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Shelf life and sensory analysis comparison of alginate and chitosan edible coating incorporating avocado extract applied to minimally processed apples

Adrielle Aparecida Paulista Ribeiro[1], Rafaela Cristina Sanfelice[2], Geoffroy Roger Pointer Malpass[1], Mônica Hitomi Okura[1] and Ana Claudia Granato Malpass[1*]

[1] *Universidade Federal do Triângulo Mineiro, Avenida Doutor Randolfo Borges Junior, 1400, CEP: 38064-200, Uberaba-MG, Brazil*

[2] *Universidade Federal de Alfenas, Rodovia José Aurélio Vilela, BR 267, Km 533, 11999, CEP: 37715-400, Poços de Caldas-MG, Brazil*

* Corresponding author, E-mail: ana.malpass@uftm.edu.br

Abstract

The purpose of the present study was to evaluate *Persea americana* (avocado) in relation to the pulp composition, moisture content, lipids present, proteins, and ash. The *P. americana* pulp extract was prepared by solvent extraction using pure ethanol, and the chemical composition of the extract was evaluated by GC-MS. The resulting extract was subsequently incorporated in alginate and chitosan-based edible coatings on minimally processed Fuji apples. The edible coatings were evaluated microscopically, for shelf-life improvement and by sensory analysis. The alginate-based coatings were able to inhibit enzymatic browning, improve the appearance of the minimally processed apple samples and obtained the best results in all aspects evaluated in the sensory analysis. The incorporation of *P. americana* pulp extract contributed to the improvement the shelf life of minimally processed apples for 15 days and the fruits covered with the alginate-based edible coating incorporated with *P. americana* pulp extract had the best scores in acceptance and appearance.

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Introduction

Apple is one of the most cultivated and consumed fruits in the world, with more than 2,500 species, with the Fuji variety being one of the most cultivated[1]. According to the FAO[2], Brazil is one of the largest apple producers in the world, having produced 1.2 billion tons of the fruit. However, this volume produced is not always consumed in its entirety. According to the United Nations[3] annually around the world, about 1.3 billion tons of food are lost, especially in Latin America and the Caribbean and these wasted foods could feed more than 30 million people. Therefore, new preservation technologies are needed to extend the shelf life of these foods.

Currently, it is observed that consumers are more concerned and attentive with regard to the composition of processed foods. Thus, the food industry, in response to this, has developed products known as Minimally Processed Foods (MPFs). These are submitted to operations such as drying, cutting, sanitizing, centrifuging, and conditioning in appropriate packaging, guaranteeing their sensory, nutritional and microbiological properties until purchase[4]. For MPF foods, packaging is the most important step, as it allows transport to the point of sale or distribution[5].

The packaging must consist of a material that contributes to maintaining the physicochemical, functional, and sensory characteristics of the food, does not interact with the product and protects it from external damage of a chemical, physical and biological nature[5]. Thus, edible coatings emerge as natural and eco-friendly alternatives to preserve the quality and prolong the shelf life of such products[6].

However, edible coatings are mostly non-active, and therefore additives are used to bring functionality, such as antimicrobial and antioxidant activities. Aloui et al.[7] studied alginate-based edible coatings incorporating grapefruit extract and essential oil to improve the quality of table grapes. Coatings incorporating grapefruit extract were effective in reducing weight loss and maintaining grape firmness during storage. Coatings incorporating essential oil and grapefruit extract were able to preserve the antioxidant activity of treated grapes, in addition to reducing the incidence of rot in inoculated fruits. Peretto and coworkers[8] found that the edible coating of alginate enriched with carvacrol and methyl cinnamate applied to fresh strawberries led to a significant reduction in fruit spoilage relative to the uncoated control. The shelf life of the coated fruit was 11 d while the uncoated fruit was 7 d, demonstrating superior performance in terms of firmness, color retention and weight, in addition to reducing losses. Medina-Jaramillo et al.[9] developed alginate coatings incorporated with carvacrol for application on blueberries. Coated blueberries were preserved for 21 d of storage in comparison with the uncoated ones. Coating formulations

with 0.09% of carvacrol was the most effective in preventing mesophilic aerobic bacteria and molds/yeasts growth on the fruits during the storage.

