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CROP SCIENCE

Edible Coating with Jambolan (*Syzigium cumini* L.) Peel and Leaf Extracts to Reduce Color Changes, Mass Loss and to Increase β-Carotene Retention of Minimally Processed Papaya

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Abstract: The aim of the study was to evaluate the effect of the jambolan (*Syzigium cumini* L.) peel and leaves extract added in pectin based edible coating onto color changes, mass loss and β-carotene retention of minimally processed papaya during storage at 5 ±2 °C for 9 days. Leaves and peels were crushed in water in solid:liquid ratio, 1:1.5 and 1:2.0 and filtrered to obtain vegetable extracts. After, aqueous solutions were prepared adding 2% of pectin and 5% of extract. The mass loss increasing in all treatments evaluated in this work, being that in the final of 9 days of storage, control and P2 (peel 2%) treatment presented the highest loss, 6.23 and 10.12% respectively. The L1.5 (leaf 1.5%) treatment was the one presented the lowest percentage of mass loss (3.8%). The values of (a_f^* / a_0^*) of the control samples reduced significantly during the storage period, reaching 21% of reduction after 9 days. Coating with vegetable extract from jambolan peel and leaf reduced the loss of β-carotene in minimally processed papaya during the storage, being that the treatment P1.5 provided the highest retention value of the compound. The results demonstrated that the studied coating set with extracts (peel and leaves) of jambolan, was efficient to preserve the color, the mass loss and the β-carotene content of the minimally processed papaya.

Key words: Conservation, papaya, pectin coating, quality, vegetable extract.

INTRODUCTION

Brazil is one of the world's leading producers of papaya (*Carica papaya* L.). In 2017, the national production was 1.5 million tons, corresponding to 11.6% of the world papaya production (FAOSTAT 2017). Papaya is a fruit rich in carotenoid, vitamin C and minerals salt that presents relatively long harvest period, but also presents high perishability (Garcia et al. 2014), which contribute to low shelf life of the fruit and discard of the same. According to Food and Agriculture Organization of the United Nations, 45% of fruits produced worldwide are discarded in the trash for a lot of reasons, contributing to the statistics of 1.3 billion tons of food wasted in the world yearly (FAO 2014).

Minimal processing of fruits have been studied over the last years with the purpose of increasing fruit consume, reducing high post-harvest losses and aggregating value to the final product, avoiding, therefore, the waste (Alzamora et al. 2016). Minimal processing consists of submitting fruits and vegetables to treatments that it alters its original form, maintaining fresh quality of the product. For this purpose, physical alteration as washing, peeling, slicing, cutting with seed removal, or chemical treatment can be employed. However, unlike most food processing techniques, that stabilize the shelf-life of products, minimal processing can increase perishable of fruits, once upon that cutting and peeling change fruit integrity, enhancing metabolic process and deteriorating the color and nutrient of food (Yousuf et al. 2018). Edible coatings are enhancing importance in this area as potential treatment to reduce the effects induced for these actions (Mantilla et al. 2013).

Edible coating is a film forming biopolymer applied directly into the surface of the food that presents as one of the objective to isolate the coated product from environment and create a barrier to gases and water vapor, reducing the degradation and increasing food shelf-life (Oliveira & Silva 2017, Olivas & Barbosa-Cánovas 2005). The polysaccharide is one of the sources of biopolymers much used to elaborate edible coatings. Some researchers related that edible coating based on polysaccharide, as pectin (Sanchís et al. 2016), enhanced the minimally processed papaya quality during the storage.

Pectin is a linear polysaccharide, soluble in water, that can be classified as low methoxyl (25–50 %) or high methoxyl (50–80 %) pectin. Low methylated pectin form gel stable to moisture and heat in presence of calcium ions (Thakur et al. 1997). Pectin based edible coating presents good oxygen barrier property (Silva et al. 2015) and it is indicate as good option to reduce oxidative reactions in minimally processed food (Valdés et al. 2015).

