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Application of biofilms in the post-harvest conservation of pequi (*Caryocar brasiliense* Camb.)

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Pequi (*Caryocar Brasiliense Camb*.), fruit characteristic of the Brazilian cerrado, has sensory and nutritional characteristics, pleasant flavor and aroma and considerable presence of lipids and fiber, essential for human consumption. The aim of this study was to assess the post-harvest conservation of this fruit by using different sources of biofilms as a means to ensure the maintenance of fruit physical and chemical characteristics in order to increase its shelf life. Fruits were submitted to four treatments: control (no coating) (T1); 0.5% w/w carnauba wax (T2); 1% w/w cassava starch (T3) and 1.5% w/w xanthan gum (T4) stored during 15 days at BOD at 22 ± 0.1°C and submitted every three days to analyses of titratable acidity, soluble solid, pH, turgor pressure, vitamin C, weight loss and physical structure by scanning electron microscopy. The pH levels and turgor pressure showed expected values for control and coated pequi fruits. The vitamin C, titratable acidity, soluble solids contents and weight loss showed that coatings did not achieve satisfactory results. However, fruits coated with cassava starch showed the best conservation results during the experimental period.

Key words: Coatings, shelf life, storage.

INTRODUCTION

The Brazilian cerrado has great variety of climate, soil, flora and fauna. This biome has many native plant species that have been exploited for medical purposes and human consumption. In this region, fruits are consumed fresh or as products such as juices, liqueurs and ice creams (Cardoso et al., 2013). *Caryocar Brasiliense* Camb. (Caryocaraceae) is a tree distributed in the Cerrado region that produces pequi, appreciated for its sensory characteristics: color, flavor and aroma and for its nutritional characteristics: high contents of fiber, lipids and total energy (Aguilar et al, 2012; Cardoso et al, 2013). It is reported by Rodrigues et al. (2011) that pequi fruits showed durability nine to twelve days during storage.

After harvest, fruits are subject to biological, chemical and physical changes that may occur during handling,

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transport, processing and storage (Wu, 2010). Seeking to minimize changes in fruit quality, physical and chemical factors and gaseous treatments such as applying wax, biofilms, atmosphere modification, can be applied to the product (Mahajan et al., 2014).

The use of coating in fresh fruits is a post-harvest technology that combined with atmosphere changes or temperature control delays the senescence period of fruits (Maftoonazad et al., 2007). Edible films and coatings are an alternative by reducing the availability of oxygen, breathing, water loss and the fruit oxidation reaction rate (Kerdchoechuen et al., 2011).

Coatings are produced from protein molecules, hydro-colloids, lipids or combinations of these components (Kim et al., 2012). Biofilms made with protein raw-materials present good results in the maintenance of the physical properties of fruits and block gas exchange; however, biofilm made with lipids and hydrocolloids are suitable in the formation of gels and water retention (Kim et al., 2012). The thickness of the biofilm layer applied to the product must be carefully calculated, because when too thin, it does not prevent water loss and when too thick, it can lead to the development of unpleasant flavor caused by fruit deterioration (Silva et al., 2012).

Carnauba wax is extracted from the leaves of a native plant to Northeastern Brazil (*Copernicia prunifera*) (Hai et al., 2014). This wax assigns brightness, reduces loss of material and can be removed with water, if necessary (Malgarim et al., 2007).

Cassava starch is considered the most suitable raw material in the manufacture of edible biofilms for forming resistant and transparent film, being efficient barrier to water loss, providing good appearance and intense shine, making fruits and vegetables commercially attractive (Bobbio and Bobbio, 1984; Cereda et al., 1992; Vila, 2004). Cassava starch is produced in large scale in Brazil and is an attractive product due to its low cost when compared to other commercial waxes (Oriani et al., 2014).

Gums affect mass viscosity, which is an essential feature for the quality of a coating (Fiszman and Salvador, 2003). Xanthan gum has high viscosity and is responsible for forming stable solutions that contribute as stabilizer, thickener and emulsifier in food products (Mohamed et al., 2013).

In this context, the aim of this study was to assess the post-harvest conservation of this fruit by using different sources of biofilms as a means to ensure the main-tenance of fruit physical and chemical characteristics in order to increase the shelf life.

MATERIALS AND METHODS

Pequi fruit were manually harvested in January 2013 in a farm located in Lagoa Santa, metropolitan region of Belo Horizonte - MG (19° 37 '45 "S and 43° 53' 23" W) and carefully transported to the Laboratory of Fruits and Vegetables of IF Goiano - Rio Verde Campus, which were coated using carnauba wax ® Aruá BR18% v/v, cassava starch ® Amafil and xanthan gum ® Primmax Wax 40 purchased from the local trade.

