Abstract: The aim of this study was the preparation and characterization of chitosan/gelatin films with grape seed extract. Films were prepared by mixture of chitosan/gelatin/grape seed extract in 1:1:1 and 1:1:2 ratios (w/w/w). Grape seed extract (GSE) presence decreases the water quantity and increases the thermal stability of films as observed in thermogravimetric curves. Scanning electronic microscopy images show a homogeneous surface without undissolved materials and GSE does not change the appearance of the film. GSE influences the water vapor permeability (WVP) as values increase from 2.20 $\times$ 10^{-9} g m^{-1} s^{-1} Pa^{-1} to 2.48 $\times$ 10^{-6} g m^{-1} s^{-1} Pa^{-1}. This change in values is related to the crosslinking effect of proanthocyanidin that weakens the direct associations of chitosan and gelatin, increasing the water molecules diffusion.

Palavras-chave: Chitosan, Collagen, Grape seed extract

INTRODUCTION

Chitosan is a polysaccharide derived from the partial deacetylation of chitin found in the shells of crustaceans, mollusks and exoskeleton of insects. Chitosan has amino groups in its structure, which in acid solution acquire positive charge that allow electrostatic interaction with proteins and nucleic acids. It is for this reason that this polymer has important applications in the medical, food and pharmaceutical area\(^{1}\).

Gelatin is produced by the partial hydrolysis of collagen, the main constituent of animal skins, bones, tendons and connective tissue. Gelatin is a heterogeneous mixture of proteins with average molecular weight ranging from 20,000 to 250,000 Da, depending on the degree of hydrolysis of collagen.
Gelatin is a biomaterial that has no taste or odor and is transparent. Moreover, it is biocompatible and biodegradable, have plasticity and adhesion, promoting cell growth and adhesion\(^{(2)}\).

Polyelectrolyte complexes (PEC) are formed when solutions of macromolecules carrying opposite charges are mixed. Essentially, this is the result of electrostatic interactions between both polyions\(^{(3)}\). So, in this study two biopolymers, chitosan (C) and gelatin (G) are used as polycation and polyanion, respectively, to prepare PEC and to evaluate the physical and thermal properties. In addition, grape seed extract was used to verify the effect in the properties of this PEC. It is know that proanthocyanidin, which is the main component present in grape seeds, is antioxidant and have certain biological effects that promote improvements in vascular endothelial growth, aiding in tissue healing process\(^{(4)}\). Proanthocyanidins belong to the class of flavonoids. They are oligomeric compounds, formed by catechin and epicatechin molecules (Figure 1).

![Chemical structures of: A, Epicatechin and B, Catechin.](image)

**Figure 1.** Chemical structures of: A, Epicatechin and B, Catechin.

Chitosan/gelatin films associated with grape seed extract can be applied in the food industry as a coating material on the preservation of fruit. In the area of biomaterials, their use is directed to the improvement in the wound healing process.

**MATERIALS AND METHODS**

**Raw materials**

Chitosan was obtained in our laboratory by demineralization, deproteinization and deacetylation of squid pens from *Loligo sp*, as described earlier\(^{(5)}\). Values of degree of acetylation (DA) and molecular weight (MW) of
chitosan were determined by potentiometric titration and capillary viscosimetry method respectively, as described by Raymound et al.\(^{(6)}\). DA calculated value was 9.05 % ± 0.35 and MW was 4.28x10\(^5\)\(^{(5)}\).

Gelatin (Sigma-Aldrich) and grape seed extract were acquired in a local commerce.

**Films preparation**

A 2% (w/w) chitosan solution was prepared by dissolution in 1% acetic acid (w/w). Gelatin solution (2% w/w) was gelatinized in water at 80°C during 30 min. PEC was obtained by mixture between both solutions in 1:1 ratio with vigorous agitation. PEC was labeled as CG. Grape seed extract solution (water/alcohol) was slowly added to PEC at 1% or 2% (w/w), and mixtures named as CGE1 and CGE2. Films were prepared at ambient temperature by solvent evaporation technique (casting method) in Teflon® molds.

**Characterization**

**Termogravimetry (TG).** Thermogravimetric (TG/DTG) curves were obtained with a TGA Q50 module (TA Instruments) with a heating rate of 10°C min\(^{-1}\) between 25 and 800°C, in synthetic air atmosphere. Sample sizes were about 10 mg.

**Film thickness.** Film thickness was measured using a Micrometer (Model M110-25, Mitutoyo MFG. Co. Ltd., Japan). Thickness measurements were taken at 10 different points along the film and the average was used to calculate the water vapor permeability.

