

Development and Characterization of Soy Protein Isolate Emulsion-Based Edible Films with Added Coconut Oil for Olive Oil Packaging: Barrier, Mechanical, and Thermal Properties

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Abstract Emulsion-based edible films made of soy protein isolate (SPI), virgin coconut oil (VCO), and soy lecithin (SL) and plasticized with glycerol were prepared using the casting method. The effect of VCO and SL concentrations in SPI films and their in-between interaction were studied through the evaluation of physical (moisture and opacity), mechanical (elongation and tensile strength), water vapor permeability, and thermal properties. The response surface methodology was used to identify the most significant factors in the properties studied. The applicability of SPI emulsion-based films was evaluated as a package for olive oil to be used in small portions. The oxidative stability of the packaged olive oil was monitored by peroxide analyses during 28 days. The incorporation of VCO and SL decreased the moisture content and increased the elongation of the SPI emulsion-based films when compared to the SPI film without these components (control). The opacity of the films increased with the addition of VCO into the protein-based films, but not with the addition of SL or a combination of both constituents. By the other hand, the water vapor permeability was not improved by the incorporation of VCO, SL, or a combination of both. The peroxide value of the olive oil stored in SPI emulsion-based film sachets increased rapidly during the seven first days of storage. After this period, the peroxides increased relatively slow up to 28 days of storage. The peroxide values of the

packaged olive oil did not reach the maximum limit recommended by the Codex Alimentarius. Based on these results, this work may be useful for the technological enhancement of emulsion-based films or for food packaging applications.

Keywords Emulsion-based edible film · Soy protein isolate · Soy lecithin · Virgin coconut oil · Olive oil packaging

Abbreviations

SPI	Soy protein isolate
VCO	Virgin coconut oil
SL	Soy lecithin
Gly	Glycerol
FFS	Film-forming solution
TS	Tensile strength
ELO	Elongation at break
WVP	Water vapor permeability
TGA	Thermogravimetric analysis

Introduction

In the past few years, there was an increased interest in developing environmentally friendly packaging, as those produced from renewable resources, especially in the field of bioplastics. Bioplastics or edible films are commonly produced using proteins (Blanco-Pascual et al. 2014; Chang and Nickerson 2014; Hammann and Schmid 2014; Gofferje et al. 2014), lipids (Chiumarelli and Hubinger 2014), polysaccharides (Antonioni et al. 2014; Jost et al. 2014; Pan et al. 2014a), or mixed composite blends (Kowalczyk and Baraniak 2014; Marquez et al. 2014; Pan et al. 2014b; Winkler et al. 2015). In general, polysaccharides and proteins provide mechanical

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stability and good gas barrier properties, while fats are used to minimize water transmission (Pavlath and Orts 2009).

Proteins have several functional properties for film formation due their structure and intrinsic properties. They present multiple sites for chemical interactions due to the high diversity of amino acid functional groups and charged areas along the protein chains, which, in turn, result in interactive forces capable of improving the stability of films (Hammann and Schmid 2014; Dangaran et al. 2009). Recent reports suggested the use of soy protein isolate (SPI) as a matrix of edible films (Ou et al. 2005; Cho et al. 2007; Denavi et al. 2009; Atarés et al. 2010; Kokoszka et al. 2010; González et al. 2011; Friesen et al. 2015; Hopkins et al. 2015) due to the significant production of soy protein in the world and its recognized ability of forming edible films. The SPI comprises a mixture of proteins, of which approximately 90 % are globulins: 7S (β -conglycinin) and 11S (glycinin) are the main fractions of globulins, corresponding to approximately 37 and 31 % of the total extractable protein (Cho and Rhee 2004). The SPI is known for its good gelling capacity and emulsifying characteristics (Nishinari et al. 2014), which means it can improve the incorporation of hydrophobic components into solutions where it is contained, as in the case of solutions for making composite or blend films. Thus, the structural and functional properties of the SPI make it a potential element for the development of edible films.

The SPI edible films are transparent and flexible, exhibit good oxygen barrier (Krochta and Miller 1997; Cho et al. 2007), have low cost, and are of biodegradable origin and available worldwide. However, protein-based edible films are sensitive to moisture and exhibit poor water vapor barrier (Guilbert et al. 1996; Al-Hassan and Norziah 2012). The permeability of water vapor or gases depends on their movement through a biopolymer film (diffusion)—which is related to the permeant film affinity—and their dissolution through this biopolymer (solubility) (Pavlath and Orts 2009; Miller and Krochta 1997). Thus, the incorporation of components having lower affinity with the diffusing molecule can decrease this rate, for example dispersing lipids in the protein matrix (Bertan et al. 2005b; Yang and Paulson 2000; Limpisophon et al. 2010; Zahedi et al. 2010), since lipids present nonpolar or hydrophobic characteristics. Other strategies can also be employed for the improvement of the water vapor barrier of films, such as applying cross-linking agents (Schmid et al. 2014), applying additional film layers (Cho et al. 2010; Anker et al. 2002), and combining different plasticizers (Wan et al. 2005). Among these techniques, the incorporation of lipids appears to be a promising alternative; since it requires only one step in the manufacturing process, there is high availability of waxes and lipids and some lipids may also exerted additional benefits to the film structure.

Coconut oil is extensively used for food and industrial purposes (Marina et al. 2009a). It shows excellent oxidative

stability since more than 90 % of its fatty acids are saturated. Virgin coconut oil (VCO) is known for its important antioxidant activity (Marina et al. 2009b; Seneviratne et al. 2009). Emulsifiers are essential in the formation of protein-based films containing lipid molecules (Zhao 2012). Aiming the formation of a homogeneous film structure, several authors have suggested the use of surfactants such as soy lecithin and yucca extract (Andreuccetti et al. 2011), Tween 40 (Kowalczyk and Baraniak 2014), glycerol monostearate, and Tween 60 and 80 (Bravin et al. 2004). Soy lecithin is an amphoteric surfactant; it is composed of one or two fatty acid groups (hydrophobic) linked to a glycerol chain, a phosphate and alcohol group (hydrophilic) (Stauffer 2005).

Natural biopolymer-based packaging materials have a good potential for enhancing food stability, safety, and quality (Garcia et al. 2010; Chen et al. 2012). Edible pouches and sachets made from heat-sealable biodegradable films can be considered as novel packaging systems, once they permit a controlled release of added active compounds, such as flavors, antioxidants, and antimicrobial agents (McHugh and de Avena-Bustillos 2012).

