Influence of Hydrolysis Degree on the Functional Properties of Salmon Byproducts Hydrolysates

G.A. GBOGOURI, M. LINDER, J. FANNI, AND M. PARMENTIER

ABSTRACT: Protein hydrolysates from salmon heads were obtained by enzymatic treatment with Alcalase® 2.4L. Response surface methodology (RSM) allowed optimization of temperature, enzyme/substrate, and pH leading to various hydrolysates (11.5% to 17.3% hydrolysis degree [DH]) and protein recovery ranging from 47% to 70%. Size exclusion chromatography of hydrolysates showed that small peptides increased only at high DH. The nitrogen solubility index (NSI) of hydrolysates was higher than 75% over a wide range of pH values, whereas hydrolysates with high DH had the best solubility. Emulsifying capacity, emulsion stability, and fat absorption capacity were better when DH was low.

Keywords: fish protein, enzymatic hydrolysis, functional properties, RSM

Introduction

According to the last FAO reports, a huge effort has been deployed to improve world fish production. Production increased from 120 million tons in 1996 to 128.8 million tons in 2001 (FAO 1998) and came from both from capture and aquaculture. The consequence was that the byproducts or wastes were estimated at 50% of the production (Jeon and others 1999; Kristinsson and Rasco 2000a). This is a considerable loss considering that the frame (heads, edges, tails) accounts for about 22% of the raw material and may contain a significant quantity of nutritional resources, for example, proteins, lipids, and minerals.

Because of the growing request for fish proteins, a particular interest should be focused on the underuse of the proteinic resources (Liaset and others 2000). Byproducts of fish-processing plants, such as downgraded whole fish, heads, skin, and frame bones, are currently used to produce food proteins at low cost (Quaglia and Orban 1987a, 1987b; Liaset and others 2000; Gilberg and others 2002). Kristinsson and Rasco (2000a) reviewed studies on fish proteins and their properties and concluded that this field was promising regarding nutritional, biological, and pharmaceutical aspects.

To achieve this purpose, the use of commercial enzymes is the gravitational means to obtain proteinic hydrolysates. Enzymatic methods constitute an essential part of the processes used by modern companies to produce a large and diversified range of products for human and animal consumption. Studies have shown that these methods are efficient to separate the protein hydrolysates from the lipid fraction and the insoluble residues and make it possible to produce peptides with specified functional and nutritional properties (Quaglia and Orban 1987b; Liceaga and Li-Chan 1999; Kristinsson and Rasco 2000a, 2000b; Liaset and others 2000, 2003). These researchers have shown that enzymatic methods improved functional and sensorial properties without lack of nutritional value. However, enzymes should be properly chosen and used under suitable conditions (temperature, pH, enzyme/substrate (E/S) ratio). Various kinds of proteases are described in the literature; for example, Quaglia and Orban (1987a) used Alcalase® 0.6, Neutrase® and papain to solubilize sardine proteins; Kristinsson and Rasco (2000a, 2000b) studied Alcalase, Flavourzyme®, Corolase® for the production of protein hydrolysates of salmon fillets; Liaset and others (2000) used Alcalase, pepsin, Neutrase, and Kojizyme® to hydrolyze carcasses of tilapia and salmon. More recently, Guerard and others (2001, 2002) used Alcalase to produce tuna waste hydrolysats.

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In the 1st part of the present work, the enzymatic extraction of proteins from salmon heads to obtain nonbitter hydrolysates was undertaken with a strict control of the proteolytic reaction. The relative importance of temperature, E/S, and pH on responses (hydrolysis degree [DH] and yield) was studied by using a quadratic model and response surface methodology (RSM). In the 2nd section, functional properties (solubility, emulsifying capacity, stability of the emulsion, fat absorption capacity) of the different hydrolysates were characterized in detail.

Materials and Methods

Substrate and enzyme

A local plant (Les Salaisons Maritimes André Ledun, Cany-Barville, France) provided 100 kg Atlantic salmon heads (*Salmo salar*). Regularly preserved at –20°C, samples (2 kg) were thawed at +4 °C over 24 h before being crushed with a domestic grinder (S.A.S. Groupe SEB Moulinex, Ecully, France).

The food-grade enzyme Alcalase 2.4L, an endopeptidase from *Bacillus licheniformis*, was provided by Novo Nordisk (Bagsvaerd, Denmark). This enzyme was selected on the basis of its ability to produce hydrolysates of specific functional properties.

Chemical analysis

Fresh crushed heads and freeze-dried hydrolysates were analyzed for crude protein (N × 6.25) content by Kjeldahl method

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(AOAC 1997) in a nitrogen analyzer (Gerhadt Vapodest 50, Gerhardt GMBH KG, Bonn, Germany). Lipid content was determined by the method of Bligh and Dyer (1959) for substrate and by the Soxhlet method with petroleum ether for hydrolysates. Moisture was quantified by drying sample overnight at 103 °C; ash was determined after heating the sample overnight at 550 °C; and mineral content was analyzed by atomic absorption spectrophotometry (Perkin-Elmer, model 1100, Paris, France).

Native protein from fresh crushed heads was obtained according to Kristinsson and Rasco (2000b). The substrate was treated in the same conditions as for preparation of hydrolysates except that no protease was added (discussed subsequently).

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Preparation of hydrolysates

Enzymatic hydrolysis was performed in a stirred batch and thermostated reactor (1L) where raw material was suspended in distilled water. The hydrolysis is outlined in Figure 1. The adjustment of pH with 4 *M* NaOH was done for 15 min by mixing. The enzyme solution was then added, and the reaction was allowed to continue for 2 h under constant stirring at 600 rpm. The pH was kept constant by automatically adding 4 *M* NaOH during hydrolysis according to the pH-stat method. The volume of alkali was recorded to allow calculation of the degree of hydrolysis (DH).