**Water vapor permeability (WVP).** Water vapor permeability of the films was calculated as described by Garcia et al.\(^{(7)}\). Each film sample was fixed in a circular hole of 0.007 m\(^2\) containing anhydrous calcium chloride (0% humidity). The cups were weighted and placed at 75% relative humidity (NaCl saturated solution) and 25°C in a humidity chamber. The cups were weighted 6 times over a period of 9 h. The water vapor transferred through the films at different time intervals was determined from the weight gain of the cups. A curve of weight change (g) versus time (min) was obtained to determine the slope, used for the calculation of water vapor transmission rate (WVTR) as shown in Equation A:
where $m/t$ is the slope of the curve and $A$ is the permeation area of the sample (m²). Therefore, the water vapor permeability (WVP) for the films was determined using Equation B:

$$WVP = \frac{WVPR \cdot t}{sp(RH_1 - RH_2)}$$

where $t$ is the film thickness (mm), $sp$ is the water vapor pressure at 25°C, $RH_1$ is the relative humidity inside the chamber (75 %) and $RH_2$ is the relative humidity inside the cup (0 %).

**Scanning electron microscopy (SEM).** The morphology was observed using a ZEISS LEO 440 OXFORD detector (model 7060) operating with an electron beam of 20 kV. Before the examination, the films were covered with a thin gold layer of 6 nm thick using a sputter Coating System BAL-TEC MED 020 at a pressure of 2.00x10⁻² mbar in the chamber, current of 60 mA, and a deposition rate of 0.60 nm s⁻¹.

**RESULTS AND DISCUSSION**

Films were homogeneous, flexible and easily removed from cast support. Thickness varied between 0.087 and 0.128 mm, as shown in Table 1.

<table>
<thead>
<tr>
<th>Film</th>
<th>Thickness (mm)</th>
<th>WVP (g m⁻¹ s⁻¹ Pa⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>0.087 ± 0.011</td>
<td>2.20 x 10⁻⁹</td>
</tr>
<tr>
<td>CGE1</td>
<td>0.077 ± 0.011</td>
<td>1.15 x 10⁻⁹</td>
</tr>
<tr>
<td>CGE2</td>
<td>0.128 ± 0.026</td>
<td>2.48 x 10⁻⁶</td>
</tr>
</tbody>
</table>

Both biodegradability and good gas barrier properties are the main features of polysaccharide-based films. The use of grape seed extract in films formulations affects their barrier properties, due to the chemical interaction between biopolymers and extract compounds.
The WVP values increases from $2.20 \times 10^{-9} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ to $2.48 \times 10^{-6} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$, after GSE addition (Table 1). WVP is highly dependent on the quantity of amorphous and crystalline zones and some specific interactions between functional groups of chitosan, gelatin and the water molecules\(^8\). The change in WVP values is related to the crosslinking effect of proanthocyanidin that weakens the direct associations of chitosan and gelatin, increasing the water molecules diffusion. Additionally, higher concentration of hydrophilic GSE, offering more free OH-hydrophilic positions to water molecules, reason why CGE2 showed high WVP value.

Thermogravimetric analysis is a simple and accurate method for studying the decomposition pattern and the thermal stability of polymers. Thermogravimetric curves (Figure 1) shows the thermal decomposition of chitosan/gelatin films in air atmosphere. The weight loss occurs in three steps: the first one refers to the loss of structural water (25-200°C); the second, (200-400°C), corresponds to a complex process including dehydration of saccharide rings, depolymerization, and decomposition of the acetylated and deacetylated units of polymer\(^9\) and the third stage, 400-750°C, to the carbonization of polymeric materials. The close similarity of TG curves for films prepared with or without grape seed extract indicated that GSE does not changes the behavior of weight loss.
Table 2 shows the weight loss and the $T_{\text{onset}}$ point obtained by termogravimetry of the chitosan/gelatin films and films containing of grape seed extract. GSE addition decreases the water quantity and increases thermal stability of films. The extract stabilizes the polymer chains, increasing the energy required for degradation and consequently, increase the decomposition temperature.

**Table 2. Weight loss and the $T_{\text{onset}}$ point of films**

<table>
<thead>
<tr>
<th>Films</th>
<th>% Weight loss</th>
<th>$T_{\text{onset}}$ ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25-200°C</td>
<td>200-400°C</td>
</tr>
<tr>
<td>CG</td>
<td>23.9</td>
<td>41.3</td>
</tr>
<tr>
<td>CGE1</td>
<td>20.5</td>
<td>42.7</td>
</tr>
<tr>
<td>CGE2</td>
<td>20.8</td>
<td>42.5</td>
</tr>
</tbody>
</table>

For CG film, the SEM image shows a homogeneous surface without undissolved materials (Figure 3A). After grape seed extract addition, a rough surface was observed only for CGE2 (Figure 3C). This appearance is attributed to the grape seed presence in film formulation, behavior already observed earlier\(^8\). CGE1 film showed a homogeneous surface probably because the quantity of grape seed is not sufficient to change their characteristic.
CONCLUSION

Grape seed extract can be associated with chitosan/gelatin to prepare films. Changes in physical and thermal properties were observed. The increase in thermal stability of films can be attributed to the stabilization effect of grape seed extract increasing the temperature required for degradation. SEM images show a homogeneous surface without undissolved materials and GSE does not change the appearance of the film. The increase of water molecules diffusion is related to the crosslinking effect of proanthocyanidin.

ACKNOWLEDGEMENTS

M.M.H. acknowledges the financial support of PNPD/Capes.
REFERENCES


