BOLETIM DO INSTITUTO DE PESCA
ISSN 1678-2305 online version
Artigo Científico

EMULSIFIED FILMS PRODUCED WITH PROTEINS EXTRACTED FROM WHITEMOUTH CROAKER BYPRODUCTS: DEVELOPMENT AND CHARACTERIZATION

Lorena Limão VIEIRA[1]
Cleidiane da Silva ARAÚJO[1]
Eleda Maria Paixão Xavier NEVES[1]
Jáira Thayse Sousa BATISTA[1]
Maria Regina Sarkis PEIXOTO JOELE[2]
Lúcia de Fátima Henriques LOURENÇO[1]

[1]Universidade Federal do Pará – UFPA, Programa de Pós-graduação em Ciência e Tecnologia de Alimentos, Laboratório de Produtos de Origem Animal, Rua Augusto Corrêa, 01, Campus Universitário do Guamá, Guamá, CEP 66075-110, Belém, PA, Brasil. E-mail: luciahl@ufpa.br (corresponding author).

[2]Instituto Federal de Educação, Ciência e Tecnologia do Pará - IFPA, Rod. BR 316, Km 63, s/n, Campus Castanhal, Setor Rural, CEP 68740-970, Castanhal, PA, Brasil.

Received: January 22, 2018
Approved: March 29, 2018

ABSTRACT

This study aimed to develop and characterize biodegradable films from myofibrillar proteins from whitemouth croaker (*Micropogonias furnieri*) using stearic acid (SA) to improve its technological properties. The proteins extracted were lyophilized, characterized, and used to prepare the films. The filmogenic solutions were dried on a silicone stand at 35 °C for 14 h in an incubator oven according to the casting method. The region with the best mechanical, physical, and barrier properties in the films was defined by a full factorial design. The lyophilized myofibrillar proteins (LMP) had protein content of 96.03%. The analysis of the experimental design results indicated the best conditions to prepare the optimized film were 2.84% LMP, 3.18% SA, and 78.41% SDS (sodium dodecyl sulfate). The control films were prepared with 2.84% LMP and 30% plasticizer. The optimized film had significantly lower water vapor permeability (5.87E-11 g m m^{-2} s^{-1} Pa^{-1}), higher tensile strength (6.35 MPa), and lower elongation (235.60%) compared with control ($p \leq 0.05$). It also had lower transparency values and excellent UV barrier property, which indicates a tendency to opaque. Thermal stability was good and the microstructure revealed a structural change in the filmogenic matrix, confirmed by x-ray diffraction, which indicates influence of SDS and SA on film crystallinity. Solubility increased by 22% and swelling decreased slightly in the optimized film compared to the control. The results obtained represent a positive contribution with the use of fish byproducts by applying alternative, sustainable technologies.

Keywords: film; myofibrillar proteins; stearic acid; dodecyl sodium sulfate.

FILMES EMULSIONADOS PRODUZIDOS COM PROTEÍNAS EXTRAÍDAS DE SUBPRODUTOS DE CORVINA: DESENVOLVIMENTO E CARACTERIZAÇÃO

RESUMO

O objetivo deste trabalho foi desenvolver e caracterizar filmes biodegradáveis de proteínas miofibrilares de aparas da filetagem da corvina (*Micropogonias furnieri*) utilizando ácido esteárico (AE) para melhorar suas propriedades tecnológicas. As proteínas extraídas foram liofilizadas, caracterizadas e utilizadas na elaboração dos filmes. As soluções filmogênicas foram secas em suporte de silicone a 35°C por 14 horas em estufa incubadora, de acordo com o método *casting*. A região com as melhores propriedades mecânicas, físicas e de barreira dos filmes foi definida por planejamento fatorial completo. As proteínas miofibrilares liofilizadas (PML) apresentaram teor proteico de 96,03%. A análise dos resultados do planejamento experimental indicou que as melhores condições para elaborar o filme otimizado foram: 2,84% PML, 3,18% AE e 78,41% SDS (dodecil sulfato de sódio). Os filmes controles foram elaborados com 2,84% de PML e 30% de plastificante. O filme otimizado apresentou significativa diminuição da permeabilidade ao vapor de água (5,87E-11 g m m^{-2} s^{-1} Pa^{-1}), com maior resistência a tração (6,35 MPa) e menor elongação (235,60%) quando comparado ao controle ($p \leq 0,05$. Apresentando também menores valores de transparência e excelente propriedade de barreira UV, indicando tendência ao opaco. Apresentou boa estabilidade térmica e a microestrutura revelou mudança estrutural na matriz filmogênica, confirmado pela difração de raio-x, indicando a influência do SDS e AE na cristalinidade do filme. Houve aumento de 22% na solubilidade e ligeira diminuição do intumescimento do filme otimizado em relação ao controle. Os resultados obtidos representam contribuição positiva com o aproveitamento de subprodutos de peixe aplicando tecnologias alternativas e sustentáveis.

Palavras-chave: filme; proteínas miofibrilares; ácido esteárico; dodecil sulfato de sódio.

INTRODUCTION

Excessive use of synthetic packaging materials from non-renewable sources and their disposal in the environment has been causing growing concern and requires alternatives to control and/or decrease the use of this type of material. On the other hand, the fish industry produces large amounts of byproducts such as skins, scales, and parings during fish filleting. Fish filleting parings contain proteins that can be used to create biodegradable films with several applications, besides minimizing the environmental impact generated by the disposal of these residues (PASCHOALICK *et al*., 2003; PIRES *et al*., 2011; HALAL *et al*., 2016; TAO *et al*., 2015).

Myofibrillar proteins, obtained from fish muscle, have been studied and applied to prepare films (ROSTAMZAD *et al*., 2016) and figure among the most widely used natural biopolymers as they are able to form a continuous, cohesive matrix (ZAVAREZE *et al*., 2012). Researches show that films prepared with fish proteins also have good technological properties (CHINABHARK *et al*., 2007; PRODPRAN *et al*., 2007; ARTHARN *et al*., 2009; LIMPAN *et al*., 2010; ZAVAREZE *et al*., 2012; ZAVAREZE *et al*., 2014; TAO *et al*., 2015; PHAKAWAT *et al*., 2015; WENG and ZHENG, 2015; KAEWPRACHU *et al*., 2016; ROSTAMZAD *et al*., 2016).

The use of biodegradable films as food packaging is a global trend that has significantly expanded in recent years, driving studies on the development and characterization of such films (KHWALDIA *et al*., 2010). Biodegradable films obtained from biological materials act as a barrier against external elements, protecting packaged foods from physical and biological damage, decreasing compound volatilization and moisture loss, and extending product shelf life.

Films prepared with proteins have excellent mechanical and optical properties and are good barriers to O_2 and CO_2 in environments with low relative humidity. However, in the presence of high moisture, the film may become susceptible to deterioration, which is not technologically viable (GALLO *et al*., 2000; DAVANÇO *et al*., 2007). The water vapor barrier property is considered one of the most important because, depending on the water vapor transfer rate, spoilage processes may begin in the packaged food (PRODPRAN *et al*., 2007; CARPINÉ *et al*., 2015).

Researches on the preparation of composite films (added with hydrophobic components) have been carried out to improve permeability, strength, flexibility, and nutritional value. A hydrophobic component is added to the film-forming suspension of emulsified composite films, where the lipid component acts as water vapor barrier and the protein or polysaccharide provide the mechanical characteristics required for a good film and oxygen barrier (ANKER *et al*., 2002). When the lipid is applied onto the protein film layer, the resulting film is called bilayer (GALLO *et al*., 2000).

Among the lipid materials studied, waxes, long-chain saturated fatty acids, and fatty alcohol were the most effective in providing moisture barrier properties to hydrocolloid films (YANG and PAULSON, 2000). However, emulsifying or surfactant agents are often required to improve the stability of lipid particles in the protein matrix. Surfactants are compounds that have interface activity between two phases, such as air-water, oil-water, and on the surface of solids as they have two distinct regions of the same molecule: one polar hydrophilic and the other non-polar hydrophobic (DAVANÇO *et al*., 2007).

This study aimed to develop and characterize biodegradable films from myofibrillar proteins from byproducts (parings) from whitemouth croaker (*Micropogonias furnieri*) processing using stearic acid (SA) to improve its technological properties.

METHODS

Filleting parings from whitemouth croaker (*Micropogonias furnieri*) were donated by the industry G-Pesca, located in the city of Bragança, PA, Brazil. The parings were placed in polyethylene packaging, stored in an isothermic box with flake ice, and transported to the laboratory. They were then hygienized by immersion in 5 ppm (mg L^{-1}) chlorinated water for 5 min and the spines and skin residues were removed and manually pressed to remove excess water and obtain the muscle. Next, the muscle was ground in a cutter food processor (Filizola, São Paulo, SP, Brazil) for 60 s, placed in polyethylene bags, vacuum-packaged, and frozen at - 18 °C.

