



Use of solid lipid nanoparticles (SLNs) in edible coatings to increase guava (*Psidium guajava* L.) shelf-life

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ABSTRACT

The objective of this work was to prepare solid lipid nanoparticles (SLNs) based on the hot lipid dispersion method using a rotor–stator device in order to obtain a submicronic system and evaluate the effect of SLN-xanthan gum coatings on the guava shelf life. Candeuba® wax was used as a component of SLN. The coating was formulated with xanthan gum (4 g/L) and polyethylene glycol (5 g/L) in order to form a continuous film retaining the SLN. The SLN concentrations were varied from 60 to 80 g/L and were compared with the control and xanthan gum. These were evaluated ($n=3$) every third day. The film-forming dispersion was applied by dipping the guavas and storing them under refrigeration at 10 °C and 85% RH for 30 days. Every 5 days guava samples were transferred to room temperature (25 °C) in order to assess the effect of maturation on the changes in weight loss, chemical quality, texture and color. It was established that Candeuba® wax as a SLN maintained the quality of guavas, and that the SLN concentration in the film formation depends on the characteristics of the fruits. The best results were obtained with SLN concentrations of 60 and 65 g/L since at these concentrations, guavas showed the lowest range of weight loss and preserved the best quality compared to the fruits processed at concentrations above 70 g/L. In addition high contents of SLN cause physiological damage and also delay the maturation which can be observed by the greenness color without changes.

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1. Introduction

Nowadays, nanotechnology has quickly emerged as one of the most promising and attractive research fields in food industry. Nanoemulsions and nanoparticles may contribute to barrier properties and functionality of coatings for fruit preservation since these systems show an increased surface area. Actually, submicronic systems allow better distribution and homogeneity on the fruit skin with several advantages for various applications and uses (Rao & McClements, 2012; Zambrano-Zaragoza, Mercado-Silva, Gutiérrez-Cortez, Castaño-Tostado, & Quintanar-Guerrero, 2011).

Solid lipid nanoparticles (SLNs) are lipid colloidal submicronic systems that have been developed to encapsulate and deliver lipophilic functional components. SLNs are typically prepared using the hot homogenization process where a lipid and an aqueous surfactant solution are homogenized at a temperature above the melting point of the lipid

to produce an oil–water nanoemulsion. This hot nanoemulsion is then cooled at room temperature leading to the formation of solid particles (Quintanar-Guerrero, Fessi, Allémann, & Doelker, 1996; Vitorino, Carvalho, Almeida, Sousa, & Pais, 2011). SLNs are systems with high of level technological potential in different areas, including food industry.

Guava (*Psidium Guajava* L.) is an important fruit crop in the subtropical regions. Mexico is the second largest producer of guava in the world. In declining nature, short postharvest life and susceptibility to chilling injury limit the potential for its commercialization (Mercado-Silva, Benito-Bautista, De los & García-Velasco, 1998). Guava, being a highly perishable fruit, undergoes rapid postharvest ripening within few days under ambient conditions. However, its storage below 10 °C may cause severe chilling injury symptoms in the form of skin surface pitting and flesh browning (Gonzalez-Aguilar, Zacarias, Perez-Amador, Carbonell, & Lafuente, 2000; Singh & Pal, 2008a; Wang, Duan, & Hu, 2009). For this reason, guava is an excellent model to evaluate the effectiveness of submicron-sized systems since it is a highly perishable raw fruit. Researchers have made continuous attempts to delay the softening process of detached guava by making

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use of different technologies; a few of them have been proved to be effective in some parameters such as texture or color but not in others like flavor and physiological aspects. Technologies like modified atmosphere packaging, controlled atmosphere storage, surface coating and shrink wrapping have the same problems and do not involve the best quality parameters (Pal, Ahmad, Roy, & Singh, 2004; Singh & Pal, 2008b). The use of edible waxes together with low temperatures can increase the shelf life by creating an internally modified atmosphere that modulates fruit metabolism and moisture loss. The covers are often made with polysaccharides, lipids or proteins. Polysaccharides allow the passage of water vapor and prevent gas permeation, while waxes and oils prevent the passage of water vapor and they are permeable to gases (Saucedo-Pompa et al., 2009). Besides, the use of xanthan gum as edible coating on fresh fruits has been little explored. Its mechanical properties and its ability to retain aroma and flavor are very attractive, thus, we believe that it is an interesting option to be used as a coating or support for other additives (Chen & Nussinovitch, 2000; García-Ochoa, Santos, Casas, & Gómez, 2000). The aim of this work was to develop SLN-xanthan gum as a high-stability film-forming coating by hot lipid dispersion using a rotor–stator device, evaluating its effect on the shelf life on guavas, to establish its real potential in food coating technology.

2. Materials and methods

2.1. Materials

Pluronic® F-127 (poloxamer-407 Mw 9,840–14,600) was used as stabilizer, propylene glycol, (Mw 76.09 g mol⁻¹ ρ = 1.036 g/cm³) was the plasticizer and xanthan gum from *Xanthomonas campestris* (Mw $\approx$ 2 $\times$ 10⁶ g/mol and μ = 7627 mL/g) was used as film-forming material, they were provided by Sigma-Aldrich Chemical S.A. de C.V. (State of Mexico, México). Candeuba®S wax (carnauba wax and candelilla wax mixture, melting point 82–86 °C) was obtained from Multiceras S.A. de C.V. (Mexico). This wax was selected for this study due to its combined characteristics, Candeuba® wax provides a high barrier to humidity and carnauba wax gives high brightness and good emulsification properties. Sodium hydroxide was purchased from J.T. Baker (New Jersey, USA). Distilled water was obtained from a MilliQ® equipment (Millipore Corp., Massachusetts, USA).

