LWT - Food Science and Technology 72 (2016) 251–260

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Development of flexible bactericidal films based on poly(lactic acid) and essential oil and its effectiveness to reduce microbial growth of refrigerated rainbow trout

Zahra Javidi, Seyed Fakhreddin Hosseini*, Masoud Rezaei

Department of Seafood Processing, Faculty of Marine Sciences, Tarbiat Modares University, P.O. Box 46414-356, Noor, Iran

ARTICLE INFO

Article history:
Received 28 November 2015
Received in revised form
12 March 2016
Accepted 26 April 2016
Available online 2 May 2016

Keywords:
Antimicrobial film
Flexibility
Poly(lactic acid) PLA
Origanum vulgare L. essential oil
Rainbow trout

ABSTRACT

The main shortcoming of poly(lactic acid) (PLA) for food packaging applications is its brittleness. The aim of this work was to evaluate the potential use of *Origanum vulgare* L. essential oil (OEO) in PLA-based matrices to obtain developed bio-based films with enhanced mechanical properties as well as antimicrobial performance. The PLA-essential oil composite films were more flexible than neat PLA films; so that up to 6-fold increase in the elongation-at-break values (2.82–16.78%) were acquired when 0.5% (w/w) oil was added to the polymer matrix. The addition of essential oil decreased the PLA glass transition temperature (T_g), as the result of the polymer plasticization, and led to modification of the tensile behavior. AFM results confirmed that the PLA surface microstructure was drastically influenced in terms of roughness parameters (R_a and R_q) when OEO was added. The films' antimicrobial activities were induced by incorporating essential oil, which were more effective against the bacteria in direct contact method than a vapor phase. However, in both of test methods, *Staphylococcus aureus* was the most sensitive bacterium to OEO-containing films, while *Escherichia coli* was the most resistant. Then, efficacy of bioactive film included with 1.5% (w/w) essential oil to reduce microbial growth of rainbow trout during chilled storage was evaluated; the results indicated that antimicrobial PLA film was effective against all the microorganisms tested in this study. These results suggest that the developed PLA films with active substance could be used in designing antimicrobial packaging materials.

1. Introduction

Petroleum-based plastics used for food packaging had some disadvantages such as declining oil and gas resources, rising price of crude oil, environmental concerns for their degradation or incineration and global warming (Siracusa, Rocculi, Romani, & Rosa, 2008). In addition, consumer toxicity risks about monomers or oligomers migrating to edible materials present safety problems (Jamshidian, Tehrany, Imran, Jacquot, & Desobry, 2010). These ecological, economic, health and safety challenges have motivated researchers and producers to replace petroleum-based polymers with biodegradable polymers.

One of the most widely used biopolymers is polylactic acid (PLA) which is miscible, biodegradable and thermoplastic, made from renewable sources (Armentano et al., 2015). PLA is the fastest growing market in the plastic industry, credited with a 10–30% increase depending on the sources (http://www.carbios.fr/en/press-releases/show/52). The increasing interest in developing PLA-based films for food packaging purposes is not only because of the need for substitution of many petroleum-based polymers (Erdohan, Çam, & Turhan, 2013; Mascheroni, Guillard, Nalin, Mora, & Piergiovanni, 2010), but also because of its beneficial physical and mechanical properties (Mascheroni et al., 2010). Nevertheless, practical applications of PLA are often limited by its drawbacks such as low plasticity, low heat resistance, high modulus and fragile behavior (Armentano et al., 2015). Therefore, expansion of PLA functions in the packaging industry requires overcoming these problems (Mascheroni et al., 2010).

Various lipid materials such as oils, fats, fatty acids, and waxes have been mixed with other biopolymer-based film-forming solutions to obtain the desirable properties of component materials to improve gloss, permeability characteristics, strength, flexibility, and general performance of bio-based films (Zhang, Rempel, &

* Corresponding author.
E-mail addresses: hosseinisf@modares.ac.ir, hosseinisf@gmail.com (S.F. Hosseini).

http://dx.doi.org/10.1016/j.lwt.2016.04.052
0023-6438/

Mclaren, 2014). In this regard, the inclusion of plant essential oils in biodegradable film-forming solutions offers an interesting alternative. Essential oils (EOs) similar to other fats, may improve the tensile properties such as flexibility of biopolymer films due to its plasticizing effect. Meanwhile, active compounds like EOs can be added to the films in order to improve their functional properties, such as water vapor permeability, as well as to introduce antimicrobial properties (Teixeira et al., 2014).

The essential oil of *Origanum vulgare* L. (OEO) compared with other essential oils, is considered for its antioxidant and antimicrobial activity (Burt, 2004). These actions are primarily related to two phenols: carvacrol and thymol (main components of this essential oil) and the monoterpene hydrocarbons *p*-cymene and γ-terpinene which exist at low concentrations (Burt, 2004; Hosseini, Rezaei, Zandi, & Farahmandghavi, 2016).

Due to the presence of many antimicrobial compounds in EOs, biodegradable films activated with these bactericidal agents are highly useful to minimize the growth of post-processing contaminant microorganisms, extending shelf life of food and improving food safety (Sánchez Aldana, Andrade-Ochoa, Aguilar, Contreras-Esquivel, & Nevárez-Moorillón, 2015).