Edible coating also can be used as vehicle for a varied amount of food additives, including antioxidants compounds (Melo et al. 2017). Edible coatings have been reported to provide a semi-permeable barrier to gases and water vapor and also improve mechanical properties, thus delaying the natural senescence, minimizing water loss and keeping the structural integrity of coated product intact (Tabassum & AliKhan 2020). Antioxidant addition in edible coating presents as purpose to protect the fruit against undesirable oxidative reactions that would result in nutrients reduction and color change during storage (Vargas et al. 2008).

The interest in natural antioxidant has gained more and more space due to growing concern of consumers with health. Many researchers have pointed plant extract as natural antioxidant alternative to be added in edible coating (Yousuf et al. 2018). Some researchers have reported the presence of antioxidant in extract from agro-industrial waste as jambolan peel (Costa et al. 2013) or even leaf from some plants as jambolan (Timbola et al. 2002).

Jambolan (*Syzigium cumini* L.) is a plant originated from India that bears fruit in the form of berries, with white pulp and dark peel. In Brazil it is considered wild and its fruit is poorly marked (Stefanello et al. 2011), despite of the fruit to be considered rich in antioxidant compounds (Tavares et al. 2016), but in US and other European countries, jambolan fruit is considered as a delicacy, being used in the production of health drinks, juices, squashes and jellies (Li et al. 2009).

Tavares et al. (2016) identified about 74 phenolic compounds in jambolan fruit. Jambolan peel presents phenolic compounds higher than one found in açaí and anthocyanin content higher than one obtained in jabuticaba peel or pitanga (Costa et al. 2013). Jambolan leaves are rich in flavonoids and tannins and their extracts are used in traditional medicine for diabetic treatment (Timbola et al. 2002). The higher content of antioxidant compounds in jambolan peel and leaf can be a good alternative of natural antioxidant. This work aimed to study the effect of jambolan (*Syzigium cumini* L.) peel and leaves extract added in pectin based edible coating onto color changes, mass loss and β-carotene retention of minimally processed papaya during storage at 5 ±2 °C for 9 days.

MATERIALS AND METHODS

Materials

Jambolan peels and leaves were collected in January – March 2018 from sites on the Maringá State University, located in the Umuarama city (Paraná, Brazil). Papayas of the Formosa cultivar (*Carica papaya*) from Paraná State (Brazil) were purchased in March/2018 on local market (Umuarama, Paraná, Brazil). Ripe fruits with orange peel weighing approximately 1.8 kg were used in the experiments. Low methoxylated pectin (GRINDSTED® LA 210, Danisco, Cotia, SP) and calcium chlorite (Nuclear) were used to prepare the coatings applied to papaya slices.

Methods

Bioactives compounds extraction and coating preparation

Jambolan leaves and fruits were washed in running water and after dried with absorbing paper. The peel was separated from pulp and crushed with distillate water, for 5 min, in two different solid:liquid ratio, 1:1.5 w/v (P1.5 (peel 1.5%)) and 1:2.0 w/v (P2 (peel 2%)). The leaves were also crushed with distillate water, for 5 min, in the same solid:liquid ratio, 1:1.5 w/v (L1.5 (leaf 1.5%)) and 1:2.0 w/v (L2 (leaf 2%)). The obtained extracts were filtered through Whatman N° 1 filter paper and added, separately, in pectin solution.

The pectin aqueous solution was prepared in thermostatic bath (Simétrica, SI/6Aneis/18L, Brasil) at 50 °C until obtains a homogeneous mixture. After the solution was cooled at 25 °C, jambolan leaves or peel extracts were added, in order to obtain solution with 2% (w/w) of pectin and 5% (w/w) of extract. This solution was used to coat the samples. Five treatments were studied: control (without coating), and coating with P1.5, P2, L1.5 and L2.

Minimally processing and coating

The minimal processing was performed at a temperature of about 20 °C with the previously sanitized utensils in a solution of organic chlorine (dichlorocyanurate) at the concentration of $2 g.L^{-1}$. The selected whole papayas were cleaned and sanitized with a solution of organic chlorine at the concentration of $2\ g.L^{-1}$ for 15 min, peeled manually, seeded and cut into 2.5 cm side cubes. The ends of papaya were discharged to maintain the samples uniformity.

