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Edible coatings based on hydroxypropyl methyl cellulose and beeswax in pinecone

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ABSTRACT: The pinecone (*Annona squamosa* L.) is an Annonaceae that provides much appreciated fruit. However, these fruits have a limited shelf life due to their intense metabolic activity after harvest. The application of post-harvest conservation techniques, such as edible coatings, has been used to minimize the deleterious effects after the harvest of fruits, such as pinecone. The objective of this work was to evaluate the effects of coatings based on hydroxypropyl methyl cellulose (HPMC) and beeswax (BW) on the quality of pinecones stored at room temperature of 20 °C, from the local market in the city of Juazeiro, BA, Brazil. The fruits were selected for the absence of damage and sanitized by immersion in chlorinated water. The coatings were prepared by diluting the HPMC solution in deionized water at 90 °C under stirring, followed by cooling and addition of BW in different proportions, stearic acid, and glycerol. The coatings were applied to the surface of the fruits by immersion in the film-forming solution and dried at room temperature of 20 °C. The HPMC-based coatings associated with BW maintained the firmness and the whitening index of the pulp of the cones for a longer time, as well as led to lower activity of the enzymes polyphenoloxidase (PPO) and peroxidase (POD) during storage.

Key words: browning; coating; storage

Revestimentos comestíveis à base de hidroxipropil metil celulose e cera de abelha em pinha

RESUMO: A pinha (*Annona squamosa* L.) é uma anonácea que fornece frutos bastante apreciados. No entanto, esses frutos apresentam uma vida útil limitada devido a sua atividade metabólica intensa após a colheita. A aplicação de técnicas de conservação pós-colheita, como revestimentos comestíveis, tem sido empregada para minimizar os efeitos deletérios após a colheita de frutos, como a pinha. O objetivo do trabalho foi avaliar os efeitos de recobrimentos a base de hidroxipropil metil celulose (HPMC) e cera de abelha (CA) na qualidade de pinhas armazenadas a temperatura ambiente de 20 °C, provenientes do mercado local da cidade de Juazeiro, BA, Brasil. Os frutos foram selecionados quanto a ausência de danos e higienizados por meio da imersão em água clorada. Os recobrimentos foram preparados através da diluição da solução de HPMC em água deionizada a 90 °C sob agitação, seguida de resfriamento e adição da CA em diferentes proporções, ácido esteárico e glicerol. Os recobrimentos foram aplicados na superfície dos frutos através da imersão destes na solução filmogênica e secos a temperatura ambiente de 20 °C. Os recobrimentos a base de HPMC associados a CA mantiveram a firmeza e o índice de embranquecimento da polpa das pinhas por mais tempo, assim como acarretou menor atividade das enzimas polifenoloxidase (PPO) e peroxidase (POD) ao longo do armazenamento.

Palavras-chave: escurecimento; recobrimento; armazenamento

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Introduction

The pinecone (*Annona squamosa* L.) is an anonaceous tree that provides highly appreciated fruit and is cultivated in dry regions. The mesocarp of its fruit is white or yellow in color, with a pleasant aroma, very sweet, being used both for consumption in natura and in the manufacture of juices, ice cream, jams, and jellies, standing out among other tropical fruits of superior commercial relevance (Ambika et al., 2019).

Although the fruits are considered tasty, production and marketing are still limited due to high perishability stemming from post-harvest metabolic processes (Reges et al., 2018; Ambika et al., 2019). These exhibit restrictions as to their distribution to foreign markets, these being accelerated ripening, making them very soft, making them difficult to handle, and extremely succinct preservation. Because it is a climacteric fruit, the changes that cause loss of firmness and fruit darkening are due to the instantaneous rise in the rate of ethylene biosynthesis and the activity of oxidative enzymes such as polyphenoloxidase (PPO) and peroxidase (POD) throughout ripening (Wijesinghe et al., 2017).

In order to extend the post-harvest life of these fruits, edible coatings are recommended because the effects resulting from metabolic processes are delayed without changes in the physiology of the fruit (Ambika et al., 2019). The filmogenic solution based on hydroxypropyl methyl cellulose (HPMC) is composed of cellulose ethers, beeswax, (lipid barrier to water vapor), stearic acid (emulsifier), and glycerol (higher elasticity), present a high potential in aroma retention, acts as a barrier to oxygen, minimizing the rate of respiration, water loss and enzymatic browning (Pinsetta Junior et al., 2019).

