Characterization and Application of Nanomaterials (2022) Volume 5 Issue 2
doi:10.24294/can.v5i2.1686

ORIGINAL RESEARCH ARTICLE

Edible alginate-based coating in combination with nanoencapsulated eugenol and its preservative effect on the shelf life of tomato (*Solanum lycopersicum*)

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ABSTRACT

Deficiencies in postharvest technology and the attack of phytopathogens cause horticultural products, such as tomatoes to have a very short shelf life. In addition to the economic damage, this can also have negative effects on health and the environment. The objective of this work is to evaluate an active coating of sodium alginate in combination with eugenol-loaded polymeric nanocapsules (AL-NP-EUG) to improve the shelf life of tomato. Using the nanoprecipitation technique, NPs with a size of 171 nm, a polydispersity index of 0.113 and a zeta potential of −2.47 mV were obtained. Using the HS-SPME technique with GC-FID, an encapsulation efficiency percentage of 31.85% was determined for EUG. The shelf-life study showed that the AL-NP-EUG-treated tomatoes maintained firmness longer than those without the coating. In addition, the pathogenicity test showed that tomatoes with AL-NP-EUG showed no signs of damage caused by the phytopathogen Colletotrichum gloesporoides. It was concluded that the formulation of EUG nanoencapsulated and incorporated into the edible coating presents high potential for its application as a natural nanoconservative of fruit and vegetable products such as tomato.

Keywords: Shelf Life; Edible Coatings; Eugenol; Polymeric Nanoparticles; HS-SPME

ARTICLE INFO

Received: 29 June 2022
Accepted: 11 August 2022
Available online: 22 August 2022

1. Introduction

Mexico is the producer of agrifood products currently among the world's top 10. Tomato (*Solanum lycopersicum*) is one of Mexico's most important fruit and vegetable products. Unfortunately, its very nature, the deficiencies in post-harvest technology and the attack of phytopathogens cause that, in general, fruit and vegetable products have a very short shelf life and post-harvest losses of up to 50% of total production, according to information from the Food and Agriculture Organization of the United Nations[1]. In recent years, the use of natural preservatives in the food industry has become a trend due to consumer demand for natural products. In particular, essential oils (EOs) are secondary metabolites of aromatic plants and have a wide range of biological activities (e.g. antioxidant and antimicrobial) that have made them emerge as an alternative for the control and reduction of postharvest losses[2,3]. In fact, the antimicrobial properties of EOs and their components have been exploited to control fungi and phytopathogenic bacteria[4,5]. Eugenol (EUG) is a phenolic derivative commonly known as

clove essence, which is extracted from the EOs of pepper, bay, cinnamon and camphor, among others. EUG has been shown to exhibit significant antimicrobial activity against bacteria and fungi[6], moreover, the U.S. Food and Drug Administration (FDA) has classified it as a GRAS (Generally Recognized As Safe) substance and it has been approved by the European Commission as a food additive[7]. Unfortunately, the application of compounds such as EUG, as food additives, has limitations as they exhibit strong lipophilic character, high volatility, are insoluble in water and are easily deteriorated by environmental factors, such as light and oxygen, making it difficult to incorporate them into commercial products[8,9]. In recent years, nanotechnology in the food industry has presented an important development, offering new alternatives to overcome these impediments. Nanoencapsulation involves the incorporation, adsorption, solubilization or dispersion of bioactive compounds (e.g., EUG) in or on a nanoscale polymeric structure. Incorporation of these biocompounds into polymeric nanoparticles (NPs) based on preformed polymers (e.g., Eudragit L 100-55) can protect them against degradation, thus improving their physical and chemical stability. In addition, the combination of these NPs with so-called edible coatings (RC) has emerged as an important alternative in food preservation. An RC is defined as a thin, continuous layer of some material that is incorporated on the food. Alginate (AL) is a linear glycosidic anionic polysaccharide consisting of monomeric units of D-mannuronate and L-guluronate and is obtained mainly from two sources: brown algae (Phaeophyceae) (40% of dry matter) and bacteria[10,11]. This polymer has been used in the food industry as a coating or packaging material, in addition, it is also recognized by the FDA as a GRAS substance. The incorporation of nanomaterials and antimicrobials, including essential oils and their components, in CR has been studied to give new properties to the coating and to improve the safety and shelf life of fruits and vegetables[12,13]. In this context, a combined system of NPs, CRs and compounds exhibiting biological activity, such as EUG, may have potential as an alternative to synthetic agrochemical preservatives.

Therefore, the present study focuses on evaluating the efficacy of a combined NP-EUG system with an alginate RC to extend the shelf life of tomato (*Solanum lycopersicum*) and inhibit the phytopathogenic action of Colletotrichum gloesporoides.

