Effect of adding fatty acids and surfactant on the functional properties of biodegradable films prepared with myofibrillar proteins from acoupa weakfish (Cynoscion acoupa)

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Abstract
This research aimed to assess the properties of biodegradable films prepared with lyophilized myofibrillar proteins (LMP) from fish filleting residues, fatty acids (stearic, palmitic, and caproic), and surfactant (SLS). The films were characterized to assess the effects of adding those components. Adding fatty acids and SLS resulted in more flexible films with higher elongation values compared to the LMP film. The films prepared with 5% stearic acid and 10% SLS and with 10% palmitic acid and 20% SLS had higher tensile strength compared to the LMP film. Solubility reached 100% in the films added with 10% fatty acids with and without SLS. Increasing the concentration of fatty acids and SLS led to less transparent films. Microscopy analysis showed changes in the morphological structure of the films added with fatty acids and SLS, resulting in whitish films when greater SLS contents were used.

Keywords: myofibrillar protein film; compound film; stearic acid; palmitic acid; caproic acid; sodium lauryl sulfate.

Practical Application: Biodegradable packaging to extend the shelf life of food.

1 Introduction

The environmental impact of non-biodegradable plastic residues is a growing global concern. With the goal of partially replacing this type of material, researches have been under way to find renewable, ecologic polymeric materials (Kaewprachu et al., 2018). Such researches to develop biodegradable packagings contribute to decreasing environmental pollution.

The use of byproducts to prepare biodegradable films with protective characteristics and/or fungicidal and bactericidal action may be a promising alternative in preservation systems, which is highly important to the food industry besides contributing to reducing environmental impacts by using byproducts from the fishing industry (Mali et al., 2006). Natural biopolymers such as proteins and polysaccharides are promising raw materials as they are abundant, renewable, economical, and able to form a continuous matrix (Kaewprachu et al., 2016).

The residues and byproducts of fish industry processing may reach up to 70% of the initial weight of fish and are considered high-quality raw materials with low commercial value, which mostly go unused and cause ecological, sanitary, and economic harm (Pires et al., 2011). On the other hand, the food industry is constantly seeking new strategies to extend the shelf life of foods. Edible films and coatings have been considered technologies that may potentially reach such goals by providing microbiological safety and protecting foods from the influence of external factors (Pires et al., 2011).

The proteins of animal origin most commonly used in the formation of biodegradable films are collagen and myofibrillar proteins from fish and cattle (Souza et al., 2004; Raghavan & Kristinsson, 2008; Limpan et al., 2010; Zavareze et al., 2012). Fish proteins are able to form networks, which results in films with proper plasticity and elasticity and good oxygen barrier properties, however, with poor water vapor barrier properties, which may be changed by adding hydrophobic compounds, plasticizers, and additives (Andreuccetti et al., 2010; Zavareze et al., 2012).

The functionality and behavior of films depend mainly on their mechanical and transport properties, which, in turn, depend on the film’s composition, its formation process, and method of application onto the product (Andreuccetti et al., 2010). However, when materials with different hydrophobicity are mixed, adding an emulsifier (surfactant) is required to allow for homogenous dispersion of the hydrophobic material on the hydrophilic protein matrix (Andreuccetti et al., 2010). Thus, the preparation of compound films has used sodium lauryl sulfate (SLS) (Davanço et al., 2007).

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Stearic, palmitic, and caproic acids, among others, are currently used as hydrophobic additives in the formulation of edible films due to their good film-forming characteristics (Rojas-Argudo et al., 2009). According to Caba et al. (2012), adding palmitic-stearic acid blends may reduce the water vapor permeability (WVP) of the films. Adding stearic acid also leads to lower water absorption while not impacting biodegradability (Lodha & Netravali, 2005).

Thus, the present study aimed to assess the properties of biodegradable films prepared with myofibrillar proteins from fish filleting residues, fatty acids, and surfactant.

