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Physicochemical Properties of Isolated Peptides from *Hwangtae* (yellowish dried pollack) Protein Hydrolysate

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Abstract

Fish protein hydrolysates (FPHs) with different degrees of hydrolysis by treatment with alcalase, pronase, flavourzyme and trypsin and isolated peptide were prepared from *Hwangtae* (yellow dried pollack, *Theragra chalcogramma*). *Hwangtae* protein hydrolysate was fractionated according to the molecular weight into six major types of APO1 (1.3 kDa), APO2 (1 kDa), APO3 (<1 kDa), APACE (<1 kDa), APG1 (70 kDa) and APG2 (70 kDa) isolated from the hydrolysate using consecutive chromatographic methods. Soluble peptide were produced from *Hwangtae* and evaluated for their nutritional and functional properties. Some functional properties of FPHs were assessed and compared with those of egg albumin or the soybean protein. APO2 had the highest nitrogen solubility value (94.2%), emulsion capacity and emulsion stability of the Alaska Pollack peptide ranged from 12.4 to 39.5 (mL of oil per 200 mg of protein) and 44.0% to 77.5%, respectively. Highest and lowest fat adsorption values were observed for APG1 (9.9 mL of oil per gram of protein) and APO3 (3.8 mL of oil per gram of protein), respectively.

Key words: *Hwangtae*, hydrolysate, physicochemical properties, nitrogen solubility, emulsifying properties, fat absorption

INTRODUCTION

Hwangtae is preparing by removing internal organs and drying with thawing in the daytime and freezing at night under sub-zero, -10°C, and gradually drying from December to the next April for around 5 months from *Myungtae* in Korea. The head, skin and bones are not removed from the fish and it is dried in the breeze as they hang from wooden logs. After 5 months of hanging up to dry and 80% water loss due to evaporation, the fish is considered matured for harvesting. When dried well, *Myungtae* turns yellow, so it is called *Hwangtae* (yellowish dried pollack), it is prepared not only from dried whole fish but also sliced fillet called *Hwangtae-che* for various ways to cook source such as soup, saute, panfry, roast, mixing with spice, steaming, stew, deep fry etc. Removal of *Hwangtae* head, skin and bones is necessary for preparing *Hwangtae-che*, only 50~60% of the production is used for human consumption as whole dried fish. During processing and packaging of *Hwangtae-che*, the rest of the parts and generated debris has been discarded as by-produts. The major fraction of the by-product is used for animal feed production and fertilizers, but the by-products contain valuable protein and calcium in addition to mineral and vitamins. Althrough the nuturitional value of these wastes is high, no substantial attempt is being made to utilize these potentially valuable resources. Thus novel means of processing are required to convert the underutilized and limited materials, into a more marketable and acceptable form. It may be possible to produce a broad spectrum of food ingredients or industrial products for a wide range of applications in fish processing, such as produce fish powders or fish protein hydrolysates that may be used as carbon and/or nitrogen source for biomass and metabolite production.

Utilizing proteolytic enzymes, fish protein hydrolysates (FPHs) can be prepared in controlled conditions with peptides having new and/or improved properties that may be used as food ingredients. Many enzymes

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have been described for the hydrolysis of fish proteins (papain, Alcalase®, Neutrase®, Flavourzyme®, Protamex®, etc.) (1). However, the substrate is one of the major factors influencing the hydrolysis (2). Kristinsson and Rasco (3) mentioned that Alcalase® was the most desirable enzyme in hydrolyzing fish proteins. The enzymatic hydrolysis of native proteins improved its functional and rheological properties, including solubility, emulsifying and foaming characteristics (4) and offered interesting opportunities for food applications. Also, the protein-rich seafood by-products can have a range of dynamic properties and could poteintially be used as food binders, emulsifiers, and gelling agents (5). Functional properties can be defined as the overall physicochemical properties of peptide from hydrolysis enzymes in food systems during processing, storage and consumption (6). The changes in functional properties of native proteins are related to peptides and free amino acids produced by enzymatic hydrolysis, which are mainly characterized by a lower molecular mass, exposure of hydrophobic groups and by an increased number of ionic groups (7).

