Food Packaging and Shelf Life 11 (2017) 31–39

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: http://www.elsevier.com/locate/fpsl

Improving ham shelf life with a polyhydroxybutyrate/polycaprolactone biodegradable film activated with nisin

Juan Pablo Correa[a,b,*], Vanesa Molina[c], Mariana Sanchez[c], Cecilia Kainz[c], Patricia Eisenberg[b,c], Mariana Blanco Massani[a,c]

[a] National Council of Scientific and Technical Research-CONICET, Av. Rivadavia 1917 (C1033AAJ), Buenos Aires, Argentina
[b] National University of San Martín – UNSAM 3iA. Campus Miguelete, Av. 25 de Mayo y Francia (CP 1650), San Martín, Buenos Aires, Argentina
[c] National Institute of Industrial Technology-INTI, Av. Gral. Paz 5445, San Martín (B1650WAB), Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 11 June 2016
Received in revised form 15 November 2016
Accepted 21 November 2016
Available online xxx

Keywords:
Biodegradable nanocomposite films
Active packaging
Nisin
Ham
Shelf life extension

ABSTRACT

Environmental polution and food shelf life extension are issues of global concern. In this work, biodegradable Polyhydroxybutyrate/Polycaprolactone (PHB/PCL) films and organo-clays (Cloisite® 30 B and 10A) based nanocomposites were prepared. Tensile and thermal properties, water vapor barrier and activation with nisin were studied. Organo-clays addition promoted a reinforcement effect of the polymer blend, increasing barrier properties and degradation temperature. The optimal parameters for nisin adsorption to PHB/PCL film were 4000 IU cm^{-3}, 40 °C and 10 min. Organo-clays exerted antimicrobial activity against *Lactobacillus plantarum* CRL691; nevertheless, their inclusion into the polymer blend did not lead to antimicrobial films. Nisin adsorption to PHB/PCL film was not afected by clays presence. PHB/PCL nisin activated film was effective against *L. plantarum* CRL691 (used as processed meat spoilage bacterium model) inoculated on sliced ham, thus extending its shelf life. PHB/PCL blend and its nanocomposites activated with nisin showed potential for their application in processed meat packaging.

1. Introduction

Spoilage bacteria lead to economic losses in meat processing industry (Giaouris et al., 2014). To overcome this problem, and according to an increased negative perception towards chemical agents, natural antimicrobial agents have been extensively screened and tested for their effectiveness in meat and processed meat foods (Woraprayote et al., 2016).

Active packaging containing bacteriocins showed the ability to inhibit unwanted microorganisms in various meat products (Blanco Massani et al., 2014; Marcos, Aymerich, Garriga, & Arnau, 2013; Woraprayote et al., 2013). In contrast to plant extracts and other natural antimicrobial preservatives, bacteriocins present colorless, odorless, and tasteless characteristics. These characteristics make bacteriocins good candidates for their application in active packaging, since their use does not interfere with sensory quality of food products (Elsser-Gravesen & Elsser-Gravesen, 2013; Woraprayote et al., 2016).

Regulatory requirements for active packaging technologies in the United States are not very different from the requirements for conventional antimicrobial additives. The material exerting antimicrobial effect on food through migration or controlled release would constitute a "direct additive" and would be subject to FDA regulatory requirements (Restuccia et al., 2010). For the European Union, regulation 450/2009/EC (European Commission, 2009), set legal basis for the correct use, safety and marketing of active packaging. The use of the active substance must accomplish a technological need and active substances migrating from the packaging shall comply with the conventional rules laid down in the EU Directives (European Commission, 2008; European Commission, 2011a; European Commission, 2011b; Restuccia et al., 2010).

