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Physical, antioxidant and antimicrobial properties of chitosan films containing *Eucalyptus globulus* essential oil

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ABSTRACT

In this work active films from chitosan containing *Eucalyptus globulus* (EG) essential oil (0, 1, 2, 3 and 4% (v/v)) were developed by casting and solvent-evaporation method. Then, the physical properties, the total phenolic content, the antioxidant and antimicrobial activities of different films were assessed, to define if the chitosan films incorporated with EG essential oil could be used as natural active films for food use. The antioxidant activity was determined with three different analytical assays (DPPH, NO and H_2O_2). The agar disc diffusion method was used to determine the antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida parapsilosis*. In fact, incorporating EG essential oil into chitosan-based films significantly decreases moisture content and water-solubility. These results can be explained by the films microstructure, which was analyzed by scanning electron microscopy. The antioxidant properties proved to be significantly enhanced with increasing EG essential oil concentration. The microbiological assessment confirmed antimicrobial efficiency of chitosan films EG containing essential oil. The mentioned results proved the potential of this original material which could be used as active films due to its excellent antimicrobial and antioxidant activities.

1. Introduction

Biodegradable films made of polysaccharides, proteins and lipids have currently a variety of advantages over synthetic materials, such as biodegradability, edibility, biocompatibility and environmentally friendly properties (Petersen et al., 1999). These films are loaded with many functional ingredients, such as antioxidants, antimicrobial agents, flavors, spices and colorants which improve the functionality of the packaging materials via adding novel or extra functions (Salmieri & Lacroix, 2006).

Chitosan is a linear polysaccharide of randomly distributed β-(1-4)- linked D-glucosamine and N-acetyl-D-glucosamine, it has been recommended due to its biocompatibility, biodegradability and non-toxicity properties (Guldas, Akpinar-Bayizit, Ozcan and Yilmaz-Ersan, 2010). Chitosan with a high degree of deacetylation (>75%) and an important molecular weight have shown the strongest antibacterial effects in aqueous solutions regardless of the type of acid used for solubilization (No, Park, Lee, & Meyers, 2002). However, the acid used for film preparation significantly affects the film properties. Acetic and formic acid–based films have shown the highest tensile strengths followed by the films prepared with lactic, propionic, and citric acids (Begin & Van Calsteren, 1999). Park, Marsh, and Rhim (2002) confirmed that increasing chitosan molecular weight improved the films strength, but did not significantly affect their water and vapor permeability. Furthermore various additives have been examined for their potential in order to modify chitosan films properties, for example the use of glycerol as plasticizer aiming at decreasing their hydrophilicity (Vargas, Albors, Chiralt, & González-Martínez, 2009). In addition, chitosan offers immense advantages as an edible packaging material owing to its good film-forming properties (Kim et al., 2011; Ruiz-Navajas, Viuda-Martos, Sendra, Perez-Alvarez, & Fernández-López, 2013). However, antimicrobial properties may become inconsiderable when chitosan is in a form of insoluble films (Ouattara, Simard, Piette, Begin, & Holley, 2000). There are several natural ingredients, added to edible films, that present antioxidant or

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antimicrobial properties such as nisin or lysozyme (Pranoto, Rakshit, & Salokhe, 2005), essential oils (Ruiz-Navajas et al., 2013) or fruit extracts (Genskowsky et al., 2015). Essential oils from plant extracts are natural antimicrobial agents; incorporation of essential oil in edible films may not only enhance the films antimicrobial properties but also reduce water-solubility, vapor-permeability and slow lipid oxidation of the product (Ojagh, Rezaei, Razavi, & Hosseini, 2010).

Eucalyptus species are aromatic and medicinal plants belonging to the Myrtle family. The antimicrobial and antioxidant properties of *Eucalyptus* species essential oil are widely used in pharmaceutical and cosmetic products, flavoring and preservation of several foods. A number of studies have demonstrated the antimicrobial properties of Eucalyptus essential oils against a wide range of microorganisms (Ait-Ouazzou et al., 2011; Santos et al., 201). These studies, however, are focused on a few Eucalyptus species, especially *Eucalyptus globulus* (EG) oil, which has been shown to have a wide spectrum of antimicrobial activity (Pereiraa, Dias, Vasconcelos, Rosaa & Saavedra, 2014; Vázquez et al., 2008).

The aim of this study was to evaluate chitosan films incorporated with EG essential oil (1%, 2%, 3% and 4% (v/v)), in order to examine their physical properties, their antioxidant activity and the growth inhibition of some bacterial strains to assess the ability of chitosan films containing EG essential oil to enhance food safety.