HPMC perfectly suits the particularities of the pinecone, delaying fruit ripening by controlling the respiration rate (Oliveira et al., 2018). However, the objective of this work was to evaluate the effects of HPMC and beeswax based coatings on the quality of pinecones stored at 20 °C.

Materials and Methods

Pinecone fruit (*Annona squamosa* L.) was sourced from the local market in the city of Juazeiro, BA, Brazil. The fruits were selected for color, firmness, size, and absence of damage. The fruits were handled carefully in plastic boxes, previously sanitized. Soon after, they were taken to the Laboratório de Análise de Alimentos - Centro de Ciências e Tecnologia Agroalimentar – Universidade Federal de Campina Grande. After one day at 20 °C, the pinecones were selected again for better uniformity of the samples. The pinecones were washed in running water and sanitized by immersing the fruit in cold, chlorinated water (200 mg L^{-1} of free chlorine - sodium dichloroisocyanurate - Neoclor) for 10 minutes.

The fruits were subjected to the control (deionized water); HPMC + 10% BW and HPMC + 20% BW treatments for 0, 2, 4, and 6 days. The pinecones were individually immersed in the coatings for 1 minute and placed to dry in an environment with a controlled temperature of 20 ± 2 °C and RH of 50%. Then they were distributed in polystyrene trays and stored at room temperature of 20 °C for a period of six days. The edible coatings used had a final solids concentration of 6% (Formiga et al., 2019) and the wet basis composition of the coatings is shown in Table 1.

The coatings were prepared according to the methodology described by Navarro-Tarazaga et al. (2011), with modifications. HPMC stock solution at 5 g $100g^{-1}$ (m/m) (HPMC - Methocel, Dow Chemical, USA) was prepared by diluting HPMC in 1/3 (v/v) deionized water at 90 °C under constant stirring using a mechanical stirrer, then cooled in an ice bath to 20 °C. Soon after, BW (Synth, Brazil) was added, at concentrations of 10 and 20 g $100g^{-1}$ (dry basis) and deionized water. Stearic acid (SA) (Synth, Brazil) was added as an emulsifier in the ratio BW:SA (5:1, w/w) and glycerol (Synth, Brazil) as a plasticizer in the ratio HPMC:glycerol (2:1, w/w). The mixture was heated in a microwave oven to 90 ± 2 °C and homogenized for 1 minute. The solution was cooled in an ice bath to 20 °C, allowed to stand for 45 minutes, and stored under refrigeration.

Fruit firmness was evaluated using a brand digital penetrometer (SoilControl), with a 3.0 mm tip. Four readings were taken per fruit at the equatorial region, totaling 12 repetitions per treatment. The results were expressed in Newton (N). The accumulated mass loss was determined with a semi-analytical balance (SSR 600 - Bel) and this was calculated as the difference between the initial and final mass of the fruit, divided by the initial mass. The results were expressed as a percentage (%).

The change in coloration of the fruit mesocarp was determined with a Minolta CR-300 colorimeter, with a D 65 light source, 8 mm aperture. The L* (Lightness) (100 = white; 0 = black), a* (positive = red; negative = green), b* (positive = yellow; negative = blue) values were determined with four readings on opposite sides. The coloration parameters (L*, a*, and b*) were used in the determination of the pulp whitening and browning index using the following equations: $100 - [(100 - L)^2 + (b)^2]^{0.5}$ and $100 \times (Z - 0.031)/0.172$; $Z^* = [(1.75 \times L^*) + a^*]/[(15.465 \times L^*) + (a^* - 3.02) \times b^*]$, respectively.

Titratable acidity (TA) was determined according to the methodology described by Ryan & Dupont (1973). The results are expressed in percent citric acid, equivalent to the amount of 0.1N NaOH spent in the titration. The total soluble solids were quantified according to the methodology described by the IAL (2008). The results were expressed in °Brix. The ascorbic acid content was estimated by titration, using the Tillmans reagent, according to the method (365/IV) described by the IAL (2008). The results were expressed as mg ascorbic acid $100g^{-1}$ MF.

Table 1. Edible coatings based on HPMC and beeswax (g $100g^{-1}$, bu).

