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The effect of enzymatic hydrolysis time and temperature on the properties of protein hydrolysates from Persian sturgeon (*Acipenser persicus*) viscera

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ABSTRACT

Protein hydrolysate was prepared from the viscera of Persian sturgeon (*Acipenser persicus*), a major sturgeon species in the Caspian Sea. Hydrolysis was performed at three different temperatures (35, 45 and 55 °C), pH 8.5, using commercially available Alcalase® and an enzyme to substrate ratio of 0.1 AU/g viscera protein over a 205 min incubation period. Protein and lipid content of the hydrolysate were 65.82%, and 0.18%, respectively. Protein recovery and degree of hydrolysis ranged from 34.97% to 61.96% and 13.32% to 46.13%, respectively. The highest degree of hydrolysis was observed at 55 °C after 205 min ($p < 0.05$). The amino acid score of the hydrolysates was similar to that of the FAO/WHO reference protein. It is revealed that Persian sturgeon visceral protein hydrolysate amino acid fulfils adult human requirements. Based on National Research Council guidelines, phenylalanine is the first limiting amino acid in the hydrolysate. However, the hydrolysate has the potential for application as an ingredient in formulated diets.

1. Introduction

Much of the protein-rich byproducts from seafood processing plants are discarded without any attempt at recovery. At the same time many processors are no longer allowed to discard their offal directly into the sea, resulting in a very high cost of refining the material before it is discarded. To meet the need of the seafood processing industry, an alternative for discarding these byproducts should be developed (Kristinsson & Rasco, 2000a).

Every year over 132 million tons of fish are harvested, of which 29.5% is converted into fish meal (FAO, 2006). Possibly more than 50% of the remaining fish tissue is considered to be non-edible waste material. With a dramatically increasing world population and a world catch of fish of more than 100 million tons per year, there is obviously an increased need to utilise our sea resources with more intelligence and foresight (Kristinsson & Rasco, 2000a). By applying enzyme technology for protein recovery in fish processing, it may be possible to produce a broad spectrum of food ingredients or industrial products for a wide range of applications. This would utilise both fisheries byproducts, secondary raw materials and, in addition, underutilised species that would otherwise be discarded. Fish viscera, one of the most important byproducts, are a rich source of protein and polyunsaturated lipids but with low storage stability if not frozen or otherwise preserved (Raa, Gildberg, & Strom, 1983).

Enzymatic modification of proteins using selected proteolytic enzyme preparations to cleave specific peptide bonds is widely used in the food industry (Mullally, O'Callaghan, FitzGerald, Donnelly, & Dalton, 1994). The most common commercial proteases reported used for the hydrolysis of fish protein are from plant sources such as papain (Hoyle & Merritt, 1994; Shahidi, Han, & Syniwiecki, 1995) or from animal origin, such as pepsin (Viera, Martin, Sampaiao, Omar, & Gonsalves, 1995), chymotrypsin and trypsin (Simpson, Nayeri, Yaylayan, & Ashie, 1998). Enzymes of microbial origin have been also applied to the hydrolysis of fish proteins. In comparison to animal- or plant-derived enzymes, microbial enzymes offer several advantages, including a wide variety of available catalytic activities, and greater pH and temperature stabilities (Diniz & Martin, 1997). Generally, Alcalase® 2.4 L-assisted reactions have been repeatedly favoured for fish hydrolysis, due to the high degree of hydrolysis that can be achieved in a relatively short time under moderate pH conditions, compared to neutral or acidic enzymes (Aspmo, Horn, & Eijsink, 2005; Benjakul & Morrissey, 1997; Bhaskar, Benila, Radha, & Lalitha, 2008; Hoyle & Merritt, 1994; Kristinsson & Rasco, 2000a, 2000b; Shahidi et al., 1995).

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Hydrolysing protein can also improve intestinal absorption (Kristinsson & Rasco, 2000a), and be used as a source of peptides, such as peptone, for ingredients in microbial growth media (Gildberg, Batista, & Strom, 1989).

The work to date on fish protein hydrolysate (FPH) has been somewhat sporadic, with most research conducted on industrial and animal feed application, and some directed to the potential of using powdered hydrolysates in food formulations, with some fish protein hydrolysates having excellent functional properties (Kristinsson & Rasco, 2000a).

Persian sturgeon, one of the most important sturgeon fishes on the south coast of the Caspian Sea, is caught for both meat and caviar production. Sturgeon processing waste is usually discarded, without any recovery except for the swim bladder. Also the notochord is recovered, and kept as frozen or dried, to use in soups or as a nutritional supplement. Sturgeon viscera is rich in protein, which can be used as animal feed, and its proteins may potentially serve as a human food ingredient. However, there have been no reported attempts studying the effect of enzymatic hydrolysis on sturgeon viscera protein. In this study the effects of time and temperature on the hydrolysis of sturgeon visceral proteins were investigated.

