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Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*)

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Protein hydrolysates were prepared from male and spent capelin (*Mallotus villosus*) using commercially available Alcalase, Neutrase and papain. Short-time autolysis of proteins by endogenous enzymes in fish viscera was also investigated and compared to procedures of accelerated enzymatic hydrolysis. While protein recovery varied from 51·6 to 70·6% for commercial enzymes, a yield of 22·9% was obtained for autolyzed products. All methods of preparation afforded products containing about 71–78% proteins after dehydration. Alcalase served best for preparation of capelin protein hydrolysates (CPH). Thus, products of Alcalase-assisted hydrolysis of capelin proteins were further assessed for their nutritional and functional characteristics. The amino acid composition of CPH was similar to that of the starting capelin, except for methionine and tryptophan which were present in smaller amounts. The products had excellent solubility (≥84%) over a pH range of 2–11. Incorporation of CPH (up to 3%) in meat model systems resulted in an increase of 4% in cooking yield and inhibition of oxidation (determined by the 2-thiobarbituric acid test) by 17·7–60·4%.

INTRODUCTION

Capelin (*Mallotus villosus*) is a small silvery fish related to smelt. It is found in abundance in the cold waters of the Arctic and sub-Arctic in both the Atlantic and Pacific regions. In the eastern Atlantic, schools of capelin are located from western Norway to northern Russia. They are also found around Iceland and Greenland. In North America, they exist primarily from Hudson Bay to Nova Scotia, but they are most abundant around Newfoundland and Labrador. In the Pacific, they occur from Alaska to Juan de Fuca Strait and from the Sea of Chukotsh to Japan and Korea.

Capelin is an important forage fish in the northwestern Atlantic and is consumed by many predators. Small quantities of capelin have traditionally been used fresh, dried or salted for food purposes. Female roe capelin (referred to as 'shishamo') has become an important product in the Japanese market. However, capelin is still considered as being under-utilized; the male and spent capelin are generally dumped, although some have been used for the production of pet food, fish meal and silage. Marination of male capelin and the production of fish sauce has recently been accomplished in Newfoundland. In addition, the preparation of snack foods from capelin has been tested. There is a general desire to better-utilize capelin and all species of latent fish that are currently being reduced to animal feed.

The application of enzyme technology in fish processing has attracted considerable interest for converting fish processing wastes and under-utilized species into protein concentrates. One of the potential uses of enzymes for the modification and improvement of protein functionality is through controlled hydrolysis. While nutritional and safety criteria are important in developing unconventional proteins as a food source, their successful adaptation and acceptance by the food industry and consumers depend on their sensory and functional properties. A broad spectrum of products may be produced for a wide range of applications. The treatment of fish or fish processing wastes with proteolytic enzymes represents an interesting alternative to the mechanical separation of flesh from bones (Mohr, 1977) and chemical methods for the preparation of fish protein concentrates.

Biochemical production of fish protein hydrolysates may be carried out by employing an autolytic process or an accelerated hydrolysis method (Mohr, 1977). The autolytic process depends on the action of digestive enzymes of the fish itself. The process lasts from a few days to several months. There are no enzyme costs involved and it is a simple operation. However, prolonged digestion may adversely affect the functional properties of the resultant hydrolysate, and such products are generally used in feed formulations. Excellent reviews on the autolysis of fish have been published

(Kubota & Sakai *et al.*, 1978; Raa & Gildberg, 1982; Gildberg *et al.*, 1984; Mukundan *et al.*, 1986). On the other hand, accelerated hydrolysis using commercial proteases offers many advantages over the autolysis, since it allows control of the hydrolysis and the properties of the resultant products. A variety of enzymes from animal, plant or microbial sources may be employed (Hale, 1969, 1974; Cheftel *et al.*, 1971; Wessels & Atkinson, 1973; Mackie, 1974; Hevia *et al.*, 1976; Mohr, 1977; Hale & Bauersfield, 1978; Sikorski & Naczk, 1981; Haard *et al.*, 1982). However, accelerated hydrolysis is generally a more complex process, and the cost of enzymes may influence the economy and commercial viability of the process. This study was designed to examine the use of commercially available proteolytic enzymes for the preparation of capelin protein hydrolysate (CPH), to establish optimum processing conditions for enzymatic hydrolysis and to investigate selected functional properties of the resultant products. The hydrolysis of capelin proteins by autolysis was also examined.

MATERIALS AND METHODS

Materials

Male and spent capelin (*Mallotus villosus*) were obtained from fish processing plants or directly from fishermen in the Avalon Peninsula of Newfoundland. Whole fish were ground and homogenized immediately before use or were kept frozen at –60°C until used.

Chemicals and reagents

All chemicals and/or reagents used in this work were food-grade or reagent-grade. Alcalase and Neutrase were obtained from Novo Enzymes (Bagsvaerd, Denmark), and papain was purchased from Sigma Chemical Co. (St Louis, MO, USA).

