Biochemical and Functional Properties of Herring *(Clupea harengus)* Byproduct Hydrolysates

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ABSTRACT: The functional, nutritional, and antioxidative properties of hydrolyzed herring and herring byproducts (head and gonad) were evaluated. All freeze-dried herring fish protein hydrolysate (FPH) powders were light yellow and contained 77% to 87% protein. The degree of hydrolysis was 18.3%, 13%, 13%, and 10.1%, respectively, for head, whole fish, body, and gonad after 75 min digestion. All FPH powders had desirable essential amino acid profiles and mineral contents. The emulsifying capacity and stability of all FPH powders were lower than those of egg albumin and soy protein; the fat adsorption was comparable to that of egg albumin. The antioxidative activity of whole herring FPH was highest, followed by that of body, gonad, and head.

Keywords: antioxidant activity, enzyme hydrolysates, fish byproducts, functional properties, herring

Introduction

Large amounts of protein-rich byproducts from the seafood industry are discarded or processed into fish meal. Novel processing methods are needed to convert seafood byproducts into more profitable, marketable products. Many of these protein-rich seafood byproducts have a range of dynamic properties (Phillips and others 1994) and can potentially be used in foods as binders, emulsifiers, and gelling agents. Soy and milk proteins are widely used in many segments of the food industry, whereas amino acids and peptides are gaining use in energy drinks and other applications (O'Donnell and Dornblaser 2002).

Proteins from fish processing byproducts can be modified to improve their quality and functional characteristics by enzymatic hydrolysis (Shahidi 1994). Utilizing proteolytic enzymes, fish protein hydrolysates (FPHs) can be prepared with the peptides having new and/or improved properties. Extensive proteolysis using different enzymes can result in different hydrolysate properties including sensory characteristics such as bitterness (Lin and others 1997). A large number of proteolytic enzymes, including commercially available exopeptidases and endopeptidases, can be used to make hydrolysates. Some have obtained a food-grade status and are complied with standards imposed by international regulatory agents. The application of an endopeptidase to produce FPH from the whole herring has been reported (Hoyle and Merritt 1994; Liceaga-Gesualdo and Li-Chan 1999). However, there is a lack of information on FPHs from herring-processing byproducts and their functional, nutritional, and antioxidant properties.

A total of 32509 metric tons of herring were harvested in Alaska in 2000 (Crapo and Bechtel 2003). Most herring harvested in Alaska is processed into herring roe, a valuable commodity in the Japanese market. However, the male herring and spent female are generally used as byproducts in fish meal. The objective of this study was to develop and characterize FPHs from whole herring, herring body, herring head, and herring gonad that may be used as functional ingredients, nutritional supplements, and antioxidant agents. This research aims at elucidating opportunities for processors to make value added products from byproducts of herring roe processing.

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Materials and Methods

Materials

Fresh herring (*Clupea harengus*) were obtained from commercial fish processing plants in Kodiak, Alaska, U.S.A. Fish were immediately processed and fish parts, that is, whole herring (WH), body (HB) (only head and gonads were removed), heads (HH), and gonads (HG) were vacuum-packaged and stored at –40 °C until analyzed. The alcalase enzyme with activity of 2.4 Anson Units per gram (AU/g) was purchased from Novo Nordisk (Franklinton, N.C., U.S.A).

Preparation of fish protein hydrolysate

WH, HB, HH, and HG were thawed at 4 °C overnight and minced in a Hobart mincer (K5SS, Hobart Corp., Troy, Ohio, U.S.A.). Hydrolysis conditions were similar to those documented by Liceaga-Gesualdo and Li-Chan (1999) and Hoyle and Merritt (1994). A 500-g portion of each fish part was mixed with an equal volume of distilled water and homogenized in a Waring blender (Waring Products Div., New Hartford, Conn., U.S.A.) for about 2 min. The mixture was stirred and adjusted to pH 8.0 and 50 °C for optimal alcalase activity (Novo Nordisk 1995). The alcalase enzyme was added to the mince protein at 0.5% wt/wt. The mixture was continuously stirred for 60 min at 50 °C and then the temperature was increased to above 85 °C for 15 min to inactivate the enzyme. The hydrolysate was centrifuged at 2560 × *g* for 15 min and the soluble aqueous fraction decanted, freeze-dried, sealed in vacuum bags, and stored at 4 °C until used.

