

# Calcium Lactate Effect on the Shelf Life of Osmotically Dehydrated Guavas

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**Abstract:** The effect of calcium lactate on osmodehydrated guavas in sucrose and maltose solutions was monitored during storage under passive modified atmosphere for 24 d at 5 °C. Sample texture and color characteristics, microbial spoilage, sensory acceptance, structural changes, and gas composition inside the packages were periodically evaluated. Calcium lactate inhibited microbial growth on guavas, with yeast and mold counts in the order of  $10^2$  CFU/g throughout storage. The calcium salt reduced respiration rate of guava products, showing  $O_2$  and  $CO_2$  concentrations around 18% and 3% inside the packages. A firming effect on fruit texture, with up to 5 and 2 times higher stress and strain at failure values and tissue structure preservation could also be attributed to calcium lactate use. However, fruits treated with calcium lactate, osmodehydrated in maltose and sucrose solutions, showed sensory acceptance scores below the acceptability limit (4.5) after 13 and 17 d of storage, respectively.

**Keywords:** calcium, guava, osmotic dehydration, quality characteristics, shelf life

## Introduction

The application of calcium salts, before or after harvest, is a common method used to delay ripening and senescence, and to prevent physiological disorders in several fruits (Lara and Vendrell 1998). These salts have also been widely used for the structural preservation of processed tissues (Luna-Guzmán and others 1999; Mastrángelo and others 2000; Quiles and others 2004) and, more recently, to prepare functional or fortified fruit and vegetable products (Fito and others 2001; Barrera and others 2004; Anino and others 2006).

Two main ways of calcium application in fresh fruits and vegetables have been reported: dipping treatments and impregnation processes. Dipping treatments are commonly used for fresh products, especially those more perishable, such as leafy vegetables. It usually consists of product soaking, with or without mechanical agitation, followed by the removal of excess washing solution. This treatment is gentler to the product than the impregnation techniques that may cause tissue damage and metabolic stress. Impregnation processes, such as the osmotic dehydration process, aim the modification of food composition through partial removal of water and impregnation with solutes, without affecting the materials integrity. The driving force of the process is the osmotic pressure gradient between the sample and solution, the latter usually a sugar source. Impregnation offers broad applications in fruit and vegetable processing and one of its advantages is the development of reformulated products by impregnation with different calcium sources (Martín-Diana and others 2007).

Calcium has been related to the structural preservation of vegetable tissues since calcium ions form bridges between free carboxyl groups of pectin chains, resulting in strengthening of the cell wall and maintenance of product texture (Jackman and Stanley 1995). Calcium treatments have resulted in improvements in product texture and have shown a protective effect on tissue structure in several kinds of processed fruits and vegetables, such as melon (Luna-Guzmán and others 1999; Mastrángelo and others 2000), apple (Quiles and others 2004; Alandes and others 2006), guava (Pereira and others 2007), carrot (Rico and others 2007a), peach (Manganaris and others 2005), raspberry, and blackberry (Sousa and others 2006).

Calcium salts may also help to control the development of enzymatic browning when applied in combination with ascorbic acid (Gorny and others 2002; Lee and others 2003; Varela and others 2007), inhibit color changes during storage (Saftner and others 2003), reduce the incidence of chilling injury (Gerasopoulos and Drogoudi 2005; Hewajulige and others 2006; Manganaris and others 2007), and decrease the respiration rate, retarding the metabolic activity of vegetable tissues (Luna-Guzmán and others 1999; Saftner and others 2003; Recasens and others 2004; Aguayo and others 2008).

Another benefit derived from calcium treatments is the development of enriched or functional foods, through the incorporation of high quantities of calcium to the fruit and vegetable matrix (Fito and others 2001; Alzamora and others 2005; Anino and others 2006). A fortified apple tissue was obtained by Anino and others (2006) using impregnation techniques. According to the authors, the  $Ca^{2+}$  amount incorporated into the apple matrix would satisfy about 23% to 62% of the Adequate Daily Intake, in 200 g of fruit.

Adequate calcium intake has been associated with reduced risk of osteoporosis, hypertension, colon cancer, kidney stones, and lead absorption (Weaver 1998). Fruits and vegetables are the potential sources of calcium, and increasing the calcium content in these commodities may represent an interesting way of adding value to vegetable products (Martín-Diana and others 2007).

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An antimicrobial effect has been also related to the use of calcium salts. A reduction of microbial growth rate was observed in some fruits and vegetables (Izumi and Watada 1994; Luna-Guzmán and Barrett 2000; Martín-Diana and others 2005; Aguayo and others 2008). However, in some cases, the inhibitory effect of calcium cation was attributed to the increase of tissue resistance to microbial infection, due to the stabilization and strengthening of the cell wall, rather than to an antimicrobial action of calcium (Izumi and Watada 1994).

Thus, the objective of this work was to verify the effect of calcium lactate on shelf life of osmotically dehydrated guavas, by evaluating texture and color characteristics, microbial spoilage, and sensory acceptance of the fruits during 24 d of storage. Structural changes, by light microscopy, and gas composition inside the packages were also evaluated during the shelf life study, and related to guavas quality characteristics.

## Materials and Methods

### Materials

Red guavas (*Psidium guajava* L.) of the Paluma cultivar (Pereira and others 2007) supplied by Val Fruit Industry (Vista Alegre do Alto, SP, Brazil) were used in the trials approximately 4 to 5 d after harvest, when the fruits reached a suitable ripening grade. Fruit sampling was based on ripening grade (7 to 8 °Brix and 80% of skin yellowness), shape, and size (7.8 ± 0.4 cm length, 6.7 ± 0.2 cm dia, 1.0 ± 0.1 cm pericarp thickness, and weight of 152.0 ± 7.8 g). The raw material used showed a pH of 3.8 ± 0.1 and moisture content of 86.4 ± 0.9% (wet basis).

