

## **Amazonian tuber starch based films incorporated with silver nanoparticles for preservation of fruits**

Filmes à base de amido de tubérculo da Amazônia incorporados com nanopartículas de prata para preservação de frutas

Películas a base de almidón de tubérculo amazónico incorporadas con nanopartículas de plata para la conservación de frutas

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### **Abstract**

Films and coatings based on natural polymers are used to conserve nutritional quality of fruits, vegetables and also delay their ripening. The purpose of this study was to develop films with starch extracted from *Dioscorea altissima* Lam. (dunگوی) incorporated with silver nanoparticles for coating and preserving fruits. The films obtained by cast were characterized visually, by Scanning Electron Microscope, by Atomic Force Microscopy, by X-Ray Diffraction and by Fourier-Transform Infrared Spectroscopy. Antimicrobial activity and technological properties were also

evaluated. The coating of camu-camu fruits [*Myrciaria dubia* (Kunth) McVaugh] was carried out by immersing them in the filmogenic solution, followed by their physicochemical and microbiological analysis. The films with silver nanoparticles showed transparency, flexibility, spherical clusters and a higher average roughness. A reduction in thickness, solubility and water vapor permeability was also observed. Antimicrobial action against *Staphylococcus aureus* and *Escherichia coli* was proved as well. The fruits coated with films exhibited delay in ripening, with maintenance of quality and longevity. Uncoated fruits showed greater wilting and wrinkling. The starch film incorporated with silver nanoparticles was effective for preserving camu-camu fruit.

**Keywords:** Antimicrobial activity; *Dioscorea altissima*; Nanoparticles; Starch film.

### Resumo

Filmes e revestimentos à base de polímeros naturais são usados para conservar a qualidade nutricional de frutas, vegetais e também retardar seu amadurecimento. O objetivo deste trabalho foi desenvolver filmes com amido extraído de *Dioscorea altissima* Lam. (cará-de-espinho) incorporado com nanopartículas de prata para revestimento e preservação de frutas. Os filmes obtidos por casting foram caracterizados visualmente, por Microscópio Eletrônico de Varredura, Microscopia de Força Atômica, Difração de Raios-X e Espectroscopia de Infravermelho com Transformada de Fourier. A atividade antimicrobiana e as propriedades tecnológicas também foram avaliadas. O revestimento dos frutos de camu-camu [*Myrciaria dubia* (Kunth) McVaugh] foi realizado por imersão em solução filmogênica, seguida de suas análises físico-químicas e microbiológicas. Os filmes com nanopartículas de prata apresentaram transparência, flexibilidade, aglomerados esféricos e maior rugosidade média. Uma redução na espessura, solubilidade e permeabilidade ao vapor de água também foi observada. A ação antimicrobiana contra *Staphylococcus aureus* e *Escherichia coli* também foi comprovada. Os frutos revestidos com filmes exibiram atraso no amadurecimento, com manutenção da qualidade e longevidade. Frutos não revestidos apresentaram maior murchamento e enrugamento. O filme de amido incorporado com nanopartículas de prata foi eficaz na preservação do fruto do camu-camu.

**Palavras-chave:** Atividade antimicrobiana; *Dioscorea altissima*; Nanopartículas; Filmes de amido.

### Resumen

Películas y recubrimientos a base de polímeros naturales son utilizados para conservar la calidad nutricional de frutas, verduras y también para retrasar su maduración. El propósito de este estudio fue desarrollar películas con almidón extraído de la *Dioscorea altissima* Lam. (ñame) incorporado con nanopartículas de plata para el recubrimiento y conservación de frutas. Las películas obtenidas por fusión se caracterizaron visualmente, por medio de Microscopio Electrónico de Barrido, mediante Microscopía de Fuerza Atómica, con Difracción de Rayos X y por Espectroscopia Infrarroja con Transformada de Fourier. Actividad antimicrobiana y propiedades tecnológicas también fueron evaluadas. El recubrimiento de frutos de camu-camu [*Myrciaria dubia* (Kunth) McVaugh] se realizó sumergiéndolos en la solución filmógena, seguido por el análisis fisicoquímico y microbiológico. Las películas con nanopartículas de plata mostraron transparencia, flexibilidad, acumulaciones esféricas y un mayor promedio de rugosidad. También se observó una reducción en el espesor, la solubilidad y la permeabilidad al vapor de agua. Asimismo, acción antimicrobiana contra *Staphylococcus aureus* y *Escherichia coli* fue evidenciada. Los frutos recubiertos con películas exhibieron retraso en la maduración, con mantenimiento de calidad y longevidad. Los frutos sin recubrir mostraron mayor marchitez y arrugas. La película de almidón incorporada con nanopartículas de plata fue eficaz para preservar a la fruta de camu-camu.