Extracting lyophilized myofibrillar proteins

The methodology proposed by ZAVAREZE *et al*. (2012) was used, with modifications, to obtain the lyophilized myofibrillar proteins (LMP). The ground muscle was added with five volumes of 50 Mm NaOH at 7 °C for 5 min, centrifuged at 10,000 rpm for 3 min at 4 °C in a refrigerated centrifuge (Thermo Fisher, Multifuge X1R), and filtered. This process was repeated three times. After those steps, the myofibrillar proteins were placed onto stainless steel trays, frozen at -18 °C, and lyophilized (Liotop, L101) at -60 °C for 48 h. The lyophilized proteins were then sieved (Tyler 20 0.84 mm mesh), weighed, vacuum packaged, and kept at -18 °C. Myofibrillar protein yield was obtained from the ratio between the lyophilized protein and the initial amount of raw material (parings), expressed in g LMP 100 g^{-1} muscle (KAEWRUANG *et al*., 2013).

Characterizing the raw material (parings) and LMP

Analyses of proteins, lipids, moisture, and ash (AOAC, 1997) were carried out on the raw material (parings) and on the myofibrillar proteins.

Experimental design

In order to obtain the optimized film, a full factorial 2^3 experimental design was carried out with 17 experiments (Table 1), being eight factorial assays (combination between levels ±1), three assays at the central point (three variables at level 0), and six assays at the axial levels ±α. The input (independent) variables were concentrations of LMP, AE, and SDS. The dependent variables were water vapor permeability (WVP), tensile strength (TS), and percent elongation (E). The definition of the levels of the

Table 1. Coded matrix of the full factorial 2^3 design for whitemouth croaker myofibrillar protein film optimization.

Assays	Independent variables			Dependent variables (Responses)		
	LMP	SA	SDS	WVP (g m m^{-2} s^{-1} Pa^{-1})	TS (MPa)	E (%)
1	1.5 (-1)	10 (-1)	60 (-1)	7.67E-11	2.06	215.76
2	1.5 (-1)	10 (-1)	80 (+1)	7.52E-11	2.44	217.66
3	1.5 (-1)	30 (+1)	60 (-1)	5.02E-11	4.41	219.32
4	1.5 (-1)	30 (+1)	80 (+1)	6.98E-11	3.69	218.53
5	2.5 (+1)	10 (-1)	60 (-1)	1.15E-10	3.64	248.16
6	2.5 (+1)	10 (-1)	80 (+1)	8.26E-11	5.01	239.06
7	2.5 (+1)	30 (+1)	60 (-1)	1.20E-10	3.89	237.45
8	2.5 (+1)	30 (+1)	80 (+1)	7.55E-11	3.71	227.89
9	1.15 (-1.68)	20 (0)	70 (0)	4.84E-11	2.77	221.84
10	2.84 (1.68)	20 (0)	70 (0)	7.23E-11	4.27	240.51
11	2 (0)	3.18 (-1.68)	70 (0)	6.40E-11	3.38	245.05
12	2 (0)	36.82 (1.68)	70 (0)	6.97E-11	4.21	233.08
13	2 (0)	20 (0)	53.18 (1.68)	1.22E-10	3.49	233.03
14	2 (0)	20 (0)	86.82 (1.68)	9.22E-11	3.80	227.65
15	2 (0)	20 (0)	70 (0)	9.31E-11	3.16	236.51
16	2 (0)	20 (0)	70 (0)	9.13E-11	3.46	238.22
17	2 (0)	20 (0)	70 (0)	8.63E-11	3.12	240.20

LMP: Lyophilized myofibrillar proteins; SA: Stearic acid; SDS: Dodecyl sodium sulfate; WVP: Water vapor permeability; TS: Tensile strength; E: Elongation.

variables studied was based on the literature (DAVANÇO *et al*., 2007; LIMPAN *et al*., 2010).

The response surface methodology was used to verify the behavior of the system, combining the independent and dependent (response) variables. For each response, the significance of the variables or interactions in the polynomial equation described (Equation 1) was verified. After the exclusion of the non-significant effects, the equations and influence plots of the independent variables on the dependent ones were obtained. The results enabled establishing the concentrations of myofibrillar proteins, fatty acid, and surfactant to obtain a film with low WVP and high TS and E values. Thus, some criteria were followed based on the restrictions mentioned for the minimum, intermediate, and maximum values of each response aiming to find the optimal operational values of the indepen dent variables that simultaneously meet the requirements of the response variables (desirability function).

$$Y = \beta_0 + \beta_1(LMP) + \beta_{11}(LMP)^2 + \beta_2(SA) + \beta_{22}(SA)^2 + \beta_3(SDS) + \beta_{33}(SDS)^2 + \beta_{12}(LMP\,x\,SA) + \beta_{23}(SA\,x\,SDS) + \beta_{13}(LMP\,x\,SDS) \quad (1)$$

Film preparation

The films were prepared according to the methodology described by DAVANÇO *et al*. (2007) with adaptations. Initially, the filmogenic solutions with different concentrations of LMP, SA, and SDS were prepared and 30% glycerol was added to each solution according to the full factorial design (Table 1).

The pH of the solution was adjusted to 12 with 2 M NaOH, the solution was homogenized at 10,000 rpm for 5 min (Turratec/Tecnal, TE-102), and then placed in a water bath at 70 °C/30 min. According to the casting method, 130 mL of the filmogenic solution obtained were added to a silicone support with 22 cm diameter and 3 cm height and dried in an incubator oven (Quimis, Q315M) at 30 °C for 16 h. Next, the films were placed in polyethylene packaging and stored at room temperature. For comparison purposes, a control biofilm was prepared with the same concentrations of LMP and plasticizer as the optimized film, however, with no added SA or SDS.

Characterization of the optimized film

Thickness

Film thickness was measured using a digital micrometer with 0.001 mm resolution (Insize, model IP54). Eight random spots were measured around each film while observing a distance of 60 mm from the edge (ZAVAREZE *et al*., 2012).

Mechanical properties

Film TS and E were measured in a texture analyzer (QTS, Brookfield) at room temperature (ASTM, 1996). The initial gap of the grips and probe velocity were 50 mm and 1 mm.s^{-1}, respectively

(ZAVAREZE *et al*., 2012) and the samples were cut into 100 mm x 25 mm strips. TS was calculated as TS = Fm/A (Fm=maximum force at the moment of film rupture (N) and A=area (m^2) of the film cross-section). Elongation was calculated as $E=d_f/d_{initial}$ x 100 (d_f=total distance at the moment of rupture (mm) and $d_{initial}$=initial distance of 50 mm of grip separation).

Water vapor permeability

The modified American Society for Testing and Materials (ASTM, 1996) method, described by ARFAT *et al*. (2014), was used. The films were placed onto a glass permeation container with 4.5 cm diameter and 7.0 cm height containing 10 g silica gel (0% RH; 0 Pa water vapor pressure at 30 °C) and fixated with silicone adhesive. Next, the permeation containers were placed in desiccators with distilled water at 30 °C (99% RH; 4,244.9 Pa vapor pressure at 30 °C) and weighed every hour for 10 h. WVP (g m^{-1} s^{-1} Pa^{-1}) was calculated by $WVP=W.X/At.\Delta P$ (W=weight gain by the desiccant (g); X=film thickness (mm); A=surface area of the exposed film (m^2); t=incubation time (h); ΔP=partial pressure difference (Pa)). Three specimens were used for the tests.

Solubility and swelling

In order to determine solubility, the initial dry matter of the films was established in an oven at 105 °C/24 h and they then were immersed in 50 mL water. The system was stirred in a refrigerated shaker incubator (Cielanb, model CE-725B) at 150 rpm for 24 h at 25 °C. After this period, the samples were removed and dried (105 °C for 24 h) to determine the dry matter undissolved in water (GONTARD *et al*., 1994).

In order to determine swelling, a sample 2 cm in diameter was weighed, immersed in 75 mL distilled water, and placed in an incubator oven (Quimis, Q315M) at 25 °C for 6 h. After this procedure, excess water in the films was removed with filter paper and the sample was weighed again. Swelling was determined as the weight gain of the sample divided by the initial weight of dry solids (LEE *et al*., 2004).

Light transmittance and transparency

Light transmittance was measured in the ultraviolet and visible ranges (200 and 800 nm) in specimens cut into rectangles and placed in the inner side of the cuvette. Transparency was determined at 600 nm and calculated by $T=-logT/e$ (T=transmittance at 600 nm; e=film thickness in mm) according to the ASTM D1746 method cited by ARFAT *et al*. (2014). Both parameters were quantified using a spectrophotometer (Biospectro, model SP-22).

Color parameters

Biofilm color was determined using a MINOLTA CR-310 colorimeter to obtain parameters L* (luminosity), a* (red intensity), and b* (yellow intensity), while values of C* (chroma), h* (hue angle), ΔE (total color difference), and ΔC (difference in chroma values) were calculated.

Differential Thermal Analysis (DTA) and Thermogravimetry (TG)

The thermal analyses were carried out in a SHIMADZU DTG-60AH device at 25 to 600 °C using heating rate of 10 °C/min and nitrogen flow at 40 mL/min in an alumina crucible. Data were acquired and treated in the software TA60 version 2.21.

