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Restructured products from tilapia industry byproducts: The effects of tapioca starch and washing cycles

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A B S T R A C T

The tilapia fileting industry generates large amounts of nutritionally significant waste material, and the recovery of this material is important. The manufacture of restructured products from mechanically recovered fish meat (MRFM) obtained from tilapia fileting allows the use of proteins of high biological value that would otherwise be discharged into the environment. The objective of this study was to evaluate the effect of washing cycles (either one cycle or five cycles) and of the addition of tapioca starch (20% vs. a no-starch control) on the characteristics of surimi obtained from MRFM produced by the tilapia industry and destined for use in restructured products. To evaluate the quality attributes of the product, the structure of a surimi protein matrix and its relationship to selected physicochemical parameters and morphological characteristics was assessed. Both the number of washing cycles and the starch addition were found to influence the moisture, protein and lipid content of the MRFM surimi. Higher whiteness was found after five washing cycles. Because the tapioca starch acted as a stabilizer, the fat globules were more stable and well distributed, and an emulsion with better properties resulted. A homogeneous network of fat globules linked to the protein matrix by a layer of tapioca starch was formed. Another advantage of this approach is that tapioca starch is gluten free. This property is important for specific groups in the population, e.g., celiac-intolerant consumers.

Keywords: Mechanically recovered fish meat; Surimi; Microstructure; Color; Chemical composition; Lipid oxidation

1. Introduction

The commercial processing of foods of aquatic origin requires the removal of the bones, skin, head and viscera (byproducts), which represent approximately 60–70 g/100 g of the total weight of the fish (Taskaya and Jaczynski, 2009). The development of technology for protein recovery from the byproducts of fileting offers many benefits because this technology facilitates a more responsible use of the available resources for human food and reduces the environmental stresses associated with the disposal of the processing byproducts (Jaczynski, 2005).

Fileting byproducts can be transformed into high-value products through the use of restructuring technology. This technology can be applied to obtain novel products based on the use of an array of additives to improve the mechanical and functional properties of the material (Ramirez et al., 2011). Surimi consists of stabilized myofibrillar proteins obtained from mechanically deboned fish flesh that is washed with water and blended with cryoprotectants (Park and Lin, 2005). The methods used to concentrate myofibrillar proteins in surimi production can be adapted for use in restructured products. Note, however, that the loss of freshness sustained by fileting byproducts compromises the quality of the surimi produced from these byproducts.

Abbreviations: MDA, malondialdehyde; MRFM, mechanically recovered fish meat; TBARS, thiobarbituric acid-reactive substances; TCA, trichloroacetic acid.

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Washing the mechanically recovered meat is a critical step in the production of surimi. The amount of water required and the number of washing cycles are determined by the fish species, the condition of the fish and the product quality required (Lee, 1984). The washing procedure is the key to the quality of the surimi produced. Washing not only removes fat and undesirable materials but also, more importantly, increases the concentration of myofibrillar protein, thereby improving the gel-forming ability of the surimi (Nopianti et al., 2011). However, the repeated washes that are applied during surimi processing require increased amounts of freshwater and cause severe contamination of the wastewater (Park and Lin, 2005). In this context, the number of washing cycles is one of the most important steps in surimi production, especially if fileting byproducts are used.

Viscoelasticity is an important quality of surimi products. The ingredients used to prepare surimi significantly influence the rheological properties of the product (Sarker et al., 2012). Starch has been considered the most important ingredient in surimi seafood products due to its effects on the textural and physical characteristics of surimi fish protein gels (Burey et al., 2008; Hunt et al., 2009). Starches promote the formation of a continuous matrix by interacting with water and protein in the fish paste, and they play an important role in improving the mechanical and functional properties of surimi (Ramirez et al., 2011). Furthermore, starch is added to surimi because of its water-binding ability. The starch serves to maintain gel strength in the face of a decrease in the water content of the surimi. It also improves stability during refrigerated or frozen storage (Lee, 1984). The biological origin of the starch used in surimi and surimi products has an important influence on the resulting physico-chemical and functional properties of the material (Sarker et al., 2012).

Starch is commonly added to surimi at a level of 4–12%. The most frequently used starches include wheat, corn, potato, waxy maize and tapioca (Hunt et al., 2010).

