



# Characterization of multilayered and composite edible films from chitosan and beeswax

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## Abstract

Chitosan-based edible films were prepared and subjected to cross-linking reactions using sodium tripolyphosphate and/or to beeswax coating on both films interfaces. In addition, chitosan–beeswax emulsion-based films were produced. The goal of these modifications of the chitosan films was the improvement of their barrier to water vapor and to decrease their affinity to liquid water maintaining or improving the mechanical and optical properties of the original chitosan films. The cross-linking with tripolyphosphate decreased both the water vapor permeability and the water absorption capacity to about 55% and 50% of that of the original chitosan films, respectively. However, there was an increase in the films stiffness, revealed by the increased Young modulus from 42 kPa up to 336 kPa. The multilayered wax–chitosan–wax films exhibited a similar improvement of the barrier properties to water vapor, with the advantage of maintaining the mechanical properties of the original chitosan films. However, these wax-coated films showed a higher water absorption capacity, which is believed to be a consequence of water entry into small pores between the film and the wax layers. Regarding the film samples subjected to cross-linking and further coating with beeswax, a similar behavior as the uncoated cross-linked films was observed. The emulsion-based composite films were characterized by a substantial decrease of the water vapor permeability (40%), along with a decrease in their stiffness. Regarding the optical properties, all films presented a yellowish color with similar values of lightness, chroma, and hue.

## Keywords

Chitosan; beeswax, edible films, water vapor permeability, cross-linking, coating

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## INTRODUCTION

The food industry today is focusing on alternative packaging films derived from natural biopolymers due to the negative effect of the plastic packaging materials. Natural biopolymer films have the advantage over synthetic films because they are biodegradable and can be derived from renewable materials. According to their major components, edible films can be divided into

three categories: hydrocolloids (polysaccharides and proteins), lipids, and composites (Valencia-Chamorro et al., 2011; Vermerien et al., 1999). The polysaccharides are the most extensively used biopolymers for the production of edible films, because films made from

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polysaccharides are expected to be good oxygen and carbon dioxide barriers due to their tightly packed and ordered hydrogen-bonded network structure. Unfortunately, they have poor water vapor barrier properties, which results from their hydrophilic nature. Several strategies have been tested to enhance the water resistance and barrier properties of these edible films, such as the inclusion of inorganic impermeable particles, polymer cross-linking, blends with hydrophobic polymers and lipids, and production of multilayered films (Alves et al., 2010).

Among the various carbohydrate materials available for the development of edible films, special attention is given to chitosan due to its unique properties. Chitosan is a polysaccharide derived from chitin, the second most abundant polymer, and is mainly composed of 2-amino-2-deoxy- $\beta$ -D-glucopyranose repeating units. The importance of chitosan is based on its antimicrobial properties, bioactivity, and film-forming properties. Chitosan films are usually plasticized to improve their manipulation and flexibility (Rivero et al., 2009; Suyatma et al., 2005; Xu et al., 2005) or cross-linked to limit their solubility and improve mechanical properties (Remuñán-López and Bodmeier, 1997). These films, like the other polysaccharide films, are often coated with a second or even third layer to improve the water vapor barrier properties. From the edible hydrophobic components, alkanes and waxes are usually used. Waxes are supposed to be more resistant to water vapor flux than most other lipid components (Navarro-Tarazaga et al., 2008; Peroval et al., 2002). The beeswax use in the edible films is especially interesting because of the chemical composition of the beeswax, which consists of a high percent of esters of long-chain fatty alcohols and acids and smaller percent of long-chain hydrocarbons and free fatty acids (Auras et al., 2009). Another alternative is the preparation of composite films where the advantage of each component can be used to improve the physical, mechanical, and barrier properties of the obtained edible films (Garcia et al., 2006; Sanchez-Gonzalez et al., 2010; Vargas et al., 2011).

Recent studies on layered or blend composite films are mainly centered on two-component films. Information on multilayered wax–chitosan–wax or composite chitosan–wax films is poorly available. The aim of this study was to improve the water vapor barrier properties of chitosan films by decreasing their affinity to liquid water, while maintaining suitable characteristics in terms of color and mechanical (tensile strength and elongation) properties. Three modification techniques were studied for reaching that goal: cross-linking of chitosan films with sodium tripolyphosphate (TPP), coating the chitosan films with beeswax, and preparation of wax–chitosan emulsions.

Sodium tripolyphosphate is a multipurpose GRAS food substance (FDA, 2012). It was chosen due to its nontoxicity and easy controlling of the cross-linking ratio just by adjusting the pH value of the TPP solution. Beeswax was used to develop multilayered wax–chitosan–wax films and wax–chitosan emulsion-based films due to its availability and easy handling.

## MATERIALS AND METHODS

### Materials

Chitosan (>85% deacetylation degree) was purchased from Golden-Shell Biochemical Co., Ltd., China. Beeswax was provided by the beekeeping center at the Instituto Superior de Agronomia in Lisbon, Portugal. All the other chemicals used in the experiments were of analytical grade and were purchased from Panreac Chimica S.A.U., Spain.

