radical scavenging activity, extracts were prepared by mixing samples with 80% ethanol at a ratio of 1:10 (w/v). The mixture was stirred at room temperature for 3 h and then centrifuged at 3,200 ×*g* for 10 min at 4℃. The supernatant was collected and used for antioxidant activity analyses.

2.5.2. TPC

The TPC of extracts from different soybean samples was

measured using the Folin-Ciocalteu method (Folin and Ciocalteu, 1927). Briefly, 0.2 mL of ethanol extract solution was mixed with 0.2 mL of Folin-Ciocalteu phenol reagent (Junsei, Tokyo, Japan) and allowed to react at room temperature for 3 min. Subsequently, 0.4 mL of 10% Na_2CO_3 solution (OCI, Seoul, Korea) and 4 mL of DW were added, and the mixture was allowed to react for 1 h at room temperature in the dark. The absorbance was measured at 725 nm. TPC was calculated from a standard curve prepared using gallic acid (Sigma-Aldrich Co.) and expressed as mg gallic acid equivalents (GAE) per 100 grams.

2.5.3. DPPH radical scavenging activity

DPPH radical scavenging activity of the ethanol extracts was evaluated using a slightly modified method of Lee et al. (2010). A total of 200 μL of 0.2 mM DPPH solution was mixed with 100 μL of sample extract in a 96-well microplate. The reaction mixture was incubated at room temperature in the dark for 30 min, and absorbance was measured at 517 nm. DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A - B}{C}\right) \times 100$$

where A is the absorbance of the reaction mixture containing the test sample and DPPH solution, B is the absorbance of the blank containing distilled water instead of DPPH solution, and C is the absorbance of the control containing distilled water instead of the test sample.

2.6. Statistical analysis

All experiments were conducted in triplicate, and the results are expressed as mean values ± standard deviation. Statistical analyses for all data were performed using IBM SPSS Statistics software (version 23.0, IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was applied to determine significant differences among samples at a significance probability of $p < 0.05$. Differences between mean values were evaluated using Duncan's multiple range test.

3. Results and discussion

3.1. Soluble protein content, NS, and DH

Table 1 presents the soluble protein content, NS, and DH of soybean hydrolysates prepared using different enzyme treatments. Overall, enzyme treatment markedly increased soluble protein content and NS, accompanied by a higher DH, compared with the untreated samples.

In *Daewon* soybean, the soluble protein content increased from 22.60 mg/g in the untreated sample to 31.60 mg/g and 31.12 mg/g following enzyme A and enzyme B treatments, respectively, corresponding to an increase of approximately

Table 1. Soluble protein content, nitrogen solubility, and degree of hydrolysis of soybean protein hydrolysates prepared using different enzyme combinations

Cultivar	Enzyme treatment[1)]	Soluble protein content (mg/g)	Nitrogen solubility (%)	Degree of hydrolysis (%)
Daewon	Untreated	22.60 ± 0.35[2)b3)]	9.90 ± 0.35[c]	0.00 ± 0.00[c]
	Enzyme A	31.60 ± 0.96[a]	13.54 ± 0.43[b]	37.03 ± 0.53[b]
	Enzyme B	31.12 ± 0.98[a]	15.07 ± 0.07[a]	40.57 ± 1.01[a]
Seoritae	Untreated	24.00 ± 0.15[c]	18.14 ± 0.42[b]	0.00 ± 0.00[c]
	Enzyme A	31.79 ± 0.87[b]	18.80 ± 0.51[b]	31.13 ± 0.55[b]
	Enzyme B	33.82 ± 0.65[a]	21.34 ± 0.49[a]	33.45 ± 0.90[a]
Seomoktae	Untreated	33.55 ± 1.02[c]	17.48 ± 0.44[c]	0.00 ± 0.00[b]
	Enzyme A	36.04 ± 0.88[b]	18.36 ± 0.28[b]	27.16 ± 0.11[a]
	Enzyme B	38.78 ± 0.70[a]	22.60 ± 0.37[a]	26.74 ± 0.29[a]

[1)]Enzyme A consisted of Protamex and Flavourzyme (2:1), and enzyme B consisted of Alcalase and Flavourzyme (1:1).
[2)]All values are mean ± SD (n = 3).
[3)]Values with different letters in the same soybean cultivar are significantly different at $p < 0.05$ by Duncan's multiple range test.