Formulations	HPMC [a]	BE [b]	Glycerol	SA [c]
HPMC+10% BE	3.52	0.60	1.76	0.12
HPMC+20% BE	3.04	1.20	1.52	0.24

[a]HPMC = hydroxypropyl methyl cellulose; [b]BW = beeswax; [c]SA = stearic acid.

The phenolic compounds (FC) were quantified by the spectrophotometric method, using the Folin-Ciocalteu reagent, according to the methodology described by Waterhouse (2002). For FC extraction 1 g of pulp was weighed, then the samples were macerated and diluted in 50 mL of distilled water, left to stand for 30 minutes and then filtered. A 0.7 mL aliquot of the filtrate was transferred to test tubes, adding 1.425 mL of water and 0.125 mL of Folin-Ciocalteau. The tubes were shaken, and after 5 minutes 0.25 mL of 20% sodium carbonate was added. The tubes were allowed to stand for 30 minutes in a water bath at 40 °C. Readings were taken in a spectrophotometer (SP 1105 - Spectrum) at 765 nm absorbance. The results were expressed as mg gallic acid $100g^{-1}$ of fresh sample.

Polyphenoloxidase (PPO) enzyme activity (EC 1.10.3.1) was determined according to the methodology described by Wissemann & Lee (1980), Aydin & Kadioglu (2001), adapted by Costa (2009). The reaction system was composed of 0.01 mL of the enzyme extract, 0.3 mL of 200 mM catechol, and 1.19 mL of 0.2 M sodium phosphate buffer (pH 6.5). The reaction took place in a water bath at 30 °C for 3 minutes, the readings monitored at 475 nm.

Peroxidase (POD) enzyme activity (EC 1.11.1.7) was determined as described by Wissemann & Lee (1980), Aydin & Kadioglu (2001), adapted by Costa (2009). The reaction system was composed of 0.015 mL of the enzyme extract, 0.5 mL of 26 mM hydrogen peroxide, 1 mL of 40 mM guaicol, and 1.485 mL of 0.2 M sodium phosphate buffer (pH 6.5). The reaction took place in a water bath at 25 °C for 5 minutes, the readings monitored at 420 nm.

The experiment was conducted in an entirely randomized design (ERD), in a factorial scheme 3 (treatments) × 4 (storage periods) with three repetitions of one fruit for each treatment. At time 0 (T0) only the control samples were analyzed, and at the other times (T2, T4, and T6) all treatments were analyzed, totaling 21 fruits. The results were submitted to analysis of variance (ANOVA) and the means were compared by Tukey test, considering a significance level of 5% probability, using Agroestat software, version 1.0 (Barbosa & Maldonado, 2015). In relation to the storage time, regression analyses were performed, the polynomial models were selected observing the significance of the F test and the equations were adjusted according to the highest R^2 value.

Results and Discussion

Throughout the storage period, the firmness values decreased. The fruits covered with HPMC+BW remained firmer throughout storage compared to the control (Figure 1A). However, the fruits submitted to the treatment with HPMC+20% BW showed maintenance of firmness higher than the other treatments until the fourth day of storage, on the other hand, at six days of storage they presented firmness similar to the control.

The maintenance of firmness in the fruit of the HPMC+20% BW treatment, is probably related to the reduced O_2 and

