Published by Avanti Publishers

Journal of Chemical Engineering Research Updates

ISSN (online): 2409-983X

Characterization of Filmogenic/Edible Covering Based on Pectin Extracted from Cajá (*Spondias mombin*) Applied to Coating Green Acerolas (*Malpighia emarginata*)

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ARTICLE INFO

Article Type: Research Article

Keywords:
Pectin
Edible coating
Acerola (*Malpighia emarginata*)

Timeline:
Received: September 25, 2021
Accepted: November 05, 2021
Published: November 28,2021

Citation: Asevedo EA, de Oliveira Júnior SD, dos Santos ES. Characterization of Filmogenic/Edible Covering Based on Pectin Extracted from Cajá (*Spondias mombin*) Applied to Coating Green Acerolas (*Malpighia emarginata*). J Chem Eng Res Updates, 2021; 8: 36-47.

DOI: https://doi.org/10.15377/2409-983X.2021.08.3

ABSTRACT

Filmogenic coatings can be used as a post-harvest strategy to extend shelf life and ensure improvements in fruit quality and safety given their perishable nature. Due to their edibility, the composition of the coverings is a determining factor for their application, and for this reason, it is interesting and desirable that the constituents come from natural sources. The objective of the present study was to develop and characterize a pectin-based film extracted from cajá peel (*Spondias mombin*) and verify its efficiency as an edible coating in postharvest acerolas (*Malpighia emarginata*). The film was characterized in terms of mechanical properties by Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM); as well as in terms of chemical properties using the analysis of structural bonds by Fourier Transformed Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD). In addition, the antimicrobial activity against two bacteria was evaluated. The analyzed film was presented as a dense membrane, with the presence of pores, fissures, and a very rough surface. The degree of esterification of pectin extract from cajá peel was 44%, and for this reason it was classified as low methocxyl (LM) pectin. The filmogenic solution presented antimicrobial activity against the bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The applicability of the edible coating was tested on green acerolas, monitoring their maturation stage through analyzes such as weight loss, titratable acidity, and total soluble solids. At the end of the 7 days of storage, acerolas with the application of the coating showed 8.97% weight loss while acerolas without coating showed 9.89%; the percentage of total soluble solids was 7.68% higher for acerolas with the coating, as well as ascorbic acid content was higher for the protected fruits, indicating that the coverage favored the delay in the maturation of acerolas.

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1. Introduction

Brazil annually produces around 44 million tonnes of fruit, occupying the third position in the ranking of global production, according to the CNA (Confederation of Agriculture and Livestock of Brazil). Acerola is one of the commercially exploited fruits, with a well-structured production chain primarily in Brazil's northeastern region [1]. This tropical fruit stands out as one of the richest natural sources of ascorbic acid (Vitamin C), as well as phytonutrients such as phenolic carotenoids, anthocyanins and flavonoids, associated with the prevention of degenerative diseases due to its wide antioxidant potential [2]. Due to the high perishability of acerola, this fruit is also part of another scenario: approximately 30% of the produced fruits are discarded in Brazil [3]. Food waste is a global problem and even its reduction was chosen as a priority in the goals established by United Nations for 2030 in the pursuit of sustainable development [4].

In view of the need to reduce food waste, filmogenic coatings have been widely explored by the scientific community as a simple alternative that can avoid post-harvest fruit physiological and phytopathological losses. Filmogenic coatings work as a semi-permeable protective barrier around the fruit surface, responsible for decreasing the rates of gas exchange, solute and moisture loss to the atmosphere external to it without the occurrence of anaerobic fermentation processes [5]. In addition to delaying ripening and physicochemical changes, this method has the potential to prevent the development of microorganisms, improve the visual appearance of fruits by increasing their brightness, and can be safely consumed as part of the product [6].

According to Riva *et al.* [5], polysaccharide-based filmogenic coatings are widely disseminated throughout the literature, and their application to stone fruits is the most promising. Polysaccharides are highly available, allergen-free and normally soluble in water, in addition to having a well-ordered and well-compacted structure, due to the hydrogen bonds between the chains, which give them excellent mechanical properties and make them stand out as a barrier to gases. Among the less explored polysaccharides for this purpose, pectin, a linear polymer of α-D-galacturonic acids esterified in methyl groups, stands out [7]. Este polissacarídeo pode ser facilmente encontrado na natureza, como por exemplo em frutas presentes em abundância na região nordeste do Brasil, como o cajá (*Spondias mombin* L.) [8].