Mature green guavas cv. “Media China” from the State of Michoacan, Mexico was chosen for this study. The fruit was carefully selected according to size (radial diameter, 4.6 to 5.6 cm) and color uniformity, according to the description by Mercado-Silva et al. (1998). The storage temperature was 10 °C (RH ~85%). Sampling period was carried out every other day during 34 days and thereafter at 25 °C for 5 days to evaluate the maturity development.

2.2. SLN preparation

SLNs were prepared using the hot high shear stirring method (Solans, Izquierdo, Nolla, Azemar, & García-Celma, 2005). The lipid phase was prepared with 100 g/L of Candeuba® wax melted by heating at 90 °C. The aqueous phase consisted of Pluronic® F-127 solution (5 g/L) which was heated to the same temperature. The melted wax was dispersed in the aqueous phase using a high shear stirrer (Ultra-Turrax T50, IKA®, Staufen, Germany with a S25N-25 G, IKA disperser element) to obtain an oil-in-water nanoemulsion at 10,000 rpm/10 min for 3 cycles, leaving the solution to stand for 5 min repose between the cycles and finally cooling it down at room temperature to obtain the SLN dispersion.

2.3. Determination of particle size, polydispersion index (PDI) and zeta potential (ζ)

Particle size distribution and PDI were determined (at 25°C) using the laser light scattering technique at a 90° fixed angle by using a Z-sizer 4

equipment (Zetasizer Nano Series Malvern Ltd, France). In general 0.3 mL of SLN dispersion was diluted in 10 mL Milli-Q® water in order to obtain the volume frequency histograms. Measurements were performed in triplicate.

The ζ was estimated for all the systems prepared using a Z-sizer 4 at 90° (Zetasizer Nano Series Malvern Ltd, France) after appropriate dilution. The values were normalized with polystyrene standard dispersion (ζ = -55 mV). Measurements were made in triplicate at 25 °C.

2.4. Scanning electron microscopy (SEM)

The morphological analysis of SLN was performed after removing the excess of stabilizer by three centrifugations at 30,000 rpm for 50 min. A drop of this concentrated suspension was spread on a microfilter membrane (200 nm) and it dried under vacuum at RT. The samples were placed on stubs and coated with a gold layer (~20 nm thickness) (Coater® JFC-1100 JEOL, Tokyo, Japan) and they were finally observed under a high vacuum scanning electron microscope (JSM-6400® SEM JEOL, Tokyo, Japan).

To perform this test, it was necessary to dehydrate the fruit by dipping a portion of the guava peel in ethanol for 24 h draining and drying the sample at room temperature. Each portion with different SLN treatments was placed on a slab and analyzed by SEM according to the technique described above.

2.5. Preparation of the film-forming dispersions

The SLN coating formulations were prepared from dilutions of the initial SLN suspension with 100 g/L of Candeuba® wax (see Section 2.2). Six systems (60, 65, 70, 75 and 80 g/L and xanthan gum) were made in order to study the effect of SLN concentration on the preservation of raw guava. To obtain a coating providing a better barrier to gases and aroma retention, constant concentrations of xanthan gum (4 g/L) and polyethylene glycol (5 g/L) were used. The guavas were coated by dipping for 1 min. They were dried at room temperature for 1 h and were immediately transferred to refrigeration storage at 10 °C.

2.6. Skin color development

Guava external color was evaluated with a Minolta Colorimeter (Model CR-300, N.Y., USA), as stated the CIE (Commission Internationale de l'Eclairage), L, a, and b coordinates were recorded using C illuminant and a 2° standard observer as reference system. L is lightness, a (- green to + redness), b (blueness to yellowness) and hue angle ($h = \tan^{-1} (a/b)$). Each measurement was taken at two locations on the equatorial zone. A total of 21 fruits from each treatment were tested. All measurements were made in triplicate.

2.7. Texture analysis

The fruit firmness was measured at the equatorial region using an Instron-Universal Testing Machine (Model 4411, Instron, Massachusetts, USA). A plunger with diameter of 6 mm was used to puncture the fruit tissue to a depth of 5 mm for the determination of firmness at a crosshead speed of 150 mm/min using a 50 N load cell. Three fruits per replication, each punctured on both sides, were subjected to firmness testing.

2.8. Quality attributes

Changes during storage were evaluated comparing different attributes like weight loss, development of skin color, titratable acidity, total soluble solids and textural analysis.

For the determination of weight loss guavas were weighed after coating every other day during the storage period. Weight loss was expressed as the percentage loss of initial total weight. Measurements

were performed in triplicate for each treatment. The total soluble solids (TSS) were recorded with a refractometer (Leica® AR 200, USA). The values were corrected to 20 °C. The titratable acidity (TA) was determined by titrating the fruit juice, after diluting it with distilled water, against 0.1 N NaOH solution using phenolphthalein as an indicator to the end point of pH 8.1. The titratable acidity was expressed as percentage of citric acid (Singh & Pal, 2009).

