

Effect of the Addition of Carnauba Wax on Physicochemical Properties of Chitosan Films

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Films and edible coatings of natural polymers have been proposed as an attractive alternative for conventional plastic packaging because of their excellent biodegradability and biocompatibility. However, natural polymers, like chitosan, are hydrophilic and present poor water barrier properties. The addition of wax or other hydrophobic substances decreases water permeability of natural polymers but can increase their opacity. The objective of this work was to produce biofilms based on chitosan and different concentrations of carnauba wax and analyze their optical and barrier properties. The films were obtained by dissolving chitosan in acetic acid. Carnauba wax was incorporated into film-forming solutions at 0, 15, 30, 40 and 50% (w/w). The opacity increased with higher concentrations of wax, the film with 50% of wax showed the highest value with an increase of 10.5% compared to the control film. The water vapor permeability decreased from 2.73 g.mm/kPa.m².h (0% of wax) to 0.77 g.mm/h.kPa.m² (40% of wax). The solubility decreased to about 60% over the control films, and the contact angle increased from 53° to 83°, in film with 0 and 50% of wax, respectively.

Keywords: *biofilms, chitosan, carnauba wax, opacity, water vapor permeability*

1. Introduction

Over the years, many studies have been conducted with the goal of increasing the shelf life of fruit by applying edible coatings. These coatings can provide many advantages such as improved appearance, antimicrobial properties, selective permeability for gases (CO₂ and O₂), low water vapor permeability, good mechanical properties, non-toxicity, non-polluting properties and low cost¹.

The antifungal and antioxidant activities of chitosan have been studied by several authors, along with their film formation ability. These characteristics make it interesting use as an active biodegradable packaging material that can be used to improve storage capacity of perishables^{2,3}. Furthermore, chitosan films tend to have fat and oil resistance and low permeability to gases, but do not have resistance to water permeability⁴.

The addition of lipids reduces the water vapor permeability of films or coatings, but can affect its transparency and mechanical properties. Lipids used in coating formulation for preserving minimally processed products are stearic acid, palmitic acid and certain vegetable oils such as soybean and sunflower oil. Waxes such as carnauba, candelilla and bee are also used, showing better gas and moisture barrier properties than coatings containing only fatty acids⁵.

In the studies of Chiumarelli and Hubinger⁵, when they used cassava starch and carnauba wax edible coatings

to preserve fresh-cut apples verified that there was an improvement in their water vapor permeability, solubility, among other analyzes. Bilayer films composed of cellulose ether and beeswax⁶, lipids and wheat gluten⁷, zein and waxy sorghum / carnauba wax⁸ and sodium caseinate and paraffin⁹ were analyzed as effective barriers for water vapor transport¹⁰. Using chitosan as polymer was reported that when it was mixed in beeswax and coated in strawberries there was a decrease in weight loss and senescence, color retention and texture, titratable acidity, pH, soluble solids and sugars, taste and visual appearance, improvement in antimicrobial properties and reduced respiration rate^{11,12}.

The purpose of the present study was to develop edible biofilms based on chitosan and carnauba wax, mixtures were made with the chitosan solution 1% (w/v) and different concentrations of carnauba wax: 0, 15, 30, 40 and 50% (w/w), in order to improve its solubility, water vapor permeability, contact angle without damaging its optical properties.

2. Materials and Methods

2.1 Materials and reagents

Chitosan (degree of deacetylation of 85%) was purchased from Polymar (Industry and Com Imp & Exp Ltda - Brazil). Carnauba wax type I (MW = 144.859) was kindly provided by the company ORTAL LTDA (Mossoró - RN - Brazil).

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Acetic acid (Glacial PA), with purity of 99.8%, used to dissolve chitosan was obtained from Proquimicos (Brazil). Glycerol, used as plasticizer, was purchased from Chemical Dynamics Inc. Contemporary (Brazil). Tween 20, surfactant responsible for reducing the surface tension of the solutions and to form an emulsion, was obtained from VETEC (Brazil). All chemicals and reagents were used without pretreatment.

2.2 Preparation of films

Chitosan solution of 10 g/L was prepared by dissolving chitosan 1% (w/v) in a solution of acetic acid 1% (w/v) and deionized water, under moderate stirring. The homogenization of the solution was carried out for 24 h at room temperature (29°C). Glycerol was added in a chitosan-plasticizer ratio of 2:1 for all chitosan solutions¹. Carnauba wax was incorporated into film-forming solutions at 0, 15, 30, 40 and 50% (w/w) and Tween 20 was added as a surfactant in a 1:0.5 wax/surfactant weight ratio. The amounts of carnauba wax and Tween 20 were defined according to preliminary tests and based on literature data^{1,12}. The lipid-free film was prepared as control film. The final film-forming solution was homogenized using a magnetic stirrer with heating (Model LUCA-0851 / Group Lucadema - Brazil) at 1000 rpm for 10 min until 85 °C (melting wax). Then, 60 ml of each solution were transferred to an acrylic plate of 15 cm diameter. The plates were placed on a level surface and the films were naturally dried at room temperature (29°C) for 48 hours. The dried films were manually removed from the plates. Assays were performed in triplicate.

