Radiation Physics and Chemistry 80 (2011) 1406–1413

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Improved postharvest quality in patagonian squash (*Cucurbita moschata*) coated with radiation depolymerized chitosan

Maria Alicia Pugliese [a], Maria Teresa Goitia [a], Mariana Yossen [b], Norma Cifone [a], Enrique Agulló [a], Noemi Andreucetti [c,*]

[a] Laboratorio de Investigaciones Básicas Aplicadas en Quitina, Departamento de Química, Universidad Nacional del Sur. Avenida Alem 1253, B8000CPB Bahía Blanca, Argentina
[b] Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), CONICET-Universidad Nacional del Litoral, Ruta Nacional 168-Paraje ''El Pozo'', 3000 Santa Fe, Argentina
[c] Laboratorio de Radioisótopos, Departamento de Química, Universidad Nacional del Sur, Avenida Alem 1253, B8000CPB Bahía Blanca, Argentina

ARTICLE INFO

Article history:
Received 25 April 2011
Accepted 5 July 2011
Available online 13 July 2011

Keywords:
Gamma irradiation
Depolymerized chitosan
Cucurbita moschata
Squash
SEC-MALLS-RI

ABSTRACT

Different molecular weight chitosans were evaluated on the decay of coated Anquito squashes (*Cucurbita moschata*) as well as the maintenance of the fruit quality along five storage months. The original chitosan (Mw=391 kDa, 83% DD), was depolymerized by gamma radiation. Apart from chain scission, other chemical changes were not detected by FTIR or UV–vis analyses. The molecular weight characterization of chitosans was done by size exclusion chromatography with dual light scattering and concentration detection (SEC-MALLS-RI). The coating effectiveness was evaluated on the following parameters: fungal decay incidence, weight loss, firmness, total reducing sugar, soluble solid, flesh color, carotene content, pH and titratable acidity. No sign of fungal decay was observed in squashes coated with 122 and 56 kDa chitosans, which were also the most effective treatments in reducing the weight loss. The chitosan with Mw=122 kDa was also the best treatment considering firmness, internal aspect, sugar and carotene content. Then, radiation degraded chitosan was better in *C. moschata* preservation than the original chitosan.

1. Introduction

The Butternut squash, a variety of *Cucurbita moschata*, is also commercially known in Argentina as "Anquito" or "calabacita". Because this variety has a sweet, nutty taste, it has become very popular during the last years (de Escalada Pla et al., 2007). For this reason, nearly 800 hectares of the lower valley of the Rio Negro River, in the northeast of Patagonian region, are cultivated using the Anquito squash variety. The Rio Negro supplies the water volumes for the development of the agriculture in this semi-arid region, by means of a complex channel irrigation system. Most of the produced Anquito is commercialized into the principal markets of Argentina and the surplus is exported to Europe.

Taking into account the habitual postharvest practices, the Anquito squashes from the Rio Negro are stored in farms, piled up and covered with a layer of straw inside plastic bags, so as to avoid the squash dehydration or ripening produced by climate changes. Also, as a consequence of fungi development, different commercial fungicides have been used to extend shelf life.

The fact that consumers around the world demand high quality food without chemical preservatives, gives rise to the search of new natural antimicrobials. Because of this fact, there are many works in the literature, related to the use of chitosan as the base of many edible films and coatings. The chitosan, a natural cationic polysaccharide composed of β-1-4-linked glucosamine units with various quantities of n-acetylated glucosamine residues, is a by-product of chitin extracted from shrimp waste. It is known that the chitosan-based films or coatings are non-toxic and safe, and they exhibit antifungal activity prolonging the storage life and controlling the decay of strawberries, tomatoes, baby carrots, peaches, pears, kiwifruit, litchi, apples, honey tangerine fruits, among others vegetables. (Zhang and Quantick, 1997; Jiang and Li, 2001; Chien et al., 2007a, 2007b; Badawy and Rabea, 2009).

