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OBTAINING BIOPOLYMERS FROM CO-PRODUCTS AND THEIR APPLICATION IN TOMATO (*SOLANUM LYCOPERSICUM*) PRESERVATION

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Abstract

The research objective was to obtain co-products from the agroindustry to elaborate edible coatings based on biopolymers (7 treatments, combining starch, pectin, whey and cinnamon essential oil) and to evaluate their effect on tomato (*Solanum lycopersicum*) preservation. Vitamin C content, pH, respiration rate, total soluble solids (TSS) content, weight loss, titratable acidity (TA) and firmness were evaluated. Tomatoes were evaluated every 72 h for 18 days. TA, TSS and pH showed slight differences for some sampling days. Still, at the end of the study, no significant differences were found between treatments (p>0.05), all values being suitable for tomato consumption. The highest respiration rate was found at 9 days, corresponding to the climacteric peak. Uncoated tomatoes had the highest respiration rate. The coating positively affected weight loss and firmness of the tomatoes, and did not modify the ascorbic acid content. It is concluded that treatment 7 (starch and essential oil) and treatment 4 (starch, pectin and whey) had a greater effect and better preservation on tomato characteristics.

Keywords: starch, pectin, post-harvest losses, biopolymers, whey.

Resumen

La investigación tuvo como objetivo obtener co-productos de la agroindustria para elaborar recubrimientos comestibles a base de biopolímeros (7 tratamientos, combinando almidón, pectina, suero de leche y aceite esencial de canela) y evaluar su efecto en la conservación de tomate (*Solanumlycopersicum*). Se evaluó el contenido de vitamina C, pH, tasa de respiración, contenido de sólidos solubles totales (SST), pérdida de peso, acidez titulable (AT)y firmeza. Los tomates fueron evaluados cada 72 h por un periodo de 18 días. AT, SST y pH mostraron ligeras diferencias para algunos días de muestreo, pero al final del estudio no se encontraron diferencias significativas entre tratamientos (p>0,05), siendo todos los valores aptos para el consumo de tomate. A los 9 días se tuvo la tasa de respiración más alta que corresponde al pico climatérico. Los tomates sin recubrimiento presentaron mayor tasa de respiración. El recubrimiento tuvo efecto positivo en la pérdida de peso y firmeza de los tomates, y no modificó el contenido de ácido ascórbico. Se

concluye que los tratamientos 7 (almidón y aceite esencial) y el tratamiento 4(almidón, pectina y suero) tuvieron mayor efecto y mejor conservación en las características del tomate.
Palabras clave: Almidón, pectina, pérdidas postcosecha, biopolímeros, suero de leche.

Introduction

In the world, approximately 20% of fruit and vegetable products are lost in the handling at harvest, inappropriate packaging or packing, inadequate transport routes, and short storage periods (Castro et al., 2017).

Tomato (*Solanum lycopersicum*) is cultivated in different countries because of its important contribution to fiber, vitamins, antioxidant capacity and lycopene content (Angarita, 2012). However, it is susceptible to mechanical damage and the presence of fungi, making it necessary to preserve its quality; for this reason, the use of bio-coatings made from polymeric materials is a viable alternative (Zhang et al., 2020) due to their antimicrobial and antifungal effects, which act as a barrier, reducing (Atarés & Chiralt, 2016a). They act as a barrier, reducing microbiological alterations and extending stability by reducing the exchange of moisture, lipids, volatiles and gases between the food and the environment (Tian et al., 2020)

The solutions that make the coatings are elaborated based on polysaccharides, proteins and lipids, or a mixture of them (Atarés & Chiralt, 2016b). Therefore, they are effectively used on fruits and vegetables to prevent damage and are employed as active packaging, not consumed without causing damage to health.

Polymers are mostly obtained from processing other products, either as co-products or waste products (Commission for Environmental Cooperation-CEC, 2017). These are the main products not used in agribusiness. So there is a trend to use by-products to increase their value as raw materials or sources of various substances or metabolites.

Obtaining biopolymers from co-products can be employed to prolong shelf life and improve post-harvest quality (Zhang et al., 2020)and(Ma & Wang, 2016)because they provide an odorless and transparent film with low permeability to oxygen and other gases and active compounds that help in antifungal control (Ortega-Toro et al., 2017).

On the other hand, starch presents a poor hydrophilic character and little water vapor barrier power (Teixeira et al., 2015). Therefore, research is being carried out to obtain starch blends with other biopolymers. (Nouraddini et al., 2018). Pectin is a polysaccharide in plant tissues mainly composed of galacturonic acid (Canteri et al., 2012). Reports show the use of pectin to elaborate biocoatings due to its capacity to transport antimicrobial compounds (Kumar et al., 2020). Likewise, whey is a residue that contains many nutrients, mainly composed of protein (Ryan & Walsh, 2016; y Kolev Slavov, 2017).

Research on edible coatings based on whey proteins are good barriers to O_2, their mechanical properties improve considerably, by the addition of a plasticizing agent, such as glycerol, in the same way, the action of lipids on the control of water vapor permeability has been included, obtaining good results combined with beeswax (Mohd et al., 2019).

The objective of the work was to obtain biopolymers from agroindustry co-products such as cocoa shell pectin, whey and malanga starch and to evaluate the effect of the coatings on the shelf life of red tomato stored at room temperature in the humid tropics.