Among hundreds of bacteriocins, nisin is the only commercial bacteriocin approved for food applications (European Commission, 2011a; Elsser-Gravesen & Elsser-Gravesen, 2013). Its mechanism of action involves initial interaction with the membrane and further

Abbreviations: PHB, polyhydroxybutyrate; PCL, polycaprolactone; IU, international units; AU, arbitrary units; Clo 30B, cloisite® 30B; Clo 10A, cloisite® 10A; OOT, oxidation onset temperature; DSC, differential scanning calorimeter; XRD, X-ray diffraction; WVP, water vapor permeability; RIA, relative inhibition areas; PLA, polylactic acid; MMT, montmorillonite; LAB, lactic acid bacteria.

* Corresponding author at: National Council of Scientific and Technical Research-CONICET, Av. Rivadavia 1917 (C1033AAJ), Buenos Aires, Argentina.
E-mail address: jcorrea@inti.gob.ar (J.P. Correa).

http://dx.doi.org/10.1016/j.fpsl.2016.11.004
2214-2894/

lipid II binding. In this way, a stabilized poration complex is formed in the target site and at the same time, sequestration of lipid II causes cell wall biosynthesis inhibition. This dual mode of action, in which inhibition of peptidoglycan synthesis and pore formation are most efficiently combined, makes nisin a very potent antimicrobial agent and impedes the emergence of resistant strains (Islam, Nagao, Zendo, & Sonomoto, 2012).

Bacteriocins adsorption on polymer surfaces offers a way for setting up antibacterial systems. Other strategy is adding antimicrobial agents into polymers. In this regard, commercially montmorillonites modified with quaternary alkyl ammonium salts Cloisite® 10A and 30 B showed potent antimicrobial activity; thus, active packaging obtained with these and other modified clays was reported (Nigmatullin, Gao, & Konovalova, 2008; Rhim, Hong, & Ha, 2009; Tornuk, Hancer, Sagdic, & Yetim, 2015).

Polyhydroxybutyrate (PHB) is a renewable thermoplastic material exhibiting intermediate oxygen and water permeability compared to petroleum based polymers. Also, PHB is more attractive than other biopolyesters like polylactic acid (PLA) in terms of barrier properties (Corre, Bruzaud, Audic, & Grohens, 2012). Nevertheless, PHB has limited food packaging applications due to its cost, narrow processability window, stiffness and brittleness properties. Blending PHB with other polymers offers a way to obtain materials with enhanced properties. Polycaprolactone (PCL) is a good candidate for this purpose due to its biodegradable nature, its flexibility, toughness and thermal stability (Lovera et al., 2007). Aditionally, in order to improve PHB mechanical, gas barrier, and thermal properties, nanofillers such as organo-clays could be incorporated at low filler content (less than 5% by weight) and nanoscale distribution (Bordes, Pollet, Bourbigot, & Avérous, 2008; Botana, Mollo, Eisenberg, & Torres Sanchez, 2010). However, the incorporation of nanofillers has raised concerns among several researchers, regarding the effects of particles migration from the nanocomposites to the packaged food. The European regulation on plastic materials and articles intended to come into contact with food (European Commission, 2011c) is rather specific with regard to nanoparticles and states that risk assessment of materials in nanoform has to be performed on a case-by-case basis, until more information is known in relation to this new technology. According to Souza and Fernando (2016), nanoparticles could have potential to migrate to the packaged foodstuff, but migration assays and risk assessment are still not conclusive. This scarce information regarding migration is related to the lack of suitable and validated test methods for the identification, characterization and detection of nanoparticles in complex matrices as food (EFSA, 2011). Even when in vitro toxicological research on neat clays is of high interest nowadays, studies dealing with nanocomposites containing clays are still scarcer. Maisanaba et al. (2015) reported that different clays have their own cytotoxic profile with dependence on the experimental conditions (type of clay, modifier, cell line, concentrations used, etc). Despite neat clay Cloisite 30 B showed high toxicity effects (Maisanaba et al., 2015), particularly for a Organoclay/Poly(butylene adipate-co-terephthalate) nanocomposite, no citotoxicity was observed with 10% of clay incorporation (Fukushima, Wu, Bocchini, Rasyida, & Yang, 2012). Similar results have been revealed for in vitro and in vivo toxicity of organo-clays/PLA nanocomposites (Maisanaba et al., 2014a; Maisanaba et al., 2014b). These authors also concluding that organo-clays/polymer based nanocomposites need to be studied in greater detail, regarding safety issues, in order to support their conclusions.