Eshghi et al.[10], investigated the effect of chitosan-based edible coatings with and without ghatti gum to improve the biophysical and safety properties of 'Rishbaba' grape (*Vitis vinifera* L.). All coatings positively controlled berry softening, berry discoloration, berry drop and rachis browning. The mixture of chitosan and C3G3 ghatti gum (1% chitosan-1% ghatti gum) showed better effects in delaying weight loss and titratable acidity and sensory scores compared to other treatments. This C3G3 formulation showed the greatest effect on all measured parameters and is recommended as a useful edible coating combination to improve the quality of postharvest properties of the grape. Pinzon et al.[11] used composite films made from banana-chitosan starch and aloe vera gel at different gel concentrations. The authors show that the inclusion of the gel can significantly reduce fungal caries, increasing the shelf life of strawberries for up to 15 d of storage in the highest AV gel concentration (20%), maintaining their physicochemical properties, such as color and firmness. Weight loss was reduced by 5% compared to uncoated fruit. Li et al.[12] evaluated a chitosan coating in addition to films of nanomaterials such as silicon and titanium dioxide to detect changes in fresh blueberry fruit at commercial storage temperature. The titanium-based nanocoating presented the most adequate values for weight loss, titratable acidity and recoat index. While the silicon-based nanocoating showed the least change in acidity, anthocyanin and minimized the growth of aerobic mesophilic populations, yeasts and molds. The chitosan coating maintained its lightness and recorded the highest ascorbic acid content.

In this work, the avocado pulp extract was used as it is of great importance for the fruit industry in several regions around the world. The world production of avocado was estimated at 5.6 million tons in 2016 and in the same year, Brazil ranked sixth in world production, producing 195.5 thousand tons, occupying an area of 10,855 hectares[13]. Due to the huge volume of avocado produced, it is interesting to take advantage of this fruit and reduce the production of waste, generate income and enjoy all the benefits present in the avocado pulp. Avocado oil has a characteristic antioxidant and antimicrobial action, the antioxidant action is due to the composition, which contains carotenoids, phytosterols and tocopherols[14]. Thus, the objective of this work was to develop edible coatings based on alginate and chitosan incorporating avocado pulp extract to preserve the quality, protect against the action of microorganisms and to prolong the shelf life of minimally processed apples, whilst preserving sensory attributes.

Materials and methods

Raw material characterization

The avocados were purchased from a supermarket in the city of Uberaba-MG-Brazil, weighed and separated into: peel, pulp, and stone. In this material, the average weight and percentage of peel, pulp and stone were determined.

Centesimal composition of avocado pulp

The fresh avocado pulp was characterized by physicochemical methods, according to the official methodology of the Association of Official Analytical Chemists (AOAC)[15], by determination of moisture, proteins, lipids, and ash contents. All analyzes were performed in triplicate.

Water content determination

Ten gram of the sample was weighed in a porcelain capsule and the sample was heated for 6 h at 105 °C. The sample was then cooled in a desiccator to room temperature, heated and cooled repeatedly until a constant mass was obtained, and the moisture percentage was calculated according to Eqn (1).

$$Umidity\ \% = \frac{\text{Sample mass (g)} - Dry\ Sample\ mass\ (g)}{\text{sample mass (g)}} \times 100 \quad (1)$$

Protein determination

One gram of the sample was weighed, transferred to a Kjeldahl flask, 25 mL of concentrated sulfuric acid (96%) and ~6 g of the catalytic mixture was added. The sample mixture was heated on an electric plate until the solution turned blue-green and was free of undigested material. The sample was subsequently heated for 1 h and allowed to cool. The material from the Kjeldahl flask was quantitatively transferred to a distillation flask, 10 drops of phenolphthalein indicator and 1 g of zinc powder were added. The flask was immediately coupled to the distillation equipment and connected to an erlenmeyer flask containing 25 ml of 0.05 M sulfuric acid and 3 drops of the methyl red indicator. Sodium hydroxide solution (30%) was added to the vial containing the digested sample, which was then heated to boiling and distilled to obtain ~250 ml of distillate. The excess sulfuric acid was titrated with sodium hydroxide (0.1 M) using methyl red indicator. The percentage of proteins was calculated according to Eqn (2).

$$Proten\ \% = \frac{\text{Volume spent on the titration (mL)} \times 0.14 \times Correction\ factor*}{\text{Sample weight}} \quad (2)$$

* Correction factor considered equal to 6.25.

