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Original research article

Valorization of pulp and viscera from hybrid sorubim and pacu by production of protein concentrate using alkaline solubilization

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ABSTRACT

Fish protein concentration (FPC) by alkaline solubilization has proven to be an alternative method in adding value to muscle-based materials. The aim of this work was to evaluate the influence of different processes (decanting/sieving or centrifugation), reagents (sodium bicarbonate or sodium hydroxide) and washing cycles (1–4) on the quality of protein concentrates obtained from mechanically separated meat (MSM) and viscera of hybrid sorubim (*Pseudoplatystoma reticulatum* × *P. corruscans*) and pacu (*Piaractus mesopotamicus*) fishes. The protein concentrate was obtained by washing the viscera or MSM during up to four cycles (4:1 solution:meat ratio). In each cycle, the liquid waste and fat were removed by decanting/sieving or centrifugation. Moisture, crude protein, crude fat and crude ash contents were determined after each washing cycle. The moisture and protein contents increased immediately after the first washing up to 77.88% (dry basis). The lipid content decreased with the cycles down to 2.99% (dry basis). Higher FPC yield was obtained for MSM during the centrifugation process compared to the decantation/sieving process. Two weight yields above 100% were found in procedures with $NaHCO_3$ and MSM of hybrid sorubim and pacu, probably due to the larger capacity of gel formation during protein concentration. Differences related to the fish species were more evident than the processes and utilized reagents.

1. Introduction

Fish farming in Brazil is considered more recent compared to other livestock activities. Among the main species produced and industrialized in the Brazilian state of Mato Grosso do Sul are the hybrid sorubim (*Pseudoplatystoma reticulatum* × *P. corruscans*) and pacu (*Piaractus mesopotamicus*). The hybrid sorubim is often found in the Paraguay-Uruguay Rivers Basin and San Francisco River Basin. It presents great acceptance due to the excellent palatability and the absence of scales and intramuscular thorns in its flesh (Gonçalves & Carneiro, 2003). The pacu is a high commercial value native species that stands out due its easy reproduction and management in a captive environment (Miyazaki & Pitelli, 2003). The ease of obtaining larvae through induced breeding and good adaptation to artificial feeding contributed to make this species the second most cultivated native fish in Brazil (Povh et al., 2009).

The amount of discard from fish processing can range from 20% to 70% of the total weight of fish, depending on the specific fishery and processing methods involved (Ozogul et al., 2021). These residues could be a natural resource of valuable components, such as proteins and proteases. The main by-products derived from fish processing include carcasses, viscera, skin, bones and scales (Bhaskar & Mahendrakar, 2007).

The mechanically separated meat (MSM) of fish is a flesh pulp with pasty and uniform texture obtained from eviscerated and cleaned fish carcasses mechanically separated by means of a machine that removes fish bones from meat. The MSM of fish may serve as raw material for the manufacture of sausages, nuggets, burgers, pasta, among others

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(Cavenaghi, Hashinokuti, Albuquerque, & Fonseca, 2018).

Differently of the MSM of fish, the fish protein concentrate (FPC) is an odorless white paste that combines the myofibrillar proteins set of flesh raw material, obtained from successive washings of fish carcasses or MSM of fish, which remove blood, pigments, sarcoplasmic proteins, soluble components, lipids and other substances which can catalyze protein and lipid degradation (Alcalde, Cavenaghi, & Fonseca, 2017). In contrast to surimi, the flesh doesn't receive the addition of cryoprotector agents.

The most effective method of eliminating fat and increasing the gel strength and protein content is utilizing alkaline solutions in the washing of pulp (Benjakul, Visessanguan, & Kwalumtharn, 2004) due to the solubilization of muscle sarcoplasmic proteins which facilitates the removal of the heme pigments and improves the flavor by removal of the carboxyl compounds, including carboxyl groups, carboxylic acid side chains, and carboxylate anions (Cortez-Vega, Fonseca, & Prentice, 2012). Due to these relevant characteristics that help to maintain the quality of the fish protein against lipid oxidation and the shelf-life of the derived food products, the FPC is a good alternative to the MSM (Rebouças, Rodrigues, de Castro, & Vieira, 2012) and the residues (Hisano et al., 2013) from the fish processing.

The aim of this work was to evaluate the influence of different processes (decanting/sieving or centrifugation), reagents (sodium bicarbonate or sodium hydroxide) and washing cycles (1, 2, 3 and 4) on the quality of protein concentrates obtained from MSM and viscera of hybrid sorubim and pacu.

