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Mechanical properties and water vapour permeability of hydrolysed collagen–cocoa butter edible films plasticised with sucrose

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ABSTRACT

The aim of this study was to develop and characterise edible films produced from hydrolysed collagen and cocoa butter and plasticised with sucrose. The mechanical properties, water vapour permeability, opacity and morphology of the films were characterised. The film composition that yielded the best results was used to produce a coating for application in chocolate panned products. A water-based coating with desirable barrier properties that could replace shellac is important for the environment as well as health, and also because chocolate products have great appeal among children. The films obtained were easily manageable and flexible. Sucrose reduced tensile strength (TS), while hydrolysed collagen at concentrations above 15% increased it. Cocoa butter resulted in less-resistant films. The elongation at break values (EAB%) were higher for films containing higher sucrose concentrations. The water vapour permeability (WVP) ranged from 0.32 to 0.63 g mm m^{-2} h^{-1} kPa^{-1}. For the same concentration of cocoa butter, the WVP was directly affected by the thickness of the film, i.e., the greater the thickness, the higher the WVP. Cocoa butter increased film opacity, while sucrose decreased it, particularly at concentrations above 17.5%. High concentrations of hydrolysed collagen produced films with more homogeneous surfaces. The brightness of the product with the coating developed in this study was attractive; however the brightness of the product with shellac was considered more intense. The properties of these films indicate that they are promising systems for coating chocolate panned products.

1. Introduction

The food industry regularly faces challenges related to the preservation of the properties of processed products. Preserving the characteristics of food products, such as by optimising process conditions, using preservatives or developing protection systems such as packaging, films and coatings, is an on-going area of study (Thakhiew, Devahastin, & Soponronnarit, 2010).

Studies of alternative systems for food protection that utilise biopolymers have increased significantly because these substances are entirely biodegradable and often edible and protect against environmental effects. The package also can play an active role in the food (nutrition and carrier substances of interest) (Mayachiew & Devahastin, 2010).

Sothornvit and Krochta (2000) and Dangaran and Krochta (2003) reported that edible films made from whey protein isolate and plasticised with sucrose were excellent oxygen barriers and were flexible, tough and highly glossy. According to Dangaran, Renner-Nantz, and Krochta (2006), all these characteristics are desirable for coating applications. Lee, Dangaran, and Krochta (2002) determined that whey protein isolate coatings plasticised with sucrose provided more gloss to panned chocolate candies than those plasticised with glycerol, propylene glycol or polyethylene glycol, although a significant loss of gloss was observed during storage. Dangaran et al. (2006) evaluated the addition of crystallisation inhibitors to a whey protein isolate-sucrose high-gloss coating and concluded they were effective in preventing the cracking of coatings and loss of gloss caused by sucrose crystallisation during product storage.

According to Krochta (2002), protein-based films have good mechanical resistance but are hygroscopic. The addition of hydrophobic compounds can improve the water vapour barrier but can also decrease the mechanical resistance of the films. When materials with different hydrophobicities are mixed, an emulsifier must

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be added to enable the homogeneous dispersion of the hydrophobic material in the hydrophilic protein matrix.

In the confectionery industry, the gloss and the moisture barrier of chocolate-covered products are conferred by the application of shellac, which is the refined form of lac, a resinous secretion of the Lac insect. Shellac is used in solutions of ethyl alcohol or isopropyl. According to Lee, Dangaran, Krochta, Guinard, and Krochta (2002), although shellac provides desirable gloss and functions as a good moisture barrier, many disadvantages are associated with its use for glazing confectioneries. Alcohols are miscible with the fats and oils contained in chocolate and may cause an undesirable bitter taste and off-flavours. Using alcohol as a solvent also produces volatile organic compounds, which are hazardous to the environment.

The development and characterisation of water-based edible films that could replace shellac with desirable barrier properties is important for the environment and for health especially because chocolate panned products have great appeal among children.

Lee, Dangaran, Krochta, Guinard, et al. (2002) studied the consumer acceptance of whey-protein-coated chocolate as compared with shellac-coated chocolate, and the results demonstrated a high potential for the use of whey protein isolate (WPI) as a chocolate glaze alternative to shellac. The results also showed that a significant majority of the consumers were not familiar with the term "shellac." Thus, the majority of the consumers were neutral in their attitude towards products containing shellac, resulting in "no change" in their purchase intent for those products containing shellac. However, a significant majority of the consumers expressed "strong dislike" or "dislike" for those products associated with beetle exudates, significantly decreasing their purchase intent.

