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Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases

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Abstract

Fish protein hydrolysate (FPH) was produced from fish soluble concentrate (FSC), a by-product from canned fish industry, by using Flavourzyme™ and Kojizyme™. Hydrolysis conditions were optimized by using a response surface methodology (RSM). The model equations were proposed with regard to the effects of temperature (T), time (t), and enzyme concentration (E) on the degree of hydrolysis (DH). The optimum values for Flavourzyme™ concentration, substrate concentration, temperature, and hydrolysis time were found to be 50 LAPU/g protein, 20% (w/w), 45 °C, and 6 h, respectively (LAPU; Leucine Aminopeptidase Unit). While those values for Kojizyme™ were 40 LAPU/g protein, 20% (w/w), 50 °C, and 6 h, respectively. Kojizyme™ enhanced the formation of some bitter-taste amino acids such as tryptophan during hydrolysis process whereas Flavourzyme™ did not. The spray-dried FPH produced with Flavourzyme™ contained high protein content (66%). The bitterness of FPH was less than that of 1 ppm caffeine solution.

Keywords: Fish protein hydrolysate; Fish soluble concentrate; Bitterness

1. Introduction

Fish soluble is a by-product from canned tuna processing. It is made from tuna pressed liquids which is processed into a concentrated form of fish soluble concentrate (FSC). This FSC contains high protein and amino acids contents, which are useful for aquaculture uses and animal feed. In order to increase the utilization of FSC, hydrolysis of protein with proteolytic enzymes can provide FSC into more marketable and value-added products of fish protein hydrolysate (FPH; Jeon, Byun, & Kim, 2000; Kristinsson & Rasco, 2000a; Liaset, Lied, & Espe, 2000; Liceaga-Gesualdo & Li-Chan, 1999; Shahidi, Han, & Synowiecki, 1995).

Benjakul and Morrissey (1997) studied the production and composition of protein hydrolysate from Pacific whiting solid waste (PWSW) by using commercial enzymes Alcalase and Neutrase. The optimum conditions for PWSW hydrolysis were 20 AU Alcalase/kg, pH 9.5, 60 °C, and 1 h reaction time. Their hydrolysate had a high protein content and amino acids composition comparable to fish muscle. Guérard, Dufossé, De La Broise, and Binet (2001) used Alcalase to hydrolyse proteins from tuna waste. Their studies revealed that the tuna protein hydrolysate performed effectively as a nitrogenous source in microbial growth media.

The production of seafood flavors from under utilized fish species, using protein hydrolysis, is very challenging in order to ensure a high organoleptic quality (Shahidi, 1998). An enzymatic process using specific protease has been developed to produce flavorants from seafood by-products (Baek & Cadwallader, 1995). However, the hydrolysis of protein is often accompanied with

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flavor defects such as bitterness and off-flavor (Kilara, 1985).

Bitterness is the major problem affecting the sensory acceptability of protein hydrolysate. Several attempts have been made to limit the formation of bitterness through controlling the degree of hydrolysis (Adler-Nissen, 1984), using plastein reaction (Synowiecki, Jagielka, & Shahidi, 1996), or using specific enzymes such as exopeptidases (Blortz, Bohrmann, Maier, & Müller, 1999; Slattery & Fitzgerald, 1998). The bitterness is due to the exposure of interior hydrophobic amino acid side chains. The amino acids that create a bitter taste are valine, isoleucine, phenylalanine, tryptophan, leucine, and tyrosine (Pedersen, 1994). Both Kojizyme™ and Flavourzyme™ are the endo- and exopeptidase enzymes mixtures. These commercial enzymes can minimize the bitterness in the hydrolysed product (Imm & Lee, 1999; Liaset et al., 2000).

The objectives of this study were to optimize reaction conditions for the hydrolysis of fish soluble concentrate, to determine the bitterness and the compositions of the fish protein hydrolysate.

2. Materials and methods

2.1. Materials

Fish soluble concentrate (FSC) containing 50% minimum solids content as specified by the manufacturer (T.C. Union Agrotech Co. Ltd., Bangkok, Thailand) was used as a substrate. Commercial endo- and exopeptidase enzyme mixtures, Flavourzyme™ (declared activity of 1000 LAPU/g) and Kojizyme™ (declared activity of 800 LAPU/g) were purchased from Novo Nordisk (Denmark). One LAPU (Leucine Aminopeptidase Unit) is the amount of enzyme which hydrolyses 1 μmol of L-leucine-*p*-nitroanilide per minute. Both enzymes have an optimum pH range of 5–7.

2.2. Experimental design for optimization

A $4 \times 5 \times 6$ factorial in randomized complete block design (RCBD) was used to obtain the combination of values that optimized the response. To identify optimum levels of three variables, the response surface methodology was applied. The three variables studied were temperature, enzyme concentration, and hydrolysis time. The levels used are shown in Table 1. The selection of these factors was based on a preliminary study of enzymatic hydrolysis conducted by the enzyme manufacturer. The studies were preliminary performed by incubation of enzymes and fish soluble concentrate at 55 °C and pH 6.0 with working volume of 1 l while stirring at 200 rpm. It was assumed that the estimated response surface $\hat{y}$ can be described with the aid of a second order model:

Table 1
Factors and conditions used in the optimization experiments

Factors	Code	Conditions
Temperature (°C)	x_1	45, 50, 55, and 60
Enzyme concentration[a] (% w/w)	x_2	1, 2, 3, 4, and 5
Hydrolysis time (h)	x_3	1, 2, 3, 4, 5, and 6

[a] Per gram of protein dry weight in substrate.

$$\hat{y} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (1)$$

The variable $\hat{y}$ is a predicted response, x_1, x_2, and x_3 are independent variables, b_0 is an offset term, b_1, b_2, and b_3 are linear effects and b_{12}, b_{13}, and b_{23} are interaction effects (Khuri & Cornell, 1987). The model evaluated the effect of each independent variable to a response. Analysis of the experimental design and calculation of predicted data were carried out using Statistical Analysis System: SAS software (SAS Institute, Cary, NC, USA) to estimate the response of the independent variables (Little, Feund, & Spector, 1991). The surface plots were produced by Sigma Plot program (Jandel Scientific, 1989, Corte Madera, CA, USA).

2.3. Enzymatic hydrolysis

The FSC was diluted to 20% (w/w) solids content with distilled water. The pH of diluted FSC mixture was in the range of 5.9–6.0 which is optimum for both enzymes. One liter diluted FSC was placed in a 2-l beaker which was in a waterbath (Heto-Holten, Denmark) and preincubated for 30 min at the desired temperature. A specific protease was added to the beaker, and the mixture was stirred at 200 rpm during reaction using a stirrer (RW 20n, IKA Laboratechnik, Malaysia). Aliquots (25 ml) of hydrolysate were removed at time intervals of 1, 2, 3, 4, 5, and 6 h and immediately heated at 85 °C for 15 min to terminate the enzyme reaction. The hydrolysate obtained was analyzed for the degree of hydrolysis (DH) and free tryptophan. A batch of 2 l of FSC was hydrolysed at the optimum conditions using Flavourzyme™. The hydrolysate obtained was centrifuged (Sorvall RC-3B plus, Sorvall Instrument, USA) at 4200 rpm for 1 h and filtered twice through an eight-layer sheath-cloth to remove sludge. The FPH was then dehydrated by spray drying in a laboratory spray dryer (Model Minor Spray Dryer Serial No.2627, GEA-NIRO, Denmark) with an inlet temperature of 180 °C, outlet temperature of 90 °C and feed flow rate of 27 ml/min. The scheme of the FPH production is shown in Fig. 1.