Materials and methods

Biological material

The biopolymers used were malanga starch (*Colocasia esculenta*), pectin and whey. We followed the methodology proposed by Medina et al. (2007) to obtain the starch. First, the samples were obtained from a local market, 4.5 kg of fresh white, clean and not diseased malanga, peeled and cut into cubes of approximately 3 cm^3, then blended (Oster blender) adding water in a ratio of 1:6, for a time of 2 min, thus reducing the particle size. Next, the starch slurry was filtered in sieves (80 mesh), thus eliminating the fiber and other existing particles; the filtrate obtained after sieving was left to settle for 15 h at room temperature. After this time, most of the supernatant liquid was decanted and the starch slurry was washed twice with distilled water, leaving it to settle between each wash; then it was dried in a convection oven (Felisa model FE-291) at 60 °C for 24 hours. Finally, it was ground, and sieved in 100 mesh and the starch was stored in a plastic bottle with an airtight lid for its later use.

To obtain pectin, cocoa fruit waste shells in good condition were selected, then chopped and dehydrated in the sun (room temperature) for 96 h until reaching a moisture content of approximately 10%. Once dried, they were ground (manual screw mill) to a particle size of 0.17 mm using an 80 mesh sieve. This flour was stored in jars in a dry place and was used to obtain the pectin following the methodology proposed by Sosa-Romero et al. (2016), using citric acid solution (2.8 %), pH 2.0, as an extractant agent. A 10:1 (w/w) ratio of extractant solution: peel flour was used. The acid solutions were heated for 90 min at 85 °C with constant stirring. Then they were filtered using gauze, cooling rapidly below 25 ºC. The supernatant was added 96 % ethanol and allowed to stand for 1 hour. Finally, the extracted pectin was dried at 55 °C for 20 h. The pectin extraction yield was estimated as the ratio between extracted pectin's weight and the dried peel's initial weight.

The whey used was purchased directly (5 L) from a home cheese producer in Tapachula, Chiapas, Mexico, transferred to the laboratory and stored at 4 ºC until use (no more than 12 h).

To evaluate the effect of the coatings, tomatoes were acquired at advanced physiological maturity (breaking) in the supply market of the municipality of Tapachula, Chiapas, Mexico.

Development and application of biorelayers

For the formulation of the biorelief solutions, we followed the method proposed by. Weiwei et al. (2016).. Individually, aqueous solutions of malanga starch (2.5 % w/v), cocoa shell pectin (1.25 % w/v) and whey (50 % v/v) were prepared. Each of the solutions was kept under magnetic stirring for 20 min. Once the solutions were dissolved, through a completely randomized design, they were combined to obtain six treatments as shown in Table 1. Subsequently, 1.5 % v/v glycerol was added to all the solutions and then they were brought to 90 ºC for 15 min under constant agitation; After this time, the solutions were cooled to room temperature, 0.5 % (v/v) of Tween 20 was added and homogenized for 30 min, 2 % (v/v) of cinnamon essential oil was added. The solution was taken to ultrasound in an Ultrasonic Processor© VCX 500 sonicator (500 W, 20 kHz), the tip used was 13 mm, for 5 minutes at 60 % amplitude with 5.0 s of activity and 1.0 s of inactivity.

The tomatoes were selected according to the degree of maturity and free of physical damage; they were then washed with potable water and dried for 20 min. They were then numbered in ascending order and a completely random selection was made according to the treatments evaluated. Thirty-three tomato fruits were used for each treatment. First, the respective solutions were applied to the tomato fruits using a small brush; then the fruits were allowed to dry at room temperature for 30 min. The coated fruits were then stored at room temperature of 28- 30 °C and relative humidity (RH) of 80% for 18 days. Uncoated fruits were used as control.

Table 1. ***Formulation of biopolymers using agro-industrial co-products.***

	Starch (%)	Pectin (%)	Cinnamon essential oil (%)	Serum (%)	Tween® 20 (%)	Glycerol (%)
T1	-	-	-	-	-	-
T2	-	1,25	2	50	0,5	1,5
T3	2,5	-	2	50	0,5	1,5
T4	2,5	1,25	2	50	0,5	1,5
T5	2,5	1,25	2	-	0,5	1,5
T6	-	1,25	2	-	0,5	1,5
T7	2,5	-	2	-	0,5	1,5

Shelf life of tomato fruits

For each treatment, 3 fruits (21 in total per sampling) were taken at random every 72 hours for 18 days (6 samplings) to determine the post-harvest quality of the fruits, evaluating titratable acidity content, pH, total soluble solids (°Brix), weight loss (%), ascorbic acid content, CO2 production (respiration) and firmness.

a. Determination of titratable acidity

The tomatoes were crushed in a mortar, then 1 g of sample was weighed on a balance, then 10ml of distilled water in an Erlenmeyer flask was added to the sample and three drops of phenolphthalein were added, followed by titration with NaOH 0.1N, until a permanent pink tone and the volume spent was noted. Finally, the percentage of acidity was calculated with the following formula(Romero et al., 2005)where: N = normality of the NaOH solution, V = ml of NaOH used in the titration, peX= equivalent weight of citric acid (0.064), W= weight of the sample. The results are expressed as citric acid content (%).

