Biochemical and Functional Properties of Atlantic Salmon (*Salmo salar*) Muscle Proteins Hydrolyzed with Various Alkaline Proteases

Hordur G. Kristinsson*,† and Barbara A. Rasco‡

Institute for Food Science and Technology, The School of Fisheries, University of Washington, Seattle, Washington 98105

Protein hydrolysates (5, 10, and 15% degrees of hydrolysis) were made from minced salmon muscle treated with one of four alkaline proteases (Alcalase 2.4L, Flavourzyme 1000L, Corolase PN-L, and Corolase 7089) or endogenous digestive proteases. Reaction conditions were controlled at pH 7.5, 40 °C, and 7.5% protein content, and enzymes were added on the basis of standardized activity units (Azocoll units). Proteases were heat inactivated, insoluble and unhydrolyzed material was centrifuged out, and soluble protein fractions were recovered and lyophilized. Substrate specificities for the proteases was clearly different. Protein content for the hydrolysates ranged from 71.7 to 88.4%, and lipid content was very low. Nitrogen recovery ranged from 40.6 to 79.9%. The nitrogen solubility index was comparable to that of egg albumin and ranged from 92.4 to 99.7%. Solubility was high over a wide range of pH. The water-holding capacity of fish protein hydrolysates added at 1.5% in a model food system of frozen minced salmon patties was tested. Drip loss was on average lower for the fish protein hydrolysates than for egg albumin and soy protein concentrate, especially for Alcalase hydrolysates. Emulsification capacity for fish protein hydrolysates ranged quite a bit (75−299 mL of oil emulsified per 200 mg of protein), and some were better than soy protein concentrate (180 mL of oil emulsified per 200 mg of protein), but egg albumin had the highest emulsifying capacity (417 mL of oil emulsified per 200 mg of protein). Emulsification stability for fish protein hydrolysates (50−70%) was similar to or lower than those of egg albumin (73%) or soy protein concentrate (68%). Fat absorption was greater for 5 and 10% degrees of hydrolysis fish protein hydrolysates (3.22−5.90 mL of oil/g of protein) than for 15% hydrolysates, and all had greater fat absorption than egg albumin (2.36 mL of oil/g of protein) or soy protein concentrate (2.90 mL of oil/g of protein).

Keywords: *Enzymatic hydrolysis; fish protein hydrolysates; functional properties; alkaline proteases; endogenous enzymes; Azocoll*

INTRODUCTION

Adding enzymes to hydrolyze food proteins is a process of considerable importance that can improve the physicochemical, functional, and sensory properties of the native protein without prejudicing its nutritive value. Hydrolyzing protein can also improve intestinal absorption. Commercially viable means for totally utilizing aquatic animals and using them as food have not been successful. Around 100 million tons of fish per year are being harvested, close to the maximum sustainable yields, of which 29.5% is transformed into fishmeal (Rebeca et al., 1991). More than 15% of the biomass harvested is considered processing waste and is not used as food. Vast amounts of protein-rich byproducts from the seafood industry are discarded without any attempt of recovery. Until recently, processors in many locations are no longer permitted to discard their offal directly to the aquatic environment. This results in refining costs for the material before discarding. Alternative uses for these materials must be developed. With a dramatically increasing world population and the danger of overfishing, there is a great need to utilize our sea resources with more intelligence and foresight. The application of enzyme technology to recover and modify fish protein may produce a broad spectrum of food ingredients or industrial products for a wide range of applications better utilizing protein byproducts.

Most of the initial work on fish protein hydrolysis was performed in the 1960s, most of it on fish protein concentrate (FPC) as a cheap nutritious protein source for developing countries (Kristinsson and Rasco, 2000a). Enzymatic hydrolysis of fish protein has been employed as an alternative approach for converting underutilized fish biomass into edible protein products, instead of animal feed or fertilizer (Suzuki, 1981; Diniz and Martin, 1996). The shortcomings of most of those studies are that they completely lack comparison data among different enzymes at the same activity level and often fail to characterize the final degree of hydrolysis of the product that is subject to functional analysis. A wide array of species have been studied in making fish protein hydrolysates (FPH). These include hake (*Urophycis chuss*) (Cheftel et al., 1971; Hale, 1972; Yanez et al., 1976), shark (*Isurus oxyrinchus*) (Limonta et al., 1981; Onodenalore and Shahidi, 1996), sardine (*Sardina pilchardus*) (Quaglia and Orban, 1987a,b; Quaglia and

* Author to whom correspondence should be addressed [fax (978) 281-2618; e-mail hordur@foodsci.umass.edu].

† Present address: Department of Food Science, University of Massachusetts at Amherst, Marine Foods Laboratory, Marine Station, Gloucester, MA 01930.

‡ Present address: Department of Food Science and Human Nutrition, Washington State University, P.O. Box 646376, Pullman, WA 99164-6376.

10.1021/jf990447v CCC: $19.00
Published on Web 03/02/2000

Orban, 1990; Sugiyama et al., 1991), herring (*Clupus harengus*) (Hoyle and Merritt, 1995), crayfish (Baek and Cadwallader, 1995), lobster (*Panulirus* spp.) (Vieira et al., 1995), capelin (*Mallotus villosus*) (Shahidi et al., 1995), dogfish (*Squalus acanthias*) (Diniz and Martin, 1996), Pacific whiting (*Merluccius productus*) (Benjakul and Morrissey, 1997), and Atlantic salmon (*Salmo salar*) (Kristinsson, 1998; Kristinsson and Rasco, 2000b). Many of those studies have given FPH with excellent functional properties such as high solubility (Quaglia and Orban, 1987a,b; Hoyle and Merritt, 1995; Vieira et al., 1995; Onodenalore and Shahidi et al., 1996; Kristinsson, 1998), good emulsifying properties (Kristinsson, 1998), high fat adsorption (Shahidi et al., 1995; Onodenalore and Shahidi, 1996; Kristinsson, 1998), good foaming properties (Shahidi et al., 1995; Onodenalore and Shahidi, 1996), and good water binding (Shahidi et al., 1995; Onodenalore and Shahidi, 1996; Kristinsson, 1998). These studies include a variety of proteolytic enzymes and processing conditions, making comparison difficult.

The choice of substrate and protease employed and the degree to which the protein is hydrolyzed can greatly affect the physicochemical properties of the resulting hydrolysates (Mullally et al., 1995). Enzyme substrate specificity is also important to hydrolysate functionality because it strongly influences its molecular size and the hydrophobic/hydrophilic balance; the broader the specificity, the smaller are the peptides produced and the more complex the peptide profile becomes (Gauthier et al., 1993; Mullally et al., 1994).

The aim of this study was to evaluate the biochemical and functional properties of salmon muscle protein hydrolyzed by different alkaline proteases, Alcalase 2.4L, Flavourzyme 1000L, Corolase PN-L, and Corolase 7089, and an endogenous enzyme extract added at the same activity level based upon Azocoll units to 5, 10, and 15% degrees of hydrolysis (DH). These results may be useful in at least two ways: (1) finding new means to utilize byproducts from the growing Atlantic salmon and trout aquaculture industry and (2) providing alternative means to utilize byproducts from wild Pacific salmon. The implications of this study can be transferred to any other fish substrate.

MATERIALS AND METHODS

Material. Fresh farm-raised 2-year-old Atlantic salmon (*Salmo salar*) was kindly donated by Seafreeze Inc. (Seattle, WA). The fish were eviscerated and filleted, and the pyloric ceca were collected and immediately frozen at −40 °C in sealed polyethylene bags, until needed for enzyme extraction. The fillets were deboned, skinned, and kept at −40 °C in sealed polyethylene bags along with meat collected from the fish frames. Prior to the enzymatic hydrolysis, the meat was minced to a uniform consistency in a Hobart mincer. The proximate composition of this mince was determined according to AOAC (1990).

Chemicals and Reagents. The enzyme preparations were of food grade and were chosen on the basis of their potential to hydrolyze fish protein and produce highly functional hydrolysates. Alcalase 2.4L (endoproteinase from *Bacillus licheniformis*) and Flavourzyme (endoproteinase and exopeptidase from *Aspergillus oryzae*) were donated by Novo Nordisk (Bagsvaerd, Denmark). Corolase PN-L (endoproteinase and exopeptidase from *Aspergillus sojae*) and Corolase 7089 (endoproteinase from *Bacillus subtilis*) were donated by Rohm Enzymes (Somerset, NJ), and an endogenous enzyme mixture was extracted from the salmon pyloric ceca.