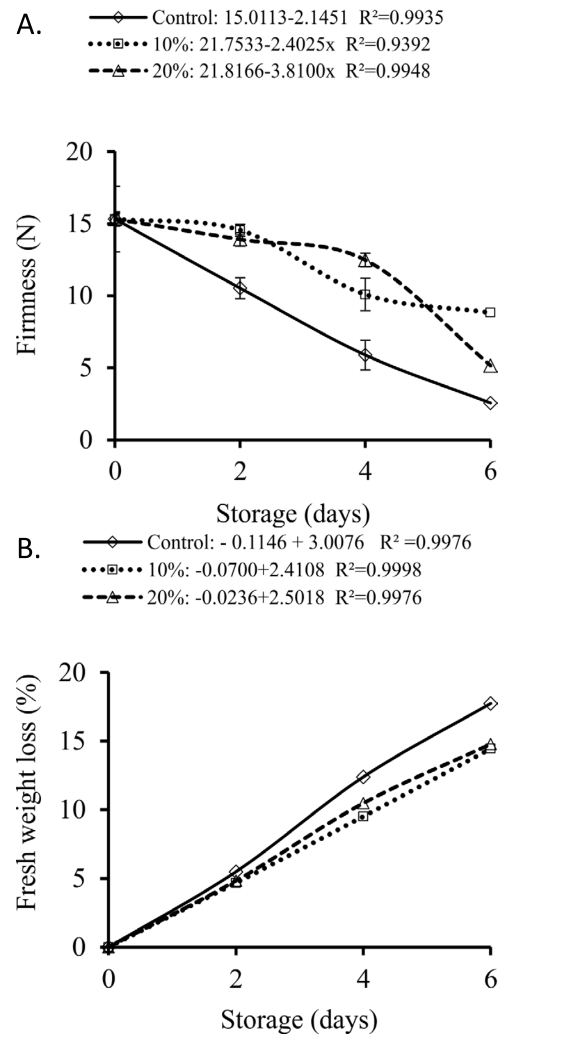

Figure 1. Effect of coatings based on hydroxypropyl methyl cellulose (HPMC) and beeswax (BW) on firmness (A) and loss of fresh mass (B) of pinecones stored for six days at 20 °C and 50% RH.

CO_2 flux. This change slows the speed of fruit ripening and senescence, because reduced ethylene biosynthesis results in reduced respiratory activity, acid consumption, coloration changes, membrane degradation processes, and loss of firmness (Reges et al., 2018).

Throughout storage, the accumulated mass loss (ML) was accentuated in the fruits of all treatments, being this more intense in the control. Until the second day of storage, mass loss was similar for all treatments (Figure 1B). At the end of storage, it was observed that the coating containing HPMC+10% BW in its composition provided a lower ML compared to the control, allowing the maintenance of turgidity of the pinecones.

The coatings act as a semi-permeable barrier to water vapor movement, due to surface transpiration elevating the diffusion of solutes. Consequently, this phenomenon results in mass loss that occurs by the outflow of water vapor to the surrounding medium due to the water vapor pressure difference between the atmosphere and the fruit surface (Reges et al., 2018; Formiga et al., 2019). The beeswax

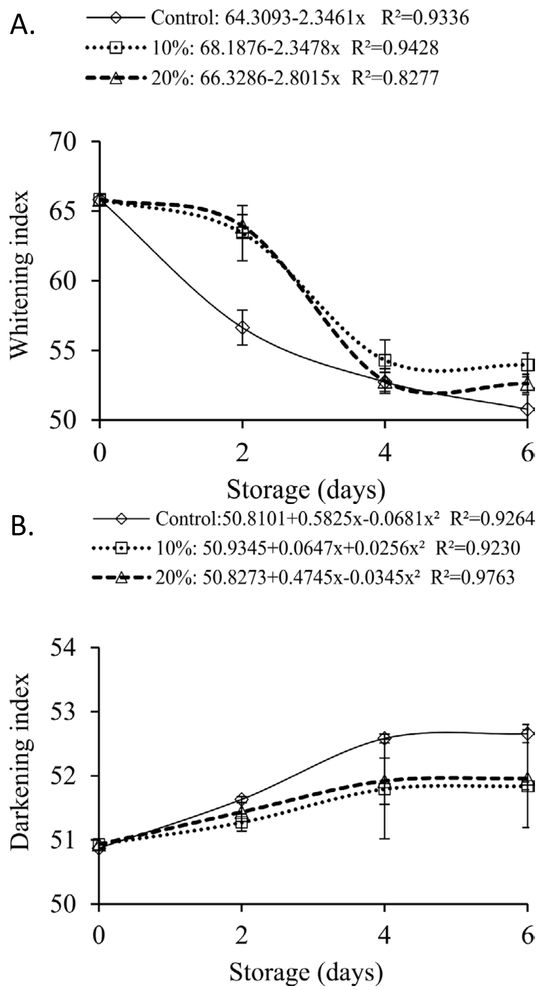

Figure 2. Effect of coatings based on hydroxypropyl methyl cellulose (HPMC) and beeswax (BW) on the evolution of the whiteness index (A) and browning index (B) of pinecones stored for six days at 20 °C and 50% RH.

inserted in the coating acts as a hydrophobic agent, modifying the water vapor permeability characteristics, reducing the accumulated mass loss (Oliveira et al., 2018; Formiga et al., 2019).

