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Films of chitosan and chitosan-oligosaccharide neutralized and thermally treated: Effects on its antibacterial and other activities

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ABSTRACT

The present study focuses on the effects of heat and neutralization treatments on solubility, water vapour permeability and antimicrobial activity of chitosan (Ch) and chitosan/chitooligosaccharide (ChO)-based films. ChO films showed stronger antimicrobial activity against *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Serratia liquefaciens and Lactobacillus plantarum* than Ch films, indicating that this effect is attributed to the presence of chitooligosaccharides (COS) in the films. Heat and neutralization treatments decreased significantly the solubility of chitosan films and gave rise to a sharp loss in their antimicrobial activity. The incorporation of COS in chitosan films increased the inhibitory effect against the studied microorganisms without affecting significantly the water vapour permeability of the films. Thus, it is possible to get a more insoluble chitosan film with high antimicrobial activity by means of incorporation of COS combined with heat or neutralization treatments.

1. Introduction

Nowadays there is an increasing interest in biodegradable/compostable polymers from renewable sources due to environmental problems caused by conventional food packaging materials (Leceta, Guerrero, & de la Caba, 2013). The problems in disposing of huge quantities of waste generated by non-biodegradable food packaging have led to the study of biopolymers as materials to be used as films and coatings in food packaging (Azeredo, Miranda, Ribeiro, Rosa & Nascimento, 2012).

Development of materials from biopolymers for different applications have been a hot topic for several years, due to increasing prices of petroleum, a non-renewable resource with diminishing quantities (Ruban, 2009; Souza, et al., 2010), and increasing environmental concerns. This approach will continue playing an important role in the food industry (Satyanarayana, Arizaga, & Wypych, 2009).

Today, the use of polymers from renewable sources in food packaging is growing. The tendency is to use natural compounds to enlarge the shelf-life of all types of food increasing the preservation and protection from oxidation and microbial spoilage.

The natural polymers used in food packaging have the advantages to be available from replenishable resources, biocompatible, biodegradable, and all these characteristics lead to ecological safety (Prashanth & Tharanathan, 2007).

In this context, chitosan and its chitooligosaccharides (COS), which are known to possess multiple functional properties, have attracted considerable interest due to their biological activities and potential applications in the food, pharmaceutical, agricultural and environmental industries. Both have inherent antimicrobial activity owing to the fact that long positively charged chitosan molecules interact with negatively charged bacteria (Zivanovic, Chi, & Draughon, 2005). Chitosan is a versatile material with proved antimicrobial activity. Three antibacterial mechanisms have been proposed (Goy, de Britto & Assis, 2009): i) the ionic surface interaction resulting in wall cell leakage (Liu, Du, Wang, & Sun, 2004). In this model, the interaction is mediated by the electrostatic forces between the protonated NH_3^+ groups and the negative residues; ii) the inhibition of the mRNA and protein synthesis via the penetration of chitosan into the nuclei of the microorganisms (Sebti,

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Martial-Gros, Carnet-Pantiez, Grelier, & Coma, 2005). The chitosan molecules are assumed to be able to pass through the bacteria cell wall and reach the plasma membrane; and iii) the formation of an external barrier chelating metals and provoking the suppression of essential nutrients to microbial growth (Cuero, Osuji, & Washington, 1991). It is well known that chitosan has excellent metal-binding capacities where amine groups are responsible for the uptake of metal cations by chelation. It is likely that all events occur simultaneously but at different intensities.

Besides, chitosan is a non-toxic compound and another fascinating advantage of this compound is the film-forming capacity that it presents, which allows its application directly as a film or as a coating without the necessity of a carrier matrix (Fernandez-Saiz, Soler, Lagaron & Ocio, 2010). Chitosan films are regarded as biofunctional material, well tolerated by living tissues, particularly applicable as edible films/coatings to prolong shelf-life and preserve quality of fresh foods.

Moreover, there is a growing interest to develop materials with antimicrobial properties in order to prevent alterations in food caused by microbial spoilage.

On the other hand, as food quality and safety are major concerns in the food industry, there is also a need for an efficient method for the delivery of preservatives into foods. Addition of compounds directly into food is an established practice with some disadvantages. Instant addition of antimicrobials in formulation often results in instant inhibition of non-desired microorganisms. However, the surviving microorganisms will continue growing, especially when the concentration of antimicrobials added to the formulation will get depleted. This may be due to complex interactions with the food matrix, or by natural degradation over time causing short shelf-life. To overcome this issue, antimicrobial packaging can be considered a modern technology that could have a significant impact on shelf-life extension and food safety. Use of antimicrobial agents in food packaging can control the microbial population and targets specific microorganisms to provide higher safety and quality products. Many classes of antimicrobial compounds have been evaluated in film structures, synthetic polymers and edible films. Among them, COS have received much more interest because they are not only water-soluble, but also possess distinctive biological activity such as antifungal and antibacterial activity, immuno-enhancing effects, and antitumor effects. Studies on the biological activities of chitosan and its oligomers have been increasing, as no single type of chitosan or its oligomers exert all of the above mentioned activities. Moreover, different chitosan derivatives and enzymatic products have different structures and physicochemical properties, which may result in novel bioactivities or novel findings in known bioactive compounds (Xia, Liu, Zhang, & Chen, 2011).