We reported in a previous paper that antioxidative and ACE inhibitory peptides isolated from enzymatic hydrolysate of *Hwangtae* and isolated peptides have functional properties such as antioxidative activities and angiotensin inhibitory activities. Ground *Hwangtae* was hydrolyzed using two proteolytic enzymes (Pepsin and Alcalase) which produced five soluble active peptides. Two different peptides (APO1 and APO2) with strong antioxidative activity were isolated from the hydrolysate and were composed of 16 and 13 amino acid residues, respectively. Both peptides contained a Gly residue at the C-terminus and the repeating motif Gly-Pro-Hyp. The peptide (APACE) obtained from enzymatic hydrolysates of *Hwangtae* exhibited ACE inhibitory activity. The APACE peptides was composed of 4 amino acid residues (Gly-Leu-Leu-Pro). Also isolated, two 70 kDa peptides (APG1 and APG2) were found to have an associated gelatinoytic activity (8). The objective of this study was to characterize *Hwangtae* protein prepared from by-products and evaluate physicochemical properties of isolated peptides from *Hwangtae* hydrolysate, improving their functional quality and bioability for potential uses as fuctional ingredients.

MATERIALS AND METHODS

Mateirals

Hwangtae was kindly donated from village of Yongdaeri (Inje country, Korea), vacuum packaged and stored at -40°C until further processed. Enzymes [Alcalase® 2.4L (EC 3.4.21.62), Flavourzyme® (EC 3.4.11.1), Pronase E (EC 3.4.24.4), Pepsin (EC 3.4.23.1), Trypsin (EC 3.4.21.4)] were purchased from Sigma Co. (St. Louis, USA). All other used reagents used were of the highest graded available commercially.

Proximate chemical composition

A proximate chemical composition of *Hwangtae* was determined according to the AOAC standard methods (9). Crude protein content was determined by the Kjeldhal method. Ash content was determined by calcination in a furnace at 550°C and the moisture content was determined by drying in an oven at 105°C for 24 hr. Fat content was determined using dichloromethyl ether on an automated ASE-200 fat extractor (Dionex Corporation, Sunnyvale CA, USA)

Amino acid analysis

Amino acid profiles were determined by the AAA Service of Institute of Animal Resources (Kangwon National University, Chuncheon, Korea). Samples were hydrolyzed with 6 N HCl and 2% phenol at 110°C for 22 hr. Amino acids were quantified using a Beckman 6300 analyzer (GMI, Inc., Ramsey, MN, USA) with post-column ninhydrin derivatization. Tryptophan and cysteine content were not determined. Samples for mineral analysis were ashed overnight at 550°C. Ashing residues were digested overnight in an aqueous solution containing 10% (v/v) hydrochloric acid and 10% (v/v) nitric acid. The ratio of TEAA (total essential amino acids) to TAA (total amino acids) was calculated using the content of Thr, Val, Met, Ile, Leu, Phe, Lys, and His on milligram of amino acid per gram of total amino acid residue basis using the equation developed by Sathivel and Betchtel (10).

Preparation of fish protein hydrolysate

The *Hwangtae* was mixed with an equal amount of distilled water and homogenized in a Moulinex® blender for about 2 min. Soluble protein powders were made according to the method of Sathivel et al. (11). A 500 g portion of each ground sample was mixed with an equal volume of distilled water (23°C) and homogenized in a Waring blender (Waring Products Div., New Hartford, CT, USA) for 2 min. The mixture was continuously stirred for 60 min at 85°C. During heating, fat cells were ruptured, releasing oil into the liquid phase. The heated suspension was centrifuged at $2560 \times g$ for 15 min, resulting in three separate phases, the semi-solid phase at the bottom containing insoluble protein, the heavier liquid phase in the middle containing soluble proteins, and the lighter liquid phase at the top containing crude lipids. The heavier liquid middle-layer was

separated, collected, and freeze-dried.