**Palabras clave:** Actividad antimicrobiana; *Dioscorea altissima*; Nanopartículas; Película de almidón.

## 1. Introduction

In response to the growing of demanding consumers for fresh foods, with guaranteed safety and a ready-to-eat concept, a lot of research work is being carried out to maintain quality, and improve post-harvest viability. Biodegradable coating films Ag nanoparticle-based are an eco-friendly, simple, and efficient technological alternative for reducing microbial contamination and increasing the storage time for products of plant origin (Pilon *et al.*, 2015).

Biodegradable polymers such as starch predominate in the production of coating films, due to the availability of different sources such as tubers, leaves, rhizomes, seeds and fruits, which are easy to process and have a low production cost (Mateescu, Dimov, Grumezescu, Gestal & Chifiriuc, 2015). Biodegradable films are commonly made via casting, although in large-scale manufacturing, semi-automatic molding and spread coating are used (Versino, Lopez, Garcia & Zaritzky, 2016).

Among the plant species of Amazon rainforest, *D. altissima* is a tuber that has stood out with a significant production of 100 kg/plant in 18 months. In addition, *D. altissima* is not toxic, has excellent nutritional value, and prebiotic action,

characteristics that promote its use as food (Teixeira *et al.*, 2016).

The productivity of the *D. altissima* and its therapeutic importance are factors that stimulate its cultivation. Moreover, in *D. altissima*, the content of available starch and resistant starch corresponds to approximately 70.0% and 10.0%, respectively. These characteristics boost the biotechnological and commercial exploitation of this vegetal as raw material (Silva *et al.*, 2019).

An alternative use of *D. altissima* starch consists of making films for the conservation of food products, a technology still unavailable for the fruits of the Amazon region. To increase efficiency, these films can be incorporated with silver nanoparticles (AgNPs) obtained by biogenic synthesis. This green technology is less aggressive to the environment, economically viable, and has demonstrated antimicrobial efficiency against several microorganisms (Silva *et al.*, 2017; Silva-Vinhote *et al.*, 2017).

Bacteria, algae, actinomycetes, filamentous fungi and yeasts are explored in bioprocesses for the production of AgNPs. Silver nanoparticles are commonly used in multiple applications due to their properties such as chemical and thermal stability, conductivity, size, shape, surface and volume ratio and high catalytic activity. Silver nanoparticles are used as deodorants and disinfectants, incorporated in fabrics, bandages, coatings and food packaging; in addition, they are used to purify drinking water and, currently as insecticide to control pest attack in agriculture (Tyagi, Tyagi, Gola, Chauhan & Bharti, 2019; Pandey, Klerk, Kim, Kang & Fosso-Kankeu, 2020).

Another raw material of great prominence in the Amazon that makes part of native people diet is *Myrciaria dubia* (Kunth) McVaugh, a wild species that grows on the banks of rivers and lakes (Santos *et al.*, 2018). The fruits are known as camu-camu, have a globose shape, and red color. The high levels of anthocyanins and vitamin C content have promoted the application of this Amazonian vegetal species in local gastronomy, as well as in pharmaceutical and cosmetics industries (Nascimento & Carvalho, 2012).

Although camu-camu has nutritional and socioeconomic importance, there is no post-harvest conservation technique available to maintain the characteristics of the fruits for its commercialization in natura. The objective of this research was to prepare coating films formulated with the starch of *D. altissima*, incorporated with AgNPs from actinomycetes mediated synthesis, as a new technological option for the conservation of fresh camu-camu.

## 2. Methodology

### 2.1 Obtaining AgNPs

AgNPs solutions preserved at 4°C were used in the preparation of starch-based films and were selected based on significant antimicrobial activity among others previously reported (Silva-Vinhote *et al.*, 2017). The AgNPs that the synthesis was mediated by *Streptomyces parvulum* DPUA 1549 were ceded by the Nanoparticle Bank of the DPUA Cultures Collection/UFAM.

### 2.2 Preparation of starch-based films and incorporation of AgNPs

To prepare the films by casting, 4 g of *D. altissima* starch was diluted in 100 mL of distilled water containing 3 g of glycerol. The filmogenic solution was heated until 95 °C. After complete gelatinization and cooling of the solution, AgNPs (2 µg/mL) were added and 10 mL of this solution was spread on Petri dishes (140 mm X 150 mm). These dishes were maintained at 40 °C in an oven with forced air circulation for 24 h. Two types of films were produced: standard (without AgNP) and test. The films were stored in glass bottles with airtight lid for further analyses (Fayaz, Balaji, Girilal, Kalaichelvan, & Venkatesan, 2009; Pagno *et al.*, 2015).

## **2.3 Characterization of films**

### **2.3.1 Determination of film thickness**

The thickness of the films was determined using a digital caliper in three random points and the results were expressed as averages (Pagno *et al.*, 2015).

