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*Research Article*

# Protein hydrolysate waste of whitemouth croaker (*Micropogonias furnieri*) as a way of adding value to fish and reducing the environmental liabilities of the fishing industry

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**ABSTRACT**. As an alternative use of natural marine resources, processing waste from whitemouth croaker was used as raw material for the production of fish protein hydrolysate (FPH). The biometric characterization of the raw material was determined, and the optimal conditions of the protease established using DH (degree of hydrolysis) as the criterion. Bromatological analyses were performed according to AOAC methods. The DH were determined by the TCA (trichloroacetic acid) method and expressed as a percentage of solubilized protein after hydrolysis compared to the total protein in raw material. The average yields of viscera, heads and carcasses were 7.4, 36.3 and 56.3%, respectively. The optimal conditions for enzyme action on the viscera were: 55°C, hydrolysis time of 90 min, and enzyme concentration of 0.4 mg $mL^{-1}$. For the muscle, the conditions established were: 45°C, 90 min, and 0.8 mg $mL^{-1}$. The dry matter of hydrolysate from viscera yielded 73.5% supernatant (soluble fraction) and 27.5% precipitate (insoluble fraction), while that from muscle yielded 45.7% and 54.3%, respectively. The hydrolysate from viscera presented 86.0% proteins, 0.4% lipids and 4.4% ashes in the soluble fraction, and 51.0% proteins, 25.8% lipids and 5.1% ashes in the insoluble fraction. The hydrolysate from muscle presented 86.0% proteins, 0.4% lipids and 9.0% ashes in the soluble fraction, and 87.0% proteins, 4.4% lipids and 2.4% ashes in the insoluble fraction. A lower degree of hydrolysis of hydrolysate from muscle than that produced from viscera was observed, demonstrating the different interactions between the enzyme and the residues studied.

**Keywords**: *Micropogonias furnieri*, fish protein hydrolysate, fish waste, protease.

# Hidrolizado proteico de desechos de corvina (*Micropogonias furnieri*) como una manera de agregar valor a los peces y reducir los pasivos ambientales de la industria pesquera

**RESUMEN**. Como una alternativa de uso de los recursos marinos naturales, se emplean como materia prima los residuos del procesamiento de la corvina, para la producción de hidrolizado de proteínas de pescado (FPH). Se determinó la caracterización biométrica de la materia prima y se establecieron las condiciones óptimas de la proteasa, usando el GH (grado de hidrólisis) como criterio. Los análisis bromatológicos se realizaron de acuerdo con los métodos de AOAC. El GH se determinó por el método de TCA (ácido tricloroacético) y se expresó en porcentaje (%) de proteína solubilizada después de la hidrólisis, en comparación con la proteína total de la materia prima. Los rendimientos medios de vísceras, cabezas y cuerpos fueron de 7,4; 36,3 y 56,3% respectivamente. Las condiciones óptimas para la acción enzimática sobre las vísceras fueron: 55°C, tiempo de hidrólisis de 90 min y concentración enzimática de 0,4 mg $mL^{-1}$. Para el músculo, las condiciones establecidas fueron 45°C, 90 min y 0,8 mg $mL^{-1}$. La materia seca del hidrolizado de vísceras produjo 73,5% de sobrenadante (fracción soluble) y 27,5% precipitado (fracción insoluble), mientras que del músculo se obtuvo 45,7% y 54.3%,

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respectivamente. El hidrolizado de vísceras presentó 86,0% de proteínas, 0,4% de lípidos y 4,4% de cenizas en la fracción soluble, y 51,0% de proteínas, 25,8% de lípidos y 5,1% de cenizas en la fracción insoluble. El hidrolizado a partir de músculo presentó 86,0% de proteínas, 0,4% de lípidos y 9,0% de cenizas en la fracción soluble, y 87,0% de proteínas, 4,4% de lípidos y 2,4% de cenizas en la fracción insoluble. Se determinó un menor grado de hidrólisis en el hidrolizado en el músculo, que el producido por las vísceras, mostrando diferentes interacciones entre la enzima y los residuos analizados.

**Palabras clave**: *Micropogonias furnieri*, hidrolizados de proteína de peces, residuos de pescado, proteasa.

## INTRODUCTION

The whitemouth croaker (*Micropogonias furnieri*) is an important demersal fishing resource of the coastal region of the southeast/south of Brazil (Carneiro *et al.*, 2005). Inventories of the species show a distribution between 23ºS and 29ºS (southeast stock) and between 29ºS and 33ºS (southern stock). Both regions already have high levels of exploitation (Carneiro *et al.*, 2000).

