

Research Article

Preparation and physical properties of calcium pectinate films modified with sunflower wax

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In this study, the preparation of films made from calcium pectinate (CP) and sunflower waxes (SFW) by casting is described. The optical properties, water resistance, thermal behavior, and rheological properties of SFW/CP films were examined. At higher SFW content irregular surfaces were obtained. Films made from a 2% aqueous pectin solution (PA) showed the best inclusion of the lipid material. Compared to SFW-free films, SFW/CP films were less transparent, being the SFW concentration the responsible for the loss of transparency. The swelling index was affected by SFW inclusion and PA concentration. In general, water adsorption was not affected by SFW concentration, but it was affected by the environmental conditions. The inclusion of SFW did not improve the moisture barrier efficiency of the films. The thermal behavior of SFW/CP films revealed a melting peak in the range of 70–80°C attributed to the melting of SFW. The rheological analysis showed G' values between 2.98×10^5 and 71.9×10^5 Pa for films stored at lower water activities, and they ranged from 3.07×10^5 to 38.4×10^5 Pa at higher environmental humidity. In both cases, G' values were affected by SFW inclusion. A linear viscoelastic region (LVR) was observed for all the samples.

Practical applications: A flexible and adequate film for handling and wrapping was obtained in this study. The addition of SFW to the CP films network was possible, and the films were easily prepared. This could be a potential application for waxes obtained from sunflower oil refining processes.

Keywords: Edible films / Low methoxyl pectin / Sunflower waxes

Received: March 7, 2014 / Revised: May 29, 2014 / Accepted: June 5, 2014

DOI: 10.1002/ejlt.201400117

1 Introduction

Certain food processes produce large amounts of surplus and waste that are recycled into useful materials such as pectins and waxes. One of the uses of these materials is in the

formulation of edible films. Pectins consists of a complex set of polysaccharides that are present in most primary cell walls and are particularly abundant in the non-woody parts of land plants. Pectin is a major component of the middle lamella, where it helps to bind cells together, but is also found in primary cell walls. Their composition varies with the source and extraction conditions used during isolation and purification, location, and other environmental factors [1].

Waxes can be found naturally in fruits and seeds, constituting a significant byproduct during the refining process of several vegetable oils. Particularly, crude sunflower oils contain partially and completely crystallizable aliphatic waxes consisting of esters of long-chain fatty acids and long-chain alcohols, having 42 to 58 carbon atoms [2, 3]. Finding new applications for waxes obtained from sunflower oil refining processes may increase their market value. One of their most recognized uses is in the preparation of edible films, which are generally designed using biological materials

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Abbreviations: a_w , water activities; CP, calcium pectinate films; DSC, differential scanning calorimeter; G' , storage moduli; G'' , loss moduli; LVR, linear viscoelastic region; PA, pectin-film forming solutions; SEM, electron microscopy; SFW, sunflower waxes; SFW/CP, cross-linked pectin film with sunflower waxes; T%, light transmission; TGA, thermogravimetric analysis; TSM, total soluble matter; VC, variation coefficients; WVP, water vapor permeability; WVTR, water vapor permeation; WVTR, water vapor transmission rate; γ^* , strain value at the limit of linearity

such as proteins, polysaccharides, lipids, and their derivatives [4, 5]. Films have long been used to prevent moisture migration between food and the environment, and also between compartments of different water activities (a_w°) within food.

Specific functional properties of edible films can be tailored. Film development has focused on the inclusion of water-barrier lipids to obtain less permeable films to water vapor, and proteins to obtain more permeable films [6]. Due to their hydrophobicity, films based on proteins and polysaccharides have poor barrier properties against moisture, but their mechanical properties are generally good [7, 8]. The use of low-methoxyl pectinate to cover the surface of certain foods has been proposed because it is edible and provides an attractive, non-stick surface. Moreover, pectinate can be applied by dip coating to form a uniform film on the object [4]. Conversely, lipid films are good moisture barriers, but they are usually opaque, relatively inflexible, unstable (rancidness), and waxy tasting. Improved film performances are obtained with a multicomponent system where hydrocolloids (proteins or polysaccharides) form a continuous and cohesive network, and the hydrophobic substances (lipids) provide the moisture barrier properties [7, 9]. Lipids can form a layer over the hydrocolloid matrix (bilayer films) or can be dispersed into the matrix (emulsified films). While the application of lipid-based barriers to processed food has been limited, the use of such barrier technology for both the extension of the shelf life of processed foods and the creation of innovative foods has great potential.

The techniques of film formation, properties, and application of edible films and coatings have been reviewed in the literature [10–14]. Other authors have determined that gas and water vapor permeation properties in edible films are affected by polymeric materials that provide the structural matrix, and plasticizers which impart flexibility [15].

The objectives of the present work were (a) to develop composite films based on low-methoxyl pectin and sunflower wax (SFW), (b) to evaluate their structure, optical properties and water resistance, and (c) to determine their thermal behavior and rheological properties.

2 Material and methods

2.1 Materials

Low-methoxyl citric pectin (LM 104) AS was provided by GENU PECTIN (Denmark) and used as the film-forming substance. This pectin is cross-linkable with divalent cations such as Ca^{2+} and Mg^{2+} . A filter cake obtained from the dewaxing process of crude sunflower oil consisting of waxes, oil, and filter aid was provided by a local factory and used to obtain purified sunflower waxes (SFW) that were used as the lipid component of the film. The purification procedure consisted of washing with n-hexane at 50°C for 30 min, and

then filtering the hot solution to eliminate insoluble particles. After this process, a mixture mainly of waxes and oil was obtained. The waxes were then purified by successive washings with cold hexane.

Glycerol was obtained from Fisher Scientific (98–99% purity, analytical reagent grade) and used as plasticizer. Calcium chloride anhydrous was obtained from J.T. Backer Inc. (Phillipsburg, NJ) (98–99% purity, analytical reagent grade) and used as the cross linking material.

2.2 Preparation of the film

Pectin-film forming solutions (PA) were prepared by hydrating the pectin at RT and dissolving it in distilled water to reach the desired concentration (1, 2, or 3 wt%) using a water bath at 80°C for 15 min. Once the pectin was completely dissolved, 2 g of glycerol (plasticizer) were added, followed by the appropriate amount of SFW (0, 0.2, or 0.4% wt/PA weight). The solution (PA/SFW) was homogenized using a high-intensity ultrasonic generator (HIU, Misonix S-3000, Qsonica, Newtown, CT) with a 1/8" diameter tip (amplitude of the tip = 12 μm) for 5 min. During the emulsification process, the samples were maintained inside the water bath at 80°C to avoid wax crystallization.