2.9. Statistical analysis

Significance and statistical differences were analyzed using Minitab 14 (Minitab Inc., State College, PA, USA). ANOVA was performed to compare the different SLN concentrations in the coated samples. Means were tested using the Tukey's test ($p < 0.05$).

3. Results

3.1. Particle size, polydispersion index and zeta potential

Fig. 1 shows the behavior of particle size as a function of the numbers of homogenization cycles. The first cycle exhibited three populations; most (65.6% intensity fraction) were 326 nm in size on average, and the third cycle showed one population with a mean particle size of 239 nm. Table 1 summarizes the changes in PDI, which showed a pattern similar to that observed for the particle size. After four cycles, the polydispersion index was greater than 0.3, indicating a wide distribution with small and large particles in the SLN suspension, which increases the probability of system instability (Lemarchand, Couvreur, Vauthier, Costantini, & Gref, 2003). The ζ is a measure of the degree of repulsion between adjacent and similar charged particles in the dispersion (Attama, Schicke, Paepenmüller, & Müller-Goymann, 2007; Awad et al., 2008; Noriega-Pelaéz, Mendoza-Muñoz, Ganem-Quintanar, & Quintanar-Guerrero, 2011). Table 1 shows that there are important changes in ζ apparently due to the high energy introduced into the dispersion causing surface modification in the particle's charge. The dispersions were stable during eight weeks at 4 °C except for the fifth cycle. Thus, it was established that with three cycles it was possible to obtain stable submicronic dispersions that can be easily incorporated into the coating formulations.

3.2. Morphological analysis

Fig. 2 shows the SLN formed in the initial suspension (10 g/L Candeuba® wax), circled and marked with an arrow. These SLN

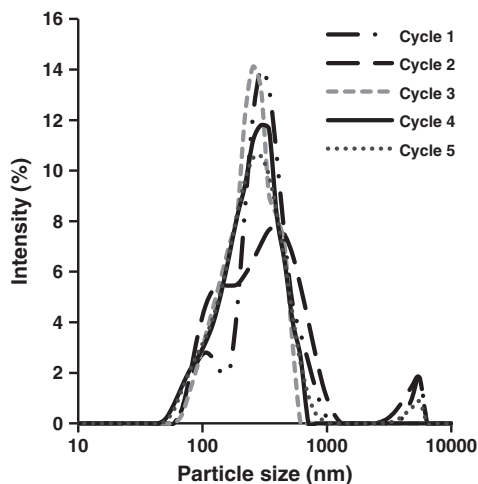


Fig. 1. Particle size distribution as a function of the number of cycles.

Table 1

Particle size, polydispersion index (PDI), and zeta potential (ζ) for each homogenization cycle.

Homogenization cycle	Particle size (nm)	Polydispersion index (PDI)	Zeta potential (ζ) (mV)
1	326 ± 04 ^a	0.57 ± 0.08 ^a	−22.2 ± 3.3 ^a
2	290 ± 25 ^a	0.35 ± 1.35 ^b	−24.5 ± 3.4 ^a
3	239 ± 13 ^b	0.18 ± 0.50 ^c	−25.7 ± 1.1 ^a
4	238 ± 06 ^b	0.22 ± 0.40 ^c	−24.2 ± 4.1 ^a
5	222 ± 07 ^c	0.25 ± 1.00 ^c	−15.2 ± 5.6 ^b

Values in columns labeled with different letters are significantly different ($p < 0.05$).

micrographs evidence the presence of nanoparticles with a solid matrix without irregularities. Particle size shows a good correlation with the particle sizes obtained by dynamic light scattering. There was no evidence of floc aggregates or fused nanoparticles, suggesting good dispersion stability.

3.3. Guava surface characterization

Fig. 3 shows the micrographs of the guava surfaces treated with different coatings. The micrograph in Fig. 3(a) is a representative image of the guava skin surface which was used as a reference in the testing of treatments, and was compared with the micrographs of the coatings. Fig. 3(b) shows the formation of a xanthan gum coating homogeneously distributed on the guava skin. The micrographs in Fig. 3(c) and (d) corresponding to 60 and 65 g/L of SLN, respectively, evidence the presence of a film with submicron spherical bodies on the guava surface. Apparently during the film formation, the water transport causes an accumulation of SLN around the lenticels without occlusion. Fig. 3(d) shows aggregation of SLN around the lenticels, apparently this SLN concentration prevents fruit transpiration. Furthermore, this SLN arrangement can explain the functionality of these coatings. In contrast the coatings with 70 g/L of SLN (Fig. 3(e)) show several micropores formed by SLN accumulation, which begin to decline with the appropriate transpiration of fruit, this is evident throughout the film.

When the SLN concentration was 80 g/L a continuous film with large pores was observed. Then, the SLN forms wide lipid regions that limit the water evaporation and the transpiration process forms superficial holes on the film. These images reveal an impermeable behavior that may limit gas exchange.

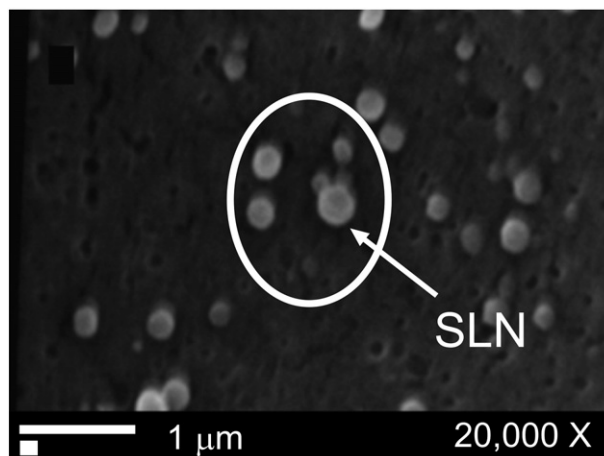


Fig. 2. Morphological structure of solid lipid nanoparticles (100 g/L Candeuba® wax) at 20,000 $\times$.

