Food Research International 99 (2017) 650–659

Contents lists available at ScienceDirect

Food Research International

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

Supercritical impregnation of cinnamaldehyde into polylactic acid as a route to develop antibacterial food packaging materials

Carolina Villegas[a], Alejandra Torres[a,*], Mauricio Rios[a,b], Adrián Rojas[a,b], Julio Romero[b], Carol López de Dicastillo[a], Ximena Valenzuela[a], María José Galotto[a], Abel Guarda[a]

[a] Laboratory of Food Packaging (LABEN), University of Santiago de Chile (USACH), Technology Faculty, CEDENNA, Center for the Development of Nanoscience and Nanotechnology, Chile
[b] Laboratory of Membrane Separation Processes (LabProSeM), Department of Chemical Engineering, Engineering Faculty, University of Santiago de Chile, Santiago, Chile

ARTICLE INFO

Keywords:
Supercritical impregnation process
Cinnamaldehyde
Polylactic acid (PLA)
Antimicrobial active packaging

ABSTRACT

Supercritical impregnation was used to incorporate a natural compound with antibacterial activity into biopolymer-based films to develop active food packaging materials. Impregnation tests were carried out under two pressure conditions (9 and 12 MPa), and three depressurization rates (0.1, 1 and 10 MPa min^{-1}) in a high-pressure cell at a constant temperature equal to 40 °C. Cinnamaldehyde (Ci), a natural compound with proven antimicrobial activity, was successfully incorporated into poly(lactic acid) films (PLA) using supercritical carbon dioxide ($scCO_2$), with impregnation yields ranging from 8 to 13% w/w. Higher pressure and slower depressurization rate seem to favor the Ci impregnation. The incorporation of Ci improved thermal, structural and mechanical properties of the PLA films. Impregnated films were more flexible, less brittle and more resistant materials than neat PLA films. The tested samples showed strong antibacterial activity against the selected microorganisms.

In summary, this study provides an innovative route to the development of antibacterial biodegradable materials, which could be used in a wide range of applications of active food packaging.

1. Introduction

In the last decades, there has been an increasing interest in the development of renewable materials with biodegradable properties in an attempt to contribute to the sustainable development and to reduce the environmental impact associated with petroleum-based plastics (Queiroz & Collares-Queiroz, 2009). Poly(lactic acid) (PLA) is one of the most important commercially available bio-based and biodegradable thermoplastic polyesters (Inkinen, Hakkarainen, Albertsson, & Sodergard, 2011). Until the last decade, the main uses of PLA have been limited to medical applications because of its high cost, low availability and limited molecular weight. However, with the advance of new technologies and lower processing costs, PLA is being used in other commodity areas like packaging, textiles and composite materials (Drmright, Gruber, & Henton, 2000; Hughes, Thomas, Byun, & Whiteside, 2012; Lim, Auras, & Rubino, 2008; Mano, Gomez-Ribelles, Alves, & Salmeron-Sanchez, 2005).

PLA has been extensively used for active packaging development. Active packaging is an innovative concept where package, product and environment interact in order to prolong shelf life or enhance safety and/or sensory properties, contributing to preservation of the products quality (Ahvenainen, 2003; Suppakul, Miltz, Sonneveld, & Bigger, 2003). Active materials with antioxidant or antimicrobial properties have gained considerable attention, since they are one of the most promising alternatives to traditional packaging, in order to extend shelf life of food products (Al-Naamani, Dobretsov, & Dutta, 2016; López de Dicastillo, Bustos, Guarda, & Galotto, 2016; Qin et al., 2015; Ramos, Jiménez, Peltzer, & Garrigós, 2012; Râpă et al., 2016; Wu et al., 2014). In addition, the demand for the use of natural additives has produced in recent years a clear increase in the number of studies based on antimicrobial plant extracts, such as carvacrol, thymol, cinnamaldehyde, olive leaf extract, and resveratrol, which are generally recognized as safe in food industry (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; du Plooy, Regnier, & Combrinck, 2009; Erdohan, Çam, & Turhan, 2013; Ramos et al., 2012; Tunc & Duman, 2011; Wu et al., 2014).