Preparation of Endogenous Extract. This crude enzyme mixture was prepared by first thawing the frozen pyloric ceca at 2 °C, weighing 400 g, and mixing with 800 mL of cold (4 °C) buffer solution (10 mM Tris, 10 mM $CaCl_2$, pH 8.0). The mixture was homogenized in a Waring blender for 30 s following centrifugation at 10000*g* at 2 °C for 15 min. The supernatant was collected. Granular ammonium sulfate [$(NH_4)_2SO_4$] to give 10% saturation (66 g) and calcium chloride ($CaCl_2$) to give 50 mM were added slowly to the supernatant, with continuous stirring, and the solution was left to stand for 1 h on ice. The solution was then centrifuged for a second time at 10000*g*, 2 °C, for 15 min, and the supernatant was collected and stabilized by adding 20% (v/v) glycerol. The endogenous extract was kept frozen at −70 °C in 50 mL PVC tubes.

Enzyme Activity Assay. The hydrolytic activity of the enzymes and the endogenous extract was tested using a synthetic substrate, Azocoll (Calbiochem-Novabiochem, La Jolla, CA), according to the methods of Dean and Domnas (1983) and Ferreira and Hultin (1994) with some modification as follows. Precisely 50 mg of Azocoll was added to 0.1 N sodium phosphate buffer (pH 7.5) to give a total volume of 5.0 mL. The Azocoll suspension was transferred to an Aquatherm water bath (New Brunswick Scientific Inc., New Brunswick, NJ) at 40 °C. The proteases to be tested were added at between 10 and 140 μL, and the tubes were covered with a strip of Parafilm and the contents mixed by inverting three times. The tubes were then placed in the 40 °C water bath for 15 min and inverted three times at time 0, 5, and 10 min. The reaction was terminated by placing the tubes on ice for 5 min. The reaction mixture was then filtered through Whatman No. 1 filter paper and absorbance measured in a Bausch and Lomb Spectronic 21 spectrophotometer at 520 nm. The results were expressed as Azocoll units per gram of enzyme preparation; one Azocoll unit is defined as that amount of enzyme which produced an absorption of 0.1 at 520 nm under the conditions described above. The temperature and pH used for the assay were the same as for the protein hydrolysis experiment. All enzymes were assayed using Azocoll from the same batch. This way it was possible to get an estimate of the quantity of each enzyme needed to obtain a uniform level of proteolytic activity for all of the enzymes under the reaction conditions tested.

The endogenous proteolytic activity of the substrate was also measured using this Azocoll method, using a diluted extract prepared from salmon muscle mince.

Enzymatic Hydrolysis. The substrate was prepared by homogenizing salmon muscle mince with distilled water to a protein substrate of 7.5% (%N × 6.25) to give 300 g. The reaction vessel (Pyrex 6947) containing the reaction mixture was put into an Aquatherm water bath (New Brunswick Scientific Inc.) at 40 °C. The mixture was stirred using a Welch 5230 stirrer (WM Welch Scientific Co., Chicago, IL) at 70% output of 140 V. The pH of the mixture was adjusted to 7.5 using 1 N NaOH. A reaction pH of 7.5 was chosen because it allowed for good dissociation of the α-NH groups.

Enzyme preparations (in sodium phosphate buffer, pH 7.5) were first slowly added to the substrate at the same activity level, 29264 AzU (Azocoll units) to examine their kinetics on the substrate as presented by Kristinsson and Rasco (2000c). To reach the set degrees of hydrolysis, it proved to be necessary to add certain enzymes at a higher activity. For Alcalase, 175584 AzU (0.262 g) was used to reach 10% DH and 292640 AzU (0.437 g) to reach 15% DH. For Corolase PN-L, 146320 AzU (5.575 g) was used to reach 10% DH and 204848 AzU (7.805 g) to reach 15% DH. For Flavourzyme, 143318 AzU (2.403 g) was used to reach both 10 and 15% DH. For Coroalse 7089 29264 AzU (2.540 g) was used to reach all DH levels and likewise for the endogenous extract (24.165 g). Controls were reaction mixtures to which no enzymes were added. The hydrolysis process is outlined in Figure 1. The pH was monitored and 0.5 N NaOH base added to maintain a constant pH of 7.5. The amount of base added was recorded at set time intervals and used to calculate the DH. The DH is calculated from the volume and molarity of base or acid used to maintain constant pH and is expressed as the percent ratio of the numbers of peptide bonds broken (*h*) to the total numbers of

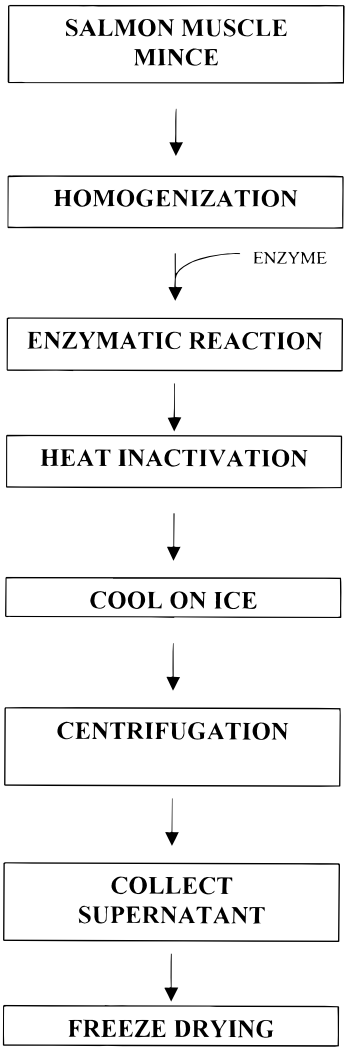

Figure 1. Enzymatic hydrolysis process for FPH from salmon muscle mince.

bonds per unit weight according to the formula (Adler-Nissen, 1986)

$$\%\mathrm{DH} = \frac{h \times 100}{h_{\mathrm{tot}}} = \frac{BN_B}{\alpha h_{\mathrm{tot}} \times \mathrm{MP}} \times 100$$

where B = base consumption in mL (or acid in the case of acid proteases); N_B = normality of the base (or acid); α = average degree of dissociation of the α-NH groups (0.5516, see below); MP = mass of protein in grams (%N × 6.25), 22.46 g; and h_{tot} = the total number of peptide bonds in the salmon protein substrate (7.501 mequiv/g).

The degree of dissociation is calculated as

$$\alpha = 10^{\mathrm{pH}-\mathrm{p}K}/(1 + 10^{\mathrm{pH}-\mathrm{p}K})$$

The pK value varies significantly with temperature but is relatively independent of the substrate as such. The pK values at different temperatures (T in Kelvin) can be calculated according to the method of Steinhardt and Beychok (1964):

$$\mathrm{p}K = 7.8 + \frac{298 - T}{298T} \times 240$$

When the reaction had reached the set degree of hydrolysis (5, 10, or 15%), it was terminated by immersing the reaction vessel into a water bath (95 °C) for 15 min with occasional stirring to inactivate the proteolytic enzymes. The reaction mixture was directly transferred to 250 mL centrifugation bottles, put on ice in a refrigerator, and cooled to room temperature. The reaction mixture was then centrifuged in a Beckman model J2-21M induction drive centrifuge at 10000*g* for 10 min at 2 °C. The supernatant was collected, frozen at −70 °C, and later freeze-dried in glass bottles in a Virtis freeze-dryer. After freeze-drying, argon gas was injected into the bottles, and they were immediately capped to protect the samples from oxidation. Samples were kept at 4 °C until needed for functional tests.

Proximate Analysis of Substrate and Hydrolysates. The substrate and selected hydrolysate products were analyzed for protein using a modified $CuSO_4/TiO_2$ mixed catalyst Kjeldahl method according to method 4.2.03 (AOAC, 1990), moisture content was determined according to the convection-less oven method 24.003 (AOAC, 1990), total mineral content was determined according to the direct ash method 14.006 (AOAC, 1990), and lipid content was determined according to the modified acid hydrolysis method 948.15 (AOAC, 1990). Nitrogen recovery was calculated as the amount of protein (%N × 6.25) present in the hydrolysates relative to the initial amount of protein present in the reaction mixture. This is an important measurement because a maximum recovery is desired in the production of hydrolyzed food proteins.

