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# Effect of modified starch and gelatin by-product based edible coating on the postharvest quality and shelf life of guava fruits

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# Abstract

To improve storability and to maintain the qualitative characteristics of guava fruit, by an intelligent and sustainable approach, a factorial experiment was conducted in a completely randomized design with three replications. The experimental treatments were guavas with an edible coating based on modified starch and a gelatin by-product (T1), supplemented with peppermint essential oil at three different concentrations (0.5%, 1%, and 1.5%) (T2, T3, and T4), and a control (TC) consisting of guava without coating. All treatments were stored until 15 days at room temperature ( $25 \pm 1$  °C), and characteristics were measured every 3 days. The results were analyzed by regression analysis and Tukey's post hoc test,  $p \le 0.05$ ). The application of a modified-starch based coating and gelatin by-product, with or without peppermint essential oil, was efficient at prolonging the shelf-life of fruit to 15 days after harvest and a little ripening related to the concentration of added peppermint oil. Considering the physicochemical aspects, the best treatment was T1 and considering the microbiological aspects, the best treatments were T3 and T4.

**Keywords:** *Psidium guajava* L; postharvest; shelf-life; edible coating; food waste; peppermint oil; sustainability; natural polymer. **Practical Application:** The use of inexpensive and straightforward edible coatings increases the shelf-life of guavas.

# **1** Introduction

Guava (*Psidium guajava* L.) is a tropical fruit that is widely consumed and appreciated for its high nutritional value, especially for its content of antioxidants, vitamin C, dietary fiber, and minerals (Flores et al., 2015). It is an economically important crop, especially in tropical and subtropical regions of the world. Brazil occupies the fourth position among world producers of guava, behind India, Pakistan, and China, according to the FAO report; however, in global trade, Brazilian participation is only 2%, as its production is mainly directed to domestic consumption (Agrianual, 2014; Altendorf, 2018).

Fruit and vegetable quality are influenced by extrinsic, as a production environment, handling during harvest at various supply chain stages and package and intrinsic factors to the food itself, such as visual appearance, texture, firmness, food safety, sensory and nutritional properties. All these attributes are of interest to the consumers. The diverse range and characteristics of fruit and vegetables fresh and their inherently perishable nature warrant specific attention to their production conditions, agronomic management, pest and disease control, harvesting techniques, and postharvest handling systems (Food and Agriculture Organization, 2020). Because produce continues to respire during storage, it consumes oxygen from within the packaging and emits carbon dioxide, slowing down the aging process and extending shelf life (Vitón et al., 2020). As a result, the postharvest shelf-life of guava is limited to 3-4 days at a temperature of  $25 \pm 2$  °C, thus making transportation to more distant consumer centers difficult (Silva et al., 2012). Despite the modernization of production systems and distribution of perishable products in recent decades, postharvest losses in Brazil continue to be persistent and relevant (Henz, 2017).

New technologies are necessary to reduce postharvest losses to guaranteeing extended fruit quality for the Brazilian guava to reach the international market, meet the increased domestic market demand, and reach consumers' tables. Novel food packaging techniques promotes food quality and safety. Edible materials as consumable wrapping (film) or coating around the food could reduce the waste. As edible materials examples are Nanotechnology that provides bioactive, antimicrobials, vitamins, antioxidants, and nutrients (Suhag et al., 2020). Active packaging plays an essential role in the packaged foods by desirably interacting with food. They provide technological functions, such as, releasing scavenging compounds as provide antimicrobials, antioxidants, remotion of harmful gases as oxygen

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and water vapor, in the form of natural-origin antioxidants including plant extracts, essential oils,  $\alpha$ -tocopherol, ascorbic, and citric acid. The use of peppermint essential oils (EO) makes the edible coating studied an active packaging to increase the functionality of edible packaging (Trajkovska Petkoska et al., 2021; Hassan et al., 2018).

