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Characterization of active chitosan films as a vehicle of potassium sorbate or nisin antimicrobial agents

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ABSTRACT

Antimicrobial and biodegradable active films were obtained by incorporating potassium sorbate (PS) and nisin (NIS) into chitosan matrix films. The diffusion mechanism of antimicrobial compounds through chitosan matrix was studied, applying Fick's Second Law and the Power Law. Films were produced by casting and the antimicrobial agents were added at different concentrations. They were also characterized by moisture content, water solubility, mechanical properties, contact angle, and microstructure. It was observed that the tensile strength decreased by 49.7% for PS films, and elongation increased by 206% for NIS films, when comparing 10.0 with 2.5 mg/g films. The presence of antimicrobial agents provided a significant increase in contact angle. The modeling fitted to the PS-chitosan films and the diffusivity coefficients did not show significant differences for different compound concentrations. The diffusion coefficients of PS ($1.909–2.292 \times 10^{-13}$ m^2/s) were approximately two times higher than the diffusion coefficient of NIS active films.

1. Introduction

Active packaging systems are based on the incorporation of active compounds aiming for improvements in their functionality, such as antimicrobial activity, antioxidant activity and others. These systems have been used successfully to increase the shelf life of processed foods, satisfying the consumer demands for minimal processing of food products, with quality and safety (Abreu, Cruz, & Losada, 2012; Shen, Wu, Chen, & Zhao, 2010). An antimicrobial packaging system can be obtained by incorporating natural or synthetic antimicrobial agents in a coating and food packaging films. These agents are released slowly into the food in order to prevent microbial growth (Mascheroni, Guillard, Nalin, Mora, & Piergiovanni, 2010). Several authors have already demonstrated their efficiency over the last decade (Bahram et al., 2014; Dashipour et al., 2015; Irkin & Esmer, 2015; Li et al., 2017; Luz et al., 2017; Nam, Scanlon, Han, & Izydorczyk, 2007; Nobile, Conte, Incoronato, & Panza, 2008; Ojagh, Rezaei, Razavi, & Hosseini, 2010).

Antimicrobial agents applied in food products could be incorporated into polymeric films, forming active packaging films. Potassium sorbate is a GRAS additive salt derived from sorbic acid, and is widely used in the food industry due to its antimicrobial activity, against fungi, yeasts and some bacterias (Diblan & Kaya, 2017; Mehyar, Al-Qadiri, & Swanson, 2012; Pranoto, Rakshit, & Salokhe, 2005; Sayanjali, Ghanbarzadeh, & Ghiassifar, 2011; Valencia-Chamorro, Palou, del Río, & Pérez-Gago, 2008). It has been used to inhibit the growth of fungi and yeasts, and verified against *Staphylococcus aureus*, *Clostridium botulinum*, Salmonella and pseudomonas (Zamora & Zaritzky, 1987). Films with potassium sorbate may inhibit pathogenic contamination and increase the shelf life of food products, as verified by several authors (Appendini & Hotchkiss, 2002; Barzegar, Azizi, Barzegar, & Hamidi-Esfahani, 2014; Juck, Neetoo, & Chen, 2010; Türe, Gällstedt, & Hedenqvist, 2012). Another antimicrobial agent used in food products is nisin. It is a hydrophobic and cationic polypeptide, a bacteriocin, produced from strains of *Lactococcus lactis*, which is naturally found in raw milk and fermented foods (Irkin & Esmer, 2015). Nisin exhibits antimicrobial activity toward a wide range of Gram-positive bacteria, but shows little or no activity against Gram-negative bacteria, yeasts and molds (Kallinteri, Kostoula, & Savvaidis, 2013). The advantage of bacteriocins is that they are thermostable, hypoallergenic and easily degraded by proteolytic enzymes in the human gastrointestinal tract (Abreu et al., 2012).

According to the composition and arrangement of their filmogenic

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matrix, active films are responsible for releasing active agents incorporated into the external medium. Knowledge of the diffusion of active compounds in the food system provides a better understanding of the preservatives' transport mechanisms. Furthermore, the preservative's diffusivity values are essential to predict the shelf life of food products after packaging (Mohan et al., 2017; Ozdemir & Floros, 2003).

The initial microorganism contamination in packed food products occurs at the surface. The diffusivity controls the gradual release of antimicrobial agent to the food surface, and it will remain there in a high concentration, extending the shelf life and decreasing the concentration of antimicrobial compounds in the whole food (Irkin & Esmer, 2015; Pranoto et al., 2005). The antimicrobial compounds reduce, inhibit or retard the growth of microorganisms, and their controlled liberation into food can be extended to the food transport and storage (Appendini & Hotchkiss, 2002; Quintavalla & Vicini, 2002). The mathematical modeling of its mass transfer is necessary to understand and optimize the active packaging film.

