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Active films based on thermoplastic corn starch and chitosan oligomer for food packaging applications

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ABSTRACT

Antimicrobial biodegradable films based on thermoplastic corn starch (TPS) and chitosan oligomers (CO) were obtained in order to develop a package prototype for perishable food products. Active films were fabricated thermo-compressing a sandwiched structure constituted by a thin layer of an oligomers enriched solution between two TPS films, previously made by melt-mixing and hot-pressing. Regarding enriched solution, it was obtained by dissolving oligomers in diluted acetic acid. Final material was characterized, determining its physical and optical properties, as well as, studying its microstructure. By diffusion assays it was demonstrated the capability of CO to migrate from the active film towards the aqueous simulant media. Moreover, oligomers were able to diffuse from the matrix, regardless the aqueous medium acidity. Experimental data of diffusion assays were fitted using a mathematical model, estimating diffusion coefficients at three studied pH values (3, 5, and 7). Active film was used to obtain sachets to package perishable foods such as strawberries, ricotta, and flavored breads, which were stored for 7 days under controlled conditions. Antimicrobial capacity of active sachets was corroborated through molds and yeast counts in the stored food products. Additionally, it was demonstrated that CO incorporation to the packaging material resulted in a more efficient way to inhibit microbial development than the spraying technique.

1. Introduction

Perishable foods are naturally susceptible to spoilage produced by microorganism attacks, for this reason, some method of protection is necessary to avoid product deterioration during the entire supply chain. Hence, antimicrobial agents are usually used to extent food shelf-life. Nowadays, the use of natural compounds to preserve food products is exponentially growing due to the awareness about synthetic chemical additives from consumers. In this regard, Lucera, Costa, Conte, and Del Nobile (2012) reported the use of essential oils derived from plants (e.g., basil, thyme, oregano, cinnamon, clove, and rosemary), animal enzymes (e.g., lysozyme, lactoferrin), microbial bacteriocins (nisin, natamycin), organic acids (e.g., sorbic, propionic, citric acid) and biopolymers (chitosan). Several methodologies were employed to protect foods by using antimicrobials: they can be incorporated during food processing, coated on products surface or included into the packaging material. Among direct methods, dipping, impregnation, coating, and spraying are the different ways of application of active agents at industrial scale because of its simplicity, low cost, and versatility (López, Giannuzzi, Zaritzky, & García, 2013). However, one of its disadvantages is the efficiency loss of the agent functionality, which forces the use of relatively concentrated active solutions. In this sense, the main drawbacks associated to spraying technique are the occurrence of secondary reactions between active agents with food components, decreasing the method effectiveness. Some food substances, such as proteins, proteases, lipids, salts, and metal ions, can interfere in the interaction of antimicrobials by reacting directly with them or by interacting with their target microorganism (Juneja, Dwivedi, & Yan, 2012; Touch, Hayakawa, & Commins, 2009). Besides, antimicrobial compounds could migrate quickly into the bulk product, leaving its surface unprotected (Pranoto, Rakshit, & Salokhe, 2005). Therefore, the development of active packaging materials containing antimicrobials could be an interesting alternative to optimize the agent activity and efficiency. It is important to highlight that antimicrobial agents must gradually diffuse through a polymeric matrix towards the food product. This gradual release allows maintaining an effective concentration of

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antimicrobial agent on food surface for longer periods of time, being an advantage over the spraying technique (Appendini & Hotchkiss, 2002). Active agent diffusion from the packaging material is affected by several factors. Within this context, Kuorwel, Cran, Sonneveld, Miltz, and Bigger (2013) claimed that this phenomenon depends on: i) film preparation method, ii) intrinsic agent characteristics, iii) agent-polymer chemical interactions, iv) changes in packaging film induced by the agent presence, v) inherent matrix characteristics, vi) composition, water activity and pH of the food, and vii) storage conditions.

Nowadays, there is a continuously growing interest in the use of biodegradable materials to develop food active packaging, not only for ecological reasons but also from a practical point of view (Srinivasa & Tharanathan, 2007). In this sense, starch is a promising alternative because of its low cost, world availability, and functionality. Starch processing under high temperature and shear stress, in the presence of plasticizers, allows obtaining thermoplastic starch (TPS), (Castillo et al., 2013). Several research papers of starch active films for food packaging can be found in the literature. For example, potassium sorbate incorporation into tapioca starch—glycerol edible films prevented external *Z. bailii* contamination and controlled yeast growth in an acidified (pH 4.5) high water activity (aw = 0.980) semisolid product (Flores, Haedo, Campos, & Gerschenson, 2007). Besides, Barzegar, Azizi, Barzegar, and Hamidi-Esfahani (2014) demonstrated that starch–clay nanocomposite films which contained potassium sorbate also had antimicrobial property against *Aspergillus niger*. On the other hand, chitosan and lauric acid were added to starch films at different proportions evidencing an inhibitory effect on *Bacillus subtilis* and *Eschericha coli* growth (Salleh, Muhammad, & Pahlawi, 2014). Glycerol plasticized potato starch films containing bioactive proteins (lactoferrin and/or lysozyme) were developed by Moreno, Atarés, & Chiralt, (2015). In this work the authors stressed that even though proteins incorporation affected films structural and physical properties, antimicrobial action against *Escherichia coli* and coliforms was observed. Besides, essential oils such as linalool, carvacrol, or thymol have been incorporated to starch based materials as active compounds (Cano, Cháfer, Chiralt, & González-Martínez, 2015; Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2011; Kuorwel et al., 2013; Mehdizadeh, Tajik, Razavi Rohani, & Oromiehie, 2012). Particularly, Kuorwel et al. (2013) studied the migration of these antimicrobial agents from starch-based film samples into isooctane as a fatty-food simulant and demonstrated their high release efficiencies, allowing to extend food shelf-life and to reduce microbial contamination.