b. pH determination

The pH of the tomato was determined by crushing the sample in a mortar and weighing 1 g of sample on the digital balance, 10 ml of distilled water was added and finally, the potentiometer (Starter 2100 brand) was placed, allowing the pH value to stabilize on the display of the equipment.

c. Soluble solids determination

The juice was extracted from the fruit and then a drop of the sample was placed on the prism of the refractometer (brand Atago- A524794), observing the value recorded by the instrument. The results are expressed in ºBrix.

b. Firmness

For the measurement of tomato fruit firmness, an Extech 575044 penetrometer was used to measure the firmness of three parts of the fruit and the value was recorded in Newton (N).

c. Weight loss

To determine the weight loss of tomato fruit, a Sartorius 225S-100-DA analytical balance with a measuring range of 0 to 250 g was used to weigh the fruit each day of evaluation. The loss of each fruit was calculated individually considering the weight on the day of sampling according to the weight at the beginning of the study.

d. Determination of ascorbic acid

2 ml of ascorbic acid standard solution (1mg/ml) were pipetted for which an Erlenmeyer flask of 250 ml was used, 10 ml of acetic acid at 10 % and 50 ml of distilled water were added. The content of the flask was titrated with the solution of 2,6 dichlorophenol indophenol, until it presented a slightly pink color for at least 15 s. The mg of ascorbic acid of the samples were calculated according to the volume used in the titration of one ml of indicator after titrating 10 ml volumes of tomato aqueous extract.

e. Respiration (CO2 produced)

To determine the respiration rate, a container with 424.05 cm3 was used, which was connected to two hoses at the top, where the tomato fruits (approximately 250 g) were placed. First, the container was hermetically covered for one hour. After that time, the hose was uncovered and connected to the IAQ-CALC model 8762 respirometer probe, which measures the amount of CO2 accumulated in the container. From this data, the amount of CO2 produced was calculated and the results are expressed as mg/kg/h.

Data analysis

The data obtained were subjected to analysis of variance (ANOVA) and subsequent comparison of means by Tukey's test (α=0.05). All analyses were processed using the statistical program Infostat Professional© version 2019.

Results and discussion

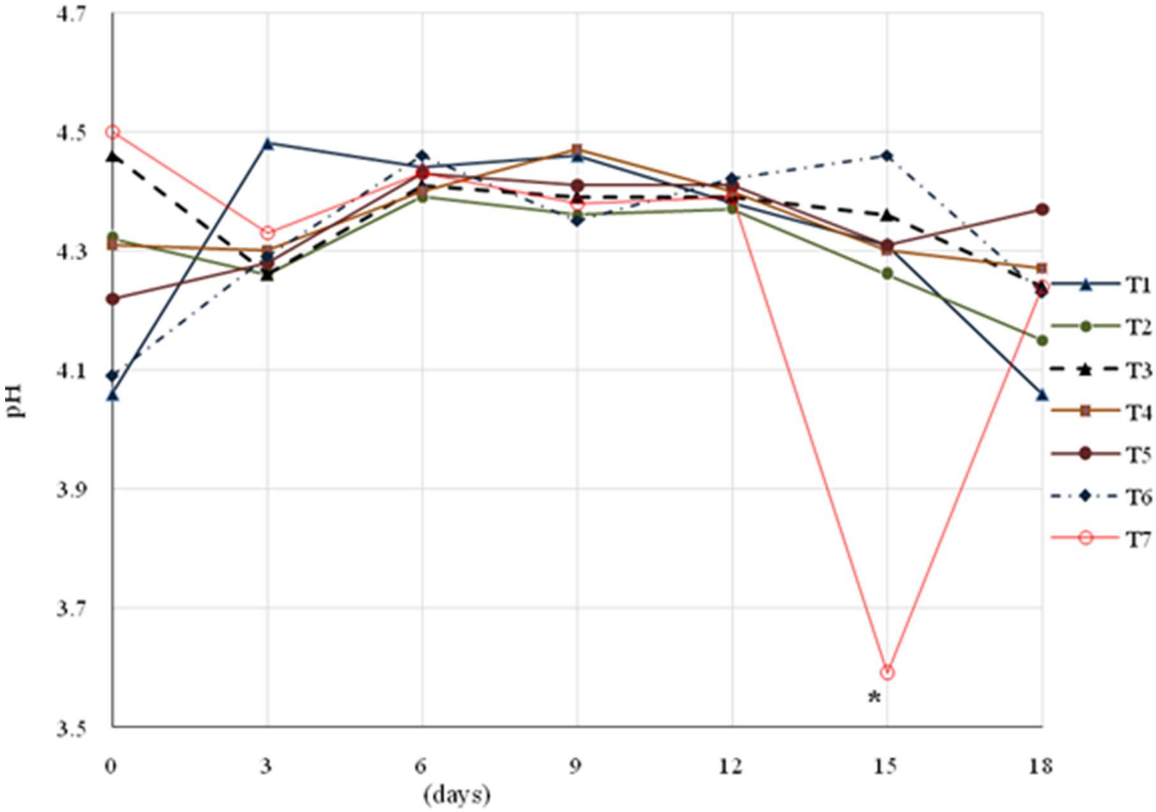

Figure 1. Evolution of pH in tomato fruits with bio-coating.
Source: Own elaboration

The large and small dairy and agricultural processing companies generate a large amount of waste derived from the different processes they give to raw materials; many of these companies do not give added value to the waste they generate and throw it into the environment, which becomes a pollutant for the environment since these wastes have different compounds that by the action of microorganisms begin to degrade, which is why in recent years several researchers are conducting studies to give added value and obtain by-products from the waste generated(Beltrán-Ramírez et al., 2019). Biotechnology allows the reuse of agro-industrial wastes through a series of processes to be converted into products or inputs of commercial interest. Therefore, the use of agroindustrial by-products has incidence and importance in preserving the environment, considering the development of technologies oriented towards a sustainable transformation of natural resources.