The development of biopolymer packaging systems is one of the most fashionable trends to improve the food market. Biopolymers obtained from agricultural commodities and/or food waste products have emerged as an option with regard to their film-forming capacity as an environmentally friendly technology (Espitia, Du, Avena-Bustillos, Soares, & McHugh, 2014). Chitosan is a natural polymer with a linear structure consisting of β-(1-4)-2-acetamide-2-deoxi-D-glucan units, being commonly described in terms of deacetylation degree. Chitosan forms excellent films, with favorable characteristics such as high flexibility and high resistance to manipulation (Silva, Lopes, Silva, & Yoshida, 2016; Srinivasa, Ramesh, Kumar, & Tharanathan, 2003). Some researches have shown the chitosan films properties, including as a gas barrier (Aguirre-Loredo, Rodríguez-Hernández, Morales-Sánchez, Gómez-Aldapa, & Velazquez, 2016; Butler, Vergano, Testin, Bunn, & Wiles, 1996; Kurek, Guinault, Voilley, Galić, & Debeaufort, 2014; Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011; Yoshida, Oliveira, & Franco, 2009), and mechanical properties (Aljawish et al., 2016; Elsabee & Abdou, 2013; Shahbazi, Rajabzadeh, Ettelaie, & Rafe, 2016; Yoshida et al., 2009).

The objective of this study was to develop and characterize chitosan active films, incorporating two different antimicrobial compounds, potassium sorbate (PS) and nisin (NIS), and to compare their diffusion mechanisms at different concentrations, according to their physicochemical properties.

2. Materials and methods

2.1. Materials

Chitosan (degree of deacetylation 89%, molar mass = 165.62 g/mol) donated by Polymar (Brazil); glacial acetic acid (LabSynth, Brazil); potassium sorbate (PS) (Plury Química Ltda, Lot 01070901, Brazil) and nisin (NIS) (Danisco, Nisaplin®, Brazil) were used.

2.2. Production of chitosan active films

The films were produced according to the methodology proposed by Yoshida et al. (2009), where chitosan (2 g/100 g film-forming solution) was dispersed in aqueous solution containing acetic acid (1% v/v). The solution was maintained under magnetic stirring (SOLAB agitator, model SL-91, Brazil) at 500 rpm for 2 h (25 ± 2 °C), for complete chitosan solubilization. Then, antimicrobial agents potassium sorbate (PS) or nisin (NIS) were added, after previous solubilization in water, at the concentrations of 2.5, 5.0, 7.5 and 10.0 mg/g chitosan. The final pH of the chitosan solution containing PS or NIS was approximately 4.2. The film-forming solution was dispersed on an acrylic plate (12 cm × 12 cm) and submitted to forced air drying (Marconi, MA-035, Brazil) at 30 °C for 24 h. Films without antimicrobial agent were used as control. Chitosan films were stored in desiccators containing saturated sodium bromide solution in order to control the relative humidity (58%, 25 ± 2 °C).

2.3. Moisture content

The moisture content was determined according to Gontard, Duchez, Cuq, and Guilbert (1994). Samples of approximately 0.01 g were dried (Fanem, Model 515, São Paulo, Brazil) at 105 °C for 24 h.

2.4. Water solubility

Water solubility of chitosan film was determined after 24 h of distilled water immersion, following the methoology proposed by Gontard et al. (1994). Disc-shaped film samples (approximately 2 cm in diameter) were immersed in distilled water (50 ml) and kept under mechanical stirring using a shaker (MA420 – Marconi, Brazil) for 24 h at 25 ± 2 °C. After this period, samples were dried for 24 h at 105 °C to determine the final dry mass. The solubility, expressed in terms of dissolved dry mass, was determined according to Equation (1) (Gontard et al., 1994):

$$S = \frac{|m_0 - m_f|}{m_0} 100 \quad (1)$$

where S is the water solubility (g/100 g film); m_0 is the dry mass of the sample (g); and m_f is the final mass of the sample after immersion for 24 h in water and drying (g).

2.5. Mechanical properties

The mechanical properties (tensile strength (TS), elongation at break (EB) and Young's modulus (E)) were determined by tensile tests using a texturometer (TA Instruments, TA.XT Plus, USA). The samples were stored in desiccators with NaBr, for 7 days at 25 ± 2 °C. The test was performed according to ASTM method D882-10 (ASTM International, 2012). The properties were determined through the curve generated after analysis. Three independent experiments were carried out, analyzing a total of 24 samples.

2.6. Contact angle

The contact angle of the films was determined using the Attension Theta Lite tensiometer (Biolin Scientific AB, Sweden) following Shahbazi et al. (2016). The measurement was recorded after 35 s of deposition with the aid of the software Attension Theta (version 4.1.9.8).

2.7. Scanning electron microscope (SEM)

Samples (25 mm × 100 mm) were cut and stored in a desiccator with silica for 10 days. Surface analysis was performed by attaching the sample to the equipment carrier (SEM TM-3000 – Hitachi). To evaluate the internal structure of the films, they were immersed in liquid nitrogen, followed by their fracture, and then fixed in support. The analysis was performed using 5 kV electron beams for surface analysis, and 15 kV for internal structure analyses.

2.8. Compounds diffusion

The diffusion of potassium sorbate (PS) and nisin (NIS) was analyzed following a method adapted from Yoshida, Bastos, and Franco (2010). Samples of active films (2 cm × 2 cm) were immersed in 100 ml of distilled water and kept under stirring (170 rpm, 25 °C ± 2) using a shaker table (Marconi, model MA420, Brazil). Aliquots were withdrawn at pre-established times. After the removal of each aliquot, an equal volume was added back to maintain a constant volume. The solute concentration (PS and NIS) released from the active film to the solution