### **2.3.2 Determination of water vapor permeability**

Water vapor permeability was determined by ASTM's E96 / E96 gravimetric method (American Society for Testing and Materials-ASTM, 2010). The rate of water vapor permeability was determined at 23 °C and 75% RH. The weight gain of anhydrous chloride inside aluminum capsules, kept closed with the films was quantified on a Mettler analytical balance; model AT 400 (Greifensee, Switzerland), with a resolution of 10<sup>-4</sup> g.

### **2.3.3 Determination of humidity**

The humidity was determined by drying the film specimens in forced air circulation oven, at 105° C, until constant weight (Association of Official Analytical Chemists - AOAC, 2006).

### **2.3.4 Determination of solubility**

The water solubility of the films was determined according to the method of Gontard, Duches, Cuq & Guilbert (1994). The films of known humidity were weighed, submerged in 50 mL of distilled water and kept under stirring at 26 °C. After 24 hours, the samples were dried at 105 °C for 4 hours and weighed. The results were expressed as percentages (%).

### **2.3.5 Scanning Electron Microscopy**

The Scanning Electron Microscopy (SEM) of the films was performed in a compact scanning electron microscope; model JSM-6010LA, Jeol (Akishima, Japan). The samples were fixed to the sample holder with double-sided adhesive tape. An acceleration potential of 15 kV and 20 kV was used in the analyses (Campos *et al.*, 2017).

### **2.3.6 Atomic Force Microscopy**

The Atomic Force Microscopy (AFM) of the films was performed in an Agilent equipment, model AFM/SPM 5500 Dynamics (Santa Clara, USA), with a resonance frequency of 320 kHz and a constant force of 42 N/m. A fragment of the films was added to the commercial silica cantilever and dried by evaporation at 25 °C. Images were treated in the WSXM software v.5.0 (Horcas & Fernández, 2007). The topography image was analyzed to obtain the average height of the peaks, and roughness medium square (RMS) parameters using Gwyddion software version 2.19. The RMS was defined as the standard deviation of the elevation values z, within the determined area.

### **2.3.7 X-Ray Diffraction**

The X-Ray Diffraction (XRD) analysis of the films was determined on a STADI-P diffractometer (Darmstadt, Germany) with molybdenum anode (MoK $\alpha$ 1,  $\lambda = 0.7093 \text{ \AA}$ ), at 25 °C, under 40 mA current and 50 kV voltage. The X-ray photons were captured by a Mythen 1K detector (Baden-Daettwil, Switzerland) by scanning 2 $\theta$  from 5 to 60 ° and scanning speed of 0.471 °. min<sup>-1</sup>.

### 2.3.8 Fourier-Transform Infrared Spectroscopy

The Fourier-Transform Infrared Spectroscopy (FTIR) spectra were obtained using a Agilent Cary 630 spectrophotometer (Santa Clara, USA), in the spectral range of 700 to 4000  $\text{cm}^{-1}$ , with a resolution of 7  $\text{cm}^{-1}$  (Berté, 2013).

### 2.4 Quantification of total silver in films

The quantification of total silver in films, the samples were digested at 90 °C for four hours, in 98% (v/v) nitric acid (Rolim *et al.*, 2019). The quantification of total silver was determined on a model 7900 ICP-MS spectrophotometer from Agilent Technologies (Hachioji, Japan).

### 2.5 Determination of antimicrobial activity

The antimicrobial activity of the films incorporated with AgNPs was determined by the agar diffusion method (Fayaz *et al.*, 2009), against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* CBAM 001 and *Candida albicans* DPUA 1706. The cultures of the test microorganisms were performed on Sabouraud agar (yeasts) and Mueller-Hinton agar (bacteria). For the inoculum, a cell suspension was previously prepared with sterile distilled water, the yeast it was standardized at 105 CFU/mL and bacteria at 108 CFU/mL using spectrophotometer Uv-Vis spectra (UV-VIS Cary 50 Probe Agilent). From each suspension, 100  $\mu\text{L}$  was transferred to Mueller-Hinton (MHA) and Sabouraud (SAB) agar surfaces, respectively. After absorption of the suspension, the discs of the 8 mm films were transferred to the culture medium surface. As a control, streptomycin and itraconazole solution (50  $\mu\text{g}/\text{mL}$ ) was used for bacteria and yeasts, respectively. The experiments were maintained at 37 °C for 24 h and were performed in triplicate and the inhibition halo expressed in millimeters.

### 2.6 Preservation of fruits with coating films

#### 2.6.1 Obtaining and sanitizing the fruits

The camu-camu [*Myrciaria dubia* (Kunth) McVaugh] was obtained from Lake Iripixi, located in the city of Oriximiná-Pará. The fruits were sanitized with 1% (v/v) hypochlorite solution for 20 minutes, followed by washing them under running water to remove the hypochlorite excess (Siddiq, Ahmed, Lobo & Ozadali, 2012). The fruits were classified into four degrees of physiological ripeness: green (S1), reddish green (S2), greenish red (S3) and purple (S4). The fruits on stage 2 of ripeness were chosen for the coating analyses.