World production of soybeans is about 315.05 million tons, and Brazil is the second largest producer (94.50 million tons) behind only of the USA (108.01 million tons) (USDA 2015). Due to the significant production of soy protein in Brazil and its recognized ability to form edible films, SPI was chosen as the main matrix in this work. The VCO was incorporated to the film solution in order to improve the water vapor permeability and mechanical properties (elongation) of the SPI edible films. Soy lecithin was chosen as the surfactant agent, since it is a by-product of soybean oil processing. Thus, the objective of this study was to develop, characterize, and study an application of emulsion-based edible films made of soy protein isolate with added virgin coconut oil and soy lecithin. Emulsion-based films were characterized with respect to their mechanical, thermal, and barrier properties. The applicability of the SPI emulsion-based edible films was evaluated in the form of a sealed pack for olive oil.

Material and Methods

Material for Film Production

Commercial SPI (>90 % protein content; Bremil Food Products Industry Ltda., Brazil) was used as the matrix of the film. Anhydrous glycerol (Gly, ≥ 99.5 % purity; Panreac, Spain) was used as the plasticizer. Distilled water and ethanol (96 % purity; Panreac, Spain) were used as the solvent. VCO (Copra Food Industries, Brazil) was incorporated in the emulsion-based edible films, and soy lecithin (SL; Imcopa Import, Export and Oil Industry S.A., Brazil) was employed as the surfactant.

Preparation of the SPI Emulsion-Based Films

The edible films were prepared using the casting method. The concentration of SPI was fixed in 6.5 % (*w/w* film-forming solution), and the glycerol content was based on SPI content at 2.5:10 (Gly:SPI, mass basis). Ethanol (20 % *w/w* film-forming solution) and distilled water (completing 100 % *w/w* film-forming solution) were applied as the solvent. Ethanol was used in the emulsion-based films to solubilize soy lecithin and also to accelerate the drying process of the film-forming solutions. Table 1 shows the different film formulations studied, varying VCO and SL content. Preliminary experiments were conducted to determine the threshold concentration of SPI, plasticizer (glycerol), surfactant (SL), and VCO in order to produce flexibility on handling films that did not show phase separation and VCO or glycerol exudation, and were also easy to remove from the supports (plates).

Tests were performed with SPI concentrations ranging from 5.0 to 9.0 % (*w/w*). The forming solution with protein concentrations lower than 6.0 % resulted in films with fragile and brittle texture, while film solutions containing above 7.0 % protein became highly viscous after heating, which hampered their removal from the plates. Thus, the concentration of soy protein isolate was set at 6.5 %. The mass ratio of plasticizer was fixed in 2.5:10 (Gly:SPI), since films produced with concentrations of plasticizer lower than 2.5:10 were fragile and broke easily during their removal from the support. The surfactant was added to the matrix at 1:10, 2:10, and 3:10 (SL:Gly) mass ratios, according to the study performed by Andreuccetti et al. (2009). Higher amounts of soy lecithin were not applied due to the increased difficulty of solubilizing it and to avoid the increasing formation of bubbles in the film-forming solution. In the first tests, the proportion of surfactant was fixed at 3:10 (SL:Gly) and increasing mass ratios of virgin coconut oil (VCO:SPI) were added to the formulation (0.1:10, 0.4:10, 0.7:10, and 1:10). As a result, the film was visually

homogeneous for ratios up to 0.7:10 (VCO:SPI). The incorporation of VCO into the SPI matrix was not possible without surfactant. Control films were prepared from solutions containing only SPI, glycerol, water, and ethanol, in order to compare it with those containing VCO and SL.

The film-forming solution (FFS) was prepared by dispersing the SPI in distilled water at 25 °C. The pH of the solution was adjusted to 9.00 ± 0.05 (NaOH, 5 N), and the solution was stirred for 60 min. Meanwhile, SL was solubilized in ethanol under magnetic agitation for 90 min at 25 °C. After this, the SL solution, the glycerol, and the VCO were added to the SPI solution, which was heated in a thermostatic bath (Ethik Technology) until the FFS reached 70 ± 3 °C. This temperature was chosen in order to partially disrupt the tertiary and quaternary structure of SPI and to expose its hydrophobic chains. Then, the FFS was homogenized (Polytron PT 3100D) firstly at 13,500 rpm for 1 min, followed by 20,500 rpm for 2 min, according to the methodology described by Rezvani et al. (2013). Fixed volumes of FFS (Table 1) were poured on acrylic plates (14 cm × 14 cm) and dried at 25 ± 3 °C for 24 h. During the step of film drying, the acrylic plates were maintained in a plain table with adjustable feet in order to minimize irregularities in film thickness. After being dried, the films could be easily removed from the plates without using lubricant and without any additional effort. All films were conditioned in desiccators (25 ± 3 °C, 57 ± 3 % relative humidity, maintained using a saturated $Mg(NO_3)_2$ solution) during 7 days prior to the analyses so the films could reach moisture equilibrium. This methodology of conditioning was previously reported by some authors (Fakhoury et al. 2012; Galus et al. 2012; Andreuccetti et al. 2009; Ozdemir and Floros 2008; Bertan et al. 2005b), who suggested a minimum of 48 h of film conditioning in these conditions.

Properties of the Emulsion-Based Edible Films

Film Thickness Film thickness was determined using a digital micrometer with a sensitivity of 1 μm (Mitutoyo, Japan). Measurements of film specimens were taken at ten random points. The results were expressed as the mean of random measurements made in each type of film.

Moisture Circular film specimens (20 mm in diameter) were weighed and dried at 105 °C for 24 h in a convection oven. The weight loss of each sample was determined, and the moisture content was calculated as the percentage of water removed from the films. Analyses were performed in triplicate.