2 Material and methods

2.1 Obtaining acoupa weakfish byproduct

The residues of filleting (muscle trimming) of acoupa weakfish (Cynoscion acoupa), were obtained from a fish factory located in the municipality of Vigia, Pará, Brazil, were shipped packaged and stored in ice boxes. In the laboratory, the residues were hygienized with chlorinated water (5 ppm) at 4 °C for 5 min and the skins, spines, and other materials were removed to obtain the muscle mass, which was vacuum packaged and stored in a freezer at -18 °C.

2.2 Obtaining Lyophilized Myofibrillar Proteins (LMP)

The methodology proposed by Limpan et al. (2012) was used, with modifications, to obtain the lyophilized myofibrillar proteins (LMP). The ground muscle (Sire cutter, Filizola, Brazil) was mixed at a 1:3 (muscle:acid) ratio with a 0.05% metaphosphoric acid (HP0₃) solution at 4-5 °C. The muscle mass was mixed with three volumes of distilled water at 5 °C, centrifuged at 10,956 g for 2 min in a refrigerated centrifuge (Thermo Scientific, Multifuge X1R, Germany), and then filtered. The material retained was mixed (1:5) for 5 min with a 50 mM sodium chloride solution and filtered, which was performed twice. After those steps, the myofibrillar proteins obtained were spread over stainless steel trays, frozen at -22 °C, and lyophilized at -60 °C for 48 h (Liobras, Liotop L101, Brazil).

2.3 Biodegradable film preparation

The films were prepared according to Davanço et al. (2007) and Limpan et al. (2012) with modifications. The pH of the protein solution (w/v) was adjusted to 11.0 with 2 M NaOH and then the fatty acids (stearic, palmitic, and caproic) and the surfactant (SLS) were added at different concentrations, besides glycerol as plasticizer (Table 1). The solutions obtained were homogenized at 10,000 rpm for 5 min in a Turrurate (Tecnal, TE-102, Brazil) disperser and then placed in a water bath (Tecnal, TE-057, Brazil) for 30 min at 70 °C to obtain the filmogenic solutions. 120 mL of each solution obtained were placed in silicone recipients (22 cm diameter/2.5 cm height) to maintain uniformity and repeatability of the measures. The solutions were then dried in a BOD air circulation oven (Quimis, 0315M16, Brazil) at 26 °C for 17 h. After drying, the films were vacuum packaged (Fastvac, F200, Brazil) and stored at 25 °C.

2.4 Biodegradable film characterization

Thickness

Film thickness was measured using a digital micrometer with 0.001 mm resolution (Insize, IP54, Brazil) (Zavareze et al., 2012).

Transparency value

The light transmittance of the films was measured in a spectrophotometer (Thermo Scientific, Evolution 60, USA) at 600 nm according to the method by Shiku et al. (2004). The analysis was performed in triplicate and the transparency value was calculated using Equation 1 (Han & Floros, 2010).

Table 1. Percentage of the compositions used to prepare the biodegradable films.

<table>
<thead>
<tr>
<th>Samples</th>
<th>LMP (%)</th>
<th>Glycerol (%)</th>
<th>SA (%)</th>
<th>PA (%)</th>
<th>CA (%)</th>
<th>SLS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMP</td>
<td>1</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LMP/5% SA</td>
<td>1</td>
<td>50</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LMP/5% PA</td>
<td>1</td>
<td>50</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LMP/5% CA</td>
<td>1</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>LMP/10% SA</td>
<td>1</td>
<td>50</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LMP/10% PA</td>
<td>1</td>
<td>50</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LMP/10% CA</td>
<td>1</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>LMP/5% SA/5% SLS</td>
<td>1</td>
<td>50</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>LMP/5% PA/5% SLS</td>
<td>1</td>
<td>50</td>
<td>-</td>
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<td>5</td>
</tr>
<tr>
<td>LMP/5% CA/5% SLS</td>
<td>1</td>
<td>50</td>
<td>-</td>
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<td>5</td>
<td>5</td>
</tr>
<tr>
<td>LMP/5% SA/10% SLS</td>
<td>1</td>
<td>50</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>LMP/5% PA/10% SLS</td>
<td>1</td>
<td>50</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>LMP/5% CA/10% SLS</td>
<td>1</td>
<td>50</td>
<td>-</td>
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<td>5</td>
<td>10</td>
</tr>
<tr>
<td>LMP/10% SA/20% SLS</td>
<td>1</td>
<td>50</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>LMP/10% PA/20% SLS</td>
<td>1</td>
<td>50</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>LMP/10% CA/20% SLS</td>
<td>1</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