The industrialization of the species generates almost 45% waste by evisceration alone. When utilized adequately, this waste is mainly transformed into fish meal (Stori, 2000; Silva *et al.*, 2014). The remaining protein content in the fish waste may constitute raw material for obtaining higher value-added products, such as protein concentrates and hydrolysates. In addition to the added value, this type of use of fish waste helps reduce environmental liabilities and increase the supply of high nutritional value products.

Fish protein hydrolysate (FPH), as designated by the Food and Agriculture Organization (FAO), can be obtained by the digestion of the proteins contained in waste from fish processing (Xu *et al*., 2016). The lack of low cost products with high nutritional value has led to the use of FPH as an alternative to the use and incorporation of animal proteins in other food sources, both for animal and human consumption (Salas-Mellado *et al.*, 2007; Silva *et al.*, 2014).

The FPH preparation process involves grinding the raw material, followed by adding water and mixing to obtain a viscous mass and a chemical or enzymatic hydrolysis (Batista, 2011). Enzyme hydrolysis is a friendlier alternative to replace the chemical process, and is suitable for solubilization of the protein. This process results in a soluble fraction, which contains more hydrolyzed and polar proteins and other precipitates with a lower degree of hydrolysis and more hydrophobic, which can also be used as a source of protein. Other than these, an oil fraction and an insoluble fraction, rich in minerals, are obtained too. The hydrolyzed protein will constitute an ingredient to be incorporated into feed or processed foods (Furlan & Oetterer, 2002; Duan *et al*., 2010).

This study aims to develop a process for obtaining a protein hydrolysate from whitemouth croaker (*M. furnieri*) residues.

## MATERIALS AND METHODS

Whole whitemouth croakers were acquired in the Municipal Fish Market and also directly from the fishing vessel Caixa D 'Steel II, both in the town of Itajaí, Santa Catarina, Brazil. Samples were transported in cool boxes to the UNIVALI Laboratory of Biochemistry (Vale do Itajaí University), where they were kept in freezer at -20°C until analysis.

The yield analysis was performed with twelve whole whitemouth croakers. The total lengths (TL) were determined in a 60 cm icthyometer. After biometrics, the guts and heads were removed, leaving only the carcass, from which samples of 300 g of muscles fillets were taken, for subsequent use in the hydrolysis assays. The viscera were completely separated, providing another raw material for the hydrolysis. The samples for hydrolysis were separated into four lots, each lot comprising muscle and viscera samples of three different fish. The samples were stored in identified aseptic plastic bags and stored in a freezer at -20°C until the time of hydrolysis.

The samples were thawed in the refrigerator at 8°C for 24 h, then 100 g of muscle (m) or viscera residue (v), separately, were homogenized in a blender, with 400 mL water (ratio 1:4; m:v). For each homogenized sample, 500 mL of solution were obtained, and of this total volume, 30 mL were used for the bromatological determination (protein, ash, moisture and lipids), with the analyses being carried out in triplicate. Part of the solution was used to establish ideal conditions for the enzymatic hydrolysis tests. The remaining solution was stored frozen at -20ºC until the time of testing under optimum hydrolysis conditions.

The tests to establish optimal parameters for the hydrolysis were conducted with bacterial protease from *Bacillus licheniformis* and *B. amyloliquefaciens* (Protamex® Novozymes A/S) by the micro-assay method, using the enzyme at a ratio of 1:500 (E/P-Enzyme/Fish) as a standard solution for testing the

enzyme concentrations for wastes of whitemouth croaker. 2.5 mL of the homogenate was incubated in fresh material, and tested at temperatures of 40, 45, 50, 55 and 60°C; the reaction times were determined at 30, 60, 90, 120, 200 and 360 min, with concentrations of enzyme solution of 0.1, 0.2, 0.4, 0.8, 1.2 and 2.4 mg $mL^{-1}$, and four replicates per treatment (independent experiments). Once the reaction time had elapsed, a 50 uL aliquot of the hydrolysate was placed in wells containing 100 uL of trichloroacetic acid (TCA 5%), to precipitate insoluble higher molecular weight proteins. They were then centrifuged at 1889x g for 5 min, withdrawing 50 uL aliquots from the protein supernatant, which were then quantified by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. The degree of hydrolysis (DH) was determined by the ratio of solubilized proteins in the hydrolysate (PS) and total protein (TP) present in the raw material, determined by the Kjeldahl method, and the results were expressed as a percentage (%).