Functional Properties of Protein Hydrolysates. At least three separate experiments for each product were conducted for functional properties. In addition, two reference proteins were used: purified egg albumin, product 0440-04 (J. T. Baker Inc., Phillipsburg, NJ), and soy protein concentrate powder Promine DS (Central Soya Inc., Fort Wayne, IN). All results are reported on a protein content basis.

1. Solubility. The modified nitrogen solubility index (NSI) procedure of Morr et al. (1985) was used to measure the solubility of 500 mg protein hydrolysate samples. The solubility of the hydrolysate was expressed as the fraction of soluble N to total N and was calculated as

$$\text{protein solubility (\%)} = \frac{[\text{supernatant pr. concentration (mg/mL)} \times 50] \times 100}{\text{sample wt (mg)} \times [\text{sample pr. cont. (\%)}/100]}$$

The hydrolysate sample with the greatest solubility was further tested at a pH range of 2−11 to evaluate the effects of pH.

2. Emulsifying Capacity. Because the FPH emulsions were of low viscosity, the most accurate way to measure the emulsifying activity was to use an oil titration method similar to that of Webb et al. (1970) with some modifications. A model system was made by dissolving freeze-dried fish protein hydrolysates corresponding to 200 mg of protein in 20 mL of 0.1 M NaCl solution in a tared 400 mL beaker. A stirrer propeller attached to a motorized stirrer (Welch Scientific Co.) was immersed in the solution but not touching the bottom of the beaker. A 250 mL buret filled with 100% pure soybean oil (Hunt-Wesson Inc., Fullerton, CA) was placed above the beaker, and a tube was attached so the oil could be delivered into the beaker. A pair of electrodes was also immersed in the solution. The electrodes were connected to an A. W. Sperry DM-7A multimeter (A. W. Sperry Instruments, Inc., Hauppauge, NY), which measured the electrical resistant (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 immediately delivered into the beaker at ∼0.7 mL/s, generating an oil-in-water emulsion at 22−23 °C. Precise control of blender speed, rate of oil delivery, and emulsion temperature were essential for repeatability. 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, the oil delivery was stopped and the oil volume measured by weighing the beaker and calculating the quantity in milliliters by correcting for oil density (ϵ = 0.9112 g/mL). The emulsifying capacity was expressed as milliliters of emulsified oil per 200 mg of protein.

3. Emulsifying Stability. The emulsifying stability of each hydrolysate was evaluated according to the method of Yasumatsu et al. (1972) as modified by Miller and Groninger (1976) with some additional modifications to increase its repeatability and accuracy. Exactly 500 mg of freeze-dried fish protein hydrolysate was weighed and transferred into a 250 mL beaker. The sample was dissolved in 0.1 M NaCl to a total volume of 50 mL to make a 1% protein solution. To this was added 50 mL of soybean oil. A stirrer propeller attached to a motorized stirring unit (Welch Scientific Co.) driven by a rheostat was immersed in the mixture, which was then 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, and then the milliliters of aqueous volume to total volume was read. Emulsion stability was expressed as

$$\%\ \text{emulsifying stability} = \frac{(\text{total vol} - \text{aqueous vol}) \times 100}{\text{total vol}}$$

4. Fat Absorption/Oil-Holding Capacity. The ability of the hydrolysate to bind oil was measured according to the method of Shahidi et al. (1995) with some modifications. A 500 mg sample of freeze-dried hydrolysate was put into a 50 mL centrifuge tube. To the tube was added 10 mL of soybean oil, and the mixture was thoroughly mixed with a small steel spatula. The mixture was kept for 30 min at room temperature (23 °C), with mixing every 10 min, and then centrifuged for 25 min at 2000*g* in a Beckman model TJ-6 centrifuge (Spinco Division, Beckman Instruments Inc., Fullerton, CA). Free oil was then decanted and the fat absorption of the sample determined from the weight difference. The fat absorption was reported in terms of milliliters of fat adsorbed by 1 g of protein.

5. Water-Holding Capacity. Atlantic salmon muscle mince was used to measure water-holding capacity. The mince was kept on ice in a refrigerator at all times during its preparation. Exactly 1.5 g of FPH sample was mixed with 98.5 g of mince (1.5% FPH) and also for the egg albumin and soy protein concentrate. The control was mince containing no additive. The mince containing the protein hydrolysates was formed into uniform patties (1 cm × 8 cm, $n = 3$) weighing ~30 g. The patties were then transferred to individual zip-lock polyethylene bags packed again in larger bags to ensure protection from outside moisture. The patties were then transferred to a −20 °C freezer for 48 days.

After the storage period, the patties were removed and water loss was measured. The patties were carefully removed from the bags and placed on a plastic mesh (mesh size 0.5 × 0.5 cm), where they were left to thaw at room temperature for 2 h. The patties were then weighed in a tared plastic cup, and the weight difference of the patties was expressed as percent weight loss per 1.5% protein addition.

Molecular Characterization of the Hydrolysates. SDS−PAGE electrophoresis was performed on all samples according to the discontinuous electrophoresis method of Laemmli (1970) using a 4% stacking gel and 15% acrylamide gel. Protein content of samples was determined according to the method of Bradford (1976) using bovine serum albumin as a standard. The electrophoresis was performed in a water-cooled electrophoresis apparatus (OWL Instruments) using an LKB Bromma power supply. All chemicals were from Sigma Chemical Co. (St. Louis, MO).

The protein standard used was composed of aprotinin (6100 Da), α-lactalbumin (14200 Da), trypsin inhibitor (20100 Da), trypsinogen (24000 Da), carbonic anhydrase (29000 Da), glyceraldehyde-3-phosphate dehydrogenase (36000 Da), egg albumin (45000 Da), and bovine plasma albumin (66000 Da).

Statistical Analysis. To determine the differences in mean values based on the two to three experimental replications of each measurement, an analysis of variance and Tukey's studentized range test were used. Significance level was determined at the 95% probability level. The computer programs SYSTAT and Microsoft EXCEL were employed for data processing and statistical analysis on the results as well.

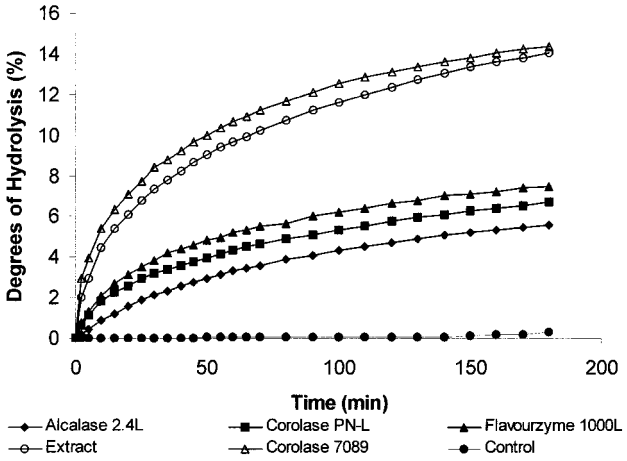

Figure 2. Enzymatic hydrolysis of salmon muscle mince by alkaline proteases and an endogenous enzyme extract (pH 7.5, 40 °C, 180 min, 7.5% substrate concentration, $n = 2$).

Table 1. Proximate Composition of Atlantic Salmon (*S. salar*) Mince and Fish Protein Hydrolysates

composition	Atlantic salmon mince (%)	Alcalase 10% DH (%)	end. extract 10% DH (%)
protein	20.32 ± 0.47	88.39 ± 0.55	71.67 ± 0.19
lipid	2.90 ± 0.37	0.23 ± 0.12	0.06 ± 0.04
moisture	75.17 ± 0.99	0.92 ± 0.22	4.21 ± 0.37
ash	0.91 ± 0.02	8.96 ± 0.13	22.34 ± 0.87

RESULTS AND DISCUSSION

The functionality of hydrolysates is a major factor in their success as functional food ingredients. To use a protein hydrolysate in a food system, it has to have chemical characteristics that function appropriately in that particular system. Protein hydrolysates thus have to be carefully produced under controlled conditions to a specific degree of hydrolysis and the products characterized to examine their functional properties to determine what purposes they might serve in food systems. A successful specific application of a functional protein ingredient or an additive used to enhance a single specific functional property may, however, not be transferrable to other food systems.