The enzymatic reaction mixture was prepared by adding 500 mL of buffer solution with pH 2.0 (pepsin) to 1 g of freeze-dried soluble protein from *Hwangtae*. The mixtures were then initially pre-incubated and digested with pepsin at 37°C for 24 hr (Enzyme-to-substrate ratio [E/S]: 1/50). The pH of the mixture was adjusted to the optimum activity value for the other hydrolysis enzyme (Alcalase, Flavourzyme, Pronase E, Pepsin, Trypsin). The hydrolysis was performed as described below at pH 7.0. The enzyme was added to the reaction to give an enzyme/substrate ratio of 1:50. After the required digestion time, the reaction was stopped by heating the solution at 80°C for 20 min to inactivate the enzyme. The fish hydrolysate was centrifuged at 5000×*g* for 20 min to separate insoluble and soluble fractions. Finally, the soluble phase was freeze dried. The degree of hydrolysis (%) was calculated as the ratio of degraded weight of substrate to its initial weight.

The hydrolysates were fractionated in cartridges with molecular weight cut-offs (MWCO) of 30, 10, 5, 3, and 1 kDa (Millipore Pelicon XL Biomax, Bedford, MA, USA). The cartridges were assembled in the ascending order of MWCO increment, and the hydrolysates were pumped with pressure (Millipore Labscale TFF system, Millipore System Division).

Purification of the active peptides

The isolation and purification of active peptide was carried by the method of Je et al. (12). The lyophilized hydrolysate was loaded onto a SP Sephadex C-25 ion-exchange column (4.0×40 cm), equilibrated with 20 mM sodium acetate buffer (pH 4.0), and eluted with a linear gradient of NaCl (0～1 M) in the same buffer at a flow rate of 60 mL/h. The active fractions were pooled and lyophilized immediately. The lyophilizate was further purified on Sephadex G-25 gel filtration column (2.5×90 cm) and equilibrated with distilled water. The column was eluted with distilled water and 5.0 mL of fractions were collected at a flow rate of 60 mL/h. The fraction exhibiting antioxidative activity was further purified using reversed-phase high performance liquid chromatography (RP-HPLC) on a Primesphere 10 C_{18} (20 mm×250 mm) column with a linear gradient of acetonitrile (0～35% in 30 min) containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 2.0 mL/min. The active peak was concentrated using a centrifugal evaporator. The peak representing the active peptide was rechromatographed on a ODS (10 mm×250 mm) column using a linear gradient of acetonitrile (0～15% in 30 min) containing 0.1% TFA at a flow rate of 2.0 mL/min.

Physicochemical properties

Three separate experiments: nitrogen solubility, emulsifying properties and fat absoption for all purified peptides from *Hwangtae* hydrolysate were conducted and results reported on a protein content basis. Nitrogen solubility was determined following the procedure of Morr et al. (13). A 500 mg aliquot of purified peptides from *Hwangtae* hydrolysate was dispersed in 50 mL of 0.1 M NaCl at pH 7.0. The solution was stirred for 1 hr at 25°C and centrifuged at 2560×*g* for 30 min. The supernatant was analysed for nitrogen content using a Leco FP-2000 Nitrogen Analyzer (Leco Co., LTD, USA). Percent nitrogen solubility (%) of peptides samples was calculated as [Supernatant nitrogen content/ Total sample nitrogen content]×100%.