Regarding the pH of tomato fruits, it was observed during the 18 days of evaluation that the evolution was similar for all treatments with no significant difference ($p>0.05$) except day 15 in which a difference is found in treatment 7 concerning the other treatments ($p<0.05$) as shown in Figure 1. This may be because the tomato fruits coated with this treatment were not yet ripe. These results are in agreement with those of(Garcia et al., 2014), who states that the change in pH could be attributed to metabolic changes and water loss, the variation of pH in tomato fruits is a parameter that increases with ripening and storage time; while for fresh tomatoes it can vary between 4.17 to

4.59 (Cantwell et al., 2006). These values reported by other researchers are similar to those recorded in the present investigation.

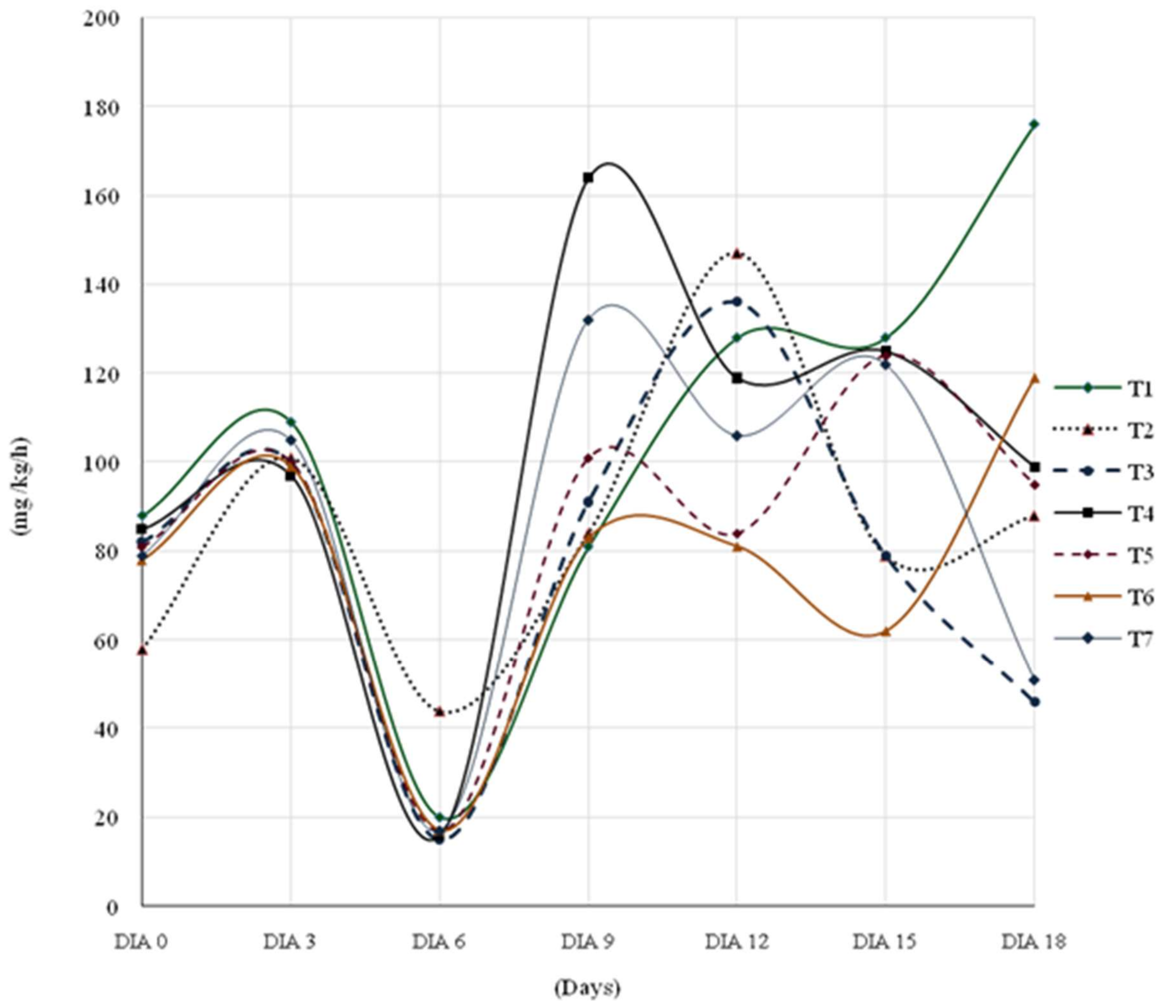

Figure 2. Variation in respiration rate of bio-coated tomatoes during storage.
Source: Own elaboration

