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Antibacterial performance of solvent cast polycaprolactone (PCL) films containing essential oils

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ABSTRACT

The increase of consumer demand for higher quality and longer shelf-life in foods, while reducing the use of non-compostable packaging materials, has encouraged research on biopolymers incorporating natural antimicrobial compounds. Cinnamaldehyde (CNMA) and allyl isothiocyanate (AITC) were incorporated into polycaprolactone (PCL) films by solvent casting. The release study was carried out by means of ATR and transmission FTIR spectroscopy and showed high volatility of the essential oils during the film forming process. While only negligible quantities of AITC were retained in the polymer matrix after film curing, the release of CNMA was prolonged for at least 50 h at room temperature. PCL films incorporating 10 wt.-% and 20 wt.-% CNMA were further investigated using both a macrodilution technique and a vapour diffusion technique. MICs against *Salmonella enterica*, and *Listeria monocytogenes* in the liquid phase were determined to be 5.87 and 4.49 mM, for films containing 10 and 20 wt.-% of CNMA with regard to the polymer in solution. The influence of relative humidity (RH) and temperature on the antimicrobial performance of the active films was investigated in the vapour phase. RH was not found to play a key role in the release and antimicrobial performance of the films, while decreasing temperatures resulted in a considerable increase in the antimicrobial effect. In a sealed environment, a concentration of less than 5.34 mg CNMA/L air from PCL with 20 wt.-% CNMA was able to cause complete inhibition of bacterial growth at 4 °C and 10 °C during at least 30 days. These results suggest that the combination of cold storage with biodegradable polyesters incorporating CNMA or other EOs could be an interesting approach in active packaging technologies.

1. Introduction

The globally increasing demand for minimally processed, easily prepared and ready-to-eat 'fresh' food products has encouraged manufacturers to develop alternative technologies to thermal processing of foods as a means to reduce pathogenic bacterial growth and prevent recontamination during the post-processing steps. In this sense, biopreservatives are a wide range of natural products that can be used to reduce or eliminate pathogen populations while increasing food quality. Among this type of antimicrobials, essential oils (EOs) have long been applied as flavouring agents in foods, and due to their content in antimicrobial compounds, they have potential as natural agents for food preservation (Burt, 2004).

Antimicrobial activity of EOs relies on an unspecific mechanism of action involving their attachment to cell membrane, disruption of enzymes and membrane potential at different levels. The active compound Allylisothiocyanate (AITC), for example, has been shown to inhibit several bacterial pathways, involving the enzymes thioredoxin reductase and acetate kinase (Luciano & Holley, 2009) and produces metabolite leakage (Lin, Preston Iii, & Wei, 2000). Cinnamaldehyde (CNMA) is known to inactivate acetyl-coA-carboxylases (Burt, 2004; Meades et al., 2010), and to disrupt metabolism (Amalaradjou & Venkitanarayanan, 2011; Bouhdid et al., 2010). Even at subinhibitory concentrations, CNMA has been found to decrease bacterial motility (Amalaradjou, Narayanan, & Venkitanarayanan, 2011), cell attachment and biofilm formation (Upadhyay et al., 2012). In addition to its inherent antimicrobial activity, CNMA has recently been found to possess antiinflammatory (Youn et al., 2008) and antioxidant (Salmieri & Lacroix, 2006; Shan, Cai, Brooks, & Corke, 2011) properties, which could further add value to its possible implementation as an additive in the food area. CNMA and AITC can be found as major compounds in

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the EO of cinnamon and mustard, horseradish or wasabi, respectively, or can be artificially synthesized (Delaquis & Sholberg, 1997).

Due to these beneficial properties, the use of EOs or their main active compounds for food applications is a growing field of interest, either in washing solutions or incorporated into packaging materials (Burt, 2004). This latter option has attracted much attention among packaging manufacturers and demanding consumers since EOs and their compounds are categorized as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration. Some antimicrobial films containing EOs or their compounds have already been evaluated for food-packaging applications, such as polyethylene (PE), low-density polyethylene (LDPE), polypropylene (PP) or polyvinyl chloride (PVC) films (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2011). The non-biodegradable origin of these polymers goes against the current trend towards reducing the waste management problems associated with the use of plastics in packaging. Therefore, incorporation of natural antimicrobials into polymers has gained much attention in the last years, as a means to produce 100% biodegradable materials with antimicrobial activities for food packaging or other applications.

In this sense, the antimicrobial activity of essential oils incorporated into chitosan (Chen, Jin, Gurtler, Geveke, & Fan, 2012; Ouattar, Simard, Piett, Bégin, & Holley, 2000), soy protein isolate (Gamage, Park, & Kim, 2009), polylactides (PLA) (Jin & Niemira, 2011), starch (Romero-Bastida, Zamudio-Flores, & Bello-Pérez, 2011), alginate based (Salmieri & Lacroix), pectin based (Du et al., 2009) or cellulose (Caillet, Millette, Turgis, Salmieri, & Lacroix, 2006; Sanla-Ead, Jangchud, Chonhenchob, & Suppakul, 2012) films has recently been evaluated. Polycaprolactone (PCL), which has been approved by the United States Food and Drug Administration (FDA) in many applications, has been widely adopted as a suitable biomaterial because of its relatively slow degradability, good biocompatibility, and superior rheological and viscoelastic properties (Woodruff & Hutmacher, 2010).

