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Dr. Harvey A. Villa-Vélez

Centro de Ciências Exatas e Tecnologicas, Universidade Federal do Maranhão, São Luís-MA, Brasil

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*Correspondence:
Willian Renzo Cortez-Vega
williamvega@ufgd.edu.br

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Evaluation of protein isolate obtained from byproducts of hybrid sorubim (*Pseudoplatystoma reticulatum* x *Pseudoplatystoma corruscans*)

Avaliação de isolado proteico obtido de bioprodutos de sorubim híbrido (Pseudoplatystoma reticulatum x Pseudoplatystoma corruscans)

[1] Rosiane de Souza SILVA, [1] Barbara Matias Moreira dos SANTOS, [1] Sandriane PIZATO, [1] Gustavo Graciano FONSECA and [1,*] William Renzo CORTEZ-VEGA

[1]Federal University of Grande Dourados, Laboratory of Bioengineering. Rod. Dourados km 12, Itahum – 79804970, Dourados, MS - Brazil.

ABSTRACT

Industrial fish processing generates large quantities of protein-rich byproducts, which are usually discarded because of their low commercial value. It is therefore very important to find feasible alternatives to make use of this protein-rich raw material. The aim of this study was to obtain protein isolate from the byproducts of the fish hybrid sorubim (*Pseudoplatystoma reticulatum* x *Pseudoplatystoma corruscans*). After obtaining the fish protein isolate (FPI) from hybrid sorubim, the proximate composition of the raw material and of the isolate were determined and the functionality of the FPI was also evaluated. The mechanically separated meat (MSM) of hybrid sorubim contained 73.98% moisture, 37% lipids (dry basis-d.b.), 56.85% protein (d.b.) and 6.13% ash (d.b.). The protein isolate contained 5.61% moisture, 95.21% protein (d.b.), 3.06% lipids (d.b.) and 1.71% ash (d.b.). As for functionality, the isolate showed high solubility (88.5%), good water holding capacity (20.11 mL) and low oil holding capacity (2.03 mL). The process was considered efficient, in view of the high protein concentration and low moisture and lipids levels obtained in the isolate. The high water holding capacity and solubility confirm the promising potential of fish protein isolate from hybrid sorubim for use in the production of high added-value products.

Keywords: Solubility. Fish protein. Composition. Industrial processing.

RESUMO

O processamento industrial de pescado gera grandes quantidades de subprodutos ricos em proteínas, que geralmente são descartados devido ao seu baixo valor comercial. Por isso, é muito importante encontrar alternativas viáveis para utilizar esta matéria-prima rica em proteína. O objetivo deste estudo foi obter isolado proteico dos subprodutos do pescado surubim híbrido (*Pseudoplatystoma reticulatum* x *Pseudoplatystoma corruscans*). Após a obtenção do isolado proteico de pescado (IPP) a partir do surubim híbrido, determinou-se a composição proximal da matéria-prima e do isolado proteico e também avaliou-se a funcionalidade do IPP. A carne mecanicamente separada (CMS) de surubim híbrido apresentou 73,98% de umidade, 37% de lipídios (base seca – b.s), 56,85% de proteína (b.s.) e 6,13% de cinzas (b.s.). o isolado proteico continha 5,61% de umidade, 95,21% de proteína (b.s.), 3.06% de lipídios (b.s.) e 1,71% de cinzas (b.s.). quanto a funcionalidade, o isolado apresentou alta solubilidade (88,5%), boa capacidade de retenção de água (20,11 mL) e baixa capacidade de retenção de óleo (2,03 mL). O processo foi considerado eficiente, tendo em vista a alta concentração proteica e os baixos níveis de umidade e lipídios obtidos no isolado. A alta capacidade de retenção de água e a solubilidade confirmam o potencial promissor do isolado proteico de pescado de surubim híbrido para uso na produção de produtos de alto valor agregado.

Palavras-chave: Solubilidade. Proteína de pescado. Composição. Processamento industrial.