Approximately 30 g of PA/SFW solution were poured into 9 cm glass Petri plates and allowed to dry at 50°C overnight. As the water evaporated from the PA/SFW solution, a thin film of pectin and SFW was obtained on the surface of the glass Petri dishes. For cross-linking, dried films were immersed in approximately 30 g of a 2% calcium chloride solution and allowed to stand for 30 minutes. The calcium reacts with the galacturonic acid in the pectin film forming a cross-linked water-insoluble calcium pectinate film (CP). The glass Petri dishes containing the cross-linked pectin film with SFW (SFW/CP) were rinsed three times with distilled water. Three films were prepared for each concentration.

After peeling the SFW/CP films from the glass Petri plates, they were stored in sealed desiccators with controlled water activity (a_w°). Saturated solutions of different salts were used to achieve specific a_w° values: MgCl_2 ($a_w^\circ = 0.333$), NaBr ($a_w^\circ = 0.577$), and KNO_3 ($a_w^\circ = 0.95$) at 25°C.

2.3 Film thickness

Thickness of the films was measured using a micrometer with a sensitivity of 0.001 mm at five random positions. Average micron thickness and SDs (SD) were then determined for each film. The average value was subsequently used in determining the water vapor permeability (WVP) and transparency.

2.4 Microscopy

For the micro-structural analysis of the SFW/CP films, the specimens were studied with a scanning electron microscope

(LEO EVO 40XVP, Cambridge, 2004). The samples were fixed to a cover slip, coated with a thin gold film in a sputter coater (Pelco 91000). Digital images were collected at 5.0 kV using 10 000 \times magnification.

2.5 Optical properties

The light transmission (T%) of the films was measured using a SpectraMax 190 spectrophotometer (Molecular Devices, Sunnyvale, CA) with a flat-bottom 96-well plate for optical microscopic observations without lids. Three rectangular filmstrips were cut for each film treatment and attached at the bottom of the wells. T% was measured in the visible spectrum (from 400 to 800 nm). The procedure was carried out twice for each film sample.

The transparency of the films was calculated by the following equation [16]:

$$\text{Transparency} = A_{600}/x,$$

or

$$\text{Transparency} = -\log(T_{600})/x$$

where A_{600} is the absorbance at 600 nm, T_{600} is the transmittance at 600 nm, and x is the average film thickness (mm). The result for each film concentration is the average of six measurements.

2.6 Water resistance

2.6.1 Swelling index

Each film was cut in 2.5 cm \times 2.5 cm squares, weighed (w_1) and immersed in distilled water (25°C) for 2 min. Then, wet samples were wiped with filter paper to remove excess liquid and weighed (w_2) [17]. The swelling index of the film was calculated as

$$\text{Swelling index}(\%) = 100(w_1 - w_2)/w_1$$

where w_2 and w_1 are the weights of the wet and the air-dried samples, respectively. Three independent determinations were taken for each film treatment, and the average value and SD were reported.

2.6.2 Water adsorption

The ability of the films to adsorb water was determined by measuring the weight gain of a specimen (expressed as a percentage) after being stored at 95% relative humidity (over a saturated solution of KNO₃ in distilled water, $a_w^o = 0.95$) in a desiccator at 25°C for 2 weeks [18]. The amount of adsorbed water was calculated as

$$((w_t - w_0))/w_t \times 100\%,$$

where w_0 is the weight of the specimen before the water adsorption experiment, and w_t is the weight after conditioning. Two independent determinations were taken for each film treatment, and the average value and SD were reported.

2.6.3 Film solubility (total soluble matter, TSM)

Film solubility was determined as the percentage of SFW/CP film dry matter solubilized after immersion for 24 h in distilled water [19]. The initial dry matter was determined as the percentage weight change after drying the film at 105°C for 24 h on glass plates. The absence of solid residue on the plate after removing the film confirmed that there was no loss of wax. Film specimens that had been stored at 25°C and $a_w^o = 0.577$ were weighed (≈ 0.0001 g) and immersed in 50 mL beakers of distilled water. The beakers, covered with Parafilm "M" wrap (American National Can, Greenwich, CT), were periodically stirred for 24 h at 25°C; the agitation was performed slowly by hand. After the immersion period, the films were then taken out and dried under the same conditions used to determine the initial dry matter (at 105°C for 24 h). Different film specimens were used to determine initial dry matter and soluble matter to avoid heating the film sample prior to incubation in water.

The weight of solubilized dry matter was calculated by subtracting the weight of dry matter not solubilized from the weight of the initial dry matter. This was calculated from the percentage of dry matter and the weight of the film. Results were reported as a percentage of dry matter. Water solubility and the initial dry matter of each film treatment were determined in triplicate. Each independent determination was the mean value of three measurements from three samples (specimens) taken from the same film.

2.6.4 Water vapor transmission rate determination (WVTR) and water vapor permeation (WVP)

Water vapor transmission rate (WVTR) was determined gravimetrically according to ASTM method E 96–95 [20]. Briefly, a glass container (diameter = 1.7 cm) with anhydrous CaCl₂ desiccant was covered with the films on the top and sealed with Parafilm "M" wrap (American National Can, Greenwich, CT). The container was conditioned at 95% relative humidity in a desiccator at 22°C and weighed at desired time points.

After the water transfer reached the equilibrium state (the gain in mass between two successive weightings was <5%), WVTR (mg/day cm²) was calculated from the weight increase of the container over time according to:

$$\text{WVTR} = w/(tA),$$

where w is the increase (mg) in mass, t (day) is the duration of the experiment, and A is the permeation area (2.27 cm²). Differences in film thickness were ignored in this study.

Water vapor permeability (WVP) ($\text{mg} \cdot \text{mm}/\text{cm}^2 \cdot \text{day} \cdot \text{kPa}$) was calculated using the following equation:

$$\text{WVP} = \text{WVTR} \times x / \Delta P,$$

where x is the average film thickness (mm), and ΔP is the water vapor pressure differential across the film (kPa). The results were analyzed by linear regression. WVP tests were repeated at least four times for each film.

2.7 Thermal behavior

The thermal behavior of the samples was studied using a DSC 2910 differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE, USA). Five to 15 mg of sample were placed on an aluminum DSC pan and hermetically sealed. Samples were placed in the DSC chamber, set at 25°C and kept at this temperature for 2 min. Then, the samples were heated at 5°C/min to 250°C to evaluate the melting behavior. DSC tests were repeated at least three times for each film.

