

ENZIMATIC HYDROLYSIS OF THE RAW MUSCLE OF NURSE SHARK, *Gynglimostoma cirratum*

Hidrólise enzimática do músculo cru do cação-lixá,
Gynglimostoma cirratum

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ABSTRACT

This paper reports studies on the hydrolysis of the raw muscle of nurse shark, *Gynglimostoma cirratum*. The material was washed three times with distilled water and diluted in water in a 1:2 muscle/water ratio. The pH was maintained at 7.5 and pancreatin, papain or protease were added in the proportions of 0.1%, 0.2%, 0.3%, 0.4% and 0.5% of the total weight of shark muscle. The hydrolysis took place in an agitated aqueous medium at 40°C. The environmental pH was measured at 5, 10, 15, 30, 60, 90 and 120 min. If required, 0.1% NaOH was added to keep the pH at 8.5. The enzymatic reaction rate was determined by the degree of hydrolysis. The statistical analysis showed there to be significant differences in the results as a function of the enzymes, times and enzymatic concentrations used. The best results were produced with the enzyme pancreatin; optimal reaction times were found at 90 min. and 120 min., and optimal enzymatic concentrations were found at 0.4 and 0.5%. Cost factors suggest the use of pancreatin at 0.4%. By choosing a reaction time of 90 min. there will be less risks of bacterial contamination.

Key words: *Gynglimostoma cirratum*, hydrolysis, fish concentrate, protein.

RESUMO

Este trabalho descreve o processo de preparação do hidrolisado do músculo cru do cação-lixá, *Gynglimostoma cirratum*. O músculo foi filetado e a carne lavada cinco vezes com água destilada, seguindo-se a homogeneização com água na proporção de 1:2 (p/v) e ajuste do pH da mistura em 7,5 e então adicionou-se pancreatina, papaína ou protease nas concentrações de 0,1%, 0,2%, 0,3%, 0,4% e 0,5% em relação ao substrato. A hidrólise se processou a 40°C em banho-maria com agitação mecânica durante os tempos de 5, 10, 15, 30, 60, 90 e 120 min. A velocidade da reação enzimática foi medida pelo grau de hidrólise conforme o método descrito por Adler-Nissen & Olsen (1979). Os resultados estatísticos demonstraram que há diferença significativa entre os tratamentos enzimáticos, apontando os melhores resultados para a pancreatina a 0,4% e 0,5%, e o tempo entre 90 min. e 120 min. Levando-se em consideração os gastos com a enzima e a exposição do produto a um longo período e a temperatura favorável ao crescimento bacteriano, sugere-se como melhor resultado a concentração de 0,4% e o tempo de 90 min.

Palavras chaves: *Gynglimostoma cirratum*, hidrólise, concentrado de peixe, proteína.

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INTRODUCTION

In Ceará State, Brazil the harvest of nurse shark *Gynghimostoma cirratum* in 1992 was 204,1 tons, as reported by the Brazilian Institute for the environment and Natural Renewable Resources, at Fortaleza. Sharks are important components of the by-catch in tuna fishing, but only a small share of total production is made use of, what explains their relatively low amount as target species of the artisanal fisheries in Northeastern Brazil.

Protein hydrolysis is characterized by the breakdown of the peptide links of proteins by proteolytic enzymes, which results in a mixture containing protein, peptides and amino acids. The functional properties of this mixture are superior to those of the native protein, and its absorption by the organism is more accentuated. The concentration of protein in the hydrolysate can be raised by the elimination of water and fat. In general, fish hydrolysates contain 85-95% protein, 2-4% fat, and 6-7% ash. Of these components, the most important are the protein residues, such as amino acids and soluble peptides in water (Meinke *et al.*, 1982).

Several studies have been reported on the production of fish protein hydrolysates (Venugopal 1994; Vieira *et al.*, 1995; Ahmed & Mahendrakar 1996; Diniz & Martin, 1997; Gurgel & Vieira, 1997 and 2000), including fermented ones (Fangbenro & Bello-Olusoji, 1997; Gurgel *et al.*, 2000). The objective of the present paper is to test the efficiency of several proteolytic enzymes on the hydrolysis of fish muscle.

MATERIAL AND METHODS

Raw material

Filets of the shark *Gynghimostoma cirratum* were used in the experiments. By comparing the filet and the whole shark weight, it was possible to obtain the yield of the filet, which was expressed in percentage.