Enzymatic Hydrolysis. When the enzymes were added at the same activity levels to compare hydrolytic efficiencies, Corolase 7089 and the endogenous extract were the most efficient and Alcalase was the least efficient (Figure 2). The shape of the hydrolysis curve is typical of those previously published for fish protein hydrolysis (Hevia et al., 1976; Quaglia and Orban, 1987a,b; Baek and Cadwallader, 1995; Onodenalore and Shahidi, 1996) and milk protein hydrolysis (Mahmoud et al., 1992; Mutilangi et al., 1995). To achieve all three degrees of hydrolysis, it was necessary to use higher enzyme activity for some enzymes, as presented under Materials and Methods. No proteolytic activity was measured for the substrate alone at pH 7.5 (Figure 2).

Proximate Composition and Nitrogen Recovery. The freeze-dried FPH was a white to light yellow powder with almost no fishy odor and a slight fishy taste accompanied by a metallic taste possibly due to the relatively high mineral content. Table 1 shows the proximate composition of the substrate and protein hydrolysates containing the lowest and highest protein

Table 2. Protein Composition and Nitrogen Recovery for FPH at Different DH[a]

	protein content (%)			nitrogen recovery (%)		
FPH	5% DH	10% DH	15% DH	5% DH	10% DH	15% DH
Alcalase	86.92 ± 0.91[a]	88.39±.0.55[a]	88.12±.0.79[a]	46.83	57.03	74.64
Flavourzyme	84.26 ± 0.51[a]	82.71 ± 0.68[b]	78.95 ± 0.20[c]	42.92	58.06	79.89
Corolase PN-L	79.32 ± 0.05[a]	74.90 ± 0.39[b]	72.88 ± 0.72[c]	41.56	56.29	67.39
Corolase 7089	86.48 ± 0.33[a]	85.33 ± 1.57[ab]	82.41 ± 0.71[b]	51.95	61.79	74.50
end. extract	79.12 ± 0.02[a]	71.67 ± 0.19[b]	73.41 ± 0.14[c]	40.65	48.59	68.59

[a] Means with the same superscript letter in a row are not significantly different ($p > 0.05$).

contents. The ash content was relatively high in both hydrolysate samples measured but considerably higher for the endogenous extract hydrolysates. This is interesting because both samples are generated at the same DH and should contain the same amount of added base. Two factors might account for this. First, the endogenous extract was added in higher amounts than the commercial enzymes, due to lower activity, and the preparation contained CaCl, NaOH, and HCl. Second, it is possible that more acid/base was added to the runs using the endogenous extract when the initial pH was adjusted. The minced muscle had a low ash content, and each run was made from the same batch of mince, and it is very unlikely that it was the cause of this difference. Ash content is frequently high in fish protein hydrolysates (Benjakul and Morrisey, 1997; Onodenalore and Shahidi, 1996; Shahidi et al., 1995; Vieira et al., 1995). The endogenous extract hydrolysate also had a higher moisture content than that produced by Alcalase. The lipid content was low in the hydrolysates because lipids were most likely centrifuged out with insoluble protein fractions and substrate was relatively lean.

The protein content of the hydrolysates varied with both enzyme treatment and degree of hydrolysis (Table 2). The hydrolysates produced by Corolase PN-L and the endogenous extract had the least protein. Alcalase treatment resulted in hydrolysates with the highest protein content, followed by Corolase 7089 and Flavourzyme. Results here are similar to those of other published studies on FPH that have ranged from 63.4 to 90.8% protein (Yanez et al., 1976; Quaglia and Orban, 1987b; Yu and Fazidah, 1994; Shahidi et al., 1995; Cui, 1996; Onodenalore and Shahidi, 1996). Hydrolysates from land animal muscle such as mechanically deboned turkey (Fonkwe and Singh, 1996) and broiler chicken heads (Surowka and Fik, 1992) are close to the protein content in this study. Similar ranges of protein content as presented here have also been achieved with chemical hydrolysis to make FPC (Moorjani et al., 1968; Sen et al., 1969). To increase protein content, the ash content of hydrolysates may be reduced by ion exchange chromatography.

Nitrogen recovery increased as the hydrolysis progressed, and recovery varied among the enzyme treatments (Table 2). Nitrogen recovery was 40.65−51.95% for hydrolysates at 5% DH, increasing to 48.59−61.79% for 10% DH hydrolysates and to 67.39−79.89% at 15% DH. These recoveries are higher than those reported by others at the same DH. The nitrogen recovery at 5 and 10% DH was relatively low, with the hydrolysates produced by the endogenous extract having the lowest recovery and those produced by Corolase 7089 having the highest. At 15% DH the recovery was relatively high. Hydrolysates produced by Flavourzyme contained close to 80% of the initial protein/nitrogen. A lower nitrogen recovery of the endogenous extract is supported by previous studies. For example, a protein recovery of 22.9% was achieved for capelin hydrolyzed by endogenous enzymes under acid conditions (Shahidi et al., 1995). Autolysis by endogenous enzymes in red hake gave a lower protein yield compared to commercial enzymes (Hale, 1972). Studies using commercial neutral and alkaline proteases to hydrolyze fish substrate generally result in higher nitrogen recovery than endogenous enzymes (Shahidi et al., 1995; Benjakul and Morrissey, 1997).

Alkaline proteases have reportedly a greater capability to solubilize fish protein compared to neutral and acidic proteases, with the exception of pepsin (Sugiyama et al., 1991). As the proteolytic breakdown progresses, more soluble and suspendible peptides are released into the system, the size and chemical characteristics of these peptides depending in part on the enzyme specificity and the DH, with increasingly smaller fractions being produced at higher DH. In this study at lower DH, such as 5% DH, relatively large insoluble protein fractions remain in the reaction mixture. These insoluble large fractions are centrifuged out after protease inactivation, leaving the smaller soluble peptides in the supernatant. Many of the larger peptide fractions may also be bound to lipids in the mixture and removed with the lipids during centrifugation.

Electrophoresis reveals that hydrolysates have a markedly different peptide profile at different DH (Figure 3); peptide sizes decreased with increased hydrolysis, as observed by Quaglia and Orban (1990), Cui (1996), and Benjakul and Morrissey (1997). The maximum peptide size was ∼20 kDa, and the peptide profile was strongly enzyme dependent due to differences in substrate specificity.

Solubility of Hydrolysates. Solubility is one of the most important physicochemical and functional properties of protein hydrolysates (Kinsella, 1976; Mahmoud, 1992). A low solubility may cause an unattractive appearance and a sandy mouthfeel of the final product (Petersen, 1981). Table 3 provides solubility values for the hydrolysates and reference protein. The solubility of the hydrolysates was very high at pH 7.0 and 0.1 M NaCl, in every case >90%, with the majority of the hydrolysates being between 95 and 100% soluble (Table 3). The solubility of egg albumin was similar to that of FPH. Soy protein concentrate (SPC) was markedly less soluble than the hydrolysates, having only ∼20% solubility. This excellent solubility of FPH compared SPC indicates that it may have many potential applications in formulated food systems. Increases in solubility are expected with an increase in hydrolysis. For our FPH there is, however, no significant difference in the solubility with increasing DH. At 5% DH, the hydrolysates reached a maximum solubility that remained unchanged at higher DH (Figure 3). This solubility is most likely due to the fact that soluble and suspendible peptides were collected and freeze-dried, whereas unhydrolyzed insoluble fractions were centrifuged out.

