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OPTIMIZATION OF THE SURIMI PRODUCTION FROM MECHANICALLY RECOVERED FISH MEAT (MRFM) USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

The by-products generated from industrial filleting of tilapia surimi can be used for the manufacture of surimi. The surimi production uses large amounts of water, which generates a wastewater rich in organic compounds (lipids, soluble proteins and blood). Optimizing the number of washing cycles will contribute to a more sustainable production. A mathematical model of mechanically recovered tilapia meat (*Oreochromis niloticus*) for the processing of surimi (minced fish washing cycles and tapioca starch addition) based on two quality parameters (texture and moisture) was constructed by applying the response surface methodology (RSM). Each factor had an important effect on the moisture and texture of surimi. This study found that the optimal formulation for producing the best surimi using the by-products of tilapia filleting in manufacturing fish burger were the addition of 10% tapioca starch and three minced fish washing cycles. A microstructural evaluation supported the findings of the mathematical model.

PRACTICAL APPLICATIONS

The use of mechanically recovered fish meat (MRFM) for the production of surimi enables the utilization of the by-products of filleting fish. However, the inferior quality of the surimi produced from MRFM in relation to that produced with fillets necessitates the addition of starch; secondly, surimi production consumes a large volume of water. RSM provides a valuable means for optimizing the number of washing cycles and starch amounts utilized in fish burger production. Tapioca starch, widely produced in Brazil, has desirable characteristics (surface sheen, smooth texture, neutral taste and clarity in solution) for use in MRFM-produced surimi.

INTRODUCTION

In 2010, the tilapia was responsible for 40% of aquaculture production of freshwater fish in Brazil (FAO 2012), and that most of the tilapias were used to produce fillet. The average tilapia fillet yield varies from 30–38% and industrial filleting generates significant amounts of by-products. The development of technology for protein recovery from filleting by-products offers many benefits, because this technology facilitates the more responsible utilization of available resources for human food and it reduces the environmental stresses associated with the disposal of processing by-products (Jaczynski 2005). The recovered muscle protein can be used to manufacture value-added products such as surimi.

Surimi is minced fish flesh that has been washed to remove most of the lipids, blood, enzymes and sarcoplasmic proteins, which is then stabilized using cryoprotectants to allow frozen storage (Lee 1984; Park and Lin 2005; Julavittayanukul *et al.* 2006).

The utilization of fish industry by-products, such as surimi, provides an important opportunity for the industry because it could potentially generate additional revenue as well as reducing the costs of disposing of these materials (UNEP 2000). However, surimi requires the use of large volumes of freshwater and it produces high levels of contaminated wastewater (Martín-Sánchez *et al.* 2009).

The number of washing cycles, the ratio of water : fish, and the washing time are important factors determining the quality of surimi gel. The amount of water required and the number of washing cycles is determined by the fish species, the fish condition and the product quality required (Lee 1984).

The texture, color and odor composition of the final product is greatly improved when impurities are removed by washing (Park and Lin 2005). These functional properties are the major factors responsible for the final acceptance of surimi-based products by consumers (Tabilo-Munizaga and Barbosa-Canóvas 2004; Park and Morrissey 2005). However, rising utility costs, limited water sources and pollution have prompted surimi manufacturers to consider minimizing water usage and reducing wastewater disposal (Park and Lin 2005). The number of washing cycles and ratio water : fish are important parameter for texture, color and odor, and the use of mechanically recovered meat usually requires a larger amount of water.