In recent years, many packaging materials have been developed from alternative and renewable sources, such as edible coatings produced using polysaccharides (starch, pectin, cellulose, chitosan and carrageenan), proteins (gelatin, casein, and ovalbumin), lipids (waxes), or a combination of several polysaccharides (Luvielmo & Lamas, 2012). Starch-based coatings are natural coatings that pose an eco-friendly technological solution by reducing both the dependence on fossil resources and the product's carbon footprint, when compared with conventional plastic packaging materials, in addition to being biodegradable and low cost (Chen et al., 2019a; Kumar & Neeraj, 2019; Molavi et al., 2015, Nešić et al., 2019, Suhag et al., 2020).

However, starch-based coatings are weak barriers to the entry of water vapor due to the hydrophilic characteristics of starch (Ghanbarzadeh & Almasi, 2011). Yet, modified starch (MS) has a higher capacity to retain water and resist retrogradation. The use of modified starch combined with other polymers such as gelatin from by-products of the food industry, and essential oils (EO), with an antibacterial and microbial effect, helps to reduce these limitations and can be a low-cost alternative with great potential to increase the shelf life of guava and improve its postharvest quality (Mohammadi et al., 2015; Grande-Tovar et al., 2018).

Then, the present study evaluated the efficacy of an edible coating produced with modified starch added of gelatin byproduct from the meat industry, and different concentrations of peppermint essential oil in the postharvest conservation of guava (*Psidium guajava* L.) over a storage period of 15 days. The firmness, appearance, physicochemical (pH, acidity, °Brix) and microbiological aspects were evaluated.

# 2 Materials and methods

This study was a cross-sectional study with laboratory analyses performed over 15 days of storage studies of guava fruit. A factorial experiment was conducted in a completely randomized design with three replications. The experimental treatments were guavas with an edible coating based on modified starch and a gelatin by-product (T1), supplemented with peppermint essential oil at three different concentrations (0.5%, 1%, and 1.5%) (T2, T3, and T4), and a control (TC) consisting of guava without coating. All treatments were stored until 15 days at room temperature ( $25 \pm 1$  °C), and characteristics were measured every three days.

# 2.1 Material

The guava (*Psidium guajava* L.) fruits of the Paluma variety used in the study were selected to with harvested at maturation stage 2 (light green, about 80% ripened, according to the scale of Cavalini et al., 2006) directly from the place of cultivation of producers in the District of Ceraíma, rural area of Guanambi-BA (latitude 14° 17' 06"S and longitude 42° 42' 49"W; average altitude of 525 m). A total of 300 units of fruits (40 kg) were

transported from the field to the laboratory. It was wasted 30 fruits, and them 225 fruits were used for Physico-chemical analyses, 30 for microbiological studies and 15 for appearance.

Modified starch by physical process (thermal heating) was obtained from a commercial source (Docina Nutrição LTDA, Juiz de Fora, MG, Brazil), the gelatin type B, produced from raw cowhide and bone, via an alkaline process from by-products of the bovine industry, kindly provided by the State University of Campinas (UNICAMP, São Paulo, Brazil), and BioEssência<sup>®</sup> brand essential oil of peppermint (*Mentha piperita*) was purchased at a drugstore. Analytical grade Glycerol (Dinâmica) was used as the vehicle.

# 2.2 Coating production and application

Coatings were produced according to Valencia et al., (2016), with adaptations. Thirty grams of modified starch (MS) was added to 1000 mL of distilled water. This solution was heated at 70 °C for 20 minutes under stirring until complete starch gelatinization and named as hydrolyzed modified starch (HMS). Ten grams of gelatin were hydrated with 100 mL of distilled water and kept at rest for one hour. This solution was heated to 85 °C for 20 minutes on a hot plate with the addition of 1.5 g of glycerol/100 g of hydrated gelatin and named as Hydrated Gelatin (HGe).

Four types of edible coverings were developed (T1, T2, T3, and T4). T1 was composed of a gel formed from HMS + HE, and T2 to T4, were composed of the T1 gel added with three different concentrations of EO (v: m), EO (mL)/100 g of glycerol) (0.5%, 1%, and 1.5%) for each treatment (Table 1) at room temperature and homogenized by stirring. Nine samples guava were subjected to each treatment at each time.