Opacity Opacity measurements were performed by spectrophotometry, according to Gontard et al. (1992), Cho and Rhee (2004), Cho et al. (2007), and Zahedi et al. (2010). A spectrum of each film specimen (1 × 4 cm) was recorded using a UV-VIS spectrophotometer (UV-1800; Shimadzu, Japan). The

Table 1 Film-forming solution (FFS) formulations

Formulation ^a	SL:Gly (<i>w/w</i>)	VCO:SPI (<i>w/w</i>)	FFS (mL)
A	3:10	0.7:10	20.0
B	3:10	0.4:10	20.4
C	3:10	0.1:10	20.9
D	2:10	0.7:10	20.3
E	2:10	0.4:10	20.8
F	2:10	0.1:10	21.3
G	1:10	0.7:10	20.7
H	1:10	0.4:10	21.2
I	1:10	0.1:10	21.7
Control	0:10	0:10	22.32

^a All formulations contain soy protein isolate (SPI) (6.5 % *w/w*), ethanol (20 % *w/w*), glycerol, and 2.5:10 (Gly:SPI)

area under the absorbance curve ranging from 360 to 800 nm was calculated as the opacity of the films, which were expressed as absorbance unit (AU)×nanometer/micrometer.

Water Vapor Permeability The water vapor permeability (WVP) test was performed according to a modification of the Standard Method E96/96M-13 (ASTM Standard E96/E96M 2013). Circular test cells (cups) of 0.06 m (internal diameter)×0.035 m (depth) with an exposed film area of 0.0028 m² were used. The cells were filled with anhydrous calcium chloride, and the film specimens were sealed over the circular opening of the test cups. The cups were placed in desiccators containing saturated NaCl solution (25 °C, 75 % relative humidity (RH)). The gain of weight of the cells was monitored every 24 h during 7 days. The experiments were performed in triplicate, and the WVP was calculated using Eq. (1).

$$\text{WVP} = \frac{Gx}{tAS(R_1 - R_2)} \quad (1)$$

where G/t is the rate of humidity absorbed (g s⁻¹), A is the permeation area (m²), x is the average thickness of the film specimen (m), S is the partial pressure of water vapor at the test temperature (Pa), and $(R_1 - R_2)$ is the gradient of relative humidity of the environment containing the calcium chloride (0 % RH) and the saturated NaCl solution (75 %).

Mechanical Properties The tensile strength (TS, MPa) and elongation at break (ELO, %) of the films were measured on a Brookfield CT3 Texture Analyzer (Brookfield Engineering Laboratories, Inc., USA), according to the Standard Method D882-12 (ASTM Standard D882 2012). Rectangular film strips of 60 mm length and 25 mm width were used as the test specimen. The initial grip separation was set at 20 mm, and the crosshead speed was set at 1 mm s⁻¹. TS was calculated using the equation $TS = F/A$, where F is the force (N) at maximum load and A is the initial cross-sectional area (m²) of the film. The ELO (%) was determined by dividing the film elongation at rupture by the initial gauge length. Measurements for each film were repeated at least ten times, and the results are expressed as mean values.

Thermogravimetric Analysis The thermal stability and degradation of films were analyzed by thermogravimetric analysis (TGA) (model 4000; PerkinElmer, USA). Samples weighing about 10–15 mg were scanned from 25 to 600 °C at a heating rate of 10 °C/min. Nitrogen was used as purge gas at (20 mL/min) in order to avoid thermo-oxidative reactions. Thermal analysis was carried out on the control film and on the emulsified edible film that presented the highest concentration of surfactant and coconut oil (formulation A).

Film Pouch Production for Olive Oil Packaging

Pouches were made with the emulsion-based edible films in order to pack olive oil as a condiment that could be easily used in small portions. The methodology was based on the study of Souza et al. (2011), applying some modifications. The films were cut in 100 mm×40 mm pieces, and two of these were taken and sealed on three sides using a VC999K3/CH9100 sealer (Switzerland), creating a pouch. Samples of olive oil weighing approximately 5.0±1.0 g were used to fill the pouch, which was subsequently sealed to form a four-side sealed pouch, as shown in Fig. 1. A control pack was produced using SPI, Gly, ethanol, water, and SL (1:10, SL:Gly), without VCO, in order to check whether the incorporation of VCO could have some additional effect or negatively influence the stability of the packed olive oil. The control pack was submitted to the same storage conditions of the VCO-containing pouches. The film pouches containing olive oil were stored in desiccators at controlled RH (60±3 %), maintained using a saturated Mg(NO₃)₂ solution. These desiccators were stored at 30 °C in photoperiod conditions (EL 202, 20-W lamp; EletroLab, Brazil).

The shelf life of the pouched olive oil was monitored for 28 days. The oxidative stability of the olive oil was determined by the peroxide value (POV) according to the AOCS official method Cd 8-53 (AOCS 2001).

Statistical Analysis

Statistical analysis was conducted using the analysis of variance (one-way ANOVA, $p < 0.05$) procedure available in the STATISTICA® software (StatSoft, Tulsa, USA), version 10. Statistically different means were analyzed using Tukey's post



Fig. 1 Sachet for olive oil packaging, produced from emulsified films made of SPI, glycerol, ethanol, SL, and VCO

hoc test. The data were fitted to a second-order equation (Eq. (2)) as a function of the dependent variables.

$$Y_i = b_0 + b_1 \cdot SL + b_2 \cdot VCO + b_{12} \cdot SL \cdot VCO + b_{11} \cdot SL^2 + b_{22} \cdot VCO^2 \tag{2}$$

where b_n are constant regression coefficients, Y_i is the dependent variable (physical, mechanical, and water barrier properties), and SL and VCO are the independent variables. Response surface graphics were constructed in order to check the influence of independent variables on the properties of emulsion-based films. From the proposed model, we considered only the significant parameters that described the behavior of the dependent variables. The results of Eq. (1) fitting were not presented for those dependent variables where the model was not able to reproduce experimental data.

Results and Discussion

Properties of the Emulsion-Based Edible Films

Moisture Content The water content of films is an important parameter, since the water can act as a plasticizer in the polymeric matrix (Sothornvit and Krochta 2005). The moisture content of the control and the emulsified edible films obtained are described in Table 2. The moisture content of control film was $11.65 \pm 1.17\%$, while for the emulsified films, it ranged from $6.22 \pm 0.53\%$ (formulation D) to $9.86 \pm 0.36\%$ (formulation A). The moisture of the emulsified films was significantly lower than the control film ($p < 0.05$). Among the

emulsified films, it was found that lower water contents were reached in the intermediate SL concentration (2:10, SL:Gly).

