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EFFECT OF POLYMER MIXTURE ON BIOPLASTIC DEVELOPMENT FROM FISH WASTE

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ABSTRACT

The bioconversion of protein from the fishing industry waste into bioplastics allows the valorization of biological material, reducing the production of waste and consequently of negative environmental impacts through the use of synthetic packaging. The objective of this work was to develop biodegradable films from the mixture of gelatin and myofibrillar fish proteins. Proteins (myofibrillar and gelatin) were extracted from filleting residues from king weakfish (*Macrodon ancylodon*) from industrial fishing. The properties of the blend films were compared to those of individual protein films. It was found by scanning electron microscopy that there was good compatibility between the two polymers. Mechanical analyzes showed that myofibrillar proteins films were the most resistant to traction, but less flexible; characteristics contrary to those of gelatin. The mixing films presented the lowest values of water vapor permeability and solubility were transparent, and mechanically strong and flexible, confirming the improvement of the properties by mixing the polymers. Fourier transform infrared spectroscopy revealed that there was interaction between the myofibrillar protein chains and gelatine driven by hydrogen bonds, thus forming cohesive and reinforced matrix, which resulted in good thermal resistance of the films. The mixture between polymers improved the technological properties of the elaborated biodegradable films, making its application feasible as food packaging.

Key words: blended films; fish gelatin; fish myofibrillar protein; technological properties.

EFEITO DA MISTURA DE POLÍMEROS NO DESENVOLVIMENTO DE BIOPLÁSTICO A PARTIR DE RESÍDUOS DE PEIXE

RESUMO

A bioconversão de proteínas de subprodutos da indústria pesqueira em bioplástico possibilita a valorização do material biológico, reduzindo a produção de descarte e consequentemente, de impactos ambientais negativos do uso de embalagens sintéticas. O objetivo deste trabalho foi misturar gelatina e proteínas miofibrilares de pescada gó (*Macrodon ancylodon*) com intuito de desenvolver filmes biodegradáveis com propriedades tecnológicas aprimoradas. As propriedades dos filmes de mistura foram comparadas com as de filmes dos polímeros individuais. A espectroscopia de infravermelho com transformada de Fourier revelou boa compatilidade química entre gelatina e proteínas miofibrilares na formação do filme de mistura. A interação entre os polímeros foi conduzida por ligações de hidrogênio, formando película coesa e reforçada. Os filmes de mistura além de apresentarem os menores valores de permeabilidade ao vapor de água e solubilidade, também foram transparentes e, termicamente resistentes. Assim a mistura entre gelatina e proteínas miofibrilares possibilitou a formação de películas biodegradáveis com propriedades tecnológicas superiores às produzidas com os polímeros separados.

Palavras-chave: filmes biodegradáveis; gelatina de peixe; proteína miofibrilar de peixe; propriedades tecnológicas.

INTRODUCTION

Conventional petrochemical-based food packaging present excellent mechanical properties and stand as a good gas and water barrier. However, their biodegradability and the potential transfer of toxic agents to food have led several studies to investigate the development of biopolymer-based packaging. In addition to promoting environmental sustainability, this solution would ensure food safety (Bravin et al., 2006; López-Rubio et al., 2008; Nur Hanani et al., 2014).

Much research aimed at the development of biodegradable packaging used proteins as raw materials, such as soy protein (Guerrero et al., 2011), wheat gluten (Ansorena et al., 2016), and fish proteins (Alfaro et al., 2014; Romani et al., 2018). The use of proteins is due to its excellent capacity to form film, as protein chains form intra- and intermolecular bonds, and to provide the adequate properties for the end use of packaging by means of chemical, physical, or enzymatic changes (Hammann and Schmid, 2014). In addition, the use of such biopolymers in the manufacture of packages, such as coating films, is interesting not only because it is environmentally friendly, but also because it has characteristics such as comestibility, biocompatibility, texture and aesthetic properties, extensibility (Blanc et al., 2019).

Proteins, especially from animal origin, are the most used biomaterials in the food industry, and have been widely explored. The choice and/or combinations of proteins as to their intrinsic characteristics as, structure and molecular weight, amino acid composition, chemical interactivity; associated with chemical environment conditions such as temperature and pH, favors the development of structure and and nanostructures that aim at the availability of bioactive compounds, functional foods and also active packaging (Bourbon et al., 2019).

The use of (myofibrillar and gelatin) proteins from fish byproducts to develop biodegradable packaging allows for the reduction of environmental impact, as they represent an alternative to replace petrochemical packagings. Recent studies show that biodegradable films from these polymers have promising mechanical and barrier properties for use in the food industry. However, these properties have only been achieved with the addition of oil and/or plastifiers to the formulation (Araújo et al., 2018; Silva et al., 2018).

In spite of the good water vapor permeability and resistance to traction, due to the rigidity of myofibrillar protein films, a significant amount of plastifier, glycerol or sorbitol, must be added to the formulation for adequate flexibility. However, these hydrophilic substances reduce the film's water vapor barrier and increase its solubility (Chinabhark et al., 2007; Prodpran et al., 2007). On the other hand, fish gelatin films are transparent and flexible, which makes them excellent materials for encapsulation and dispersion of bioactive components (Wang et al., 2007). Nevertheless, individual gelatin films tend to bloat and dissolve in contact with highly humid foods (Gómez-Guillén et al., 2011).

