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Whitemouth croaker (*Micropogonias furnieri*) protein hydrolysates: chemical composition, molecular mass distribution, antioxidant activity and amino acid profile

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Abstract

The objective of the present work was to develop and evaluate whitemouth croaker's (*Micropogonias furnieri*) protein hydrolysates by using the proximate composition, the degree of hydrolysis, antioxidant activity, molar mass distribution profile and amino acid composition. Fillets of whitemouth croaker were hydrolysed with the enzyme alcalase 2.4 L (2/100, m/m, enzyme/protein) by varying only the time, thus hydrolysates after 2, 4 and 8 h of hydrolysis were obtained. The hydrolysates were subjected to freeze-drying. The protein content of the hydrolysates ranged from 78.28 to 82.30% (dry basis), where the hydrolysate from the longest hydrolysis time showed the highest degree of hydrolysis (32.1%) and oxidation inhibition force by the ABTS and DPPH methods (98.35% and 54.11%, respectively). Because of the higher inhibitory activities of the hydrolysates, they could be a promising alternative as bioactive additives suitable for application in foods to improve their stability and extend their shelf life..

Introduction

In the last decades, fish protein hydrolysates have been the subject of many scientific investigations since they are considered an important source of bioactive proteins and peptides (Je *et al*., 2009; Kim and Wijesekara, 2010; Najafian and Babji, 2012). Protein hydrolysates are products resulting from the cleavage of proteins into peptides of various sizes obtained by chemical or enzymatic processes. Bioactive peptides are short sequences of approximately 3-20 amino acids that contain important biological activities such as antihypertensive, immunomodulatory, antithrombotic, antioxidant, anticancer and antimicrobial. (Vercruysse *et al*., 2005; Di Bernardini *et al*., 2011). Some studies have shown that fish protein hydrolysates are potential sources of antioxidant peptides (Bougatef *et al*., 2010; You *et al*., 2010). Because of this, antioxidant peptides are an alternative to the use of synthetic antioxidants because the former offers less health risk than the latter (Lin and Liang, 2002).

The antioxidants from peptides of fish proteins are considered safe molecules, with low molecular mass, easy absorption, low cost and high activity (Sarmadi and Ismail, 2010). The antioxidant properties of fish peptides are closely related to amino acid composition, sequence and molecular hydrophobicity (Ren *et al*., 2008; Bougatef *et al*., 2010). You *et al*. (2010) reported that antioxidant peptides have metal chelating activity, the ability to donate electrons or hydrogens, which allows them to interact with free radicals by avoiding or terminating reactions in chains.

Several factors might influence the enzymatic hydrolysis process, among them, the selection of the enzyme and hydrolysis conditions. The control of these parameters is fundamental in the release and functionality of the peptides, especially those with antioxidant properties. Several studies point to time as a determinant factor in the quality of peptides. Thus, reactions with longer time result in peptides with greater antioxidant power. By considering the present work, it is believed that prolonged reactions are not necessary to obtain these products, since hydrolysates from short-time reactions were similar in quality to medium- and long-time reactions (Sbroggio *et al*., 2016).

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Laroque *et al*. (2008) reported that the selection of the enzyme is an important factor to be considered in the enzymatic hydrolysis since it determines the yield and functionality of the peptides. The enzyme alcalase (alkaline bacterial endopeptidase produced from the submerged fermentation of *Bacillus licheniformis*) has been regarded as one of the best enzymes to hydrolyse fish proteins (Bhaskar *et al*., 2007; Guerard *et al*., 2010).

