

Full Length Research Paper

Evaluation of the postharvest quality of Cagaita fruits (*Eugenia dysenterica* DC.) coated with chitosan and associated with refrigeration

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Cagaita fruits are subject to seasonality and perishability. This work aims to use scanning electron microscopy (SEM) to evaluate the physicochemical characteristics, texture, color and physical structure of cagaita fruits coated with different chitosan concentrations. The fruits were divided as follows: T0 (uncoated fruits), T1 (fruits coated with 1% (v/v) chitosan), T2 (fruits coated with 2% (v/v) chitosan) and T3 (fruits coated with 3% (v/v) chitosan). They were analyzed at 0, 10, 20 and 30 days of storage. Titratable acidity and soluble solids content showed no conservation of fruit characteristics; they showed better results for uncoated fruits, as well as weight loss, vitamin C and peak strain. The color of cagaita fruits confirmed ripening during storage regardless of treatment. Scanning electron microscopy showed that the film solution did not adhere, as desired, to the cell wall of fruits. As the results of fruits coated with 3% pectin were close to control, further studies should be carried out with higher coating percentages so that the fruit quality is maintained during storage.

Key words: Physical structure, film solution, quality, shelf life.

INTRODUCTION

Cerrado is the second largest biome in South America occupying an area of 25% of the Brazilian territory. It has extremely rich flora, averaging 1000 species of trees,

3,000 species of herbs and shrubs and 500 vines (Roesler et al., 2007). Due to the heterogeneity of the plant, some fruits of Cerrado are little studied, despite

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Abbreviations: SEM, Scanning electron microscopy, DPIP, 2,6-dichlorophenol indophenols sodium.

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having great economic potential and nutrition (Santos et al., 2012). Cagaita (*Eugenia dysenterica* DC.), a typical fruit of the Brazilian savanna, has fragile shell, green and light yellow color, juicy pulp, pleasant flavor, but slightly acidic (Santos et al., 2012). The fruit has important nutritional characteristics represented by vitamin C, as well as polyunsaturated fatty acids such as linoleic acid, with contents higher than those found in coconut and olive oils and linolenic acid, with contents higher than those found in sunflower, soybean and peanut (Nietsche et al., 2004). Being a regional fruit that is subject to seasonality and due to few studies conducted to increase its shelf life, cagaita is not available for consumption throughout Brazil. However, whenever available, it is consumed fresh or incorporated into food products such as sweets, juices, liqueurs and jellies (Santos et al., 2012).

In search of new technologies to preserve the quality of cagaita fruits, structural, sensory and nutritional changes should be minimized through physical, chemical and gaseous treatments that can be applied to the product (Mahajan et al., 2014). One of these applications relates to the use of edible coating combined with modified atmosphere and/or temperature control (Maftoonazad et al., 2007). Such edible coatings help to reduce oxygen availability, breathing, water loss and oxidation reaction rate (Kerdchoechuen et al., 2011). Chitosan is a biopolymer that can be used as edible coating because of its nontoxic characteristics; it forms biodegradable films and prevents microbial activity (Soares et al., 2011). In the post-harvest quality, chitosan is responsible for reducing breathing rate, ethylene production and transpiration of plants, and contributes to the fungicidal properties of fruits (Luvielmo and Lamas, 2012).

This work aimed to evaluate the physicochemical characteristics, texture, color and physical structure of cagaita fruits coated with different chitosan concentrations by scanning electron microscopy (SEM).

MATERIALS AND METHODS

Cagaita fruits were collected at Fazenda Gameleira, municipality of Montes Claros de Goiás - GO (16°06'20" S and 51°17'11" W), 592 m above sea level on September 14, 2014; they were subsequently transported to the Laboratório de Frutas e Hortaliças, Instituto Federal Goiano - Rio Verde Campus. Initially, fruits were selected by size, color and absence of mechanical damage and surface stains. Subsequently, they were sanitized with chlorinated water at 150 ppm for 15 min and dried at room temperature. Then, fruits underwent four treatments as follows: control treatment, T0 (fruit receiving no chitosan coating), T1 (fruits coated with 1% (v/v) chitosan), T2 (fruits coated with 2% (v/v) chitosan) and T3 (fruits coated with 3% (v/v) chitosan). The preparation of film solutions was carried out from chitosan solubilization in glacial acetic acid and water (800 ml of water and 50 ml of glacial acetic acid); pH was adjusted to 4.00, with sodium hydroxide solution (0.1 mol/L). For the distinct treatments, the following amounts of chitosan were added: 0 g (control, T0); 5 g (treatment 1); 10 g (treatment T2); 20 g (treatment T3). Subsequently, fruits were immersed in chitosan solution for about 1 min and allowed to dry naturally. Treatments

were placed in Styrofoam trays with dimensions of 150 × 150 × 18 mm. Trays were placed in BOD with controlled temperature at 12°C ± 0.1. They were evaluated at 0, 10, 20 and 30 days in three replicates with six fruits each. In each day of analysis, 72 fruits were evaluated. In the three replicates, each repetition possessed six fruits in the tray. The following parameters were analyzed: weight loss, titratable acidity and soluble solids, ascorbic acid, texture (through peak strain), color and physical structure by scanning electron microscopy (SEM). Digital scale with accuracy of four places was used to measure weight loss, and the results were expressed as percentage of the original weight through the reserved lot for analysis, using the same fruits. The lot used for weight loss was separated from the lot used for physical and chemical analysis. The fruits of physical-chemical analysis were discarded each day of the analysis, since the lot used for weight loss was maintained until the 30 days analysis. That is, they were removed from the heavy weight, and BOD was stored again. Titratable acidity quantification was obtained by titrating the filtered juice with NaOH solution (0.01 N), and the results were expressed as % citric acid by method No. 986.13 (AOAC, 1992). The soluble solids content, expressed in °Brix, was evaluated by reading the juice in refractometer Atago N-2E according to AOAC standard No. 983.17 (1992). Ascorbic acid was determined by volumetric oxidation-reduction, titrating samples with a 2,6-dichlorophenol indophenols sodium (DPIP) solution by AOAC method No. 967.21 (2000). All reagents used were of Neon Comercial Ltda in São Paulo - SP - Brazil.