To Arroyo et al. (2019) although promising most proteins do not have a set of properties suitable for the specific use, it being necessary to add modifying agents or the combination of two or more components to obtain the desired characteristics. A great number of methods have been studied with the objective of improving the characteristics of biodegradable packaging, such as the addition of oil containing antimicrobial agents (Arfat et al., 2014b) and antioxidants (Uranga et al., 2018), supercritical impregnation (Souza et al., 2014), and polymer mixing (Hoque et al., 2011; Arfat et al., 2014a; Abdelhedi et al., 2018). Mixing two or more polymers has proven efficient in the search for films with superior technological properties, such as low water vapor permeability, and highly resistant, flexible, transparent, and bioactive films (Abdelhedi et al., 2018). Hosseini et al. (2016) produced films with low oxygen and water vapor permeability by mixing fish gelatin and polylactic acid. Films from fish biopolymers had their properties enhanced when mixed with myofibrillar protein and commercial fish gelatin in different proportions. The mix provided the films with improved mechanical and water vapor barrier properties compared to pure polymer films (Arfat et al., 2014a).

Therefore, the aim of this research is to study the mixture of biopolymers as a tool to develop biodegradable films with improved technological properties.

MATERIAL AND METHODS

Raw materials

The byproducts (filleting scrapings and skin) of king weakfish (*Macrodon ancylodon*) fillet production have been donated by Ecomar Ltda. fishing industry, located in the city of Vigia, state of Pará, Brazil. The residues were washed in chlorinated water (5 ppm) at ± 4 °C. The scrapings were ground for 1.5 minutes in a cutter (Filizzola, Sire Cutter). After removing the scales, the skins were cut into pieces of approximately 4×4 cm. The samples were then vacuum sealed and frozen at -26 °C ± until the moment of extraction of the myofibrillar proteins and gelatin.

Myofibrillar protein extraction

The methodology proposed by Zavareze et al. (2012) was used, with some modifications, to extract the myofibrillar proteins. For deodorization, the ground king weakfish muscle was mixed with phosphoric acid at 0.02% in the proportion of 1:3 ($m.v^{-1}$) for 15 minutes. The solution was then filtered through faille fabric. The resulting solids were washed in distilled water at 7 °C in the proportion of 1:3 ($m.v^{-1}$) for removal of excess acid, having then been filtered again.

The resulting material was mixed with a sodium chloride solution 50 Mm at ± 7 °C in the proportion of 1:3 ($m.v^{-1}$) for 5 minutes, followed by centrifugation at 10,000 rpm for 4 min at ± 4 °C in a refrigerated centrifuge (Thermo Fisher, Multifuge X1R). The process was repeated twice and the resulting paste (myofibrillar proteins) was spread onto stainless steel trays, frozen at ± -26 °C and lyophilized at ± -60 °C for 48 hours in a lyophilizer (Liotop, L101). Following lyophilization, the material was sifted (Tyler 35 with 0.42 mm opening) and the king weakfish myofibrillar proteins (FMP) were obtained.

Gelatin extraction

The methodology proposed by Bueno et al. (2011) was used, with some modifications, to extract the gelatin from the king weakfish skin. About 20 g of skin were mixed with 100 mL of NaCl solution 0.6 M, agitated in a Shaker incubator (Cielanb, model CE-725B) at 85 rpm and 25 °C ± for 15 minutes. The skin was then drained and washed in running water. Then, a solution of NaCl solution 0.3 M ($1:5\ m.v^{-1}$) was added to the skin, which was agitated at 85 rpm and 25 °C ± for 15 minutes and immediately drained and washed in running water. Afterwards, acetic acid

0.02 M was used in the proportion of 1:5 ($m.v^{-1}$) under agitation for 1 hour. The skin was drained and washed in running water.

Then, distilled water in the proportion of 1:5 ($m.v^{-1}$) was added to the skin, which was brought to a water bath (TECNAL, TE-057) for 12 hours at 50 °C ± and filtered through faille fabric. The resulting solution was spread onto stainless steel trays, frozen at - ± 26 °C ± for 48 hours, lyophilized at -60 °C ± for 36 hours, after which the lyophilized king weakfish gelatin (FG) was obtained.

Preparation of films

The methodology proposed by Zavareze et al. (2012) was used, with some modifications, to prepare the "pure" myofibrillar protein (FMP) and gelatin (FG) films . These films have been prepared by polymer suspension at 1.0 and 3.0% (m/v), pH adjusted to 3.0 with hydrochloric acid (HCl) 2M and glycerol at 20% as a plastifier. The solution was homogenized at 10,000 rpm for 5 minutes in an homogenizer (Turratec, Tecnal, TE-102). Then, it was warmed up to 70 °C ± in water bath (Tecnal, TE-057) for 40 minutes. The resulting filmogenic solution was filtered, spread onto silicone trays of 22 cm in diameter and dried in a ventilated oven incubator (Quimis, Q315M) at 30 °C ± for 18 hours.