In the development of the experiment, fruits were initially selected by size, color and absence of mechanical damage and surface stains. Subsequently, they were sanitized with chlorinated water (100 mg.L^{-1}), and dried, the fruit being immersed in water and the water heated to the boiling point, for fifteen minutes. The film solutions were prepared as follows: carnauba wax was diluted in distilled water up to the desired concentration and homogenized; cassava starch and xanthan gum were solubilized in water previously heated to 70°C for the gelation of biofilms.

Subsequently, fruit were immersed for one minute in the respective concentrations that correspond to: control (no treatment) (T1); 0.5% v/v carnauba wax (T2); 1% v/v cassava starch (T3) and 1.5% v/v xanthan gum (T4), and these concentrations were previously tested. Fruit were removed from filmogenic solutions and allowed to dry naturally.

Fruit from the different treatments were stored in Styrofoam trays with measures of 150x150x18 mm. Trays were placed in BOD at controlled temperature of 22 ± 0.1°C and evaluated at 0, 3, 6, 9, 12 and 15 days in three replications of five fruit each. The parameters analyzed were: fresh weight loss, turgor pressure, content of soluble solids and titratable acidity, ascorbic acid, pH and physical structure analysis by scanning electron microscopy (SEM).

For weight loss, six fruits from each treatment were reserved, which allowed evaluating the evolution of this parameter during the storage period, always using the same fruits. Weight loss was assessed with a digital scale and the results were expressed as a percentage in relation to the original weight.

The turgor pressure was determined by applanation technique using a horizontal applanator (Calbo and Nery, 1995) and results were expressed as kgf.cm^{-2}. The titratable acidity values were obtained by titrating the filtered juice with NaOH solution (0.01 N), and results were expressed as % citric acid according to method No. 986.13 (AOAC 1992). In determining the content of soluble solids, expressed in °Brix, the filtered juice was read in refractometer Atago N-2E according to AOAC method No. 983.17 (1992). The ascorbic acid content was determined by volumetric redox titrating of samples with 2,6-dichlorophenol-indophenol sodium solution (DCFI), according to AOAC method No. 967.21 (2000). The pH was determined using potentiometer Bel Engineering, model W3B according to AOAC technique No. 981.12 (1992).

The analysis of the physical structure of pequi fruit consisted of the removal of fruit epicarp, which was dried at 60°C for 12 h and stored in desiccator. For analysis of scanning electron microscopy (SEM), samples were placed on stabs, coated with a thin layer of gold and micrographed. Evaluation was performed at the Laboratory of Multiuser High-Resolution Microscopy, Instituto de Física- Universidade Federal de Goiás. Scanning Electron Microscope, Jeol, JSM -. 6610, equipped with EDS, Thermo scientific NSS Spectral Imaging was used.

The experimental design was completely randomized and the analysis of results was performed using 4x6x3 factorial design consisting of four treatments, six storage times and three replications for each tray analyzed, resulting in nine replicates at each treatment and study day. The models were selected according to the coefficient of determination and their significance was determined through the F test.

The averages obtained for analyses of total soluble solids, titratable acidity, pH, weight loss and turgor pressure were compared by the Tukey test at 5% probability, using the SISVAR software.

RESULTS AND DISCUSSION

Total titratable acidity from control (Table 1) showed a

Table 1. Mean titratable acidity (%) values of pequi fruits (*Caryocar brasiliense* Camb.) in control (T1) and coated with carnauba wax (T2), cassava starch (T3) and xanthan gum (T4).

Days of storage	T1	T2	T3	T4
0	0.0022bC	0.0040aAB	0.0045aA	0.0037aB
3	0.0043aAB	0.0040aAB	0.0035aAB	0.0046aAB
6	0.0029aBC	0.0026aB	0.0023aB	0.0033aB
9	0.0036bBC	0.0064aA	0.0038bAB	0.0035bB
12	0.0058aA	0.0036bAB	0.0037bAB	0.0055aA
15	0.0043aAB	0.0050aAB	0.0040aA	0.0044aAB

Different lowercase letters in line show significant differences between lines and capital letters indicate significant differences between columns using the Tukey test at 5% probability.

Table 2. Average pH values for pequi fruits (*Caryocar brasiliense* Camb.) in control (T1), coated with carnauba wax (T2), cassava starch (T3) and xanthan gum (T4).