Texture, analyzed by the peak strain, was determined with the help of Brookfield texturometer, model CT3 texture analyzer. This technique consists of a uniaxial compression test at high deformation of samples using a cylindrical acrylic plate (model TA3/1000) at compression speed of 1 mm/s and 50% of sample deformation. The results are expressed in N/m². Color (L*, a*, b*) of cagaita fruits was analyzed in Hunter Lab colorimeter model Color Quest II. The evaluation used 10° observation angle and D65 as standard illuminant, which corresponds to natural daylight. The results were expressed as L*, a* and b* values, where L* (luminosity or brightness) values range from black (0) to white (100), a* values range from green (-60) to red (+60) and b* values range from blue (-60) to yellow (+60). The characterization of the physical structure of cagaita fruits consisted of removing the epicarp, drying at 60°C for 12 h and storing in desiccator. For scanning electron microscopy analysis (SEM), samples were placed on stubs, coated with a thin layer of gold and micrographed. Evaluation was performed at the multiuser Laboratório de microscopia de Alta Resolução at the Instituto de Física, Universidade Federal de Goiás using Scanning Electron Microscope, Jeol, JSM - 6610, equipped with EDS, Thermo scientific NSS Spectral Imaging. Statistical analysis consisted of a 4 × 4 × 3 factorial design, with four treatments (control, 1, 2 and 3% chitosan), four storage times (0, 10, 20 and 30 days) and three replicates for each fruit tray analyzed. This results in nine replicates of each treatment studied by completely randomized design. The models were selected according to the determination coefficient and its significance was tested by the F test. The mean values for weight loss, titratable acidity, soluble solids, ascorbic acid, texture and color analyses were compared by the Tukey test at 5% probability with the help of the SISVAR software.

RESULTS AND DISCUSSION

Weight loss

Cagaita fruits showed similar average weight loss results (Figure 1). Throughout the storage period control fruits showed the lowest weight loss values. The fruits of

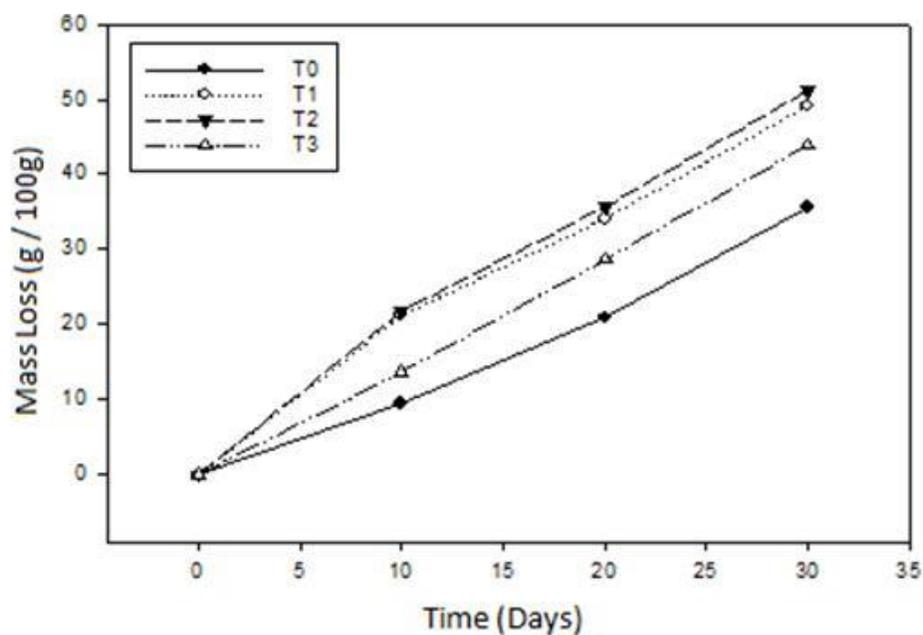


Figure 1. Weight loss for control fruits (T0) and treatments with 1% (v/v) (T1), 2% (v/v) (T2) and 3% (v/v) (T3) chitosan during storage of cagaita fruits under BOD at $12^{\circ}\text{C} \pm 0.1$.

treatment T2 (fruits coated with 2% chitosan) had the highest mass loss rate compared to the other treatments. Chitosan has an undesirable effect on the weight loss of cagaita fruits, where the smallest weight loss was observed in control fruits. However, fruits coated with 3% chitosan showed lower weight loss when compared to fruits coated with 1 and 2% chitosan (Figure 1). The non-protective effect of chitosan coating was also reported by Santos et al. (2008) in peach fruits. Although chitosan has the capacity of forming semi-permeable films that act by modifying the internal atmosphere and reducing loss by transpiration and dehydration (Santos et al., 2008), some authors report that hydrophilic molecules are not significant barriers to the dissemination of water vapor (Azeredo et al., 2010). Botrel et al. (2007) reported that components such as lipids and proteins contribute to decreased weight loss when added to coatings, since the matrix becomes more compacted with increasing amounts of amylase, thereby decreasing the coating permeability to water vapor. These authors reported that weight loss in coated garlic is due to the low barrier property of the coating to fruit transpiration and dehydration, as reported in this work. The smaller weight loss presented by fruits with 3% chitosan when compared to other treatments may be related to the coating thickness, because when too thin, coating can contribute to moisture loss (Silva et al., 2012).