Lipid determination

Approximately 2 g of the dry sample was weighed and transferred to the Soxhlet-type extractor device and kept under heating for 6 h (2–3 drops per second). The material was removed from the extractor and the flask with the extracted residue was transferred to an oven at 105 °C, for ~3 h. The sample was cooled in desiccator to room temperature. The percentage of lipids was calculated according to Eqn (3).

$$Lipid\ \% = \frac{\text{Lipid mass (g)}}{\text{Sample mass (g)}} \times 100 \quad (3)$$

Ash determination

Approximately 5 g of the sample was weighed in a porcelain capsule and elevated to a muffle at 550 °C. After cooling, the sample was weighed, and the percentage of ash was calculated according to Eqn (4).

$$Ash\ \% = \frac{\text{Ash weight (g)}}{\text{Aample weight (g)}} \times 100 \quad (4)$$

Extraction of avocado pulp

The fresh avocado pulp was submitted to drying in an oven at 60 °C and was ground to obtain a bran. Then, the avocado

pulp bran was extracted by solvent extraction with pure ethanol over 2 d. After filtration, the extract was concentrated in a rotary evaporator.

Gas chromatography with mass spectrometry (GC-MS)

The GC-MS analysis was performed as described by Posetti et al.[16]. The apparatus employed was a Shimadzu 2010 High Resolution Gas Chromatograph coupled to a Mass Spectrometry Detector. An Agilent DB-5MS column (30 m × 0.25 mm – 0.25 μm) was employed. The following run conditions were used: Injector Temperature: 220 °C, Splitless Injection Mode, 2 min sampling time, linear velocity 15.7 Psi Pressure, Total flow of 19.4 mL·min^{-1}, Column flow of 1.49 mL·min^{-1}, Linear velocity of 45.0 cm·s^{-1}, Purge flow of 3.0 mL·min^{-1}, Split ratio of 10, Gradient Mode column temperature of 80–280 °C. The Mass Spectrometry Detector parameters were 200 °C ion source temperature, 280 °C interface temperature, 3 min solvent cut-off time, 3 min initial detection time, a detection time of 17.0 min, SCAN acquisition mode, 0.25 s acquisition time, SCAN mass/charge ratio (m/z) from 40 to 600 and 1 μL injection volume[16].

Minimum inhibitory concentration (MIC)

MIC determination was performed using 96-well microdilution plates (12 columns and 8 rows) and three microorganisms, *Bacillus cereus* (ATCC11778), *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 35218), according to Methods for Dilution Antimicrobial Susceptibility Tests[17]. Initially, the dilutions were carried out in test tubes where BHI broth and the tested extract were added. The dilutions were made in the following percentages: 100% extract, 95%, 90%, 85%, thus up to 5% extract. In each well of the plate, 300 μL of the previously diluted solution was added to the test tubes except for the last two columns, where the wells were only filled with BHI broth, for the positive control (using chlorhexidine 2%) and the control negative. After all wells were filled, the microorganisms were added, each in its respective plate, only the last column did not receive the microorganism, as this was the negative control. The plates were incubated at 37 °C for 24 h. The MIC is the lowest concentration that completely inhibits the growth of the microorganism, that is, in which turbidity is not observed in the medium in the well[4,16,17].

Preparation of coatings

In the development of coatings, avocado pulp extract at 5% was used. The chitosan-based edible coating was elaborated according to de Araújo et al.[18]: 2% (m/v) Chitosan were dissolved in 1% (v/v) acetic acid and stirred under heating (40 °C) for 6 h. Subsequently, 0.75 mL of glycerol/q of chitosan was added, and the solution was stirred for another 30 min. After this period, 5% (v/v) avocado pulp extract was added.