INTRODUCTION

In general, the market demand for fish tends towards filleted fish, which is the product preferred by consumers. The yields of this product vary according to fish size and the technological mastery of fish processing companies. However, the filleting process generates by-products, such as heads, bones, skin, scales and carcasses, which are often discarded, resulting in environmental pollution. Thus, alternative protein sources such as fish processing byproducts are important because, although they constitute 60% to 70% of the original raw material, they are discarded by processing companies, harming the environment (Nolsøe & Undeland, 2009). The nonuse of these byproducts means that this raw material and its great technological potential for sustainable development is wasted, since it is not applied for alternative production purposes (Bery, Nunes, Silva, Santos & Bery, 2012).

An alternative to exploit and value by-products of the fishing industry is to isolate the protein from mechanically separated meat (MSM) of fish, which is a product obtained from a single species or from a mixture of fish species with similar sensory characteristics (Cavenaghi-Altemio, Alcade, & Fonseca, 2013).

Protein isolates or concentrates have a high nutritional value and are intended as a fat-free bodybuilding product for human consumption, avoiding the intake of saturated fats that cause high cholesterol, obesity and other negative health consequences. Protein isolation is basically an extraction process aimed at obtaining an interferon-free product (more than 90% protein and less than 1% lipids), and because it is a more concentrated product, it presents different properties and characteristics of conservation and use (Lopes & Prentice, 2006).

The fish species used in this study was the hybrid sorubim (*Pseudoplatystoma reticulatum* x *Pseudoplatystoma corruscans*) because of its white flesh, firm texture, mild flavor, low fat content and absence of intermuscular bones. These characteristics satisfy current market preferences, making this species a highly attractive product (Fonseca, Cavenaghi-Altemio, Silva, Arcanjo & Sanjinez-Argandoña, 2013).

The aim of this study was to develop suitable processes to obtain a fish protein isolate (FPI), using hybrid sorubim by-products as raw material, and to evaluate its physicochemical and functional properties.

MATERIAL AND METHODS

Raw material to obtain recovered protein

The raw material used in this study was hybrid sorubim (*Pseudoplatystoma reticulatum* x *Pseudoplatystoma corruscans*) byproducts from fish processing plants in the region of Grande Dourados, MS, Brazil. The byproducts were put into a cooling box and taken to the laboratory. The fish flesh was obtained by means of mechanical meat separation, using a Baader model 694 deboner machine (Germany).

Obtaining the fish protein isolate (FPI)

The obtained mechanically separated meat of hybrid sorubim was homogenized with distilled water in a ratio of 1:9 (w/v) at 5°C for 5 min., using a propeller stirrer (Fisatom 713D, São Paulo, Brazil). After homogenization, the protein was subjected to alkaline solubilization in an ultra-thermostatic water bath (QUIMIS model 214 D2, São

Paulo, Brazil) for 20 min., under constant stirring with a propeller stirrer (Fisatom 713D, São Paulo, Brazil), using sodium hydroxide (NaOH 1 N) as the alkalizing agent, at a solubilization pH of 11, and constant controlled temperature of 5°C. After this step, the material was centrifuged at 9,000 x *g* for 20 min. (Nova Técnica, model MA 1815 centrifuge, Brazil) to separate the solubilized product into three phases: lipids, soluble proteins and insoluble proteins, thus facilitating the removal of the supernatant. The intermediate phase resulting from centrifugation, corresponding to the soluble proteins, was separated and subjected to isoelectric precipitation, using hydrochloric acid (HCl 1N). The pH was adjusted to 5.8 at 5°C under constant stirring (Fisatom 713D stirrer, Brazil) for 20 min. Another centrifugation was carried out at 9,000 x *g* for 20 min. to separate the precipitated fraction, thus facilitating the collection of the precipitate and obtaining the protein isolate. The fish protein isolate was dried in an air circulating oven (MA model 035) at 45°C for 16 h and then crushed in a double bladed grinder. The protein isolate was stored in a hermetically sealed glass container at room temperature throughout the course of this study.