Titrateable acidity content

Titrateable acidity of cagaita fruits varied during storage

(Figure 2), showing lower values on the tenth day of analysis compared to the first day of storage and subsequent higher titrateable acidity levels on the twentieth day analysis compared to the tenth day of storage. At the end of the experiment, fruits showed acidity levels similar to those reported on the tenth day of analysis. The drop in acidity levels on the tenth day of storage indicates that fruits are going through processes that lead to maturity, whereas according to Scalon et al. (2012), fruits with titrateable acidity content above 1.5% are at the climacteric peak stage. Oshiro et al. (2012) reported that the drop in acidity content is a response to increased respiratory rate and contributes to water loss of fruits. On the twentieth day of storage, an unexpected increase of the titrateable acidity content was observed for all treatments. This increase may be the result of anaerobic respiration that causes physiological disorders in fruits. The process of anaerobic respiration may indicate response to the low biofilm permeability to gases, causing the fruit to form ethanol and acetaldehyde by obtaining energy for this process, affecting the product quality (Steffens et al., 2007; Petracek et al., 2002). This increase can also occur through the release of galacturonic acid from the cell wall by the action of pectinmethylesterase and poligalac-turonase enzymes (Scalon et al., 2012). The decrease reported again on the thirtieth day of storage of cagaita fruits may be related to degradation caused by the anaerobic respiration phenomenon. Oshiro et al. (2012) reported that coating guavas with 3% chitosan does not contribute to the preservation of the titrateable acidity content.

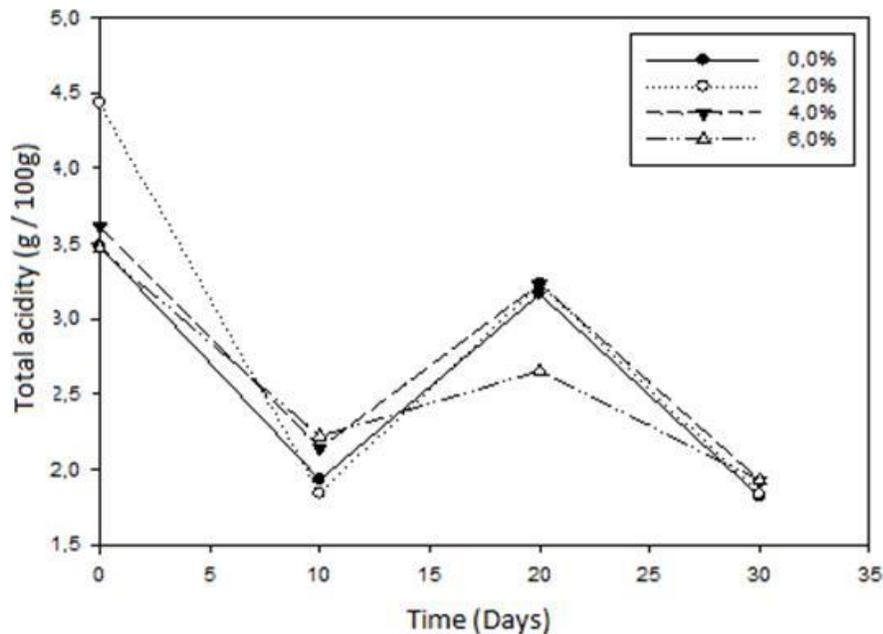


Figure 2. Titratable acidity of control fruits (T0), treatments with 1% (v/v) (T1), 2% (v/v) (T2) and 3% (v/v) (T3) chitosan during storage of cagaita fruits under BOD at 12°C ± 0.1.

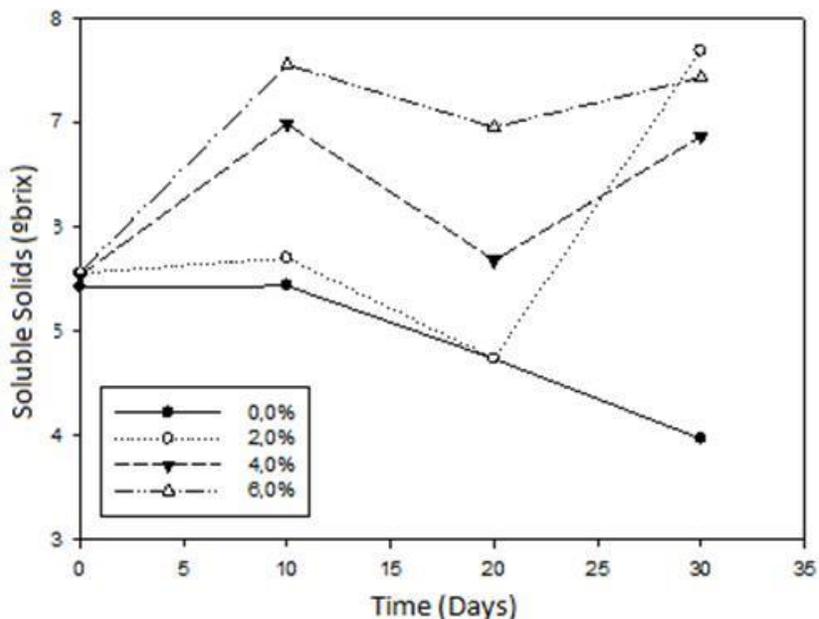


Figure 3. Soluble solids content of control fruit (T0) and treatments with 1% (v/v) (T1), 2% (v/v) (T2) and 3% (v/v) (T3) chitosan during storage of cagaita fruits under BOD at 12°C ± 0.1.

Soluble solids content

Considerable variation in soluble solids content of uncoated and coated cagaita fruits was observed during

storage (Figure 3). On the tenth day of analysis, fruits showed an increase in soluble solids content, for treatments with 2 and 3% chitosan. On the twentieth day of analysis, decreases in the quantification of this parameter

