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Enzymatic hydrolysis of cobia *(Rachycentron canadum)* meat and wastes using different microbial enzymes

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Abstract

Some proteins, beyond to their technological, functional and nutritional properties, exhibit biological activity, being one of them, the antioxidant activity, associated to bioactive peptides released after hydrolysis. The industrial processing wastes of fishes are a great source of proteins, and the cobia *(Rachycentron canadum)*, that it is a large and easily adaptable to aquaculture emerges as an option to obtain peptides. Thus, the aim of this study was to obtain peptides by hydrolyzing meat and wastes of cobia with different enzymes. Six hydrolysates through the hydrolysis of the meat and wastes with Alcalase, Flavourzyme and Protamex were obtained, wherein the last one presented greater hydrolytic capacity for the substrates, reaching a DH of 27.94% in 760 min for meat and 33.14% in 580 min for wastes. The free tyrosine content varied depending on the substrate and the enzyme, and the highest values for the hydrolysate wastes by Alcalase (8.46%) and by Protamex (6.46%) and hydrolysate meat by Protamex (6.47%). Therefore, these results indicate the potential utilization of the hydrolysates from cobia meat and wastes in food formulations.

Introduction

Cobia *(Rachycentron canadum)* is a fish species that, under suitable conditions, has extremely rapid growth rates. They adapt well to confinement, readily accept formulated feeds and are of excellent food quality, with high consumer appeal. These characteristics, along with the successful expansion and growth of cobia aquaculture in several countries, have served as stimulants for industry development elsewhere (Weirich *et al.*, 2010).

Out of the estimated 140 million tons of fish and shellfish produced each year worldwide, between 50 to 70% according to the species and the possibilities of processing, are discarded. In the past, these by-products have often been dumped or used without treatment for animal feed or as fertilizer. However, due to the worldwide decline of fish stocks, a better use of by-catch and by-products is deemed necessary (Guérard *et al*., 2010). These facts contribute to the development of alternative techniques for exploiting its by-products, or even, new ends for these.

In the last years, directing by-products to fish protein hydrolysates and isolated active ingredients is the "big dream" of the marine biotechnology industry, these products are low in volume but highly valued, and there is a tremendous potential for these innovative molecules (Thorkelsson and Kristinsson, 2009). The production by enzymatic treatment of fish protein hydrolysates (FPH) is very interesting to add value to fishery protein by-products (fish frames, heads etc.). Indeed, FPH possess good nutritional properties and biological activities for food and feed uses (Guérard *et al.*, 2007; Borseau *et al*., 2009).

A promising route is to proceed them through enzymatic hydrolysis, which, operated in mild controlled conditions influences the molecular size, hydrophobicity, and polar groups of the fish protein hydrolysates (FPH) with excellent nutritional properties and/or interesting biological activities for nutritional uses (Adler-Nissen, 1986; Kristinsson and Rasco, 2000; Picot *et al*., 2006; Klompong *et al*., 2007; Guerárd *et al*., 2007; Borseau *et al*., 2009).

The DH influences many bioactivities and functional properties (Klompong *et al.*, 2007). Then a description of the different types of hydrolysates (different DH) is done: limited (1-10%) that are used to improve functional properties of foods and extensive (above 10%) that are used as protein supplement in the diet or with a defined composition for the treatment of specific illness (Vioque *et al*., 2001).

Additional researches are needed for optimization of the enzymatic processes in order to develop hydrolysates enriched in tailored peptides suitable for the production of specific food with bioactive compounds (Guerárd *et al*., 2007; Centenaro *et al.*, 2011; 2014; Zavareze *et al.*, 2014). From that, this

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work aims to evaluate and compare FPH from cobia *(Rachycentron canadum)* meat and wastes obtained by different enzymes through the study of the hydrolysis degree.