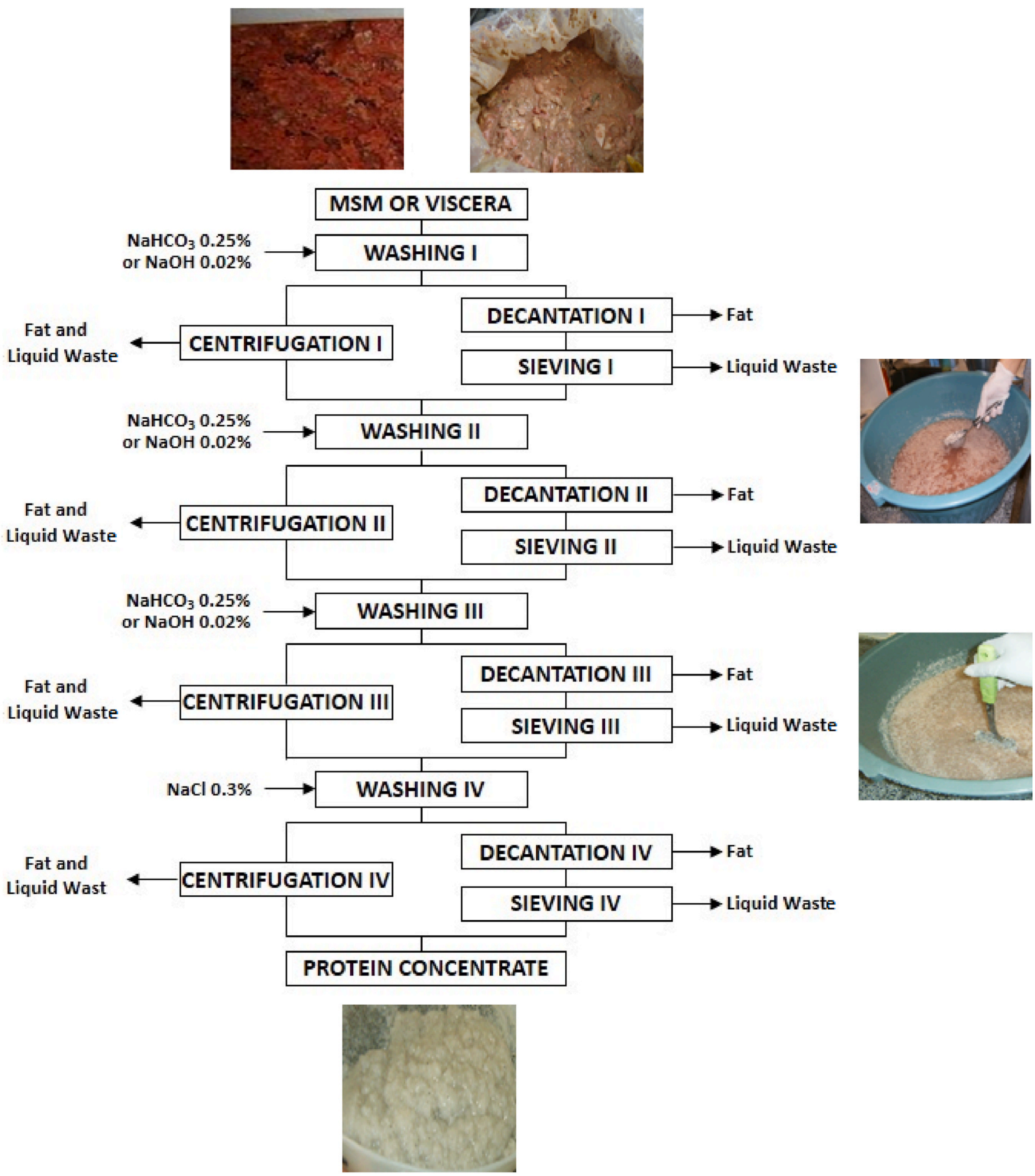

Fig. 1. Processing flowchart for the obtaining of protein concentrated (adapted from Alcalde, Cavenaghi-Altemio, & Fonseca, 2017).

2. Material and methods

2.1. Raw materials and reagents

MSM and viscera were supplied from a local fish processing plant (Mar & Terra S.A., Itaporã, Brazil) and transported under refrigerated conditions to our laboratory. The MSM was produced in 3 mm particle size using a High-Tech separator (model HT 250, Chapecó, Brazil), operating at inlet 6 °C and outlet 10 °C, from hybrid sorubim (*Pseudoplatystoma reticulatum* × *P. corruscans*) and pacu (*Piaractus mesopotamicus*) eviscerated and headed fish carcasses, 24 h after the slaughtering. Part of the raw materials was immediately utilized to obtain the FPC while another part was packaged in five-layer nylon propylene bags, and stored at −18 °C for up to 1 month for the proximate composition analysis. The sampling of the raw materials in this study was obtained ethical approval from the relevent institutional animal care and use committee (IACUC) or equivalent body.

Ranges of sodium bicarbonate (0.05%–2.0%) and sodium hydroxide (0.02%–0.5%) were evaluated, based on the pH, to define the best extraction conditions and save reagent during the washing cycles.

2.2. Protein concentrate

MSM or viscera was washed in four cycles utilizing in each cycle a washing solution:meat (viscera or MSM) ratio of 4:1 (v/w), at 7 °C as described elsewhere (Alcalde, Cavenaghi, & Fonseca, 2017) (Fig. 1). In each washing cycle, the stirring was done manually (process 1) or mechanically (process 2). 0.25% $NaHCO_3$ or 0.02% NaOH was utilized in the first, second and third washings and 0.3% NaCl solution in the last one. After each washing cycle, samples were decanted and sieved (process 1) or centrifuged (process 2).a

In process 1, samples were homogenized manually for 5 min and decanted for 10 min. The supernatant containing fat and water-soluble proteins was discarded. The final slurry was then sieved through a 1 mm plastic sieve. In process 2, samples were mechanically homogenized (500 r/min) for 5 min at 10 °C using a mechanical agitator (Fisatom model 715, São Paulo, Brazil), and centrifuged at 3400 × *g* for 15 min at 4 °C (Biovera model RB7-R, Rio de Janeiro, Brazil). The supernatant containing fat and water-soluble proteins were discarded.

The designed procedures (raw material, fish species, process for protein concentration, and washing reagent) are shown in Table 1 and detailed in Fig. 1. The masses of the samples before and after each washing cycle were recorded for weight yield calculation.

2.3. Proximate composition

Moisture, crude protein, crude fat and crude ash contents were determined in triplicate according to the methods defined by AOAC (2000). Moisture was determined by the oven drying method at 105 °C until constant weight (method 950.46), protein (N × 6.25) by the Kjeldhal method (method 928.08), fat by the Soxhlet extraction method (method 960.39) and ash by using the muffle oven technique (method 920.153). Values of protein were utilized to calculate protein yield and protein recovery.

2.4. Weight and protein yields, protein recovery, crude fat extraction and pH

Protein concentrate weight yield of all procedures was calculated from the percentage of protein concentrate obtained from the raw material used (Alcalde, Cavenaghi, & Fonseca, 2017) (Eq. (1)).

$$\text{Weight yield } (\%) = \frac{\textit{protein concentrate weight}}{\textit{viscera or MSM weight}}\ x\ 100 \quad (1)$$

Protein yield of the procedures with a minimum protein concentrate weight yield of 30% was calculated from the percentage of final protein content obtained from the initial protein content of the raw material used (Eq. (2)).

$$\text{Protein yield } (\%) = (\textit{final protein} - \textit{initial protein})\ x\ 100 \quad \text{Eq. 2}$$

Protein recovery was calculated per cycle from the protein content before and after the washing cycles (WC) (Eq. (3)) and the protein recovery obtained from the difference between the protein content at determined WC and the protein content of the raw material (cycle 0) (Eq. (4)).

$$\text{Protein recovery per cycle } (\%) = \textit{protein}_{WC} - \textit{protein}_{WC-1} \quad \text{Eq. 3}$$

$$\text{Protein recovery after cycle } (\%) = \textit{protein}_{WC} - \textit{protein}_{raw\ material} \quad \text{Eq. 4}$$

Crude fat extraction was calculated according to Eq. (5).

$$\text{Crude fat extraction } (\%) = \frac{\textit{crude fat weight}}{\textit{viscera or MSM crude fat weight}}\ x\ 100 \quad \text{Eq. 5}$$

pH was measured from the washing solution using a digital pH meter (Hanna pH21, São Paulo, Brazil). For that, different masses of the reagents used ($NaHCO_3$ or NaOH) were individually weighed and dissolved in 50 mL of water to determine the pH reached by these reagents at different concentrations (Alcalde, Cavenaghi, & Fonseca, 2017).