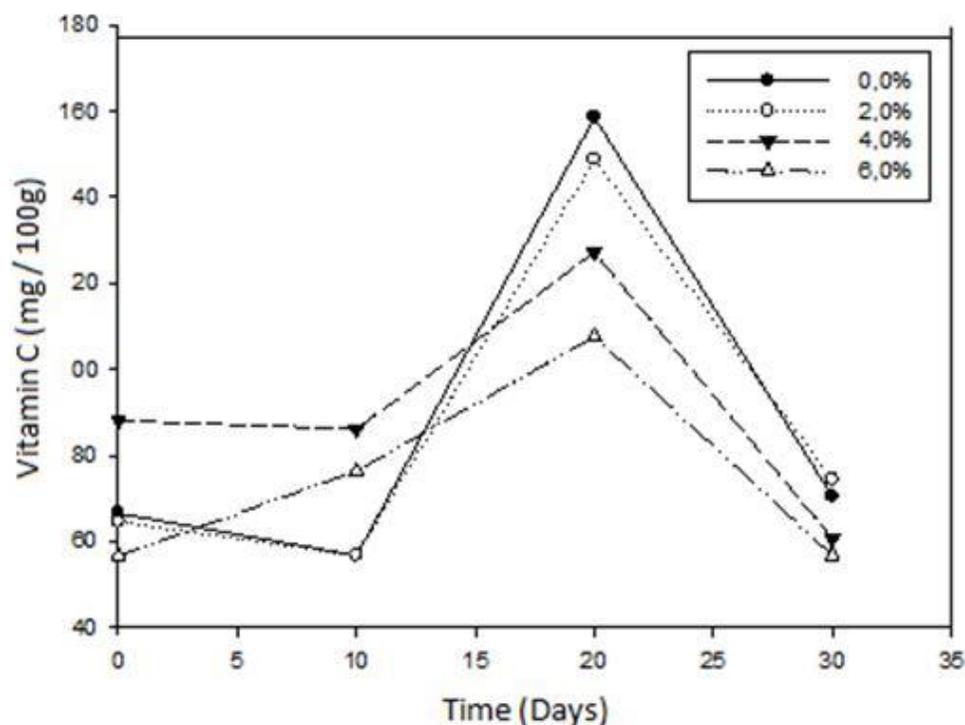


Figure 4. Vitamin C content of control fruits (T0) and treatments with 1% (v/v) (T1), 2% (v/v) (T2) and 3% (v/v) (T3) chitosan during storage of cagaita fruits under BOD at $12^{\circ}\text{C} \pm 0.1$.

for all treatments analyzed were observed, which, with the exception of uncoated fruits, increased again at the end of storage. The increase in the soluble solids content observed on the tenth and thirtieth day of analysis may be related to the characteristic of climacteric fruits, which even after harvest, still presents maturation evolution (Taiz and Zeiger, 2006). These fruits tend to increase the soluble solids content due to the biosynthesis of soluble sugars or degradation of polysaccharides (Kays, 1997). However, increase in soluble solids content may also indicate use of accumulated reserves in processes of fruit solid transformation into soluble sugars (Jeronimo and Kanesiro, 2000).

Santos et al. (2008) reported that in soluble solids analysis, an increase after a decrease may indicate concentration of solids due to loss of water during storage. On the twentieth day of storage analysis (Figures 2 and 3), with increasing acidity there was a decrease in soluble solids, which may indicate a rapid consumption of sugars through the aerobic respiration process. When this process is enhanced, future lack of sugar causes the onset of anaerobic respiration indicated by the increased sugar content and decrease in acidity values. Chien et al. (2013) determined the best results for preserving soluble solids in papaya fruits coated with 1% chitosan. Hong et al. (2012) showed a drastic increase in the soluble solids content of guavas coated with chitosan and control.

Ascorbic acid content

There were variations in the vitamin C content of stored cagaita fruits (Figure 4). At the tenth day of storage, except for fruits coated with 3% chitosan, there was a decrease in ascorbic acid content when compared to the beginning of the experiment. In twenty days of storage, fruits showed a considerable increase in vitamin C content, especially control fruits, and at the thirtieth day, the content showed a considerable drop. Reduced ascorbic acid content is related to the ripening of cagaita fruits at the tenth day of storage for fruits coated with 3% chitosan. Ripening was also analyzed for titratable acidity and soluble solids. Lee and Kader (2000) reported that, although the ascorbic acid content decreases with maturation, there may be an increasing trend due to the increase in the total volume of juice with advancing maturation process. This increase can be analyzed at the twentieth day of storage, but it is also the result of oxidative reactions that vitamin C undergoes during ripening by acting as a molecule with antioxidant properties (Carnelossi et al., 2009). Ascorbic acid has characteristics of unstable molecule that may undergo auto-oxidation into dehydroascorbic acid, which although being reversible, may cause losses in product quality (Gonçalves and Maia Campos, 2009) and lead to increased and decreased contents in stored fruits. This phenomenon can be caused by temperature used in the

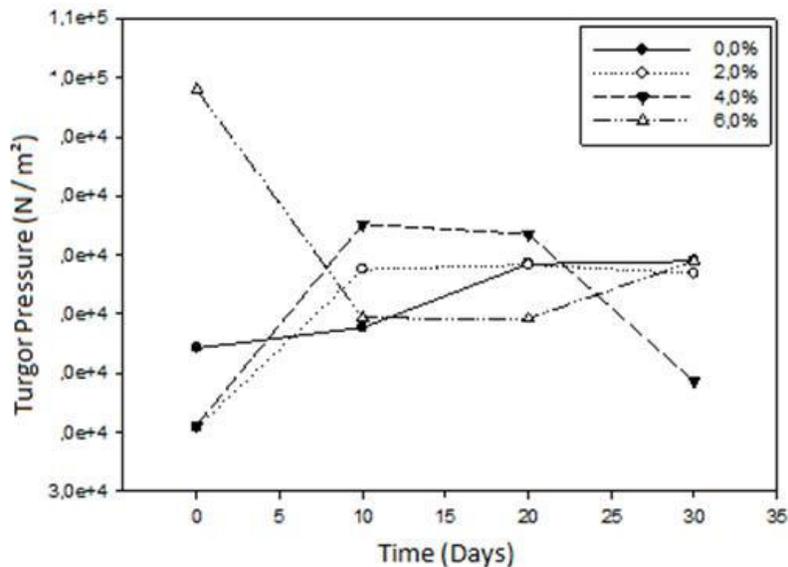


Figure 5. Peak strain of control fruits (T0) and treatments with 1% (v/v) (T1), 2% (v/v) (T2) and 3% (v/v) (T3) chitosan during storage of cagaita fruits under BOD at $12^{\circ}\text{C} \pm 0.1$.

experiment; in future work, a lower temperature storage can be used. At the end of the experiment, the decrease in ascorbic acid content can be related to chemical and physical aspects of fruits in which the concentration of phenols and ascorbic acid has positive relationship with fruit firmness, where losing firmness is a result of full maturity and fruits have lower ascorbic acid content (Blum et al., 2008). Oshiro et al. (2012) explain that the vitamin C content was better preserved in guava stored at 5°C and coated with 3% chitosan.