### Beeswax characterization

**Saponification value.** Saponification value was determined according to the procedure described by Auras et al. (2009). An amount of  $0.30 \pm 0.01$  g beeswax was weighed into a 10-mL screw-capped glass vial. Four milliliters of 4 M aqueous NaOH solution was pipetted into the vial before it was closed and placed into an oven at 100 °C for 1 h. Next, the aqueous solution was poured into a beaker. About 5 mL of distilled water was used to rinse the vial, and the water was later combined with the aqueous solution. Two drops of 1% phenolphthalein were used as an indicator. This solution was titrated against a 0.5 M aqueous HCl solution with manual shaking, and the end point was carefully monitored. Blank analyses were also performed. The saponification value (mg KOH/g) was calculated as follows:

$$\text{Saponification value} = 56.1 \times M(V_b - V_s/w) \quad (1)$$

where 56.1 is the molar mass of KOH in g/mol, M is the molarity of HCl solution in mol/L,  $V_b$  is the titrated volume of HCl solution for the blank in mL,  $V_s$  is the titrated volume of HCl solution for the sample in mL, and  $w$  is the weight of the sample in g.

**Acid value.** Acid value was determined according to the procedure described by Auras et al. (2009). The beeswax (1 g) was weighed, placed in an Erlenmeyer flask, and 50 mL of chloroform was added. The flask was placed in an ultrasonic bath for about 5 min. Two drops of 1% phenolphthalein solution were added to the solution before it was titrated with 0.05 M NaOH in methanol. The titration was continued by manually shaking the flask until the indicator turned the clear

solution a faint pink color. Blank analyses were also conducted. The acid value was expressed as mg KOH/g and calculated as follows:

$$\text{Acid value} = 56.1 \times M(V_b - V_s/w) \quad (2)$$

where 56.1 is the molecular weight of KOH in g/mol, M is the molarity of NaOH solution in mol/L,  $V_s$  is the titrated volume of NaOH in methanol used for the sample in mL,  $V_b$  is the titrated volume of NaOH in methanol used for the blank solution in milliliter, and  $w$  is the mass of the sample (g).

### Preparation of the films

Chitosan solution (0.8%, w/v) was prepared by dissolving 0.8 g of chitosan in 100 mL of acetic acid (1%, v/v) with continuous stirring at room temperature ( $20 \pm 2^\circ\text{C}$ ). After the complete dissolution of the chitosan, 0.2 g of glycerol and 0.2 g of Tween 80 were added, and the solution was stirred for another 30 min. Afterward, the chitosan solution was centrifuged for half an hour at 16,900 g (Hermle Labortechnik, Germany) to separate all the impurities. The resulting solution was transferred to a square plastic (polypropylene) casting container,  $20 \times 20$  cm, ensuring that all air bubbles were removed from the viscous medium before casting. The films were dried at  $40^\circ\text{C}$  for 48 h. The dried chitosan films were peeled off from the container before further treatment.

The cross-linking of the chitosan films was performed by immersing the films in 10 mL of sodium TPP aqueous solutions, with TPP concentrations of 0.5% and 1% (w/v). The ratio of chitosan:TPP was 1:0.3 and 1:0.6 for the 0.5% and 1% solutions, respectively. The cross-linking lasted 5 min, and then the

excess of the cross-linking solution was poured out and the films were dried at  $40^\circ\text{C}$ .

For the preparation of the multilayered films, 0.25%, 0.5%, and 1% (w/v) wax solutions were prepared by melting 0.25 g, 0.5 g, or 1 g of beeswax in 100 mL of ethanol heated to  $70^\circ\text{C}$ . The wax solutions were emulsified with 25% of Tween 80 with respect to the wax content. The chitosan films were coated by dipping the films in the heated wax solutions and then letting the chitosan film coated on the both sides to dry at room temperature.

The composite films were prepared by adding 10 g of wax to 100 mL of 1% chitosan solution and heating at  $70^\circ\text{C}$ . The wax was melted in the heated solution, and the whole mixture was homogenized for 2 min with Ultraturrax homogenizer at 8000 rpm. The emulsion was casted in plastic (polypropylene) casting containers and dried at  $40^\circ\text{C}$  for 48 h to produce composite films. All the formulations of chitosan-based films are listed in Table 1. The obtained films were conditioned in a desiccator at 50% relative humidity and  $20 \pm 2^\circ\text{C}$  for 1 week prior to analysis.

### Film characterization

**Thickness measurement.** Thickness of the films was measured using a hand-held micrometer (Alves et al., 2010). Eight thickness measurements were taken at random positions on each film, and the mean was used in calculating the film water vapor barrier and mechanical properties.

**Optical properties.** Film color was determined by Dr Lange spectro-color colorimeter calibrated with a standard white and black tile according to the procedure described by Mayachiew et al. (2010). The CIE Lab

**Table 1.** Composition and coding of the chitosan-based edible films

Sample code	Type of film	Chitosan (% w/v)	TPP:chitosan ratio	Glycerol (% w/v)	Tween80 (% w/v)	Wax (% w/v)	Coating ( $\text{mg}/\text{cm}^2$ )
CH	Monolayer film	0.8	/	0.2	0.2	/	/
0.5TPP	Crosslinked film	0.8	0.3:1	0.2	0.2	/	/
1 TPP	Crosslinked film	0.8	0.6:1	0.2	0.2	/	/
CH-0.25W	Wax-coated, multilayer film	0.8	/	0.2	0.2	0.25	1.25
CH-0.5W	Wax-coated, multilayer film	0.8	/	0.2	0.2	0.50	2.50
CH-1W	Wax-coated, multilayer film	0.8	/	0.2	0.2	1.00	5.00
TPP-0.25W	Crosslinked, wax-coated, multilayer film	0.8	0.3:1	0.2	0.2	0.25	1.25
TPP-0.5W	Crosslinked, wax-coated, multilayer film	0.8	0.3:1	0.2	0.2	0.50	2.50
TPP-1W	Crosslinked, wax-coated, multilayer film	0.8	0.3:1	0.2	0.2	1.00	5.00
CHW	Composite film	0.8	/	0.2	0.2	10	/

