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Functional Properties of Enzymatic Hydrolysate and Peptide Fractions from Perilla Seed Meal Protein

*Bo Yeon Park, Kyung Young Yoon**

Department of Food and Nutrition, Yeungnam University. Gyeongsan, Gyeongbuk 38547, Korea

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The present study aimed to decompose perilla seed meal (PSM) proteins *via* enzymatic degradation and to evaluate the functional properties of the resulting enzymatic hydrolysate and peptide fractions for the utilization of PSM, a by-product in the production of perilla seed oil. PSM protein hydrolysate was fractionated based on molecular weight using an ultrafiltration system, and the physical properties required to utilize the hydrolysate and peptide fractions as functional food components were determined. The enzymatic hydrolysate and peptide fractions showed higher solubility, oil absorption capacity, and emulsifying and foaming properties than protein isolates. In particular, peptide fractions below the 5 kDa showed high solubility, emulsifying activity, and foaming capacity. Therefore, these peptide fractions are ideal as functional raw materials and substitutes that can be used to improve the quality of various processed food products and protein supplements.

INTRODUCTION

Perilla (*Perilla frutescens var. Japonica* HARA) is a seedling of a perennial plant belonging to the family Lamiaceae and is cultivated in some Asian countries such as Korea, China, and Japan [Meng *et al.*, 2009a]. Perilla leaves are used as a fresh vegetable and to process pickles due to their unique scent. Perilla seeds were also considered as an interesting oil source for non-food uses and this variety has become widely cultivated in China and Korea. Perilla is not only used as a food ingredient but also for skin creams, soaps, and medicinal preparations, because of its recognized bioactivities, such as antioxidant, anti-allergic, anti-inflammatory, and anti-HIV-1 activity [Meng *et al.*, 2009b]. In particular, perilla oil has excellent physiological functions such as lowering blood pressure, improving thrombosis, and inhibiting cancer cell proliferation [Mohammad, 2011], and perilla cultivation is increasing due to an increase of perilla oil consumption. Perilla seed meal (PSM), a byproduct of the production of perilla oil, is expected to be very useful as a functional food component because it contains high amounts of proteins and active ingredients [Di Bernardini *et al.*, 2011].

Enzymatic hydrolysates, such as sugars, proteins, and lipids, are incorporated in the diets of individuals or patients suffering from impaired metabolic conditions. Protein hydrolysates have long been used in various processing applications, including dietary supplements for athletes and elderly people who need protein. Furthermore, they are used in animal feed to replace expensive protein sources [Schmidl *et al.*, 1994; Córdova-Murueta & García-Carreño, 2002]. Hydrolysates containing low molecular weight peptides are easier to digest and absorb than long polymeric proteins and are widely available in various foods and processed products [Megias *et al.*, 2009]. Peptides produced *via* enzymatic hydrolysis of food proteins exhibit physicochemical properties that are different from those of the original proteins because of lower molecular weights and exposure of hydrophobic residues caused by changes in the molecular structure, while maintaining physiological activity. Peptides present in food products exert biological control functions and provide nutritional value by supplying amino acids. In addition, peptides have excellent emulsifying properties and desired physical properties, such as gel-forming ability, solubility, viscosity, and emulsion properties, and strong affinity fat, and are thus ideal components of food products, such as sports drinks, dietetic foods, and health supplements [Chalamaiah *et al.*, 2012; Benitez *et al.*, 2008]. Various bioactive peptides have been produced from proteins, and their potential use as a functional food material has been proposed.

Therefore, in the present study, PSM protein hydrolysate was prepared *via* enzymatic degradation, and the peptides from the hydrolysate were fractionated based on the molecular weight using an ultrafiltration system. The amino acid composition and physical properties, including solubility, emulsification, foaming, oil retention, and water absorption properties, of the protein hydrolysate and peptide fractions were evaluated to serve as the basis for the utilization of PSM as functional ingredients in food products.

* Corresponding Author: Tel: +82-53-810-2878; Fax: +82-53-810-4666;
E-mail: yoonky2441@ynu.ac.kr

MATERIAL AND METHOD

Materials

The PSM was obtained from Queensbucket Co. (Seoul, Korea) and ground and stored in a deep freezer (MDF-435, Sanyo, Tokyo, Japan) at –42°C for use in subsequent experiments. The proximate composition of PSM was measured according to methods of Bhattacharjee *et al.* [2013] and Park & Yoon [2014] with some modifications. Moisture content was measured using an infrared moisture analyzer (FD-720, Kett, Tokyo, Japan). The contents of crude protein and crude fat were determined using a Micro Kjeldahl system (Distillation Unit B-323, Buchi, Flawil, Switzerland) and an auto fat extraction system (Soxtec 2050, Foss, Sweden), respectively. Crude fiber content was analyzed with a raw fiber extractor (Fiber test F-6, Raypa, Spain). Crude ash content was determined by weighing the remaining inorganic residue after being completely burned at a temperature of 550°C in a muffle furnace. The total carbohydrate content (%) in the PSM was determined by the method involving the summing up of the total values of crude protein, crude fat, crude fiber, and crude ash constituents of the sample and subtracting it from 100. The moisture content of PSM was 26.55%, and crude protein, crude fiber, crude fat, crude ash, and carbohydrate contents were 45.50, 30.74, 18.69, 4.81, and 0.27 % dry weight, respectively.