Texture

The texture of cagaita fruits was quantified by peak strain, which indicates the force required to break the cell walls of fruits. The peak strain is variable during storage (Figure 5). This parameter has not presented the desired values for any of the treatments, with decreases and increases during the storage period. Control fruits showed the best peak strain results, which varied less compared to the other treatments during the storage period. Fruits with 3% chitosan, which at the beginning of the storage period showed interesting texture values, confirmed more consistent bark and showed a significant decrease in texture values with increasing storage time. This significant decrease can be related to the ripening of fruits through a series of enzymatic reactions related to climacteric respiration and ethylene production (Castricini, 2009). The subsequent increase in peak strain is confirmed by the firmness of the cell wall due to the lack of degradation of insoluble protopectins to form

soluble pectic acid and pectin in fruits (Maftoonazad and Ramaswamy, 2008). According to literature, the decrease in texture value can also be the result of hydrolases acting on the cell wall (Vicentini et al., 1999).

Fruit color

The brightness of cagaita fruits varied similarly for all treatments analyzed (Figure 6), showing an increase in the average L^* value up to the tenth day of storage, and after, values decreased up to the end of the experiment. The L^* parameter is related to brightness, which ranges from 0 (completely dark) to 100 (completely clear). With the application of chitosan, fruits had a matte appearance on the first day of storage, not as glossy as expected for cagaita fruits. With advancing storage, coated fruits were similar to uncoated fruits because chitosan remained with matte appearance. The cell walls of cagaita fruits showed symptoms of dryness, detached films with brittle appearance and depressions in the pulp, indicating that the coating film did not properly adhere to the cell wall of fruits. The matte appearance contributed to the decrease in fruit brightness values. This drop can also indicate that cagaita fruits undergone maturation processes and degradation reactions may have occurred, which contributed to the darkening of the fruit surface. Reis et al. (2006) reported that Japanese cucumbers also showed a slight increase in brightness and, later, values decreased up to the end of the analysis. The a^* parameter varied similarly for control fruits and for those added with 3% chitosan; T3 fruits showed the lowest

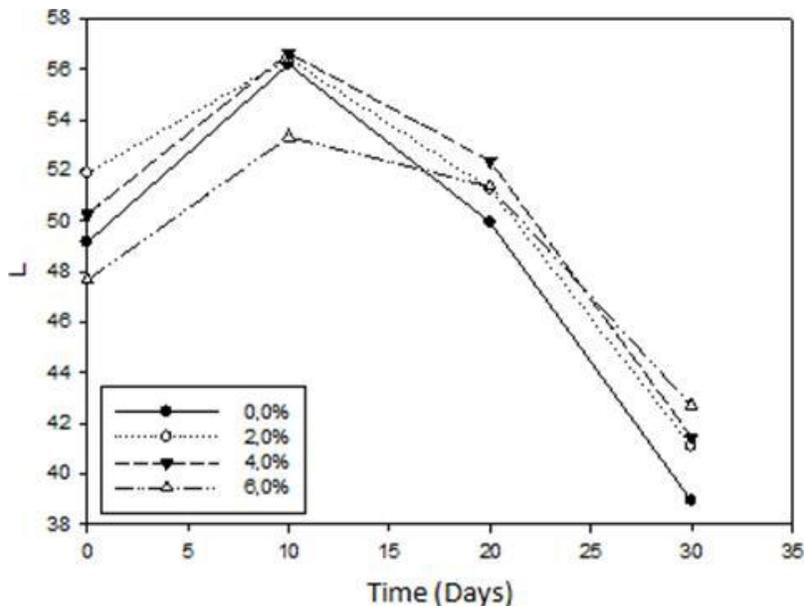


Figure 6 - L* parameter for control fruits (T0) and treatments with 1% (v/v) (T1), 2% (v/v) (T2) and 3% (v/v) (T3) chitosan during storage of cagaita fruits under BOD at 12°C ± 0.1.

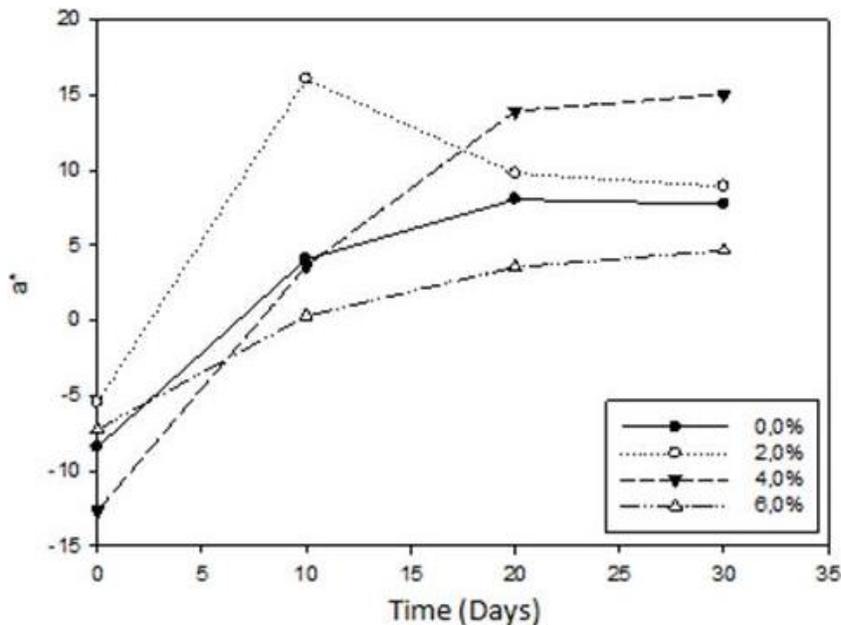


Figure 7. a* parameter of control fruits (T0) and treatments with 1% (v/v) (T1), 2% (v/v) (T2) and 3% (v/v) (T3) chitosan during storage of cagaita fruits under BOD at 12°C ± 0.1.