The alginate-based edible coating was prepared according to Santos et al.[4] and de Araújo et al.[18]: 1.29% (w/v) of sodium alginate was dissolved in distilled water under heating (70 °C) and mechanical stirring until the mixture was clear. Then, 1.16% (m/v) of glycerol and 5% of the avocado pulp extract were added. To promote the crosslinking of the polymer matrix, a solution containing 2% (m/v) of calcium chloride, 1% (m/v) of ascorbic acid and 1% (m/v) of citric acid was used.

Application of the edible coatings

The edible coatings were applied to 'Fuji' variety apples, purchased in a supermarket in the city of Uberaba, Brazil. The chosen apples were ripe and selected manually, analyzing the redness, average size and if there were no signs of injury. The fruits were washed and sanitized in 0.5% chlorinated water (v/v) for 15 min, then peeled and cut into slices of approximately 25 g. The apple slices were submerged in the coating solutions and left to rest to allow for dripping. The fruits coated with the alginate-based coating were submerged in the solution 2% (m/v) of calcium chloride, 1% (m/v) of ascorbic acid and 1% (m/v) of citric acid to promote polymeric crosslinking. Once dried, samples were packaged separately in non-toxic plastic bags, sealed and stored at 3 °C for the analysis period (15 days performing microbiological analysis every 5 d). Four treatments were obtained:

- AA1 – apple + alginate-based coating without the extract.
- AA2 – apple + alginate-based coating with the extract.
- AC1 – apple + chitosan-based coating without the extract.
- AC2 – apple + chitosan-based coating with the extract.

Microbiological evaluation of the samples (shelf life)

For the standard plate count, 25 g of the sample were added to 225 mL saline solution (0.1%), followed by homogenizing and proceeding with the serial dilution up to 10-4. Afterwards, 1 mL of each dilution was inoculated into sterile, empty Petri dishes, pouring 20 mL of molten Plate Count Agar medium. The medium was homogenized with the inoculum through movements in the shape of a figure eight and, after the medium had solidified, the plates were incubated at 37 °C for 24 h[19]. For the determination of thermotolerant coliforms, the technique of multiple tubes by the most probable number (MPN/g) was used. A 10-1 dilution was performed, homogenizing 25g of sample with 225 mL of peptone water (0.1%) and from the serial dilutions (10-2 and 10-3), 1 mL of each dilution was seeded. The medium used was 0.1% peptone water and incubated at 35 °C for 24 h. From the reading of the positive tubes (with growth and gas production in the Durham tubes), confirmation was performed with Brilliant Green Bile Broth under the same time and temperature conditions recommended by the American Public Health Association (APHA)[20]. The determination of the Most Probable Number of Thermotolerant Coliforms was carried out using the technique of multiple tubes, with Broth Escherichia coli (EC). For positive tubes for thermotolerant coliforms, culture subcultures were performed into tubes with EC Broth. After sowing, the sample was incubated at 37 °C for 24 h, in a water bath, with a series of three tubes for each subculture[21]. Subsequently, an elevation of the positive EC Broth tubes was striated on plates containing the Eosin Methyl Blue medium and incubated at 37 °C for 24 h to confirm the growth of *Escherichia coli* characterized by the growth of black flowering colonies. As for the evaluation of *Salmonella* ssp, 25 g of each sample were homogenized in 225 mL of broth for *Salmonella* enrichment and incubated at 30 °C, after 20–24 h 1 mL aliquots were transferred to tubes containing 9 mL of Tetrathionate Broth Base and incubated at 30 °C for 20–24 h. The sample was then, striated to a Petri dish containing XLD Agar and incubated at 37 °C for 24 h[19,22].

Sensory analysis

The sensory analysis was approved by the Research Ethics Committee of UFTM (process number 2,108.891), using the acceptance test employing a 9-point hedonic scale that contains the defined terms situated between 'I really liked' and 'I disliked extremely', as described by Santos et al.[4] and Posetti et al.[16]. The sensory analysis was performed in a laboratory that has a specific area for sample preparation and individual, climate-controlled booths. The tasters were instructed to read the Informed Consent Form and after clarifying any doubts that they had about the object of the study, the form was signed in two copies, one of which remained with the taster and the other with the researcher. This analysis involved the participation of 50 untrained tasters aged between 18–63 years, composed of university employees and students. The samples were characterized microbiologically and physiochemically before sensory analysis. The apple samples slices of approximately 25 g (a coating-less control sample apple without any coating), an AA1 sample, an AA2 sample, an AC1sample and an AC2 sample) were served individually in plastic dishes, and duly identified with numerical codes of three random digits. Samples were served simultaneously and were at 8 °C. Tasters were instructed to identify the sample codes on the sensory analysis form, taste the samples from left to right, arranged on the tray, record the score according to the hedonic scale score for each attribute: color, aroma, texture and taste and finally rinse your mouth with water before tasting the next sample.