Characterization of the FPI

Proximate composition

The proximate composition of the FPI was determined according to the official methods of analysis (AOAC, 2000). The moisture content was determined by the gravimetric method (AOAC 950.46) in an oven at 105°C. The total nitrogen content was determined by the Kjeldahl method (AOAC 928.08), while the crude protein content was calculated by multiplying by factor 6.25. The lipid content was determined by the Soxhlet method (AOAC 960.39) and the crude ash content by gravimetric method (AOAC 920.153) in muffle furnace at 500-600°C. The analyses were performed in triplicate.

Solubility

Solubility was determined according to the method proposed by Chalamaiah, Rao & Jyothirmayi (2010) and Tadpitchayangkoon, Park and Yongsawatdigul (2010), with variations in pH (3, 5, 7, 9 and 11). Protein solubility was calculated using Eq. 1.

$$\text{Solubility (\%)} = \frac{\text{Amount of protein in the supernatant}}{\text{Amount of protein in the sample}} \text{ x } 100$$

Eq. (1)

Water holding capacity (WHC)

Water holding capacity (WHC) was determined according to method proposed by Regenstein, Jauregui and Baker (1984), based on the difference between the weight of the wet sample and that of the dry sample. The result was expressed in mL of water per gram of protein, according to Eq. 2.

$$\text{WHC} = \frac{\text{Amount of removed water (mL)}}{\text{Original protein mass (g)}}$$

Eq. (2)

Oil holding capacity (OHC)

The oil holding capacity (OHC) was determined according to the method described by Fonkwe and Singh (1996). The result was expressed as the amount of oil retained (mL) per gram of protein contained in the sample, based on Eq. 3.

$$\text{OHC} = \frac{\text{Added oil} - \text{removed oil(mL)}}{\text{Protein mass (g)}}$$

Eq. (3)

Statistical analysis

The experiments were carried out in triplicate. Results were expressed as mean and standard deviation values. The results were evaluated using analysis of variance (ANOVA) and Tukey's multiple comparison test, with the significance level of 5%, using Statistica 7.0 software.

RESULTS AND DISCUSSION

Proximate composition

The lipid, protein and moisture contents of the MSM and FPI samples differed significantly. In fact, the amount of protein in the FPI was practically double that contained in the raw material. The high protein content of the raw material indicates that the protein concentrating process used here was effective, and that it is a possible alternative to add value to fish industry waste. Table 1 describes the proximate composition of the MSM and FPI of hybrid sorubim.

Table 1. Proximate composition of the mechanically separated meat (MSM) and fish protein isolate (FPI) of hybrid sorubim

Compound	MSM		FPI	
	%(w.b.)*	%(d.b.)*	%(w.b.)*	%(d.b.)*
Moisture	73.98±1.34a	-	5.61±0.01b	-
Proteins	15.84±0.18a	56.85±0.18	90.82±0.8b	95.21±0.8
Lipids	10.31±1.16a	37.00±1.16	2.92±0.08b	3.06±0.08
Ashes	1.71±0.02a	6.13±0.02	1.64±0.06a	1.71±0.06

*Average of 3 replicates ± standard deviation; w.b.: wet basis; d.b.: dry basis.

Different letters in the same line indicate that MSM and FPI statistically differ by Tukey's test P>0.05 for the corresponding compound.

The lipid content of the protein isolate obtained here was lower than that of the MSM (Table 1), since most of the lipids were separated together with the insoluble protein fraction during the first centrifugation. These components become separated due to differences in density and solubility during centrifugation, associated with the solubilization temperature (5°C), which contributes to separate the fatty layer (Kristinsson & Ingadottir, 2005). Lowering the lipid content is very important to reduce

the susceptibility of the protein isolate to lipid oxidation (Nolsøe & Undeland, 2009). Research conducted by Undeland et al. (2002) also demonstrated the efficiency of lipids removal achieved in acid and alkaline solubilization processes. Similarly, Hisano et al. (2013) reported 4.71% (dry basis) of lipids in hybrid sorubim (*Pseudoplatystoma reticulatum* x *P. corruscans*) viscera protein concentrate.

As can be seen in Table 1, the protein content of FPI was 95.21% (dry basis), which is higher than the 72.34% and 88.28% protein content found by Martins, Costa and Prentice-Hernández (2009) in fillet and waste from the fish whitemouth croaker (*Micropogonias furnieri*), and the 88.74% (dry basis) reported by Hisano et al. (2013) for hybrid sorubim viscera protein concentrate. Freitas et al. (2015) reported a protein content of 93.11% (dry basis) in protein isolate from whitemouth croaker byproducts, which is also lower than that obtained in this study.