It is known that, during the ripening process, there is an increase in the amount of ethylene produced, this increase is accompanied by an increase in the fruit respiration rate (typical of climacteric fruits). Data from the literature indicate that after harvest, biochemical changes still occur in the fruit, and the rate of respiration depends on factors that influence the rate of fruit respiration(Horvitz, 2017). The rate of respiration depends on factors such as fruit variety, storage temperature, storage humidity, fruit age, etc. Figure 2 shows the respiration curve of tomatoes with and without biocoatings. It shows that on day 3 there was an increase in the amount of CO2 produced, possibly due to the stress of fruit handling, followed by a drastic decrease on day 6, and day 9 presented the highest peak, possibly the climacteric peak. From that day on, the biochemical characteristics that make the product suitable for consumption are developed and then aging or overripening begins until the product deteriorates, although on day 18, treatment 1 (Control) is the one with the highest respiration rate. However, the values obtained do not agree with(Valle & Rodriguez, 2019). The difference may be due to the storage conditions (temperature) and storage time.

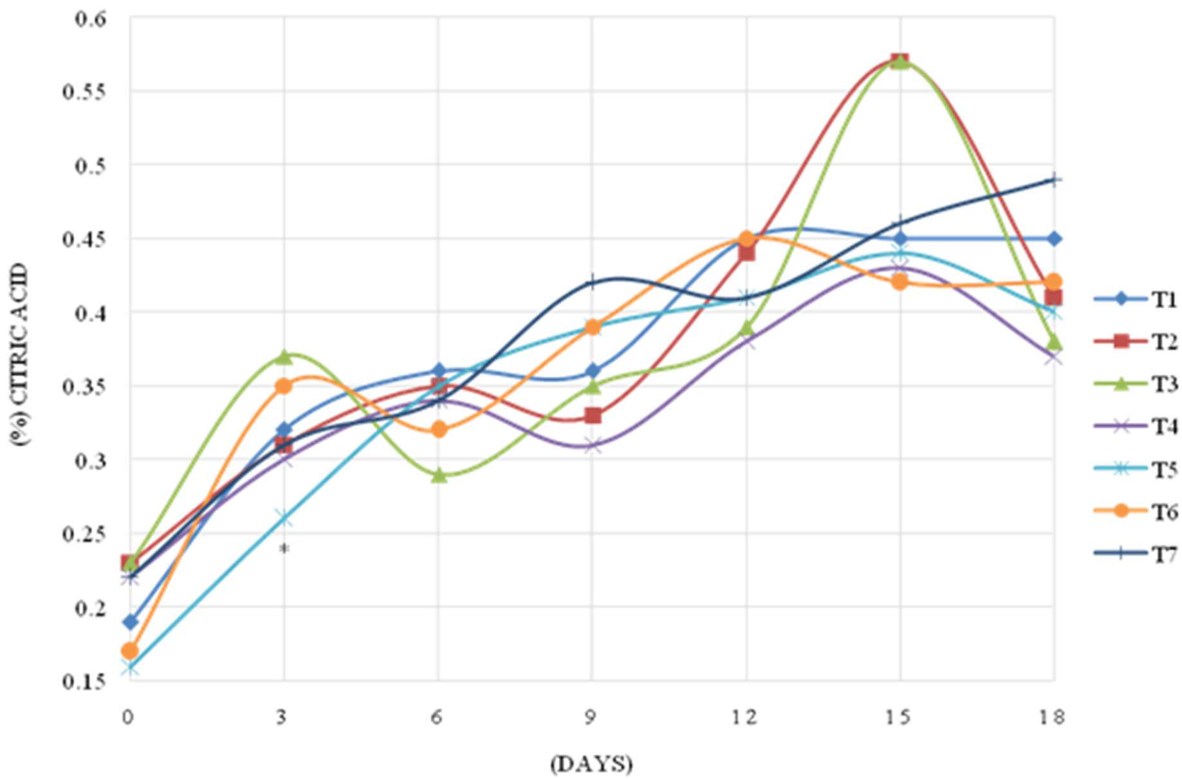

Figure 3. Variation of tomato acidity with bio-coating.
Source: Own elaboration

In Figure 3, it can be observed that on day 3 there was a significant difference between treatments ($p<0.05$). This may be because on these days, the ripening process was different; for the rest of the days there is no difference, having an average acidity of 0.39 %; however, the highest acidity was reported on day 15 with a moderate acidity of 0.5 % that corresponded to treatments 2 and 3. Later the acidity began to decrease as observed in Figure 3; these results agree with what was reported by (Duma et al., 2017). Furthermore, the results obtained are in agreement with those of the authors of the study "Acidity level is a factor that affects the stability of vitamin C during storage in fruits and vegetables," in which they state that the level of acidity is a factor that affects the stability of vitamin C during storage in fruits and vegetables. (Valle & Rodriguez, 2019)who reported that they found an average of 0.6 % titratable acidity in tomato fruits.