Systematically, in this work, cajá was chosen as a natural source for the extraction of pectin that will be used in the production of the filmogenic coating. In this way, concomitantly with the caja appreciation, this strategy makes it possible to increase the quality and shelf life of acerola with little added expense to the process, making this alternative more affordable. Therefore, this study will evaluate two alternatives that may contribute to the achievement of goals established by the United Nations for Sustainable Development.

2. Material and Methods

2.1. Cajá Peel Residue

The cajá peel residue was obtained on the market located in Natal (RN/Brazil). The material was dried at 50°C for 48 h, thus reduced to powder in a mill Willye type (TE-680).

2.2. Pectin Extraction

The methods described by McCready and McComb [9] and by Kratchanova *et al*. [10] were adapted for pectin extraction tests. Each 1.0 g of the cajá peel extract was hydrated with 70 mL of distilled water and adjusted to pH 2.5 with 1.0 M citric acid. The acidified mixture was kept for 2 h, at 80°C under magnetic stirring. Then, the solution was centrifuged at 25°C, for 10 min at 2500 rpm, the supernatant was collected and ethyl alcohol 95% was added in the ratio of 1:2 v/v (supernatant/ ethyl alcohol) and rested for 1 h. The pectin was separated through vacuum filtration and dried at 50°C.

2.3. Obtaining Filmogenic Coverage of Natural Pectin

Pectin extracted from the cajá peel was dissolved in distilled water (2.0% m/v), with 0.5% (m/m) of glycerol and the solution was submitted to magnetic stirring at 80°C for 2 h. The film was obtained from wrapped filmogenic solution in polypropylene plates, and dried at 50°C for 24 h.

2.4. Analysis of Structural Bonds by Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis used spectra from 400 to 4000 cm^{-1}, achieved by using the equipment Shimadzu IR Tracer 100 (Perkin Elmer, USA).

In addition, the quantitative determination of the degree of esterification (DE) of pectin extracted from cajá peel was calculated according to Fellah *et al*. [11], using Eq. (1):

$$\%DE = \frac{ECG}{TEC} \times 100 \quad (1)$$

In Eq. (1), ECG refers to esterified carboxyl groups and TEC to total esterified carboxyl groups and carboxylate groups.

2.5. Scanning Electron Microscopy (SEM)

The pectin film sample was analyzed on the scanning electron microscope (Phillips XL - 30ESEM, USA), by using a 15 kV electron beam. The electronic micrographs were taken ~~at~~ with magnifications of 150 and 400 times.

2.6. X-Ray Diffraction (XRD)

The film's crystallinity was evaluated through XRD spectroscopy (XRD-6000, Shimadzu, Japan), Cu-Kα radiation, 30.0 kV and 15 mA, with a rate of 2.0 degrees per minute to a 2θ-continuous scan with an interval of 4.0 - 70.0°.

2.7. Atomic Force Microscopy (AFM)

The morphological surface of the raw film was analyzed by using a Shimadzu brand (SPM-9700). The analysis was performed on the dynamic mode (tapping mode) with a scan speed of 1 Hz and with the resolutions of X, Y, with 0.2 nm and Z with 0.01 nm with a piezoelectric tube component.

2.8. Inhibition of Microbial Growth

The antimicrobial activity of filmogenic solution was assessed by means of determining inhibitory concentrations against model strains of Gram-positive bacteria *Staphyloccoccus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 27853) [12].

Initially, inoculums were prepared by taking three to four colonies of the strain, isolated on Muller-Hinton broth and diluted at 0.85% saline solution until reaching their respective logarithmic growth phases, as measured by the optical density (OD) of the medium at 595 nm. Samples of diluted cultures were then added to 96-well plates, mixed with filmogenic solution at different concentrations (5 and 10 mg/mL) and after 24 h incubation at 35°C, the OD of each well was measured at 595 nm using ELISA reader (Epoch-Biotek, USA). Solution control of the suspensions of microorganisms in Muller Hinton broth was carried out too [13].

2.9. Fruit Collection

The green acerolas used in the applicability test were obtained at the Supply Center of Rio Grande do Norte S/A (CEASA-RN, geo-position: -5.82445000, -35.22450000) in 2018.