Polyethylene terephthalate (PET) containers with hinged lids and 350 mL capacity (Galvanotek, Carlos Barbosa, RS, Brazil), with oxygen transmission rate (O<sub>2</sub>TR) of 6 cm<sup>3</sup> standard temperature and pressure (STP)/package per day at 25 °C and vapor transmission rate (WVTR) of 0.243 g/package per day at 38 °C and 90% relative humidity were used as packaging system (Pereira and others 2004).

### Processing and storage

The guavas were washed with tap water and immersed in a 5.5 g/L solution of chlorinated sanitizer (165 ppm active chlorine) for 10 min (Diversey Lever, São Paulo, SP, Brazil). The sanitized fruits were peeled using a 20 g/L NaOH solution at 65 °C for 50 s, washed with tap water and immersed again in a 5.5 g/L solution of chlorinated sanitizer for 10 min. The guavas were then cut into halves and the seeds removed.

Guava halves (one half guava per flask) were soaked in sucrose (Copersucar União, Piracicaba, SP, Brazil) and maltose (Maltegiill® 63/82, Cargill SA, Mairinque, SP, Brazil) solutions at 60 °Brix. Calcium lactate was added to these solutions at a concentration of 15 g/L (Pereira and others 2007).

The solutions with the samples were placed in a thermostatic shaker (TE 420, Tecnal, Piracicaba, SP, Brazil) at 120 rpm and 40 °C for 2 h. The mass ratio of fruit to solution of 1:10 was used in the experiments in order to keep constant the sugar solution concentration along the osmotic treatments. After this process, the samples were rinsed with 2 g/L solution of chlorinated sanitizer (60 ppm active chlorine) and placed on absorbent paper to remove excess solution (Pereira and others 2007).

The osmotically dehydrated guavas were packed in PET containers to promote a passive modification of the atmosphere around the product. Two guava halves, weighing approximately 70 g, were packed in each PET container and were stored at 5 °C for 24 d.

The guavas were evaluated with respect to texture and color characteristics, microbial spoilage, and sensory acceptance after 0, 7, 13, 17, 21, and 24 d of storage. Guavas microscopic features and the gas composition inside the packages were also evaluated and related to quality characteristics of the fruits.

The samples were named SUCROSE and SUC+Ca (osmotically dehydrated in sucrose solution without and with addition of calcium lactate), MALTOSE and MAL+Ca (osmotically dehydrated in maltose solution without and with addition of calcium lactate) and showed moisture content after process of 73.6 ± 0.6%, 72.3 ± 0.5%, 70.5 ± 1.7%, and 70.9 ± 1.6% (wet basis), respectively.

### Gas composition analysis

An oxygen/carbon dioxide analyser Dual Head Space (Model 650, Mocon, Minneapolis, Minn., U.S.A.) was used for analysis of the gas composition inside the packages. Three packages from each treatment were taken and the mean values were reported.

### Texture evaluation

The guavas texture characteristics were evaluated by uniaxial compression tests using a Universal Testing Machine (TA.XT2i Texture Analyser, Stable Micro Systems, Godalming, Surrey, England). Stress and strain at failure values were determined using a 30 mm dia lubricated acrylic plate at a crosshead speed of 1 mm/s until 70% sample deformation. A 10-mm dia cylindrical sample removed from the center of the guava halves was used for this assay and was analyzed at room temperature (23 ± 1 °C). Force and height data were converted to Hencky stress ( $\sigma_H$ ) and strain ( $\varepsilon_H$ ), according to Eq. (1) and (2), and the stress and strain at failure were determined from the peak of the stress-strain curve.

$$\sigma_H = \frac{F(t)}{A(t)} \quad (1)$$

$$\varepsilon_H = -\ln\left(\frac{H(t)}{H_0}\right) \quad (2)$$

where  $F(t)$ ,  $A(t)$ , and  $H(t)$  are the force, product area, and height at time  $t$ , respectively, and  $H_0$  is the initial product height.

Five guava halves from each treatment were taken and the mean values were reported.

### Color measurements

Color measurements were performed using a Hunterlab colorimeter (Reston, Va., U.S.A.).  $L^*$  (luminosity),  $a^*$  (green [−] to red [+]), and  $b^*$  (blue [−] to yellow [+]) coordinates were obtained using a D65 illuminant and 10° observer angle as the reference system. The color parameters were determined for the internal part of the fruits. Five guava halves from each treatment were taken and the mean values were reported.

### Microbiological analysis

Microbiological analyses were carried out during storage to determine the yeast and mold count and the lactic acid bacteria count, considered the main spoilage microorganisms able to develop in the product due to the pH value shown by the guava (pH of 3.8 ± 0.1). The coliform group and *Salmonella* spp. were also determined at zero storage time, to evaluate the hygienic conditions of the raw material and processing. The microbial counts were done in triplicate, according to Downes and Ito (2001) methodology, and the mean values were reported.

## Sensory analysis

Sensory acceptance tests during storage were carried out in a standardized test room. The samples were presented in monadic form in white saucers labeled with 3-digit random number codes. Color, aroma, flavor, texture, and overall impression of the samples were evaluated by 30 panelists, who were guava consumers and representative of the target public, using a 9-cm unstructured hedonic scale anchored with "I dislike very much" on the left side and "I like very much" on the right side. An average score of 4.5 was considered the limit of acceptability. The intention to purchase the samples was also evaluated (Meilgaard and others 1999).

