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ScienceDirect
Bioresource Technology 99 (2008) 335–343
BIORESOURCE TECHNOLOGY

Optimization of enzymatic hydrolysis of visceral waste proteins of Catla (*Catla catla*) for preparing protein hydrolysate using a commercial protease

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Received 6 November 2006; received in revised form 14 December 2006; accepted 14 December 2006
Available online 15 February 2007

Abstract

Protein hydrolysate was prepared from visceral waste proteins of Catla (*Catla catla*), an Indian freshwater major carp. Hydrolysis conditions (viz., time, temperature, pH and enzyme to substrate level) for preparing protein hydrolysates from the fish visceral waste proteins were optimized by response surface methodology (RSM) using a factorial design. Model equation was proposed with regard to the effect of time, temperature, pH and enzyme to substrate level. An enzyme to substrate level of 1.5% (v/w), pH 8.5, temperature of 50 °C and a hydrolysis time of 135 min were found to be the optimum conditions to obtain a higher degree of hydrolysis close to 50% using alcalase. The amino acid composition of the protein hydrolysate prepared using the optimized conditions revealed that the protein hydrolysate was similar to FAO/WHO reference protein. The chemical scores computed indicated methionine to be the most limiting amino acid. The protein hydrolysate can well be used to meet the amino acid requirements of juvenile common carp and hence has the potential for application as an ingredient in balanced fish diets.

Keywords: Protein hydrolysate; Fish viscera; Alcalase; Fish waste; Optimization

1. Introduction

The world fish production has almost stagnated and presently stands at 132 mmt (FAO, 2006). Fish sources once appeared to be inexhaustible and by-products arising out of fish processing were looked as worthless garbage and discarded without an attempt of recovery (Kristinsson and Rasco, 2000a; Gildberg, 2002). Although some by-products like shrimp waste are being utilized today, a huge amount is still being discarded creating both disposal and pollution problems. Annual discards of fish industry is estimated to be approximately 20 million tonnes (or 25% of the total production) per year (Rustad, 2003).

Unlike the seafood processing sector, fresh water fish or the inland fisheries sector in general is un-organized, especially in developing countries and hence poses a different level of waste disposal problems. The major by-products arising out of fish processing include viscera, skin, scales, bones and bone frames (in case of surimi production) and they constitute as high as 70% of the original raw material (Benjakul and Morrisey, 1997). The fact that these by-products are rich in protein and fat make them more perishable. As per one estimate, visceral waste alone contributes to the tune of 3,00,000 tonnes (Mahendrakar, 2000). Further, fish processing wastes including viscera have been reported to be good source of proteins including enzymes and fats (Gildberg, 2001; Arnesen and Gildberg,

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0960-8524/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2006.12.015

2007; Bhaskar et al., 2007a), good substrates for lactic acid fermentation (Gao et al., 2006) and source of protease producing bacteria (Bhaskar et al., 2006). If these biological compounds can be recovered, it would serve the dual purpose of recovery of these biomolecules and reducing the pollution problems associated therewith.

Fish viscera constitute approximately 20% of the fresh water fish biomass, and are a rich source of protein and polyunsaturated lipids. These are low quality raw materials or wastes, which, if not utilized may cause environmental, health and economical problems (Vidotti et al., 2003). One way to add value to proteinaceous fish waste is to convert it into hydrolysate (Aspmo et al., 2005). Enzymatic proteolysis and solubilisation of proteins from various sources has been studied extensively and described by several researchers (Kristinsson and Rasco, 2000a,b; Liaset et al., 2000; Nilsang et al., 2005; Bhaskar et al., 2007b). Enzymes hydrolyze proteins, thereby allowing the nitrogen to be more soluble, to free amino acids, thus making the hydrolyzed mass the most available amino acid source (Espe et al., 1989; Vidotti et al., 2003). Also, such hyrolysates can find use as ingredients in aquaculture feeds (Vidotti et al., 2003; Nilsang et al., 2005) and as an effective nitrogen source in microbial growth media (Guerard et al., 2001).

