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The Effect of Enzyme, Time and Temperature on the Properties of Hydrolyzed Protein of Viscera Grass Carp (*Ctenopharyngodon idella*)

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Abstract

The purpose of this study was to investigate the effect of enzyme kind, time and temperature on the properties of viscera (*Ctenopharyngodon idella*) hydrolyzed proteins. In this study, viscera was subjected to hydrolysis by alkalase, pepsin and flavourzyme commercial enzymes. Recycling efficiency and degree of hydrolysis obtained proteins and antioxidant activity DPPH in different conditions of the hydrolysis process at 15, 30, 60 min and three temperatures of 35, 45, 55 °C and between three enzymes with three replicates reviewed and compared. To investigate the existence or absence of significant differences between the values of each index, two-way analysis of variance and comparison of mean traits were used by Duncan test. For all enzymes used in this study, by increasing the time and temperature of hydrolysis, the amount of protein recovery and degree of hydrolysis and antioxidant activity increased, and the highest of these two indices for all three enzymes at 55 °C and in the time was 60 min and the lowest was observed at 35 °C and at 15 min. Also, the results showed that among the three enzymes studied, alkalase was similar in temperature to different pepsin and flavourzyme enzymes, with protein recovery and higher degree of hydrolysis and antioxidant activity. The highest protein digestion was related to the alkalase enzyme at 55 °C and 60 min (54.52±0.11%), and the lowest was obtained at 35 °C and 15 min. The results showed that the use of alkalase enzyme to produce hydrolyzed protein from grass carp was better in terms of recycling efficiency and degree of hydrolysis and antioxidant activity.

Keywords: Enzyme, Viscera, Hydrolyzed protein, Grass carp

Introduction

Seafood processing, generates a significant amount of wastes. During processing, much is done to maximize the yield of directly edible products but the production of waste or by-products is inevitable (Shabanpour *et al*., 2017). Much of this is generally discarded as waste or as low-value products; this is the case for the carp head, viscera and tail (Razavi Shirazi, 2001). Fish by-products contain valuable protein and lipid fractions as well as vitamins and minerals (Ovissipour *et al*., 2012). There are many options for seafood waste management that could help to resolve these problems. Enzymatic hydrolysis is one of the methods for recovery of valuable components from fish by-products. Enzymatic hydrolysis is one of the most efficient methods to recover protein and thus to increase the commercial value of such biomass (Ovissipour *et al*., 2009). Fish protein hydrolysates have good foaming and

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emulsifying properties, thus may be used as emulsifying and emulsion stabilizing ingredients in a variety of products as well as aid in the formation and stabilisation of foam-based products (Pezeshk *et al.*, 2017). The objective of this work is to investigate the effect of time hydrolysis (15, 30 and 60 min) of grass carp by-products (viscera) using alkalase, pepsin and flavourzyme.

Material and methods

Fresh grass carp were obtained from a local fish market immediately after processing and brought to the laboratory, stored at 4 °C for <2 h before experimental work. In this study, viscera was subjected to hydrolysis by alkalase, pepsin and flavourzyme commercial enzymes. Recycling efficiency and degree of hydrolysis obtained proteins and antioxidant activity DPPH in different conditions of the hydrolysis process at 15, 30, 60 min and three temperatures of 35, 45, 55 °C and between three enzymes with three replicates reviewed and compared. These enzymes were chosen because of their availability and their apparent suitability for industrial use. The hydrolysis was performed in a 4:l closed glass vessel stirred with a marine impeller (150 rpm). Viscera were mixed with warm (55 °C) water at a weight ratio 1:1. When the temperature of the mixture was 55 °C, the enzymatic hydrolysis was started by adding 0.1% (by weight of raw material) each of enzyme. After different hydrolysis times: 35, 45 and 55 min, enzyme inactivation was done by microwave heating for 5 min at a temperature higher than 90 °C. The bones were separated from the hydrolysate mixtures by sieving and the hot mixtures were centrifuged in 1:l batches at 2250×g for 15 min. Two fractions were obtained after centrifugation: the sludge (non-water-soluble part) on the bottom and fish protein hydrolysate (FPH, water-soluble compounds). The fractions were separated by decanting. Both fractions were freeze-dried. The hydrolysis was performed in duplicate (Kristinsson & Rasco, 2000).

