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Ag-chitosan nanocomposites in edible coatings affect the quality of fresh-cut melon

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ABSTRACT

The effect of incorporating Ag-chitosan nanocomposites into chitosan coatings on the quality of fresh-cut melon over 13 d at 5 °C has been studied. The respiration rate of fresh-cut melon was reduced after coating treatments. Particularly, the coating with red claw crayfish-extracted chitosan (including red claw crayfish-extracted Ag-chitosan nanocomposites) showed lower RR increments during storage compared to that of the rest of coatings. Coated samples reached a steady-state atmosphere within packages of 12.6–16.2 kPa of CO_2/2.3–3.7 kPa of O_2 after 9–10 d. Softening was prevented in advanced storage periods by the coating with red claw crayfish-extracted chitosan (including red claw crayfish-extracted Ag-chitosan nanocomposites). The colour, soluble solids content, sucrose, glucose and fructose, pH, TA, and citric and malic acids were not greatly affected by the coating treatments. Furthermore, the coating with red claw crayfish-extracted chitosan (including red claw crayfish-extracted Ag-chitosan nanocomposites) showed the highest total vitamin C content after 13 d at 5 °C compared to the rest of the coating treatments. The coated samples were better sensory-scored than were the uncoated samples. Particularly, the coating with red claw crayfish-extracted chitosan (including red claw crayfish-extracted Ag-chitosan nanocomposites) showed lower translucency, which is the most important visual alteration in fresh-cut melon. Only the coating with red claw crayfish-extracted chitosan (including red claw crayfish-extracted Ag-chitosan nanocomposites) induced a microbicidal reduction (0.6 log units) from days 10 to 13. We conclude that the coating with red claw crayfish-extracted chitosan (including red claw crayfish-extracted Ag-chitosan nanocomposites) has the potential to be applied in the fresh-cut industry to extend the shelf-life of these products.

1. Introduction

Melon is the fifth most important fruit worldwide in terms of production (31,166,896 t) (FAOSTAT, 2018). Melon is sweet (mainly glucose, fructose and sucrose), is balanced with mild acidic flavour (mainly citric and malic acids), and has nutritional contents such as vitamin C (Albuquerque et al., 2006). Melon consumption may be increased when it is offered as a fresh-cut product to the actual consumer, who usually has very limited time for food preparation. However, the shelf-life of fresh-cut products is limited, especially that of fruit. Edible coatings (thin layers of material on the product surface) have been proposed to extend of the shelf-life of fresh-cut products (Olivas and Barbosa-Cánovas, 2005). Edible coatings can extend the shelf-life of these products by decreasing the moisture and solute migration, respiration, gas exchange, and oxidative reactions, as well as by inhibiting the development of physiological disorders (Vargas et al., 2008). Nanotechnology provides an additional technology for improving the properties of edible coatings (Rojas-Graü et al., 2009).

Chitosan was obtained from the exoskeleton of crustaceans (red claw crayfish and crab shells), fungal cell walls, and other biological materials (Jianglian and Shaoying, 2013). Chitosan is a biopolymer that is obtained from chitin after deacetylation. It is non-toxic, biodegradable in the human body, and not allergenic to shell fish-allergic individuals (Waibel et al., 2011). Chitosan is organoleptically and functionally biocompatible with fresh-cut fruit (Artés-Hernández et al., 2017). Furthermore, it has a high antimicrobial activity that is associated with its polycationic nature (Jianglian and Shaoying, 2013). Several intrinsic factors of chitosan, primarily the degree of

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deacetylation (DD), influence its positive charge density, which directly affects its antimicrobial activity. The polycationic nature of chitosan also allows for the incorporation of additional antimicrobial compounds and their slow release (Dhall, 2013). Interest in the antimicrobial activity of chitosan has led to the formulation of several mixed nanosystems in which it is formulated with metals or becomes a carrier for natural or synthetic compounds, which have an intrinsic antimicrobials (Perinelli et al., 2018). Chitosan nanoparticles show higher antimicrobial activity than do chitosan coarse solutions, although such mechanisms of action have not been fully explained (reviewed by Perinelli et al., 2018). Furthermore, the antimicrobial activity of chitosan nanoparticles is enhanced when they are loaded with metals; Ag^{+}–chitosan nanoparticles have shown higher antimicrobial activity (against Gram + and Gram–bacteria) than chitosan coarse solutions or other chitosan nanoparticles containing different metal ions, such as Cu^{2+}, Zn^{2+}, Mn^{2+} or Fe^{2+} (Du et al., 2009). The antimicrobial activity of Ag is related to its ability to bind to microbial DNA, proteins and enzymes (Cavaliere et al., 2015). Ag presence as metallic nanoparticles were found to be responsible of the antimicrobial effect of this element rather than Ag^{+} ions (Kumar-Krishnan et al., 2015). Smaller silver nanoparticles showed higher antimicrobial activity due to their higher specific surface area that allows for a faster release rate of Ag^{+} ions (Sotiriou and Pratsinis, 2010). In addition, the incorporation of Ag nanoparticles (nanocomposites) into chitosan matrixes improved mechanical, water vapour barrier and the antimicrobial properties of traditional chitosan coatings (Rhim et al., 2006). Ag-chitosan nanocomposites still allow for edible coatings to be obtained, with high elasticity being owed to a strong interaction between the nanocomposites and the polymer, producing a crosslinking effect, which leads to a reduction in the polymer of its free volume and molecular mobility (Medina-Ramirez et al., 2009). Nevertheless, the potential beneficial effects of Ag-chitosan nanocomposites application as edible coatings on the quality of fresh-cut products has not yet been addressed.