LMP: Lyophilized myofibrillar protein; SA: stearic acid; PA: palmitic acid; CA: caproic acid; SLS: sodium lauryl sulfate.
Where: VT is the value of transparency, T_oo is transmittance at 600 nm, and x represents the film thickness (mm). Higher transmittance values represent lower film transparency.

**Water Vapor Permeability (WVP)**

The modified American Society for Testing and Materials (1989) method described by Shiku et al. (2004) was used. The films were placed in a glass permeation beaker containing dry silica gel (0% RH; 0 Pa water vapor pressure at 30 °C) with engine sealant (Orbived, Orbi Quimica, Brazil). Next, the beakers 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. Film WVP was calculated using Equation 2.

$$WVP = \frac{W - I}{A \times t \times \Delta P}$$

Where: WVP: water vapor permeability (g·mm/m²·d·kPa); W: weight gain of the beaker (g); I: film thickness (mm); A: exposed film surface area (m²); t: time of gain (d); ΔP: vapor pressure difference through the film (4.2449 kPa at 30 °C).

**Water solubility**

To assess solubility, the samples were cut into disks 2 cm in diameter, placed in an oven at 105 °C for 24 h, and weighed. Then, the dry films were immersed in containers with 50 mL water. These were stirred in a refrigerated shaker incubator (Lucadema, model Luca-223, Brazil) at 150 rpm for 24 h at 25 °C. The samples were then filtered and the retained fraction was dried (105 °C for 24 h) to determine the amount of material not dissolved in water using Equation 3 (Gontard et al., 1994).

$$SOL(\%) = \frac{M_i - M_f}{M_i} \times 100$$

Where: SOL (%): percentage of material solubilized; M_i: initial mass of the sample (g); M_f: final mass of the sample (g).

**Mechanical properties**

Methodology ASTM D882-91 (American Society for Testing and Materials, 1996) was used to verify the tensile strength and elongation at break of the films using a texture analyzer (Emic, DL 500, Brazil). Tensile strength (TS) and percent elongation (%E) were calculated by Equations 4 and 5, respectively.

$$TS = \frac{F_m}{A}$$

Where: TS: tensile strength (MPa); F_m: maximum force at the moment of film rupture (N); A: film cross-sectional area (m²).

$$E(\%) = \frac{d_{\text{final}}}{d_{\text{initial}}} \times 100$$

Where: E: elongation (%); d_{final}: final distance at the time of rupture (cm); d_{initial}: initial gap distance (5 cm).

**Scanning Electron Microscopy (SEM)**

The analyses were performed in a digital scanning electron microscope (Zeiss, LAO 1430, Brazil). The samples were metallized with gold using coating time of 1.5 min. The analysis conditions for the secondary electron images were: electron beam current = 90 µA, constant acceleration voltage = 10 kV, and work distance = 15 mm.

**Statistical analysis**

The data were submitted to analysis of variance (ANOVA) and Tukey's test at 5% (p≤0.05) significance level. The statistical analyses were carried out using the software STATISTICA 7 for Windows.

**3 Results and discussion**

**3.1 Biodegradable film characterization**

The characterization of the films prepared with different formulations is presented in Table 2. Higher contents of acids and surfactant led to thicker films. This behavior was also observed by Oliveira et al. (2012) in gelatin films when greater fatty acids and surfactant contents were added to the solution. According to Hosseini et al. (2016), thickness depends on film composition and processing parameters.