Starches promote the formation of a continuous matrix by interacting with water and protein in the fish paste and they have an important role in improving the mechanical and functional properties of surimi (Ramirez *et al.* 2011). Furthermore, starch is added to surimi to maintain gel strength with a reduction in surimi content, because of its water-binding ability, while it also improve stability during refrigerated or frozen storage (Lee 1984). A 4–12% starch level is commonly added to surimi and the most frequently used starches include wheat, corn, potato, waxy maize and tapioca (starch produced from treated and dried cassava root) (Hunt *et al.* 2010). Different botanical starch sources have different effects on the texture of surimi starch gels (Yang and Park 1998). In Brazil, tapioca starch is widely used in the baking industry because of its special characteristics in starch gelatinization and the added advantage of not containing gluten. Tapioca starch is used in meat industry for its surface sheen, smooth texture, neutral taste and clarity in solution (Zhang and Barbut 2005). The cassava plant originated in the Brazilian Amazon rainforest and it was adopted as a staple food in the African and Asian continents, which are now the main producers of this raw material (Maieves *et al.* 2011).

Response surface methodology (RSM) is a useful statistical technique that has been applied to complex variable processes where its principal advantage is the reduced number of experimental runs required to generate sufficient information to provide a statistically acceptable result (Jeong *et al.* 2009).

The present study tested the number of washing cycles and the starch concentration as independent variables when optimizing shear stress and moisture in tilapia surimi for fish burger production.

MATERIALS AND METHODS

Materials

Experiments were performed at Universidade Estadual Paulista, UNESP, Brazil. Minced fish of tilapia was obtained from a commercial abattoir. The meat was removed from tilapia carcasses that were produced and slaughtered at the site and that belonged to the same production lot. 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 fillet removal. Once sliced, the fish carcasses were passed through a deboning machine (High Tech, HT 250, Chapeco, SC, Brazil) to remove muscle attached to bones, resulting in a product known as mechanically recovered fish meat (MRFM). The MRFM was packaged and frozen in a freezing tunnel at −25C and then stored in a freezer at −18C. Samples were transported in cold boxes to keep them frozen. On arrival at the laboratory, they were kept in a freezer (–18C) for 48 h.

Surimi Preparation

Surimi was prepared using a manual process. The MRFM was kept under refrigeration at 5C for 24 h before being handled. After thawing, it was subjected to a different number of washing steps (1, 3 or 5) using four volumes of cold distilled water. The water temperature during washing was maintained at approximately 5C using crushed ice. After each wash, the mince was manually pressed on cotton. At the end of processing, 1% sucrose was added as a primary cryoprotectant and 2% sodium chloride was used as a flavor enhancer, which masked the sweetness, because Brazilians dislike surimi-based products with strong sweet taste. The treatment with 10 or 20% of starch addition was done slowly until homogenization. Adding starch was performed to simulate the fish burger production according Fogaça (2009). The addition of cryoprotectants (sucrose), flavor enhancer (sodium chloride) and texture enhancer (starch) was performed using an electric mixer (Arno, Planetaria, São Paulo, Brazil), then surimi was frozen.

Surimi Gel Preparation

Surimi samples were thawed and placed in ham forms during baking and the induction of surimi gelatinization.

Each sample was directly cooked to a gel in a bath (Novatecnica, NT 249, Piracicaba, SP, Brazil) and heated at 90C for 30 min. After cooking, samples were cooled using crushed ice for 15 min to stop the process.

Shear Stress

The shear stress of fish ham was measured using an SMS TA-XT PlusTexture Analyzer (Stable Micro Systems, UK) and blade attachment (Warner-Bratzler shearing device) with a load cell of 5 kg and a crosshead speed of 12 cm/min at a distance of 1.5 cm. Cubes (1 cm^3) were compressed in the axial direction until they completely cut the sample. The computer program Texture Expert Exceed version 2.5 was used for data collection (25 measurements per treatment) and shear stress calculation (expressed in KPa). Shear stress was carried out at room temperature (20–22C).

Moisture

The moisture content was determined based on the weight difference of 2 g surimi before and after heating in an oven (Fanen, São Paulo, Brazil) for 24 h at 105C (method 950.46) (Association of Official Analytical Chemists 2005). All samples were stored at −18C and thawed at 5C for a period of 24 h prior to analysis.