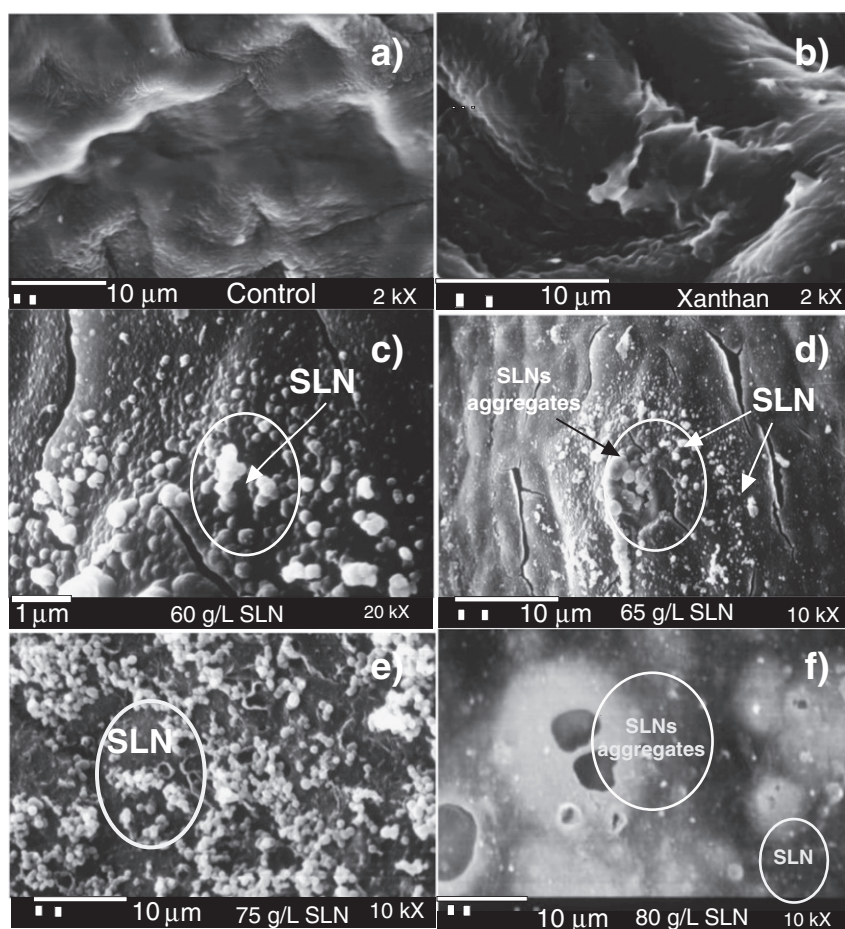


Fig. 3. Micrographs of guava surface as a function of treatments: (a) control, (b) xanthan gum (4 g/L), (c) SLN 60 g/L, (d) SLN 65 g/L, (e) SLN 70 g/L and (f) SLN 80 g/L. Samples with SLN had 4 g/L of xanthan gum.

3.4. Skin color development

Fig. 4(a–b) shows a box plot graph that represents the range of measurements during the storage period in function of the color changes (hue angle) associated to the refrigeration storage and those transferred at room temperature (25 °C). It was observed that all guavas started with a light green maturity stage (116° hue angle). Fig. 4(a) shows that control guavas achieved a yellow color (92° hue angle) at 24 days of refrigeration storage. In the guavas coated with xanthan gum, the hue angle decreased very similarly to that of control guavas at 5 days of refrigeration storage, with a subsequent decrease showing that xanthan gum apparently helped to keep the quality of the fruits stored under refrigeration up to 19 days, deteriorating after this time. The samples coated with 60, 65 and 70 g/L of SLN did not show significant differences between them ($\alpha = 0.05$), with an average hue angle, of 94, 95 and 97, respectively, at the end of refrigeration storage. In contrast, guavas with 75 and 80 g/L of SLN did not show important changes in the hue angle (116°) during refrigeration.

Fig. 4(b) shows the changes in the hue angle following the transferring of the refrigerated samples to room temperature (25 °C). Control guavas, achieved a yellow color at 10 days, and a hue angle of 91°. Additionally, the guavas coated with xanthan gum showed a gradual decrease in the hue angle, reaching the characteristic yellow color at 24 days. The guavas coated with 60 and 65 g/L of SLN did not show statistically significant differences regarding the hue angle, achieving a value of 92° at the end of the storage period. The samples with 70 g/L had no apparent change in the average hue angle (95°) after 10 days of storage. Fig. 4(b) also shows that the samples with 75 and 80 g/L of

SLN do not have color changes from green to yellow, with an average hue angle of 105–100°, suggesting metabolic damage.

