Short Communication:

Development and characteristics of spray-dried hydrolysates from limited hydrolysis of shark meat

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Abstract

The recovery of underutilized fish biomass through enzymatic hydrolysis is an alternative that could contribute to alleviating the problem of malnutrition in developing countries. In this study, a proteolytic enzyme was used to catalyze the hydrolytic cleavage of peptide bonds to produce a fish protein concentrate from shark meat, an underutilized marine species, under conditions of limited hydrolysis. After the enzymatic reaction, the protein was recovered through separation and dehydration. The resulting product was a stable and edible powder that could be utilized as a protein supplement in human nutrition.

The final product exhibited high protein (> 82%) and low-fat concentrations (< 0.9%). Its amino acid profile was comparable to that of the original shark muscle, indicating that the nutritional balance was retained. Additionally, the physical characteristics of the spray-dried hydrolysates produced from limited hydrolysis of shark meat using the bacterial enzyme Alcalase, suggest potential applications as a protein supplement in protein-rich carbonated beverages.

Keywords: *Squalus acanthias*, Proteolytic reaction, Protein recovery, Food conversion, Food ingredients.

Introduction

The recovery of protein from underutilized fish biomass through enzymatic hydrolysis is a method that could contribute to alleviating the problem of malnutrition among low-income populations in developing countries[21]. It could be considered as one of the most promising technologies for maximizing commercial value and increasing the profitability of underutilized or discarded fish species, such as those from by-catch fisheries[28].

In this process, enzymes are used to recover the muscle protein by cleaving the peptide bonds within it. The resulting material from the proteolytic degradation is a liquid slurry containing the hydrolyzed protein, which consists of smaller polypeptides and amino acids. Their concentration depended on the degree of hydrolysis (DH) achieved. The liquid hydrolysate can be further processed into a stable powder using spray dehydration and can be converted into an ingredient for incorporation in food systems. Such a product is known as fish protein hydrolysate (FPH). FPHs possess desirable properties and high nutritional value[12,15,22], making them good alternatives for protein enrichment of other food products.

The physical properties and nutritional quality of a new protein source are important in food systems. In fact, the incorporation of this high-protein food ingredient into formulations depends on the properties they exhibit. For example, dispersibility and hygroscopicity are two important physical properties, particularly of protein powders that influence their behavior in liquid foods like soups and beverages. These properties also determine the potential limitations and applications of the ingredient in different food systems to impart desirable characteristics [4, 13, 14].

The spiny dogfish is an underutilized shark species found in the North Atlantic and Northeast Pacific. While it has good nutritional quality[19], its market value is low due to limited human consumption, making it a potential raw material for conversion into a protein-rich food ingredient via enzymic modification. This study describes the hydrolysis of dogfish meat using the commercial protease Alcalase, including the proximate and essential amino acid compositions of the resulting protein hydrolysate, along with the physical properties of the spray-dried powder.

Material and Methods

Raw material: Spiny dogfish (*Squalus acanthias*) was supplied by Fishery Products International Ltd., St. John's, Newfoundland. The fish was caught along the southwest coast of Newfoundland, Canada and immediately eviscerated on board. The dogfish was transported to the laboratory under chilled conditions and stored at -25 ºC until use.

Enzyme: Alcalase® is a food-grade serine endopeptidase produced by a strain of *Bacillus licheniformis*, a bacterium known for its ability to produce thermostable enzymes. Alcalase® was provided by Novozymes A/S (formerly Novo Industri), Bagsvaerd, Denmark[23, 24, 29].

Preparation of the hydrolysate: Hydrolytic reactions were performed in a 250-mL polyethylene vessel immersed in a constant temperature water bath, following the method of Diniz and Martin[10]. During reactions, pH was maintained at 8.0 by adding 0.2 N NaOH. The reaction flask containing ground dogfish muscle, deionized water and phosphate buffer, making up an initial substrate concentration (S %) of 8 % of protein (N x f), was placed in the preheated water bath. A 5-min homogenization was allowed for adjustment of pH, through the addition of NaOH and of temperature (55

°C). Then, the enzyme was added (3.7%, w/w) and the reaction proceeded with constant agitation at 200 rpm. The volume of NaOH needed to maintain pH constant during the hydrolytic reaction was recorded to calculate the degree of hydrolysis.