Although the MSM of hybrid sorubim contained a larger quantity of organic elements, its crude ash content (wet basis) of 1.71% differed very little from that of the FPI, which was 1.64%. This can be explained by the accumulation of inorganic solutions employed to adjust the pH in the extraction process of protein isolate (Fontana, Centenaro, Palezi & Prentice-Hernández, 2009). Cortez-Vega, Bagatini, Souza and Prentice (2013a) also reported finding only a slight difference in the crude ash contents of MSM and protein isolate of Whitemouth croaker, *i.e.*, 1.19% and 1.29%, respectively.

Moisture is one of the critical factors in the protein isolate extraction process. Differences in moisture of raw materials are expected due to the differences in species composition. However, the higher the moisture content, the higher the concentration of myofibrillar proteins, which occurs due the sequential washings with sodium bicarbonate solutions that increase pH, generating repulsion between protein groups and the water entrapment by hydrophilic residues of myofibrillar proteins (Cortez-Vega, Fonseca, Feisther, Silva and Prentice, 2013b, Cortez-Vega, Fonseca & Prentice, 2015). Here the moisture was reduced to 5.61% (Table 1) due to the 16 h drying period at 45 °C, after the protein isolation. It was reported elsewhere moisture contents of 80.9% for Whitemouth croaker and 74.7% for anchovy (*Engraulis anchoita*) when utilized the alkaline solubilization process for protein recovery (Freitas et al., 2015). Moreover, a low moisture content is favorable to the process for obtaining isolate-based products, since the resulting gel will be of higher quality and greater strength (Rawdkuen, Sai-Ut, Khamsorn, Chaijan & Benjakul, 2009, Cavenaghi-Altemio et al., 2013).

Solubility

Figure 1 depicts the solubility of the hybrid sorubim protein isolate as a function of pH. Solubility was found to be low at the pH near the isoelectric point of the proteins (29.47%), while the highest solubility (88.5%) was attained at an extreme pH of 11.0. Fontana et al. (2009) found higher values than those of this study in protein concentrates of Whitemouth croaker byproducts, but proteins also showed low solubility near the isoelectric point (32.5% pH 5.0) and high protein solubilization at extreme pH (97.5% at pH 11.0). In a study of proteins recovered from fish, Freitas et al. (2016) also obtained low solubility near the isoelectric point and high solubility at pH 11.0.