#### 2.6.2 Coating of the fruits

For coating the fruits, *D. altissima* starch (4% w/v) was firstly diluted in distilled water. Subsequently, glycerol (3% w/v) was added and heated to 95 °C until complete gelatinization. The cooled solution was added to the colloidal solution of silver nanoparticles (2  $\mu\text{g}/\text{mL}$ ) from the nanoparticle bank of the DPUA Culture Collection (Fayaz *et al.*, 2009; Pagno *et al.*, 2015). The fruits were completely immersed in filmogenic solutions for 5 seconds at 25° C two times consecutively. Then, the fruits coated with the standard film formulated with starch and glycerol (CF), the fruits coated with the test film incorporated with AgNPs (CFN) and the control fruits without any coating film (WF) were kept under refrigeration ( $5 \pm 2$  °C). After 12 days the fruits were evaluated according to their visual appearance, physical injuries and the presence or absence of decay.

### 2.7 Determination of pH, titratable acidity, total soluble solids and weight loss

The pH of the fruits was measured using a digital pH meter, model PHS-3E. Acidity expressed as citric acid was determined by titration with 0.1M NaOH solution, using 1% (w/v) alcoholic phenolphthalein solution. The content of soluble

solids, expressed in degrees Brix ( $^{\circ}$ Brix), was measured by direct reading of a double scale Incoterm portable refractometer (AOAC, 2006). The fruits were weighed with an analytical balance to calculate the loss of mass (Fakhouri *et al.*, 2007). Analyses were performed on the 1<sup>st</sup>, 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> days.

## 2.8 Microbiological analysis

The microbiological analysis of the fruits was performed the 1<sup>st</sup>, 4<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> days to assess the presence of Coliforms at 45  $^{\circ}$ C, *Escherichia coli*, *Salmonella* sp., yeast and filamentous fungi (Loy-Hendrickx *et al.*, 2018).

## 2.9 Statistical analysis

The data from the experiments carried out in triplicate and were subjected to analysis of variance. The means were compared by the Tukey test ( $p < 0,05$ ), using the Minitab version 17.0 software.

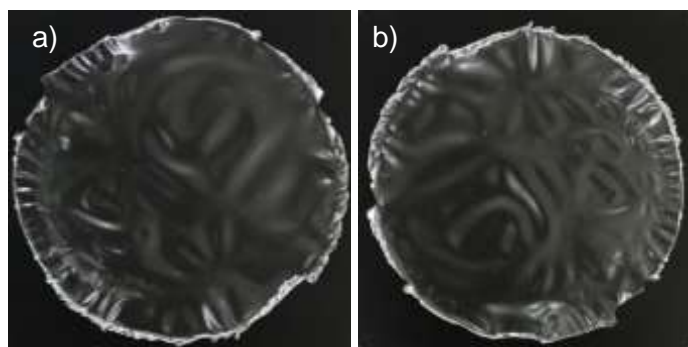
# 3. Results and Discussion

## 3.1 Characterization of coating films

### 3.1.1 Visual aspect, Humidity, Solubility, Thickness, Water vapor permeability of coating films

The visual analysis of the films showed that the addition of AgNPs did not interfere in the filmogenic capacity of *D. altissima* starch. The evaluated films showed transparency, flexibility and absence of any cracks when removed off the plates (Figure 1). According to Fakhouri *et al.* (2007) edible films used to coat fresh foods must be transparent, odorless and tasteless, characteristics that do not interfere with the product's sensory properties.

**Figure 1.** Coating films of *Dioscorea altissima* and glycerol. a) Standard film; b) Test film.



Source: Authors.

The characteristics of the coating films are shown in Table 1. The humidity values in the standard film and in the test films were 8.22% and 11.16%, respectively. The values determined by Kanmani and Rhim (2014) in gelatin films without and with AgNPs were 16.9% and 17.3%, respectively. These results related to the increase in humidity in films with AgNPs are similar to those obtained in the present study. The variation in humidity in the samples may be related to the reduction of the interaction between the amylose and amylopectin chains, a factor that allows the absorption of water by free hydroxyl groups. Additives incorporated in the films can influence the increase or decrease in the humidity of the starch films (Dantas *et al.*, 2015).

The incorporation of AgNPs in films with *D. altissima* starch also caused a reduction of 15.3%, 7.6%, 15.68% and 24.67% in thickness, solubility, water vapor and permeability, respectively. Thickness control is an important parameter in film production, as it directly influences water vapor permeability and solubility (Farias, Carvalho, Takeiti & Ascheri, 2012). The

low solubility of films incorporated with AgNPs is an indicator of integrity and water resistance, and the coated products would not disintegrate easily in contact with water (Basiak, Lenart & Debeaufort, 2017).