The blended films (BL) obtained from blending myofibrillar proteins and gelatin have been prepared according to the methodology proposed by Arfat et al. (2014a), with some changes . They were prepared in the concentrations of 1.0 and 3.0% ($m.v^{-1}$), whereas polymers were mixed in the proportion of 1:1 (FMP:FG) and with the same amount of glycerol used in the pure films (20%).

The gelatin (FG) had been previously dissolved into 50 mL of distilled water and reserved. The myofibrillar proteins (MFP) were solubilized into 100 mL of water and added to the plastifier (glycerol at 20%). Then, the pH of the solution was adjusted to 3.0 with HCl 2M and homogenized (Turratec, Tecnal, TE-102) at 10,000 rpm for 5 minutes. Afterwards, the gelatin was added to the myofibrillar protein solution, homogenized again and brought to a water bath at 70 °C ± for 40 minutes. The resulting solution was filtered through faille fabric, spread onto the silicone support measuring 22 cm in diameter, and dried under the same conditions as the pure films.

Determining the properties of the films

Film thickness

The measurement of film thickness was taken with the aid of a digital micrometer (Insize, model IP54) from eight random points around each film, all at a distance of 60 mm from the edges (Zavareze et al., 2012).

Mechanical properties

The resistance to traction (RT) and percent elongation at break (E) of the films were determined according to the ASTM D882-91 methodology (ASTM, 1986) with the aid of a texturometer (QTS, Brookfield). The initial grip separation and speed were 30 mm and 1 $mm.s^{-1}$, respectively, and the testing specimens of each film type measured 2.5 cm × 3.0 cm. The maximum force (N) and the final extension at break were used to calculate the RT and E, respectively.

Water vapor permeability

The water vapor permeability (WVP) was determined according to the modified ASTM D882-95 method described by Arfat et al. (2014a). The film was attached to the opening of a beaker with 4.5 cm in diameter and 7.0 cm in height containing 10g of silica gel at 0% UR; 0Pa of water vapor pressure at 30 °C. The sample set was placed in a desiccator containing distilled water and kept in a ventilated oven incubator (Quimis, Q315M) at 30 °C at 99% UR and 4244.9 Pa of water vapor pressure at 30 °C ± for 10 hours. The WVP was calculated by means of Equation 1.

$$PVA = \frac{W.X}{A.t.\Delta P} \quad (1)$$

where: WVP = water vapor permeability ($g.m^{-1}.\ s^{-1}.\ Pa^{-1}.$); W = weight gain in desiccant (g); X = film thickness (mm); A = exposed biofilm surface area (m^2); t = incubation time (hours); ΔP = partial pressure difference (Pa). Three specimens of each film were used in the WVP test.

Film solubility

The methodology proposed by Gontard et al. (1996) was used to measure film solubility. The films were cut into discs of 2 cm in diameter and the dry matter was determined in an oven at 105 °C ± for 24 hours. The dry samples were immersed in 50 mL of water. The system was agitated in a refrigerated Shaker incubator (Cielanb, CE-725B) at a speed of 150 rpm for 24 hours at 25 °C. Afterwards, the samples were removed and dried (105 °C for 24 hours) in order to determine the dry matter not dissolved in water. The solubility percentage was calculated by means of Equation 2.

$$S = \frac{mi - mf}{mi}.100 \quad (2)$$

where: S = solubility (%); mi = initial dry matter (g); mf = final dry matter (g).

Light transmission and transparency

The ultraviolet light and visible spectrum barrier of the films was measured within the 200-800 nm range with the aid of a spectrophotometer (Biospectro, model SP-22) as described by Han and Floros, 1997. The transparency of the films was determined at 600 nm by means of Equation 3, according to ASTM D1746 method (Han and Floros, 1997).

$$Transparency = \frac{log\%T}{e} \quad (3)$$

where: T = transmittance at 600 nm; e = biofilm thickness (mm).

Film colors

The color of the films was determined using a digital colorimeter (Minolta, CR 310). The parameters of L* (luminosity), a* (intensity of the red pigment), b* (intensity of the yellow pigment), C*, h* (hue angle).

Thermogravimetric analysis (TG/DTG)

The films were analyzed with the aid of a thermogravimetric analyzer (Shimadzu, DTG-60AH) under nitrogen atmosphere with a flow rate of 20 mL.min^{-1}. The samples were warmed up from ambient temperature (± 25 °C) to 600 °C at a heating rate of 10 °C.min^{-1}. Record keeping and data treatment were carried out on software TA60 version 2.21 (Shimadzu).

Fourier-transform infrared spectroscopy (FTIR)

The infrared absorption spectroscopy was carried out with a Fourier-transform spectrometer (Bruker, Vertex 70v). The spectra were obtained in the spectral range of 400-4000 cm^{-1} and 32 scans with resolution of 4 cm^{-1} were collected.

Microscopic characterization of the film

The microstructure of the films was determined with a scanning electron microscope (Tescan, Vega3) with acceleration voltage of 15 kV. The samples were metalized with gold to allow the electric conductivity required for the formation of images with a sputter coater (Quorum Technologies, SC7620). The micrographies were captured with a 7000x zoom for the surface and 4000x for the cross-section of the film.

Statistical analysis

The results were submitted to analysis of variance (ANOVA) carried out with software Statistica® version 7.0 (STATSOFT Inc., 2004).