3.5. Texture analysis

Fig. 5(a–b) presents the firmness variation as a function of storage conditions. Fig. 5(a) shows that control samples had an important decrease of firmness from 40.8 N to 24.7 N within ten days. The samples coated with xanthan gum had loss firmness at 15 days. Samples with 60 and 65 g/L of SLN maintained the best texture with a minimum compressive force of 14.3 N after 30 days of refrigeration. Fig. 5(b) shows the changes in firmness for refrigeration storage plus five days at room temperature. The control and xanthan gum coated guavas had the maximum loss of firmness (19 N) at 5 days after transferring at room temperature. On the other hand, samples with 60 and 65 g/L of SLN had the best behavior during refrigeration storage; perhaps they show changes at room temperature enhancing the proper maturity of the fruit. The guavas with 75 and 80 g/L did not show statistical differences at room temperature. However, they had a rapid decrease of firmness, which can be attributed to the dehydration and physiological disorders generated on the fruit in these samples.

3.6. Quality changes

3.6.1. Weight loss

Fig. 6 presents the physiological weight loss of guavas for the different treatments. Fig. 6(a) shows that the control guavas had the highest weight loss at rate of 0.61 g/day ($R^2 = 0.98$) during the first 19 days,

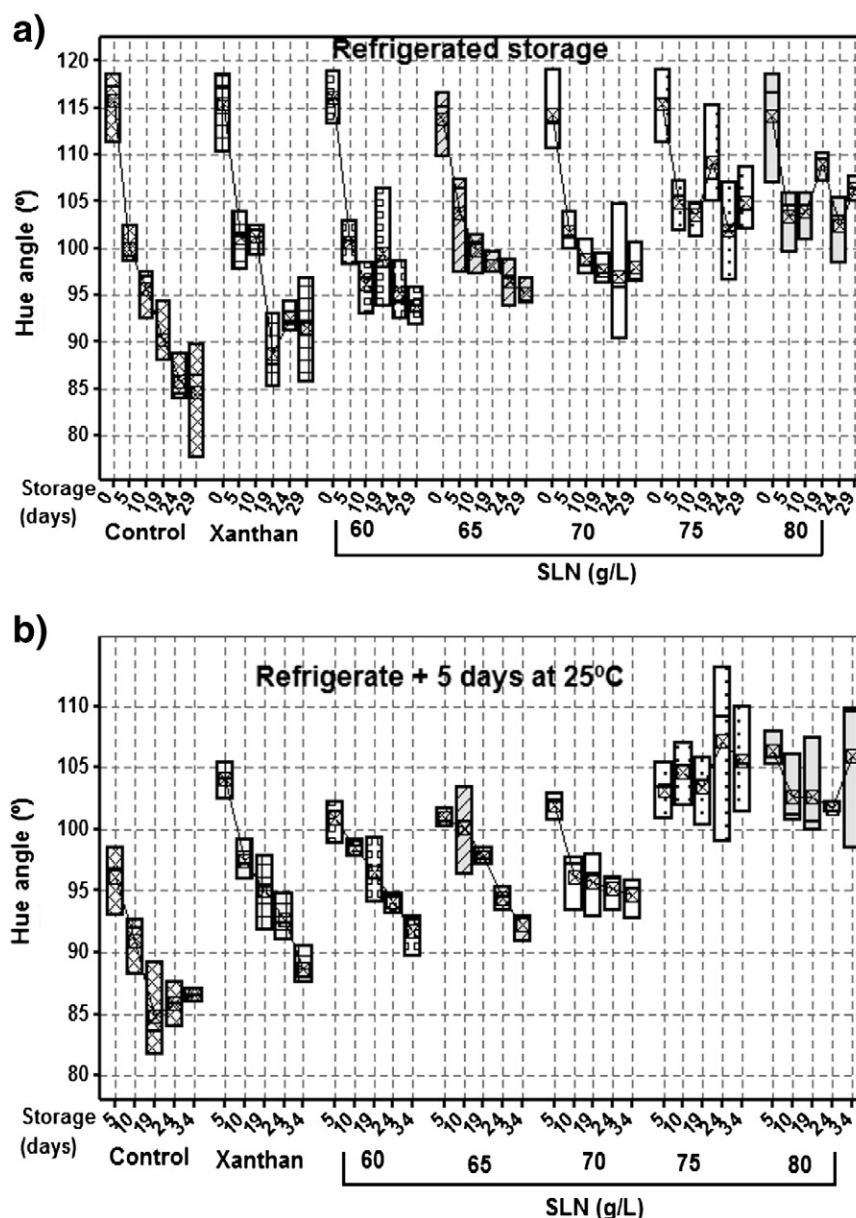


Fig. 4. Hue angle changes: (a) guavas at refrigeration storage and (b) refrigeration storage + 5 days at 25 °C (room temperature).

which decreased at 0.14 g/day after 22 days of refrigeration. On the other hand, guavas treated with xanthan gum, showed a weight loss of 0.45 g/day ($R^2=0.84$). The guavas treated with 60, 65 and 70 g/L of SLN showed lower water loss rates 0.31, 0.23, and 0.23 g/day respectively, hence, they are capable to retain water during storage period avoiding dehydration of the fruit. The samples treated with 75 and 80 g/L did not show a statistically significant weight decrease (0.418 g/day $R^2=0.98$) compared to those treated with xanthan gum ($\alpha=0.05$).