2.10. Applicability Test

The acerolas were submerged in the filmogenic solution for 1 min and then stored, at room temperature (21.0 ± 1.0°C) and a relative humidity of 85 ± 5%. The physicochemical analyzes (weight loss, titratable acidity and total soluble solids) of acerolas uncoated and coated with filmogenic solution of natural pectin from cajá peel were performed on days 1, 3, 5 and 7 of storage. To perform some analyses, the fruit was crushed and homogenized with distilled water to obtain the diluted acerola pulp.

2.10.1. Weight Loss

The weight loss was determined by gravimetry according to Correa *et al* [14] and calculated by Eq. (2):

$$\text{Weight loss } (\%) = \frac{w_o - w_{(t)}}{w_o} \, x \, 100 \tag{2}$$

In Eq. (2), w_0 corresponds to the weight of the acerola at time zero and w_t to the weight after the corresponding days.

2.10.2. Titratable Acidity (TA)

Titratable acidity was determined by titration of diluted pulp from acerola with 0.1 M NaOH, according to the technique established by Instituto Adolfo Lutz [15] and the results were expressed in grams of ascorbic acid/100g of fruit, according to Eq. (3):

$$\text{TA } (\%) = \frac{\text{V x F x M x PM}}{\text{10 x P x n}} \text{ x } 100 \tag{3}$$

In Eq. (3), V is the volume of ascorbic acid in mL, F the correction factor and M the molarity of NaOH solution, P the weight of the analyzed fruit in gram and n the number of ionizable hydrogens.

2.10.3. Soluble Solids (SS)

The soluble solids (SS) content in the diluted pulp was determined in a Digital Refractometer (Hanna Hi 96801), with values expressed in °Brix [17].

2.11. Statistical Analysis

All the experiments were carried out in triplicate. Statistical analysis was performed using the Software Statistica v. 7.0 (StatSoft, OK/USA). Analysis of variance (ANOVA) combined with the Tukey test was applied to establish statistical significance ($p < 0.05$).

3. Results and Discussion

3.1. Analysis of Structural Bonds by Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the natural pectin-based film is presented in Figure **1** and from this analysis it is possible to detect the presence of the main constituent groups of the film.

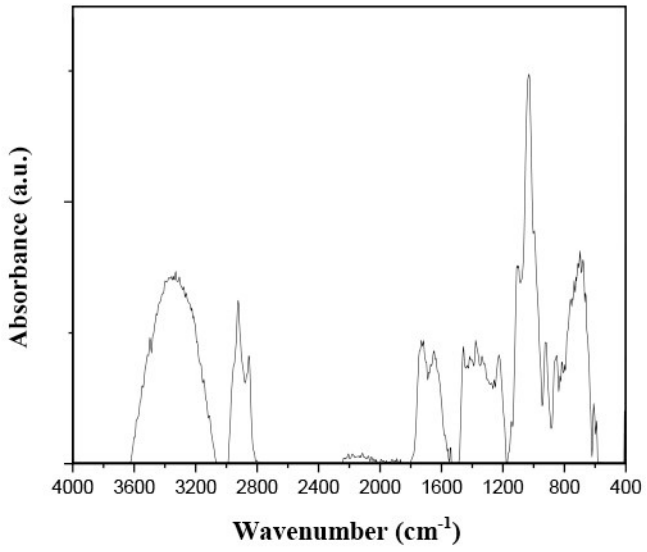


Figure 1: FTIR spectra of the natural pectin-based film.

The first bands between 3100 and 3500 cm^{-1} is attributed to existence of groups O-H–due to the inter- and intramolecular hydrogen bonds of the galacturonic acid chain, main component of pectin. The second band, centered around 2900 cm^{-1}, corresponds to the vibrations of the C-H bond. The band between 1790 and 1730 cm^{-1} is associated with the esterified carboxylic groups (-$COOCH_3$) and the bands between 1660 and 1590 cm^{-1} is associated with free carboxylic groups (-COOH) [16]. Absorption bands between 1100 and 1200 cm^{-1} are associated to the C-O-C bond and C-C bonds [18].

The pectin behavior depends both on the quantity of ionic groups bonded to it and on its distribution along the main chain. Thus, it is important to determine the degree of esterification (DE) of pectin in order to predict its behavior in solution [19]. The Figure **2** presents the bands of FTIR spectra that were used to determine the DE.