The aim of this study was to develop and characterise edible films based on hydrolysed collagen, sucrose and cocoa butter for application on the surface of chocolate panned products to provide protection and brightness.

2. Materials and methods

2.1. Materials

The following materials were used to produce the films: hydrolysed collagen (Hidrogel® B50, supplied by Gelita South America) and pectin (Genu® pectin type B rapid set-Z, supplied by CP Kelco). Sucrose, cocoa butter, soy lecithin and glucose syrup 40DE were obtained from a local market.

2.2. Preparation of films

The films were prepared by dehydrating film-forming solutions with casting technique. In the experimental design, the hydrolysed collagen concentration ranged from 6.6 to 23.4%, sucrose concentration from 11.6 to 28.4% and cocoa butter concentration from 4.64 to 15.4%. The film-forming solutions also included 3% pectin, 3% glucose syrup (as an inhibitor of sucrose crystallisation) and soy lecithin (3.5% over the amount of cocoa butter). Preliminary tests demonstrated that film solutions without pectin resulted in phase separation, with low emulsification of the components.

Glucose syrup and soy lecithin were added to the distilled water and heated to 65 °C with stirring for 5 min. Sucrose and pectin were added, and stirring was continued for an additional 5 min, followed by the addition of hydrolysed collagen with constant stirring for another 5 min. The cocoa butter was then added, and the mixture was stirred at 500 rpm for 5 additional min. Distilled water was added until the solid content reached 30%.

The film-forming solutions were stored at 65 °C for 1 h to eliminate air bubbles. The filmogenic solution obtained was dispersed on plates (15 cm diameter) and kept at room temperature (25 °C) for 20 h to dry. The time required to dry the films was visually determined to obtain an easy peeling from the plates.

Before analysis, the dry films were stored in desiccators containing saturated sodium bromide (NaBr) solution with 58% Relative Humidity (RH) at 22 °C for 4 days. The thicknesses of the films were measured with a manual micrometer (Mytutoyo, Tokyo, Japan).

2.3. Tensile strength and elongation tests

The mechanical properties were evaluated by conducting tests according to ASTMD 882 (ASTM, 1995). The analyses were performed with a Texturometer TA.XT2 (Stable Microsystems, Surrey, England) with a 5 kg load cell. Films were cut into strips that were 25 mm wide and 100 mm long. The separation grip distance and crosshead speed were 50 mm and 1 mm/s, respectively. A stress as a function of distance was applied until the rupture occurred. The results of the tensile strength (TS) and elongation at break (EAB) tests were expressed in MPa and percentage (%), respectively. Each test trial per film consisted of 5 replicate measurements. All measurements described were performed at room temperature immediately after the removal of the films from the desiccators.

2.4. Water vapour permeability

The water vapour permeability (WVP) of the films wasdetermined at 25 ± 0.5 °C according to the ASTM E 96/E 96 M-05 method (ASTM, 2010). The insides of the cells were filled with anhydrous calcium chloride (0% RH). The cells were covered with the conditioned films, sealed and placed in a chamber with air circulation at 75 ± 2%RH. The cells were weighed six times during a 72 h period, and the determinations were replicated 4 times for each batch of films. The data were recorded on a weight gain vs. time graph. The coefficient of the straight line obtained by linear regression was determined, and the WVP of the films was determined as follows: $WVP = (WVTR \cdot L)/\Delta P$, where WVTR is the water vapour transmission rate through the film, calculated from the slope of the straight line divided by the exposed film area (m^2); L is the mean film thickness; and ΔP is the partial water vapour pressure difference across the two sides of the film (ASTM, 2010).

2.5. Opacity

Film opacity was determined with a Minolta CR140 colorimeter according to Eq. (1) as described in ASTM D523 (1999), and 10 determinations were made 4 samples of each test, after calibration with standard black and white backgrounds.

$$O_p = O_{pb}/O_{pw} \times 100 \quad (1)$$

O_p represents the percentage film opacity; O_{pb} represents the film opacity against a black background; and O_{pw} represents the film opacity against a white background.

2.6. Film morphology and visual aspects

Morphology was evaluated by optical microscopy with a Mod. Olympus BX4, and images were captured (100× magnification) with an Olympus Q-Color3 digital camera. Visual aspects such as homogeneous surface, phase separation and brittle zones were also observed.

2.7. Chocolate panned product production

The chocolate was applied to rice crisp balls by using a 5 L coating pan. The dark chocolate was melted and held at the desired controlled temperature for panning. Untempered chocolate was used.

The melted dark chocolate was poured uniformly across the revolving centres with a ladle until a uniform chocolate layer was built up on the centres. Cold air was applied to set the coating. This was repeated until the desired amount of coating on the centres had been obtained.

2.8. Coating application

The film composition with lower opacity was used to produce a coating solution that was applied to the surface of chocolate panned products. The sensory brightness of this product was compared to a control product to which shellac was applied.

The coating was applied as described by Dangaran et al. (2006). The film solution was applied to 1000 g batches of chocolate panned products by using a 5 L coating pan. The coating solution was added in 8 aliquots of 5 mL each. After each addition, the batch was tumbled in the coating pan to evenly distribute the coating solution on the surfaces of the product. The pan was then stopped, and the coating was dried with forced ambient air for 10 min. This process was repeated until the batch weight was increased by 1.2%. Shellac was applied to the surface of the control product after polishing.

2.9. Sensory evaluation

A paired comparison test with 30 consumers was used. The purpose was to determine whether two samples differed with respect to a specified attribute, in this case, the brightness of a product with shellac and the brightness of a product with the coating that showed the best results in this study.

2.10. Statistical design

A full factorial design (2^3) was adopted to determine the influence of three independent variables, at two levels each, on film properties. The complete design consisted of 17 experiments with 3 central and 6 axial points, which were included to estimate the pure error of the analysis and to predict the lack of fit of the models. The three independent variables were hydrolysed collagen concentration (x_1), sucrose concentration (x_2) and cocoa butter concentration (x_3) in the casting solution (Table 1). Variable levels were chosen from preliminary studies. The responses under observation were water vapour permeability (Y_1), opacity (Y_2), tensile strength (Y_3) and elongation at break (Y_4).

The Statistica® 10 (StatSoft Inc., Tulsa, USA) program was used for the analysis of variance, regression coefficient calculation and response surfaces. The statistical analyses were reported with 95% confidence intervals.

Table 1
Real levels of independent variables.

Independent variables (%)	Coded variables	−1.68	−1	0	+1	+1.68
Hydrolysed collagen	x_1	6.60	10	15	20	23.40
Sucrose	x_2	11.60	15	20	25	28.40
Cocoa butter	x_3	4.64	8	10	12	15.36

3. Results and discussion

Table 2 shows the experimental values and coded levels of the independent variables according to the central composite design and the results obtained.

3.1. Tensile strength (TS)

According to Fig. 1, in general, sucrose reduced the TS, while hydrolysed collagen increased the TS. In the range studied, cocoa butter exhibited a slight tendency to reduce the TS. Bertan, Tanada-Palmu, Siani, and Grosso (2005) pointed out that the addition of fatty acids weakened gelatine-based films, which may indicate that the protein phase has a higher tensile strength than the lipid phase and that the increased concentration of lipids causes a reduction in the tensile protein phase.

Similar results were obtained by Yang and Paulson (2000), who observed that the TS of films composed of gelan and a blend of stearic and palmitic acid or bees wax decreased sharply as the lipid concentration was increased. Other authors, including Gallo, Debeaufort, Callegarin, and Voilley (2000) and Gontard, Duchez, Cuq, and Guilbert (1994), have observed similar phenomena.

Statistical analysis of the TS response yielded a regression model significant at $p < 0.05$ within the range studied. Except for the quadratic term of the variable cocoa butter ($x3$ (Q)) and the interactions of collagen and sucrose ($x1\ x2$), all others parameters of the model were significant, and the model could be developed with the coded variables. Therefore, the coded model is expressed by Eq. (2).

$$Y = 1.67 + 0.38x_1 + 0.18x_1^2 - 0.66x_2 + 0.21x_2^2 - 0.26x_3 - 0.36x_1x_3 + 0.27x_2x_3 \quad (2)$$

The ANOVA data obtained when considering the significant coefficients resulted in a R^2 of 83%, and the calculated regression F-value was higher than the tabulated value, indicating good reproducibility of the experimental data.

