



Physicochemical, total phenols and pectin methylesterase changes on quality maintenance on guava fruit (*Psidium guajava* L.) coated with candeuba wax solid lipid nanoparticles-xanthan gum



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ABSTRACT

The objective of this work was to evaluate the application of candeuba wax solid lipid nanoparticles (SLN) and xanthan gum (XG) as coatings on guava, and their effect on the fruit's physicochemical and nutritional parameters, complementing a previous publication carried out by Zambrano-Zaragoza et al. (2013). The concentrations of SLN were selected according to those reported as the most (65 g/L) and least (75 g/L) efficient in post-harvest life preservation, and were compared to a coating of XG and untreated control samples. According to results, the submicron-sized systems used in the coatings with a particle size range of 267–344 nm, a polydispersity index < 0.2, and zeta potential of –22.8 to –30 mV remained stable during 8 weeks of storage. The best results were from the fruits coated with 65 g/L of SLN and stored at 10 °C, as they showed the lowest O₂ and CO₂ respiration rates and, consequently, less weight loss. They also had the best retention of ascorbic acid and total phenol content, with less change in fruit color compared to the control guava and those coated only with XG. These findings indicate that this batch continued their natural maturation process, but at a slower rate than the other samples. The firmness was affected by the activity of the enzyme pectin methylesterase, but results show that the 65 g/L coating was efficient in maintaining fruit texture. In contrast, the 75 g/L coating produced epoxy in the fruit, causing physiological damage. Finally, the guava coated with XG only had a maturation rate similar to that of the control fruit.

1. Introduction

Guava (*Psidium guajava* L.) is in great demand because of its flavor and nutritional composition, but is susceptible to chilling injury when stored at temperatures below 8 °C and has a short post-harvest life when conserved at room temperature. It is widely-consumed in its fresh state, but is also incorporated into processed products because of its palatable flavor and various dietary benefits for consumers (Lee, Choi, Cho, & Kim, 2010). Guava is rich in antioxidant activity, perhaps due to its high ascorbic acid (AA) and total phenol content (TPC), as these phytochemicals are ubiquitous in this fruit. Guava is also rich in pectic substances (Da Silva Cerqueira Leite, Tadiotti, Baldochi, & Oliveira,

2006). The enzyme pectin methylesterase (PME; EC: 3.1.1.11), also known as pectin esterase, catalyzes hydrolysis of the methoxyl group of pectins, producing pectin acid (Barnavon et al., 2001). The decrease in the degree of pectin methoxylation may, in turn, trigger various processes that can affect fruit texture and firmness (Tieman & Handa, 1994). Also, the gas exchange that occurs in the pericarp plays a major role during fruit maturation. Guava fruit consumes O₂, which then acts as a substrate for several metabolic processes that produce ripening, but is later released as CO₂. One method often used in fruit conservation involves edible films and coatings that function by regulating gas exchange (Salgado, Ortiz, Musso, Di Giorgio, & Mauri, 2015). There is evidence that the gas barrier properties of edible films and coatings can

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be improved through the addition of natural waxes (Bourtoom, 2008), these lipids present a lower O₂ and CO₂ permeability than the proteins and polysaccharides often used as continuous matrix (Bai, Hagenmaier, & Baldwin, 2003) and the fruit pericarp by itself. Low concentrations of O₂ around the fruit decrease the oxidation process and respiration rates maintaining the quality and nutritional compounds in fruits (Vaughn & Duke, 1984); Two common oxidation reactions in plants are: 1) those carried out by the polyphenol oxidase enzyme that catalyzes polyphenol compounds to o-diquinones; these metabolites are relevant in terms of food flavor (bitter, sweet, pungent, astringent) and aroma (Tomás-Barberán & Espín, 2001). 2) Ascorbic acid losses, the vitamin C degradation mainly proceed the aerobic pathway and depends upon oxygen concentration (Burdurlu, Koca, & Karadeniz, 2006). For these reasons, the addition of solid lipid nanoparticles (SLN) made with candeuba wax to such coatings promises better control by modifying the atmosphere around the fruit. Zambrano-Zaragoza et al. (2013) developed an edible coating based on SLN that, when applied to guava, lengthened the fruit's shelf-life. SLN are colloidal dispersions with particle sizes of 100–500 nm (Weiss et al., 2008) whose important features include low toxicity and excellent physical stability. SLN typically consist of three essential components: a solid lipid, a surfactant, and water. Lipid compounds are generally regarded as safe (GRAS) and have proven biocompatibility and biodegradability, since they are physiological lipids that occur naturally in organisms. Stabilizers often used in preparing SLN include alkyl aryl polyether alcohols and the triblock copolymers composed of polyoxypropylene (Pluronic® F68, F127), (Gupta, Kesarla, Chotai, Misra, & Omri, 2017). Pluronic F127 is an amphiphilic block copolymer that consists of two hydrophilic ethylene oxide blocks and one hydrophobic propylene oxide block. It has been approved for use in alimentary products by the U.S. Food and Drug Administration (FDA) (Rao et al., 2015). The properties of the surface area of nanometric systems differ in behavior from those of micrometric-sized systems, as they show high diffusion rates with low viscosities that facilitate their transport and application. Previously, Zambrano-Zaragoza et al. (2013) reported that adding low concentrations of SLN (60–65 g/L) to a xanthan gum (XG) coating matrix allow guava to continue maturing, but more slowly, since at those concentrations the SLN do not completely cover the fruit skin, thus permitting adequate transpiration and regulation of metabolic processes and reducing quality changes (firmness, skin color) and quantity losses in nutritional compounds, nevertheless the analysis of respiration rate and its effect in PME activity, TPC and AA were not conducted in this study, these authors also stated that the addition of high SLN concentrations (above 70 g/L) cause physiological damage and delay the maturation. The main aim of the present study was to evaluate the effect of SLN coatings at the concentration reported as “the best” (65 g/L) in the previous work on respiration rate, PME activity and TPC, and their relation to the effectiveness of nanocoatings on the regulation of maturation and physicochemical behavior of guava since no evidence about it has been reported, contrasting the findings with the addition of high concentrations of SLN (75 g/L).