Emulsifying capacity (EC) was measured by an oil titration method similar to that of Webb et al. (14). Each peptide sample (100 mg) was dissolved in 10 mL of 0.1 m NaCl solution in a tared 200 mL beaker. A motorized stirrer/homogenizer (B25 Laboratory High-shear Emulsifier, Shanghai BRT equipment tehchnology Co., LTD, China) was immersed in the solution in the beaker. A separatory funnel filled with 100% pure soybean oil was placed above the beaker. A pair of electrodes connected to a multimeter (True RMS multimeter, John Fluke Co., Everett, WA, USA), was immersed in the solution to measure the electrical resistance (Ohms) in of the emulsion. The solution was first stirred at 60% output of a 120 V rheostat for 20 s to make a homogenized solution and to obtain a constant resistance reading. The output was then increased to 100% and the oil was immediately dispensed from the separatory funnel into the beaker at 0.5 $mL \cdot s^{-1}$, generating an oil-in-water emulsion at 25°C. A sudden increase in resistance was observed when the oil capacity of each peptide emulsion reached a maximum value and the emulsion collapsed to form a water-in-oil emulsion. At that point, oil delivery was stopped and the oil volume was measured by weighing the beaker and the quantity was calculated in millilitres by correcting for the oil density (0.93211 $g \cdot mL^{-1}$). EC values are expressed as milliliters of emulsified oil per 100 mg of protein.

Emulsifying stability (ES) was evaluated according to the method of Yatsumatsu et al. (15). A 500 mg samples were transferred into 250 mL beakers and dissolved in 50 mL of 0.1 m NaCl, and then 50 mL of soybean oil was added. The homogenizer equipped with a motorized stirrer driven by the rheostat was immersed at 50 mL depth of the mixture, and operated for 2 min at 100% output at 120 V to make an emulsion. From the emulsion, three 25 mL aliquots were immediately taken and

transferred into three 25 mL graduated cylinders. The emulsions were allowed to stand for 15 min at 25°C and then the aqueous volume was investigated. ES (%) was calculated as [(total volume − aqueous volume)/ total volume] × 100%

The fat adsorption (FA) capacites of the peptide samples were determined by placing 500 mg peptide samples into 50 mL centrifugal tubes and adding 10 mL of soybean oil. The samples were thoroughly mixed with a small steel spatula, kept for 30 min at 25°C with intermittent mixing every 10 min, and then centrifuged at 2560 × *g* for 25 min. Free oil was then decanted and the fat absorption of the sample determined from the weight difference. FA was expressed in terms of millilitres of fat adsorbed by 1 g of peptide

Statistical analysis

Statistical analysis of data was performed by SAS software (SAS 2000) using Fisher's least significant difference procedure (LSD) and analysis of variance (ANOVA). All data were determined at least in duplicate and all were averaged.

RESUTS AND DISCUSSION

Proximate chemical composition

The proximate compositions of *Hwangtae* are listed in Table 1. Song and Mok (16) reported that the proximate composition of fresh Alaska pollack fish was: Moisture 79.6%, ash content 1.2%, protein 18.2% and lipid 0.6%. The protein contents of the *Hwangtae* (43.3%) were higher than those (18.2%) reported for fresh fish of Alaska pollak protein. The *Hwangtae* contained higher ash (38.0%) and lower moisture content (12.5%) than fresh Alaska pollack, which might significantly contribute to stability (Table 1). The *Hwangtae* showed high levels of ash, which indicated the accumulation of inorganic compounds such as calcium. Karawita et al. (17) reported that Alaska pollack fish bones may have the potential to be a good source of bioavailable calcium that could be extracted and used as a nutritional supplement. Also, calcium forms biologically unavailable compounds with many of the anions present in foods, however it can be yielded as a soluble bioavailable form of calcium in Alaska pollack.