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38-40%. In *Seoritae* soybean, soluble protein content increased from 24.00 mg/g to 31.79 mg/g (enzyme A) and 33.82 mg/g (enzyme B), representing an increase of approximately 32-41%, with enzyme B showing the highest value. For *Seomoktae* soybean, soluble protein content increased from 33.55 mg/g in the untreated sample to 36.04 mg/g and 38.78 mg/g following enzyme A and enzyme B treatments, respectively, corresponding to an increase of approximately 7-16%. Overall, enzymatic hydrolysis significantly enhanced soluble protein content across all soybean varieties, with enzyme B treatment producing the greatest increase.

Alcalase is known to exhibit strong endopeptidase activity, efficiently cleaving internal peptide bonds, whereas Flavourzyme possesses exopeptidase activity that further hydrolyzes peptide termini. The combination used in enzyme B is therefore considered to promote a greater reduction in molecular weight and increased exposure of hydrophilic amino acid residues, resulting in improved protein solubility (Kristinsson and Rasco, 2000). These findings are consistent with the results reported by Tavano (2013), who demonstrated that an Alcalase-Flavourzyme enzyme system effectively enhances the solubility of plant proteins.

In addition, varietal differences in soluble protein content are likely attributable to differences in soybean storage protein composition, particularly the relative proportions of glycinin and β-conglycinin, as well as inherent structural characteristics of the proteins (Damodaran, 1996). Taken together, the results indicate that enzymatic treatment, especially the combined use of Alcalase and Flavourzyme, is an effective strategy for improving the solubility of soybean proteins, suggesting its potential application in the development of plant-based protein beverages and functional food ingredients.

The NS values ranged from 9.90 to 15.07% for *Daewon* soybean, 18.14 to 21.34% for *Seoritae* soybean, and 17.48 to 22.60% for *Seomoktae* soybean. In *Daewon* soybean, NS increased from 9.90% in the untreated sample to 15.07% following enzymatic treatment, corresponding to an increase of approximately 52%. Similarly, *Seoritae* soybean exhibited an increase from 18.14% to 21.34% (approximately 18% increase), while *Seomoktae* soybean showed an increase from 17.48% to 22.60% (approximately 29% increase). Overall, enzymatic treatment significantly enhanced NS in all samples, with enzyme B consistently showing higher NS values than enzyme A, indicating more effective protein solubilization and nitrogen release.

Although NS values of legumes are generally reported to be approximately 90% (Sul et al., 1998), all samples in the present study exhibited relatively low NS values (< 25%). This discrepancy is likely attributable to heat-induced protein denaturation during the soybean steaming process. Previous studies have shown that prolonged steaming markedly reduces NS, decreasing from approximately 83% to around 20% within 20 min (Anderson, 1992), and to approximately 28% after 30 min of heat treatment (Rackis, 1981). Such reductions are associated with protein denaturation, which exposes hydrophobic groups and promotes protein aggregation through hydrophobic interactions and disulfide (S-S) bond formation (Kim, 2014). Despite the overall low NS values, enzymatic treatment significantly increased NS, suggesting that enzymatic hydrolysis partially disrupted heat-induced protein aggregates and generated soluble peptides and free amino acids, thereby improving NS.

The DH values of untreated samples were set to 0.00% as a baseline for comparison. It should be noted that the DH values reported in this study represent the enzymatic contribution to protein hydrolysis relative to the non-treated samples. Although steaming may induce protein denaturation or structural modification, its independent effect on baseline hydrolysis-related changes was not separately quantified; therefore, this should be considered a limitation of the present study.

Following enzymatic treatment, DH increased markedly in all soybean cultivars. In *Daewon* soybean, DH increased to 37.03% with enzyme A and 40.57% with enzyme B, with enzyme B exhibiting a significantly higher DH. Similarly, *Seoritae* soybean showed higher DH values with enzyme B (33.45%) compared to enzyme A (31.13%). In contrast, *Seomoktae* soybean exhibited DH values of 27.16% and 26.74% with enzyme A and enzyme B, respectively, showing no significant difference between enzyme treatments. These results are consistent with previous findings reporting that Alcalase produces a higher DH than Protamex in lentil protein hydrolysates (García-Mora et al., 2014). Although both Alcalase and Protamex are classified as endopeptidases, they differ in substrate specificity and accessibility to internal peptide bonds. In particular, Alcalase is known to possess broad substrate specificity, enabling more efficient cleavage of internal peptide bonds and thereby promoting protein hydrolysis (Arteaga et al., 2020). The absence of significant

differences in DH between enzyme treatments in *Seomoktae* soybean may be attributed to cultivar-specific protein composition and protein-protein interactions that limit enzyme accessibility. In general, a higher DH indicates that proteins are effectively converted into low-molecular-weight peptides and free amino acids, which may enhance digestibility, absorption, and solubility. However, excessive hydrolysis can negatively affect emulsifying properties and sensory quality. Therefore, appropriate control of the DH is essential to optimize the functional properties of protein-based food ingredients (Adler-Nissen, 1986; Kristinsson and Rasco, 2000).