Preparation of perilla seed meal (PSM) protein isolate

The PSM protein isolate was prepared according to the alkaline extraction and acid precipitation method of Gnanasambandam & Heitiarachchy [1995] with slight modifications. The PSM was added with distilled water at a ratio of 1:10 (w/v) and stirred in a shaking water bath (BS-11, JeioTech, Seoul, Korea) at 25°C for 1 h; the pH of the mixture was adjusted to 10.0 with 1 N NaOH. The resulting slurry was incubated at 25°C for 1 h and centrifuged for 17,000 ×*g* for 30 min at 4°C to obtain the supernatant. The supernatant was adjusted to pH 4.0 with 1 N HCl and incubated at 25°C for 30 min to precipitate the proteins. The precipitated proteins were then collected by centrifugation at 16,000 ×*g* for 30 min, and the residue was homogenized with a homogenizer (AM-1, Nihonseiki Kaisha LDD, Nissei, Japan) by the addition of distilled water equivalent to twice the weight of the residue. The pH of the mixture was adjusted to 7.0, then the mixture was lyophilized using a freeze dryer (FD-1, Eyela, Tokyo, Japan), and used as the protein isolate for hydrolysate preparation.

Preparation of PSM protein hydrolysate and membrane fractions

The PSM protein hydrolysate was prepared following the method of our previous study to find optimal hydrolysis conditions [Park & Yoon, 2018]. As a result of previous experiments, optimal hydrolysis conditions of the PSM protein isolate were determined to be pH 7.0, hydrolysis temperature of 50°C, hydrolysis time of 4 h, and enzyme concentration of 10 U using Flavourzyme (Novo Nordisk Co., Bagsvaerd, Denmark). Thus, the following hydrolysis conditions were used in the present study: PSM protein isolates (5% w/v) were suspended in 25 mL of 0.1 M phosphate buffer (pH 7.0) in a reaction vessel equipped with a stirrer and hydrolyzed with 10 U of Flavourzyme at 50°C for 4 h. After enzymatic hydrolysis, the reaction mixture was boiled at 95°C for 10 min to inactivate the enzyme and then centrifuged at 18,000 ×*g* for 20 min. A part of the supernatant (hydrolysate solution) was passed through ultrafiltration membranes with varying molecular weight cut-offs (3, 5, and 10 kDa) using an Amicon Stirred Ultrafiltration Cell (8050, Millipore, Bedford, MA, USA). The permeate from each molecular weight cut-off membrane was collected as <3, 3–5, 5–10, and >10 kDa peptide fractions. The hydrolysate solution and all permeates were freeze-dried and stored at –42°C for subsequent determination of physical properties.

Amino acid analysis

Amino acid composition of protein isolate, hydrolysate, and peptide fractions was determined using an amino acid analyzer (Biochrom 30, Biochrom, Cambridge, UK). The sample (30 mg) was mixed with 3 mL of 6 N HCl and then hydrolyzed at 110°C for 24 h. The hydrolyzed mixture was filtered with a glass wool to remove the residue. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator (R-124, Buchi, Flawil, Swizerland) at 45°C, and then diluted to 100 mL with a sodium citrate buffer (pH 2.2). The filtrate filtered through a 0.45 μm membrane filter (Millipore, Billeria, MA, USA) was used as a sample for amino acid analysis. The amino acid content was expressed as a percentage of total amino acids in the sample.

Solubility measurements

The solubility of each sample was measured following the method of Nalinanon *et al.* [2011] with some modifications. The sample was mixed with distilled water at 1:100 (w/v) and stirred for 1 h. Each dispersion was adjusted to pH 2, 4, 6, 8, and 10 and subsequently centrifuged at 4,000 × g for 20 min. The protein content of the supernatant was measured by performing a bicinchoninic acid assay [Jang *et al.* 2016] and expressed as the percentage of the total protein content in the sample, using the following equation (1):

$$\text{Solubility (\%)} = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times 100 \quad (1)$$

Water and oil absorption capacity

Water and oil absorption capacities were measured according to the method of Beuchat [1977]. Each sample (1 g) was mixed with 10 mL of distilled water, vortexed, and incubated at room temperature for 30 min. After centrifugation at 3,000 ×*g* for 15 min, the volume of the supernatant was measured using a 10-mL graduated cylinder. Water absorption capacity (WAC) and oil absorption capacity (OAB) were expressed as g of distilled water or soybean oil adsorbed to 1 g of sample.

