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Research note

Use of chitosan solutions for the microbiological shelf life extension of papaya fruits during storage at room temperature

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ABSTRACT

Chitosan solutions were used to extend the microbiological shelf life of papaya fruits during storage at room temperature. The fruits were coated using different chitosans (molecular weights of 150 or 300 kDa) and, stored for 20 days under ambient conditions. Evolution curves of mesophilic bacteria, yeasts and molds on papaya fruits during the storage period were constructed for coated and uncoated samples. These curves were represented by the Gompertz model, in order to estimate the shelf life of papaya fruits. The results showed that the 150 kDa chitosan solution was more adequate to preserve the papaya fruits. After 10 days of storage, the Log (CFU/g) of mesophilic bacteria and yeasts and molds were, respectively, 1.3 and 2 times lowest for coated fruits. The use of 150 kDa chitosan solutions extended in about 4–7 days the shelf life of papaya fruits, during the storage at room temperature.

1. Introduction

Papaya (*Carica papaya* L.) is a native fruit of tropical America and is widely distributed in all areas of the tropical world. It is produced mainly for the consumption of fresh fruit, juice, or jams. Papaya fruit is the most popular fruit in Brazil, which is currently the world's largest producer, responsible for a quarter of the global production and the third largest exporter (Cia, Pascholati, Benato, Camili, & Santos, 2007). As a tropical commodity, the storage has serious limitations, which results in their rapid deterioration and high incidence of rots. It is estimated that the papaya postharvest losses due to microbiological diseases account for almost 93% of the losses (Bautista–Baños, Sivakumar, Bello–Pérez, Arce, & Hernández–López, 2013). Given the susceptibility of papaya to postharvest diseases is high, a careful management is necessary (Lima et al., 2013).

Some methods are reported in order to avoid the postharvest losses of papaya (and others fruits), and consequently, extend its shelf life, such as, application of fungicides, heat treatment, irradiation, use of biocontrol agents, use of natural coatings (essential oils, chitosan) and others (Ali, Muhammad, Sijam, & Siddiqui, 2010; Bautista–Baños et al., 2013; Cia et al., 2007; Maqbool et al., 2011; Rojas–Graü, Oms–Oliu, Soliva–Fortuny, & Martín–Belloso, 2009). In addition, the use of chitosan coatings outward due to its relative low–cost, effectiveness, ease of application and antimicrobial properties (Kim et al., 2011; Wang & Gao, 2013). These coatings act as barriers to water loss and gas exchange by creating a micro–modified atmosphere around the product (Han et al., 2014; Rojas–Grau et al., 2009; Zhang, Yu, Xiao, Wang, & Lei, 2014).

The use of chitosan coatings has been successfully investigated to extend the shelf life of several food products, such as, mangoes (Djioua et al., 2010), water caltrop (Zhan, Hu, & Zhu, 2011), Chinese water chestnut (Peng, Li, & Yin, 2013), eggs (Pujols et al., 2014), fruit–based salad (D'Amato, Sinigaglia, & Corbo, 2010) and Eksotika II papaya (Ali et al., 2010). In spite of this, there is a lack of information regarding the use of chitosans with different molecular weights as coatings, to extend the papaya shelf life. The literature shows that the biological activities of chitosan including antimicrobial and antioxidant activities are dependent on molecular weight (Li, Lin, & Chen, 2014).

This work aimed to use chitosan solutions with different molecular weights (150 and 300 kDa) in order to extend the microbiological shelf life of papaya fruits during its storage at room temperature.

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2. Materials and methods

2.1. Preparation and characterization of chitosans

Chitosans were obtained from shrimp wastes (*Penaeus brasiliensis*) according to our previous work (Weska, Moura, Batista, Rizzi, & Pinto, 2007). In brief to obtain a chitosan paste, shrimp wastes were submitted to demineralization, deproteinization, deodorization, deacetylation and purification steps. Chitosan paste was dried using two different techniques, in order to obtain chitosan powder with 150 and 300 kDa, respectively. Chitosan powder with molecular weight of 150 kDa was obtained by spouted bed drying, with particle size ranging from 68 to 75 μm (Dotto, Souza, & Pinto, 2011). Chitosan powder with molecular weight of 300 kDa was obtained by tray drying (Halal, Moura, & Pinto, 2011), being the samples ground (Wiley Mill Standard, model 03, USA) and sieved until the discrete particle size ranging from 68 to 75 μm. The molecular weight values of the samples were determined by the viscosimetric method (Gupta & Jabrial, 2006).

Chitosan powder samples were characterized according to the moisture and ashes content (AOAC, 2000). The deacetylation degree (DD) was estimated by Fourier transform infrared spectroscopy (FTIR) (Shimadzu, Prestige 21, Japan), based on the absorbance of amide I band ($A_{C=O}$) at 1655 cm^{-1} and hydroxyl groups (A_{-OH}) at 3450 cm^{-1} (Sabnis & Block, 1997). The crystallinity index (CI) was assessed by X–ray powder diffractometry (XRD) (Rigaku, Miniflex 300, Japan), using the intensities of the peaks at 110 lattices (I_{110}, at $2\theta \approx 20°$corresponding to maximum intensity) and at $2\theta \approx 16°$ (amorphous diffraction, I_{am}) (Al Sagheer, Al–Sughayer, Muslim, & Elsabee, 2009). Scanning electron microscopy (SEM) was carried out to verify the structural, morphological and textural characteristics of the chitosan samples (Goldstein et al., 1992). The images were obtained at 10 kV with magnification of 5000 × (Jeol, JSM–6060, Japan).

