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Properties of gelatin-based films with added ethanol–propolis extract

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ABSTRACT

Considering the possibility of using propolis as a natural bioactive compound, and the growing interest in active and biodegradable packaging materials, gelatin-based films plasticized with sorbitol and added of ethanol–propolis extract (EPE) were produced. Four different concentrations of EPE (0, 5, 40 or 200 g/100 g of gelatin) were analyzed. The effect of concentrations of EPE were evaluated on: mechanical properties, solubility, moisture content, water vapor permeability, scanning electron microscopy and infrared spectroscopy characteristics, stability of polyphenol concentrations, and antimicrobial activity against *Staphylococcus aureus*. EPE incorporation to the films promoted reduction in rupture tension and water vapor permeability, besides other microstructural changes, when compared with the control films (0 g of EPE/100 g of gelatin). Activity against *S. aureus* was observed in films with 40 and 200 g of EPE/100 g of gelatin. These films kept their antimicrobial activity and polyphenol concentration for 177 days of storage.

1. Introduction

Considering the current demand of consumers for more natural and potentially biodegradable packaging materials (Emiroğlu, Yemiş, Coşkun, & Candoğan, 2010), and the constant concern in preventing microbiological deterioration of foods (Chen, Wang & Weng, 2010), there is growing interest in films produced from natural macromolecules. Besides being biodegradable, these films may serve as support for antimicrobial additives, and may enable these active compounds to be released on the surface of the food (Chen et al., 2010), promoting improved food safety for consumers and increased shelf life of ready-to-eat products.

Selection of antimicrobial agents for application in films or edible packaging materials should be based on food-grade compounds and should preferentially be made of nature materials, mainly due to the adverse effects that synthetic substances cause to the environment and consumer health (Chen et al., 2010).

Several natural compounds have been proposed to be used in biodegradable films due to their antimicrobial activities, such as organic acids and nisin (Eswaranandam, Hettiarachchy, & Johnson, 2004; Sivarooban, Hettiarachchy, & Johnson, 2008), neem (Jagannath, Radhika, Nanjappa, Murali, & Bawa, 2006), propolis (Pastor, Sánchez-González, Cháfer, Chiralt, & González-Martínez, 2010), and essential oils (Altiok, Altiok, & Tihminlioglu, 2010; Gómez-Estaca, López de Lacey, López-Caballero, Gómez-Guillén, & Montero, 2010; Maizura, Fazilah, Norziah, & Karim, 2007; Ojagh, Rezaei, Razavi, & Hosseini, 2010; Pranoto, Salokhe, & Rakshit, 2005; Rojas-Graü et al., 2007; Seydim & Sarikus, 2006). Among these different substances that have been tested as antimicrobial agents in biodegradable films, essential oils have an important role, once they have the ability to inhibit growth of pathogenic and spoilage microorganisms commonly associated with food products. This activity is due to their chemical composition, more specifically to the presence of phenol compounds, such as flavonoids and phenolic acids (Altiok et al., 2010; Dadalioglu & Evrendilek, 2004; Maizura et al., 2007).

Therefore, considering the potential of substances rich in phenolic compounds in acting as efficient natural antimicrobial agents in polymer matrices, propolis is a widely available natural substance that could be a good candidate. Ancient people, such as Egyptians, Greeks, Romans and Incas already used the biological properties of propolis, which is widely used in popular medicine nowadays, either as a pure compound or combined with other products (Sforcin & Bankova, 2011). Growing interest in propolis has been shown in many scientific studies (Gardana, Scaglianti, Pietta, & Simonetti, 2007; Mello, Petrus, & Hubinger, 2010; Miguel, Nunes, Dandlen, Cavaco, & Antunes, 2010; Silva, Souza, Matta, Andrade, & Vidal, 2006). The name propolis is used to describe a complex mixture of resinous, gummous and balsamic substances collected by honey bees from plant sprouts, flowers and exudates, to which bees add saliva, wax, and pollen to elaborate the final product (Ghisalberti, 1979). Countless biological properties

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have been associated with propolis, such as antibacterial, antifungal, anti-inflammatory, antitumor, and antioxidant actions (Carvalho et al., 2011; Chaillou & Nazareno, 2009; Kalogeropoulos et al., 2009; Paulino et al., 2008). However, in spite of the antimicrobial properties, the use of propolis in foods is still limited, mainly because of its strong and characteristic flavor which may alter sensory characteristics of the foods. Thus, propolis application in biodegradable packaging may be an alternative because of its antimicrobial properties.

Therefore, based on the scientific studies that demonstrate the wide therapeutic potential of propolis, besides the reduced number of scientific studies that apply this substance as an additive in polymer films, the objectives of this study were to produce and characterize gelatin-based films with added concentrations of ethanol–propolis extract (EPE), and to analyze the antimicrobial activity of these films, as well as the effect of the length of storage on polyphenol concentration and antimicrobial activity of propolis on the biopolymer matrix.

2. Materials and methods

2.1. Materials

Gelatin type A (GELITA do Brasil Ltda., Brazil) and sorbitol (as plasticizing agent, Nuclear) and ethanol (Sinth) were used in the production of the films. Ethanol–propolis extract was produced with type 12 resin (Star Rigel, Raffard – SP). Reagents used in polyphenol analysis were Folin–Ciocalteu (Sigma–Aldrich) and anhydrous sodium carbonate (Sinth). Brain heart infusion (BHI) broth (BD), bacteriological peptone (BD), and nutrient agar (Merck) were used in the evaluation of antimicrobial activity. *Staphylococcus aureus* (ATCC 25923) used in the microbiological analyses was provided by Fundação André Tosello culture collection (Campinas).