After determining the optimal incubation conditions, larger amounts of protein hydrolysate was produced, with 90 min of incubation at 55°C and the enzyme solution at a concentration of 0.4 mg $mL^{-1}$ for the viscera and 90 min at 45°C and enzyme concentration of 0.8 mg $mL^{-1}$ for the muscle. The precipitate and soluble fraction were separated by centrifugation at 2600x for 15 min, and frozen following the separation of the fractions. The samples were then vacuum dried and the yields and chemical composition determined, as well the degree of hydrolysis of the hydrolysates.

In the chemical composition, the moisture content, lipid, ash and protein were determined using the methodology described by AOAC (1995). Moisture was determined in an oven at 105°C for 24 h and measured gravimetrically. The lipid content was determined prior acid hydrolysis with hydrochloric acid: methanol 1:1 at 60°C for 60 min, followed by extraction by adding petroleum ether-lipids. The ether phase was transferred to another, previously weighed tube, and evaporated to dryness leaving dry lipid. The ether wash was performed three times in each tube. The lipid content was determined by the residual ether mass fraction in relation to the initial mass of the dried hydrolysate. Total protein was determined by the Kjeldahl method (N x 6.25), according to AOAC (1995) modified by Galvani & Gaertner (2006). The ash content was determined in a muffle oven at 850°C for four hours and evaluated gravimetrically. All analyses were performed in triplicate.

## RESULTS

The weights of the entire whitemouth croakers (PT), the viscera, the heads, and the carcasses showed averages of 1389.1, 103.8, 504.8 and 774.1 g, respectively (Table 1). In the analysis of yield from the viscera, the heads and the carcasses in relationship to the whole fish, the averages were 7.5%, 36.3% and 55.7% (Table 1).

The lipid content in the viscera resulted in 10.7%, while for muscle, the lipid content was 1.5% (Table 2). In the analyses of crude protein, the levels were 67.1% in the viscera and 84.3% in the muscles. The ash contents were 4.3% and 4.6% for muscle and viscera, respectively. In the mass balance, the total recovered from the viscera was 82.1%, while for muscle it was 90.4%.

The enzyme activity was determined at different temperatures, for the two tissues, independently. The optimum temperature for the enzymatic hydrolysis was 55°C for the viscera and 45°C for the muscle (Fig. 1).

The incubation time for the reaction to reach a higher degree of enzymatic hydrolysis for viscera was 90 min (Fig. 2). Although muscle showed a higher degree of hydrolysis over longer periods (200 and 360 min), this difference does not usually compensate for the costs of maintaining the temperature, unless the need for hydrolysates with biological activities justifies it.

The degree of hydrolysis was determined for different enzyme concentrations against the same substrate concentration. The highest degree of hydrolysis was obtained at a concentration of 0.4 mg enzyme $mL^{-1}$ of incubation medium for viscera (Fig. 3). To muscle could be recommended 0.8 mg, given the higher degree of hydrolysis. However, it cannot be justified by the cost (Fig. 3). Although used at relatively small concentrations, the cost of the enzyme is impressive for a production process, because it is a consumable ingredient, and is not recovered.

Analysis of composition of the soluble fraction of the viscera and hydrolyzed muscle samples tested, as expected, resulted at higher lipid content to viscera and higher protein content for the musculature (Table 3). As

**Table 1.** Biometrics and carcass weight of whitemouth croakers (*Micropogonias furnieri*) (n = 12). SD: standard deviation, CV: coefficient of variation.

| Variable | Average ± SD | CV (%) |
|---|---|---|
| Length (cm) | 51.2 ± 2.66 | 5.19 |
| Weight (g) | 1389.1 ± 164.70 | 11.86 |
| Viscera weight (g) | 103.8 ± 25.98 | 25.02 |
| Head weight (g) | 504.8 ± 67.90 | 13.45 |
| Carcass yield (g) | 774.1 ± 91.28 | 11.79 |
| Viscera yield (%) | 7.5 ± 1.50 | 20.18 |
| Head yield (%) | 36.3 ± 2.40 | 6.60 |
| Carcass yield (%) | 55.7 ± 2.21 | 3.96 |

**Table 2.** Composition (%) in lipids, protein and ash samples of viscera and muscle of whitemouth croaker (*Micropogonias furnieri*). SD: standard deviation, Total recovered: sum of the percentages of lipids, protein and ash.