Whitemouth croaker (*Micropogonias furnieri*) is considered a low commercial value type of fish in Brazil with wide acceptance by the consumers. It is the second most captured species in the state of São Paulo, Brazil in 2015 of about 1,545.6 tons (Instituto de Pesca de São Paulo, 2016). Chao *et al*. (2015) mention that more than 40,000 tons were captured throughout Brazil. In the present work, this fish was used to produce protein hydrolysate since it possesses high protein content and has affordable price and availability throughout the year. Despite the large volume available in the market, this species presents some restrictions that affect its commercial value, such as low yield in muscle and the susceptibility to parasites (Luque *et al*., 2010; Martins *et al*., 2014). According to Curcho *et al*. (2009), whitemouth croaker has 77.9% moisture, 19.6% protein, 1.06% ash and 1.46% lipid. It can thus be an excellent source in the production of protein hydrolysates. The reaction time is one of the factors that influence the production and quality of protein hydrolysates. Thus, the objective of the present work was to investigate the effect of hydrolysis duration (2, 4, 8 h) on whitemouth croaker hydrolysate production in terms of the degree of hydrolysis, antioxidant activity, chemical composition, molecular mass distribution and amino acid profile.

Materials and methods

Materials

Fresh fish were bought from a local market in the city of Pelotas, State of Rio Grande do Sul, Brazil. Afterwards, they were taken to the laboratory of the Federal University of Pelotas where they were filleted and then used to obtain protein hydrolysates. The enzyme Alcalase 2.4L was purchased from Novozymes Latin America (Araucária, Brazil). All reagents used in the present work were of analytical grade.

Preparation of protein hydrolysate

The hydrolysates were prepared according to Zavareze *et al*. (2014) with modifications. The fresh fish were transported to the Federal University of Pelotas in icebox, where they were cleaned, gutted and filleted. Fish fillets were frozen at -24°C for the total period of approximately 1 w (beginning to end of analysis). Hydrolyses were performed in triplicates and on different days due to the complexity of its steps. All the hydrolysates were from the same fish batch. Fillets of whitemouth croaker were ground and homogenised with distilled water at 1:6 (m/v) ratio, heated at 80°C for 20 min. The hydrolysis conditions were 2/100 (m/m) enzyme/protein ratio; pH = 8; temperature of 50°C; stirring at 300 rpm. The pH-Stat method was used with 0.2 mol/L sodium hydroxide constant solution. The amount of this added solution was written down at 10-min intervals to determine the degree of hydrolysis. Hydrolysates from 2, 4 and 8 h of reaction were prepared. Following the hydrolysis, the solution was inactivated by heating (85°C for 15 min). Next, it was centrifuged at 4°C for 30 min in order to remove the insoluble protein and lipid fractions. The soluble fraction was freeze-dried at -60°C for 30 h.

Proximate analysis

The chemical compositions of the fish muscle and protein hydrolysate were evaluated according to AOAC (2000). Briefly, the moisture content was determined in an oven at 105°C until constant mass (gravimetric method no. 950.46). The protein content was determined by quantifying the total nitrogen of the sample by the Kjeldahl method using a 6.25 conversion factor (Kjeldahl method no. 928.08). The lipid content was obtained by the Soxhlet method (Soxhlet method no. 960.39) and the ash content was determined in a muffle at 500-600°C (gravimetric method no. 920.153). All analyses were performed in triplicate.

Degree of hydrolysis

The degree of hydrolysis, defined as the number of hydrolysed peptide bonds, was expressed in hydrolysis equivalents (h), in relation to the total number of peptide bonds before the reaction (h_{total}) according to the method described by Adler-Nissen (1985). The degree of hydrolysis was calculated according to Equation 1.

$$DH = \frac{(B \times N_b)}{(MP \times \alpha \times h_{total})} \times 100 \qquad \text{Equation 1}$$

where B was the volume (mL) of the base consumed during the hydrolysis to maintain pH constant; N_b was the molar concentration of the base; MP was the mass of the protein (g); α was the degree of dissociation; and h_{total} was the total number of peptide bonds prior to the reaction.

