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Active packaging using *Scenedesmus* sp. and agar delays maturity and maintains post-harvest quality of 'Paluma' guavas

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Abstract

The use of new technologies is important for the preservation of guava, especially in reaching long-distance markets, being indispensable to associate storage techniques to increase the durability of fresh fruit. We evaluated the efficiency of edible coatings based on agar and *Scenedemus* sp. on the quality and post-harvest conservation of 'Paluma' guava. The experiment consisted of a randomized design with four replicates. The treatments (T) were composed of mixing concentrations between agar and *Scenedesmus* sp.: A: (0% + 0%); B: (0% + 0.5%); C: (0% + 1%); D: (0% + 2%); E: (3% + 0%); F: (3% + 0.5%); G: (4% + 0%) and H: (4% + 0.5%) applied by immersion. At the end of 11 days of storage at 10 °C ± 2ºC and 65% ± 5% RH, the fruit was analyzed. There was a significant effect ($P < 0.05$) of the coating based on *Scenedesmus* sp. on the brightness (L*) of the peel and pulp of guava, hue (°h) and chromaticity (C*) of the peel, loss of fresh mass, firmness of the pulp, ascorbic acid, titratable acidity, pH, SS/AT ratio, and total sugars. We observed a lower maturation and maintenance of the post-harvest quality of 'Paluma' guava with the active packages corresponding to treatments F and H. This included the maintenance of the indexes L*, C* and h of peel color, retention in the loss of fresh mass, in the firmness and maintenance of organic acids.

Keywords: biological coating, conservation, post-harvest, *Psidium guajava* L.

Introduction

Guava (*Psidium guajava* L.) is a crop of economic importance, mainly in tropical and subtropical regions. The variety "Paluma" has a red color pulp, commercialized *in natura* or processed as sweets and juices. However, the fruit matures rapidly after harvest, occurring as a series of biochemical events that result in alterations in texture, color, flavor, and aroma (Onias et al., 2018; Germano et al., 2019).

Guava has an intense metabolism during ripening, quickly coming into senescence. The post-harvest period is characterized by large quality loss, preventing long storage periods. Generally, the post-harvest useful life is three and four days when stored at 28 to 30°C (Murmu and Mishra, 2017).

There is a growing interest in the use of new technologies to prevent the rapid deterioration of food. The application of edible coatings on fruits surface together with a lower storage temperature is one method used and diffused worldwide (Costa et al., 2017).

Edible coatings for guava are diverse, such as those formulated from cashew gum and carboxyl methyl cellulose (Forato et al., 2015), chitosan and cassava starch added with rosemary (Aquino et al., 2015), tamarind seed starch and pomegranate seed oil (Onias et al., 2018), araruta starch (Teodosio et al., 2018a), and microalgae (Teodosio et al., 2018b).

Microalgae receive special attention for the formulation of edible coatings because of their multiple benefits to human health (Michalak et al., 2017). *Scenedesmus* sp. is a high-quality nutritional green alga for the food industry (Gong et al., 2019) that contains essential amino acids and strong levels of proteins, lipids, macroelements, and microelements (Cheban et al., 2015; Abdulsamad and Varghese, 2017). *Scenedesmus* sp. is a rich source of antimicrobial and anticancer compounds (Marrez et al., 2019). Therefore,

Scenedesmus sp. is a promising component as edible coatings increase the shelf life of fruit. Its composition builds a film on the surface of the fruit, possessing rheological and functional characteristics (Teodosio et al., 2018b), besides adding nutritional properties.
This study evaluates the efficiency of the use of edible coatings based on agar and *Scenedemus* sp., on quality and post-harvest preservation of 'Paluma' guava.

Results and discussion

The coating based on *Scenedesmus* sp. significantly influenced the brightness (L *) of the guava peel and pulp, hue (ºh) and chromaticity (C *) of the peel, loss of fresh mass, pulp firmness, ascorbic acid, titratable acidity, pH, SS/TA ratio and total sugars. However, the hue (ºh) and chromaticity (C *) of the pulp and soluble solids were not affected by the studied coatings (Table 1).