Fig. 1 shows that the TS decreases as the concentration of sucrose (plasticiser) increases. Hydrolysed collagen increased the TS, particularly at concentrations above 15%. Andreuccetti, Carvalho, and Grosso (2010) reported that an increase in the plasticiser concentration produced a decrease in the TS value of gelatin-based films, regardless of the type of hydrophobic plasticiser evaluated. Chiumarelli and Hubinger (2012) also observed a reduction in TS with increases in plasticiser concentration in cassava starch–carnauba wax films.

Cocoa butter reduced the TS and affected the influence of hydrolysed collagen on increases in TS, mainly when the hydrolysed collagen was added at concentrations lower than 15%. Only the combination of low concentrations of cocoa butter combined with high concentrations of hydrolysed collagen resulted in slightly larger TS (Fig. 2). Chiumarelli and Hubinger (2012) reported that the TS was increased only in films without added lipids.

The films TS decreased with increasing concentrations of sugar and cocoa butter. The results showed that this effect could be minimized by adding sugar at concentrations below 16% and cocoa butter at concentrations below 12% (Fig. 3).

The tensile strength results obtained in this study are much lower than those reported in the literature for gelatin-based films, with tensile strength values close to 100 MPa, but are similar to those observed by Dangaran and Krochta (2007) for whey protein films. Al-Hassan and Norziah (2012) reported that sago starch-gelatin edible films plasticised with glycerol exhibited a tensile strength ranging from 1.28 MPa to 1.67 MPa.

In general, sucrose and cocoa butter reduced the TS, while hydrolysed collagen increased it. By analysing only hydrolysed

Table 2
Responses of dependent variables to the film-forming compositions.

Run	Coded values			Real values (X)			Responses (Y)			
	X_1	X_2	X_3	Hydrolysed collagen (%)	Sucrose (%)	Cocoa butter (%)	WVP[a]	OP (%)	TS (MPa)	EAB (%)
1	−1	−1	−1	10	15	8	0.52	24.45	2.47	20.40
2	1	−1	−1	20	15	8	0.34	23.45	4.87	11.34
3	−1	1	−1	10	25	8	0.63	20.55	1.28	23.64
4	1	1	−1	20	25	8	0.41	20.83	1.80	44.14
5	−1	−1	1	10	15	12	0.42	27.72	2.49	27.16
6	1	−1	1	20	15	12	0.32	26.51	2.17	22.08
7	−1	1	1	10	25	12	0.51	21.97	1.12	69.78
8	1	1	1	20	25	12	0.55	22.66	1.45	39.82
9	−1.68	0	0	6.6	20	10	0.50	22.71	1.30	25.18
10	1.68	0	0	23.4	20	10	0.56	21.23	2.61	24.74
11	0	−1.68	0	15	11.6	10	0.46	26.56	2.82	17.08
12	0	1.68	0	15	28.4	10	0.62	21.11	1.26	62.26
13	0	0	−1.68	15	20	4.6	0.50	19.45	1.50	54.96
14	0	0	1.68	15	20	15.4	0.39	23.15	1.28	21.88
15	0	0	0	15	20	10	0.43	22.57	1.71	38.52
16	0	0	0	15	20	10	0.39	22.45	1.65	40.02
17	0	0	0	15	20	10	0.50	23.67	1.87	47.44

[a] WVP (Water vapour permeability in g mm m^{-2} h^{-1} kPa^{-1}).

collagen in the absence of cocoa butter, it was observed that hydrolysed collagen concentrations of greater than 15% would be more effective. However, when the influence of cocoa butter was analysed along with hydrolysed collagen, concentrations of hydrolysed collagen of greater than 20% were necessary to obtain the same range of TS.

3.2. Elongation at break (EAB%)

The highest percentages of EAB were observed for those films containing sucrose concentrations greater than 20%. However, statistical analysis did not demonstrate a significant regression model ($p < 0.05$) within the range studied. The trend observed in this study was also observed by Sobral and Bergo (2007), who reported an increase in EAB when glycerol concentrations were increased. The authors highlighted that this behaviour was due to the plasticiser effect of glycerol, i.e. the plasticisers reduce the interactions between adjacent chains in the biopolymer, leading to increased mobility and, consequently, film flexibility. Run 7 and run 12 (Table 2), which utilised high levels of sucrose (plasticiser), resulted in higher percentages of EAB and lower TS.

Dangaran and Krochta (2007) reported that films plasticised with sucrose exhibit decreased TS and EAB during storage due to the conversion of amorphous sucrose to crystalline sucrose over time, resulting in brittle films that weakened and cracked.