Autolytic degradation was also conducted under identical experimental conditions, but without enzyme addition, so any hydrolysis observed was due to endogenous enzymes in the dogfish muscle. Reactions were terminated by heating the solution to 90 °C for 15 min, which assured the inactivation of the enzyme[23] and the separation of the oil from substrate. The resultant slurry was centrifuged at 2800 × g for 20 min and the lipid layer was skimmed off. The volume of the supernatant was recorded and analyzed for nitrogen content using the Kjeldahl method. Powdered freeze-dried muscle, which contained concentrated native dogfish protein, was also used as the unhydrolyzed control sample.

Determination of degree of hydrolysis and protein recovery: The degree of hydrolysis was calculated using the method of Adler-Nissen[2-4]. The amount of crude protein in each hydrolysate was compared to the amount of protein in the raw material to determine protein recovery yield[30].

Dehydration: The soluble protein hydrolysates were spray-dried using a Büchi 190 spray-dryer (Büchi Laboratoriums - Technik AG, Flawil/Schweiz) with an air inlet temperature of 120°C and outlet temperature of 90°C. The feed rate was 0.4 L/hr. Moisture content was determined by drying samples in a forced air oven at 103-105 °C for 24 h. The spray-drying yield was calculated as the mass ratio of spray-dried powder to the total solids content in the soluble protein hydrolysate. The dry powders were stored in air-tight containers at 5 °C until use.

Analytical methods: The moisture content was calculated as the percentage of weight loss upon drying[1]. Ash was determined by incinerating the dried residue in a Muffle furnace at 550°C for 24 h[1]. Total lipids were estimated by extraction with chloroform:methanol:water mixture as described by Bligh and Dyer[6] and modified by Ke et al.[16] Nitrogen determinations were based on the Kjeldahl method described by A.O.A.C.[1]. The content of crude protein in samples was calculated by multiplying the percentage of nitrogen by a factor (f) of 6.25. Non-protein nitrogen (NPN) content was measured as the percent fraction of total Kjeldahl nitrogen that was not precipitated in a 10 % trichloroacetic acid (TCA) solution, according to Ke et al.[16]

Amino acid analysis was performed using a 121-MB Amino Acid Analyzer (Beckman, Palo Alto, CA) on the spray-dried enzymatically hydrolyzed shark muscle according to Penke et al.[25] Amino acid score (AAS), as defined as the content of the limiting amino acid in the food protein, was expressed as the percentage of the limiting amino acid content in the reference amino acid pattern (g/100 g protein) recommended by WHO/FAO/UNU[31].

Dispersibility was measured following the method of Rakesh and Metz[26], with minor modifications. Protein samples were dispersed in distilled water (10 g/L) and the pH was adjusted to 7.0 using 0.5 N HCl or 0.5 N NaOH. The solution was mixed for 60 minutes using a magnetic stirrer, then left to stand for 120 minutes. A 5-mL aliquot of supernatant was dried at 103°C to a constant weight. Dispersibility was expressed as the percentage of suspended solids relative to the initial sample weight.

Hygroscopicity of powders was determined gravimetrically, following the method of Cai and Corke[7] with minor modifications. Pre-weighed samples were placed in a desiccator containing saturated sodium chloride and allowed to equilibrate at 25 °C. Hygroscopicity was calculated as the percentage of moisture gained to reach equilibrium from the initial moisture content. All analytical determinations were run in triplicate with at least three determinations for each experiment.

Statistical analysis: The data were subjected to One-way analysis of Variance (ANOVA) using Minilab Statistical Software, release 6.1. Duncan's New Multiple Range Test (DNMRT) was performed to determine significant differences between samples at the 5% probability level.

Results and Discussion

Degree of hydrolysis and protein recovery: The concentration of solids in the spray-dried powder was 2.8 times higher (57.74 ± 0.35 %) than the total solids content in the soluble protein hydrolysate (20.43 ± 0.44 %). Figure 1 shows the hydrolysis curves for the proteolysis of the dogfish using Alcalase and the control. The hydrolysis observed in the control sample was due to the endogenous enzymes naturally present in the fish muscle. The shape of the hydrolysis curve is typical of enzyme-assisted reactions on food proteins, such as casein, gelatin, maize and soy isolates and wheat gluten[4].

Protein recovery from the original substrate is also shown in figure 1 with 77.93 % of the protein being recovered from the shark muscle. It is reasonable to establish a hydrolysis time of 120 min, since a more extensive hydrolysis could lead to a hydrolysate with a bitter taste. It is suggested that future research explores the application of immobilised enzymes in the hydrolysis of these proteins, which would allow the reuse of the enzyme and reduce the processing costs of the hydrolysates.