Figure 2 presents the response surface of the emulsion-based films' moisture content as a function of SL and VCO. It was found that the concentration of VCO and SL had a significant effect on the reduction of moisture. However, the moisture was not affected by the interaction of these variables. Thus, the model considering only the significant parameters is given by Eq. (3). It can be observed graphically and by Eq. (3) that the SL content was the most influencing parameter on the reduction of film moisture.

$$\text{Moisture content}(\%) = 13.529 - 6.280 \text{ SL} - 5.959 \text{ VCO} + 1.705 \text{ SL}^2 + 7.016 \text{ VCO}^2 \tag{3}$$

where the errors of the parameters of Eq. (2) are $b_0 \pm 1.053$, $b_1 \pm 1.118$, $b_2 \pm 2.515$, $b_{11} \pm 0.276$, and $b_{22} \pm 3.072$ with $R^2 = 0.715$.

In its native state, the polar groups of the SPI are unavailable because the hydrophobic region of the SPI protein is enclosed by a hydrophilic region, maintained by non-covalent forces and disulfide bonds (Qu et al. 2015). The processing conditions applied for the production of SPI emulsion films in this study, namely alkaline conditions (Qu et al. 2015), heating (Keerati-u-rai and Corredig 2010), and the homogenization process (Damodaran 2008), can partially or fully denature the proteins and expose their functional groups, cleave disulfide bonds, and expose hydrophobic and sulfhydryl groups. After this process, probably the tertiary and quaternary structures were disrupted, exposing the hydrophobic side chains of the SPI and making them available to bind to VCO and the non-polar side of the lecithin molecule. Since soy lecithin is a

Table 2 Physical and water barrier properties of SPI control and SPI emulsion-based films

Formulation ^a	SL:Gly (w/w)	VCO:SPI (w/w)	Moisture content (%)	Opacity (AU × nm/μm)	WVP (g m ⁻¹ s ⁻¹ Pa ⁻¹)
A	3:10	0.7:10	9.86 ± 0.36 ^{bC}	6.10 ± 0.64 ^{bCDE}	(8.13 ± 0.39) × 10 ^{-11bCD}
B	3:10	0.4:10	8.18 ± 0.19 ^{aBC}	5.99 ± 0.17 ^{bCDE}	(10.10 ± 0.04) × 10 ^{-11cE}
C	3:10	0.1:10	9.55 ± 0.65 ^{bC}	3.93 ± 0.17 ^{aA}	(5.99 ± 0.71) × 10 ^{-11aA}
D	2:10	0.7:10	6.22 ± 0.53 ^{aA}	6.28 ± 0.26 ^{cDE}	(9.27 ± 0.55) × 10 ^{-11aDE}
E	2:10	0.4:10	7.26 ± 0.37 ^{bAB}	5.23 ± 0.24 ^{bBC}	(9.01 ± 0.25) × 10 ^{-11aDE}
F	2:10	0.1:10	7.36 ± 0.11 ^{bAB}	3.94 ± 0.19 ^{aA}	(9.11 ± 0.14) × 10 ^{-11aDE}
G	1:10	0.7:10	8.50 ± 0.57 ^{aBC}	6.31 ± 0.12 ^{cE}	(6.75 ± 0.58) × 10 ^{-11aAB}
H	1:10	0.4:10	7.56 ± 0.62 ^{aAB}	5.41 ± 0.50 ^{bBCD}	(7.61 ± 0.31) × 10 ^{-11aBC}
I	1:10	0.1:10	8.28 ± 0.54 ^{aBC}	4.01 ± 0.04 ^{aA}	(12.0 ± 0.06) × 10 ^{-11bF}
Control	0:10	0:10	11.65 ± 1.17 ^D	4.53 ± 0.18 ^{AB}	(5.57 ± 0.30) × 10 ^{-11A}

Moisture content, opacity, and WVP are means ± standard deviation of each formulation (n=3). Means in the same column with different superscript letters are significantly different by Tukey's HSD test (p < 0.05): uppercase letters (comparison between all formulations) and lowercase letters (comparison between fixed concentrations of lecithin)

SL soy lecithin, VCO virgin coconut oil

^a All formulations contain soy protein isolate (SPI) (6.5 % w/w), ethanol (20 % w/w), glycerol (Gly), and 2.5:10 (Gly:SPI)

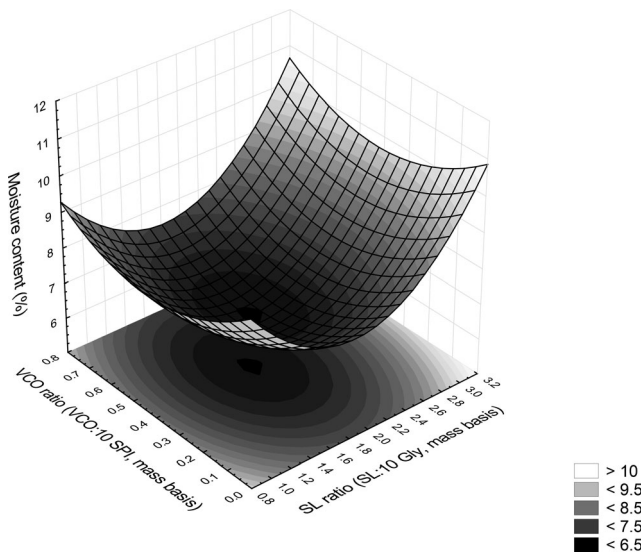


Fig. 2 Moisture content of SPI emulsion-based edible films as a function of the SL and VCO

predominantly lipophilic surfactant (Bueschelberger 2004), the VCO and the hydrophobic chains of the SPI can link to these domains, while the hydrophilic chains of the protein can bind to the polar side of the lecithin (phosphate and alcohol group) and to glycerol. As a result, there are fewer free domains available to bind to the water molecules. Thus, a reduction in the moisture content of SPI emulsion-based films was expected, as also observed by Peng et al. (2013) after the incorporation of lipids and surfactant in films. Indeed, the incorporation of SL and VCO (within the range of this study) resulted in a moisture reduction of the emulsified films when compared to the control one. The moisture reduction can be considered as a positive result, since a decrease in the water content of the structure may increase the stability of the pack during storage. On the other hand, the water can act as a plasticizer and improve the flexibility of edible films. So, the reduction in the moisture content can be considered as a negative or positive result, depending on the purpose for which it is intended for packaging.