The films containing only fatty acids at higher concentrations (10%) had lower transparency (p≤0.05) compared to the LMP film, as reported by Arfat et al. (2014). That can be explained by the dispersion of light due to the presence of droplets of the lipid compound emulsion in the protein matrix. According to Cerqueira et al. (2012) and Tongnuanchan et al. (2014), the addition of lipid components directly impacts film appearance by making them less transparent (more opaque). According to Acosta et al. (2015), transparency may be strongly related to the migration of the hydrophobic component during film preparation, which may be evidenced by the reduction or loss of transparency.

In the films containing SLS, it was observed that increasing the concentration of fatty acids from 5 to 10% led to a significant increase in transparency (p≤0.05), resulting in less transparent films except when stearic acid was used. The presence of the SLS surfactant led to better interaction of the protein matrix with palmitic and caproic acids at the lowest concentrations, resulting in significantly more transparent films. Polymer transparency and opacity are related to the matrix composition as well as the organization and rearrangement of components (Acosta et al., 2015), Davanço et al. (2007) found similar behavior as the one in the present study, with the transparency of compound gelatin film being impacted by the addition of SLS surfactant. Other authors also found that surfactant contributes to improving the transparency of compound films depending on their concentration and homogeneity of components (Chen et al., 2009).

The films containing LMP and fatty acids had lower WVP values compared to those containing only LMP. However, the film with 10% caproic acid, of shorter chain length, differed (p≤0.05) due to the better interaction of this component with the biopolymers in the matrix. According to Brandelero et al. (2013),
non-polar or hydrophobic substances are commonly added to improve the water vapor barrier properties of hydrophilic biopolymers.

It was observed that the films containing SLS at 5% and 20% had higher WVP values (p≤0.05) compared to those with LMP, except for the film with caproic acid, which had no significant difference. The low solubility of SLS in water and its lipophilic nature may have led to non-homogeneous distribution at that concentration and, consequently, of the hydrophobic component. The same was reported by other authors, who related WVP to the increase in surfactant solubility in the medium (Andreuccetti et al., 2011; Chen et al., 2009).

Davanco et al. (2007), when using only fatty acids (stearic and caproic) in compound gelatin films, found higher WVP values than when 70% SLS surfactant was added, indicating an increase in hydrophobicity. In the present study, lower SLS concentrations were used, which may not have been enough for homogenous distribution due to its low dispersion and weak stability in the emulsion system and/or interaction of the hydrophobic components with the SLS surfactant in the protein matrix.

Water permeability in compound films depends on several factors such as chemical structure of the lipid components added, degree of organization of those components, free space through which the water molecule may permeate (Acosta et al., 2015; Bertan et al., 2005), and the nature of the surfactant used (Andreuccetti et al., 2011; Peng et al., 2013).

It was found that adding 10% fatty acids (stearic, palmitic, and caproic) with or without SLS increased (p≤0.05) film solubility. Bertan et al. (2005) prepared gelatin films with stearic, palmitic, and lauric acids and observed that increasing the concentration of the lipid components reduces molecular interactions among the protein chains, thus de-stabilizing the structure an increasing the solubilization of the components in water. Fakhouri et al. (2003), when using long- and short-chain fatty acids (palmitic, myristic, caproic, and capric) at different concentrations, found no significant difference in the solubility of gelatin-based films.

Adding 5 and 10% surfactant decreased (p≤0.05) the solubility of films regardless of the percentage of fatty acids used. When LMP films were compared with those added with SLS, it was found that increasing the concentration of the surfactant from 10 to 20% increased solubility, except for the film with palmitic acid. The higher solubility may be related to the formation of micelles due to the presence of SLS, which lowers the surface tension of the protein solution above a certain critical micelle concentration. According to Rufino et al. (2011), surface tension decreases when the concentration of surfactant in the aqueous medium increases, leading to the formation of micelles, which are aggregated amphipathic molecules with their hydrophilic portions positioned outwards and the hydrophobic portions positioned inwards.