Microbiological activity of chitosan has been detected for many bacteria, (Muzzarelli et al., 2001; No et al., 2002; Zheng and Zhu, 2003; Liu et al.,2006) and fungi (Hernández-Lauzardo et al., 2008; Chien et al., 2007b; Hernández-Muñoz et al., 2006). However, there are yet some controversial results about the antimicrobial and antifungal activities and their relation with the chitosan molecular weight. Tikhonov et al. (2006) considered that part of this uncertainty arises from the fact that various researchers have been using chitosans depolymerized by different chain degradation methods. For example, differences were found between the antimicrobial

* Corresponding author. Tel.: +54 291 4595100; fax: +54 291 4595160.
E-mail address: andreuce@criba.edu.ar (N. Andreucetti).

0969-806X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.radphyschem.2011.07.003

activities of a low molecular weight chitosan (5 kDa) chemically depolymerized with other of similar molecular weight obtained by enzymatic hydrolysis, and these differences were explained in relation to the modification in the n-acetyl distribution into the chitosan chains.

Then, based on the uncertainty present in the antimicrobial action mechanism, the specification of the method used to change the native chitosan and its physicochemical characterization would be important before its application.

In a previous experience, Cifone (2006) studied the effect of coating Anquito squashes from the lower valley of Rio Negro with different concentrations of a chitosan extracted from shrimp bio-waste of the fishery industry (Mw=391 kDa and 83% DD). The results of this study verified that the optimal chitosan concentration was 0.2% (w/v), because it provided the best results, maintaining postharvest quality and improving antifungal properties.

Besides chitosan concentration, some coating properties, relating to the gas permeability and the antifungal effect, are also very dependent on the molecular weight of polymer. (Chen and Hwab, 1996; Zhang and Quantick, 1997; Hernández-Lauzardo et al., 2008; Badawy and Rabea, 2009). It is known that the molecular weight of polysaccharides can be modified by gamma irradiation; so as a result different molecular weights can be obtained by depolymerization of the original chains. Apart from radioinduced scission in specific (glycosidic bond) sites, any other significant changes in the chemical structures of gamma-treated chitosan were not detected (Choi et al., 2002; Hai et al., 2003).

The aim of this research was to study the effect of the chitosan with different molecular weights on the postharvest decay, and the quality maintenance of the coated Anquito squash (*C. moschata*) during storage. For this purpose, the original chitosan (Cifone, 2006) was irradiated within a dose range from 7 to 100 kGy in a solid state. Afterwards, the depolymerized chitosans with 10, 45 and 100 kGy doses were selected for this work. Chemical modifications of chitosans were characterized by UV and FTIR spectroscopy, and the molecular weight changes were characterized by size exclusion chromatography with dual light scattering and concentration detectors (SEC-MALLS-RI). Anquito squashes were coated using 0.2% (w/v) chitosan solutions and stored at 20 °C and 55% relative humidity throughout five months. Changes in visual quality parameters, weight loss, firmness, total reducing sugar, soluble solids, flesh color, carotene content, acidity and pH were measured during storage. At the end of the storage period, all marketable squashes were cut down and internal characteristics such as pulp color, dryness and general appearance of the fruits were also evaluated.

2. Materials and methods

2.1. Chitin and chitosan

The shrimp waste contains the chitin, a nitrogen containing polysaccharide, among other compounds. The chitin is a high molecular weight linear polymer of n-acetyl-D glucosamine, insoluble in most of the solvents. The extraction of the chitin was done in the Laboratory of the Basic and Applied Research on Chitin (LIBAQ, Chemistry Department, Universidad Nacional del Sur). After that, the chitin was deacetylated to produce the chitosan, a derivative soluble in dilute acetic acid solution. This process was carried out in 45% sodium hydroxide solution over 90 min at 90 °C, in a stirred 10 l batch reactor. From these reaction conditions, an 83% DD (degree of deacetylation) was obtained, based on the first derivative UV method applied to the spectra of the n-acetylglucosamine standards and the chitosan, using a Shimadzu UV/Visible 1603 spectrophotometer (Tan et al., 1998).