3.3. Water vapour permeability (WVP)

There were no apparent trends in WVP as a function of film composition. At the same concentration of cocoa butter, the thickness of the film had a greater influence on WVP than the actual composition of the film. The thickness of the films ranged from 0.153 to 0.234 mm.

The WVP results ranged from 0.32 to 0.63 g mm m^{-2} h^{-1} kPa^{-1}. Gelatine films with added hydrophilic plasticisers have been reported to have WVP coefficients of 0.54–0.95 g mm m^{-2} h^{-1} kPa^{-1} (Thomazine, Carvalho, & Sobral, 2005); 0.30–0.59 g mm m^{-2} h^{-1} kPa^{-1} (Vanin, Sobral, Menegalli, Carvalho, & Habitante, 2005); 0.17–0.38 g mm m^{-2} h^{-1} kPa^{-1} and 0.44–1.23 g mm m^{-2} h^{-1} kPa^{-1}

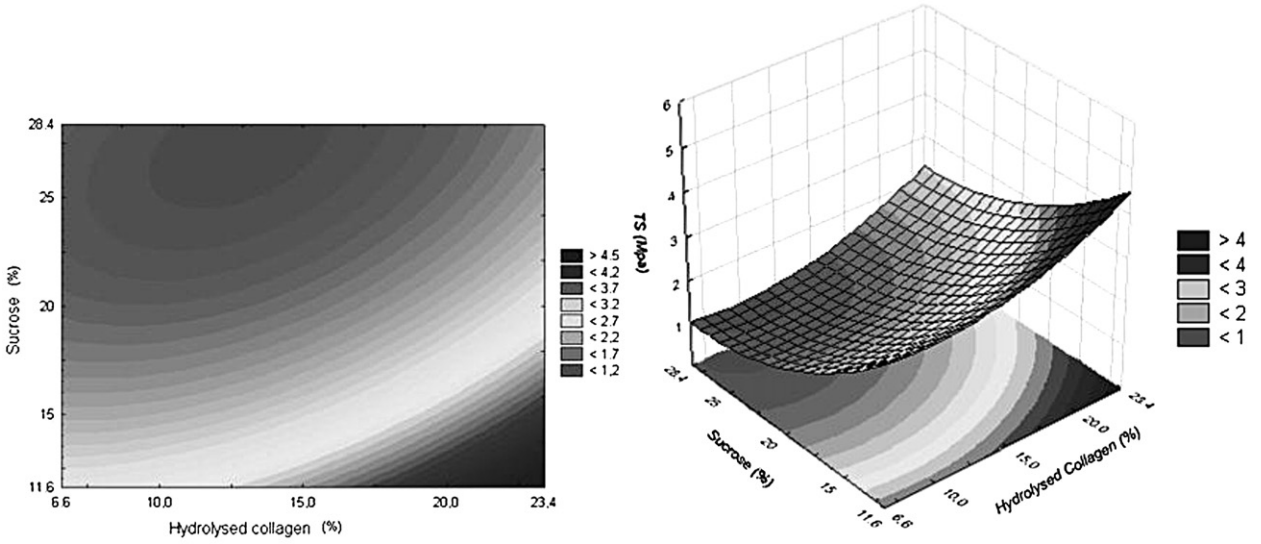

Fig. 1. Response surface and contour plot of experimental values obtained for Tensile Strength (TS) of the films as a function of sucrose and hydrolysed collagen concentration.

