PREPARATION AND CHARACTERISATION OF ZEIN AND CHITOSAN EDIBLE FILM

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RESUMO – O objetivo deste trabalho foi desenvolver uma blenda de zeina-quitosana e estudar o efeito do calor sobre a quitosana no processo de elaboração de filme. As soluções de zeina e de quitosana foram preparadas separadamente. Duas soluções de quitosana foram produzidas uma aquecida a 80 ºC durante 1 h, e outra só agitada durante 1 h e sem aquecimento. Espessura, cor, solubilidade em água, ângulo de contacto e as propriedades mecânicas dos filmes foram analisados. Os dados indicaram que o aquecimento da solução de quitosana não melhorou as características físicas do filme. Em vez disso, os filmes zeina-quitosana preparados com quitosana sem aquecimento apresentaram melhores resultados para a solubilidade em água, ângulo de contato e propriedades mecânicas.

ABSTRACT – The aim of this work was to develop zein/chitosan blends and study the heat effect on chitosan in the film-making process. The zein and chitosan solutions were prepared separately; two different chitosan solutions were produced, one heated at 80 ºC for 1 h, and another just stirred for 1 h and unheated. Thickness, color, water solubility, contact angle and mechanical properties of the blend films were investigated. The data indicated that heating the chitosan solution did not improve the physical characteristics of the film. Instead, the zein-chitosan films prepared with unheated chitosan showed better results for water solubility, contact angle and mechanical properties.

PALAVRAS CHAVE: zeina; quitosana; formação de filme; aplicação para alimentos.

KEYWORDS: zein; chitosan; blend; film formation; food packaging application.

1. INTRODUCTION

Environmental pollution as a consequence of waste from packaging plastics has recently become a major issue (Paramawati et al., 2001). The use of biodegradable, environmentally friendly and renewable materials, such as proteins, thermoplastic starch and other polysaccharides has steadily increased in food packaging. These biopolymers have partly replaced the polymers synthesized from petroleum-based feedstock (Forato et al., 2013).

Zein is extracted from corn during industrial processing or production of bioethanol. Because it is a natural polymer with interesting properties, it has a high potential to be a bioplastic. Thus, it has
received a lot of interest as a packaging film for direct contact with food (Sanchez et al., 2015; García et al., 2013; Forato et al., 2013; Biswas et al., 2009; Sessa et al., 2013). More than 50% of the amino acid residues in zein are hydrophobic (~1.36 J mol\(^{-1}\)) which makes it one of the few proteins that can be solubilized in aqueous ethanol (60 - 90%) but not in pure aqueous solutions (Hu et al., 2015; Sant’Ana et al., 2012). Zein film serves as a better barrier against water vapor and volatile compounds than other protein-based edible films (Cheng, Wang and Weng, 2015). However, pure zein films are quite fragile, so it is necessary to add a plasticizer to reduce the intermolecular forces among the polymer chains and increase the flexibility of the film, but it causes a reduction in the barrier property (Forato et al., 2013, Paramawati et al., 2001).

The mixtures of proteins with lipids or polysaccharides can improve the film properties. In particular, polysaccharides have shown advantages when compared to other polymeric materials, particularly due to their multifunctionality and biodegradability. For example, the films prepared from chitosan are clear, strong, and flexible, provide adequate barriers to oxygen, as well as antimicrobial protection (García et al., 2013). In view of the desirable properties of chitosan and zein, the formulation of a blend of these two materials would seem to be attractive.

Nonetheless, the mechanical and barrier properties of blend films depend on many factors such as formulation, processing conditions, and the state of film formation at a particular temperature and water activity level (Panchapakesan et al., 2012). Thus, the method of film preparation can directly influence the properties of the material obtained. There is some disagreement among the chitosan solubilization for film preparation; some authors suggested heating the chitosan solution, while others gave the opposite suggestion (Bhuvaneshwari et al., 2009; García et al., 2013). In this study, we aim to reexamine zein/chitosan blends, study the heat effects on the chitosan film formation, and evaluate the properties of the resulting films.

2. MATERIAL AND METHODS

2.1. Material

Zein (regular grade) was obtained from Flo Chemical Corporation (Ashburnham, MA, USA) and chitosan (low molecular weight) from Sigma Aldrich (St. Louis, MO, USA). Glacial acetic acid and glycerol were obtained from Dinâmica (Huixtla, Brazil) and Tween\(^{®}\) from Oxiteno (Sao Paolo, Brazil).