Adding palmitic and caproic acids at 10% decreased (p≤0.05) the TS of the protein films compared to those with lower concentration (5%) and the LMP film. The reduction in TS as more hydrophobic components are added to the matrix was expected since lipids may impact the protein-protein interactions or lead to the segregation of the lipid phase (Saurabh et al., 2016). Valenzuela et al. (2013) also observed lower TS values as lipids were added to the matrix.

The film with 5% stearic acid and 10% SLS and the film with 10% palmitic acid and 20% SLS had greater TS (p≤0.05) compared to the LMP film. In the present research, it was observed that increasing the concentration of SLS in some formulations (Table 2) led to greater TS. However, according to Rhim et al.

### Table 2. Results of the transparency, water vapor permeability, solubility, and mechanical (tensile strength and percent elongation) analyses of the films composed of myofibrillar protein, fatty acids, and surfactant (SLS).

<table>
<thead>
<tr>
<th>Film types</th>
<th>Thickness (mm)</th>
<th>Transparency value</th>
<th>WVP (g.mm/m²d.KPa)</th>
<th>SOL (%)</th>
<th>TS (MPa)</th>
<th>E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMP/10% PA</td>
<td>0.054 ± 0.00</td>
<td>2.10 ± 0.04</td>
<td>6.95 ± 0.35</td>
<td>92.48 ± 0.28</td>
<td>1.52 ± 0.20</td>
<td>72.00 ± 6.20</td>
</tr>
<tr>
<td>LMP/5% PA</td>
<td>0.057 ± 0.00</td>
<td>1.70 ± 0.06</td>
<td>7.26 ± 0.09</td>
<td>65.33 ± 0.66</td>
<td>2.71 ± 0.22</td>
<td>69.15 ± 6.74</td>
</tr>
<tr>
<td>LMP/5% CA</td>
<td>0.058 ± 0.00</td>
<td>1.60 ± 0.09</td>
<td>7.48 ± 0.75</td>
<td>59.76 ± 0.06</td>
<td>2.38 ± 0.20</td>
<td>58.80 ± 5.98</td>
</tr>
<tr>
<td>LMP/10% AS</td>
<td>0.059 ± 0.00</td>
<td>4.50 ± 0.00</td>
<td>7.89 ± 0.29</td>
<td>100.00 ± 0.00</td>
<td>3.22 ± 0.12</td>
<td>191.40 ± 12.55</td>
</tr>
<tr>
<td>LMP/10% CA</td>
<td>0.054 ± 0.01</td>
<td>4.50 ± 0.49</td>
<td>6.94 ± 0.76</td>
<td>100.00 ± 0.00</td>
<td>1.40 ± 0.07</td>
<td>120.15 ± 8.04</td>
</tr>
<tr>
<td>LMP/10% PA</td>
<td>0.055 ± 0.01</td>
<td>3.60 ± 0.07</td>
<td>5.89 ± 0.85</td>
<td>100.00 ± 0.00</td>
<td>1.17 ± 0.18</td>
<td>129.35 ± 10.30</td>
</tr>
<tr>
<td>LMP/5% SA/5% SLS</td>
<td>0.075 ± 0.01</td>
<td>2.70 ± 0.38</td>
<td>13.40 ± 0.30</td>
<td>34.14 ± 2.13</td>
<td>2.04 ± 0.16</td>
<td>116.75 ± 9.91</td>
</tr>
<tr>
<td>LMP/5% PA/5% SLS</td>
<td>0.063 ± 0.00</td>
<td>1.40 ± 0.04</td>
<td>11.20 ± 0.86</td>
<td>37.40 ± 1.98</td>
<td>1.85 ± 0.40</td>
<td>86.75 ± 7.69</td>
</tr>
<tr>
<td>LMP/5% CA/5% SLS</td>
<td>0.058 ± 0.00</td>
<td>1.40 ± 0.12</td>
<td>10.01 ± 0.78</td>
<td>37.87 ± 1.68</td>
<td>1.79 ± 0.31</td>
<td>93.95 ± 8.70</td>
</tr>
<tr>
<td>LMP/5% SA/10% SLS</td>
<td>0.057 ± 0.01</td>
<td>2.60 ± 0.12</td>
<td>7.20 ± 0.57</td>
<td>47.10 ± 0.05</td>
<td>3.09 ± 0.47</td>
<td>179.50 ± 12.41</td>
</tr>
<tr>
<td>LMP/5% CA/10% SLS</td>
<td>0.061 ± 0.00</td>
<td>2.40 ± 0.50</td>
<td>7.41 ± 0.15</td>
<td>36.92 ± 0.97</td>
<td>2.37 ± 0.37</td>
<td>163.95 ± 10.97</td>
</tr>
<tr>
<td>LMP/5% CA/5% SLS</td>
<td>0.058 ± 0.00</td>
<td>2.10 ± 0.18</td>
<td>6.88 ± 0.23</td>
<td>50.42 ± 0.91</td>
<td>1.78 ± 0.05</td>
<td>133.04 ± 9.86</td>
</tr>
<tr>
<td>LMP/10% SA/20% SLS</td>
<td>0.079 ± 0.00</td>
<td>2.50 ± 0.11</td>
<td>11.03 ± 0.23</td>
<td>100.00 ± 0.00</td>
<td>2.11 ± 0.13</td>
<td>172.60 ± 8.99</td>
</tr>
<tr>
<td>LMP/10% PA/20% SLS</td>
<td>0.077 ± 0.00</td>
<td>3.50 ± 0.02</td>
<td>10.57 ± 0.30</td>
<td>100.00 ± 0.00</td>
<td>2.82 ± 0.45</td>
<td>134.95 ± 8.24</td>
</tr>
<tr>
<td>LMP/10% CA/20% SLS</td>
<td>0.070 ± 0.00</td>
<td>3.10 ± 0.08</td>
<td>9.51 ± 0.58</td>
<td>100.00 ± 0.00</td>
<td>2.56 ± 0.19</td>
<td>142.60 ± 8.15</td>
</tr>
</tbody>
</table>