Guavas were washed with water, sanitized with sodium hypochlorite solution at 100 ppm for 10 minutes, rinsed in chlorinated water at three ppm, and placed in plastic trays to dry at room temperature ( $26 \pm 2$  °C). The coating treatments were immersed for 1 minute in the coatings and returned to the trays to dry at room temperature ( $26 \pm 4$  °C) for 1 hour. Subsequently, the coated fruits were stored in a Biochemical Oxygen Demand (BOD) refrigerator (SL-200, Solab, Piracicaba, São Paulo, Brazil) at 25 °C ± 0.5 °C for up to 15 days.

# 2.3 Physico-chemical analyses

Physico-chemical analyses were carried out in triplicate with approximately 100 g of pulp per triplicate, every 3 days, at

Table 1. Control and experimental treatments were applied to guava.

Description of Components
uncoated
HMS + HGe
HMS + HGe + EO (0.5%)
HMS + HGe + EO (1.0%)
HMS + HGe + EO (1.5%)

HMS: hydrated and heat-modified starch; HGe = by-product of hydrated, heated gelatin and added of glycerol; EO: peppermint essential oil. n=255 units of fruits.

0, 3, 6, 9, 12, and 15 days, for each treatment, and each analysis (loss of mass, acidity, pH, °Brix, and firmness), totaling 45 fruits per analysis, per day, and 225 fruits in total. Each sample was homogenized in a semi-industrial blender.

The relative loss of fruit mass was verified over time concerning the initial mass, according to method 934.06 (Association of Official Analytical Chemists, 2012). The pH determination was measured in 10 g of sample diluted in 100 mL of distilled water in potentiometer equipment (model 8650, AZ brand, AZ Instrument Corp, Taichung City, Taiwan) (method 017/IV) (Instituto Adolfo Lutz, 2008). The total acidity (%) determination was performed using potentiometric volumetry technique (method 942.15 B) (Association of Official Analytical Chemists, 2012). The total soluble solids (°Brix) were determined in a portable digital refractometer (model AR-200, Tecnal, Piracicaba, Brazil) and adjusted to 20 °C, according to method 315/IV (Instituto Adolfo Lutz, 2008). The Firmness (N) measurement was performed on a manual texturometer (model FR-5120, brand Fruit Firmness Tester, Tamil Nadu, India), with 6 mm probe. The measurements were performed in each fruit at two points on opposite sides, in the fruits equatorial zone. The probe had the speed of 1 mm/s with a penetration of 10 mm and returned to the initial position. The results were expressed in Newtons (N) and represented the maximum force expressed in the penetration of the fruit (Juhaimi et al., 2012).

#### 2.4 Microbiological analyses

The analysis was performed in thirty fruits units. About 25 g of each sample were homogenized in a Stomacher apparatus with 225 mL of peptone water in aseptic packaging (10<sup>-1</sup>) and two subsequent dilutions were made. The analyses were performed in triplicate on the first and last day of the experiment (days 0 and 15), according to APHA (American Public Health Association, 2015). It was analyzed filamentous fungi with counting of fungi and yeasts, the Total count of aerobic mesophilic bacteria. The results were expressed in colony forming units per gram (CFU/g).

# 2.5 Appearance

Fruit appearance was monitored visually and registered in images. Guavas were observed for 15 days and the presence of imperfections and spots on the fruits recorded photographically with the aid of a 13-megapixel mobile phone with f/1.9- and 8-megapixel apertures, which captures high-quality images, even in low-light environments.

# 2.6 Experimental design and statistical analysis

The experiment was conducted in an internally randomized delineation in a 5 x 6 factorial scheme (five treatments x six duration times), for the physicochemical and microbiological evaluations. The data obtained were submitted to the tests for normality and homogeneity first and after by analysis of variance (ANOVA) and the Tukey post hoc test at the 5% level of significance ( $p \le 0.05$ ). To verify interactions between factors, a simple linear regression test and adjustments were used, with the aid of the statistical software program R.