Tapioca starch has been used in surimi products because it provides cohesive, elastic-matrix-consistent seafood (Mason, 2009). Tapioca starch is the highly concentrated (>80% starch) product obtained when water is used to extract the starch from cassava. The cassava plant originated in the Brazilian Amazon rainforest and has been adopted as a staple food in Africa and Asia. These continents are now the leading producers of this raw material (Maieves et al., 2011).

In Brazil, tapioca starch is widely used in the baking industry because of its special starch gelatinization properties and because of its added attractiveness as a gluten-free product. Tapioca starch is used in the meat industry because it produces a surface sheen and a smooth texture, has a neutral taste and is clear in solution (Zhang and Barbut, 2005).

Brazilian consumers habitually eat restructured products from the poultry industry, and we believe that restructured fish products can also be well accepted. The objective of this study was to evaluate the effect of wash cycles and of the addition of starch on the characteristics of the surimi obtained from MRFM produced by the tilapia industry. This evaluation addressed the potential use of the surimi in restructured products.

2. Materials and methods

2.1. Fish

The experiments reported here were performed at Universidade Estadual Paulista (UNESP), Brazil. The meat was removed from tilapia carcasses that were produced and slaughtered at the site and that belonged to the same production lot. The fish were deprived of food for 24 h and then killed by heat shock (using water and ice at a 1:1 ratio) before gutting and heading prior to filet removal. After filet removal, the fish carcasses were passed through a deboning machine (High Tech, HT 250, Chapecó, SC, Brazil) to remove the muscle attached to the bones. The resulting product constitutes the MRFM. The MRFM was packaged and frozen in a freezing tunnel at −25 °C, then stored in a freezer at −18 °C. The samples were transported in cold boxes to ensure that they would remain frozen. On arrival at the laboratory, they were held in a freezer (−18 °C).

2.2. Surimi preparation

Surimi was prepared using a manual process. The MRFM was kept under refrigeration at 5 °C for 24 h before handling. After thawing, it was subjected to wash steps (either one or five steps) with four volumes of cold distilled water (pH = 7). The water temperature during washing was maintained at approximately 5 °C with crushed ice. After each wash, the MRFM was manually pressed in cotton. The material from each washing treatment (one or five washing cycles) was then divided into two equal portions. Tapioca starch (20%, w/w) was added to one portion from each washing treatment. The 20% (w/w) tapioca starch addition was performed slowly while the MRFM was homogenized. At the end of processing, 1% (w/w) sucrose was added as a primary cryoprotectant, and 2% (w/w) of sodium chloride was used as a flavor enhancer to mask the sweetness.

The sucrose, sodium chloride and tapioca starch were mixed with the MRFM. An electric mixer (Arno, Planetária, São Paulo, Brazil) was used to combine these ingredients. According to the information furnished by the manufacturer, the chemical composition of tapioca starch is as follows: moisture, 12.6%; protein, 0.4%; carbohydrates, 86.8%; and dietary fiber, 0.2%.

The samples were stored at −18 °C until analysis.

2.3. Surimi gel preparation

The surimi samples were thawed and approximately 100 g of each treatment were placed in steel forms for baking and for the induction of surimi gelation. Each sample in triplicate was exposed to heat in a bath (NT 249, Novatecnica, Piracicaba, SP, Brazil) at 90 °C for 30 min. After cooking, the samples were cooled in crushed ice for 15 min to stop the process. The samples were then packaged and frozen until analysis.

2.4. Physical and chemical analyses

The moisture content of the product was measured by determining the difference between the initial weight (2.0 g) of a surimi sample before heating in an oven (Fanem, São Paulo, Brazil) and the weight of the sample after heating for 16 h at 105 °C (method 950.46) (AOAC, 2005). The total nitrogen content was determined by the Kjeldahl procedure (method 981.10), and the protein content was estimated using a conversion factor of 6.25 (AOAC, 2005). The lipid content was determined by extraction with chloroform and methanol according to the method of Folch et al. (1957). All wet surimi samples were stored at −18 °C and thawed at 5 °C for 24 h before analysis. Four surimi samples were taken for each treatment, and all analyses were performed in triplicate.