RESULTS

The films obtained from king weakfish proteins (myofibrillar and gelatin) were homogeneous, thin, and transparent in appearance, in addition to being flexible and elastic. Thus, they are visually similar to conventional films.

Film properties

Table 1 shows the results regarding the physical, chemical, mechanical, and barrier properties of the pure and blended films (BL).

The thicknesses of FG, FMP, and BL king weakfish films were different from one another ($p \leq 0.05$). The gelatin films (FG) were the thickest compared to the myofibrillar protein (FMP) and blended films (BL) in the same proportions. It was also observed that the thickness of the films increased proportionally to the polymer concentration in the formulations. Among the three types, the BL at 1% of concentration was the thinnest, whereas the FG 3% was the thickest. This shows that the miscibility and interaction between the gelatin and myofibrillar proteins in the blended film network was positive.

The resistance to traction (RT) and percent elongation at break (%E) of the films in Table 1 shows that the FMP films presented the highest values of RT, followed by the FG films. In the same polymer concentrations, the BL films presented the lowest RT values, which suggests that they are more malleable and flexible than the others. As to %E, the lowest values were found in the FMP films, considered as tensile films, a characteristic of films prepared exclusively with myofibrillar proteins (Hoque et al., 2011). On the other hand, the FG films presented %E values about four times higher, which makes them superior to FMP. The BL films presented average values in this parameter (315.65 ± 3.06 and 384.80 ± 1.28) compared to pure films, reaching elasticity four times higher than the FMP films. By correlating RT and %E, it is observed that the BL films presented promising characteristics, as it presents resistance and elasticity.

The best WVP values were found in the films with lower polymer concentrations. The association of FG with FMP has proven beneficial for the development of biodegradable films with low water vapor permeability. The results show that the BL films presented the lowest values for this property (2.69 ± 0.16 and 3.12 ± 0.22) and can be up to two times lower than the WVP of pure films. The same behavior is observed with regard to the solubility parameter. The BL films were the least soluble ones, up to four times less soluble than the FG films. No difference was found ($p \leq 0.05$) between the BL and FMP films in this parameter. However, when analyzed in association with WVP, it is observed that the BL films had their physical and barrier properties enhanced. The positive

Table 1. Properties of gelatin films (FG), myofibrillar proteins (FMP) and blended (BL) at different concentrations.

Film composition	Thickness (mm)	RT (MPa)	Elongation (%)	WVP (x 10^{-11} g.m^{-1}.s^{-1}.Pa^{-1})	Solubility (%)
FG 1%	0.037 ± 0.002d	2.75 ± 0.53e	538.26 ± 3.96a	5.48 ± 0.52a	93.75 ± 1.67a
FG 3%	0.128 ± 0.001a	3.13 ± 0.02e	459.10 ± 2.89b	5.40 ± 0.16a	74.08 ± 2.74b
FMP 1%	0.036 ± 0.001d	9.58 ± 0.51b	114.97 ± 0.31f	5.41 ± 0.54a	27.40 ± 2.92c
FMP 3%	0.087 ± 0.003c	12.31 ± 0.42a	126.58 ± 1.24e	4.26 ± 0.12b	25.46 ± 3.45c
BL 1%	0.031 ± 0.002e	5.26 ± 0.43d	315.65 ± 3.06d	3.12 ± 0.22c	27.04 ± 0.57c
BL 3%	0.106 ± 0.002b	6.49 ± 0.36c	384.80 ± 1.28c	2.69 ± 0.16c	23.11 ± 2.09c

Means followed by the same letters in a column indicate do not differ significantly ($p \leq 0.05$); *RT: resistance to traction;**WVP: water vapor permeability.

interaction between the gelatin and myofibrillar protein polymers in the BL films, governed mainly by hydrogen bonds (cf. FTIR), resulted in high density and, consequently, reduced solubility of the blended films.

Color and transparency

Transparency and color are essential characteristics of food packaging, where the appearance of the contents drives consumers' purchase decision. The parameters (L*, a*, b*, C*, and h) allow for the quantitative and qualitative characterization of the pure and blended films. Table 2 shows that both pure films (FG and FMP) and blended films (BL) are characterized by the predominance of the yellow pigment (b*), confirmed by the tone angle h*, over 100º—i.e. closer to the chromatic coordinate b*, just as the color intensity and distinction is ratified by the increase in chroma C*. Taking into account that the films were produced under acid conditions (pH 3.0), the increase in coordinate b* is explained by the fact that acid conditions stimulate the formation of yellow pigments. It was observed that, in all films, polymer concentration increased proportionally to the increase in yellow pigment and decrease in luminosity (L). However, the BL films were clear and more transparent (lower values), which are desirable features for food packaging.

Thermal analyses

The thermogravimetry (TG) and derivative thermogravimetry (DTG) of the pure and blended films are shown in Figure 1. The thermal stability of films can be analyzed under temperature by means of the mass loss measurement. The mass loss stages, represented by peaks, are more easily perceived by means of the DTG curve.

The TG/DTG curves show that all of the films presented thermal degradation in two stages. The first stage was observed between 30.18 and 52.32 °C, peaking at 228 °C. This loss of mass corresponds to water volatilization, whether free or adsorbed from the film mesh, as well as to glycerol components and proteins with low molecular weight (Hoque et al., 2011). The second stage took place between 232.44 and 301.17 °C. This may be attributed to the degradation of proteins with high molecular weight establishing strong bonds in the film matrix formation.