# **3 Results**

#### 3.1 Loss of mass

There was an increase in the mass loss percentage over time in the fruits of all treatments, but after the sixth storage day, mass lost by the control samples ( $T_0$ ) was significantly higher vs. all other samples ( $p \le 0.05$ ). However, throughout the experiment, there was no significant difference (p > 0.05) in mass loss between experimental treatments ( $T_1$  to  $T_4$ ). The loss of mass was affected ( $p \le 0.05$ ) by the interaction between the studied factors, but in the control, treatment was more affected with a 24.34% loss of mass at the end of storage, while the others treatments demonstrated the same loss of mass (%) (Figure 1).

# 3.2 pH

The pH was different between the treatments according to ANOVA and Tukey test (Table 2).

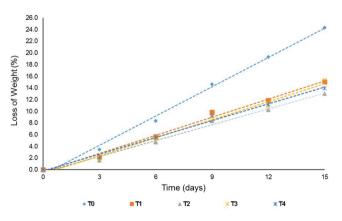
There was a significant increase in pH with increasing storage time, mainly between  $(T_0)$  and  $(T_4)$ . Guavas coated with the highest concentration of EO  $(T_4)$  had the highest pH value (4.09), with a significant difference over the other treatments. The EO at a concentration of 1.5% raised the pH.

#### 3.3 Titratable acidity

The titratable acidity and levels of total soluble solids were affected (p  $\leq$  0.05) by the interaction between the factors studied, being more affected by the control treatment, while the other treatments were not effect (Figure 2 A, B). In all treatments there were reductions in acidity and increasing of soluble solids over time. The control treatment (T<sub>0</sub>, uncoated) showed the greatest reduction in acidity (0.38%).

#### 3.4 Firmness

The firmness was affected ( $p \le 0.05$ ) (Tukey) by the storage time and also by the different types of coatings, but not (p>0.05)

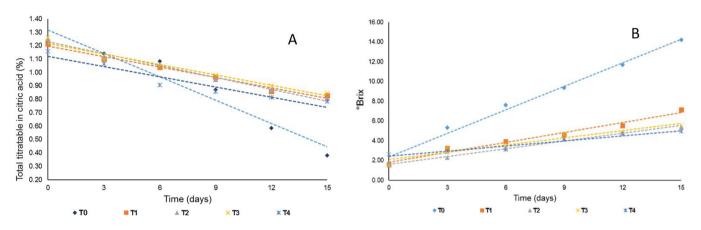


**Figure 1**. Loss of mass (%) in uncoated guavas and those coated with starch, gelatin by-product, and different concentrations of peppermint oil. n=45. T0 = uncoated; T1 = hydrated and heat-modified starch (HMS) mixed with hydrated, heated gelatin by-product and added glycerol (HGe); T2 = HMS + HGe + 0.5% peppermint essential oil (EO); T3 = HMS + HGe + 1.0% (EO); T4 = HMS + HGe + 1.5% (EO).

Treatments	Coating type	pН	Firmness
T	Uncoated	3.92 ± 0.0933 b*	8.86 ± 2.1645 a
T,	HMS + HGe	$4.00 \pm 0.1280$ ab	16.34 ± 5.3757 b
T <sub>2</sub>	HMS + HGe+ EO (0.5%)	3.98 ± 0.0849 ab	16.90 ± 4.2393 b
T,	HMS + HGe+ EO (1.0%)	3.94 ± 0.1127 b	16.61 ± 3.6138 b
T,	HMS + HGe+ EO (1.5%)	$4.09 \pm 0.1209$ a	14.78 ± 2,6890 b
Average		$3.99 \pm 0.1079$	$14.70 \pm 3.6164$

Table 2. Average values of pH and firmness in uncoated guavas and those with different coatings, observed throughout the experimental storage monitoring period (15 days, at room temperature).

\*Expressed as average  $\pm$  standard deviation. Averages followed by the same letter, in the column, do not differ significantly by the Tukey post hoc test ( $p \le 0.05$ ). T0 = uncoated; T1 = hydrated and heat-modified starch (HMS) mixed with hydrated, heated gelatin by-product and added glycerol (HGe); T2 = HMS + HGe + 0.5% peppermint essential oil (EO); T3 = HMS + HGe + 1.0% (EO); T4 = HMS + HGe + 1.5% (EO). n=45 for pH and n=45 for Firmness.