Lipid oxidation was evaluated from the formation of thiobarbituric acid reactive substances (TBARS) according to Vyncke (1970) for samples of 10 g of surimi. A 5-ml aliquot of the distillate was used for color development and was measured at 532 nm using a spectrophotometer (UVmini 1240, Shimadzu, Tokyo, Japan). The malondialdehyde (MDA) concentration was calculated based on the calibration curve obtained using 1,1,3,3-tetraethoxypropane, a precursor of MDA. The results were expressed as mg MDA per kg of surimi.

Soluble nitrogen was determined using trichloroacetic acid (TCA) according to Stefansson et al. (2000). Protein in muscle was precipitated by addition of 10% TCA. After filtration, the amount of nitrogen compounds soluble in TCA was measured using the semi-macro Kjeldahl method.

The instrumental color was determined using a color spectrophotometer (Minolta, CM20001, Osaka, Japan) at an angle of 90° at room temperature (25 °C). Values of a^* and b^* were based on the CIELAB system (Hunter, 1975). The L^* value denotes luminosity ($L^*=0$ is black, $L^*=100$ is white); a^* denotes the color in a range from green (−a) to red (+a); and b^* denotes the color in a range from blue (−b) to yellow (+b). Brightness or whiteness (w) was calculated as $w=L^*-3b^*$ according to the Hunter Lab system, with 18 readings taken for each surimi formulation.

2.5. Scanning electron microscopy (SEM)

The morphology of the sample was observed with scanning electron microscopy (Jeol, JSM 5410, MD, USA). The samples were fixed in 2.5% buffered glutaraldehyde and post-fixed in 1% osmium tetroxide for 2 h. They were then washed in PBS, dehydrated in ethanol and dried at the critical point using CO_2. The samples were metalized with pale-gold ions for electron microscopy. Four samples were prepared for each treatment.

2.6. Statistical analysis

The data were analyzed using SAS version 6.12 (SAS Institute Inc., Cary, NC, USA). The main effects of wash cycles and tapioca starch levels and of their interaction on the quality parameters of the surimi were determined with a Tukey–Kramer test. Differences were considered significant at $P<0.05$.

3. Results and discussion

3.1. Chemical composition

The chemical composition of the surimi was affected by the number of wash cycles and by starch addition (Table 1). The surimi produced with one wash cycle showed a small increase in moisture with a resulting decrease in the levels of protein and lipids. For the surimi produced with five wash cycles, the moisture increased by ten percentage points. These results are expected because washing removes some of the protein and because an excess number of washes concentrate the myofibrillar proteins, which retain water and increase the final moisture content. The addition of 20% starch and the use of one wash cycle caused a decrease in the moisture content of approximately 10–12% (Table 1). This decrease occurred because starch filled the interstitial spaces previously occupied by water. A similar effect was observed in surimi prepared from Alaska pollock (*Theragra chalcogramma*); the addition of 5% corn starch resulted in an approximate 9% decrease in moisture compared with the original raw material (Couso et al., 1998). However, the addition of 20% starch with five washing cycles resulted in a considerably smaller decrease in the moisture content (<5%).

During the preparation of surimi, a substantial decrease in protein occurs due to the leaching of sarcoplasmic proteins during the washing steps. Both the number of washing cycles and the starch addition influenced the protein content of the surimi. The variation observed in the protein values (Table 1) is consistent with previous observations by Kirschnik and Macedo-Viegas (2009), who found a 15.13% protein level for unwashed minced material and an 8.93% level for protein slurry. They attributed this decrease to the removal of most of the soluble proteins. For the MRFM tilapia surimi presented here, one wash produced a 6% decrease in protein in relation to MRFM, whereas five washes produced a 20% decrease, in relation to MRFM. The addition of 20% starch caused a significant decrease in the protein level, whereas the addition of 20% starch and five washing cycles decreased the protein level to a value less than 10%. Brazilian legislation drafted by the Ministry of Agriculture, Livestock and Supply states that breaded products can contain 30% carbohydrates and at least 10% protein (Brasil, 2012). Accordingly, the protein level is less than the required value in this case (i.e., 20% starch and five washing cycles).

The lipid content of the minced tissue was 7.63%. This value is high compared with tilapia filets, but it is less than the value of approximately 8.57% reported by Rawdkuen et al. (2009). The high lipid content of minced tissue is due to the large amount of fat in tilapia ventral muscles. The combination of washing and starch addition affected the lipid content. Starch addition reduced the lipid content by 16% for one wash and by 49% for five washes in relation to MRFM (Table 1).