Table 1. Proximate composition of *Hwangtae*

	Content (%)			
	Moisture	Crude ash	Crude protein	Lipid
Hwangtae	12.5 ± 0.2	38.0 ± 0.3	43.3 ± 0.2	0.4 ± 0.1

Amino acid analysis

The amino acid composition of *Hwangtae* consisted of higher concentrations of aspartic acid, glutamic acid, serine, glycine, alanine, and histidine in *Hwangtae* than *Myungtae* (19). A high concentration of aspartic acid and glutamic acid residues was also observed in *Hwangtae* protein, and was 13.2% and 17.1%, respectively, of the total amino acids. These amino aicds, accouting for 30.3% of total amino acid content, might contribute to the calcium binding property of the *Hwangtae* protein, similar to results of the characterization of the amino acid composition in fish backbone of Alask pollack (17). Aspartic acid and glutamic acid maintain the solubility and ionic character of proteins (free carboxyl group makes it acidic and hydrophilic). The negatively charged side chains of these amino acids function as the binding sites for minerals and enhance calcium-binding capacity (17). The glycine content is typical of the amino acid profile of collagen which is a connective protein in the fish. Futhermore, the serine and alanine were furnished binding sites for Ca-binding phosphorprotein such as osteocalcin (17).

The essential amino acid contents (miligram of amino acid per gram of protein) of *Hwangtae* were higher than the recommended values for human adults (FAO/WHO (20). As a percent of total amino acids (TAA), the total essential amino acid content (TEAA) required for adult human was lower for *Hwangtae* (34.26%) than *Myungtae* (43.83%). But the nutrition values for *Hwangtae* was higher than values for fresh fish within a species and its processing product, because the protein contents of *Hwangtae* was 2.4 fold higher than that of reported for protein contents of *Myungtae* (Table 2). Human diets contain a wide variety of proteins from different sources. It is generally accepted that the relative concentration of dietary essential amino acids is the major factor determining the nutritional value of food protein (18). Proteins derived from meat and poultry muscle are also very high quality and fish muscle proteins are equally nutritious (21). Fish muscle contains an excellent amino acid composition, is an excellent source of many nutrients, and is an excellent source of nutritive and easily digestible proteins (22,23). Because fresh fish is extremely perishable, the utilization of fish as a basic raw food material presents unique food processing problems but the proteins and isolated peptides derived from stockfishs such as *Hwangtae* are considered to be nutritionally superior to those from fresh fish because they contain a better balance of the dietary essential amino acids and convenience of processing.

Table 2. Amino acid compositions of *Hwangtae*

	Amino acid (mg/g protein)		
	Hwangtae	*Myungtae*[1)]	EAA[2)]
Asp	131.2±3.2	92.6	
Thr*	44.0±2.0	43.5	9
Ser	52.5±3.5	38.4	
Glu	169.6±4.3	134.6	
Pro	34.3±1.9	34.2	
Gly	95.3±3.8	45.7	
Ala	92.5±5.3	64.1	
Val*	43.5±1.3	42.7	13
Met*	17.5±1.7	34.2	17
Ile*	27.3±2.4	37.6	13
Leu*	50.1±1.1	77.0	19
Tyr	21.0±1.6	35.6	
Phe*	29.4±0.6	38.6	
Lys*	85.5±1.7	86.4	16
His*	43.4±0.9	32.3	
Arg	57.5±1.4	57.6	
TEAA	340.7±1.8	392.3	
TAA	994.6±3.1	895.1	
TEAA/TAA (%)	34.26	43.83	

Data expressed as mg of amino acid per g protein, Tryptophan and cystein were not determined.
*Essential amino acid.
TEAA, total essential amino acid; TAA, total amino acids.
[1)]Amino acid composition of *Myungtae* (Korean Health Industry Development Institute, 2004).
[2)]Essential amino acid requirements for adult human (FAO/WHO, 1990).