**Figure 2.** (A) Regression between titratable acidity in uncoated guavas and those coated with starch, gelatin by-product and different concentrations of peppermint oil; (B) Total Soluble Solids (°Brix) in guavas uncoated and those coated with starch, recycled gelatin, and different concentrations of peppermint oil. n=45. T0 = uncoated; T1 = hydrated and heat-modified starch (HMS) mixed with hydrated, heated gelatin by-product and added glycerol (HGe); T2 = HMS + HGe + 0.5% peppermint essential oil (EO); T3 = HMS + HGe + 1.0% (EO); T4 = HMS + HGe + 1.5% (EO).

by the interaction between the factors, fitted by the linear regression model ( $R^2 = 0.5210$ ) (Figure 3).

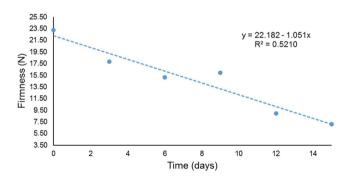
The firmness declined linearly in all treatments over storage time, with a decrease to a decrease to as low as 6.50 N at the end of the 15 days (Figure 3). Since there was no significant interaction between treatments and firmness, the coating factor was studied in isolation, using the Tukey Test (p < 0.05) (Table 2).

There was a significant difference ( $p \le 0.05$ ) between uncoated and coated treatments (Table 2). Greater mean values of firmness were obtained for coated treatments (T1 to T4), varying between 14.61 and 16.90.

# 3.5 Microbiological analysis (filamentous fungi and yeasts and mesophilic aerobic bacteria)

The current legislation does not recommend limits for counting filamentous fungi and yeasts and aerobic mesophilic bacteria in fresh fruits. For this reason, the microbiological standards established for food in general were used, which advocates a limit of counts up to  $10^5$  CFU/g for foods suitable for human consumption (Table 3) (Brasil, 2019a, b).

The treatments ( $T_0$ ,  $T_1$ , and  $T_2$ ) presented counts of filamentous fungi and yeasts but within the consumption patterns (not exceeding 10<sup>5</sup> CFU/g counts). On the other hand,  $T_3$  did not



**Figure 3**. Linear regression of the interaction between the Firmness (N) in the uncoated and coated guavas, n=45.

show growth in the samples analyzed at day zero, but at the end of the 15 days, a count of filamentous fungi was observed, demonstrating that the oil concentration at 1% was not effective for its inhibition. On the other hand, the concentration of EO at 1.5% ( $T_4$ ), was more effective, with the absence of colony growth at storage times of 0 and 15 days (Table 3). The growth behavior of bacteria colonies was similar to that of filamentous fungi in  $T_3$  and  $T_4$  treatments. With EO addition in the coating, the formation of colonies of aerobic mesophilic bacteria was reduced.

Filamentous fungi and yeasts (CFU/g) to 25 °C							
Days	T0	T1	Τ2	Т3	Τ4		
0	2.0 x 10 <sup>1</sup>	8.0 x 10 <sup>3</sup>	$1.0 \ge 10^{1}$	<10	<10		
15	1.8 x 10 <sup>3</sup>	$2.0 \ge 10^{1}$	$3.0 \times 10^3$	$3.0 \ge 10^3$	<10		
Mesophilic aerobic bacteria (CFU/g) to 35°C							
Days	T0	T1	Τ2	Т3	T4		
0	$2.0 \ge 10^{1}$	8.0 x 10 <sup>3</sup>	$1 \ge 10^{1}$	<10	<10		
15	1.8 x 10 <sup>3</sup>	2.0 x 10 <sup>1</sup>	3.0 x 10 <sup>3</sup>	3.0 x 10 <sup>3</sup>	<10		

Table 3. Filamentous fungi and yeasts (25 °C) and Aerobic mesophilic bacteria (35 °C), performed at storage times 0 and 15 days in guavas uncoated and with different edible coatings.