2.5. Statistical analysis

The *Statistica® 5.5* (*Stasoft*, USA) program was used to calculate the analysis of variance (ANOVA). The Tukey test was used to determine the differences between the samples in the range of 90% confidence ($P < 0.1$).

Table 1
Design and nomenclature of the procedures carried out and the obtained yields.

Procedure	Raw material	Fish species	Process	Reagent	Weight yield (%)
A[a]	MSM	hybrid sorubim	centrifugation	$NaHCO_3$	101.06 ± 0.34
B[a]	MSM	hybrid sorubim	decanting/sieving	$NaHCO_3$	33.03 ± 2.23
C	MSM	hybrid sorubim	centrifugation	NaOH	21.45 ± 1.34
D	MSM	hybrid sorubim	decanting/sieving	NaOH	2.98 ± 0.75
E[a]	MSM	pacu	centrifugation	$NaHCO_3$	116.86 ± 2.48
F[a]	MSM	pacu	decanting/sieving	$NaHCO_3$	66.94 ± 0.06
G[a]	MSM	pacu	centrifugation	NaOH	69.88 ± 1.48
H[a]	MSM	pacu	decanting/sieving	NaOH	44.29 ± 0.97
I[a]	viscera	hybrid sorubim	centrifugation	$NaHCO_3$	47.54 ± 1.50
J[a]	viscera	hybrid sorubim	decanting/sieving	$NaHCO_3$	45.73 ± 0.24
K[a]	viscera	hybrid sorubim	centrifugation	NaOH	37.42 ± 2.81
L[a]	viscera	hybrid sorubim	decanting/sieving	NaOH	49.04 ± 0.52
M	víscera	pacu	centrifugation	$NaHCO_3$	26.02 ± 0.19
N[a]	viscera	pacu	decanting/sieving	$NaHCO_3$	30.26 ± 1.25
O	viscera	pacu	centrifugation	NaOH	20.88 ± 0.33
P	viscera	pacu	decanting/sieving	NaOH	28.38 ± 0.64

[a] Procedures whose yields (above 30%) were considered for further investigations. The analyses were performed in triplicate and the results presented as mean and standard deviation. MSM: mechanically separated meat.

Table 2
Proximate composition of the MSM and viscera from hybrid sorubim and pacu.

Parameter	MSM(HS)	MSM(P)	Vis(HS)	Vis(P)
Moisture (%)	74.54 ± 0.74	76.26 ± 0.17	72.08 ± 0.09	52.83 ± 4.81
Protein (%)*	46.26 ± 1.18	59.10 ± 0.64	39.82 ± 2.69	4.82 ± 1.51
Lipid (%)*	41.23 ± 0.36	31.54 ± 1.34	51.34 ± 0.79	78.90 ± 1.79
Ash (%)*	2.38 ± 0.19	5.06 ± 0.20	3.51 ± 0.02	1.70 ± 0.02

MSM: mechanically separated meat; Vis: viscera; HS: hybrid sorubim; P pacu. The analyses were performed in triplicate and the results presented as mean and standard deviation. *Dry basis.

3. Results

The proximate composition of the MSM and the viscera from hybrid sorubim and pacu are shown in Table 2.

Different concentrations of sodium bicarbonate and sodium hydroxide were evaluated in order to define the best extraction conditions. The optimum pH of 9.02 was achieved for the sodium bicarbonate at 0.25% and of 12.9 for the sodium hydroxide 0.5%. It was observed that with sodium bicarbonate the pH did not drastically vary in the range of 0.05%–2.0% (Fig. 2).

The weight yields of the different procedures used for obtaining protein are shown in Table 1. To be considered feasible and receive a more detailed analysis, a minimum yield of 30% was stablished, which was achieved in the procedures A, B, E, F, G, H, I, J, K, L and N. In analyzing viscera as raw material, it was first observed that for the pacu species (procedures M, N, O and P), yields were below 30%, except for procedure N (decanting/sieving, $NaHCO_3$) that was 30.26%.

The protein yields ranged from 116.8% (procedure F: pacu MSM, decanting/sieving, $NaHCO_3$) to 998.4% (procedure N: pacu viscera, decanting/sieving, $NaHCO_3$). Procedures did not differ between themselves ($P > 0.1$), except for the procedure N that presented a protein yield six times higher than the average of the other procedures (Fig. 3).

The proximate composition after each washing to the selected procedures with MSM (A, B, E, F, G, H) and viscera (I, J, K, L, N) are shown in Tables 3 and 4, respectively.

The moisture contents in all the procedures reached higher values than initial samples (Table 2), between 80% and 95% in processing MSM (Table 3) and between 58% and 95% with viscera (Table 4). As observed in the procedures, the moisture content significantly increased ($P < 0.1$) from the first washing (between 58.17% and 88.12%) to the fourth washing (between 84.30% and 94.76%).

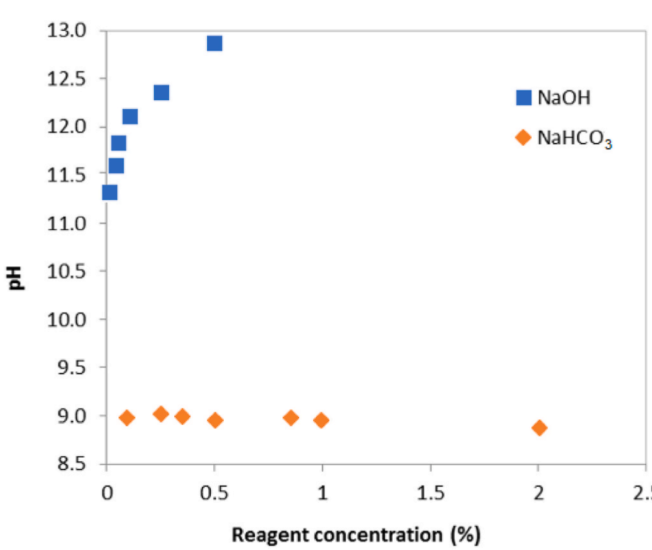

Fig. 2. Evaluation of different concentrations of sodium bicarbonate and sodium hydroxide to define the best conditions for protein extraction.