Papaya pieces were immersed on the coatings (pectin with extracts) for 1 minute, after, to activate the gelling of pectin, the coated samples were immersed in a solution of calcium chlorite (Nuclear) 2% (w/v) for 30 seconds. The excess of coating was removed by leaving the basket containing the samples to drain for 3 minutes. Control samples were not immersed in coating solution. After, all the pieces (uncoated and coated) were submitted to drying on a convective oven (Marconi, model MA 035) at 25 °C for 20 min.

The samples for each treatment were packaged in unrecycled PET (polyethylene terephthalate) containers, with cover (SANPACK), standardizing the number of five samples per package. After, the packages with the samples were stored in refrigerated conditions at 5 ± 2 °C for 9 days. Analyses were performed in triplicate on the processing day (day 0) and after 3, 5, 7 and 9 days of storage.

Analytical methods

Mass loss, color analysis and β-carotene content of samples were performed to evaluate the effect of edible coating in quality of minimally processed papaya. The mass loss

(ML) was obtained in triplicate, by taking the difference between the initial weight of the papaya minimally processed (M_0) and the weight obtained at the end of each storage time (M_f) (Eq. 1).

$$ML = \frac{M_0 - M_f}{M_0} x100 \quad \text{(Eq. 1)}$$

The carotenoids content of the samples with or without coating was determined in duplicate, by spectrophotometric measurement, using an UV - Spectrophotometer (FEMTO, 700 plus, São Paulo) calibrated at 453nm, following method described by Rodriguez-Amaya & Kimura (2004). It was determined the β-carotene content in μg.g^{-1} of fresh product and the retentions of β-carotene were determined according to Murphy et al. (1975) as described by Eq. 2.

$$R_{et}(\%) = \left(\frac{C_f M_f}{C_0 M_0}\right) \times 100 \quad \text{(Eq. 2)}$$

Where R_{et} is the retention of β-carotene after a time storage, C_f is the amount of β-carotene in the samples at a determined day, and C_0 is the amount of β-carotene in the fresh samples (at day 0), in μg.g^{-1} of fresh product; M_f is the mass of samples at a determined day and M_0 is mass of fresh samples (at day 0), in grams.

The color of the samples uncoated (control) and coated was determined in five replicates using a colorimeter (Konica Minolta, model CR-400) previously calibrated on white surface. The parameters L*, a* and b* were obtaining. To minimize the influence of the initial conditions of the raw material on the tests, the results for color were normalized by the ratio between the experimental measurement performed on the sample after a period of storage (L_f^*, a_f^*, b_f^* and the corresponding fresh sample (L_0^*, a_0^*, b_0^* - day zero of storage) (Silva et al. 2011).

Statistical methods

The Statistica 7.0 (StatSoft, Inc., Tulsa, OK) program was used to calculate the analysis of variance (ANOVA). The Tukey test was used to determine the differences between the edible coatings in the range of 95% confidence using the Assistat software. The evaluation was performed from data obtained in triplicates, and the results were presented by the mean ± standard deviation.

RESULTS AND DISCUSSION

Mass loss

Table I presents mass loss values of minimally processed papaya uncoated (control) and coated with pectin + jambolan peel or leaf extract stored at 5 ± 2 °C during 9 days.

Observing in Table I, it is noted that occurs an increasing in mass loss (%) in all treatments evaluated in this work, being that in the final of 9 days of storage, control and P2 (coating with 2% jambolan peel extract) treatment presented the highest loss, 6.23 and 10.12%, respectively. The P1.5 and L2 treatments did not present statistical difference in the end of storage. The L1.5 (coating with 1.5% jambolan leaf extract) treatment was the one presented the lowest percentage of mass loss (3.8%).

Mass loss of fruit during storage occurs because of evapotranspiration and respiration of the fruit and which is intensified with minimal processing treatment (Zillo et al. 2018). Protein and polysaccharides based coatings trend to act as a barrier to water vapor in minimally processed fruits, reducing the mass loss during the storage (Olivas & Barbosa-Cánovas 2005). The transmission of water vapor of fruit through the edible coating occurs by activing diffusion where the vapor dissolves on the coating at the side of fruit and for difference of

Table I. Mass loss (%) of minimally processed papaya, uncoated and coated, storage at 5 °C for 9 days.