Material and Methods

Enzymes and Reagents

The enzymes used were Alcalase (endopeptidase of *Bacillus licheniformis*), and Flavourzyme (mixture of exopeptidase and endoprotease from *Aspergillus oryzae*), provided by Novozymes Latin America Brazil; and Protamex (mixture of endo and exopeptidase from *Bacillus* sp.) supplied by Sigma - Aldrich Co (St. Louis, MO, USA). The other reagents were of analytical grade (A.R.).

Obtaining cobia meat and wastes

The cobia juveniles, provided by the Aqualíder Maricultura Ltda (Recife PE, Brazil) were transported in ice-filled containers to the Laboratory of Food Technology at Federal University of Rio Grande, in Southern Brazil. The fishes then, were immediately washed with chlorinated water (2 g/l), beheaded, eviscerated and the muscle and waste were separated, minced in a mechanical meat separator (MMS) (HIGH TECH HT/2500-Brazil), to remove the skin and spines, thus obtaining the meat and wastes that were placed in plastic containers and stored frozen at −18 ± 2°C (CONSUL CHB/53), pending use. Before the use, the waste was then centrifuged at 8667 x g for 30 min in order to reduce the lipid content (top of supernatant) of the proteins (precipitated and liquid).

Proximate chemical composition

The proximate composition of the samples was performed as recommended by the AOAC (2000) methodologies. The analyses were conducted for moisture, protein, lipid and ash, with n° 960.39, 992.15, 925.30 and 923.03, respectively.

SDS-PAGE electrophoresis

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was performed by the method of Laemmli (1970). The cobia meat and wastes samples were diluted to a final protein concentration of 0.2% in a buffer 0.5 M Tris pH 6.8, containing β-mercaptoethanol, denatured by heating at 90°C for 5 min. Electrophoresis was performed on SDS, with a gradient gel 4 to 12% (w/v) (Vertical System Electrophoresis 10 x 10 cm). The gel was stained with Coomassie blue (0.1% w/v) and the excess dye incorporated into the gel removed by distaining with 25% methanol and 10% acetic acid. The qualitative analysis for the identification of the bands was realized through the images of the gels revealed using the software Gelanalyzer (GelAnalyzer, 2010).

Obtaining enzymatic hydrolysates

The enzymatic hydrolysis of the different samples was based on the procedure described by Kristinsson and Rasco (2000), using portions of meat and waste that were suspended in distillated water (1:8 solid/liquid). Prior to the hydrolysis process, the endogenous enzymes contained in the meat and wastes were inactivated in a water bath at 85 °C for 15 min. The hydrolysis reaction was conducted in triplicate in a glass reactor, double wall, connected to a thermostatic bath (TC/102 BROOKFIELD - USA), using three separate enzymes (1:10 U/g protein), which corresponded in weight/weight to 0.10; 4.33 to 1.19% for the respective enzymes Alcalase (pH 8.0, 50 °C, 99.75 U/g), Flavourzyme (pH 7.0, 50 °C, 2.07 U/g) and Protamex (pH 7.0, 40 °C, 8.41 U/g), (Sigma, 1999; Lowry *et al*., 1951), under controlled optimal conditions and depending on the predetermined specific activity of each enzyme (μmol/min/g protein) (Jung *et al.*, 2006).

The DH was monitored throughout the process according the pH-stat method, and when it became constant, the reactions were terminated, inactivating the enzyme by heating the mixture (90 °C/10 min) in a thermostatic bath (QUIMIS, model 218.2 - Brazil). After cooling to room temperature, the hydrolysates were then centrifuged (3220 x g for 20 min) (BIOSYSTEMS MPW-350/350-R - Brazil), the supernatants from each hydrolysate were filtrated, lyophilized (Liotop L108, Brazil) and stored at -18 ± 2 ºC for further analysis.