mean values (Figure 7). For T2, variation is greater than that reported for T0 and T3 and for T1; variation did not follow the same behavior of other fruits analyzed. For low a* values, even negative, samples showed a more

greenish coloration and for high a* values, samples showed red-purple color. The increased red values may be the result of maturation. According to Chitarra and Chitarra (2005), the color of fruits is related to the

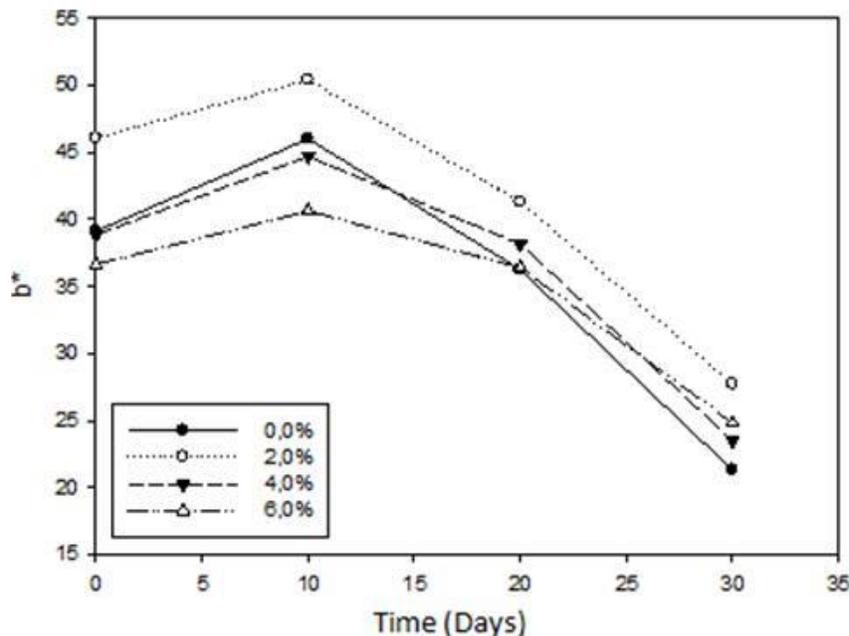


Figure 8. Color parameter b^* of control fruits (T0) and treatments with 1% (v/v) (T1), 2% (v/v) (T2) and 3% (v/v) (T3) chitosan during storage of cagaita fruits under BOD at $12^{\circ}\text{C} \pm 0.1$.

uniformity of the maturation stage. Santos et al. (2008) assessed the quality of peach cv. *Douradão* treated with 1% chitosan solution and found that the values of red coloration intensity decreased in peaches treated with chitosan, similarly to cagaita fruits coated with 3% chitosan in this study. Color parameter b^* showed similar variation for all treatments, with greater variation for cagaita fruits coated with 1% chitosan (Figure 8). After the experiment, control fruits showed the lowest b^* values. This parameter varies so that when low, including negative values, the sample shows blue color and when high, the sample is yellowish. Cagaita fruits showed no incidence of blue color on the outer surface and had a mixed green and yellow color. The decrease in yellow color during storage indicates that, correlating the changes in the green color, fruits underwent chlorophyll degradation and synthesis of yellow and red pigments that can be biosynthesized carotenoids (Vianna-Silva et al., 2008). The synthesis of these compounds is not interesting, as they indicate that cagaita fruits matured during the analysis, even coated fruits, and fruits coated with 1% chitosan showed the lowest incidence.

Scanning electron microscopy

The physical structure of cagaita fruits in the control treatment indicates the presence of heterogeneous surface (Figure 9), with formation of small bubbles and crusts indicated by arrows at the top and bottom of the

image. The cell wall of the fruit does not show smooth appearance and can determine sites of gas exchange, water loss and entry of microorganisms that cause a drop in post-harvest quality and shelf life (Wu, 2010). Cagaita fruits coated with 1% chitosan (Figure 10) showed heterogeneous surface with incidence of pores. The presence of pores, especially in the amount indicated in the lower image shows that the cell walls of fruits can be more prone to gas exchange and water loss, which will result in loss of fruit quality, and such fact may occur with control fruits. Control fruits and those with 1% chitosan in the cell wall showed cracks on the physical structure. Such cracking may be due to the fact that wax production is disconnected from fruit growth, causing an imbalance of these parameters that may cause the formation of an interconnected network of channels on the fruit surface (Roy et al., 1994). The physical structure of cagaita fruits with 2% chitosan (Figure 11) indicates that the fruit surface was covered by a coating layer that has caused the appearance of higher points in relation to the remainder of the cover, indicated by arrows on the upper images. These points can be the result of misapplication of chitosan solution or poor adhesion to the cell wall of fruits. However, the lower image shows that there is formation of a network by the film solution, which may indicate that the solution adhered to the cell wall of fruits and when applied under the ideal conditions can contribute to maintaining product quality. The surface of cagaita fruits coated with 3% chitosan was more homogeneous compared to other physical structures