Antioxidant activity

The antioxidant activity of the hydrolysates was evaluated by the sequestering capacity of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical, as described by Brand-Williams *et al*. (1995) with some modifications. The solution of the compound was prepared by dissolving 2.4 mg DPPH in 100 mL methanol. A stock solution was prepared and refrigerated for approximately 24 h. The DPPH working solution was adjusted to an absorbance of about 0.97 by adding methanol. In order to obtain the extract, 1 g sample was weighed and diluted in 20 mL 50% methanol. Then, the solution was homogenised in Ultra Turrax® (IKA®, T18 digital) at 7,500 rpm for 1 min, centrifuged (Eppendorf Centrifuge, 5430) at 7,000 rpm for 20 min, and cooled to 4°C. Then, 3.9 mL DPPH working solution was mixed with 100 µL sample. The solution was homogenised for 1 min in a vortex (Phoenix, AP-56) and kept in the dark for 24 h. Following this, reading was performed using a spectrophotometer (Jenway, 6700 UV-Vis) at 517 nm. The capture of the 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) radical (ABTS) was performed according to the methodology of Re *et al*. (1999). The same sample concentration (0.005 g/4 mL) was used for the two analyses (DPPH and ABTS).

Distribution profile of molar mass of the hydrolysates

The distribution profile of the molar mass of the hydrolysates was evaluated by high performance size exclusion chromatography (HPSEC) according to the methodology of Buggenhout *et al*. (2013) with some modifications. A Yarra SEC-2000 column and cytidine (0.24 kDa), blue dextran (2 kDa), cytochrome C (12 kDa), albumin (66 kDa) and phosphorylase b (97 kDa) standards were used. Extraction protocol: non-denaturing medium (NDM) and denaturing medium (DM + mercaptoethanol). A 10-mg sample of protein hydrolysate was extracted with 10 mL sodium phosphate buffer (0.05 mol/L, pH 6.8) containing: (1) 2.0% NDM; and (2) 2.0% NDM and 1% mercaptoethanol. The material was centrifuged at 10,000 g for 10 min, and filtered (polyethersulfone 0.45 µm, Millipore). Test protocol: injection of 10 µL, flow of 0.35 mL/min, column temperature of 30°C, mobile gradient: 50 mM sodium phosphate buffer, pH 6.8 containing 2.0% NDM.

Amino acid profile of the hydrolysates

The amino acid profiling of the hydrolysates was carried out at the Chemistry Centre of the Medical School of Ribeirão Preto (USP, Ribeirão Preto-SP, Brazil). The evaluation of the amino acids was performed according to the methodology of Spackman *et al*. (1958). The amino acids were obtained by hydrolysis of the peptides with 6 mol/L HCl for 22 h at 110 ± 1°C according to Spackman *et al*. (1958). The analysis was performed by HPLC-amino acid analysis (PTC-AA) (LDC-Analytical, USA).

Statistical analysis

The treatments were performed in triplicate, and the results obtained were statistically analysed using Analysis of Variance (ANOVA) and Tukey's test at 5% of significance.

Results and discussion

Chemical composition

The muscle of whitemouth croaker yielded 79.3% moisture, 14.9% protein, 1.2% lipid and 0.8% ash. Curcho *et al*. (2009) found very close results of 77.9% moisture, 19.6% protein, 1.06% ash and 1.46% lipids. The chemical composition of the fish might vary according to the type of muscle, gender, age, time of the year, habitat and diet (Luzia *et al*., 2003). Table 1 shows the chemical composition of the whitemouth croaker protein hydrolysates obtained after 2, 4 and 8 h of reaction. The protein content of the hydrolysates ranged from 78.3 to 82.3%; however, they presented significant differences. The hydrolysate with the shortest reaction time displayed the highest protein content. Regarding the moisture content, no significant differences were observed between the hydrolysates. In relation to the ash content, it was observed that the longer the reaction time, the greater the ash content. Some authors have reported that the high ash content of the hydrolysates is related to the use of acidic or basic solutions to adjust the pH of the medium (Dong *et al*., 2005; Pacheco-Aguilar *et al*., 2008; Choi *et al*., 2009). Chalamaiah *et al*. (2010), studying hydrolysates of meriga proteins (*Cirrhinus mrigala*) using alkaline enzyme, found 85.0% protein, 6.1% lipids, 5.2% humidity and 3.7% ash. Bougatef *et al*. (2009) reported that the chemical properties of hydrolysates depend on several factors such as chemical reagents, type of enzyme, substrate, pH, temperature, incubation time and enzyme concentration. According to the literature, fish protein hydrolysates have protein contents between 60 and 90%, and ash between 0.45 and 27%. The high protein content present in fish protein hydrolysates is due to the solubilisation of proteins during the hydrolysis and the removal of insoluble solid matter by means of the centrifugation process (Chalamaiah *et al*.,