Cinnamaldehyde (Ci) is a biologically active compound present in the essential oil of the genus *Cinnamomun*, which is the responsible for the distinctive aroma and flavor of cinnamon (Lauw, Zhong, & Webster, 2016). It has also been widely used to give a cinnamon flavor and/or aroma to medical products, cosmetics, and perfumes (Hooth et al.,

* Corresponding author.
E-mail address: alejandra.torresm@usach.cl (A. Torres).

http://dx.doi.org/10.1016/j.foodres.2017.06.031
Received 3 March 2017; Received in revised form 13 June 2017; Accepted 17 June 2017
Available online 20 June 2017

2004; Nostro et al., 2012). Ci is categorized as Generally Recognized as Safe (GRAS) by the US Food and Drug Administration as well as by the current European Legislation for materials intended to be in contact with food (EU N10/2011 Regulation). Additionnally, is an active inhibitor of bacterial (Quale, Landman, Zaman, Bumey, & Sathe, 1996; Shreaz et al., 2010), yeast and filamentous growth (Chang, Chen, & Chang, 2001; Shreaz et al., 2010). It exerts inhibitory effects on the activity of ATPases (Quale et al., 1996), cell wall biosynthesis (Usta, Kreydiyyeh, Barnabe, Bou-Moughlabay, & Nakkash-Chmaisse, 2003), and alteration of the membrane structure and integrity (Xie, Fang, & Xu, 2004). Accordingly, Ci and its derivatives been proven for antifungal activity againts several pathogenic fungi (Qin et al., 2015).

On the other hand, there are different techniques for incorporating additives or active substances in plastic films, such as solvent casting (Hu, Chen, & Wang, 2012; Quintero, Rodriguez, Bruna, Guarda, & Galotto, 2012; Rodriguez et al., 2014), coating (Cerisuelo, Gavara, & Hernández-Muñoz, 2014; Hauser & Wunderlich, 2011), electrospinning (Cerqueira et al., 2016; Fabra, Lopez-Rubio, & Lagaron, 2013; Fabra, López-Rubio, & Lagaron, 2015; López de Dicastillo et al., 2017) and melting extrusion, which is the most method used in the industry for the inclusion of active additives into polymer matrices (Byun, Kim, & Whiteside, 2010; Galotto, Valenzuela, Rodriguez, Bruna, & Guarda, 2012; Ramos et al., 2012; Torres, López de Dicastillo, et al., 2014). However, this process has disadvantages, such as the possible volatilization or degradation of the active agent (s) because of the high temperature values used during the plastic processing (Torres, Romero, et al., 2014; Torres et al., 2017). In this way, the incorporation of these compounds into polymeric matrices by means of supercritical impregnation process has been reported as an efficient alternative for this purpose (de Souza, Dias, Sousa & Tadini, 2014; Goñi, Gañán, Strumia, & Martini, 2016; Markovic, Milovanovic, Radetic, Jokic, & Zizovic, 2015; Torres et al., 2017). Carbon dioxide is the most common compound used as supercritical fluid, since it is not expensive, generally regarded as safe (GRAS-status), non-toxic and chemically inert, capable of dissolving a wide range of organic molecules under supercritical conditions (supercritical conditions; $P > Pc = 73.8$ bar and $T > Tc = 304.15$ K) (Torres, Romero, et al., 2014).

Supercritical impregnation process is advantageous for it allows (i) impregnation of a large number of different natural and synthetic-based polymers if they swell when in contact with supercritical carbon dioxide ($scCO_2$); (ii) it is particularly advantageous for impregnating hydrophobic molecules such as essential oils; (iii) controlled its solute load and depth of impregnation can be tuned by changing the conditions of the process; (iv) it generates final products that are free from organic solvent residues, as $scCO_2$ is released as a gas after depressurization and (v) the technique allows working at relatively mild conditions in an oxygen-free environment, which is often desirable if the objective is impregnating natural-based compounds with biological activity (de Souza, Dias, Sousa, & Tadini, 2014).

This technique has been largely used in the development of materials for applications in the biomedical field (Braga et al., 2008; Costa et al., 2010; Dias et al., 2011; Masmoudi, Azzouk, Forzano, Andre, & Badens, 2011) and, more recently, for the development of active polymers for food packaging employing natural compounds (de Souza et al., 2014; Goñi et al., 2016; Hassabo, Nada, Ibrahim, & Abou-Zeid, 2015; Markovic et al., 2015; Milovanovic et al., 2016; Rojas et al., 2015; Rojas et al., 2017; Torres, Romero, et al., 2014; Torres et al., 2017; Varona, Rodríguez-Rojo, Martín, Cocero, & Duarte, 2011).