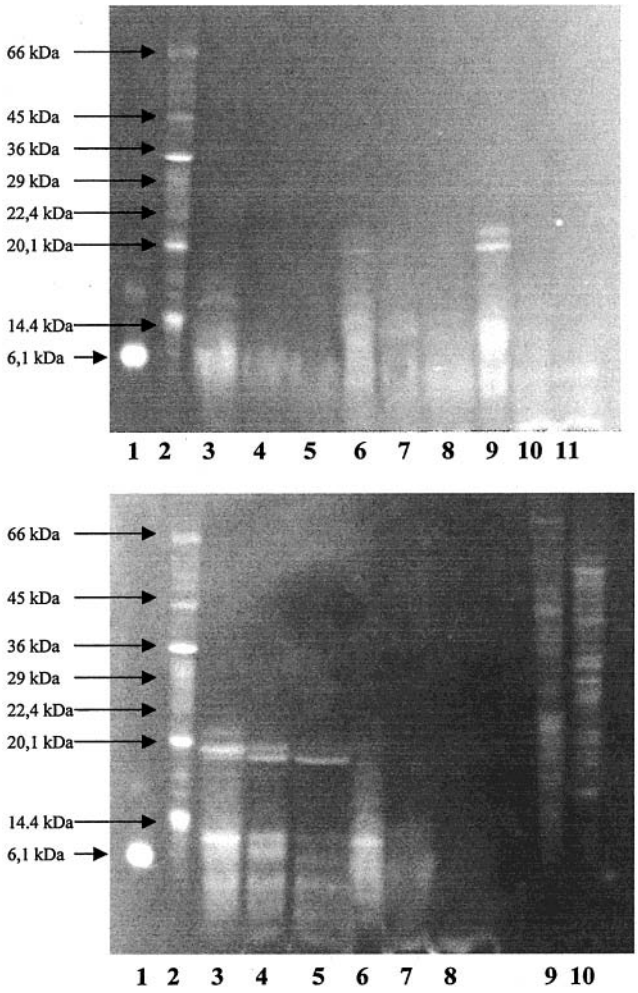

Figure 3. Electrophoresis of the FPH and control protein: (top) lane 1, aprotenin; lane 2, SDS Mark VII marker (14.4−66 kDa); lane 3, 5% DH FPH from Alcalase; lane 4, 10% DH FPH from Alcalase; lane 5, 15% DH FPH from Alcalase; lane 6, 5% DH FPH from Corolase PN-L; lane 7, 10% DH FPH from Corolase PN-L; lane 8, 15% DH FPH from Corolase PN-L; lane 9, 5% DH FPH from Flavourzyme; lane 10, 10% DH FPH from Flavourzyme; lane 11, 15% DH FPH from Flavourzyme; (bottom) lanes 1 and 2, same as above; lane 3, 5% DH FPH from endogenous extract; lane 4, 10% DH FPH from endogenous extract; lane 5, 15% DH FPH from endogenous extract; lane 6, 5% DH FPH from Corolase 7089; lane 7, 10% DH FPH from Corolase 7089; lane 8, 5% DH FPH from Corolase 7089; lane 9, egg albumin; lane 10, SPC.

Table 3. Nitrogen Solubility Index of FPH at Different DH and Reference Protein[a]

	nitrogen solubility index (%)		
FPH	5% DH	10% DH	15% DH
Alcalase	99.73 ± 0.25[a]	98.73 ± 0.57[a]	95.25 ± 0.26[b]
Flavourzyme	97.60 ± 0.65[a]	93.25 ± 0.55[b]	95.02 ± 0.57[c]
Corolase PN-L	96.51 ± 1.43[a]	96.29 ± 0.74[a]	92.41 ± 0.93[b]
Corolase 7089	94.43 ± 1.10[a]	93.30 ± 0.90[a]	96.64 ± 0.61[b]
end. extract	96.22 ± 0.58[a]	97.44 ± 2.84[a]	95.50 ± 1.35[a]
control protein			
egg albumin[b]	95.61 ± 0.86		
SPC[b]	20.08 ± 2.24		

[a] Means with the same superscript letter in a row are not significantly different ($p > 0.05$). [b] DH is unknown.

Previous studies on protein hydrolysate solubility report an increasing solubility at increasing DH as seen here (Petersen, 1981; McNairney, 1984; Cui, 1996). This connection between solubility and DH is believed to be primarily due to the decrease in peptide size, because smaller and more soluble peptides are produced at higher DH. The proper salt concentration is important for solubility, and proteins tend to be very soluble at favorable NaCl conditions. Increased solubility is partly through the formation of sodium salts of carbonyl groups of proteins, COONa (Venugopal and Shahidi, 1994).

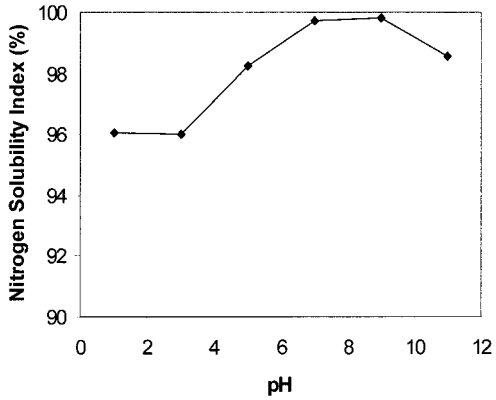

Figure 4. Solubility of salmon protein hydrolysate in 0.1M NaCl made with Alcalase (5% DH) over a pH range of 1−11.

Intact fish proteins are commonly believed to lack solubility in water (Venugopal and Shahidi, 1994), although recent studies have shown that fish protein can be quite soluble at very low ionic strength (Stefansson and Hultin, 1994; Feng and Hultin, 1997). Enzymatic breakdown of protein involves a major structural change in that the protein is gradually cleaved into smaller peptide units, having increasingly higher solubility than the intact protein. This increased solubility is partly due to the smaller peptide size but, most importantly, to the delicate balance of hydrophilic and hydrophobic forces of the peptides. The smaller peptides from myofibrillar protein are expected to have proportionally more polar residues, with increased ability to form hydrogen bonds with water and increasing solubility compared to that of the intact protein.

Solution pH is also important because pH influences the charge on the weakly acidic and basic side-chain groups; thus, proteins and protein hydrolysates generally exhibit lowest solubility at their isoelectric point and highest solubility when maximally charged (at low and high pH). Figure 4 shows the effect of pH on the solubility of 5% DH hydrolysate from Alcalase, which was the most soluble. FPH solubility was high at all pH values, indicating that the hydrolysate could have many useful applications in a variety of food systems. Salmon hydrolysates in this study had solubility similar to that of enzymatically hydrolyzed herring muscle protein when hydrolyzed by Alcalase and papain (Hoyle and Merritt, 1995) and Alcalase-hydrolyzed sardine muscle protein (Quaglia and Orban, 1987b). Alcalase produced the most soluble hydrolysates in our study at 5 and 10%. Other studies with FPH have shown solubility as a function of pH, in that solubility is slightly decreased around the isoelectric point (Vieira et al., 1995; Onodenalore and Shahidi, 1996). Excellent solubility of other muscle food protein hydrolysates as presented here has been observed with turkey meat (Fonkwe and Singh, 1996) and chicken head meat (Surowka and Fik, 1992, 1994).

There are no published data on functional properties of hydrolysates made with Corolase PN-L, Flavourzyme, Corolase 7089, and endogenous alkaline proteases. The endogenous extract compared reasonably with com-

Table 4. WHC of Salmon Mince Patties Containing 1.5% FPH at Different DH and Reference Protein[a]

FPH	water loss[b] (%)		
	5% DH	10% DH	15% DH
Alcalase	0.96 ± 0.28^{a1}	0.92 ± 0.37^{a1}	1.24 ± 0.39^{a}
Flavourzyme	1.79 ± 0.22^{a2}	1.92 ± 0.23^{a2}	2.17 ± 0.12^{a2}
Corolase PN-L	$2.13 \pm 0.22^{a2,3}$	2.09 ± 0.07^{a2}	$2.30 \pm 0.21^{a2,3}$
Corolase 7089	$2.74 \pm 0.37^{a3,4}$	$2.62 \pm 0.51^{a2,3}$	2.61 ± 0.13^{a3}
end. extract	$2.29 \pm 0.51^{a2,3,4}$	2.94 ± 0.03^{a3}	2.81 ± 0.53^{a3}
control protein			
egg albumin[c]	$2.98 \pm 0.43^{2,3}$		
SPC[c]	2.52 ± 0.51^{3}		
no addition	2.95 ± 0.46^{3}		

[a] Means with the same superscript letter in a row are not significantly different ($p > 0.05$). Means with the same superscript number in a column are not significantly different ($p > 0.05$). [b] Protein ingredient was added at 1.5% (w/w) basis to minced salmon patties. [c] DH is unknown.

mercial proteases in solubilizing the salmon mince, in contrast to the extensive study performed by Hale (1972) on autolysis of hake.

Water-Holding Capacity (WHC). The functional properties of proteins in a food system depend in part on the water−protein interaction. WHC refers to the ability of the protein to imbibe water and retain it against gravitational force within a protein matrix, such as protein gels or beef and fish muscle, and it is positively correlated with water-binding capacity (Damodaran, 1996). Several protein ingredients are well proven water-holding additives in muscle foods, including soy protein ingredients that can bind water at up to 5 times their weight. Fish proteins are not widely used as water-binding agents. The ability of freeze-dried FPH to bind and hold water in salmon patties at a 1.5% addition level as a model food system is given in Table 4. Enzyme treatment affected WHC, but it was unaffected by DH. Alcalase-treated salmon proteins had the greatest WHC. Alcalase hydrolysis yielded peptides with lower molecular weight than the other enzymes (Kristinsson, 1998). Corolase 7089 generated hydrolysates of molecular weight almost as low as those generated by Alcalase (Kristinsson, 1998); however, the WHC of these hydrolysates in the model was low. These enzymes all have different specificities yielding hydrolysates with different peptide makeups. The differences in peptide makeup might contribute to the differences observed. Alcalase and Corolase 7089 both have primarily endoprotease activities, whereas the others have a mix of both exo- and endoprotease activities. The exact activity for these is not known, whereas the endogenous extract was found to have mostly chymotrypsin activity (Kristinsson and Rasco, 2000b).

The reference proteins, especially egg albumin, were less effective in binding water in the minced patties than FPH. Egg albumin treated mince resulted in the highest water loss value of 2.98%, similar to that with no treatment (2.95%). SPC is frequently used to improve water binding, but in this application was found to be less effective than Alcalase hydrolysates at all DH and Flavourzyme at 5% DH. These FPH could therefore be suitable to use in several formulated meat systems. The only published studies on water-holding ability of FPH are on the cooking yield of meat systems treated with FPH, but drip losses were not reported. Adding capelin (Shahidi et al., 1995) and shark FPH (Onodenalore and Shahidi, 1996) in a pork model system has shown that a moderate increase in cooking yield is accompanied by an increase in FPH addition. A significant increase in cooking yield was also observed for a sardine protein concentrate made by ethanol extraction when added to hamburgers (Vareltzis et al., 1990)

Table 5. Emulsifying Capacity of FPH at Different DH and Reference Protein[a]

FPH	mL of oil/200 mg of protein		
	5% DH	10% DH	15% DH
Alcalase	192.51 ± 8.47^{a}	105.86 ± 1.33^{b}	74.80 ± 4.09^{c}
Flavourzyme	191.91 ± 1.73^{a}	119.76 ± 3.65^{b}	93.73 ± 1.82^{c}
Corolase PN-L	222.14 ± 10.78^{a}	209.17 ± 9.89^{a}	104.53 ± 10.11^{b}
Corolase 7089	234.79 ± 5.71^{a}	184.03 ± 11.45^{b}	100.42 ± 7.64^{c}
end. extract	298.77 ± 4.26^{a}	236.29 ± 19.00^{b}	154.96 ± 2.78^{c}
control protein			
egg albumin[b]	417.27 ± 13.34		
SPC[b]	179.84 ± 14.09		

[a] Means with the same superscript letter in a row are not significantly different ($p > 0.05$). [b] DH is unknown.

Table 6. Emulsifying Stability of FPH at Different DH and Reference Protein[a]

FPH	% emulsified		
	5% DH	10% DH	15% DH
Alcalase	70.3 ± 0.60^{a}	61.0 ± 1.0^{b}	50.7 ± 5.0^{c}
Flavourzyme	67.3 ± 2.3^{a}	55.7 ± 2.1^{b}	50.3 ± 3.3^{b}
Corolase PN-L	68.0 ± 1.0^{a}	61.5 ± 1.3^{b}	57.1 ± 3.2^{b}
Corolase 7089	70.2 ± 1.0^{a}	61.1 ± 1.0^{b}	50.8 ± 2.4^{c}
end. extract	69.7 ± 2.5^{a}	67.2 ± 0.8^{a}	58.4 ± 1.1^{b}
control protein			
egg albumin[b]	73.0 ± 1.0		
SPC[b]	68.0 ± 0.0		

[a] Means with the same superscript letter in a row are not significantly different ($p > 0.05$). [b] DH is unknown.

Emulsifying Properties. Tables 5 and 6 show emulsifying capacity and stability for FPH and reference protein. Egg albumin had the highest emulsifying capacity and highest stability. SPC had reasonably good stability. Hydrolysates produced with the endogenous enzymes had significantly higher ($p > 0.05$) emulsifying capacity at all DH than the other hydrolysates. Corolase 7089 and Corolase PN-L produced hydrolysates with the next best capacity to emulsify soybean oil and water. The emulsifying capacity dropped significantly with change in DH, except for Corolase PN-L 5% and 10% DH. This roughly correlates with the changes in peptide size observed during hydrolysis (Figure 3). Smaller peptides may have reduced emulsifying properties (Chobert et al., 1988). A positive correlation between surface activity and peptide length has been found (Jost et al., 1977), and it is generally accepted that a peptide should have a minimum length of >20 residues to possess good emulsifying and interfacial properties (Lee et al., 1987). The large difference in emulsification properties seen between hydrolysates at the same DH is due to differences in enzyme specificity and hydrophobicity (Gauthier et al., 1993). The endogenous extract yielded hydrolysates that retained some larger peptides (~20000 Da) at all DH (Figure 3). These larger peptides may be responsible for the high emulsifying capacity observed for the hydrolysates. Corolase 7089 had small peptide units at all DH but still exhibited relatively high emulsifying capacity. Flavourzyme hydrolysates had an electrophoresis profile similar to that of the endogenous extract hydrolysates at 5% DH (Figure 3); however, they had poor emulsification ability at 5% DH. Corolase PN-L hydrolysates with a majority of its small peptides (Figure 3) still exhibited a high capacity to emulsify oil and water. Corolase 7089 and Corolase PN-L had similar emulsification capacities,

although their substrate specificities were different. There is thus not a clear connection between peptide size and emulsification, indicating that the physicochemical makeup of the peptides may play an important role in the difference in protein functionality observed.

The emulsion stability for all hydrolysates was greater at 5% DH than at higher DH. At 5% DH, the emulsions were most stable with no significant difference observed among the different hydrolysates. The endogenous extract hydrolysate produced the most stable emulsion at 10 and 15% DH. At 15% DH the emulsion droplets were very large and irregular and the emulsion broke down very rapidly. These large droplets indicate a poor ability to form emulsions. Egg albumin gave the most stable emulsion, and the SPC was more effective in stabilizing the emulsion as compared to forming it. In general, larger peptides and those with the proper balance of hydrophilic and hydrophobic groups are most effective.

Many different factors account for the difference observed between the hydrolysates in ability to both form and stabilize emulsions. A few detailed studies have shown that smaller molecular weight peptides have less emulsifying ability as a result of reduced peptide surface hydrophobicity (Kato and Nakai, 1980; Li-Chan, 1984; Quaglia and Orban, 1990; Mahmoud et al., 1992; Cui, 1996). The results here also show that at increasing DH, the emulsifying capacity and stability decreases, and it is therefore assumed that the hydrolysates have less surface hydrophobicity at increasing DH. Peptide behavior is complex and not easy to explain, and synergistic effects of peptides on emulsifying properties have been noted (Gauthier et al., 1993). Small peptides diffuse rapidly, adsorb at the interface, and are less efficient in reducing the interfacial tension because they cannot unfold and reorient at the interface, like proteins or hydrolysates with higher molecular weight (Turgeon, 1991), explaining in part the poor results seen at 15% DH.

Protein solubility also plays an important role in emulsification because rapid migration to and adsorption at the interface are critical (Chobert et al., 1988), although 100% solubility is not required (Damodaran, 1996). All hydrolysates at 5−15% DH had high and similar protein solubilities, making it hard to distinguish among them on this basis. The hydrolysates produced by the endogenous extract seem to best represent those attributes and could possibly be successfully used as emulsifiers in a meat model system such as sausages.