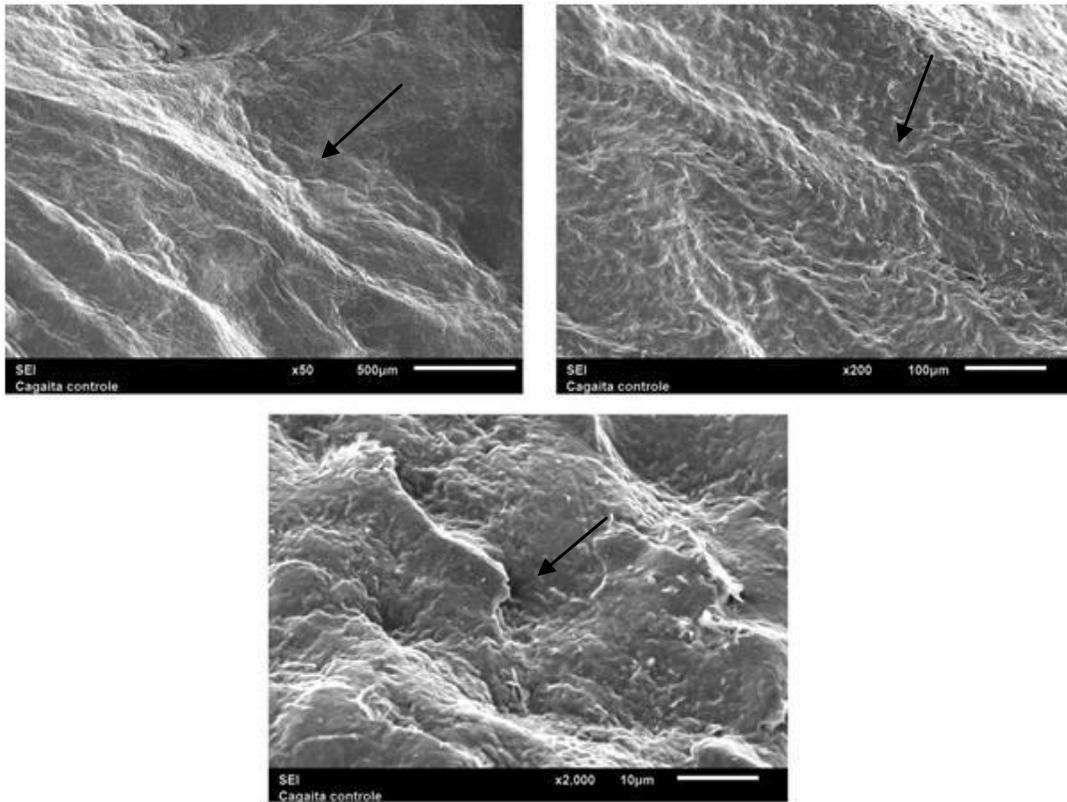


Figure 9. Physical structure (SEM) of control cagaita fruits (*Eugenia dysenterica* DC.). Fruit epicarp surface images.

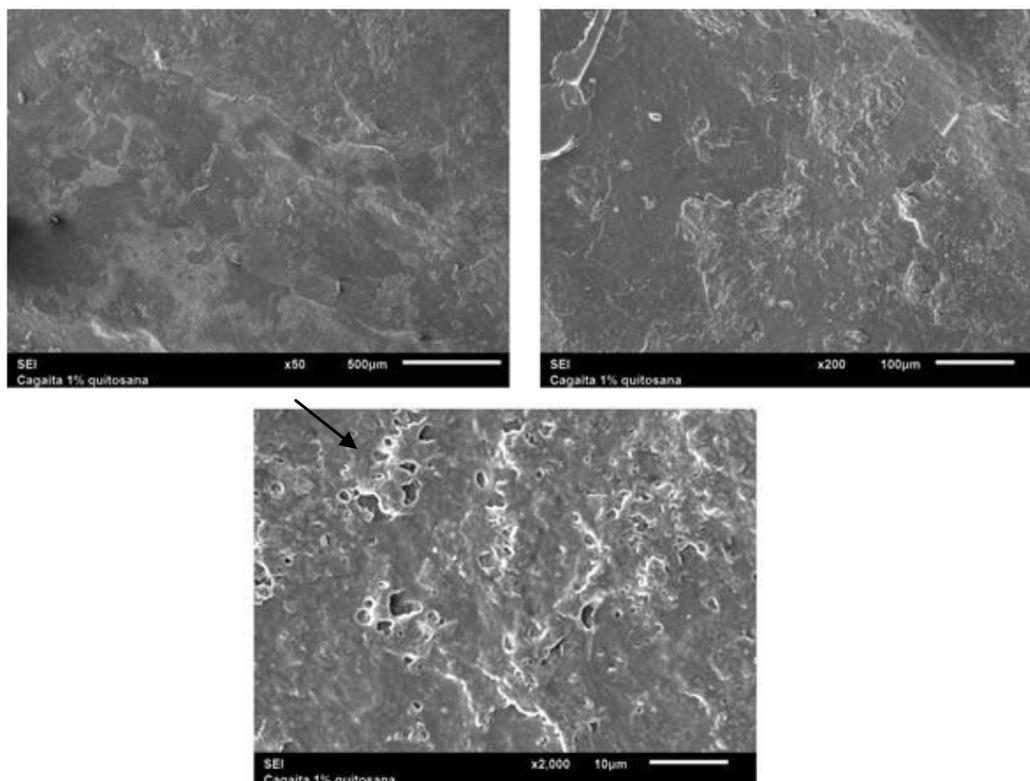


Figure 10. Physical structure (SEM) of cagaita fruits (*Eugenia dysenterica* DC.) coated with 1% chitosan. Fruit epicarp surface images.