2.2. Film preparation

Zein and chitosan solutions were prepared separately. Two different batches of chitosan solution were processed, one heated at 80 °C for 1 h, and another just stirred for 1 h and unheated. Two corresponding films were prepared and named Z/Q-h (with heated chitosan solution) and Z/Q (with unheated chitosan solution).

Chitosan and zein films were formulated by mixing previously prepared solutions of chitosan (5% chitosan in 1 N acetic acid solution (w/v), heated or unheated), zein (10%, zein/ethanol (w/v)), Tween 80 emulsifier (2%), and glycerol. The amount of glycerol was calculated to give 1:3 dry weight ratios of glycerol : zein+chitosan. The solution was mixed for 10 min at 10,000 rpm using an Ultra-Turrax homogenizer (Model T25, IKA, Campinas, Brazil) and then degassed. The pH of the mixture was kept at a value of ~ 5.6. The films were prepared by the casting method and dried in an air circulation oven at 33 °C for 3 h.

2.3. Film characterization
Thickmess: Measurements were conducted using a digital micrometer (Digimatic model, Mitutoyo, Brazil) with a range of 0-25 mm and an accuracy of ± 0.01 mm. The thickness reported was the arithmetic average of eight measurements made randomly along each sample evaluated.

Color: The color parameters L* (Light), a* (−green/+red degree), b* (−blue/+yellow degree) of the films were determined with a ColorQuest XE colorimeter and Easy Match QC software (Hunter Lab, Reston, VA, USA).

Solubility: The water solubility determination was carried out on 3 cm x 3 cm film pieces based on the method proposed by Gontard et al. (1994), with some modifications. Previously dried and weighed samples were immersed in 50 mL of distilled water for 24 h at 25 °C with stirring (75 rpm). The dry weight of the remaining film pieces was obtained after filtration on previously dried and weighed filter paper and was used to calculate the insoluble matter as a percentage of the initial dry weight (g/100 g). All the dry weights (of the initial and final film pieces and the filter paper) were determined after drying at 105°C for 24 h using a fan-assisted convection oven (Quimis model Q 31 4M22, Brazil).

Water contact angle measurements: Droplets of deionized water were dispensed onto the surface of the zein/chitosan films. A photograph of each droplet was taken 2 s after contact with the surface using a Digidrop instrument (GBX, Dublin, Ireland), and the instrument’s software was utilized to measure the stable contact angle at the water zein/chitosan film interface.

SEM analysis: Morphological characterization was performed by SEM (Zeiss DSM, model 940A). Samples were sputter coated with thick platinum layers using an evaporator (Electron Microscopy Sciences, Hatfield, PA, USA) and examined on the SEM using an accelerating voltage of 15 kV and a magnification of 1000x.

Mechanical analysis: This was conducted according to Method D882-09 (ASTM, 2009) on a mechanical tester (EMIC DL 3000) on 12.8-cm strip film samples. The initial grip separation and cross-head speed were set to 10 cm/min. Both force (N) and deformation (mm) were recorded during extension. Tensile strength was calculated by dividing the required force for film rupture by the cross-sectional area, and elongation at break was calculated as the percentage increase in sample length. The elastic modulus was calculated from the slope of the stress–strain curve in the elastic deformation region. The reported values are the averages of five measurements.

3. RESULTS AND DISCUSSION

The results on thickness, color, water solubility, contact angle and mechanical properties are shown in Table 1. The Z/Q and Z/Q-h film thicknesses were 0.118 and 0.131 mm, respectively.

Color is a parameter that affects the acceptance or rejection of a product by consumers; hence, when films are developed to be used for food packaging, the final appearance of the material to be covered, such as transparency and color, is preferably not changed by the manufacturer (Sánchez et al., 2015). In general, a film with a higher brightness has a higher relative L* value. According to the L*, a* and b* values for Z/Q and Z/Q-h films shown in Table 1, sample Z/Q has a L* value of 57.13 and sample Z/Q-h 49.89; these results reflect the fact that heating of chitosan caused the Z/Q-h film to be slightly darker. According to a* and b* values, both films exhibited some yellow color. Earlier, Cheng, Wang and Weng (2015) formulated zein/chitosan (unheated chitosan) films and found similar L* values (65.41 and 55.08).

Water solubility of the films produced in the present work showed similar results. There seemed to be a very small difference in the solubility values (35.9% for Z/Q-h, and 34.9% for Z/Q). According to Pena-Serna and Lopes-Filho (2013), low water solubility is a highly desirable characteristic in order to maintain product integrity in packaging. The same authors found 12.5% water solubility for zein film with 30% of glycerol.
Table 1 – Results of Z/Q and Z/Q-h films characterization: thickness, color, water solubility, contact angle and mechanical properties (mechanical strength and elastic modulus).