Several researchers have studied the emulsifying properties of FPH. As in our study FPH's ability to form and stabilize emulsions is generally found to decrease as the DH increases (Quaglia and Orban, 1990; Cui, 1996), although the opposite was observed by Spinelli et al. (1972) on rockfish. Most previous studies reveal a rather poor emulsification ability of FPH (Miller and Groninger, 1976; Vieira et al., 1995; Shahidi et al., 1995; Onodenalore and Shahidi, 1996), contrary to what we found. It is, however, very hard to compare these studies with ours because hydrolysis is performed at different conditions with different enzymes, and some fail to define the DH of the product. The emulsifying properties of the hydrolysates in our study were superior to those of any products in earlier studies, especially at 5% DH, as a result of the substrate specificity of the enzymes chosen.

Table 7. Fat Absorption of FPH at Different DH and Reference Protein[a]

FPH	mL of oil/g of protein		
	5% DH	10% DH	15% DH
Alcalase	5.98 ± 0.20^a	5.12 ± 0.32^b	3.86 ± 0.04^b
Flavourzyme	5.71 ± 0.06^a	3.22 ± 0.05^b	2.95 ± 0.05^c
Corolase PN-L	7.07 ± 0.02^a	3.31 ± 0.07^b	2.86 ± 0.02^c
Corolase 7089	5.88 ± 0.11^a	3.88 ± 0.09^b	3.77 ± 0.06^b
end. extract	4.48 ± 0.04^a	3.78 ± 0.02^b	2.89 ± 0.06^c
control protein			
egg albumin[b]	2.36 ± 0.03		
SPC[b]	2.90 ± 0.05		

[a] Means with the same superscript letter in a row are not significantly different ($p > 0.05$). [b] DH is unknown.

Fat Absorption. The capacity of a protein hydrolysate to absorb fat/oil 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. The FPH exhibited good fat absorption and could very well be used in such applications. The fat absorption was measured for hydrolysates and reference protein samples (Table 7) and shows that there is generally a good correlation between the emulsifying properties and oil-holding capacity for the FPH. The 5% DH hydrolysates have significantly greater ability to bind the soybean oil than hydrolysates at 10 and 15% DH and egg albumin or SPC. Egg albumin had the poorest ability to bind fat but a high capacity to emulsify and stabilize an oil−water emulsion. Albumin did not disperse well in the soybean oil and had a tendency to clump. SPC had a poor ability to bind fat. There was a progressive drop in fat absorption as the DH increased. Limited research has been conducted on fat absorption properties of FPH. Fat absorption levels of capelin protein hydrolysates (Shahidi et al., 1995) and shark protein hydrolysates (Onodenalore and Shahidi, 1996) are the only ones published so far. Both studies fail to define the units used to express fat absorption, and it is thus impossible to compare them to the results of this study. The mechanism of fat absorption is attributed mostly to physical entrapment of the oil, and the higher bulk density of the protein, the greater the fat absorption (Kinsella, 1976). Five percent DH hydrolysates have considerably higher bulk density than the other hydrolysates, with Corolase PN-L hydrolysates having the highest. Enzyme substrate specificity also appears to play a major role in hydrolysate fat binding abilities (Haque, 1993).

Conclusion. FPH had excellent functional properties compared to those of well-established food proteins. By utilizing proteases with different substrate specificities, hydrolysates of predetermined, markedly different functional properties could be produced, each with uses in a variety of food systems. In general, the lower the DH, the greater the emulsion formation, emulsion stability, and oil absorption, but solubility was high at all DH. The hydrolysates, especially at 5% DH, compared well with the reference protein and other studied FPH and food proteins. A unique amphiphilic nature of the hydrolysates might explain their high functionality.

LITERATURE CITED

Adler-Nissen, J. *Enzymic Hydrolysis of Food Proteins*; Elsevier Applied Science Publishers: Barking, U.K., 1986.

AOAC. *Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990.

Baek, H. H.; Cadwallader, K. R. Enzymatic hydrolysis of crayfish processing by-products. *J. Food Sci.* **1995**, *60*, 929−934.

Benjakul, B.; Morrissey, M. T. Protein hydrolysates from Pacific solid wastes. *J. Agric. Food Chem*. **1997**, *45*, 3423−3430.

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. **1976**, *72*, 248−254.

Cheftel, C.; Ahern, M.; Wang, D. I. C.; Tannenbaum, S. R. Enzymatic solubilization of fish protein concentrate: Batch studies applicable to continuos enzyme recycling processes. *J. Agric. Food Chem*. **1971**, *19*, 155−161.

Chobert, J.-M.; Bertrand-Harb, C.; Nicolas, M.-G. Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *J. Agric. Food Chem*. **1988**, *36*, 883−892.

Cui, H. The influence of degree of hydrolysis on the structural and functional properties of fish protein peptic hydrolysates. Master's Thesis, University of Washington, Seattle, WA, 1996.

Damodaran, S. Amino Acids, Peptides, and Proteins. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Dekker: New York, 1996.

Dean, D. D.; Domnas, A. J. The extracellular proteolytic enzymes of the mosquito-parasitizing fungus *Lagenidium giganteum*. *Exp. Mycol*. **1983**, *7*, 31−39.

Diniz, F. M.; Martin A. M. Use of response surface methodology to describe the combined effects of pH, temperature and E/S ratio on the hydrolysis of dogfish (*Squalus acanthias*) muscle. *Int. J. Food Sci. Technol*. **1996**, *31*, 419−426.

Feng, Y.; Hultin, H. O. Solubility of the proteins of mackerel light muscle at low ionic strength. *J. Food Biochem*. **1997**, *21*, 479−496.

Ferreira, N. G.; Hultin, H. O. Liquefying cod fish frames under acidic conditions with a fungal enzyme. *J. Food Process. Preserv*. **1994**, *18*, 87−101.

Fonkwe, L. G.; Singh, R. K. Protein recovery from enzymatically deboned turkey residue by enzymic hydrolysis. *Proc. Biochem*. **1996**, *31* (6), 605−616.

Gauthier, S. F.; Paquin, P.; Pouliot, Y.; Turgeon, S. Surface activity and related functional properties of peptides obtained from whey proteins. *J. Dairy Sci*. **1993**, *76*, 321−328.

Hale, M. B. *Making Fish Protein Concentrate by Enzymatic Hydrolysis*; NOAA Technical Report NMFS SSRF-675; U.S. Department of Commerce: Seattle, WA, 1972.

Haque, Z. U. Influence of milk peptides in determining the functionality of milk proteins: A review. *J. Dairy Sci*. **1993**, *76*, 311−320.

Hevia, P.; Whiteker, J. R.; Olcott, H. S. Solubilization of a fish protein concentrate with proteolytic enzymes. *J. Agric. Food Chem*. **1976**, *24*, 383−386.

Hoyle, N.; Merritt, J. H. Quality of fish protein hydrolysates from herring (*Clupea harengus*). *J. Food Sci*. **1995**, *59*, 76−79.

Jost, R.; Monti, J. C.; Pahud, J. J. Partial enzymatic hydrolysis of whey protein by trypsin. *J. Dairy Sci*. **1977**, *60*, 1387−1393.

Kato A.; Nakai, S. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochim. Biophys. Acta* **1980**, *624*, 13−20.

Kinsella, J. E. Functional properties of proteins in foods: a survey. *Crit. Rev. Food Sci. Nutr*. **1976**, *8* (4), 219−280.

Kristinsson, H. G. Reaction kinetics, biochemical and functional properties of salmon muscle proteins hydrolyzed by different alkaline proteases. Master's Thesis, University of Washington, Seattle, WA, 1998.

Kristinsson, H. G.; Rasco, B. A. Fish protein hydrolysates: production, biochemical and functional properties. *CRC Crit. Rev. Food Sci. Nutr*. **2000a**, *40* (1), 43−81.

Kristinsson, H. G.; Rasco, B. A. Hydrolysis of salmon muscle proteins by an endogenous enzyme mixture extracted from Atlantic salmon (*Salmo salar*) pyloric caeca. *J. Food Biochem*. **2000b**, in press.