2.3 Film properties

The film thickness was measured with a micrometer (Mitutoyo, model MDC-25M/Japan) with sensitivity of 0.001 mm. Three thickness measurements were carried out on each film¹³.

Optical parameters of films were measured with a MINOLTA colorimeter (model CR-10 (Konica Minolta Sensing, INC., Japan)). Film samples were placed on a white standard background and on a black standard background, by acquiring the parameters a^* , b^* and L^* .

Where L^* , ranges from 0 (black) to 100 (white); a^* , from green (-) to red (+); and b^* , from blue (-) to yellow (+)^{14,15}.

The opacity values were calculated according to Eq. 1¹⁶:

$$Op = \frac{L_{black}}{L_{white}} \times 100 \quad (1)$$

Where Op is the film opacity (%), L_{black} is the luminosity of the film against a black background, and L_{white} is the luminosity of the film against a white background.

And the color difference (ΔE) was calculated by Eq. 2¹⁷.

$$\Delta E = \sqrt[3]{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \quad (2)$$

For solubility, small pieces of film (2 x 2 cm) were dried in an oven at 105°C for 24 h to obtain the initial dry mass of the film. After drying, films were placed into erlenmeyers containing 20 ml of distilled water. Erlenmeyers were covered and gently shaken for 24 h. The remaining pieces of film were dried in an oven at 105°C for 24 h to obtain the final dry mass of the film. The percentage of solubility (% SOL) of the films was calculated using the Eq. 3:

$$SOL = \frac{M_i - M_f}{M_i} \times 100 \quad (3)$$

Where SOL is the percentage of solubilized material, M_i is the initial mass and M_f is the final mass of the films^{1,18}.

A modification of the ASTM E96-05 (McHugh et al.)¹⁹ gravimetric method was used to measure water vapor permeability (WVP)²⁰. Upon drying, films were chosen for WVP testing based on lack of physical defects such as cracks, bubbles or pinholes. The films were fixed on permeation cells containing distilled water (100% RH). Cells were maintained at 29°C within desiccators containing silica. Cell weight was recorded at 1 hour intervals and 6 measurements were collected. Water vapor transmission rate (WVTR) was determined from the slope obtained from the regression analysis of weight loss data as a function of time, once the steady state was reached, divided by the film area, Eq. 4. The WVP was calculated using Eq. 5^{20,21}.

$$WVTR = \frac{m}{t * A} \quad (4)$$

$$WVP = \frac{WVTR * L}{\Delta P} \quad (5)$$

Where WVTR is the water vapor transmission rate (g/h.m²), WVP is the water vapor permeability (g.mm/h.kPa.m²), m is the mass of water that passes through the film (g), L is the thickness of the film (mm), t is the time at which mass loss occurs (hours), A is the exposed area of the film (m²) and ΔP is the partial pressure difference of water vapor, at 29°C (kPa).

The contact angle measurements, based on the sessile drop technique^{22,23,24}, were performed in an apparatus composed basically of a mobile base, a camera (VP 540 s, Intelbras) and a pipette. In analyzes, three drops of distilled water were deposited on the surfaces of the films, which were on the sample holder. The calculation methodology was based on the transformation of images, determining the angle formed by the intersection of the liquid-solid. The software used to calculate the contact angle was the SurfTens 4.5. The tests were performed at an ambient temperature of 25±2°C.

All data were collected at least in triplicate. The significant difference between averages was established by the Duncan method with a level of statistical significance lower than 5%.

3. Results and Discussion

3.1 Optical properties

The color parameters (Opacity, a^* , b^* , L^* , ΔE) of the films tested are summarized in Table 1.

In this work, films with carnauba wax were more opaque than films with 0% of wax, (Table 1). An increase in Op of films after the incorporation of solid fat, including wax, has been reported by different authors^{25,26,27,28}. Due to their physical state, the solid particles of lipids cause a lack of morphological homogeneity in the films emulsions, and thus causing visible light scattering through the films, resulting in their opacity²⁹. Despite the increase in Op with the addition of lipid, this growth was not very marked. The addition of 30% of wax, for example, only implied a 6% (approximately, 13% for 0% of wax and 19% for 30%) increase in relation to the control films.