Emulsifying properties

The emulsifying activity was determined following the method of Yim & Lee [2000] with modifications. Each sample (1 g) was mixed with 100 mL of distilled water, dispersed by vortexing, and the pH of the dispersion was ad-

justed to 2, 4, 6, 8, and 10. Then, 20 mL of the dispersion was added to 20 mL of soybean oil and dispersed for 5 min to form an emulsion. The emulsion was centrifuged at 1,100 ×*g* for 5 min, and the height of the emulsified layer and the total height of the tube were determined. The emulsifying activity was measured according to the following equation (2):

$$\text{Emulsifying activity (\%)} = \frac{\text{Height of emulsified layer}}{\text{Height of total contents in the tube}} \times 100 \quad (2)$$

For the measurement of emulsion stability, the emulsion was prepared by the same method as that used for emulsifying activity. The emulsion was then heated in a hot water bath at 80°C for 30 min, cooled to 15°C and centrifuged at 1,100 ×*g* for 5 min, and the emulsion layer was measured. Emulsion stability was expressed using equation (3):

$$\text{Emulsion stability (\%)} = \frac{\text{Height of emulsified layer after heating}}{\text{Height of the initial emulsified layer}} \times 100 \quad (3)$$

Foaming properties

Determination of foaming capacity was determined by a modified method of Sathe & Salunkhe [1981]. The sample (1 g) was added to 99 mL of distilled water, and each solution was adjusted to pH 2, 4, 6, 8, and 10, respectively. The mixture was transferred to a graduated cylinder and bubbled at 10,000 rpm for 30 s using a homogenizer (AM-1, Nihonseiki Kaisha LDD, Nissei, Japan), and the corresponding increase in volume (mL) was measured.

Foam stability was measured by comparing the foam volume at constant time to the initial foam volume of samples. In other words the foam was formed in the same method as that of foaming capacity and the initial volume of the bubble was measured. The foam was allowed to stand for 30 and 60 min at room temperature, and its volume was measured again. Foam stability was calculated according to equation (4):

$$\text{Foam stability (\%)} = \frac{B - A}{B} \times 100 \quad (4)$$

where A represents the volume after standing (mL), and B represents volume before whipping (mL).

Statistical analysis

Results were expressed as the mean and standard deviation of triplicate experiments. Statistical analysis was performed using SPSS (Ver. 21, Chicago, IL, USA) statistical program. Statistical significance was considered at $p<0.05$. Significant differences between the mean values of the tests were verified by conducting Duncan's multiple range test.

RESULTS AND DISCUSSION

Amino acid composition

The amino acid composition of protein isolate, hydrolysate, and peptide fractions is presented in Table 1. All samples were characterized by high levels of aspartic acid (7.92±13.26–13.26±1.13%) and glutamic acid (19.61±27.03–28.61±0.24%), *i.e.* negatively charged amino acid, which would suggest that PSM protein hydrolysate possessed acidic characteristic. In addition, all samples contained high levels of lysine and arginine, and, especially, the lysine content of <3 kDa fraction was 14.02±4.52%, which was significantly the highest in the samples. It is important to note that lysine is nutritionally important as cases of growth retardation in children increase as a consequence of deficiency in dietary lysine [Tan *et al.*, 2014]. All samples also contained the high amount of the essential amino acids (EAA), and the EAA content of peptide fractions increased with decreasing molecular weight. For example, EAA contents of protein isolate, hydrolysate, >10 kDa, 5–10 kDa, 3–5 kDa, and <3 kDa peptide fractions were 29.26±0.11, 30.93±1.98, 30.34±0.51, 37.75±0.03, 38.18±0.17, and 44.91±0.76%, respectively. Hence, the results suggested that hydrolysate and peptide fractions could be used as an alternative source for cereal-based foods which are low in lysine. These results indicated also that peptide fractions obtained from PSM could be used as dietary protein supplements within food formulations to design protein products. Protein isolate and hydrolysate showed the higher levels of glutamic acid, arginine, and glycine contents compared to peptide fractions, while peptide fractions except for >10 kDa fraction had higher contents of lysine, leucine, valine, and phenylalanine than protein isolate and hydrolysate. As a result, the peptide fractions except for the >10 kDa fraction showed significantly higher contents of hydrophobic amino acids (25.9±0.91–30.79±3.79%) than the protein isolate (18.73±0.07%) and hydrolysate (22.57±1.06%). Amino acid composition has been reported to influence the structure, hydrophobicity, and functionality of peptides [Kimatu *et al.*, 2017]. Therefore, it could be expected that the physicochemical and functional properties of protein isolate and hydrolysate would be different from these of the peptide fractions.

Solubility

The solubilities of the hydrolysate and peptide fractions obtained from PSM proteins were measured to evaluate the potential use of PSM in various food applications. Results were compared relative to the protein isolates (Table 2). The solubility of protein isolate, hydrolysate, and peptide fractions obtained *via* ultrafiltration ranged from 22.81±0.61% to 39.21±1.27%, 38.84±1.39% to 57.35±1.97%, and 31.79±0.93% to 61.73±1.77%, respectively. The solubilities of the hydrolysate and peptide fractions were relatively higher than those of protein isolates, and fractions with smaller molecular weights showed higher solubilities. In particular, the <3 kDa peptides showed the highest solubilities among peptide fractions under all pH conditions, and the highest solubility (61.73±1.77%) was observed at pH 10.0. These results are consistent with the previous findings by Lee *et al.* [1995], in which proteins with smaller molecular sizes were found to exhibit higher solubility. The peptide permeates prepared *via* ultrafiltration and papain modification treatment of soy protein were almost completely soluble at all pH values, except for pH 5.0 [Wu *et al.*, 1998]. In general, the degradation of proteins into smaller peptides leads to the forma-

TABLE 1. Amino acid composition of protein isolate, hydrolysate, and peptide fractions obtained from perilla seed meal.