Enzymatic hydrolysis

The scheme for the production of protein hydrolysate from capelin is given in Fig. 1. The ground fish sample was mixed with an equal amount of water and homogenized in a Waring blender for about 2 min. The pH of the mixture was adjusted to the optimum activity value for each enzyme. The hydrolysis was performed under different conditions with respect to temperature, pH and concentrations of substrate and enzyme. The pH of the mixture was kept constant by continuous addition of a 4 M NaOH solution to the reaction mixture. The reaction was stopped by lowering the pH to 3–4 in order to deactivate the enzyme. Hydrolysis conditions for the preparation of CPH are summarized in Table 1.

The degree of hydrolysis (DH%), defined as the percent ratio of the number of peptide bonds broken (h) to the total number of bonds per unit weight (h_{tot}), in each case, was calculated from the amount of base consumed (Adler-Nissen, 1986), as given below:

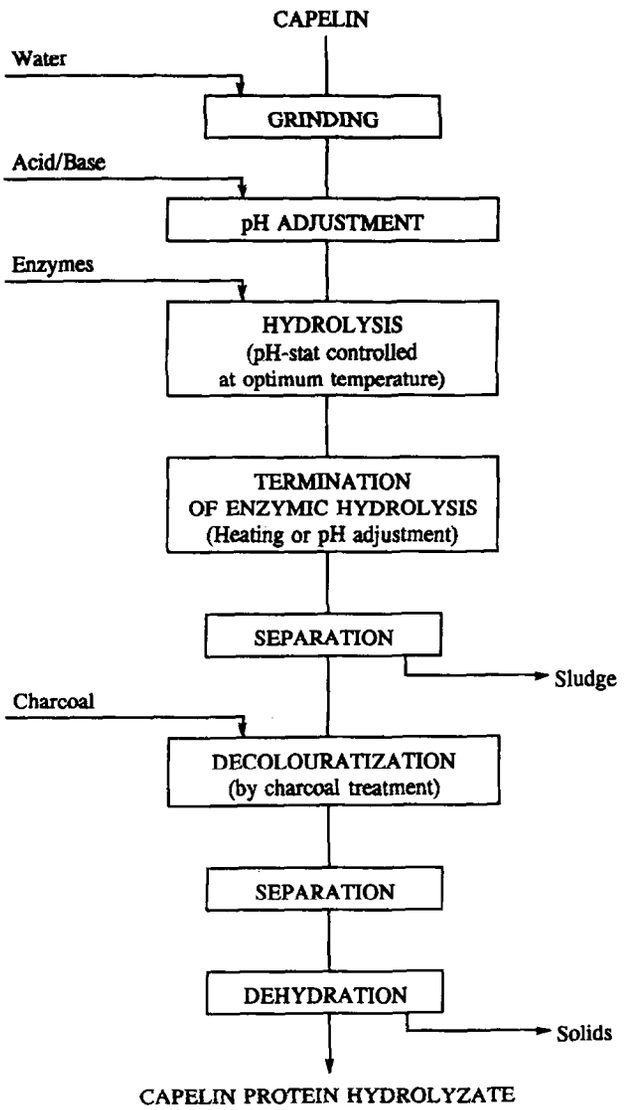

Fig. 1. Flowsheet for the production of capelin protein hydrolysate (CPH).

Table 1. Hydrolysis conditions of capelin protein by different enzymes

Hydrolysis condition	Enzyme system			
	Alcalase	Neutrase	Papain	Autolysis
Temperature (°C)	45–65	50–55	45–65	25
Reaction pH	8·5	7·0	6·1	3·0
Inactivation pH[a]	4·0	3·0	3·0	As is
[E]/[S][b]	30	30	1–4	—

[a]Inactivation was carried out for 30 min, possibly with heating, except for autolysis which was only heated for 10 min at 70°C.
[b]The enzyme concentration ([E]/[S]) is expressed as AU/kg Kjeldahl N × 6·25 for Alcalase and Neutrase and as percent weight ratio of enzyme to fish sample for papain.

$$DH\% = \frac{h \times 100}{h_{tot}} = \frac{B \times N_b \times 100}{\alpha \times M_p \times h_{tot}}$$

where B is the amount of base consumed (litres), N_b is the normality of base, M_p is the mass (kg) of protein

(N × 6·25) and α is the average degree of dissociation of the α-NH_2 groups expressed as

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

The sludge was removed by suction filtration. Removal of colored and odoriferous matters was achieved using charcoal (1%, w/v; 30 min stirring at 55°C). The pH of the decolorized hydrolysate was adjusted to 6·0 using 4 M NaOH followed by dehydration by freeze-drying or spray-drying. The ratio of total analyzable Kjeldahl nitrogen in the final product to that originally present in ground fish was calculated as the yield of protein recovery.