Simultaneously, one SFW/CP (0.4%/1%) film sample was sent to the TA Instruments Applications Laboratory. The analyses made by this external laboratory were:

-Thermogravimetric analysis (TGA): experimental methods and analysis: to determine the % mass loss in the temperature range requested for the DSC test. Knowing the decomposition profile gives insight into the best conditions to use when testing a sample in the DSC (i.e., pan selection, upper temperature limit, etc.)

-DSC experimental methods and analysis: to determine the transition temperatures and heats of reaction/transition. The sequence for DSC analysis was:

cycle 1: equilibrate at 25.00°C, ramp 10.00°C/min to 200.00°C, end of cycle 1

cycle 2: isothermal for 1.00 min, end of cycle 2,
cycle 3: ramp 10.00°C/min to 25.00°C, mark end of cycle 3,
cycle 4: isothermal for 1.00 min, mark end of cycle 4,
ramp 10.00°C/min to 250.00°C, end of method.

2.8 Rheological analysis

The rheological characteristics of the SFW/CP films were determined using an AR-G2 controlled stress dynamic rheometer (TA Instruments) with stainless steel parallel plates (40 mm diameter). Oscillatory tests (small deformation) were performed at 25°C by means of a strain sweep trial. Constant frequency of 1 Hz was used, and strain values were controlled from 0.003 to 0.8%.

2.9 Statistical Analysis

Significant differences were analyzed using ANOVA (two- and three-way tests) and a Holm-Sidak post-hoc test ($\alpha = 0.05$). Statistical analysis was performed using Stratigraphics statistical analysis software for PC, Sigma Plot for Windows Version 8.01 (2002 SPSS Inc., Chicago, IL, USA).

3 Results and discussion

3.1 Film thickness

The SFW/CP films obtained were flexible and adequate for handling and wrapping. Film thickness ranged from 61 to 417 μm and was obviously influenced by the pectin content in the PA (Table 1). As pectin content increased, thicker films were obtained. Variation coefficients (VC) were between 7

Table 1. Film thickness (μm), light transmission (T%), and transparency (A_{600}/mm) of SFW/CP films

Composition SFW %/PA %	Thickness (μm)		T (%)		Transparency (A_{600}/mm)
	$a_w^0 = 0.333$	$a_w^0 = 0.577$	$a_w^0 = 0.333$	$a_w^0 = 0.577$	
0/1	74 ± 23 ^a	61 ± 14 ^a	72.79 ± 5.68 ^c	66.77 ± 3.12 ^{de}	2.29 ± 0.65 ^b
0.2/1	79 ± 29 ^a	81 ± 11 ^a	14.77 ± 5.05 ^b	6.47 ± 1.30 ^{bc}	12.78 ± 2.54 ^c
0.4/1	133 ± 32 ^a	137 ± 50 ^a	5.10 ± 1.52 ^a	6.64 ± 1.15 ^{abc}	9.25 ± 1.01 ^d
0/2	203 ± 25 ^b	305 ± 86 ^b	73.88 ± 1.60 ^c	58.64 ± 8.00 ^d	0.64 ± 0.05 ^a
0.2/2	183 ± 61 ^{bc}	305 ± 90 ^b	20.21 ± 1.97 ^c	7.18 ± 1.46 ^{bc}	4.00 ± 0.58 ^c
0.4/2	295 ± 69 ^c	340 ± 23 ^{bc}	3.55 ± 0.54 ^a	4.05 ± 0.74 ^a	4.25 ± 0.60 ^c
0/3	361 ± 81 ^d	401 ± 61 ^c	71.94 ± 1.58 ^c	67.27 ± 4.40 ^c	0.41 ± 0.05 ^a
0.2/3	163 ± 29 ^{ac}	417 ± 112 ^c	25.08 ± 2.18 ^d	8.74 ± 1.37 ^c	3.51 ± 0.98 ^c
0.4/3	320 ± 114 ^{cd}	411 ± 92 ^c	6.49 ± 0.37 ^a	5.27 ± 1.14 ^{ab}	3.42 ± 0.34 ^c

Thickness values are the mean of five replicates ± SD.

Transmission values are the mean of six replicates ± SD.

Transparency values are the mean of twelve replicates ± SD.

Any two means in the same column followed by the same superscript letter are not significantly different ($p > 0.05$) by the Holm-Sidak method.

and 37% independently of film formulation. The higher VC values could be mainly due to the marked structural differences between film surfaces, as it will be discussed below in the microstructure (Section 3.2).

Films stored over saturated solutions of MgCl_2 ($a_w^\circ = 0.333$) showed a statistically significant interaction between PA and SFW concentration ($p = 0.004$), but this was not observed ($p = 0.823$) in films stored over saturated solutions of NaBr ($a_w^\circ = 0.577$). In the latter films, the effect of the different PA concentrations was not dependent on the level of SFW present. As a result, films stored over saturated solutions of NaBr did not show significant differences among the different levels of SFW ($p = 0.270$).

The results obtained in these two environments suggested a possible influence of humidity on the analyzed property. In order to confirm these results, a film formulated with solutions of 1% PA and 0.2% SFW was stored over a saturated solution of KNO_3 ($a_w^\circ = 0.95$), rendering a film thickness of $213 \pm 64 \mu\text{m}$. The statistical analysis revealed significant differences among the three analyzed humidity values ($p = 0.006$).

3.2 Microstructure

Electron microscopy (SEM) was used to visualize the top surface (open to the environment when drying) of each type of film (Fig. 1). Noticeable differences in film microstructure were observed. The inclusion of SFW caused surface roughness, which was more notable with increasing SFW content. Films obtained from 1% PA were brittle and fragile, and in the SEM images it was possible to observe the cracks on the surface, as shown in Fig. 1A. The surface of the films without SFW and with a pectin content of 2 and 3% appeared to be similar (Fig. 1B and C): these films were thicker and less

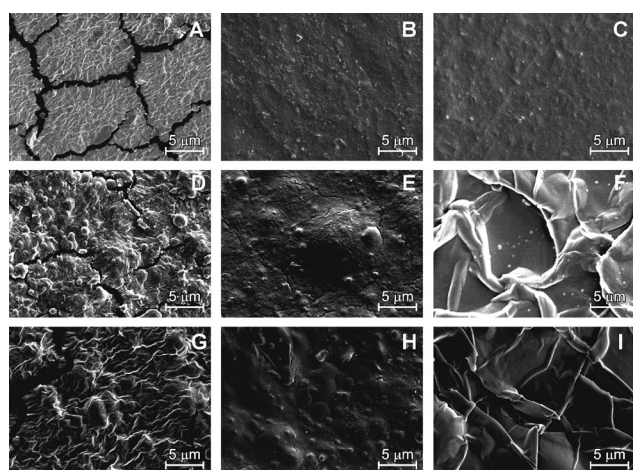


Figure 1. SEM photograph of films: (A) 1% PA, (B) 2% PA, (C) 3% PA, (D) 0.2% SFW/1% PA, (E) 0.2% SFW/2% PA, (F) 0.2% SFW/3% PA, (G) 0.4% SFW/1% PA, (H) 0.4% SFW/2% PA, and (I) 0.4% SFW/3% PA. Magnifications of 10 000 \times .