T0 = uncoated; T1 = hydrated and heat-modified starch (HMS) mixed with hydrated, heated gelatin by-product and added glycerol (HGe); T2 = HMS + HGe + 0.5% peppermint essential oil (EO); T3 = HMS + HGe + 1.0% (EO); T4 = HMS + HGe + 1.5% (EO). n=30.

The microorganism culture figures are in the Supplementary Material (Supplementary Material)

# 3.6 Appearance

The fruits showed visual changes as imperfections, spots, and changes in the skin color over the experimental time (Figures 4).

The fruits started the experiment standardized as to the degree of ripeness (Figure 4A). On 3<sup>rd</sup> day, T0 fruits (Figure 4B) showed color change from green to yellow, and T4 fruits had ripened slightly, remaining so until the sixth day. At 9<sup>th</sup> day (Figure 4D), a T0 fruit ripened, and T4 continued to ripen slowly, followed by the beginning of ripening and alterations in T3 fruits, with small spots on their skin. On the 12<sup>th</sup> day (Figure 4E), all the T0 fruit ripened, and the repining of T4 and T3 continued. On day 15 (Figure 4F), it was observed that treatments T4 and T3 were more mature than those of T2 and T1.

#### **4** Discussion

The loss of mass verified in the different treatments studied (around 5%), is acceptable for fresh fruits, since greater losses can reduce their acceptance in the market (Vitón et al., 2020). The loss of mass in the uncoated fruit over storage time, can be explained by the high rates of transpiration and respiration from the physiological metabolism of the fruit combined with the loss of water retention of the fruit, resulting in loss of mass and fruit wrinkling (Chitarra & Chitarra, 2005; Lufu et al., 2020). The addition of EO to the coating made no difference in the loss of fruit mass; therefore, it did not improve the moisture barrier properties. The same was observed in strawberries, with the addition of the lipid compounds oleic acid and peppermint EO in concentrations of 0.5% (v/v) and 0.2% (v/v), respectively (Leite et al., 2015).

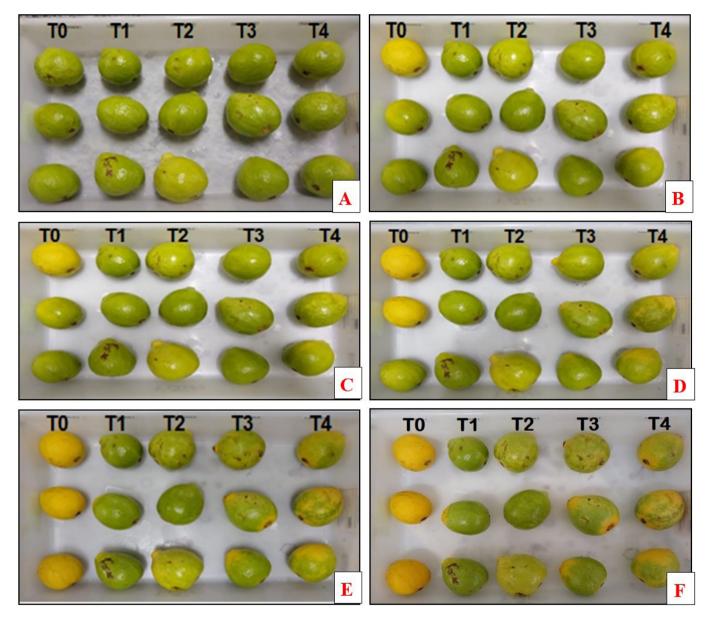
It was observed that during storage, there was a small pH variation and a significant increase until 15<sup>th</sup> day. In general, the pH tends to increase during fruit ripening, explained by the consumption of organic acids during ripening due to the respiratory activity of cells this change is considered a natural process after harvest (Pareek, 2016).