Yoksan and Chirachanchai (2010) presented that the chitosan films incorporated with AgNPs showed a decrease in water vapor permeability. Films used to cover fresh fruits and vegetables require low permeability to water vapor, as the loss of moisture reduces the metabolic activity of the food, causing loss of mass, dryness, change in color and texture (Sánchez-González, Arab-Tehrany, Cháfer, González-Martínez & Chiralt, 2014). Ortega, Gianuzzi, Arce & Garcia (2017) reported that the filling of the spaces present in the polymeric matrix by the AgNPs increase the tortuosity of the film and hinder the movement of the permeating material.

**Table 1.** Characteristics of coating films.

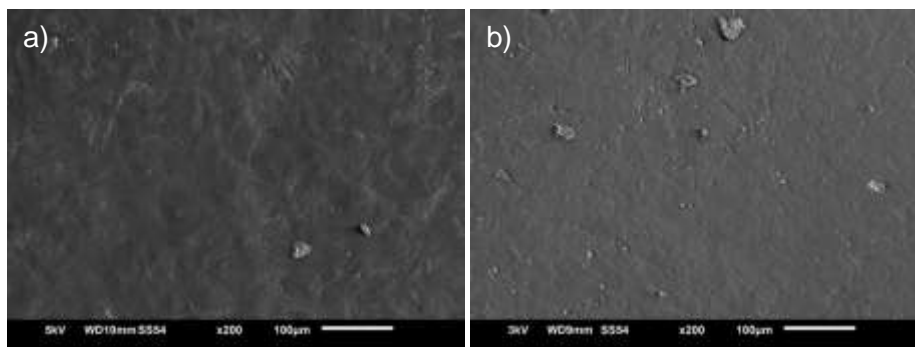
	Standard Film	Test Film
Humidity (%)	(8.2±0.3) <sup>b</sup>	(11.2±0.5) <sup>a</sup>
Solubility (%)	(57.8±0.9) <sup>a</sup>	(53.4±0.9) <sup>b</sup>
Thickness (mm)	(0.3±0.0) <sup>a</sup>	(0.2±0.0) <sup>b</sup>
WVP (g.mm.m <sup>-2</sup> .d.kPa)	(4.9±0.1) <sup>a</sup>	(4.1±0.0) <sup>b</sup>
Grammage (g/m <sup>2</sup> )	(7.7±0.2) <sup>a</sup>	(5.8±0.2) <sup>b</sup>

Means that do not share the same letter are significantly different by the Tukey Test ( $p < 0.05$ ). WVP=Water Vapor Permeability.  
 Source: Authors.

### 3.1.2 Scanning Electron Microscopy

Figure 2a and Figure 2b shows that the standard films have a smooth and homogeneous surface. However, in films incorporated with AgNPs, spherical clusters were observed. A similar result was reported by Oliani *et al.* (2016) in polyethylene films formulated with AgNPs.

**Figure 2.** *Dioscorea altissima* starch films viewed in SEM. a) Standard film; b) Test film.

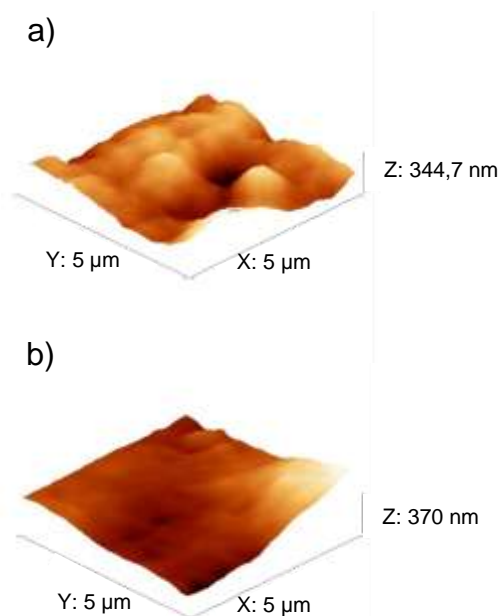


Source: Authors.

### 3.1.3 Atomic Force Microscopy

The three-dimensional images obtained by Atomic Force Microscopy (AFM) confirm the results obtained by SEM (Figure 3a and Figure 3b). The *D. altissima* films incorporated with AgNPs present a higher average roughness of 16.40% when compared to the standard film. Ortega *et al.* (2017) mentions that high concentrations of AgNPs used in coating films tend to form agglomerates and, consequently, generates a rougher surface.

**Figure 3.** Three-dimensional micrographs of *D. altissima* starch films. a) Standard film; b) Test film.



Source: Authors.