The mass loss temperatures differed among films due to their composition and polymer concentration (Table 3). Among the films, FMP 1% presented the highest degradation temperatures (52.32-301.17 °C), followed by BL 1% (41.38-258.24 °C). The mass loss in the first stage was higher for the FMP 1% film and, in the second stage, the highest mass loss was found in film BL 1% (57.20%). All of the films presented residual mass at 600 °C (22.12-32.76%). However, the FMP 3% film presented the highest percent (32.76%), which means it has proven to be the most thermally stable.

Fourier-transform infrared spectroscopy

The spectra of FG, FMP, and BL films are shown in Figure 2. The spectra of all films presented the highest vibrations within the 3279-3263 cm^{-1} wavelengths, which corresponds to amide A (hydrogen-bonded NH stretching vibrations); 2941-2903 cm^{-1}, which corresponds to amide B, which represents CH and NH_3 stretching vibrations; 1643-1628 cm^{-1}, to amide I (C=O/hydrogen bond coupled to COO group stretching molecular vibrations), and 1549-1520 cm^{-1} to amide II (bending vibration of N=H groups and stretching vibration of C=N). Among the blended specimens, the BL 3% film presented a lower amide A vibrational spectrum,

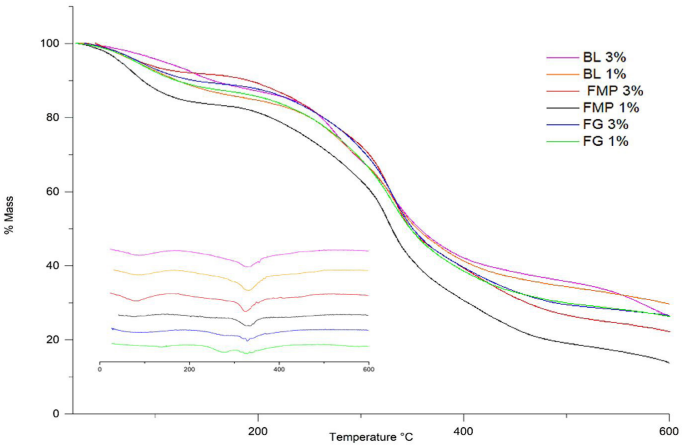

Figure 1. TG/DTG curves of gelatin (FG), myofibrillar protein (FMP), and blended (BL) films in different concentrations.

Table 2. Color and transparency parameters of gelatin films (FG), myofibrillar proteins (FMP) and blended (BL) at different concentrations.

Film	Color parameters					
	L*	a*	b*	C*	h	Transparency
FG 1%	89.51 ± 0.18a	-4.59 ± 0.07b	6.20 ± 0.58d	7.72 ± 0.51d	126.63 ± 0.09ab	3.40 ± 0.06d
FG 3%	88.56 ± 0.44b	-4.80 ± 0.04a	7.13 ± 0.06c	8.57 ± 0.07c	124.13 ± 0.13b	6.03 ± 0.03a
FMP 1%	87.11 ± 0.22cd	-3.88 ± 0.02f	6.42 ± 0.19cd	7.50 ± 0.17d	121.09 ± 0.75c	5.21 ± 0.01b
FMP 3%	86.78 ± 0.12d	-4.25 ± 0.02c	10.56 ± 0.08a	11.39 ± 0.07a	111.92 ± 0.20e	5.04 ± 0.01c
BL 1%	89.42 ± 0.22a	-4.02 ± 0.01e	5.31 ± 0.28e	6.66 ± 0.17e	127.51 ± 0.11a	1.84 ± 0.01e
BL 3%	87.64 ± 0.18c	-4.14 ±0.01d	8.43 ± 0.08b	9.39 ± 0.07b	116.17 ± 0.20d	1.00 ± 0.01f

Means followed by the same letters in a column indicate do not differ significantly ($p \leq 0.05$).

Table 3. Thermal properties of pure and blended (BL) films prepared with king weakfish gelatin (FG) and myofibrillar proteins (FMP).

FILM	Stage 1		Stage 2		Residue (%)
	Onset (°C)	% mass	Onset (°C)	% mass	
FG 1%	34.28	18.35	252.42	53.65	27.99
FG 3%	33.55	23.43	254.02	54.45	22.12
FMP 1%	52.32	26.07	301.17	47.29	26.64
FMP 3%	31.31	18.69	232.44	48.55	32.76
BL 1%	41.38	20.28	258.24	57.20	22.52
BL 3%	30.18	21.92	254.63	54.26	23.82