3.2. Surimi quality parameters

The TBARS values indicate the extent of lipid oxidation. The TBARS values in Table 2 demonstrates that washing reduced the lipid oxidation rate relative to that of MRFM. However, the results for one and five washes did not differ. Washing MRFM can prevent lipid oxidation in addition to removing much of the fat contained in fish muscle; washing also removes primary and secondary products of oxidation (Eymard et al., 2009).

An analysis of soluble nitrogen showed that the high values found for MRFM decreased with the number of washes (Table 2). The results of the soluble nitrogen analysis procedure should be expressed without multiplying by 6.25 as suggested by Afonso and Sant'Ana (2008). Soluble nitrogen values refer to not only sarcoplasmic protein, peptides and free amino acids but also residues of other nitrogen compounds, including nucleotides. These results demonstrate that washing inhibits protein degradation and improves product stability.

The colorimetric results for the MRFM tilapia surimi gel are shown in Table 3. Washing produced a decrease in the lightness (L^*) of the MRFM regardless of the number of washing cycles, whereas the addition of starch increased the L^* value significantly. The redness-greenness value (a^*) of the MRFM was significantly decreased by each type of washing cycle and by starch addition. Decreases occurred in the positive a^* values (even in the most strongly positive values) because the washes eliminated the colored pigments, especially the myoglobin contained in red muscle and blood. Moreover, the starch neutralized the red color. The washing cycles produced a significant decrease in the yellowness-blueness (b^*) values. However, the original b^* value of the MRFM did not differ from the b^* values found for the treatments with 20% starch addition.

Whiteness is an important aspect of the quality of surimi base products (Chen, 2002). Generally, the market demands surimi with high L^*, low b^* and high w values (Hsu and Chiang, 2002). High L^* values were obtained with five washing cycles regardless of the addition of starch. The use of 1 or 5 washing

Table 1 – Changes in moisture, protein and lipid as a function of washing cycles and starch addition.

Washing cycles	Starch (%)	Moisture (%)	Protein (%, wet weight)	Lipid (%, wet weight)
0	0[a]	73.87 ± 0.15c	15.87 ± 0.33a	7.60 ± 0.53a
1	0	75.24 ± 0.43b	14.10 ± 0.22b	6.39 ± 0.62b
1	20	65.14 ± 0.20e	11.58 ± 0.32c	5.99 ± 0.36b
5	0	83.24 ± 0.18a	12.07 ± 0.76c	5.38 ± 0.58b
5	20	71.86 ± 0.14d	8.93 ± 0.03d	3.10 ± (0.5)c

[a] MRFM.
Values are mean ± standard deviation, $n = 4$. Means followed by different letters in columns differ significantly between treatments ($P < 0.05$).

Table 2 – Changes in TBARS and soluble nitrogen as a function of washing cycles and starch addition.

Washing cycles	Starch (%)	TBARS (mg MDA/kg surimi)	Soluble nitrogen (gN/100 g surimi)
0	0[a]	0.77 ± 0.02a	1.69 ± 0.02a
1	0	0.58 ± 0.06b	1.01 ± 0.12b
1	20	0.52 ± 0.02b	0.82 ± 0.03c
5	0	0.53 ± 0.08b	0.21 ± 0.02d
5	20	0.47 ± 0.05b	0.20 ± 0.09d

[a] MRFM.
Values are mean ± standard deviation, $n = 4$. Means followed by different letters in columns differ significantly between treatments ($P < 0.05$).
TBARS, thiobarbituric acid reactive substances.

cycles produced a significant decrease in the b^* value. Starch addition did not influence the b^* value. As expected, the washing cycles caused the whiteness of the surimi to increase. Independent of starch addition, surimi with higher whiteness was obtained with five washing cycles.

3.3. *Surimi gel microstructure*

Particle morphology has substantial effects on consumer perception. For this reason, it is important to understand particle morphology and to use appropriate techniques to study particle properties (Burey et al., 2008).

Photomicrographs of MRFM are shown in Fig. 1(a–d). The surface is a protein lattice structure and regions of (Fig. 1a) high and (Fig. 1b) low protein content can be distinguished, with occasional fragments of muscle fiber (Fig. 1c). The minced tissue was subjected to the same heat treatment as surimi and it is possible to visualize more homogeneous regions (Fig. 1d). The presence of fat droplets (Fig. 1d) is in agreement with the highest lipid percentage in minced tissue according to chemical analysis (Table 1).