CH: chitosan; TPP: sodium triphosphate; W: wax.

scale was used: lightness,  $L$ , and chromaticity parameters  $a^*$  (red-green) and  $b^*$  (yellow-blue) were measured. Assays were performed by placing the film samples over the standard white tile. Samples were analyzed in five repetitions. The color differences ( $\Delta E$ ), chroma ( $C$ ), and hue ( $h$ ) values were calculated by the following equations:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (3)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (4)$$

$$h^0 = \tan^{-1} \frac{b^*}{a^*} \quad (5)$$

where  $L^*$ ,  $a^*$ , and  $b^*$  values were the color parameters of the films, while the  $L_0^*$ ,  $a_0^*$ , and  $b_0^*$  values were the color parameters of the white tile.

**Moisture content.** Moisture content of the films was determined by measuring the weight loss upon drying in a vacuum oven at  $70 \pm 1^\circ\text{C}$  until constant weight (dry sample weight). Samples were analyzed in triplicates, and results were expressed as mean values.

**Water absorption capacity.** In order to determine the water absorption capacity or swelling degree of the films, three samples from each film were dried under vacuum at 50 mbar for 48 h at  $40 \pm 1^\circ\text{C}$  as described in the ASTM D5229/D2229M (2004) method. After weighing, the samples were slowly immersed in water or 1% acetic acid (14.5 mL), with 0.5 mL of sodium azide solution (1 ppm) to avoid the growth of fungi. The samples were taken out of the liquid, blotted with absorbent paper to remove the excess of water or acetic acid from the surface, and weighed again. The procedure was repeated until reaching a constant mass in two consecutive measurements (during 4 days), and the absorption capacity of the films was calculated:

$$\text{absorption capacity} = \frac{m_s - m_i}{m_i} \quad (6)$$

where  $m_s$  is the mass of the immersed film (g) and  $m_i$  is the mass of the dried film (g).

**Water sorption isotherms.** Water sorption isotherms were determined by the gravimetric method for selected films chosen as representative of each group of modified chitosan films (chitosan film, CH, chitosan film coated with 0.5% wax solution, CH-0.5 W, chitosan film cross-linked with 0.5% TPP and coated with 0.5% wax solution, TPP-0.5 W, and composite film, CHW). Film samples with dimensions of  $20 \times 20$  mm were previously dried at  $60^\circ\text{C}$  during 48 h. The samples were then placed in desiccators with different relative humidities, imposed by the use of saturated saline solutions. The experiment was carried out at  $25 \pm 2^\circ\text{C}$ . The samples were weighed after equilibration for 3 weeks. The relation between the equilibrium moisture content and the water activity of the films was predicted using three isotherm models presented in Table 2. The constants of the various sorption models were obtained by nonlinear fitting using the software package Scientist<sup>TM</sup>, from MicroMath<sup>®</sup>. These parameters proved to be very useful in the physicochemical description of the films. The goodness of the fit of each model was evaluated by calculating the mean relative percentage deviation modulus,  $P$  (%), and the standard error,  $S$ , which are defined as follows:

$$P = \frac{100}{N} \sum_{i=1}^N \frac{X_{exp} - X_{cal}}{X_{exp}} \quad (10)$$

$$S = \sqrt{\frac{\sum_{i=1}^{N_{exp}} (X_{exp} - X_{cal})^2}{N_{exp.data} - N_{param}}} \quad (11)$$

where  $exp$  is experimental value,  $cal$  is calculated value, and  $param$  is parameter.

**Water vapor permeability.** The water vapor permeability was measured gravimetrically according to the ASTM E96/E96M-12 (1993) protocol. The films were sealed with silicone to the top of a glass petri dish with a diameter of 5 cm. The water vapor permeability was measured using films conditioned previously at a relative humidity of 54%. A fan was used to promote the circulation of air inside the desiccator, in order to minimize the mass transfer resistance of the air boundary layer above the membrane. The room temperature and

**Table 2.** Isotherm models used for experimental data fitting

Model	Mathematical expression	Equation	References
Guggenheim-Anderson-De Boer	$X = X_m C K a_w / [(1 - K a_w) (1 - K a_w + C K a_w)]$	7	van den Berg and Bruin, 1981
Iglesias-Chirife	$X = A + B [a_w / (1 - a_w)]$	8	Bouquet et al., 1978
Oswin	$X = A [a_w / (1 - a_w)]^B$	9	Oswin, 1946

X: equilibrium moisture content ( $g_{water}/g_{dry\ mass}$ );  $X_m$ : monolayer value ( $g_{water}/g_{dry\ mass}$ ); C, K, A, B: constants.

the relative humidity outside the petri dish were measured over time using a thermohygrometer. The water vapor molar flux ( $N$ ) was determined by weighing the petri dish in regular time intervals for 24 h. The water vapor permeability was calculated as follows:

$$WVP = \frac{N \cdot \delta}{\Delta P_w} \quad (12)$$

where  $\delta$  is the film thickness (m) and  $\Delta P_w$  is the water vapor difference between both sides of the film.