The enzyme was inactivated by heat treatment with live steam injection (95 °C, 10 min). The medium was coarsely filtered to retain the bones, and the liquid phase was clarified by centrifugation at 2000 × *g* for 30 min in a J2-HS Beckman centrifuge (Beckman Instruments, Gagny, France). A subsequent centrifugation was carried out to separate the oil fraction from the aqueous phase containing the hydrolysate.

Table 1—Experimental domain and level distribution of the variables used in the hydrolysis of salmon heads with Alcalase 2.4L.

Independent variables	Symbol	Levels	Experimental values
Temperature (°C)	X1	5	4s9 52 55 58 61
Enzyme/substrate (%)	X2	7	3.5 4.0 4.5 5.0 5.5 6.0 6.5
pH	X3	3	7.0 7.5 8.0

Table 2—Doehlert uniform shell design for 3 variables (X1: temperature, X2: enzyme/substrate, and X3: pH) and responses for salmon heads hydrolysis by Alcalase 2.4L[a]

Experiment	Temp. (°C)	E/S	pH	DH (%)	Yield (%)	Protein content (%)
1 c	61	5.0	7.5	14.26	69.1	83.30
2	49	5.0	7.5	12.20	61.5	83.00
3	58	6.5	7.5	12.90	63.7	81.00
4 a	52	3.5	7.5	11.50	61.8	82.30
5	58	3.5	7.5	13.00	60.0	80.00
6	52	6.5	7.5	13.30	47.7	81.25
7 d	58	5.5	8.0	17.30	71.0	78.86
8	52	4.5	7.0	10.80	51.7	83.80
9	58	4.5	7.0	11.60	59.1	85.30
10	55	6.0	7.0	12.00	57.6	85.70
11	52	5.5	8.0	15.80	61.8	83.00
12	55	4.0	8.0	14.70	63.1	83.00
13 b	55	5.0	7.5	13.00	62.5	81.50
14	55	5.0	7.5	12.50	62.2	82.50
15	55	5.0	7.5	12.40	63.0	82.00

[a]Experiment nr followed by a, b, c, d are extensively described in the text.

Calculation of the degree of hydrolysis (DH)

The hydrolysis was carried out using the pH-stat method described by Adler-Nissen (1977). The DH (%) was calculated from the volume and the molarity of alkali used to maintain constant pH:

$$\text{DH (\%)} = \frac{B \times N_B}{\alpha \times M_p \times h_{tot}} \times 100$$

where B is alkali consumption (mL); N_B is the molarity of the alkali; α is the average degree of dissociation of NH2; M_p is the mass of protein (N × 6.25); h_{tot} is the total number of peptide bonds in the protein (7.5).

The degree of dissociation of α-amino groups was computed from:

$$\alpha = \frac{10^{pJ-pK}}{1 + 10^{pJ-pK}}$$

The pK value is dependent on temperature and can be calculated according to the following equation:

$$\text{pK} = 7.8 + \frac{298 - T}{298T} \times 2400$$

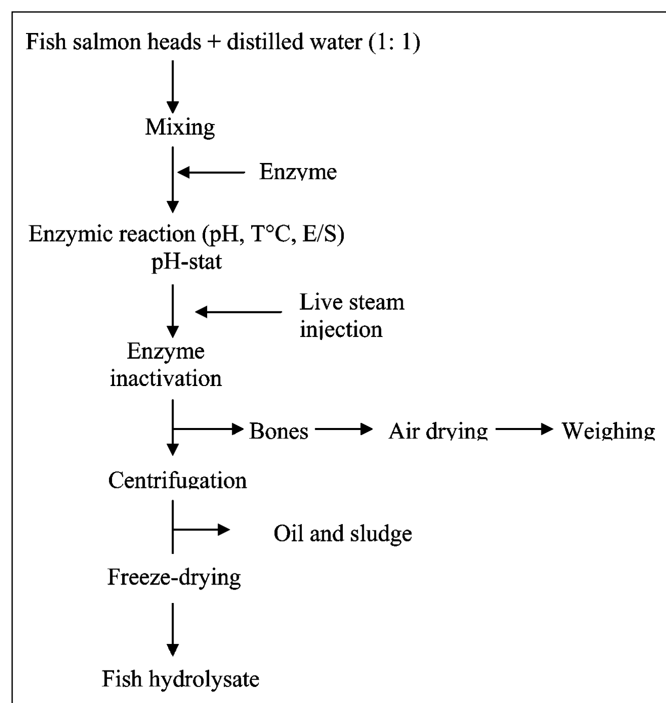

Figure 1—Scheme of the hydrolysis process of salmon heads with Alcalase 2.4L.

Doehlert uniform shell design and statistics

Experiment I. According to preliminary studies based on a survey of the effective parameters, a Doehlert experimental design was selected, using NEMROD® software (Mathieu and Phan Tan Luu 1998). This matrix displayed a uniform distribution of the points within the experimental domain and allowed a number of distinct levels for each variable. The processing variables investigated were temperature (X1, 5 levels), enzyme/substrate (X2, 7 levels), and pH (X3, 3 levels) and are displayed in Table 1. The total number of points (N) for 3 factors (k) based on $N \geq k^2 + k + 1$ was 13. Fifteen experiments were carried out; the 13th assay was per-

formed at the center of the experimental domain and was repeated twice (14, 15) to estimate the residual variance (Table 2).