Amino acid	Protein isolate	Hydrolysate	>10 kDa	5–10 kDa	3–5 kDa	<3 kDa
Aspartic acid	10.40±0.08^{b}	13.26±1.13^{a}	7.92±0.22^{d}	10.44±0.35^{b}	9.67±0.20^{c}	10.84±0.21^{b}
Threonine	2.85±0.19^{bc}	2.89±0.12^{b}	2.43±0.05^{d}	3.44±0.30^{a}	3.43±0.04^{a}	2.64±0.14^{c}
Serine	4.73±0.11^{cd}	4.85±0.01^{c}	5.07±0.03^{b}	5.73±0.11^{a}	5.82±0.13^{a}	4.62±0.19^{d}
Glutamic acid	27.03±0.07^{b}	25.59±0.01^{c}	28.61±0.24^{a}	22.33±0.33^{d}	20.5±0.53^{e}	19.61±0.04^{f}
Proline	n.d	n.d	n.d	n.d	n.d	n.d
Glycine	6.92±0.04^{a}	5.73±0.01^{b}	5.97±0.09^{b}	4.41±0.23^{d}	4.98±0.07^{c}	4.06±0.60^{e}
Alanine	3.70±0.07^{c}	4.73±0.07^{b}	3.57±0.19^{cd}	4.59±0.36^{b}	4.84±0.24^{b}	5.59±0.39^{a}
Cystine	n.d	n.d	n.d	n.d	n.d	n.d
Valine	3.62±0.09^{cd}	3.45±0.21^{c}	2.98±0.01^{d}	4.64±0.25^{b}	4.63±0.11^{b}	5.71±0.46^{a}
Tryptophan	n.d	n.d	n.d	n.d	n.d	n.d
Methionine	1.98±0.02^{bc}	1.75±0.15^{c}	3.40±0.11^{a}	2.01±0.11^{bc}	1.00±0.01^{d}	2.30±0.91^{b}
Isoleucine	1.42±0.05^{c}	1.95±0.60^{abc}	1.70±0.47^{bc}	2.04±0.34^{ab}	2.16±0.01^{ab}	2.47±0.71^{a}
Leucine	4.11±0.04^{e}	5.25±0.41^{d}	5.10±0.16^{d}	6.30±0.30^{c}	7.47±0.04^{b}	8.02±0.71^{a}
Tyrosine	2.86±0.05^{a}	2.38±0.28^{a}	2.91±0.13^{a}	2.52±0.04^{a}	3.13±0.11^{a}	2.38±1.33^{a}
Phenylalanine	3.90±0.10^{d}	5.45±0.07^{c}	3.57±0.04^{e}	6.32±0.24^{b}	6.66±0.10^{ab}	6.71±0.61^{a}
Histidine	3.59±0.08^{ab}	4.04±1.29^{a}	3.49±0.00^{ab}	3.15±0.09^{b}	3.63±0.01^{ab}	3.05±0.22^{b}
Lysine	7.80±0.21^{bc}	6.16±2.15^{c}	7.68±0.02^{bc}	9.84±0.36^{b}	9.19±0.04^{b}	14.02±4.52^{a}
Arginine	15.09±0.09^{a}	12.54±0.64^{bc}	15.59±0.02^{a}	12.23±0.27^{c}	12.87±0.35^{b}	7.99±0.65^{d}
HHA	18.73±0.07^{d}	22.57±1.06^{c}	20.32±0.39^{c}	25.90±0.91^{b}	26.77±0.07^{b}	30.79±3.79^{a}
PCAA	26.48±0.38^{b}	22.74±0.23^{a}	26.75±0.07^{b}	25.23±0.01^{b}	25.70±0.39^{b}	25.06±3.64^{b}
NCAA	37.43±0.01^{b}	38.85±1.14^{a}	36.53±0.01^{c}	32.77±0.68^{d}	30.16±0.33^{e}	30.45±0.25^{e}
TEAA	29.26±0.11^{d}	30.93±1.98^{c}	30.34±0.55^{cd}	37.75±0.03^{b}	38.18±0.17^{b}	44.91±0.76^{a}

The amino acid content was expressed as the mean ± SD (n = 3) as a percentage of the total amino acids. Values with different letters in the same row are significantly different at $p<0.05$. HHA, hydrophobic amino acids-Ala, Vla, Ile, Leu, Phe, Try, Pro, Met and Cys; PCAA, positively charged amino acids-Arg, His and Lys; NCAA, negatively charged amino acids- Asp and Glu; TEAA, total essential amino acids. n.d, not detected.

TABLE 2. Solubilities of protein isolate, hydrolysate, and peptide fractions obtained from perilla seed meal at varying pH conditions.

