

Production and characterization of starch composite films with silver loaded zeolite

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Abstract

Clinoptilolite zeolite was modified by ionic exchange with silver and employed in the production of cassava starch composite films by casting method. The obtained films were evaluated in terms of mechanical, antimicrobial and microstructure properties as well as water vapor permeability and color. The starch films containing silver loaded zeolite showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. The composite films with unloaded zeolite did not show antimicrobial activity. The composite films with unloaded zeolite and silver loaded zeolite presented an increase in the tensile strength, confirming the action of zeolite as reinforcement agent for the polymeric matrix. A decreased in water vapor permeability was observed for both treatments. The use of silver loaded zeolite should be a viable alternative as biodegradable active packaging due to its reinforcement and antimicrobial characteristics.

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Keywords

Antimicrobial activity

Mechanical properties

Active packaging

Microstructure

Water vapor permeability

Introduction

The depletion of fossil resources and the pollution problem caused by petrol based non-biodegradable plastic materials have led to a renewed interest in the research of biodegradable and/or compostable materials from natural biopolymers. In this context, the development of biodegradable films for packaging materials is an interesting perspective (Belibi *et al.*, 2013; Peelman *et al.*, 2013).

Films produced with biopolymers from renewable sources have the ability to carry active compounds (Muñoz *et al.*, 2012) thus, they can be used as active packaging for food. Active packaging may contain substances that interact with the packaged product (Bitencourt *et al.*, 2014; Suppakul *et al.*, 2003; Pereira de Abreu *et al.*, 2012) such as antioxidants and antimicrobials, increasing food safety and quality. These technologies may increase shelf-life and reduce the risk of contamination by pathogens due to the slow and continuous diffusion of antimicrobial agent from packaging material to food surface (Appendini and Hotchkiss, 2002; Moraes *et al.*, 2007; Sung *et al.*, 2013).

Silver is a metal with antimicrobial properties against bacteria, fungi, protozoa, and certain viruses.

At low concentrations it does not present toxicity and can reduce problems related to resistant bacteria (Rivera-Garza *et al.*, 2000). Its application in food packaging (Fortunati *et al.*, 2011; Llorens *et al.*, 2012) and edible films (An *et al.*, 2008) has been the subject of many studies. The ion exchange method is widely used to prepare Ag-zeolite, in which silver nitrate is used as an ion-exchange solution and zeolites as carrier materials (Zhou *et al.*, 2014). This is a more cost-effective alternative than the direct use of silver compounds such as silver nitrate solution and silver plate (Ferreira *et al.*, 2012). Furthermore Ag-zeolite can be incorporated into films formulation being active as antimicrobial agent at all film surface (Zampino *et al.*, 2011), as well as controlling silver diffusion rate from the polymeric material to food surface. This may increase potential applications since smaller amounts of silver are released to food (Muñoz *et al.*, 2012).

Polymers with enhanced mechanical performance can be obtained through the incorporation of inorganic materials into the polymeric matrix and zeolites can be used for this purpose (Ciobanu *et al.*, 2007). Unlike traditional fillers, the conditions of zeolite synthesis can be greatly changed providing a broad range of potential properties (e.g. antibacterial

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properties, volatile organic compounds adsorption and catalytic properties) and the ability to tailor the functions of composite materials (Kamışoğlu *et al.*, 2008).

The investigation of the antimicrobial activity of polymeric composites, containing Ag-zeolite, against *Staphylococcus epidermis*, *Staphylococcus aureus* and *Escherichia coli* with the polymers: polyvinyl chloride (Zampino *et al.*, 2011), polyurethane (Kamışoğlu *et al.*, 2008; Kaali *et al.*, 2010), polyethylene (Boschetto *et al.*, 2012) and poly(lactic acid) (Fernández *et al.*, 2010) has already been reported. Although the application of Ag-zeolite in starch based composite films was not investigated. Considering the potential use of zeolite as additive in film production, the objective of this study was to produce cassava starch films added of Ag-zeolite, and evaluate their mechanical, barrier, microstructural and antimicrobial properties.

Materials and Methods

Material

The films were produced using native cassava starch (Indemil, Paranaíba, Brazil), commercial glycerol (Dinâmica, Brazil), clinoptilolite zeolite (Si/Al ratio of 6.4, 325 mesh, Zeocel, Portugal) and silver nitrate with 99.8% of purity (Synth, Brazil). *Escherichia coli* (IAL339) and *Staphylococcus aureus* (IAL1875) cultures were employed in antimicrobial activity analysis.