Days	Treatments				
	Control	P1.5	P2	L1.5	L2
3	3.5 ± 0.2^{bB}	3.1 ± 0.2^{bBC}	4.6 ± 0.5^{bA}	2.5 ± 0.5^{cC}	3.3 ± 0.1^{bBC}
5	3.8 ± 0.9^{bAB}	3.3 ± 0.6^{bAB}	4.8 ± 0.5^{bA}	3 ± 0.2^{bcB}	3.4 ± 0.3^{bAB}
7	4 ± 0.5^{bAB}	2.6 ± 0.1^{bC}	4.9 ± 0.2^{bA}	3.4 ± 0.1^{abBC}	3.8 ± 0.7^{abABC}
9	6 ± 0.4^{aB}	4.5 ± 0.5^{aC}	10.2 ± 0.8^{aA}	3.8 ± 0.2^{aD}	4.6 ± 0.3^{aC}

Numbers are the mean ± standard errors of three replications. Means with the same lowercase letter, in the same column, did not differ significantly at p≤0.05 (5%) according to the Tukey test, for the days variable. Means with the same capital letter, in the same line, did not differ significantly at p ≤0.05 according to Tukey test, for the treatment variable. Where: Control - piece of papaya not immersed in coating solution; P1.5 - coating with pectin more 1.5% jambolan peel extract; P2 - coating with pectin more 2% jambolan peel extract; L1.5 coating with pectin more 1.5% jambolan leaf extract; L2 - coating with pectin more 2% jambolan leaf extract.

concentration runs through the edible coating to the other side (lower concentration). The structure and coatings characteristic, determine how will be the dissolution of the water vapor on the coating (Han & Gennadios 2005). It was possible to observe that the increase of extract concentration, both peel and leaves, in edible coating formulation increased mass loss in stored samples. The increase of added extracts concentration can have influenced the chemical interaction existent between the blocks of galacturonate of pectin and calcium ions, increasing water vapor permeability of the edible coating.

To compare jambolan peel extract with leaves extract, it was possible to verify that coating with leaves extract provided less mass loss in papaya minimally processed during the storage than coating with peel extract. Research performed by Mohamed et al. (2013), demonstrated that it is possible to extract oil from the jambolan leaf. Oil presence in edible coating can act reducing its water vapor permeability (Wang et al. 2011). The lowest mass loss observed in the samples may be related to the presence of a small fraction of oil resulted from the crushing of the jambolan leaf during obtaining extract.

Color

Color is considered one of the most important and crucial attributes associated with fresh fruit. This property exerts great influence on the acceptability of the product by the consumer and for this reason the color of edible coatings applied on fruit surface did not provoke undesirable visible change in the food appearance (Galus & Lenart 2013).

Figure 1 (a) presents parameter *L** ratio (L_f^* / L_0^*) of minimally processed papaya coated and uncoated (control), during storage at 5 ±2 °C.

The lightness (Figure 1 (a)) of the samples decreased approximately 6–16 % after 9 days of storage, being the higher reduction verified in control samples (16%). The lightness of samples treated with P2, L1.5 and L2 did not statistically differ at the end of the evaluated period.

The darkness of minimally processed fruit during storage period can be related to action of polyphenol oxidase (Chevalier et al. 2018) that in contact with oxygen of the environment potentiates browning oxidative reactions. The use of edible coating must have minimized the contact between the surface of the papaya and oxygen, decreasing oxidative browning of samples during storage. This effect can be