Scanning Electron Microscopy (SEM)

The microstructure of gels prepared using the different methods was determined with a SEM. Samples with a thickness of 5 mm were fixed using 2.5% buffered glutaraldehyde and post-fixed in 1% osmium tetroxide for 2 h. They were then washed in phosphate buffered saline, dehydrated in ethanol and dried to the critical point using CO_2. Samples were metalized with pale-gold ions prior to electron micrography.

Design and Statistical Analysis

The experimental design was a three-level Central Composite Design (Box and Draper 1987) that included 18 runs divided into two blocks at each time (Table 1). The central point was replicated six times (five within a block) and the two-level factorial portion was replicated twice. The responses were analyzed by fitting RSMs (second order polynomials) after coding both factors, i.e., washing cycles (x_1) and starch amount (x_2), such that their levels were centered at zero and their maximum and minimum levels were 1 and −1, respectively. The "lm" function from R (R Development Core Team 2011) was used for the calculations. *F*-tests from the analysis of variance were used for deciding for parsimonious fitted models following the hierarchy principle applied to the polynomial terms (Wu and Hamada 2009). Significance was attained for *P*-value smaller than 0.05.

RESULTS

In the analysis of the response variable moisture, the analysis of variance and associated *F*-tests for model effects indicated significant linear effects of the number of washing

TABLE 1. EXPERIMENTAL DESIGN (X_1, X_2), MOISTURE (M) AND SHEAR FORCE (SF) ACCORDING TO THE NUMBER OF SAMPLE WASHINGS (W) AND THE PROPORTION OF ADDED CASSAVA STARCH (S)

Treatment*	$x_1 = \frac{W-3}{2}$	W	$x_2 = \frac{S-10}{10}$	S (%)	y_1 M (%)†	y_2 SF (KPa)†
W1S0	−1	1	−1	0	75.24 (0.43)	32.5 (5.9)
W1S20	−1	1	1	20	65.14 (0.20)	32.0 (5.7)
W5S0	1	5	−1	0	83.24 (0.18)	43.0 (11.6)
W5S20	1	5	1	20	71.86 (0.14)	42.4 (15.7)
W3S10	0	3	0	10	72.07 (0.27)	17.1 (5.5)
W3S10	0	3	0	10	78.54 (0.65)	22.4 (10.2)
W3S10	0	3	0	10	72.51 (0.89)	15.0 (5.6)
W3S10	0	3	0	10	72.38 (0.20)	16.3 (6.4)
W3S10	0	3	0	10	74.79 (0.36)	14.4 (5.6)
W1S0	−1	1	−1	0	72.61 (0.30)	18.0 (6.9)
W1S20	−1	1	1	20	64.57 (0.88)	16.6 (4.1)
W5S0	1	5	−1	0	78.88 (0.61)	24.7 (10.3)
W5S20	1	5	1	20	71.20 (0.93)	17.9 (5.3)
W3S10	0	3	0	10	68.53 (1.66)	18.0 (3.7)
W1S10	−1	1	0	10	69.31 (0.69)	11.5 (2.1)
W5S10	1	5	0	10	75.70 (0.29)	11.0 (1.0)
W3S0	0	3	−1	0	75.44 (0.97)	11.4 (1.7)
W3S20	0	3	1	20	66.84 (0.09)	20.1 (2.9)

* Does not correspond to the randomized order.

† Mean (standard deviation). We used 25 readings for texture ($n = 25$) and four moisture analyses in triplicate ($n = 12$) per test.

TABLE 2. SECOND ORDER MODEL PARAMETER ESTIMATES FOR MOISTURE ($R^2 = 0.89$)