Statistical analysis

Data were analyzed using Analysis of Variance (F test) at 5% probability and once the significant effect of the treatment was verified, the Tukey test was applied at 5% probability. Statistical analyzes were performed using the SISVAR computer program, developed by Ferreira[23].

Results

Raw material characterization

The raw material characterization is important to observe how the fruit varies, besides being possible to see how significant the by-products are. The average percentage of each component and the average values and standard deviation of pulp, peel, seed and fruit, where the accuracy of the measuring equipment was 0.01g (Table 1). Emphasizing that the variety of avocado used was Fortuna.

Table 1. Average weight and average percentage of components.

Attributes	Seed mass (g)	Pulp mass (g)	Pell mass (g)	Total mass (g)
Average weight	105.05 ± 6.07	452.56 ± 39.00	51.34 ± 0.05	609.06 ± 45.19
Average percentage	17.25%	74.30%	8.45%	–

Proximate composition of the avocado pulp

The samples were separated and the analyzes were performed in triplicate, the precision of the measuring equipment was 0.0001 g (Table 2).

Avocado pulp extraction, Minimum Inhibitory Concentration (MIC) and evaluation of the chemical composition of the obtained extract

The extraction had a yield of approximately 8%, considering the input of fresh raw material, that is, with high water content. The minimum inhibitory concentration values for the extract obtained against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* are shown in Table 3.

Regarding the chemical composition, 12 peaks were obtained in the GC analysis that were identified by MS and Table 4 shows the compounds present in the avocado pulp extract.

Microbiological evaluation – shelf life

The samples of fresh apples (without any coating, only minimally processed) and coated with alginate or chitosan-based coating with and without avocado pulp extract, were evaluated for 15 d, every 5 d and the results are shown in Table 5.

Sensory analysis

The statistical analysis of the results obtained in the sensory analysis are summarized in Table 6. It was observed that the coated apples were well accepted by the panelists, except for sample AC1 (apple + chitosan-based coating without extract) which obtained in the global evaluation a mean less than 5, which symbolizes neither liked nor disliked in the hedonic scale used.

Discussion

Raw material characterization

The seed represented 17.25% of the total fruit mass, the skin is thin and represented on average 8.45% of the average fruit weight, it is noted that the skin weight did not vary significantly between samples, as each fruit was relatively similar in size.

Proximate composition of the avocado pulp

The pulp is mostly water, the average found among the samples was 83.4% moisture on a wet basis. The amount of protein present in the pulp was small – a 100 g portion of 'Fortuna' avocado pulp has an average of 1.16 g of protein, this represents 1.55% of the daily value that should be consumed in a 2,000 kcal (8,400 kJ) diet.

The amount of lipids presents in the 'Fortuna' avocado pulp was quite significant, averaging 53.62% in dry matter, and 8.89% in whole matter. These values are important as it is possible to determine the best extraction method and yield, in addition to identifying the type of variety used.

The amount of ash present in the pulp was 0.53 g for every 100 g of fresh 'Fortuna' avocado pulp, this number represents

Table 2. Results of the proximate composition of the Fortuna avocado pulp.

Sample	Water content (%)	Protein (%)	Dry matter lipids (%)	Whole matter lipids (%)	Ashes (%)
Pulp	83.4 ± 0.19	1.16 ± 0.12	53.62 ± 1.67	8.89 ± 0.26	0.53 ± 0.011

Table 3. MIC of the studied extract.