Fraction	Solubility (%)				
	pH 2.0	pH 4.0	pH 6.0	pH 8.0	pH 10.0
Protein isolate	37.81±0.88^{cA}	22.81±0.61^{dB}	37.86±1.07^{cA}	38.43±0.57^{dA}	39.21±1.27^{eA}
Hydrolysate	54.98±1.54^{aA}	38.24±1.39^{aB}	55.39±1.66^{abA}	56.25±1.66^{bA}	57.35±1.97^{cA}
>10 kDa	50.27±1.58^{bA}	31.79±0.93^{cB}	48.51±1.45^{bA}	49.58±1.48^{cA}	51.92±1.59^{dA}
5–10 kDa	50.63±2.31^{bB}	32.22±0.94^{cC}	50.49±1.59^{bB}	51.41±1.54^{cB}	56.04±1.60^{cA}
3–5 kDa	55.05±1.84^{aA}	36.40±0.95^{bB}	55.52±1.74^{abA}	56.34±1.68^{abA}	58.16±1.66^{bA}
< 3 kDa	55.87±1.72^{aB}	39.82±1.07^{aC}	58.48±1.70^{aAB}	59.66±1.72^{aAB}	61.73±1.77^{aA}

Mean±SD (n=3). Values with different small letters in the same column are significantly different at $p<0.05$. Values with different capital letters in the same row are significantly different at $p<0.05$.

tion of more soluble products [Klompong *et al.*, 2017], since smaller peptides have increased the accessibility of hydrophilic groups, and facilitated the reaction of hydrophilic amino acid to the aqueous medium [Nguyen *et al.*, 2017; Sarabandi *et al.*, 2018]. The high solubility of the peptides obtained *via* ultrafiltration using molecular weight cut-off membranes is presumably caused by the exposure of all hydrophilic groups of small molecules to the aqueous environment [Wu

et al., 1998]. Regarding the solubilities of the samples under various pH conditions, all samples showed the lowest solubilities at pH 4.0, and solubility increased at pH 2.0 and above pH 6.0. The above results are consistent with those reported by Tan *et al.* [2014], who showed that the solubilities of soybean and pinto bean protein isolates were the lowest at the pH range from 4.0 to 5.0. The low solubilities of protein hydrolysates and peptides at pH 4.0 were attributed to the low solubilities of amino acids and proteins at isoelectric point, leading to the formation of precipitates [Singh *et al.*, 2005]. Several factors are known to influence protein solubility, including surface charges, pH, molecular size, ionic strength and type, and temperature of solvent. The pH is considered the most significant factor influencing protein solubility [Kinsella, 1979], and alkali treatment usually improves the solubility of proteins [Pearson, 1994]. Peptides obtained from the PSM protein hydrolysate showed higher solubilities than those of the protein isolates and thus could be used as components of various food products.

Water and oil absorption capacity

Water absorption capacities (WACs) of protein isolate, hydrolysate, and ultrafiltration fractions obtained from PSM are shown in Table 3. The highest WAC of protein isolates was 1.23±0.47 g/g, while the WAC of the hydrolysate was 0.68±0.05 g/g. The WACs of peptide fractions ranged from 0.72±0.07 to 0.76±0.16 g/g, with no significant differences among the peptide fractions. The interactions of protein with water are important in relation to dispersibility or wettability, water absorption and binding, swelling, viscosity, gelation, and surfactant properties [Lee, 2015]. The oil adsorption capacities (OACs) of the hydrolysate (2.45±0.21 g/g) and peptide fractions (2.43±0.25–2.79±0.08 g/g) were higher than those of the protein isolates (1.36±0.15 g/g), and the OAC of the peptide fraction tended to increase with decreasing molecular weight. Manak *et al.* [1980] reported that the OAC of soy protein isolates prepared *via* ultrafiltration was 2.52 mL/g, which is similar to those of the peptide fractions obtained *via* ultrafiltration. Hermansson [1975] reported that the OAC of proteins is influenced by pH and ion concentrations and is dependent on protein composition, amino acid ratios, and the presence of carbohydrates. In addition, oil/water holding capacity relies on surface properties, overall charge density, thickness and hydrophobic/hydrophilic nature of the food particle [Dehnad *et al.*, 2016]. From the above results, the peptide fractions from PSM showed lower WAC and higher OAC than protein isolate, which is considered to be related to the result that the peptide fraction with the smaller molecular weight had the higher content of hydrophobic amino acids. In other words, it is presumed that the hydrophobic peptides exposed by enzymatic hydrolysis are due to increased binding to the oil as compared to the interaction with water [Tan *et al.*, 2014].

Emulsifying properties

Protein emulsifying activity is the ability of the protein to participate in emulsion formation and to stabilize the newly created emulsion. Emulsifying properties are useful functional characteristics which play an important role in the development of new sources of plant protein products for uses as foods [Cabra *et al.*, 2008; Dehnad *et al.*, 2016]. The emulsifying activities of protein isolate, hydrolysate, and peptide fractions obtained from PSM are shown in Table 4. The emulsifying activity of protein isolate ranged from 40.18±0.58% to 52.46±0.28%. The emulsifying activity of the hydrolysates at various pH conditions ranged from 48.58±2.63% to 64.87±0.76%, while those

TABLE 3. Water and oil absorption capacities of protein isolate, hydrolysate, and peptide fractions obtained from perilla seed meal.