Days of storage	T1	T2	T3	T4
0	7.40aA	7.16aA	7.39aA	6.52bB
3	7.46aA	7.51aA	7.62aA	7.32aA
6	7.69aA	7.51aA	7.54aA	7.62aA
9	7.59aA	7.23aA	7.53aA	7.44aA
12	6.53abB	6.07bB	6.72aB	6.51abB
15	4.90cC	5.40bC	5.92aC	6.06aB

Different lowercase letters in line show significant differences between lines and capital letters indicate significant differences between columns using the Tukey test at 5% probability.

growing trend in the first three days of storage in relation to the other treatment (T1); however, between 3rd and 9th days, there was fall, and rising again at the end of the storage period. For treatment T2, there was a fall only on the sixth day and increase at the end of the storage period. Fruits coated with cassava starch (T3) decreased total acidity up to the sixth day, after this period, the values increased up to the end of analyses.

The lining of the order is to prevent the physical and physicochemical changes during ripening of fruits. Table 1 indicates values for different fruits with and without coating during storage. Falls delivered on fruit in some analysis of the moments were also found by Agostini et al. (2009) for jabuticabas stored in different packaging.

The ninth and twelfth day of storage showed considerable differences in the titratable acidity levels of pequi fruits, occurring at a peak of this parameter for fruits coated with carnauba wax (9th day), control and xanthan gum (12th day). This peak can be characteristic of the occurrence of anaerobic respiration. Anaerobic respiretion induces the development of physiological disorders, so it is essential to know if biofilms keep the oxygen levels in the fruit through their permeability. In biofilms with low permeability, fruits obtain energy through anaerobic respiration with ethanol and acetaldehyde formation, affecting their quality (Steffens et al, 2007; Petracek et al., 2002).

The increase in acidity may be also attributed to the release of galacturonic acids from the cell wall that increase during fruit ripening by the action of enzymes pectin methyl esterase and polygalacturonase (Scalon et al., 2012). According to Reis et al. (2006), during the respiration process, organic acids are generated and volatilize. The treatments probably had an effect in delaying or concentrating this volatilization, allowing fruits to remain more acidic due to maturation. The degradation of the cell wall of the fruit contributes to the loss of firmness which can be analyzed by mass of data loss during storage.

Fruits treated with cassava starch showed lower titratable acidity values (TA) during the two days of storage period, indicating that it is possible that this biofilm is the best for the preservation of fruit quality. A small decrease in TA values for guava stored at room temperature (25 ± 4°C), treated with 2% cassava starch was also verified by Oliveira and Cereda (1999). Pequi fruits from control and treatments T2 and T3 showed no significant differences in pH values (Table 2) up to the ninth day of storage. Fruits from treatment T4 had similarities with the other treatments between the third and ninth days of analysis. After this period, all treatments decreased pH values.

The pH values can be related to the titratable acidity levels. On the sixth day, fruits had an increase in pH,

Table 3. Average turgor pressure values of pequi fruits (*Caryocar brasiliense* Camb.) in control (T1), coated with carnauba wax (T2), cassava starch (T3) and xanthan gum (T4).

Days of storage	T1	T2	T3	T4
0	3.70aA	3.58aA	3.70aA	3.26bB
3	3.73aA	3.76aA	3.81aA	3.66aA
6	3.85aA	3.76aA	3.77aA	3.81aA
9	3.80aA	3.62aA	3.77aA	3.72aA
12	3.27abB	3.04bB	3.36aB	3.26abB
15	2.45cC	2.70bC	2.96aC	3.03aB

Different lowercase letters in line show significant differences between lines and capital letters indicate significant differences between columns using the Tukey test at 5% probability.

Table 4. Mean values of soluble solids for pequi fruits (*Caryocar brasiliense* Camb.) in control (T1), coated with carnauba wax (T2), cassava starch (T3) and xanthan gum (T4).

Days of storage	T1	T2	T3	T4
0	6.67aA	5.00aC	7.33aA	6.00aA
3	8.67aA	10.0aB	8.33aA	9.00aA
6	8.67bA	16.0aA	7.33bA	8.67bA
9	7.00aA	7.33aBC	7.00aA	6.67aA
12	8.67abA	9.17aB	8.67abA	5.67bA
15	9.67aA	7.00aBC	9.00aA	8.67aA

Different lowercase letters in line show significant differences between lines and capital letters indicate significant differences between columns using the Tukey test at 5% probability.

which characterizes maturation. The increase in pH and decrease in acidity are characteristic of the use of organic acids by fruit to obtain energy, causing maturation, in which there is an increase in respiration rate, synthesis of hormones and cell wall degradation (Soares Júnior et al., 2008).