X-Ray Diffraction (XRD)

The samples were placed onto the glass stand of the device and exposed to Cu radiation (Kα1=1.540598 Å) submitted to 40 kV voltage and 40 mA current of l=1.78897 x 10^{-1} nm in a BRUKER D8 Advance diffractometer with Bragg-Brentano geometry and LynxEye detector with 0.4 s scan time in the Bragg-Brentano geometry of 5<θ<60, 0.6 mm divergence slit, 2.5° Soller slit, and Kβ Ni filter. The diffractograms were collected with 0.02° step angle and 0.4 s step time.

Film surface microscopic characterization

The upper surface microstructure of the film was analyzed in a scanning electron microscope (SEM) (Tescan, VEGA3) with acceleration voltage of 5 kV, electron beam current of 85-90 μA, and 15 mm work distance for the secondary electron images. The samples were metallized twice with gold/palladium with 5 mA for 120 s in a Quorum Technologies SC7620 sputter coater.

Statistical analysis

The results underwent statistical analysis using the software Statistica® 10.0 (STATSOFT, 2011) by assessment of effects, analysis of variance, and response surface.

RESULTS

The raw material extracted and lyophilized had high myofibrillar protein content (96.03% dry basis), important for the formation of the biopolymer matrix, and low lipid content (0.94% dry basis), which were partially removed in the successive rinsings during extraction and centrifugation. However, ash concentration (2.21% dry basis) compared to the raw material/parings (2.88% dry basis) did not decrease much due to the presence of salt residues from the myofibrillar protein extraction process. Yield (13.25%) is highly influenced by the extraction of myofibrillar proteins, which eliminates lipids, minerals, bloods, sarcoplasm proteins, and stromal proteins. Moisture decreased between the raw material *in natura* (7.21% dry basis) and the lyophilized myofibrillar proteins (0.06% dry basis) due to the lyophilization process.

Film optimization

The results of the full factorial 2^3 design for whitemouth croaker myofibrillar protein film optimization is presented in Table 1.

Figure 1a shows the WVP level curves generated by the models proposed (Table 2) considering the mean points of the effects. It is noteworthy that this study expects to obtain low-permeability

films. The lowest WVP values are obtained at 60 to 80% SDS and 3.18 to 10% SA (or above 30%) combined with 1.5% LMP. A negative correlation of SDS with WVP can be observed, i.e., when the concentration of this surfactant increases, WVP decreases. The opposite effect occurs with LMP concentration on WVP, where higher protein concentrations increase permeability.

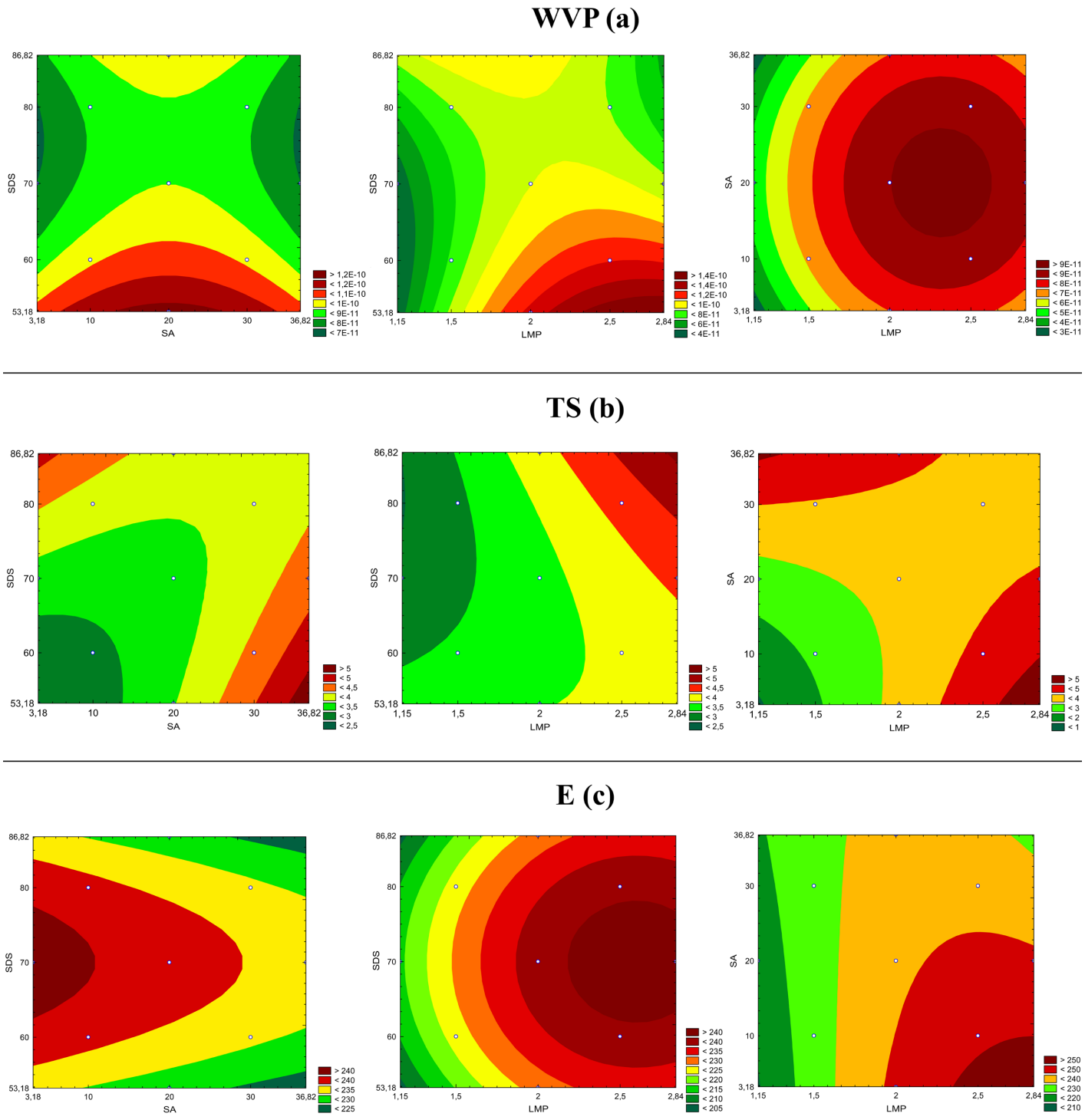

Figure 1. Level curves showing the effects of lyophilized myofibrillar protein (LMP), dodecyl sodium sulfate (SDS), and stearic acid (SA) on the optimized whitemouth croaker film: (a) water vapor permeability (WVP), (b) tensile strength (TS), (c) and percent elongation (E).

Figure 1b shows the negative influence of the SA x SDS interactions since the highest TS values are observed with the highest SDS and low SA concentrations. A similar behavior was observed for the LMP x SA interaction. The LMP x SDS interaction indicates the region of the highest levels (+1) in both effects favor the maximum TS value. This study aims to obtain films with high TS values and the best combination obtained for the three effects are SDS (60 to 80%), SA (3.18 to 15% or 25% to 36.82%), and LMP (2% to 2.84%).

Figure 1c shows that the highest E values are at the central point of SDS (60% to 80%) associated with the region with low SA level (3.18% to 10%) and high LMP level (2% to 2.84%). The increase in whitemouth croaker LMP concentration improves the mechanical properties (TS and E) of the film (Figure 1b and 1c).

Definition of the optimal process conditions: desirability function

After the statistical analysis of the results, criteria were adopted to determine the minimum, intermediate, and maximum values of each response of the independent variables that simultaneously meet the requirements of the response variables (desirability function) with high values for the mechanical properties (TS and E) and low values for permeability (WVP).

Based on the results of the desirability function, it was verified that, in order to obtain the maximum value of this global function (1.0), LMP, SA, and SDS concentrations must be 2.84%, 3.18%, and 78.41%, respectively. The film prepared with these values will have minimum WVP values (5.64E-11 g m^{-1} s^{-1} Pa^{-1}) and maximum TS (5.88 MPa) and E (253.58%) values.

Characterization of the optimized film

The results of mechanical, physical, barrier, and color characterization of the control and optimized films are presented in Table 3.

The parameters thickness and breaking force of the films analyzed (optimized and control) did not significantly differ (Table 3). The optimized film had the highest TS and the lowest E compared with control ($p \leq 0.05$). Adding SA and SDS impacted WVP reduction in the optimized film ($p \leq 0.05$) as the control film had 31% lower WVP.

Table 2. Reduced model for WVP, TS, and E as a function of the independent variables, F-test, R^2, and P.