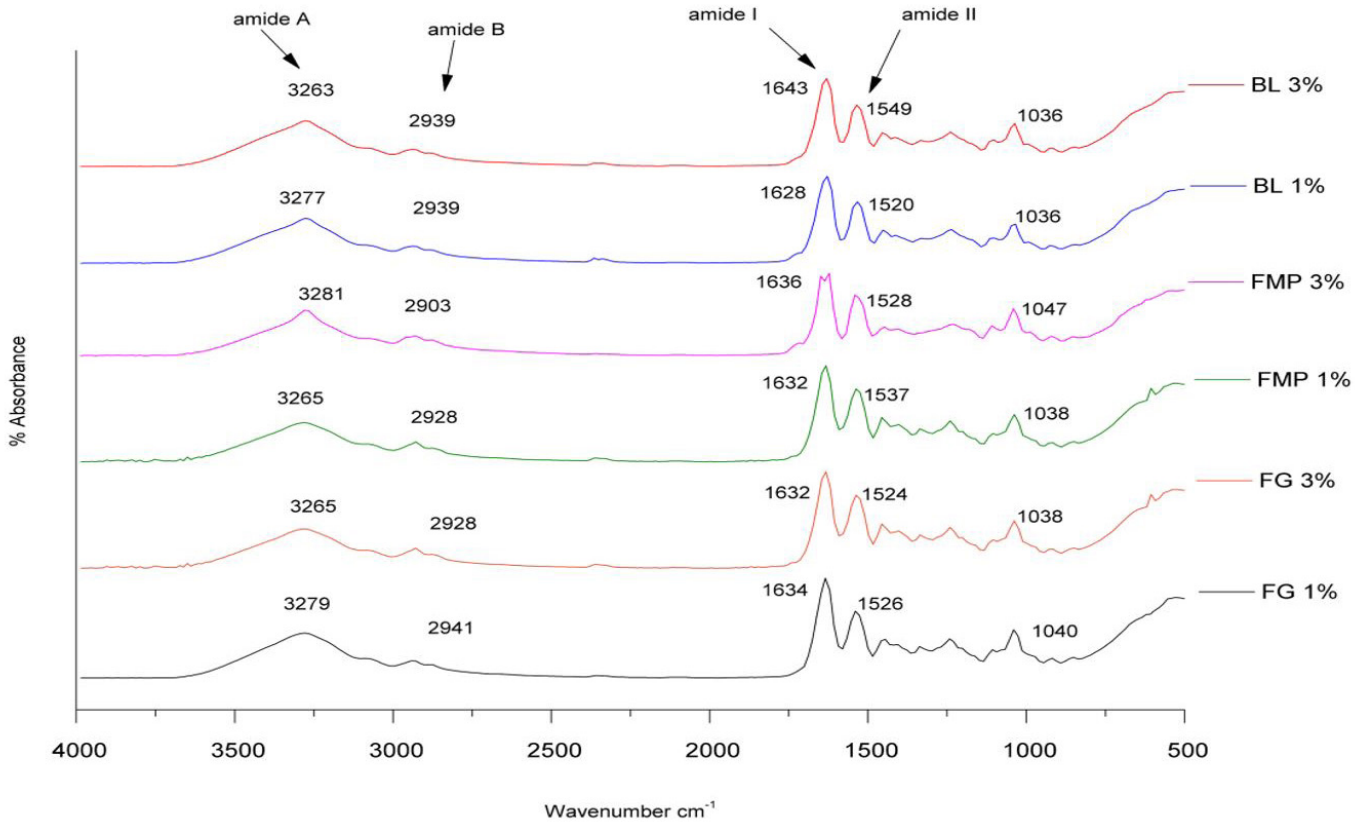

Figure 2. FTIR spectra of gelatin (FG), myofibrillar protein (FMP), and blended (BL) films in different concentrations.

which is possibly due to the positive interaction between the gelatin and myofibrillar protein chains (Nur Hanani et al., 2014).

The highest peak of amide I was found in the BL 3% film. The absorption range between 1600-1700 cm^{-1} is mentioned in the literature as key for the study of protein secondary structure (Muyonga et al., 2004). The spectra allow for analysis of the possible interactions between the biopolymers in the matrix of the BL films.

An absorption band within the 1047-1036 cm^{-1} range was observed in all films; it is probably due to the bond between plastifier (glycerol) and the polymers composing the films.

Microscopic characterization of the films - MEV

The electron micrographies of the pure and blended films are shown in Figure 3. Upon observation with the naked eye, both the pure and blended films seemed homogeneous, smooth, and continuous. Nevertheless, when analyzed by microscopy, it was observed that both FG film specimens presented a coherent surface; however, they were rugged and presented insoluble particles. Fissures and orifices are observed in the cross-section.

The FMP films are visibly dense, presenting accumulated insoluble dry matter with small gaps in the cross-section. The BL film micrographies have shown a favorable compatibility between