Opacity The opacity values of the emulsion-based films are presented in Table 2. The opacity of control film was 4.53 ± 0.18 ($\text{AU} \times \text{nm}/\mu\text{m}$). The incorporation of VCO:SPI in the ratio of 0.1:10 at all concentrations of SL showed no significant difference in the opacity of films with respect to the control. Among the emulsified films, there was a linear trend on increasing the opacity, as the concentration of VCO:SPI is increasing, but not with the addition of SL, as also verified in the data fitting (Fig. 3). Since there was no significant influence of SL content on opacity, the model was simplified to a function of VCO only, as shown in Eq. (4).

$$\text{Opacity} = 3.230 + 7.765 \text{ VCO} - 4.974 \text{ VCO}^2 \quad (4)$$

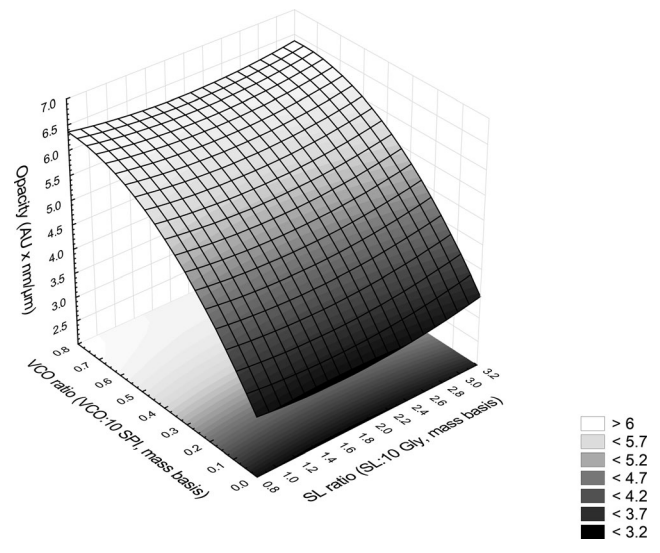


Fig. 3 Opacity of SPI emulsion-based edible films as a function of SL and VCO

where the errors of the parameters of Eq. (3) are $b_0 \pm 0.200$, $b_2 \pm 1.272$, and $b_{22} \pm 1.554$ with $R^2 = 0.896$.

This increase in opacity can be explained by the solidification of VCO at room temperature. The VCO has precise melting characteristics: at 21 °C, it is hard and brittle, a typical behavior of oils or fats with a predominance of lauric acid (45.9 to 50.3 % of a total of 90 % saturated fatty acids) (O'Brien 2009). An increased opacity, due to the addition of hydrophobic components, was also observed by Wang et al. (2014) and Bertan et al. (2005b). Thus, it was concluded that VCO (0.7:10, VCO:SPI) had a significant effect on increasing the opacity of SPI films. Opacity is a desired parameter in some packages, as those for foods containing photosensitive compounds, since it can reduce the passage of light.

Water Vapor Permeability Several properties can influence the permeability of the biopolymer films, such as the chemical structure of the components, the interstitial space between the molecules, the organization of the molecules in the film structure, the orientation or alignment of the protein chains in the structure, and the formation of intermolecular bonds among the chains of the proteins (cross-linking) and biopolymer blends (composite or emulsified films) (Miller and Krochta 1997). Thus, the films of this study were incorporated with VCO and SL to check if the permeability of the control film could be improved and how the components could exert influence on these properties. The results of WVP for the SPI control and SPI emulsion-based films are described in Table 2.

Effect of the Combined Action of SL and VCO In general, for the emulsified films, WVP values ranged from $(5.99 \pm 0.71) \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ (formulation C) to $(12.0 \pm 0.06) \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ (formulation I). The combined incorporation of VCO and SL did not decrease the water

permeability of the films when compared to the control. It was expected that the hydrophobic portion of the surfactant should decrease the rate of solubility and diffusion of water molecules through the film structures (Rhim et al. 2002). Similarly, the use of VCO should also decrease the water permeability due to their hydrophobic nature. However, this effect was not found. According to Gontard et al. (1994), if materials with high steric dimensions (such as SL) are employed at the composite film formulations and if these components are not associated with the protein chains, the structural matrix of the films could stretch. So, a small degree of irregularity in a film can exponentially increase the diffusion rate (Pavlati and Orts 2009) since there is an increase of interstitial spaces between the protein chains, allowing water molecules to move more easily through the structure.

Effect of the Addition of SL The effect of the addition of SL was checked by comparing the control film to the emulsified films using fixed proportions of VCO. The first assays involved the application of the lower proportion of VCO (0.1:10, VCO:SPI) and three concentrations of SL (1:10, 2:10, and 3:10, SL:Gly), formulations I, F and C, respectively. It was found that the three formulations evaluated showed higher WVP than the control film ($5.57 \pm 0.30 \times 10^{-11} \text{ g}^{-1} \text{ m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$). However, in formulation C, these values were significantly equal to the control ($p < 0.05$). For these systems containing 0.1:10 (VCO:SPI), the WVP of the emulsified films decreased with the increasing lecithin content.

When keeping the proportion of VCO in 0.4:10 or 0.7:10 (VCO:SPI), there was an increase in the WVP for higher amounts of lecithin. As the emulsion formed in this work is an oil-water type (due to lecithin characteristics), this may have led to an inhomogeneous distribution of lecithin and VCO in the polymer matrix. Soy lecithin is a hydrophobic surfactant type, and because of this, it is less soluble in the continuous hydrophilic phase of the film-forming emulsion. Maybe for these reasons, inconsistent results of WVP were obtained. The expected tendency for these data was the decrease in WVP with increasing SL and VCO. Andreuccetti et al. (2011) evaluated the effect of the surfactants SL and yucca extract (*Yucca schidigera*) on the WVP of gelatin-based edible films. Films containing yucca extract presented lower values of WVP when compared to the lecithin-based ones. The authors attributed this behavior to the low solubility of lecithin in water, which, in turn, may have generated a non-homogeneous distribution of the surfactant in the matrix.

Effect of VCO Addition The increasing addition of VCO only showed a positive effect on reducing the films' permeability at the lecithin ratio of 1:10 (SL:Gly). For the

3:10 (SL:Gly) ratio, the best WVP value was obtained for the lowest concentration of VCO applied, 0.1:10 (VCO:SPI). Bravin et al. (2004) concluded that the concentration and type of lipid exert different effects on the water vapor barrier of film composites of corn starch and methylcellulose. They found that the WVP of these edible films was significantly reduced by the addition of soybean oil (10 to 20 %) but was not reduced when using cocoa butter. To this behavior, it was attributed to the formation of discontinuous zones, which were probably crystallization related. The water vapor transmission through emulsion-based or composite films still depends on the nature (crystal arrangement and chain length) and dispersion of the lipid in the matrix (Gallo et al. 2000; Bertan et al. 2005b; Bravin et al. 2004) and on the type of surfactant applied (Peng et al. 2013; Andreuccetti et al. 2011; Bravin et al. 2004).