**Mechanical properties.** The mechanical properties were studied using a TAXT2 (Stable Micro Systems, London, England). For the tensile tests, film strips (20 mm × 70 mm) were attached on tensile grips A/TG, and force (N) and deformation were recorded during extension at 0.2 mm/s from an initial effective length of 50 mm. Tensile strength,  $TS$ , and elongation at break,  $E$ , were determined in accordance with ASTM D-882-91 (1996).  $TS$  was calculated by dividing the maximum load by the initial cross-sectional area of the sample and expressed in MPa.  $E$  was calculated as a ratio of the elongation at the point of sample rupture to the initial length of a sample as a percentage. The Young's modulus, calculated as ratio between the tensile strength and elongation in the elastic deformation region of the stress–strain curve, was also expressed in MPa. Seven replicates were measured.

For the puncture test, five sample squares (20 × 20 mm) of the films were cut out from the conditioned films. The puncture was carried out with probe (P/2) with 2 mm diameter, while the film squares were fixed down on perforated heavy duty platform. The initial distance was set to be 5 mm. The films were punctured in the center with cross-head speed of 1 mm/s, and the force and distance necessary to puncture the films were recorded. Puncture tension was calculated by dividing the maximum force by the cross-sectional area of the probe, while the deformation was calculated as a ratio of the deformation at the point of sample rupture to the set initial distance as a percentage.

### Statistical analysis

Descriptive statistics and one-way analysis of variance (ANOVA) were performed on all parameters to evaluate significant differences among the samples at 95% confidence interval according to Tukey's test using Minitab 15 statistical software.

## RESULTS AND DISCUSSION

### Film formation and appearance

The films were easy to peel from the casting container and also easy for further handling and treatment.

Transparent, homogeneous, thin, flexible, and easy to handle films were prepared from the 0.8% chitosan solution. The addition of the glycerol and Tween 80 to the chitosan solution resulted in films with a slight yellow appearance. The beeswax, used in the multilayered and composite films, was a yellowish waxy solid with density of 850 kg/m<sup>3</sup> at room temperature, acid value of 16.12 ± 1.2 mg KOH/g, and saponification value of 92.01 ± 1.56 mg KOH/g. The melting point of the wax was around 61 °C.

The different types of the prepared edible films are given in Table 3. The application of the waxy coatings to chitosan films to obtain the multilayered films introduced a mat look to the films with decreased transparency, while the emulsion of chitosan and beeswax composite films became with a more intensive yellow-brownish color.

The applied cross-linking procedure was based on the diffusion of tripolyphosphate into the chitosan-based film. A shrinking of the films was observed during the cross-linking step, which increased as the TPP concentration in the cross-linking solution was higher. Furthermore, the cross-linking step made the films glossier, harder, and slightly brittle.

### Film characterization

**Films thickness.** The thickness of the edible films is highly important for all physical properties of the films. In the intent to produce films with similar thickness values for all the formulations of chitosan-based films used in this research, the volume of each film formulation used in casting of the films was always the same, 20 ± 1 mL. The average thickness values for all chitosan-based films were from 30 to 114 μm (Table 3). The cross-linked chitosan films exhibited reduced thickness, while the wax-coated films had slightly increased thickness. Only the multilayered films prepared with a 0.25% wax solution revealed a significantly higher thickness at the confidence level of  $p < 0.05$  when compared to the rest of the chitosan-based films. The thickness of the composite films was similar to the one of pure chitosan films.

**Optical properties.** The optical properties of the edible films are very important attributes because they may directly influence the consumer's acceptability of the product. Transparent films are less likely to induce changes on the appearance of the products. The color parameters (lightness,  $L$ ; color saturation,  $C$  and hue,  $h$ ) for all film formulations are presented in Table 4. The lightness of the film samples was quite similar. The only film with a significant difference at  $p < 0.05$  level was the composite film, which was also noticeable from the visual appearance of the films.

**Table 3.** Effect of cross-linking, coating, and emulsion technique on film thickness and appearance

Sample code	Film thickness (μm)	Film appearance
CH	52 ± 7	Transparent, yellowish, elastic, perfect, easy to peel, and easy to handle films that are completely intact with smooth surface
0.5TPP	43 ± 4	Transparent, yellowish, glossy, hard, and easy to handle films that are completely intact with smooth surface
1 TPP	30 ± 2	Transparent, yellowish, very glossy, nonelastic, very hard, brittle, and difficult to bend or handle
CH-0.25W	85 ± 6	Mat, yellowish, elastic, soft, easy to peel, and easy to handle films with smooth waxy surface
CH-0.5W	55 ± 2	Mat, light yellow, soft, easy to peel, and easy to handle films with smooth and intact waxy surface
CH-1W	66 ± 7	Mat, light yellow to white, soft, easy to peel, and easy to handle films with smooth and intact waxy surface
TPP- 0.25W	114 ± 7	Mat, yellowish, hard, and easy to handle films with smooth and intact waxy surface
TPP-0.5W	48 ± 7	Mat, light yellow, hard, and easy to handle films with smooth and intact waxy surface
TPP-1W	54 ± 7	Mat, light yellow to white, hard, and easy to handle films with smooth and intact waxy surface
CHW	60 ± 7	Transparent, yellow-brown films, weak, but easy to peel, and easy to handle films

CH: chitosan; TPP: sodium tripolyphosphate; W: wax.