Proximate composition

Moisture content was determined by placing approximately 2 g of sample into a pre-weighed aluminum dish. Samples were then dried in a forced-air convection oven at 105°C overnight or until a constant weight was reached (AOAC, 1990). The total crude protein (N × 6·25) content of samples was determined using the Kjeldahl method (AOAC, 1990). Total lipids in each sample were extracted with a mixture of chloroform and methanol as described by Bligh and Dyer (1959). The content of minerals (expressed as percent ash content) was determined by charring approximately 2 g of sample in a crucible over a Bunsen burner and then heating in a muffle furnace at 550°C until the ash had a white appearance (AOAC, 1990).

Amino acid composition

The amino acid composition of lyophilized and powdered samples was determined by digestion in 6 M HCl at 110°C (Blackburn, 1968). The acid was then removed under vacuum and the resultant dried material was reconstituted with a pH 2·2 lithium citrate buffer. The amino acids were then quantified using a Beckman 121 MB amino acid analyzer. Cysteine and methionine were subjected to performic acid oxidation prior to their digestion in 6 M HCl and were determined as cysteic acid and methionine sulphone, respectively (Blackburn, 1968). Analysis of tryptophan was performed by hydrolysis of the sample in 3M mercaptoethanesulphonic acid at 110°C under vacuum (Penke *et al.*, 1974).

Functional properties of protein hydrolysates

Solubility

The solubility of protein hydrolysate was determined according to the method of Chobert *et al.* (1988) with slight modifications. A sample of protein hydrolysate (1%, w/v) was dispersed in distilled water and its pH was adjusted by the addition of a 6M HCl or NaOH solution. After standing for 3 min at room temperature, the mixture was centrifuged at 12000 *g* for 20 min. The solubility was determined by measuring the content of protein in the filtrate (AOAC, 1990). Absorbance values were read at 534 nm. The nitrogen solubility was determined by measuring the content of Kjeldahl nitrogen in the filtered solution (AOAC, 1990) and expressed as percent soluble protein at a given pH.

Moisture and fat adsorption

For the determination of the moisture adsorption, a 1–2 g accurately weighed sample was left, as a thin layer at 22°C, in an open aluminum tray at a given relative humidity (RH%) for a period of 24–48 h. The maximum percent weight gain was then recorded (Dev & Quensel, 1986). Slow addition of water to lyophilized CPH was also monitored to establish the minimum quality of water required to form a ball, a paste or a solution from the powder.

For the determination of fat adsorption, a 2 g sample was transferred into a 50-ml centrifuge tube, 12 ml of soybean oil were added and the mixture was thoroughly mixed with a glass-rod. The sample was then kept for 30 min while mixing every 5 min for 30 s, after which it was centrifuged for 25 min at 2000 *g*. Free oil was decanted and the fat adsorption of the sample was determined from the weight difference data.

Water-holding capacity and cooking yield of meat model systems

A 20-g sample of freshly ground meat, with or without added hydrolysate, was transferred into a pre-weighed 50-ml centrifuge tube and homogenized with 20 g of water. Tubes were covered with aluminum foil and placed in a boiling water bath for 20 min. The mixture was then cooled to room temperature and centrifuged for 15 min at 3000 *g*. The juice released was decanted and the sample was blotted over a Whatman No. 1 filter paper and transferred back into the tube. The percent cooking yield was calculated from the weight difference data.

Alternatively, the water-binding capacity of CPH in meat systems was determined according to NOVO (1987) with slight modifications. Frozen pork was thawed overnight in a refrigerator and treated with salt at a 3% (w/w) level. It was then homogenized with the addition of ice (20%, w/w) followed by the addition of protein hydrolysate and mixing. The homogenate was then transferred into a glass test tube, capped and kept at 5°C for 2 h before heating to 95–97°C for 2 h. After cooling to room temperature under a cold stream of water, the container was opened and the amount of free drip water was measured.

Emulsifying properties

To a 3·5-g sample, 50 ml of distilled water and 50 ml of soybean oil were added. The mixture was then homogenized for 30 s using a Polytron homogenizer (setting 4) and divided into four equal parts in four centrifuge tubes. The tubes were then centrifuged for 5 min at 2000 *g* and the emulsifying capacity was calculated as the ratio of emulsified versus total volume.

Emulsion stability of the samples was determined by

heating the mixtures for 30 min at 80°C, followed by dividing them into four equal portions in four centrifuge tubes. Tubes were then centrifuged at 2000 *g* for 5 min. The volume of emulsion and the total volume were recorded. Emulsion stability was expressed as percent emulsifying capacity after heating.