| | Viscera Average ± SD | Muscle Average ± SD |
|---|---|---|
| Crude protein (N x 6,25) | 67.1 ± 6.38 | 84.3 ± 1.87 |
| Lipid | 10.7 ± 4.38 | 1.5 ± 0.63 |
| Ash | 4.3 ± 0.89 | 4.6 ± 1.23 |
| Total recovered | 82.1 | 90.4 |

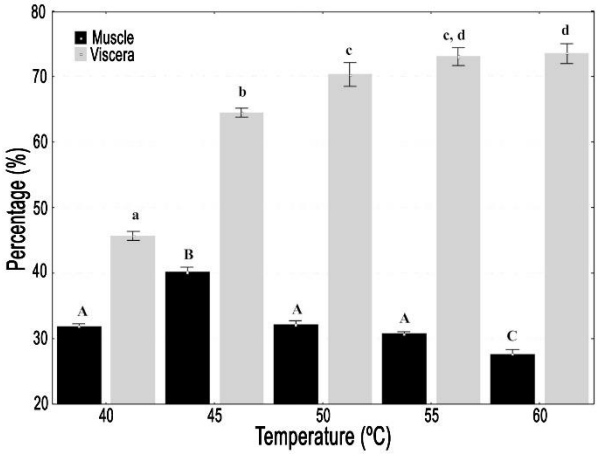


**Figure 1.** Degree of hydrolysis (%) for waste muscle and viscera relative to incubation temperatures (°C). Different letters (upper case for muscle and lower case for viscera) mean significance ($P < 0.05$).

for the insoluble fraction, the lipid content in viscera was 22.9%, while for muscle, it was 4.6% (Table 3).

The levels of crude protein were 49.7% in the viscera and 87.3% in the muscle. The ash contents were 7.1% and 2.4% for both muscle and viscera. In the mass balance, the total percentage recovered in the viscera in the soluble fraction was 87.5%, while for the muscle it was 92.1%, *versus* 79.7% for the viscera and 94.3% for the muscle in the insoluble fraction. The yield of the soluble fraction, which is the dry mass of the fraction recovered in the dry mass of initial raw material, 69.7% for viscera and 48.7% of muscle, with 79.7% of the total being recovered for the viscera and 94.3% for the muscle in the insoluble fractions (Table 3).

The degree of hydrolysis to the hydrolysates produced under optimum kinetic conditions was 87.40% in the viscera and 78.6% in the muscle (Table 4).

## DISCUSSION

The whitemouth croakers presented fairly homogeneous lengths of about 50 cm each and weighed close

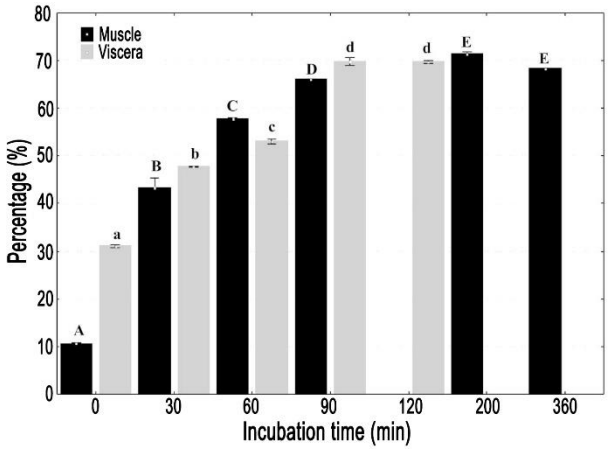


**Figure 2.** Degree of hydrolysis (%) to the residue of muscle and viscera, relative to the incubation time (in minutes). Different letters (upper case for muscle and lower case for viscera) mean significance ($P < 0.05$).

to 1400 g. This size can be considered good development, as the minimum permitted length for capture is 25 cm (Ministério do Meio Ambiente, 2005). The other thing that compromises carcass yield is the head, representing about 1/3 of the total weight. On the other hand, the weight of the viscera, which is just over 7%, can be considered small when compared to species such as arabaiana (*Seriola dumerlii*), tuna (*Thunnus* spp.), mackerel (*Scomberomorus mackerel*) and cation (*Carcharrhinus* spp.), in which the viscera can represent up to 15% of the body weight (Bery *et al.*, 2012).