Proximate composition of shark protein hydrolysate: The composition of the shark protein hydrolysate is presented in table 1. The protein content (Nitrogen × 6.25) was 82 %, which is 4.3 times higher than the protein content of shark muscle on a wet weight basis. The increase in the protein content in the spray-dried hydrolysate is a consequence of the simultaneous decrease in water and fat content through spray dehydration. Fat content decreased significantly 0.84%, nearly 6.6 times lower than the lipid

content of dogfish muscle. This reduction can be attributed to the removal of the lipid layer after centrifugation.

On the other hand, the ash content increased as a consequence of the addition of NaOH during the hydrolytic reaction to maintain pH, a trend observed in other controlled hydrolytic reactions using alkaline proteases[5,17,20]. The moisture content was found to be 3.92%. The relatively low amount of NPN (1.06 g/100g) is expected, since washing and soaking the small pieces of fillet, procedure performed in this study, is known to reduce urea and other NPN constituents[8].

The amino acid composition of the shark protein hydrolysate revealed that all essential amino acids were present (Figure 2), indicating high nutritional value. Its amino acid composition meets the requirements for adults and, except for tryptophan, for preschool children[11]. Lysine and threonine levels were higher than the FAO/WHO protein standard[11]. This endorses the use of shark protein hydrolysate as a protein supplement in food systems rather than vegetable proteins, which generally contain low levels of Lys and Thr. The presence of all essential amino acids indicates high biological value, digestibility studies should be carried out to confirm its suitability for food systems.

Physical properties of the spray-dried powder: Dispersibility was notably enhanced to 91.88% for the hydrolyzed sample (FPH) compared to 14.45% for the unhydrolyzed control and 86.19% for the autolytic digestion by endogenous enzymes (Figure 3). Both the autolysed and hydrolysed samples exhibited high dispersibility values; however, the FPH showed statistically higher dispersibility ($P < 0.05$) than the autolysed sample. The control had significantly lower dispersibility than all the other samples.