## Light microscopy

Samples (5 mm × 5 mm × 3 mm) from the flesh tissue of guavas were fixed in 40 g/L glutaraldehyde in phosphate buffer (0.2 mol/L, pH 7.0) with 40 g/L added sucrose, and dehydrated in a graded ethanol series. Dehydrated samples were embedded in hydroxyethyl methacrylate historesin (Leica Microsystems, Jung, Heidelberg, Germany) and sectioned using a rotary microtome (820 Spencer Microtome, American Optical Corp, New York, N.Y., U.S.A.). Sample sections measuring 8 μm were stained with Toluidine Blue O in acetate buffer (0.1 mol/L, pH 4.7) and examined using an Olympus BX 51 light microscope (Olympus Optical CO., Tokyo, Japan). For each treatment, 2 samples from different fruit were used.

## Statistical analysis

The results were statistically evaluated by the analysis of variance (ANOVA) to determine significant differences between the samples, using the software STATISTICA 5.0 (StatSoft, Inc., Tulsa, Okla., U.S.A.). Analysis of the means was performed using the Tukey procedure at  $P < 0.05$ .

## Results and Discussion

### Gas composition

The PET packages provided a slight passive modification of the atmosphere around the guavas osmotically dehydrated in sucrose solutions, with or without the calcium lactate addition (Figure 1A). For guavas osmotically dehydrated in maltose solutions with calcium lactate addition similar behavior was also observed. The gas equilibrium between the product respiration rate and the gas permeability of the package was noticed at the beginning of storage for these samples, after 7 d of storage, with oxygen and carbon dioxide concentrations reaching levels of about 18% and 3%, respectively (Figure 1A). However, a significant increase in the carbon dioxide level and decrease in the oxygen level was seen around the guavas osmotically dehydrated in maltose solutions without calcium lactate addition, reaching values around 20% of CO<sub>2</sub> and 7% of O<sub>2</sub> at the end of storage (Figure 1B).

According to Rico and others (2007b), a practical approach to evaluate the respiration rate, when comparing similar samples, can be carried out by monitoring the headspace composition in the packages. The oxygen and carbon dioxide concentrations inside the headspaces are related to the metabolic state of samples. In this way, we may conclude that the calcium lactate was able to reduce the respiration rate of osmotically dehydrated guavas in maltose solution, as also observed by some authors in other fruits (Luna-Guzmán and others 1999; Saftner and others 2003; Recasens and others 2004; Aguayo and others 2008), resulting in a lesser modification of gas composition inside the packages.

## Texture characteristics

A significant increase in guavas stress and strain at failure was verified with the addition of calcium lactate on osmotic solutions, resulting in a harder and more resistant to deformation fruit. However, these texture characteristics remained constant throughout the storage period for all treatments used, showing no statistically significant differences ( $P > 0.05$ ), and were not influenced by the type of osmotic agent, sucrose or maltose (Figure 2).

The firming effect provided by calcium salts has also been observed by several researchers (Luna-Guzmán and others 1999; Mastrángelo and others 2000; Manganaris and others 2005; Sousa and others 2006; Pereira and others 2007; Rico and others 2007a) and explained by the linkage of calcium ions with cell wall and middle lamella pectin of vegetable tissues. Bridges formation between pectin molecules stiffens the tissue, increasing the resistance to deformation (Lewicki and others 2002).

The maintenance of vegetable tissue texture during storage in modified atmosphere packages has also been observed by other researchers, such as Martínez-Ferrer and others (2002), Ali and others (2004), Villanueva and others (2005), and Serrano and others (2006). The gas composition inside the packages, with low oxygen and high carbon dioxide contents, reduces the respiration rate of fruits and vegetables and consequently inhibit the metabolic process associated with ripening and senescence. Moreover, the enzymes responsible for changes in texture properties