Although the methodology proposed did not lead to a decrease in the WVP of the emulsion-based films when compared to the control, the WVP values obtained in this study were slightly lower than those from other studies involving soy protein films (Denavi et al. 2009) and soy protein isolate films incorporated with beeswax, span 20, and glycerol (Chao et al. 2010), for example. Other studies involving different protein-based films showed similar trends, such as films produced with whey protein and glycerol (Ramos et al. 2013) as well as gelatin, triacetin, lauric acid, and a blend of stearic and palmitic acid (Bertan et al. 2005a). However, the WVP values of this study were higher than those obtained in films involving other matrices, for example quinoa protein-chitosan-sunflower oil edible film (Valenzuela et al. 2013), chitosan-virgin coconut oil film (Binsi et al. 2013), and synthetic films, such as polyethylene films (low-density polyethylene (LDPE)) (Peychès-Bach et al. 2009, 0.14×10^{-11} to $0.28 \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$).

The addition of VCO and/or SL had no positive effect on reducing the film WVP when compared to the control one. This behavior probably occurred due to the difference in the structural and chemical nature of the components used in the formulations. The incompatibility of the components may have limited the incorporation of VCO and SL to the protein matrix, leading to the formation of heterogeneous zones. These zones may have been formed by the concentration of lipids in a region or by the remaining hydrophilic ends of the surfactant or glycerol, which have affinity to humidity. The structural volume of SL and the lipophilic nature of the surfactant in an aqueous medium may also had led to the formation of cavities, facilitating the permeation of water in the structure of the films. The second-order model did not reproduce adequately the experimental data of WVP for the emulsified films.

Table 3 Mechanical properties of SPI control and SPI emulsion-based films

Formulation ^a	SL:Gly (w/w)	VCO:SPI (w/w)	Thickness (μm)	TS (MPa)	ELO (%)
A	3:10	0.7:10	70.0±3.0	8.09±0.77 ^{aA}	211.94±9.79 ^{cD}
B	3:10	0.4:10	73.0±2.0	12.19±0.43 ^{cE}	136.77±8.88 ^{aA}
C	3:10	0.1:10	69.0±3.0	9.44±0.76 ^{bBC}	155.92±12.29 ^{bAB}
D	2:10	0.7:10	69.0±3.0	7.65±0.48 ^{aA}	250.01±27.84 ^{cD}
E	2:10	0.4:10	69.0±3.0	8.65±0.81 ^{bAB}	213.69±27.09 ^{bC}
F	2:10	0.1:10	70.0±2.0	10.89±1.23 ^{cD}	168.64±16.39 ^{aB}
G	1:10	0.7:10	73.0±2.0	7.70±0.38 ^{aA}	273.94±15.04 ^{bD}
H	1:10	0.4:10	69.0±2.0	9.71±0.57 ^{cC}	162.71±15.91 ^{aB}
I	1:10	0.1:10	67.0±3.0	8.36±0.50 ^{bA}	154.17±13.81 ^{aAB}
Control	0:10	0:10	64.0±3.0	12.05±0.61 ^E	103.75±16.78 ^A

TS and ELO values are means±standard deviation of each formulation ($n=10$). Means in the same column with different superscript letters are significantly different by Tukey's HSD test ($p<0.05$): uppercase letters (comparison between all formulations) and lowercase letters (comparison between fixed concentrations of lecithin)

SL soy lecithin, VCO virgin coconut oil, TS tensile strength, ELO elongation at break

^a All formulations contain soy protein isolate (SPI) (6.5 % w/w), ethanol (20 % w/w), glycerol (Gly), and 2.5:10 (Gly:SPI)

Mechanical Properties The investigated mechanical properties—ELO (%) and TS (MPa)—of the control and emulsion-based edible films are shown in Table 3. Regardless of the concentration of SL used, by increasing the proportion of VCO from 0.1:10 to 0.7:10 (VCO:SPI), there was also an increase in the elongation of the films, comparing to the control one (103.75±16.78 %). Monedero et al. (2009) reported an increase in elongation of SPI films added with oleic acid and attributed this behavior to the plasticizing effect of the acid in the matrix.

From the adjusted second-order model, it was found that the SL and VCO content had significant effect on increasing the elongation of the emulsion-based films, as shown in Fig. 4.

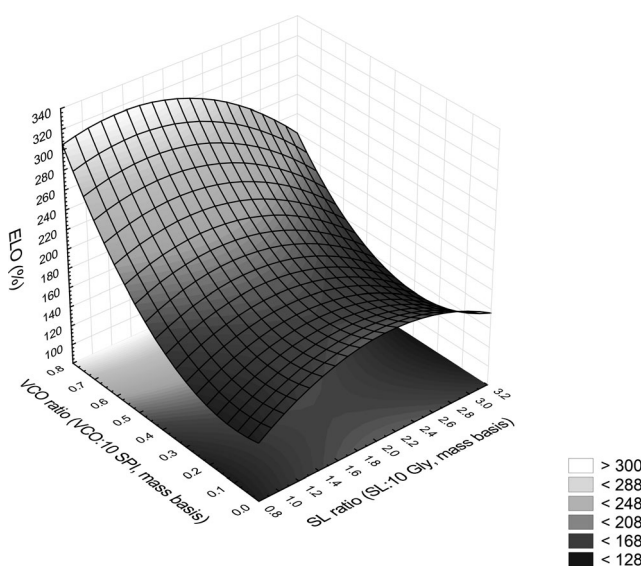


Fig. 4 Elongation at break (ELO) of SPI emulsion-based edible films as a function of SL and VCO

The model considering the significant parameters is described by Eq. (5).

$$\text{ELO}(\%) = 56.16 + 120.96 \text{ SL} - 56.28 \text{ SL VCO} - 28.20 \text{ SL}^2 + 320.61 \text{ VCO}^2 \quad (5)$$

where the errors of the parameters of Eq. (4) are $b_0 \pm 17.80$, $b_1 \pm 19.54$, $b_{12} \pm 9.91$, $b_{11} \pm 4.73$, and $b_{22} \pm 26.15$ with $R^2 = 0.815$.