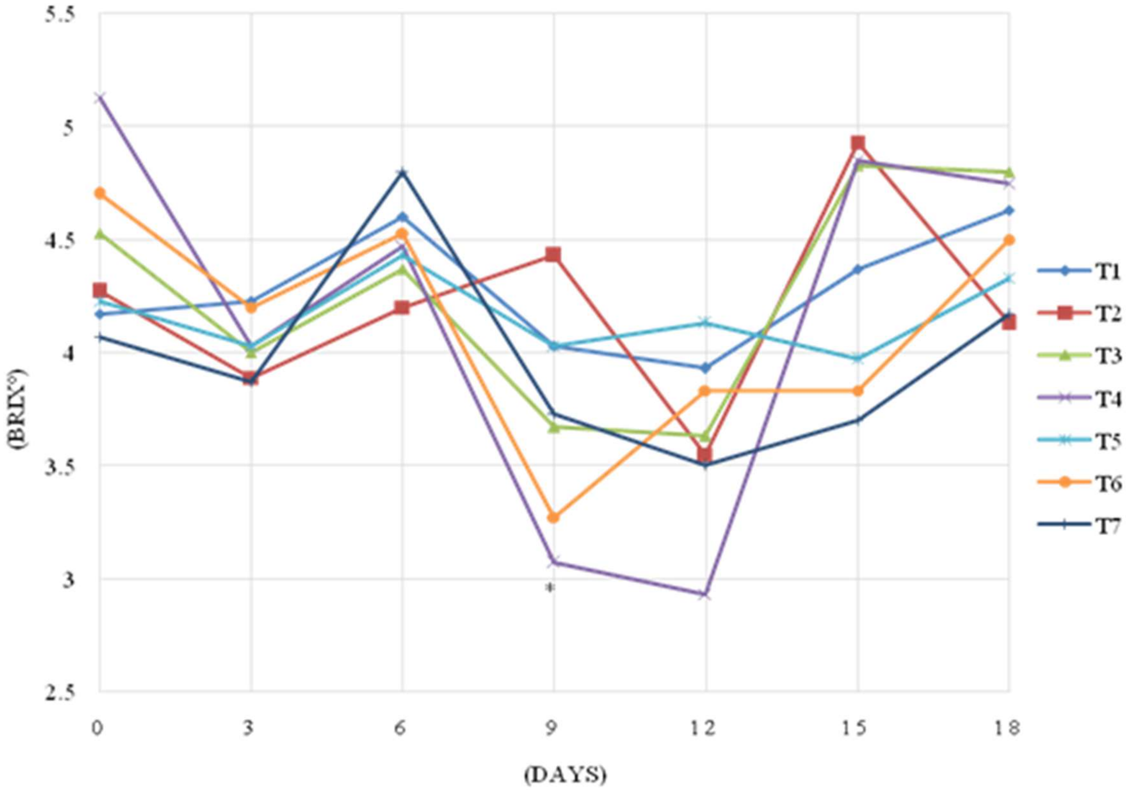

Figure 4. Variation of soluble solids content during storage of bio-coated tomato.
Source: Own elaboration

The contents of total soluble solids (ºBrix) observed in Figure 4 reveal that only on day 9 is there a significant difference between treatments; for the rest of the days, there is no difference; in addition, it can be observed that the TSS values are within the range to be considered as products that are fit to be consumed, the values that have been obtained in the research are similar to what is stated in Figure 4(Segura et al., 2009)who says that the tendency of TSS during storage is ascending, TSS vary between 4.0 and 7.5 °Brix. Therefore, the soluble solids during the storage process of fruits tend to increase, which coincides with what was reported by Garcia (2015).

