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Development and characterization of active films based on starch-PVA, containing silver nanoparticles

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ABSTRACT

In order to obtain antimicrobial packaging films, starch-PVA-based films with silver nanoparticles (AgNPs) have been developed and characterized as to their physical and antimicrobial properties and silver release kinetics to polar (A, B, C and D1) and non-polar (D2) food simulants. Antimicrobial activity against two bacteria, *Listeria innocua* and *Escherichia coli,* and two fungi, *Aspergillus niger* and *Penicillium expansum,* was studied. Silver-loaded starch-PVA films exhibited antimicrobial activity against the tested microorganisms, which depended heavily on the concentration of AgNPs. Their addition only led to notable physical changes in the colour and transparency of the films, which underwent significant changes and turned brownish-yellow and opaque, this being more notable when the silver concentration rose. Silver was released into aqueous simulants in its entirety within the first 60 min of contact. In the non-polar simulant (oleic acid), the release capacity of the films drastically decreased, being the only case where the established limit (60 mg/kg simulant) was met. As a consequence, the use of the developed films as food packaging materials should be restricted to fat-rich foodstuffs.

1. Introduction

About one third of the food produced for human consumption is lost or wasted worldwide, this being approximately 1300 million tonnes per year (FAO, 2011). These losses take place along the food supply chain due to physical, chemical and biological factors. For example, as a result of microbial growth, off-odors and changes in the aroma, color, and texture can be accelerated. Additionally, some microorganisms and their toxins may cause food recalls and serious foodborne outbreaks (Corrales, Fernández, & Han, 2014, Chap. 7).

The considerable pressure placed on achieving a reduction in these losses has increased the interest in developing new packaging materials which lead to the retardation of deterioration, the extension of the shelf-life, and the quality maintenance of the foodstuff. The incorporation of natural active substances in film matrices is a current alternative means of preventing food spoilage (Lanciotti et al., 2004).

Heavy metals from mineral sources have been used in the form of salts, oxides, and colloids for thousands of years because of their antimicrobial properties. These metals can be incorporated into food-contact polymers to enhance the mechanical and barrier properties and to extend food shelf life (Pal, Tak, & Song, 2007). Of the metals, silver exhibits a higher degree of toxicity to microorganisms while being less toxic to mammalian cells in minute concentrations (Rai, Yadav, & Gade, 2009). Silver has strong inhibitory or bactericidal effects for a broad spectrum of bacteria, fungi, and viruses (Ghosh et al., 2010; Li et al., 2010; Mohanty et al., 2012). Moreover, its high thermal stability, low volatility and cost of production are remarkable (Duran, Marcarto, de Souza, Alves, & Esposito, 2007, Martínez-Abad, Lagarón, & Ocio, 2014).

Silver in its metallic state is an inert material, but it can react with the environmental moisture to provide silver ions. The catalytic oxidation of metallic silver and the reaction with dissolved monovalent silver ion probably contribute to the bactericidal effect (Martínez-Abad, 2014; Pal et al., 2007; Rai et al., 2009). In spite of that, the exact mechanism of the action of silver species is not well known. Some studies describe it as based on the morphological and structural changes found in the bacterial cells (Rai et al., 2009). The mechanism of action of metallic silver, silver ions and silver nanoparticles (AgNPs) is linked with its interaction with the thiol group (-SH) compounds found in the respiratory enzymes of bacterial cells. For example, the interactions of silver with L-Cysteine residues cause the denaturation and loss of enzymatic functions (Feng et al., 2000; Liau, Read, Pugh, Furr, & Russell, 1997; Martínez-Abad et al., 2014). The mode of

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antibacterial action of AgNPs is probably similar to that of silver ions (Mohanty et al., 2012) and different authors report that the antimicrobial effect of silver nanoparticles depends on their size and shape (Ghosh et al., 2010; Rai et al., 2009; Raimondi, Scherer, Kotz, & Wokaun, 2005).

Wet chemical reduction is the most frequently applied method for the synthesis of AgNPs. For the chemical synthesis, the use of different radiation sources and/or a combination of different strong reducing agents have been applied in the presence of stabilizers in order to prevent the unwanted agglomeration of the colloidal forms (Mohanty et al., 2012; Neto, Ribeiro, & Zucolotto, 2008). Most of these methods, which make use of strong reducers, lead to environmental toxicity risks. As an alternative, the green synthesis of AgNPs has been developed, which involves the selection of a solvent medium and environmentally-friendly reducing agents and stabilizers (Raveendran, Fu, & Wallen, 2003; Sharma et al., 2009Sharma, Yngard, & Lin, 2009). Some authors followed these steps by using sunlight or UV radiation (Pourjavadi and Soleyman, 2011; Vimala et al., 2011), reducing biopolymers such as poly(vinyl alcohol) (Bryaskova, Pencheva, Kale, Lad, & Kantardjiev, 2010), poly(vynil pyrrolidone) (Morales, Morán, Quintana, & Estrada, 2009), gelatin (Darroudi et al., 2011; Pourjavadi & Soleyman, 2011), starch (Torres-Castro, González-González, Garza-Navarro, & Guana-González, 2011), poly(ethylene glycol) (Vimala et al., 2011), or even plant extracts (Mohapatra, Kuriakose & Mohapatra, 2015; Roy et al., 2015Roy, Sarkar, & Ghosh, 2015). The preparation of nanoparticles within biopolymers provides several advantages due to the fact that macromolecular chains possess a large number of hydroxyl groups that can complex the metal ion, thus enabling a good control of the size, shape and dispersion of nanoparticles, increasing biocompatibility and biodegradability, and giving rise to species that are less toxic to mammalian cells (Mohanty et al., 2012).