Degree of hydrolysis (DH)

The DH was determined by the pH-stat method, according to Adler-Nissen (1986) apud Geirsdottir (2009). During hydrolysis, the pH was maintained at the desired value by addition of NaOH 0.2 N, whose volume was recorded. DH was calculated according to equation 1.

$$DH(\%) = \frac{BN_B}{\alpha h_{tot} \times MP} \times 100 \quad (1)$$

Where: h_{tot} is the number of peptide bonds, with 8.6 mol equiv/kg for fish (Adler-Nissen, 1986) apud Geirsdottir (2009), B(mL) is the volume of base consumed during hydrolysis to maintain constant pH, and N_B the normality of base; MP is the protein mass (g, determined by N x the Kjeldahl factor), and α is the degree of dissociation.

Analysis of free tyrosine

It was determined the content of the amino acid tyrosine of the suspension before hydrolysis and after heat inactivation of the enzyme used in the reaction (at the end of hydrolysis) according Sigma (1999). Was considered the difference before and after the procedure and quantified by standard tyrosine curve. This is also an indirect way to check if there was hydrolysis.

Statistical analysis

Results were expressed as mean ± standard deviation, submitted to Analysis of Variance (ANOVA) and Tukey's test applied for comparison of means ($p < 0.05$).

Results and Discussion

Proximate chemical composition

Many researches about the cobia can be found, however, most of them are only about its cultivation in aquaculture (Chou *et al.*, 2001; Zhou *et al.*, 2005; Liu *et al.*, 2009; Nguyen *et al.*, 2014). These researches provide a wide variation in the composition of this fish, as found by Chuang *et al.* (2007, 2010). The moisture content of the fish meat is generally negative if correlated with fat content, because when the flesh of the fish accumulates fat, its moisture level tends to decrease and vice versa. These phenomena have been demonstrated in several studies (Hirano *et al.*, 1980; Morishita *et al.*, 1987; Date and Yamamoto, 1988; Shiau *et al.*, 1997), and agree with the results obtained in this work, in which the wastes showed lower moisture contents (64.55%) than meat (69.17%), and higher fat content (18.92 and 12.04%, respectively). Also the sum of fat and moisture (about 80 %) has been used as a thumb rule to check these values.

The lipid content was high in meat and wastes, and this is a compound that highly interferes on the hydrolysis, especially by the addition of NaOH during the process, which can cause a reaction of saponification (Allinger, 1971). Hydrolysis tests were performed, but the reaction did not happen with the residue, because of its high lipid content. It probably happens because of the inhibition of the enzymes, due to the lipid oxidation, as found by Matsushita *et al.* (1970). So, the removal of some fat from the residue by using a physical process (centrifugation) was done, achieving a reduction of over 20% and, consequently, the increase of the protein content of the residue to about 25%, making easier and improving the hydrolysis of this substrate.

Table 1. Proximate chemical composition of cobia meat and wastes

Sample	Moisture	Protein (%)		Lipids (%)		Ash (%)	
	(%)	W.B.	D.B.	W.B.	D.B.	W.B.	D.B.
Meat	69.2 ± 0.9	16.3 ± 1,6	52.7^{a} ± 1.9	12.0 ± 0.1	39.0^{c} ± 0.3	1.5 ± 0.1	4.9^{a} ± 0.4
Wastes	64.5 ± 0.8	10.6 ± 0.2	29.8^{c} ± 0.5	18.9 ± 0.2	53.4^{a} ± 0.1	1.6 ± 0.3	5.1^{a} ± 0.5
Defatted waste	-	-	37.5^{b} ± 0.6	-	41.4^{b} ± 0.1	-	5.5^{a} ± 0.2

All data was expressed as average (mean ± standard deviation, n = 4). Equal lowercase letters on the same column indicate that there is no significant difference between the samples ($p < 0.05$). W.B.: wet basis; D.B.: dry basis.