2012). Most studies have shown that fish protein hydrolysates have a lipid content of less than 5% and a moisture content of less than 10%. The main reason for the low lipid content of the hydrolysates is the centrifugation process which removes the lipids and the insoluble protein fractions (Bueno-Solano *et al*., 2009). Chemical content of whitemouth croaker protein hydrolysates obtained with different reaction times can be seen in Table 1.

Table1. Chemical composition (on dry basis) of whitemouth croaker muscle hydrolysate hydrolysed at different durations.

Hydrolysis time (h)	Moisture (%)	Proteins (%)	Lipid (%)	Ash (%)
2	3.7 ± 0.4[a]	82.3 ± 0.8[a]	4.4 ± 0.1[a]	9.5 ± 0.1[c]
4	4.1 ± 0.0[a]	80.4 ± 0.9[b]	4.1 ± 0.1[ab]	10.5 ± 0.1[b]
8	4.2 ± 0.1[a]	78.3 ± 0.2[c]	3.5 + 0.3[b]	11.7 ± 0.1[a]

[a]Different letters in the column denote significant difference by Tukey's test ($p < 0.05$).

Degree of hydrolysis (DH)

Bougatef *et al*. (2010) reported that the degree of hydrolysis is a crucial parameter to produce protein hydrolysates since it measures the extension of protein degradation by the protease. It is an indicator widely used to compare different protein hydrolysates. The results of the degree of hydrolysis obtained from the whitemouth croaker muscle proteins with different reaction times are shown in Table 2. The degree of protein hydrolysis, obtained from the muscle of whitemouth croaker after 2, 4 and 8 h reaction was 22.6, 26.7 and 32.1%, respectively (Table 2). The enzyme displayed better activity in the first 60 min of reaction. After that, the degree of hydrolysis varied slowly. This indicates that the highest percentage of peptides was released within the first hour of reaction. García-Moreno *et al*. (2014), hydrolysing muscle of different species of fish: sardine (*Sardina pilchardus*), horse mackerel (*Trachurus mediterraneus*) and axillary seabream (*Pagellus acarne*) using enzymes subtilisinae trypsin in proportion of 3% of enzyme/substrate for a period of 4 h, found degree of hydrolysis of 13, 2 and 21.0%, respectively. Zavareze *et al*. (2014) produced protein hydrolysate of whitemouth croaker muscle using flavourzyme enzyme in a 1:5 substrate/buffer ratio with 2% of enzyme/substrate. The authors obtained the degree of hydrolysis of 28.5%. Benítez *et al*. (2008) reported that the degree of hydrolysis might be influenced by several factors, such as substrate concentration, enzyme/substrate ratio, incubation time, pH, temperature and nature of the enzyme, characterised by its specific activity and type of activity. According to Polanco-Lugo*et al*. (2014), protein hydrolysates can be divided into three groups: a) hydrolysates with a low degree of hydrolysis (<10%), used to improve the functional properties of proteins; b) hydrolysates with variable degree of hydrolysis, used as flavouring agents; and c) hydrolysates with a high degree of hydrolysis (> 10%), used in specialised nutrition and bioactive compounds. Therefore, the hydrolysates obtained in the present work were considered to be of high hydrolysis degree, and because of that they might be used as bioactive compounds with specific functions such as opioid, immunoregulatory, antiulcer, anticarcinogenic, antihypertensive, antitumor, antimicrobial and antioxidant activity. They can also be used as special-purpose foods (Korhonen and Pihlanto, 2006; Lee *et al*., 2010).