Fresh fish are highly perishable products and their deterioration is mainly from the biological reactions such as oxidation of lipids, protein degradation or decomposition mediated by endogenous enzymes, and the metabolic activities of microorganisms (Rezaei & Hosseini, 2008). These activities lead to a short shelf life in fish and other seafood products. Rainbow trout (*Oncorhynchus mykiss*) are highly susceptible to deterioration and microbial spoilage during storage. Given that the shelf life of refrigerated rainbow trout fillet is relatively short and that there is a growing tendency of consumers for fresh fish, the development of new preservation methods, which permit shelf-life extension of fresh fish, is required.

In previous studies, PLA were used as polymer matrices to produce active food packaging films with the combination of natural extracts or essential oils which could come from plants (Erdohan et al., 2013; Llana-Ruíz-Cabello et al., 2015; Mascheroni et al., 2010; Tawakkal, Cran, & Bigger, 2016). The present study makes a contribution to develop active bio-packaging films using hydrophobic compounds such as essential oil, which could improve the mechanical and physical properties as well as induced antimicrobial function to the PLA films. Moreover, the efficacy of PLA-essential oil film on the microbiological characteristics of rainbow trout during refrigerated storage was evaluated.

2. Materials and methods

2.1. Materials

Polylactic acid (PLA), 2002 D, was purchased in pellet from Natureworks® Co., (USA). Oregano essential oil (100% pure) and chloroform were obtained from NewDirections Aromatics Inc. (Hampshire, UK) and Merck Chemicals Co. (Darmstadt, Germany), respectively. For antimicrobial tests, Tryptic Soy Agar (TSA) and Brain Heart Infusion (BHI) Broth, Violet Red Bile Dextrose (VRBD) Agar and de Man Rogosa Sharpe (MRS) Agar were purchased from Quelab Laboratories Inc. (Montreal, Quebec, Canada). Bacterial stock such as *Staphylococcus aureus* (PTCC 25923), *Listeria monocytogenes* (PTCC 19118), *Escherichia coli* (PTCC 1330) and *Salmonella enteritidis* (PTCC 138) were provided from Persian Type Culture Collection (Tehran, Iran). Sub culturing was carried out on monthly basis to maintain bacterial livability. All strains were preserved in BHI added with 30% glycerol at −20 °C when used.

2.2. GC-MS analysis of oregano essential oil

Essential oil was analyzed in an Agilent 6890 gas chromatograph interfaced to an Agilent 5973 N mass selective detector (Agilent Technologies, Palo Alto, USA). Oregano oil was separated by a capillary column HP-5MS (30 m × 0.25 mm id, 0.25 mm film thickness; Restek, Bellefonte, PA). Essential oil components were identified based on the comparison of their mass spectra with the spectra from Wiley7n.L libraries. The relative percentage for the main components was calculated from the peak areas.

2.3. Preparation of antimicrobial films

PLA-based films were provided by solvent casting method as reported by Jamshidian et al. (2012) with some modifications. Since PLA is very hygroscopic, PLA pellets were previously dried in oven at 90 °C for 3 h. Then, a solution of 3.5% (w/w) PLA was prepared in chloroform and then 0.5, 1 and 1.5% (w/w) of OEO based on PLA dry matter were added to solution and homogenized at 22,000 rpm for 1 min using a homogenizer (UltraTurrax IKA, T25, Germany). Afterward, the polymer solution was cast into a glass petri dishes and the solvent was allowed to evaporate in a hood in a dark place over 3 days at ambient temperature. The final thickness of 40 ± 2 μm was determined in all samples except the 1.5% (w/w) OEO formulation concentration, which was 50 ± 1 μm.

2.4. Characterization of the films

2.4.1. Mechanical properties

An universal testing machine (TVT-300Xp, Perten, Sweden) was used to determine the tensile strength (TS), elongation-at-break (EAB) and elastic modulus (EM) of the bioactive films according to the ASTM D882-02 standard procedure (ASTM, 2002). Film samples (rectangular strips of 100 × 20 mm) were conditioned in an environmental chamber for 48 h at 23 ± 2 °C and 53 ± 2% RH before testing. Tests were performed on at least five samples of each material with an initial grip separation and mechanical crosshead speed was set at 50 mm and 5 mm/min, respectively.

2.4.2. Water vapor permeability (WVP)

WVP values of films were calculated gravimetrically via ASTM E96-05 method (2005). Circular glass cups with a diameter of 50 and a depth of 10 mm were applied. The samples were cut into circles and sealed onto the cup mouth containing 6 ml distilled water (100% RH, 2.337×10^3 Pa vapor pressure at 20 °C), stored in a desiccator at 20 °C and 0% RH (0 Pa water vapor pressure) containing silica gel. The water transported through the film was measured every 2 h during 10 h by the weight loss of the glass permeation cell. The measured WVP of the films was calculated using the following equation:

$$WVP = (WVTR \times L)/\Delta p$$

Where WVTR is the water vapor transmission rate (g mm/kPa h m^2) calculated from the slope of the straight line divided by the exposed film area (m^2), L is average film thickness (mm), and ΔP is the partial water vapor pressure variance (kPa) through two sides of the film. Three replicates of each film were tested.

2.4.3. Measurement of contact angle (CA)

CA measurements of water on the surface of samples were carried out by using a sessile drop method with a PG-X goniometer (PG-X, Switzerland). The contact angle was measured by adding a droplet of ultrapure water (~5 μL) onto the film surface with a precision syringe. Five measurements were performed for each film