Coloring
The fruits of treatments G and H, that is, four percent agar with or without Scenedesmus sp., had a less shiny, greenish, and opaque peel compared to the other treatments. Uncoated fruits (A) and those covered with 0.5 percent of Scenedesmus sp. (B) had a brighter shell at the end of the experiment (Table 2). Uncoated fruits tended to become brighter, less green, and more yellow due to the degradation of chlorophyll pigments and the production of carotenoids (Santos et al., 2018). The brightness of the peel is a very important aspect in the ripening process of climacteric fruits, such as guava. Ripening increases the brightness of the peel that consequently becomes more attractive to the consumer. The use of four percent agar with or without Scenedesmus sp. interfered with the external color, delaying the increase in brightness during storage. Teodosio et al. (2018a) also found that the color of guavas covered with arrowroot starch were more conserved when compared to the fruits of the control treatment.
The active packaging did not cause interference in the pulp color so the values of the variables L *, ºh, and C * were similar between treatments, an indication of stability between treatments regarding internal and biochemical processes of pigment formation (Table 2).

Fresh mass loss
The most significant losses of mass occurred in treatments A, B, and D. Treatment F significantly reduced fruit mass loss concerning the other treatments, equivalent to the preservation of 75.95 percent of the fresh fruit mass (Table 3). This likely happened due to the semi-permeable barrier established by the coating, making it difficult to transfer water molecules to the environment and consequently reducing the amount of ethylene produced, delaying the ripening of the fruits (Pellá et al., 2020). A study of coatings based on microalgae by Onias et al. (2018) and Teodosio et al. (2018a) observed that the uncoated samples presented a rapid rate of degradation, while the fruits with coatings had a longer shelf life. Microalgae-based coatings protected the fruit against excessive loss of water to atmosphere.

Firmness of the fruits
The treatments E, G, and H presented the greatest firmness, showing an increase equivalent to 50.99 percent compared to uncoated fruits. The retention of firmness of guavas in those treatments may be a result of agar in the coating formulation since two of the treatments do not involve *Scenedesmus* sp. (Table 3). Hong et al. (2012), using two percent chitosan, found higher firmness on coated treatments, indicating that coatings constituted by polysaccharides tend to improve fruit firmness.
The firmness of the guava is an important indicator of quality and longer life, being relevant in the acceptance of guava by the consumer (Aquino et al., 2015). This is essential for quality maintenance. The absence of coating led to an increase in water loss, which likely results in a broken cell wall and substantial tissue senescence (Velickova et al., 2015). Coatings based on carbohydrates and other biopolymers can potentially decrease respiration rates and modify the internal atmosphere of fresh fruits and vegetables, acting as selective barriers for O_2 and CO_2 (Hernández-Muñoz et al., 2006).

Ascorbic acid
The highest ascorbic acid content occurred in fruits covered with 0.5 percent *Scenedesmus* sp. (B) (93.64% ascorbic acid), which is statistically equal to the C coating with one percent *Scenedesmus* sp. (78.17 percent ascorbic acid). Agar-based coatings had lower values of ascorbic acid – 54.46 percent, 53.67 percent, and 51.98 percent, respectively –for coatings with three percent agar (E), three percent agar with the addition of 0.5 percent *Scenedesmus* sp. (F), and four percent of agar (G). The H treatment had the lower acid ascorbic content, four percent agar and 0.5 percent *Scenedesmus* sp. (49.10). Our result demonstrates that microalgae-based coatings were more efficient in maintaining the ascorbic acid content in guava fruits than coatings with agar (Table 3). In guava, the increased activity of oxidizing enzymes during storage, such as ascorbic acid oxidase, polyphenol oxidase, peroxidase, and catalase, oxidizes ascorbic acid to dehydroascorbic acid, thus reducing its content. Microalgae generally contain secondary metabolites, constituents of high antioxidant levels. Given it is a living material, it could act in conjunction with the enzymes present in the fruit and delay its activity. This could likely maintain the ascorbic acid content of the fruit (Singh et al., 2005).