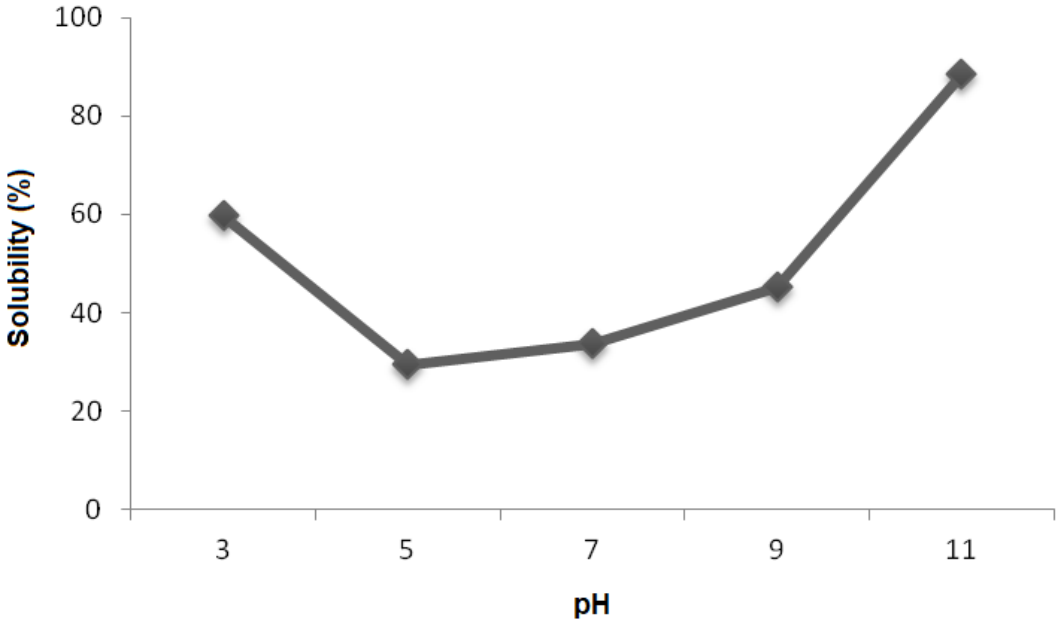

Figure 1. Solubility of the hybrid sorubim protein isolate (FPI) as a function of pH

Low protein solubility is caused by protein denaturation, which in turn is induced by changes in pH levels (Rawdkuen et al., 2009). The lowest solubility of proteins usually occurs at their isoelectric point, where protein-protein interactions increase because the electrostatic forces of the molecules are minimal, and less water interacts with protein molecules (Freitas et al., 2015). High protein solubility, on the other hand, occurs because, as the pH becomes more alkaline, there is a predominance of negative charges, with stronger interactions between protein molecules and water, and also stronger repulsive forces between protein molecules, significantly increasing their solubility (Fontana et al., 2009).

Solubility, which is one of the most important properties of proteins, varies considerably as a function of pH and ionic strength (Brasileiro, Cavalheiro, Prado, Anjos & Cavalheiri, 2012). Similar results have been reported for other fish species such as sardines, catfish and Whitemouth croaker (Fontana et al., 2009). The high solubility of recovered protein indicates its potential application in formulated food, giving the prepared product an attractive appearance and a pleasant mouthfeel (Sathivel, Bechtel, Babbitt, Nesgulescu & Reppond, 2004).

Water holding capacity

Figure 2 shows the water holding capacity (WHC) curves as a function of the pH of the FPI. WHC and solubility showed the same protein profile, *i.e.*, WHC increased at pH 3.0 (10.8 mL), decreased at pH 5.0 (4.45 mL) and then increased again at pH 7.0 (6.37 mL) and 9.0 (12.33 mL), with the highest value (20.11 mL) obtained at pH 11.0.

As expected, the WHC measured at pH 5.0 was lower than that obtained at the other pH levels. This was explained by Kinsella (1987), who stated that the ability of protein to bind with water decreases due to intermolecular interactions and the formation of large protein clusters, and may also be affected by other properties, such as solubility.