Due to their distinctive nature, the guts and muscles have characteristic composition, whereby the former has more lipids and the latter, more protein. In fish considered lean, such as a whitemouth croaker, almost all the fat accumulates in the liver and gonads, while a higher amount of protein is found in the muscle (Sales & Monteiro, 1988; Ogawa & Maia, 1999; Centenaro *et al.*, 2009). Ash content exceeding 4% was similar for the muscles and viscera, and higher than the 1.2% reported by Centenaro *et al.* (2009). As the whitemouth croaker is a fish whose habitat is associated with the seabed, it is possible that the feed may contain small amounts of sediment. There are other factors that can influence the proximal composition of the whitemouth croaker, such as tissue type, sex, age and season (Yeannes & Almandos, 2003). The sample itself may be the cause of this difference. A particular region of the body may have more pimples and/or scales, which would increase the ash content. According to Chaguri (2013) the chemical composition of marine fish may be influenced by both exogenous factors including location, fishing season, sex, age of animals, domestication level, availability and quality of food, salinity and temperature, and endogenous factors, through the

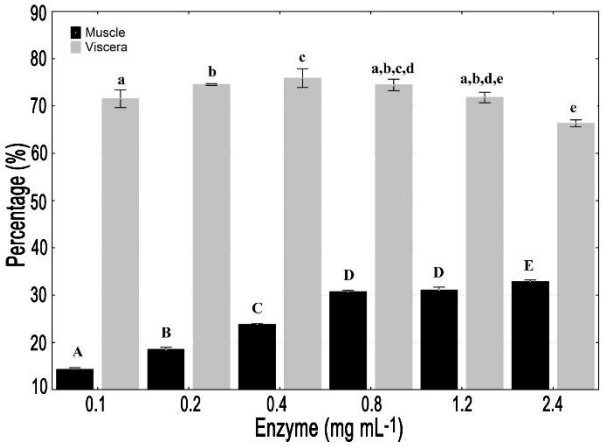


**Figure 3.** Degree of hydrolysis (%) to the residue of muscle and viscera relative to enzyme concentration (mg $mL^{-1}$). Different letters (upper case for muscle and lower case for viscera) mean significance ($P < 0.05$).

mobilezation of lipids and proteins to the gonads during the reproductive period.

To enable the production of fish protein hydrolysates, it is necessary to optimize the hydrolysis conditions, adapting the technical, economic and environmental aspects. In terms of process, temperature and incubation time, as well as enzyme concentration, are basic factors that must be available. In the temperature tests of enzymatic hydrolysis, the optimal temperatures were 55°C to 45°C for viscera and muscle, respectively. An increase of 10°C for hydrolysis of the viscera may be associated with various kinds of proteins contained in this residue, in contrast to the more homogeneous muscles. The residue, named herein as viscera, incorporates various organs such as the heart, stomach, gut, gonads, liver and swim bladder, which consist of sarcoplasmic proteins, liquids and enzymes present in the digestive apparatus. Moreover, the plasticity of the protease to exert its hydrolytic activity at different temperatures is emphasized. For the tissues studied, it took 90 min of incubation for the reaction to reach a higher degree of enzymatic hydrolysis. Increasing the incubation time does not increase the degree of hydrolysis, and could represent an unnecessary cost.

In this phase of the study, it was noticed that at time zero, the viscera showed 30% hydrolysis, while in the muscle was only 10%. This result may be partly linked to the fact that the viscera already contain endogenous proteases that are available in the environment with the disruption of tissues. Another possibility is that the fish wastes contain large amounts of micro-organisms including producers of proteases, especially in the viscera (Beerli *et al.*, 2004).

As the enzymes represent a significant portion of the hydrolysate production costs, the process should be optimized by using the lowest possible amount of enzyme. Again, the nature of the tissues was decisive for the amount of enzyme to be used. The highest degree of hydrolysis was observed at a concentration of 0.4 mg enzyme $mL^{-1}$ of incubation medium to viscera and 0.8 mg $mL^{-1}$ for muscle. The lowest amount of enzymes needed for the hydrolysis of the viscera may be associated with the presence of endogenous enzymes. Even at a concentration of 0.1 mg $mL^{-1}$, the hydrolysis of viscera residue is already high compared to that of the muscle. The same behavior was observed in the test to determine the best incubation time, when at time zero, there was a high degree of hydrolysis in waste viscera.