Responses	Model	$F_{calculated}$		R^2	p (%)
		Regression	L.F.		
WVP	8.99E-11 + 1.18E-11 (LMP) - 0.92E-12 (LMP^2) - 6.98E-12 (SA^2) - 7.96E-12 (SDS) + 7.19E-12 (SDS^2) - 1.19E-11 (LMP SDS)	79.27	7.45	0.895	3.47
TS	3.26 + 0.45 (LMP) + 0.29 (SA) - 0.59 (LMP SA) - 0.28 (SA SDS)	30.70	3.10	0.976	5.22
E	238.65 + 8.25 (LMP) - 3.69 (LMP^2) - 2.75 (SA) - 3.99 (SDS^2) - 3.29 (LMP SA)	84.10	3.62	0.829	0.34

Regression: F_{TAB} = 19.33 (WVP); F_{TAB} = 19.25 (TS); F_{TAB} = 19.29 (E); Lack-of-fit: F_{TAB} = 19.37 (WVP); F_{TAB} = 19.396 (TS); F_{TAB} =19.385 (E).

Table 3. Mechanical, physical, barrier, and color characterization of the control and optimized films.

Determinations	**Results**	
	Control[1]	**Optimized[2]**
Film thickness (mm)	0.147±0.0029[a]	0.176±0.0181[a]
Breaking force (N)	14.98±1.5628[a]	17.45±0.9526[a]
Tensile strength (MPa)	3.99±0.7608[b]	6.35±0.8857[a]
Elongation (%)	364.93±4.3306[a]	235.60±11.3589[b]
WVP (g m m^{-2} s^{-1} Pa^{-1})	1.92E-10±1.72E-11[a]	5.87E-11±6.71E-12[b]
Solubility (%)	34.66±1.1633[b]	42.24±0.2786[a]
Film swelling ($m_{water}/m_{dry\ solids}$)	29.45±3.038[a]	24.85±0.7093[a]
Color parameters		
L*	89.17±0.5290[a]	87.34±0.4235[b]
a*	-4.51±0.2014[a]	-5.71±0.0741[b]
b*	11.25±0.6106[b]	19.32±0.8757[a]
h*	111.83±0.4001[a]	106.48±0.8001[b]
C*	12.13±0.6390[b]	20.15±0.8309[a]
ΔE	7.16±0.6683[b]	13.86±0.9479[a]

[1] Control film with 2.84% LMP and 30% plasticizer; [2] Optimized film with 2.84% LMP, 30% plasticizer, 3.18% stearic acid, and 78.41% SDS. The same letters in different columns do not differ according to Tukey's test at 5% significance ($p \leq 0.05$).

The solubility of the control film was 22% lower than that of the optimized one ($p \leq 0.05$). Water solubility of the films is directly related to the intermolecular interactions among its components, according to their structure and chemical characteristics (hydrophilicity and hydrophobicity), thus, the solubility of the optimized film was expected to decrease with the addition of SA and SDS.

Table 3 shows that the integrity of the optimized film was reduced by approximately 16%, with no significant difference compared with control.

Color parameters were impacted by SA and SDS addition to the film ($p \leq 0.05$). Color difference (ΔE) in the optimized film was greater than in the control, which indicates a more opaque film.

Light transmittance and transparency

Table 4 shows the effect of adding SDS and SA to the optimized film based on whitemouth croaker myofibrillar proteins on the barrier properties against radiation in the UV/Vis region as a function of transmittance at different wavelengths (200-800 nm) and transparency at T600 mm^{-1}.

In the wavelength range assessed, the transmittances obtained for the optimized film were significantly lower (Table 4), which indicates less transparent films with barrier property in the UV/Vis region. This property is considered important for food packaging since UV light causes oxidative spoilage of foods, leading to nutrient loss, loss of color, and off flavors (MARTINS *et al*., 2012). The optimized film had lower transparency ($p \leq 0.05$) than the control since the lower the value, the higher the transparency (Figure 2). Thus, adding SA and SDS directly impacted the light transmittance and transparency of the films.

Differential Thermal Analysis (DTA) and Thermogravimetry (TG)

The thermal behavior of the control and optimized films are shown in Figure 3 and the events described, in Table 5. Both films had four stages (events) of mass loss. The first event,

Table 4. Light transmittance and transparency of the control and optimized films.

Film	Light transmittance (%)								Transparency (T600 nm^{-1})
	Wavelength (nm)								
	200	280	350	400	500	600	700	800	
Control[1]	59.23[a]	27.30[a]	53.80[a]	63.47[a]	75.67[a]	77.47[a]	81.73[a]	81.50[a]	0.76±0.019a
Optimized[2]	0.00[b]	0.00[b]	4.73[b]	8.20[b]	15.03[b]	19.47[b]	26.30[b]	30.47[b]	4.07±0.319[b]

[1] Control film with 2.84% LMP and 30% plasticizer; [2] Optimized film with 2.84% LMP, 30% plasticizer, 3.18% stearic acid, and 78.41% SDS. Different letters in the same column do not differ according to Tukey's test at 5% significance ($p \leq 0.05$).

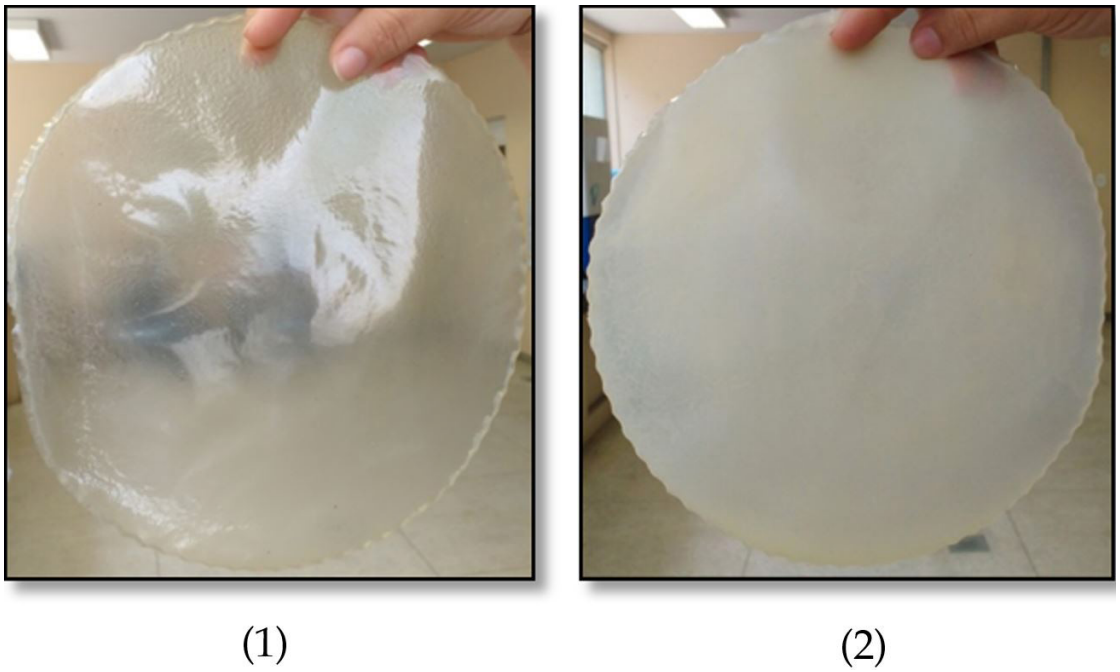

Figure 2. Control film with 2.84% LMP and 30% glycerol (1) and optimized film with 2.84% LMP, 30% glycerol, 3.18% SA, and 78.41% SDS (2).

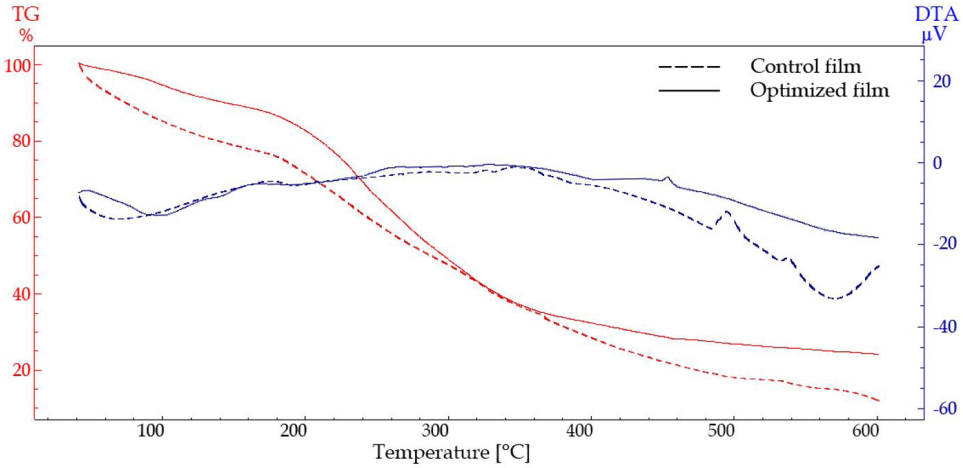

Figure 3. TG and DTA curves of the control and optimized films.

Table 5. Thermal events related to the thermogravimetric analysis of the control and optimized films.