Alternatively, emulsifying activity (EA) of CPH was evaluated by the spectroturbidity procedure of Pearce and Kinsella (1978) with slight modifications. A 30-ml sample of 1·0% CPH in water was emulsified with 10 ml of soybean oil (volume of dispersed phase, π = 0·25). The mixture was homogenized for 30 min in a stainless container using a Waring blender at room temperature. Aliquots were immediately pipetted off from the emulsion and diluted 500-fold into 0·1% (w/v) sodium dodecyl sulphate (SDS) in 0·1 M NaCl, pH 7·0. The tubes were inverted three times to obtain homogeneous mixtures. Absorbances were then recorded at 500 nm. The EA was expressed as emulsifying activity index (EAI) as given below:

$$EAI = 2T/\pi c$$

where turbidity (T) = 2·3A/l [A is the absorbance at 500 nm and l is the light path (m)], π is the oil phase volume = 0·25 and c is the concentration of protein (1 %) before emulsion was formed.

In an alternate approach, emulsion stability was then determined using emulsions prepared above after a 24-h standing period at room temperature. The emulsions were heated at 80°C for 30 min, cooled to room temperature and the turbidity was read (EAI, 80°C). The absorbances of the aliquots prior to heating, with or without a 24-h standing, were also read (EAI, 20°C). Emulsion stability (Pearce & Kinsella, 1978) was calculated as given below:

$$\Delta EAI(\%) = (EAI_{max} - EAI_{80°C}) \times 100/EAI_{max}$$

where EAI_{max} is the maximum value (denoting best) emulsion stability obtained for t_0 to $t_{24\ h}$. These latter values did not differ significantly from one another.

Whippability and foaming stability

CPH (3 g) was dispersed in 100 ml of distilled water and the mixture was homogenized for 1 min using a Polytron homogenizer at setting 4. The mixture was then poured into a 250-ml graduated cylinder and the total volume was read. Whippability was expressed as percentage volume increase upon whipping, and foaming stability was calculated as the volume of foam remaining after 0·5, 10, 40 and 60 min quiescent periods.

Evaluation of antioxidant properties of CPH in meat model systems

Antioxidant activity of a typical CPH was evaluated in a meat model system. Ground pork was homogenized with 20% water (w/w) and 0·5–3·0% (w/w) CPH. Mixtures were subsequently cooked to an internal temperature of 75°C for 40 min in a thermostated water bath. After thermal processing, samples were cooled to room temperature and homogenized in a Waring blender followed by storage in plastic bags at 4°C until used.

The oxidative state of samples containing CPH was evaluated by the 2-thiobarbituric acid (TBA) test according to the distillation method of Tarladgis *et al.* (1964). In all cases a 10-g freshly ground and homogenized meat sample was transferred into a 500 ml round-bottom flask containing 97·5 ml of distilled water and 2·5 ml of 4 M HCl solution along with a few drops of DOW antifoam A and several glass beads. The slurry was then distilled under atmospheric pressure and 50 ml of the distillate was collected over a 20 min period. Equal volumes (5 ml each) of each of the distillates were mixed with 5 ml of a 0·02 M aqueous solution of the TBA reagent and were heated in a boiling water bath for a 35-min period. After cooling, the absorbance of the resultant pink-colored chromogen was read at 532 nm.

To convert the absorbance readings at 532 nm to TBA numbers, 1,1,3,3-tetramethoxypropane was used to construct a standard curve. A factor of 8·1 was obtained for converting the A_{532} values to the so-called TBA numbers defined as milligrams of malonaldehyde equivalents per kilogram of sample.

Calculation of protein efficiency ratio values

Protein efficiency ratio (PER) values of capelin and CPH were calculated according to the equations developed by Alsmeyer *et al.* (1974) and Lee *et al.* (1978). These equations are given in Table 2.

Statistical analysis

The RSREG (Response Surface REGgression) procedure of the Statistical Analysis System (SAS, 1990) was used to fit a quadratic polynomial equation to the experimental data. The fitted polynomial regression equations were expressed graphically by using the Surface Plot program of SAS at the Computer Centre of Memorial University of Newfoundland.

Analysis of variance and Tukey's studentized range test (Snedecor & Cochran, 1980) were used to determine differences in mean values based on data collected from 3–5 replications of each measurement. Significance was determined at a 95% level of probability.

Table 2. Prediction equations for the calculation of protein efficiency ratio (PER)

Equation no.	Equation[a]
1	–0·684 + 0·456 [Leu] –0·047 [Pro]
2	–0·468 + 0·454 [Leu] –0·104 [Tyr]
3	–1·816 + 0·435 [Met] + 0·780 [Leu] + 0·211 [His] –0·944 [Tyr]
4	0·08084 [ΣAA_7] –0·1094
5	0·06320 [ΣAA_{10}] –0·1539

[a] ΣAA_7 = Thr + Val + Met + Ile + Leu + Phe + Lys; ΣAA_{10} = ΣAA_7 + His + Arg + Tyr.