The TS of the films ranged from 7.65±0.48 MPa (formulation D) to 12.19±0.43 MPa (formulation B). For the higher and lower ratios of VCO (0.1:10 and 0.7:10, VCO:SPI), the tensile strength showed the opposite behavior of elongation; that is, the incorporation of VCO into the SPI matrix caused a decrease in TS values. This mechanism can be explained by the partial replacement of polymers by lipids in the matrix, since the interactions of only polar polymer molecules are stronger than those involving only lipid molecules and also stronger than those between lipids and polar polymer molecules (Yang and Paulson 2000). The addition of lipids in the matrix by means of an emulsion may induce the development of discontinuities in the polymer network of the structures (preferential break points), leading to a decrease in TS (Bravin et al. 2004). The decrease in TS due to the addition of lipids into the matrix was also observed by Valenzuela et al. (2013), Atarés et al. (2010), and Yang and Paulson (2000). The second-order model did not reproduce adequately the experimental data of TS for the emulsified films.

A positive effect on the elongation of the films was found due to the incorporation of VCO and SL in SPI films. These components must have acted as plasticizers or lubricants in the protein structure of the films, resulting in the increase of flexibility and the reduction of tensile strength.

Thermogravimetric Analysis Thermogravimetric analysis and derivative thermogravimetric (DTG) curves of the control film and SPI emulsion-based edible film (formulation A) are illustrated in Fig. 5.

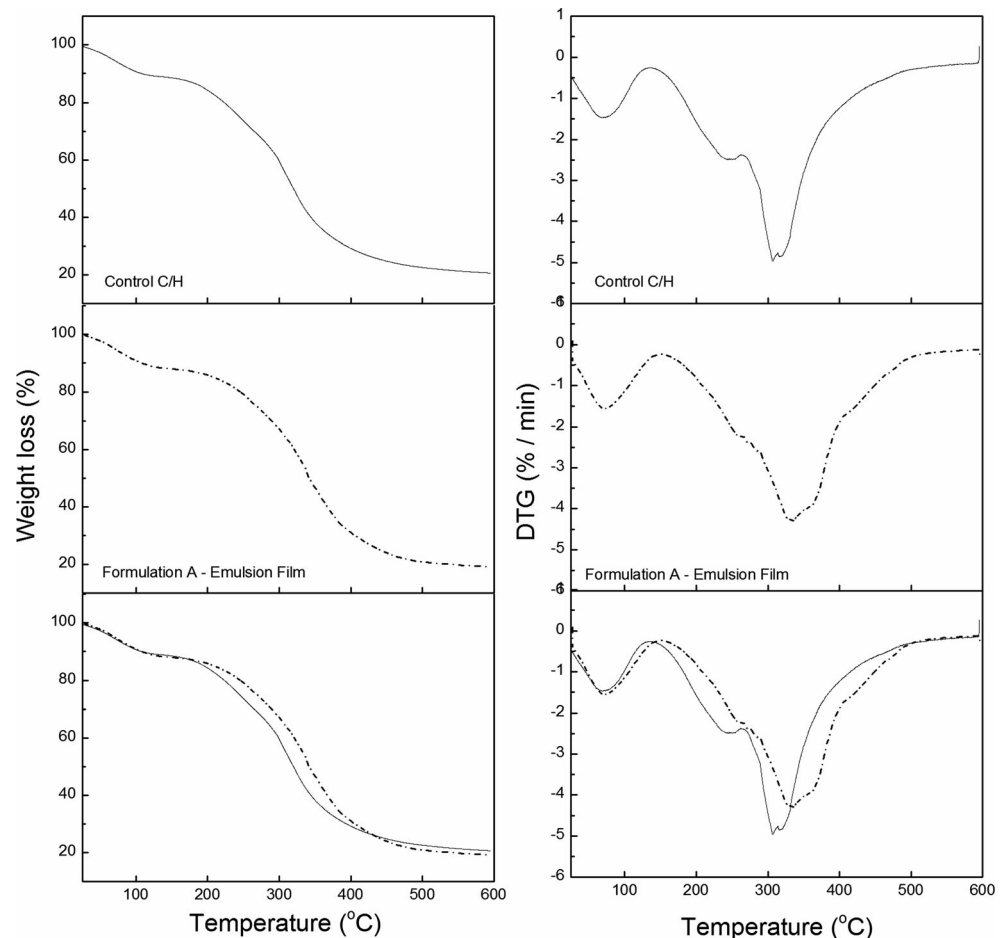
For both films, four well-defined stages of weight loss were observed. In the first stage, similar weight losses (Dw_1) were found, corresponding to 5.6 and 5.3 % at temperatures (Td_1) of 70.80 and 72.04 °C, for the control and formulation A films, respectively. Guerrero et al. (2011) and Su et al. (2010) observed that up to 100 °C, the weight loss at the first stage is related to the moisture loss of films. The second stage was verified at temperatures (Td_2) of 266.95 and 257.01 °C for the control and formulation A films, respectively. In this case, formulation A showed the lower weight loss (Dw_2) (22.4 %) than the control film (30.5 %). This stage was associated with beginning of SPI film degradation, whereas the main loss occurs at the range of 270–380 °C (Song et al. 2013). For film A, Td_2 value can be attributed to the thermal decomposition of coconut oil. The onset temperature of pure coconut oil is 257 °C and involves the rupture of oxygenated hydrocarbons present in the oil into volatile lower molecular hydrocarbons, carbon dioxide, and carbon monoxide (Jayadas and Nair 2006).

The third stage (306.83 and 333.12 °C) and fourth stage (318.14 and 364.8 °C) are probably associated to the degradation of soy protein. Kumar et al. (2010) attributed the decomposition of soy protein and loss of glycerol in the range of 300–400 °C. Tongnuanchan et al. (2014) associated a thermal degradation in the range of 301.29–334.48 °C to the protein fractions in fish gelatin film incorporated with essential oils and surfactants.