In this way, the aim goal of this work was the development of active biodegradable PLA-based films impregnated with cinnamaldehyde at different process conditions to be used for food packaging purposes. Quantification of cinnamaldehyde, thermo-mechanical and structural characterization and antibacterial properties of the impregnated samples were investigated and the results are presented.

2. Materials and methods

2.1. Reagents and strains

Poly(lactic acid) (PLA), Ingeo™ Biopolymer 2003D (specific gravity 1/4 1.24; MFR g/10 min (210 °C, 2.16 kg)), was purchased from Natureworks® Co.; Minnetonka (USA). Merck (Darmstadt, Germany) supplied absolute ethanol (99.9% HPLC grade). Ci (≥ 99.5%) was purchased from Aldrich® Chemistry (St. Louis, MO, USA). Methanol, ethanol and acetonitrile HPLC grade were supplied by Merck. Carbon dioxide was supplied by Linde (Santiago, Chile). The food-borne microbial strains, Gram-negative *Escherichia coli* (O157:H7) and Gram-positive *Staphylococcus aureus* (ATCC 25923), were obtained from the Laboratory of Biotechnology and Applied Microbiology (LAMAP) of the University of Santiago of Chile.

2.2. Preparation of active PLA/Ci materials

2.2.1. Extrusion of PLA films

PLA powder, previously dried at 60 °C for 24 h, was melt-extruded through a 20 mm co-rotating laboratory Scientific Labtech LTE20 twin-screw extruder (Samutprakarn, Thailand). The temperature profile of extruder from Zone 1 to Zone 5 was kept between 175 and 200 °C with a die temperature of 200 °C. The screw speed and the feed were fixed at 30 rpm, and the films were collected in a Scientific Labtech LBCR-150 chill roll attachment (Samutprakarn, Thailand) at 1.8 rpm.

2.2.2. Supercritical impregnation of Ci in PLA films

Supercritical fluid impregnation of Ci in PLA films were performed using the apparatus schematically described in Fig. 1. This process was carried out into a 100 mL high-pressure cell. Cinnamaldehyde (1.0 mL) was placed at the bottom of a vessel in a glass container. PLA plastic films (200 cm^2, 77.8 ± 5.2 μm average thickness) were separated by a metal support and placed into the cell. CO_2 was loaded in the system by means of a ISCO 500D syringe pump operated at a constant pressure rate during the impregnation runs. The experiments were carried out under two conditions of pressure (9 and 12 MPa) and three depressurization rates (0.1; 1 and 10 MPa min^{-1}) (Table 1) at a constant temperature of 40 °C for 3 h. The temperature of the high-pressure cell was controlled using a thermostatic electric resistance around the cell.

2.3. Characterization of active impregnated PLA films

2.3.1. Quantification of Cinnamaldehyde in the plastic films

In order to study the influence of different processing conditions, the effective concentration of Ci in the films was determined after each supercritical impregnation process. The analysis was performed using a method of dissolution and precipitation of the modified polymer (Stoffers et al., 2004), followed by a detection and quantification of the active compound carried out through high performance liquid chromatography (HPLC).

The sample (0.1 g of film) was dissolved into a centrifuge tube with 20 mL of chloroform at room temperature. After that, 30 mL of methanol were added to produce the precipitation of the polymer. Subsequently, the polymer was insolated by centrifugation (4500 rpm for 10 min) and the liquid phase was analyzed by a high-performance liquid chromatography (HPLC). Chromatographic analysis was performed next in a HPLC (Hitachi LaChrom Elite, Dallas, TX, USA) equipped with a Hitachi L-2455 diode array detector and a Hitachi L-2200 autosampler. The chromatographic column used was an Inertsil ODS-3 C18, 5 μm, 4.6 × 250 mm. The mobile phase consisted of a mixture of acetonitrile and distilled water (40:60) at a flow rate of 2 mL min^{-1}, with an injection volume of 5 μL. The oven temperature was constant at 40 °C. The detection of cinnamaldehyde was performed at 275 nm. The calibration curve was constructed for peak area against the cinnamaldehyde concentration of standard solutions from 6 to