brittle, not showing cracks on the surface. Films containing SFW were more flexible, but still the presence of cracks was observed at 1 and 2% PA (Fig. 1D, E, G, and H). Thus, films with 3% PA with or without SFW did not show cracks possibly due to a pectin capacity to retain water in concordance with higher swelling indices, as shown in Section 3.4.1. In addition, they presented a hilly appearance caused probably by wax droplets dispersed throughout the film surface (Fig. 1D–I). The increase in pectin content in SFW/CP films originated different surfaces. The microstructure of films made with 2% PA and SFW seemed more homogenous and with fewer irregularities than those of 3% PA films (Fig. 1E and H). Films with SFW and 3% PA showed irregular surfaces with more ripples or protruding strips (Fig. 1F and I).

3.3 Optical properties

A more thorough understanding of the structure of films can be obtained by analyzing their optical properties. Transparent films are used as coatings to preserve the original appearance of wrapped or coated food; this property is relevant since it has a direct impact on the appearance of the coated product.

In order to evaluate the differences between CP films without waxes and those prepared with added SFW, light transmission (T%) was measured in the visible spectrum (Fig. 2). SFW concentration was the most important factor in transparency loss. An increase in SFW concentration caused a decrease in the film light transmission. The same results were observed for some emulsified films formulated with different compounds [21, 22]. Comparing CP films without SFW and those with SFW, the latter were less transparent (photograph, Fig. 3).

In Fig. 2 it is also possible to observe that the light transmission (T%) was affected by the storage condition, being lower for films stored at $a_w^\circ = 0.577$. To confirm this observation, the effect of humidity on film transparency was evaluated using transmittance measurements at 600 nm. Tests were carried out on SFW/CP films made with 1% PA and 0.2% SFW concentrations, stored over saturated salt solutions of $a_w^\circ = 0.333$, $a_w^\circ = 0.577$, and $a_w^\circ = 0.95$. The statistical analysis revealed that this property was highly affected by the environmental conditions ($p = 0.002$). Based on these results, films stored at $a_w^\circ = 0.333$ and $a_w^\circ = 0.577$ were used to compare the effect of storage conditions on film transparency using statistical tools. Table 1 summarizes the transparency and light transmission values of the films with different compositions stored at different relative humidity environments. For films stored over saturated solutions of MgCl_2 ($a_w^\circ = 0.333$) with 0.4% SFW and without SFW, the transmission did not show significant statistical differences ($p \geq 0.142$) for all the PA concentrations studied; however, different pectin contents significantly affected the transmission of films with 0.2% SFW content ($p < 0.029$). For films stored over saturated solutions of NaBr ($a_w^\circ = 0.577$), highly

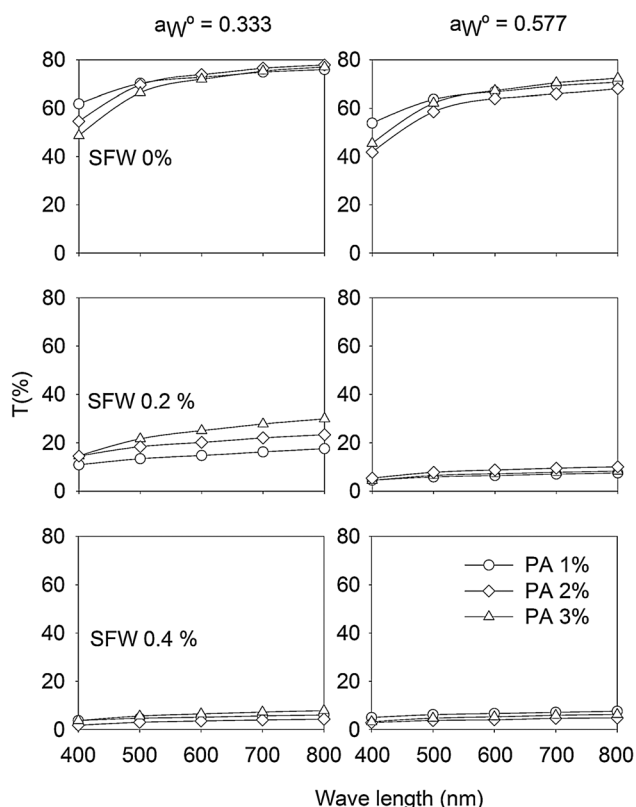


Figure 2. Percentage of transmittance (T%) in the visible spectrum (400–800 nm) of SFW/CP films.

significant differences were observed ($p < 0.0001$) when SFW content was analyzed.

The transparency of films was relatively similar to that of synthetic polymer films (low density polyethylene: 3.05, oriented polypropylene: 1.67, polyester: 1.51, polyvinylidene chloride: 4.58, [14]). Statistical analysis did not report differences among the transparency of films for different storage conditions ($p = 0.659$). Thus, the data reported are an average of both results (Table 1). As it was mentioned above, SFW and PA concentrations affected the transparency of the films, causing an increase in this value as SFW concentration increased and PA concentration decreased.

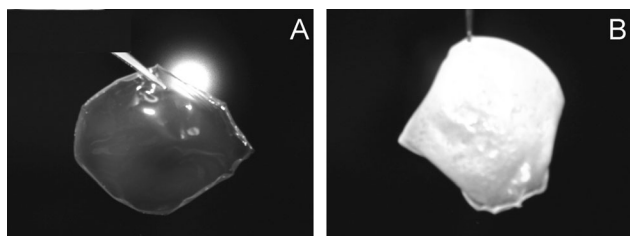


Figure 3. Photograph showing the appearance of CP (A) and SFW/CP films (B).

3.4 Water Resistance

3.4.1 Swelling index

The swelling index was expressed as the hydrated weight of the film related to that of the film conditioned to its moisture content. Only a few minutes of storage in distilled water was sufficient to induce considerable swelling of the films, as it can be inferred from the data reported in Table 2. The ANOVA test revealed an interaction between PA and both SFW concentration and water activity of the environment where the films were stored ($p < 0.001$). Therefore, in order to avoid an ambiguous interpretation of the results, the statistical analysis was performed for each environmental condition.

