



## Effect of treatments and washing cycles on the quality of Nile tilapia (*Oreochromis niloticus*) protein concentrate

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### Abstract

The extraction of proteins from wastes reduces production costs and environmental pollution. The aim of this work was to evaluate the effect of two treatments involving decanting/sieving or centrifugation and the number of washing cycles on the quality of protein concentrate obtained from mechanically separated meat (MSM) of Nile tilapia (*Oreochromis niloticus*). Results were analyzed in terms of final yield and proximate composition (moisture, protein, fat and ash) after each washing cycle. Moisture did not vary statistically with the treatments and after the third washing cycle. However, the process involving centrifugation was more efficient for protein concentration because the final protein content increased 2.0 folds (79.82%, dry basis) and fat decreased 6.1 folds (8.29%, dry basis). After four washing cycles, it was obtained a protein concentrate with 79.82% protein, 8.29% lipid and 0.45% ash (dry basis), and 80.0% yield, using the centrifugation procedure. Visual whiteness was highly improved after four washing cycles using both processes. It was concluded that the centrifugation process with four washing cycles was the most appropriate method for producing protein concentrate from MSM of Nile tilapia.

**Keywords:** Fish residues; centrifugation; sieving; proximate composition; protein concentrate.

## 1 | INTRODUCTION

The fish processing generates a large amount of waste that are usually utilized for fish meal production, if not discarded. These residues represent a valuable source of nutrients that can be recycled (Kirschnik and Macedo-Vieira 2009; Cabral *et al.* 2012; Menegazzo *et al.* 2014), e.g. by using mechanical deboners to obtain mechanically separated meat (MSM; Cortez-Vega *et al.* 2013). It also represents a good option for the processing industry because the MSM of fish can be utilized to elaborate a wide range of products, e.g. fish burger, sausages, breaded and

canned fishes, fish strips, nuggets, among others (Cavenaghi-Altemio *et al.* 2013; Bartolomeu *et al.* 2014).

Another option is the obtaining of fish protein concentrate, which is a product derived from muscle or MSM, after successive washings, consisting of a crude myosin extract of high quality nutritious and excellent functionality. After the addition of cryoprotectants, it receives the name of surimi (Cortez-Vega *et al.* 2012). The washing process can improve the quality and functional characteristics of the MSM of fish, removing sarcoplasmic proteins,

inorganic salts, low-molecular weight substances, lipids, and blood components and other substances which can catalyze protein degradation, lipid oxidation and cause undesired coloration of the final product (Cortez-Vega *et al.* 2015). However, the washing leads to the loss of soluble protein and other nutrients, generating abundant liquid effluent (Nolsøe *et al.* 2007).

The ideal number of washings cycles and the most adequate treatments for obtaining the protein concentrate is still not unanimity among researchers. In this way, the aim of this work was to evaluate the effect of different processes (decanting/sieving or centrifugation) and washing cycles (1, 2, 3 or 4) on the quality of the protein concentrate obtained from MSM of Nile tilapia.