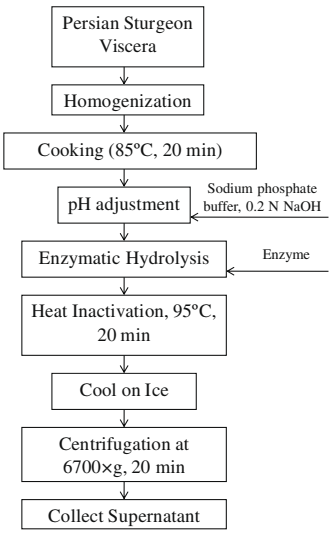

Fig. 1. Enzymatic hydrolysis process flow chart for the preparation of fish protein hydrolysates.

2. Materials and methods

2.1. Materials

Persian sturgeon (*Acipenser persicus*) was caught from the south coast of the Caspian Sea in Sari, Iran. The fish were immediately transferred to the laboratory, where viscera were removed and kept at −20 °C until use. Prior to the hydrolysis process, viscera were thawed overnight in a refrigerator at 4 ± 1 °C.

2.2. Enzymes

Alcalase® (with a declared activity of 2.4 AU/g and a density of 1.18 g/ml) is a bacterial endoproteinase from a strain of *Bacillus licheniformis*. It was provided by the Iranian branch of the Danish company Novozyme, and stored at 4 °C until use. All chemical reagents used for experiments were of analytical grade.

2.3. Preparation of fish protein hydrolysate

The Persian sturgeon protein hydrolysate production scheme is given in Fig. 1. The fish viscera were first minced in a Moulinex® blender, and then heated at 85 °C for 20 min to inactivate endogenous enzymes (Guerard, Guimas, & Binet, 2002). The cooked viscera were mixed with sodium phosphate buffer 1:4 (w:v) and homogenised in a Moulinex® blender for about 2 min at ambient temperature. The pH of the mixture was adjusted to the optimum activity of Alcalase, pH 8.5, by adding 0.2 N NaOH. Alcalase was added to the substrate based on its enzyme activity (0.1 AU/g protein). All reactions were performed in 250 ml glass vessels, in a shaking incubator (Jaltajhiz, Iran) with constant agitation (200 rpm) at three different temperatures: 35, 45 and 55 °C (Bhaskar et al., 2008). After each treatment, the reaction was terminated by heating the solution at 95 °C for 20 min (Guerard et al., 2002), assuring the inactivation of the enzyme. The hydrolysate were then cooled on ice to room temperature and centrifuged at 6700*g* at 10 °C for 20 min in a Hettich D-7200 (Tuttlingen, Germany) centrifuge, to collect the supernatant.

2.4. Chemical composition

Moisture content was determined by placing approximately 2 g of sample into a pre-weighted aluminium dish. Samples were then dried in an oven at 105 °C until a constant weight (AOAC, 2005). The total crude protein ($N \times 6.25$) in raw materials was determined using the Kjeldahl method (AOAC, 2005). Total lipid in sample was determined by Soxhlet extraction (AOAC, 2005). Ash content was estimated by charring in a predried sample in a crucible at 600 °C until a white ash was formed (AOAC, 2005).

Protein in the fish hydrolysates was measured by the Biuret method in the supernatant following centrifugation (Layne, 1957), using bovine serum albumin as a standard protein. Absorbance was measured at 540 nm in a UV/vis spectrophotometer. The Gerber method was applied to measure total lipid content of the hydrolysate (Collares, Gonçalves, & Ferreira, 1997). Protein recovery was calculated as the amount of protein present in the hydrolysate relative to the initial amount of protein present in the reaction mixture.

2.5. Degree of hydrolysis

Degree of hydrolysis was estimated according to the method of Hoyle and Merritt (1994). To the supernatant, one volume of 20% trichloroacetic acid (TCA) was added, followed by centrifugation at 6700*g* at 10 °C for 20 min to collect the 10% TCA-soluble materials. The degree of hydrolysis (DH) was computed as:

$$\%DH = 100 \times (10\%\ \text{TCA} - \text{soluble } N_2 \text{ in the sample/total } N_2 \text{ in the sample})$$

2.6. Amino acid composition

Sample preparation was conducted by hydrolysis with 6 M HCl at 110 °C for 12 h and derivatisation using phenyl isothiocyanate prior to HPLC analysis. The total amino acids and free amino acids were analysed by the Pico Tag method (Waters Corporation, Milford, MA), using a Pico Tag column (3.9 × 150 mm; Waters) at a flow rate of 1 ml min^{-1} with UV detection. Breez® software was applied to data analysis.

2.7. Chemical score

The chemical score of the protein hydrolysate was computed according to Bhaskar et al. (2008), relative to the essential amino acid (EAA) profile in a standard protein as described by FAO/