The coating based on HPMC and BW significantly influenced the development of pulp coloration of pinecone fruit (Figure 2A). The pericarp whitening index differed from the control for the fruits coated with HPMC + BW, they showed slower evolution of the pulp coloration, that is, they remained white for a longer time when compared to the control fruits, showing averages of 59.4 and 58.8 for HPMC+10% and 20% BW, respectively, with the decreasing averages for the control, ranging from 65.8 to 50.78.

Until the second day, the fruit pulp darkening index showed similar behavior for all treatments. On subsequent days, HPMC+10% BW treatment reduced pulp darkening during storage (Figure 2B). The edible coating associated with BW concentrations decreases the activity of enzymes responsible for pulp browning such as POD and PPO (Figure 5A and 5B), turning them white. POD and PPO act in plants due to disruption of membrane integrity with complete oxidation of phenolic substrates, giving rise to brown, black or reddish-brown pigments called melanins (Cruz et al., 2019; 2021). As such, the edible coatings in this work reduced the internal O_2 of the fruits by reducing the oxidation of phenolic substrates (Formiga et al., 2019).

The soluble solids (SS) values increased until the fourth day of storage. The control fruits presented the lowest SS values in this period. In fruits treated with HPMC+BW, SS contents were highest in fruits from the HPMC + 20% BW treatment and fruits treated with HPMC + 10% BW showed the lowest SS contents on the sixth day of storage (Figure 3A).

The increase in SS is related to the conversion of starch into soluble sugars. The employment of barriers, such as coatings, forms a modified atmosphere that results in increased CO_2 and a reduced O_2, which can lead to decreased carbohydrate metabolism and delayed starch conversion (Reges et al., 2018; Formiga et al., 2019).

The titratable acidity values increased until the fourth day of storage, decreasing on the sixth day of evaluation in the control fruits, while the titratable acidity in the fruits coated with HPMC+BW remained constant, presenting values around

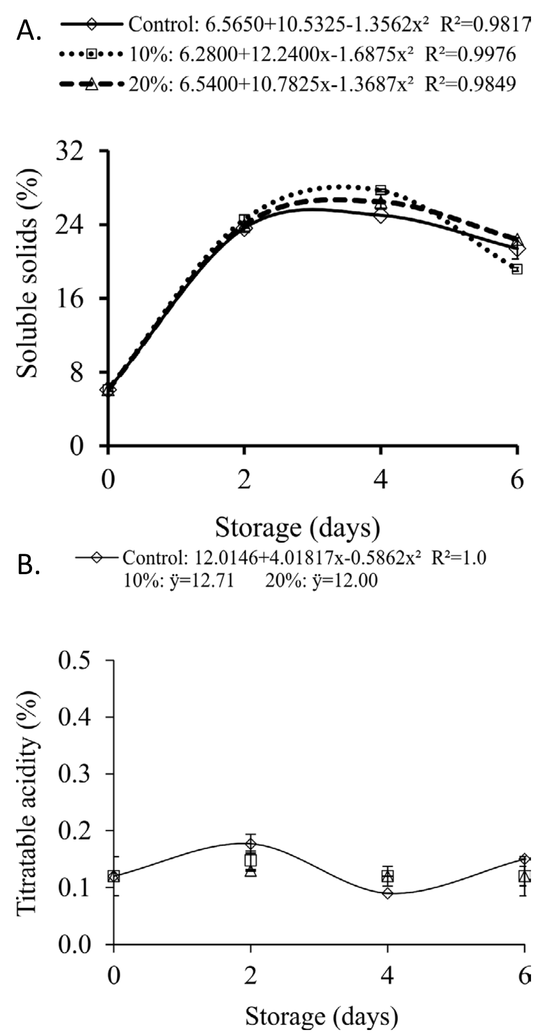

Figure 3. Effect of coatings based on hydroxypropyl methyl cellulose (HPMC) and beeswax (BW) on soluble solids (A) and titratable acidity (B) of pinecones stored for six days at 20 °C and 50% RH.

12.00 (Figure 3B). This behavior indicates that the fruits accumulated organic acids by the use of the coating, indicating delayed fruit ripening, since the decline in acidity is directly linked to the natural ripening process (Germano et al., 2019).