2. Materials and methods

2.1. Chemicals

Chitosan from shrimp shells (≥75%, deacetylated) was purchased from Sigma–Aldrich Chemical Co. All chemicals were of analytical grade and also purchased from Sigma–Aldrich Chemical Co. (St. LouisMo).

2.2. Essential oil extraction from E. globulus

Fresh plant leaves were collected; the leaves were subjected to steam distillation using a Clevenger-type apparatus. Briefly, the plant leaves were completely immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapor and finally collected after decantation. The distillate was isolated and dried in a Rota-vapor. The oil was stored at 4 °C (Ait-Ouazzou et al., 2011).

2.3. GC–MS analysis

Analysis of the essential oil was performed using a Hewlett Packard 5890 II GC, equipped with a HP 5972 mass selective detector and a HP-5 MS capillary column (30 m × 0.25 mm id, film thickness 0.25 μm). For GC/MS detection, an electron ionization system, with ionization energy of 70 eV, a scan time of 1.5 s and mass range 40–300 amu, were used. Helium was the carrier gas at a flow rate of 1.2 mL/min. Injector and transfer line temperatures were set at 250 and 280 °C, respectively. Oven program temperature was the same with GC analysis. Diluted samples (1/100 in hexane, v/v) of 1.0 μL were injected manually and in the splitless mode. The identification of the compounds was based on mass spectra (compared with Wiley 275.L, 6th edition mass spectral library) or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature as described by Adams (2001). Further confirmation was done from Kovats Retention Index data generated from a series of n-alkanes retention indices (relative to C9–C28 on the BP-1).

2.4. Preparation of chitosan films

Chitosan-based films were produced according to the casting method as reported by Ojagh et al. (2010) method with some modifications. Film-forming solutions of chitosan were prepared by dissolving 2% (w/v) of chitosan in an aqueous solution H_2O (1% (v/v) of glacial acetic acid) while stirring on a magnetic stirrer-hot plate at 50 °C. The resultant chitosan solution was filtered through a Whatman filters paper and followed by vacuum filtration to eliminate any undissolved particles. After filtration the solution was returned to the magnetic stirrer/hot plate and glycerol was added with a concentration of 0.5 mL/g chitosan as a plasticizer. The plasticizer was mixed into the solution for 30 min. 0.2% (v/v) of Tween 80 was added to the essential oil as an emulsifier to facilitate its dispersion in film forming solution. After 15 min of stirring, EG essential oil was added to chitosan solution to reach final concentrations of 0%–4% (v/v). The film forming solutions were degassed for 5 min, and 45 mL were casted on the center of circular glass plates (Ø15 cm). After drying the films at room temperature during four days, they were peeled from the plates. Dried films were conditioned at 4 °C prior to testing.

2.5. Surface color measurements

Film color was determined by a colorimeter (Spectraflash 600 plus, Data-color International, USA). The CIE color values recorded were: L^* = lightness (0 = black, 100 = white); a^*($-a^*$ = greenness, $+a^*$ = redness); and b^*($-b^*$ = blueness, $+b^*$ = yellowness). Color differences (ΔE) were calculated by the following equation (Eq. (1)):

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

2.6. Moisture content

Moisture content of films was determined by measuring weight loss of films, upon drying in an oven at 110 °C until a constant weight was reached (dry sample weight).

2.7. Film solubility in water

Pieces of film of 1 × 3 cm^2 were cut from each film and weighed to the nearest 0.0001 g. The solubility in water of the different chitosan films was measured from immersion assays under constant agitation in 50 mL of distilled water for 24 h at 25 °C. The remaining pieces of film after immersion were dried at 110 °C to constant weight (Final dry weight). The initial dry weight was determined by thermal processing at 110 °C to constant weight. Solubility in water (%) was calculated by using the following Eq. (2).

$$\text{Water} - \text{solubility}(\%) = \left(\frac{\textbf{Initial dry weight} - \textbf{Final dry weight}}{\textbf{Initial dry weight}}\right) \times 100 \quad (2)$$

2.8. Scanning electron microscopy

The surface morphology of different films were assessed by observation with a Scanning Electron Microscopy (SEM) using a Jeol JCM 5000 microscope operating at 10 kV acceleration under moderate vacuum, thus avoiding the need for metallization of the samples. A Sodemat microscope equipped with VisioCal software application was used to visualize the oil droplets in the films.