**Table 4.** Color parameters of the edible films

Sample code	L	E	C	h
CH	92.2 ± 2.8 b	15.6 ± 0.5 b	17.0 ± 0.5 b	82.2 ± 2.6 a
0.5TPP	90.1 ± 2.7 b	12.1 ± 0.4ab	14.2 ± 0.4ab	81.5 ± 2.5 a
1 TPP	92.1 ± 2.7 b	10.5 ± 0.3 a	13.0 ± 0.4 a	83.4 ± 2.4 a
CH-0.25W	94.6 ± 2.8 b	19.3 ± 0.3 c	21.3 ± 0.6 c	83.8 ± 2.5 a
CH-0.5W	95.8 ± 2.9bc	20.8 ± 0.6 c	23.1 ± 0.7 c	84.0 ± 2.5ab
CH-1W	99.5 ± 2.9 c	22.5 ± 0.7 c	25.3 ± 0.7 d	84.2 ± 2.5ab
TPP- 0.25W	95.9 ± 2.9bc	15.9 ± 0.5 b	17.9 ± 0.5 b	82.8 ± 2.5 a
TPP-0.5W	96.6 ± 2.9 c	15.2 ± 0.4 b	17.4 ± 0.5 b	83.0 ± 2.5 a
TPP-1W	98.4 ± 2.9 c	14.8 ± 0.4 b	17.0 ± 0.5 b	83.0 ± 2.5 a
CHW	81.1 ± 2.4 a	19.0 ± 0.6 c	21.2 ± 0.6 c	85.4 ± 2.5 a

CH: chitosan; TPP: sodium tripolyphosphate; W: wax.

Values in the same column with alphabets mean that the values are significantly different (p < 0.05).

From the visual inspection, all films presented a yellowish color, which is reflected on a hue value near 90°. The addition of wax, either as a coating or emulsified within the polymeric matrix, did not change significantly the hue value measured for most samples. Regarding the color saturation, although the values did not change substantially, there was a tendency to increase its value for the wax-containing films, due to the yellow color of wax. The calculated values for overall color change ( $\Delta E$ ) revealed the color differences due to the different film formulations. The values of  $\Delta E$  were low, but still revealing that all

films change the color of the white tile, especially those with wax coatings showing a mat look and loss of shininess.

**Water absorption capacity.** The resistance of films to aqueous solutions was determined through the absorption capacity in distilled water and acetic acid solution (Table 5). It was evident that most of the films had higher absorption capacity in the acetic acid solution than in distilled water. The degree of absorption capacity of chitosan films in distilled water was  $1.21 \pm 0.247\%$ , which is similar to the results for

**Table 5.** Absorption capacity of films in water and acetic acid

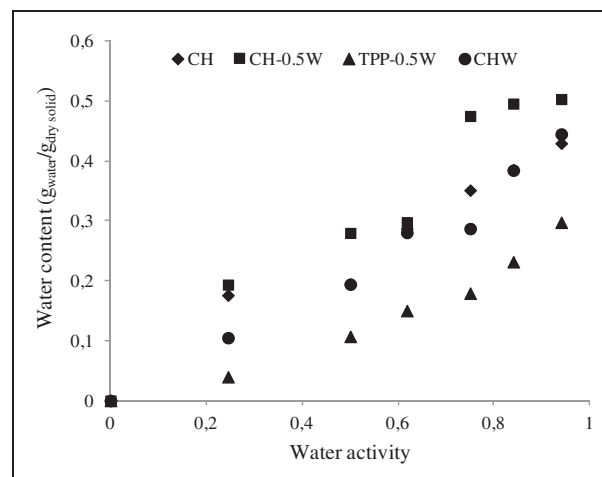
Sample code	Absorption capacity (%)	
	Water	Acetic acid
CH	1.21 ± 0.247	2.83 ± 0.346
0.5TPP	1.28 ± 0.101	1.08 ± 0.094
1 TPP	0.61 ± 0.086	0.89 ± 0.153
CH-0.25W	6.22 ± 0.900	11.52 ± 0.674
CH-0.5W	4.64 ± 0.672	5.89 ± 0.943
CH-1W	1.22 ± 0.269	5.57 ± 0.178
TPP- 0.25W	5.82 ± 0.257	7.23 ± 0.047
TPP-0.5W	4.62 ± 0.176	6.95 ± 1.042
TPP-1W	1.44 ± 0.163	1.09 ± 0.055
CHW	0.68 ± 0.097	4.30 ± 1.055

CH: chitosan; TPP: sodium triphosphate; W: wax.

absorption capacity of chitosan films in distilled water of 1.73% reported by Mayachiew et al. (2010).

One of the objectives of the cross-linking was to improve the stability of chitosan film under acidic media, because chitosan can be easily dissolved in acid solution, which limits its practical use. The cross-linking of chitosan films was carried out with TPP, which can interact strongly with chitosan at pH values around and below neutral since it is highly negatively charged, while chitosan is highly positively charged (Mi et al., 1999). Liu et al. (2004) reported that non-cross-linked chitosan membrane dissolved in aqueous solutions, while the cross-linked membrane could resist dissolving even in acid solution with pH 2. That result implied that cross-linking with TPP was effective enough to improve the chemical stability of the chitosan film and reduce the swelling properties of chitosan-based films.