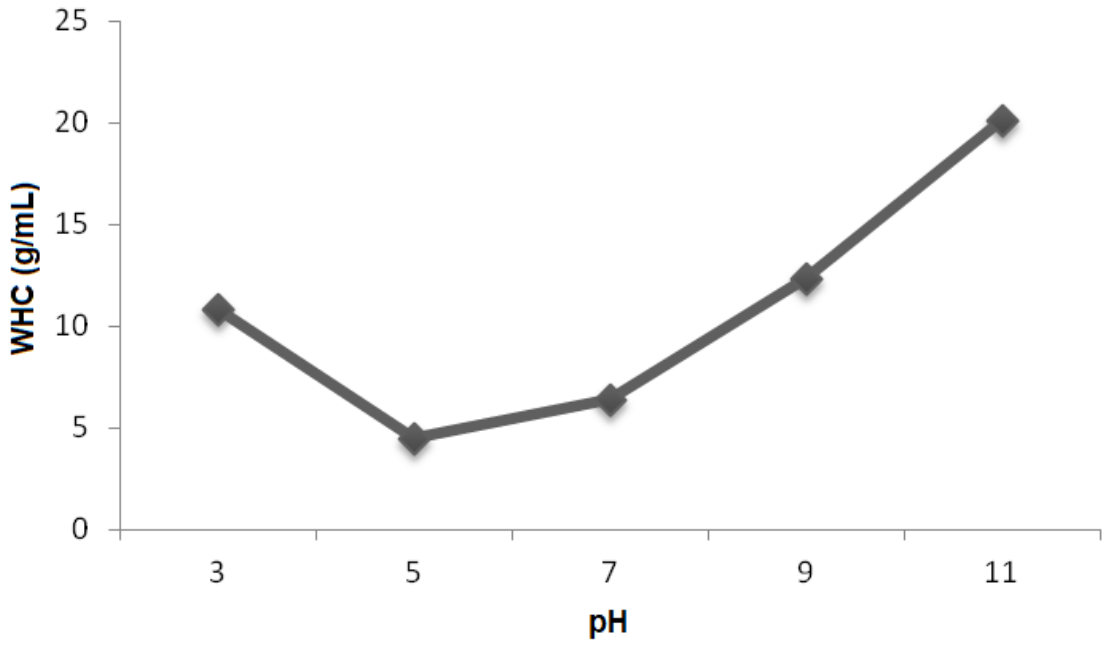

Figure 2. *Water holding capacity (WHC) of the hybrid sorubim protein isolate (FPI)*

At pH 11.0, Fontana et al. (2009) reported maximum values of 21.9 and 22.9 mL of water per gram of protein for Whitemouth croaker protein isolates obtained, respectively, by acid and alkaline solubilization. The values they reported are similar to those found in this study.

At pH levels far from the isoelectric point of proteins, the predominance of charges with the same signal causes repulsive forces to separate protein molecules, leaving more space to be filled by water molecules, thus increasing the WHC. At pH levels below 5.0 and above 7.0, water molecules combine with the polar groups of proteins and the WHC tends to increase (Menezes, Zanette, Souza, Cortez-Vega & Prentice, 2015).

Water holding capacity is an important phenomenon of food, given that small amounts of absorbed water do not act as a solvent, but contribute to increase viscosity (Cândido, Nogueira & Sgarbieri, 1998).

Oil holding capacity

The average oil holding capacity (OHC) was 2.03±0.06 (mL oil/g protein), which was lower than the OHC of 13.17±0.11 (mL oil/g protein) in Whitemouth croaker MSM reported by Ferreira, Freire, Souza, Cortez-Vega, and Prentice, (2013). Freitas et al. (2015), who evaluated the OHC of protein isolate obtained from byproducts of Argentine anchovy, reported a value of 7.27±0.64 mL oil/g protein, which was also higher than the OHC found in this study.

The low OHC found in this study indicates low hydrophobicity of the protein isolate, considering that large numbers of hydrophobic regions in proteins favor their interactions with oil. According to Fontana et al. (2009), OHC varies as a function of

the number of hydrophobic groups exposed to proteins, and the nonpolar chains of proteins probably have an affinity with the hydrophobic chains of oil molecules, contributing to oil absorption.

The oil holding mechanism is due mainly to the physical capture of oil by the protein and is a very important functional characteristic, required mainly by the industry of meat and emulsified products (Brasileiro et al., 2012).

Fat deserves attention due to its varying content, which is directly reflected in the stability of the emulsion and in oxidative processes. It is therefore important to know the product's OHC (Menezes et al., 2015).

CONCLUSIONS

The process employed to obtain fish protein isolate from hybrid sorubim byproducts can be considered efficient, given that, compared to the raw material, the end product contained a high concentration of proteins and low moisture and lipid contents, which may represent interfering factors in the process of conservation and use of the isolate. As for functional properties, the FPI presented high water holding capacity and high solubility. These characteristics suggest that fish protein isolate from hybrid sorubim can potentially be used to produce high added-value products and also to develop edible films and coatings, contributing to reduce the environmental impacts of the fish processing industry and providing a viable and low cost alternative.

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AUTHOR CONTRIBUITION

Rosiane de Souza Silva – Carried out the analyzes of this study and elaborated the scientific article

Barbara Matias Moreira dos Santos - Performed the analyzes of this study

Sandriane Pizato - Carried out the analyzes of this study and elaborated the scientific article

Gustavo Graciano Fonseca – Co-Adviser professor of this work and supported the preparation of the article

William Renzo Cortez-Vega - Adviser professor of this work and elaborated the scientific article

CONFLICT OF INTEREST

The authors declare that there is not conflict of interest.

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