Modification of zeolite with silver

Zeolite was modified with silver nitrate by ion exchange according to the method described by Boschetto *et al.* (2012). Three grams of clinoptilolite zeolite and 50 mL of silver nitrate aqueous solution (5% w/v) was kept under magnetic stirring at 40°C for 16 h. The solution was filtered, washed with distilled water and the zeolite was dried at 100°C for 24 h.

Film production

Starch films were produced by casting according to the method described by Mali *et al.* (2005) and Araújo *et al.* (2015) with some modifications. A film-forming solution with 3% (w/w) of solids (starch and glycerol) was prepared and the silver loaded zeolite (Ag-Z) or unloaded zeolite (Z) was added substituting the respective amount of solids at the filmogenic solution: 0, 0.1 and 0.5% (w/w). Glycerol was used as plasticizer and the concentration employed was 20 g of glycerol/100 of starch. All the components (starch, glycerol, water and Ag-Z or Z) were weighed, and

the final mixture was stirred for 10 min in Ultraturrax (IKA, T25 model, Brazil) at 10,000 rpm. Finally the solution was heated until 80°C (up to 20 min) for starch gelatinization, poured onto plates (25 cm × 37 cm) and dried in a forced air oven (40°C, 16 h, Cienlab, Brazil).

Thickness and density

The film thickness was measured with a digital micrometer (Starrett, Brazil) at ten different locations on the films. The density of the films was determined according to Müller *et al.* (2011). Film samples with dimension of 20 mm x 20 mm were kept in a desiccator with anhydrous calcium chloride (0% RH) for 14 days and were then weighed.

Color measurements

The color parameters (L , a , b) (Hunterlab system) of the films were determined using a colorimeter (Mini Scan Ez) with an illuminant D65 (daylight) and a visual angle of 10°. The final results were expressed in terms of color difference between the control films and zeolite and Ag-zeolite films (ΔE), according to Equation 1. In this equation ΔE is color difference, L is lightness, a is redness ($+a$) or greenness ($-a$), and b is yellowness ($+b$) or blueness ($-b$).

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \text{ (Equation 1)}$$

Mechanical properties

The tensile tests were performed with a texture analyzer (Stable Micro Systems, TA XTplus model, England) based on the American Society for Testing and Material standards (ASTM D882-02, 2002). Ten samples of each formulation with dimension of 50 mm x 20 mm were previously stored at 25°C and 53% RH for 48 h. The crosshead speed was 0.8 mm/s and the initial distance between the grips was 30 mm. The results were expressed as tensile strength (MPa), elongation at break (%), and Young's modulus (MPa).

Water vapor permeability

Water vapor permeability (WVP) was determined gravimetrically in duplicate, according to the ASTM E96-00 standard, with some modifications, under a relative humidity gradient of 33 – 75%. The films were previously conditioned in desiccator at 25°C and 53% RH for 48 h.

Scanning Electron Microscopy

The microstructure of the films was analyzed with a scanning electron microscope (Philips, FEI Quanta 200 model, Japan). The samples were immersed in liquid nitrogen, fractured and gold-

Table 1. Density, color difference and mechanical properties of the starch composite films added of zeolite and Ag-zeolite.

Sample	ρ (g/cm ³)	ΔE	T (MPa)	MY (MPa)	ϵ (%)
Control	1.43 ± 0.003 ^a	-	3.7 ± 0.6 ^b	87 ± 23 ^c	81.2 ± 46.7 ^a
Z 0.1	1.28 ± 0.004 ^a	3.36	10.1 ± 0.9 ^a	345 ± 14 ^b	14.1 ± 1.9 ^b
Z 0.5	1.09 ± 0.002 ^b	9.08	10.9 ± 0.5 ^a	505 ± 82 ^a	1.5 ± 0.5 ^c
Ag-Z 0.1	1.37 ± 0.006 ^a	16.74	11.3 ± 0.9 ^a	398 ± 29 ^b	12.2 ± 1.9 ^b
Ag-Z 0.5	1.02 ± 0.003 ^b	49.48	12.8 ± 0.8 ^a	621 ± 33 ^a	1.6 ± 0.3 ^c

ρ = Density; ΔE = color difference; T = Tensile strength; MY = Young's modulus; ϵ = Elongation at break.

^{a, b, c} Means with different small letters in the same column indicate significant difference by Tukey test ($p < 0.05$).

coated (sputter coater Bal-Tec, SCD-050 model, Balzers, Liechtenstein). The samples were analyzed with accelerating voltage of 20 kV.