Degree of hydrolysis (DH) time course

Three separate experiments were performed to determine the effect of hydrolysis time on DH using the method of Hoyle and Merritt (1994). An equal volume of water was added to a 50-g portion of fish mince, and the mixture was adjusted to pH 8.0 and 50 °C. The alcalase enzyme was added to the mince protein at 0.5% wt/wt. At the end of each hydrolysis time of 0, 15, 30, 45, 60, and/or 75 min, an aliquot (50 mL) was removed and mixed with 50 mL of 20% trichloroacetic acid (TCA) to obtain 10% TCA-soluble nitrogen and 10% TCA-insoluble nitrogen and then centrifuged at 2560 × *g* for 15 min. The supernatant was decanted and analyzed for nitrogen by a combustion method using the Leco FP-2000 nitrogen analyzer (LECO Corp., St. Joseph, Mich., U.S.A.) that was calibrated with ethylenediaminetetraacetic acid as outlined in the Leco FP-2000 nitrogen analyzer manual.

The degree of hydrolysis (DH) was calculated as:

$$DH = \frac{[10\%\ TCA - \text{soluble N in the Sample}]}{\text{Total N in the Sample}} \times 100$$

Proximate composition

FPH samples were analyzed in triplicate for moisture and ash using the AOAC standard methods 930.15 and 942.05, respectively (AOAC 1995). The lipid content was determined in triplicate using the Leco FA-100 analyzer (LECO Corp.). The nitrogen content was determined in triplicate using the Leco FP-2000 nitrogen analyzer. The protein content was calculated as percent nitrogen times 6.25.

Amino acid and mineral analysis

Amino acid analysis was conducted at the AAA Service Laboratory Inc. (Boring, Oreg., U.S.A.). All FPH samples for amino acid analysis were hydrolyzed in 6 *N* HCl and 2% phenol at 110 °C for 22 h. Samples for cysteine analysis were oxidized with performic acid before hydrolysis. Amino acids were quantified using the Beckman 6300 sodium hydrolysate method with post column ninhydrin derivatization.

The mineral content of all FPHs was determined in triplicate by the acid digestion method involving microwave technology (CEM microwave, MDS-2000, CEM Corp., Matthews, N.C., U.S.A.). A 0.5-g FPH sample was placed in a vessel, and 6 mL HNO_3 was added. The sealed vessel was heated until digestion was completed. The sample was cooled for 5 min. The inductively coupled argon plasma machine (Model CIROS, SPECTRO Analytical Instruments, Kleve, Germany) was used to analyze the mineral content.

Color and sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Color of the FPH powders was determined using a Minolta chromameter (Model CR-300, Minolta Co., Ltd, Osaka, Japan) and reported as L^*, a^*, and b^*. The SDS-PAGE was performed in a Mini-Protein electrophoresis unit (Bio-Rad Laboratories, Richmond, Calif., U.S.A.) under reducing conditions according to Laemmli (1970), using a 10% acrylamide separating gel and a 4% acrylamide stacking gel. The molecular mass standards were purchased from Bio-Rad Laboratories. The proteins were visualized from the gels stained with Coomassie blue.

Functional properties of herring protein hydrolysates

Three separate experiments for each FPH powder were conducted for determination of functional properties. Purified egg albumin (J.T. Baker Inc., Phillipsburg, N.J., U.S.A.) and soy protein concentrate powder (Central Soya Inc., Fort Wayne, Ind., U.S.A.) were used as protein references. All results were reported on a protein content basis.