A full quadratic model (Eq. 1) containing 10 coefficients including the interaction terms was assumed to describe the relationships between the following responses: (1) DH (Y1), which should be controlled to avoid product bitterness according to Saha and Hayashi (2001) and Gilberg and others (2002). (2) The hydrolysis yield (Y2), expressed as amount of nitrogen in the hydrolysate over initial nitrogen content.

$$\eta_k = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1+i}^{3} \beta_{ij} X_i X_j$$

where η_k refers to the dependent variables; β_0 is the constant coefficient; X_i are the coded independent variables; β_i are the linear coefficients; β_{ij} are the second-order interaction coefficients, and β_{ii} are the quadratic coefficients. Results are valid within the experimental domain.

Experiment II. Functional properties were studied especially as a function of DH (11.50, 13.00, 14.26, and 17.30) with 4 experiments named a, b, c, and d, respectively (Table 2).

Data analysis

Data were computed using NEMROD including ANOVA and canonical analysis to obtain interaction data between the process variables and responses.

Characterization of hydrolysates by size-exclusion chromatography

The molecular weight distribution of peptides for each sample was analyzed by size exclusion using low-pressure chromatography system. The procedure was carried out on a Sephadex® G50 Fine column (Sigma) (100 cm height × 1-cm internal dia, 70 cm^3 bed volume) at 25 °C, with exclusion limits of 1500 to 30000 Da. The mobile phase was Tris-HCl 50 m*M*, pH 7.5. The sample of freeze-dried hydrolysates and native protein were diluted in distilled water (14 mg/mL), and filtered through a 0.45-μm Millipore filter. An aliquot of 0.5 mL was injected and eluted at 0.14 mL/min.

The column was calibrated with standard proteins (Sigma): bovine serum albumin (66000 Da), carbonic anhydrase bovine erythrocyte (29000 Da), cytochrome C (12400 Da), aprotinin (6500 Da), and human parathyroid hormone (2808 Da). All standards were dissolved at 1 mg/mL in distilled water and eluates were monitored at 210 nm.

Determination of amino acid composition

Total amino acid composition of freeze-dried samples was determined after hydrolysis in 6 *M* HCl with phenol (1%) at 150 °C for 60 min, in Pico-Tag system (Waters, Milford, Mass., U.S.A.). The phenylisothiocyanate (PITC®) amino acid derivatives were eluted on HPLC Applied Biosystems Model 172 A (Applera Corp, Foster City, Calif., U.S.A.) equipped with a PTC RP-18 column (2.1 mm × 22 cm). Sodium acetate (45 m*M*, pH 5.9) and sodium acetate (105 m*M*, pH 4.6; 30%), and acetonitrile (70%) were used as buffers.

Functional properties of hydrolysates

Nitrogen solubility index (NSI). The solubility of freeze-dried hydrolysates and freeze-dried native fish protein was determined by the standard AOAC method modified by Morr and others (1985). About 1% of suspensions of each sample were prepared in 0.1 *M* NaCl (w/v) at different pH values (3.0 to 11.0) adjusted with NaOH or HCl. The suspensions were stirred for 1 h and centrifuged at 20000 × *g* for 15 min. The supernatant was filtered through Whatman paper nr 1, and the nitrogen content in the total and in the soluble fraction was analyzed by the Kjeldahl method (AOAC 1997). All experiments were done in triplicate. The nitrogen index in each sample was calculated as:

$$\text{NSI (\%)} = \frac{\text{supernatant nitrogen concentration}}{\text{total nitrogen concentration}} \times 100$$

Emulsifying capacity (EC). Samples were checked for EC according to the method of Vuillemard and others (1990) based on oil titration. Protein dispersions (50 mL) were prepared in distilled water from 0.02% to 0.20% and adjusted to pH 7.0 with 0.1 *M* NaOH or HCl. The process was carried out in a glass vessel connected with a refrigerated water bath to keep the temperature of emulsion constant at 25 °C during processing. A flask of corn oil (Epi d'Or-Lesieur, Asnières-sur-Seine, France) was installed on a balance pan and the oil was added at about 17 g/min flow rate using a peristaltic pump. Emulsion conductivity was recorded continuously using a Tetracon 96-1.5 electrode connected to a Microprocessor conductivity-Meter LF 196 (WTW, OSI, France), fitted to an Ultra-Turrax® T25 (IKA®, Staufen, Germany) set at 20000 rpm. The addition of oil was stopped when the conductivity dropped.

Emulsifying capacity was expressed as the ratio of emulsified oil minus the blank over the amount of proteins in sample. All experiments were done in triplicate.

The blank was the quantity of oil (21.6 g) added before the phase inversion in 50 mL distilled water.

Emulsion stability (ES). The method of Yasumatsu and others modified by Miller and Groninger (1976) was used for ES determination. The sample (500 mg) was dissolved in 0.1 *M* NaCl made up to a total volume of 50 mL to make a 1% protein solution. Corn oil (50 mL) was added, and the emulsion was stirred with an Ultra-Turrax T25 at 20000 rpm for 2 min. From the emulsion, 3 aliquots of about 25 mL were transferred into 3 25-mL graduated cylinders. The emulsions were allowed to stand for 15 min, and the volume of the exuded aqueous phase was measured. Each determination was performed in triplicate.

ES was expressed as:

$$\text{\% Emulsifying stability} = \frac{\textit{Total volume} - \textit{aqueous volume}}{\textit{Total volume}} \times 100$$

Fat-holding capacity. The capacity of the hydrolysates to hold oil was determined according to the modified method of Shahidi and others (1995). Ten mL of corn oil was added to 500 mg of sample into a centrifuge tube. The mixture was kept for 30 min at room temperature (23 °C) and mixed thoroughly with a small spatula every 10 min. After centrifugation (25 min; 2000 × g), the free oil was decanted and the fat adsorption was determined from the weight difference and expressed as milliliters of fat adsorbed per gram of protein. All measurements were done in triplicate.