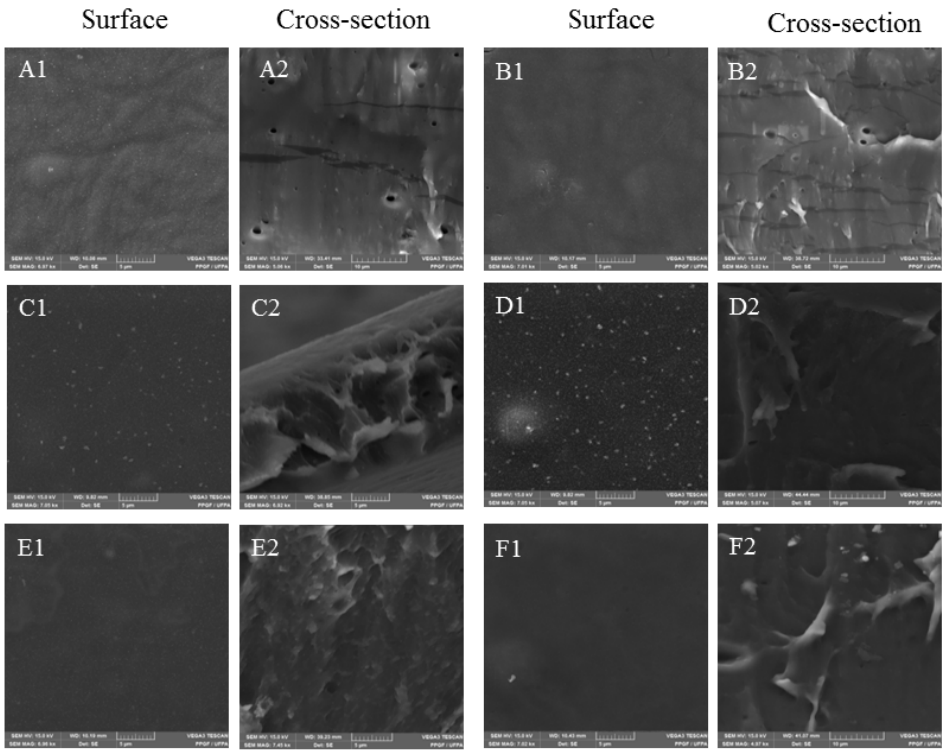

Figura 3. Micrographs of the films' surface (5000x) and cross-section (7000x): FG1% film: (A1) and (A2); FG3% film: (B1) and (B2); FMP1% film: (C1) and (C2); FMP3% film: (D1) and (D2); BL1% film: (E1) and (E2); BL3% film: (F1) and (F2).

FG and FMP. It was observed that blended films have a smooth and homogeneous surface without accumulation or particles, which characterizes a coherent and ordered structure. The mesh of the films did not vary among polymer types. Notably, the BL films formed denser and more organized meshes.

DISCUSSION

Overall, FMP films are thinner, due to the strong disulfide bonds in the protein matrix, which establish a constant density. It was noted that increased thickness is significantly associated with increased polymer concentration in the formulation of films ($p \leq 0.05$). Although this change is clearly associated with the volume of polymers in the films' matrix, it is also attributed to a potential absorption of water, as the number of polar groups presented on the protein surface in the films' mesh favors water absorption in the medium (Krochta, 2002).

The thickness of films is strongly related to their mechanical properties—the less thick it is, the less force is required to break it. That is, the RT value is reduced, whereas elasticity is increased, which also affects film solubility (Table 1). The values found in this study were higher than those reported by Hoque et al. (2011) and Arfat et al. (2014a), who investigated blends of fish gelatin and mung bean protein and of fish gelatin and myofibrillar proteins, respectively.

The resistance to traction (RT) and percent elongation at break (%E) of the films studied show that the FMP films are harder, presenting high RT and lower %E values. According to Mirzakhani et al. (2015), the presence of high contents of glutamine and asparagine amino acids in the fish myofibrillar proteins provide for double hydrogen bonds (85 kj mol^{-1}) between the peptides of the protein chain, which makes them less stretchable. However, by combining the myofibrillar protein with gelatin in the BL films, it was observed that the films gain in flexibility, although they continue to present significant RT values. In face of the interaction between polymers, the double bonds give way to simple hydrogen bonds (25 kj mol^{-1}), which are weaker, thus reducing the density and shear strength among peptides. This effect might have been favored by the action of pH 3.0 used in the film formulations. Nuanmano et al. (2015) observed the same effect upon the addition of fish gelatin as a plastifier in red tilapia myofibrillar protein films.

It was also observed that the increase in the level of polymers used in the formulation was followed by an increase in RT values. This is probably due to the increased number of protein chains and consequent increase in intermolecular interactions of the films' mesh (Kaewprachu et al., 2016).

Water vapor permeability is one of the most important features for applicability of biodegradable films. Overall, fish protein-based films present low water vapor barrier, which hinders their use in packaging. Nevertheless, WVP is strongly associated with the characteristics of the polymer and its interactions with associated polymers. When strong inter- and intramolecular interactions occur among polymers, crossed disulfide and hydrophobic bonds may take place, resulting in low WVP (Kaewprachu et al., 2016). As water migration across the film is also governed by its mesh, thickness must be taken into consideration, as thicker films with dense structure prevent water migration, thus reducing WVP (Oujifard et al., 2013).

Water solubility of biodegradable films is highly dependent on their polymer structure or the bonds established between them, in the case of blended films (Kaewprachu and Rawdkuen, 2014). As expected, the FG films presented high solubility; firstly, due to the spiral structure acquired by the collagen following partial hydrolysis during gelatin extraction; then, to its hydrophilic amino acid composition (Hoque et al., 2011; Yao et al., 2017). Using glycerol as a plastifier in the formulation of films has contributed to this effect as, in addition to being hydrophilic, glycerol increases the gelatin peptide chains mobility, making them more soluble (Ekrami and Emam-Djomeh, 2014).

