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Development of a multilayer antimicrobial packaging material for tomato puree using an innovative technology

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Etil-N-Lauroyl-L-arginine (PubChem CID: 188214)
trans-cinnamaldehyde (PubChem CID: 637511)

ABSTRACT

In the present study, a new innovative procedure to incorporate an antimicrobial agent in a multilayer active material was used to prepare several antimicrobial packaging materials. For this purpose, the activity of six antimicrobial substances was evaluated and three of them were selected as active agents. Among the active materials prepared, those containing polyurethane adhesive free of isocyanates and different concentrations of cinnamon essential oil as active agent were the most effective. Therefore, these materials were studied in depth. Migration assays demonstrated that cinnamaldehyde was released from these active packaging materials to the food simulant. However, the degradation of cinnamaldehyde in the food simulant was also observed. Finally, the antimicrobial activity of the material was evaluated in tomato puree, obtaining high efficiency for *Escherichia coli* O157:H7 and *Saccharomyces cereviase* but not for *Mucor mucedo*.

1. Introduction

Active packaging has emerged as an efficient technology to protect the packaged food from microbial proliferation (Appendini & Hotchkiss, 2002; Cooksey, 2005). A great number of studies have demonstrated that antimicrobials films improve the safety and quality of food products reducing the amount of preservatives that come in contact with the food (Biji, Ravishankar, Mohan, & Srinivasa Gopal, 2015; Cha & Chinnan, 2004; Vermeiren and Debevere, 2002). The protective action of most of these materials is based on the release of volatile or non-volatile antimicrobial substances from the packaging material to the food, by direct contact, or to the space that surrounds the food where they do the preservative action.

The incorporation of the antimicrobial agents into the packaged material can be achieved by adding them directly into the polymers or by coating them onto polymer surfaces (Gutiérrez, Batlle, Sánchez, & Nerín, 2010; Vermeiren and Debevere, 2002). However, these methods could present some disadvantages that can limit the production of the material on a large scale. Coating increases the cost of production due to additional steps and technical changes in the film manufacturing process (Cooksey, 2005). The direct incorporation on the polymer by extrusion does not need additional steps of production but requires the addition of higher amounts of active compounds mainly due to two reasons. First, the extrusion processes require high temperatures that might degrade or evaporate part of the antimicrobial

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compound. Second, in the extruded films the antimicrobials are distributed through all the thickness of the material and therefore can be released to the outer side of the material, leading to a considerable loss of active agent.

Multilayer flexible packaging is widely used in food industry. Films containing layers of different materials provide desirable properties such as high barrier, mechanical strength or heat sealability that no single material possesses (Goulas, Riganakos, & Kontominas, 2003). Furthermore, this type of packaging materials has also been proposed to control the release of active compound from the packaging towards the packaged food. It would requires basically an outer layer with high barrier properties that would prevent the loss of active substances to the environment and an inner layer with appropriate thickness, chemical composition and structure that would control the flux of active agent release (Buonocore, Conte, Corbo, Sinigaglia, & Del Nobile, 2005).

In this work, a new antimicrobial multilayer material based on a commercial packaging for tomato puree has been developed using an innovative procedure to incorporate the antimicrobial agent in the material. It consists of incorporating the antimicrobial substance into the adhesive layer that joins different laminating materials. This new and simple methodology does not increase the cost of production, since it does not require additional steps or technical changes in the manufacture process. Furthermore, the high barrier aluminum central layer of the tomato packaging prevents the loss of antimicrobial substance to the environment.

The main objective of this work is to demonstrate that the new procedure developed is useful to create an efficient antimicrobial packaging for tomato puree. With this purpose the antimicrobial activity of the new films was determined and the release of the active compounds was characterized.

2. Materials and methods

2.1. Microbial strains

The following bacteria, mold and fungi strains were selected for their relevance in tomato sauce spoilage: *Escherichia coli* O157:H7 CECT 5947, *Saccharomyces cerevisiae* ATCC 7753, *Fusarium oxysporium* CECT 20201, *Mucor mucedo* CECT 20115 and *Penicillium expansum* CECT 2278. Bacteria were subcultured in tryptic soy agar (TSA) at 37 °C for 24 h, yeast in potato dextrose agar (PDA) at 25 °C for 48 h and fungi in PDA at 25 °C for 7 days.