Results and Discussion

Many factors such as pH, duration, temperature, substrate, and enzyme concentration are known to affect the course of proteolysis. Then, RSM was effective in replacing the traditional kinetic experiments to quantify the effects of each parameter and interaction between factors. It is well known that the one factor at a time ignores interactions between factors, which are simultaneously present.

Chemical composition

The proximate composition of the raw material and hydrolysates are displayed in Table 3. The raw material contained high amounts of proteins and lipids up to 15% and 20%, respectively. Compared with the chemical analysis of fillets (Kristinsson and Rasco 2000b), these byproducts are a potential source of proteins and lipids. The proteolysis led after centrifugation to a defatted hydrolysate containing about 82% protein (dry basis). However, the ash content (dry basis) in the hydrolysate was 21% higher than in the raw material (dry basis). This was a consequence of the addition of alkali required to control the pH during hydrolysis (Liceaga and Li-Chan 1999; Kristinsson and Rasco 2000c).

Enzymatic hydrolysis

The pH-stat method is commonly used to monitor hydrolysis of proteins. This method indicates very little variation among replicates. This is very important when predetermined properties at a specific DH% are required. The hydrolysis was achieved within 120 min and exhibited typical curves (Figure 2). Other authors, such as Quaglia and Orban (1987a, 1978b), expected this for sardine, Liceaga and Li-Chan (1999) for herring, and Kristinsson and Rasco (2000c, 2000d) for salmon fillet muscle. In these experiments, different final DH were obtained after 120 min of hydrolysis, depending on the experiment conditions of the experiments.

Figure 3 shows the effect of enzyme addition after 1 h of reaction. Fresh enzyme addition (100% and 400% of the original enzyme concentration) led to a small increase of DH, from 12.5% to 15%. From a technical point of view, this would mean that the optimal DH could be achieved within 1 h.

Data analysis of the experimental Doehlert design showed that all the factors investigated (temperature, E/S, pH) were significant, with $P > 0.05$ and a high regression coefficient, $r^2 > 0.91$. The quadratic model used displayed particularly significant interaction between temperature and E/S (10.27**) (Table 4). Canonical analysis performed on quadratic polynomial models using the NEMROD software led to predict optimal conditions to increase the DH. Contour plots for DH (%) response are displayed in Figure 7. A maximal value of 17.2% for the DH was obtained under the following conditions: E/S = 5.2%; temperature, 57 °C; and pH 8.0 (Figure 7c and 7d).

Table 3—Proximate composition of freeze-dried hydrolysates and control

Content	Salmon heads (% wet basis)	Minced salmon muscle[a] (% wet basis)	Hydrolysate 11.5% DH (% dry basis)	Hydrolysate from salmon frames[a] 10% DH
Protein (NT × 6.25)	15.0 ± 0.1	20.3 ± 0.4	82.3 ± 1.9	71.6 ± 0.2
Lipid	20.0 ± 0.4	2.9 ± 0.4	0.8 ± 0.02	0.06 ± 0.04
Moisture	67.8 ± 1.3	75.1 ± 0.9	5.3 ± 0.2	4.2 ± 0.4
Ash	2.6 ± 0.6	0.9 ± 0.02	10.4 ± 1.1	22.3 ± 0.9
K	0.14	nd	1.2	nd
Na	0.013	nd	1.3 to 2.5	nd
Mg	0.042	nd	0.02	nd
Ca	0.13	nd	—	nd
Dry bones	5.5 ± 0.2	nd	—	nd

[a]Kristinsson and Rasco 2000b.

Table 4—Regression coefficient of predicted quadratic polynomial models[a]

Variables		DH (%)	Yield (%)
Intercept		12.63***	62.58***
Linear			
Temperature	b1	0.94*	5.74***
E/S	b2	0.75*	–1.45*
pH	b3	2.73***	5.61***
Quadratic			
b11		0.59	2.75*
b22		–0.14	–6.63**
b33		1.48	–1.82*
Interaction			
b12		–1.09	10.27**
b13		0.81	–2.53*
b23		0.48	–2.54***
R^2		0.97	0.91

[a]*1% < α ≤ 5%; **1‰ ≤ α < 1%; ***α < 1‰.

Peptide molecular range of hydrolysates

Fish protein hydrolysates were separated using gel chromatography on Sephadex G 50 F to analyze peptide size composition. The chain length of peptides, which are depending on the DH, is of

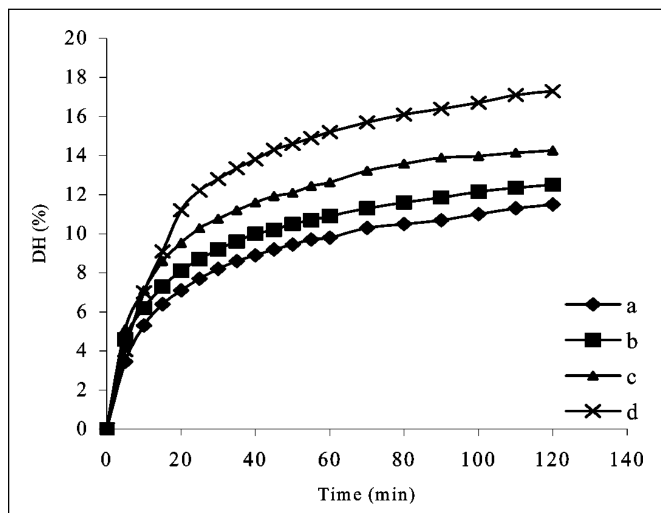

Figure 2—Enzymatic hydrolysis of salmon heads by Alcalase 2.4L in different conditions: (a) 52 °C, pH 7.5, E/S 3.5%; (b) 55 °C, pH 7.5, E/S 5%; (c) 61 °C, pH 7.5, E/S 5%; (d) 58 °C, pH 8, E/S 5.5.