At the end of the storage period, control fruits had the lowest pH values in relation to the other treatments, as discussed by Sánchez et al. (2014) for Castilla mulberry fruits with edible coating and Rathore et al. (2009) in mango fruits coated with packaging and fungicide, ethylene absorbent and agent for preventing ripening.

The fruits coated with xanthan gum were the only ones that were significantly different from control fruits at the beginning of storage (Table 3). However, after the first day of analysis, all the fruits were not significantly different until the ninth day of storage. Between the twelfth and fifth day, the fruits began to differ for all treatments, with lower values for the start. After the analyses, the fruits coated with xanthan gum showed the lowest reduction in turgor pressure values 5.7% in relation to pressure at the beginning of storage, while treatments T1, T2 and T3 showed reductions of 33.8, 24.6 and 20%, respectively. This behavior may be related to the variability of the physical characteristics of native pequi fruits (Vera, 2004).

Untreated fruits showed greater reduction in turgor pressure by 33.8%. This result suggests that the great water loss of this treatment has contributed to reduction in turgor pressure during storage, and weight loss was analyzed, in relation to control fruits which showed higher weight loss.

Tessarioli Neto et al. (1998) also observed the drastic reduction of this characteristic in untreated fruits beet variety "early wonder" during storage. According to Sams (1999), changes in physical and chemical characteristics are related to changes in pulp firmness, being affected by water content, turgor pressure, composition and chemical constituents of the cell wall. Turgor pressure is a major component related to the firmness of fruits and vegetables (Calbo and Nery, 1995) and the monitoring of this parameter has shown to be effective in determining loss of pulp firmness and shriveling.

Fruit softening is associated with ripening, which involves a series of enzymatic reactions due to climacteric fruit respiration and ethylene production (Castricini, 2009). Loss of firmness during fruit ripening occurs through the conversion of insoluble pectins into soluble pectins, which causes loss of fruit resistance (Chitarra and Chitarra, 2005).

Table 4 shows that T1, T3 and T4 did not differ significantly ($p<0.05$) for the soluble solids content. However, control fruits and those coated with cassava starch were more stable against variation of soluble

Table 5. Average vitamin C values of pequi fruits (*Caryocar brasiliense* Camb.) in control (T1), coated with carnauba wax (T2), cassava starch (T3) and xanthan gum (T4).

Days of storage	T1	T2	T3	T4
0	121.65aB	97.61aC	134.41aB	150.45aAB
3	156.57aB	177.56aABC	143.60aB	168.35aAB
6	146.72bB	283.09aA	128.77bB	148.45bAB
9	121.30aB	140.35aBC	123.56aB	120.33aB
12	203.06abB	248.74aAB	191.95abAB	135.74bAB
15	402.08aA	240.27bAB	256.84bA	236.33bA

Different lowercase letters in line show significant differences between lines and capital letters indicate significant differences between columns using the Tukey test at 5% probability.

solids compared to treatments with carnauba wax and xanthan gum. Stability in the soluble solids content is interesting to show that the fruit remained with the same initial conditions, indicating lower ripening.

However, Bashir and Abu-Goukh (2003) reported that the increase in sugar content occurs up to the time when the fruit reaches the climacteric peak, and after these values are reduced. Table 4 shows that fruits with carnauba wax had more characteristic climacteric fruit indexes, and for the other coatings, this index was lower, indicating better preservation of nutritional and sensory plant characteristics.

Fruits coated with carnauba wax showed increased soluble solids content after six days of storage, which relates to the increase in acidity titratable, characterizing, according to Knee and Bartley (1981), fruit ripening due to biosynthesis or degradation of polysaccharides. This increase in soluble solids can be as a result from the transformation also of accumulated reserves in the formation and development of solids into soluble sugars (Jerônimo and Kanesiro, 2000).

On the ninth day of analysis, fruits of T2 showed decreased soluble solids content, which could be due to catalytic reactions that characterize senescence, or to the storage atmosphere that reached critical oxygen levels, causing anaerobic respiration (Soares Júnior et al., 2008), as in the titratable acidity in this time of analysis. The increase in soluble solids content was also observed by Maciel et al. (2004) when biofilms were used in the conservation of acerola.

Coatings affect the vitamin C contents (Table 5), showing significant increase over the storage period regardless of coating used. The vitamin C content varied materially during the storage for uncoated and coated fruit. The application of biofilms did not contribute to this parameter remained similar during the analysis.