RESULTS AND DISCUSSION

The yields of protein hydrolysate using different proteolytic enzymes are given in Table 3. Although many factors affect the yield of hydrolysis, the type of enzyme used had a marked effect on the yield and properties of the final product. Neutrase had a much lower activity than Alcalase and is suitable only when a low degree of hydrolysis is preferred. At the same enzyme concentration ([E]/[S]) and reaction temperature, during a 3-h hydrolysis period, the yield of protein recovery (AN/TN) for Neutrase was lower than that for Alcalase and was always less than 60%. Papain at a 2–4% (w/w) addition level gave a higher protein recovery than Neutrase.

High protein recovery by Alcalase and its low cost may provide an incentive for using it in commercial operations. Thus, functional properties of protein hydrolysates so prepared were further evaluated in order to determine their suitability in different industrial and/or food applications.

Mechanism of enzyme hydrolysis

The control of enzymatic hydrolysis is related to the mechanism of proteolytic reactions involving a soluble enzyme and an insoluble substrate in the form of comminuted particles of fish tissue. The kinetics of enzymic reaction can easily be studied in a pH-controlled experiment. The amount of alkali or acid used to keep the pH constant is proportional to the number of peptide bonds broken (Adler-Nissen, 1986). The hydrolysis of the fish particles was characterized by an initial rapid phase, during which a large number of peptide bonds were hydrolysed (Fig. 2). The rate of enzymatic hydrolysis was subsequently decreased and reached a stationary phase when no apparent hydrolysis took place. The corresponding percentage of total protein recovery is shown in Fig. 3. Thus, the bulk of soluble protein hydrolysate was released during the initial phase of hydrolysis. It is of interest to note that no increase in the release of soluble hydrolysates was observed when additional enzyme was added to the reaction mixture during the stationary phase of the hydrolysis. Possible inhibition of protein hydrolysis by the presence of products or total cleavage of all susceptible peptide bonds by the enzyme may be contemplated. A high concentration of soluble peptides in the reaction mixture reduces both the rate of hydrolysis and the recovery of soluble proteins. Thus, removal of hydrolysate from the reaction mixture is expected to enhance the hydrolysis rate and recovery of proteins.

Table 3. Protein recovery and compositions of hydrolysates using various proteolytic enzymes[a]

Enzyme	Protein recovery[b] (%)	Composition (%)[c]			
		Protein[c]	Lipid	Moisture	Ash
Papain	57·1 ± 2·13	78·3 ± 1·92	0·39 ± 0·02	5·32 ± 0·24	17·7 ± 1·80
Neutrase	51·6 ± 1·92	71·2 ± 0·83	0·21 ± 0·02	5·26 ± 0·11	21·5 ± 0·53
Alcalase	70·6 ± 1·50	72·4 ± 0·70	0·18 ± 0·03	6·34 ± 0·11	20·8 ± 1·82
Endogenous	22·9 ± 1·66	74·4 ± 1·14	1·51 ± 0·02	5·78 ± 0·21	17·7 ± 1·23
Whole capelin	—	13·9 ± 0·21	3·56 ± 0·27	79·1 ± 0·08	2·41 ± 0·02

[a]The ratio of enzyme to substrate was 30 AU/kg proteins in initial material except for papain, which was 2% (w/w). The reaction was carried out at 55°C except for autolysis which was at 25°C. Data are mean values of triplicate determinations ± standard deviation.
[b]Based on Kjeldahl determinations (N × 6·25).
[c]Calculated on a dried weight basis.

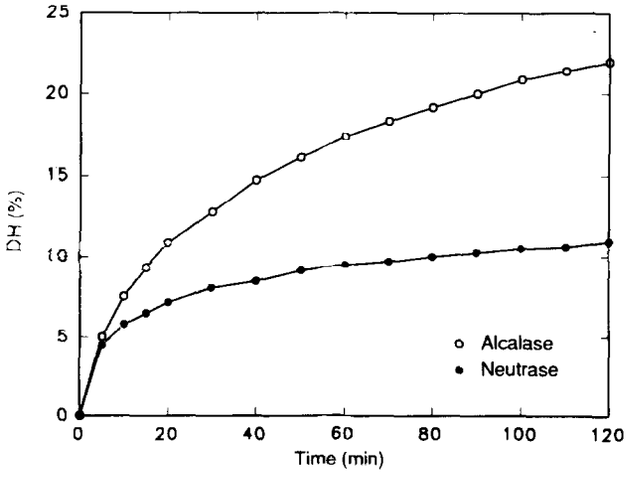

Fig. 2. Dependence of the degree of hydrolysis (DH%) on ground whole capelin on the length of hydrolysis period and type of enzyme.