2. Materials and methods

2.1 Materials

Alginate (PM 216.12 g·mol^{-1}) and EUG (Eugenol Reagent Plus 99%) were purchased from Sigma Aldrich® brand. Polyvinyl alcohol (Mowiol 4-88 with a PM 26.000 g·mol^{-1} and hydrolysis degree of 88%) was kindly donated by Omya AG. Commercial tomatoes (*Solanum lycopersicum*) which presented homogeneous characteristics of color, texture and size were used for the shelf-life tests. Acetone and methanol were purchased from TEDIA® brand. Eudragit L 100-55 polymer (Methacrylic acid: Ethyl acrylate (1:1), PM 320,000 Da) was kindly donated by Evonik Industries®. Sodium hydroxide was purchased from MERCK® brand and 75 µm Carboxen/Polydimethyl- siloxane SPME (CAR/PDMS) fiber from Supelco-Sigma Aldrich brand®.

2.2 Nanoformulation with eugenol and its incorporation into an edible coating

NPs with EUG were prepared by the nanoprecipitation technique[14]. For this purpose, 4 mL of organic phase containing Eudragit L 100-55 polymer (55 mg) and EUG (60 mg) dissolved in acetone were injected into an aqueous phase (25 mL) containing 0.5% (w/w) PVA surfactant. The diffusion of the organic phase into the aqueous phase induced the aggregation of the Eudragit L 100-55 polymer and thus the encapsulation of the EUG (NP-EUG) inside it. Finally, the solvent was removed with using a rotary evaporator (Control Laborota 4003, Heidolph Instruments, GER). NPs without EUG (NP-BCO) were obtained following the same procedure described above.

The average particle size and poly-dispersity index (PI) of NP-EUGs were measured at a scattering angle of 90 degrees using dynamic light scattering, whereas, zeta potential measurement was

performed by laser doppler microelectrophoresis (Zetasizer Nano-ZS90, Malvern Instruments, UK).

For the formation of the edible coating, AL was used as the forming agent. The solid AL was incorporated into an aqueous dispersion of NP-EUG with magnetic stirring until complete dissolution.

2.3 Analysis of the nanoformulation by gas chromatography with flame ionization detector (GC-FID) and headspace mode solid phase microextraction (HS-SPME)

To determine the percent encapsulation efficiency (%EE), the NP-EUG dispersion was centrifuged (Allegra 64R Centrifuge, Beckman Coulter, USA) and the sediment formed by the NP-EUGs was subjected to the HS-SPME technique using a carboxen/polydimethylsiloxane (CAR/PDMS) fiber of 75 µm coating thickness to quantify the EUG in the NPs by GC-FID (Clarus 480, Perkin Elmer, USA). A capillary column (Elite-5, Perkin Elmer, USA) (30 m × 0.25 mm × 0.25 µm) was used for the chromatographic method. The injector and detector temperatures were 270 °C. The oven temperature was programmed as follows: 70 °C for 1 min, increased by 30 °C·min^{-1} to 190 °C, increased by 10 °C·min^{-1} to 210 °C and finally increased by 20 °C·min^{-1} to 270 °C and held for 1 min. The flow rate of helium carrier gas (99.999% purity, INFRA®) was 1 mL·min^{-1}. Subsequently, the percent encapsulation efficiency (%EE) was calculated using the following formula:

$$\%EE = (EUGc/EUGt) \times 100 \quad (1)$$

Where EUGc is the amount of EUG quantified in the NP-EUGs (mg) and EUGt is the amount of total EUG (mg) used in the organic phase of the nanoformulation.

2.4 Evaluation of the preservative effect of nanoformulation in combination with an edible coating on the shelf life parameters of tomato

Tomatoes (*Solanum lycopersicum*) with homogeneous characteristics of color (ripening), size and without mechanical damage were selected for the application of the different treatments. They were washed with distilled water and dried. The fruits were distributed in five groups of three tomatoes each. The first group was used as CONTROL (no treatment). The second group was put in contact by immersion with a 0.5% (w/w) AL solution (ALG) for 1 min. The third group was applied by immersion, under the same conditions as above, a RC treatment of AL with EUG without nanoencapsulation (ALG-EUG). The fourth group was treated with a NP dispersion without active, i.e., without EUG (NP-BCO). Finally, the fifth group was immersion-treated with an aqueous dispersion of the 0.5% (w/w) RC of AL with the nanoformulation with EUG incorporated (ALG-NP-EUG). All groups were maintained at 25 °C and 35% humidity for 16 days. After this time, each group underwent the evaluations mentioned below:

Evaluation of firmness. Firmness of tomatoes was measured by using a Texture Analyzer (CT3 Texture Analyzer, Brookfield-Ametek, USA) equipped with a cylindrical probe of 2 mm diameter. Firmness was expressed in Newton (N).

Evaluation of color change. Color values (CIE L* a* and b*) of tomatoes were determined by direct measurement of the fruit surface using a colorimeter (ColorFlex EZ, HunterLab, USA). Total color change (ΔE) was measured using the following equation:

$$\Delta E^* = ((L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2)^{1/2} \quad (2)$$

Where ΔE* is the total color change. L^*_1/L^*_2 is initial brightness/lightness obtained. a^*_1/a^*_2 is initial red-green color/red-green color obtained and b^*/b^*_2 is initial yellow-blue color/yellow-blue color obtained.

Evaluation of total soluble solids content. The total soluble solids (TSS) content of tomato juice was obtained directly by refractometry (Abbemat 200, AntonPaar, AUT).