Tritratable acidity and hydrogen potential (pH)
The highest acidity was found in (H) treatment, although with values similar to other treatments, which may not be perceptible to the palate. The higher value of citric acid in (H) treatment may be associated with the lower ripening, as observed in color indexes and firmness of the fruit (Table 3). After harvesting and during storage, the concentration of organic acids generally decreases due to the use of these compounds as a substrate for breathing, phenomena that lead to fruit senescence (Lubna et al., 2012).
Fruits without coating (A) had a higher pulp pH, indicative of lower acidity, higher consumption of organic acids through metabolism – that is, a more active metabolism (Table 3). The pH likely increased because organic acids were used during the breathing process during storage (Han et al., 2004). Additionally, it is reported that the pH reduction in guavas is related to the senescence of the fruit, which may be due to the use of organic acids during the breathing process (Martinez-Ferrer et al., 2002; Chitarra and Chitarra, 2005).

Table 1. Results of the analysis of variance for color parameters (L*, ºh, C*) of the peel and pulp, fresh mass loss (%, FML), firmness (N, FP), ascorbic acid (% ascorbic acid, AA), titratable acidity (% citric acid, TA), pH, soluble solids (%, SS), SS/TA ratio and total sugars (mg $100g^{-1}$, TS), 'Paluma' submitted to different coatings based on agar and *Scenedesmus* sp. during storage at 10 ± 2 °C and 65 ± 5% RH, after 11 days.

FV	Medium Square							
	DF	L* peel	ºh peel	C* peel	L* pulp	ºh pulp	C* pulp	FML
Coating	7	212.21**	61.87**	90.79**	6.28*	1.54^{ns}	3.24^{ns}	28.68**
Residue	24	42.44	10.69	8.68	2.62	1.15	2.02	2.84
	DF	FP	AA	TA	pH	SS	SS/TA	TS
Coating	7	80.14*	998.02**	0.21**	0.02*	1.18^{ns}	6.16**	36.09**
Residue	24	31.37	91.25	0.03	0.006	0.57	1.14	6.06

** Significant at 1%; *Significant at 5%; ^{ns} Not significant at 5%.

Table 2. Luminosity (L*), hue angle (ºh), chromaticity (C *) of the peel and pulp in 'Paluma' guavas submitted to different coatings based on agar and *Scenedesmus* sp. during storage at 10 ± 2 °C and 65 ± 5% RH, after 11 days.

	Peel			Pulp		
Coatings	L*	ºh	C*	L*	ºh	C*
A	67.99ª	103.38^{b}	48.91ª	58.49^{ab}	35.55ª	38.88ª
B	67.46ª	102.14^{b}	47.13ª	57.97^{ab}	35.85ª	39.92ª
C	65.97^{ab}	105.37^{ab}	47.40ª	57.95^{ab}	35.40ª	39.49ª
D	66.38^{ab}	102.31^{ab}	45.58^{ab}	55.90^{b}	34.97ª	39.07ª
E	63.66^{ab}	106.71^{b}	46.15ª	60.09ª	36.30ª	37.88ª
F	54.54^{ab}	108.90^{ab}	38.90^{bc}	58.42^{ab}	34.67ª	39.12ª
G	51.36^{b}	111.54^{a}	38.00^{c}	59.31^{ab}	35.80ª	37.94ª
H	51.75^{b}	111.94^{a}	37.29^{c}	59.21^{ab}	34.51ª	37.29ª
CV (%)	10.65	3.07	6.75	2.77	3.03	3.68
Total Average	61.14	106.53	43.67	58.42	35.38	38.70

Equal letters in the columns do not differ from each other by the Tukey test at 5% probability. (A): 0% agar + 0% *Scenedesmus* sp.; (B): 0% agar + 0.5% *Scenedesmus* sp.; (C): 0% agar + 1% *Scenedesmus* sp.; (D): 0% agar + 2% *Scenedesmus* sp.; (E): 3% agar + 0% *Scenedesmus* sp.; (F): 3% of agar + 0.5% of *Scenedesmus* sp.; (G): 4% agar + 0% *Scenedesmus* sp. and (H): 4% agar + 0.5% *Scenedesmus* sp.

Table 3. Fresh mass loss (%, FML), firmness of pulp (N, F), ascorbic acid content (% ascorbic acid, AA), titratable acidity (% citric acid), pH, soluble solids (%, SS), SS/TA ratio and total sugars (mg $100g^{-1}$, TS) in 'Paluma' guavas submitted to different coatings based on agar and *Scenedesmus* sp. during storage at 10 ± 2 ° C and 65 ± 5% RH, after 11 days.