In this work, a PHB/PCL blend, with a high (50%) content of renewable polymer and organo-clays (Cloisite® 30 B and 10A) PHB/PCL based nanocomposites were activated with nisin in order to develop antimicrobial films with enhanced material properties. The effects of the type of organo-clay (Cloisite® 30 B and 10 A) on the intercalation of polymeric chains into clay galleries, and on the final mechanical properties of the biodegradable nanocomposites were studied. Nisin adsorption at different temperatures was investigated for the PHB/PCL matrix looking for the best conditions to obtain an antimicrobial biodegradable film. Nisin adsorption on PHB/PCL matrix with and without clays was studied and compared to find out possible synergistic antimicrobial effects. Finally, the ability of nisin activated PHB/PCL films to inhibit *Lactobacillus plantarum* CRL691 (used as processed meat spoilage bacterium model) was explored for cooked ham during 4-week period.

2. Materials and methods

2.1. Materials

Polyhydroxybutyrate (PHB) homopolymer commercial grade "Biocycle 1000" was obtained from PHB Industrial S.A., Brazil, in the form of powder with a weight average molecular weight of approximately 600,000 g/mol. Polycaprolactone (PCL) commercial grade "FB-100" was obtained from Perstorp, United Kingdom, in the form of pellets with a weight average molecular weight of 100,000 g/mol. Organo-clays Cloisite® 30 B and 10A (Clo 30 B and Clo 10A) were obtained from Southern Clay Products, USA.

2.2. Bacterial strains and growth conditions

Lactobacillus plantarum CRL691 was grown in (Man, Rogosa and Sharpe) MRS broth (Britania, Argentina) at 30 °C. The strain, kindly transferred by CERELA-CONICET (Argentina), was maintained and stored at −20 °C in 0.15 g cm^{-3} of glycerol until use.

2.3. Nanocomposite films preparation

Materials were dried during 8 h under vacuum at 80 °C (PHB and organo-clays) and 40 °C (PCL). For this study, PHB/PCL in a 50/50% (w/w) proportion was used as the blend with the highest content of renewable polymer without detrimental consequences on mechanical properties and processabilty, due to the stiffness and high flowability of PHB. The 50/50 PHB/PCL blend and organo-clay based nanocomposites with a Clo10A and Clo30 B fixed content at 5% (w/w) were prepared by melt intercalation in an internal mixing chamber Brabender Plasticorder (30 cm^3) at 165 °C and 50 rpm rotor speed for 5 min. Nanocomposites were labeled as PHB/PCL/Clo10A and PHB/PCL/Clo30B. Films were obtained by compression molding at 175 °C using an 8 min molding cycle.

2.4. Polymer and nanocomposites characterization

2.4.1. Oxidation onset temperature (OOT)

OOT was performed by dynamic scanning in a differential scanning calorimeter (DSC) Mettler 822e/500/1473 using the method described in ASTM E 2009 (American Society for Testing and Materials, 2014a). Measurements were carried out under an oxygen atmosphere and analyzed from 30 °C to 350 °C at a heating rate of 10 °C/min. Results of two determinations were averaged and informed.

2.4.2. X-Ray diffraction (XRD)

Organo-clays structure and their PHB/PCL composites were evaluated with XRD measurements. XRD patterns were taken with a Phillps PW 1730/10 X-ray diffractometer, operated at 40 kV and 30 mA, equipped with Cu Ka radiation at a wavelength of 0.1546 nm. Diffraction data was collected over a 2θ range of 1°–10°, with a step width of 0.02° and a counting time of 2.0 s/step.