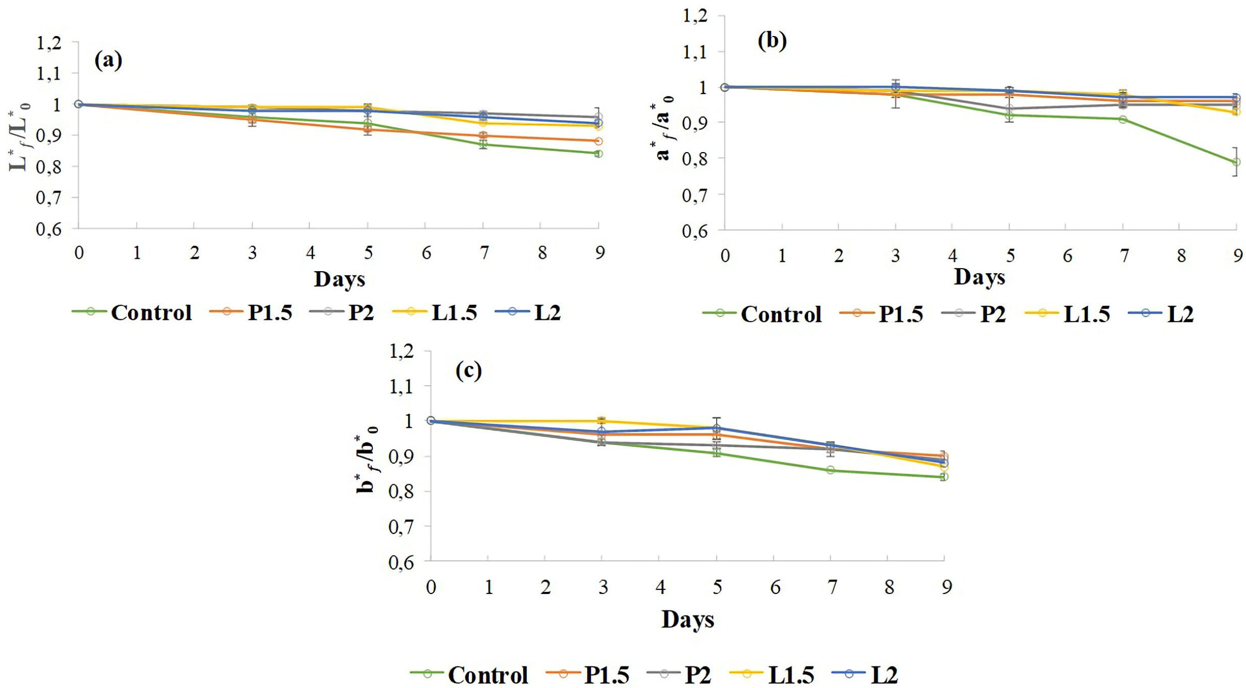

Figure 1. Lightness parameter L^* ratio (L^*_f / L^*_0) (A), parameter *a ratio (a^*_f / a^*_0) (B) and parameter *b** ratio (b^*_f / b^*_0) (C) of minimally processed papaya coated and uncoated (control).**
Numbers are the mean ± standard errors of three replications. p≤0.05 (5%) according to the Tukey test. Where: Control - piece of papaya not immersed in coating solution; P1.5 - coating with pectin more 1.5% jambolan peel extract; P2 - coating with pectin more 2% jambolan peel extract; L1.5 coating with pectin more 1.5% jambolan leaf extract; L2 - coating with pectin more 2% jambolan leaf extract.

attributed to pectin capability to form strong chemical interaction with calcium ions resulting resistance network structure that difficult the access of oxygen to the food surface (Rhim 2004). Moreover, extracts added in the coating can have reduced the oxygen contact in the papaya surface, since jambolan peel and leaves are rich in antioxidant compounds (Costa et al. 2013, Timbola et al. 2002) which present frees radical-scavenging capacity. Oms-Oliu et al. (2008) also observed that pectin-based coatings with Nacetylcysteine and glutathione added as antioxidants reduced the browning of fresh-cut pears storage at 4 °C for 14 days. Sanchís et al. (2016) observed that persimmon slices coated with pectin + citric acid (antioxidant agent) present the same lightness than uncoated samples after 9 days storage at 5 °C. In this work it is possible to observe that jambolan peel and leaves extracts used as natural and cheap antioxidant agent have a significant effect on lightness of minimally processed papaya after 9 days storage at 5 °C. Rais et al. (2019), working with jujube fruit (*Ziziphus lotus* L.), observed that the extract of this fruit has a potent antioxidant effect. Similar results regarding the antioxidant potential were found in the present work, demonstrating that fruits with a high content of phenolic compounds can act as antioxidants and help maintain the color of minimally processed products.