		Avocado pulp extract
Escherichia coli	Bactericide	80%
	Bacteriostatic	75%
Staphylococcus aureus	Bactericide	80%
	Bacteriostatic	75%
Bacillus cereus	Bactericide	45%
	Bacteriostatic	40%

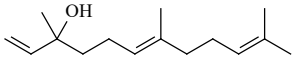
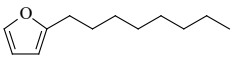
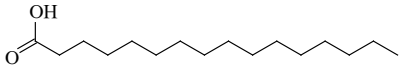
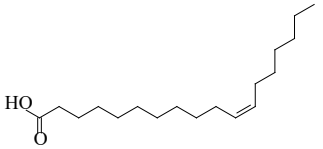
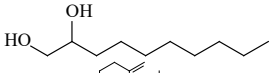
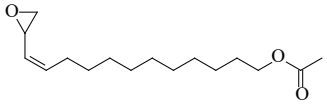
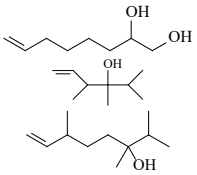
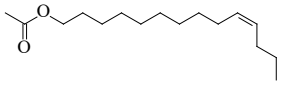
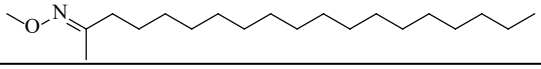
the total mineral content that can be used as a measure of identity and quality.

Avocado pulp extraction, Minimum Inhibitory Concentration (MIC) and evaluation of the chemical composition of the obtained extract

The extract presented better antimicrobial activity against *B. cereus*. Against other microorganisms, the bactericidal and bacteriostatic action were equivalent.

Chen et al.[24], reported that 2-octylfuran were obtained as Maillard reaction products from mushroom hydrolysate and that this compound was responsible for the caramel-like flavor of the product. The authors also reported that Maillard reaction products not only contributed to the flavor, but also

Table 4. Chemical composition of avocado pulp extract.

Retention time (min)	Compound	Structure
7.825	(6*E*)-3,7,11-trimethyl-1,6,10-dodecatrien-3ol	
10.154	2-octylfuran	
11.758	palmitic acid	
13.433	ci-vaccenic acid	
13.933	1,2-decanediol	
15.025	3-methyl-2-(2-methylene-cyclohexyl)-butan-2-ol	
15.504	(13,14-epoxy)-tetradec-11-en-1-ol acetate	
15.651	7-octene-1,2-diol	
15.942	2,3,4-trimethyl-5-hexen-3-ol	
16.225	2,3,6-trimethyl—7-octen-3-ol	
16.425	10-methyl-(11*E*)-tridece-1-ol acetate	
16.762	O-methyloxime-(2*E*)-nonadecanone	

to antioxidant and antimicrobial effects, and also played an important role in the improvement of functional properties of the products.

Table 5. Standard count on plates.

	Day 0 (CFU)	Day 05 (CFU)	Day 10 (CFU)	Day 15 (CFU)
In natura apple	3.2×10^3	5.7×10^5	1.5×10^6	2.0×10^6
AA1	<3.0	<3.0	2.0×10^4	1.0×10^5
AA2	<3.0	<3.0	1.0×10^2	3.8×10^2
AC1	8.0×10^2	5.0×10^4	3.5×10^5	6.8×10^5
AC2	<3.0	3.0×10^4	4.8×10^5	6.6×10^5

Awang-Jamil et al.[25] obtained high levels of palmitic acid in extracts of medicinal plants with high antimicrobial activity, suggesting this compound is responsible for the biological activity. Other authors also related the antimicrobial and antifungal activity to palmitic acid[26–28]. Additionally, Robles et al.[29] explained organic acids play a main role in maintaining the quality and nutritional value of food, and contribute to the sensory (flavor, color, and aroma) and healthy properties (antioxidant and antimicrobial activity) of all types of food (fruits, vegetable, mushrooms and beverages). Golebiowski et al.[30] also reported the antimicrobial activity of organic acids.

Okukawa et al.[31] reported antibacterial and antifungal activity of 1,2-alkanediols, such as 1,2-decanediol. The authors

Table 6. Averages, standard deviations and results of statistical tests to compare the evaluated attributes.