However, the initial increase in titratable acidity in the control treatment fruits likely occurred by the synthesis of polygalatonic and phenolic compounds in response to accelerated metabolism during fruit ripening (Germano et al., 2019). On the fourth day of storage, there was probably greater consumption of organic acids as a result of the respiratory process of the fruit, causing the reduction in titratable acidity values (Formiga et al., 2019).

In the control fruits the ascorbic acid (AA) content increased until the second day, with a high reduction on the consecutive days of evaluation. Similar behavior was seen in fruits coated with HPMC+10% and 20% BW with an increase in AA content until the second day with a subsequent reduction, which was lower than the control (Figure 4A).

By the second fia of storage, the increase in AA content in the control fruit occurred given the need for the fruit to detoxify the tissues from EROs developed in the course of ripening (Jiang et al., 2018). Also, the maturation of the fruits significantly influences the AA content, which may show an increasing, decreasing behavior or remain unchanged during storage. The higher availability of sugars from control fruits promotes ascorbic acid synthesis via glycolysis (Formiga et al., 2019).

The content of phenolic compounds (FC) increased over the storage period in all treatments. However, the coated fruits showed a tendency to higher FC production compared to the control, there is an increment trend in the content of phenolic compounds for the treatment with HPMC+20% BW, showing an average of 193.07 mg 100g^{-1} (Figure 4B).

The induction of FC synthesis promoted in the pinecones coated with HPMC+ 10% and 20% BW, can be explained by the fact that the coating provided stress on the fruit by O_2 scarcity, as a defense mechanism of the fruit (Formiga et al., 2019).

The action of the enzyme polyphenoloxidase (PPO) had the greatest increase in the control fruits, in the first two days of storage, standing out from the coated fruits (Figure 5A). The treatment with HPMC+20% BW resulted in lower PPO activity until the second day, showing similar activity as the HPMC+10% BW coated fruits on the fourth day of storage, followed by a sharp decrease. While in the treatment with HPMC + 10% BW a stabilization of the activity was observed at the end of the storage period, resulting in higher PPO activity. The results show that the use of the coating with 20% BW in its composition was more efficient in reducing the PPO activity and consequently reducing the reactions responsible for the browning of the pulp.

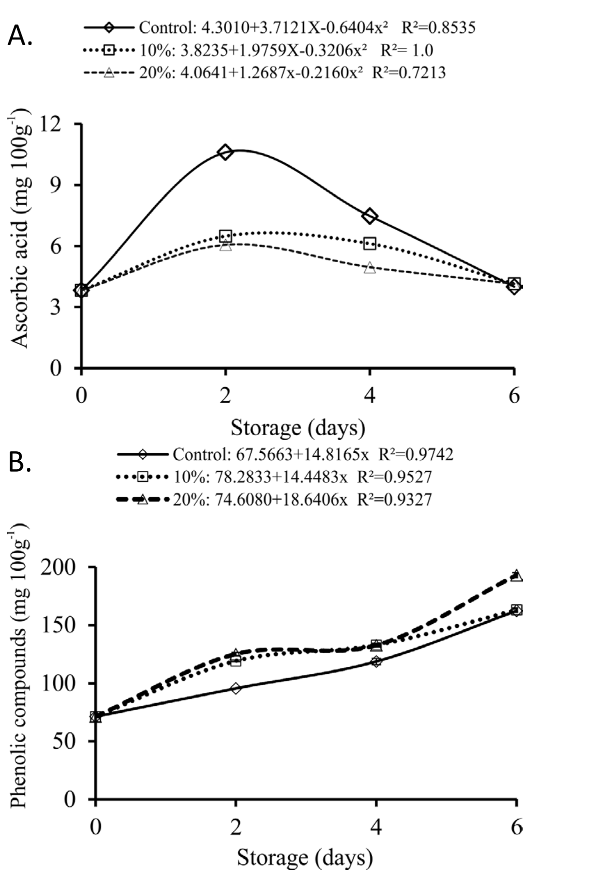

Figure 4. Effect of coatings based on hydroxypropyl methyl cellulose (HPMC) and beeswax (BW) on the ascorbic acid (A) and phenolic compounds (B) contents of pinecones stored for six days at 20 °C and 50% RH.