Satisfactory pH values for fruit pulp must be between 3.5 and 4.2 per the Identity and Quality Standards recommended in Normative Instruction no. 1 (Brasil, 2000). Despite being obtained from the whole fruit, the values observed in the present study were in accordance with the legal standards for pulp. Uncoated guavas and coated guavas based on cassava starch and chitosan showed pH values between 3.85 and 4.10, with small significant differences between treatments over storage time (de Aquino et al., 2015), as occurred in the present study. Coatings based on O-carboxymethyl chitosan and oregano EO in guavas showed greater pH variations (between 4.1 and 4.7) (Tavares et al., 2018).

The percentage of titratable acidity is a criterion widely used to verify the quality of fruits, as they show the organic acids, that are used as substrate for cellular respiration, synthesis of phenolic compounds and volatile aromas of fruits. The organic acid content tends to decline as the fruit ripens (Batista-Silva et al., 2018; Formiga et al., 2019).

In climacteric fruits, as guavas, there is oxidation of organic acids and an increase in soluble solids with the respiration process. Thus, the acidity of the fruits is expected to decrease over the storage time (Yahia & Carrillo-Lopez, 2018). This reduction is related to ethylene production, that begins soon after fruit development is complete in climacteric fruits, until it reaches a maximum peak, just before the peak of respiration (Perdones et al., 2012). Ethylene triggers the fruit ripening process. However, it is possible to further slow ripening with the use of postharvest strategies, such as natural coatings like those used in the present study, which prevented the increase in fruit acidity (Thakur et al., 2019). Thus, the influence of coatings on the maintenance and stability of the total titratable acidity of the fruits was observed when compared with uncoated fruits. Acidity was also maintained in guavas coated with cassava starch and chitosan (de Aquino et al., 2015), differing significantly from that of uncoated guavas.

Treatments ( $T_2$ ,  $T_3$ , and  $T_4$ ) with different added concentrations of peppermint EO, showed a lower ripeness over time. They also promoted a lower concentration of sample soluble solids (when compared with  $T_1$ , without added E0). The content of soluble solids (SS) represents the water-soluble compounds present in fruits, such as sugars, water-soluble vitamins, acids, amino acids, and some pectin. It generally increases with the repining of the fruit due to biosynthesis or degradation of polysaccharides, in addition to the proportional loss of fresh mass, which concentrates the total soluble solids. This increasing observed corroborated



**Figure 4**. Appearance analysis for guavas coated and uncoated at times 0 (A), 3 (B), 6 (C), 9 (D), 12 (E), and 15 days (F), for treatments T0 to T4. T0 = uncoated; T1 = hydrated and heat-modified starch (HMS) mixed with hydrated, heated gelatin by-product and added glycerol (HGe); T2 = HMS + HGe + 0.5% peppermint essential oil (EO); T3 = HMS + HGe + 1.0% (EO); T4 = HMS + HGe + 1.5% (EO). n=15.

with the decreasing in the acidity of the fruits over storage time. As the fruit ripens, there is a rapid loss of acidity, making a sweeter the fruit (Rodrigues et al., 2020).

Firmness is a practical way to assess the stage of fruit ripeness and is fundamental in determining the shelf-life and the market value of a fruit, in addition to being one of the main elements judged by the consumer at the time of purchase (Chen et al., 2019b; Hong et al., 2012). The firmness decreased observed in the uncoated could be explained by the metabolism increasing, acceleration of cell wall degradation, softening of the pulp, besides the degradation of starch and constituent molecules of fruit cell walls, such as cellulose, hemicellulose, and pectin, due the action of cell wall enzymes such as pectinmethylesterase, polygalacturonase, and cellulase (Soradech et al., 2017; Li et al., 2014). However, the applied coatings demonstrated effectiveness in preventing the decrease in the firmness of guavas. Moreover, the addition of EO oils did not change fruit firmness. The results observed for firmness agree with the results for loss of mass. The reduction in loss of mass also contributed to the stability increased of fruit firmness (de Aquino et al., 2015). The greater stability of the fruit presented can offer even more resistance to transport (Cerqueira et al., 2011).