<table>
<thead>
<tr>
<th>Film</th>
<th>Thickness (mm)</th>
<th>Color</th>
<th>Water solubility (%)</th>
<th>Contact angle</th>
<th>Mechanical strength (MPa)</th>
<th>Elastic modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z/Q</td>
<td>0.118</td>
<td>57.13</td>
<td>-0.33</td>
<td>7.93</td>
<td>34.9</td>
<td>70.9</td>
</tr>
<tr>
<td>Z/Q-h</td>
<td>0.131</td>
<td>49.89</td>
<td>-0.82</td>
<td>10.95</td>
<td>35.9</td>
<td>63.9</td>
</tr>
</tbody>
</table>

High values of contact angle were found in the present work: Z/Q 70.9 and Z/Q-h 63.9. A similar value (67) was found by Biswas et al. (2009) in zein film solubilized in 90:10 ethanol/water without glycerol. Water solubility and contact angle are related to film hydrophobicity. Thus, the greater the contact angle, the greater the hydrophobicity (therefore, lower water solubility) (Pena-Serna and Lopes-Filho, 2013). These results are expected, since corn–zein has a hydrophobic character due to its high content of non-polar amino acids (Tihminioglu et al., 2010).

Figure 1 – Contact angles formed by droplets of deionized water on zein-chitosan films surface: (A) Z/Q and (B) Z/Q-h.

Figures 2a and b show the SEM photomicrographs of the surfaces of Z/Q and Z/Q-h, respectively. They revealed that the surfaces are granular with some pore being present. In contrast, Zhang et al. (2015) studied the surface of chitosan-based films with various concentrations of vanillin and observed that the film of pure chitosan and with 0.5% – 10% concentration of vanillin are smooth and have a compact structure.

Figure 2 – SEM micrographs of the surface of the zein-chitosan films: (A) Z/Q and (B) Z/Q-h. The analysis conditions entailed 15.0 kV, magnification 1000x, and 5 µm range.
García et al. (2013) prepared edible films of zein-chitosan using heated chitosan solution (varying the zein and chitosan concentration from 25 to 75%) and made quantitative roughness measurements from AFM images. The films exhibited a coarser surface when the zein concentration in the film increased. The increasing roughness can be related to protein aggregation, as zein molecules are more likely to aggregate in more concentrated solutions. Guo et al. (2005) prepared a solution of zein dissolved in 70% ethanol, deposited it onto a newly cleaved mica surface, and dried it by flowing air; he then observed the surface morphology. Many zein globules of non-uniform size were seen after heating the solution in aqueous ethanol solution (70%) and depositing it on mica. They believed that these globules were aggregates of zein molecules. The non-uniform size may result from different degrees of aggregation.

Moreover, Guo et al. (2005) suggested that when the concentration of zein solution reaches a certain value, proteins agglomeration, hydrogen bonding, and hydrophobic interactions will increase, thereby maintaining the meshwork and leading to the formation of the zein film. Wang et al. (2003) and García et al. (2013) indicated that zein aggregation through hydrophobic interactions is promoted by changing the solution polarity, which can be achieved by changing the solution pH.

Mechanical strength (MS) reflects the maximal tensile force per original cross-sectional area that the film can sustain before breakage while elastic modulus (EM) reflects the ductile behavior of the material (Chen, 2011). In the present work better MS and EM results were found for Z/Q (3.9 MPa and 185.3 respectively) than Z/Q-h. Therefore, heating the chitosan solution prior to film formation decreased the mechanical properties of the film.

The mechanical properties of Z/Q and Z/Q-h films were superior to zein/chitosan films reported in the literature. Cheng, Wang and Weng (2015) made zein-chitosan films (unheated chitosan) with or without the addition of phenolic compounds and found tensile strength values varying from 0.73 to 2.59 MPa. García et al. (2013) prepared edible films of zein-chitosan, using heated chitosan solution (varying the zein and chitosan concentration from 25 to 75%) and obtained elastic modulus values of 65 – 78 MPa, lower than those found in this work.

4. CONCLUSION

Edible films of zein and chitosan blends were prepared and evaluated. Heating the chitosan solution during film formation did not improve the physical properties of the film. Instead, the zein-chitosan films prepared with unheated chitosan showed better results for water solubility, contact angle and mechanical properties.

5. REFERENCES