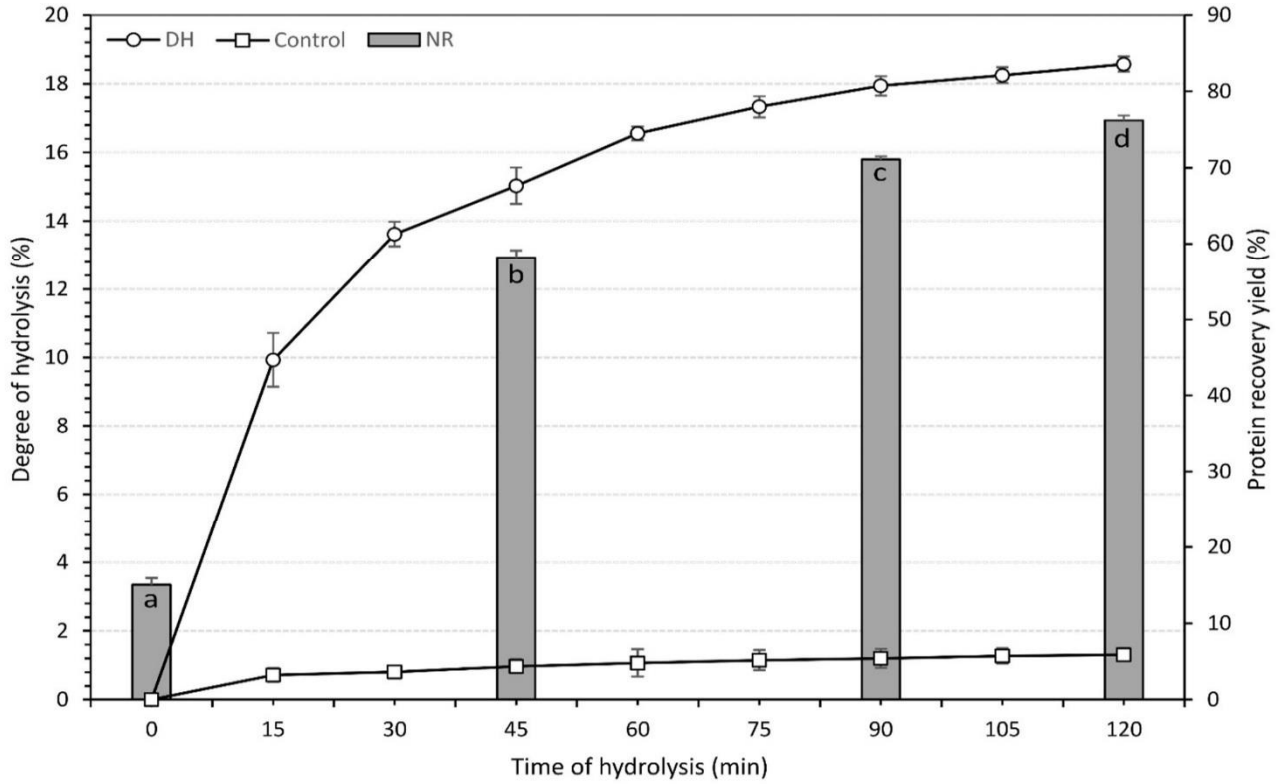

Figure 1: Degree of hydrolysis and protein recovery yield from shark meat using Alcalase®. Reaction conditions: T: 55 ºC; pH: 8.0; E/S: 3.7 %. (○): Alcalase-assisted reaction; (□): No enzyme addition (control). Columns represent protein recovery values. Values in columns with different letters are significantly different ($P < 0.05$).

Table 1
Proximate composition of shark (*Squalus acanthias*) meat and its protein hydrolysate.

Components	Composition (g/100g)	
	Shark meat	Shark hydrolysate
Ash	1.11 ± 0.05	13.15 ± 0.14
Lipids	5.58 ± 0.09	0.84 ± 0.03
Moisture	75.73 ± 0.20	3.92 ± 0.27
Total Nitrogen	3.08 ± 0.11	13.12 ± 0.05
Non-protein nitrogen	0.47 ± 0.04	1.06 ± 0.09