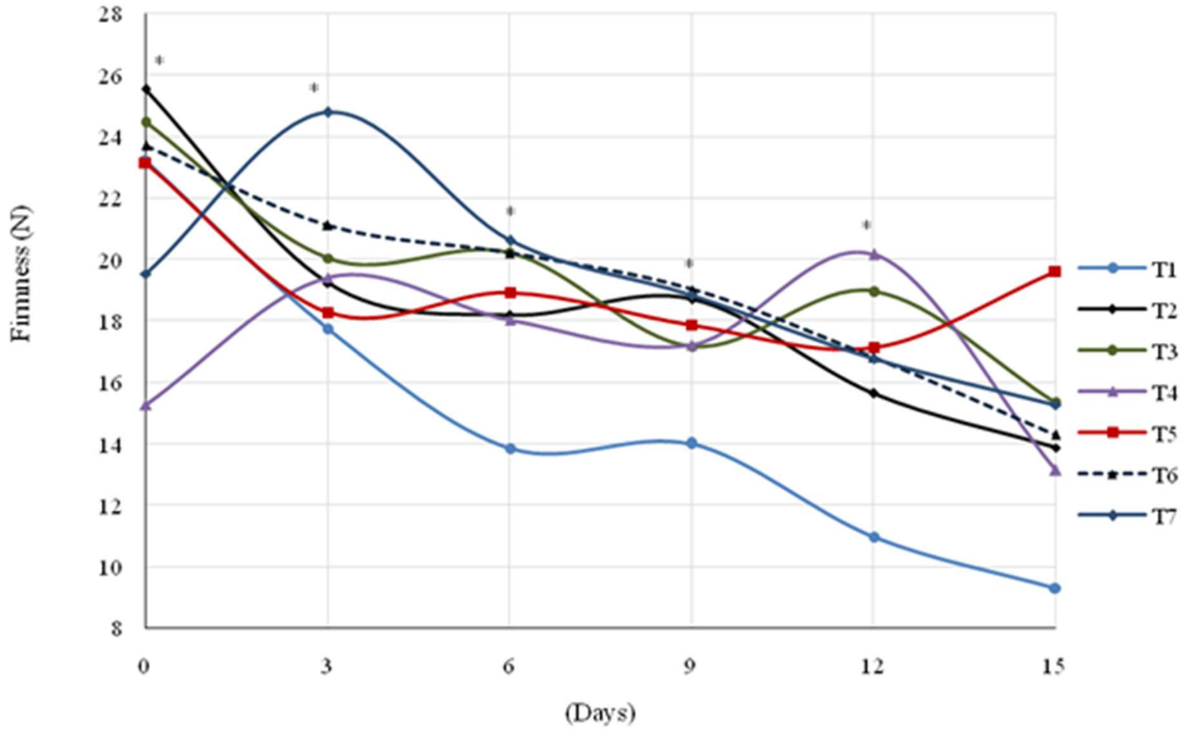

Figure 5. Variation of firmness in bio-coated tomato fruits.
Source: Own elaboration

The firmness or hardness of tomato fruits decreased during storage, in this case, there was a significant difference between treatments ($p<0.05$) between the first 12 days of evaluation, as shown in Figure 5. However, the fruits that lost more firmness were those of treatment 1 (Control). The loss of firmness is related to the oxygen content in the atmosphere, being more effective in delaying the changes in those with a lower concentration of this gas, which was also observed by other researchers; (Mekhled et al., 2020).The researchers concluded that the fruit maturity stage at harvest substantially influences fruit flesh firmness and the levels of lipophilic and hydrophilic antioxidants in ‘Red Rose’ tomato fruit grown in a protected environment.

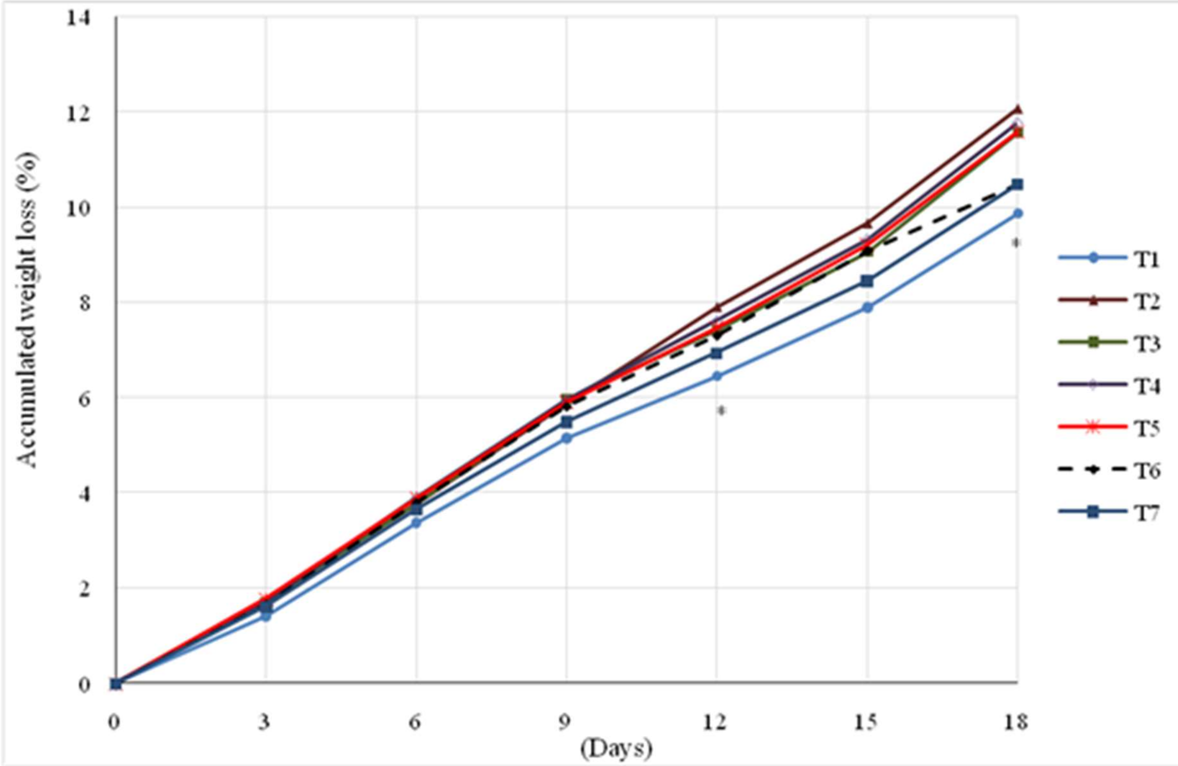

Figure 6. Evolution of cumulative weight loss of bio-coated tomato.
Source. Own elaboration

During the 18 days of storage, it is found that if there was a significant difference between treatments for weight loss on days 12 and 18 ($p<0.05$), from this it can be observed in Figure 6 that the control was the one that lost the least weight 10 % and the fruits that had the treatments lost about 11 % weight. This case can be explained by the fact that the fruits with the different treatments had some burns due to the action of the essential oil since the concentration in the solutions was high and this caused the fruits to have stress due to damage, which led to a greater loss of water in the fruit. It is reported that the factors determining weight loss in fruits and vegetables are the surface area/volume ratio, the fruit wall's nature and the fruit's condition. (Khalid et al., 2017). However, in the present investigation, the loss of water by transpiration was due to the condition of the fruit, in addition to the state of the fruit(Thompson et al., 1998). In the present investigation, however, the loss of water by transpiration was due to the condition of the fruit, and it is stated that water is lost to the environment as water vapor moving from the intercellular spaces in the parenchyma to the outside atmosphere. According to the values obtained in the laboratory, which are shown in Figure 6, it can be deduced that the weight loss of the tomato is uniform. It is considered that the weight loss was around 10% from the first day to the last day of evaluation.