After the ninth day of storage, vitamin C levels increased in all treatments, and this increase was more pronounced for uncoated fruits, where there was greater weight loss. Although the vitamin C content decreases in citrus fruits, other fruits show an increase tendency, which can be explained due to the offsetting effect to the water loss with advancing maturity, concentrating the fruit juice (Carvalho and Manica, 1994; Lee and Kader, 2000).

Increased vitamin C levels in control fruits indicate that biofilms used in coated fruits did not show adequate gas permeability for the conservation of this parameter. Dang et al. (2010) reported that cherries coated with chitosan biofilms showed an increase in vitamin C content during storage due to the film permeability, contributing to the reduction in the enzyme activity and ascorbic acid oxidation in fruits.

Literature data indicate vitamin C stability according to the fruit. Cardello and Cardello (1998) observed considerable reduction of this vitamin for 14 ripening days of "Haden" mango. Lee and Kader (2000) reported increased levels in peach and papaya, and reduction in apple and mango with advanced ripening.

As shown in Figure 1, the equation of development over time and and its correlation coefficient for weight loss, and control (Yc), carnauba wax (Ycc), cassava starch (Yf) and xanthan gum (YGX) is presented.

Fruits coated with xanthan gum presented the best results for weight loss when compared to the other treatments. Antunes et al. (2003) showed that weight loss is due to water eliminated by the processes of fruit transpiration.

Weight loss that occurs during storage is a limiting factor for the marketing of any fruit. In general, fruits have rapid weight loss, causing skin wrinkling, making the fruit not visually attractive to consumers, even if internally, the fruit has good conservation condition. In the case of coatings, the intention of using them in pequi fruits was to maintain the visual quality of the fruit, minimizing weight loss.

Hagenmaier and Baker (1994) reported that carnauba wax is an interesting biofilm for reducing the loss of fresh weight in fruits; however, this behavior was not observed in this study. This result may be due to the thickness of films used in the coating of pequi fruit. Figures 2, 3, 4 and 5 show the physical structure by scanning electron microscopy (SEM) of fruits without treatment, treated with carnauba wax, cassava starch and xanthan gum, respectively.

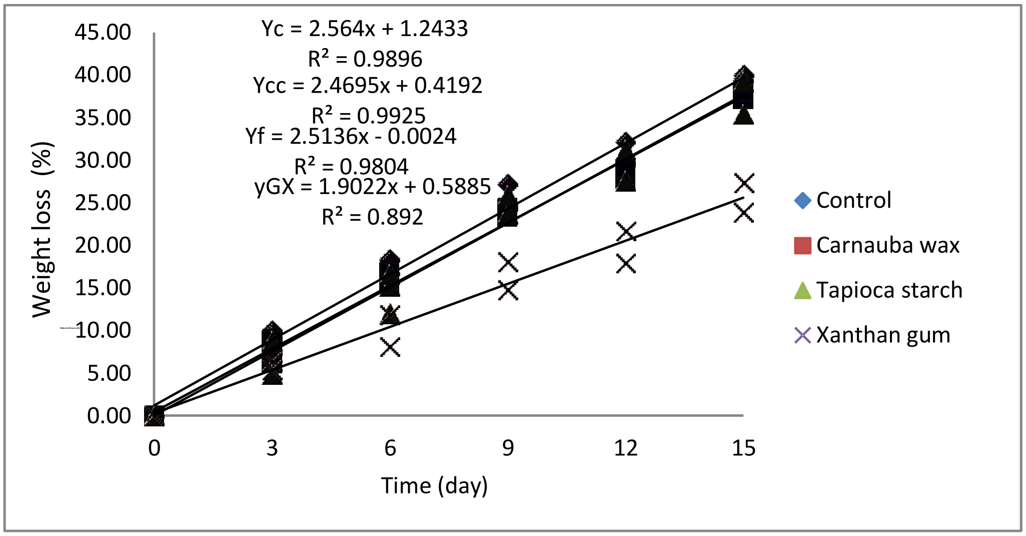

Figure 1. Development over time, equation correlation coefficient and its mass loss for fruit pequi (*Caryocar brasiliense* Camb.) in the control (Yc), as carnauba wax (Ycc), tapioca starch (Yf) and xanthan gum (YGX).