In this work, by using a 1% w/v TPP solution, the water absorption of the chitosan films in distilled water (1 TTP) decreased to about 50% of the value observed for the films without cross-linking (CH). The use of 0.5 and 1% w/v TPP solution for producing cross-linked chitosan films, 0.5 and 1 TTP, led to decreased water absorption in acetic acid for 68 and 62% when compared to control chitosan films. A higher water absorption capacity for the multilayered films was observed, when compared to the single chitosan films, reaching values as high as 11.52% in acetic acid for CH-0.25W. This fact may attribute to water entrapped in small pores between the film and the wax layers. Gällstedt and Hedenqvist (2004) reported that small cracks in the wax, caused by the clamping, allowed slow penetration of water through the lipid coating, which ended with expansion and further absorption of water into the substrate. The chitosan film TPP-1W, which was first

**Figure 1.** Experimental moisture content of the films as a function of the water activity.

crosslinked with 0.5% TPP solution and then wax-coated, exhibited the same behavior as the crosslinked chitosan film 0.5 TPP proving that 1% wax-coating solution enabled producing multilayered films without any cracks in the wax layer that would increase the absorption capacity.

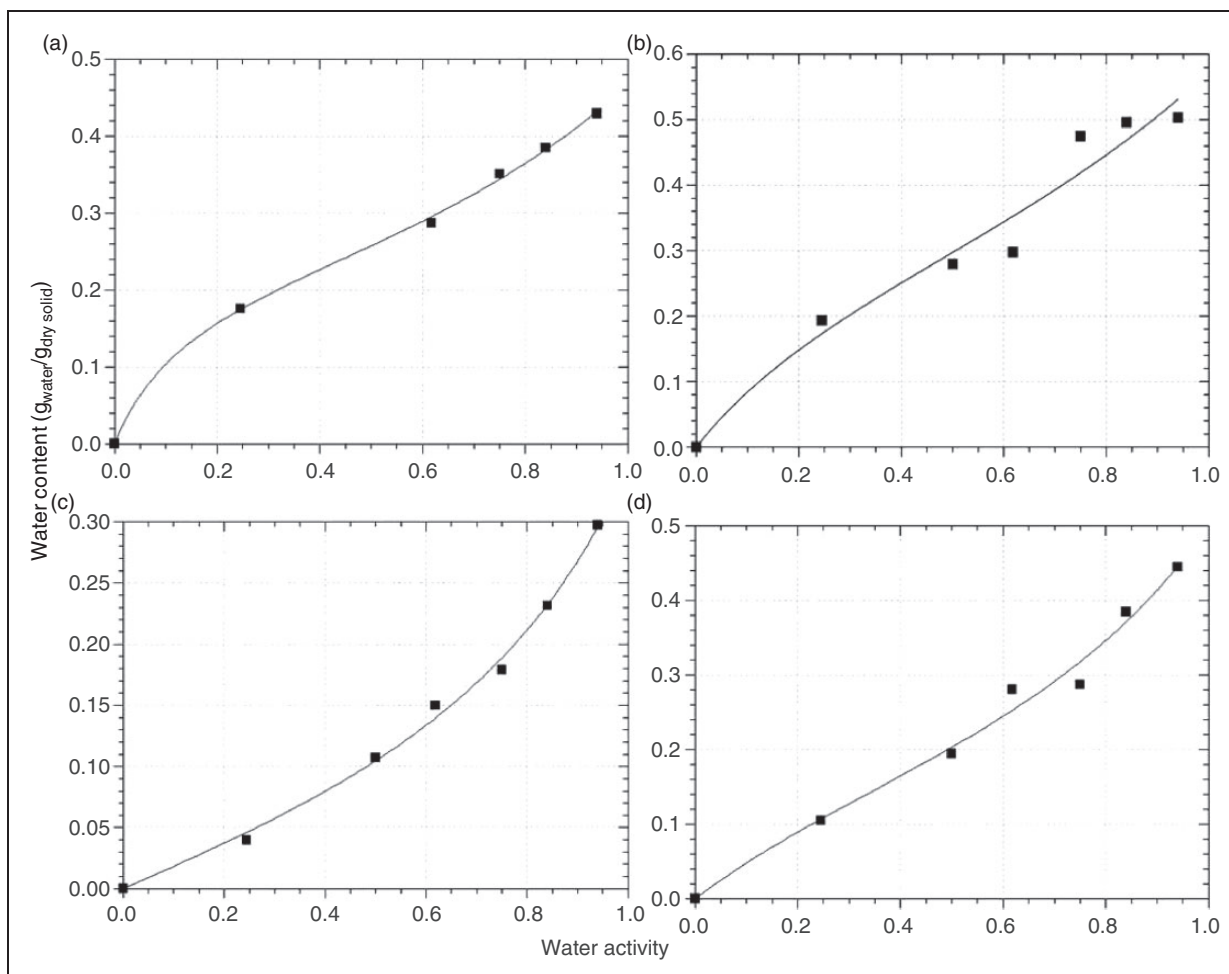
The composite films (CHW) showed a significantly lower absorption capacity in water compared to the other films, which did not happen when immersed in the acetic acid solution.

**Water sorption isotherms.** The experimental results for the moisture content of all films are presented in Figure 1, while the calculated constants and the goodness of the fit of the sorption models tested are listed in Table 6. The curves for the chitosan-based films followed linear rather than sigmoidal pattern. From the results, it was obvious that the experimental values could be fitted by all models. It is usually considered that a good fit is reached when P values <10%. Since in most of the cases, P values were between 0.4 and 8.5%, and the correlation criteria for the films were very satisfactory. The GAB model was the one that best correlated with the experimental results (Figure 2). The equilibrium moisture content for chitosan films is usually in the range from 0.03 to 0.15 g<sub>water</sub>/g<sub>dry solid</sub> depending on the chitosan origin and the manufacturing process (Bourlieu et al., 2009). The monolayer moisture content ( $X_m$ ) for the chitosan and the modified chitosan-based films was in the range between 0.142 and 0.336 g<sub>water</sub>/g<sub>dry solid</sub>. Similar values for the monolayer moisture content of edible films were reported by Chowdhury and Das (2012) and de la Caba et al. (2012).