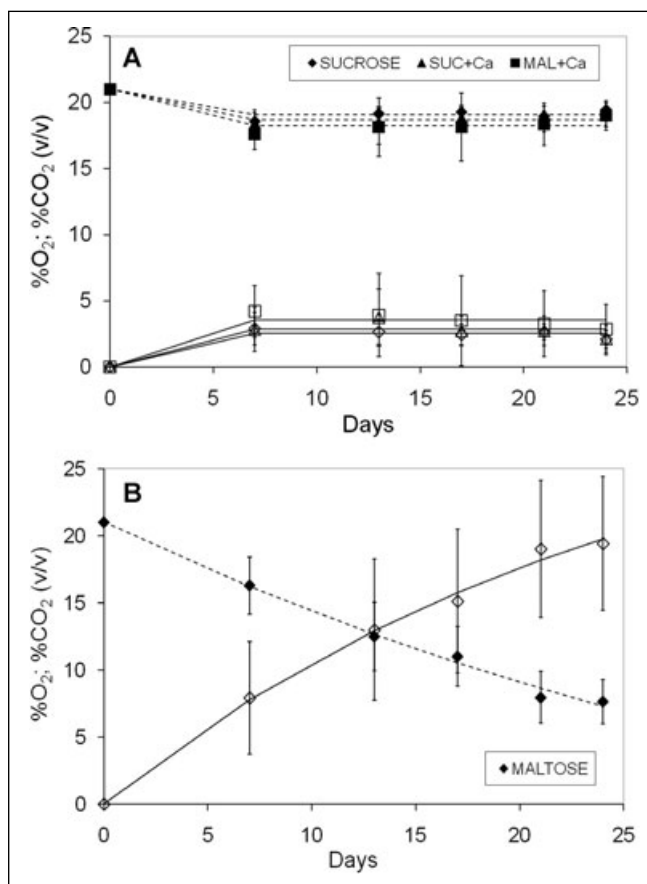


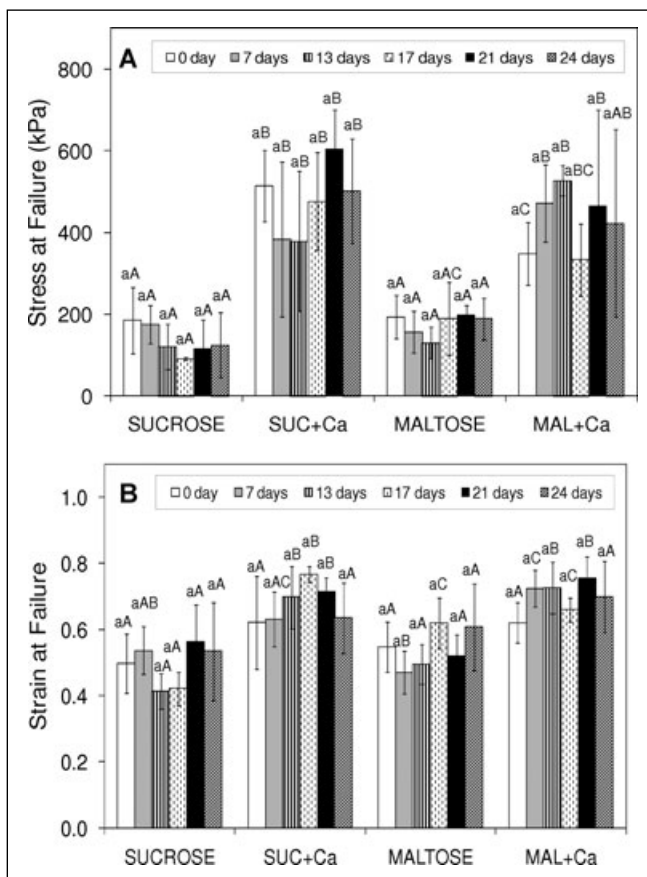
Figure 1—Concentrations of oxygen (closed symbols) and carbon dioxide (open symbols) inside the PET packages during storage. SUCROSE and SUC+Ca: osmotically dehydrated in sucrose solution without and with addition of calcium lactate; MALTOSE and MAL+Ca: osmotically dehydrated in maltose solution without and with addition of calcium lactate.

during ripening, as polygalacturonase and pectinesterase, may have their activity suppressed in these storage conditions (Ali and others 2004).

## Color

The guavas' color was not affected by the use of calcium lactate nor by the sugar used in osmotic treatment, and remained practically constant throughout the 24 d of storage, showing  $L^*$ ,  $a^*$ , and  $b^*$  values around 24, 18, and 7, respectively. Only a slight decrease in  $a^*$  parameter was observed at the beginning of storage for all osmotic treatments, but was kept constant from the 7th day of storage. This change in  $a^*$  parameter may be attributed to osmotic solution remaining on fruit surface after the osmotic process, and color stabilization only after some days of storage. A slight increase in  $b^*$  parameter was also observed at the end of storage, mainly for guavas treated with calcium lactate, resulting in yellowing of the fruit after long storage times.

The color changes of vegetable tissues are also associated with metabolic processes that occur during ripening and senescence. Since these processes are inhibited in low oxygen and high carbon dioxide conditions, the color characteristics of fruits and vegetables were maintained during storage under modified atmosphere packages. Furthermore, most pigments found in vegetable tissues are degraded by exposure to oxygen.



**Figure 2—Stress (A) and strain (B) at failure of guavas during storage.** SUCROSE and SUC+Ca: osmotically dehydrated in sucrose solution without and with addition of calcium lactate; MALTOSE and MAL+Ca: osmotically dehydrated in maltose solution without and with addition of calcium lactate. Mean separation by Tukey ( $n = 5$ ). Different letters indicate statistically significant differences at  $P < 0.05$  (lower case = sample variation during storage; upper case = variation among different sample treatments during a fixed storage time).

Serrano and others (2006) also observed the color maintenance of broccoli heads during storage under modified atmosphere conditions, with low oxygen and high carbon dioxide content, retarding the yellowing process of broccoli inflorescences. Pereira and others (2004) and Rodrigues and others (2006) verified the effect of low oxygen concentration, provided by the modified atmosphere packaging, on color maintenance of guavas and papayas, avoiding the natural pigments degradation along the storage.

With respect to the action of calcium lactate on color characteristics, Castelló and others (2010) also verified that the addition of calcium lactate on osmotically dehydrated strawberries did not lead to significant variations in the color coordinates  $L^*$ ,  $a^*$ , and  $b^*$ .

## Microbial spoilage

The guavas subjected to different osmotic treatments were in accordance with the microbiological standards established by the Resolution RDC No. 12 of 2 January 2001—National Health Surveillance Agency (Brazil 2008). Salmonella was not found in 25 g samples analyzed and coliform bacteria were below 10 CFU/g. These results indicate that the hygienic practices and the sanitization process applied were effective. With regard to yeast and mold count and lactic acid bacteria count, the Brazilian legal requirements do not specify limits for this type of product. However, the development of these microorganisms was evaluated, as they are considered the main spoilage microorganisms able to grow in guavas due to the pH value shown by the fruit.

The yeast and mold counts of guavas osmotically dehydrated in sucrose solutions with or without calcium lactate addition were lower than the detection limit of the method ( $\leq 1.0 \times 10^2$  CFU/g), and remained unchanged during the 24 d of storage at 5 °C (Figure 3).

Slight alterations in yeast and mold counts were observed in guavas osmotically dehydrated in maltose solution with calcium lactate addition during storage, but still showing values in the order of  $10^2$  CFU/g. However, the guavas osmotically dehydrated in maltose solution without calcium lactate addition showed high yeast and mold counts immediately after the osmotic process, with values around  $10^4$  CFU/g before product storage. Microbial growth was inhibited in the first days of storage (after 7 d) due to packaging under passive modified atmosphere and refrigeration, but a yeast and molds proliferation was observed from the 13th day, showing values above  $10^3$  CFU/g till the end of storage.