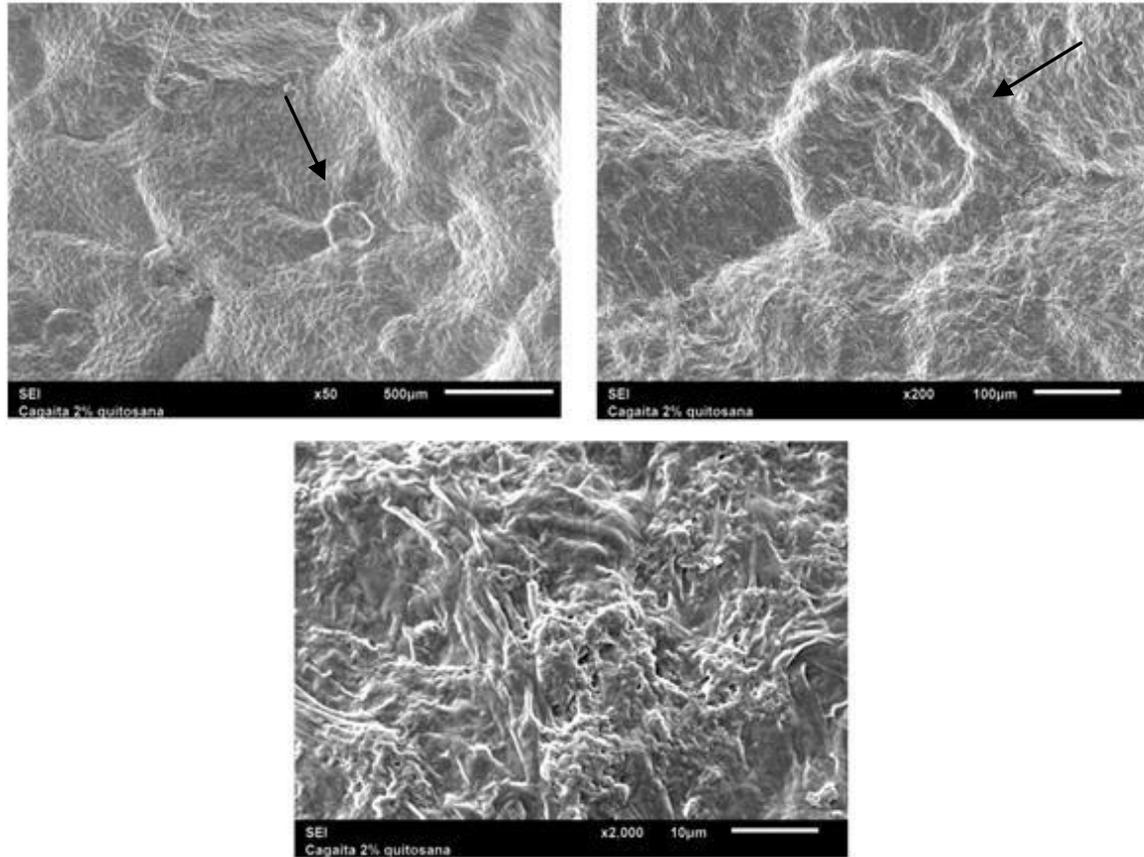


Figure 11. Physical structure (SEM) of cagaita fruits (*Eugenia dysenterica* DC.) coated with 2% chitosan. Fruit epicarp surface images.

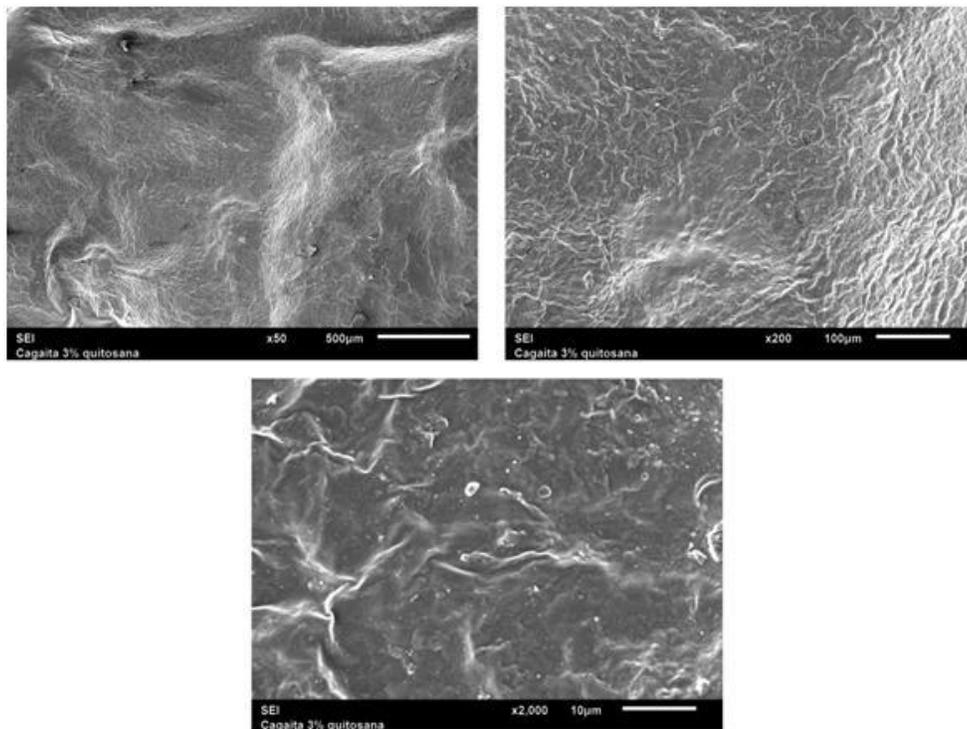


Figure 12. Physical structure (SEM) of cagaita fruits (*Eugenia dysenterica* DC.) coated with 3% chitosan. Fruit epicarp surface images.

analyzed in this work (Figure 12). Despite the presence of heterogeneous points, there is no incidence of bubbles or pores on the cell wall, which may indicate that such treatment is the most effective in maintaining fruit quality and increasing shelf life. The analysis of the physical structure of fruits through scanning electron microscopy contributes to evaluate the morphology of films due to detailed images of the cell wall surface (Freire et al., 2009). The drying process of the film and the nature of the hydrocolloid that change the interaction of components such as polysaccharides, plasticizers and water was also analyzed (Meneguim, 2012).

Conclusion

Chitosan solutions did not show the expected result in the conservation of cagaita fruits, and all parameters had values close to those analyzed in control fruits. However, this occurrence may be related to the presence of pores and poor adhesion of the solution to the cell wall of fruits as demonstrated in the physical structure analysis. Fruits that showed the best results, along with control fruits, were those coated with 3% chitosan, which suggests that further studies with higher chitosan concentrations could obtain more desired results for the conservation of fruit quality.

Conflict of interests

The authors did not declare any conflict of interest.

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