2.2. Extraction and characterization of ethanol–propolis extract (EPE)

In the preparation of EPE (Nori et al., 2011), 30 g of propolis type 12 resin, ground beforehand, were mixed with 100 mL of ethyl alcohol (80 mL/100 mL of solution), and the mixture was stirred at 50 °C for 30 min. After extraction, the mixture was stored (10 °C) for 24 h. After that, it was filtered to produce EPE. Total polyphenol concentration in EPE was carried out by Folin–Ciocalteu spectrophotometric method (Woisky & Salatino, 1998) using gallic acid as the standard. Aliquots of 0.5 mL of EPE were diluted (1:1000) in ethyl alcohol (80 mL/100 mL of solution), and after that, 2.5 mL of the Folin–Ciocalteu reagent diluted in water (1:10) were added. The resulting solution was left standing for 5 min, and then 2.0 mL of Na_2CO_3 (4 g/100 mL of solution) were added to it. The final solution was left standing for 2 h away from light, and readings were carried out in a spectrophotometer (Biochrom Libra S22 – Cambridge, England) at 740 nm. Results were expressed in mg of gallic acid equivalent/g of EPE.

2.3. Film production

Films were produced with type A gelatin (2 g/100 g of filmogenic solution), which was hydrated (25 °C, 30 min) and then solubilized (55 °C, 15 min) in a thermostatic bath. After thorough solubilization, the plasticizing agent sorbitol (30 g/100 g of gelatin) was added, and the solution was magnetically stirred (1 min) and kept for 15 min at 55 °C, until the solubilization of sorbitol was complete. After this stage, the filmogenic solution was cooled to 40 °C and EPE was added at concentrations equal to 5, 40, or 200 g/100 g of gelatin. Ethyl alcohol (15 g/100 g of filmogenic solution) was added to complete the solubilization of the extract in the filmogenic solution. Solutions were spread in 12 × 12 cm^2 polyethylene plates (50 g solution/plate). The filmogenic solutions were dried in a forced air oven at 30 °C, for 24 h. Film thickness was determined as the arithmetic mean of ten random measures carried out with a digital micrometer (0.001 mm resolution, Mitutoyo). Film thickness was controlled by means of a mass/area ratio and kept constant at 0.086 ± 0.008 mm.

2.4. Characterization of bioactive films

Before characterization, films were placed in desiccators containing saturated NaBr solution (relative humidity = 58%) at fixed temperature (25 °C) for 5 days. Characterization of the films was carried out in an acclimatized room (25 °C, relative humidity between 55 and 65%).

2.4.1. Microstructure of the films

Superficial and internal microstructure of the films was analyzed (Carvalho & Grosso, 2004) using scanning electron microscopy (Leica LEO 440i microscope at 15 kV). For the analysis of internal microstructure, films were fractured in liquid nitrogen.

2.4.2. Fourier transform infrared spectroscopy (FTIR)

Analyses (Vicentini, Dupuy, Leitzelman, Cereda, & Sobral, 2005) were carried out using a Perkin Elmer spectrophotometer (Spectrum One). Films were directly placed on the reading area. Sixteen scans were carried out in the spectrum range of 400 a 4000 cm^{-1}, at a 2 cm^{-1} resolution. Scanning spectra were collected and analyzed using the equipment software.

2.4.3. Mechanical properties

Mechanical properties of the films were evaluated by tensile tests (Paschoalick, Garcia, Sobral, & Habitante, 2003), using a texture analyzer TA.XT2 (TA Instruments). Samples of the films (15 × 100 mm) were placed in specific tensile grips. The initial distance of separation and velocity were fixed at 80 mm and 0.9 mm/s, respectively. Tensile strength (T) and elongation (E) were directly obtained in the tension vs. elongation curves, and elastic modulus (EM) was determined by calculating the angular coefficient in the linear part of the curve.

2.4.4. Water solubility

The analysis of water solubility (Gontard, Duchez, Cuq, & Guilbert, 1994) was carried out in film samples cut as discs (diameter = 2 cm). Samples were immersed in distilled water (50 mL) and kept under mechanical stirring (Marconi-MA141 stirring table) for 24 h at 25 °C. After this period, samples were dried (105 °C, 24 h) and weighed. Final dry mass of the samples was then determined. Initial mass was determined by the moisture of the samples. Solubility was expressed in terms of dissolved dry mass.

2.4.5. Water vapor permeability

Water vapor permeability (WVP) was determined according to the ASTM E96-95 method (ASTM E96-95, 1995), with a relative humidity gradient equal to 100%. Films were fixed by means of a perforated ring in aluminum cells containing silica gel (exposed area of 12.29 cm^2). These cells were placed in desiccators containing distilled water and kept at 25 °C (±0.2 °C) in an incubator (BOD TE 390, Tecnal) provided with electronic temperature control. Gain of mass in the system (cell + film) was determined in regular intervals for a period of 101 h using a semi-analytical scale (Marte, AS2000), and water vapor permeability was calculated with the equation presented below: