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Edible coating with microalgae and modified atmosphere packaging for post-harvest conservation of tomatoes

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ABSTRACT

Tomato fruits are highly perishable. In this sense, adopting techniques to maintain and extend its shelf life is essential. Recent studies have used microalgae as an edible coating for fruit, as it is a nutrient-rich alternative and reduces fruit mass loss and respiration, delaying senescence. The aim of this study was to evaluate the application of microalgae-based coatings with or without the use of modified atmosphere packaging, polyvinyl chloride (PVC), in post-harvest tomato conservation. The design used was completely randomized, in a 4x2 factorial arrangement, with four coatings (no coating, coating composed of *Spirulina platensis* sp., *Chlorella* sp. and *Scenedesmus* sp.) and two conditions (with and without PVC), totalizing 8 treatments, with four replicates, consisting of one fruit each. Stored for 7 days at 10±2ºC and 55±5% UR and evaluated at harvest and on the last day of storage. The fruits coated with *Chlorella* sp. without PVC and *Scenedesmus* sp. associated with PVC, showed the lowest mass losses, representing a reduction of 73.79% and 78.47%, respectively, in relation to the control. In addition to mass loss, the levels of ascorbic acid (18.91 and 16.97 mg/100 g), citric acid (4.02 and 4.01), respectively, and the SS/AT ratio also stood out. The microalgae *Chlorella* sp. and *Scenedesmus* sp. can be used in coating 'Santa Clara' tomato fruits to maintain their physicochemical characteristics over 7 days of storage. The use of PVC film coating helped maintain these characteristics, reducing the perishability of the fruits.

Keywords: *Solanum lycopersicum*, *Spirulina platensis* sp., *Scenedesmus* sp., *Chlorella* sp., post-harvest conservation.

RESUMO

Revestimento comestível com adição de microalgas e embalagem com atmosfera modificada para a conservação pós-colheita do tomate

Os frutos do tomateiro têm alta perecibilidade havendo a necessidade da adoção de técnicas que mantenham e prolonguem sua vida útil pós-colheita. Estudos recentes têm utilizado microalgas como recobrimento comestível em frutos, por ser uma alternativa rica em nutrientes e reduzir perda de massa e respiração do fruto e assim retardar a senescência. Objetivou-se avaliar a aplicação de revestimentos a base de microalgas com ou sem o uso de embalagem com atmosfera modificada (policloreto de vinila) na conservação pós-colheita de tomate. O delineamento utilizado foi inteiramente casualizado, em arranjo fatorial 4x2, com quatro revestimentos (sem revestimento, revestimento com *Spirulina platensis* sp., *Chlorella* sp. e *Scenedesmus* sp.) e duas condições [com e sem PVC (policloreto de vinila)], totalizando 8 tratamentos, com quatro repetições, constituída por um fruto cada. Armazenados por 7 dias a 10±2ºC e 55±5% UR e avaliados na colheita e no último dia de armazenamento. Os frutos recobertos com *Chlorella* sp. sem o PVC e *Scenedesmus* sp. associado ao PVC, tiveram as menores perdas de massa, representando redução de 73,79% e 78,47%, respectivamente, em relação ao controle. Além da perda de massa, também tiveram destaque nos teores de ácido ascórbico (18,91 e 16,97 mg/100 g). As microalgas *Chlorella* sp. e *Scenedesmus* sp. podem ser utilizadas no revestimento de frutos do tomateiro 'Santa Clara' para manter suas características físico-químicas ao longo de 7 dias de armazenamento. A utilização do recobrimento com filme PVC auxilia na manutenção dessas características, diminuindo a taxa de perecibilidade dos frutos.

Palavras-chave: *Solanum lycopersicum*, *Spirulina platensis* sp., *Scenedesmus* sp., *Chlorella* sp., conservação pós colheita.

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Tomato is one of the main vegetables, fruit type, grown in Brazil and the second worldwide crop production (Bello *et al*., 2020). However, due to some aspects, such as weight loss, excessive maturity and high respiration rate, which lead to quick senescence, this vegetable has a short shelf life after harvest and an early fruit quality deterioration, resulting in a loss of 21% of the total production (Pirozzi *et al*., 2020).

One way to maintain greater durability concerning the shelf life of various fruits is the use of plastic packaging film, reducing transpiration and modifying the surrounding atmosphere, which is a functional protection against surface abrasion (Sandri *et al*., 2015). Since this kind of packaging is not biodegradable, it generates a great quantity of solid residues, though.

Given the above, an increasing number of studies on natural resources to produce biodegradable edible coatings have been developed recently. These coatings create a semipermeable barrier which interferes in the metabolic process, minimizing the gas exchanges between the fruits and the atmosphere, mainly oxygen and carbon dioxide, and also humidity and organic solutes (Zhao, 2019). They can be formulated with cassava starch (Nunes *et al*., 2017), chitosan (Yan *et al*., 2019) and, according to recent studies, with microalgae in fruit trees (Oliveira *et al*., 2018; Onias *et al.,* 2018; Teodósio *et al*., 2020, 2021).

The advantage of using microalgae is that, in addition to prolonging the vegetable shelf life, this microscopic algal species contains carbohydrates, mineral salts, high protein content, vitamins, antioxidants and unsaturated fatty acids, serving as a food supplement (Silva *et al*., 2019). Microalgae, like Sp (*Spirulina platensis*)*, Ch* (*Chlorella sp.*) and *Sd* (*Scenedesmus sp.*), are rich in these compounds, promote porous plastification and interaction with protein complexes, such as PHBV (Polihidroxi Butirato Valerato), which allows the formation of mechanical and interactive structure chains on the surface of the fruit, regulating transpiration and fruit mass loss (Oliveira *et al*., 2018; Onias *et al*., 2018; Teodósio *et al*., 2020). In addition, they have antitumor, anti-inflammatory, antimicrobial, antioxidant and anticoagulant properties (Silva *et al*., 2019).

However, further studies to investigate if these edible coatings can replace or be used associated with non-biodegradable packaging, helping preserve the post-harvest quality, are necessary (Azeredo *et al*., 2012). The authors believe that studies on the use of edible coatings and plastic films (PVC) on fruits of different species are essential to select the best form of post-harvest conservation, maintaining quality.

In this sense, this study aimed to evaluate the effect of edible coatings based on *Spirulina platensis*, *Chlorella sp.* or *Scenedesmus sp*., associated or not with modified atmosphere using PVC to extend the post-harvest shelf life of 'Santa Clara' tomatoes.

MATERIAL AND METHODS

Materials

'Santa Clara' tomatoes were harvested in a commercial orchard in the municipality of Alexandria, Rio Grande do Norte, Brazil (6°24'15"S, 38°0'35"W, 303 m altitude). Fruit ripening stage III, orange yellow skin color (ºh 52.19±1.67) was harvested at 6 a.m., stored in Kraft cardboard boxes lined with shredded paper, in order to reduce impact and friction during transportation, and taken to Laboratório de Tecnologia de Pós-Colheita de Frutos e Hortaliças of Universidade Federal de Campina Grande (UFCG), Pombal, Paraíba, Brazil. In the laboratory, the fruits were selected uniforming the color, absence of mechanical damage, stains or any phytosanitary problem.

The microalgae biomass, *Chlorella* sp., *Scenedesmus obliquo* or *Spirulina platensis*, were provided by the company J.H. de Lima, CNPJ 23.176.796/0001-33.

Coating preparation

Coatings were formulated using agar-agar© for culinary use in powder form (Vovó Nize) (2%), polysorbate 20 (Tween©) PS (VETEC) (1 mL/L), pomegranate seed oil (0.3 mL/L), obtained from pomegranate fruit seeds at commercial maturity stage (90 days), produced at hinterland of Paraíba and microalgae (0.05%). The dispersions were prepared under heating at 70°C on a hotplate (Bio AGa10L, 7Lab) under constant agitation in order to dissolve the agar-agar completely. The microalgae biomass and pomegranate seed oil were added after the temperature was lowered to 35ºC under constant agitation (Figure 1).

Coating application

The fruits were washed under running water, immersed in sodium hypochlorite solution (0.1 mL/L) for 15 min, then rinsed again under drinking water and air dried (Anvisa, 2020).

The fruits were immersed in the coating solution at 30ºC to avoid causing harm, and then placed to dry in empty containers previously sanitized with neutral detergent and running water for 2 h. After the fruits were dried, the authors picked up 4 of these fruits and placed them in shallow styrofoam trays (150x150x18 mm). We recovered with PVC (polyvinyl chloride, thickness 0.01 mm) the treatments in which the environmental conditions were changed (25ºC, 55±5%), and stored them in biological oxygen demand (BOD) incubator chamber at 10±2ºC and 55±5% (ELETROlab®, EL131/4) relative humidity, measured daily using a digital thermo-hygrometer (Incoterm, 7664.01.0.00).

Experimental design

Completely randomized design was used, in a 4x2 factorial arrangement, with four treatments (no coating, coating with *Spirulina platensis, Chlorella sp.* or *Scenedesmus sp.*) and two conditions [with and without PVC (polyvinyl chloride)], totalizing 8 treatments, with four replicates, consisted of one fruit each.

Analyses

External appearance: evaluated by trained referees using subjective visual rating scale, based on Ferreira *et al*. (2010), with modifications. For the skin, the authors adopted the following scale: 1= orangish yellow; 2= orangish red; 3= red; 4= ripe red; 5= rotten red.

Coloration: calculated using the L*, a* and b* color system, by reflectometry, using a reflectometer (Konica Minolta, Chroma meter CR-400). For the skin, two readings were taken at the equatorial region of the fruit. For the pulp, two readings were taken in the central region of the fruit cut into halves. The equipment was calibrated to a standard white plate, following the manufacturer's instructions. Using the values L*, a* and b*, the hue angle, ºh= 90-arctang(a*/b*, and chroma saturation index, C* (C*= a*2+b*2)$^{1/2}$; were calculated.

Fresh mass loss (%): calculated by the difference between the initial mass and the final mass of the fruits at the final storage, weighed using an electronic scale, 0.01 g precision, (Bel, S2202H) and the results expressed in percentage (%).

Pulp firmness (N): determined using a digital penetrometer (Instrutherm, PTR-300), measured at two opposite points, at the equatorial region of the fruit, after removing the skin, with the aid of a 8 mm tip, according to (AOAC, 2016);

Soluble solids (SS, %): determined in the pulp by direct reading using a digital refractometer (Digital Refractometer) (AOAC, 2016);

Titratable acidity (AT, % citric acid): determined by titration of 1 g pulp and afterwards titration under constant agitation, with 0.1 M NaOH sodium hydroxide solution, the results expressed in % citric acid, (IAL, 2008);

SS/AT ratio: calculate based on the quotient between the two variables;

pH: determined by direct reading in the homogenized pulp using a digital benchtop pH meter (Digimed, DM-22) (IAL, 2008);

Ascorbic acid (mg/100g): was determined by titration of 1 g pulp diluted to 49 mL oxalic acid, performing titration, under constant agitation, with DFI solution. The results were expressed in mg/100 g, according to Tillmans method (AOAC, 2016).

Statistical analysis

Data obtained in this study were submitted to variance analysis (F test) at 5% probability, and when the results were significant, the averages were compared by Tukey test at 5% probability using software SISVAR 5.6 (Ferreira, 2014).

RESULTS AND DISCUSSION

In harvest, as the experiment was installed, a sampling consisting of 16 fruits was carried out. This sampling presented physico-chemical characteristics of tomatoes 'Santa Clara'. The fruits showed average mass of 104.74 g. The fruits were orangish yellow color, hue angle of 52.19, skin luminosity and chroma (48.74 and 45.93), respectively.

The fruits obtained in this experiment showed ascorbic acid contents lower than those reported by Caliman *et al*. (2010) in Santa Clara cultivar which showed 17.71 mg/100 g fruit fresh mass.

Titratable acidity of the fruits was 0.62% citric acid, considered to be of high quality, since the content was higher than 0.32% citric acid (Eloi *et al*., 2011). The value found was superior to the one observed by Ferreira *et al*. (2010), who found 0.21% citric acid in tomatoes 'Santa Clara'.

The average hydrogen potential was 4.54, close to the one found by Machado *et al*. (2018). Raupp *et al*. (2009) reported that pH around 4.5 is an optimal fruit conservation value. Soluble solid contents were in average 4.82%. SS/AT ratio showed an average of 7.65, being considered lower than the value presented by Sandri *et al*. (2015), 13.74 in harvesting minitomato Sweet Grape.

The coatings and the two conditions studied in this experiment significantly influenced pulp luminosity (L*), fruit mass loss (PMF), soluble solids (SS), soluble solids and titratable acidity ratio (SS/AT) and ascorbic acid (AA).

Coatings based on microalgae significantly influenced the skin chroma (C*) and titratable acidity (ATT). Whereas PVC packaging significantly influenced hydrogen potential (pH).

Coatings based on *Chlorella* sp. with or without PVC minimized the tomato mass loss in relation to the control. Fruits coated with *Chlorella* sp. without PVC showed lower mass loss (6.87) in relation to the control (31.9), representing a 78.47% reduction. The use of coating associated with PVC also provided a reduction in mass loss, with a reduced efficiency, though, 60.58% (Table 1).

Table 1. Fruit mass loss (PMF), soluble solids (SS), soluble solids and titratable acidity ratio (SS/AT), ascorbic acid content (AA) and luminosity (L)* of tomatoes 'Santa Clara' packaged with PVC and edible coating with microalgae (*S. platensis, Chlorella* sp. or *Scenedesmus* sp.) after 7 days of storage under refrigeration (10±2ºC and 55±5% UR). Pombal, UFCG, 2020.

Coating	PMF		SS		SS/AT		AA (mg/100 g)		L*	
	(-)PVC	(+)PVC	(-)PVC	(+)PVC	(-)PVC	(+)PVC	(-)PVC	(+)PVC	(-)PVC	(+)PVC
San	31.9aA	9.69aB	4.95aA	4.67aA	2.75cA	2.48bA	15.03aA	13.58cA	27.98aB	49.84aA
Ch	6.87cA	3.82bB	4.70aA	4.70aA	11.99abA	11.56aA	16.00aB	18.91aA	28.86aB	48.41aA
Sp	10.02bA	9.04abA	4.85aA	4.72aA	12.55aA	12.41aA	14.06aB	16.97abA	28.40aB	51.23aA
Sd	13.32bA	4.32aB	4.32aB	5.07aA	10.79bB	12.65aA	16.25aA	15.03bcA	28.17aB	51.33aA
CV(%)	12.45	6.75	8.37	9.14	3.89	12.45	6.75	8.37	9.14	3.89
Average	10.9	4.75	9.65	15.73	50.54	10.9	4.75	9.65	15.73	50.54

*Averages followed by the same lowercase letter in the column and uppercase letter in the line do not differ from each other by Tukey test ($p<0.05$). San: sanitized fruits; Ch: coating with *Chlorella* sp.; Sp: coating with *Scenedesmus* sp.; CV: variation coefficient.

Besides the *Chlorella* sp.-based coating, the one based on *Scenedesmus* sp. also showed to be effective in reducing the mass loss when used with PVC, representing an efficiency of 73.79% in relation to the control (Table 1). Possibly, the coatings may have avoided moisture evaporation, which provided a barrier between the skin and the environment, resulting in a lower water loss, minimizing wilting and spoilage of the fruit (Nawab *et al*. 2017). The use of PVC may have worked as a second layer between the fruit and the environment, which explains lower losses in some cases.

The lowest soluble solid content (SS) was obtained in fruits coated with *Scenedesmus* sp. without PVC (4.32%) in relation to fruits without coating and PVC, showing slower maturation of the tomato fruits, since lower quantity of SS indicates the efficiency in controlling fruit maturation which hydrolyses starch into sugar (Moreira *et al*., 2007).

SS/AT ratio showed significant difference between edible coatings with and without PVC. In the two conditions (with and without PVC) the lower SS/AT ratios were obtained in the sanitized fruits. In the fruits without packaging, coating composed of *Spirulina platensis* stood out (12.55), not differing from *Chlorella* sp. (11.99) and *Scenedesmus* sp. (10.79). The fruits which were not sanitized showed the lowest values for SS/AT ratio (2.74), representing significant increases in relation to the ones which were sanitized, 78.17, 77.15 and 74.61%, respectively. The authors observed that the fruits which were packaged in PVC showed differences between the studied coatings and sanitized fruits which showed the lowest values (2.48), in average, this increase represented about 79.69% in relation to the sanitized fruits (Table 1).

SS/AT ratio represents a quality indicator, since this is considered a reference to taste. The increase in SS/AT ratio is related to fruit maturation process, this is due to an increase in SS (Table 1) and a decrease in acidity (Table 2). The maturation process and consequent increase in SS/AT ratio is due to a degradation of polysaccharides and increased oxidation of tricarboxylic acids in the respiratory process of fruits (Teodosio *et al*., 2021). Studies on tomatoes for fresh consumption treated with cassava starch and corn starch, greater control of gas exchange with the environment was noticed, providing a less rapid maturation, maintaining the balance between sugars and organic acids for up to 12 days of storage (Menezes *et al*., 2017).

Table 2. Titratable acidity (ATT), chroma (C*) and hydrogen potential (pH) of tomatoes 'Santa Clara' packaged with PVC and edible coating with microalgae (*S. platensis*, *Chlorella* sp. or *Scenedesmus* sp.) after 7 days of storage under refrigeration (10±2ºC and 55±5% UR). Pombal, UFCG, 2020.

Coating	ATT	C*
Sanitized fruits	1.84 a	42.20 a
Chlorella sp.	0.40 b	43.94 ab
S. platensis sp.	0.38 b	42.06 c
Scenedesmus sp.	0.40 b	42.71 bc
DMS	0.05	1.78
Average	0.76	43.48
Packaging	**pH**	
With PVC	4.46 b	
Without PVC	4.59 a	
DMS	0.12	
Average	4.52	

*Averages followed by the same lowercase letter in the column do not differ significantly by Tukey test (p<0.05). DMS: Minimum significant difference.

Fruits using edible coatings of *Chlorella sp.* and *S. platensis* with PVC showed the highest ascorbic acid contents, 18.91 and 16.97 mg/100 g, respectively, a difference of 28.19 and 19.98% in relation to uncoated fruits. The use of PVC promoted higher pH value. The highest pH values can be an indicative of lower formation of organic acids associated with fruit respiration (Onias *et al*., 2018). The values found

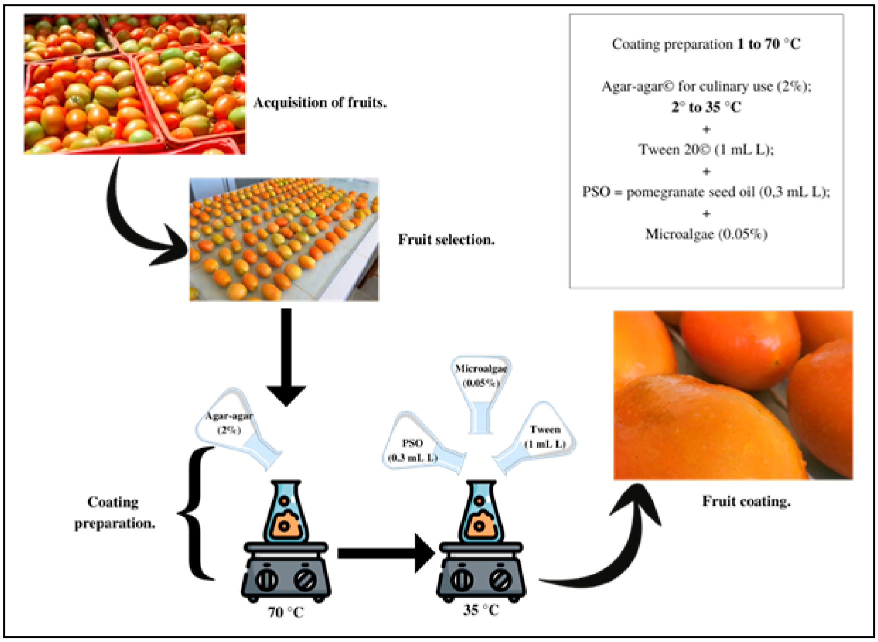

Figure 1. Flowchart representing how the coatings were prepared. Pombal, UFCG, 2020.

are within interval from 4.24 to 4.52 found by Ferreira *et al*. (2010) evaluating the quality of post-harvest tomatoes 'Santa Clara'.

Pulp luminosity was not influenced by the studied coatings. However, the fruits associated with PVC, regardless of the coating used, showed the highest values and brightest pulp compared to fruits without PVC (Table 1). Probably the second protection layer (PVC) preserved the brightness of the fruits for a longer time, which is interesting since fruits with a shiny appearance are more consumed.

The titratable acidity of the fruits was statistically higher in the sanitized fruits compared to the other coatings, with no significant interference related to the use or not of PVC (Table 2). The fruits coated with the other treatments, Sp., Ch., and Sd. showed the lowest acidity. Nevertheless, a greater acidity was observed under the effect of these treatments using concentrations of 1.3 and 5% of cassava starch in the coating of papaya fruits; this fact can be explained due to the formation of a higher semipermeable barrier around fruits, causing a lower rate of maturity (Otoni *et al*., 2011). This fact can be explained since the cell wall enzymes are released at different times, due to the nature of the pectic substances in each coating and other cell wall components (Oliveira *et al*., 2015). On the other hand, the lowest acidity observed in coated fruits may be due to the metabolism of the fruit, which is intensified when handled, leading to the consumption of organic acids, making ripening process faster, since the reduction in the acidity content is caused by the decrease in the activity of enzymes in respiratory process (Gonçalves *et al*., 2020).

Fruits coated with *S. platensis* showed the lowest values of skin chroma in relation to the uncoated ones (Table 2). The fruits with higher values of chroma are those with more vivid skin color, indicating fruit ripening process. Considering that the fruits coated with *S. platensis* were the only ones statistically different from the control, this could be an indicative of conservation of the color of these fruits.

Coatings based on *Chlorella* sp. and *Spirulina platensis* can be applied to preserve the physicochemical characteristics of tomatoes 'Santa Clara' stored for seven days under refrigeration (10±2ºC and 55±5%). PVC packaging can be used to increase luminosity and help preserve the physicochemical characteristics of tomatoes 'Santa Clara' during seven days of storage.

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