3.2. WHC and OHC

The WHC and OHC of soybean hydrolysates are presented in Table 2. For *Daewon* soybean, the WHC of the untreated sample was 4.06 g/g, which was significantly higher than those of enzyme A (3.20 g/g) and enzyme B (3.12 g/g), with no significant difference between the two enzyme treatments. Similarly, *Seoritae* soybean exhibited the highest WHC in the untreated sample (4.30 g/g), while enzyme A (3.52 g/g) and enzyme B (3.47 g/g) showed significantly lower values without a significant difference between enzyme types. In *Seomoktae* soybean, the untreated sample also showed the highest WHC (4.32 g/g), followed by enzyme B (3.52 g/g) and enzyme A (3.44 g/g).

Table 2. Water-holding and oil-holding capacities of soybean protein hydrolysates prepared using different enzyme combinations

Cultivar	Enzyme treatment[1)]	Water-holding capacity (g/g)	Oil-holding capacity (g/g)
Daewon	Untreated	4.06 ± 0.04[2)a3)]	2.30 ± 0.02[a]
	Enzyme A	3.20 ± 0.05[b]	1.95 ± 0.01[b]
	Enzyme B	3.12 ± 0.07[b]	1.94 ± 0.00[b]
Seoritae	Untreated	4.30 ± 0.08[a]	2.29 ± 0.03[a]
	Enzyme A	3.52 ± 0.01[b]	1.88 ± 0.00[b]
	Enzyme B	3.47 ± 0.05[b]	1.84 ± 0.02[b]
Seomoktae	Untreated	4.32 ± 0.03[a]	2.20 ± 0.05[a]
	Enzyme A	3.44 ± 0.01[c]	1.87 ± 0.02[b]
	Enzyme B	3.52 ± 0.03[b]	1.85 ± 0.03[b]

[1)]Enzyme A consisted of Protamex and Flavourzyme (2:1), and enzyme B consisted of Alcalase and Flavourzyme (1:1).
[2)]All values are mean ± SD (n = 3).
[3)]Values with different letters in the same soybean cultivar are significantly different at $p < 0.05$ by Duncan's multiple range test.

Overall, untreated samples exhibited significantly higher WHC than enzymatically treated samples across all soybean varieties. This trend is consistent with previous reports showing a reduction in WHC following enzymatic hydrolysis of quinoa flour (Srinivasu and Eligar, 2024). The decrease in WHC after enzymatic treatment is likely due to the breakdown of protein macromolecules into low-molecular-weight peptides, which reduces the three-dimensional structural spaces available for water binding. Nevertheless, the WHC values of soybean hydrolysates obtained in this study were relatively higher than those reported for yellow soybean and black soybean proteins (3.14 g/g and 2.90 g/g, respectively), indicating comparatively favorable water-binding properties (Dzudie and Hardy, 1996; Gupta et al., 2018).

The OHC showed a similar trend, with untreated samples exhibiting the highest values in all soybean varieties. The OHC of *Daewon* soybean was highest in the untreated sample (2.30 g/g) and significantly decreased following enzyme A (1.95 g/g) and enzyme B (1.94 g/g) treatments. In *Seoritae* soybean, OHC values decreased from 2.29 g/g in the untreated sample to 1.88 g/g and 1.84 g/g following enzyme A and enzyme B treatments, respectively. *Seomoktae* soybean showed a comparable pattern, with the untreated sample exhibiting the highest OHC (2.20 g/g), followed by enzyme A (1.87 g/g) and enzyme B (1.85 g/g). The reduction in OHC following enzymatic hydrolysis is attributed to structural degradation of proteins and rearrangement of hydrophobic regions, which diminishes the ability of proteins to interact with and retain oil (Agrawal et al., 2017). These results are consistent with previous results indicating that protein hydrolysates generally exhibit lower OHC than native proteins (Adler-Nissen, 1986; Kristinsson and Rasco, 2000).

Although soybean hydrolysates exhibited lower WHC and OHC compared to untreated samples, this reduction suggests a moderation of excessive water and oil binding, which can improve dispersion and processing stability (Kristinsson and Rasco, 2000). Such properties are advantageous for preventing excessive viscosity development and lipid entrapment, thereby enhancing the applicability of soybean hydrolysates as ingredients in liquid foods and functional food formulations (Wouters et al., 2016). Consequently, the soybean hydrolysates produced in this study exhibit more balanced water- and oil-holding characteristics than native soybean proteins, supporting their potential use as ingredients in liquid foods, functional products, and high-protein food applications.