The nitrogen solubility was determined following the procedure of Morr and others (1985). A total of 500 mg of each FPH sample was dispersed in 50 mL of 0.1 *M* NaCl at pH 7.0; the solution was stirred for 1 h at 25 °C and centrifuged at 2560 × *g* for 30 min. The supernatant was analyzed for nitrogen using the Leco FP-2000 Nitrogen Analyzer. The solubility of FHPs, defined as the amount of soluble nitrogen from the total nitrogen, was calculated as follows:

$$\text{Nitrogen solubility (\%)} = \frac{\text{Supernatant nitrogen concentration} \times 100}{\text{Sample nitrogen concentration}}$$

Emulsifying capacity was measured by an oil titration method similar to that of Webb and others (1970). A total of 200 mg of FHP samples was dissolved in 20 mL 0.1 *M* NaCl solution in a tared 400-mL beaker. A motorized stirrer of a homogenizer (model 6-105-AF, Virtis Co, Gardner, N.Y., U.S.A.) was immersed in the solution in the beaker. A separatory funnel filled with 100% pure soybean oil (Hunt-Wesson Inc., Fullerton, Calif., U.S.A.) was placed above the beaker. A pair of electrodes connected to a multimeter (True RMS multimeter, John Fluke Co., Everett, Wash., U.S.A.) was immersed in the solution to measure the electrical resistance (in ohms) 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 get 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/s, generating an oil-in-water emulsion at 25 °C. A sudden increase in resistance was observed when the oil capacity of the FPH 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 measured by weighing the beaker and calculating the quantity in milliliters by correcting for oil density (0.9112 g/mL). The emulsifying capacity was expressed as milliliters of emulsified oil per 200 mg protein.

Emulsifying stability was evaluated according to the method of Yasumatsu and others (1972); 500 mg of FHP samples was transferred into a 250-mL beaker 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 a rheostat was immersed in the mixture, and mixed 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 23 °C, and then the milliliter of aqueous volume was read against the total emulsion volume (25 mL). Emulsion stability (%) was calculated as [(total volume – aqueous volume)/total volume] × 100.

The fat absorption (FA) of the FPH samples was measured according to the method of Shahidi and others (1995). A total of 500 mg sample of each FHP was put into a 50-mL centrifuge tube and 10 mL soybean oil was added. The sample was thoroughly mixed with a small steel spatula, kept for 30 min at 25 °C and mixed every 10 min, and then centrifuged for 25 min at 2560 × *g*. Free oil was then decanted and the fat absorption of the sample determined from the weight difference. The fat absorption was expressed in terms of milliliters of fat absorbed by 1 g of protein.

Antioxidative analysis

The antioxidative activity of the FPH was determined by the thiocyanate method (Osawa and Namiki 1981; Shih and others 2002). Food grade antioxidants, α-tocopherol, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) (Sigma Chemical Co., St. Louis, Mo., U.S.A.), were used as references. Each FPH (0.2 mg) was added to a solution containing 0.13 mL linoleic acid, 10

Table 1—Proximate composition of whole herring and herring byproducts[a,b]

Composition (%)	WH	HB	HH	HG
Protein	14.5 ± 0.1c	16.9 ± 0.5b	13.1 ± 0.3d	18.0 ± 0.5a
Fat	8.8 ± 0.5b	6.6 ± 0.7c	10.9 ± 0.6a	3.4 ± 0.2d
Moisture	73.9 ± 0.6b	74.4 ± 0.1b	72.4 ± 0.2c	77.5 ± 0.8a
Ash	3.0 ± 0.2b	2.0 ± 0.2c	3.9 ± 0.3a	1.4 ± 0.0d

[a]Means with different letters in each row are significantly different ($P < 0.05$). Values are means and SD of triplicate determinations.
[b]HB, herring body; HG, herring gonad; HH, herring head; WH, whole herring

Table 2—Proximate composition of whole herring and herring byproduct hydrolysates[a,b]

Composition (%)	WHH	HBH	HHH	HGH
Protein	84.4 ± 0.5b	87 ± 0.2a	85.2 ± 0.4b	77 ± 0.4c
Fat	1.2 ± 0.2a	0.4 ± 0.1b	1.2 ± 0.2a	1.5 ± 0.2a
Moisture	4.3 ± 0.2b	3.0 ± 0.1d	3.5 ± 0.2c	6.2 ± 0.3a
Ash	10.3 ± 0.3b	10.1 ± 0.1b	10.1 ± 0.3b	15.3 ± 0.1a

[a]Means with different letters in each row are significantly different ($P < 0.05$). Values are means and SD of triplicate determinations.
[b]HBH, herring body hydrolysate; HGH, herring gonad hydrolysate; HHH, herring head hydrolysate; WHH, whole herring hydrolysate.