The enzymatic hydrolysis process tends to keep the fraction insoluble most concentrated in lipids, partly as a consequence of the release of hydrolysis products of proteins into the soluble medium. It is possible that the lower breakdown of particles containing lipid favors their precipitation on centrifugation. Viscera hydrolysates have the soluble fraction with the highest concentration of protein, with most lipids present in the insoluble fraction. This did not occur with muscle, in which both fractions showed similar high protein levels, reflecting the nature of the raw material. In this tissue the hydrolysis yield was about 50%, while the hydrolysate in the gut transformed into soluble fraction reaches almost 70%. This may be related, in part, to the fact that the viscera are already pre-hydrolyzed, unlike the muscles that, which are more retained.

In the soluble fraction of the hydrolyzed muscle and viscera, the lipid levels (0.3% and 5.1%) reflect the contents of the original raw material (1.5% and 10.7%). It is possible that the hydrolysis process may have released compounds of an emulsifying nature, causing a part of the lipid to remain in the soluble fraction. Peptides and proteins, in turn, may present non-polar fractions, thus attracting hydrophobic chains of the fat molecule, resulting in adsorption (Zavareze *et al.*, 2009). With increasing hydrolysis time, there is greater fragmentation of the proteins, causing them to become increasingly soluble in the middle and with less fat molecules associated with them. The major protein, containing more adsorbed fats, ends up precipitating with the force applied to the centrifugation (Cândido & Sgarbieri, 2003). In the centrifugation process, separation of the aqueous phase occurs, containing the soluble proteins, and the insoluble precipitate, containning proteins and fats (Timm-Heinrich *et al.*, 2007).

**Table 3.** Composition analysis of the soluble and insoluble fractions of the hydrolysate of the viscera and muscle of whitemouth croaker (*Micropogonias furnieri*). Results expressed as average % ± standard deviation. *Sum of the percentages of lipids, protein and ash, **Dry mass of the recovered fraction after hydrolysis.

| | Viscera | | Muscle | |
|---|---|---|---|---|
| | Soluble | Insoluble | Soluble | Insoluble |
| Crude protein (N x 6,25) | 75.7 ± 5.6 | 49.7 ± 4.8 | 83.9 ± 3.7 | 87.3 ± 2.0 |
| Lipids | 5.1 ± 4.0 | 22.9 ± 3.2 | 0.3 ± 0.2 | 4.6 ± 0.9 |
| Ash | 6.7 ± 1.8 | 7.1 ± 2.5 | 7.9 ± 1.1 | 2.4 ± 0.5 |
| Total recovered* | 87.5 | 79.7 | 92.1 | 94.3 |
| Fraction yield** | 69.7 | 30.3 | 48.7 | 51.3 |

**Table 4**. Degree of hydrolysis (DH) of the viscera and muscle of whitemouth croaker (*Micropogonias furnieri*). SD: standard deviation.

| | Viscera | Muscle |
|---|---|---|
| | Average ± SD* | Average ± SD* |
| DH (%) | 87.4 ± 7.00 | 78.6 ± 10.64 |

The lipids may also have affected the hydrolysate yield viscera, acting as an inhibitor of enzymatic action. The protein content in the soluble fraction of viscera hydrolysate was lower (75.7%) than that found in muscle (83.9%). According to Diniz & Martin (1999), because of the specificity of the enzyme activity on the hydrolysis, soluble protein presents attractive functional properties, such as solubility and dispersibility, with no destruction of amino acids, retaining the nutritional value of protein. It may therefore constitute an ingredient to be incorporated into processed foods and even foods for human consumption. According to Furlan & Oetterer (2002), the soluble fraction of the enzymatic protein hydrolysates can be considered an optimal source of lysine, arginine, glycine, alanine and proline, modifying the functional properties of food, when are used as a source of amino acids and small peptides. It could also be effective as a milk substitute in feed for calves and pigs, and as a protein supplement in feed for fish, poultry and domestic animals (Sgarbieri, 1996). According to Centenaro & Salas-Mellado (2008) the protein content of the whitemouth croaker hydrolysate varied, in ten trials, from 49.7 to 80.3%.

Even when the waste has passed through the hydrolysis process, the mineral content in the soluble part is still high, generating higher levels of ash than those found in the materials in nature. The ash content remained at about 7%, below the 16.8% reported by Martins *et al.* (2009). These differences stem from the strategy of separating the soluble and insoluble phases, as well as the nature and characteristics of the feedstock. If the raw material contains whole fish, containing skin, bones and scales, it is expected that the mineral matter content will be higher. Another caution is given relating to the methodology of analysis, since the partial incineration of the samples may overstate the ash content. In relation to the insoluble fraction, the ash content showed differences depending on the raw material (7.1% to 2.4% and the viscera into the muscle). In this analysis, it was observed that there was a large amount of sedimentary material in the pellet of the viscera, which may explain the higher ash content found in the insoluble fraction of the hydrolysate. However, the reasons why the mineral residue levels are similar in soluble and insoluble fractions of the guts, and higher in the soluble fraction of the muscles, remain to be clarified.