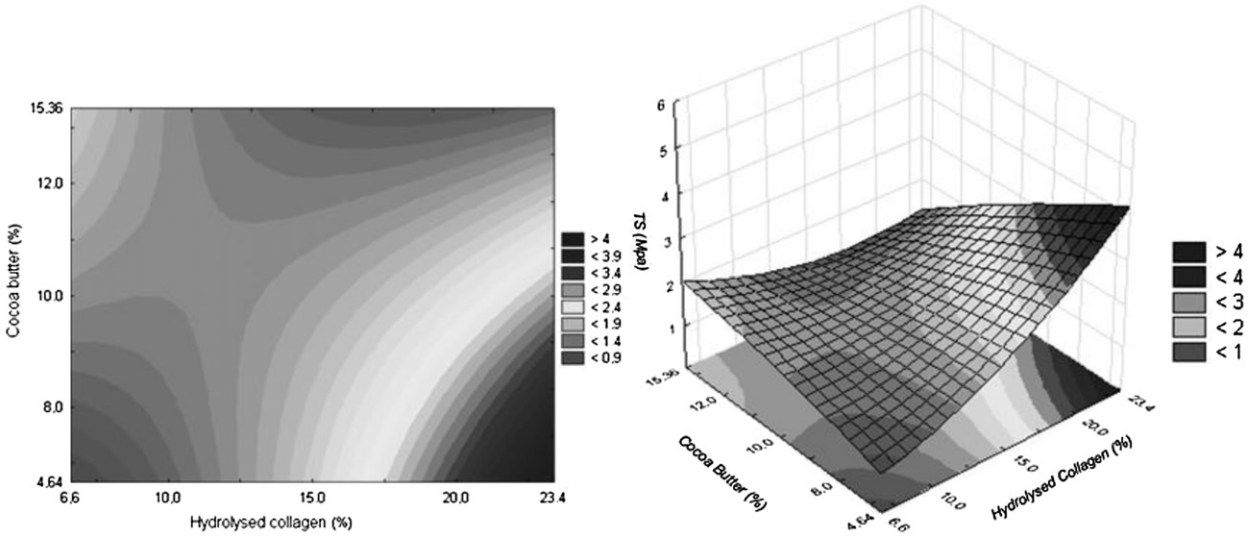

Fig. 2. Response surface and contour plot of experimental values obtained for Tensile Strength (TS) of the films as a function of cocoa butter and hydrolysed collagen concentration.

(Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006). Yoo and Krochta (2011) observed WVP coefficients of approximately 4.25 ± 1.0 g mm m^{-2} h^{-1} kPa^{-1} for whey protein-polysaccharide blended edible films.

An increase in WVP with increasing film thickness was also observed by Mali, Grossmann, García, Martino, and Zaritzky (2004), in which the WVP of starch films plasticised with yam were influenced by the linear effects of the thickness. According to Park and Chinnan (1995), the coefficients of WVP can change with the thickness of the film due to structural changes caused by the swelling of the hydrophilic matrix that will affect the structure of the film and cause internal tensions that influence its permeation.

Some studies have demonstrated that the addition of hydrophilic low-molecular-weight plasticisers reduces the protein–protein interaction, leading to increased molecular mobility and thus facilitating the migration of water vapour (Orliac, Rouilly, Silvestre, & Rigal, 2003; Rodríguez, Oséas, Ziani, & Maté, 2006; Sobral, Menegalli, Hubinguer, & Roques, 2001; Thomazine et al., 2005). This facilitates the reorganisation of the protein network, which becomes less dense and has a large free volume, permitting greater diffusion of water through the film matrix (Sobral et al., 2001).

3.4. Opacity (Op)

Opacity is directly related to the film appearance and colour. Statistical analysis of the *O*p response yielded a regression model significant at $p < 0.05$ within the range studied. The significant parameters were the linear effects of sucrose ($x2$(L)) and cocoa butter ($x3$(L)). Eq. (3) presents the model obtained.

$$Y = 23.00 - 1.85x_2 + 1.16x_3 \quad (3)$$

The ANOVA data considering the significant coefficients resulted in a R^2 of 79.6%, and the calculated regression F_{test} was higher than the F_{listed}, indicating good reproducibility of the experimental data.

According to Fig. 4, the opacity of the films increased with increasing cocoa butter concentration. The addition of sucrose made the films less opaque, especially at concentrations greater than 17.5%.