The aim of this study was to develop and characterize novel antimicrobial materials of PCL as a way to evaluate the suitability of this polymer for controlled diffusion of the natural biocide agents CNMA and AITC. Antibacterial activity of the films with best performance was investigated in liquid and vapour phase against *Salmonella enterica* and *Listeria monocytogenes*, two of the most common foodborne pathogens.

2. Materials and methods

2.1. Materials

PCL grade FB100 with a density of 1.1 g/cm^3 and a mean molecular weight of 100,000 g/mol was kindly supplied in pellet form by Solvay Chemicals, Belgium. The antimicrobial compounds, cinnamaldehyde and allyl isothiocyanate, were purchased from Sigma–Aldrich (Germany), with a purity of 93 and 95% respectively. The surfactants Tween 20 and Tween 80 were purchased from Sigma–Aldrich.

2.2. Bacterial strains and their MICs and MBCs

L. monocytogenes CECT 5672 and *S. enterica* CECT 554 were obtained from the Spanish Type Culture Collection (CECT; Valencia, Spain).

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were estimated by the broth macrodilution method (M-26A) according to the National Committee for Clinical Laboratory Standards guidelines. Briefly, 100 μl of mid-exponential phase cultures were inoculated in 10 ml of Tryptic Soy Broth (TSB) added with different concentrations (from 0.5 to 6 mM) of the antimicrobial compounds diluted in DMSO. Cultures were incubated at 37 °C in a shaker incubator for 24 h and optical density (OD) was measured at 600 nm for MIC determination. For MBC determination, after 24 h incubation in contact with the antimicrobial compound at 37 °C, cultures were spread on Trypticasein Soy Agar (TSA) for plate counts.

2.3. Preparation of bioactive PCL composites

PCL films with 10 or 20 wt.-% of cinnamaldehyde and allyl isothiocyanate (related to the dry weight of PCL) were prepared by a solvent casting technique (hereinafter referred to as PCL–CNMA [10%] and PCL–CNMA [20%], respectively). Briefly, 1 g of the polymer beads and 0.1 g or 0.2 g of the EOs were dissolved in 20 g of chloroform at room temperature. The solution was cast onto Petri dishes and allowed to dry at room temperature to yield films of a dry thickness of 70–75 μm.

2.4. Transmission and attenuated total reflectance Fourier transform infrared (FTIR) analysis

Transmission FTIR experiments were recorded in a controlled chamber at 21 °C and 40% RH using a Bruker (Rheinstetten, Germany) FTIR Tensor 37 equipment. The spectra were taken at 4 cm^{-1} resolution during 1 s averaging a minimum of 10 scans. To determine the concentration of bioactives in the films and monitor the release of the active compounds, a calibration curve was obtained by recording the transmission IR spectra of KBr plates containing increasing CNMA concentrations ranging from 0.07 wt.-% to 0.13 wt.-%. The detailed procedure for obtaining these plates can be found elsewhere (Martínez-Sanz, Olsson, Lopez-Rubio, & Lagaron, 2012). ATR–FTIR spectra were collected coupling an ATR accessory GoldenGate of Specac Ltd. (Orpington, UK) to the FTIR equipment.

2.5. Antimicrobial activity of PCL films in liquid phase

The antibacterial activity of the PCL–CNMA [10%] films was tested against the two aforementioned bacterial foodborne pathogens. Films were aseptically cut and weighed and then placed on 10 ml of TSB, containing 5×10^5 CFU/ml, to achieve concentrations from 5 to 25 mg/mL. Antimicrobial activity was determined by the broth macro-dilution test as explained above. To promote contact of the antimicrobial compounds with the pathogens, Tween 20 and Tween 80 at 0.1% and 0.5% (v/v) were added to the tubes containing 10 mg/mL of PCL–CNMA [10%] films. Controls without antimicrobial compounds but with the evaluated Tween concentration were also analysed.

2.6. Antimicrobial activity of PCL films in vapour phase

Tests were carried out with PCL films containing 10 and 20% of cinnamaldehyde. TSA plates spread with 0.1 mL 10^4 CFU/ml of each bacterial inoculum were incubated at different temperatures (37 °C, 20 °C, 10 °C and 4 °C) and relative humidities (0%, 53%, 75% and 100%) in a desiccator (7.5 L of free volume in the headspace) with a Petri dish containing the PCL films (70–75 μm thick, 11 cm diameter, 650–700 mg weight). Plates without lids were incubated from 24 h to 28 days depending on the conditions and, thereafter, bacterial counts were recorded. The maximum contents of CNMA in the free volume of air were calculated considering the CNMA contents in the film as measured directly after its formation.

2.7. Statistical analysis

The significance of differences among the mean viable count numbers determined after the various treatments were decided by