### 3.1.4 Fourier-Transform Infrared Spectroscopy

The FTIR spectra of standard films and test films showed similar bands and small displacements that can be explained due to the interaction of AgNPs and starch. An intense band was observed at  $3293\text{ cm}^{-1}$  that refers to the hydroxyl group present in the starch and in the plasticizer (Bergo, Sobral & Prison, 2010). The peaks at  $1648\text{ cm}^{-1}$  indicate the presence of water bound to the polymer (Sueiro, Faria-Tischer, Lonni & Mali, 2016). The bands found in the  $1000\text{ cm}^{-1}$  region are related to the crystalline structure of starch (Ferreira, Passos & Marques, 2015).

### 3.1.5 X-Ray Diffraction

Analysis of the XRD films showed that the standard and the test films presented peaks at diffraction angles close to each other. These data confirm the presence of starch extracted from type B tubers in the preparation of *D. altissima* films. Similar results were found by Chevirón, Gouanvé & Espuche (2016) in potato starch-based films.

### 3.2 Silver concentration in films

The analysis performed in the ICP-MS showed that the AgNPs incorporated films had silver concentration of  $152.50\text{ }\mu\text{g/mL}$ . Li, Li, Zhang, Yun & Qin (2018) reported that this concentration is lower than that recommended for food by the European Food Safety Authority. The possibility of migration of AgNPs from packaging to food is very low. In addition, studies reporting the possible toxicological effects of AgNPs levels in food as a result of contamination through packaging are still scarce (Simbine *et al.*, 2019).



### 3.3 Film antimicrobial activity

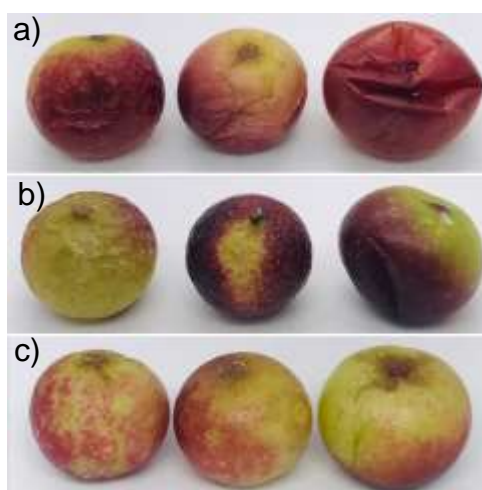
The films incorporated with AgNPs showed bactericidal action against *S. aureus* (9 mm) and bacteriostatic action against *E. coli* (7 mm). However, *C. albicans* did not express sensitivity. Similar data were observed by Silva-Vinhote *et al.* (2017). Yoksan and Chirachanchai (2010) reported that AgNPs attach themselves to the surface of the bacterial cell membrane and disrupts its integrity, causing damage to the metabolism and death of the microorganism.

### 3.4 Preservation of fruits with coating films

#### 3.4.1 Visual aspect of the fruits

The visual evaluation of the fruits after 12 days of storage showed that the fruits without film (WF) showed wrinkling, with inadequate physical characteristics for consumption and commercialization. The fruits coated with the standard film without AgNPs (CF) showed deterioration and those with silver nanoparticles (CFN) showed delay in ripening and preserved their integrity, which is a favorable aspect for commercialization of fresh foods (Figure 4a, Figure 4b and Figure 4c). Similar data were observed for minimally processed melons, packed in trays made of cellulose and AgNPs (Assis & Britto, 2014). Coating films promote the filling of possible aperture on the fruit peel, reducing the permeation of O<sub>2</sub> inside the fruit and consequently reducing ethylene production and maturation (Sanches, Silva, Moreira, Costa & Cordeiro, 2017).

**Figure 4.** Fruits (camu-camu): a) without coating film (WF); b) with standard film without AgNPs (CF); c) with test film incorporated with AgNPs (CFN) after 12 days of storage.

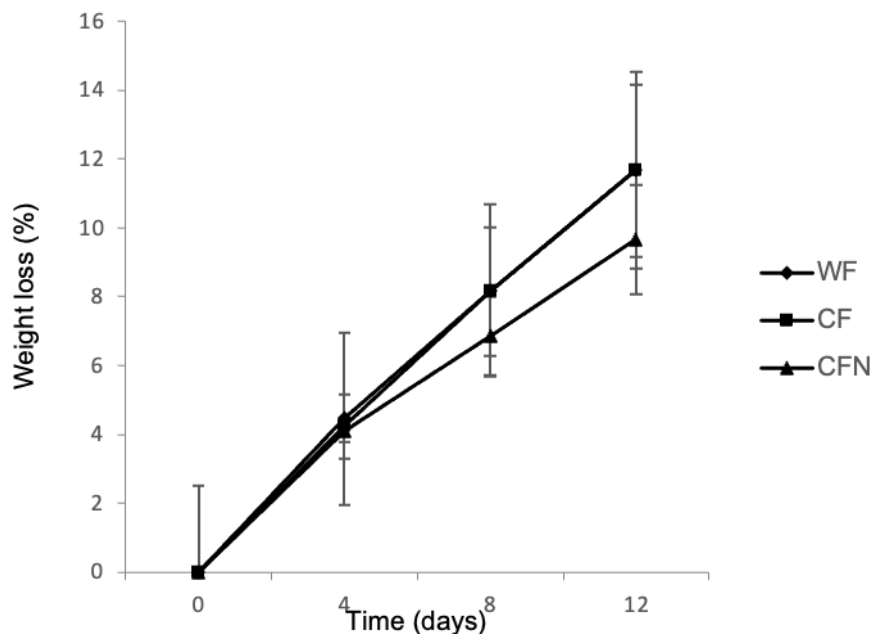


Source: Authors.