WVP: water vapor permeability; SOL: Solubility; TS: tensile strength; E: elongation. LMP: lyophilized myofibrillar protein; SA: stearic acid; PA: palmitic acid; CA: caproic acid; SLS: sodium lauryl sulfate. The same letters in the same column indicate no significant difference at p≤0.05 among the means obtained through Tukey’s test.
Pereira et al. (2002), adding SLS to films lowers TS, which is related to the formation of weaker structures due to the lack of hydrophobic interactions close to the protein molecular chains, thus favoring SLS-protein hydrophilic interactions. Davanço et al. (2007) observed a significant reduction in tensile strength when SLS surfactant was added to a compound gelatin film containing stearic and caproic acids.

All formulations, except for the films with 5% fatty acids, had significantly (p≤0.05) greater elongation (%E) values than the LMP film (Table 2). Among the films added only with fatty acids, raising the concentration of hydrophobic substances to 10% led to significantly (p≤0.05) higher elongation values, which shows the fatty acids in the matrix acted as plasticizer or lubricant to make the films more flexible. However, Péroval et al. (2002) found the addition of fatty acids to arabinoxylan films decreased elongation and argued that some lipids are unable to form a cohesive, continuous matrix, thus leading to lower elongation.

3.2 Film Scanning Electron Microscopy (SEM)

The SEM of the LMP film (Figure 1) shows a homogenous structure, which confirms the aggregation of proteins to form a dense, continuous network (Limpan et al., 2010). It also shows the presence of small air bubbles due to foaming during the homogenization of the solution, which did not impact WVP. However, the cracks (fissures) on the surface of the films (Figure 1) may have been produced by the vacuum treatment applied to the sample prior to microscopy, causing the loss of free glycerin, which is not completely miscible with myofibrillar proteins (Monterrey-Quintero and Sobral, 2000), thus reducing %E (Table 2).