3.6.2. Titratable acidity (TA) and total soluble solid (TSS)

Table 2 summarizes the TA and TSS changes of guavas transferred to room temperature for five days. Control guavas had their maximum TSS at 19 days. No, statistical difference between 60 and 65 g/L of SLN was observed, both concentrations had a delayed ripening by increasing the TSS up to 29 days plus 5 days at room temperature storage, contributing to increase the shelf life of the guava. Initially, the samples with 70 g/L of SLN had no problems with the development of TSS until day 19 of refrigeration storage plus 5 days at room temperature. Guavas with 75 and 80 g/L of SLN showed delayed maturation the most of the time.

Samples with xanthan gum only and those with 60 and 65 g/L of SLN showed a reduction in the TA up to 29 days of storage with an average of 0.53 mg citric acid/g of the fruit, with a slight increase at day 30. In contrast, the samples with 70 g/L of SLN showed a decrease in acidity up to 12 days of storage. The TA begins to vary as a result of the coating concentration of SLN. Guavas with concentrations of 75 and 80 g/L of SLN had an irregular behavior in TA associated with the disorders caused by using higher SLN concentrations.

Finally, Fig. 7 summarizes the changes in the visual aspect of guavas at different times after storage at room temperature (25 °C) for the control and the two most effective treatments (60 and 65 g/L of SLN), in addition to those that presented physiological damage (80 g/L).

4. Discussion

The use of high shear stress (10,000 rpm/5 min) at high temperature (90 °C) allowed to obtain SLN of Candeuba® wax, with particle sizes below 400 nm and a homogeneous distribution since the third cycle. It is important to point out that when larger particles were used, system aggregation and irregular coatings were obtained,

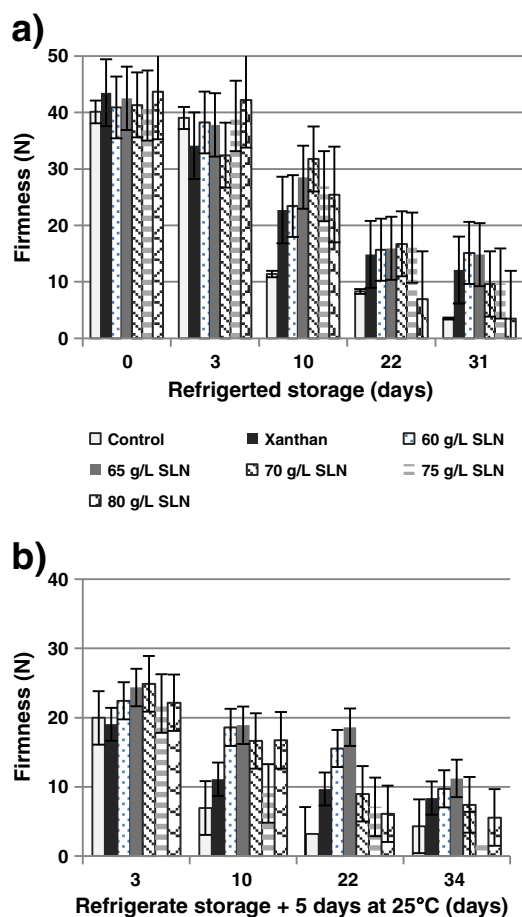


Fig. 5. Firmness loss during storage: a) refrigeration storage and b) refrigeration storage + 5 days at 25 °C (room temperature). The error bar shows the standard deviation.

showing that the best particle size distribution was for the third cycle (239 nm) displaying a constant distribution ($PDI=0.18$). These suspensions were stable during storage guaranteeing the preservation and the functionality of the dispersion. Variations were observed for the fourth and the fifth cycles; this behavior can be attributed to the energy absorbed by the dispersion due to excess homogenization, causing destabilization of the system. According to Shi, Li, Wang, Li, and Adhikari (2011) the excess of energy used to perform the homogenization cycles may have contributed to an increase of particle coalescence. The ζ value defines the stability of the dispersion from an electrostatic point of view (Noriega-Peláez et al., 2011). When ζ potential is high, the repulsive forces prevail, suggesting the formation of a deflocculated suspension (Mirhosseini, Tan, Hamid, & Yusof, 2008; Weiss-Angeli et al., 2008). It is well accepted that dispersions will be stable if the absolute value of ζ is 30 mV, which can be used in the coating. It is important to point out that all SLNs used in this study were obtained with three homogenization cycles (Schramm, 2005).

Xanthan gum coatings with different SLN contents (60, 65, 70, 75 and 80 g/L), were evaluated for the physicochemical, color and textural changes associating them with maturity and metabolic activity (Pérez-Gago, González-Aguilar, & Olivas, 2010). Apparently, SLNs have potential applications due to their distribution in the coating on guava surface. The most effective SLN concentrations were 60 and 65 g/L where the fruits showed a slower change of color, textural and physicochemical parameters; also, they do not show any physiological damage at the moment of transferring to room temperature for five days, with successful maturation development attributed to the homogeneous SLN distribution in the coating formed on the guava skin allowing the fruit respiration. Fig. 7 shows the visual aspect of guavas