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3.3. FA and FS

The FA and FS of hydrolysates from *Daewon*, *Seoritae*, and *Seomoktae* soybeans subjected to enzymatic treatment are summarized in Table 3. The FA decreased markedly following enzymatic treatment in all soybean varieties. Specifically, the FA of *Daewon* soybean decreased from 5.00% in the untreated sample to 2.00% and 2.47% after enzyme A and enzyme B treatments, respectively. Similarly, *Seoritae* soybean exhibited the highest FA in the untreated sample (6.00%), whereas substantially lower values were observed following enzyme A (2.98%) and enzyme B (3.00%) treatments. In *Seomoktae* soybean, FA was highest in the untreated sample (6.25%) and decreased to 3.00% after both enzyme A and enzyme B treatments.

The FS decreased over time in all samples. For *Daewon* soybean, the foam formed in the untreated sample remained stable for up to 10 min but gradually decreased thereafter, with complete foam collapse observed at 60 min. In contrast, the enzyme A-treated sample showed a reduction in FS to 82.22% at 10 min, followed by partial retention of foam (41.11%) at both 30 and 60 min. The enzyme B-treated sample exhibited a more rapid decline, with FS decreasing to 50% at 10 min, remaining until 30 min, and disappearing completely by 60 min.

In *Seoritae* soybean, the untreated sample retained 28.97% of the initial foam volume after 60 min, whereas FS in enzyme-treated samples decreased sharply, resulting in complete foam collapse at 60 min. A similar trend was observed for *Seomoktae* soybean, where the untreated sample maintained 23.17% of foam after 60 min, while enzymatic treatment led to a rapid loss of FS. Notably, in the enzyme B-treated *Seomoktae* sample, complete foam collapse occurred within 30 min. Overall, except for *Daewon* soybean, enzymatic treatment resulted in a pronounced reduction in foam stability in *Seoritae* and *Seomoktae* soybeans.

These findings are consistent with the results of Severin and Xia (2006), who reported that increasing degrees of hydrolysis reduced both FA and FS in whey proteins treated with Alcalase and Protamex. Effective foam formation and stability require proteins to rapidly adsorb at the air-water interface and form a cohesive, elastic interfacial film. However, peptides generated by enzymatic hydrolysis are less capable of forming continuous and mechanically stable interfacial films, thereby impairing foam formation and stabilization (Van der Ven et al., 2002). Therefore, the observed reductions in FA and FS of soybean hydrolysates in this study are attributed to enzyme-induced protein structural modifications and molecular weight reduction.

3.4. EA and ES

The EA and ES of hydrolysates from *Daewon*, *Seoritae*, and *Seomoktae* soybeans subjected to enzymatic treatment are presented in Table 4. For *Daewon* soybean, EA was significantly highest in the untreated sample (67.56%) and

Table 3. Foaming ability and foam stability of soybean protein hydrolysates prepared using different enzyme combinations

Cultivar	Enzyme treatment[1)]	Foaming ability (%)	Foam stability (%)		
			10 min	30 min	60 min
Daewon	Untreated	5.00 ± 0.82[2)a3)]	100.00 ± 0.00[a]	82.22 ± 13.70[a]	0.00 ± 0.00[b]
	Enzyme A	2.00 ± 0.00[b]	82.22 ± 13.70[a]	41.11 ± 6.85[b]	41.11 ± 6.85[a]
	Enzyme B	2.47 ± 0.02[b]	50.00 ± 0.00[b]	50.00 ± 0.00[b]	0.00 ± 0.00[b]
Seoritae	Untreated	6.00 ± 1.63[a]	86.90 ± 10.24[a]	57.94 ± 6.83[a]	28.97 ± 3.41[a]
	Enzyme A	2.98 ± 0.82[b]	82.22 ± 13.70[a]	41.11 ± 6.85[a]	0.00 ± 0.00[b]
	Enzyme B	3.00 ± 0.82[b]	100.00 ± 0.00[a]	25 ± 20.41[a]	0.00 ± 0.00[b]
Seomoktae	Untreated	6.25 ± 1.02[a]	86.90 ± 10.24[a]	57.94 ± 6.83[a]	23.17 ± 2.73[a]
	Enzyme A	3.00 ± 0.82[b]	100.00 ± 0.00[a]	25.00 ± 0.00[b]	0.00 ± 0.00[b]
	Enzyme B	3.00 ± 0.82[b]	72.22 ± 20.79[a]	0.00 ± 0.00[c]	0.00 ± 0.00[b]

[1)]Enzyme A consisted of Protamex and Flavourzyme (2:1), and enzyme B consisted of Alcalase and Flavourzyme (1:1).
[2)]All values are mean ± SD (n = 3).
[3)]Values with different letters in the same soybean cultivar are significantly different at $p < 0.05$ by Duncan's multiple range test.