## 2 | METHODOLOGY

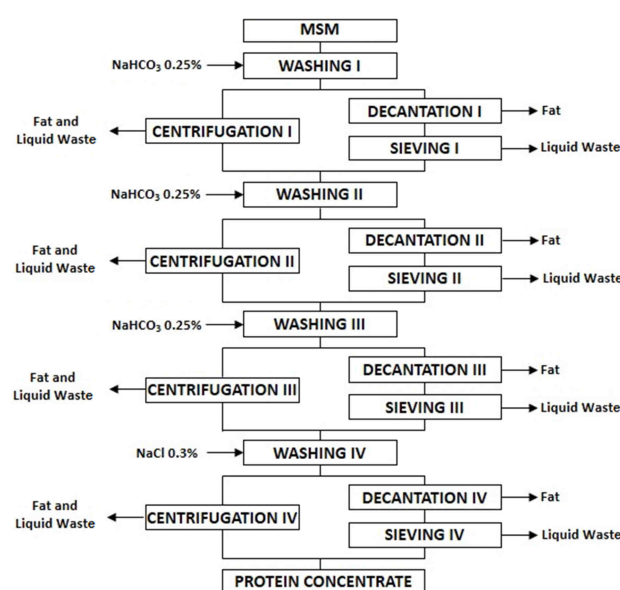
### 2.1 | Raw materials

Mechanically separated meat (MSM) of Nile tilapia (*Oreochromis niloticus*) was supplied from a local fish processing plant (Mar & Terra S.A., Itaporã, Brazil) and transported under refrigerated conditions to the Laboratory of Bioengineering, where was kept at  $-18\text{ }^{\circ}\text{C}$  for up to 1 month, for the production of protein concentrate. The MSM was produced in 3 mm particle size using a Baader separator (Baader model 694, Lübeck, Germany), operating at inlet  $6\text{ }^{\circ}\text{C}$  and outlet  $10\text{ }^{\circ}\text{C}$ , from Nile tilapia fish carcasses, 24 h after the slaughtering (Cavenaghi-Altémio *et al.* 2013).

### 2.2 | Protein concentrate

MSM was washed in four cycles utilizing in each cycle a washing solution:MSM ratio of 4:1 (v/w), at  $7\text{ }^{\circ}\text{C}$ . In each washing cycle, the stirring was done manually (process 1) or mechanically (process 2). It utilized 0.25%  $\text{NaHCO}_3$  as washing solution in the first, second and third washings and 0.3%  $\text{NaCl}$  in the fourth one. After each washing cycle, samples were decanted and sieved (process 1) or centrifuged (process 2).

In process 1, samples were homogenized manually by pressing in a cotton tissue 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 rpm) for 5 min at  $10\text{ }^{\circ}\text{C}$  using a mechanical agitator (Fisatom model 715, São Paulo, Brazil), and then centrifuged at  $3,400 \times g$  for 15 min at  $4\text{ }^{\circ}\text{C}$  (Biovera model RB7-R, Rio de Janeiro, Brazil). The supernatant containing fat and water-soluble proteins were discarded. The experimental schema is shown in Figure 1. The masses of the samples before and after each washing cycle were recorded for yield calculation.



**FIGURE 1** Processing flowchart for protein concentrated obtaining from MSM of Nile tilapia. MSM: mechanically separated meat.

### 2.3 | Yield and pH

The yield of protein concentrate was calculated from the percentage of protein concentrate obtained from the raw material used. Yield (%) = (protein concentrate weight / MSM weight) x 100.

pH was measured from the washing solution using a digital pH meter (Hanna pH21, São Paulo, Brazil). For that, different masses of  $\text{NaHCO}_3$  were individually weighed and dissolved in 50 ml of water to determine the pH reached by these reagents at different concentrations.

### 2.4 | Proximate composition

After each washing cycle to obtain the protein concentrate from MSM of tilapia, moisture, crude protein, crude fat and crude ash contents were determined in triplicate according to the methods described by AOAC (1995). Moisture was determined by the oven drying method at  $105\text{ }^{\circ}\text{C}$  until constant weight (method 950.46), protein by the Kjeldahl method (method 928.08), fat by the Soxhlet method (method 960.39) and ash by using the muffle furnace technique (method 920.153).

### 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.

### 3 | RESULTS AND DISCUSSION

The proximate composition of the unwashed MSM of Nile tilapia is shown in Table 1. The literature reports variables compositions (in dry basis) for MSM of Nile tilapia, e.g. 79.83% moisture, 75.01% protein, 14.43% lipids and 6.69% ash (Kirschnik and Macedo-Viegas 2009), 78.31% moisture, 64.96% protein, 28.91% lipids, 5.12% ash (Kirschnik *et al.* 2013), and 73.87% moisture, 60.73% protein, 29.09% lipids (Fogaça *et al.* 2013). As the MSM com-

position varies in function of the composition of the raw material (Cortez-Vega *et al.* 2015), it will be reflected in the number of washings necessary for obtaining a high-quality protein concentrate. The same was observed for Nile tilapia filleting residues, with 77.24% moisture, 76.80% protein, 19.6% lipid, 4.48% ash (Rebouças *et al.* 2012), and minced Nile tilapia, with 75.47% moisture, 52.02% protein, 42.97% lipids, 2.69% ash (de Oliveira Filho *et al.* 2010).

**TABLE 1** Proximate composition of the unwashed MSM of Nile tilapia and after one, two, three or four washing cycles, utilizing decanting/sieving or centrifugation procedures.

