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Characterization of antimicrobial polylactic acid based films

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ABSTRACT

Olive leaf extract (OLE) (*Olea europaea* L.), which has antimicrobial effect on many food pathogens, was incorporated as antimicrobial agent into polylactic acid (PLA) films. Antimicrobial activities of films were tested against *Staphylococcus aureus*. Increasing amount of the OLE in the film discs from 0.9 mg to 5.4 mg caused a significant increase in inhibitory zones from 9.10 mm to 16.20 mm, respectively. Moreover, incorporation of OLE and/or increasing the amount in the film formulation significantly enhanced the water vapor permeability (WVP). The water solubility and the degradation rates of films increased up to 19.3% and 22.4%, respectively. Thus, OLE incorporated PLA films have a prospectively potential in antimicrobial food packaging to reduce post-process growth of *S. aureus* with improved properties.

1. Introduction

Antimicrobial packaging can be considered one of the most innovative ways to inhibit microbial growth on foods while maintaining quality, freshness, and safety. Although there has been a rising interest in this research area, the availability of antimicrobials and new polymer materials, regulatory concerns, and appropriate testing methods limit the developments (Appendini and Hotchkiss, 2002). Antimicrobial packaging is highly regulated around the world and researchers must take these regulations into consideration. The component showing the antimicrobial effect, for example, must be considered as a food additive and must meet the food additive standards. Several studies have focused on the use of antimicrobial plant extracts, such as olive leaf extract (OLE), since these are generally classified as GRAS (Generally Recognized As Safe) in the food industry (Ayana and Turhan, 2009).

The knowledge of the medicinal properties of the olive tree (*Olea europaea*) date back to the early 1800's and its efficiency as a medicine is directly related with its polyphenols. These polyphenols can inhibit the growth of *Staphylococcus aureus* (Pereira et al., 2007) which is an important pathogen due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance (Le Loir et al., 2003). Among the food poisoning events, staphylococcal poisoning is the most common one. In all cases of staphylococcal food poisoning, one of the foodstuffs or ingredients is contaminated with an enterotoxin producing *S. aureus* strain. Many foods can serve a good growth medium for *S. aureus*, and have been implicated in staphylococcal food poisoning (Le Loir et al., 2003). There are two ways to prevent staphylococcal poisoning occurring after applying a sufficient thermal process. The first way is avoiding contamination of the food with *S. aureus* and the second way is inhibition of the microbial growth before the living organism number reach the critical level if the contamination occurs. Therefore, antimicrobial films containing olive leaf extract may offer an interesting and effective way to control the growth of food pathogens like *S. aureus* on foods.

An antimicrobial substance must be released from the matrix structure of the package to food surface to show an antimicrobial effect. Thus, the interaction between the antimicrobial substance and the polymer used for obtaining an antimicrobial package is crucial. To the best of our knowledge, there is only one study in the literature revealing the effects of OLE incorporation into methylcellulose film on the antimicrobial efficiency and film properties (Ayana and Turhan, 2009).

Biobased/biodegradable polylactic acid (PLA) as a packaging material is an increasing research area, not only because of the need to replace many petroleum-based polymers, but also because of its useful physical and mechanical characteristics (Mascheroni et al., 2010). There are limited studies investigating the antimicrobial efficiency of nisin (Jin et al., 2009), propolis (Mascheroni et al., 2010), lemon extract, thymol, lysozyme (Del Nobile et al., 2009) incorporated into PLA films; therefore, the present study explore the possibility of using OLE as an antimicrobial agent for the PLA films.

PLA films can be produced by various techniques: injection molding, thermoforming and film casting (Lim et al., 2008). Though

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solvent-casting method has been commonly used for the preparation of biopolymer films, it is normally not feasible for commercial film production. It is difficult to scale up and it incorporates several steps in order to produce an acceptable biopolymer film, which results in a relatively long processing time. On the other hand, for heat-sensitive antimicrobials, solvent compounding may be a more suitable method for their incorporation into polymers (Appendini and Hotchkiss, 2002).

The natural antimicrobial agent incorporation into a polymeric material for the development of antimicrobial food packaging materials is a new subject. Most of the natural antimicrobial agents show low resistance to excessive heat treatments applied during processing. Thus, the extrusion may not be suitable for the production of natural antimicrobial agent incorporated packaging materials. On the other hand, we believe that the increasing number of the studies revealing the potential of the plant extracts as antimicrobial agents will stimulate the development of new antimicrobial packaging material production process.

The objective of this study was to develop a new OLE incorporated antimicrobial and degradable PLA based packaging material and to investigate the effects of OLE on water vapor permeability, mechanical and thermal properties, water absorption, water solubility and degradability rates of the films.

2. Materials and methods

2.1. Materials

Polylactic acid (PLA; NatureWorks®, 4042-D) was obtained from Cargill Dow (Minneapolis, USA). The 4042-D was nominally made with 92% L-lactide and 8% D-lactide units. The molecular weight was 74,000 g/mol and the density was 1.25 g/cm^3. In this study, ethanol (EtOH, Merck, Darmstadt, Germany) and chloroform (Chl, Ridel-de Haen, Honeywell, USA) were used as solvent; glycerol (Gly, Carlo Erba, Milan, Italy) was used as plasticizer. Methanol (MeOH, Merck, Darmstadt, Germany) and Chl were used for extraction. Tyriptic Soy Broth (TSB), Tyriptic Soy Agar (TSA) and Mueller Hinton Agar (MHA) were used for microbiological analysis and purchased from Merck. Olive leaves (*Olea europaea* L.) were picked up in May in Bozön region, Mersin, Turkey and their extracts were used as antimicrobial agents.