mL of 99.0% ethanol, and 10 mL of phosphate buffer (pH 7.0), and distilled water added to achieve a total volume of 25 mL. The mixed solution was incubated in a conical flask at 40 °C. On the 5th day of inhibition, a peroxide value was determined using the thiocyanate method. A 0.2-mL sample mixture was added to 9.4 mL ethanol (75%) and 0.2 mL aqueous ammonium thiocyanate (30%), and then 0.2 mL of ferrous chloride solution (20 m*M* in 3.5% HCl) was added sequentially. After stirring 3 min, the absorbance was measured at 500 nm. Methanol was used as a control. The antioxidative capacity of inhibiting the peroxide formation in linoleic acid was expressed as follows:

$$\text{Inhibition \%} = \{1 - [(\text{absorbance of sample at 500 nm})/(\text{absorbance of control at 500 nm})]\} \times 100$$

Statistical analysis

Statistical significance of observed differences among means of experimental results was evaluated by analysis of variance (SAS 2002), followed by post-hoc Tukey's studentized range test.

Results and Discussion

Proximate composition

The proximate composition of whole herring and herring byproducts is given in Table 1. The herring and herring byproducts contained 72.4% to 77.5% moisture, and the protein content was 14.5%, 16.9%, 13.1%, and 18% for WH, HB, HH, and HG, respectively. The fat content of WH, HB, and HH, ranging from 6.6% to 10.9%, was significantly ($P < 0.05$) higher than that (3.4%) of HG. The proximate composition of the FPH samples derived from whole herring, herring body, herring head, and herring gonad is shown in Table 2. The whole herring hydrolysate (WHH), herring body hydrolysate (HBH), and herring head hydrolysate (HHH) had high protein contents ranging from 84.4% to 87%, whereas herring gonad hydrolysate (HGH) had a significantly lower value of 77%. These results are similar to those of Liceaga-Gesualdo and Li-Chan (1999) and Hoyle and Merritt (1994) who reported the protein content of herring FPH ranging from 77% to 87.9%. The fat content was ≤ to 1.5% in all hydrolysate samples because lipid was removed during the centrifugation step before freeze-drying. The ash content of WHH, HBH, and HHH was approximately 10% compared with 15% of HGH, which was significantly higher ($P < 0.05$). Other investigators have reported FPH with ash contents of 9% to 22% (Onodenalore and Shahidi 1996; Benjakul and Morrisey 1997; Liceaga-Gesualdo and Li-Chan 1999).

Degree of hydrolysis

The time courses for hydrolysis of whole herring, heads, gonads, and body are shown in Figure 1. There was a steady increase in DH% with increased time for all samples. The DH% after 75 min of hydrolysis was 18.3%, 13%, 13%, and 10.1%, respectively, for HH, WH, HB, and HG. Mackie (1982) stated that after an initial rapid phase of hydrolysis, the rate of hydrolysis tends to decrease, entering a stationary phase. In this study, there was an initial rapid rate of hydrolysis followed by a decreasing rate of hydrolysis. The shape of the hydrolysis curve is similar to those previously published for herring protein hydrolysates (Hoyle and Merritt 1994; Liceaga-Gesualdo and Li-Chan 1999). The yield of hydrolysate was not calculated. However, the data from Figure 1 provide a preliminary estimate of the amount of fish protein that can be hydrolyzed.

Table 3—Color *L, *a**, *b** values of whole herring and herring byproduct hydrolysates[a,b]**

Samples	*L**	*a**	*b**
WHH	89.4 ± 1.3a	3.3 ± 0.1b	8.0 ± 0.4c
HBH	84.3 ± 0.2b	2.8 ± 0.1c	13.4 ± 0.8b
HHH	79.3 ± 2.5c	4.2 ± 0.2a	14.0 ± 0.5b
HGH	74.6 ± 3.9d	3.1 ± 0.2b	18.0 ± 1.5a

[a]Means with different letters in each column are significantly different ($P < 0.05$). Values are means and SD of triplicate determinations.
[b]HBH, herring body hydrolysate; HGH, herring gonad hydrolysate; HHH, herring head hydrolysate; WHH, whole herring hydrolysate.