To the insoluble fraction, the yields were 30.3% for the viscera and 51.3% for the muscle. These results are consistent with the higher degrees of hydrolysis observed in the viscera hydrolysates (87.4%), resulting in higher yields of soluble fraction, similar to those reported by Centenaro & Salas-Mellado (2008), who report 80.3% hydrolysis for whitemouth croaker.

A greater degree of hydrolysis for the viscera compared to the muscle was observed in preliminary testing in our laboratory. In these tests, the volume of solution used was 2 mL. When the hydrolyzed was produced in batch (400 mL), it was found that the degree of hydrolysis was higher in the muscle. This difference can be partially explained by an interference of lipids in the batch experiments, which was not observed in the preliminary tests, given the lower volume of the solution. Schmidt & Salas-Mellado (2009) reported that the high amount of lipids leads to a lower degree of hydrolysis. The author also argue that a high amount of lipids could form complexes protein/lipids, which would be more resistant to enzymatic hydrolysis.

It was there for concluded that the waste (muscle and viscera) of whitemouth croaker (*M. furnieri*) are suitable for production of fish protein hydrolysate (FPH), which have optimum features of appearance and

chemical composition, adding value and reducing damage to the environment.

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## REFERENCES

Association of Official Analytical Chemists (AOAC). 1995. Official methods of analysis. Association of Official Analytical Chemists, Arlington, 1850 pp.

Batista, I. 2011. Hidrolisado proteico de pescado. In: A. A. Gonçalves (ed.). Tecnologia do pescado: Ciência, Tecnologia, Inovação e Legislação, São Paulo, 608 pp.

Beerli, E.L., K.M.C. Beerli & P.V.R. Logato. 2004. Silagem ácida de resíduos de truta (*Oncorhynchus mykiss*), com a utilização de ácido muriático. Ciênc. Agrotec., 28(1): 195-198.

Bery, C.C.S., M.L. Nunes, G.F. Silva, J.A.B. Santos & C.S. Bery. 2012. Estudo da viabilidade do óleo de vísceras de peixes marinhos (*Seriola dumerlii)* arabaiana, (*Thunnus* ssp.*)* atum, (*Scomberomorus cavala)* cavala e (*Carcharrhinus* spp.*)* cação, comercializados em Aracaju - SE para a produção de biodiesel. Rev. Gein. Ges. Inov. Tecnol., 2: 297-306.

Cândido, I.M.B. & V.C.J. Sgarbieri. 2003. Enzymatic hydrolysis of Nile tilapia (*Oreochromis niloticus*) myofibrillar proteins: effects on nutritional and hydrophilic properties. Sci. Food Agric., 83: 937-944.

Carneiro, M.H., P.M.G. de Castro, S.L. dos S. Tutui & G.C.C. Bastos. 2005. *Micropogonias furnieri* (Desmarest, 1823). Estoque Sudeste. In: M.C. Cergole, A.O. Ávila-da-Silva & C.L.D.B. Rossi-Wongtschowski (eds.). Análise das principais pescarias comerciais da Região sudeste/Sul do Brasil: dinâmica oopulacional das espécies em explotação. Série Documentos REVIZEE: Score Sul, São Paulo: Instituto Oceanográfico, Universidade de São Paulo, São Paulo, pp. 94-100.

Carneiro, M.H., L. Fagundes, A.O. Ávila-da-Silva & M.R. Souza. 2000. Ambientes marinhos explorados pelas frotas pesqueiras de Santos e Guarujá (SP). Anais V Simpósio de Ecossistemas Brasileiros: Conservação-ACIESP, Vitória, 109(1): 83-91.

Centenaro, G.S. & M. Salas-Mellado. 2008. Influência das concentrações de enzima e de substrato no grau de hidrólise e no conteúdo proteico de hidrolisados enzimáticos de whitemouth croaker *(Micropogonias furnieri)*. B. CEPPA, Curitiba, 26: 61-70.