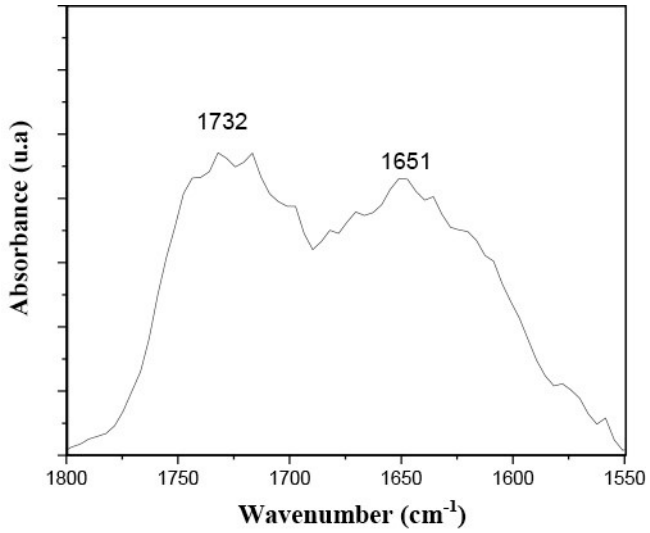


Figure 2: FTIR spectra to the natural pectin-based film to the determination of the Degree of Esterification (DE).

The DE determination for the natural pectin-based film through the integration of the bands of the esterified (COOR) and free (COO—) carboxylic groups showed result equal to 44% and consequently it was classified as low methocxyl (LM) pectin. A very close result was found by Sousa *et al*. [21], which extracted pectin with organic acid too and it presented a DE of 41%.

According to Turquois *et al*. [20], the DE of 50% is used as a parameter of reference, since pectins are, commercially, classified as high-methoxy (HM) when they contain over 50% of their carboxylic groups esterified and low-methoxy (LM) when values equal or under 50% of these groups are esterified. It should be highlighted that pectins classified as LM have higher ability to form gels, and this characteristic is desirable for this work.

3.2. Film Microstructure

Through the Scanning Electron Microscopy (SEM) is possible to visualize the surface of the natural pectin-based film, the presence of pores, the separation of the components used in the formulation of the film and possible imperfections. Figure **3** shows the micrographs obtained from the film.

The Figure **3** showed that the film was in the form of membranes with some less dense regions noticeable in the darker areas, with pores and small cracks. Monterrey-Quintero and Sobral [22] and Tavares *et al*. [23] suggested that the black spots indicated in Figure **3** were microbubbles embedded in the matrix or spaces occupied by glycerol before the drying process. Spada *et al*. [24] suggested that cracks in the structure may be related to sample storage conditions, drying by using forced convection or high temperatures. Oliveira Junior *et al*. [25] reported that the synthetic pectin-based film had a smooth surface, with less porosity and a well-homogenized material. However, the formation of a discontinuous structure and, consequently, exposure of the matrix may be responsible for reducing the passage of oxygen through the films, since the gases are non-polar and therefore have little affinity for the polysaccharide matrix [26].

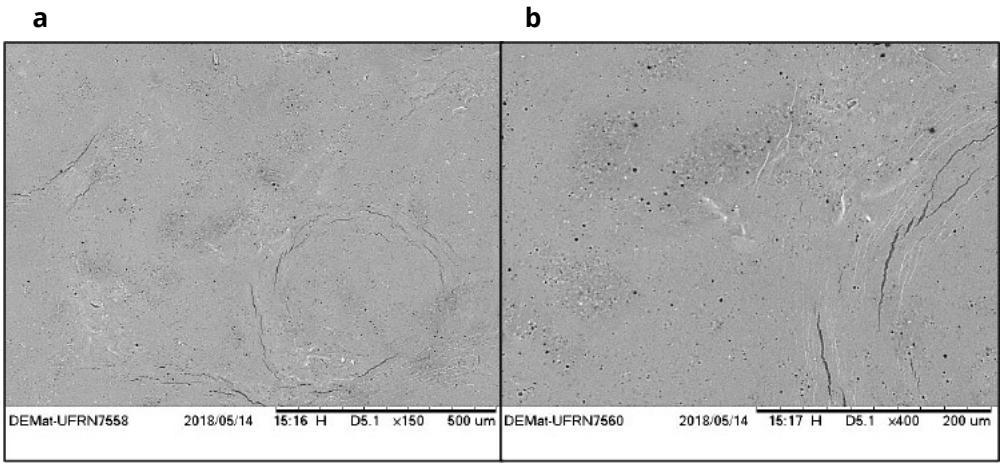


Figure 3: Micrographs of the Scanning Electron Microscope (SEM) from the natural pectin-based film with magnifications of (**a**) 150 times and (**b**) 400 times.