Preparation of *Hwangtae* hydrolysate

The curve of hydrolysis degree of pepsinolytic *Hwangtae* hydrolysates prepared with four kinds of hydrolysis enzymes after 12 hr of incubation is shown in Fig. 1. After 2 hr, the hydrolysis of the *Hwangtae* protein was characterized by an initial rapid phase; the hydrolysis degree of drying fish protein ranged from 25.3 to 31.5% during which a large number of peptide bonds were hydrolyzed. The rate of enzymatic hydrolysis subsequently decreased, and then the enzymatic reaction reached a steady-state phase in all enzymatic hydrolysis, in which no apparent further hydrolysis took place. The alcalase treatment had the highest percent of hydrolysis degree (29.7%) and pronase treatment had a similar percent of hydrolysis degree value (28.1%) after one hour (Fig. 1). Pepsin is generally considered one of the best enzymes to solubilize fish protein. The acid enzyme pepsin has been most successful in solubilizing fish protein. Liu and Pigott (24) produced a high quality, fluffy, water-soluble fish protein hydrolysate by pepsin hydrolysis of rockfish fillets. The pepsinolytic hydrolysate product was a creamy white, nonhygroscopic, water-soluble hydrolysate with low lipid content but had very poor nutri-

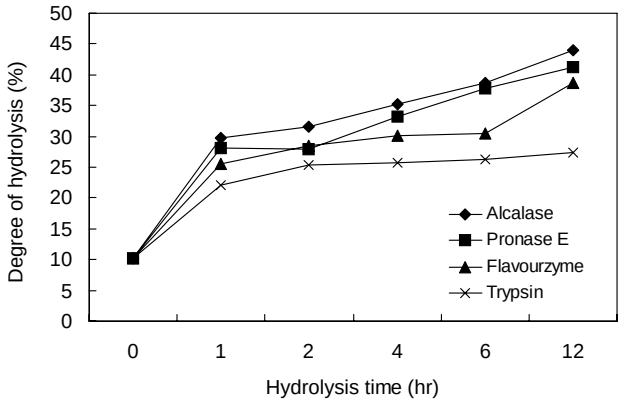

Fig. 1. Hydrolysis degree of enzymatic hydrolysates from pepsin treated hydrolysate incubated with various enzymes for different times in Alaska pollack (*Hwangtae*).

tional value. Enzyme hydrolysis was dependent on the substrate concentration. Cheftel et al. (25) used pronase to hydrolyze fish protein concentrate and found that the rate constantly decreased with time. However, proteolysis did not follow first-order kinetics with respect to concentration of the peptide bonds. This may be due to the multitude of possible substrates in fish protein concentration, the number of different proteolytic enzymes present, the inhibitory effects of substrate or self-digestion, as well as the different specificities that are known to be present in Pronase. Alcalase produced a hydrolysate with a markedly reduced bitterness and less fishy odor. Alcalase, an alkaline enzyme produced from *Bacillus licheniformis* and developed by Novo Nordisk (Bagsvaerd, Denmark) for the detergent industry, has been proven repeatedly by many researchers to be one of the best enzymes used to prepare functional fish protein hydrolysate and other protein hydrolysates (26). Alcalase treated hydrolysates exhibited superior protein recovery (70.6%) compared with the alkaline protease Neutrase and papain. Alcalase-treated hydrolysates also had the lowest lipid content (0.18%) and excellent functional properties (2). Proteases are characterized further by their hydrolyzing mechanism into endoproteinases or exopeptidases. The endoproteinases cleave/hydrolyze the peptide bonds within protein molecules, usually at specific residues, to produce relatively large peptides. The process of using added endoproteinases offers many advantages because it allows good control of the hydrolysis and thereby, the properties of the resulting products (2). However, for an enzyme preparation to be effective for hydrolyzing protein, both exopeptidases and endoproteinases are required. Many studies have shown that proteolytic preparations containing exopeptidases and endoproteinases produce less bitter peptides than single proteases (27,28). The use of exopeptidases, as opposed to endo-

proteinases, can also be helpful in overcoming the bitterness in fish protein hydrolysates, particularly exopeptidases such as flavourzyme in this study that split off hydrophobic amino acids from bitter peptides. In food protein hydrolysis, endoproteinases are always used, but occasionally endoproteinases are combined with exopeptidases to achieve a more complete degradation (26).