The high yeast and mold counts of guavas osmotically dehydrated in maltose solution may be attributed to the initial count of maltose syrup used in the osmotic solution, with values up to  $5.0 \times 10^2$  CFU/g, as specified by the sugar supplier, resulting in the proliferation of these microorganisms during the osmotic process. Nevertheless, the presence of calcium lactate in the maltose solution was able to inhibit microbial growth in the fruits. This microbial contamination in maltose-treated guavas may have promoted an increase in the respiration rate of the fruits, resulting in a great modification of the atmosphere inside the PET packages throughout shelf life, as observed in Figure 1.

An antimicrobial effect of calcium salts on fresh-cut cantaloupe was also observed by Luna-Guzmán and Barrett (2000), but the authors suggested further studies to confirm the action of calcium ion on yeast and mold development in fruits. Izumi and Watada (1994) reported that treatment with calcium chloride reduced the microbial growth rate in minimally processed carrots during storage. However, as the calcium chloride effect was noted only

after several days of storage, the inhibitory effect of calcium cation was attributed to the increase in tissue resistance to microbial infection, due to the stabilization and strengthening of the cell wall, rather than to an antimicrobial action of calcium ion.

In the present study, the antimicrobial effect of calcium lactate was evidenced, since the guavas treated with maltose solution showed high yeast and mold counts, around  $10^4$  CFU/g, immediately after the osmotic process and the calcium lactate addition on this solution resulted in an immediate decrease in the fruits microbial contamination, showing values lower than or equal to  $10^2$  CFU/g throughout storage.

Martín-Diana and others (2005) also observed an inhibitory effect of calcium lactate on microbial growth in fresh-cut lettuce and carrot. Studying the efficacy of calcium lactate as a washing treatment in comparison with chlorine treatments (sodium hypochlorite solutions), the researchers observed similar antimicrobial effects for both washing treatments, with a reduction on the mesophilic and psychrotrophic counts of the vegetables.

Another important inhibitory effect on microbial growth was observed due to storage under passive modified atmosphere and refrigeration. The lower oxygen availability and higher carbon dioxide content inside the packages, associated with the low storage temperature, may have caused the death of a large part of microorganisms present in guavas treated with maltose solution. However, due to the microorganisms ability for readaptation to new environmental conditions, the yeast and mold population continued to proliferate during shelf life.

The microbial stability promoted by modified atmosphere and refrigeration has been observed by several researchers, such as Gil and others (2002), Bai and others (2003), Pereira and others (2004), and Villanueva and others (2005). However, some authors concluded that storage temperature was a more critical factor than modified atmosphere packaging in microbial control, suggesting the use of low storage temperatures with a suitable gas composition to achieve the microbial stability of minimally processed products (Gil and others 2002; González-Aguilar and others 2004).

With regard to lactic acid bacteria, the counts were lower than the detection limit of the method ( $\leq 1.0 \times 10$  CFU/g) and remained unchanged during the 24 d of storage at 5 °C for all treatments studied, not showing the development of this microorganism throughout shelf life.

## Sensory acceptability

The guavas subjected to different osmotic treatments showed no statistically significant differences ( $P > 0.05$ ) regarding the main sensory attributes evaluated at the beginning of shelf life (Figure 4A). Only the color of guavas osmotically dehydrated in maltose solution with calcium lactate addition showed a slightly lower score, but with average scores above the acceptability limit (4.5).

After 13 d of storage (Figure 4B), a significant decrease in sensory acceptance was observed for guava treated with maltose and calcium lactate, showing average scores below the acceptability limit for almost all the sensory attributes evaluated, leading to sample rejection.

During this storage time, a consumer preference for guava osmotically dehydrated with sucrose solution was also noted. Aroma, flavor, and overall impression scores for this sample were significantly superior to scores of sample osmotically treated with sucrose and calcium lactate and that osmotically treated with maltose ( $P < 0.05$ ); with slight differences observed between these 2 latter samples. However, the guava osmotically dehydrated in sucrose solution with calcium lactate addition presented some scores below the acceptability limit after 13 d of storage. On the 17th day of storage (Figure 4C), this behavior was intensified and the sample was rejected.

After 21 d of storage (Figure 4D), the guava osmotically dehydrated with maltose solution was not accepted by the consumers, showing low scores mainly in relation to flavor and overall impression. On the other hand, the sensory attributes of guava osmotically dehydrated in sucrose solution presented good sensory scores up to the end of storage, 24 d, showing a good sensory acceptance by the consumers.

The purchase intention of consumers confirmed the preference for guavas osmotically dehydrated in sucrose solution, showing values of around 75% throughout the whole storage period. For guavas subjected to the other osmotic treatments consumers purchase intention was above 50% only at the beginning of storage.

The poor sensory acceptance of guavas treated with calcium lactate may be associated with the firming effect provided by calcium ions on the texture characteristics of the fruits (Figure 2). But, according to the consumers' comments, the rejection of samples was mainly due to their salty taste that probably masked the guavas sweetness, thus impairing the fruits flavor. This effect seems to