Parameter	Estimate	Standard error
Intercept	71.09	1.00
Block effect	2.64	0.97
x_1	3.40	0.63
x_2	–4.58	0.63
x_1^2	0.96	1.14
x_2^2	–0.41	1.14
$x_1.x_2$	–0.12	0.70

cycles (P-value = 0.0002) and starch content (P-value <0.0001). There was no evidence of curvatures with respect to both factors or any interaction between them (P-values = 0.4606, 0.7272 and 0.87334, respectively). Parameter estimates and their standard errors are presented in Table 2. The fitted equation, averaging over the two blocks, resulted in: $\hat{y}_1 = 72.41 + 3.40x_1 - 4.58x_2$, where $\hat{y}_1$ is the fitted response moisture. The fitted model indicated that moisture increases with washing cycles and decreases with starch addition (as expected). The contours and plane surface for the fitted response moisture are shown in the first row of Fig. 1. The estimates show that any level of moisture between 65 and 80% can be obtained by varying the starch level and the number of washing cycles. Moisture levels between 75 and 78% can be achieved by reducing the starch level if the number of cycles is low or vice-versa.

Analysis of variance for the response shear stress showed strong evidence of curvature effects for both washing cycles (P-value = 0.0026) and starch (P-value = 0.0108) factors. Again, there was no evidence of interaction between the two factors (P-value = 0.7406). Parameter estimates and standard errors are presented in Table 3. The fitted equation, averaged over the two blocks, resulted in: $\hat{y}_2 = 12.70 + 2.84x_1 - 0.06x_2 + 5.53x_1^2 + 10.03x_2^2$, where $\hat{y}_2$ is the fitted response shear stress. Contours and surfaces for the estimated response are shown in the second row of Fig. 1. A region of minimum response can be seen from these plots and the partial derivatives of the shear stress with respect to washing cycles and starch addition indicated that the estimated shear stress attained the minimum when starch amount was around 10 and washing cycles around 2.5 which means in practice, two or three cycles.

TABLE 3. SECOND ORDER MODEL PARAMETER ESTIMATES FOR SHEAR STRESS ($R^2 = 0.78$)

Parameter	Estimate	Standard error
Intercept	6.20	2.85
Block effect	13.00	2.79
x_1	2.84	1.81
x_2	–0.06	1.81
x_1^2	5.53	3.28
x_2^2	10.03	3.28
$x_1.x_2$	–0.69	2.04

In accordance with the model in optimum conditions for tilapia surimi production, the shear force predicted is 12.5 KPa while in practice conditions, using three washing cycles, the shear force determined is 17.2 ± 2.86 KPa.

The highest values for shear stress were observed with 0 and 20% starch and one or five washing cycles. The protein structure of surimi with 0% starch and one washing cycle was intact and the fat droplets remained within the protein network (Fig. 2A). The protein structure was surrounded by starch granules while the fat droplets appeared to be wrapped in starch granules (20% starch and one washing cycle) (Fig. 2B). With five washing cycles, 0% starch it was found that the protein structure contained gaps, probably due to changes in protein composition and the elimination of soluble proteins, which consequently increased the concentration of myofibrillar proteins (Fig. 2C), while with five washing cycles, 20% starch that the structure was now formed of a mass of protein, starch and fat droplets (Fig. 2D).

The surimi gel produced with 10% starch and three washing cycles had an intermediate structure with amorphous starch granules that were probably gelatinized, while the fat droplets were evenly distributed throughout the surimi structure (Fig. 3).

DISCUSSION

Since the surimi produced in this study was to be used for fish burger production, it was desirable that the product had a soft texture with a low shear force. Burgers formulated from blends, in particular tapioca starch, present improved tenderness and in particular lowered shear force (Troy *et al.* 1999). Based on these considerations, we estimated that the shear stress was minimized when the starch amount was approximately 10% with approximately 2.5 washing cycles, which in practice meant three cycles (Fig. 1, second row). A micrograph of this treatment (Fig. 3) shows that the presence of fat globules and a starch network surrounded by the protein matrix promoted the soft texture.

The use of three washing cycles during the preparing of surimi from tilapia fillets, with a fish-to-water ratio of 1:4 (w/v) or 1:3 (w/v), is recommended by several authors (Rawdkuen *et al.* 2009; Duangmal and Taluengphol 2010; Ingadottir and Kristinsson 2010; Mahawanich *et al.* 2010).