Figure 1 (b) and Figure 1 (c) present parameter *a** ratio (a^*_f / a^*_0) and *b** ratio (b^*_f / b^*_0), respectively, of minimally processed papaya coated and uncoated (control), during storage at 5 ± 2 °C. It is possible to observe that despite jambolan peel presents dark color and jambolan leaves present green color, the extract added in coating did not change the initial color and neither lightness of the coated samples in comparison with control samples (Figure 1a, b and c).

The values of (a_f^*/a_0^*) of the control samples reduced significantly during the storage period, reaching 21% of reduction after 9 days. Coated samples presented the lowest reduction in the *a** parameter (3-7%) and did not present significant difference among treatments. Tabassum & Alikhan (2020), working with minimally processed papaya coated with alginate and modified atmosphere observed a decrease in the values of Chroma *a**, and that the control sample presented major decreased for this parameter. These results agree with the present study, which also observed decreases in the Chroma *a** over the days of storage for the control sample.

Storage time also reduced yellowish color (b_f^*/b_0^*) of samples, being that control samples presented 16% of reduction and coated samples 10-12%. It was not observed significant difference (p>0.05) among L1.5, L2, P1.5 and P2 treatment.

The major carotenoids of the papaya pulp (as lycopene, β-cryptoxanthin, β-carotene) are typically red-orange pigments (Kimura et al. 1991). Oxidation of these carotenoids results in change of color parameters *a** and *b** of the papaya (Garcia et al. 2014). The lowest change in values of (a_f^*/a_0^*) and (b_f^*/b_0^*) of the coated samples (Figure 1 (b and c) suggest that pectin coating with jambolan peel and leaves extract addition exerted protective effect against oxidation of the carotenoids in papaya. This result is confirmed with β-carotene content expressed in Table II.

Table II. Carotenoids content (µg/g) of minimally processed papaya (uncoated and coated) and carotenoids retention (in parenthesis - %) of samples during 9 days of storage at 5 °C.

Days	Treatments				
	Control	**P1.5**	**P2**	**L1.5**	**L2**
0	42.6±2^{bA}	42.6±2^{bA}	42.6±2^{bA}	42.6±2^{aA}	42.6±2^{abA}
	(-)	(-)	(-)	(-)	(-)
3	41.4±1^{bC}	46.9±3.5^{aAB}	50.6±1.5^{aA}	36.2±0.2^{bD}	44.1±1.5^{aB}
	(94±2.3%)^{aB}	(106±8.0%)^{aA}	(109±3.2%)^{aA}	(82±0.5%)^{aC}	(97±2.6%)^{aB}
5	47.7±1.5^{aA}	45.5±0.5^{aAB}	49.4±0.6^{aA}	37.7±2.9^{abC}	41.3±1.1^{abcC}
	(94±3.0%)^{aB}	(97±1.2%)^{bAB}	(103±1.3%)^{bA}	(83±6.4%)^{aC}	(89±2.4%)^{bBC}
7	44.3±0.4^{abA}	42.3±2.4^{abA}	40±1.3^{bA}	41.7±4.4^{aA}	40.2±1.3^{bA}
	(68±0.7%)^{bB}	(93±5.3%)^{bA}	(83±2.6%)^{cA}	(87±9.2%)^{aA}	(82±2.7%)^{cA}
9	34.8±2.9^{cC}	48±0.5^{aA}	40.4±0.7^{bB}	42.5±2.4^{aB}	44.5±0.1^{aB}
	(60±5.1%)^{cC}	(94±0.9%)^{bA}	(75±6.0%)^{dB}	(82±4.7%)^{aB}	(85±2.1%)^{cAB}

Numbers are the mean ± standard errors of three replications. Means with the same lowercase letter, in the same column, did not differ significantly at p≤0.05 (5%) according to the Tukey test, for the days variable. Means with the same capital letter, in the same line, did not differ significantly at p ≤0.05 according to Tukey test, for the treatment variable. Where: Control - piece of papaya not immersed in coating solution; P1.5 - coating with pectin more 1.5% jambolan peel extract; P2 - coating with pectin more 2% jambolan peel extract; L1.5 coating with pectin more 1.5% jambolan leaf extract; L2 - coating with pectin more 2% jambolan leaf extract.