All procedures showed an increase in the protein content from the raw material just after the first washing (Tables 2–4). For the MSM of hybrid sorubim the content increased from 46.26% to 77.88% (dry basis) in procedure A and to 70.23% (dry basis) in the procedure B. For MSM of pacu the content increased from 59.10% to 64.84%, 60.81%, 70.09% and 66.03% in the procedures E, F, G and H, respectively. Similarly to the hybrid sorubim viscera, the protein content increased from 39.82% in the raw material to 69.16%, 74.75%, 73.74% and 67.69% (dry basis) from the procedures I, J, K and L, respectively. For pacu viscera the content significantly increased ($P < 0.1$) from 4.82% to 13.75% (dry basis) in the procedure N.

Protein recovery was statistically higher ($P > 0.1$) for the first washing cycle of procedures A, B, I, J, K and L (Fig. 4A). If considered the protein recovery by comparing the protein obtained after a determined washing cycle and the protein from the corresponding raw material, then procedures I and K were the ones the achieved the maximal protein recovery, both after the third washing cycle, with 46.5 and 48.9%, respectively (Fig. 4B).

After each washing cycle lipids were reduced or remained constant (without significant difference, $P > 0.1$) (Tables 3 and 4). A wide variation of final lipid contents was found in this work ranging from 2.99% to 28.72% (dry basis). Among the raw materials studied, the lowest lipid contents were found in MSM (31.54% for MSM of pacu and 41.23% for MSM of hybrid sorubim; dry basis) (Table 2). On the other hand, the highest values were found in viscera (78.90% for pacu viscera and 51.34% for hybrid sorubim viscera; dry basis) (Table 2).

Regarding the percentage of crude fat extracted (dry basis), best results were obtained for hybrid sorubim viscera (81.3%–94.2%), followed by MSM of hybrid sorubim (80.8%–85.5%), pacu viscera (63.6%, procedure N), and finally, the MSM of pacu (40.9%–56.4%). With the procedure J (hybrid sorubim viscera, decanting/sieving, $NaHCO_3$) it was achieved the highest percentage of crude fat extraction (94.2%) and also the lowest final crude fat content in the protein concentrate (2.99%).

The ash content in most of the procedures significantly increased ($P < 0.1$) from the first to the last washing, varying in the range of 1.81%–8.45% (dry basis) (Table 4).

4. Discussion

4.1. Raw materials

The values for MSM of hybrid sorubim obtained here are above those found elsewhere for mandi-pintado *Pimelodus britskii* fillets, with 68.71% moisture, 21.05% protein, 14.09% lipids and 1.13% ash, in dry basis (excluding moisture) (Veit et al., 2011), and MSM of Nile tilapia *Oreochromis niloticus* (except for moisture, which was higher - 77.24%), with 21.18% protein, 4.67% lipids and 1.03%, in dry basis (Rebouças et al., 2012). It was reported elsewhere 69.48% moisture, 20.51% protein, 12.17% lipids, and 2.88% ash for pacu fillets (Cortez Netto et al., 2010), which are lower values in comparison to those obtained here for the MSM of pacu.

Regarding viscera proximate composition, examples of moisture, proteins, lipids and ash contents, respectively, reported in the literature are: 58.0%, 6.8%, 35.5% and 3.0% for carp viscera (Bhaskar & Mahendrakar, 2007); 77.46%, 15.76%, 4.89% and 1.90% for sardine viscera (Kechaou et al., 2009); and 36.80%, 20.20%, 49.20% and 2.73% for cachama blanca *Piaractus brachyponum* viscera (Bermudez, Rodriguez, Ocampo, & Peñuela, 1999).

It can be observed from the literature and the results obtained here that the protein and lipid contents are inversely proportional, and among the species studied, the hybrid sorubim viscera had the highest protein content. The low value of protein obtained for pacu is due to the accumulation of visceral fat by this species, impairing the protein concentrate obtaining. Studies conducted by Signor et al. (2010) with pacu grewn in net pounds, fed with different levels of protein and energy, showed differences in the percentage of visceral fat among the

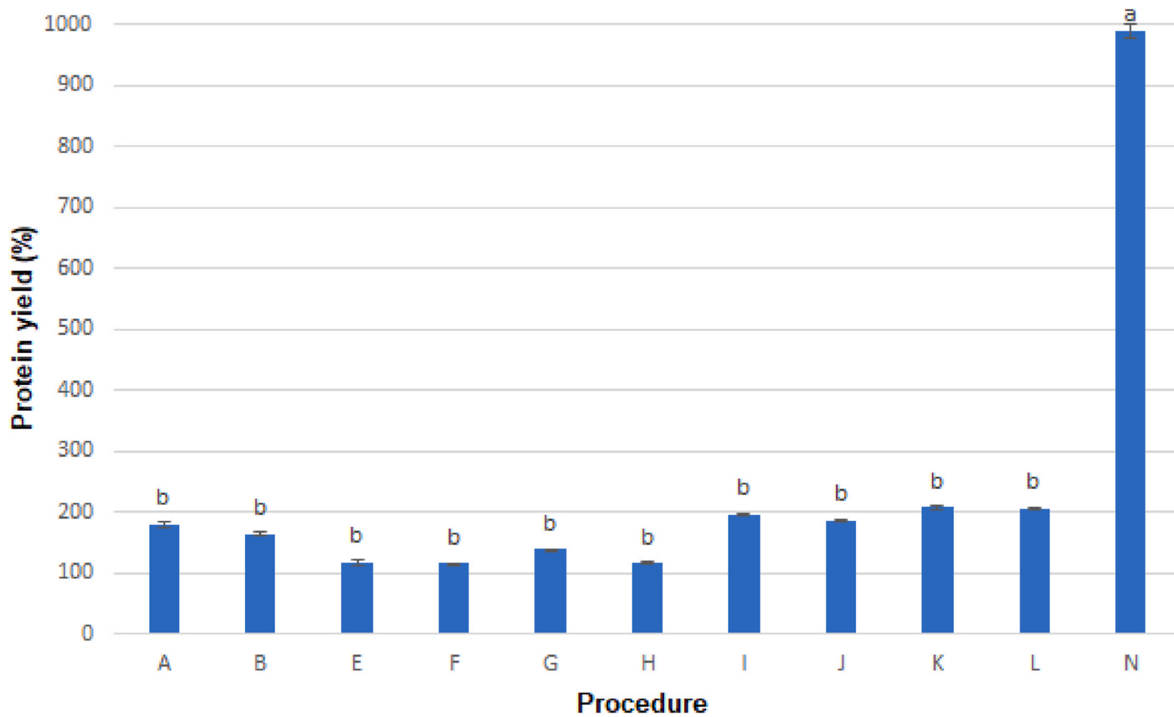

Fig. 3. Protein yield for different procedures according to Table 1.

energetic levels evaluated.