The addition of starch can enhance the textural characteristics of breaded or restructured products made with surimi. There is an increasing interest in identifying

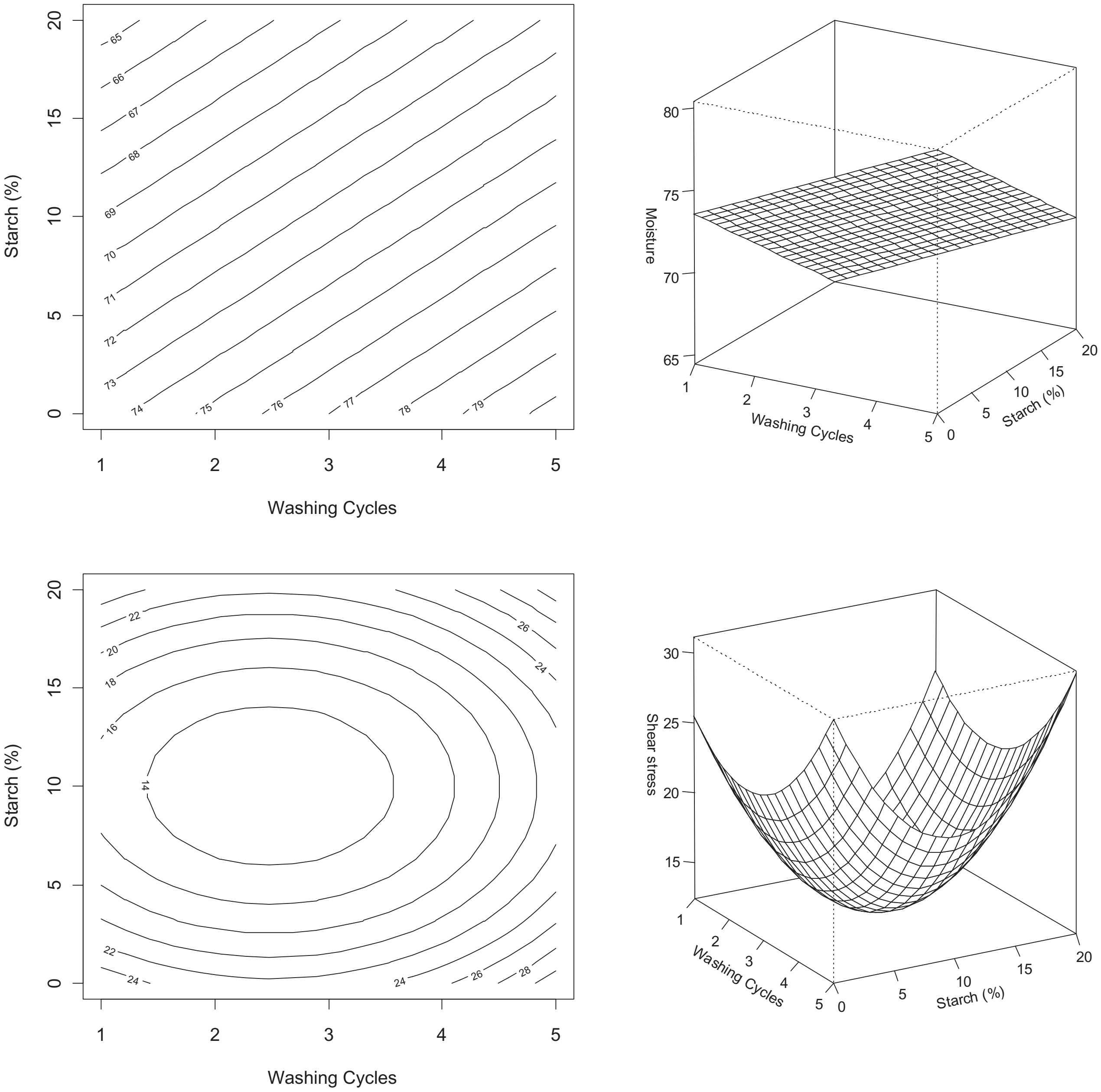
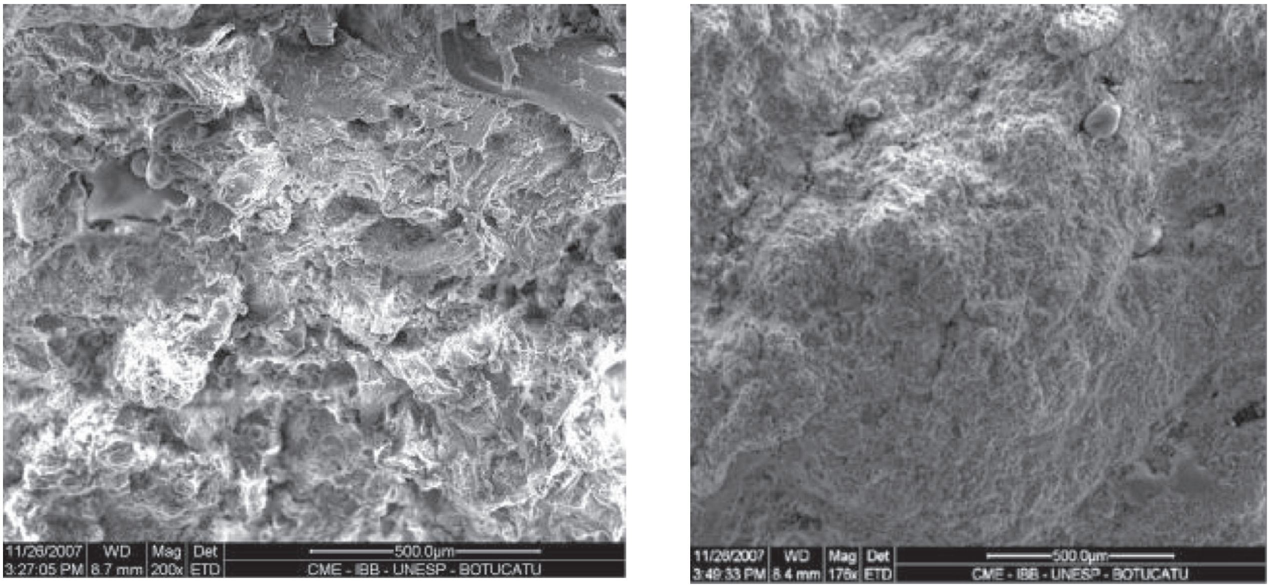