The higher water vapor adsorption was observed for the uncrosslinked beeswax chitosan films (CH-0.5W),

**Table 6.** Sorption models parameters

Sample code	C	K	X <sub>m</sub>	R <sup>2</sup>	P (%) equation (10)	S equation (11)
<i>GAB</i>						
CH	13.45 ± 3.478	0.55 ± 0.039	0.22 ± 0.020	0.999	0.9	0.03
CH-0.5W	5.93 ± 2.499	0.49 ± 0.102	0.34 ± 0.079	0.990	0.4	0.05
TPP-0.5W	1.88 ± 0.099	0.67 ± 0.094	0.14 ± 0.064	0.998	2.0	0.01
CHW	3.86 ± 1.011	0.62 ± 0.140	0.22 ± 0.108	0.996	0.5	0.02
<i>Iglesias-Chirife</i>						
CH	0.18 ± 0.053	0.02 ± 0.008	/	0.894	8.5	0.08
CH-0.5W	0.12 ± 0.045	0.05 ± 0.007	/	0.965	6.9	0.12
TPP-0.5W	0.08 ± 0.029	0.01 ± 0.004	/	0.910	6.7	0.05
CHW	0.16 ± 0.050	0.02 ± 0.008	/	0.899	3.2	0.08
<i>Oswin</i>						
CH	0.26 ± 0.015	0.20 ± 0.031	/	0.995	2.3	0.04
CH-0.5W	0.28 ± 0.025	0.24 ± 0.044	/	0.988	3.7	0.06
TPP-0.5W	0.12 ± 0.008	0.32 ± 0.030	/	0.996	12.5	0.02
CHW	0.19 ± 0.019	0.31 ± 0.045	/	0.990	5.6	0.04



**Figure 2.** Experimental moisture sorption data for selected films fitted according to the GAB sorption model: (a) chitosan film (CH), (b) chitosan film coated with 0.5% wax solution (CH-0.5W), (c) chitosan film cross-linked with 0.5% TPP and coated with 0.5% wax solution (TPP-0.5W) and (d) composite film (CHW).

which is in agreement with the results obtained for the water absorption capacity (Table 5). However, the cross-linking step of the chitosan films before being coated with beeswax (TPP-0.5 W) seemed to hinder water adsorption, since these films were the ones exhibiting the lower equilibrium moisture content. Furthermore, the inclusion of beeswax within the chitosan matrix in the emulsion-based films (CHW) did not change substantially the equilibrium moisture content of the single chitosan films (CH).

**Water vapor permeability.** Edible films are used as a barrier to moisture transfer between food and surrounding atmosphere to prevent product's dehydration. Therefore, water vapor permeability is a key parameter to be studied. A reduction of this parameter for the chitosan films was expected to be obtained by their modification with cross-linking, coating, and preparation of composite films. The results are shown in Table 7. It may be observed that the cross-linking of the chitosan films resulted in cross-linked films (0.5 TPP and 1 TPP) that exhibited about two times lower water vapor permeability than the original chitosan films (CH). It is known that edible waxes, like the beeswax, are considerably more resistant to moisture transport than most of the other lipid polymers (Kester and Fennema, 1986). Hence, it was expected that the moisture-barrier properties of the hydrophilic chitosan would be significantly improved by the application of wax coating. The results confirmed that the wax coating decreased the permeability of the initial chitosan films (CH) from  $4.15 \times 10^{-11} \text{ mol m/m}^2\text{s Pa}$  to  $1.49 \times 10^{-11} \text{ mol m/m}^2\text{s Pa}$  (CH-1 W). In addition, the concentration of the wax-coating solution, upon application on the chitosan film, significantly influenced the barrier properties of the

multilayered films. The permeability decreased from  $3.09 \times 10^{-11} \text{ mol m/m}^2\text{s Pa}$  (CH-0.25 W) to  $1.49 \times 10^{-11} \text{ mol m/m}^2\text{s Pa}$  (CH-1 W). The increase of the wax concentration in the wax formulation enabled the formation of more continuous wax layers on the chitosan films, leading to a higher barrier to water vapor. The same trend was observed within the wax-coated crosslinked chitosan films, except for the TPP-0.25 W films where higher permeability value ( $4.79 \times 10^{-11} \text{ mol m/m}^2\text{s Pa}$ ) was observed. This fact is probably due to its higher thickness, which was two times of the other films (Table 3). According to equation (11), the water vapor permeability is directly proportional to the films thickness. Furthermore, in contrast to different types of hydrophobic synthetic polymeric materials, the water vapor permeability of films prepared from biopolymers, such as chitosan, is highly dependent on their thickness due to their hydrophilic nature (Rivero et al., 2009).

Similar results of improved barrier properties by application of lipid layers were reported by several other authors, using different waxes, such as beeswax, paraffin wax, and carnauba wax as a coating of the polysaccharide edible films (Debeaufort and Voilley, 1995; Martin-Polo et al., 1992; Weller et al., 1998).

The last modification of the chitosan films was the use of the emulsion technique under heating to produce composite chitosan films by direct incorporation of the beeswax in the film-forming solution. The temperature of  $70^\circ\text{C}$  was necessary for melting the beeswax. The resulting emulsion-based composite films (CHW) presented a water vapor permeability 2.5 times lower than that of the chitosan films (CH) and similar to that of the films coated using 1% wax ethanolic solution (CH-1 W and TPP 1 W).