Although the incorporation of carnauba wax resulted in higher values of a^* , these did not differ statistically. The parameter b^* had a slight increase, and the L^* did not show great differences between them. Additionally, the films showed a yellowish color and according to Casariego et al.³⁰, is the parameter b^* that describes the color of chitosan based films due to the natural yellowish color of chitosan, furthermore this effect was also due to the yellow color inherent in carnauba wax. Studies by Kowalczyk et al.²¹ demonstrated similar behaviors when using films of soy protein and candelilla wax. The ΔE of the biofilms grew with the increase of the wax concentration; similar results were also obtained by Monedero et al.¹⁷ when they incorporated beeswax into soy protein.

3.2 WVP and thickness

Table 2 shows the influence of the carnauba wax concentration on chitosan films WVP and thickness.

The addition of carnauba wax affected the films thicknesses, where a significant increase was observed when wax was incorporated into the formulation (Table 2), ranging from 0.0364-0.0858 mm. When the wax content increases the higher molecular contact between the chitosan and wax compounds may weaken the aggregation forces of the polymer chain, making the matrix more open^{1,3,31}. Other authors reported the same behavior with the addition of lipids to filmogenic solutions. Hromišet al.¹ reported an increase of 0.0592-0.4718 mm in chitosan films with the addition of beeswax and caraway essential oil. Hosseini et al.²⁰ reported an increase of 0.0612 - 0.0889 mm in films of chitosan and gelatin incorporating oregano essential oil.

Regarding WVP, the control film showed the highest WVP, since film without wax has hydrophilic character facilitating a greater passage of water vapor. The addition of 15% of wax was not sufficient to change the hydrophilicity of the film, not showing significant difference in relation to the control films. Films with 30% of wax showed superior water vapor resistance with a decrease of 63% compared to control films, which indicates that the addition of 30% of wax resulted in biofilms with hydrophobic character that hindered the passage of water vapor through them. Films with 40% of wax had the best reduction of WVP (0.77g.mm/h.kPa.m²). Therefore, the increase in the concentration of carnauba wax decreased the hydrophilicity of the films, as the hydrophobic character of the carnauba wax strongly influenced the behavior of the water vapor barrier. Similar results were observed by Kowalczyk et al.²¹ in films with candelilla wax and soy protein with final WVP of 1.45g.mm/h.kPa.m², and candelilla wax and potato starch with final WVP of 1.07g.mm/h.kPa.m².

In the film with 50% of wax a different behavior was observed, showing a WVP higher than the films with 30 and 40% of wax. It has been suggested that the incorporation of apolar components in solid state until a certain amount can

Table 1. Mean values for colour coordinates (a^* , b^* , c^* , L^*), Op and ΔE for different carnauba wax percentages.

Wax (%)	Op (%)	a^*	b^*	L^*	ΔE
0	13.56 ^{bc} ± 0.060	-8.36 ^b ± 0.25	10.73 ^{bc} ± 0.15	87.10 ^a ± 0.00	17.18 ^{bc} ± 0.17
15	17.12 ^{bc} ± 0.005	-4.16 ^a ± 0.15	15.90 ^{ac} ± 1.04	83.90 ^{bc} ± 0.62	21.24 ^{ac} ± 1.12
30	19.42 ^{ac} ± 0.004	-4.4 ^a ± 0.48	17.80 ^{ac} ± 1.05	83.90 ^{bc} ± 0.40	22.97 ^a ± 1.27
40	19.91 ^{ac} ± 0.010	-3.76 ^a ± 0.06	18.20 ^{ac} ± 1.02	83.26 ^{bc} ± 0.65	23.29 ^a ± 1.18
50	24.11 ^a ± 0.006	-3.63 ^a ± 0.81	22.63 ^a ± 0.67	81.43 ^{bc} ± 0.42	25.72 ^a ± 2.39

Different lowercase letters in superscript mark significantly different mean values within the same column.

Table 2. Mean values for water vapor permeability (WVP) and thicknesses for different carnauba wax percentages.

Carnauba wax (%)	Thickness (mm)	WVP
0	0.0364 ^{bc} ± 0.002	2.18 ^a ± 0.16
15	0.0542 ^{ac} ± 0.001	2.05 ^a ± 0.02
30	0.0678 ^{ac} ± 0.002	0.85 ^{bc} ± 0.20
40	0.0724 ^a ± 0.003	0.77 ^{bc} ± 0.07
50	0.0858 ^a ± 0.003	1.12 ^{ac} ± 0.20

Different lowercase letters in superscript mark significantly different mean values within the same column.

cause more perturbations in the matrix of the films, creating a larger number of voids at the polymer-wax interface, which facilitates the transfer of water molecules inside the film^{32,33}.