2. Materials and methods

2.1. Materials

Pluronic® F-127 (poloxamer-407 Mw 9840–14,600 g/mol) was the stabilizer, xanthan gum derived from *Xanthomonas campestris* (Mw ≈ 2 × 10⁶ g/mol and $\eta_{\text{int}} = 7627$ mL/g) was used as the film-forming material, and propylene glycol (Mw 76.09 g/mol, $\rho = 1.036$ g/cm³) was the plasticizer. These materials were purchased from Sigma-Aldrich Chemical® S.A. de C.V. (State of Mexico, Mexico). Candeuba® wax (melting point 84 ± 2 °C) was acquired from Multiceras® S.A. de C.V. (Monterrey, Mexico). Distilled water was obtained using Milli-Q equipment (Millipore® Corp. Massachusetts, USA). All other chemicals were of analytical grade and were used without further purification.

2.2. Biological material

Green mature guavas (*Psidium guajava* L.) var. Media china from Calvillo, Aguascalientes, were purchased – free of coatings– in Cuautitlan, Mexico two days after harvested; 424 guavas were selected based on maturity, color, size, and uniformity of shape, considering an initial color of 115 ± 5°Hue. The fruits chosen were then randomly divided into 4 batches of 106 fruits each one, the coatings applied were 65 g/L of SLN-XG, 75 g/L of SLN-XG, XG (0.4 g/L) and control samples (uncoated). Three guavas were analyzed for respiration rate, PME activity, firmness, TPC and AA; for total color difference (ΔE) and weight loss (WL) 5 fruits were tested.

2.3. Solid lipid nanoparticle (SLN) preparation

The SLN were prepared using the hot high-shear stirring method described by Zambrano-Zaragoza et al. (2013). Briefly, the lipid phase was prepared with 100 g/L of candeuba® wax melted at 85 °C. The aqueous phase consisted of a Pluronic® F-127 solution (50 g/L), also at 85 °C. The melted wax was dispersed into the aqueous phase using a high-shear stirrer (Ultra-Turrax® T5; KikalborTechnik, Germany, with a S25N-25G, IKA® disperser element), and then cooled to 25 °C to obtain the SLN dispersion.

2.4. Determination of particle size (Ps), the polydispersity index (PDI), and zeta potential (ζ)

The particle size (Ps), polydispersity index (PDI) and zeta potential (ζ) of the SLN were measured during a period of 8 weeks. Ps distribution and PDI were determined using the laser light-scattering technique at 90° fixed angle, while ζ was estimated by electrophoretic mobility using a Z-sizer 4 (Zetasizer Nano Series, Malvern, Ltd., France) after appropriate dilution in Milli-Q water. Values were normalized by polystyrene standard dispersion ($\zeta = -55$ mV). Measurements were made in triplicate at 25 °C.

2.5. Coating-forming dispersions

Xanthan gum (XG) was dispersed in water at a concentration of 4 g/L. Incorporation was performed with a variable-speed agitator (Eurostar Power Control Visc, IKA®, WERKE). The edible coatings with SLN and XG were prepared from dilutions of the initial SLN suspension (100 g/L). Three systems (65 and 75 g/L of SLN-XG, and only XG at 4 g/L) were used. All dispersions contained 5 g/L of propylene glycol.

2.6. Application of coatings

The fruit was coated by dipping for 2 min in the coating-forming dispersion, followed by 1 min of draining. Then they were left to dry at 25 °C for 4 h. Immediately afterwards, they were transferred to refrigerated storage at 10 °C and RH ≈ 85% for 5 weeks. 10 guavas from each batch were taken from the refrigerator twice a week, 5 fruits were analyzed on the same day and the rest were stored 5 days at room temperature for further analysis. In this study, 32.6 g of coating were necessary to coat 1 kg of guava; therefore, every guava coated with the 65-g/L formulation contained 2.10 g of candeuba wax and those with 75-g/L contained 2.43 g. Every coated guava also contained 13.04 mg of xanthan gum and 16.3 mg of propylene glycol.

2.7. Quality attributes

Weight loss was expressed as the percentage decrease from initial weight, 5 guavas were tested each sampling day. Changes in color were evaluated in the fruit surface with a colorimeter Agrocólor® (Apollinaire Ltd, Agrotechnology, France) to determine the parameters R (Red), B (Blue) and G (Green), which were then transformed to the

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