Procedure	Washing cycle	Moisture (%)	Protein (%)*	Lipid (%)*	Ash (%)*
MSM	-	75.34 <sup>a</sup> ± 0.82	39.87 <sup>a</sup> ± 1.85	50.45 <sup>a</sup> ± 3.76	0.22 <sup>a</sup> ± 0.01
Decanting/ Sieving	1	79.83 <sup>a,b</sup> ± 2.54	32.55 <sup>a</sup> ± 4.23	49.98 <sup>a</sup> ± 2.98	0.20 <sup>a</sup> ± 0.07
	2	85.16 <sup>b,c</sup> ± 2.18	39.62 <sup>a</sup> ± 0.74	30.54 <sup>a</sup> ± 1.24	0.33 <sup>b</sup> ± 0.05
	3	92.49 <sup>c</sup> ± 4.76	63.21 <sup>b</sup> ± 4.86	21.98 <sup>b</sup> ± 0.98	0.41 <sup>c</sup> ± 0.02
	4	90.95 <sup>c</sup> ± 3.67	64.32 <sup>b</sup> ± 0.32	15.36 <sup>b</sup> ± 3.65	0.49 <sup>c</sup> ± 0.11
MSM	-	75.34 <sup>a</sup> ± 0.82	39.87 <sup>a</sup> ± 1.85	50.45 <sup>a</sup> ± 3.76	0.22 <sup>a</sup> ± 0.01
Centrifugation	1	79.99 <sup>a,b</sup> ± 1.43	52.47 <sup>b</sup> ± 0.11	33.23 <sup>b</sup> ± 4.00	0.27 <sup>a</sup> ± 0.01
	2	86.55 <sup>b,c</sup> ± 4.65	57.15 <sup>c</sup> ± 1.79	22.94 <sup>c</sup> ± 3.40	0.26 <sup>a</sup> ± 0.01
	3	92.87 <sup>c</sup> ± 2.65	67.60 <sup>d</sup> ± 1.22	12.54 <sup>d</sup> ± 2.56	0.32 <sup>a,b</sup> ± 0.07
	4	92.05 <sup>c</sup> ± 2.78	79.82 <sup>e</sup> ± 0.36	8.29 <sup>d</sup> ± 0.07	0.45 <sup>b</sup> ± 0.10

\*Dry basis. MSM: Mechanically separated meat. The analyses were performed in triplicate and the results presented as mean and standard deviation. Different letters (superscript) within the same column (and block) indicate significant differences ( $P < 0.1$ ).

The obtained yields were 22% for the process evolving decanting/sieving and 80% for the process evolving centrifugation. The yield after the washing process is mainly influenced by the water removal efficiency and amount of fat in the MSM, but here it was basically a reflex of the utilized process, as the initial MSM for both processes presented the same composition. Replacing the first centrifugation with sieving improves the yield of protein isolate, but worsens the quality of the produced gel. Replacing the second centrifugation with filtration has no influence on the yield or the quality of the protein isolate. Different yields of protein concentrates have been reported, e.g., 18.34% from Nile tilapia residues (Vidal *et al.* 2009), 25% from residual Nile tilapia carcasses (Barreto and Beirão 1999), 44.75% from Nile tilapia fillet frames (Mello *et al.* 2010), 67.9% from minced tilapia muscle (Rawdkuen *et al.* 2009), and 63–68% (Sary *et al.* 2009) and 84.7% from MSM of Nile tilapia (Kirschnik and Macedo-Viegas 2009).

The variation of the proximate composition with the washing cycles is shown in Table 1. The moisture contents statistically increased with the washings up to the third cycle. After that, variation was not statistically significant ( $P > 0.1$ ; Table 1). It was expected because NaCl solution was utilized for the fourth washing cycle, which, besides increasing the ionic strength, facilitates the elimination of the excess of washing water (Cortez-Vega *et al.* 2013).