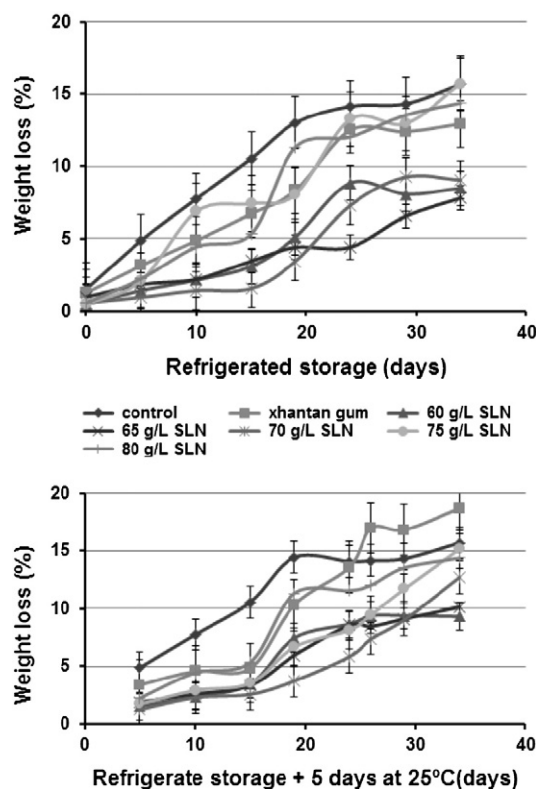


Fig. 6. Weight loss during storage: a) refrigeration storage and b) refrigeration storage + 5 days at 25 °C (room temperature). The error bar shows the standard deviation.

for the best SLN concentrations (60 and 65 g/L) compared to those with physiological damage (80 g/L of SLN); establishing that at 60 and 65 g/L of SLN, the nanoparticles, distribution is homogeneous. Additionally, these SLN concentrations did not fully cover the fruit's skin having a selective permeability that only slows the rate of the metabolic processes, increasing the storage time of the fruit. On the other hand the increase of the SLN concentration to 80 g/L probably inhibits the transpiration process removing moisture from the film formed by the SLN-xanthan gum complex, thus promoting SLN aggregation and resulting in the inhibition of the physiological process of product.

The hue angle is the best form to express color evolution. Regarding guava preservation, it has been established that the use of plastic packaging increases the shelf life of guava without any apparent physiological damage (Jacomino, Bron, De Luca Sarantópoulos, & Sigrist, 2005). In our study, the samples with 60 and 65 g/L of SLN showed a reduction in color changes because of the delay of chlorophyll degradation by reducing the metabolism of guava, which results in delayed color changes (Espinoza-Zamora, Baez-Sañudo, Saucedo-Veloz, & Mercado-Silva, 2010; Jain, Dhawan, Malhotra, & Singh, 2003). The samples with 80 g/L of SLN remained in green maturity because at this concentration the wax caused damage to the fruit keeping it in green state with loss of luminosity.

The weight loss is indicative of a fruit dehydration process due to transpiration and it involves water transfer from the cell to the surrounding atmosphere, thus representing a way to evaluate coating efficiency in the preservation of quality (Pérez-Gago et al., 2010). In this work xanthan gum was used as a film former, acting as a gas exchange barrier that did not modify the water transport, so, the aim of introducing SLN into the coating is to create a lipophilic environment capable of acting as a barrier against water, as happened in the samples with 60 and 65 g/L. In the case of the samples with 75 and 80 g/L, there was a limited exchange of water vapor, which caused physiological damage. This effect can be explained by the hydrophilic nature of the gum, which absorbs water and removes it through

Table 2

Physicochemical changes in guava with SLN coating under refrigeration storage + 5 days at 25 °C.

Time (days)	Control	XG	60 g/L SLN	65 g/L SLN	70 g/L SLN	75 g/L SLN	80 g/L SLN
<i>Acidity titratable (TA) (% citric acid)</i>							
5	0.71 ± 0.03 ^a	0.57 ± 0.01 ^a	0.74 ± 0.02 ^a	0.74 ± 0.04 ^a	0.76 ± 0.02 ^a	0.56 ± 0.02 ^a	0.76 ± 0.04 ^a
10	0.57 ± 0.02 ^b	0.57 ± 0.02 ^a	0.57 ± 0.03 ^b	0.58 ± 0.02 ^b	0.52 ± 0.01 ^b	0.58 ± 0.02 ^a	0.57 ± 0.01 ^b
19	0.56 ± 0.07 ^b	0.47 ± 0.04 ^b	0.67 ± 0.01 ^c	0.60 ± 0.08 ^b	0.68 ± 0.02 ^c	0.75 ± 0.09 ^b	0.82 ± 0.04 ^c
29	0.44 ± 0.05 ^c	0.71 ± 0.01 ^c	0.69 ± 0.04 ^c	0.76 ± 0.04 ^c	0.79 ± 0.02 ^d	0.76 ± 0.03 ^b	0.64 ± 0.02 ^d
34	0.45 ± 0.01 ^c	0.57 ± 0.01 ^a	0.43 ± 0.04 ^d	0.86 ± 0.01 ^d	0.56 ± 0.12 ^b	0.75 ± 0.02 ^b	0.72 ± 0.08 ^a
<i>Total solid soluble (TSS) (°Bx)</i>							
5	9.1 ± 0.1 ^a	7.9 ± 0.2 ^a	8.4 ± 0.2 ^a	9.1 ± 0.3 ^a	9.5 ± 0.4 ^a	9.2 ± 0.3 ^a	8.4 ± 0.1 ^a
10	12.1 ± 0.2 ^b	13.0 ± 0.4 ^b	11.2 ± 0.3 ^b	11.7 ± 0.5 ^b	10.5 ± 0.3 ^a	9.9 ± 0.6 ^a	10.4 ± 0.2 ^b
19	13.3 ± 0.3 ^c	11.8 ± 1.4 ^c	12.5 ± 0.3 ^c	12.9 ± 0.3 ^c	11.2 ± 0.6 ^b	10.2 ± 0.3 ^a	6.5 ± 1.2 ^c
29	11.2 ± 1.6 ^d	9.8 ± 0.4 ^d	12.0 ± 0.5 ^c	11.7 ± 1.3 ^b	9.3 ± 0.3 ^a	8.5 ± 0.9 ^b	6.5 ± 0.5 ^c
34	5.4 ± 1.2 ^e	9.5 ± 0.2 ^d	11.0 ± 1.1 ^b	11.2 ± 0.6 ^b	9.4 ± 1.0 ^a	10.5 ± 0.6 ^a	–