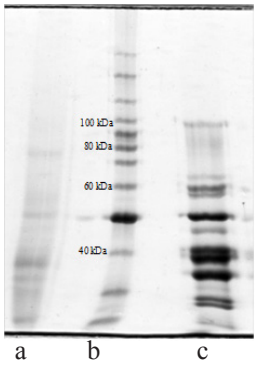

Figure 1. Eletrophoresis of cobia waste (a), standard (b) and meat (c)

Electrophoresis

Figure 1 shows the electrophoretic profile from both cobia wastes and meat, against a pattern that has a molecular weight protein range between 25 and 220 kDa (Figure 1b). Figure 1a shows that the cobia wastes proteins are below 80 kDa, however the meat proteins (Figure 1c) presents proteins from 100 kDa to below 25 kDa, being impossible to determine if there were proteins below this value, due to the limit of the method under these conditions. Most of the proteins found in meat are concentrated about 40 kDa, in agreement with the results found by Silva *et al.* (2014b), who when assessing waste from the processing of tilapia (carcass, without viscera), found an electrophoretic profile, in which most proteins are around 40 kDa, and between 66 and 80 kDa, with a pattern that has a molecular weight protein range between 6 to 200 kDa.

It is possible that the waste presents smaller protein than meat due to the autolytic process that depends on the action of the natural digestive enzymes, occurring in the fish itself (Möhr, 1980). The determination of the specific activity of the

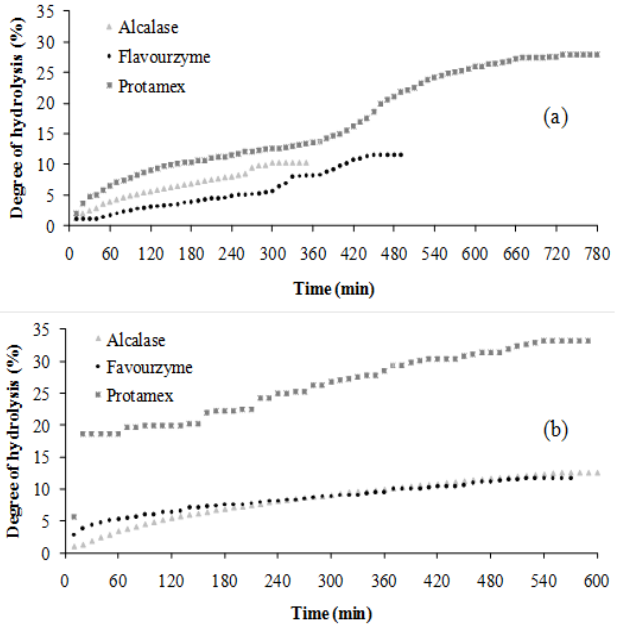

Figure 2. Graph of the hydrolysis degree of meat (a) and wastes (b), with Alcalase, Flavourzyme and Protamex

enzymes was performed to adjust the parameters of hydrolysis, utilizing tyrosine and protein content, and so, being able to mutually compare the activity of each enzyme at each substrate, using the same proportion in enzymatic activity per protein mass. With the same objective, the endogenous enzymes contained in meat and wastes were inactivated, making the action of enzymes easier, not affecting the characteristics of the resulting products, and allowing them to be reproducible (Benkajul *et al*., 1997; Martins *et al*., 2009).

Enzymatic hydrolysis

Hydrolysis had their degree followed during the process (Figures 2a and 2b). The kinetic study of the enzymatic hydrolysis behavior can allow the determination of the obtained product, and so being possible to produce a hydrolyzate with well-defined profile of peptides, having high functionality and nutritional value compared to chemical isolates, whether acid or alkali (Martins *et al.*, 2009).

It was possible to prove in the Figure 2 that the curves showed an intense hydrolysis in the beginning of the process, in which a great number of peptide bonds per time unit were cleaved and a great ratio of soluble material was released in the solution; with a reduction in the reaction rate along the time because the most compressed proteins are hydrolysated more slowly, concomitant with the available substrate, which decreased over the reaction time, a common behavior in this kind of reaction, already reported by several other authors (Adler-Nissen, 1979; Chen *et al., 1*995; Márquez and Vázquez, 1999; Nguyen *et al*., 2011). An exception to this rule has been taken when the meat was hydrolysated with Flavourzyme.