Fig. 2(I a and b) shows the surface of a surimi sample. The surface shows air holes and includes sporadically and irregularly distributed portions of muscle containing two to three muscle fibers, as described by Moreira et al. (2006). The presence of these fibers indicates that one wash was not sufficient to fully denature the muscle structure and that even after heat treatment to induce surimi gel formation, there was no denaturation of myofibrillar protein. The presence of empty and relatively clear cells indicates a loss of residual fat caused by the lack of a thickener and stabilizer (e.g., tapioca starch). This feature can be observed in small reticular areas scattered on the surface of the surimi and connected by very thin fibers, indicating a low degree of aggregation (Tabilo-Munizaga and Barbosa-Cánovas, 2005). Although this layer of relatively homogeneous fibers is characteristic of thermally induced gel (Aguilera and Stanley, 1999), it does not provide product stability. The absence of muscle fibers and a more homogeneous structure with large fat globules were observed in the surimi that was washed five times (Fig. 2II b). These features show that the product lacks stability after five washing cycles. If the emulsion is not stable, fat is not trapped in the network; then, because a formless mass results, the texture of the product is a problem. Alveoli were also observed in the product after five washings. The presences of alveoli may be related to increased water retention during processing (Tabilo-Munizaga and Barbosa-Cánovas, 2005) and to the occlusion of air expansion during cooking.

Fig. 3 shows a surface covered by fat globules within a protein matrix, characteristic of an emulsion. The tapioca starch acted as a stabilizer, and the fat globules were more stable and well distributed (Fig. 3I a). These factors yielded a better emulsion. An emulsion is a mixture of immiscible liquids, one of which is dispersed in the other in the form of small droplets. For meats, such systems comprise two phases, a dispersed phase formed by fat particles and a continuous phase formed

Table 3 – Color parameters as a function of washing cycles and starch addition.[a]

Washing cycles	Starch (%)	L^*	a^*	b^*	w
0	0[b]	65.06 ± 1.67c	4.96 ± 0.93a	8.37 ± 1.09b	39.83 ± 3.00e
1	0	58.75 ± 0.52d	2.24 ± 0.54b	5.56 ± 0.95b	42.06 ± 3.03d
1	20	71.62 ± 0.61b	2.00 ± 0.43b	8.44 ± 0.32a	46.29 ± 1.20c
5	0	58.79 ± 0.73d	0.27 ± 0.42d	2.15 ± 0.94c	52.34 ± 3.27a
5	20	74.58 ± 0.78a	1.50 ± 0.17c	8.05 ± 0.43a	50.44 ± 1.75b

[a] Wet samples.
[b] MRFM.
Values are mean ± standard deviation, $n = 4$. Means followed by different letters in columns differ significantly between treatments ($P < 0.05$).