Table 4. Emulsifying activity and emulsion stability of soybean protein hydrolysates prepared using different enzyme combinations

Cultivar	Enzyme treatment[1)]	Emulsifying activity (%)	Emulsion stability (%)
Daewon	Untreated	67.56 ± 0.43[2)a3)]	61.87 ± 1.71[a]
	Enzyme A	50.28 ± 0.68[b]	63.21 ± 0.59[a]
	Enzyme B	43.79 ± 2.40[c]	54.59 ± 1.77[b]
Seoritae	Untreated	65.92 ± 2.10[ns4)]	67.42 ± 3.67[a]
	Enzyme A	65.22 ± 5.83	66.67 ± 0.60[a]
	Enzyme B	62.78 ± 6.80	59.48 ± 1.67[b]
Seomoktae	Untreated	65.75 ± 0.75[a]	69.21 ± 2.99[ns]
	Enzyme A	62.63 ± 1.23[b]	69.96 ± 4.21
	Enzyme B	64.25 ± 0.16[ab]	65.39 ± 1.04

[1)]Enzyme A consisted of Protamex and Flavourzyme (2:1), and enzyme B consisted of Alcalase and Flavourzyme (1:1).
[2)]All values are mean ± SD (n = 3).
[3)]Values with different letters in the same soybean cultivar are significantly different at $p < 0.05$ by Duncan's multiple range test.
[4)]ns, not significant.

decreased following enzyme A (50.28%) and enzyme B (43.79%) treatments. In contrast, *Seoritae* soybean showed no significant differences in EA among the untreated (65.92%), enzyme A-treated (65.22%), and enzyme B-treated (62.78%) samples. Similarly, *Seomoktae* soybean exhibited the highest EA in the untreated sample (65.75%), with a slight decrease observed after enzyme B (64.25%) and enzyme A (62.63%) treatments.

Regarding ES, *Daewon* soybean showed comparable values in the untreated (61.87%) and enzyme A-treated (63.21%) samples, whereas a significantly lower value was observed in the enzyme B-treated sample (54.59%). In *Seoritae* soybean, the untreated (67.42%) and enzyme A-treated (66.67%) samples exhibited the highest ES, while enzyme B treatment resulted in a reduced stability of 59.48%. For *Seomoktae* soybean, ES did not differ significantly among the untreated (69.21%), enzyme A-treated (69.96%), and enzyme B-treated (65.39%) samples.

Overall, an increasing DH was associated with a tendency toward reduced EA and ES across all soybean varieties. This trend is consistent with previous reports showing a decline in emulsifying properties of lentil protein hydrolysates following enzymatic hydrolysis (Rezvankhah et al., 2021). The observed reduction in emulsifying performance is attributed to an increased proportion of hydrophilic peptides generated during hydrolysis, which alters the hydrophile-lipophile balance of proteins and consequently diminishes the interfacial activity required for stable emulsion formation and maintenance (Fathollahy et al., 2021).

3.5. Turbidity

The apparent absorbance at 600 nm (A_{600}) of protein hydrolysates from *Daewon*, *Seoritae*, and *Seomoktae* soybeans under different enzymatic treatments are presented in Table 5. Within the tested concentration range of 10-50 mg/mL, A_{600} increased with increasing concentration in all samples. In *Daewon* soybean, A_{600} values ranged from 2.41 to 3.00 in the untreated sample, 2.25 to 3.05 following enzyme A treatment, and 2.15 to 3.04 following enzyme B treatment. For *Seoritae* soybean, A_{600} values ranged from 2.27 to 3.49 (untreated), 2.38 to 3.54 (enzyme A), and 2.39 to 3.54 (enzyme B). Similarly, *Seomoktae* soybean exhibited A600 values of 2.49-3.63, 2.43-3.50, and 2.32-3.55 for the untreated, enzyme A-treated, and enzyme B-treated samples, respectively.

The A_{600} values observed in this study were markedly higher than those typically reported for soybean protein isolate (SPI) at lower concentrations ($A_{600} < 0.5$ at approximately 0.5-1.0%, w/v) (Kinsella, 1979). This discrepancy is likely attributable to the relatively high sample concentrations employed, where incomplete solubilization and the presence of protein aggregates enhance light scattering (Damodaran, 1996; Petruccelli and Añón, 1994).