Coatings	FML(%)	F(N)	AA(%)	TA(%)	pH	SS(%)	SS/TA	TS(mg $100g^{-1}$)
A	10.52ª	14.24^{b}	70.83^{bcd}	1.23^{b}	3.66ª	9.99^{a}	8.09^{ab}	6.25^{c}
B	8.15^{ab}	13.71^{b}	93.64ª	1.23^{b}	3.45^{b}	10.73^{a}	8.74^{ab}	7.55^{bc}
C	$5.49b^{cd}$	15.71^{b}	78.17^{ab}	1.16^{b}	3.47^{b}	10.89^{a}	9.53ª	8.88^{abc}
D	7.30^{abc}	12.82^{b}	72.71^{abc}	1.45^{ab}	3.48^{ab}	11.35^{a}	8.08^{ab}	10.29^{abc}
E	3.59^{cd}	27.84ª	54.46^{cd}	1.41^{b}	3.54^{ab}	12.81^{a}	7.80^{abc}	12.83^{ab}
F	2.53^{d}	17.00^{b}	53.67^{cd}	1.17^{b}	3.50^{ab}	13.63^{a}	8.49^{ab}	13.90ª
G	6.50^{bc}	27.63ª	51.98^{cd}	1.56^{ab}	3.51^{ab}	12.76^{a}	6.91^{bc}	12.38^{ab}
H	3.73^{cd}	29.05ª	49.10^{d}	1.82ª	3.62^{ab}	14.23ª	5.45^{c}	14.23ª
CV (%)	28.23	14.89	14.57	12.53	2.30	7.20	13.58	22.82
Total average	5.97	20.12	65.57	1.38	2.53	10.54	7.89	10.79

Same letters in the columns do not differ from each other by the Tukey test at 5% probability. (A): 0% agar + 0% *Scenedesmus* sp.; (B): 0% agar + 0.5% *Scenedesmus* sp.; (C): 0% agar + 1% *Scenedesmus* sp.; (D): 0% agar + 2% *Scenedesmus* sp.; (E): 3% agar + 0% *Scenedesmus* sp.; (F): 3% of agar + 0.5% of *Scenedesmus* sp.; (G): 4% agar + 0% *Scenedesmus* sp. and (H): 4% agar + 0.5% *Scenedesmus* sp.

Soluble solids, total sugars and ratio (SS/TA)

Guava is a fruit with low levels of starch. Therefore, it is expected that there will be no major changes in the content of soluble solids during ripening. The coverings studied did not significantly influence the content of soluble solids. Only total sugars and the SS/TA ratio were significantly influenced. Soluble solids and total sugars were higher in H and F coatings. Total sugars did not differ statistically from the C, D, E, and G. Coating B and the control treatment (A) had the lowest values of soluble solids and total sugars (Table 3). The coatings delayed the ripening process and consequently preserved guava fruits during the refrigerated storage period, while the control fruits matured faster, with the beginning of senescence at the 10th day of storage. The decline in SS of control fruits can be attributed to the use of soluble solids as a respiratory substrate (Sahu et al., 2020). According to Oliveira et al. (2018), the faster ripening in control fruits is likely due to the consumption of sugars as a substrate for breathing.

The ratio (SS/TA) is an important quality variable in the post-harvest period as it expresses the balance between sweetness and acidity, representing, in part, a pleasant sensation in the consumer's taste. That is, the higher its value, the better the degree of sweetness in the fruit (Dias et al., 2011). Treatment C obtained the highest average of SS/TA but did not statistically differ from treatments B, D, E, and F. Control A did not statistically differ from the G and H coatings that obtained the lowest SS/TA averages (Table 3). The lowest SS/TA occurred because treatment C presented the lowest acidity and one of the highest values of soluble

solids, leading to palatability. Therefore, it shows that the fruits of this study show a delay in ripening, demonstrated by the slower increase in SS and minor titratable acidity.

Materials and methods

Fruit acquisition and sanitization

The fruits were obtained in an orchard close to the city of Aparecida, Paraíba, Brazil. Guavas were harvested manually in the early hours of the morning. A previous selection was made in the field, avoiding fruits with damages and symptoms of disease. Afterward, the fruits were packed in a single layer in containers previously coated with paper to minimize the impact and friction then transported to the Laboratory of Post-Harvest Technology of the Federal University of Campina Grande (UFCG) in Pombal, Paraíba. In the laboratory, we selected the fruits based on size and color uniformity, discarding those uneven and those that presented apparent damage due to transportation. The fruits were washed with one percent neutral detergent solution and, after rinsing, sanitized with sodium hypochlorite solution at 100 mg L^{-1} chlorine for 15 minutes and dried in the open air.