XG = xanthan gum; all system with SLN containing 4 g/L of xanthan gum and 5 g/L of propylene glycol.

Values in columns labeled with different letters are significantly different ($p < 0.05$).

the ambient (García-Ochoa et al., 2000). Furthermore the increasing levels of TSS in guava samples after transferring to room temperature for 5 day post refrigeration storage indicated good maturity development (Jain et al., 2003; Mercado-Silva et al., 1998; Ortiz-Hernandez, Espinoza-Hernández, Saucedo-Veloz, & Mercado-Silva, 2010; Singh & Pal, 2008a, 2008b). The TSS decreased during the ripening process, but it increased again in over ripened fruit according to Singh & Pal (2008a, 2008b) and Mercado-Silva et al. (1998). The lower sugar content in the treated fruits may be explained by the high respiration rate displayed by the fruits that consumed sugars (Espinoza-Zamora et al., 2010). It is clear that the application of 60 and 65 g/L of SLN was the most effective way to stop the conversion of the acids present in the fruit into sugar. Thus, these coatings create a new atmosphere on surface of guava (Bassetto, Jacomino, Pinheiro, & Kluge, 2005).

The decrease in acidity of control guavas during storage might be due to a rapid utilization of acids by respiration, as a result of maturity (Table 2), demonstrating that the said decrease is depended on many factors, including SLN aggregation in the coating's surface (Singh & Pal, 2008b; Mercado-Silva et al., 1998; Jain et al., 2003). In contrast, the guavas coated with 75 and 80 g/L showed delayed maturation. For this reason SLNs are presented as a viable alternative in the

development of coatings with the advantage of being submicronic in size, in addition to contributing to homogenously cover the skin of the fruit being more effective when used in large size coatings.

5. Conclusions

The SLNs prepared with Candeuba® wax were stable with particle sizes larger than 300 nm. These SLNs allowed the development of coatings using xanthan gum as a polysaccharide base. These coatings applied by dipping on the guavas evidenced that SLNs have potential applications in fruit preservation. The micrographs demonstrated that the SLN-xanthan gum coating was homogenous on guava surface and that in the case of 60 65 g/L, the SLN did not completely cover the skin of the fruit, which enabled adequate transpiration and regulating the metabolic process on the fruits, thus increasing the storage period and preserving its quality features, as evidenced by the changes in the hue angle, physiological loss of weight and texture. High concentrations of SLN caused physiological damage due to the lipophilic nature of the wax changing the internal atmosphere on the surface of the guava. These findings indicate that the use of nanotechnology can improve the functionality of edible films for food applications. It is clear that

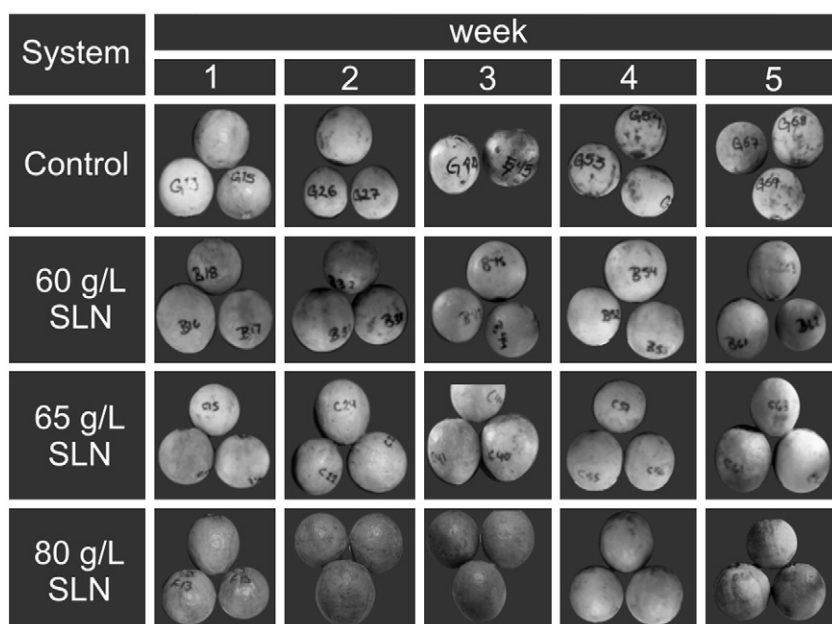


Fig. 7. Visual aspect of guava refrigeration storage + 5 days at 25 °C as a function of weeks of storage, including the control, the best conditions (60 and 65 g/L of SLN) and the guavas with physiological damage (80 g/L).

the potential use of SLN in edible coatings could be applied easily to other fruits and vegetables and it could represent the introduction of a new type of coating helping to minimize the senescence of different products.

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