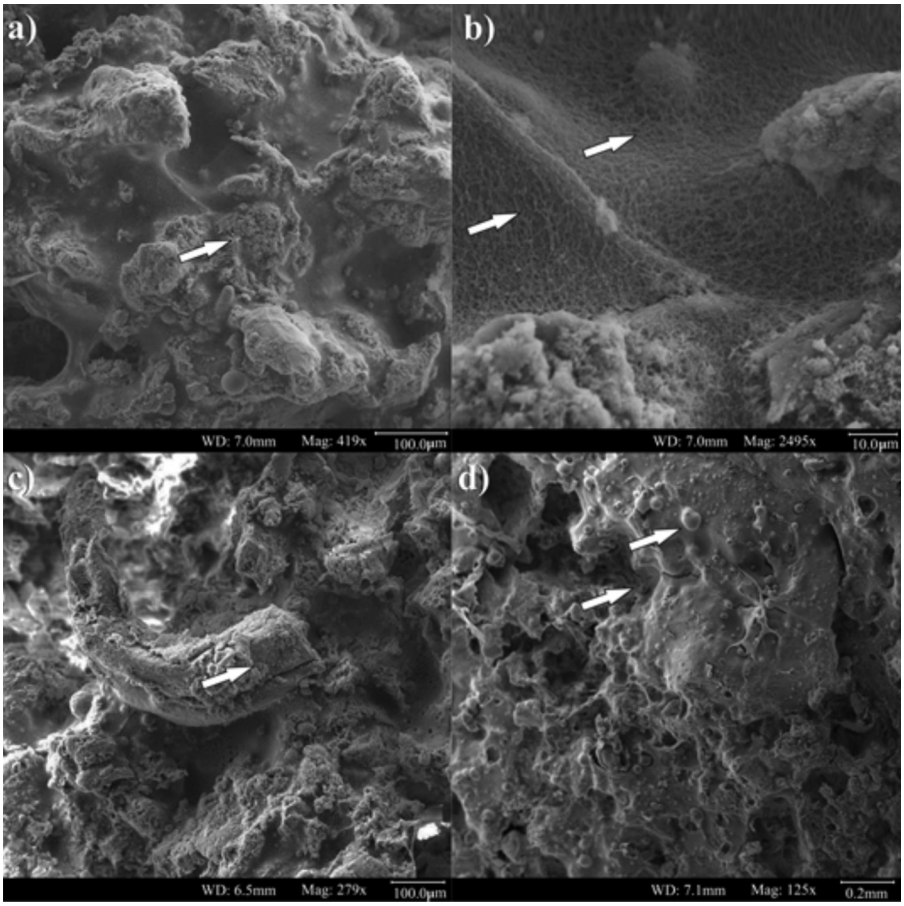


Fig. 1 – Mechanically recovered fish meat (MRFM) (a) region of high protein density; (b) region of low protein density; (c) detail of muscle fibers; (d) homogeneous surface with fat droplets.

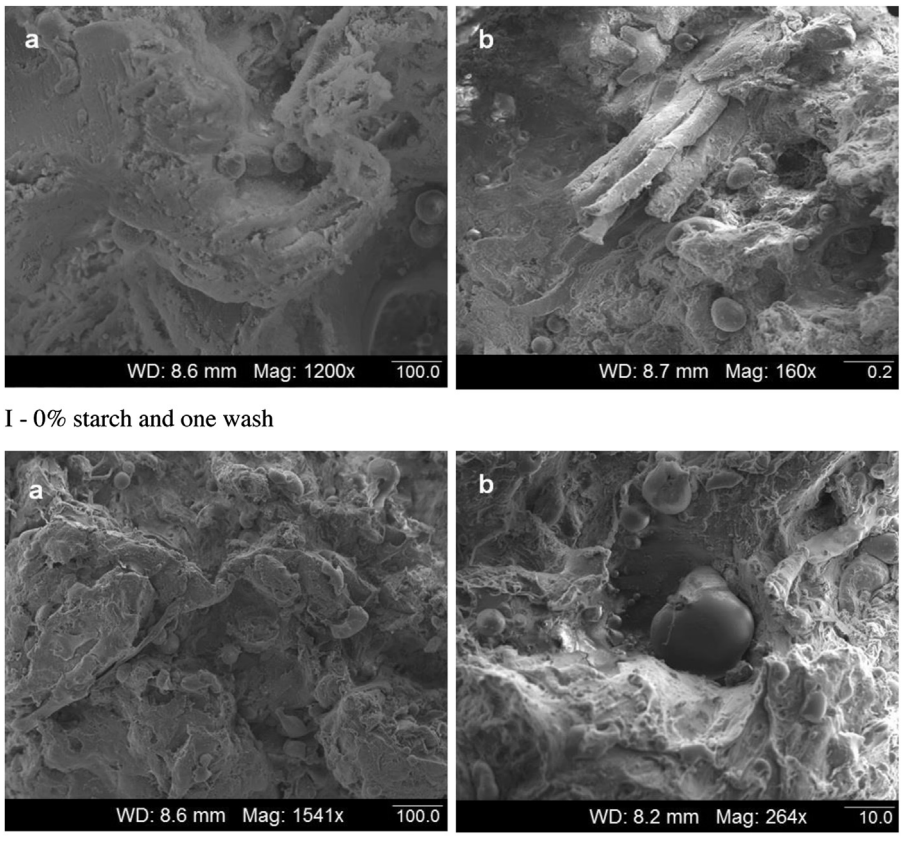
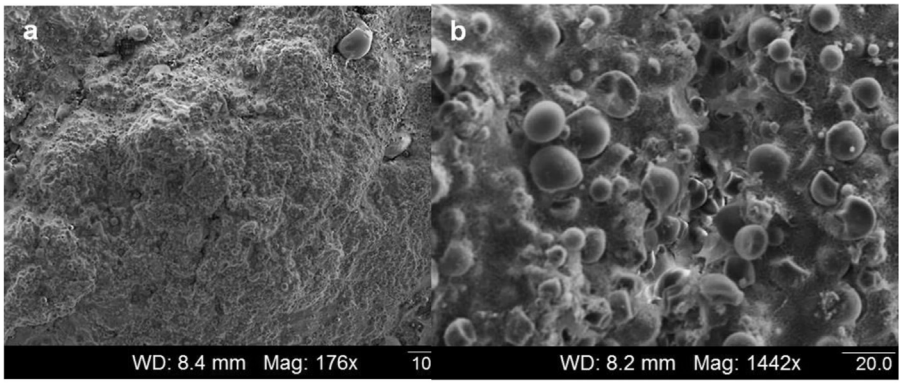
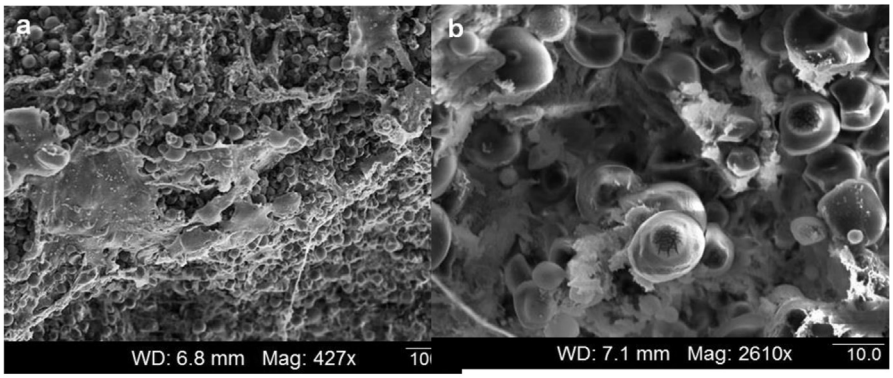