According to Krinstinsson and Rasco (2000), the enzymatic hydrolysis of the meat proteins is defined by a rapid initial stage, in which many peptide bonds are broken. After that, this hydrolysis rate decreases and reaches a stationary stage, when no apparent hydrolysis happens. Möhr (1980) also defends the hypothesis that the global reaction involves at least two stages. In the first, the enzyme molecules associate themselves with the fish particles. Subsequently, the hydrolysis occurs, resulting in the liberation of soluble peptides and amino acids.

Diniz and Martin (1997), studying the hydrolysis of dogfish proteins with the Alcalase 2.4L enzyme, suggested that the constant DH decreasing behavior can happen due to a deactivation or inhibition of the enzyme by inhibitory peptides that are constantly solubilized during the hydrolysis. Such behavior also indicated possible enzyme deactivation over time, due to a low stability at high temperatures (around 45 °C) during several hours, and an enzyme auto-hydrolysis possibility. This observation, along with the obtained results, makes it possible to conclude that the form of the hydrolysis curve can be explained because of the lack of available peptide bonds combined with a partial deactivation of the enzyme during the course of the hydrolysis.

All the hydrolysis presented a second stage of intense reaction. This characteristic is usually assigned as an enzyme or substrate addition effect (Guérard *et al*., 2001), that might have been caused by the release of protein parts with greater ability to act for the enzyme or by the release of more cofactors with the ability to increase the number of active enzymes.

Alcalase showed lower capacity to act in the initial of the process on the wastes protein when compared to the other enzymes (Flavourzyme and Protamex) due to smaller time and DH. Piotrowicz (2012), when hydrolyzing mechanically separated anchovy flesh in the proportion of 1.0% w/w enzyme/protein at fixed times of 1 and 5 h obtained a higher DH with the Alcalase enzyme than with Flavourzyme and Protamex, reaching about 80% at the highest time (5 h). Since the quantity of enzyme used in this work was 10 times smaller, this could explain the difference between both works. The Alcalase presented higher affinity than Flavourzyme and Protamex, however, it should be highlighted that the end of the process and enzymatic activities were not measured. At 270 min (4.5 h) of the meat proteolysis with Alcalase, the DH presents an intense increase with a rate of 3 to 4 times more than the other times.

In the Figure 2, it was possible to notice that the Flavourzyme enzyme had difficulties to hydrolyze the meat proteins in the beginning of the process, wherein in 10 min of process it reached 1.14% of DH, stabilizing with no hydrolysis for 30 min. At some moments, it is possible to see that there are rates close zero, while at others there are intensive peaks, where the most prominent is between 310 and 330 min. To this oscillation, it is believed that the Flavourzyme enzyme, for being an mixture of exopeptidase and endoprotease EC 3.4.11.1 (NC-IUBMB, 1992), has cleaved the leucine located at the N-terminal ends in the first 10 min, stabilizing the reaction until it could start to break the end bonds that had other amino acids, which have less affinity. After starting to search again for leucine N-terminal bonds that may have been released during the hydrolysis, this behavior might have repeated for many times, until the enzyme has lost its activity, or some other factor, already mentioned, may have influenced the performance of the enzyme in the hydrolysis of cobia muscle, the exact cause this behavior is not evident (Diniz and Martin, 1997).

It is also important to point out the impact of the enzyme specificity, which is a key factor in influencing the characteristics of the hydrolysis, nature and composition of the produced peptides. The proteolysis can act sequentially, releasing only one peptide each time, or through the formation of intermediates that are further hydrolyzed until smaller peptides as the hydrolysis progresses, which is often called "zipper mechanism" (Panyam and Kilara, 1996). This phenomenon may have occurred during this hydrolysis.