Kristinsson, H. G.; Rasco, B. A. Kinetics of enzymatic hydrolysis of Atlantic salmon (*Salmo salar*) muscle proteins by alkaline proteases and an endogenous enzyme mixture. *Proc. Biochem*. **2000c**, submitted for publication.

Laemmli, U. K. Cleavage of structure proteins during the assembly of head of bacteriophage T4. *Nature* **1970**, *277*, 680−685.

Lee, S. W.; Shimizu, M.; Kaminogawa, S.; Yamaguchi, K. Emulsifying properties of a mixture of peptides derived from the enzymatic hydrolysates of β-casein. *Agric. Biol. Chem*. **1987**, *51*, 161−165.

Li-Chan, E.; Nakai, S.; Wood, D. F. Hydrophobicity and solubility of meat proteins and their relationship to emulsifying properties. *J. Food Sci*. **1984**, *49*, 345−350.

Limonta, B. A.; Behnke, U.; Ruttloff, H. Herstellung von fischproteinhydrolysate (Manufacture of fish protein hydrolysate). GDR Patent 148437, 1981.

Mahmoud, M. I.; Malone, W. T.; Cordle, C. T. Enzymatic hydrolysis of casein: effect of degree of hydrolysis on antigenicity and physical properties. *J. Food Sci*. **1992**, *57*, 1223−1229.

McNairney, J. Modification of a novel protein product. *J. Chem. Technol. Biotecnol*. **1984**, *34B*, 206−214.

Miller, R.; Groninger, H. S. Functional properties of enzyme-modified acylated fish protein derivatives. *J. Food Sci*. **1976**, *41*, 268−272.

Moorjani, M. N.; Nair, R. B.; Lahiry, N. L. Quality of fish protein concentrate prepared by direct extraction of fish with various solvents. *Food Technol*. **1968**, *22* (12), 1557−1561.

Morr, V.; German, B.; Kinsella, J. E.; Regenstein, J. M.; Van Buren, J. P.; Kilara, A.; Lewis, B. A.; Mangino, M. E. A collaborative study to develop a standardized food protein solubility procedure. *J. Food Sci*. **1985**, *50*, 1715−1718.

Mullally, M. M.; O'Callaghan, D. M.; FitzGerald, R. J.; Donnelly, W. J.; Dalton, J. P. Proteolytic and peptidolytic activities in commercial pancreatin protease preparations and their relationship to some whey protein hydrolysate characteristics. *J. Agric. Food Chem*. **1994**, *42*, 2973−2981.

Mullally, M. M.; O'Callaghan, D. M.; FitzGerald, R. J.; Donnelly, W. J.; Dalton, J. P. Zymogen activation in pancreatic endoproteolytic preparations and influence on some whey protein characteristics. *J. Food Sci*. **1995**, *60* (2), 227−233.

Mutilangi, W. A. M.; Panyam, D.; Kilara, A. Hydrolysates from proteolysis of heat-denatured whey proteins. *J. Food Sci*. **1995**, *60* (5), 1104−1109.

Onodenalore, A. C.; Shahidi, F. Protein dispersions and hydrolysates from shark (*Isurus oxyrinchus*). *J. Aquat. Food Prod. Technol*. **1996**, *5*, 43−59.

Petersen, B. R. The impact of the enzymatic hydrolysis process on recovery and use of proteins. In *Enzymes and Food Processing*; Elsevier Applied Science Publishers: London, U.K., 1981; pp 149−175.

Quaglia, G. B.; Orban, E. Enzymic solubilisation of proteins of sardine (*Sardina pilchardus*) by commercial proteases. *J. Sci. Food Agric*. **1987a**, *38*, 263−269.

Quaglia, G. B.; Orban, E. Influence of the degree of hydrolysis on the solubility of the protein hydrolsyates from sardine (*Sardina pilchardus*). *J. Sci. Food Agric*. **1987b**, *38*, 271−276.

Quaglia, G. B.; Orban, E. Influence of enzymatic hydrolysis on structure and emulsifying properties of sardine (*Sardina pilchardus*) protein hydrolysates. *J. Food Sci*. **1990**, *55* (6), 1571−1573.

Rebeca, B. D.; Pena-Vera, M. T.; Diaz-Castaneda, M. Production of fish protein hydrolysates with bacterial proteases; Yield and nutritional value. *J. Food. Sci*. **1991**, *56*, 309−314.

Sen, D. P.; Sayanarayana Rao, T. S.; Kadkol, S. B.; Krishnaswamy, M. A.; Venkata Rao, S.; Lahiry, N. L. Fish protein concentrate from bombay-duck (*Iharpoden nehereus*) fish: Effect of processing variables on the nutritional and organoleptic qualities. *Food Tecnhol*. **1969**, *23*, 683−688.

Shahidi, F.; Han, X.-Q.; Synowiecki, J. Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). *Food Chem*. **1995**, *53*, 285−293.

Spinelli, J.; Koury, B.; Miller, R. Approaches to the utilization of fish for the preparation of protein isolates; enzymic modifications of myofibrillar fish proteins. *J. Food Sci.* **1972**, *37*, 604−608.

Stefansson, G.; Hultin, H. O. On the solubility of cod muscle protein in water. *J. Agric. Food Chem*. **1994**, *42*, 2656−2664.

Steinhardt, H.; Beychok, S. Interaction of protein with hydrogen ions and other small ions and molecules. In *The Proteins*; Neurath, H., Ed.; Academic Press: New York, 1964; Vol. 2, pp 139−304.

Sugiyama, K.; Egawa, M.; Onzuka, H.; Oba, K. Characteristics of sardine muscle hydrolysates prepared by various enzymic treatments. *Nippon Suisan Gakkaishi* **1991**, *57* (3), 475−479.

Surowka, K.; Fik, M. Studies on the recovery of proteinaceous substances from chicken heads: I. An application of neutrase to the production of protein hydrolysate. *Int. J. Food Sci. Technol.* **1992**, *27*, 9−20.

Surowka, K.; Fik, M. Studies on the recovery of proteinaceous substances from chicken heads: II—Application of pepsin to the production of protein hydrolysates. *J. Sci. Food Agric.* **1994**, *65*, 289−296.

Suzuki, T. *Fish and Krill Protein: Processing Technology*; Applied Science: London, U.K., 1981.

Turgeon, S. L.; Gauthier, S. F.; Paquin, P. Interfacial and emulsifying properties of whey peptide fractions obtained with a two step ultrafiltration process. *J. Agric. Food Chem*. **1991**, *39*, 673−676.

Vareltzis, K.; Soultos, N.; Zetou, F.; Tsiaras, I. Proximate composition and quality of a hamburger type product made from minced beef and fish protein concentrate. *Lebensm. Wiss. -Technol.* **1990**, *23* (2), 112−115.

Venugopal, V.; Shahidi, F. Thermostable water dispersions of myofibrillar proteins from Atlantic Mackerel (*Scomber scombrus*). *J. Food Sci.* **1994**, *59* (2), 265−268.

Viera, G. H. F.; Martin, A. M.; Saker-Sampaiao, S.; Omar, S.; Goncalves, R. C. F. Studies on the enzymatic hydrolysis of Brazilian lobster (*Panulirus spp.*) processing wastes. *J. Sci. Food Agric.* **1995**, *69*, 61−65.

Webb, N. B.; Ivey, F. J.; Craig, H. B. The measurement of emulsifying capacity by electric resistance. *J. Food Sci.* **1970**, *35*, 501−504.

Yanez, E.; Ballester, D.; Monckeberg, F. Enzymatic fish protein hydrolyzate: chemical composition, nutritive value and use as a supplement to cereal protein. *J. Food Sci.* **1976**, *41*, 1289−1292.

Yatsumatsu, K.; Sawada, K.; Moritaka, S. Whipping and emulsifying properties of soybean products. *Agric. Biol. Chem*. **1972**, *36* (5), 719−727.

Yu, S. Y.; Fazidah, S. Enzymic hydrolysis of proteins from *Aristichthys noblis* by protease P "Amano"3. *Trop. Sci.* **1994**, *34*, 381−391.

Received for review May 3, 1999. Revised manuscript received December 9, 1999. Accepted December 29, 1999.

JF990447V