The Figure **4** shows the diffractogram of the natural pectin-based film obtained from X-Ray diffraction analysis.

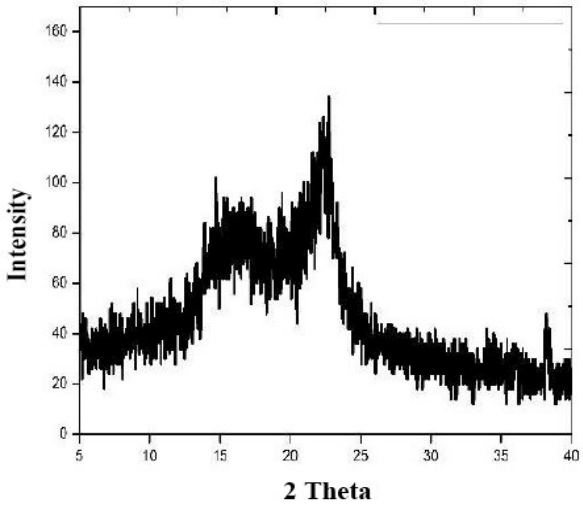


Figure 4: Diffractogram of the microstructural characterization of the natural pectin-based film.

Between 15° and 20°, the shoulder can be seen, which receives this nomenclature because it is not as intense as the peak, and at 23° there is a relatively expressive peak, thus revealing a poorly crystalline polymer structure. The result obtained was similar by Andrade *et al*. [27], for commercial pectin, however, there is a difference in the intensity of the peak at 23° which is named as a shoulder in the referring works. This indicates that the natural pectin-based film has a more crystalline material than commercial pectin and can be justified by the presence of cellulose in the cajá peel extract. This hypothesis is supported by the results of XRD from celullose obtained by Santos *et al*. [28].

Figure **5** presents another results about film microstructure, the micrograph of the topographic study was obtained through Atomic Force Microscopy (AFM).

The maximum roughness value of the analyzed film was 262.48 nm. When compared with the result obtained by Eça *et al*. [29], in which obtained, for the commercial pectin-based film, a maximum roughness of 130 nm, it is concluded that the natural pectin-based film is a considerably roughened film. The same behavior was observed in the films that contain fruit extract (acerola, papaya, cashew, strawberry and pequi) with commercial pectin. The addition of extracts to the films increases their roughness.

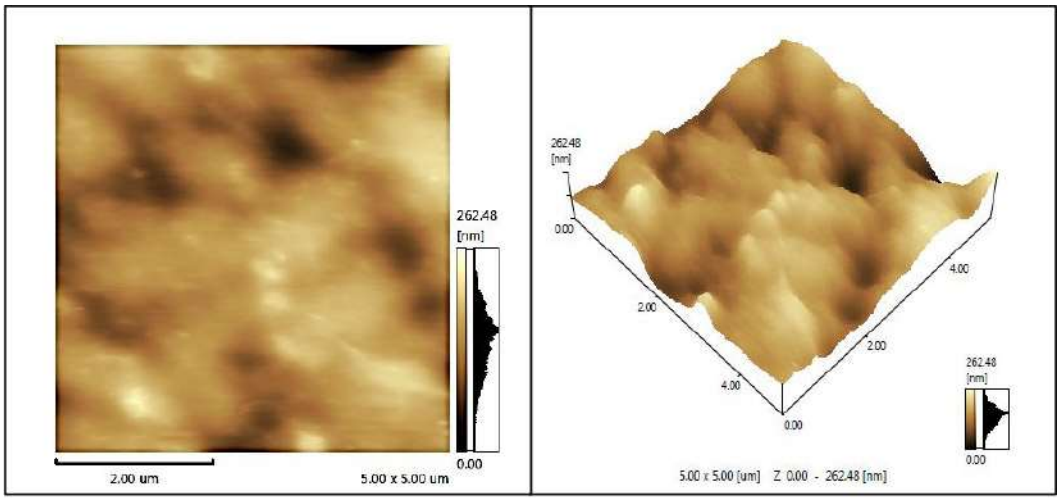


Figure 5: Topographic images of film through Atomic Force Microscopy (AFM) of the natural pectin-based film.

This fact can be explained by the presence of the cajá bark extract and glycerol in the film which, through thermoplasticization leads to the formation of a highly mobile biopolymer matrix, vulnerable to interaction with the environment. Since glycerol is responsible for breaking the forces inter and intramolecular structures established between the constituents of the film matrix [30]. Linked to this, as observed in SEM, the presence of pores in the film also can be explained by presence of caja extract on film matrix. This fact draws attention to the need to carry out some treatment on the film to better purify its components.