Table 2. Degree of hydrolysis of whitemouth croaker muscle hydrolysate hydrolysed at different durations.

Time (min)a	Degree of hydrolysis (%)[a]		
	Hydrolysate - 2h	Hydrolysate - 4h	Hydrolysate - 8h
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
30	13.5 ± 0.8	11.7 ± 0.6	12.4 ± 0.6
60	18.3 ± 0.6	16.3 ± 0.3	17.8 ± 0.9
90	20.7 ± 0.0	19.4 ± 0.1	21.0 ± 0.9
120	22.6 ± 0.1	21.6 ± 0.1	23.3 ± 0.8
150		23.3 ± 0.1	25.0 ± 0.6
180		24.6 ± 0.2	26.3 ± 0.5
210		25.7 ± 0.3	27.3 ± 0.4
240		26.7 ± 0.4	28.2 ± 0.4
270			28.9 ± 0.4
300			29.5 ± 0.4
330			30.0 ± 0.3
360			30.5 ± 0.4
390			31.0 ± 0.4
420			31.3 ± 0.5
450			31.7 ± 0.4
480			32.1 ± 0.5

[a] Mean of three replicates ± standard deviation.

Antioxidant activity of hydrolysates

The hydrolysates of muscles of whitemouth croaker were lyophilised and their antioxidant activity was assessed by means of the sequestering capacity of DPPH free radical and sequestering activity of ABTS free radical. The same sample concentration (0.005 g/4 mL) was used for the two analyses (DPPH and ABTS). The antioxidant activity of the hydrolysates with different reaction times is shown in Table 3. Significant differences were found between the protein hydrolysates, both by the capture of the DPPH radical and by the capture of the ABTS radical. The hydrolysate with the longest reaction time showed a higher degree of hydrolysis (32.1%) and a greater inhibition activity of ABTS and DPPH

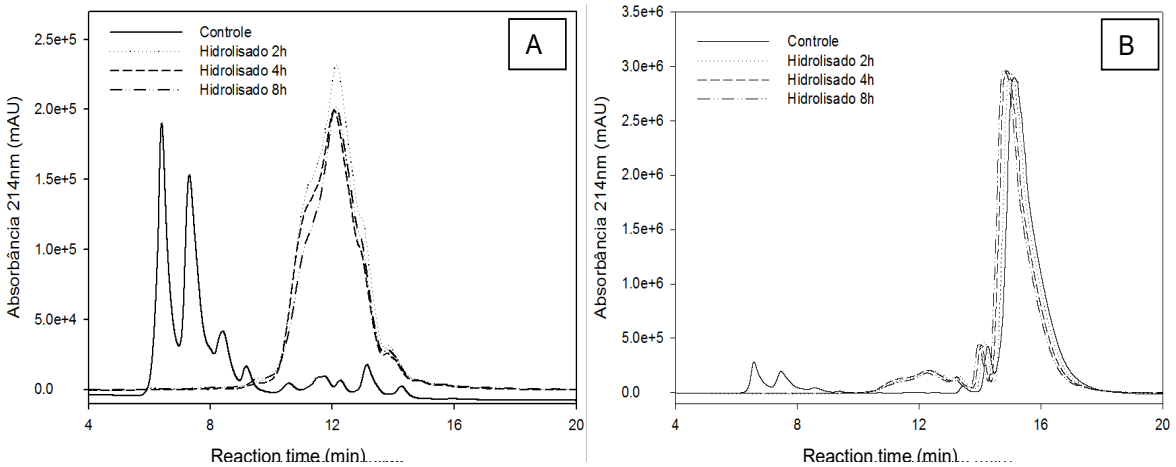

Figure 1. Profiles of molecular mass distribution of whitemouth croaker muscle hydrolysates hydrolysed at different durations. A: Extraction in non-denaturing medium (2.0% SDS); B: Extraction in denaturing medium (2.0% SDS and 1% mercaptoethanol).