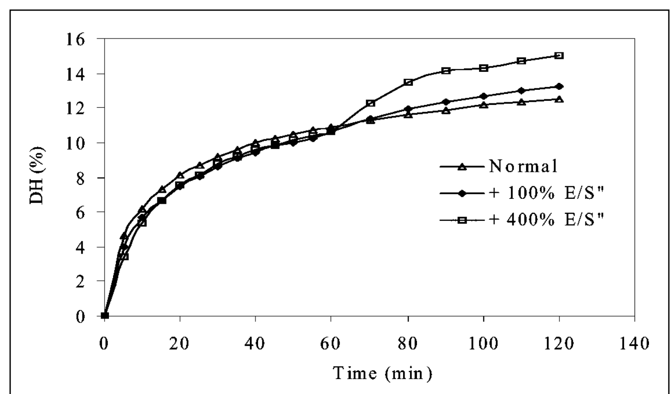

Figure 3—Effect of fresh enzyme addition (Alcalase) during the course of hydrolysis in a proportion of 100% and 400% of original enzyme concentration after 1 h. Initial: E/S 5% (w/w protein), 55 °C, pH 7.5.

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special interest in relation with organoleptic and functional characteristics. This is because properties such as solubility, emulsion capacity, and bitterness depend at least in part on molecular size (Mohr 1980). Bitterness of hydrolysates is mainly caused by hydrophobic oligopeptides and is less intense by using Alcalase instead of Neutrase, papain, and pepsin (Gildberg and others 2002).

Chromatograms of various hydrolysates after 300 min of elution are displayed in Figure 4. The molecular size distribution of the 4 hydrolysates was similar although the relative proportions of the peaks varied accordingly with the DH. From chromatographic data (Table 5), it can be seen that the average molecular weight of the peptides is below 13200 Da and that the peptide range repartition depends on DH. Hydrolysates with low DH (11.5%) were characterized by a high percentage of peptides with molecular weights ranging from 4200 Da to 13200 Da. As expected, this fraction decreased as the DH increased. However, the proportion of peptide molecular range of hydrolysates with DH 12.5%, 14.26%, 17.3% was not significantly different, despite various experimental conditions.

Functional properties

Solubility. The solubility of hydrolysates and control was expressed as the percentage of soluble nitrogen compounds with respect to total nitrogen in each sample. The solubility was measured in the pH range of 3 to 11, as shown in Figure 5. All hydrolysates were more soluble than native protein and indicated nitrogen solubility values higher than 75%. The enhancement of solubility is because of the smaller molecular size of hydrolysates compared with the intact protein (Chobert and others 1988; Linder and others 1996; Mahajan and Dua 1998; Kristinsson and Rasco 2000b). It should also be reminded that proteins and hydrolysates are salted-in, partly through the formation of sodium salt of carboxyl groups.

A relationship between solubility and DH value was observed. As expected, hydrolysates with high DH values showed higher solubility than hydrolysates with low DH values. That was in agreement with the results found by Linder and others (1996) on veal bone hydrolysates and confirmed by gel chromatography (discussed previously).

The balance of hydrophilic and hydrophobic forces of peptides is another important cause of solubility enhancement. The smaller peptides from myofibrillar proteins are expected to have proportionally more polar residues, with the ability to form hydrogen bonds with water and increasing solubility.

The solubility of the hydrolysates was high at pH 6 to 7, in every case higher than 90%, and was low at pH 3 to 4. Kristinsson and Rasco (2000b) observed similar results with salmon fillet hydrolysates. The pH influences the charge on the weakly acidic and basic

Table 5—Distribution of peptide classes from salmon head hydrolysates

		Area (%)			
Retention time (min)	Molecular weight (Da)	DH 11.5%	DH 12.5%	DH 14.3%	DH 17.3%
450 to 500	1382 to 2430	13.7	29.1	30.3	30.4
400 to 450	2430 to 4272	38.9	44.6	42.6	42.2
300 to 400	4272 to 13200	47.4	26.3	27.3	27.4