The Food and Drug Administration/Centre for Food Safety and Applied Nutrition (FDA/CFSAN – USA) accepted the use of silver nitrate as a food additive in bottled water and silver zeolites for use in all types of food-contact polymers (FDA, 2010), while in the European Regulation silver is accepted under 94/36/EC Directive as a colouring agent (E-174) with no restrictions. Silver is one of the most widely used antimicrobial additives in polymer films for food packaging applications (Martínez-Abad, Sánchez, Lagarón, & Ocio, 2012). This approach has been tested on a wide variety of biopolymer matrices including hydroxyl propyl methyl cellulose (de Moura, Mattoso, & Zucolotto, 2012), agar (Ghosh et al., 2010; Rhim, Wang, & Hong, 2013), (poly)vinyl alcohol (Bryaskova et al., 2010; Sedlarik, Galya, Sedlarikova, Valasek, & Saha, 2010), gelatin (Kanmani & Rhim, 2014) or blends such as chitosan-cellulose (Lin et al., 2015), starch-clay (Abreu et al., 2015) or chitosan-PVA-Glutaraldehide (Vimala et al., 2011).

Previous studies revealed that blend films based on starch-PVA presented several advantages over pure starch films. The incorporation of PVA into gelatinized starch matrices implied the formation of interpenetrated polymer networks with beneficial effects on the mechanical and water barrier properties of the films, these becoming much more extensible and stable during storage (Cano et al., 2015). These results suggest that starch-PVA-based films could be a proper alternative for the development of active films containing silver nanoparticles. These silver particles might be able to improve the physical properties of films and to control the food spoilage. To the best of our knowledge, no studies about the blend starch-PVA-silver nanoparticles have been published.

In the development of silver-loaded films, knowledge of the release kinetics of the active compound is needed in order to ensure that it complies with the current legislation for food packaging materials (Commission Regulation EU 10/2011), while assuring antimicrobial effectiveness.

The aim of the work was to develop active starch-PVA-based films which are able to deliver silver species. In this sense, the release kinetics of silver from starch-PVA films to different food simulants as well as their physical properties and antimicrobial activity against two bacteria *Listeria innocua* and *Escherichia coli* and two fungi, *Aspergillus niger* and *Penicillium expansum* were studied.

2. Materials and methods

2.1. Materials

Pea starch (S) was purchased from Roquette Laisa España S.A. (Benifaió, Valencia, Spain), poly(vinyl alcohol) (PVA)(M_w: 89,000–98,000, degree of hydrolysis >99%, and viscosity: 11.6–15.4 cP) and silver nitrate ($AgNO_3$) were obtained from Sigma Aldrich Química S.L. (Madrid, Spain) and glycerol, magnesium nitrate-6-hydrate ($Mg(NO_3)_2$), ethanol, 98% glacial acetic acid and oleic acid were provided by Panreac Química S.A. (Castellar de Vallès, Barcelona, Spain).

2.2. Preparation of film forming dispersions

Films were obtained by means of the solvent casting procedure after the preparation of film forming dispersions (FFDs) following the methodology described by Cano et al. (2015). Starch (2% w/w) was dispersed in an aqueous solution at 95 °C for 30 min, while being stirred, to induce starch gelatinization. Thereafter, the dispersion was homogenized using a rotor-stator homogenizer (Ultraturrax D125, Janke and Kunkel, Germany) at 13,500 rpm for 1 min and 20,500 rpm for 3 min. Afterwards, PVA was incorporated into the previously gelatinized starch dispersion in a S:PVA ratio of 2:1 and the dispersion was maintained at 90 °C for 30 min under stirring. Finally, glycerol was added at a starch:glycerol ratio of 1:0.25, on the basis of previous studies (Jiménez, Fabra, Talens, & Chiralt, 2012).

Starch-PVA film forming dispersions containing silver nanoparticles were obtained by the reduction of silver nitrate salts using UV light (Monge, 2009) in the starch-PVA dispersion itself, taking advantage of the stabilizing properties of the polymers (Torres-Castro et al., 2011). The synthesis can be summarized as follows: different amounts of 40 mM $AgNO_3$ were added to the previously described starch-PVA dispersions so as to obtain different S:$AgNO_3$ ratios: 1:0.006, 1:0.06, 1: 0.16 and 1:0.32. Each mixture was maintained at 90 °C for 30 min under stirring and UV radiation till the dispersion turned brown due to the formation of AgNPs. Finally, glycerol was also added in the same ratio as in the control film. The reduction of silver nitrate into an AgNPs formation was monitored by using a DU 730 spectrophotometer (Thermo Scientific, England) at 420 nm.

2.3. Film formation

From the above described silver starch-PVA dispersions, dried films were obtained by means of the casting method. Newly obtained dispersions were poured into a Teflon plate at a surface density of solids of 85 g m^{-2}. For antimicrobial tests, films were casted into Petri dishes, by using the same amount of the film-forming dispersion. Films were dried at 22 °C and 45% HR for 48 h and afterwards, peeled off the casting surface. They were conditioned at 25 °C and 53% RH in a chamber using a Mg $(NO_3)_2$ saturated solution until further analysis. The film thickness was measured at six random positions with a Palmer digital micrometer to the nearest 0.0025 mm, reaching values of between 0.058 and 0.067 mm. The control film (S-PVA) and four silver-loaded films were obtained with the increasing amounts of $AgNO_3$