**Table 7.** Mechanical and water vapor permeability properties of the films obtained from the tensile and puncture test

Sample code	Tensile test			Puncture test		Water vapor permeability $\times 10^{11} \text{ (mol m/m}^2\text{s Pa)}$
	Tension (MPa)	$\varepsilon$ (%)	Young's modulus (MPa)	Tension (MPa)	$\varepsilon$ (%)	
CH	$10 \pm 2.1$	$24 \pm 2.1$	$42 \pm 3.8$	$74 \pm 7.7$	$14 \pm 1.1$	$4.1 \pm 0.7$
0.5TPP	$35 \pm 3.8$	$10 \pm 1.3$	$336 \pm 17.5$	$83 \pm 6.8$	$5 \pm 0.5$	$2.4 \pm 0.3$
1 TPP	$28 \pm 3.5$	$10 \pm 1.5$	$278 \pm 13.5$	$85 \pm 5.5$	$4 \pm 0.2$	$2.3 \pm 0.4$
CH-0.25W	$13 \pm 2.7$	$19 \pm 2.8$	$66 \pm 7.4$	$57 \pm 4.7$	$18 \pm 1.8$	$3.1 \pm 0.5$
CH-0.5W	$11 \pm 1.8$	$20 \pm 2.9$	$56 \pm 6.8$	$54 \pm 4.3$	$12 \pm 2.5$	$2.6 \pm 0.3$
CH-1W	$5 \pm 0.8$	$26 \pm 3.5$	$20 \pm 1.8$	$67 \pm 7.8$	$18 \pm 3.9$	$1.5 \pm 0.1$
TPP-0.25W	$24 \pm 3.5$	$7 \pm 0.9$	$355 \pm 12.3$	$95 \pm 8.5$	$5 \pm 0.7$	$4.8 \pm 0.8$
TPP-0.5W	$23 \pm 2.2$	$5 \pm 0.7$	$425 \pm 18.8$	$106 \pm 7.2$	$7 \pm 0.5$	$3.3 \pm 0.6$
TPP-1W	$30 \pm 3.6$	$8 \pm 0.6$	$361 \pm 10.9$	$108 \pm 9.9$	$6 \pm 0.4$	$1.3 \pm 0.2$
CHW	$1 \pm 0.1$	$25 \pm 2.5$	$4 \pm 0.7$	$23 \pm 3.4$	$19 \pm 1.9$	$1.7 \pm 0.2$

CH: chitosan; TPP: sodium triphosphate; W: wax.

**Mechanical properties.** The mechanical properties of the edible films, i.e. their tensile strength and elongation at break, should be adjusted in such a way for them to withstand the normal stress encountered during their application and handling. The results for the mechanical properties of the films are presented in Table 7. From the results, it was obvious that different treatments resulted in films with both improved and worsened mechanical properties. The cross-linking of chitosan films produced considerably stiffer films, evidenced by the increase of tensile strength at break (from 10 MPa for CH to 28 MPa for 1 TPP), decrease of elongation at break (from 24% for CH to 10% for 1 TPP), and increase of Young's modulus (from 42 MPa for CH to 278 MPa for 1 TPP). The same behavior was observed in the puncture tests, where there was a tendency to increase the tension at puncture and a significant decrease of the elongation at puncture (from 14% for CH to 4% for 1 TPP). The incorporation of lipids in the film formulation either as coating or as composite could negatively affect the mechanical properties of the films. In this study, the beeswax coating of chitosan films did not affect significantly ( $p < 0.05$ ) the tensile strength at break, the elongation at break or the Young's modulus of the original chitosan films. The same was observed for the tension and elongation at puncture. The exception are the multilayered films prepared with a 1% wax solution, which showed to be less stiff, revealed by the lower tensile strength at break (5 MPa) and Young's modulus (20 MPa). The cross-linked and wax-coated films exhibited the same behavior as the uncoated cross-linked films. The different concentration of the wax in the coating solution did not affect the increased stiffness induced by the cross-linking step. Beeswax as a coating did not interfere with the structure of the original chitosan films, which presents the major contribution to the overall mechanical resistance, and therefore it did not affect the mechanical properties of the multilayered films. In the composite films, the incorporation of the beeswax into the polymeric matrix introduced discontinuities in the polymer structure, which affected both the barrier and mechanical properties and led to less stiff films.

## CONCLUSION

The properties of the original chitosan films were modified by (a) cross-linking with sodium tripolyphosphate, (b) beeswax coating on both interfaces of the films, (c) beeswax coating after the cross-linking, and (d) inclusion of beeswax into the polymeric matrix.

Both cross-linking with tripolyphosphate and beeswax coating on both interfaces of the films increased significantly the barrier properties of the films to water vapor. However, while the multilayered wax-chitosan-

wax films maintained the mechanical properties of the original chitosan films, the crosslinked ones revealed an increase of their stiffness. The films subjected to beeswax coating after the cross-linking have shown a similar behavior to that of the uncoated cross-linked films. The emulsion composite films presented simultaneously a substantial decrease of their water vapor permeability and stiffness.

Regarding the behavior in contact with liquid water, the water absorption capacity of the multilayered films was much higher, which is believed to be a consequence of water entry into small pores between the film and the wax layers. Tailor-made multilayered films could be made by optimizing the application of the beeswax layer, namely by changing the wax formulation using plasticizers and surfactants, to obtain less brittle and more continuous wax layers over the chitosan films.

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