4.2. Reagents and concentrations

The use of reagents for the pH regulation increases the yield of the obtained protein concentrate, in comparison with the traditional process that uses only water to wash the raw material (Nolsøe, Imer, & Hultin, 2007). The pH 9.02 was very similar to the pH 8.96 observed with sodium bicarbonate at 0.5%, which is mostly reported in the literature (Cortez-Vega et al., 2012). This concentration was sufficient to achieve the desired pH for the extraction of sarcoplasmic protein and so was utilized instead of 0.5% to save reagent during washing cycles. In its turn, sodium hydroxide showed itself extremely alkaline, given that at the concentration of 0.02%, the pH already was 11.33.

High solubility and low viscosity are prerequisites for good extraction of muscle proteins and their separation of undesirable components (Undeland, Kelleher, & Hultin, 2002). The protein solubility follows a curve in the form of “U", typical of homogenized of muscle proteins (Choi & Park, 2002), where the solubility of the protein is significantly high below pH 5 and above pH 10 but remains low between pH 5 and 10. This increased solubility at extreme pH values was attributed to increased positive and negative charges of the muscle proteins at low and high pH, respectively, leading to electrostatic repulsion between proteins (Tian et al., 2017). As evidence, Kristinsson, Theodore, Demir, and Ingadottir (2005) found higher solubility in American catfish proteins at pH lower than 2.5 and greater than 11.

It was previously reported the use of $NaHCO_3$ solution (for the first and second washings and 0.3% NaCl solution for the last one) to concentrate protein from whitemouth croakers and mechanically separated chicken meat (Cortez-Vega, Fonseca, Feisther, Flôres, & Prentice, 2013), and NaOH reagent to adjust the washing solution to pH 11 and concentrate fish (Jung et al., 2004) and chicken (Jin, Kim, Choi, Park, & Yang, 2008) meat proteins. Likewise, NaOH solution (pH 10.8) was utilized for alkaline solubilization for protein concentration of fresh fillets of cod, haddock, pollock and whole mackerel (Nolsøe et al., 2007).

4.3. Weight and protein yields

Some authors found weight yield values in the range 10%–45% during fish processing for protein concentration, *e.g.* 23.12% (Peixoto, Sousa, & Mota, 2000), 13.6% and 44.75% (Mello et al., 2010) and 25% (Barreto & Beirão, 1999). Due to the high lipid content, we concluded that pacu viscera as raw material is inadequate independently of type of process or reagent.

The weight yield after the washing cycles can be influenced by the efficiency of water removal, the number of washes and type of equipment utilized (Kirschnik & Macedo-Viegas, 2009). All the procedures with MSM as raw material presented yield with centrifugation process higher than with the decantation/sieving process, when compared each pair of procedures with the same fish species and reagent, differing only the kind of process, *e.g.* the pairs A/B, C/D, E/F and G/H. Two weight yields above 100% were found in procedures A and E, with $NaHCO_3$, and MSM of (A) hybrid sorubim and (E) pacu, probably due to the larger capacity of gel formation during protein concentration, which causes water retaining and thus increases the mass. Moreover, no process was utilized for water removal.

On the other hand, with viscera as raw material the opposite behavior was observed except for the pair I/J (with $NaHCO_3$ and hybrid sorubim). These results are comparable with others reported elsewhere for cod and haddock, where the yields from sieving process, 90% and 71%, respectively, were higher than those from centrifugation process (Nolsøe et al., 2007).

A strong relationship between the protein yield and the raw material protein content was observed (MSM pacu > MSM hybrid sorubim > hybrid sorubim viscera > pacu viscera) (Fig. 3; Table 2). The same behavior was not observed neither for the weight and the protein yields nor the weight yield and the raw material protein content. It means that a heigh weight yield does not necessarily reflects into a high recovery of proteins and/or protein yield. However, the weight yield is still important because it shows the gelling capacity of the recovered proteins, which is intimately related to the quality of the protein and the potential applications. Thus, the formation of the 3D matrix may be impaired mainly due to the presence of sarcoplasmic protein that do not

Table 3
Proximate composition of the procedures A, B, E, F, G and H carried out with mechanically separated meat from hybrid sorubim and pacu.