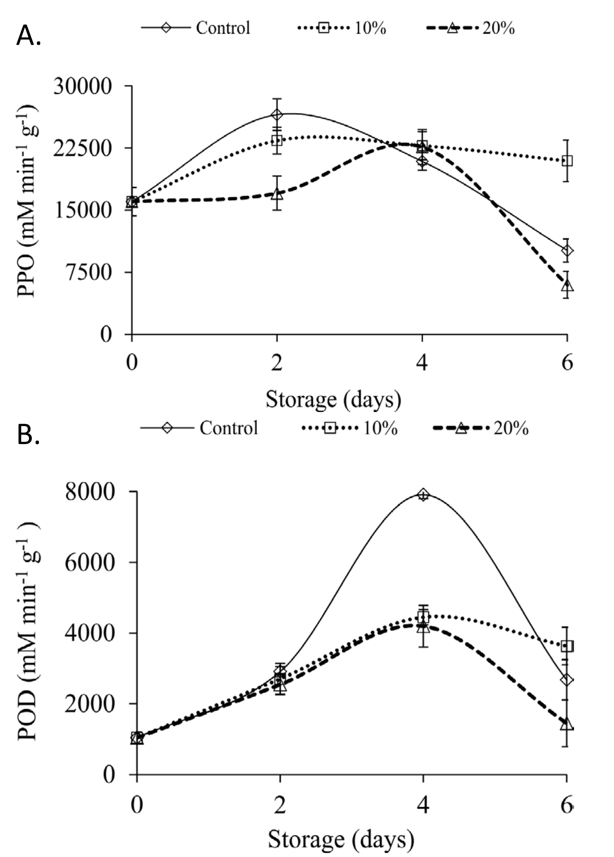

Figure 5. Effect of coatings based on hydroxypropyl methyl cellulose (HPMC) and beeswax (BW) on the activity of polyphenoloxidase - PPO (A) and peroxidase - POD (B) enzymes of pinecones stored for six days at 20 °C and 50% RH .

Changes in PPO content can occur during storage and may be associated with senescence of plant tissues. PPO is considered the key enzyme in the surface browning of vegetables. Their reaction with phenolic compounds leads to the formation of σ-quinones and, consequently, that of dark pigments (Cruz et al., 2019; 2021).

The peroxidase (POD) activity in the treatments with HPMC+10% and 20% BW differed from the control, showing lower activity of this enzyme throughout the storage period studied (Figure 5B). The coated fruits showed similar activity among themselves until the fourth day of storage, with consecutive decline (Figure 5B). However, the uncoated fruits showed peak activity on the fourth day, going from 1040.04 to 7915.50 mM min^{-1} g^{-1} from the initial day to the fourth day of storage, respectively, followed by a high decline.

In intact plant tissue of fruits and vegetables, enzymatic browning results from the inhibition of respiration during storage through the use of coatings, causing oxidative stress to the tissue, which is mediated through the generation of free radical species such as hydrogen peroxide (H_2O_2) (Csepregi et al., 2019).

The peroxidases act as organic catalysts, whose prosthetic group is iron, possessing typical activity in the oxidation reaction of phenolic compounds in the presence of hydrogen peroxide leading to the darkening of plant tissues. The resulting quinones from the reaction product, which are unstable and after non-enzymatic oxidation in the presence of O_2 polymerize forming the melanins (Cruz et al., 2019). These also act on the oxidative destruction of vitamin C and the discoloration of anthocyanins and carotenoids in the absence of unsaturated fatty acids, inducing negative flavor changes during storage (Wang et al., 2020).

Conclusions

Coatings based on hydroxypropyl methyl cellulose (HPMC) associated with beeswax (BW) increased the shelf life of pinecones stored at 20 °C for at least two days, providing an accessible support for maintaining the post-harvest quality of pinecones during the storage.

The coating formulations (10 and 20% BW) showed similar results, keeping the fruits firmer, with lower peroxidase (POD) and polyphenoloxidase (PPO) activity and lower pulp browning.

The use of these edible coatings is suggested for post-harvest preservation of highly perishable fruits such as pinecone, increasing their shelf life.

Compliance with Ethical Standards

Author contributions: Data curation: YLB, ASF, MSS, AGFS; Methodology: YLB, ASF, MSS, AGFS; Project administration: BHM, FBC, WSR; Software: ASF; Validation: RRPC, BHM, FBC, WSR; Writing – original draft: YLB; Writing – review & editing: RRPC.

Conflict of interest: The authors no have conflict of interest.

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