#### 3.4.2 Fruit weight loss

AgNPs, in addition to inhibit the growth of microorganisms in fruits, can also help to control the weight loss during storage. Figure 5 shows that the weight loss of fruits without coating film (WF), fruits coated with the standard film without AgNPs (CF) and fruits coated with the test film incorporated with AgNPs (CFN) were not significantly different ( $p < 0.05$ ) from each other until the 4<sup>th</sup> day of analysis. According to Sanches *et al.* (2017) the fruits are climacteric and have a high respiratory rate. Probably, the coating of fruits with films has reduced the respiration and consequently led to a decrease in ethylene production and loss of mass; features that contributed to a longer conservation period. The addition of AgNPs in coating films is considered an alternative to improve the mechanical, thermal, gas and water barriers of the films (Li *et al.*, 2018).

**Figure 5.** Weight loss of fruits without coating film (WF), coated with standard film without AgNPs (CF) and coated with test film incorporated with AgNPs (CFN) during 12 days of storage.

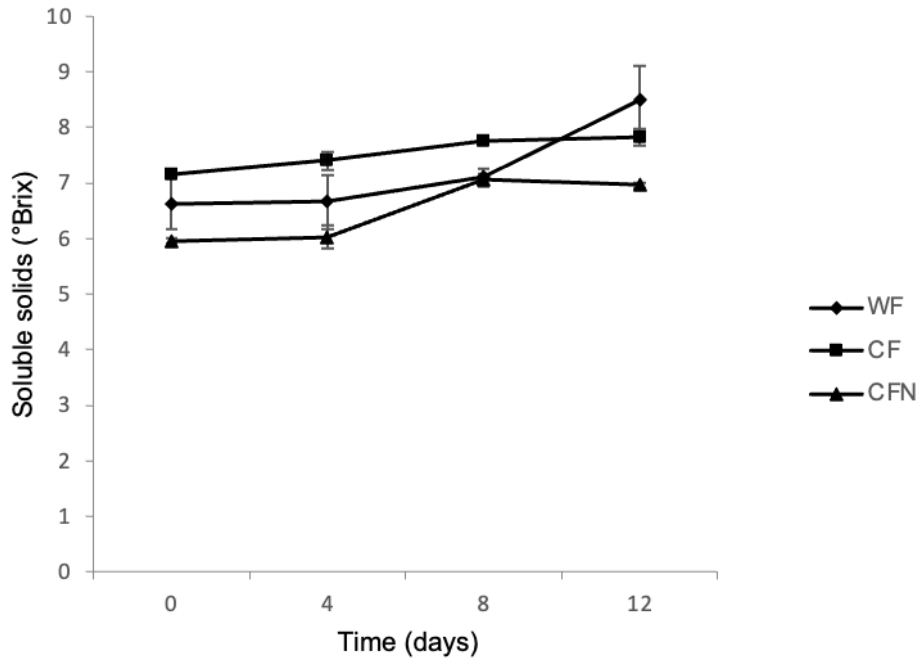


Source: Authors.

### 3.4.3 Total soluble solids

The content of soluble solids influences the flavor and acceptance of products of plant origin. In addition, it is an important parameter used to indicate the degree of fruit ripeness (Sun *et al.*, 2019). Figure 6 shows that the content of total soluble solids in all fruits exhibited no significant difference ( $p < 0.05$ ) until the fourth day of storage. At the end of the process, all samples showed a significant increase of soluble solids. When compared to time zero, an increase of 28.20%, 18.45% and 9.35% of soluble solids was observed in WF, CFN and CF, respectively. These results demonstrated that the coating films provide a novel alternative to delay ripening, and, maintain the quality and longevity of camu-camu fruits. Oliveira, Silva, Silva & Spoto (2014) also found an increase in total soluble solids in the fruits of *Myrciaria dubia*, which were not packed with BOPP (Bioriented Polypropylene) and PVC (Polyvinyl Chloride). Likewise, Fernandez, Picouet & Lloret (2010) observed that fruits packaged without the addition of AgNPs showed an increase in the content of soluble solids. The greater loss of turgidity by these unpackaged or packaged fruits without the addition of AgNPs influences the concentration of the pulp and consequently the content of soluble solids. The films with AgNPs probably decreased the ethylene synthesis and the respiratory activity of the fruits, delaying the physiological disorders and consequently their deterioration.