Preparation of the samples

The filets were washed in cold water for 6 consecutive times. Afterwards, they were homogenized with distilled water in the proportion 1:2 (w/v), and blended for 2 min. The pH of the homogenate was adjusted at 7.5, using 0.1M NaOH.

Hydrolysis

Enzymes pancreatin (p. 1750), papain (p. 3250) and protease (p. 4755) were supplied by Sigma (St. Louis, Missouri, USA.) They were added to the mixture, individually, at concentrations of 0.1%, 0.2%, 0.3%, 0.4% and 0.5% of the substrate's total weight. The enzymatic reaction took place at 40°C in an agitated water-bath for 120 min. The pH of the medium was measured

at 5, 10, 15, 30, 60, 90 and 120 min. If required, 0.1% NaOH was added to keep the pH at 8.5.

The rate for the enzymatic reactions was measured by the degree of hydrolysis, according to the method by Adler-Nissen & Olsen (1979), and expressed as percentage using the formula:

$$GH = \left[\frac{B \times NB}{MP} \cdot \frac{1}{\alpha} \cdot \frac{1}{htot} \right] 100$$

where, GH = hydrolysis degree (the percentage of the broken connections of the chains); B = volume of the added base (L); NB = normality of the added base; MP = mass of the protein of the reaction (kg); 1/α and 1/htot = constants available in the reference table.

Statistical analysis

The Analysis of Variance and the Tukey test were applied according to the methodology by Campos (1984), Singer & Andrade (1986) and Gill (1986). The SPSS/SP Program statistical package was employed.

RESULTS AND DISCUSSION

The yield of the muscle of the shark *Gynghimostoma cirratum* was 51.90%.

The degree of hydrolysis obtained by the action of pancreatin, papain and protease, on the homogenate of shark raw muscle, as a function of enzyme concentration and reaction time is shown in Figures 1 to 3. When pancreatin was used, the rate of hydrolysis increased substantially in the initial 15 minutes, stabilizing after 90 min. Similar behavior was observed in all five concentrations of pancreatin employed (Figure 1). Also, it can be seen that the best reaction time happened between 90 and 120 min., and the optimal pancreatin concentration for the reaction was between 0.4 and 0.5%. Those results

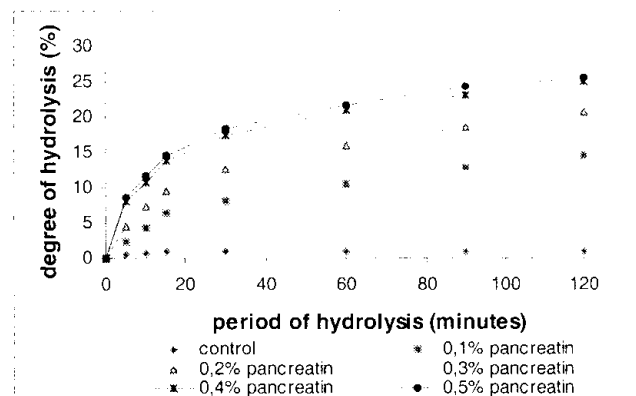


Figure 1 - Hydrolysis degree of the raw muscle of nurse shark, *Gynghimostoma cirratum*, using pancreatin at several concentration levels, pH at 7.5 and temperature at 40°C.

are expected, as increments in enzyme concentration speed up the reaction rate until the enzyme-substrate complex is saturated. Similar experiments were presented by Adler-Nissen (1982), Gouthier *et al.* (1986) and Gurgel & Vieira (1997 e 2000).

The optimal ratio of papain to muscle also produced a high rate of hydrolysis in the first 15 min. but it stabilized after 60 min. of reaction. The results were identical for papain concentrations of 0.4 and 0.5% (Figure 2). These results correspond with those found for red hake (Hale, 1973), *Urophycis chuss* (Rodriguez *et al.*, 1989) and by-products from *Sardine pilchardus* (Quaglia & Orban, 1987).

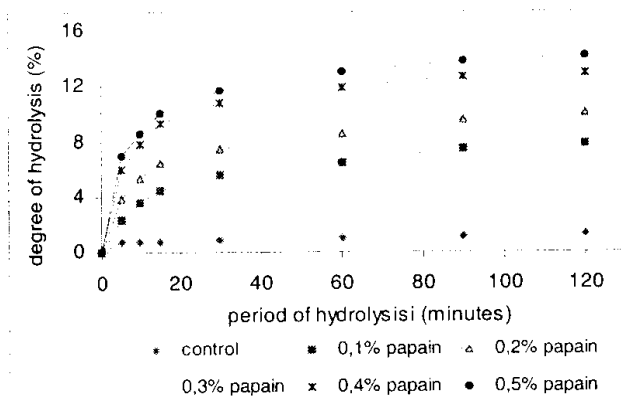


Figure 2 – Hydrolysis degree of the raw muscle of nurse shark, *Gynglimostoma cirratum*, using papain at several concentration levels, pH at 7.5 and temperature at 40°C.