2.1.1. Preparation of olive leaf (Olea europaea L.) extracts

Olive leaf extract (OLE) was used as an antimicrobial agent in PLA film formulations. The washed olive leaves were dried in a microwave oven at 800 W for 2 min. Water extraction was performed according to a method described by Ayana and Turhan (2009). For the Chl–MeOH (50/50, v/v) extraction, the ground dried leaves (20 g) were extracted with the 100 mL of Chl–MeOH mixture at 400 rpm at ambient temperature for 24 h. The extract was filtered and dried using a rotary evaporator at 65 °C to obtain OLE powder.

2.1.2. Identification of phenolics compounds present in OLE

The percentages of main phenolics and the quantity of oleuropein in water and Chl–MeOH extracted OLEs were determined by a method of Altıok et al. (2008).

2.1.3. Culture and inoculum preparation

Staphylococcus aureus (ATCC 25923) cultures were obtained from the Department of Microbiology, University of Mersin, Turkey. The bacterial cultures were grown on TSA slants and kept at 4 °C. Subculturing was carried out every fifteen days to maintain bacterial viability.

The culture density was adjusted according to Mc Farland 0.5 Standard (Barry and Thornsberry, 1991). Well isolated colonies obtained from TSA slants were inoculated into TSB medium. The broth cultures were incubated at 35 °C for 5 h. Optical densities of the inoculums were adjusted between 0.08 to 0.1 at 625 nm and the inoculums contained between 5×10^7 and 33×10^7 CFU (Colony Forming Unit) per mL.

2.1.4. Film preparation

Both PLA and antimicrobial PLA films were prepared by solvent casting method. The different amounts PLA (8–12 g PLA/100 mL solvent) were dissolved in a solvent of Chl–EtOH mixture (Chl/EtOH = 10/0–6/4, v/v) at ambient temperature for 17 h. For formation of PLA films, OLE (0.0–3.0 g OLE/100 mL total solvent) was added in 5 mL of the heated EtOH at 70 ± 5 °C and stirred on a magnetic plate for 5 min. Subsequently, 45 mL Chl was added and stirred until all the OLE was dissolved. PLA (8 g PLA/100 mL total solvent) was dissolved in the OLE solutions at ambient temperature for 17 h. Gly was used as the plasticizer (Gly/PLA = 0.5/10, w/w) in both PLA and antimicrobial PLA films. The film solutions were cast on glass plates (20 × 20 cm) with hand-operated plate coater (CAMAG, Muttenz, Switzerland) and then dried at 75 °C for 30 min. The amount of 3.0 g OLE/100 mL added to the films for antimicrobial activity was chosen as maximum; because exceeding amounts could not be removed from the surface.

2.1.5. Water vapor permeability (WVP) and mechanical properties

WVPs of films were determined gravimetrically according to ASTM, (1983) at 25 ± 1 °C and 52 ± 2% ΔRH conditions. WVP tests were repeated at least three times for each film.

Tensile strength (TS) and elongation (E) of PLA films were determined according to ASTM, (1993). Films were cut into strips of 40 × 6 mm and conditioned at 25 ± 1 °C and 53 ± 2% RH for 48 h before testing. The test was run using the texture analyzer (Model TA-XT2; Stable Micro Systems, Surrey, UK). The cross-head speed of the texture analyzer was 0.80 mm/s. TS and E were determined from stress–strain curves were plotted using software provided with the texture analyzer. Tests were repeated at least nine times for each film.

2.1.6. Antimicrobial activity of films

The antimicrobial activity test of films was carried out using the agar diffusion method (NCCLS, 2003). Film solutions (10 mL) containing 0.5–3.0 g OLE/100 mL were cast onto Petri dishes. Films were dried at 35 °C until constant weight was reached. Film samples were cut into a disc shape with a diameter of 9 mm, using a hole puncher for use in antimicrobial activity tests. The weights of the film discs were measured with an analytical balance (Sartorius BP221S, Goettingen, Germany). The OLE concentrations of the film discs were calculated using the disc weights. The film discs were placed on MHA plates, which had been previously inoculated with 0.1 mL of a pure culture of *S. aureus* approximately containing 3×10^7–33×10^7 CFU/mL. The plates were then incubated at 35 °C for 24 h. Inhibition zones, surrounding the film discs and contact areas of films with agar surfaces, were measured with a caliper. PLA films without OLE served as a control group. The tests were performed in duplicate.

2.1.7. Differential scanning calorimetry analysis

Thermal properties of PLA films were determined using differential scanning calorimetry (DSC, Perkin Elmer, PYRIS6 DSC, USA). Glass-transition temperature (T_g), cold crystallization temperature (T_{cc}), melting point temperature (T_m); enthalpy of crystallization (ΔH_c), and enthalpy of melting (ΔH_m) were measured from 4 °C to 300 °C under nitrogen atmosphere with a flow rate of 20 mL/min. The weight of samples was between 4 and 6 mg. Sam-