For films conditioned at higher a_w^o (0.577), the effect of the SFW addition was significant ($p = 0.029$) only for the films made with lower pectin content (1% PA). The swelling index was affected by PA concentration ($p < 0.05$) in the CP film and the CP/SFW film with 0.2% SFW content. For higher SFW levels, the observed swelling index was similar for all the PA concentrations ($p = 0.481$). For films conditioned at $a_w^o = 0.333$ and the same PA concentration, the addition of SFW did not cause significant differences ($p > 0.05$) in swelling. When analyzing PA concentration, the swelling index increased with pectin content regardless of the SFW content. Therefore the swelling index values for films stored at higher a_w^o were lower than those obtained for films conditioned at $a_w^o = 0.333$. This behavior may be attributed to the water retention capacity of pectin (hydrophilic substance). In the 3D structure formed by the electrostatic junctions with Ca^{+2} ions, the OH groups of galacturonic acid are free to retain water through hydrogen bridges [23]. When the films were stored in an environment with low humidity, these sites were still available to hold water, but when the films were stored at higher humidity, a high percentage of these sites were occupied by water molecules, and therefore the ability to capture water was reduced.

3.4.2 Water adsorption

The water adsorption results are shown in Table 2. Water adsorption values ranged between 41.25 and 50.16% for films conditioned at $a_w^o = 0.333$. The addition of SFW did not cause significant differences ($p < 0.953$) in water adsorption. For films stored at higher humidity, water adsorption values were lower (1.42–4.78%), indicating less ability to adsorb water in the film structure. Water adsorption was virtually null in those films made with a PA concentration of 1% and stored at $a_w^o = 0.577$, and these results were consistent with the swelling index, indicating that the water absorption capacity of the films was completely saturated. The SFW inclusion into CP films did not affect water adsorption values as expected, and no significant differences were found ($p > 0.05$).

Table 2. Swelling (%), water adsorption (%), and TSM (%) of SFW/CP films

Composition SFW %/PA %	Swelling (%)		Water adsorption (%)		TSM (%)
	$a_w^0 = 0.333$	$a_w^0 = 0.577$	$a_w^0 = 0.333$	$a_w^0 = 0.577$	
0/1	66.41 ± 14.97 ^{abcd}	21.59 ± 2.71 ^a	47.80 ± 6.26 ^{bc}	–	26.24 ± 1.80 ^a
0.2/1	62.83 ± 6.38 ^{ab}	23.15 ± 6.12 ^{ab}	47.98 ± 0.67 ^{bc}	–	27.58 ± 3.32 ^a
0.4/1	51.76 ± 5.57 ^a	41.11 ± 4.37 ^{bc}	48.98 ± 0.10 ^{bc}	–	29.56 ± 1.56 ^a
0/2	75.08 ± 5.21 ^{abcd}	47.78 ± 0.79 ^c	47.68 ± 0.42 ^{bc}	3.03 ± 2.48 ^{ab}	26.33 ± 1.22 ^a
0.2/2	76.52 ± 9.72 ^{abcd}	46.82 ± 6.83 ^c	44.63 ± 2.09 ^{ab}	4.78 ± 2.59 ^b	27.93 ± 1.94 ^a
0.4/2	64.52 ± 3.74 ^{abc}	47.44 ± 6.42 ^c	50.16 ± 1.28 ^c	4.59 ± 2.17 ^b	27.47 ± 2.08 ^a
0/3	93.47 ± 4.62 ^d	47.56 ± 3.64 ^c	41.73 ± 0.90 ^a	1.42 ± 1.31 ^{ab}	29.55 ± 0.78 ^a
0.2/3	90.96 ± 14.41 ^{cd}	48.61 ± 0.58 ^c	44.86 ± 0.20 ^{abc}	4.59 ± 2.99 ^b	28.56 ± 2.10 ^a
0.4/3	82.81 ± 10.55 ^{bcd}	41.66 ± 6.99 ^c	41.25 ± 2.66 ^a	4.17 ± 0.31 ^b	34.88 ± 3.03 ^b

Swelling index values are the mean of three replicates ± SD.

Water adsorption values are the mean of two replicates ± SD.

TSM values are the mean of three replicates ± SD.

Any two means in the same column followed by the same superscript letter are not significantly different ($p > 0.05$) by the Holm-Sidak method.

3.4.3 Film solubility (TSM)

Similarly to WVP, TSM offers an indication of the film's hydrophobicity [13]. This property, as explained in Section 2.6.3, was measured over films stored at $a_w^0 = 0.577$. Almost no significant differences ($p > 0.05$) were detected among films (Table 2). The TSM values obtained were in general between 26.24 and 29.56%, similar to the data reported by Kim et al. [13] for soy protein isolate films modified with sorghum wax. For films formulated with 3% PA and 0.4% SFW, the TSM value was slightly higher (34.88 ± 3.03%). This fact was probably due to the loss of SFW particles that were not well incorporated into the film matrix.

3.4.4 Water vapor transmission rate and water vapor permeation

The effect of SFW addition on CP films was studied in films made with 1% PA due to their greater flexibility. The experimental values for the water vapor permeated through the films are shown in Fig. 4. In the first 13 days of the assay, the regressions lines had a null linear coefficient, and the correlation coefficients (r^2) for all the reported data were 0.95 or higher. Table 3 reports the slopes $\Delta w/\Delta t$ and the corresponding WVTR. Although there seems to be evidence that the addition of SFW caused a small decrease in the mean values of the WVTR variable, no significant differences were detected ($p = 0.418$). These results may be associated with the morphology of the films and the SFW crystal structure. SEM micrographs revealed the presence of cracks in the surfaces of films made from 1% PA.

The barrier properties of edible films strongly depend on film composition. It is well known that the ability of hydrophobic substances to retard moisture transfer depends

on the homogeneity of their final distribution on the hydrophobic matrix [24]. Gontard et al. [25] determined WVP for various edible bilayer films including beeswax, paraffin wax, carnauba wax, insect wax, monoglycerides, and palm oil as the lipid layer. They found that waxes provided excellent moisture barriers compared to monoglycerides or palm oil, and WVTR decreased significantly as the concentrations of waxes increased.