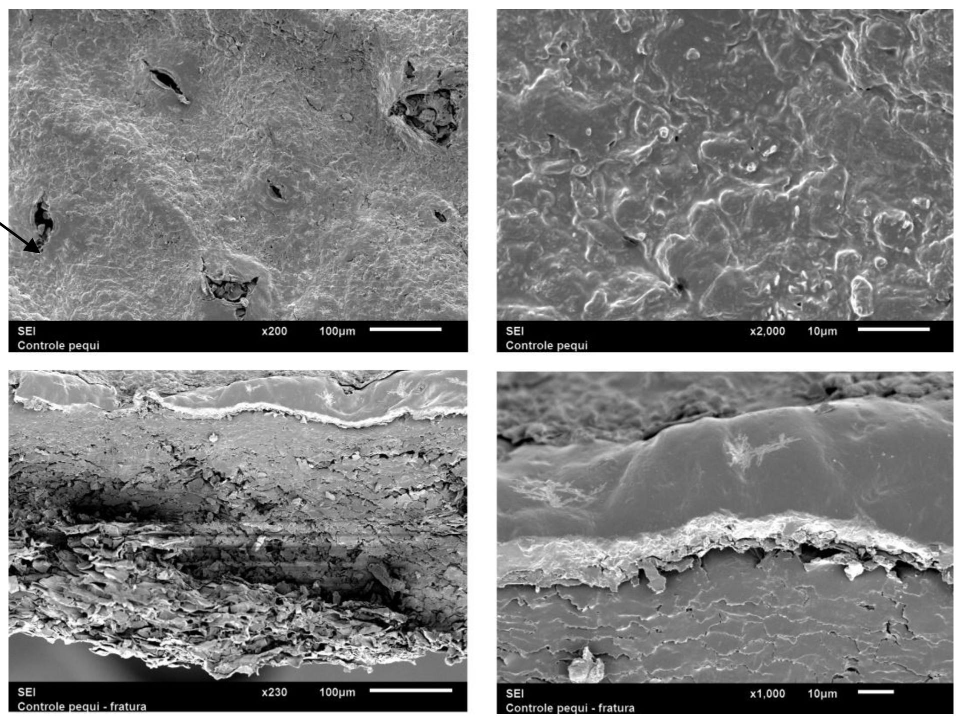

Figure 2. Physical structure (SEM) of pequi fruits (Caryocar brasiliense Camb.) in control, and the top part of the figure shows images of the surface and the bottom of the figure shows cross-sectional images.

Scanning electron microscopy of pequi fruit in control treatment shows a heterogeneous structure with small bubbles and pores in the cell wall of fruit indicated by arrow in Figure 2. There are circular structures that indicate the presence of unaligned cell wall constituents. The fracture of pequi fruit indicates the rearrangement of possible cell structures.

However, the analysis of the physical structure of pequi fruits coated with carnauba wax describes a smoother and consistent surface of the fruit cell wall, but it is likely