Figure 2 shows the SEM result of the films with LMP and fatty acids. The films with 5% fatty acids had more homogenous structure, indicating better incorporation of the acids into the protein matrix (Figure 2a, 2b, 2c). The surface of the films added with fatty acids at higher concentration (10%) had morphological alterations with the presence of fat globules (Figure 2d, 2e, 2f) caused by the failure in totally incorporating the fatty acids into the protein matrices.

The film with stearic acid (Figure 2a), of longer chain, had a more homogenous surface, which confers better barrier property...
Effect of fatty acids and surfactant on the functional properties of biodegradable fish myofibrillar protein films

(WVP). However, the film with caproic acid (Figure 2c) had cracks on its surface, lower flexibility (%E), and greater permeability to water. Davanço et al. (2007), when using stearic and caproic acids in compound gelatin films, also found alterations in film structure. According to Binsi et al. (2013), adding hydrophobic components at high ratios changes the structure of compound films.

Adding SLS to the films (Figure 3) effectively incorporated the fatty acids into the filmogenic matrices compared to the formulations with higher concentration of fatty acids (10%) and no surfactant (Figure 2d, 2e, 2f). However, in the films with 5% SLS (Figure 3a, 3b, 3c), the presence of non-solubilized surfactant particles was observed, which impacts water vapor permeability (Table 2). According to Fabra et al. (2009), the change in the structure of compound films may be attributed to the lack of miscibility of the components. However, Tongnuanchan et al. (2014) indicate that adding different surfactants contributes to the structure or morphology of the films (distribution of oil droplets).

The film with caproic acid and SLS (both at 5%) had a more uneven surface with the presence of small fat globules (Figure 3c). The suggested mechanism is that the emulsion formed was not stable enough to prevent the collapse of the acid droplets. According to Fabra et al. (2009), solvent evaporation during the drying of the film-forming solution may lead to changes in the emulsion structure due to de-stabilization phenomena such as flocculation, coalescence, and separation of the lipid phase. It is clearly seen that the film containing longer chain stearic acid (5%) with the highest concentration of SLS surfactant (5%) (10%) had a more homogenous surface, which provides it better mechanical properties.

The films with the highest concentrations of fatty acids (10%) and SLS surfactant (20%) (Figure 3g, 3h, 3i) had alterations in morphology, leading to whitish films. Davanço et al. (2007), when using stearic acid and SLS, also observed whitish color in compound gelatin films. The film with palmitic acid (Figure 3h) had a more heterogeneous structure, however, its mechanical properties were not affected and it exhibited higher tensile strength (Table 2) than the films with the same concentrations (Figure 3g, 3i).

Figure 3. Scanning electron microscopy of the films with lyophilized myofibrillar protein (LMP) and sodium lauryl sulfate (SLS). a) 5% stearic acid and 5% SLS; b) 5% palmitic acid and 5% SLS; c) 5% caproic acid and 5% SLS; d) 5% stearic acid and 10% SLS; e) 5% palmitic acid and 10% SLS; f) 5% caproic acid and 10% SLS; g) 10% stearic acid and 20% SLS; h) 10% palmitic acid and 20% SLS; i) 10% caproic acid and 20% SLS.
4 Conclusion

Film thickness changes as the components are added at different concentrations. Films with the shortest carbon chains (palmitic and caproic acids) at 5% added with 5% SLS were the most transparent whereas the lowest water vapor barrier was found for the film with 10% caproic acid compared to the LMP. %E improved when acids were used at the highest concentration (10%) and along with SLS.

Scanning electron microscopy shows that adding fatty acids and SLS at 10% positively impacted the structure of the films. The most homogenous surface was observed in the film containing stearic acid (5%), of longer chain, and 10% SLS. Films with higher concentrations of fatty acids and SLS surfactant showed morphological alteration with whitish color.

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