2.2. Antimicrobial compounds and chemicals

Four chemical preservatives and two essentials oils were tested as antimicrobials substances. The chemical preservatives benzoic acid (BA; CAS number 65-85-0), sodium metabisulphite (MB; CAS number 7681-57-4) and tert-butylhydroquinone (TBHQ; CAS number 1948-33-0) were supplied by Sigma Aldrich (Bellefonte, PA), etil-N-lauroyl-L-arginine (LAE; CAS Registry number 60372-77-2) was supplied by Lamirsa (Barcelona, Spain). The essential oils (EO) of *Cinnamomun zeylanicum* (CO; CAS Number 805-91-6) and *Origanum vulgaris* (OO: CAS Number 8007-11-2) were supplied by Argolide (Bracelona, Spain).

Trans-cinnamaldehyde (CAS number 14371-10-9), hydrocinnamaldehyde (CAS number 104-53-0) and acetic acid (CAS number 64-19-7) were supplied by Sigma-Aldrich (Bellefonte, PA).

2.3. Preparation of the antimicrobial packaging materials

Antimicrobial packaging was based on a multilayer commercial material used in tomato puree packaging. This material is composed of three laminated substrates, polyester (12 μm), aluminum (6.35 μm) and polyethylene (35 μm), joined by two adhesive layers.

To prepare the antimicrobial material, the antimicrobial substances were incorporated into the solvent based adhesive that joins the polyethylene and the aluminum layers. For this, antimicrobials in a solution of ethyl acetate were added to the adhesive. After mixing, the adhesive was applied in the polyethylene layer at a dry grammage of 3 g/m^2. Ethyl acetate was used to regulate the viscosity and therefore the grammage applied. Subsequently, the adhesive layer was air dried and joined to the aluminum-polyester layer. The active material structure is shown in Fig. 1.

Three different adhesives were tested: a diphenylmethane diisocyanate based polyurethane adhesive (A), a toluene diisocyanate based polyurethane adhesive (B) and a polyurethane adhesive isocyanate free (C). Different concentrations of BA, CO and OO were used to prepare the antimicrobial packaging materials: 31.3%, 18.5%, 10.2%, 5.4% and 0% expressed as percentage of antimicrobial on the dry weight of the adhesive.

The reticulation of the material was as follows, 3 days at 40 °C for adhesives A and B and 1 day at 25 °C for adhesive C.

All materials and adhesives were supplied by Goglio S.p.A. (Varese, Italy). According to the supplier, 31.3% was the maximum amount of antimicrobial that does not reduce the peel strength of the laminate more than 20% from the laminate without antimicrobial. The tests were made according to the ASTM F 904-98 procedure (ASTM F 904-98, 2008).

2.4. Screening of the six antimicrobials agents activity

The MIC (Minimal Inhibitory Concentration) and MBC (Minimal bactericidal Concentration) or MFC (Minimal Fungicidal Concentration) of the selected antimicrobial substances (BA, TBHQ, CO, OO, MB and LAE) were determined in acidic conditions by using a broth dilution method (Becerril, Gómez-Lus, Goñi, López, & Nerín, 2007). Briefly, serial dilutions of antimicrobials were prepared in ethanol (BA, TBHQ, CO and OO) or water (MB and LAE). 20 μL of these dilutions were mixed with 1780 mL of broth at pH 4.2 (to simulate tomato puree acidity) containing 200 μL of inoculum suspension (10^6 colony forming unit (CFU)/mL). Yeast extract broth at pH 4.2 (YE acid broth) was used for yeast and molds and tryptic soy broth at pH 4.2 (TSB acid broth) was used for bacteria. Inoculum suspensions were prepared in NaCl 0.9% and cell concentration was measured by absorbance at 625 nm. Bacteria suspensions with absorbance between 0.1 and 0.2 at 625 nm corresponds to a cell concentration of 10^8 CFU/ml, while for molds and yeasts, an absorbance between 0.2 and 0.3 at 625 nm corresponds to a cell concentration of 10^6 CFU/ml. Prepared samples were incubated 24 h at 37 °C for bacteria or 48 h at 25 °C for yeast and molds. Controls with 20 μL of ethanol or water and without antimicrobial substance were also tested. After the incubation, MIC was determined as the lowest concentration in which microorganism growth was not observed. To determine MBC or MFC, 100 μL of the non-growth suspensions were plated in the appropriate solid media above described. MBC and MFC were defined as the lowest concentration that produces at least a 3 log reduction in the number of surviving microorganism. The test was performed in triplicate.

2.5. In vitro antimicrobial activity of the multilayer materials

The antimicrobial activity of the multilayer materials was measured against *M. mucedo*, *S. cerevisiae* and *E. coli* O157:H7.

For the antimicrobial assay, bags with a food contact surface area of 70 cm^2 (7 × 5 cm) were made using the active material previously prepared. The bags were filled with 9 mL of acid broth