Samples	Attributes evaluated				
	Apparency	Aroma	Texture	Flavor	General
AA1	8.00 ± 1.28^{A}	6.88 ± 1.55^{A}	7.74 ± 1.47^{A}	6.80 ± 1.88^{A}	7.26 ± 1.23^{A}
AA2	7.86 ± 1.78^{A}	6.62 ± 1.59^{A}	7.44 ± 1.45^{A}	6.60 ± 2.12^{A}	7.04 ± 1.52^{A}
AC1	4.68 ± 1.94^{Ba}	4.94 ± 1.72^{B}	5.88 ± 2.09^{B}	4.88 ± 2.09^{B}	5.14 ± 1.59^{B}
AC2	4.42 ± 1.75^{Ba}	4.76 ± 2.07^{B}	5.76 ± 2.40^{B}	4.24 ± 2.33^{B}	4.76 ± 1.80^{B}
Control (*in natura* apple)	5.94 ± 1.89^{Bb}	6.36 ± 1.69^{A}	6.94 ± 1.70^{A}	6.90 ± 1.64^{A}	6.54 ± 1.33^{A}

Legend: Means followed by the same letter between lines do not differ significantly from each other, at 5% probability, by Tukey's test.

explained the antimicrobial activity depends on the alkyl chain length, and that 6–12 carbon 1,2-alkanediols, such as 1,2-decanediol, exhibited significant bactericidal activity.

Hyldgaard et al.[32] discussed that the antimicrobial activity of an essential oil/extract may depend on their main chemical constituents, but that evidence indicates that the inherent activity of essential oils/extracts may not depend exclusively on the proportion in which the major active constituents are present, but also on their interactions and the minor constituents in the oils/extracts, in agreement with Van de Vel et al.[33].

Thus, it can be said that the synergistic effect of the compounds present in the studied avocado extract is probably responsible for its antimicrobial activity.

Microbiological evaluation – shelf life

As can be seen in Table 5, in the samples of fresh apples, there was growth of microorganisms throughout the period analyzed. In samples AC1 and AC2, that is, minimally processed apples coated with a chitosan-based coating, with and without avocado pulp extract, there was also growth of the evaluated microorganisms and in a high concentration from the 5th day, giving a shelf life of 5 d for both samples. However, the values observed were below the values found for fresh apple samples for the same period. These results were quite unusual, since it is known that chitosan has antimicrobial activity. According to No et al.[34] chitosan displayed higher antibacterial activity, and its inhibitory effects differ with molecular weight and bacteria tested. According to the authors[34] the 470 kDa molecular weight chitosan was, in general, the most effective in reducing the growth of both gram-negative and gram-positive bacteria. Thus, in general, medium molecular weight chitosan, as used in this study, has better antibacterial activity.

Although, chitosan generally showed stronger bactericidal effects for gram-positive bacteria than for gram-negative bacteria and the MIC differed with individual bacteria and chitosan molecular weights[34]. EI-Ghaouth et al.[35] stated that chitosan acts according to a mechanism in which it reacts with the cell surface, changing cell permeability and preventing material entry or causing material leakage. This is proven by Chung et al.[36], in which it is clear that hydrophilicity was much greater in gram-negative bacteria than in gram-positive bacteria. As a result, chitosan is more effective against gram-positive bacteria than against gram-negative bacteria.

Furthermore, Azmy et al.[37] prepared chemically modified chitosan biopolymers and demonstrated that the antimicrobial activity obtained by chemically modified biopolymers was significantly superior to the activity presented by the chitosan biofilm. The authors attribute this increase in the antimicrobial activity of chemically modified chitosan biopolymers to the fact that structural changes facilitate the interaction of the biopolymer with microbial cell membranes and facilitate its process of penetration into the interior of microbial cells, in relation to the unmodified chitosan biopolymer. Thus, it is clear that natural chitosan does not have considerable antimicrobial activity and may vary with the fruit being coated. Fruits with more humid environments are likely to impair the antimicrobial activity of chitosan.

According to Assis & Hotchkiss[38] chitosan films have a high gas barrier, with greater permeation of CO_2 than O_2. But a disadvantage of chitosan is that it has a high affinity for water. Its structure contains a high number of amine (NH_2) and hydroxyl (-OH) groups, which favor water sorption. Water absorption induces swelling and consequently increases the permeation rate, which reduces long-term stability. Thus, it is possible to understand how apples coated with edible coatings based on chitosan did not inhibit microbial growth.