Carotenoids content

Table II presents carotenoids content (µg/g) of control and coated minimally processed papaya, as well as, carotenoids retention (%) during 9 days of storage at 5 ± 2 ºC.

In Table II, it is possible to observe that control samples presented significant reduction of β-carotene content with storage time, but coated samples presented little change in compound content. Retention values higher 100%, observed in coated samples with pectin + jambolan peel extract, can be related to incomplete carotenoids extraction in zero day. The complex structure of vegetable cell and its selective plasmatic membrane can hamper solvent entry into the cell. Moreover, carotenoids can associate to other components in vegetables that can also hamper its extraction (Saini & Keum 2018). Carotenoids retention higher than 100% were also observe by other authors (Tonon et al. 2007, Lago-Vanzela et al. 2013). Researchers have presented methodologies more efficient to carotenoid extraction and analysis (Saini & Keum 2018), despite this, the way how the β-carotene content of the samples was determined in this work did not compromise the comparison done between control and coated samples, once upon all samples were analyzed in the same way and the papaya used in experiment were purchased from the same batch and supplier.

It was not observed significant reductions in the β-carotene content of control samples until the 5th day of storage; however, in the seventh and ninth day it was observed a high reduction in the carotenoid content. Carotenoids are compounds very unstable and susceptible to oxidation degradation by light and oxygen exposition. Injuries performed in minimally processed fruit tissue, as peeling and slicing, accelerate carotenoids degradation during food storage (Rodriguez-Amaya & Kimura 2004). It is possible to observe that this degradation occurred with more intensity in papaya from the 5th day, which can be related to low temperature (5 °C) at which samples were stored. Falah et al. (2015) evaluated β-carotene of minimally processed papaya storage in three different conditions: room temperature at non controlled temperature (27-30 °C), at controlled room temperature (20-22 °C) and at 14-16 °C in controlled room temperature. The authors observed that papaya stored in lower temperature presents higher conservation of β-carotene content.

Coating with vegetable extract from jambolan peel and leaf reduced the loss of β-carotene in minimally processed papaya during the storage, being that the treatment P1.5 provided the highest retention value of the compound. The β-carotene content of the samples treated with P1.5 and L1.5 presented significant change after 3th day of storage. For the same period, however, significant reduction in vitamin content was observed in samples treated with P2 and L2. Despite this, after 9 days stored, samples treated with P2 and L2 still showed retention values higher than control samples. The increase in β-carotene retention is related to antioxidants compounds content present in jambolan peel and leaf extract (Costa et al. 2013, Timbola et al. 2002). Possibly, these compounds made oxygen contact in the papaya surface difficult, reducing β-carotene oxidation during the storage period.

CONCLUSION

Pectin based edible coating with jambolan peel and leaf extract were efficient to preserve the color, mass loss and β-carotene retention of minimally processed papayas during the storage for 9 days at 5 ± 2 °C.

Coated samples presented less browning and color change during the storage period than control samples. The pectin based coating with jambolan peel and leaf extract increased β-carotene retention and reduced mass loss of minimally processed samples during storage being that coating with 1.5% jambolan peel extract presented the highest retentions of the carotenoid (94% in 9 days) and the sample with 1.5% of leaf extract showed the lowest percentage of mass loss (3.8%). This study demonstrated that a simple and inexpensive technique to obtain extracts from renewable and inexpensive sources (leaf and fruit peel) can be efficient to conserve the color and β-carotene content of minimally processed papaya during storage. This technique could be applied by small agroindustries that aim to produce minimally processed foods with higher quality. The work also proposes one application for jambolan leaves extract in food area that could encourage preservation and cultivation of jambolan tree.

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Ana Carolina Barros Polido: performed the experiments; Sandriane Pizato: performed the experiments and supervised the experiment; Keila de Souza Silva: designed and supervised the experiment. All authors provided comments on initial and final drafts of the manuscript.