3.3. Inhibition of Microbial Growth

The evaluation of the antimicrobial activity of the natural pectin-based film was performed against Gram-positive bacteria *Staphyloccoccus aureus* (ATCC 6538) and Gram-negative *Pseudomonas aeruginosa* (ATCC 27853). The results obtained from Inhibition of Microbial Growth are shown in Figure **6**.

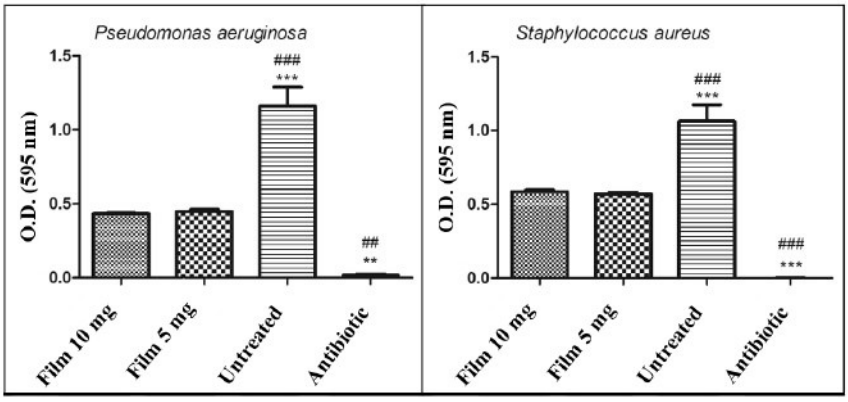


Figure 6: Inhibition of microbial growth of the *Pseudomonas aeruginosa* and *Staphylococcus aureus* tested on filmogenic solution prepared with pectin extracted from cajá peel.

The antimicrobial activity is an attractive feature of filmogenic coatings, in addition to maintaining the physicochemical properties of the fruits, the coatings can prevent contamination by pathogenic microorganisms. Such quality is found in the filmogenic solution synthesized by pectin extracted from the cajá peel.

This fact is confirmed by the results of Figure **6**, in which for both concentrations tested of filmogenic solution the growth of Gram-positive bacteria *Staphyloccoccus aureus* were in approximately 50% inhibited and 60% of the growth of Gram-negative bacteria *Pseudomonas aeruginosa* was inhibited.

Different compounds can be added to the filmogenic solution in order to induce antimicrobial activity [31]. For the film studied in this work, this characteristic can be attributed to the presence of phenolic compounds, since a direct relationship between antioxidant activities and antimicrobial activity is widely highlighted in phenolic extracts [32].

3.4. Applicability Tests

The Figure **10** shows the visual appearance of the acerolas uncoated (right) and coated (left) with the filmogenic solution during the 7 days of storage.

Analyzing the visual aspect of the fruits, it was possible to notice the difference between the maturation stage of the acerolas coated with the filmogenic solution and those of the control (without coating). The fruits showed a noticeable difference in color and stiffness, emphasizing the effectiveness of the application of the pectin-based coating extracted from the cajá peel in delaying the ripening of the acerolas.

3.4.1. Weight Loss

The weight loss of acerolas submerged in the edible coating and acerolas without coating were evaluated during the storage period (7 days) and the results are presented in Figure **7**.

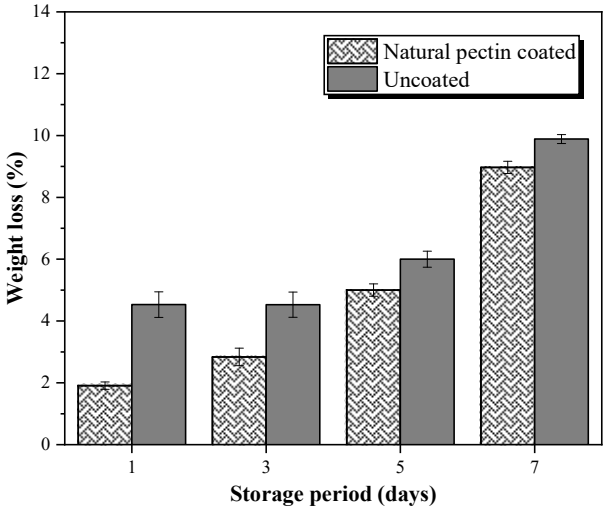


Figure 7: Weight loss percentage calculated from acerolas coated and uncoated during storage period of 7 days.

