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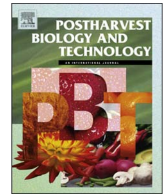
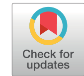


Effects of fungal chitosan nanoparticles as eco-friendly edible coatings on the quality of postharvest table grapes



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A R T I C L E I N F O

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A B S T R A C T

Chitosan edible coatings are a potential alternative for extending the postharvest life of fruit. The properties of chitosan can be enhanced when it is used in the form of nanoparticles. The aim of this study was to evaluate the in vitro antimicrobial activity and the effect of gel + fungal chitosan nanoparticles on some physicochemical properties (total soluble solids, pH, titratable acidity, reducing sugar content and moisture content) and sensory characteristics of grapes stored at 12 °C and 25 °C. The chitosan nanoparticles were prepared by ionic gelation and then underwent a heat treatment (50 °C/1200 × g/30 min). Characterization of the particles was carried out by dynamic light scattering and scanning electron microscopy. The microdilution test was used to determine the minimum inhibitory (MIC) and bactericidal concentration (MBC) of chitosan nanoparticles against food-borne pathogenic bacteria. To obtain the coatings to be tested, chitosan was solubilized in 1% acetic acid ($20\,g/L^{-1}$) and nanoparticles were subsequently added (MIC/2, MIC and 2MIC). The produced nanoparticles were spherical, had a medium size of 128.3 nm and showed an inhibitory effect against pathogenic foodborne bacteria with MIC values ranging from 2 to $3\,g/L^{-1}$. The edible chitosan nanoparticle coatings were responsible for delaying the ripening process of the grapes resulting in decreased weight loss, soluble solids and reducing sugar contents as well as increased moisture retention and preservation of the titratable acidity values and sensory characteristics. Therefore, the use of edible coatings containing chitosan nanoparticles at different concentrations (MIC/2, MIC, 2MIC) can be a promising strategy to improve the post-harvest quality of grapes.

1. Introduction

Grapes are one of the most economically important fruit, with an annual production of approximately sixty nine million tonnes (Nowshehri et al., 2015). They are considered a highly perishable, non-climacteric fruit with limited shelf life due to loss of firmness, berry drop, stem discolouration, desiccation and rot by microorganisms. Furthermore, the presence of microorganisms on the surface of the fruit can damage the microbiological safety and quality of the final product (Meng et al., 2008; Sousa et al., 2013). To control the spoilage and contamination of the food and the economic losses and risks to the health they cause, the focus of the food industry is on developing new strategies that increase shelf life and inhibit microbial contamination of food (Theron and Lues, 2007; Elsabee and Abdou, 2013).

Among the natural methods for preserving fruit, the use of edible chitosan gel coatings has been widely studied due to their antimicrobial activity and low toxicity to mammalian cells (Elsabee and Abdou, 2013; Kerch, 2015). Chitosan is a polysaccharide composed of β-1.4-D-glucosamine linked to *N*-acetyl-glucosamine residues and is naturally present in fungi cell walls or can be extracted by the deacetylation of chitin, which is a polysaccharide present in the exoskeleton of marine invertebrates. Fungi biomass is a promising eco-friendly alternative for

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obtaining chitosan because it is not affected by seasonal factors, can be produced on a large scale using industry wastes as a cheap substrate and does not contain any of the proteins that induce allergic reactions to crustaceans (Berger et al., 2014; Oliveira et al., 2014).

Chitosan edible coatings are considered one of the potential technologies to ensure the microbiological safety of foods. Nevertheless, the incorporation of nanostructures into films and coatings can improve the properties of these materials (Osheba et al., 2013; Bougnicourt et al., 2014; Pilon et al., 2014). Chitosan nanoparticles are a material with excellent physicochemical properties because they act as a more potent antimicrobial than chitosan gel. This is due to their high surface area and charge density, which effectively interact with the negatively charged surfaces of bacterial cells (Arora and Padua, 2010; Ing et al., 2012; Osheba et al., 2013; Pilon et al., 2014).

Although several studies have shown satisfactory results from the use of edible films and coatings based on chitosan gel on fruit and vegetables, there have been insufficient studies of chitosan nanoparticles incorporated into the matrix of the polymeric coatings. Therefore, due to the recognized biological potential of chitosan and its possible use in nanoparticles form, the aim of this research was to analyse, for the first time, the use of an edible fungal chitosan nanoparticle-based coating for table grape conservation.

2. Materials and methods

2.1. Materials

Grapes (*Vitis labrusca* L.) were purchased from CEASA (Supplies and Service Company of Pernambuco, Recife, Brazil) and selected according to size and colour; they showed no signs of deterioration or mechanical damage.

The chitosans (KiOfine® and KiOnutrime®) were provided by Kitozyme® Company and are both fungal chitosans from non-genetically modified *Aspergillus niger* mycelium. KiOnutrime® and KiOfine® are alternatives to crustacean-derived chitosan. The other substances used were obtained from commercial sources.