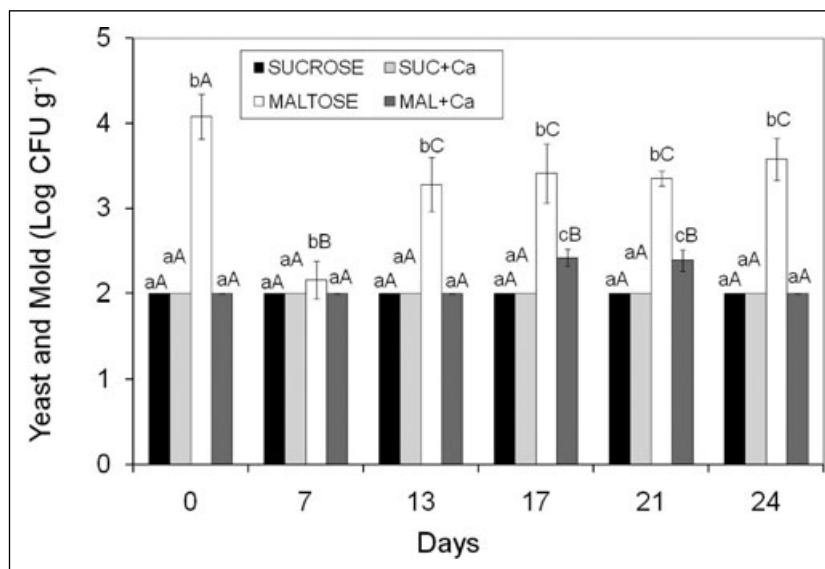


Figure 3—Yeast and mold counts of guavas during storage. SUCROSE and SUC+Ca: osmotically dehydrated in sucrose solution without and with addition of calcium lactate; MALTOSE and MAL+Ca: osmotically dehydrated in maltose solution without and with addition of calcium lactate. Mean separation by Tukey ( $n = 3$ ). Different letters indicate statistically significant differences at  $P < 0.05$  (lower case = variation among different sample treatments during a fixed storage time; upper case = sample variation during storage).

have been intensified along the storage time, probably due to the greater calcium lactate uptake in the fruits during shelf life.

For guavas osmotically dehydrated in maltose solution without calcium lactate addition, the higher yeast and mold counts observed at the beginning of storage and along the shelf life (Figure 3) may have altered some sensory characteristics of fruits. Moreover, the poor sensory acceptance of these samples may also be associated with the lower fruits sweetness, due to the low sweetener power of maltose compared with sucrose.

### Microscopic features

Fresh guava tissue showed turgid cells with a consistent cell wall structure. Cell tonoplast and plasmalemma appeared close to the cell wall, and thus could not be distinguished in the micrograph (Figure 5). However, the osmotic dehydration process using sucrose solution caused cell plasmolysis (black arrows) on guava tissue (Figure 6A). Furthermore, the structural damages were intensified along the storage time. Extensive cellular plasmolysis (black arrows) was observed and the cell collapse (white arrows) was also evident in osmotically treated guavas after 13 (Figure 6B) and 24 d (Figure 6C) of storage.

For treatment with maltose solution, a more structured cell arrangement could be observed (Figure 7A) when compared with sucrose treatment (Figure 6A), as previously observed by Pereira and others (2009), but several granules were observed in cell cytoplasm (dashed arrows), and cellular plasmolysis (black arrows) was also visible. After some days of storage, more intense structural damages seem to occur in maltose-treated samples, showing more

deformed and collapsed cells (white arrows) and extensive cellular plasmolysis (black arrows) (Figure 6B and C; and Figure 7B and C).

The use of calcium lactate on osmotically dehydrated guavas, on the other hand, was able to preserve the fruit tissue structure, as also pointed out by Mastrangelo and others (2000), Quiles and others (2004), and Pereira and others (2007) for different fruits. Calcium lactate-treated samples showed turgid cells with a thick cell wall and well-defined cellular contour, as observed for fresh

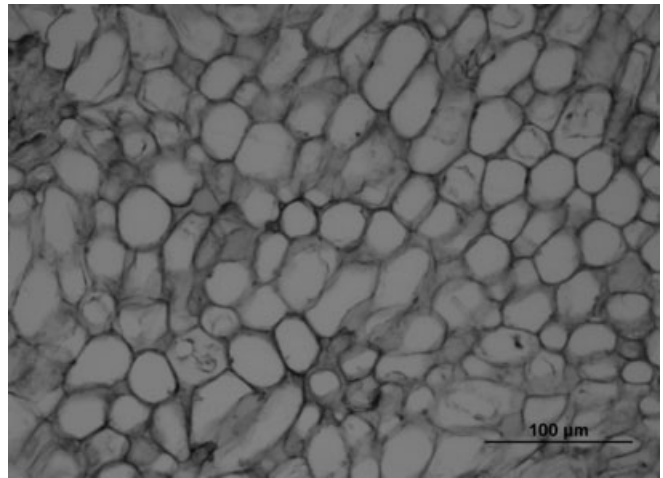


Figure 5—Parenchyma tissue of fresh guavas. Scale bar: 100 μm.