3.3 Solubility

Fig. 1. shows the influence of the carnauba wax concentration on chitosan films solubility.

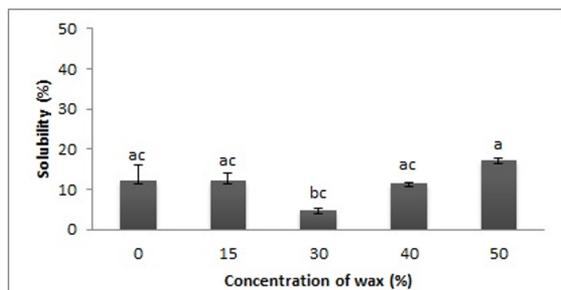


Figure 1. Mean values for solubility as a function of the carnauba wax percentage in the lipid mixture for the different polymer: lipid ratios. Different lowercase letters mark significantly different mean values.

The solubility of the pure chitosan film was 12.31%, which is in agreement with the results reported by Leceta et al.³⁴, and Hromiš et al.¹. When 15% of carnauba wax was added to the films there was no significant difference in relation to the control films. A different behavior in the solubility of the films was detected after the incorporation of 30% of wax, which caused its reduction in 60%. The reduction of solubility in films due to the incorporation of bee or candelilla waxes has been reported by Kim and Ustunol³⁰, and Soazo et al.³⁵. These authors considered that, because the total solids levels remained constant in the formulation, the incorporation of wax reduced the soluble matter present in the films, and, consequently, the solubility.

Films with 40 and 50% of wax showed higher solubility than the other films containing carnauba wax. The film with 40% of lipid had lower solubility than the control film and with 50% had higher solubility than the control film. This behavior can be explained because the surfactant (tween 20) can increase the films solubility and tween has been added in greater amounts in films with 40 and 50% of wax.

3.4 Contact angle measurements

It is possible to observe in Fig. 2, the contact angles obtained for the different filmogenic formulations.

The contact angle measurements of the films indicated that the hydrophilicity of the chitosan based films decreased with the addition of carnauba wax. Generally, low contact angle (CA < 65°) indicate a hydrophilic surface and high (CA > 65°) are characteristic of hydrophobic surfaces²⁰. Thus, the tests in the films indicated that the films with 0% and 15% of carnauba wax presented hydrophilic surfaces because they showed contact angles of 52° and 54° respectively; the films

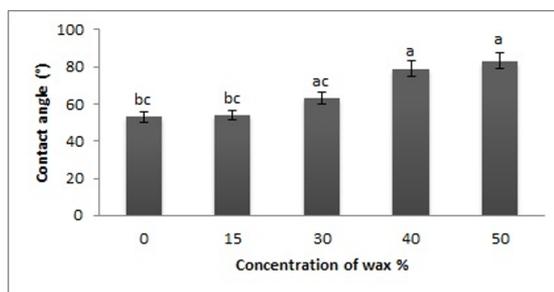


Figure 2. Mean values for contact angle as a function of the carnauba wax percentage in the lipid mixture for the different polymer: lipid ratios. Different lowercase letters mark significantly different mean values.

with 30% of wax showed angle of 63°, demonstrating the transition of the hydrophilic to the hydrophobic character. The films with 40% and 50% of wax showed angles of 78° and 83°, respectively, indicating a surface with a hydrophobic character. Similar results were reported by Hosseini et al.²⁰ that added oregano essential oil to the chitosan and gelatin matrix and obtained an increase in the hydrophobicity of the films, 58° (control films) and 80° (films with oil); and by Rezae et al.³⁶, which also led to the conclusion that the incorporation of cinnamon essential oil into chitosan films resulted in a decrease of the hydrophilicity of the composite films.

4. Conclusions

The optical and barrier properties of chitosan films were investigated when different concentrations of carnauba wax were added in their matrix. All films showed good cohesion and ability to be manipulated. Generally, increases in the amount of wax resulted in more opaque films in appearance, though with significantly improvement in barrier properties. Films with 30% of wax, for example, increased the opacity only in 6% in relation to the control films and showed good resistance to water vapor (63% of reduction in WVP). This improvement in WVP is a good result as the entry of water vapor through the packaging can cause quality and conservation damage. The contact angle increased as the wax was added, indicating the transition of the hydrophilic to the hydrophobic character of the biofilm. The solubility had a significant decrease when 30% of wax was added to the films, being a promising result because food applications require water insolubility to maintain product integrity. Therefore, chitosan film with 30% of carnauba wax showed better results among the films tested.

Thus, biofilms of chitosan in addition to providing biodegradable, antifungal and antioxidant characteristics, when carnauba wax was incorporated into its matrix, its biofilms showed improved barrier properties without damaging its optical properties, which are very promising in the application as packing.

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