Silva *et al.* (2014a), when hydrolyzing demineralized croaker bones in the same conditions, obtained an initial behavior very similar to the one found in this work (Figure 2), showing that this may be an important characteristic of the enzyme activity, which is strongly related to the substrate type. Protamex, during the meat proteolysis, provided another intense DH at 400 min. The characteristic of this enzyme is to be an endoprotease and exopeptidase, with affinity for more than one type of bond; therefore, the observed behavior may have happened due to its capacity of interacting with these bonds, since it could act on serine, leucine or phenylalanine amino acids. However, to break the last two amino acids, it needs a metallic cofactor that would modify enzyme conformation to an ionic form and so, probably losing the serine affinity. Consequently, what may have happened is that, at a certain time, the enzyme would be attached to the cofactor, and, at another time, not attached to it, also explaining the double behavior.

Wastes protein hydrolyses (Figure 2b) had different time and DH for each enzyme; for the Alcalase enzyme had time of 560 min and DH of 12.5%, for the Flavourzyme enzyme had time of 520 min and DH of 11.7% and for the Protamex enzyme had time of 540 min and DH of 33.1%. All of them showed a similar reaction time, that is not a parameter for the choice of the enzyme on this substrate, but did not present a second intense reaction during the process. This result was probably due to the substrate presenting smaller and/or more open chains (quaternary and tertiary structures) partially hydrolyzed by the endogenous enzymes presented in higher concentration in the viscera, as demonstrated in the electrophoresis (Figure 1).

Silva *et al.* (2014a) studied different protein sources from the croaker and, utilizing the enzymes Alcalase, Flavourzyme and Protamex, under the same hydrolysis conditions used in this work, has found, respectively, a DH of 6.0, 5.9 and 30.5% for the protein isolate and of 14.7, 15.5 and 21.5% for the demineralized bones, respectively. The demineralized bones demonstrated to have proteins with higher capacity to be cleaved by the enzymes than the protein isolated. The Protamex enzyme showed higher DH for both tested substrates when compared to Alcalase and Flavourzyme enzymes. This study, along with the results obtained in this work, demonstrates that the Protamex enzyme showed higher capacity to hydrolyze fish proteins, and that the between each substrate and each enzyme, there are different capacities, so the kinetic parameters must be evaluated for each hydrolysis.

Depending on the enzyme specificity, the conditions and the DH, a wide variety of peptides will be generated. The resulting hydrolyzed protein will have peculiar properties according to the newly generated peptides (Guérard *et al.*, 2002). According to concepts raised by Vioque *et al.* (2001), all hydrolysis obtained in this study can be considered of superior DH - being indicated for special purposes in foods - as well as having higher probability to present some bioactivity, which justified the use of different enzymes and substrates to perform the research about the antioxidant activity of these hydrolysis. In summary, the Protamex enzyme demonstrated the highest hydrolytic capacity for both, cobia meat and wastes protein substrates, due to the higher DH provided by the same.

Free tyrosine content

Tyrosine levels were determined at the beginning and at the end of hydrolysis, to obtain the percentage

Table 2. Percentage of free tyrosine protein hydrolysates of meat and wastes of cobia

	Tyrosine (%)	
	MEAT	WASTES
Alcalase	$2.15^{bD} \pm 0.08$	$8.46^{aA} \pm 0.10$
Flavourzyme	$2.25^{bD} \pm 0.05$	$2.63^{cC} \pm 0.06$
Protamex	$6.47^{aB} \pm 0.17$	$6.46^{bB} \pm 0.33$

All data was expressed as average (mean ± standard deviation, n = 4) values. Equal lowercase letters on the same column indicate that there is no significant difference between the samples ($p < 0.05$). Equal uppercase letters on the table indicate that there is no significant difference between the samples ($p < 0.05$).

of release, in other words, the free tyrosine content (Table 2). When analyzing the DH results, previously exposed and free of tyrosine levels (Table 2), it was verified that they can be almost directly correlated. Those that presented the higher DH, also presented higher tyrosine levels, as the cobia hydrolysis produced with the Alcalase enzyme by Amiza *et al.* (2012). They determined the hydrolysate amino acid composition. He found, for the DHs of 53.42, 70.81 and 95,63, the following values of free tyrosine percentage: 0.55, 0.59 and 0.74, respectively.