Color and odor

The FPH powders were light yellow in color (Table 3). HGH was the darkest ($P < 0.05$; $L^* = 74.6$) and most yellowish ($b^* = 18$), whereas

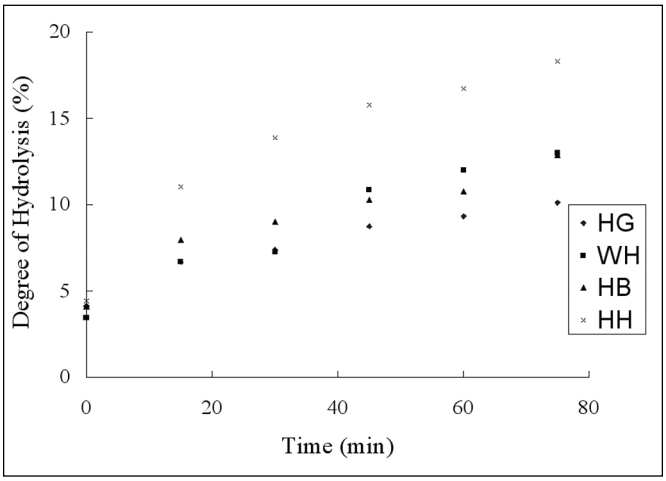

Figure 1—Effect of hydrolysis time on degree of hydrolysis (DH%) of whole herring and herring byproduct hydrolysates. Points are means of triplicate determinations.

Table 4—Amino acid composition of whole herring and herring byproduct hydrolysates[a,b]

Amino acids	WHH	HBH	HHH	HGH	EAA[c]
Cysteine	11.2	11.2	11.1	5	
Aspartic acid	91.5	93.8	89.2	86.6	
Threonine	39.6	39.3	40.4	46.7	9
Serine	42.0	41.1	45.3	44.6	
Glutamic acid	168.4	163.4	145.1	161.3	
Proline	46.2	41.0	58.9	44.7	
Glycine	75.0	69.8	95.0	84.5	
Alanine	70.4	70.1	69.4	52.9	
Valine	45.3	44.7	45.7	52.5	13
Methionine	32.1	33.3	33.1	32.8	17[d]
Isoleucine	32.4	33.2	32.6	39.5	13
Leucine	77.5	78.8	71.2	77.0	19
Tyrosine	25.1	26.3	27.4	38.8	
Phenylalanine	33.7	34.9	37.2	42.8	
Histidine	20.6	26	18.9	23.8	16
Lysine	99.2	106.6	80.4	65.5	16
Arginine	74.2	70.5	73	96.3	

[a]Data expressed as milligrams of amino acid per gram protein.
[b]HBH, herring body hydrolysate; HGH, herring gonad hydrolysate; HHH, herring head hydrolysate; WHH, whole herring hydrolysate.
[c]Suggested profile of essential amino acid requirements for adult humans by FAO/WHO (1990).
[d]methionine + cysteine.

Table 5—Mineral content (ppm) of whole herring and herring byproduct hydrolysates[a,b]

Minerals	WHH	HBH	HHH	HGH
Al	9.0 ± 2.3a	11.04 ± 4.4a	8.8 ± 2.6a	8.7 ± 1.8a
B	19.3 ± 14.2a	2.5 ± 0.2a	18 ± 10.2a	9432.1 ± 211.4b
Ca	1061 ± 135.9a	687.3 ± 51.4b	1205.7 ± 12.7a	366 ± 53.7c
Cu	4.0 ± 0.6b	2.4 ± 0.1c	3.2 ± 0.2b	6.4 ± 0.1a
Fe	41.2 ± 14.7b	16 ± 4.9c	43.5 ± 1.6ab	66.9 ± 2.2a
K	26482.7 ± 1132b	31868 ± 172a	22971.3 ± 209.7c	26915.3 ± 605.4b
Mg	2085.4 ± 387.8a	1926.7 ± 6.7a	1610.4 ± 4.6ab	1118.1 ± 136.2b
Na	13303.2 ± 1996.4c	9321.3 ± 66.6d	19830 ± 108.1b	23811.6 ± 849.8a
P	16999.8 ± 1912.8a	17103.5 ± 156.7a	10777.5 ± 66.6b	16451.2 ± 467.4a
S	10718.3 ± 1196.2b	10305.3 ± 56.5b	12849 ± 156.5a	11795.7 ± 325.3ab
Zn	12.6 ± 2.8bc	9.8 ± 0.7c	18.2 ± 0.3ab	24.5 ± 5.4a

[a]Means with different letters in each row are significantly different ($P < 0.05$). Values are means and SD of triplicate determinations.
[b]HBH, herring body hydrolysate; HGH, herring gonad hydrolysate; HHH, herring head hydrolysate; WHH, whole herring hydrolysate.