Films	Event 1		Event 2		Event 3		Event 4		Res
	Tp_1 (°C)	P_1 (%)	Tp_2 (°C)	P_2 (%)	Tp_3 (°C)	P_3 (%)	Tp_4 (°C)	P_4 (%)	(%)
Control	60.81	4.77	169.42	15.73	472.42	54.92	555.57	58.15	39.38
Optimized	87.19	3.44	159.16	9.84	402.93	58.37	449.90	61.22	34.80

T_P: Peak degradation temperature in each event; *P(%)*: mass loss observed at each thermal event; *Res(%)*: final residue of the films at 600 °C.

corresponding to endothermic peaks (61.78 and 89.36 °C) was observer at 60.8 °C (with mass loss P_1=4.77%) for the control film and 87.19 °C (with mass loss P_1=3.44%) for the optimized film.

The second event, corresponding to exothermic peaks, was observed at peak temperatures of 169.42 °C (P_2=15.73%) and 159.16 °C (P_2=9.84%) for the control and optimized films, respectively, and is related to the breakdown of the protein fractions attributed to oxidation and combustion of this organic matter.

The third event (Tp_3=472.42 °C/P_3=54.92% for the control film and Tp_3=402.93 °C/P_3=58.37% for the optimized film) and the fourth event (Tp_4=555.57 °C/P_4=58.15% for the control film and Tp_4=449.90 °C/P_4=61.22% for the optimized film) are associated with the breakdown of glycerol, SA, and SDS.

Endothermic events (383.61, 481.72, 528.39, and 566.61 °C) are observed in the control film and one peak (396.91 °C) in the optimized film due to mass loss. Exothermic peaks are also verified for both films, at 492.91 and 533.54 °C for the control and 452.15 °C for the optimized. Such anomalies observed in the TG-DTA curves were due to the combustion of organic matter, in which the sample temperature exceeds that of the oven (of reference). Moreover, both films had residual mass, which represents the content of animal charcoal at 600 °C (Table 5).

X-Ray Diffraction (XRD)

The control film has a predominance of polymer structure and a single peak can be identified, even enlarged, at an angle of 9.36° (*d* spacing=9.45 Å), which indicates the formation of a quite amorphized structure, i.e., disordered and little detectable by x-ray diffraction with very little degree of crystallinity (Figure 4).

In the optimized film (Figure 4), the crystalline regions are clearly observed in the highest intensity peak of 2Θ=6.73° (*d* spacing=13.13 Å), showing the shift of peak d001 by the presence of SDS in the structure, as well as in the four less expressive diffraction peaks (2Θ) referring to SA at angles of 20.42°, 20.77°, 21.9°, and 22.21° (4.34, 4.28, 4.06, and 4.00Å of *d* spacing, respectively). The variation in interplanar spacing in the optimized film was between D001=13.13 Å and D001=2.28 Å, indicating crystallinity as verified by the presence of well-defined peaks in the diffractogram (Figure 4). The observations indicate the influence of SDS on the crystallinity of the optimized film was greater than that of SA, suggesting the protein matrix conformation changed.

Film surface microscopic characterization

The surface micrographs of the control and optimized films are shown in Figures 5 and 6, respectively.

Visually, the control film (Figure 2) had homogeneous, transparent, flexible aspect with no evidence of air bubbles or protein clumps. However, the microscopic analysis (Figure 5) revealed that the film has heterogeneous structure with empty spaces, bubbles, and protein clumps (or clusters), besides disperse insoluble particles in low-concentration (0.05 M) saline solution.

Figure 6 shows the influence of adding SA and SDS to the structure of the optimized film. A structural change in the

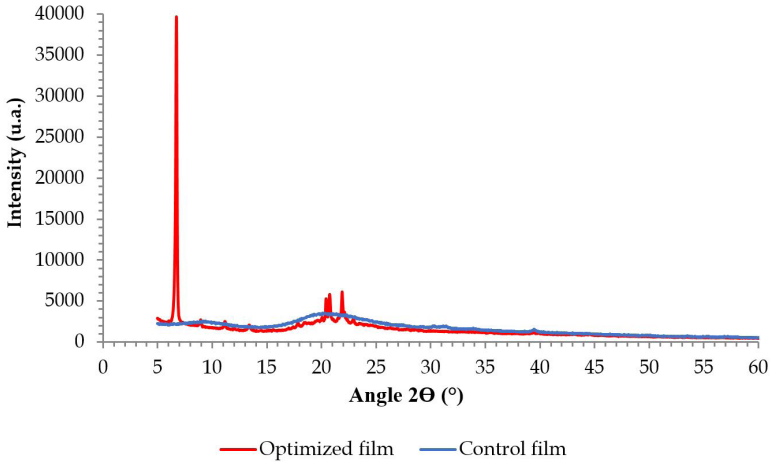

Figure 4. Diffractogram of the whitemouth croaker myofibrillar protein film, where: —Optimized film with 2.84% LMP, 30% glycerol, 3.18% SA, and 78.41% SDS; and —Control film with 2.84% LMP and 30% glycerol.

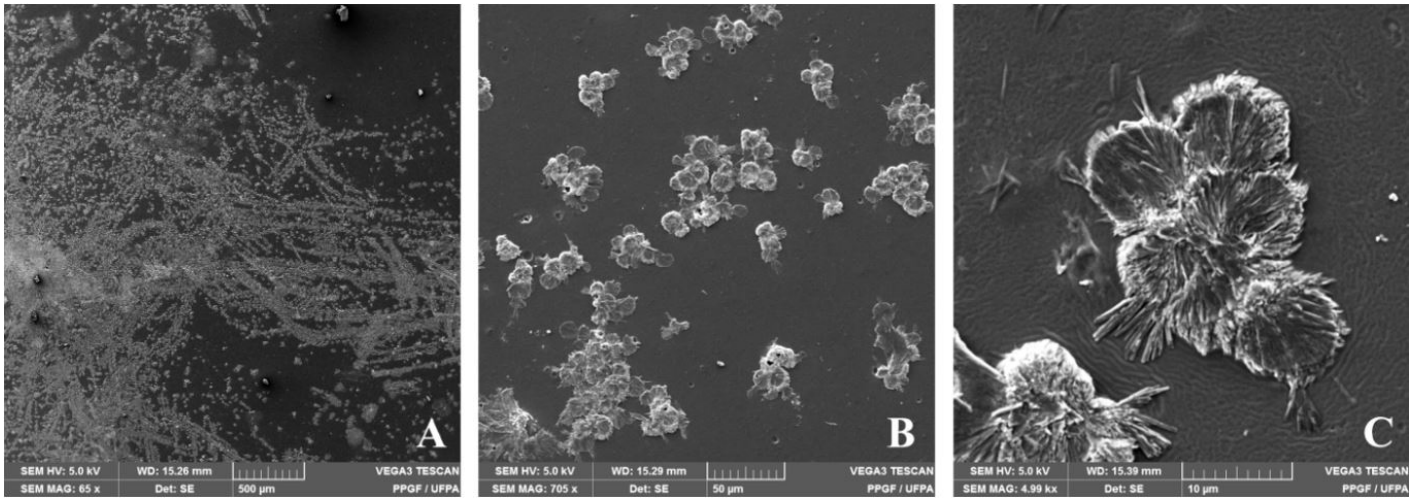

Figure 5. Microphotographs of the surface of the control film: A: 65x, B: 705x, and C≅5.000x.

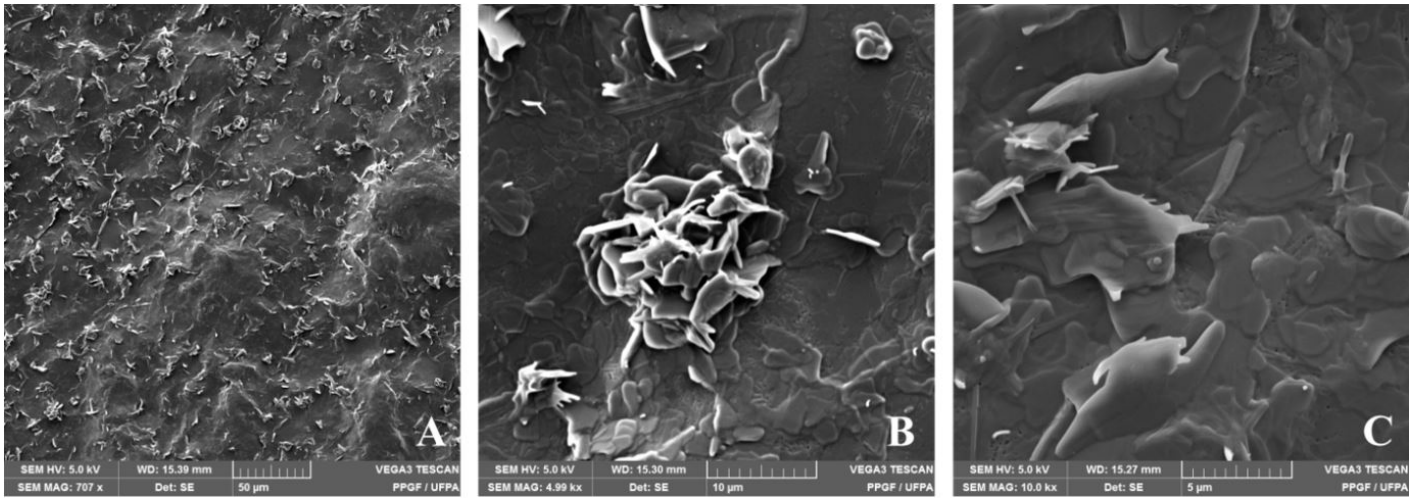

Figure 6. Microphotographs of the surface of the optimized film: A: 707x, B≅5.000x, and C: 10.000x.

filmogenic matrix can be observed compared to the control film. SDS concentration allowed incorporating SA, homogenizing the filmogenic matrix, which is compatible with the low WVP found for this film. Nonetheless, in spite of the presence of grooves and lumps in this film and of its rough, irregular surface, the film had excellent physical and mechanical characteristics.