The WVP values for the films evaluated in this work ranged between 1.83×10^3 and 4.06×10^3 mg mm kPa⁻¹ day⁻¹ cm⁻² (Table 3). Significant differences were observed for different SFW contents ($p < 0.001$), showing a WVP increase with increasing SFW content. This behavior was most likely the result of the observed difficulty in the

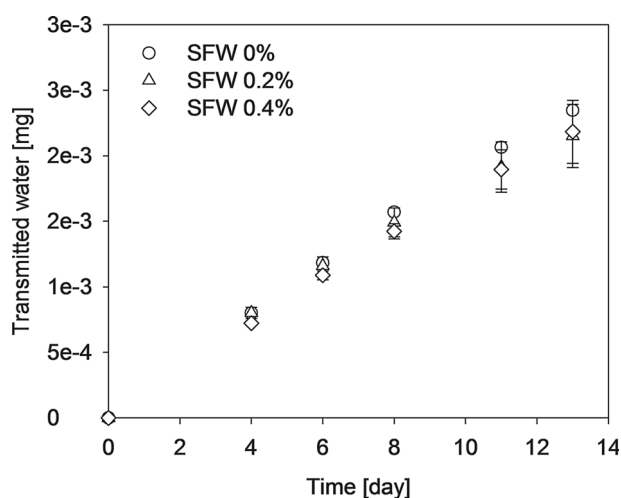


Figure 4. Water vapor permeated through films. Error bars represent SD of the mean of four measurements. Where error bars are not visible, they are less than the line thickness.

Table 3. Slopes ($\frac{\Delta w}{\Delta t}$) and correlation coefficient (r^2) of linear regression for water transmitted through the films for 15 days, WVP and WVTR for the films tested

Composition SFW %/PA %	$\frac{\Delta w}{\Delta t} 10^{-2} \left[\frac{\text{mg}}{\text{day}} \right]$	r^2	WVTR $\left[\frac{\text{mg}}{\text{daycm}^2} \right]$	WVP $10^{-3} \left[\frac{\text{mgmm}}{\text{daycm}^2 \text{KPa}} \right]$
0/1	1.82 ± 0.12^a	≥ 0.992	79.97 ± 5.36^a	1.83 ± 0.12^a
0.2/1	1.77 ± 0.06^a	≥ 0.990	78.04 ± 2.60^a	2.38 ± 0.08^b
0.4/1	1.79 ± 0.04^a	≥ 0.990	78.83 ± 1.76^a	4.06 ± 0.09^c

Values are the mean of four replicates \pm SD.

Any two means in the same column followed by the same superscript letter are not significantly different ($p > 0.05$) by the Holm-Sidak method.

incorporation of SFW in films made with 1% PA. This may be causing more disruption in the film matrix, creating an increased number of void spaces at the interface. This observation was consistent with SEM results, where cracks were detected in the film surface, allowing an easier diffusion of water. Bósquez Molina and Vernon Carter [24] reported WVP values between 1.352 and 5.29×10^{-1} ($\text{mg mm kPa}^{-1} \text{ day}^{-1} \text{ cm}^{-2}$) for edible films made from mixtures of candelilla wax with mineral oil and candelilla wax-mesquite gum. They found a close relationship between the surface morphology of coatings and their moisture barrier properties: films that had a more uniform surface morphology with fewer defects (fractures, pores) exhibited low levels of WVP.

3.5 Thermal behavior

A representative thermal behavior of SFW/CP films obtained in this study is shown in Fig. 5. A melting peak was observed at approximately 70–80°C due to the melting of SFW. At temperatures above the SFW melting peak, a second endothermic peak was observed at approximately 122°C probably due to water evaporation. An exothermic trend was observed at higher temperatures likely due to decomposition

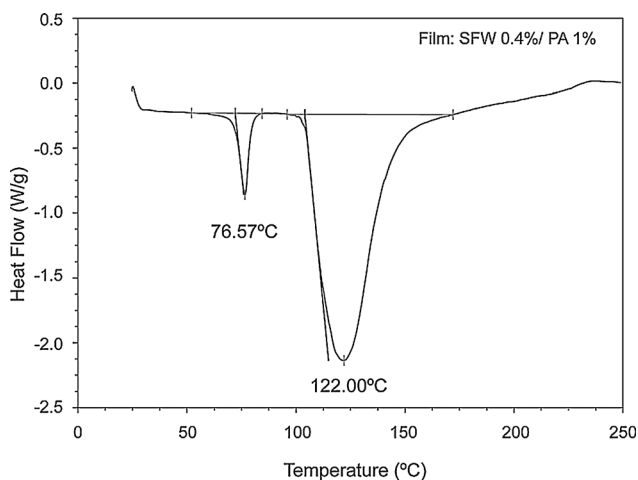


Figure 5. Representative thermal profile of SFW/CP films.

of the material. Apparently pectins do not have a transition temperature or a melting point in the temperature range tested. Iijima et al. [26] demonstrated that pectin is a crystalline polymer, but once the crystalline sample is melted, amorphous glass is formed, and this amorphous structure of pectin is stable, i.e., the sample does not crystallize by slow cooling or annealing. It is well known that amorphous polysaccharides are crystallized in the presence of water [27]. Iijima et al. [26] immersed amorphous pectin in water at 20°C for 1 month in order to examine the possibility of water-induced crystallization; however, they did not observe crystallization.

The melting parameters obtained for SFW-containing films for both environmental conditions tested ($a_w^o = 0.333$ and $a_w^o = 0.577$) are presented in Fig. 6. The melting enthalpy showed the same behavior for both conditions ($p = 0.061$). Enthalpy values were lower for films with lower SFW concentration ($p < 0.001$), and they showed a noticeable decrease as pectin content in the film increased ($p < 0.001$). This behavior may be caused by the growth inhibition of wax crystals due to the emulsifying effect of pectin.

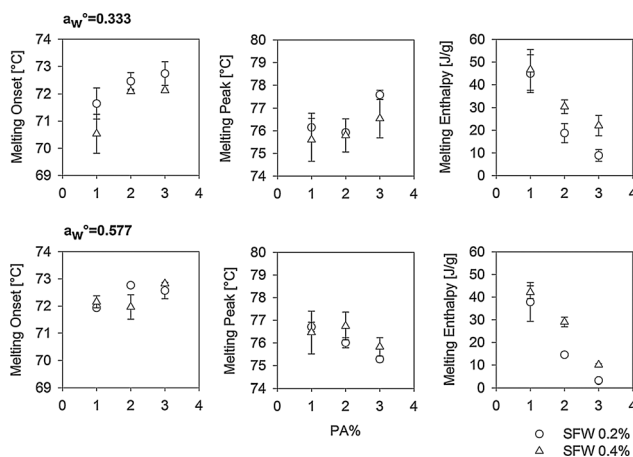


Figure 6. Melting onset (°C), melting peak (°C) and melting enthalpy (J/g) of films as a function of PA concentration (%). Error bars represent SD of the mean of three measurements from three separate film samples. Where error bars are not visible, they are less than the line thickness.