Table 6—Functional properties of whole herring and herring byproduct hydrolysates[a,b]

Samples[b]	Nitrogen solubility (%)	Emulsification capacity (mL of oil/200 mg of protein)	Emulsification stability (% emulsified)	Fat absorption (mL of oil/g of protein)
WHH	85.1 ± 6.0ab	20.6 ± 2.8c	48.6 ± 0.9d	7.3 ± 0.2a
HBH	78.6 ± 4.8b	8.2 ± 2.3c	51.8 ± 1.8cd	5.5 ± 0.4bc
HHH	84.9 ± 4.4ab	21.5 ± 1.9c	53.3 ± 1.2cd	6.1 ± 0.1b
HGH	56 ± 1.0c	11.1 ± 1.5c	54.2 ± 2.9c	3.7 ± 0.2d
Egg albumin	89.8 ± 3.5a	327.7 ± 18.1a	72.3 ± 3.4a	5.1 ± 0.8c
SPC	9.8 ± 0.1d	76.3 ± 6.8b	62.2 ± 4.2b	3.6 ± 0.3d

[a]Means with different letters in each column are significantly different ($P < 0.05$). Values are means and SD of triplicate determinations.
[b]HBH = herring body hydrolysate; HGH = herring gonad hydrolysate; HHH = herring head hydrolysate; SPC = soy protein concentrate; WHH = whole herring hydrolysate.

WHH was the lightest ($L^* = 89.4$) and least yellowish ($b^* = 8.0$). Descriptive sensory properties of the FPHs were not evaluated in this study; however, a slight fish odor and taste, accompanied by a metallic taste, were apparent in the samples.

Amino acid analysis and mineral content

Protein quality can be evaluated using biological methods such as the protein efficiency ratio or net protein utilization, or chemical methods. In this study, the essential amino acid content of the FPHs was compared with the recommendations made by FAO/WHO (1990) for adult humans. All of the herring protein hydrolysates met or exceeded the essential amino acid requirements for adult humans (Table 4). Potassium, magnesium, phosphorus, sodium, sulfur, and calcium were abundant in the FPHs (Table 5). Among FPH powders, the highest level of B, Cu, Fe, and Zn was found in HGH.

Functional properties

Solubility is one of the most important physicochemical and functional properties of protein hydrolysates (Kinsella 1976; Mahmoud and others 1992). Table 6 shows nitrogen solubility values for the FPHs and reference proteins (egg albumin and soy protein concentrate). WHH, HBH, and HHH had above 78% solubility, whereas HGH had about 56% solubility. The solubility of egg albumin was not significantly different than that of WHH and HHH. Soy protein concentrate (SPC) was less soluble than the FPHs, having a solubility of 9.8%. The high solubility of FPHs is due to cleavage of proteins into smaller peptide units that usually have increased solubility (Shahidi 1994). In addition, insoluble protein fractions were removed by centrifugation before the FPHs were freeze-dried. The high nitrogen solubility of FPHs indicates potential applications in formulated food systems by providing attractive appearance and smooth mouthfeel to the product (Petersen 1981).

The emulsifying capacity of herring FPHs was compared with egg albumin and soy protein concentrate (Table 6). FPHs had lower emulsifying capacities ($P < 0.05$) than both of the reference proteins. Emulsifying stability of FPHs ranged from 48.6% to 54.2%, compared with that of egg albumin (72.3%) and SPC (62.2%). Emulsifying stability values of 52% to 61.0% were reported for Atlantic salmon (*Salmo salar*) FPHs (10% to 15% DH) by Kristinsson and Rasco (2000).