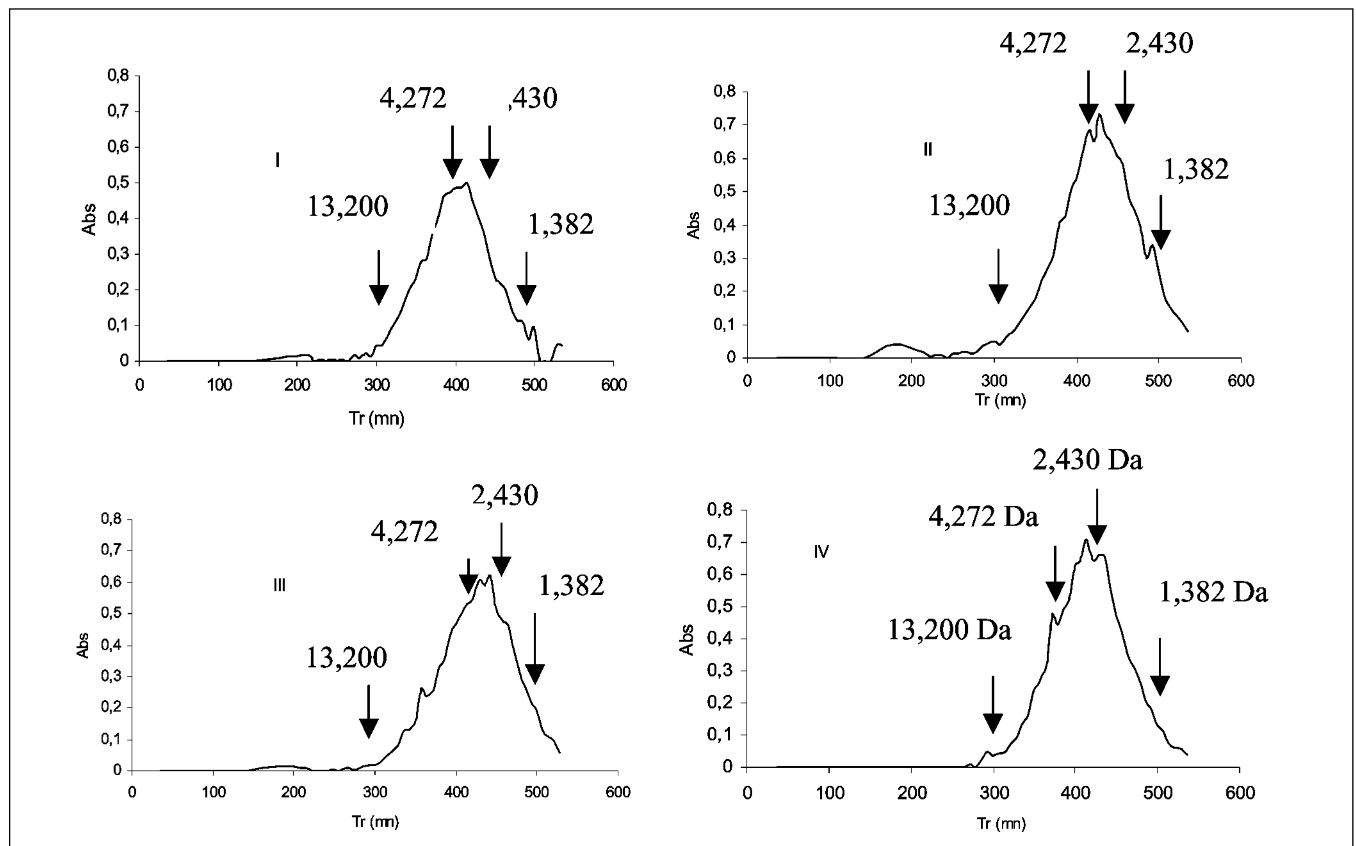

Figure 4—Size exclusion chromatographic profiles of salmon heads hydrolysates from Sephadex G50 F (1500 to 30000 Da), elution buffer: Tris-HCl 50 m*M*, pH 7.5, flow rate: 0.14 mL/min, 210 nm, (I) DH 11.5%, (II) DH 12.5%, (III) DH 14.26%, (IV) DH 17.3%.

side-chain groups, thus protein and hydrolysates display low solubility at their isoelectric point (Chobert and others 1988; Vieira and others 1995; Linder and others 1996).

Emulsifying capacity and stability of emulsions. Emulsifying capacity of hydrolysates at concentrations varying from 0.02% to 0.20% is displayed in Figure 6. It was shown that EC increased with hydrolysate concentration to a maximum and then decreased. Vuillemard and others (1990) and Linder and others (1996) reported similar results. EC increased with protein concentration until maximal EC because of difference in ratio of adsorbed protein/available protein and then decreased because high protein concentration increased the adsorption rate higher than the spreading rate (Blecker and others 1997). However, the range of DH observed (11.5% to 17.3%) was not broad enough to display a significant variation of EC for a given hydrolysate concentration. It results that high DH do not improve emulsifying capacity.

Mechanism of emulsification process of hydrolysates is attributed to adsorption to the surface of freshly formed oil droplets during homogenization and forms a protective membrane that prevents droplets coalescence. Hydrolysates are surface-active materials and promote oil-in-water emulsions because of their hydrophilic and hydrophobic groups. According to Rahali and others (2000), sites for the anchorage of peptides in the interfacial layer mostly depend on the alternative distribution of hydrophobic and charged amino acids. As observed by Quaglia and Orban (1990) and Kristinsson and Rasco (2000b), the EC of fish waste protein decreased with increasing of DH. Hydrolysates with low DH have high EC and extensive hydrolysis results in a drastic loss of emulsifying properties. Small peptides diffuse rapidly and absorb at the interface; they are less efficient in reducing the interfacial tension because they cannot unfold and reorient at the interface like large peptides. According to Kato and others (1985), the flexibility of protein (or peptide) structure may be an important structural factor governing the emulsification. It is also known that protein solubility plays an important role in emulsification because rapid migration and adsorption at the interface are critical (Chobert and others 1988).

Table 6—Emulsion stability and fat absorption of salmon head protein hydrolysates, nonhydrolyzed salmon head protein and reference protein

Salmon head protein hydrolysates (DH%)	Emulsion stability (%)	Fat absorption capacity (mL oil/g protein)
11.50%	86.6 ± 0.2	3.55 ± 0.10
12.50%	87.9 ± 0.6	3.1 ± 0.2
14.26%	85.3 ± 0.3	2.9 ± 0.1
17.30%	74.7 ± 2.7	2.8 ± 0.1
Sodium caseinate	90.3 ± 1.4	2.33 ± 0.03
Nonhydrolyzed salmon head protein	—	10.67 ± 0.60