As for alginate-based coatings, both with extract and without extract, there was no growth of microorganisms until day 5, but from the 10th day onwards growth was observed. However, for AA1 there is still a shelf life of 10 d, superior to samples coated with chitosan. For the AA2 sample, the shelf life was the longest obtained at 15 d, showing the effectiveness of the avocado pulp extract against the growth of pathogenic microorganisms.

As for the presence of *Salmonella* spp in the samples, there was no presence of this microorganism in the samples over the 15 d of study.

Sensory analysis

Analyzing each of the attributes, it was observed that regarding appearance, apples coated with alginate without and with avocado pulp extract had excellent acceptance, with an average of 8, a value that represents a great deal of pleasure. As chitosan-based coatings were not able to inhibit enzymatic browning, samples AC1 and AC2 were visually rejected by the panelists, obtaining lower scores than those of the control (*in natura* apple). The samples that showed significant differences from each other by the Tukey test, with 5% probability, were the samples with alginate-based coating, differing from the samples with chitosan-based and in natura coatings. In addition, the chitosan-based coated samples also differed from the control (*in natura* apple).

This probable enzymatic browning in minimally processed apples covered with a chitosan-based coating occurred due

to the ineffectiveness of controlling moisture transfer, due to the high-water vapor permeability of chitosan[39]. To improve water vapor barrier properties, lipids (plasticizers) are frequently incorporated into hydrocolloid-based films, as plasticizers increase the free volume of polymer structures or the molecular mobility of polymer molecules. Although, glycerol is not a good plasticizer for chitosan films because, according to Cerqueira et al.[40] the water vapor permeability of chitosan coatings increases with the increase in the concentration of glycerol used. This occurs because glycerol favors the adsorption of water molecules, which is mainly attributed to its predisposition to form hydrogen bonds, altering the polymer network, creating mobile regions with greater distances between chains, promoting the grouping of water by competing with water in active sites of the polymer matrix and reducing the intermolecular hydrogen bond between the chitosan molecules.

So, one possibility for the use of chitosan-based coatings on fruits that suffer from enzymatic browning can be the use of sorbitol, as a plasticizer. According to Cerqueira et al.[40] it decreases the permeability to water vapor due to its larger size and relatively lower hygroscopicity compared to glycerol, which might reduce the amount of water entrapped in the film matrix. Thus, the coating has a higher effective concentration of polysaccharide, reducing water mobility and reducing water vapor permeability. Another solution is the use of composite films since the functional properties of chitosan can be improved by mixing it with other hydrocolloids[39].

Regarding the aroma and flavor, the samples with chitosan-based coating did not please or displease the panelists, with an average close to 5, as the alginate-based coating made the apples lose some of their natural aroma. The samples with alginate-based coating did not obtain a good evaluation either, with averages close to 7, values close to the average obtained by the control (in natura apple), representing modern taste. As for texture, the values were not discrepant between the samples, with averages above 5 being observed, values that already demonstrate a certain interest of the panelists for all the samples.

For aroma, flavor, texture and general evaluation, the samples that showed significant differences in the Tukey test were samples AA1 and AA2, followed by samples AC1 and AC2 and finally the control sample (*in natura* apple). Thus, the edible coating based on alginate with the incorporation of avocado pulp extract obtained the best results and stands out as a technological solution for the preservation of minimally processed apples for at least 15 days.

Conclusions

The edible coatings prepared from alginate showed the best performance in the various criteria evaluated. Regarding shelf life, samples with alginate-based coating had the best results, and the sample coated with alginate-based coating and incorporating *P. americana* (avocado) pulp extract showed the best result, 15 d of shelf life. Sensorially, as the alginate-based coatings were able to inhibit enzymatic browning, considerably improving the appearance of the minimally processed apple and obtained the best results in all aspects evaluated in the sensory analysis. It can be said then, the alginate-based edible coating incorporating *P. americana* pulp extract is a viable solution for the preservation of minimally processed apples.

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Conflict of interest

The authors declare that they have no conflict of interest.

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