Proc.	Washing cycle	Moisture (%)	Protein (%)*	Lipid (%)*	Ash (%)*
A	1	86.34 ± 0.79[a]	77.88 ± 2.04[a]	13.73 ± 1.39[a]	3.54 ± 1.76[a]
	2	90.04 ± 3.27[ab]	83.87 ± 2.18[b]	7.39 ± 2.97[bc]	3.72 ± 0.21[a]
	3	91.87 ± 0.04[b]	81.62 ± 0.33[ab]	9.82 ± 0.01[b]	5.03 ± 1.53[a]
	4	91.36 ± 0.11[b]	83.06 ± 2.65[b]	5.96 ± 0.65[c]	5.82 ± 0.98[a]
B	1	88.12 ± 0.56[a]	70.23 ± 0.60[a]	20.37 ± 2.14[a]	4.63 ± 0.56[a]
	2	92.52 ± 0.08[b]	74.81 ± 1.07[b]	12.01 ± 2.21[b]	5.57 ± 0.11[b]
	3	94.25 ± 1.23[c]	76.81 ± 1.97[b]	9.79 ± 0.33[bc]	5.95 ± 0.03[bc]
	4	92.33 ± 0.37[b]	76.20 ± 1.16[b]	7.91 ± 0.09[c]	6.60 ± 0.20[c]
E	1	81.49 ± 0.01[a]	64.84 ± 3.00[a]	23.07 ± 0.61[a]	2.76 ± 0.06[a]
	2	87.11 ± 0.82[b]	70.48 ± 1.74[b]	17.77 ± 0.46[b]	2.71 ± 0.21[a]
	3	89.50 ± 0.21[c]	70.63 ± 1.09[b]	17.03 ± 0.24[b]	3.17 ± 0.01[b]
	4	89.91 ± 0.08[c]	69.27 ± 2.69[ab]	16.49 ± 0.04[b]	4.38 ± 0.07[c]
F	1	83.90 ± 0.56[a]	60.81 ± 1.44[a]	29.03 ± 0.15[a]	3.33 ± 0.11[ab]
	2	85.24 ± 1.63[a]	61.59 ± 1.68[a]	28.97 ± 0.23[a]	3.26 ± 0.29[a]
	3	88.73 ± 0.06[b]	66.02 ± 0.02[b]	25.42 ± 0.17[b]	3.72 ± 0.20[b]
	4	84.07 ± 0.78[a]	68.68 ± 0.59[c]	18.65 ± 0.45[c]	4.24 ± 0.16[c]
G	1	84.05 ± 0.29[a]	70.09 ± 1.38[a]	24.49 ± 1.39[a]	2.89 ± 0.72[a]
	2	93.86 ± 0.29[bc]	73.62 ± 4.49[ab]	13.34 ± 0.79[b]	3.15 ± 0.18[ab]
	3	94.90 ± 0.73[b]	74.42 ± 1.05[b]	13.10 ± 1.12[b]	3.78 ± 0.30[ab]
	4	92.86 ± 1.56[c]	82.53 ± 0.20[c]	13.76 ± 0.42[b]	4.56 ± 1.12[b]
H	1	82.07 ± 1.11[a]	66.03 ± 0.30[a]	25.43 ± 0.13[a]	2.51 ± 0.08[a]
	2	91.60 ± 0.18[b]	66.88 ± 0.69[ab]	23.60 ± 0.12[b]	2.13 ± 0.11[b]
	3	92.05 ± 0.17[b]	68.00 ± 0.69[bc]	23.60 ± 0.53[b]	2.25 ± 0.19[b]
	4	85.81 ± 1.24[c]	69.05 ± 0.59[c]	17.27 ± 0.35[c]	5.13 ± 0.02[c]

Proc.: procedure. The analyses were performed in triplicate and the results presented as mean and standard deviation. Different letters within the same column (and block) indicate significant differences ($P < 0.1$). *Dry basis. A, B, E, F, G and H according to Table 1.

Table 4
Proximate composition of the procedures I, J, K, L, and N carried out with viscera from hybrid sorubim and pacu.

Proc.	Washing cycle	Moisture (%)	Protein (%)*	Lipid (%)*	Ash (%)*
I	1	81.79 ± 0.60[a]	69.16 ± 1.46[a]	23.72 ± 1.00[a]	2.22 ± 0.02[a]
	2	85.43 ± 0.14[b]	76.42 ± 0.87[b]	17.64 ± 0.64[b]	2.55 ± 0.01[b]
	3	85.23 ± 0.46[bc]	86.29 ± 0.66[c]	9.17 ± 0.59[c]	2.57 ± 0.07[b]
	4	84.30 ± 0.40[c]	78.05 ± 0.51[b]	9.60 ± 0.03[c]	2.89 ± 0.08[c]
J	1	85.36 ± 0.02[a]	74.75 ± 1.16[a]	12.46 ± 0.14[a]	4.21 ± 0.08[a]
	2	90.46 ± 0.42[b]	80.68 ± 2.70[b]	6.33 ± 0.40[b]	4.26 ± 0.08[a]
	3	92.47 ± 0.24[c]	80.51 ± 0.79[b]	6.18 ± 0.56[b]	3.86 ± 0.01[b]
	4	92.93 ± 1.17[c]	74.26 ± 0.71[a]	2.99 ± 0.85[c]	5.37 ± 0.22[c]
K	1	78.49 ± 0.22[a]	73.74 ± 1.08[a]	20.82 ± 0.91[a]	1.81 ± 0.04[a]
	2	85.50 ± 0.90[b]	80.85 ± 0.68[b]	12.77 ± 0.01[b]	1.79 ± 0.08[a]
	3	93.31 ± 0.76[c]	88.70 ± 1.36[c]	9.39 ± 0.62[c]	1.69 ± 0.01[a]
	4	87.88 ± 0.59[d]	82.62 ± 1.09[b]	6.76 ± 0.28[d]	2.90 ± 0.05[b]
L	1	85.98 ± 0.66[a]	67.69 ± 1.91[a]	23.23 ± 1.37[a]	3.59 ± 0.07[a]
	2	93.31 ± 0.22[b]	77.57 ± 0.40[b]	14.39 ± 0.66[b]	2.83 ± 0.01[ab]
	3	95.86 ± 0.14[c]	78.93 ± 0.46[b]	12.28 ± 0.28[c]	2.65 ± 0.08[b]
	4	94.76 ± 0.33[d]	82.44 ± 0.49[c]	7.26 ± 0.95[d]	5.79 ± 0.75[c]
N	1	58.17 ± 1.54[a]	13.75 ± 1.08[a]	67.11 ± 0.42[a]	5.76 ± 0.26[a]
	2	69.91 ± 0.48[b]	21.20 ± 1.08[b]	48.05 ± 0.64[b]	4.92 ± 0.16[b]
	3	80.98 ± 0.35[c]	31.86 ± 2.76[c]	41.49 ± 0.60[c]	6.13 ± 0.17[a]
	4	85.60 ± 1.06[d]	47.64 ± 4.21[d]	28.72 ± 0.23[d]	8.45 ± 0.01[c]

Proc.: procedure. The analyses were performed in triplicate and the results presented as mean and standard deviation. Different letters within the same column (and block) indicate significant differences ($P < 0.1$). *Dry basis. I, J, K, L, and N according to Table 1.

contribute much to the gel formation.

4.4. Effects of process and on the number of washings on the product composition

4.4.1. Moisture

This moisture increase was expected, considering that, although the washings improve concentrate protein quality, this method also intensifies the swelling of the muscle and the difficulty of eliminating the water excess (Sebben, Beirão, Meinert, Teixeira, & Damian, 2000). The procedures carried out with viscera showed a greater range of moisture by consequence of the difference between the moisture of fresh viscera, 52.83% for viscera from pacu and 72.08% for viscera from hybrid sorubim.