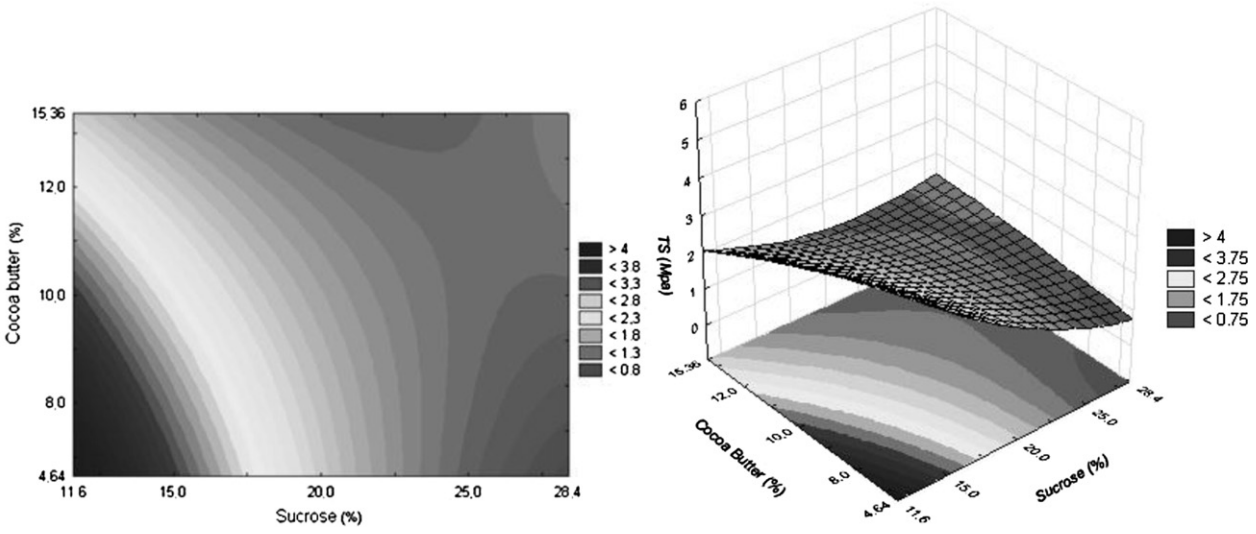

Fig. 3. Response surface and contour plot of experimental values obtained for Tensile Strength (TS) of the films as a function of cocoa butter and sucrose concentration.

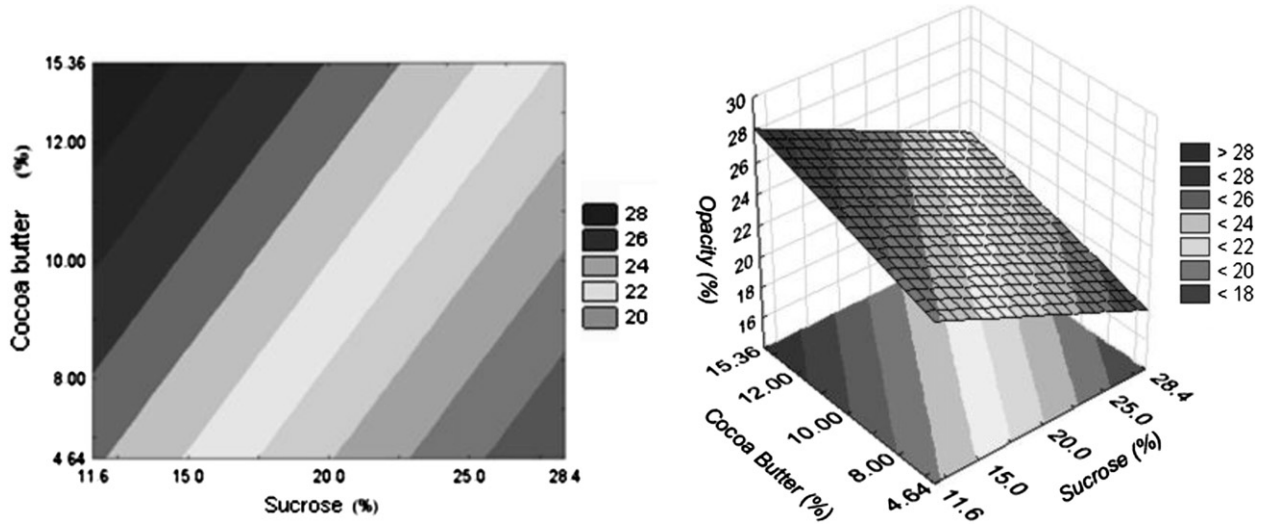

Fig. 4. Response surface and contour plot of experimental values obtained for Opacity (Op) of the films as a function of sucrose and cocoa butter concentration.

Some authors have indicated that the addition of lipids enhances the opacity of films, making them less transparent. Opacity is particularly important if the film is to be used as a food coating or food packaging (Gontard, Guilbert, & Cuq, 1992). Low opacity values indicate a transparent film. The opacity values obtained in this study are close to those ones obtained by Andreuccetti, Carvalho, and Grosso (2009) for films containing hydrophobic plasticisers. Mali et al. (2004) observed that film opacity depends on film thickness; film opacity increases with increasing thickness, and in fact, the sample with the lowest opacity was the sample with the lowest thickness. The soy lecithin emulsifier is of a brownish colour and most likely led to the formation of films with a yellowish colour. Andreuccetti, Carvalho, Galicia-García, Martínez-Bustos, and Grosso (2011) observed that the opacity values were slightly higher for lecithin-containing films compared to films containing yucca extract, and both films exhibited greater opacity than a gelatine-based film without surfactant.

