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Characterization and antimicrobial activity of cellulose derivatives films incorporated with a resveratrol inclusion complex

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ABSTRACT

The aim of this study was to develop an active film based on cellulose derivatives (hydroxyethylcellulose and cellulose acetate), while using resveratrol and its inclusion complex with hydroxypropyl-γ-cyclodextrin as active agents. These films were prepared by the casting method. The structural, mechanical, barrier, surface free energy, optical and release properties were analyzed. The antimicrobial activity of these films against *Campylobacter jejuni*, *Campylobacter coli* and *Arcobacter butzleri* was evaluated by the agar diffusion method. The anti-quorum sensing (QS) activity of films was also studied using the biosensor strain *Chromobacterium violaceum* ATCC 12472. The active films developed in this work besides being transparent, inhibit the growth of the tested strain and had anti-QS activity. This study showed the potential of these active films as a new approach to control the growth of *Campylobacter*.

1. Introduction

Microbial growth is a major concern on food products because some microorganisms can potentially cause foodborne illness (Lucera, Costa, Conte, & Del Nobile, 2012; Turhan, 2013). *Campylobacter* spp., mostly *Campylobacter coli* and *Campylobacter jejuni* are among the biggest worldwide causes of human bacterial gastroenteritis (Duarte et al. 2014). The source of human infection is mainly associated to the consumption of undercooked poultry meat and handling of raw meat (Corry & Atabay, 2001; Duarte et al. 2014). Similarly, *Arcobacter* is another pathogen commonly found in poultry meat, in particular *Arcobacter butzleri* which is considered to be a serious hazard to human health (Corry & Atabay, 2001; Wesley & Miller, 2010). Recently, the pathogenesis of bacteria linked to food spoilage has been associated to intercellular communication mechanisms like quorum sensing (QS) (Alvarez et al. 2014). So, it has been demonstrated that the regulation or inhibition of food bacterial proliferation by QS can be a good strategy for food preservation (Alvarez et al. 2014). Antimicrobial packaging is one of the active packaging concepts that are an innovative option to inhibiting microbial growth on the foods (Turhan, 2013). The antimicrobial agents are incorporated into the packaging material, maintaining their activity for a longer period of time (Lucera et al. 2012; Turhan, 2013). In the food packaging industry, films and coatings prepared from biodegradable materials and incorporated with natural antimicrobial agents are increasingly being used (Campos, Gerschenson, & Flores, 2010; El-Fawal, 2014; Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2011; Lucera et al. 2012).

Cellulose derivatives such as cellulose acetate, hydroxyethylcellulose and others, are an important source of biomaterials in food packaging (Paunonen, 2013). Cellulose derivatives films are flexible, tough, highly sensible to water and resistant to fats (Campos et al. 2010).

The use of phenolic compounds in active packaging has shown a potent antimicrobial and antioxidant activity in food (Alkan et al. 2011; Pastor, Sánchez-González, Chiralt, Chàfer, & González-Martínez, 2013). Resveratrol is a polyphenolic compound which has antimicrobial properties against several pathogens in addition to antioxidant activity (Ferreira, Silva, Queiroz, Oleastro, & Domingues, 2014; Paulo, Ferreira, Gallardo, Queiroz, & Domingues, 2010). However, resveratrol has poor solubility, is photosensitive and susceptible to oxidation (Delmas et al. 2011). Due to these problems, the complexation with molecules such as cyclodextrins (CDs) is a common procedure to increase their solubility, stability and bioavailability (Duarte et al. 2015b; López-

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Nicolás, Rodríguez-Bonilla, & García-Carmona, 2014; Silva, Figueiras, Gallardo, Nerín, & Domingues, 2014). CDs are naturally occurring cyclic oligosaccharides derived from starch and their steric arrangement results in a hydrophobic inner cavity and hydrophilic outer surface (Folch-Cano, Yazdani-Pedram, & Olea-Azar, 2014; López-Nicolás et al. 2014). In addition to the inclusion of stilbenes in several CDs, the antimicrobial and antioxidant properties of these inclusion complexes against several foodborne pathogens has been studied (Duarte, Alves, Ferreira, Silva, & Domingues, 2015a, 2015b). Recently multilayer films have gained relevance in the food packaging industry, because they combine the advantages of a single polymer into one film and compensate for their single disadvantages (Arnon, Granit, Porat, & Poverenov, 2015). Therefore, the aim of this study was to design a bilayer active film based on two cellulose derivatives (hydroxyethylcellulose (HEC) and cellulose acetate (CA)) and to use resveratrol and its inclusion complex with hydroxypropyl-γ-cyclodextrin as active agents. The physical properties, the antimicrobial and anti-QS activities of these bilayer films were then evaluated.