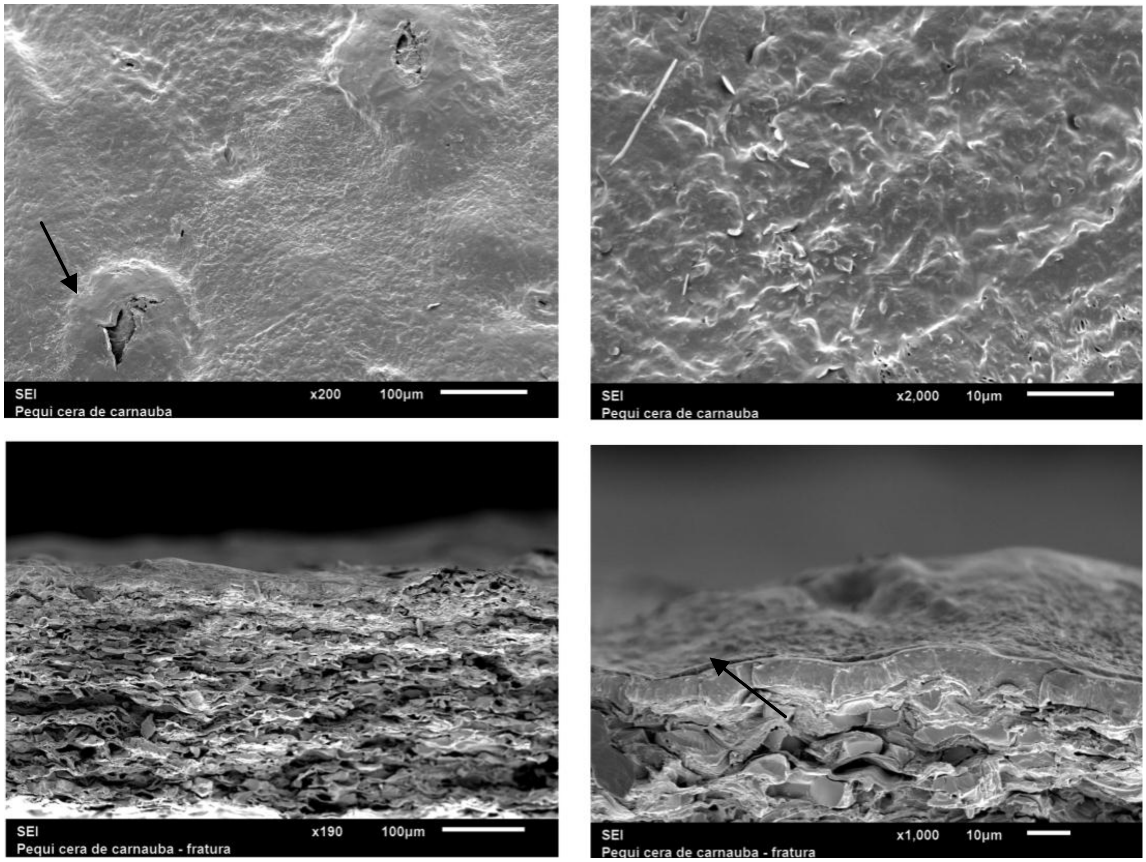

Figure 3. Physical structure (SEM) of pequi fruits (*Caryocar brasiliense* Camb.) treated with carnauba wax, and the top part of the figure shows images of the surface and the bottom of the figure shows cross-sectional images.

that the thin biofilm layer resulted in the formation of bubbles in the fruit and the presence of pores (presence of the arrow in Figure 3). These bubbles may have influence in the lower quality of fruits receiving this coating. The arrow represented in the cross section of the fruit pequi coated with carnauba wax indicates the thin biofilm layer from the surface of the fruit.

Through micrographs presented by Gonçalves et al. (2010) for peach fruit coated with carnauba wax, the formation of heterogeneous biofilm wall in fruits was observed, which contributed to the germination of *M. fructicola* and *R. stolonifer*. Fruit coated with cassava starch showed the formation of bubbles with smaller proportions and the coating surface remained smooth as arrow in Figure 4, which indicates better preservation of the quality of fruits coated with this biofilm compared to other biofilms analyzed in this work. However, fracture showed the presence of a small space between the cell wall and biofilm application, as shown in the arrow in Figure 4.

The physical structure of xanthan gum confirms the fact that its biofilm does not preserve characteristics such as titratable acidity and total soluble solids of fruits during storage due to the presence of pores on the surface which may have contributed to anaerobic respiration, represented by arrow in Figure 5. The surface with incidence of pores is convenient for water penetration (Liang et al., 2009), which contributes to weight loss of stored fruits.

The arrow in fracture of Figure 5 also indicates distance between biofilm application and the fruit cell wall, indicating a possible site for biochemical reactions. Chaisawang and Suphantharika (2006) reported through micrographs that xanthan gum shows the formation of a thin layer when used as biofilm. These authors also reported that xanthan gum has a tendency to lose viscosity, which can cause disruption of starch granules.

Conclusions

The best results for the conservation of pequi fruit were shown in cassava starch coating. However, due to process characteristic of anaerobic respiration, fruit showed unsatisfactory results for parameters titratable acidity, soluble solids and vitamin C. The best result for weight loss maintenance was shown by control fruit. Scanning electron microscopy confirmed the validity of