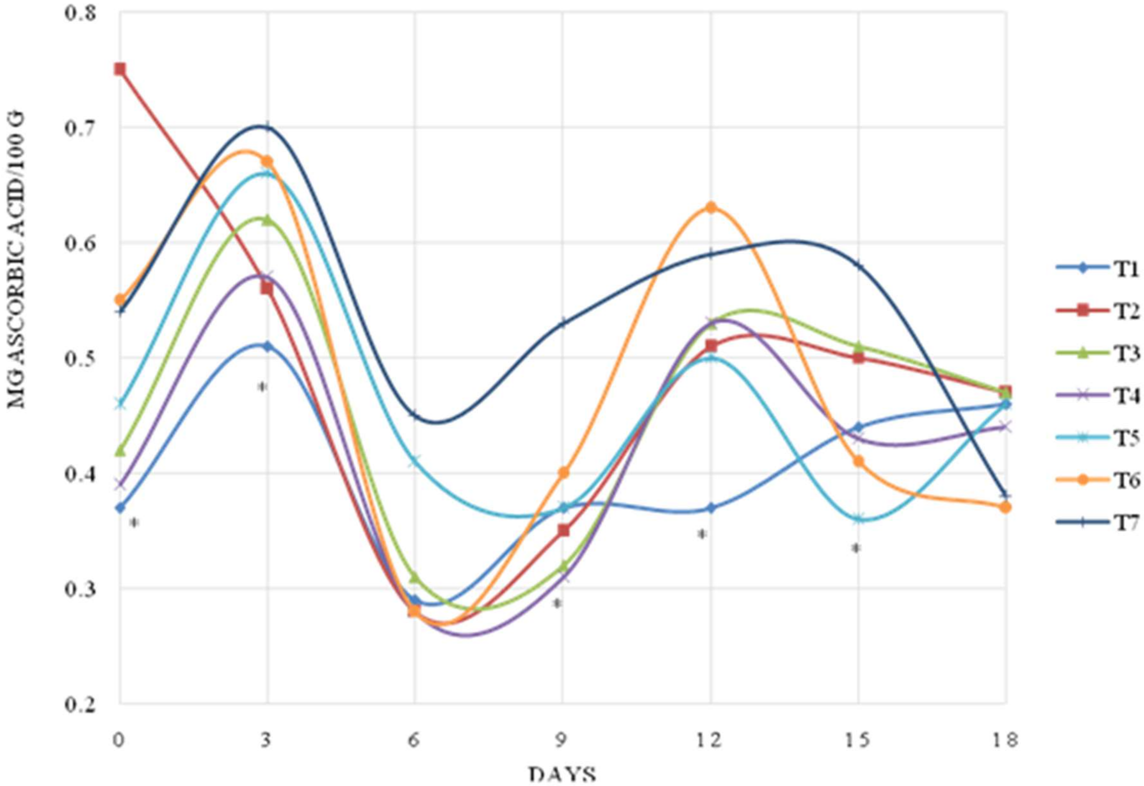

Figure 7. Variation in ascorbic acid content of tomato.
Source: Own elaboration

Fruits and vegetables, after harvesting undergo multiple biochemical changes that modify the concentrations of carbohydrates, pigments, vitamins, minerals, proteins, and flavor (Valle & Rodriguez, 2019).The vitamin C content varies with the degree of maturity of the fruits; it is maximum when the fruit is still immature; an example of this is the tomato (Petropoulos et al., 2018). Figure 7 shows a significant difference between treatments except for days 6 and 18 in which there is no difference; the differences may be due to the different stages of maturity in which the fruits were found due to the effects of the different treatments. One of the richest sources of vitamin C is camucamu with 2780 mg/100 g; compared to oranges, camucamu provides 30 times more vitamin C. Other sources of vitamin C are the fruits of the camucamu family (Barrera et al., 2010)other sources of vitamin C are vegetables such as wild cabbage 136 mg/100 g. (Ministry of Health et al., 2009). According to the World Health Organization (WHO), they recommend that the daily requirement or consumption of vitamin C is 45 mg. (Ministry of Health et al., 2009). Figure 7 shows that the vitamin C content of tomatoes is 40 mg/g, so if you want to complete the daily vitamin C diet suggested by the WHO, you would have to consume 100 g, approximately 1 to 2 tomatoes per day.

Conclusions

The edible coatings applied on tomato fruits have allowed to maintain their quality, extending the product's commercial life and improving its appearance. However, to improve the effect of biocoatings, reducing the percentage of essential oils in the solutions is recommended. In addition, implementing biodegradable and environmentally friendly products, such as polymers based on agro-industrial co-products, can be a natural alternative for elaborating edible coatings that help reduce post-harvest losses and conservation costs.

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All authors made significant contributions to the document, agree with its publication, and declare no conflicts of interest in this study.

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