# 4.1 Microbiological analysis (filamentous fungi, yeasts, and mesophilic aerobic bacteria)

In the geographic region where the study was carried out, with high temperatures and low relative moisture, the growth of filamentous fungi is favored (Garcia et al., 2015). In addition, these fungi are quite resistant to acidic pH and low water activity. Thus, all coatings were effective in reducing the microbiological count, in accordance with current legislation for fruit pulp. However, the addition of 1% and 1.5% of EO tends to be more effective at inhibiting microbial growth in the fruits.

The EO extracted from the peppermint plant has excellent antimicrobial activity and has been extensively studied and applied in the preservation of food, pharmaceuticals, and salad dressings (Liang et al., 2012; Chumpitazi et al., 2018). EO improve the appearance, microbial safety, mechanical resistance, and diffuse antimicrobial agents on the surface of food. Due to their hydrophobic character, they improve the characteristics of edible coatings by reducing perspiration and the loss of fruit mass (Murmu & Mishra, 2016; Fuciños et al., 2017).

It is suggested that the fungitoxic action against some types of microorganisms is due to the menthol active compound present in peppermint oil. Similar behavior was observed by Guerra et al., (2015), with coatings consisting of chitosan and EO of *Mentha piperita*, which strongly inhibited mycelial growth and germination of contaminating fungal spores in tomatoes. Strawberries with edible coatings based on xanthan gum and glycerol, combined with EO of peppermint also did not show fungal growth and reduced the loss of mass, color, pH, acidity, and total soluble solids, being effective in their conservation (Leite et al., 2015). EO from other plants, as *L. gracilis* Schauer (1.0% and 3.0%) used in guavas coated with an edible chitosanbased coating reduced aerobic mesophilic bacteria, molds, and yeasts during storage at room temperature (25 °C ± 4) for 10 days (de Aquino et al., 2015).

# 4.2 Analysis of appearance

The treatment without coating (T0) resulted in accelerated ripening and yellowed fruits, showing a noticeable difference in the action of the coatings. In treatment T0, all samples showed altered fruit color and wrinkling. Treatments T1 and T2 conferred the best properties on the guavas studied in terms of appearance (maintenance of green color, absence of spots, and wrinkling of the skin).

# **5** Conclusions

The attributes pH and firmness were affected by the storage time and coating, but these factors had no significant interaction during storage. The addition of EO at 0.5%, 1.0%, and 1.5% (T2, T3, and T4, respectively) did not influence the pH, titratable acidity, soluble solids, and mass loss variables, but for the inhibition of bacteria and fungi, the addition of oil at 1% and 1.5% was more effective. The use of peppermint OE is recommended in the production of coatings if the objective is to reduce the incidence of microorganisms.

Considering all the analyses performed, there was a significant difference between T0 and all experimental treatments (T1 to T4) for loss of mass, pH, acidity, °Brix, firmness, and microbiological aspects. There was a statistically significant difference in the experimental treatments with coatings in relation to pH and acidity, but the difference in pH was not considered relevant (range: 3.92-4.09). T4 had higher acidity

and a lower microbiological count for filamentous fungi and mesophilic aerobic bacteria. However, all coating treatments were microbiologically compliant.

Thus, considering all the evaluations, the application of a modified starch-based coating and gelatin by-product, with or without peppermint essential oil, was efficient at prolonging the useful life of the fruits, until 15 days after harvest at room temperature in a tropical region with a little ripening related to the concentration of added peppermint oil (ripening of T2 <T3 <T4). The combined use of all ingredients studied in the starch-based edible coating, a natural and biodegradable polymer, with a gelatin by-product and essential oil proposed is an eco-friendly, intelligent, and sustainable alternative that preserves the overall fruit quality of the product.

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# **Supplementary Material**

Supplementary material accompanies this paper.

Figure S1. Microbiological evaluation for filamentous fungi and yeast from coated and uncoated guavas at time 0.Figure S2. Microbiological evaluation for mesophilic aerobic bacteria of coated and uncoated guavas at time 0.Figure S3. Microbiological evaluation for filamentous fungi and yeasts from coated and uncoated guavas in time 15.Figure S4. Microbiological evaluation for mesophilic aerobic bacteria of coated and uncoated guavas at time 15.

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