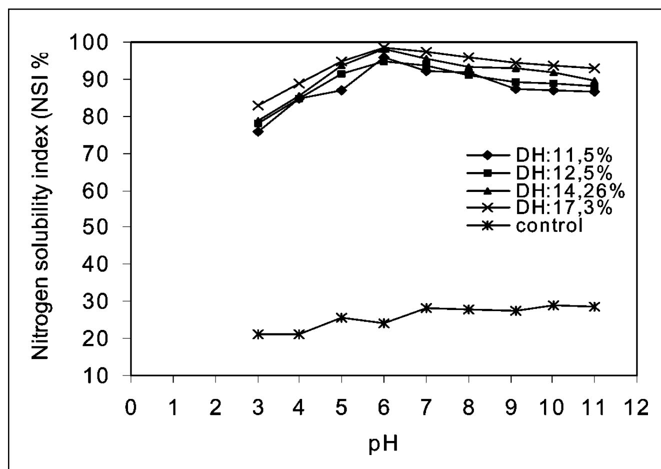

Figure 5—Solubility of salmon head protein hydrolysates obtained with Alcalase 2,4L at pH 3 to 11.

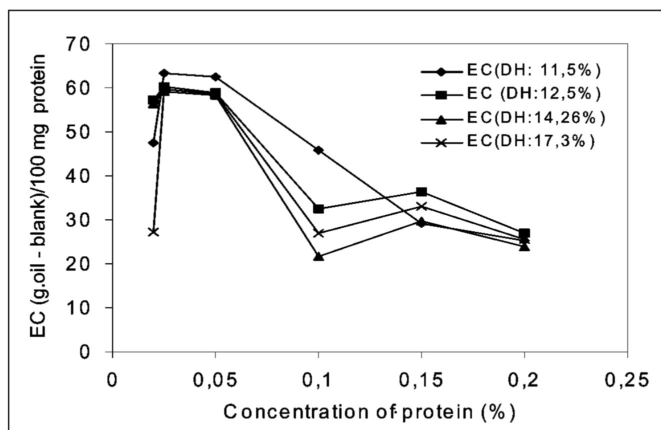

Figure 6—Emulsifying capacity of salmon head protein hydrolysates as a function of hydrolysis degree and protein concentration

Considering EC determination, Linder and others (1996) observed that several factors such as blending speed, protein source (concentration and solubility), temperature, pH, type of oil added, and water content are involved.

As for the EC, the emulsion stability (ES) decreases with wider amplitude as a function of proteolysis (Table 6). This observation confirms that better emulsifying properties are obtained at low DH. It was established that the hydrophobicity of large peptides is greater than for small peptides resulting from the same enzyme used for hydrolysis (Quaglia and Orban 1990). In the emulsion, large peptides adsorbed to the oil surface induce formation of small oil droplets, which are of higher stability than large oil droplets induced by small peptides.

Fat absorption capacity

The oil-holding capacity of hydrolysates, initial protein, and reference protein is reported in Table 6. This property that expresses the quantity of oil directly bound by the protein is of great interest because it is also an important functional characteristic especially expected by the meat and confectionery industry (Shahidi and others 1995; Onodenalore and Shahidi 1996; Periago and others 1998).

In this study, the 11.5% DH fish hydrolysate showed the best ability to bind corn oil than hydrolysates of higher DH and sodium caseinate. The nonhydrolyzed salmon protein has a fat absorption capacity significantly higher than hydrolysates and the reference protein. This could be explained by the fact that it disperses well in corn oil than other proteins.

In the case of hydrolysates, the relative drop of fat absorption as the DH increased could be because of the physical entrapment of oil and thus, the higher bulk density of protein, the more fat absorption (Kinsella 1976). Wang and Kinsella (1976) reported that the correlation between bulk density and fat absorption was up to 0.95.

Other studies indicated that hydrophobic interactions are primarily responsible for that (Haque and Mozaffar 1992; Periago

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and others 1998). Kristinsson and Rasco (2000c) confirmed this behavior and showed that the different enzymes used for hydrolysis of salmon muscle protein resulted in different fat absorption ability.

Amino acids pattern of hydrolysates

The amino acid composition does not differ significantly between the crude protein sampled before proteolysis and the different hydrolysates. Table 7 displays the patterns of crude protein,

Table 7—Amino acid pattern of salmon protein (head and frame) compared with 2 other fish protein

Amino acid	Salmon head native protein (m mol/g protein) (present study)	Salmon head hydrolysate DH 17.3% (m mol/g protein) (present study)	Salmon frame hydrolysate[a] (m mol/g protein)	Capelin protein hydrolysate[b] (m mol/g protein)	Cod protein hydrolysate[c] g/kg protein
Asn + Asp	0.62	0.61	0.61	0.37	0.34
Gln + Glu	0.78	0.75	0.86	0.46	0.46
Ser	0.35	0.37	0.40	0.40	0.53
Gly	1.69	1.10	1.45	0.68	1.51
His	0.10	0.10	0.25	0.14	0.10
Arg	0.34	0.33	0.39	0.33	0.41
Thr	0.30	0.34	0.34	0.38	0.35
Ala	0.72	0.82	0.88	0.67	0.86
Pro	0.53	0.54	0.50	0.32	0.48
Tyr	0.11	0.11	0.13	0.13	0.13
Val	0.27	0.26	0.30	0.49	0.35
Met	0.17	0.18	0.13	0.14	0.15
Ile	0.20	0.20	0.22	0.32	0.27
Leu	0.39	0.40	0.44	0.58	0.50
Phe	0.16	0.16	0.18	0.19	0.19
Lys	0.21	0.11	0.47	0.58	0.47

[a]Liaset and others 2003.
[b]Shahidi and others 1995.
[c]Liaset and others 2000.