3.5. Film morphology and visual aspects

All of the films produced were visually homogeneous, with no brittle areas, and were easily removed from the plates. The films were easily manageable and flexible.

In general, films with high levels of hydrolysed collagen exhibited more homogeneous structures and lower water vapour permeability. However, this analysis must also consider thickness, that in these cases were also the lower ones. Run 14 (Table 2), which contained the highest level of cocoa butter, resulted in a surface that seemed to not be compact, with large clusters of fat cells in certain regions (Fig. 5). Run 14 was one of five runs with the lowest observed tensile strength (Runs 3, 6, 7 and 12). In the presence of high levels of fat, a higher concentration of hydrolysed collagen may produce a more compact and homogeneous matrix. Furthermore, the fat globules may need to be smaller for a heterogeneous distribution. Among the three variables studied, the fat component presented the least homogeneous distribution in the matrix formed.

The Fig. 5a represents the image obtained for the majority of the films, i.e., components distribution homogeneous. The second micrographs, run 14 (Fig. 5b) was the only exception. It has the highest level of cocoa butter and showed a surface that seemed not be compact, with large clusters. Despite of its different appearance, this sample showed intermediate analytical results, with no significant influence in the sample characteristics.

3.6. Sensory evaluation

Run number 13 (Table 2), the one with the lowest opacity, was applied on the surface of chocolate panned products and submitted to sensory analysis with the aim of comparing its brightness to a product with shellac. The film obtained with this composition had an intermediary WVP result.

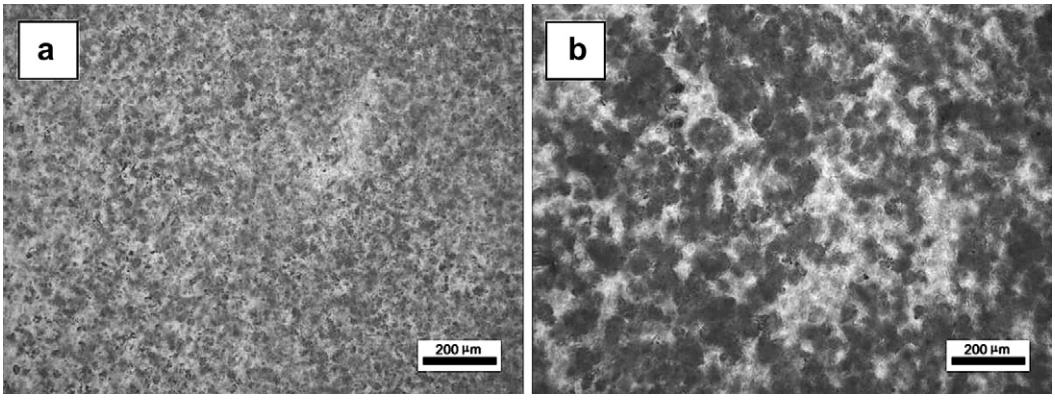

Fig. 5. Visual appearance (Optical Microscopy) of films surface. a) run 17; b) Run 14, (bars = 200 µm).

The brightness of the product with shellac was considered more intense; however, the product with the coating developed in this study also showed an attractive brightness.

4. Conclusions

The use of sucrose, hydrolysed collagen and cocoa butter proved to be feasible, resulting in flexible films. Sucrose (plasticiser) and cocoa butter reduced the TS of the films, while hydrolysed collagen at concentrations above 15% increased the TS. The elongation of the films was higher for higher concentrations of sucrose. In general, the coefficient of water vapour permeability was similar to those reported for films with hydrophilic plasticisers and was directly influenced by the thickness of the films at identical cocoa butter concentrations. The opacity of the films increased with the addition of cocoa butter and decreased with the addition of sucrose, particularly above 17.5%. With respect to morphology, high levels of hydrolysed collagen produced films with more homogeneous surfaces. The brightness of the product with the coating developed in this study was attractive; however, the brightness of the product with shellac was considered more intense. The results indicate that these films are promising systems for coating chocolate panned products, although additional investigation is necessary to evaluate the behaviour of the films during storage, particularly sucrose crystallisation.

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