Nanotechnology is constantly progressing, and associated safety regulations are being addressed. To ensure consumer safety, food consisting of engineered nanomaterials are considered to be a novel food, if they have not been used for human consumption to a significant degree within the EU before May 15, 1997 (EU, 2015). Furthermore, engineered nanomaterials contained in food must be labelled in the ingredients list (EU, 2011). Nanomaterials used in food contact materials must be explicitly authorized, although 'not all nanomaterials have new hazard properties compared with larger–sized counterparts, and therefore, a case–by–case assessment is necessary' (EFSA, 2018). Materials treated with silver to avoid microbial growth are regulated by the Biocidal Products Regulation (EU, 2012). To date, the only nanosilver product authorized in Europe is NM 300 K (agpureW10) (Schneider, 2017),

The objective of this work was to study the effects of Ag-chitosan nanocomposites on the quality of fresh-cut melon during storage at 5 °C. The effect of the chitosan source (commercial or extracted from red claw crayfishes) was also studied.

2. Material and methods

2.1. Plant material

Piel de Sapo (*Sancho cv.*) melon (*Cucumis melo*) was grown by the company Fruca (Balsapintada, Murcia, Spain) under protected conditions in the Mediterranean climate of Fuente Álamo area (Murcia, Spain). Melons were hand-harvested in August at the commercial maturity stage, and 30 km was transported to our laboratory. Plant material was carefully inspected, and fruit free from damages and with uniform size and external skin colour was selected. Fruit was stored at 5 °C with 90–95% relative humidity (RH) until the next day, when processing was conducted.

2.2. Chitosan materials

Chitosan used in this study was obtained commercially (≤75% deacetylated; Sigma Adrich, Germany) or was extracted from red claw crayfish (*Cherax quadricarinatus*). The fish were grown in an aquaculture farm (Secretaría de Sustentabilidad, Medio Ambiente y Agua; Jesús María, Aguascalientes, Mexico) in a polyvinyl chloride tube growing system, simulating a natural cave environment. They were fed with Camaronina (Purina, Vevey, Switzerland) until they were approximately 15 cm (60–70 g), when they were sacrificed by freezing (−18 °C for 24 h). Then, exoskeletons from dead red claw crayfishes were manually separated from meat and viscera. Chitosan was extracted from the exoskeletons, as described by Romo-Zamarrón et al. (2014). Briefly, the exoskeletons were dehydrated at 50 °C for 18 h within a convection oven until constant weight. Subsequently, deproteinization, demineralization and deacetylation were performed as follows. Deproteinization of exoskeletons was carried out with 2 M NaOH (1:3, *w:v*) under constant agitation in a shaking platelet for 2 h at 65 °C. Then, demineralization of the exoskeletons was conducted using 4.9 M HCl in relation 1:2 (*w:v*) with the constant agitation in a shaking platelet for 1 h at 40 °C. Finally, deacetylation was carried out with 12.5 M NaOH in relation 1:4 (*w:v*) for 1 h at 80 °C. Between each of the latter three processes, the samples were rinsed with distilled water until neutral pH was reached. The extracted chitosan showed DD and solubility in 0.12 M acetic acid of 82.45 and 96.5%, respectively.

2.3. Preparation of Ag–chitosan nanocomposites and incorporation into chitosan coatings

Ag-chitosan nanocomposites were prepared according to Medina-Ramirez et al. (2009) but with modifications. A reduction of $AgNO_3$ with $NaBH_4$ was prepared in a reactor microwave oven (CEM 908005, Matthews NC, USA). Briefly, 100 mg of chitosan (commercial or extracted) was mixed with 10 mL of 0.17 M acetic acid and was stirred for 20 min until it was completely dissolved. Then, 37.5 mg of $AgNO_3$ was added, and the mixture was placed in the microwave reactor. The microwave program was as follows: 1) heating at 90 °C min^{-1} (60 W); 2) reaction at 90 °C (60 W) for 3 min; and 3) cooling for 20 min (at 90 °C min^{-1}). The solution was further cooled in an ice bath under constant stirring. Subsequently, 2 mL of cold 0.15 M $NaBH_4$ solution was added to the solution. Then, the microwave program was repeated with a programmed dropwise addition of 2 mL of 0.25 mM ascorbic acid solution. The Ag–chitosan nanocomposites had a diameter size of 20–40 nm according to TEM (Philips Tecnai 12 microscope coupled with a camera MegaView 3; Philips, Amsterdam, The Netherlands) images (Fig. 1).

Edible coatings from commercial and extracted chitosan were prepared in acidic water (57 mM lactic acid) to a final chitosan concentration of 1.5 g L^{-1}, and the pH was adjusted to 4.2 ± 0.2 with lactic acid. The concentration was selected to allow for a uniform thickness to cover the fresh-cut melon cylinders. The pH was adjusted to 4.2 ± 0.2 because chitosan has shown higher antimicrobial activity at such pH range where the protonation degree is higher (Younes et al., 2014). Finally, glycerol was added at 2 g L^{-1} to the edible coatings as a plasticizer.

The incorporation of Ag-chitosan nanocomposites into chitosan coatings was prepared at a ratio of 1:40 (*w:v*) under constant stirring for 24 h at room temperature. Treatment descriptions and nomenclature in this study are provided in Table 1.

2.4. Processing of fresh-cut melon and storage conditions

Minimal processing was carried out in a disinfected cold room at 10 °C. Unpeeled melons were pre-washed with cold tap water (1 min; 5 °C). Whole melons were then washed with 80 mg L^{-1} of peroxyacetic acid (PAA) for 2 min (5 °C) to avoid cross contamination from the melon