The screening for a suitable enzyme in a process or experiment is very important if the product is to have predetermined properties. Thus, our choice of enzymes was alcalase for pepsinolytic hydrolysis because of its combination of efficacy and economics

Isolation and purification of active peptides

To identify the functional properties of peptides, the second-step hydrolysate of *Hwangtae* was separated by size-exclusion chromatography on a Sephadex G-25 column, cation-exchange chromatography on a SP-Sephadex C-25 and reversed-phase HPLC using the 0.1% TFA-acetonitrile system and fractionated to six peptides (8). Wilding et al. reported that the ratio of hydrophilic/hydrophobic peptides is the most important factor influencing functional properties such as whippability and emulsifying activity (29). The retention of peptides depends on their size as well as their polarity. From the RP-HPLC profiles, a decrease in the hydrophobic peptides and an increase in the peptide material eluted earlier were noted as the degree of hydrolysis increased.

Nitrogen solubility

Solubility is probably the most important factor of protein and protein hydrolysate functional properties. Many of the other functional properties, such as emulsification and foaming, are affected by solubility, and therefore it is an excellent indicator of the protein hydrolysate functionality (29). Nitrogen solubility values for the isolated peptides from *Hwangtae* are shown in Table 3. APO2 (92.4%) and APO1 (91.4%) had significantly higher solubility than other isolated peptides from *Hwangtae*. The solubilities of APO1 and APO2 were similar to those reported by Sathivel et al. (11) for egg albumin (89.8%) and higher than for soy protein concentrate (9.8%). Chobert et al. (30) reported that smaller peptide units had higher solubility than the intact proteins, and high solubility of FPHs is due to cleavage of proteins into smaller peptides that usually have increased solubility (4).

Althrough APACE and APO3 (>1.0 kDa) had a smaller or similar molecular size to that of the APO1 and APO2, the percent nitrogen solubility value of APACE (82.4%) and APO3 (78.4%) were lower than those of APO1 and APO2. A clear relationship between peptide size and percent nitrogen solubility was not found, indicating additional physicochemical properties of the proteins and peptides that may play an important role. This can be explained by the fact that hydrolysis exposes some of the hydrophobic groups to the surface. In addition, it converts some hydrophobic groups into hydrophilic ones by generating two end carbonyl and amino groups, as reported by Kristinsson and Rasco (3). APO2 and APO1 apperaed to have high nitrogen solubility values which indicated that peptides isolated from *Hwangtae* possesses potential applications in formulated food systems, such as enhancing product appearance and providing a smooth mouth feel.

Emulsifying capacity (EC) and emulsifying stability (ES)

The emulsifying properties of fish protein hydrolysate are directly connected to their surface properties, or how effectively the hydrolysate lowers the interfacial tension between the hydrophobic and hydrophilic components in food. Proteins adsorb to the surface of freshly formed oil droplets during homogenization and form a protective membrane that prevents droplets from coalescing. Hydrolysates are surface active and promote oil-in-water emulsions because they have hydrophilic and hydrophobic functional groups and are water soluble.

EC values for peptides isolated from *Hwangtae* ranged from 19 to 39.5 (mL of oil per 200 mg of protein); the APO2 hydrolysate appeared to have higher emulsifying capacity value than other isolated peptides from *Hwangtae*, similar to the nitrogen solubility results (Table 3).

Table 3. Functional properties of isolated peptides from *Hwangtae* hydrolysate

Sample	Nitrogen solubility (%)	Emulsifying capacity [mL oil (200 mg)$^{-1}$ protein]	Emulsifying stability [% emulsifier]	Fat absorbtion [mL oil g^{-1} protein]
APO1	91.4±2.1^{a}	29.1±0.2^{b}	75.9±2.5^{a}	5.9±1.3^{b}
APO2	92.4±1.7^{a}	39.5±0.3^{a}	77.5±3.3^{a}	6.3±1.7^{b}
APO3	78.4±3.4^{b}	24.4±0.2^{c}	65.2±1.9^{b}	3.8±1.5^{c}
APACE	82.4±3.2^{b}	20.6±0.3^{c}	64.6±2.0^{b}	4.1±1.2^{c}
APG1	53.2±4.9^{c}	14.7±0.2^{d}	44.6±0.6^{c}	9.9±0.8^{a}
APG2	56.4±2.4^{c}	12.4±0.3^{d}	47.3±1.5^{c}	10.8±1.9^{a}

Values are mean±SD of triplicate determinations.
Mean values with the same superscript letter in each column are not significantly different ($p>0.05$).