Film Pouch Production for Olive Oil Packaging

The storage conditions of the sealed pouches were chosen in order to estimate the susceptibility or stability of the packaged olive oil: accelerated conditions (30 °C, 60 % RH) and using photoperiod (12 h light and 12 h darkness), simulating a real storage condition. This application was performed to preliminarily and qualitatively provide an indicative of the oxygen barrier capability of the emulsion-based films, once oxygen is the main responsible for altering the quality of oils. If the quality of olive oil could be maintained, we would indirectly have an indicative of the oxygen permeability of the packaging. Olive oil oxidative stability was monitored up to 28 days

Fig. 5 Thermogravimetric curves of SPI films. Control film, 6.5 % of SPI (*w/w*), 2.5:10 (Gly:SPI); formulation A film, 6.5 % of SPI (*w/w*), 20 % ethanol (*w/w*), 2.5:10 (Gly:SPI), 3:10 (SL:Gly), and 0.7:10 (VCO:SPI)



through peroxide analysis, and the results are shown in Table 4.

The peroxide value of the olive oil stored at 30 °C increased with storage time regardless of the formulation of the film. After 7 days of storage, the POV of the olive oil increased rapidly, approximately 42.09, 33.70, and 27.82 % for control pack, formulation G, and formulation I, respectively. According to Cho et al. (2010), the lipid oxidation in the early stages of storage occurs due to the consumption of oxygen present in the headspace of the pouch. After the first 7 days, the level of hydroperoxides in the olive oil increased relatively slow up to 28 days of the storage. It is known that SPI films show low oxygen permeability values (Rhim et al. 2006; Denavi et al. 2009; Cho et al. 2010) at low and intermediate relative humidity. Cho et al. (2010) verified that the SPI films had better oxygen barrier properties than the NY/mLLDPE (nylon/metalloocene-catalyzed linear low-density polyethylene), the material usually applied for packing some condiments. Other plastics, such as LDPE and high-density polyethylene (HDPE) showed lower oxygen barrier than SPI films, as concluded by Rhim et al. (2006). Thus, in the present work, most likely the oxidation reactions ceased due to the complete consumption of oxygen in the headspace of the pouch.

There was no significant difference in the stability of the olive oil samples as a result of the different packaging formulations. In other words, the addition of VCO to the emulsion-based edible films did not exert an additional effect in reducing the oxidative rancidity when compared to the control pack containing only lecithin. Cho et al. (2010) evaluated the use of bilayer film pouches made

of corn zein and soy protein isolate to pack olive oil at 50 °C and 30 to 50 % RH. These authors concluded that after 30 days of storage, the POV of the olive oil was approximately 50 mEq/kg of oil and that these pouches were able to control lipid rancidification at dry and intermediate RH ranges. According to the Codex Alimentarius (CODEX STAN 33-1981, Rev. 1-1989, 2001), the maximum limit of POV to maintain the quality of extra virgin olive oil is 20 mEq O₂/kg. After 28 days of storage, the POV of packaged olive oil in the emulsion-based edible films did not reach the maximum limit regulated by the Codex Alimentarius.

We concluded that the SPI-based edible films could be applied as pouch packaging since the integrity of the pouch can be maintained during storage, even though studies should be made in the sense of improving their tensile strength. The peroxide values of the olive oil increased with the time of storage, independent of film formulation. However, this behavior was only pronounced in the first days of storage (first week), probably due to the oxygen present in the pouch headspace. The methodology proposed in this work was applied as a means to obtain an indicative of oxygen permeation, since this gas is the main responsible for affecting the quality of oils. Additionally, direct oxygen measurements should be made to investigate the oxygen barrier properties of these packages in order to fully understand the parameters influencing the permeation of the gas. Other strategies could be employed to maintain or prolong the stability of these films as well, such as packing the oil under vacuum or inert atmospheres.

Table 4 Peroxide value (POV) of olive oil stored in SPI emulsion-based edible films in controlled conditions (30 °C, 60±3 % RH, 12 h light and 12 h darkness)

Time (days)	Peroxide value (mEq/1000 g of sample)		
	Control pack ^a	Formulation G ^b	Formulation I ^c
0	7.590±0.011 ^{aA}	7.590±0.011 ^{aA}	7.590±0.011 ^{aA}
7	10.785±0.842 ^{aA}	10.148±0.265 ^{aAB}	9.702±0.294 ^{aAB}
14	11.545±0.521 ^{aAB}	10.657±0.081 ^{aAB}	11.418±1.501 ^{aB}
21	12.008±0.022 ^{aAB}	12.203±0.100 ^{aB}	12.562±0.794 ^{aB}
28	12.939±0.010 ^{aB}	13.197±0.976 ^{aB}	11.164±0.021 ^{aB}

Values are means±standard deviation of each analysis ($n=3$). Means with different superscript letters are significantly different by Tukey's HSD test ($p<0.05$). Lowercase letters refers to different formulations and fixed days (lines). Uppercase letters refers to a fixed formulation and different days (columns)

^a Control pack [6.5 % SPI (w/w), 20 % ethanol (w/w), 2.5:10 (Gly:SPI), 1:10 (SL:Gly), and 0:10 (VCO:SPI)]

^b Formulation G [6.5 % SPI (w/w), 20 % ethanol (w/w), 2.5:10 (Gly:SPI), 1:10 (SL:Gly), and 0.7:10 (VCO:SPI)]

^c Formulation I [6.5 % SPI (w/w), 20 % ethanol (w/w), 2.5:10 (Gly:SPI), 1:10 (SL:Gly), and 0.1:10 (VCO:SPI)]

Conclusions

In the present work, the incorporation of VCO was proposed to improve the water vapor barrier and mechanical properties of films made of SPI. In order to homogenize the system and disperse the VCO evenly, SL was used as a surfactant in the film-forming solution. Although it was not possible to improve the WVP of the films with the addition of coconut oil and surfactant, their values were, in some cases, lower than those from other SPI films found in the literature and similar to films made of some other proteins. All emulsified film formulations resulted in films with improved flexibility, which is an important property for making flexible packages. Although this increase in elongation has led to a decrease in the tensile strength, the flexibility of these films allows them to be applicable in a range of products, since the package can be adapted to the surface of the products without damaging their structure. The reduction in moisture content and increased opacity was also observed in emulsion-based films, but their effect may be considered positive or negative, depending on the destination of the packages. Thus, a most appropriate

formulation must be adjusted according to the characteristics of the food product to be packaged. The integrity of the SPI emulsion pouches was maintained during the storage period, which is an interesting attribute for future applications of these films as flexible packages. We believe that this work can contribute to the area of emulsified films, since there are few studies in which the influence of the surfactant is studied at various concentrations in a more detailed depth.

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