Several reports discuss the antimicrobial activity of chitosan, demonstrating different results depending on source of chitin, molecular weight, deacetylation degree, and the experimental methodologies used, but they all confirm that chitosan and its oligosaccharides have strong antimicrobial effects and are safe for human use. Hence, the antimicrobial characteristics of chitosan and its oligosaccharides present a profitable potential for developing natural food packaging materials and functional food-additives.

Chitosan is known to be a very hydrophilic material with very low water resistance. The biggest drawback in use of chitosan films is their hygroscopicity. In fact, this material may virtually dissolve in the presence of high moisture products. In food packaging, the dissolution of the biopolymer could compromise packaging structure, physical integrity and organoleptic or microbiological food quality aspects. Therefore, there are a number of strategies that have been used in literature, such as crosslinking or blending with a more water resistant material, to reduce its water sensitivity (Fernandez-Saiz, Lagaron & Ocio, 2009; Tang, Du & Fan, 2002). However, these alternatives to reduce the water effect on the polymer do also adversely alter its biocide properties, suggesting that both effects may well often be opposed. Therefore, further investigations on this issue are needed in order to develop formulations of chitosan with a proper balance of water resistance and antimicrobial properties.

Taking into account that there are not many reports about the effect of high temperatures and neutralization treatments on the functional properties of chitosan and its depolymerisation products (COS), we found interesting to study the effect of heat and neutralization treatments on antimicrobial activity of chitosan films alone, and with a COS incorporated in the formulation. Five representative bacteria, *Escherichia coli* and *Serratia liquefaciens* (Gram-negative), *Lactobacillus plantarum*, *Bacillus cereus*, and *Staphilococcus aureus* (Gram-positive), which are common spoilage bacteria for food contamination, have been tested.

Thus, the aim of this work is focused on analysing the addition of depolymerisation products, COS, and the effect of heat and neutralization treatments on functional properties and antimicrobial activity of chitosan-based films.

2. Materials and methods

2.1. Materials

Commercial food-grade chitosan (PubChem CID: 21896651) with a molecular weight of 169 kDa and a degree of deacetylation of 84% purchased from TRADES, S.A. (Barcelona, Spain) was utilized to obtain the films. Acetic acid (PubChem CID: 176, min. 99.8%, reagent grade, Scharlau, Spain) was used to fix the solution pH, and sodium hydroxide (PubChem CID: 14798, PA-ACS-ISO) used for neutralization, was provided by Panreac, Spain. All reagents were used as received.

Chitosan (DA 86%, *Mw* 180 KDa) from fresh North Atlantic shrimp shells (*Pandalus borealis*) (Primex, Iceland) was purified and hydrolysed according to enzymatic depolymerisation (Mengíbar, Mateos-Aparicio, Miralles, & Heras, 2013) using chitosanase from *Streptomyces griseus* (EC 3.2.1.132) (Sigma-Aldrich, St. Louis, MO, USA). COS (DA 83%, *Mw* 8.6 KDa) were separated by tangential ultrafiltration system Vivaflow 200 (Sartorius-Stedim Biotech, Goettingen, Germany) using polyetersulfone (PES) membranes with different molecular weight cut off size.

2.2. Films preparation

Chitosan films (Ch) were prepared by casting, formed by solvent evaporation and the conversion of gelled solution rapidly to a solid film. A 10 g/L chitosan solution was prepared in a 10 g/L acetic acid aqueous solution. The chitosan solution was stirred at room temperature until it was completely dissolved, and then poured into multiwall plates and dried. The films used in the subsequent experiments were dried at 45 °C and 50% relative humidity and then peeled from the plates.

Chitosan-chitooligosaccharide films (ChO) were prepared in the same conditions but starting from two solutions: A 20 g/L chitosan solution in a 10 g/L acetic acid and a 20 g/L COS solution in 10 g/L acetic acid, mixed at ratio 1:1 to get final solution of Chitosan 10 g/L-COS 10 g/L.

Two treatments were applied to the previous formed films: 1) heat treatment at 105 °C overnight and, 2) neutralization by spraying with 13 $\mu l/cm^2$ of NaOH 0.25 mol/L.