**Figure 6.** Concentration of total soluble solids in fruits without coating film (WF), coated with standard film without AgNPs (CF) and coated with test film incorporated with AgNPs (CFN) during 12 days of storage.

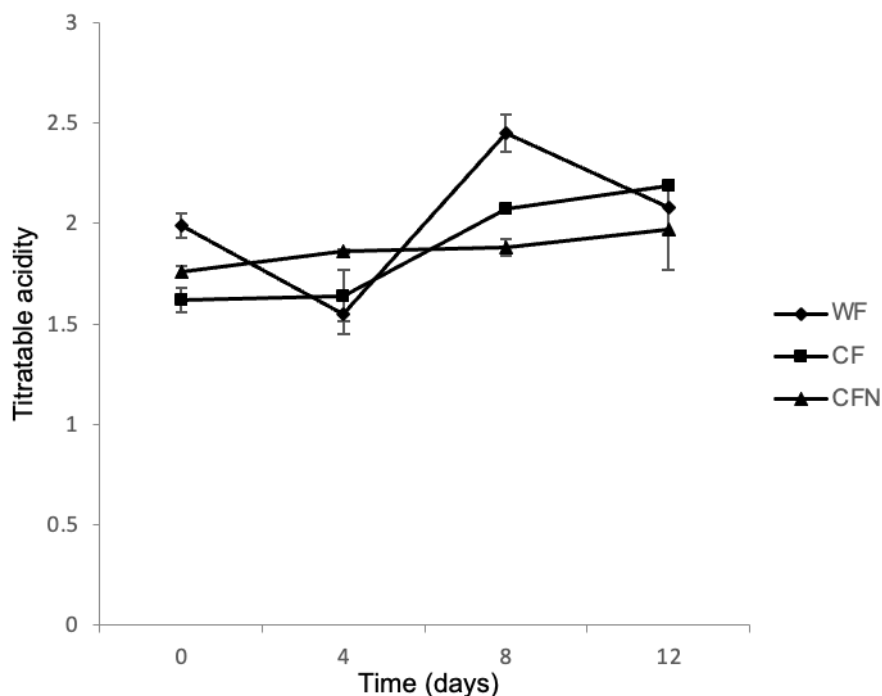


Source: Authors.

### 3.4.4 Titratable acidity and fruit pH

Throughout the storage period, the pH of the fruits remained acidic, in the range of 2.9 to 3.2. These pH values probably inhibited the growth of microorganisms in all fruits, with and without the coating films. Figure 7 shows the titratable acidity of the camu-camu fruits. In CF and CFN there was a gradual increase during the storage period. However, in WF, significant variations of this physicochemical parameter were observed. At the end of the storage period, the titratable acidity levels, in decreasing order, were 2.18%, 2.08% and 1.97%, in CF, WF and CFN, respectively. Organic acids tend to decrease with fruit ripening, as they are used as a substrate for respiration (Beber, Álvares & Kusdra, 2018). On the other hand, among other factors, the content of organic acids in fruits can also vary according to species, soil and stress conditions (Carvalho *et al.*, 2020).

**Figure 7.** Titratable acidity of fruits without coating film (WF), coated with standard film without AgNPs (CF) and coated with test film incorporated with AgNPs (CFN) during 12 days of storage.



Source: Authors.

### 3.4.5 Microbiological analysis of fruits

The data from the microbiological analysis of the fruits confirmed the absence of microorganisms. The presence of Coliforms, *E. coli*, *Salmonella* sp., yeast and filamentous fungi was not verified. These results are likely to be related to the intrinsic conditions of the fruits, such as acidic pH. The most favorable pH for the growth of most of the pathogenic microorganisms is in the range of 6.5 - 7.0 (Sanches *et al.*, 2017). Moreover, according to Arellano-Acuña, Rojas-Zavaleta & Paucar (2016), camu-camu naturally shows antimicrobial activity against *Staphylococcus aureus*, *Streptococcus mutans*, *S. sanguinis* and *C. albicans*.

## 4. Conclusion

The results showed that the films prepared with *D. altissima* starch are transparent and flexible. However, the presence of AgNPs determines the formation of roughness, being a desirable characteristic of coating films for fresh foods. Films with AgNPs have bactericidal action against *S. aureus*, bacteriostatic against *E. coli* and without expression of sensitivity by *C. albicans*. Fruits coated with the film without AgNPs present deterioration. On the other hand, films with AgNPs maintain integrity, delay ripening and increase the shelf life of fruits. As a suggestion, it is proposed to investigate the efficiency of other methods for the film's elaboration, the use of silver nanoparticles of different origins and characteristics to increase the antimicrobial activity and the shelf life of alimentary products.

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