An increase in pectin concentration caused a slight increase of the melting onset ($p < 0.05$); this suggests that pectins affect the crystallization behavior of the SFW in the film structure. The addition of SFW was significant ($p = 0.007$) for films stored at $a_w^o = 0.333$, causing a decrease in that variable ($p = 0.007$). No significant differences were found ($p = 0.198$) at higher moisture levels. The peak melting temperature was approximately 76°C , which is close to previously reported peak temperatures obtained for bulk SFW [28–30].

In order to confirm the thermal behavior of the pectin inside the SFW/CP films, one SFW/CP (0.4%/1%) film sample was sent to the TA Instruments Applications Laboratory. Figure 7 shows the results of the TGA experiment, which was performed by heating the material at a constant rate from ambient temperature to a temperature above the point of total decomposition. At 250°C the sample exhibited an excess weight loss of 25%. The chemical identities of the off-gases were unknown. The weight loss may be attributed to a combination of volatilization and decomposition of the SFW/CP film components.

The samples were tested in aluminum DSC pans with hermetic pinhole lids. If a lid without pinhole had been used with this sample at 250°C , very likely it would have leaked. The leak would be observed as an endothermic peak due to depressurization of the pan.

The melting transition was observed at $72.85 \pm 0.07^\circ\text{C}$ ($\Delta H = 42.00 \pm 0.42 \text{ J/g}$, first heat), crystallization occurred at $73.85 \pm 0.21^\circ\text{C}$ ($\Delta H = 35.25 \pm 0.92 \text{ J/g}$, cool cycle), and the melting transition in the second heat cycle occurred at $70.00 \pm 0.71^\circ\text{C}$ ($\Delta H = 39.90 \pm 0.42 \text{ J/g}$, second heat) (Fig. 8). Duplicate tests showed excellent reproducibility.

The sample underwent a true crystalline melting and recrystallization. At temperatures above the melting peak, an endothermic peak due to weight loss and an exothermic trend likely due to decomposition were observed (Fig. 8).

3.6 Rheological analysis

The desired properties of a certain food packaging material depend on its applications. In general, an edible film must

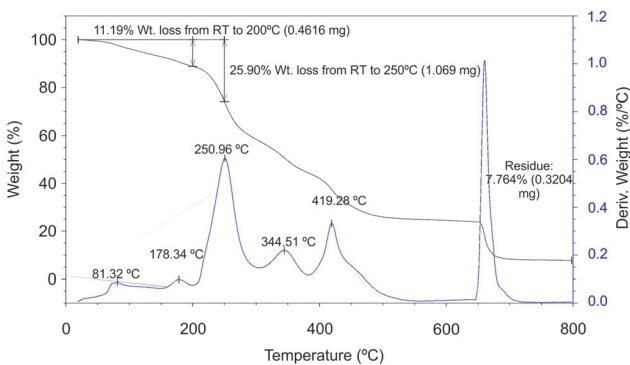


Figure 7. Result of TGA analysis.

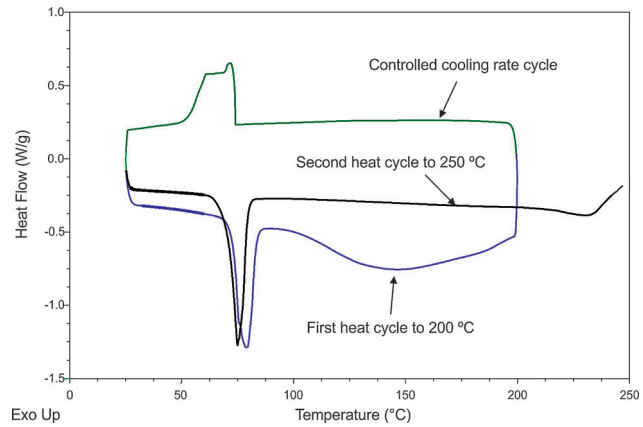


Figure 8. DSC analysis made by TA Instruments Applications Laboratory

withstand the normal stress encountered during its application, subsequent shipping and handling of the food, to maintain its integrity and also barrier properties [31]. Adequate mechanical strength ensures the integrity of a film and that minor defects such as pinholes are absent. The presence of such defects can damage the barrier property of the film. Coating can also reduce damages to food during handling and transportation. Sometimes, edible coatings and films may be used to change handling properties of materials [32].

Representative strain sweep results for the storage (G') and loss (G'') moduli for films with different pectin contents are presented in Fig. 9. A linear viscoelastic region (LVR) was observed for all the samples for strain values lower than 0.5%. The strain value at the limit of linearity (γ^*), stress values at the limit of linearity, G' , G'' , and $\tan \delta$ for the LVR are detailed in Table 4. Moduli values were obtained as an average of the LVR and as an average of two separate runs. For all the samples, G' was larger than G'' , indicating that the films behaved elastically.

The rheological analysis revealed that for films stored at lower water activity, G' values were affected by SFW addition. With increasing SFW content, G' values increased for films with 0.2% SFW. A later increase rendered a decrease in G' . The values obtained for G' were between 2.98×10^5 and $71.9 \times 10^5 \text{ Pa}$. When the environmental moisture increased, the addition of SFW to the film structure only caused a decrease in G' in films made with 3% PA. G' values in this case ranged from 3.07×10^5 to $38.4 \times 10^5 \text{ Pa}$. The films with higher SFW concentration showed defects and a non-uniform surface morphology, affecting the behavior of these films against a strain applied during the shear test.

$\tan \delta$ values ($\tan \delta = G''/G'$, Table 4) for films stored at $a_w^o = 0.333$ were only affected by PA concentration ($p < 0.001$), being lower at higher pectin content. SFW addition did not cause significant differences ($p = 0.918$) in $\tan \delta$ values. On the other hand, films stored at higher a_w^o showed a

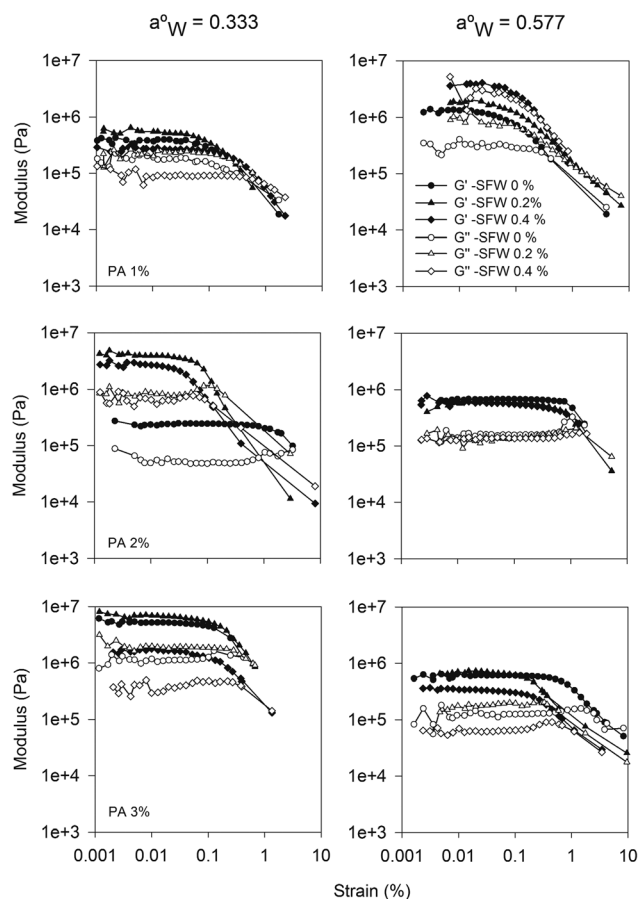


Figure 9. Storage (G') and loss (G'') moduli for SFW/CP films.