Fraction	Water absorption capacity (g/g)	Oil absorption capacity (g/g)
Protein isolate	1.23±0.47^{a}	1.36±0.15^{c}
Hydrolysate	0.68±0.05^{b}	2.45±0.21^{b}
>10 kDa	0.76±0.16^{b}	2.43±0.25^{b}
5–10 kDa	0.72±0.07^{b}	2.48±0.23^{b}
3–5 kDa	0.76±0.01^{b}	2.57±0.12^{ab}
<3 kDa	0.75±0.20^{b}	2.79±0.08^{a}

Mean±SD (n=3). Values with different small letters in the same column are significantly different at $p<0.05$.

TABLE 4. Emulsifying activities of protein isolate, hydrolysate, and peptide fractions obtained from perilla seed meal at varying pH conditions.

Fraction	Emulsifying activity (%)				
	pH 2.0	pH 4.0	pH 6.0	pH 8.0	pH 10.0
Protein isolate	52.46±0.28^{dA}	40.18±0.58^{cD}	45.79±0.45^{cC}	49.22±0.38^{cB}	49.65±0.78^{cB}
Hydrolysate	64.87±0.76^{aA}	48.58±2.63^{bC}	53.23±2.77^{abB}	54.93±3.55^{bB}	57.10±0.77^{abB}
>10 kDa	55.48±0.19^{cA}	47.77±2.74^{bB}	49.16±2.28^{bcB}	55.30±0.76^{bA}	55.33±0.27^{bA}
5–10 kDa	61.91±1.45^{bA}	49.23±3.02^{abB}	49.37±0.68^{bcB}	56.28±2.84^{abA}	56.97±5.65^{abA}
3–5 kDa	64.72±0.68^{aA}	50.67±2.74^{abC}	55.84±0.55^{aB}	56.05±0.50^{abB}	57.56±0.29^{abB}
< 3 kDa	65.74±1.12^{aA}	55.26±4.03^{aB}	56.67±5.33^{aB}	60.07±3.10^{aAB}	60.63±2.56^{aAB}

Mean±SD (n=3). Values with different small letters in the same column are significantly different at $p<0.05$. Values with different capital letters in the same row are significantly different at $p<0.05$.

TABLE 5. Emulsion stabilities of protein isolate, hydrolysate, and peptide fractions obtained from perilla seed meal at varying pH conditions.

Fraction	Emulsion stability (%)				
	pH 2.0	pH 4.0	pH 6.0	pH 8.0	pH 10.0
Protein isolate	44.04 ± 0.82^{bA}	33.18 ± 1.64^{cC}	38.81 ± 2.52^{cB}	40.90 ± 3.21^{cAB}	42.40 ± 2.29^{cAB}
Hydrolysate	54.68 ± 2.48^{aA}	38.81 ± 3.22^{bcD}	46.82 ± 2.12^{bC}	48.64 ± 2.88^{abBC}	52.07 ± 2.48^{abAB}
>10 kDa	47.05 ± 3.01^{bA}	39.90 ± 2.92^{bcB}	45.09 ± 1.19^{bA}	47.99 ± 1.50^{bA}	48.81 ± 2.89^{bA}
5–10 kDa	53.50 ± 2.18^{aA}	41.60 ± 6.40^{abC}	43.51 ± 1.94^{bBC}	47.90 ± 1.45^{bABC}	49.22 ± 1.89^{bAB}
3–5 kDa	57.28 ± 0.83^{aA}	44.25 ± 2.92^{abC}	51.29 ± 1.94^{aB}	51.30 ± 2.35^{aB}	52.48 ± 1.59^{abB}
< 3 kDa	56.61 ± 2.36^{aA}	47.45 ± 3.59^{aC}	50.78 ± 2.26^{aBC}	53.13 ± 3.40^{aAB}	54.92 ± 3.55^{aAB}

Mean±SD (n=3). Values with different small letters in the same column are significantly different at $p<0.05$. Values with different capital letters in the same row are significantly different at $p<0.05$.

TABLE 6. Foaming capacities of protein isolate, hydrolysate, and peptide fractions obtained from perilla seed meal at varying pH conditions.

Fraction	Foaming capacity (mL)				
	pH 2.0	pH 4.0	pH 6.0	pH 8.0	pH 10.0
Protein isolate	4.70 ± 0.26^{cAB}	3.70 ± 0.26^{cC}	4.20 ± 0.36^{cBC}	4.50 ± 0.35^{dAB}	4.80 ± 0.26^{cA}
Hydrolysate	6.10 ± 0.10^{bA}	5.00 ± 0.24^{bB}	5.73 ± 0.14^{bA}	5.93 ± 0.33^{cA}	6.10 ± 0.10^{bA}
>10 kDa	7.00 ± 1.00^{abAB}	5.23 ± 0.31^{bC}	5.53 ± 0.47^{bC}	5.93 ± 0.40^{cBC}	7.10 ± 0.56^{aA}
5–10 kDa	6.73 ± 0.35^{abA}	5.40 ± 0.20^{bB}	6.77 ± 0.61^{aA}	6.63 ± 0.564^{bA}	6.97 ± 0.25^{aA}
3–5 kDa	7.29 ± 0.30^{abA}	6.20 ± 0.26^{aB}	7.10 ± 0.35^{aA}	7.30 ± 0.17^{aA}	7.46 ± 0.42^{aA}
< 3 kDa	7.10 ± 0.53^{aA}	6.20 ± 0.17^{aB}	7.01 ± 0.32^{aAB}	7.29 ± 0.30^{aA}	7.40 ± 0.38^{aA}