2. Material and methods

2.1. Chemicals

trans-Resveratrol was obtained from TCI Europe N.V. and hydroxypropyl-γ-cyclodextrin (HP-γ-CD; M_w = 1580 g/mol) from Sigma-Aldrich. Cellulose acetate (CA; M_w = 100,000 g/mol; Acros Organics), hydroxyethylcellulose (HEC; Fluka), acetone (Scharlau), soybean lecithin (Tokyo Chemical Industry CO.) and glycerol (Merck) were also used.

2.2. Preparation of antimicrobial agents

A stock solution of 32 mg/mL of resveratrol was prepared in dimethylsulfoxide (DMSO). The inclusion complex (IC) of resveratrol with HP-γ-CD was prepared and quantified as previously described by Silva et al. (2014).

2.3. Bacterial strains

In this study, two reference strains (*C. coli* ATCC 33559 and *C. jejuni* ATCC 33560) and two *Campylobacter* spp. isolates (*C. coli* 873 and *C. jejuni* 225421) were used, as well as, two *Arcobacter butzleri* isolates (AB36/11 and INSA 776) and one reference strain (*A. Butzleri* LMG 10828) (Duarte et al. 2015a). All the strains were stored in Brain Heart Infusion (BHI) broth with 20% (v/v) glycerol at −80 °C and prior to testing each strain was inoculated on blood agar plates supplemented with 5% defibrinated horse blood (Oxoid, England).

2.4. Antimicrobial bilayer films preparation

The antimicrobial bilayer films were composed of an external CA layer and an internal HEC active layer and were obtained by the casting method.

2.4.1. Preparation of cellulose acetate film

CA at 1% (w/w) was dissolved in acetone and homogenized for 1 h at a room temperature. The solution was then cast on a glass Petri dish at 100 °C for 20 min.

2.4.2. Preparation of antimicrobial HEC film-forming solutions

HEC at polymer 4% (w/w) was dissolved in distilled water at 80 °C with magnetic stirring for 1 h and then cooled at room temperature. After that, soy lecithin and glycerol were added, respectively, as binder and plasticizer at 10% (w/w) of the total polymers weight (HEC and CD). Soybean lecithin was added to the solution and was homogenized with rotor-stator homogenizer (IKA T25-Digital Ultra Turrax) at room temperature for 10 min at 12,000 rpm. Then, glycerol was added and mixed under continuous magnetic stirring for 30 min. Afterwards, resveratrol was added to the solution at final concentrations of 0.05% (w/v) (RV_1;) and 0.075% (w/v) (RV_2), and the inclusion complex was added at 0.25% (w/v) (IC_1) and 0.75% (w/v) (IC_2). The mixtures were homogenized for 30 min at room temperature, added to the previously prepared CA film on a glass Petri dish and cast at 30 °C for 6 h. A film without resveratrol or IC, referred as C, and another film, named as CD, wherein the amount of the complex was replaced by the same amount of CD were prepared as control films. After casting, all these films were stored at 22 °C and 50% relative humidity (RH).

2.5. Films characterization

2.5.1. Structural properties

2.5.1.1. Basis weight. Basis weight was obtained according to ISO 536:1995 and denotes the mass per unit of area of the tested sample.

2.5.1.2. Film thickness. Thickness was measured according to ISO 534:2011 using an Adamel Lhomargy model MI 20 μm. Seven measurements were taken at different locations of each film sample and the mean values were used to calculate the mechanical properties.

2.5.1.3. Dry matter content. Dry matter content of samples was assessed by gravimetric analysis according to ISO 11465:1993 standard. The weight of films was measured before and after drying at 100 °C for 24 h and three samples of each film was used.

2.5.2. Mechanical properties

Tensile strength, elongation and Young's modulus of films were achieved on a tensile tester (Thwing-Albert Instrument Co., EJA series) based on ISO 1924/1 standard. Some changes to the conventional method were required, namely, the initial gap between grips was 5 cm and the separation rate of 5 mm/min. All the tests were performed at 23 ± 2 °C and 50 ± 5% RH. Values are the mean of six measurements in each film sample.

2.5.3. Water vapor transmission rate

The water vapor transmission rate (WVTR) was measured according to a modified ASTM E96-1995 method. The cups, which contained anhydrous calcium chloride desiccant (0% RH assay cup), were covered with the films. The mouths of the cups were sealed with melted paraffin and the test assemblies were under testing conditions of 22 °C and 50% RH. The cups were weighed periodically, to determine the amount of moisture transferred through the sample into the desiccant. WVTR was achieved in the steady state of the weight versus time graph and calculated dividing the slope of the line by the exposed film area. Three replicates from each sample were tested.

2.5.4. Contact angle and surface free energy

Contact angle measurements were carried out on an OCAH 200 DataPhysics instrument by the sessile drop method using distilled water and diiodomethane as test probe liquids to determine the surface energy parameters of each film, using the Owens-Wendt approach (Owens & Wendt, 1969). Surface tension of the used liquids can be found in the literature (Good & van Oss, 1991, Chap. 1). At least seven measurements were made and the average was