It should be noted that the absorbance values at 600 nm were relatively high across the tested concentration range and, in some cases, exceeded the linear range typically assumed for absorbance-based measurements. At elevated concentrations, increased light scattering from dispersed particles and aggregates can lead to deviations from linearity, thereby limiting quantitative interpretation (McClements, 1999). Therefore, the apparent absorbance values should be interpreted primarily as relative indicators of dispersion behavior rather than absolute quantitative turbidity values. Despite this limitation, enzyme-treated samples generally exhibited similar or slightly lower apparent turbidity compared with untreated samples at equivalent concentrations.

The slight reduction in A_{600} following enzymatic treatment may be associated with the breakdown of protein structures into smaller peptides, which can reduce aggregation and improve dispersion behavior. These characteristics suggest that enzymatic hydrolysis may enhance the applicability of

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Table 5. Apparent absorbance at 600 nm of soybean protein hydrolysates prepared using different enzyme combinations (A_{600}[1])

Cultivar	Enzyme treatment[2]	10 mg/mL	20 mg/mL	30 mg/mL	40 mg/mL	50 mg/mL
Daewon	Untreated	2.41 ± 0.04[3)a4)]	2.71 ± 0.01[a]	2.85 ± 0.02[a]	2.93 ± 0.01[a]	3.00 ± 0.00[b]
	Enzyme A	2.25 ± 0.02[b]	2.62 ± 0.01[b]	2.84 ± 0.00[a]	2.94 ± 0.02[a]	3.05 ± 0.00[a]
	Enzyme B	2.15 ± 0.02[c]	2.63 ± 0.01[b]	2.83 ± 0.01[a]	2.93 ± 0.01[a]	3.04 ± 0.02[a]
Seoritae	Untreated	2.27 ± 0.07[a]	2.56 ± 0.01[c]	2.94 ± 0.05[c]	3.27 ± 0.06[b]	3.49 ± 0.05[a]
	Enzyme A	2.38 ± 0.03[a]	2.98 ± 0.06[a]	3.34 ± 0.10[a]	3.48 ± 0.05[a]	3.54 ± 0.08[a]
	Enzyme B	2.39 ± 0.02[a]	2.79 ± 0.02[b]	3.16 ± 0.05[b]	3.39 ± 0.10[ab]	3.54 ± 0.05[a]
Seomoktae	Untreated	2.49 ± 0.04[a]	2.79 ± 0.03[a]	3.25 ± 0.06[a]	3.46 ± 0.06[a]	3.63 ± 0.04[a]
	Enzyme A	2.43 ± 0.07[ab]	2.80 ± 0.05[a]	3.07 ± 0.01[b]	3.33 ± 0.11[a]	3.50 ± 0.04[b]
	Enzyme B	2.32 ± 0.04[b]	2.75 ± 0.03[a]	3.22 ± 0.04[a]	3.44 ± 0.07[a]	3.55 ± 0.05[ab]

[1)]A_{600}, absorbance measured at 600 nm.
[2)]Enzyme A consisted of Protamex and Flavourzyme (2:1), and enzyme B consisted of Alcalase and Flavourzyme (1:1).
[3)]All values are mean ± SD (n = 3).
[4)]Values with different letters in the same soybean cultivar are significantly different at $p < 0.05$ by Duncan's multiple range test.

soybean proteins in liquid food systems by improving dispersibility without substantially increasing viscosity.

3.6. Viscosity

The viscosity values of hydrolysates obtained from *Daewon*, *Seoritae*, and *Seomoktae* soybeans following enzymatic treatment are presented in Table 6. For *Daewon* soybean, the viscosity of the untreated sample was 1.40 cP, which decreased to 1.08 cP and 1.31 cP after enzyme A and enzyme B treatments, respectively. Similarly, *Seoritae* soybean showed a viscosity of 1.40 cP in the untreated sample, whereas lower values of 1.05 cP and 1.22 cP were observed following enzyme A and enzyme B treatments. In the case of *Seomoktae* soybean, the untreated sample exhibited the highest viscosity (1.75 cP), which markedly decreased to 0.81 cP after enzyme A treatment and to 1.14 cP after enzyme B treatment.

Overall, enzymatic treatment resulted in a reduction in viscosity across all soybean varieties. This decrease is attributed to enzymatic hydrolysis-induced depolymerization of proteins, which weakens intermolecular interactions and increases fluidity of the dispersion. Chel-Guerrero et al. (2002) reported higher viscosity values for lima bean and jack bean flours, ranging from 3.00-5.00 cP and 4.00-8.75 cP, respectively, at 25°C. The lower viscosity values observed in the present study are therefore likely related to differences in raw material type and compositional characteristics.