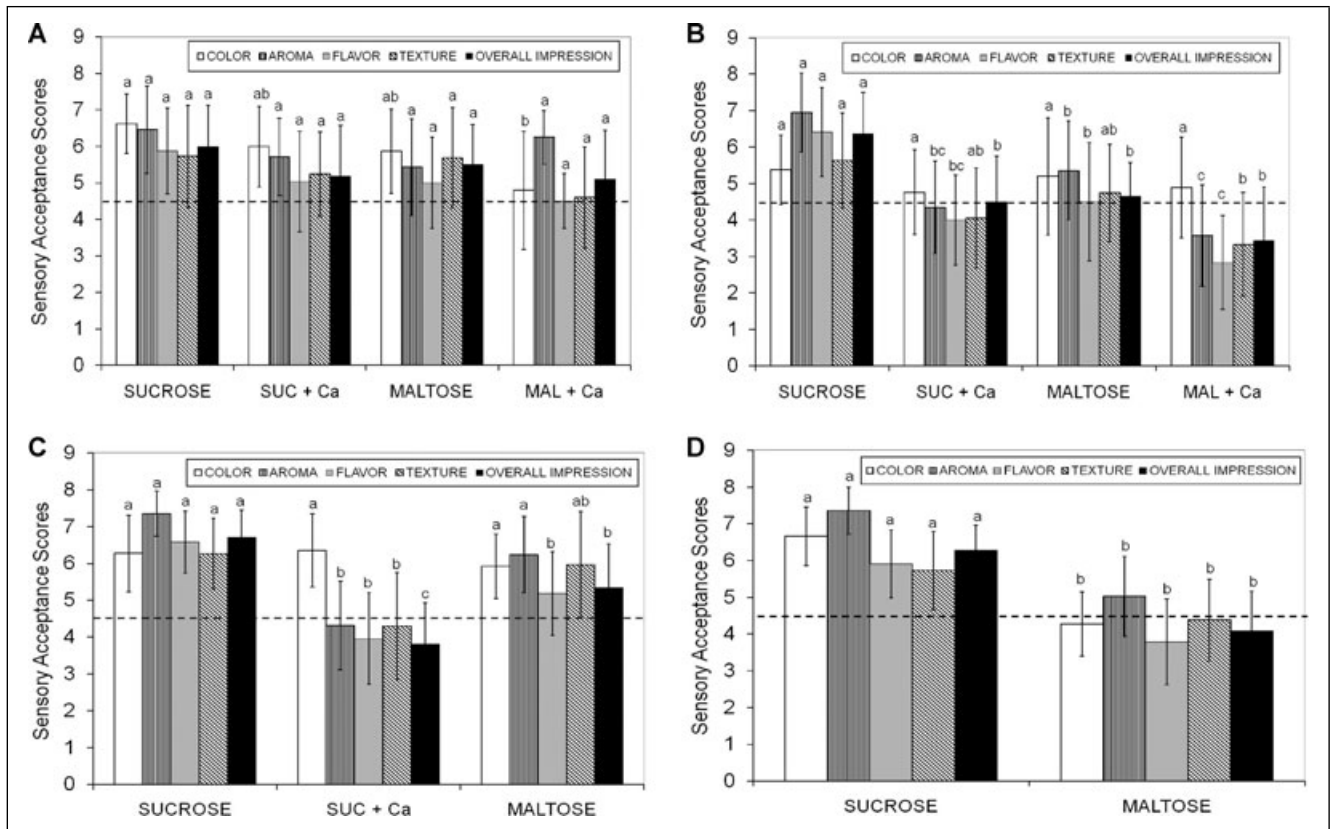


Figure 4—Sensory acceptance scores of guavas at the beginning (A), after 13 (B), 17 (C), and 21 (D) d of storage. SUCROSE and SUC+Ca: osmotically dehydrated in sucrose solution without and with addition of calcium lactate, MALTOSE and MAL+Ca: osmotically dehydrated in maltose solution without and with addition of calcium lactate. Mean separation by Tukey ( $n = 30$ ). Different letters indicate statistically significant differences between the treatments at  $P < 0.05$ .

guava (Figure 6D and 7D). Nevertheless, after some days of storage, these samples showed cellular plasmolysis and the cell damage was intensified along the time, despite the observed strengthening of the cell walls by calcium ions (Figure 6E and F; and Figure 7E and F). However, when comparing the guavas treated without (A, B, and C) and with (D, E, and F) calcium lactate in Figure 6 and 7, we can infer that the calcium lactate preserved the guava tissue structure to some extent, resulting in stronger cell walls

along the storage time, but it was unable to avoid the cellular plasmolysis.

Alandes and others (2006) also observed that the plasmalemma begins to retract in some areas of apple tissue treated with calcium lactate after 3 wk of storage, although the cell walls maintained their structure. For apples without calcium lactate treatment, besides the plasmalemma retraction toward the center of cells, in some zones the cell walls seemed to be broken.