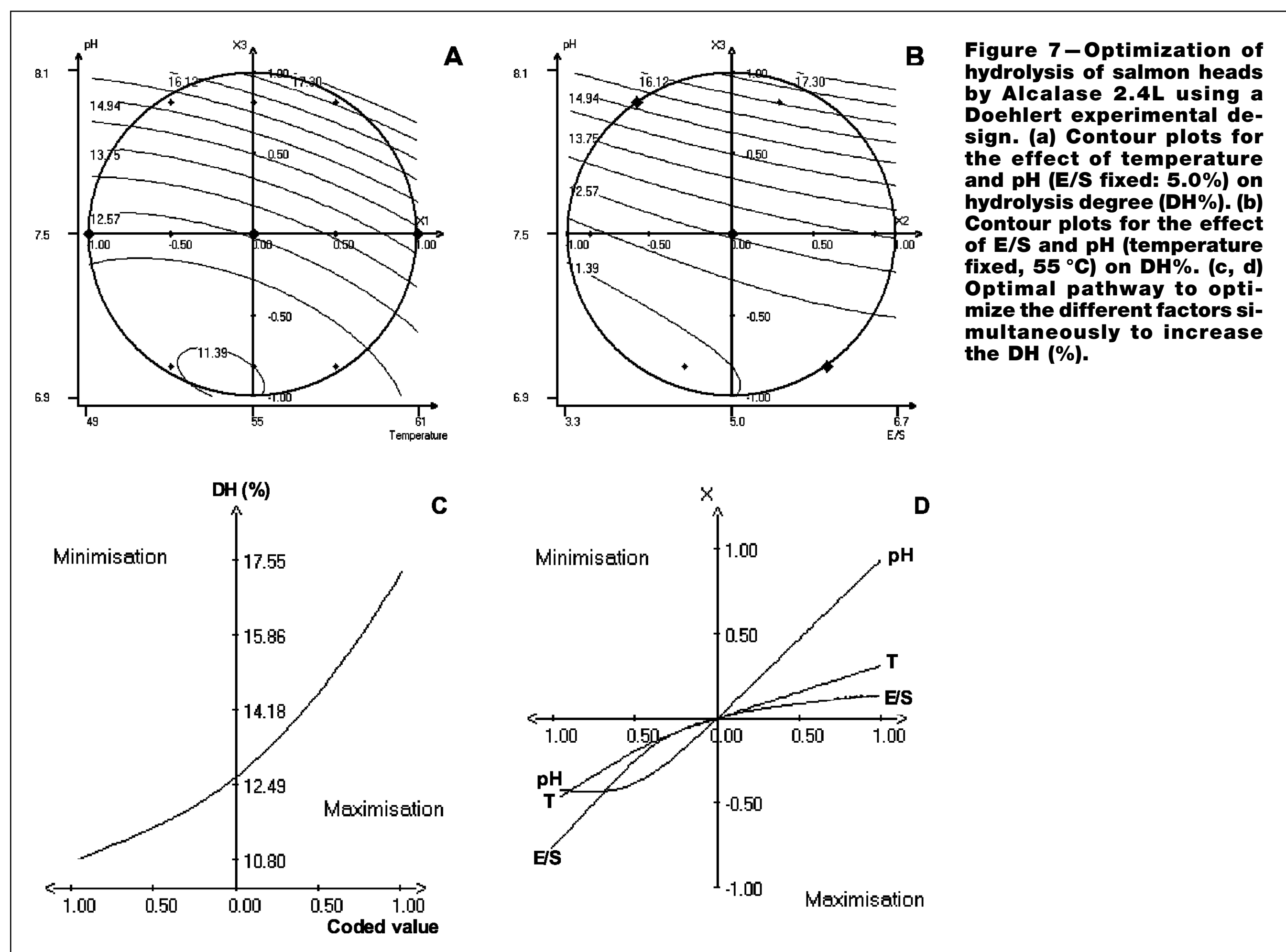

Figure 7—Optimization of hydrolysis of salmon heads by Alcalase 2.4L using a Doehlert experimental design. (a) Contour plots for the effect of temperature and pH (E/S fixed: 5.0%) on hydrolysis degree (DH%). (b) Contour plots for the effect of E/S and pH (temperature fixed, 55 °C) on DH%. (c, d) Optimal pathway to optimize the different factors simultaneously to increase the DH (%).

hydrolysate (17.3% DH), and data from other literature given for comparison, where original data have been changed to mmol/g protein for comprehension (Shahidi and others 1995; Liaset and others 2003). It appears that global compositions are very close among salmon, capelin, and cod proteins. For salmon heads, the amount of Gly appeared lower after hydrolysis with Alcalase. This was probably because of an incomplete hydrolysis of the connective tissue (data not shown). The reduction of lysine from crude protein to hydrolysates might be related to cross reactions with phospholipids or cerebrosides, which constitute major components of the brain tissue.

Conclusions

Fish protein hydrolysates have been obtained from salmon heads by using a commercial endopeptidase preparation (Alcalase 2.4L). The freeze-dried hydrolysates were quite lipid-residue free, with amounts accounting for less than 1%. On the contrary, the proteinic material was up to 82%. It was noticeable that functional properties of fish protein hydrolysates could be modified according to the DH value. Thus, extensive hydrolysis allowed high solubility, and limited hydrolysis (low DH) led to greater emulsion capacity, emulsion stability, and fat absorption. Moreover, the solubility of protein hydrolysates from salmon heads was high at all pH values, indicating potential useful applications in a variety of food formulations. It could be ascertained that just as well as the peptide size of hydrolysates, the hydrophilic-lipophilic balance plays an important role in the improvement of these properties. Therefore, the monitoring of commercial proteases could be an interesting alternative to convert fish byproducts into high-quality ingredients for industrial applications.

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