of 98.4% and 54.1%, respectively. Although the hydrolysates had different reaction times (2, 4 and 8 h), it was observed that the inhibition activity of the hydrolysates was very close for the capture of the ABTS radical. Cian, Martínez-Augustin and Drago (2012) used the same method, similar concentration of sample and found values of DPPH close to those of the present work. This suggests that the peptides that exhibit inhibitory activity for this test were released within the first two hours of reaction, after which, the proteolytic reaction continued very slowly in producing peptides with antioxidant activity. Laroque *et al*. (2008) reported that the release of antioxidant peptides might be influenced by several factors such as selection of the enzymatic treatment and hydrolysis conditions (temperature, pH, enzyme/substrate ratio and reaction time) as well as the properties of the substrate.

Table 3. Antioxidant activity of whitemouth croaker muscle hydrolysate hydrolysed at different durations.

Hydrolysis time (h)	ABTS (% Lipid oxidation inhibition)[a]	DPPH (% Lipid oxidation inhibition)[a]
2	97.2 ± 0.2[c]	40.4 ± 0.1[c]
4	97.7 ± 0.2[b]	48.0 ± 0.4[b]
8	98.4 ± 0.2[a]	54.1 ± 0.5[a]

[a]Different letters in the same column denote significant difference by Tukey's test ($p < 0.05$).

Profile of molecular mass distribution of hydrolysates

The profile of the molecular mass distribution of whitemouth croaker hydrolysates obtained at different reaction times is shown in Figure 1. All hydrolysates showed proteins with lower molecular weight than native protein (control), reducing from a dimeric and trimeric forms determined by peaks between 6 and 8 min of retention time to acidic and basic subunits observed by the presence of a huge peak with retention time between 10 and 14 min (Fig 1A). The average molecular weight of the protein fraction of the obtained hydrolysates is estimated between 2 and 34 kDa, based on the reports of Silva *et al*. (2017). Ren *et al*. (2008) hydrolysed muscle of silver carp using pancreatin and neutralised enzymes and found peptides with molecular mass lower than 3 kDa. The protein molecular weight distribution was similar between hydrolysates, regardless of hydrolysis time. Extraction at a stronger media (SDS + Mercaptoethanol) confirms the similarity of the effects of hydrolysis time on protein extractability (Fig. 1B).

Profile of hydrolysate amino acids

The amino acid profile of the whitemouth croaker hydrolysates obtained at different reaction times is shown in Table 4. It was found that glutamate, asparagine, lysine, and leucine were the most prevalent amino acids in whitemouth croaker enzymatic hydrolysates. Yang *et al*. (2011) reported that the alcalase enzyme preferentially hydrolyses the carboxy side of the peptide bonds of the amino acids glutamate, glutamine, methionine, leucine, tyrosine and lysine. By considering the presence of these amino acids in the protein of whitemouth croaker, alcalase is an excellent protease for this type of substrate. Rajapakse *et al*. (2005) reported that the antioxidant activity of protein and peptide hydrolysates is related to the hydrophobicity of the amino acids, thus, the higher the hydrophobicity, the greater the solubility in lipids and, consequently, the higher the antioxidant activity. Amino acids present in peptides such as Leu, Val, and Ala might promote the ability to sequester

free radicals, as well as those containing residues of His might exhibit strong antioxidant activity due to the decomposition of imidazole groups (Gao *et al*., 2010). Because arginine is a basic amino acid, it gives the peptide the ability to donate protons and chelate metals (Arcan and Yemenicioglu, 2010). The hydrophobic amino acids (Ala, Pro, Tyr, Val, Met, Ile, Leu and Phe) present in 2-, 4- and 8-h hydrolysates represent 40.7%, 40.9% and 40.8% of the total of amino acids, respectively (Table 4). Dong *et al*. (2008), when hydrolysing muscle of silver carp (*Hypophthalmichthys molitrix*) using enzyme flavourzyme with 1.5 and 4 h of reaction times, found 39.1% and 40.8% of hydrophobic amino acids, respectively.