The fish myofibrillar protein peptides are bonded mainly by covalent disulfide bond, considered as strong bonds. They make the film mesh more dense and compact, reducing the bonds of hydrogen with water, which makes FMP films' solubility inferior to GP's (Chinabhark et al., 2007). It was observed that the solubility of the fish gelatin and myofibrillar protein films was not different ($p \leq 0.05$) from that of the FMP films, which is probably due to the favorable compatibility between the polymers, which allowed for inter- and intramolecular interactions that makes up a coherent film matrix (Arfat et al., 2014a).

Color and transparency

The yellow color predominant in all films may be related to the presence of lipids retained in the proteins, especially the gelatin, as it is known that the lipid fraction in fish skin is significant. Despite repeated washing during protein extraction (myofibrillar and collagen), residual lipid and oxidizing agents, such as hemoglobin and myoglobin, may have been retained, which would constitute oxidation substrate and thus cause the appearance of yellow pigments (Tongnuanchan et al., 2011a). Commonly the yellowish coloration of the films is also associated with products of the maillard reaction, which is the chemical reaction between amino and carbonyl group. However, the fish muscle is poor in carbohydrates—particularly, reducing sugars (Krochta, 2002). Nevertheless, aldehydes resulting from lipid oxidation may act as a source of carbonyl for the Maillard reaction, particularly upon heating at 70 °C, which is required to obtain the filmogenic solution (Boyd et al., 1993).

The color was intensified with the increase in polymers in the films, which causes the transparency to reduce due to the concentration of polymers that keep light from penetrating the dense film layer. Similar results are portrayed by Tongnuanchan et al. (2011a) when studying the relationship between the process of lipid oxidation and the appearance of coloration in muscle protein film of red tilapia during storage and also by Abdelhedi et al. (2018) in bovine gelatin mixture films and fish protein hydrolyzate.

The FMP films are, overall, harder and more thermally resistant due to the higher amount of disulfide and hydrophobic bonds of protein chains (Tongnuanchan et al., 2011a). The degradation temperatures of BL films were superior those of FG films, which is possibly due to the interactions between FMP and FG, which resulted in a more robust film mesh—more resistant to heat. Similar results were presented by Hoque et al. (2011) and Arfat et al. (2014a), who analyzed blends of fish gelatin and mung bean protein and of fish gelatin and myofibrillar proteins, respectively.

As the initial loss is more related to evaporation of free and adsorbed water, the water contained on the surface of the film has certainly been more easily removed as the predominant bonds among the protein chains of the myofibrillar proteins are hydrophobic in nature. On the other hand, in the BL 1% film, this loss only occurred at elevated temperature (258 °C), where the strong bonds between FMP and FG were broken and the high molecular weight proteins, degraded (Kaewprachu et al., 2016; Mohajer et al., 2017).

The structural relationships observed with the pure and blended films' FTIR spectra show a decrease in the intensity of the amide A bands in the BL 3% film (3263 cm^{-1}), indicating a positive interaction between the film-forming polymers. The dislocation of the vibrational spectrum to a narrower wavelength in the amide A band suggests a broadening of the OH and NH groups interacting and forming hydrogen bonds between the molecules of the polymers in the network of the film (Hoque et al., 2011). This result reiterates the favorable compatibility between the myofibrillar and gelatin protein chains. Such interactions are reflected in the mechanical and barrier properties of the blended films (Table 1). On the other hand, the dislocation to wider wavelengths indicates hydrophobic interactions, such as those between amino and carbonyl groups (Xie et al., 2006).

Arfat et al. (2014a) report similar results with films produced with a blend of fish gelatin and myofibrillar proteins, as the spectra reported by Hoque et al. (2011) upon combining fish gelatin and mung bean protein.

As to the vibrational spectrum observed within the 1047—1036 cm^{-1} range, it is attributed to the possible interactions between the plastifier and the film matrix polymer structure, favored by the hydrogen bonds derived from glycerol OH groups, which confirms the homogeneous mixture between the polymers and glycerol (Bergo and Sobral, 2007).

Considering the micrographies of the FG films, which presents ruggedness and fissures, it may be assumed that such features reduced the RT rates and increased the %E values, making the films more elastic. On the other hand, these gaps have allowed for gas and water penetration, making them more soluble and permeable (Mirzakhani et al., 2015). Contrarily, the FMP films are denser and harder, but the presence of a large amount of insoluble particles causes agglomeration and makes their surface rough to the touch. Furthermore, the dry matter in the film mesh causes hardness and increases resistance to traction, despite reducing its solubility (Table 1). Sobral et al. (2002) made similar observations on films from tilapia myofibrillar protein.

As the BL film structure is more compact, presents few fissures and no insoluble particles, it can be stated that fish myofibrillar proteins and gelatin are favorably compatible. Molecular organization between the polymers in the BL film mesh has allowed for strong interactions between them, which provides them with promising mechanical characteristics (Table 1). Arfat et al. (2014a) observed similar results with films produced from a blend of fish gelatin and myofibrillar proteins.

CONCLUSIONS

Mixture between gelatin and myofibrillar proteins from fish waste produced films with cohesive and homogeneous matrix. The blend films had better mechanical and barrier properties when purchased with the films of the individual polymers. The good chemical interaction between the biopolymers allowed the formation of a resistant and flexible film, with low permeability to water vapor, besides being visually clear and transparent; desired characteristics in food packaging.

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