DISCUSSION

ARAÚJO (2015), when analyzing LMP from gilthead bream, found similar results as the present study, such as moisture (0.07%), proteins (96.03%), and ash (2.86%), and lower lipids (0.74%). The LMP results in the present study also neared the 94.92% d.b. and 93.22% d.b. proteins obtained by BATISTA (2016) for gilthead bream and MONTERREY-QUINTERO and SOBRAL (2000) for Nile tilapia, respectively. The yield obtained in this research was close to the one described by PEREIRA (2015) and BATISTA (2016) at 14.10% and 16.8%, respectively.

SDS improved the water vapor barrier of the films in this study, which, according to RHIM (2012), is a desirable attribute in emulsified films to be used as food packaging. A similar behavior was also observed by DAVANÇO *et al*. (2007), who reported adding surfactants was effective in increased the water vapor barrier in the film.

In the present study, the best TS values were found with high protein concentrations since an increase in the number of protein chains per surface unit usually leads to a higher number of potential intermolecular interactions (CUQ *et al*., 1996 and KAEWPRACHU *et al*., 2016).

The elongation results show that, of all effects assessed, LMP had the greatest influence on this response (Figure 1c) as the higher protein content results in increased intermolecular aggregation (KAEWPRACHU *et al*., 2016). Similar results have been reported for films made with blue marlin sarcoplasmic protein (IWATA *et al*., 2000) and tilapia myofibrillar proteins (KAEWPRACHU *et al*., 2016).

Adding SDS and SA did not impact the thickness of the optimized film, however, PRODPRAN *et al*. (2007) and OLIVEIRA *et al*. (2012) reported increased thickness in fish myofibrillar protein films added with palm oil and gelatin films with coconut oil and Tween 20 surfactant, respectively. The increase in thickness may be influenced by the non-association of the surfactant with protein chains (GONTARD *et al*., 1994). This study used protein concentrations (2.84%) which led to thicker films (0.147 and 0.176 mm). DAVANÇO *et al*. (2007) obtained 0.095 mm thickness for gelatin films added with 10% SA and 70% SDS. The type of application of the film will indicate its ideal thickness. ZAVAREZE *et al*. (2012), when analyzing films with 3% whitemouth croaker myofibrillar proteins, obtained similar TS (4.09 Mpa) and thickness (0.137 mm) as the control film (Table 3).

MONTERREY-QUINTERO and SOBRAL (2000) and PIRES *et al*. (2013), when studying king weakfish myofibrillar protein films added with thyme oil and Nile tilapia films, obtained TS values of 2.2 N and 6.67 N, respectively, which are lower than the results in this study.

KAEWPRACHU *et al*. (2016) and ROSTAMZAD *et al*. (2016) found WVP values close to those of the control film for myofibrillar protein films of tilapia and silver carp, respectively (Table 3). PIRES *et al*. (2013), when studying king weakfish protein films added with essential oils, obtained WVP of 4.2E-11 g m m^{-2} s^{-1} Pa^{-1}, similar to the value of the optimized film. DAVANÇO *et al*. (2007) stated that the permeability of biodegradable films strongly depends on their structure and that saturated fatty acids, such as SA, are the most effective in controlling moisture migration than unsaturated ones.

The solubility result of the optimized film can be compared with the studies by DAVANÇO (2006), ARTHARN *et al*. (2009), and PIRES *et al*. (2013), who also found increased solubility when adding lipids to fish-protein films. The denaturing property of SDS reduces the intermolecular forces among between polymer chains, thus destabilizing the film structure (DAVANÇO, 2006). Film solubility may also depend on factors such as species, type of muscle, and film preparation (PIRES *et al*., 2013). Nevertheless, HALAL *et al*. (2016) noticed that adding palm oil did not significantly impact the solubility of whitemouth croaker protein films.

The lower swelling is a consequence of the higher hydrophobicity of the film with SA and SDS ($p>0.05$), likely favored by a matrix with higher density and, consequently, lower molecular diffusivity of water and lower swelling capacity, which is confirmed by the low WVP value of the optimized film and by the mechanical analysis results. The swelling index in films allows verifying beforehand the degradation outlook, which is related to the level of hydration of the system.

High-solubility films, such as the ones in this study, can be used to store individual food portions that will be dissolved during preparation, to package fresh fruits and vegetables, and to be placed in trays to absorb exudates from cuts of poultry, beef, and/or fish (GONTARD *et al*., 1996; RHIM, 2012).

Several authors, when analyzing the color parameters of films added with oils and surfactants, have found similar results as the present study. PIRES *et al*. (2013) also obtained high luminosity (92.45 to 93.72%) for king weakfish protein films added with thyme oil. ARTHARN *et al*. (2009), TONGNUANCHAN *et al*. (2011b, 2015), reported that tilapia films were more yellowish than the control film as evidenced by the increase in b* and ΔE values. This characteristic may favor the protection of packaged foods against the oxidative spoilage caused by light, although films should preferably be colorless to simulate the appearance of synthetic films (MARTINS *et al*., 2012).

Adding SA and SDS to the films directly influenced their light transmittance and transparency by increasing the barrier property in the UV/Vis region, an important feature in food packaging as UV light may cause oxidative spoilage and lead to nutrient loss, loss of color, and off flavors (MARTINS *et al*., 2012). ARAÚJO (2015) and (2016) found transmittance at the same wavelength of 78.73 to 87.97% and 75.2 to 87.6%, respectively, for fish myofibrillar protein films, i.e., slightly more transparent than the

films in this study. PIRES *et al*. (2013) and HALAL *et al.* (2016) also found lower transparency in fish protein films when oils were added. That can be due to the presence of lipid droplets dispersed in the film and the physical state of the lipid (solid) at room temperature (HALAL *et al*., 2016).

The presence of marked peaks in the diffractogram of the optimized film at wide angles (WAXS) indicates the presence of crystalline regions (FAKHOURI, 2009). A similar behavior was found by CORTEZ-VEGA (2011) for whitemouth croaker protein films. AKANDA *et al*. (2015) found a peak at 2Θ=21.55° and ALMEIDA *et al.* (2012) found peaks at 2Θ=20.60, 21.71, and 24.05° for SA in fish-protein films. ELSAYED *et al*. (2014) detected a peak at 2Θ=5.27° while YU *et al*. (2011) reported a peak at 2Θ=6.5° for SDS.

The control film had higher mass loss at lower breakdown temperature than the optimized films in the first decomposition event of the thermal analyses, which may be attributed to its higher moisture control (LIU *et al*., 2009; MARTINS *et al*., 2012). That indicates greater evaporation of free water from the structure of the control film, which was also described by TONGNUANCHAN *et al*. (2014), CARPINÉ *et al*. (2015), and BATISTA (2016). The optimized film had higher hydrophobicity due to the addition of SA, which is corroborated by HALAL *et al*. (2016).

The third event is associated with the breakdown of the fraction of myofibrillar proteins of higher molecular weight and of glycerol since the boiling point of this plasticizer is around 182.0 °C (HALAL *et al*., 2016). In the optimized films, it is also associated with the thermal breakdown of SA, whose peak breakdown temperature is 236.33 °C (MATOS, 2012,) and with the thermal breakdown of SDS at 213 °C (YU *et al*., 2011).

SEM shows the action of SDS in incorporating SH into the optimized film. A similar behavior was found by DAVANÇO *et al*. (2007) when verifying the efficiency of SDS (60%) in the incorporation of SA into the filmogenic matrix because microscopy reveals fat droplets in films without the addition of this surfactant. The same was reported by ROSTAMZAD *et al*. (2016) for silver carp protein films and ARAÚJO (2015) for films based on myofibrillar proteins of gilthead bream.

SOUZA *et al*. (2004) suggested that the presence of chinks and/or irregularities in film structure may compromise its integrity by changing its functional properties and those related to film surface such as sheen and water absorption. Those factors confirm the high solubility found in this study for the optimized films as well as the results obtained for the color parameters, such as ΔE.