The difference in emulsifying properties observed among hydrolysates may have been due to hydrophobicity (Gauthier and others 1993) and peptide lengths (Jost and others 1977). The smaller peptides often have reduced emulsifying properties (Chobert and others 1988). A positive correlation between surface activity and peptide length was reported (Jost and others 1977), and a peptide should have a minimum length of 20 residues to possess good emulsifying and interfacial properties (Lee and others 1987). The molecular weights of the FPHs, which were hydrolyzed for 60 min, were analyzed by SDS-PAGE electrophoresis. Results (not shown) indicated that FPHs had a molecular weight below 14400 daltons. This observation may explain the low emulsifying capacity of the herring FPHs.

Fat binding capacity (fat absorption) is an important functional characteristic of ingredients used in the meat and confectionary industries. As shown in Table 6, WHH had the highest fat absorption capacity (7.3 mL/g protein), whereas SPC had the lowest fat absorption capacity (3.6 mL/g of protein). Both WHH and HHH had greater ability ($P < 0.05$) to bind soybean oil than did HGH, egg albumin, and SPC. We observed that HGH and SPC did not disperse well in soybean oil and that both had the tendency to clump. The fat absorption capacity range of 2.86 to 7.07 mL of oil/g protein for Atlantic salmon FPH was reported by Kristinsson and Rasco (2000). Bulk density of the protein (Kinsella 1976), degree of hy-

Table 7—Antioxidative activity of whole herring and herring byproducts hydrolysates[a,b,c]

Sample	Inhibition %[d]	Absorbance at 500 nm
Control	0.0 ± 0.0	2.8 ± 0.1a
BHA	95.6 ± 0.2a	0.1 ± 0.0e
BHT	97.5 ± 0.1a	0.1 ± 0.0e
Tocopherol	86.6 ± 6.5a	0.4 ± 0.2e
WHH	48.8 ± 4.8b	1.4 ± 0.1d
HBH	44.4 ± 2.8bc	1.6 ± 0.6cd
HHH	15.7 ± 3.2d	2.4 ± 0.1b
HGH	33.7 ± 6.1c	1.9 ± 0.2c

[a]Means with different letters in each column are significantly different ($P < 0.05$). Values are means and SD of 9 determinations.
[b]Capacity to inhibit the peroxide formation in linoleic acid at Day 5.
[c]BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; HBH, herring body hydrolysate; HHH, herring head hydrolysate; HGH, herring gonad hydrolysate; WHH, whole herring hydrolysate.
[d]Inhibition % = {1 – [(absorbance of sample at 500 nm)/(absorbance of control at 500 nm)]} × 100.

drolysis (Kristinsson and Rasco 2000), and enzyme substrate specificity (Haque 1993) appear to affect the ability of hydrolysates to bind fat.

Antioxidative activity

Lipid oxidation deteriorates flavor, color, and nutritional quality of foods (Nawar 1996), and effective natural antioxidants play an important role in the food industry. The control (without added antioxidant) had the highest absorbance value while being stored at 40 °C, indicating the highest degree of oxidation among all samples (Table 7). Among herring FPHs, WHH (48.8%) and HBH (44.4%) had much higher antioxidant activities than HHH (15.7%). The antioxidant activity of WHH and HBH was about 0.5 × less than that of α-tocopherol (86.6%), BHA (95.6%), and BHT (97.5%). Many proteins have been shown to have antioxidative activity against peroxidation of lipids or fatty acids. Kawashima and others (1979) investigated the effects of many synthetic peptides on lipid oxidation and found that some peptides having branched-chain amino acids (Val, Leu, ILe) showed antioxidative activity. Chen and Decker (1994) postulated that peptides containing basic amino acids are electron acceptors that take electrons from radicals formed during the oxidation of unsaturated fatty acids. The antioxidative activity of FPHs may function as a chain stopper for the free radical chain mechanism of unsaturated fatty acid oxidation.

Conclusions

This study demonstrates that hydrolysates derived from herring or its byproducts may potentially serve as a good source of desirable quality peptides and amino acids. The herring FPHs have desirable color, antioxidant properties, solubility, fat absorption, and emulsification stability. They can potentially compete with hydrolysates and protein powders currently available in the marketplace. Drying the herring FPHs using a freeze-dryer is relatively expensive. Alternative drying processes needs to be further investigated to lower the production cost.

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