FIG. 1. FITTED CONTOURS AND SURFACES FOR THE RESPONSE VARIABLES MOISTURE (FIRST ROW) AND SHEAR STRESS (SECOND ROW)

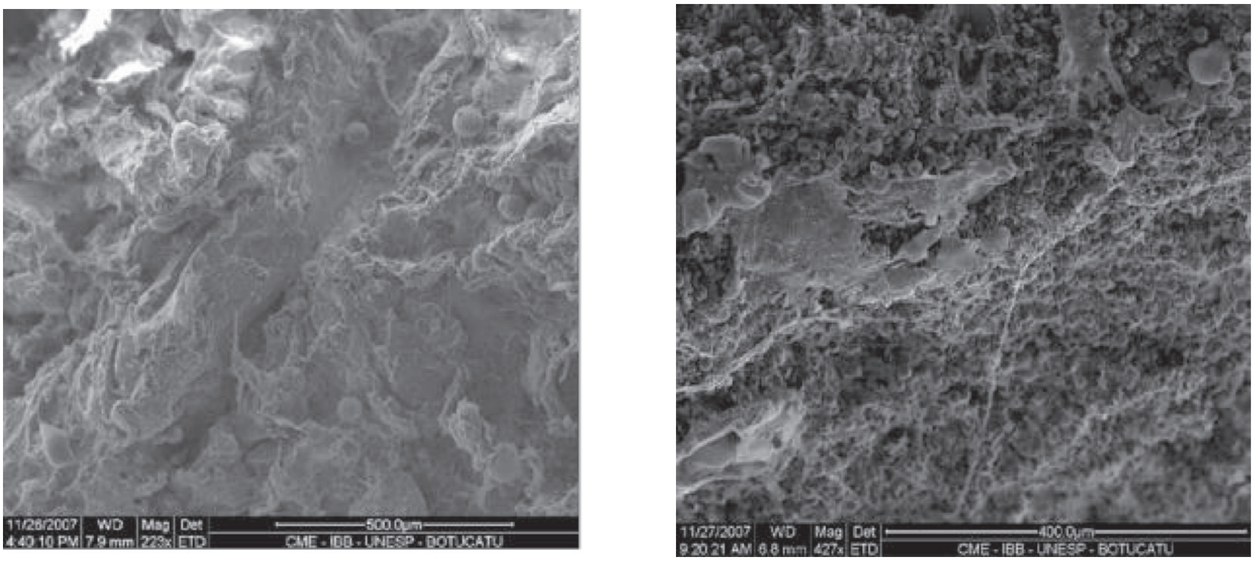
novel applications for both native and modified starches to improve the mechanical and functional properties of surimi products (Ramirez *et al.* 2011). In spite of their higher amylose content, about 18%, tapioca starches give relatively stable, clear sols on cooling, because this amylose is higher in molecular weight and may be more branched than other starches (Mason 2009). Furthermore, the addition of salt helps to disperse the fish proteins in the tapioca starch system (Chow and Yu 1997).

The U.S. Department of Agriculture has also authorized the use of a Commercial Item Description, for surimi products, whose analytical requirements specify that starch should constitute no more than 10% of the total (USDA 2008). The relevant Brazilian law, drafted by the Ministry of Agriculture, Livestock and Supply, states that breaded products can possess 30% carbohydrates and at least 10% protein (Brasil 2012). Thus, the amount of tapioca starch in the optimum condition meets both laws.



A 0% Starch, one washing cycles

B 20% Starch, one washing cycles



C 0% Starch, five washing cycles

D 20% Starch, five washing cycles

FIG. 2. MICROSTRUCTURE OF TILAPIA SURIMI GEL WITH 0 OR 20% STARCH AND ONE OR FIVE WASHING CYCLES

CONCLUSIONS

The optimizing process will contribute to sustainable production of tilapia surimi, by utilizing filleting by-products in the preparation of restructured products with high biological value, such as the fish burger and by reducing the amount of water wasted with excessive washings. The optimal conditions for tilapia surimi made with MRFM were the addition of 10% tapioca starch and three washing cycles. Further studies are needed to determine the optimum quantity of water used for washing (the water-to-fish ratio) in each cycle.

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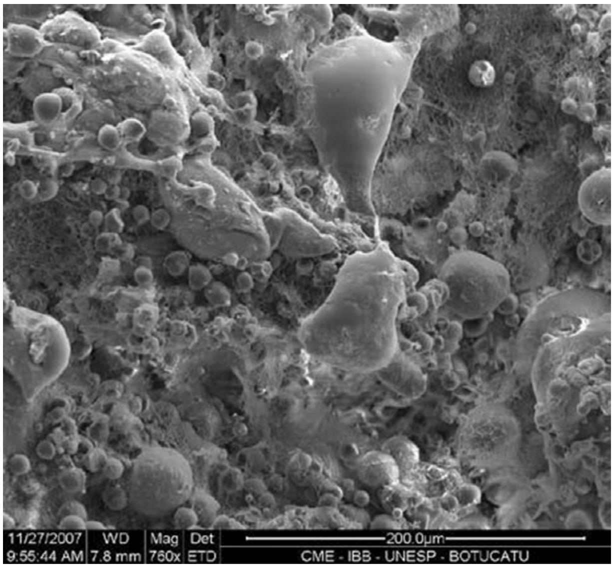

FIG. 3. MICROSTRUCTURE OF TILAPIA SURIMI GEL WITH 10% STARCH AND THREE WASHING CYCLES

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