2.2. Irradiation of chitosan

The original chitosan was irradiated in solid state, using the 60-Co source at the Centro Atómico Ezeiza (CAE) in Argentina, at room temperature. The gamma radiation doses ranged from 7 to 100 kGy, at an approximate 10 kGy/h dose rate. The doses were measured with Red/Amber Perspex Dosimeter.

The first derivative UV method was applied to the radiation depolymerized chitosans spectra and changes in the deacetylation degree were not found, comparing them with the original chitosan. Also, by means of a Nicolet 520 FTIR spectrophotometer, thin films of irradiated and original chitosans were compared. Within the precision of this method, changes were not detected in the chemical structures of the gamma-treated samples.

2.3. SEC-MALLS-RI characterization

The molecular weight changes induced by gamma irradiation on the original chitosan have been investigated by size exclusion chromatography (SEC) using a Waters Breeze HPLC with a Waters pump model 1525 in GPC mode, an automatic injector Waters 717 plus and two detectors: a Waters 2414 (Model 440) refractive index (RI) detector and, a DAWN DSP Multi Angle Laser Light Scattering (MALLS) from Wyatt Technology Corporation. Five Waters Ultra-Hydrogel (UH) 7.8 mm $\times$ 300 mm columns, with (120, 250, 500, 1000 and 2000) Å pore size and (5000–7×10^6) g/mol exclusion limit range. They are packed with hydroxylated polymethacrylate-based gel, ideal for the analysis of aqueous soluble polymers. Since the chitosan is only soluble in acidic solutions, the analyses were carried out using the following mobile phase conditions: buffer CHCOOH/CHCOONa solution, 50 mM and pH=4.50. This mobile phase has been also reported as adequate for chitosan molecular weight determination by other authors (Choi et al., 2002; Yanagisawa et al., 2006; Nguyen et al., 2009a). The sample concentration was 1 mg/mL, filtered with a 0.45 μm PTFE membrane previous to the injection. The injected volume was 200 μL, the flow rate of 0.8 mL/min and the column and detector temperatures were maintained at 30 ± 1 °C. The SEC calibration with dual multiangle-light-scattering/concentration detectors was found using the Astra software (Wyatt Technology Corp, Astra 4.70.07 Software for windows).

The SEC/RI calibration curve was obtained using seven pullulan standards (Shodex Standard P-82, No 30901, Showa Denko). Pullulan is a linear polysaccharide consisting of maltotriose units also known as α-1,4-; α-1,6-glucan. Three glucose units in maltotriose are connected by a α-1,4 glycosidic bond, whereas consecutive maltotriose units are connected to each other by a α-1,6 glycosidic bond. The accuracy of this calibration depends on the assumption of similar solution behavior of the chitosan and the chosen standard. The MALLS photodetectors were normalized using a nearly monodisperse pullulan standard (Mw=47,300; Shodex P-82) in the acetate buffer used as SEC mobile phase. The values of $dn/dc=0.146$ mL/g for pullulan, and $dn/dc=0.190$ mL/g for chitosans, (Brugnerotto et al., 2001), were related in mathematical equations required to absolute Mw determination.

2.4. Material and sample treatment

The Anquito squashes (*C. moschata* Duchesne ex Poiret) var. INTA Frontera were cultivated in the lower valley of Rio Negro River. The seeds were planted in the spring. The harvest was made in April and mature squashes underwent a curing process for two weeks at 24 °C with good air circulation after hand harvesting. Afterwards, they were sent to our laboratory. Approximately 180 very healthy fruits, within a 0.9–1.4 kg weight range, were chosen for this experience. After being carefully washed and