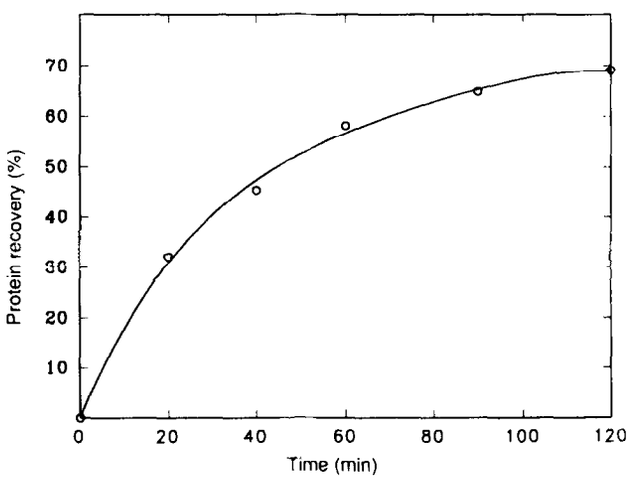

Fig. 3. Dependence of protein recovery from ground whole capelin on the length of hydrolysis period by Alcalase.

Optimization of processing conditions using Alcalase

Since Alcalase was found to be the best enzyme tested for the preparation of CPH, processing conditions for it were optimized. The three-dimensional response surfaces indicate that both the Alcalase concentration (13·9–83·5 AU/kg crude protein) and the treatment temperature (45–65°C) affect the degree of hydrolysis and thus the protein recovery (Fig. 4). The optimum temperature of the enzymic reaction depended on the length of incubation period. Therefore, the optimum temperature for a 60-min incubation period was 60°C, while that for a 120-min hydrolysis was 55°C. Hence, the reaction temperature that is selected should depend on the time and the degree of hydrolysis as well as other processing conditions.

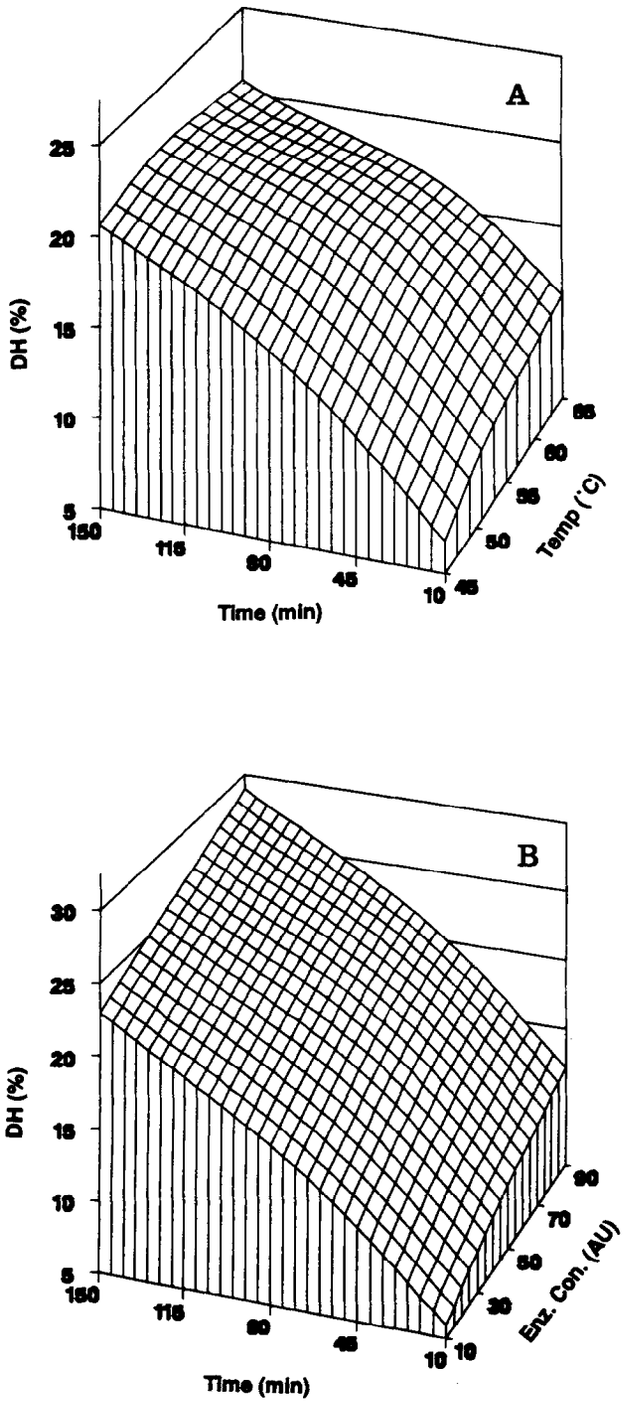

Fig. 4. Influence of processing temperature (A) and enzyme concentration (B) on the degree of Alcalase-assisted hydrolysis of capelin (DH%) over a 2·5-h period.