Traditional methods for preparation of autolytic hydrolysate like fish silage exploit the endogenous enzymes and it is rather difficult to control the autolysis by endogenous enzymes due to several factors including fish species and seasonality as well as the type and amount of enzymes (Sikoroski and Naczk, 1981). Addition of exogenous enzymes could make the hydrolytic process more controllable, apart from hastening it, thereby making it reproducible. Several factors, like pH, time, enzyme to substrate level and temperature, influence enzymatic activity co-operatively and thus, offer possibilities to control the process (Viera et al., 1995; Liaset et al., 2000). Proteolytic enzymes from plants and microorganisms have been found to be more suitable to produce fish protein hydrolysate (Shahidi et al., 1995; Benjakul and Morrisey, 1997; Guerard et al., 2001; Nilsang et al., 2005). Acid proteases, even though is better for microbial growth prevention, have only low protein yield and thus, milder enzymes at neutral and slightly alkaline condition have been used more frequently (Kristinsson and Rasco, 2000c).

Enzymes used to produce fish protein hydrolysate have at least one common characteristic; they should be food grade and if they are of microbial origin, the producing organism has to be non-pathogenic. The choice of substrate, protease employed and the degree to which the protein gets hydrolysed generally affects the physicochemical properties of the resulting hydrolysates (Mullaly et al., 1995). Alcalase – an alkaline bacterial protease produced from *Bacillus licheniformis*, has been proven to be one of the best enzyme used in the preparation of fish protein hydrolysate by many researchers (Hoyle and Merritt, 1994; Shahidi et al., 1995; Benjakul and Morrisey, 1997; Kristinsson and Rasco, 2000a,b; Guerard et al., 2001). From a technical and economical point of view, microbial enzymes like alcalase operating at alkaline pH have been reported to be most efficient in the hydrolysis of fish proteins (Dufosse et al., 1997). Further, it has been reported that fish protein hydrolysates prepared using alcalase had less bitter principles as compared to those made with papain (Hoyle and Merritt, 1994) and alcalase has been documented to be a better candidate for hydrolyzing fish proteins based on enzyme cost per activity (Kristinsson and Rasco, 2000b).

Against this background, the present investigation was undertaken to evaluate the utility of fish visceral waste as source of recoverable proteins in the form of protein hydrolysate. The objectives of the study were to optimize reaction conditions (i.e., time, temperature, pH and enzyme to substrate level) to obtain optimal degree of hydrolysis of visceral waste proteins of a freshwater fish (viz., *Catla catla* in this case) using a milder enzyme like alcalase® and to determine the amino acid composition of the protein hydrolysate prepared using the optimized conditions.

2. Materials

Visceral waste devoid of airbladder obtained by the processing of freshly harvested Indian major carp Catla (*C. catla*) collected from Mysore (Karnataka, India) fish market formed the material of the study. The visceral waste was brought to the laboratory in iced condition. The protease employed for the optimization studies were Alcalase®, (Novo Industry, Denmark; alkaline enzyme; declared activity of 0.6 Anson-U/g). All the chemicals used in different analysis were of analytical grade, unless otherwise mentioned.

3. Methods

The visceral mass was minced in a Waring blender (Stephen Mill, UM5 Universal, Hong Kong) followed by heating the visceral mass at 85 °C for 20 min to aid in inactivating the endogenous enzymes (Guerard et al., 2001) and to facilitate the removal of the fat present in the material (Bhaskar et al., 2007b). The heat treated visceral mass (HTVM) was allowed to cool and centrifuged at 10 °C for 20 min at 6000*g* to separate the oil. The separated oil was removed and the protein rich solids were collected for further studies. The protein rich solids were then extracted thrice with distilled water at 1:1 (w/v) to collect the protein extract. This protein extract (PE) was used for the optimization experiments.

Proximate composition (protein, fat, moisture and ash) of the raw material and final product was estimated as per AOAC (2002) method. All protein measurements in the samples were carried out by Kjeldahl method using Kjeltec protein analyzer (Foss–Tecator AB, Sweden).