As expected weight loss occured during acerolas storage due to respiratory process, moisture transfer and some oxidation processes [6]. As expected, over the storage time there was a loss of mass for all acerolas analyzed. However, the acerolas with edible coatings had relatively lower weight loss when compared to the percentages of uncoated acerolas. Panahirad *et al*. [31] highlighted promising results in several published works, regarding the reduction of weight loss of different fruits and vegetables with CMC and pectin-based filmogenics coating.

3.4.2. Titratable Acidity (TA)

TA values given in percentage of ascorbic acid during the storage period of acerolas coated and uncoated are shown in Figure **8**. These results indicate that on all storage days the acerolas coated had TA values higher than acerolas uncoated.

According to other studies carried out with the aim of characterizing acerola maturation, the more ripe the fruit, the lower the concentration of ascorbic acid [33,34]. In view of this fact, it can be seen that the acerola coated with the filmogenic solution of natural pectin extracted from cajá peel had a lower maturation stage on all days analyzed.

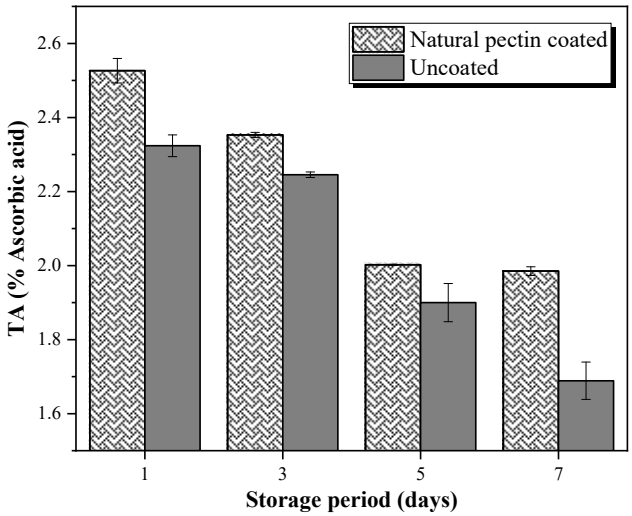


Figure 8: Titratable Acidity values given in percentage of ascorbic acid of acerolas coated and uncoated during storage period of 7 days.

3.4.3. Total Soluble Solids (TSS)

Figure **9** shows the TSS values obtained from acerolas coated and uncoated stored at room temperature for 7 days.