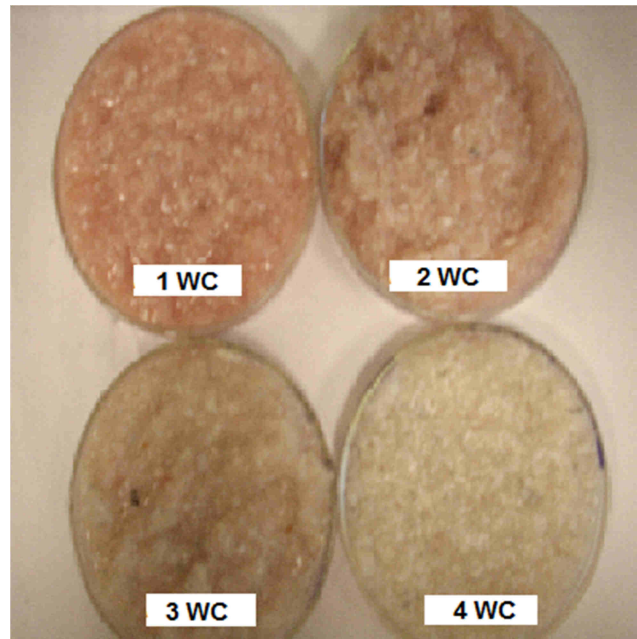
The moisture content of MSM of fish enhances up to 90–92% after repeated washing cycles (Park and Lin 2004). The moisture increases due the hydration of myofibrillar proteins that are present in large quantities in MSM, resulted from the removing the sarcoplasmic proteins during washing (Gryschek *et al.* 2003). Moisture presented here is in accordance with the findings of other authors who also used tilapia as raw material for obtaining protein concentrate, e.g., 88.78% from MSM (Kirschnik and Macedo-Viegas, 2009), and 80.82% from fillet frames (Mello *et al.* 2010).

Now comparing the results obtained with the two different processes, it observes that the moisture content remains without significant difference. It increased 15.61% with process 1 (decanting/sieving) and 16.71% with process 2 (centrifugation). This shows that both procedures equally affect the increase in the moisture content at the studied conditions.

The addition of 0.25% NaHCO<sub>3</sub> in the three initial steps was sufficient to maintain the pH near to the isoelectric point of the fish protein, which ranged 6.5 to 7, favoring the protein precipitation and separation from lipids. The process evolving centrifugation was more efficient for protein concentration because the final protein content increased 2.0 folds (to 79.82%, dry basis) while using decanting/sieving increased 1.6 fold (to 62.32%, dry basis). Moreover, fat decreased 6.1 folds (to 8.29%, dry basis) by using centrifugation against a decrease of 3.3 folds (to 15.36%, dry basis) by using decanting/sieving. The obtained lipid reduction using centrifugation was of 83.57% (from 50.45 to 8.29%, dry basis). It was superior to the 67.9% obtained with conventional protein concentration processing and somewhat similar to 85.2% and 88.6% with acid and alkali treatments, respectively, for washed minced tilapia muscle (Rawdkuen *et al.* 2009).

It was remarkable the efficiency in removing lipids from the centrifuged samples. On the other hand, the efficiency in the sieving process depended on the care of removing the fat that was separated from the protein. The combination of these operations showed itself an efficient process for obtaining protein concentrates.

The reduction in ash content favored by the loss of minerals during the leaching process due to the washing water was neutralized by the addition of NaHCO<sub>3</sub> and NaCl. The NaCl addition in the last washing cycle aids in the loss of staining of the protein concentrates (Figure 2), and provides increased ionic strength, although the main function of the NaCl is to assist in the solubilization of the myofibrillar proteins. The preparation process removed most of the materials considered non-functional, forming an extract of myofibrillar proteins with water content similar to the fish muscle, and high gelling and emulsifying capacities. The same behavior was observed elsewhere for MSM of Nile tilapia after washings (Kirschnik and Macedo-Viegas 2009; Kirschnik *et al.* 2013).



**FIGURE 2** Protein concentrate after four washing cycles (WC) using the decanting/sieving procedure.

#### 4 | CONCLUSIONS

The process involving decanting/sieving is economically interesting due to the low cost and investment, but the reduced yield does not favor its utilization. The process involving centrifugation was statistically more efficient for protein concentration indicated by the increased final protein content and decreased fat with the washings. Four washing cycles improved protein concentration as fat removing, indicating that three usual cycles are not always enough. There was no moisture gain from the third to the fourth washing cycles in none of the methods. Whiteness was highly improved after four washing cycles. The centrifugation process with four washing cycles was the most appropriate method for producing protein concentrate from mechanically separated meat of Nile tilapia due to the utmost protein recovery and successful improvement of the quality and functional characteristics of raw material.

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#### CONTRIBUTION OF THE AUTHORS

**GGF** research design; **LBA & ADCA** primary data collection; **LBA & ADCA** data analysis; **GGF** manuscript preparation.