different behavior: the $\tan \delta$ value of films made with 2 and 3% PA did not present a significant change because of the added SFW, but for lower pectin content (1%), this property suffered an increase with increasing SFW.

This suggests that films presented different viscoelastic behaviors, affected mainly by the pectin content in the film. When the pectin content was higher, the films showed a more solid/liquid behavior (low $\tan \delta$). The elastic and viscous response of the material was affected by the combined action of pectin and SFW.

4 Conclusions

The aim of this paper was to develop and characterize edible coatings made from emulsions based on low methoxyl pectin aqueous solutions and SFW. The addition of SFW to the CP films network was possible. The films were easily prepared over a period of approximately 24 h in the laboratory, but obtaining a film with a uniform surface was critical. High SFW content resulted in films with irregular surfaces with spherical wax masses that were not well incorporated. The inclusion of SFW caused a rough surface that was more notable with increasing wax content, and the best inclusion was observed in films made with 2% PA.

The SFW/CP films were opaque and their transparency was greatly affected by the SFW concentration.

The swelling index of the films was affected by the SFW inclusion and the PA concentration, but the water adsorption was more affected by the PA concentration. The inclusion of SFW did not improve the moisture barrier efficiency of the films. Thus the SFW content was a less important factor than the heterogeneous dispersion of the lipid into an emulsified film: water vapor could pass through the hydrophilic matrix.

The thermal behavior of the SFW/CP films revealed a melting peak in the 70–80°C region assigned to SFW melting. Melting enthalpy was lower in films with a lower SFW concentration, and showed a noticeable reduction with increasing pectin content in the film.

A LVR was observed for all the samples. G' values were higher than G'' , indicating that the films behaved elastically.

Table 4. Storage (G') and loss (G'') moduli and $\tan(\delta)$ in the LVR and strain values at the limit of linearity (γ^*)

Composition SFW %/PA %	$a_w^0 = 0.333$				$a_w^0 = 0.577$			
	$G' (\times 10^{-5} \text{ Pa})$	$G'' (\times 10^{-5} \text{ Pa})$	$\tan(\delta)$	$\gamma^* (\times 10^2 \%)$	$G' (\times 10^{-5} \text{ Pa})$	$G'' (\times 10^{-5} \text{ Pa})$	$\tan(\delta)$	$\gamma^* (\times 10^3 \%)$
0/1	3.62 ± 0.26^a	1.55 ± 0.45^a	0.43 ± 0.095^d	5.21 ± 0.65^{bc}	13.3 ± 0.70^c	3.20 ± 0.21^b	0.24 ± 0.001^{bc}	4.06 ± 0.001^a
0.2/1	4.99 ± 0.63^a	2.05 ± 0.24^{ab}	0.42 ± 0.006^d	4.08 ± 0.06^b	15.1 ± 3.99^c	7.54 ± 2.09^c	0.50 ± 0.004^d	3.66 ± 0.56^a
0.4/1	3.00 ± 0.45^a	1.15 ± 0.30^a	0.38 ± 0.046^{cd}	5.09 ± 0.03^{bc}	38.4 ± 0.76^d	30.4 ± 6.10^d	0.80 ± 0.001^c	5.03 ± 0.03^a
0/2	2.98 ± 0.82^a	0.784 ± 0.36^a	0.25 ± 0.037^{ab}	12.7 ± 0.15^c	5.04 ± 2.23^{ab}	1.17 ± 0.56^a	0.23 ± 0.004^b	36.1 ± 5.61^d
0.2/2	46.6 ± 9.01^{dc}	9.02 ± 1.83^d	0.19 ± 0.002^a	6.52 ± 0.06^{cd}	4.51 ± 1.40^{ab}	1.22 ± 0.25^a	0.28 ± 0.043^c	25.6 ± 0.06^c
0.4/2	34.6 ± 10.70^c	8.75 ± 2.86^{cd}	0.25 ± 0.006^{ab}	2.03 ± 0.02^a	6.26 ± 0.80^{ab}	1.50 ± 0.23^{ab}	0.24 ± 0.010^{bc}	20.3 ± 0.09^{bc}
0/3	51.2 ± 0.86^d	10.7 ± 0.63^d	0.21 ± 0.016^a	14.5 ± 2.54^c	7.51 ± 2.28^b	1.74 ± 0.71^{ab}	0.22 ± 0.021^b	46.2 ± 7.64^e
0.2/3	71.9 ± 9.81^e	21.9 ± 2.24^c	0.31 ± 0.015^{bc}	8.05 ± 0.06^d	8.27 ± 2.01^b	2.08 ± 0.49^{ab}	0.24 ± 0.025^b	20.9 ± 0.62^{bc}
0.4/3	19.6 ± 4.62^b	5.34 ± 2.40^{bc}	0.27 ± 0.058^{ab}	3.63 ± 0.58^{ab}	3.07 ± 0.59^a	0.521 ± 0.15^a	0.17 ± 0.016^a	14.5 ± 2.57^b

Values are the mean of two replicates \pm SD.

Any two means in the same column followed by the same superscript letter are not significantly different ($p > 0.05$) by the Holm-Sidak method.

Different viscoelastic behaviors were observed, which were mainly affected by the pectin content of the film. The elastic and viscous response of the material was altered by the combined action of the pectin and SFW.

The results suggest that it is possible to add SFW to CP films, but there is a limit to the amount of pectin and SFW that could be included in the coating material to still produce a satisfactory film. A flexible and adequate film for handling and wrapping was obtained in this study.

The authors acknowledge the financial support from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), ANPCyT (Agencia Nacional de Promoción Científica), Universidad Nacional del Sur, Argentina, and Department of Nutrition, Dietetics, and Food Sciences, Utah State University.

The authors have declared no conflict of interest.

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