According to Park and Lin (2005), the moisture content of MSM of fish increases from 82%−85% to 90%–92% after repeated washing cycles except for the procedure F (MSM of pacu, decanting/sieving, $NaHCO_3$) that did not increase the moisture content significantly (83.90%–84.07%).

Moisture, one of the most critical factors in the processing of protein concentrates, presented was in accordance with the findings of other authors who also used MSM of fish as raw material for obtaining protein concentrate, *e.g.* 75.87% (Peixoto et al., 2000), 81.0%–84.8% (Fontana, Centenaro, Palezi, & Prentice, 2009), 88.78% (Kirschnik & Macedo-Viegas, 2009), and 80.69% (Mello et al., 2010). However, lower moisture contents are desirable because the lower moisture content in the protein concentrate based product, the greater the possibility of formation of higher quality gel (Kuhn, Soares, Prentice, & Vendrusculo, 2003). The moisture of commercial protein concentrate (surimi) varies between 72% and 77% because the moisture content plays an important role in gelation (Park & Lin, 2005).

A decrease in the content was expected from the third to the fourth washing due to the utilization of the NaCl solution which, besides increasing the ionic strength, facilitates the elimination of the excess of washing water (Kuhn & Soares, 2002). However, three different outcomes were possible from the procedures: decrease in moisture, constant moisture, or an increase in moisture.

In this study, the procedures varied with the four parameters (raw

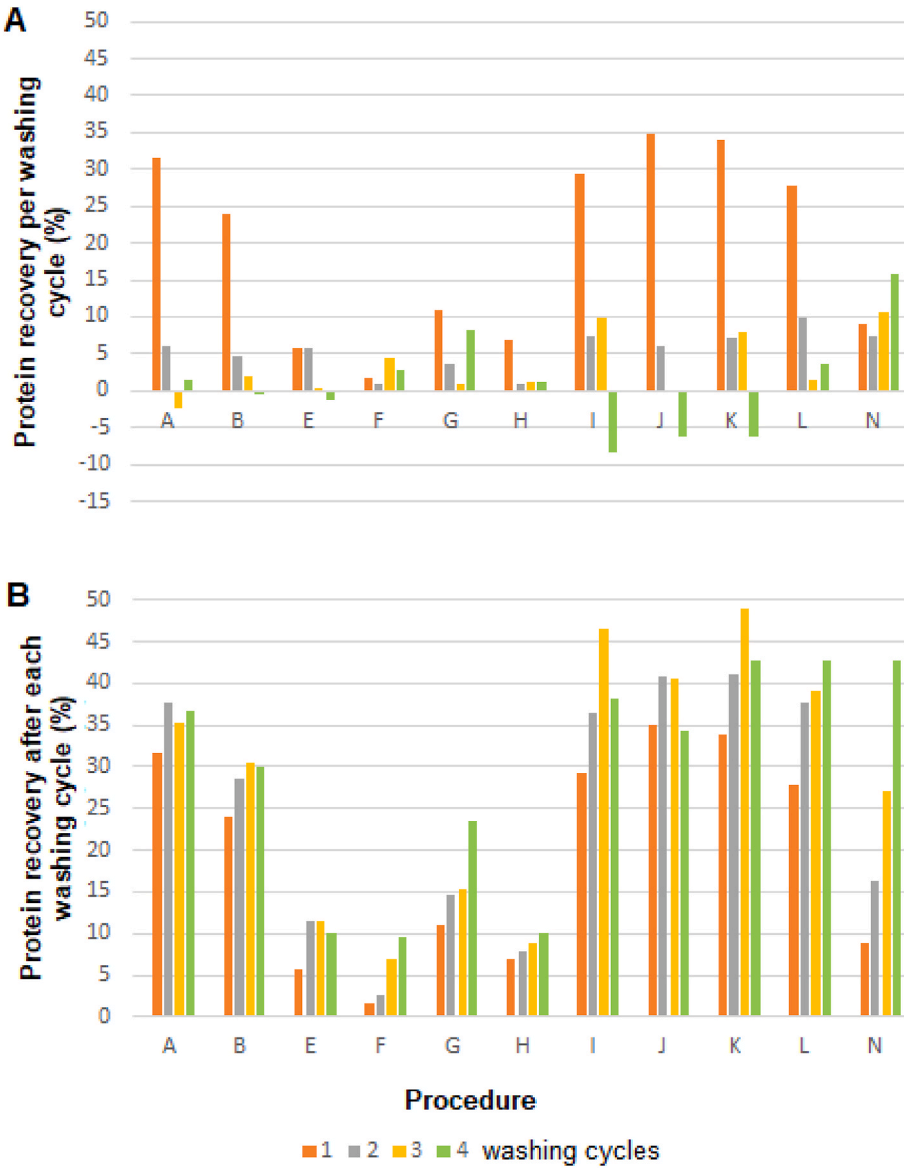

Fig. 4. Protein recovery per washing cycle (A) and after a determined number of washing cycles (B) for different procedures according to Table 1.

material, fish species, type of process and reagent). In order to interpret the obtained data and identify the parameters that played the more important role among the procedures, at least two parameters remained constant.

Comparing the procedures A and B (hybrid sorubim), with E and F (pacu), with MSM as raw material and $NaHCO_3$ as reagent, in relation to the type of process, we observed that the moisture content remains without significant difference from the third to the fourth washing in procedures A and E, in which the centrifugation/sieving process was carried out. By contrast, in procedures B and F, involving decanting/sieving, the moisture content significantly decreased ($P < 0.1$). This shows that in this comparison, the parameter that most affects the moisture content is the type of process used, and the process involving decanting and screening is more efficient for moisture reduction.

Analogously, comparing the procedures G and H (pacu), also with MSM as raw material, but with NaOH as reagent, in relation to the type of process, it was observed the decrease in moisture content was observed from the third to the fourth washing demonstrating that with MSM of pacu as raw material and NaOH as reagent, the type of process does not affect in the moisture content. As showed before, procedures C and D (hybrid sorubim) presented yields below 30% and were not studied further.

Now comparing the results obtained with hybrid sorubim viscera, I and J ($NaHCO_3$), the moisture content remained without significant difference from the third to the fourth washing, while in the procedures K and L (NaOH), the moisture content significantly decreased ($P < 0.1$). This shows that in this comparison, the parameter that most affects the moisture content is the reagent used, and NaOH is more efficient for moisture reduction.