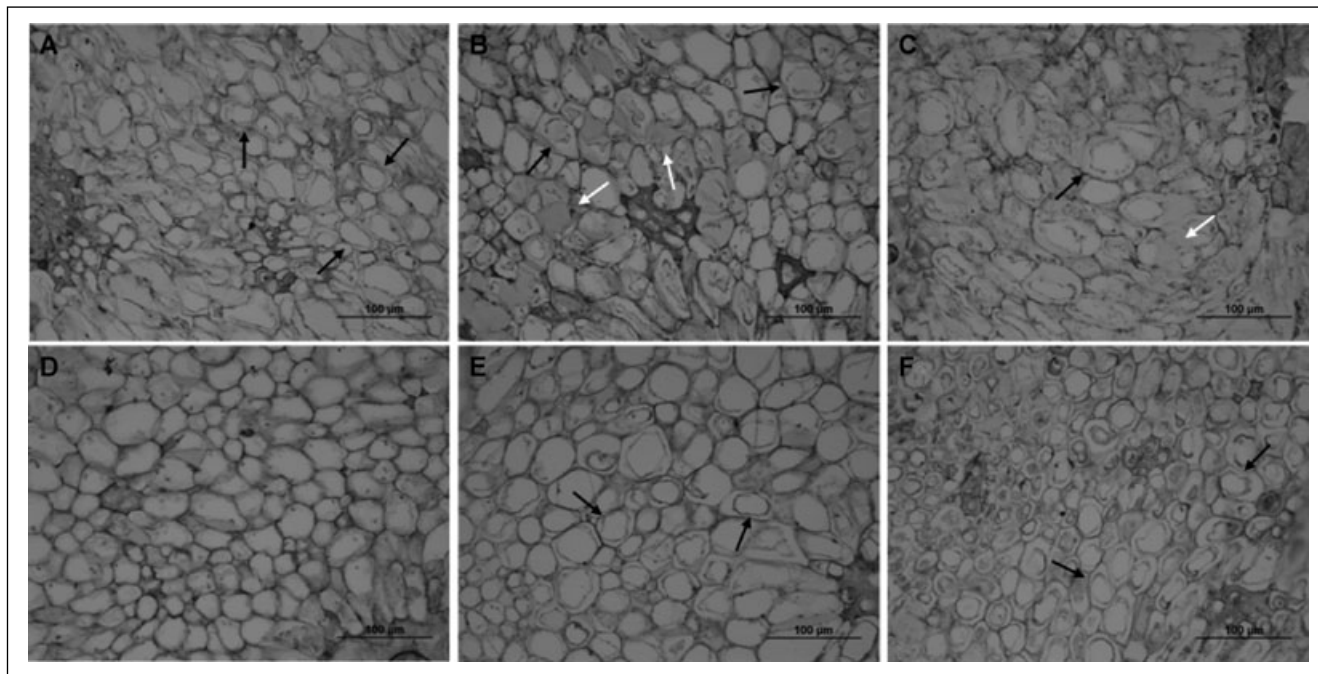


Figure 6—Parenchyma tissue of guavas osmotically dehydrated in sucrose solutions without (A, B, and C) or with (D, E, and F) calcium lactate addition during storage. (A) and (D) at the beginning; (B) and (E) after 13 d; (C) and (F) after 24 d of storage. Black arrows = cell plasmolysis; white arrows = cell collapse. Scale bar: 100  $\mu\text{m}$ .

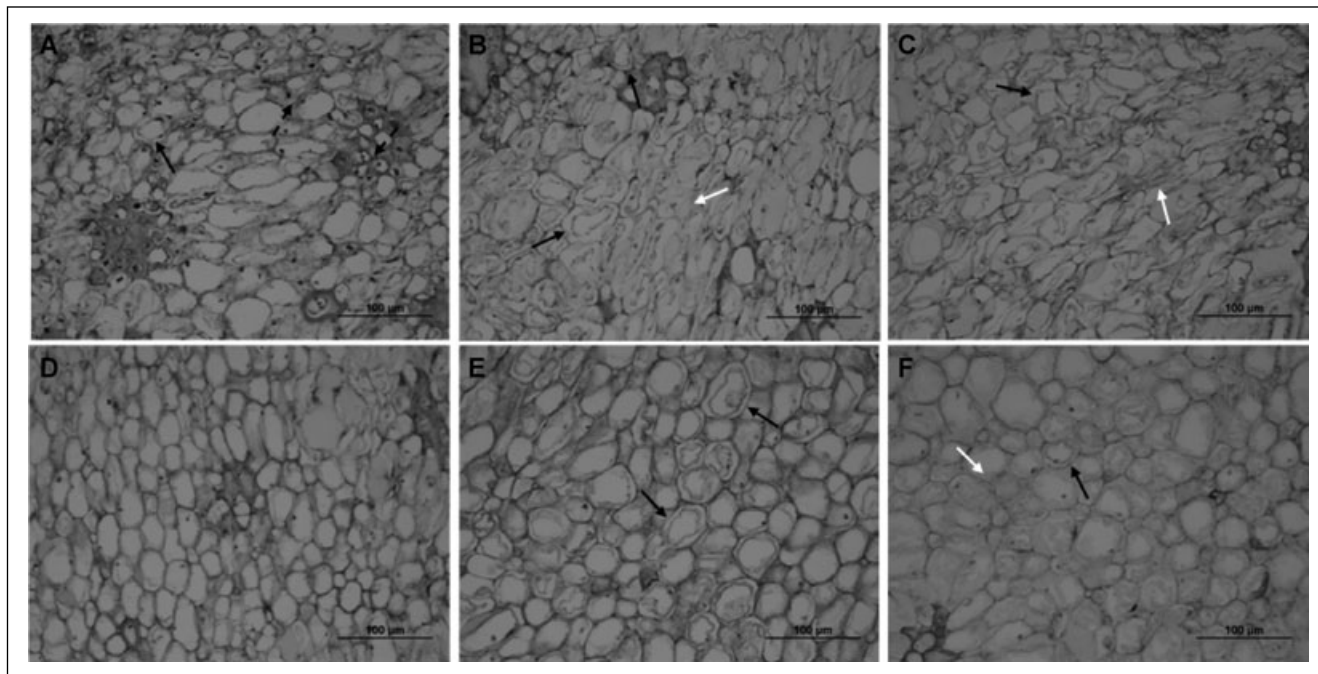


Figure 7—Parenchyma tissue of guavas osmotically dehydrated in maltose solutions without (A, B, and C) or with (D, E, and F) calcium lactate addition during storage. (A) and (D) at the beginning; (B) and (E) after 13 d; (C) and (F) after 24 d of storage. Black arrows = cell plasmolysis; white arrows = cell collapse; dashed arrows = granules on cytoplasm. Scale bar: 100  $\mu\text{m}$ .

## Conclusion

An antimicrobial effect of calcium lactate was observed on guavas osmotically dehydrated in maltose solution, and the salt was also able to reduce the samples respiration rate. A firming effect provided by calcium lactate was verified on guavas texture, resulting in a harder and more resistant to deformation fruit, and the salt did not change the guavas color characteristics. The calcium lactate was also able to preserve the guavas tissue structure to some extent, causing the strengthening of cell walls, but not avoiding the cellular plasmolysis along the storage time.

However, the use of calcium lactate on guavas osmotically dehydrated in sucrose and maltose solutions was sensory unacceptable during storage for 24 d, and only the guavas osmotically dehydrated in sucrose solution showed good scores and a great sensory acceptance by consumers until the end of storage.

The poor sensory acceptance of guavas treated with calcium lactate may be associated with the firming effect provided by calcium ions on the texture characteristics of the fruits. But, the samples rejection was mainly attributed to their salty taste that probably masked the guavas sweetness.

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