Another promising candidate as antimicrobial agent of starch packaging systems is chitosan (van den Broek, Knoop, Kappen, & Boeriu, 2015). It is a natural carbohydrate polymer obtained by the deacetylation of chitin, a major component of crustacean's shells. Chitosan presents antimicrobial and antifungal activity against a wide range of microorganisms and it has been extensively studied as food preservative (Corrales, Fernández, & Han, 2014; Srinivasa & Tharanathan, 2007). The biological activity of chitosan depends on its molecular weight, degree of deacetylation and derivatization, such as, degree of substitution, length and position of a substitute in the glucosamine units of chitosan, pH of the chitosan solution and the target organisms (Lucera et al., 2012; No, Meyers, Prinyawiwatkul, & Xu, 2007). Several models suggested that the antimicrobial activity of chitosan is a result from its cationic nature (Goy, de Britto, & Assis, 2009; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). The electrostatic interaction between positively charged R N $(CH_3)^{3+}$ sites and negatively charged microbial cell membranes, is predicted to be responsible for cellular lysis and assumed as the main antimicrobial mechanism (Goy, Morais, & Assis, 2016; Tripathi, Mehrotra, & Dutta, 2008). In accordance to Romainor, Chin, Pang, and Bilung, (2014), chitosan antimicrobial effect could be increased by reducing its molecular size. In this sense, the small size of chitosan rendered them with unique physicochemical properties such as large surface area (providing more cationic sites) and high reactivity and thus, could potentially enhance the charge interaction on the microbial surface and lead to a greater antimicrobial effect (Zhang, Pornpattananangkul, Hu, & Huang, 2010). Hussain, Singh, and Chittenden (2012) stressed that antifungal activity increases by decreasing the degree of polymerization of chitosan oligomers, which is divergent to literature data. Within this context, the use of low molecular weight chitosan as antimicrobial agent of starch packaging would be an encouraging strategy not only to increase its antimicrobial effect but also to favor its diffusion ability. The use of chitosan oligomers is doubly advantageous due to their inherent functionality as antimicrobial agent, as well as, to their natural source. Chitosan and its derivatives are obtained from fisheries waste, giving added value to these high-volume and low-cost residues (Brück, Slater, & Carney, 2010). Particularly, oligochitosan is obtained from shrimp shells wastes which are constituted by chitin, the value-added precursor of chitosan. Because chitin is an easily accessible resource, the full exploitation/bioconversion of this polysaccharide is of great interest for both, the industrial and the academic field. Thus, oligochitosan incorporation to TPS matrix would lead to a biodegradable material with an additional functionality. It is important to mention that the use of chitosan derivatives is a less investigated topic to obtain active packaging.

The aim of this work was to obtain a package prototype for perishable food products from active films based on thermoplastic corn starch (TPS) and chitosan oligomers (CO) which could extend food shelf-life. This active package would be considered as an alternative to the conventional spraying method.

2. Materials and methods

2.1. Materials

Native corn starch was provided by Misky-Arcor (Tucumán, Argentina) with an amylose content of 23.9 ± 0.7% (López, García, & Zaritzky, 2008). Chitosan oligomer (CO) was obtained by oxidative degradation of chitosan, assisted by microwave radiation, using a modification of the methodology proposed by Shao, Yang, & Zhong, (2003). The chitosan used as raw material (M_W = 468,200 g mol^{-1}) was obtained from chitin of shrimp shells wastes (*Pleoticus müller*i) through the method proposed by Zuñiga, Debbaudt, Albertengo, and Rodríguez (2010). Shrimp shells are a fraction of the wastes resulting from the fishing industry. The yield of the CO synthesis reaction was determined following the methodology proposed by Shao et al. (2003). After irradiation had been stopped, the product was cooled and filtered under reduced pressure. The obtained residue and the filter paper were dried to constant weight in an infrared dryer. By deducting the filter paper weight, the mass of the unreacted chitosan was calculated, determining the amount of water-soluble oligomers.

2.2. Characterization of chitosan oligomer

The number average molecular weight and degree of polymerization of chitosan oligomer (CO) were determined by the method of end group analysis (Shao et al., 2003). Morphological characterization was examined by Scanning Electron Microscopy (SEM) using a JEOL JSM-35 CF microscope with a secondary electron detector.

Structural characterization was carried out by Fourier Transform Infrared Spectroscopy (FTIR) using a Thermo Nicolet Nexus spectrophotometer. Samples were prepared by milling and mixing CO with KBr (Sigma–Aldrich, 99%) at 1% w/w. The mixture was pressed and a transparent sample was achieved. Spectra were obtained from 100 accumulated scans at 4 cm^{-1} resolution in the range 4000–400 cm^{-1}.

Thermal properties were determined by Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). For DSC runs, a Perkin Elmer Pyris I calorimeter was used. Approximately 10 mg of CO were weighted in hermetic pans in order to avoid water loss during the assays. An empty hermetic pan was used as reference.