CONCLUSION

Adding stearic acid and dodecyl sodium sulfate to the optimized film led to a reduction by 31% in water vapor permeability, greater tensile strength (6.35 MPa), and lower elongation (235.60%) compared with the control film ($p \leq 0.05$), producing, however, strong and flexible films. The lower transparency and more yellowish color of this film suggest it can be used in food sensitive to oxidation due to its better UV barrier property.

The optimized film had good thermal stability despite the higher mass loss compared with control, with 22% higher solubility and slightly lower swelling. The results obtained represent a positive contribution in the field of emulsified films with the use of fish byproducts and allow for the application of alternative sustainable technologies to obtain quality products, besides minimizing environmental impact.

ACKNOWLEDGEMENTS

he authors thank CAPES (Coordination for the Improvement of Higher Education Personnel) for the master's degree scholarship of the first author and CNPq (National Council of Scientific and Technological Development) for funding this research.

REFERENCES

AKANDA, M.H.; RAI, R.; SLIPPER, I.J.; CHOWDHRY, B.Z.; LAMPROU, D.; GETTI, G.; DOUROUMIS, D. 2015 Delivery of retinoic acid to LNCap human prostate cancer cells using solid lipid nanoparticles. *International Journal of Pharmaceutics*, *493*(1-2): 161-171. **http://dx.doi.org/10.1016/j.ijpharm.2015.07.042**. PMid:26200751.

ALMEIDA, E.D.P.; COSTA, A.A.; SERAFINI, M.R.; ROSSETTI, F.C.; MARCHETTI, J.; SARMENTO, V.; NUNES, R.; VALERIO, E.M.G.; ARAÚJO, A.; LIRA, A.A. 2012 Preparation and characterization of chloroaluminum phthalocyanine-loaded solid lipid nanoparticles by thermal analysis and powder X-ray diffraction techniques. *Journal of Thermal Analysis and Calorimetry*, 108(1): 191-196.

ANKER, M.; BERNTSEN, J.; HERMANSSON, A.; STADING, M. 2002 Improved water vapor barrier of whey protein films by addition of an acetylated monoglyceride. *Innovative Food Science & Emerging Technologies*, *3*(1): 87-92. **http://dx.doi.org/10.1016/S1466-8564(01)00051-0**.

AOAC – ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS 1997 Official methods of analysis of the Association of Official Analytical Chemists. 16ª ed. Washington: AOAC. 850p.

ARAÚJO, C.S. 2015 Obtenção e caracterização de biofilme a partir de proteínas miofibrilares de subproduto de peixe. Belém. 82f. (Dissertação de Mestrado. Instituto de Tecnologia, Universidade Federal do Pará). Available from: <http://ppgcta.propesp.ufpa.br/index.php/br/teses-e-dissertacoes/dissertacoes/49-2015>. Access on: 12 aug. 2016

ARFAT, Y.A.; BENJAKUL, S.; PRODPRAN, T.; OSAKO, K. 2014 Development and characterization of blend films on fish protein isolate and fish gelatin. *Food Hydrocolloids*, *39*(1): 58-67. **http://dx.doi.org/10.1016/j.foodhyd.2013.12.028**.

ARTHARN, A.; PRODPRAN, T.; BENJAKUL, S. 2009 Round scad protein-based film: storage stability and its effectiveness for shelf-life extension of dried fish powder. *Lebensmittel-Wissenschaft + Technologie*, *42*(7): 1238-1244. **http://dx.doi.org/10.1016/j.lwt.2008.08.009**.

ASTM – American Society for Testing and Materials. 1996 *ASTM D882-91: Standard Test Methods for Tensile Properties of Thin Plastic Sheeting*. Philadelphia. (Annual Book of ASTM Standards).

BATISTA, J.T.S. 2016 *Estudo de filmes biodegradáveis de proteínas miofibrilares extraídas de subprodutos da dourada (Brachyplatystoma roussauxii) com adição de quitosana*. Belém. 75f. (Dissertação de Mestrado. Instituto de Tecnologia, Universidade Federal do Pará). Available from: <http://ppgcta.propesp.ufpa.br/index.php/br/teses-e-dissertacoes/dissertacoes/202-2016>. Access on: 25 oct. 2016

CARPINÉ, D.; DAGOSTIN, J.L.A.; BERTAN, L.C.; MAFRA, M.A. 2015 Development and characterization of soy protein isolate emulsion-based edible films with added coconut oil for olive oil packaging: barrier, mechanical, and thermal properties. *Food and Bioprocess Technology*, *8*(8): 1811-1823. **http://dx.doi.org/10.1007/s11947-015-1538-4**.

CHINABHARK, K.; BENJAKUL, S.; PRODPRAN, T. 2007 Effect of pH on the properties of protein-based film from bigeye snapper (*Priacanthus tayenus*) surimi. *Bioresource Technology*, *98*(1): 221-225. **http://dx.doi.org/10.1016/j.biortech.2005.11.012**. PMid:16378726.

CORTEZ-VEGA, W.R. 2011 *Desenvolvimento de filmes nanocompósitos de isolado Protéico de corvina (**Micropogonias furnieri**) e argila organofílica*. Rio Grande. 156f. (Tese de Doutorado. Programa de Pós-graduação em Engenharia e Ciência de Alimentos, Escola de Química e Alimentos, Universidade Federal do Rio Grande). Available from: <http://repositorio.furg.br/handle/1/6162>. Access on: 25 june 2016.

CUQ, B.; GONTARD, N.; CUQ, J.L.; GUILBERT, S. 1996 Rheological model for the mechanical properties of myofibrillar protein-based films. *Journal of Agricultural and Food Chemistry*, *44*(4): 1116-1122. **http://dx.doi.org/10.1021/jf950389n**.

DAVANÇO, T. 2006 *Desenvolvimento e Caracterização de Biofilmes à base de Gelatina, Triacetina, Ácido esteárico ou Ácido capróico e Surfactantes*. Campinas. 130 f. (Dissertação de Mestrado. Faculdade de Engenharia de Alimentos, UNICAMP). Available from: <http://repositorio.unicamp.br/jspui/handle/REPOSIP/255982>. Access on: 12 dec. 2015.

DAVANÇO, T.; TANADA-PALMU, P.; GROSSO, C. 2007 Filmes compostos de gelatina, triacetina, ácido esteárico ou capróico: efeito do pH e da adição de surfactantes sobre a funcionalidade dos filmes. *Food Science and Technology (Campinas)*, *27*(2): 408-416. **http://dx.doi.org/10.1590/S0101-20612007000200034**.

ELSAYED, I.; ABDELBARY, A. A.; ELSHAFEEY, A. H. 2014 Nanosizing of a poorly soluble drug: technique optimization, factorial analysis, and pharmacokinetic study in healthy human volunteers. *International Journal of Nanomedicine, 9*(único):2943-2953.

FAKHOURI, F.M. 2009 *Bioplásticos flexíveis e biodegradáveis à base de amido e gelatina*. Campinas. 249f. (Tese de Doutorado. Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos). Available from: <http://repositorio.unicamp.br/jspui/handle/REPOSIP/256348>. Access on: 15 mar. 2016.

GALLO, J.A.Q.; DEBEAUFORT, F.; CALLEGARIN, F.; VOILLEY, A. 2000 Lipid hydrophobicity, physical state and distribution effects on the properties of emulsion-based films. *Journal of Membrane Science*, *180*(1): 37-46. **http://dx.doi.org/10.1016/S0376-7388(00)00531-7**.

GONTARD, N.; DUCHEZ, C.; CUQ, J.; GUILBERT, S. 1994 Edible composite films of wheat gluten and lipids: Water vapor permeability and other physical properties. *International Journal of Food Science & Technology*, *29*(1): 39-50. **http://dx.doi.org/10.1111/j.1365-2621.1994.tb02045.x**.

GONTARD, N.; GUILBERT, S.; GORRIS, L.G.M. 1996 Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. LWT - *Food Science and Technology*, *29*(1-2): 10-17.

HALAL, S.L.M.E.; ZAVAREZE, E.R.; ROCHA, M.; PINTO, V.Z.; NUNES, M.R.; LUVIELMO, M.M.; PRENTICE, C. 2016 Films based on protein isolated from croaker (*Micropogonias furnieri*) and palm oil. *Journal of the Science of Food and Agriculture*, *96*(7): 2478-2485. **http://dx.doi.org/10.1002/jsfa.7368**. PMid:26250365.

IWATA, K.; ISHIZAKI, S.; HANDA, A.; TANAKA, M. 2000 Preparação e caracterização de filmes comestíveis de proteínas solúveis em água de peixes. *Ciências da Pesca*, *66*(2): 372378.

KAEWPRACHU, P.; OSAKO, K.; BENJAKUL, S.; RAWDKUEN, S. 2016 Effect of protein concentrations on the properties of fish myofibrillar protein based film compared with PVC film. *Journal of Food Science and Technology*, *53*(4): 2083-2091. **http://dx.doi.org/10.1007/s13197-016-2170-7**. PMid:27413238.

KAEWRUANG, P.; BENJAKUL, S.; PRODPRAN, T.; NALINANON, S. 2013 Physicochemical and functional properties of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as affected by extraction conditions. *Food Bioscience, 2*1: 1-9.