All procedures carried out with NaOH solution as the washing reagent exhibited the same behavior, *i.e.* the decrease in moisture content from the third to the fourth washing. As shown in Fig. 2, higher pH values were reached with the NaOH than with $NaHCO_3$. Dawson, Sheldon, and Ball (1998) reported that there is a directly proportional relationship between the increase in pH of the washing solution and residual moisture content which is attributed to repulsion between like charges of protein groups (+ or -). When the pH increases, an increase in the space between the peptide chains may occur which may provide a channel for water to fill these spaces. Therefore, it is possible that the water retained during use of NaOH in the first three washes was easily removed by using NaCl in the last washing because the NaCl was more accessible.

4.4.2. Protein

The high protein content of the raw material was expected because the meat washing process promotes the removal of sarcoplasmic proteins, lipids, extractive components which do not contribute to the formation of the network structure, rendering the myofibrils purer and more concentrated (Cortez-Vega et al., 2013). The procedures F, G, L and N showed that the greater the number of washings, the higher the concentration of proteins in the final product, reaching levels of 68.68%, 82.53%, 82.44% and 47.64%, respectively. These levels were significantly different ($P < 0.1$) if compared to the first washing. The lowest content found in the studied procedures was 47.64%, achieved in procedure N (pacu viscera, decanting/sieving, $NaHCO_3$) after the fourth washing. This was due to the fact that pacu viscera also had the lowest protein content (4.82%) among the raw materials studied.

Alfaro et al. (2004) obtained surimi with protein content of 66% from MSM of hake rocket *Macrodon ancylodon* washed three times during only water in the ratio 3:1 (water:MSM) using centrifugation. On the other hand, Fontana et al. (2009) obtained a protein concentrate from corvine *Micropogonias furnieri* pulp with 97.7% of protein content, higher than the maximum level reached in the present study (88.7%). This higher level was due to the high initial content of protein of the raw material (87.1%) (Fontana et al., 2009) and the process chosen by them, in which the pulp protein is solubilized, where the obtained proteins were precipitated with the addition of NaOH until the isoelectric point (pH 5.0) and subjected to a second centrifugation. This second adjustment step of washing solution to the isoelectric point contributes to the precipitation of most proteins dissolved in the fluid obtained (Nolsøe et al., 2007).

The concentration of the protein content observed with the consecutive washings, in most of the procedures this increase was not statistically significant ($P < 0.1$). Procedures A, B, E, and J demonstrated that the washing cycles could be ended after the second washing because at that point the highest protein content was obtained. In the procedures H, I and K, the highest protein content (without significant difference with the other washings) was achieved in the third washing. Only procedures F, G, L, N needed four washings.

The excessive number of washing in the production of protein concentrates can cause great loss of myofibrillar proteins and increase in moisture of the final product (Cortez-Vega, Fonseca, & Prentice, 2015). A 35% reduction in protein content was reported in MSM of whole tilapia after two washing cycles with cold water in the ratio 3:1 (water: MSM) (Kirschnik & Macedo-Viegas, 2009).

4.4.3. Lipid

The variation in lipid content, measured in terms of crude fat, was due to the different raw materials and procedures utilized. However, the lowest levels (below 10%, dry basis) were achieved in the procedures using MSM and viscera of hybrid sorubim (procedures A, B, I, J, K and L). Alfaro et al. (2004) found a final lipid content of 10.15% for MSM of hake rocket *Macrodon ancylodon* subjected to three washing cycles with water in the ratio 3:1 (water:MSM), followed by centrifugation.

In general, the fish chemical composition is extremely variable from one species to another, and within the same species, depending on the season, the type, quality and amount of food available, the sexual maturity, age, growing conditions and part of the body analyzed (Menegazzo, Petenuci, & Fonseca, 2014).

It was expected that protein concentrates obtained from reduced fat content raw materials should also present the lower concentrations of lipids. However, it was not always observed because the different efficiencies of the procedures in relation to washing reagent solution and type of process utilized. Moreover, it is likely that the differences in the body structure between the studied species permitted an easier removing of lipids from hybrid sorubim in relation to pacu.

Monterrey-Quintero and Sobral (2000) reported a final lipid content of 2.40% for ground muscle of Nile tilapia submitted to four washing cycles with distilled water at 5 °C in a 1:2 (muscle:water) ratio, followed by sieving. Fontana et al. (2009) found lower levels of lipids (1.1%) in protein concentrates of corvina. However, as discussed above for the protein content, the chosen method promotes an efficient protein hydrolysis, facilitating the removal of lipids.

It is important to emphasizes that the low lipid content favors the protein concentrate obtaining, since the lower the fat percentage, the less it is susceptible to the lipid oxidation reactions, avoiding the formation of off flavor compounds that can interfere in the final taste of the product to be enriched (Rebouças et al., 2012).

4.4.4. Ash

The high concentration of ash was due the accumulation of salts resulting from the consecutive washings (Fontana et al., 2009; Mello et al., 2010). Ash contents within the same range were found by other authors, *e.g.* 1.5% in protein concentrate from corvina pulp (Fontana et al., 2009) and 2.4% in protein concentrate from filleting residues of piramutaba *Brachyplatystoma vaillantii*, washed twice with water and once with 2% NaCl solution at 4–5 °C and the ratio 5:1 (water:meat) (Galvão et al., 2012).

5. Conclusion

The protein concentrates are a viable alternative to add value for the fish processing waste. If the quality is appropriate, they may be utilized as intermediate raw materials for the preparation of various products. Differences related to the fish (hybrid sorubim and pacu) species were more evident than the processes (decanting/sieving or centrifugation) and utilized reagents ($NaHCO_3$ and NaOH) themselves. In three of the carried-out procedures (I, J, and K), the washing cycles influenced the quality of the final product. The protein concentrate obtained from hybrid sorubim viscera presented high quality due the high protein contents and weight yields. On the other hand, protein concentrate obtained from MSM of pacu (procedures E, F and H) presented the lowest weight yields, but considerable final protein content.

CRediT authorship contribution statement

Suéllen Patrícia Held Azambuja: Investigation, Formal analysis, Writing – original draft. **Kauyse Matos Nascimento:** Investigation. **William Renzo Cortez-Vega:** Methodology, Investigation. **Angela Dulce Cavenaghi-Altemio:** Conceptualization, Methodology, Investigation. **Jane Mary Lafayette Neves Gelinski:** Validation, Resources. **Gustavo Graciano Fonseca:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

None.

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