Mean±SD (n=3). Values with different small letters in the same column are significantly different at $p<0.05$. Values with different capital letters in the same row are significantly different at $p<0.05$.

of the ultrafiltered fractions ranged from 47.77±2.74% to 65.74±1.12%. The hydrolysate and peptide fractions obtained *via* ultrafiltration showed higher emulsifying activities than those of the protein isolates. The emulsifying activity tended to increase with decreasing molecular weight of the peptides. All samples showed the highest emulsifying activity at pH 2.0 and the lowest emulsifying activity at pH 4.0. The emulsifying activity tended to increase again at pH values above 6.0. In general, the emulsifying activity of proteins increased with higher protein solubility and was found to be significantly influenced by the pH, temperature, and ionic strength conditions [Crenwelge *et al.*, 1974; Kinsella 1979]. Wu *et al.* [1998] reported that the emulsifying activities of soy protein peptides prepared *via* papain modification and ultrafiltration were significantly higher compared to those of the protein isolates, which is consistent with our current findings. The above results suggested that hydrolysates with higher solubilities and smaller molecular weights promote diffusion and spread at oil-water interfaces [Wu *et al.*, 1998]. In addition, the findings of Lui *et al.* [1989] suggested that ultrafiltration improves the emulsifying ability by increasing the number of peptides with emulsifying power, exposing hydrophobic residues of the proteins, and increasing the balance between hydrophilicity and hydrophobicity. Moreover, the findings of Wu *et al.* [1998] suggested that solubility and molecular size, rather than surface hydrophobicity, are the major factors responsible for the strong emulsifying activity of the small peptides.

The emulsion stability of the protein isolates ranged from 33.18±1.64% to 44.04±0.82%. The emulsion stability of the hydrolysate ranged from 38.81±3.22% to 54.68±2.48% and that of the peptide fractions ranged from 39.90±2.92% to 57.28±0.83% (Table 5). The hydrolysate and peptide fractions showed relatively higher emulsion stabilities than those of the protein isolates. These results are consistent with those reported by Lui *et al.* [1989], in which the fractions fractionated *via* ultrafiltration demonstrated higher emulsion stabilities than soy. Yim & Lee [2000] also reported that <1 kDa peptides obtained *via* fractionation of soybean protein hydrolysates obtained from proteolytic enzymes of Meju showed the highest emulsifying power and emulsion stability. Emulsions with small molecular oil droplet sizes were found to be more stable than those with larger droplets [Chove *et al.*, 2002]. The emulsion stability of each sample at various pH values showed a similar trend with that of emulsifying activity. The emulsion stability was highest at pH 2.0, except for the >10 kDa fraction, and lowest at pH 4.0. Chove *et al.* [2002] reported that soy protein isolates microfiltered using the 0.1-μm membrane improved the emulsion-stabilizing ability of the resulting retentate. In addition, fractions richer in species with isoelectric point values of around 4.6 showed poor emulsifying stability in-

TABLE 7. Foam stabilities of protein isolate, hydrolysate, and peptide fractions obtained from perilla seed meal at varying pH conditions.

Fraction	Foam stability (%)									
	Standing time (30 min)					Standing time (60 min)				
	pH 2.0	pH 4.0	pH 6.0	pH 8.0	pH 10.0	pH 2.0	pH 4.0	pH 6.0	pH 8.0	pH 10.0
Protein isolate	45.5 ± 2.8^{bB}	0.0 ± 0.0	35.7 ± 1.2^{bD}	51.1 ± 2.5^{cA}	47.5 ± 1.8^{dC}	32.0 ± 1.5^{aB}	0.0 ± 0.0	28.3 ± 1.6^{bC}	37.8 ± 2.8^{cA}	34.2 ± 0.7^{nsB}
Hydrolysate	49.2 ± 6.7^{bC}	0.0 ± 0.0	35.1 ± 3.6^{bD}	77.0 ± 2.4^{aA}	59.3 ± 2.9^{bB}	32.8 ± 0.7^{aB}	0.0 ± 0.0	33.3 ± 0.5^{aC}	49.2 ± 1.5^{aA}	33.9 ± 1.5^{B}
>10 kDa	46.7 ± 2.9^{bA}	0.0 ± 0.0	34.5 ± 2.5^{bC}	67.8 ± 5.7^{bcA}	60.6 ± 3.3^{bB}	20.0 ± 2.5^{cC}	0.0 ± 0.0	23.6 ± 2.2^{cC}	41.0 ± 6.5^{bA}	31.0 ± 2.5^{B}
5–10 kDa	30.8 ± 3.5^{cD}	0.0 ± 0.0	36.8 ± 3.3^{bC}	68.2 ± 4.2^{bA}	52.9 ± 4.2^{cB}	26.2 ± 1.8^{bB}	0.0 ± 0.0	17.6 ± 1.3^{dC}	34.8 ± 1.8^{cA}	32.9 ± 2.8^{A}
3–5 kDa	56.3 ± 2.0^{aB}	0.0 ± 0.0	40.8 ± 2.1^{aC}	71.2 ± 2.5^{bA}	69.3 ± 5.3^{aA}	32.4 ± 2.9^{aB}	0.0 ± 0.0	12.7 ± 1.1^{eC}	41.1 ± 2.6^{bA}	32.0 ± 4.3^{B}
< 3 kDa	48.2 ± 1.8^{bC}	3.6 ± 0.4^{E}	42.8 ± 0.8^{aD}	72.1 ± 1.5^{bA}	60.1 ± 2.8^{bB}	30.8 ± 5.3^{aB}	0.0 ± 0.0	28.3 ± 2.9^{bC}	36.4 ± 2.0^{cA}	35.8 ± 4.0^{AB}