However, for the wastes hydrolyzed with Alcalase, this proportionality did not happen. These values depend on the amino acids (types and positions) presented on the substrate and the way that each enzyme operates (Clemente, 2000). The endoproteinase Alcalase has high specificity and strong hydrolysis ability, being able to release free amino acids, which may explain the distinct behavior of this hydrolysis. The properties of each enzyme make them provide different products for the same substrate, or, with the same substrate, different features for different enzymes.

Endoproteinases hydrolyze peptide bonds in the protein molecules in a random way, generating relatively bigger peptides. Exoproteases systematically remove the N-terminal or C-terminal amino acids or hydrolyze the peptides at the terminal bonds (Clemente, 2000). Alcalase is an alkaline enzyme which its main component, Subtilisin A (Subtilisin Carlsberg), is an endoproteinase capable of breaking the peptide bonds and is able to reduce the molecular weight and to improve the functional properties of the proteins. In addition, it generates free amino acids in protein hydrolysis (Yang *et al*., 2011), which may explain the higher levels of tyrosine of the waste hydrolyzed by this enzyme.

While the Flavourzyme is a complex of fungal proteases made of exopeptidades and endopeptidases that acts on the hydrolysis under neutral or slightly acid conditions, the Protamex is a complex of bacterial proteases, but also made of these types of peptidases. In another study, there was no proportionality between the DH and the free tyrosin content for the croaker demineralized bones hydrolysis and protein isolates with Alcalase, Flavourzyme and Protamex. In addition, it showed higher value with Flavourzyme for the first, and with Protamex for the second, being both DHs higher with the Protamex enzyme (Silva *et al*., 2014a), agreeing with what was previously exposed.

Among the amino acids that can be obtained by hydrolysis, the tyrosine may be highlighted, being a non-essential amino acid found in large amounts in vegetal and animal origin foods, and a metabolic precursor for the synthesis of the neurotransmitters catecholamine, norepinephrine and dopamine at the peripheral and central nervous system. The availability of tyrosine may be a limiting factor for the proper functioning of the central nervous system. Therefore, supplementation of tyrosine in the diet may reduce some adverse effects from stress and activation of the central nervous system (Neri *et al*., 1995; Thomas *et al*., 1999).

The determination of the free tyrosine is important as an indirect parameter to demonstrate if the hydrolysis was happening while this amino acid was being released. Some authors assign the antioxidant activity of the bioactive peptides to the presence of free tyrosine in the sequence. After hydrolyzing Yellowfin tuna and purifying the fraction with the highest bioactive potential, Jun *et al*. (2004) found the peptide with 10 components, Arg-Pro-Asp-Phe-Asp-Leu-Glu-Pro-Pro-Tyr, with 13 kDa, in which the terminal tyrosine is the most responsible for the antioxidant activity (methods of linoleic acid model system, reducing power and TBA, compared to α-tocoferol as a control), for being a powerful hydrogen-donor. In the work of Je *et al.* (2005) while hydrolyzing Alaska pollock bones, the obtained peptide sequence was Leu-Pro-His-Ser-Gly-Tyr, with molecular weight of 672 Da and they assign the antioxidant activity (methods of linoleic acid model system and reducing power, compared to α-tocoferol as a control) to the terminal tyrosine (being the hydrogen-donor), and to the histidine in the middle of the sequence (due to the imidazol ring).

Hydrolyzed protein, obtained from the cobia waste under the action of Alcalase enzyme, presented higher content of free tyrosine, statistically differing from the others, followed by the Protamex hydrolysates (both meat and wastes), which proved themselves equal at a 5% significance level.

Conclusion

The different microbial enzymes tested showed capability to hydrolyze both meat and wastes proteins of cobia. The higher hydrolytic capacity of the enzyme for the substrate was noted when the enzyme Protamex was used, where it has shown higher DH and significant content of free tyrosine, but it does not mean that these results are directly related.

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