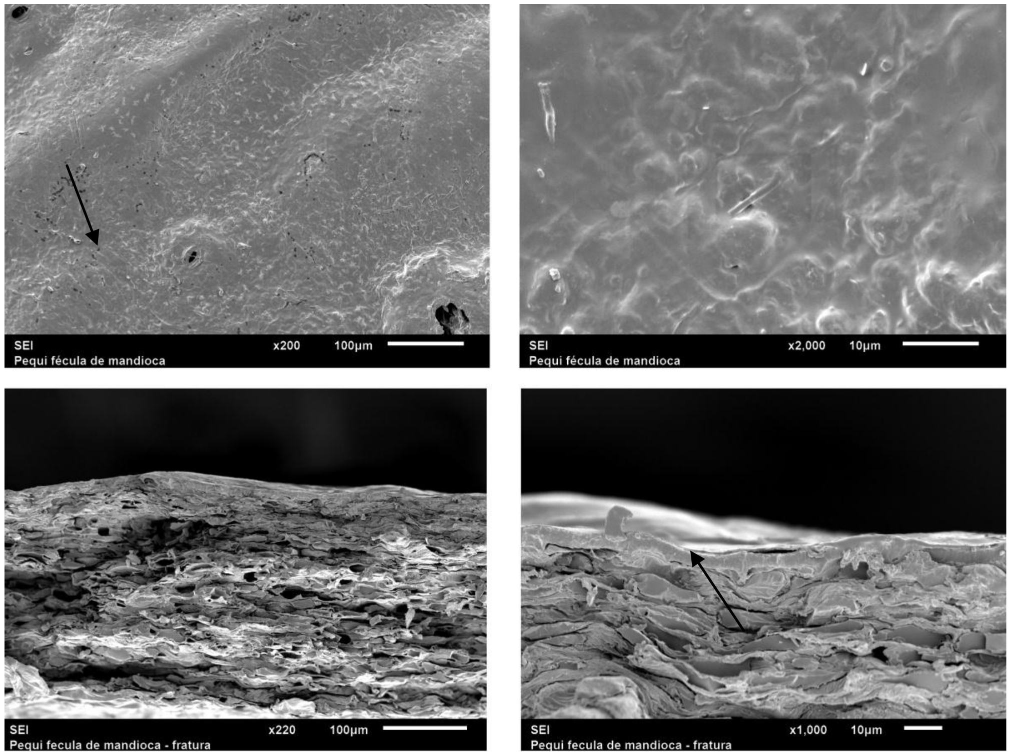

Figure 4. Physical structure (SEM) of pequi fruits (*Caryocar brasiliense* Camb.) treated with cassava starch, and the top part of the figure shows images of the surface and the bottom of the figure shows cross-sectional images.

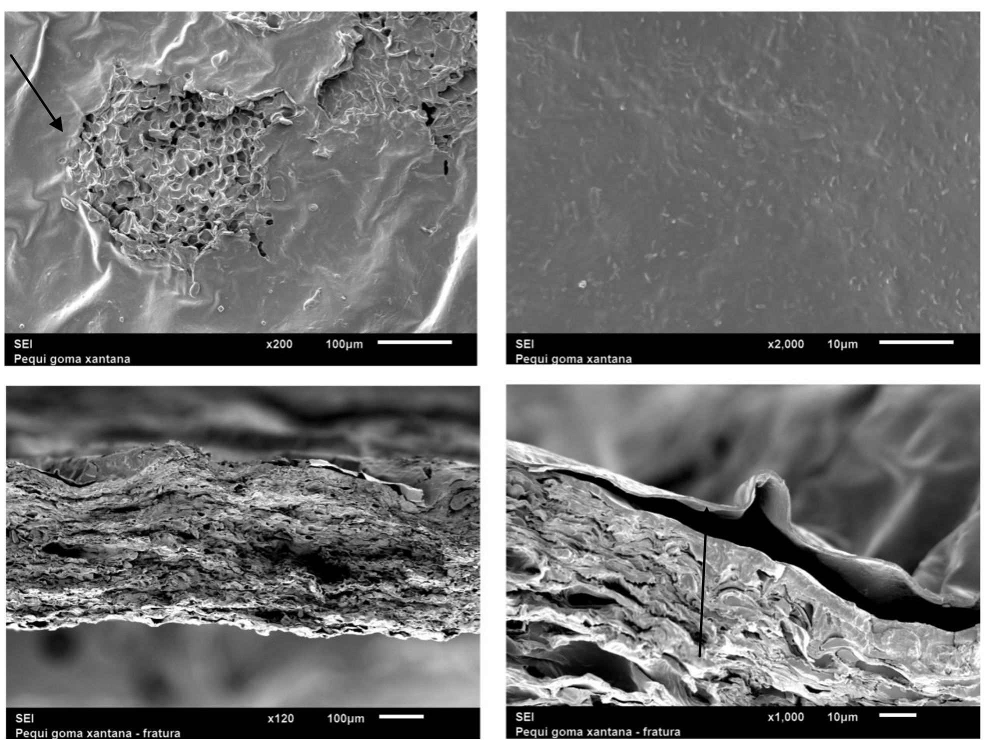

Figure 5. Physical structure (SEM) of pequi fruits (*Caryocar brasiliense* Camb.) treated with xantana gum, and the top part of the figure shows images of the surface and the bottom of the figure shows cross-sectional images.

biofilm barrier formed by cassava starch.

Conflict of interests

The authors did not declare any conflict of interest.

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