The problem with small sized peptides obtained from enzyme hydrolysis is that they are not readily soluble or dispersible in foods and have poor emulsification properties. The smaller peptides often have reduced emulsifying properties. Kuehler and Stine (31) found that whey protein hydrolyzed with Prolase yield large-molecular-weight peptides with excellent emulsifying stability and activity. Indeed, a peptide is required to have a minimum length of about 20 residues to possess good emulsifying and interfacial properties (32). However, a clear relationship between the protein and peptide size and emulsifying properties was not evident in this study. Although small peptides are highly stable and diffuse rapidly and adsorb at the interface, they are less efficient in reducing the interfacial tension by other factors such as solubility and hydrophobicity which may also play major role in emulsifying properties.

Emulsion stability refers to the ability of an emulsion to resist changes in its properties over time. ES of peptides isolated from *Hwangtae* ranged from 44.6% to 77.5%, and was well correlated with emulsifying capacity. Kuehler and Stine (31) found that enzymatically hydrolyzed whey protein yields large-molecular-weight peptides with excellent emulsifying stability and activity. Peptide molecular characteristics and peptide chain length are the major reason for the different emulsification abilities of hydrolysates, but there are many different factors excepting peptide chain length that may account for the differences observed between peptides in both the ability to form an emulsion and emulsion stability. There are many different factors such as degree of hydrolysis (33), acetylation of peptide (34) and extraction solvent (35) that may account for the differences observed between hydrolysates and peptides in both the ability to form an emulsion and emulsion stability. Also, environmental conditions such as pH, ionic strength, temperature, etc. also have an effect on the emulsification properties. Further invetstigation needs to be carried out to improve emulsifying properties to use these isolated peptides form *Hwagtae* as human dietary emulsifiers in food systems.

Emulsifying capacity and emulsion stability have been used to evaluate whether the protein powder is able to form a stable protein food containing a high level of oil. If the emulsion capacity is high, it implies that the fish protein powder is a good emulsifier for forming products such as mayonnaise.

Fat absorption (FA) capacity

Several factors may affect the ability of hydrolysates to bind fat, such as bulk density of the protein (36), degree of hydrolysis (37), and enzyme-substrate specificity (38). FA binding capacity values ranged from 3.8 to 10.8 mL of oil per gram of protein for isolated peptides from *Hwangtae*; APG1 and APG2 hydrolysates had greater amounts of high molecular weight proteins and significantly higher fat absorption capacity values (9.9 and 10.8 oil per gram of protein) than APOs and APACE (Table 3). These results were similar to that reported by Kristinsson (39) that the hydrolysates at 5% DH had significantly higher fat absorption (5.98 to 7.07 mL oil/g FPH) than 10% DH (3.22 to 5.12 mL oil/g FPH) and 15% DH (2.86 to 3.86 mL oil/g FPH) due to the larger peptide sizes in salmon protein. The fat absorption is attributed mostly to physical entrapment of the oil, and thus the higher the bulk density of the protein the more fat absorption. Fat-binding capacities of proteins correlate with surface hydrophobicity. Fat binding capacity of proteins and surface hydrophobicity by the larger peptide sizes. The capacity of a peptides and hydrolysates to absorb fat is an important attribute that not only influences the taste of the product, but is also an important functional characteristic that is required especially for the meat and confectionery industry. Peptides isolated from *Hwangtae* therefore could very well be used in such applications.

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