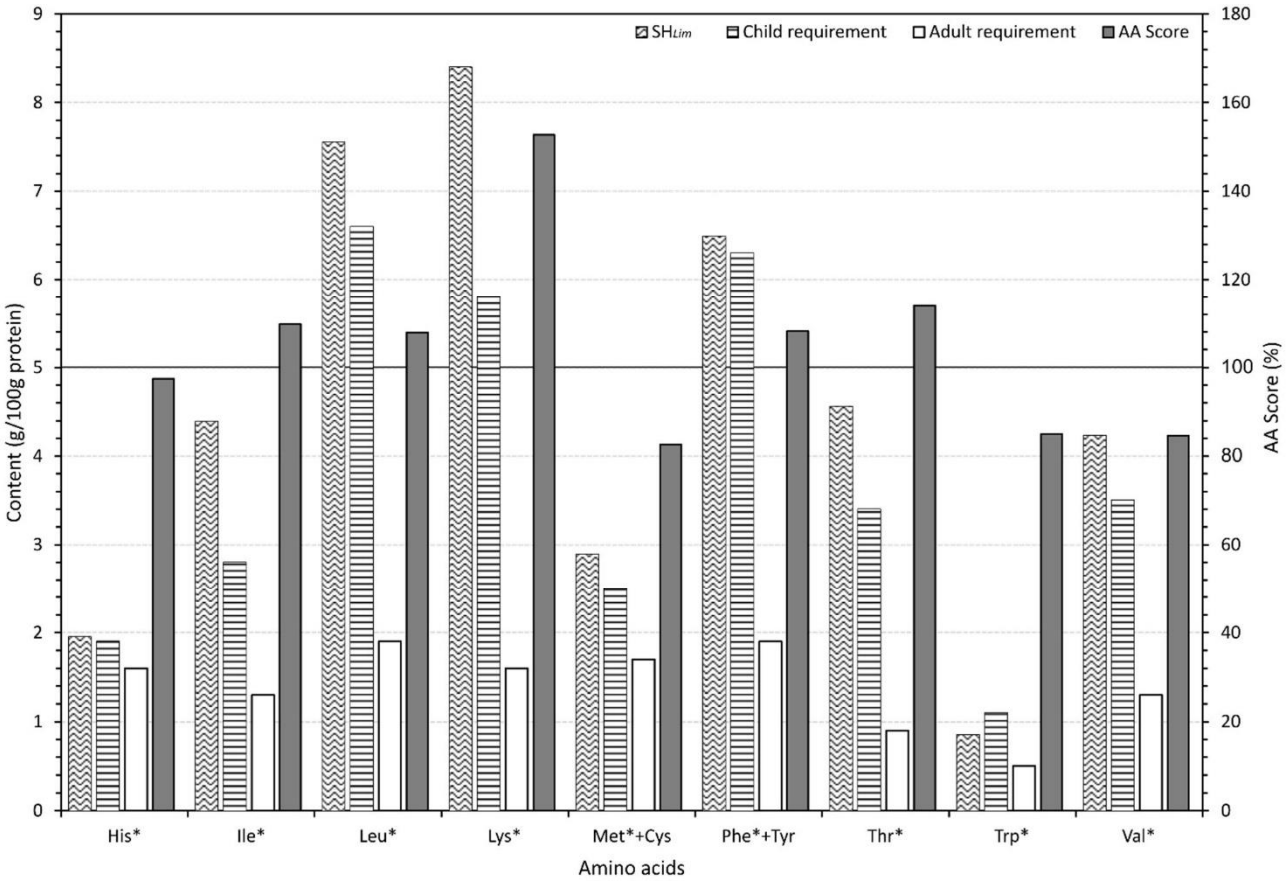

Figure 2: Amino acid composition (g/100 g crude protein) of the protein hydrolysate prepared with Alcalase® and the FAO/WHO suggested essential amino acid requirements and amino acid score (AA score)[a].
[*]Essential amino acids; SH_{Lim}: Shark hydrolysate; Suggested requirements for child and adults according to FAO/WHO[11]; [a]Amino acid scores based on the reference protein[21]: histidine 2.00, isoleucine 4.00, leucine 7.00, lysine 5.50, methionine + cystine 3.50, phenylalanine + tyrosine 4.29, threonine 4.00, tryptophan 1.00 and valine 5.00.

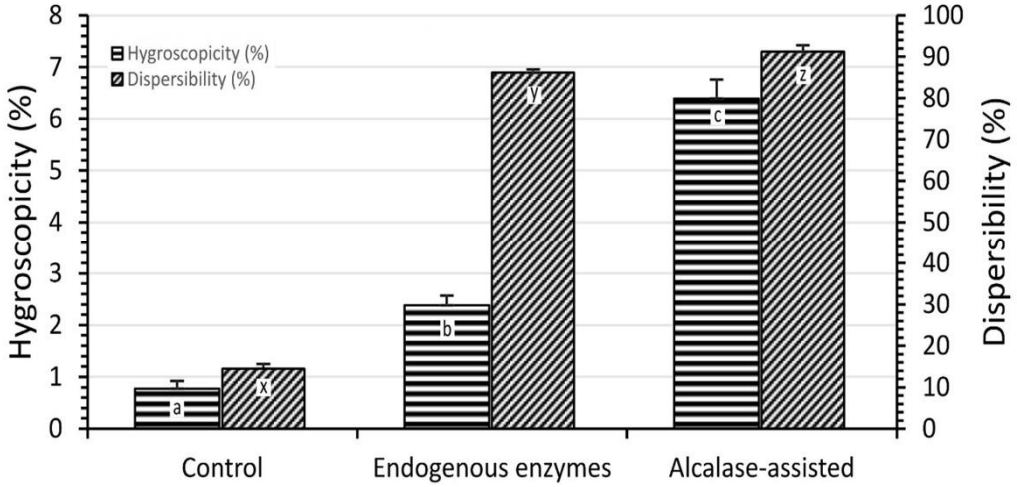

Figure 3: Hygroscopicity and dispersibility of the protein powders. Means ± standard deviation. Values in columns with different letters are significantly different (P<0.05).

In addition to the effect of proteolytic degradation on dispersibility, spray drying may have further improved dispersibility by increasing the surface area of the small particles produced through this process. The good dispersibility of this shark protein powder favours its application in the food industry and the production of composite materials. The high hygroscopicity of the protein hydrolysate (6.39%, Figure 3) may be attributed to the presence of low molecular weight peptides and/or the low initial moisture content of powders spray-dried at high temperature[9, 18]. This property could pose a problem for protein hydrolysates, but is possibly mitigated by adding carrier agents to reduce hygroscopicity and to improve stability of the hydrolysate[27].

Conclusion

The final product had high protein and low fat content. Its amino acid profile was comparable to that of the original shark muscle, suggesting that the nutritional balance was maintained. Additionally, the physical properties of the spray-dried hydrolysates produced from the limited hydrolysis of shark meat using the bacterial enzyme Alcalase indicate potential applications as a protein supplement, particularly in protein-rich carbonated beverages.

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