Table 4. Composition of amino acids of whitemouth croaker muscle hydrolysate hydrolysed at different durations.

Amino acid	Concentration (mg amino acid/g protein)[a]		
	Hydrolysate - 2h	Hydrolysate - 4h	Hydrolysate - 8h
Asparagine	105.23 ± 0.86[a]	102.80 ± 3.78[a]	105.56 ± 0.74[a]
Glutamate	173.11 ± 4.93[a]	174.65 ± 1.15[a]	169.52 ± 1.88[a]
Serine	39.47 ± 1.63[a]	38.83 ± 2.20[a]	35.66 ± 3.14[a]
Glycine	40.54 ± 0.19[a]	39.64 ± 1.71[a]	41.74 ± 0.08[a]
Histidine	23.74 ±2.46[a]	23.95 ± 0.54[a]	26.50 ± 0.86[a]
Arginine	59.36 ± 0.52[a]	58.97 ± 0.21[a]	63.18 ± 5.52[a]
Threonine	48.58 ± 0.58[a]	48.12 ± 1.84[a]	45.88 ± 1.80[a]
Alanine	57.10 ± 1.49[a]	57.40 ± 2.24[a]	56.5 ± 0.21[a]
Proline	39.33 ± 0.94[a]	40.13 ± 0.62[a]	40.64 ± 0.23[a]
Tryptophan	34.81 ± 0.66[a]	34.52 ± 0.14[a]	32.04 ± 2.17[a]
Valine	52.52 ± 1.13[a]	53.37 ± 0.51[a]	54.65 ± 1.64[a]
Methionine	39.63 ± 0.58[a]	38.74 ± 1.21[a]	37.88 ± 0.92[a]
Cysteine	5.09 ± 0.31[a]	3.92 ± 0.86[a]	4.45 ± 0.44[a]
Isoleucine	42.77 ± 2.48[a]	45.29 ± 1.30[a]	48.18 ± 2.85[a]
Leucine	96.42 ± 2.75[a]	95.75 ± 2.89[a]	97.88 ± 0.55[a]
Phenylalanine	44.43 ± 0.33[a]	44.64 ± 1.30[a]	45.09 ± 0.67[a]
Lysine	97.85 ± 1.41[a]	99.41 ± 1.65[a]	99.59 ± 1.37[a]

[a]Different letters in the same row denote significant difference by Tukey's test ($p < 0.05$).

Conclusion

Considering the concentrations of the samples tested in the present work, it was found possible to produce whitemouth croaker protein hydrolysate with expressive inhibition activity. According to the method applied in the present work, the protein content of the hydrolysates ranged from 78.3 to 82.3%, and the hydrolysate with the shortest reaction time showed the highest protein content. The degree of hydrolysis of the hydrolysed proteins of whitemouth croaker muscle was proportional to the reaction time, so the 2, 4 and 8-h hydrolysates presented 22.6, 26.7 and 32.1%, respectively. Although the hydrolysates had different reaction times (2, 4 and 8 h) and had presented significant differences for the capture of the ABTS and DPPH radicals, it was observed that the inhibition activity of the hydrolysates were close, mainly for the capture of the ABTS radical, showing that long reaction times are not needed to obtain hydrolysate with high antioxidant action. It is also noteworthy that the same sample concentrations were used for the two analyses (DPPH and ABTS). Regarding the amino acid profile, no significant difference was observed between hydrolysates after 2, 4 and 8 h of reaction. Regarding the amino acid profile, no significant difference was observed between hydrolysates after 2, 4 and 8 h of reaction. Also, similar molecular weight distribution of proteins was determined between hydrolysates, regardless of hydrolysis time, with average molecular weight between 2 and 34 kDa.

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