It is generally reported that the viscosity of water at 20°C is approximately 1 cP (Korson et al., 1969). Accordingly, the viscosities of the soybean hydrolysate solutions measured in this study were close to that of water, indicating minimal resistance to flow and water-like rheological behavior. Food ingredients exhibiting water-like viscosity are characterized by high fluidity and low perceived thickness, which contribute to improved drinkability and sensory acceptance (Tabilo-Munizaga and Barbosa-Cánovas, 2005). Moreover, such materials are less prone to sedimentation or gelation in liquid

Table 6. Viscosity of soybean protein hydrolysates prepared using different enzyme combinations

Cultivar	Enzyme treatment[1]	Viscosity (cP)
Daewon	Untreated	1.40 ± 0.00[2)a3)]
	Enzyme A	1.08 ± 0.18[b]
	Enzyme B	1.31 ± 0.09[ab]
Seoritae	Untreated	1.40 ± 0.08[a]
	Enzyme A	1.05 ± 0.32[a]
	Enzyme B	1.22 ± 0.09[a]
Seomoktae	Untreated	1.75 ± 0.00[a]
	Enzyme A	0.81 ± 0.21[c]
	Enzyme B	1.14 ± 0.00[b]

[1)]Enzyme A consisted of Protamex and Flavourzyme (2:1), and enzyme B consisted of Alcalase and Flavourzyme (1:1).
[2)]All values are mean ± SD (n = 3).
[3)]Values with different letters in the same soybean cultivar are significantly different at $p < 0.05$ by Duncan's multiple range test.

systems and allow for easy viscosity adjustment through the addition of thickeners, thereby offering high versatility for application in various liquid and functional food products (Adler-Nissen, 1986; Kristinsson and Rasco, 2000).

3.7. TPC and DPPH radical scavenging activity

TPC increased significantly following enzymatic treatment in all soybean cultivars (Table 7). In *Daewon* soybean, TPC increased from 83.06 mg GAE/100 g in the untreated sample to 108.34 and 106.75 mg GAE/100 g following enzyme A and enzyme B treatments, respectively, corresponding to an increase of approximately 28-30% (approximately 1.28-1.30-fold), with no significant difference between enzyme treatments. Similarly, *Seoritae* soybean exhibited increases from 84.54 mg GAE/100 g to 103.22 and 103.10 mg GAE/100 g, representing an increase of approximately 22% (approximately 1.22-fold). In *Seomoktae* soybean, TPC increased from 111.30 mg GAE/100 g to 122.58 and 124.29 mg GAE/100 g following enzyme A and enzyme B treatments, respectively, corresponding to an increase of approximately 10-12% (approximately 1.10-1.12-fold), again with no significant difference between enzyme treatments.

Lee et al. (2010) reported TPC of 3.01 mg/g for soybean and 3.46 mg/g for black soybean extracted with 80% ethanol, which were slightly higher than those observed in the present study. In contrast, Kim et al. (2013) reported a marked increase in TPC from 0.44 mg/g before enzymatic hydrolysis to 55.95 mg/g after hydrolysis, showing a similar increasing trend to that observed in this study. The increase in TPC following enzymatic treatment is likely attributable to the disruption of protein-polyphenol complexes during hydrolysis, which facilitates the release of polyphenolic compounds from peptide-bound forms (Kim et al., 2009). These results indicate that enzymatic treatment effectively enhances polyphenol recovery regardless of soybean cultivar, supporting the utility of enzymatic hydrolysis in developing antioxidant-enriched functional food ingredients.

Table 7. Total polyphenol content and DPPH radical scavenging activity of soybean protein hydrolysates prepared using different enzyme combinations

Cultivar	Enzyme treatment[1)]	Total polyphenol content (mg GAE/100 g)	DPPH radical scavenging activity (%)
Daewon	Untreated	83.06 ± 0.32[2)b3)]	79.04 ± 0.64[b]
	Enzyme A	108.34 ± 2.28[a]	92.69 ± 2.32[a]
	Enzyme B	106.75 ± 3.77[a]	91.21 ± 2.81[a]
Seoritae	Untreated	84.54 ± 1.40[b]	88.89 ± 0.81[b]
	Enzyme A	103.22 ± 0.28[a]	91.48 ± 0.49[a]
	Enzyme B	103.10 ± 0.98[a]	89.38 ± 0.60[b]
Seomoktae	Untreated	111.30 ± 1.68[b]	88.19 ± 0.42[b]
	Enzyme A	122.58 ± 0.85[a]	88.52 ± 0.85[b]
	Enzyme B	124.29 ± 0.32[a]	90.99 ± 0.11[a]

[1)]Enzyme A consisted of Protamex and Flavourzyme (2:1), and enzyme B consisted of Alcalase and Flavourzyme (1:1).
[2)]All values are mean ± SD (n = 3).
[3)]Values with different letters in the same soybean cultivar are significantly different at $p < 0.05$ by Duncan's multiple range test.