Experimental design and treatments

Scenedesmus sp. used in this study was produced according to Lima (2016) in tanks of organic production at Fazenda Tamanduá in Patos, Paraíba. The agar was from IMPEX Reagentes Analíticos. The coatings were prepared by aqueous solution under constant stirring and heating to a maximum temperature of 70°C. After cooling, *Scenedesmus* sp. was added in the solution under constant agitation until complete homogenized. The treatments were applied through the immersion of the fruits and draining the excess. The fruits were put in the open air to dry. After drying, the fruits were left in B.O.D at 10 ± 2 °C and 65 ± 5% RH for 11 days, when they were then analyzed.

We used a randomized design with four replications and two fruits per plot. The treatments were: A, 0% agar and 0% *Scenedesmus* sp.; B, 0% agar and 0.5% *Scenedesmus* sp.; C, 0% agar and 1% *Scenedesmus* sp.; D, 0% agar and 2% *Scenedesmus* sp.; E, 3% agar and 0% *Scenedesmus* sp.; F, 3% of agar and 0.5% of *Scenedesmus* sp.; G, 4% agar and 0% *Scenedesmus* sp., and H, 4% agar and 0.5% *Scenedesmus* sp.

Variables analyzed

Coloring: The coloring of the peel and the pulp was determined by reflectometer using a colorimeter (Konica Minolta, model chroma meter CR-400). Calibration was performed using a standard white sample, according to the manufacturer's instructions. The color parameters measured with respect to the standard plate were: Luminosity (L*), which varies from black (0) to white (100); a*, which varies from green (-60) to red (+60), and b*, ranging from blue (-60) to yellow (+60). For the values L*, a*, b*, the hue angle was calculated for the negative a*: $(^{º}h = arctang(a*/b*)(-1) + 90)$ and for a* positive: $(^{\circ}h = 90 - arctang\ (a*/b*)$ and the saturation or chromaticity index, C*: $(C* = [(a*)\,2 + (b*)\,2]1/2]$.

Fresh weight loss (%): We measured this by the difference between the mass of the fruits measured on the electronic scale on the day of installation of the experiment and at the end of storage.

Firmness (N): This was determined with the aid of a digital penetrometer (Instrutherm, model PTR-300). Measurements were made at two opposite points in the equatorial region of the fruit, after removal of the peel, performed with an 8 mm blade, according to Aoac (2012).

Titratable acidity (% citric acid): This was measured by titrating one mL of juice diluted in 50 mL of distilled water with two drops of phenolphthalein solution, under constant stirring, with 0.1 M sodium hydroxide solution (Ial, 2008).

Soluble solids (SS; %): This was determined by direct reading with a digital refractometer brand (Digital Refractometer) (Aoac, 2012).

Rátio (SS/AT): This was determined by the quotient between soluble solids and titratable acidity.

Hydrogen potential (pH): This was determined by direct reading using a digital benchtop meter (Digimed DM-22 Mark) (Ial, 2008).

Ascorbic acid (% ascorbic acid): This was measured using one mL of diluted juice to 49 mL of oxalic acid, titration under constant stirring with DFI solution according to Tilman method (Aoac, 2012).

Total sugars: This was determined by the method of Antrona, according to the procedure described by Yemn and Willis (1954) with some modifications.

Statistical Analysis: This was performed in the SISVAR software version 5.6 using analysis of variance (F test) and Tukey test at the five percent probability level (Ferreira, 2011).

Conclusion

We found a lower maturation and maintenance of the post-harvest quality of the 'Paluma' guava in the active coatings corresponding to the treatments F and H (3% of agar + 0.5% of *Scenedesmus* sp. and 4% agar + 0% *Scenedesmus* sp., respectively). The post-harvest quality of guavas in treatments F and G is a result of the maintenance of the color indexes L*, C*, and ºh in the peel, retention of fresh mass loss, and firmness and organic acids during storage.

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