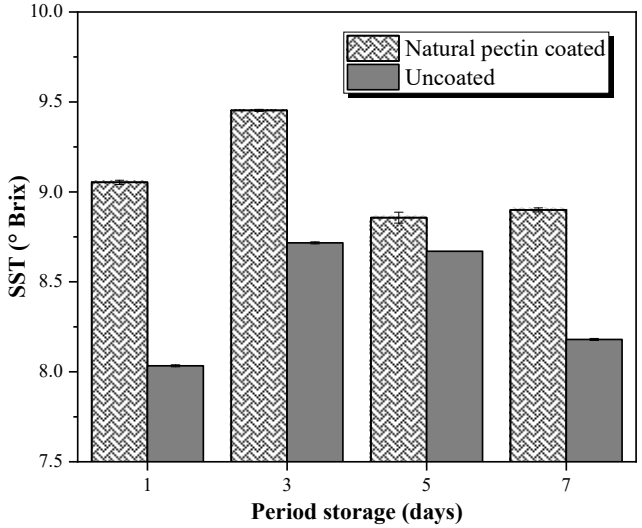
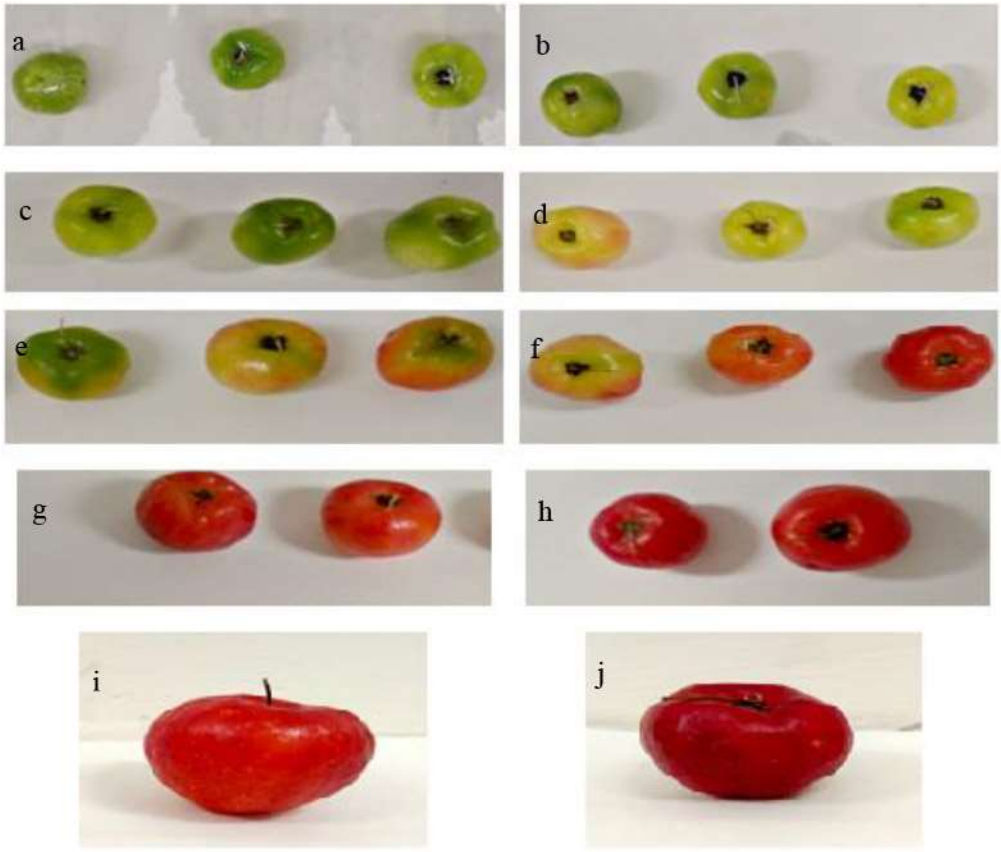


Figure 9: Total Soluble Solids (°Brix) obtained from acerolas coated and uncoated during storage period of 7 days.

In these results it is important to note that TSS values of acerolas coated with filmogenic solution of natural pectin were higher than acerolas uncoated. Such behavior is expected by the presence of pectin in the coated samples.

The observed increase in TSS value from day 1 to day 3 can be explained by the accumulation of sugars due to loss of moisture [6]. It should be highlighted that the acerolas uncoated had the higher increase (8.5%) when compared to the increase of acerolas coated.

Azeredo *et al*. [35] also evaluated the effects of coating application on fresh acerola. The authors reported that film-forming dispersions of alginate and acerola also delayed fruit ripening. In addition to this work, many others confirmed the good performance of filmogenic coatings synthesized by pectin combined or not with other compounds such as essential oil [36], sodium alginate [37], polyvinyl alcohol and acid citrus [38] applied to different fruits.



*The number of samples decreased because the fruits were used to perform the physicochemical analyzes (weight loss, soluble solids content and titratable acidity).

Figure 10: Visual appearance of the acerola at time zero with filmogenic coverage (a) and control sample (b); on the first day with filmogenic coverage (c) and control sample (d); on the third day with filmogenic coverage (e) and control sample (f); on the fifth day with filmogenic coating (g) and control sample (h) and on the seventh day with filmogenic coating (i) and control sample (j).

Conclusions

The film produced from the pectin extracted from the cajá peel presented as dense membranes, with the presence of pores, cracks and a very rough surface. Furthermore, the extracted pectin was classified as low methocxyl (LM) pectin with a degree of esterification of 44%. The film presented antimicrobial activity against the two analyzed bacteria, *Pseudomonas aeroguninosa* and *Staphylococcus aureus*.

The application of filmogenic coating on green acerola to monitor its maturation stage presented satisfactory results. The coated acerola showed less weight loss and reasonably higher titratable acidity at the end of the storage days, indicating that the coverage favored the delay in ripening.

In addition, total solid soluble values of coated acerolas were higher than uncoated acerolas due to presence of pectin on the pulp analyzed. Finally, filmogenic coating presented a great potential for industrial application for coating acerolas in order to delay ripening, increasing shelf life of fruits.

Acknowledgements

The authors would like to thank the Federal University of Rio Grande do Norte for the support, in particular the Graduate Program in Chemical Engineering, the Department of Materials Engineering and the Department of Food Engineering.

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