2.2. Preparation of chitosan solutions

Chitosan solutions (150 and 300 kDa) were prepared by diluting 5.0 g of chitosan powder in 1.0 L of 0.5 mol L^{-1} acetic acid, using moderate magnetic stirring (Marte, MAG–01H, Brazil) at room temperature for 5 h. Then, the solutions were centrifuged (Fanem, 206 BL, Brazil) at 5000× *g* for 15 min (Dotto, Moura, Cadaval, & Pinto, 2013; Souza, Monte, & Pinto, 2013), in order to remove the supernatant.

2.3. Coating and storage of papaya fruits

Commercial papaya fruits were purchased from a local market one day after the harvest. The fruits were washed with distilled water. To guarantee the same maturation degree, pH was measured and the values ranged from 5.4 to 5.6 (the pH was measured by sampling). The whole fruits were separately immersed in chitosan solutions (150 or 300 kDa) during 5 min, and after, were removed from the solutions using a sterile rod. In this way, it was estimated that the amount of chitosan applied per unit area was of 10 g m^{-2}. Then, papaya fruits were stored at room temperature during 20 days (the temperature was not controlled and, during the storage period, the room temperature ranged from 18 to 24 °C. The air relative humidity ranged from 60 to 80%). Control samples (papaya fruits without chitosan coating and also papaya fruits coated only with acetic acid) were also stored under the same conditions. The storage was performed in a room with lighting (six commercial lamps of 60 W). All experiments were made in triplicate ($n = 3$). The triplicate experiments were performed in parallel.

2.4. Microbiological analyses

Microbiological analyses of mesophilic bacteria and yeasts and molds were performed on the papaya fruits over time (0, 2, 5, 7, 10, 15 and 20 days of storage). These microbiological analyses were performed by standard methods (FDA BAM, 1998), using Plate Count Agar (PCA) (Oxoid) for mesophilic bacteria and Dichloran Rose Bengal Chloramphenicol agar (DRBC) (Oxoid) for yeasts and molds. The results were expressed in terms of CFU/g or Log (CFU/g).

2.5. Shelf life modeling

The Log (CFU/g) of mesophilic bacteria and yeasts and molds in papaya fruits along the storage time was represented by the modified Gompertz model (Zwietering, Jongenberger, Roumbouts, & van't Riet, 1990) according to Eq. (1):

$$y = k + A\exp\left\{-\exp\left[\left(\frac{2.718\mu_{max}}{A}\right)(\lambda - t) + 1\right]\right\} \quad (1)$$

where, y is the Log (CFU/g), k is the initial cell concentration (Log (CFU/g)), A is the maximum increase of cell growth attained at the stationary phase (Log (CFU/g)), μ_{max} is the maximum growth rate (Log (CFU/g)) day^{-1}, λ is the lag time (days) and t is the storage time (days).The parameters, k, A, μ_{max} and λ, were determined by the fit of Eq. (1) with the experimental data (Log (CFU/g) versus storage time) through nonlinear regression. The Quasi–Newton estimation method was used and the calculations were performed using the Statistic 7.0 software (Statsoft, USA). The coefficient of determination (R^2) and average relative error (ARE) were used to evaluate the fit quality.The microbiological shelf life (S.L.) was considered as the time necessary to reach a total cell count of 0.5×10^5 CFU/g. S.L. was estimated by the Gompertz parameters using the Eq. (2) (Corbo, Del Nobile, & Sinigaglia, 2006):

$$S.L. = \lambda - \frac{A\left\{\ln\left[-\ln\left(\frac{\log(0.5\times10^5)-k}{A}\right)\right]-1\right\}}{2.718\mu_{max}} \quad (2)$$

2.6. Tukey test

All experimental values, which need to be compared, were analyzed according to the Tukey test, with significance of 95% (Myers & Montgomery, 2002). If there is no significant difference between the values, $p > 0.05$. If there is significant difference between the values, $p \leq 0.05$.

3. Results and discussion

3.1. Chitosan characteristics

It is recognized in the literature that the antibacterial and antifungal potential of chitosan, and consequently its coating properties, are dependent of their characteristics (Pujols et al., 2014). However, the molecular weight effect is little studied. Thus, the use of chitosan solutions with different molecular weights was investigated, in order to extend the shelf life of papaya fruits. The characteristics of chitosan powder samples (which were used to obtain the chitosan solutions) with 150 and 300 kDa are shown in Table 1. The respective SEM images are shown in Fig. 1.

It was found in Table 1 that 150 kDa chitosan presented higher values of moisture content and lower values of crystallinity index than chitosan 300 kDa ($p \leq 0.05$). The deacetylation degree and