The degree of hydrolysis was evaluated as the proportion (%) of α-amino nitrogen with respect to the total N in the sample (Liaset *et al.*, 2002). Analyses were performed in duplicate.

The antioxidative activity of protein hydrolysis was determined using an indirect spectrophotometric assay, the DPPH method as described by Thiansilakul *et al.* (2007). Protein hydrolysis were dissolved in water at 0.25% concentration. 1.5 ml of protein hydrolysis solution were mixed with 1.5 ml of 0.15 mM DPPH in 96% ethanol and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm.

Results and discussion

For all enzymes used in this study, by increasing the time and temperature of hydrolysis, the amount of protein recovery and degree of hydrolysis and antioxidant activity increased, and the highest of these two indices for all three enzymes at 55 °C and in the time was 60 min and the lowest was observed at 35 °C and at 15 min. Also, the results showed that among the three enzymes studied, alkalase was similar in temperature to different pepsin and flavourzyme enzymes, with protein recovery and higher degree of hydrolysis and antioxidant activity. The highest protein digestion was related to the alkalase enzyme at 55 °C and 60 min (54.22±0.11%), the highest degree of hydrolysis and antioxidant activity were related to the alkalase enzyme at 55 °C and 60 min (25.49±0.11%) and the lowest was obtained at 35 °C and 15 min.

Ovissipour *et al.* (2009) showd that optimal processing condition based on achieving the highest degree hydrolysis was determined. It was: E/S=0.1 AU/g protein, processing time of 205 min, temperature of 55 °C with highest DH of 61.96%. Gbogouri *et al.* (2004) found that optimal processing condition based on achieving the highest degree of hydrolysis was determined. It was: E/S=5.2%, temperature of 57 °C, processing time of 2 h and pH of 8.0 for

degree of hydrolysis 17.2%. Thiansilakul *et al*. (2007) obtained hydrolysates of scad (Decapetrus maruadsi) with the enzyme Flavourzyme acting for 1 h, with DH of 60.0%. The difference in the DH values between different species may be related to the activity of the enzyme on protein chains as well as the substrate upon which the enzyme will act is closely related to the final DH value (Santos *et al*., 2011).

Table 1. Resulta of hydrolysis of viscera grass by three commercial enzymes.

T (ºC)	Enzyme	Time (min)		
		15	30	60
35	pepsin	9.30±0.08	13.33±0.07	16.44±0.07
	flavourzyme	6.95±0.20	11.76±0.10	15.42±0.13
	alkalase	10.32±0.04	15.41±0.13	18.38±0.16
45	pepsin	14.55±0.10	17.40±0.15	20.38±0.07
	flavourzyme	14.76±0.07	16.88±0.07	22.27±0.08
	alkalase	15.34±0.06	20.45±0.12	22.27±0.08
55	pepsin	17.50±0.17	19.43±0.15	23.36±0.12
	flavourzyme	16.32±0.11	20.76±0.17	22.29±0.16
	alkalase	18.82±0.14	23.33±0.12	25.49±0.11

Conclusion

The results showed that the use of alkalase enzyme to produce hydrolyzed protein from grass carp was better in terms of recycling efficiency and degree of hydrolysis and antioxidant activity. Fish protein hydrolysates can be used as food ingredients and additives that give desirable characteristics to processed food products. These include increase food storage stability and emulsifying, foaming or dispersion activities in sausages, mayonnaise, salad dressings, beverages, creams, etc. Nevertheless, further studies should be conducted in order to characterise and isolate the bioactive peptides that produce the best antioxidant and antihypertensive activities.

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