Antimicrobial activity

The agar diffusion method was employed to determine the diameter of the inhibition zone in triplicate, according to Araújo *et al.* (2015). Cultures of *Staphylococcus aureus* and *Escherichia coli* were growing in Müller Hinton broth at 35°C (0.5 of the McFarland standard scale). Plates with Muller-Hinton agar, were inoculated with *Staphylococcus aureus* and *Escherichia coli* (10^8 UFC/mL) and films discs of 5 mm of diameter were placed on the surface of the plates. Then the plates were incubated at 37°C for 24 h. Finally the formation of inhibition zone was observed. The analysis was performed in triplicate.

Statistical analysis

The results were evaluated using the analysis of variance (ANOVA) and Tukey's test at the 5% significance level ($p < 0.05$) with Statistica 7.0 software (Statsoft, Tulsa, OK, USA).

Results and Discussion

Color, density and mechanical properties

All the films showed good processability, were easily removed from the plates after drying process and were easily handled. The thickness of the films ranged from 114 to 128 μm and did not show significant difference ($p < 0.05$), suggesting adequate control of the amount of film-forming solution added to the plates, reducing the possibility of mechanical and barrier performance problems.

Regarding to materials density (Table 1), both unloaded zeolite and silver loaded zeolite presented similar results (comparing Z0.1 with Ag-Z0.1 and

Z0.5 with Ag-Z0.5), showing that zeolite modification did not interfere significantly in this property. The use of 0.1% of Z and Ag-Z did not alter density of the films, however the addition of 0.5% of Z and Ag-Z decreased significantly its value. The structure polymer type, molecular weight and the interactions between the components present in the film should be considered for this property (Pelissari *et al.*, 2013). It is possible that this concentration of zeolite was excessive and the zeolite was not properly dispersed in the continuous starch matrix, forming less dense aggregates at the polymer structure. Pehlivan *et al.* (2005) observed the formation of small pores around zeolite dispersed in polypropylene matrix which reduced the materials density.

Z and Ag-Z starch films color parameters are also presented in Table 1 and an image of the films is presented in Figure 1. Visually, the control and Z0.1 films were transparent. With the increased in zeolite concentration the films became yellowish. This explains the increase in the color difference (ΔE) from 3.36 to 9.08 for Z0.5 sample. On the other hand, the films with silver loaded zeolite (Ag-Z0.1 and Ag-Z0.5) showed brown color, being darker as higher the concentration of Ag-Z. This effect was more intense in the films containing Ag-zeolite, due to silver ions oxidation. In this case ΔE values also confirmed the color difference (up to 49.48).

The mechanical properties results (Table 1) showed a significant increase in the tensile strength (T) with zeolite addition. Considering the zeolite concentration and modification, these parameters did not influence the T values. According to the literature (Chivrac *et al.*, 2008; Müller *et al.*, 2012; Belibi *et al.*, 2013) there is significant decrease in the mechanical strength when a poor interfacial adhesion between the reinforced phase and the matrix occurs. In this study possibly the starch chains stayed between

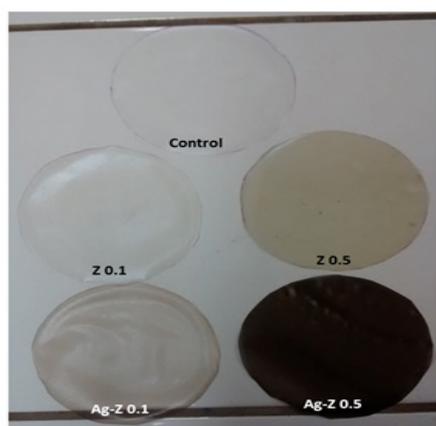


Figure 1. Starch composite films added of zeolite and Ag-zeolite.

zeolite layers increasing interfacial interaction that lead to reinforcing effect.

Increasing zeolite amount, a significant increase in the Young's modulus (MY) was noted, but elongation at break (ϵ) decreased. Similar results were observed by Plotegher and Ribeiro (2013) in starch films added of zeolite and colloidal silica, and by Belibi *et al.* (2013) in cassava starch films incorporated with beta zeolite.

The increase in stiffness of the films due to the zeolite addition, could be related to interaction between dispersed zeolite, that act as filler, and starch chain segments which reduce chain mobility and hence increase macroscopic rigidity (Chivrac *et al.*, 2008). It could also be considered that there was a synergistic effect between the glycerol and the zeolite, reducing their availability to plasticize the starch (Müller *et al.*, 2011).