KHWALDIA, K.; ARAB-TEHRANY, E.; DESOBRY, S. 2010 Biopolymer coatings on paper packaging materials. *Comprehensive Reviews in Food Science and Food Safety*, *9*(1): 82-91. **http://dx.doi.org/10.1111/j.1541-4337.2009.00095.x**.

LEE, K.Y.; SHIM, J.; LEE, H.G. 2004 Mechanical properties of gellan and gelatin composite films. *Carbohydrate Polymers*, *56*(2): 51-54. **http://dx.doi.org/10.1016/j.carbpol.2003.04.001**.

LIMPAN, N.; PRODPRAN, T.; BENJAKUL, S.; PRASARPRAN, S. 2010 Properties of biodegradable blend films based on fish myofibrillar protein and polyvinyl alcohol as influenced by blend composition and pH level. *Journal of Food Engineering*, *100*(1): 85-92. **http://dx.doi.org/10.1016/j.jfoodeng.2010.03.031**.

LIU, H.; XIE, F.; YU, L.; CHEN, L.; LI, L. 2009 Thermal processing of starch-based polymers. *Progress in Polymer Science*, *34*(12): 1348-1368. **http://dx.doi.org/10.1016/j.progpolymsci.2009.07.001**.

MARTINS, J.T.; CERQUEIRA, M.A.; VICENTE, A.A. 2012 Influence of a-tocopherol on physicochemical properties of chitosan-based films. *Food Hydrocolloids*, *27*(1): 220-227. **http://dx.doi.org/10.1016/j.foodhyd.2011.06.011**.

MATOS, F.C. 2012 *Estudo da decomposição térmica de ácidos graxos através da calorimetria exploratória diferencial*. Campinas. 107f. (Dissertação de Mestrado. Faculdade de Engenharia Química, UNICAMP). Available from: <http://repositorio.unicamp.br/bitstream/REPOSIP/266763/1/Matos_FlavioCardosode_M.pdf>. Access on: 14 sept. 2016.

MONTERREY-QUINTERO, E.S.; SOBRAL, P.J.A. 2000 Preparo e caracterização de proteínas miofibrilares de tilápia-do-nilo para elaboração de biofilmes. *Pesquisa Agropecuária Brasileira*, *35*(1): 179-190. **http://dx.doi.org/10.1590/S0100-204X2000000100020**.

OLIVEIRA, T.A.; LEITE, R.H.L.; AROUCHA, E.M.M.; NOBRE, D.M.; SANTOS, F.K.G. 2012 Biofilmes de gelatina: Efeito da adição de surfactante e ácidos graxos de óleo de coco na permeabilidade ao vapor de água. *Revista Verde*, *7*(4): 76-80.

PASCHOALICK, T.M.; GARCIA, F.T.; SOBRAL, P.J.; HABITANTE, A.M.Q.B. 2003 Characterization of some functional properties of edible films based on muscle proteins of Nile Tilapia. *Food Hydrocolloids*, *17*(4): 419-427. **http://dx.doi.org/10.1016/S0268-005X(03)00031-6**.

PEREIRA, G.V.S. 2015. *Otimização do processo de obtenção de filmes biodegradáveis com proteínas miofibrilares a partir de subprodutos de pescada amarela (**Cynoscion acoupa**)*. Belém. 78f. (Dissertação de Mestrado. Instituto de Tecnologia, Universidade Federal do Pará). Available from: <http://ppgcta.propesp.ufpa.br/ARQUIVOS/dissertacoes/2015/GLAUCE%20VASCONCELOS.pdf>. Access on: 10 aug. 2016.

PHAKAWAT, T.; BENJAKUL, S.; PRODPRAN, T.; NILSUWANA, K. 2015 Emulsion film based on fish skin gelatin and palm oil: physical, structural and thermal properties. *Food Hydrocolloids, 48*: 248-259.

PIRES, C.; RAMOS, C.; TEIXEIRA, B.; BATISTA, I.; NUNES, L.C.; MARQUES, A. 2013 Hake proteins edible films incorporated with essential oils: physical, mechanical, antioxidant and antibacterial properties. *Food Hydrocolloids*, *30*(1): 224-231. **http://dx.doi.org/10.1016/j.foodhyd.2012.05.019**.

PIRES, C.; RAMOS, C.; TEIXEIRA, G.; BATISTA, I.; MENDES, R.; NUNES, L.; MARQUES, A. 2011 Characterization of biodegradable films prepared with hake proteins and thyme oil. *Journal of Food Engineering*, *105*(3): 422428. **http://dx.doi.org/10.1016/j.jfoodeng.2011.02.036**.

PRODPRAN, T.; BENJAKUL, S.; ARTHAN, A. 2007 Properties and microstructure of films based on Protein scad round (*Decapterus maruadsi*) muscle, affected in palm oil and incorporation Chitosan. *Jornal Internacional de Macromoléculas Biológicas*, *41*(5): 60561.

RHIM, J.W. 2012 Physical-mechanical properties of agar/κ-carrageenan blend film and derived clay nanocomposite film. *Journal of Food Science*, *77*(12): N66-73. **http://dx.doi.org/10.1111/j.1750-3841.2012.02988.x**. PMid:23170836.

ROSTAMZAD, H.; PAIGHAMBARI, S.Y.; SHABANPOUR, B.; OJAGH, S.M.; MOUSAVI, S.M. 2016 Improvement of fish protein film with nanoclay and transglutaminase for food packaging. Food Packaging and Shelf Life, 7(1): 1-7. **http://dx.doi.org/10.1016/j.fpsl.2015.10.001**.

SOUZA, S.M.A.; SOBRAL, P.J.A.; MENEGALLI, F.C. 2004 Extração de proteínas miofibrilares de carne bovina para elaboração de filmes comestíveis. *Food Science and Technology (Campinas)*, *24*(4): 619-626. **http://dx.doi.org/10.1590/S0101-20612004000400024**.

STATSOFT. *Statistica for Windows, versão 10*. Tulsa: Statsoft Inc., 2011.

TAO, Z.; WENG, W.Y.; CAO, M.J.; LIU, G.M.; SU, W.J.; OSAKO, K.; TANAKA, M. 2015 Effect of blend ratio and pH on the physical properties of edible composite films prepared from silver carp surimi and skin gelatin. *Journal of Food Science and Technology*, *52*(3): 1618-1625. **http://dx.doi.org/10.1007/s13197-013-1186-5**. PMid:25745232.

TONGNUANCHAN, P.; BENJAKUL, S.; PRODPRAN, T.; SONGTIPYA, P. 2011b Characteristics of film based on protein isolate from red tilapia muscle with negligible yellow discoloration. *International Journal of Biological Macromolecules*, *48*(5): 758-767. **http://dx.doi.org/10.1016/j.ijbiomac.2011.02.017**. PMid:21382405.

TONGNUANCHAN, P.; BENJAKUL, S.; PRODPRAN, T. 2014 Structural, morphological and thermal behavior characterizations of fish gelatin film incorporated with basil and citronella essential oils as affected by surfactants. *Food Hydrocolloids, 41*: 33-43. http://dx.doi.org/10.1016/j.foodhyd.2014.03.015.

TONGNUANCHAN, P.; BENJAKUL, S.; PRODPRAN, T.; NILSUWAN, K. 2015 Emulsion film based on fish skin gelatin and palm oil: physical, structural and thermal properties. *Food Hydrocolloids, 48*: 248-259.

WENG, W.; ZHENG, H. 2015 Effect of transglutaminase on properties of tilapia scale gelatin films incorporated with soy protein isolate. *Food Chemistry, 169*: 255-260.

YANG, L.; PAULSON, A.T. 2000 Effects of lipids on mechanical and moisture barrier properties of edible gellan film. *Food Research International*, *33*(7): 571-578. **http://dx.doi.org/10.1016/S0963-9969(00)00093-4**.

YU, D.G.; ZHU, L.M.; BRANFORD-WHITE, C.; YANG, J.H.; WANG, X.; LI, Y.; QIAN, W. 2011 Solid dispersions in the form of electrospun core–sheath nanofibers. *International Journal of Nanomedicine, 6*: 3271-3280.

<jrn>ZAVAREZE, E.R.; HALAL, S.L.M.E.; MARQUES E SILVA, R.; DIAS, A.R.G.; PRENTICE-HERNÁNDEZ, C. 2014 Mechanical, barrier and morphological properties of biodegradable films based on muscle and waste proteins from the whitemouth croaker (*Micropogonias furnieri*). Journal of Food Processing and Preservation, 38(4): 1973-1981. **http://dx.doi.org/10.1111/jfpp.12173**.</jrn>

ZAVAREZE, E. R.; HALAL, S. L. M.; TELLES, A. C.; HERNANDES, P. 2012 Biodegradable films based on myofibrillar proteins of fish. *Brazilian Journal of Food Technology*, 15(n.spe): 53-57. http://dx.doi.org/10.1590/S1981-67232012005000038.