Centenaro, G.S., C.E. Prentice-Hernández & M. Salas-Mellado. 2009. Efeito da concentração de enzima e de substrato no grau de hidrólise e nas propriedades funcionais de hidrolisados protéicos de whitemouth croaker (*Micropogonias furnieri*). Quim. Nova, 32: 1792-1798.

Chaguri, M.P. 2013. Rastreabilidade em pescado: Influência do grau de domesticação, origem e sazonalidade na diferenciação de peixes. Tese Doutorado em Aquicultura, Centro de Aquicultura da UNESP-CAUNESP, São Paulo, 91 pp.

Diniz, F.M. & A.M. Martin. 1999. Hidrolisado protéico de pescado. In: M. Ogawa & E.L. Maia (eds.). Manual de pesca. Editora Varela, São Paulo, pp. 79-89.

Duan, Z., J. Wang, M. Yi & A. Yin. 2010. Recovery of proteins from silver carp byproducts with enzymatic hydrolysis and reduction of bitterness in hydrolysate. J. Food Process Eng., 33: 962-978.

Galvani, F. & E. Gaertner. 2006. Adequação da metodologia Kjeldahl para determinação de nitrogênio total e proteína bruta. EMBRAPA, Circ. Téc., 63, Corumbá, 9 pp.

Furlan, E.F. & M. Oetterer. 2002. Hidrolisado proteico de pescado. Rev. Ciên. Tecnol., 10: 79-89.

Lowry, O.H., N.J. Rosenbrough & A.L. Farr. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Martins, V.G., J.A.V. Costa & C. Prentice-Hernández. 2009. Hidrolisado protéico de pescado obtido por vias química e enzimática a partir de whitemouth croaker (*Micropogonias furnieri*). Quim. Nova, 32: 61-66.

Ministério do Meio Ambiente (MMA). 2005. Estatística da pesca 2004. Boletim da produção pesqueira anual, Brasília, 108 pp.

Ogawa, M. & E.L. Maia. 1999. Manual de pesca. Ciência Tec. Pesca, Editora Varela, São Paulo, 1: 453.

Salas-Mellado, M., C. Prentice-Hernández, K.S. Moraes & E.R. Zavareze. 2007. Produção de hidrolisado de cabrinha (*Prionotus punctatus*) e o efeito na composição química e características tecnológicas de pão. Rev. Bras. Agron., 13: 349-353.

Sales, R.O. & J.C.S. Monteiro. 1988. Estudo da composição química e rendimento de quatro espécies marinhas de interesse comercial. Ciênc. Agron. Fortaleza, 19(1): 43-47.

Schmidt, C.G. & M. Salas-Mellado. 2009. Influência da ação das enzimas alcalase e flavourzyme no grau de hidrolise das proteínas de carne de frango. Quim. Nova, 32: 55-72.

Sgarbieri, V.C. 1996. Proteínas em alimentos protéicos: propriedades, degradações, modificações. Editora Varela, São Paulo, 517 pp.

Silva, J.F.X., K. Ribeiro, J.F. Silva, T.B. Cahú & R.S. Bezerra. 2014. Utilization of tilapia processing waste

for the production of fish protein hydrolysate. Anim. Feed Sci. Technol., 196: 96-106.

Stori, F.T. 2000. Avaliação dos resíduos da industrialização do pescado em Itajaí e Navegantes como subsídio à implementação de um sistema gerencial de bolsa de resíduos. Monografia (Curso de Oceanografia), CTTMar/UNIVALI. Itajaí-SC, 145 pp.

Timm-Heinrich, M., X. Xu, S. Nielsen & C. Jacobsen. 2007. Application of antioxidants during short-path distillation of structured lipids. J. Food Lipids, 14: 244-262.

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Xu, H., Y. Mu, Y. Zhang, J. Li, M. Liang, K. Zheng & Y. Wei. 2016. Graded levels of fish protein hydrolysate in high plant diets for turbot (*Scophthalmus maximus*): effects on growth performance and lipid accumulation. Aquaculture, 454: 140-147.

Yeannes, M.I. & M.E. Almandos. 2003. Estimation of fish proximate composition starting from water contend. J. Food Compos. Anal., 16: 81-92.

Zavareze, E. da R., C.M. Silva, M. Salas-Mellado & C. Prentice-Hernández. 2009. Funcionalidade de hidrolisados proteicos de cabrinha (*Prionotus punctatus*) obtidos a partir de diferentes proteases microbianas. Quim. Nova, 32: 1739-1743.