DPPH radical scavenging activity also increased significantly following enzymatic treatment (Table 7). In *Daewon* soybean, scavenging activity increased from 79.04% in the untreated sample to 92.69% and 91.21% following enzyme A and enzyme B treatments, respectively, corresponding to an increase of approximately 15-17% (approximately 1.16-1.17-fold). For *Seoritae* soybean, enzyme A treatment showed the highest scavenging activity (91.48%), representing an increase of approximately 2.9% (1.03-fold) compared with the untreated sample (88.89%), while enzyme B treatment showed a slight increase to 89.38% (approximately 0.6% increase). In *Seomoktae* soybean, enzyme B treatment resulted in the highest scavenging activity (90.99%), corresponding to an increase of approximately 3.2% (1.03-fold) compared with the untreated sample (88.19%), whereas enzyme A treatment (88.52%) showed no significant improvement over the untreated sample.

Hong et al. (2014) reported DPPH radical scavenging activities of 77.82% for soybean and 69.03% for wild soybean extracts at a concentration of 10 mg/mL, which differed somewhat from the values observed in the present study measured at a concentration of 5%. These discrepancies are likely attributable to differences in soybean cultivars and extraction conditions. In addition, Aguilar et al. (2019) reported that enzymatic hydrolysis significantly enhanced antioxidant activity compared to non-hydrolyzed samples, which is consistent with the present findings.

The observed differences in functional and antioxidant properties among soybean cultivars may be attributed to their intrinsic compositional characteristics. In particular, the relatively higher TPC observed in *Seomoktae*, a black-seeded soybean cultivar, can be associated with the presence of seed

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coat pigments such as anthocyanins, which are well known for their strong antioxidant activity (Xu and Chang, 2008).

In addition, variations in storage protein composition, especially the ratio of glycinin to β-conglycinin, may influence functional properties including solubility, emulsifying capacity, and foaming behavior, as these protein fractions differ in structural and interfacial characteristics (Damodaran, 1996). Furthermore, differences in isoflavone content among soybean cultivars may also contribute to the observed variation in antioxidant activity, as isoflavones are recognized as major bioactive compounds with free radical scavenging properties (Messina et al., 2022).

Taken together, the cultivar-dependent differences observed in this study are likely the result of combined effects of phenolic composition, protein structure, and bioactive compound content. Moreover, the enhanced DPPH radical scavenging activity observed following enzymatic treatment is attributed to the combined effects of increased total polyphenol content and the generation of low-molecular-weight peptides with intrinsic antioxidant activity. These results demonstrate that enzymatic hydrolysis is an effective strategy for improving the antioxidant properties of soybean protein-based functional food ingredients. Consequently, soybean protein hydrolysates exhibiting high antioxidant activity show strong potential for application not only in functional beverages and protein foods for older adults but also as antioxidant-enriched ingredients in plant-based alternative protein products.

4. Conclusions

Enzymatic hydrolysis significantly improved the physicochemical and antioxidant properties of protein hydrolysates derived from Korean soybean cultivars. The use of mixed enzyme systems, particularly Alcalase-Flavourzyme, enhanced protein solubility, DH, and NS, while reducing WHC and OHC, foaming and emulsifying properties, apparent turbidity, and viscosity, indicating improved dispersibility and suitability for liquid food applications. Furthermore, the increased total polyphenol content and DPPH radical scavenging activity demonstrate that enzymatic hydrolysis is an effective approach for enhancing the antioxidant potential of soybean proteins. These findings suggest that soybean protein hydrolysates may serve as promising functional ingredients for liquid food systems. Further studies are needed to evaluate their applicability in specialized nutrition products, including elderly-friendly foods and plant-based protein formulations.

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Conflict of interests

The authors declare no potential conflicts of interest.

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Ethics approval

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ORCID

Hae Jin Kim (First author)
https://orcid.org/0009-0009-4032-9816
Eun Ji Lee (First author)
https://orcid.org/0009-0004-7195-5485
Do Wan Kim (Corresponding author)
https://orcid.org/0009-0000-8987-8490
Kyung Young Yoon (Corresponding author)
https://orcid.org/0000-0003-0626-5563

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