In contrast to the hydrolysis with pancreatin and papain, the reaction rate for protease did not stabilize (Figure 3). Similar results were observed for *Machrobrachium amazonicus* as cooked shrimp muscle (Gurgel & Vieira, 1997) and raw shrimp muscle hydrolyzed by protease (Gurgel & Vieira, 2000).

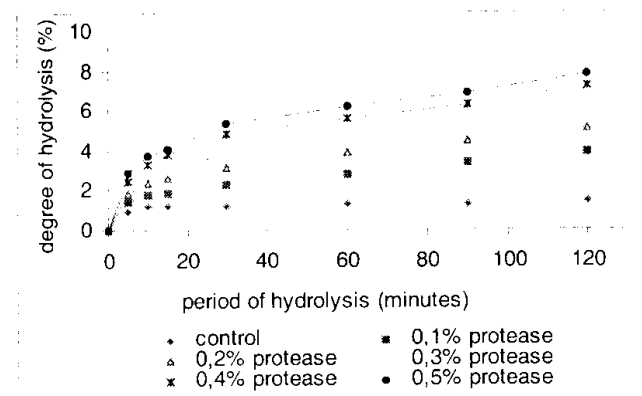


Figure 3 – Hydrolysis degree of the raw muscle of nurse shark, *Gynglimostoma cirratum*, using protease at several concentration levels, pH at 7.5 and temperature at 40°C.

The Analysis of Variance showed that there were significant differences among the values of the degree of

hydrolysis as a function of hydrolysis time, enzyme concentration, and type of enzyme (Table I). The Tukey test showed differences among the action of the enzymes papain, pancreatin and protease, with pancreatin producing the highest hydrolysis rate. The test also indicated there not to be significant differences between reaction times of 90 and 120 min. for pancreatin, and of 60, 90 and 120 min. for papain. Regarding enzyme concentrations for pancreatin and papain, no significant differences were observed at concentrations of 0.3%, 0.4% and 0.5% for papain, and concentrations 0.4% and 0.5% for pancreatin.

Table I - Analysis of Variance of the studied variables of the degree of the hydrolysis (%), of the raw muscle of nurse shark, *Gynglimostoma cirratum*.

| Source of variation | DF | MS | F |
|---------------------|----|-------|---------|
| Enzyme (E) | 2 | 456.9 | 846.3* |
| Concentration (C) | 5 | 244.1 | 452.2* |
| Interaction (Cx E) | 10 | 27.2 | 50.4* |
| Residue | 18 | 0.5 | |
| V. C. (%) | | 15.7 | |
| Time (T) | 1 | 98.6 | 1518.3* |
| Interaction (Ex T) | 2 | 15.4 | 237.1* |
| Interaction (Cx T) | 5 | 4.0 | 61.8* |
| Interaction (ExCxT) | 10 | 0.7 | 10.8* |
| Residue | 18 | 0.4 | |
| V. C. (%) | | 16.6 | |

Observations: DF = degree of freedom; MS = mean square; * = significant at 5% level of probability, for the F-test; V.C. = variation coefficient.

The above results show that the best conditions for hydrolysis of the raw muscle of nurse shark were 40°C, a pH of 7.5, a pancreatin concentration of 0.4% or 0.5%, and a time of 90 min. or 120 min.. According to Archer *et al.* (1973), microbiological contamination can occur during hydrolysis due to favorable temperature conditions for bacterial growth. In addition, a long hydrolysis liberates larger amount of peptides of low molecular weight, which give a bitter taste to the hydrolysate (Hevia 1977; Mackie, 1982). Therefore, it is recommended to choose the shorter hydrolysis time.

CONCLUSIONS

For the hydrolysis of the raw muscle of nurse shark, *Gynglimostoma cirratum*, the best results were found with pancreatin in concentrations of 0.4% and

0.5%, the former value being suggested on account of cost factors. With this enzyme concentration, optimum times for the hydrolysis of shark raw muscle were found to be 90 min., which should entail a smaller bacterial contamination, and 120 min.

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