Some properties of protein hydrolysates

The composition of protein hydrolysates depended on the type of enzyme used. However, the lipid content of all preparations was greatly reduced when compared to that in the starting material (Table 3). This may in turn enhance the storage stability of the products. Although over 90% of proteins may be recovered by simple aqueous extractions (Chu & Pigott, 1973; Shenouda & Pigott, 1975, 1976), the retention of lipids in the final product may still thwart the use of the resultant product in many food applications. However, Venugopal and Shahidi (1994) recently reported on the preparation of protein concentrates with relatively low lipid contents from Atlantic mackerel using a sequential aqueous washing process. In enzymatic hydrolysis reactions, however, rapid changes occur in the structure of fish tissues. Electron microscopy of a thin section of cod muscle reveals that the myofibrillar proteins are extensively degraded during the hydrolysis process, whereas the elaborate membrane system of the muscle cells seems to be comparatively resistant to breakdown (Mohr, 1977). As hydrolysis proceeds, these membranes tend to round up and form insoluble vesicles, thus allowing the removal of membrane structural lipids.

The amino acid composition of the CPH prepared by Alcalase-assisted hydrolysis was determined. Based on

Table 4. Amino acid composition of capelin proteins and capelin protein hydrolysate

Amino acid	Composition (%)[a]	
	Capelin protein	Protein hydrolysate
Alanine	5·57±0·04	6·00±0·01
Arginine	5·99±0·10	5·70±0·02
Aspartic acid + asparagine	8·88±0·15	9·89±0·53
Cysteine	1·33±0·01	1·34±0·00
Glutamic acid + glutamine	13·2±0·03	13·4±0·03
Glucine	5·32±0·04	5·14±0·01
Histidine	2·43±0·00	2·09±0·02
Hydroxylysine	0·09±0·00	0·17±0·02
Hydroxyproline	0·42±0·02	0·46±0·07
Isoleucine	4·72±0·08	4·25±0·04
Leucine	8·15±0·05	7·60±0·00
Lysine	8·47±0·09	8·49±0·06
Methionine	3·09±0·02	2·05±0·01
Phenylalanine	3·80±0·01	3·19±0·00
Proline	3·70±0·15	3·67±0·03
Serine	4·18±0·05	4·24±0·10
Taurine	0·73±0·00	1·66±0·01
Threonine	4·82±0·05	4·56±0·03
Tryptophan	1·07±0·01	0·43±0·01
Tyrosine	3·34±0·01	2·47±0·06
Valine	5·71±0·12	5·77±0·01

[a]Results are mean values of three determinations ± standard deviation.

the results presented in Table 4, amino acid profiles of protein hydrolysates were generally similar to that of the original capelin, except for sensitive amino acids such as methionine and tryptophan which were affected to a relatively larger extent. The content of tryptophan was reduced by approximately 60% in the final product. However, Sugiyma *et al.* (1991) reported that 97% of tryptophan was retained in the final product when sardine was used as the starting material. The PER values of the hydrolysates were 2·61–3·11, as compared with 2·86–3·25 for the starting capelin proteins (Table 5).

Selected functionalities of a typical CPH, prepared by Alcalase treatment, are presented in Table 6. The product with a DH of 12% had an excellent fat adsorption and good whippability characteristics. The solubility characteristics of CPH prepared by the use of Alcalase and Neutrase at different pH conditions are shown in Fig. 5. The percentage solubility of CPH was always ≥84%; however, the highest solubility was observed at a pH around 5. CPH prepared by different enzymes showed different solubility profiles at different pH conditions (Fig. 5). This difference may have been the result of differences in bond specificity of enzymes used.

The addition of CPH to comminuted meat increased its cooking yield (Table 6). Addition of 3% CPH resulted in an increase of approximately 4% in the cooking yield of products. Using an alternative method, a large reduction in the amount of drip water was noticed, even at 0·1% addition of CPH in the formulation

Table 5. Calculated protein efficiency ratio (PER) values of capelin and capelin protein hydrolysate (CPH) and their comparison with those for cod proteins

Equations[a]	PER		
	Capeline	CPH	Cod[b]
1	2·86	2·61	2·86
2	2·88	2·72	2·87
3	3·25	3·11	3·24
4	2·98	2·79	2·99
5	2·86	2·64	2·90

[a]See Table 2 for description of equations.
[b]From Shahidi *et al.* (1991).