Fig. 2 – Tilapia surimi gel. (I) (a) Magnification showing details of the fine fiber layer. (b) Detail of a muscle fiber. (II) (a) Surface of surimi; (b) fat globule within the alveoli.



I - 20% tapioca starch and one wash



II- 20% tapioca starch and five washes

Fig. 3 – Tilapia surimi gel. (I) (a) Wider view; (b) details of fat globules. (II) (a) Homogeneous network of fat globules linked to the protein matrix by a layer of tapioca starch; (b) magnification showing a fat globule attached to the protein matrix.

by water, together with soluble proteins, forming a matrix that encapsulates fat globules.

In the gelatinization process, the crystalline structure breaks down to form an amorphous structure as soon as the granules absorb water. The distribution of crystalline and amorphous structures is not uniform and depends on the amount of water and the cooking temperature (Couso et al., 1998). In this study, the heat treatment caused the gelatinization of the starch granules, and no crystal structures were observed; a homogeneous network of fat globules linked to the protein matrix by a layer of tapioca starch was formed (Fig. 3II b).

In general, the images showed a discontinuous texture similar to an irregular mesh network. The tissue contained air holes and included portions of muscle that occupied small, irregularly distributed areas whose characteristics were related to the number of washes during preparation, with a higher number of washes producing a smaller amount of fibers. Fat globules were distributed more evenly in samples to which starch was added, demonstrating the importance of the use of thickener in restructured products.

4. Conclusions

The results of this study showed the feasibility of producing restructured products from MRFM tilapia surimi using tapioca starch. The addition of tapioca starch, a gluten-free product, also improved the physical properties of MRFM tilapia surimi gel. In view of the current increasing incidence of celiac disease and gluten-intolerant individuals, there is a major need for the development of gluten-free products. The study of the physicochemical components of MRFM tilapia surimi to assess the intensity of the changes in protein structure produced by the use of tapioca starch could ensure that the processes and products whose feasibility was demonstrated in this study will have technological applications.

Additional aspects of this topic that should be considered are primarily those associated with water use and waste, e.g., minimizing the input of water and reusing water if possible.

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