Mean±SD (n=3). Values with different small letters in the same column are significantly different at $p<0.05$. Values with different capital letters in the row are significantly different at $p<0.05$.
ns, no significant difference

dices and emulsifying activity indices. In the present study, the lowest emulsifying activity and emulsion stability values of all samples at pH 4.0 were attributed to the isoelectric point of the PSM protein being pH 4.0.

Foaming properties

Foaming properties are physicochemical characteristics of proteins that facilitate the formation and stabilization of foams. The foaming capacity and foam stability of protein isolate, hydrolysate, and peptide fractions at different pH values are shown in Tables 6 and 7, respectively. The foaming capacities of the hydrolysate (5.00±0.24–6.10±0.10 mL) and peptide fractions (5.23±0.31–7.46±0.42 mL) were higher than those of protein isolates (3.70±0.26–4.80±0.26 mL). This result might be due to the fact that the small peptides diffuse more rapidly to the air-water interface and encapsulate air bubbles, thereby developing a foam [Wierenga & Gruppen, 2010]. The highest and lowest forming capacities of all samples were observed at pH 10.0 and pH 4.0, respectively, indicating that the foaming properties were significantly influenced by pH. Adebowale & Lawal [2003] reported that the foaming ability of a mucana bean protein concentrate was lowest at pH 4.0 and highest at pH 10, which was consistent with our result. Our current findings were also similar to these reported by Elderidge *et al.* [1963], in which the foaming capacity of soy protein was observed to be the lowest at the isoelectric point. The foaming capacity of protein improves the sensory properties of food, including the appearance and texture. Proteins with high foaming capabilities are used as foaming agents in the manufacture of food products and affect the texture and volume of bread [Kim & Ahn, 2007]. The ultrafiltration process induces structural changes in the proteins and reduces S-S interactions, which also influence bubble formation [Bang *et al.*, 1996]. In general, the foaming capacity of proteins was improved by increasing the structural flexibility *via* exposure of hydrophobic residues and by increasing the capacity to decrease surface tension [Mutilangi *et al.*, 1996]. In addition, Kinsella & Melachouris [1976] reported that protein hydrolysis exposes the hydrophobic groups of polypeptide and promotes the foam formation. Therefore, the strong foaming capacities of hydrolysate and peptide fractions from PSM protein were attributed to the exposure of hydrophobic residues to the enzymatic hydrolysis.

After standing for 30 min (Table 7), the foam stability was the highest in the 3–5 kDa fraction, and the lowest in protein isolates at various pH. The foam stability of each sample was high ranging from 51.1±2.5% to 77.0±2.4% at pH 8.0, but foam of all samples except for <3 kDa fraction was destroyed at pH 4.0. PSM protein hydrolysate showed the highest foam stability, while >10 kDa fraction showed the lowest foam stability at all pH values except pH 6.0 at standing for 60 min. In addition, the foam stability according to pH was the highest at pH 8.0 with the same tendency as at 30 min, and no foam remained at pH 4.0. These results are similar to those reported by Klompong *et al.* [2017], in which the protein hydrolysates obtained from yellow stripe trevally (*Selaroides leptolepis*) showed the lowest foam stability at pH 4, and foam stability of the hydrolysate was found to be higher at pH 6.0 or higher and eventually decreased again at pH 10.0. The low foam stability was concomitant with the low solubility at pH 4.0. Protein solubility and the pH of the dispersing medium are important factors that determine the foaming properties of proteins, especially foam stability. The reduced foam stability at very acidic or alkaline pH could be attributed to the repulsion of peptides *via* ionic repulsion [Townsend & Nakai, 1983; Klompong *et al.*, 2017].

CONCLUSIONS

The protein hydrolysate and peptide fractions produced from perilla seed meal (PSM) were found to have a very high content of essential amino acids including lysine. In addition, protein hydrolysate and peptide fractions showed superior functionality compared to PSM protein isolates. Especially <3 kDa and 3–5 kDa peptide fractions exhibited high solubility, oil absorption capacity, emulsifying activity, foam capacity, and foam stability. The results from this study indicate that peptides have the potential to be used as value-added food ingredients where functional properties

are sought after (*e.g*. protein beverage formulations, bread, emulsion *etc*.). Furthermore, it is also important to invest their physiological activity to improve utilization as functional materials in food industry.

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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