Table 6. Some functional characteristics of lyophilized capelin protein hydrolysates (CPH)

Functionality[a]	%
Fat adsorption	171 ± 2·0
Moisture adsorption[b]	
RH = 50%	1·56 ± 0·05
RH = 70%	6·23 ± 0·36
Emulsifying capacity	50·9 ± 1·2
Emulsion stability	92·0 ± 0·2
Whippability	90·0 ± 4·0
Foam stability	
0·5 min	170
10 min	10
40 min	6
60 min	0
Cooking yield	
CPH (%) 0·0	78·1 ± 0·68
0·5	78·6 ± 0·26
1·0	79·4 ± 0·19
2·0	81·3 ± 0·62
3·0	81·9 ± 0·37

[a]Slow addition of water to 100 g CPH indicated that 24·1 ± 0·20 ml water was needed to make a ball, 34·1 ± 1·00 ml to make a paste and 53·1 ± 1·06 ml to fully dissolve it.
[b]RH, relative humidity.

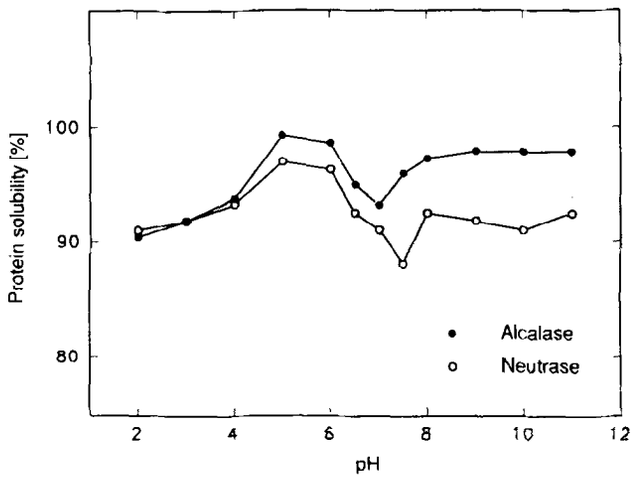

Fig. 5. Solubility of capelin protein hydrolysate (CPH) in water, as affected by pH, using an Alcalase-assisted or Neutrase-assisted hydrolysis process.

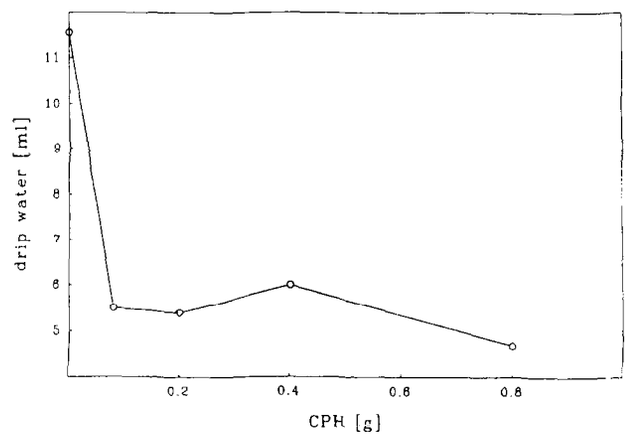

Fig. 6. Dependence of drip water on the amount of added capelin protein hydrolysate (CPH) in meat.

Table 7. Inhibition of formation of 2-thiobarbituric acid reactive substances by capelin protein hydrolysate (CPH) in cooked meats stored at 4°C[a]

CPH (%)	% inhibition (days)				
	0	1	3	5	Mean
0·5	29·4	15·9	7·3	18·3	17·7
1·0	36·0	16·5	14·6	19·9	21·8
2·0	62·3	62·3	34·6	41·2	44·4
3·0	76·4	76·4	48·2	58·1	60·4

[a]Calculated as 100 × [1– (TBA value of treated sample/TBA of untreated sample)]/TBA of untreated sample. Results were calculated from mean values of triplicate determinations.

(Fig. 6). Therefore, the method of evaluation, as well as the particular application of CPH, are important factors to be considered when using protein hydrolysates in food formulations. In addition, CPH at the 0·5–3·0% level inhibited the formation of 2-thiobarbituric acid reactive substances (TBARS) by 17·7–60·4% (Table 7). The mechanism by which CPH acts as an antioxidant may be due to a chelation effect.

Influence of autolysis on production of fish protein hydrolysates

The presence of proteolytic enzymes in the viscera of fish had a significant influence on production of hydrolysates. Endogenous enzymes alone produced hydrolysates with a protein recovery of approximately 23% at ambient temperatures after 4 h at pH 3·0 (Table 3).

The hydrolysis of ground capelin by endogenous enzymes enhanced the overall extraction of the fish protein at both acid and alkaline pH, since both acid and alkaline proteases are present in fish muscles and viscera. Pre-digestion of fish mince prior to the addition of exogenous enzymes might enhance the yield of protein extraction; however, autolytic enzymes of fish may also exert undesirable changes on the products, as it may be difficult to control the degree of autolysis during storage and processing. Furthermore, autolytic protease activity varies from species to species and depends on the season of harvest. Therefore, properties of functional protein hydrolysates so prepared may vary greatly under the same processing conditions.

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