2.2. Preparation and characterization of chitosan nanoparticles

Chitosan nanoparticles were prepared by an ionic gelation process. First, 0.5 g of fungal chitosan obtained from Kitozyme (KiOfine®, deacetylation degree of 89%, Molecular weight of 4.35×10^4, extracted from *Aspergillus niger*) was dissolved in 50 mL of 2% acetic acid and stirred for 30 min. Then, 20 mL of tripolyphosphate (TPP) solution (1%) was added to the chitosan solution dropwise (0,005 mL s^{-1}) using a peristaltic pump (Atlas Syringe Pump) under vigorous magnetic stirring at room temperature (Calvo et al., 1997). Once the dropwise addition was complete, the nanoparticle solution was stirred for an additional 2 h. Then, the chitosan nanoparticles were purified by centrifugation at 18,000 × *g* for 10 min. The supernatant was discarded, and the pellet was redispersed by vortex mixing in deionized water. The washing procedure was repeated approximately 5 times.

The final thermal treatment used to individualize and restructure the nanoparticles was performed by stirring a purified suspension of chitosan nanoparticles at 1200 × *g* for 30 min at 50 °C according to the procedure described by Bougnicourt et al. (2014). The nanoparticles were then lyophilized.

Infrared spectrometry using KBr disks in a Bruker 66 Spectrometer (Bruker Corporation Inc., Billerica, MA, USA) was used to characterize the chemical structure of chitosan and chitosan nanoparticles. The morphology and particles size of the chitosan nanoparticles were characterized by scanning electron microcopy (SEM) (SEM Quanta 200 FEI). The dry nanoparticles was placed onto an aluminium stub covered with a double-sided carbon adhesive disc (Taab, Berkshire, UK). The sample was coated with a 10 nm thick gold film using a sputter coater. The coated sample were examined using an electron acceleration voltage of 20 kV. The zeta potential of the nanoparticles was measured using a Malvern Zetasizer (model Nano ZS90, Malvern, UK). The analysis was performed at a scattering angle of 90° at 25 °C in 633 nm.

To determine the hydrodynamic diameters of the particles, 3 mL of chitosan nanoparticles solution was placed in a polystyrene cuvettes and analyzed by Dynamic Light Scattering system (Zetasizer Nano ZS90, Malvern Instruments, UK) radiating an incident light with wavelength of 633 nm to measure the intensity of the scattered light at an angle of 90° under the temperature of 25 °C. Particle size was calculated according to the Brownian movement of the nanoparticles by using the Stokes-Einstein equation (Tsai et al., 2011).

2.3. Determination of the antimicrobial activity of the chitosan nanoparticles

The determination of the antimicrobial activity of the chitosan nanoparticles against foodborne pathogenic bacteria (*Staphylococcus aureus* – ATCC 6538*, Listeria monocytogenes* – ATCC 7664*, Pseudomonas aeruginosa* – ATCC 9027*, Salmonella* spp. – ATCC 14028*, Escherichia coli* – ATCC 25922) was performed based on the microdilution test using a 96-well microplate.

The bacteria were cultivated in brain-heart infusion (BHI) broth for 24 h, at 37 °C and an initial inoculum of 10^8 CFU mL^{-1} (Berger et al., 2014). The nanoparticles (20 g L^{-1}) were dispersed in deionized water.

The chitosan nanoparticles, diluted in water, were inserted into the wells at concentrations ranging from 0 to 0,6 g L^{-1}; then, BHI broth and the inoculum of each bacteria were added such that the final volume of each well was 100 μL. The microplate was incubated at 37 °C for 24 h, and then, 30 μL of resazurin was added to each well and the plate was incubated at 37 °C for an additional 1 h. After the 1 h incubation, the colour change was analysed (blue colour – no bacterial growth, pink and purple – the presence of viable cells for growth) to determine the minimal inhibitory concentration (MIC).

After determining the MIC, the first concentration that had no visible growth and the two concentrations immediately higher were grown in petri dishes containing BHI agar for minimal bactericidal concentration (MBC) determination. Aliquots (20 μL) of negative wells (without resazurin) were taken and plated in Mueller-Hinton Agar and incubated at 37 °C for 48 h. The MBC determined corresponded to the lowest concentration at which microbial growth was not observed after incubation (Fernandes et al., 2008).

2.4. Preparation and application of edible coatings in grapes

Grapes one day after harvest were previously selected based on the uniform colour, size and firmness as well as the absence of blemishes or disease. Grapes were immersed in sodium hypochlorite (1%) for 15 min, washed with potable water and left to dry for 2 h. The grapes were then immersed for 3 min in the coating solutions containing different amounts of chitosan nanoparticles (MIC/2; MIC and 2MIC), diluted in a fixed concentration of chitosan gel (20 g L^{-1}) and left to dry for 30 min.

For the formation of chitosan gel, the microbiological chitosan (KiOnutrime®) was diluted (20 g L^{-1}) in 1% acetic acid, and the pH was adjusted to 5.8 (Shigemasa and Minami, 1996).

Each treatment included 40 grapes that were stored for 12 d at room temperature (25 °C) or for 24 d under refrigeration (12 °C) (Santos et al., 2012; Oliveira et al., 2014).

2.5. Physicochemical analyses on grapes

Coated grapes and fruits that were not coated with chitosan nanoparticles were stored at room temperature (25 °C/12 d) and under refrigeration (12 °C/24 d) and were evaluated every 6 d for weight loss and general quality aspects such as soluble solids content, titratable acidity (AT), pH, reducing sugar content and moisture content. The

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