An important observation was that the modification of the zeolite with silver by ion exchange technique did not affect the mechanical properties, confirming the fact that the modification with silver does not alter the structure or morphology of zeolite as reported in other studies (Boschetto *et al.*, 2012; Ferreira *et al.*, 2012).

Water vapor permeability (WVP)

The WVP of the films are shown in Figure 2. The addition of zeolite in starch films promoted a decrease of about 40% in the WVP. Furthermore, the increase in the zeolite concentration and its modification with silver caused no significant difference in WVP and the values ranged from 4.6×10^{-6} to 5.2×10^{-6} g/m.Pa.day. Plotegher and Ribeiro (2013) observed a reduction of about 20% in WVP of starch films added of zeolite. The authors also concluded that at higher concentration the positive effect of zeolite was diminished.

The lowering effect in WVP observed in this

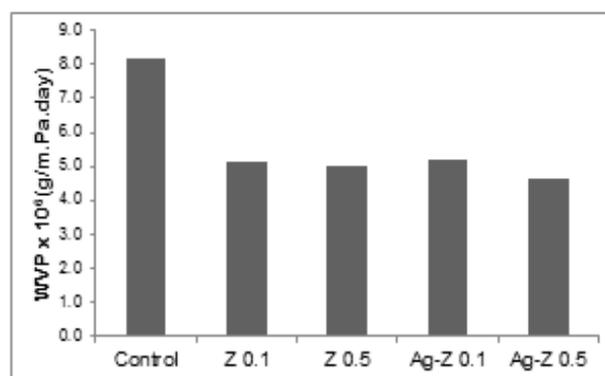


Figure 2. Water vapor permeability of the cassava starch composite films incorporated with zeolite and Ag-zeolite.

study is related to the zeolite particles distribution at polymeric matrix that can create tortuous paths that may have hindered water vapor flow through the composite. Some reports suggest that the higher the tortuosity of the system, the better the barrier property of the material (Weiss *et al.*, 2006; Faria *et al.*, 2012).

Microstructure

The films microstructure images of the surface and fracture are presented in Figure 3. The control sample showed smooth, compact and homogeneous surface and fracture. In the images of the Z0.1 and Ag-Z0.1 is possible to note the presence of zeolite particles in the fracture as well as a slightly rough surface. Differently from the other samples, at the fracture images of Z0.5 and Ag-Z0.5 films is possible to see clearly the zeolite particles with different sizes scattered in the starch matrix. As a consequence the surface was very rough compared with the control, with some zeolite agglomerates. According to Yassue-Cordeiro *et al.* (2015) when zeolite is added in film formulation, a phase separation between zeolite and starch can occur forming a composite film with two distinct phases, an organic phase of starch and an inorganic phase of zeolite.

Antimicrobial activity

The control sample and films containing only zeolite (Z0.1 and Z0.5) did not show inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*. However, Ag-Z0.1 and Ag-Z0.5 films showed antimicrobial activity and the values of inhibition zone diameter was about 1 cm for both bacteria, confirming that the antimicrobial activity was due to the presence of silver ions in the zeolite structure which were gradually released to the environment (agar plate). The antimicrobial activity of the Ag-zeolite against *Staphylococcus aureus* and *Escherichia coli* was also reported to poly(lactic acid) films (Fernández *et al.*, 2010) and polyethylene

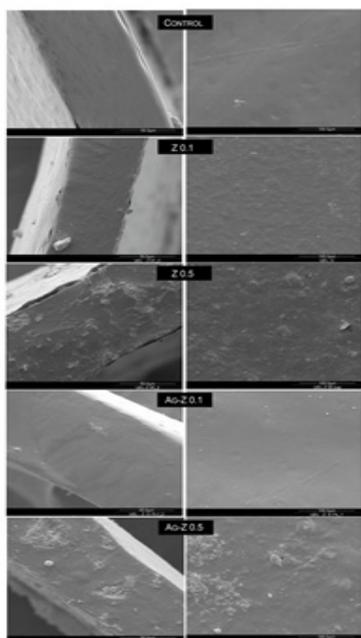


Figure 3. Fracture (left images, 1,600x) and surface (right images, 800x) microscopy images of starch composite films with zeolite and/or Ag-zeolite.

films (Boschetto *et al.*, 2012).

Conclusion

Ag-zeolite in the concentration of 0.1% improved the mechanical and barrier properties of the cassava starch films and also provided satisfactory results as an antimicrobial agent for *Staphylococcus aureus* and *Escherichia coli*. The use of Ag-zeolite as additive in the active packaging production is a promising way for food safety enhancement.

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