Evaluation of titratable acidity. For the analysis of postharvest fruit quality, titratable acidity (TA) was determined by titration of tomato juice using a 0.1 N NaOH solution until the end of titration (pH = 8.2). The result was expressed in grams of citric acid per 100 mL of juice.

All parameters were determined in triplicate at the beginning and at the end of the shelf-life study.

In addition, the results were analyzed in the statistical program Startical Product and Service Solutions (SPSS Statistics version 23) by means of an ANOVA ($p = 0.05$).

2.5 Evaluation of the protective effect of nanoformulation in combination with edible coating on tomato fruits inoculated with the phytopathogen Colletotrichum gloesporoides

The tomato fruits were cleaned and disinfected. Each of the treatments was applied by immersion. Under aseptic conditions, three wounds were made on their surface. In one of them, the phytopathogen Colletotrichum gloesporoides was inoculated by striation and another wound by puncture. The fruits were placed in a humidity chamber at 25 °C for 5 days. At the end, the absence or presence of the phytopathogen growth on the tomato was observed.

3. Results and discussion

3.1 Obtaining and physicochemical characterization of the nanoformulation with eugenol

Generally, Eudragit polymers have been used to modify drug release profiles by offering protective and sustained release properties[13]. Due to the properties exhibited by such polymers (e.g., good stability, controlled release, and taste and odor masking), their use confers protection to bioactive compounds of unstable chemical nature, not only in the pharmaceutical industry, but also in the food industry. In the present study, Eudragit L 100-55 polymer was used, which is an anionic copolymer derived from acrylic and methacrylic acid (**Figure 1**).

This polymer is proved to be very attractive for usage in the food industry, as it exhibits excellent off-flavor masking properties as well as controlled release in a pH-dependent manner[15]. In the present study, NP-BCOs with an average size of 164.2 ± 6.6 nm, a PI of 0.084 ± 0.018 and a zeta potential of −1.80 ± 0.64 were obtained. The physicochemical characteristics for the NP-EUG nanoformulation are presented in **Table 1**.

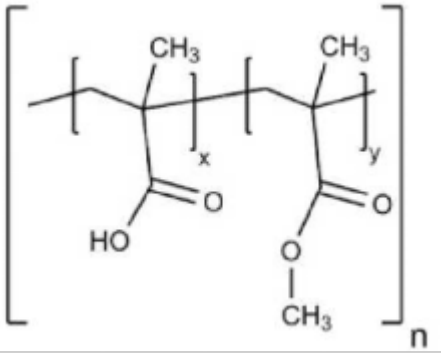

Figure 1. Basic structure of Eudragit L 100-55 polymer.

Table 1. Physicochemical characteristics of NP-BCOs and eugenol-incorporated nanoformulation obtained by the nanoprecipitation technique ($n = 3$; $\bar{x} \pm DS$)

	SIZE (nm)	IP[1]	ZETA POTENTIAL (mV)	%EE[2]
NP-BCO	164.0 ± 6.6	0.084 ± 0.018	-1.80 ± 0.64	NA
NP-EUG	171.0 ± 3.0	0.113 ± 0.036	-2.47 ± 0.61	31.85 ± 12.77

Note: [1]Polydispersity index ranging from 0 to 1. A higher value corresponds to a less homogeneous size distribution; [2]Percentage encapsulation efficiency; NA: NP without eugenol.

The nanoprecipitation technique has been successfully used for the encapsulation of EOs and their components, such as EUG[16–18]. The incorporation of these environmentally unstable components into NPs may offer advantages for their application and incorporation into commercial products in the food industry. For example, due to their nanometric size and multiparticulate character, EUG-loaded NPs can enhance NP/fruit surface interaction and subsequently gradually release the EUG[19,20]. Moreover, compared to large particles (e.g. microparticles), nanosystems present a better surface/volume ratio, therefore, it is possible to have a larger surface of the fruit in direct contact with the NPs[21]. In addition, the polymer wall of the NPs allows retaining the EUG inside the structure, thus decreasing its evaporation rate, favoring its application, increasing the residence time on the feed surface and improving the incorporation of EUG in aqueous systems[22]. The average size of the NP-EUGs (i.e. 171.0 ± 3.0 nm) was similar to that reported by Gomes *et al.*[23] who obtained PLGA

NPs with EUG incorporated with an average size of 179 nm. In addition, the authors report that in the release trials performed, a slower release rate of the EUG incorporated into the NPs was presented, which would improve their overall application.

On the other hand, PI is a parameter associated with the homogeneity of the nano-system dispersion. For this study, the IP value of NP-EUG was 0.113 ± 0.036, which indicates a high homogeneity of nanoparticles that would allow individual NP interactions (e.g. bioadhesion and EUG release) to be homogeneous on the fruit surface as well. For the Zetasizer Nano-ZS90 (Malvern Instruments) used, the PI values range from 0 to 1. A value below 0.200 indicates a homogeneous distribution of nanoparticle size[24].

The electrostatic potential at the boundary dividing the compact layer and the diffuse layer of the colloidal particles, called zeta potential, was −2.47 mV for the NP-EUGs. This negative potential can be attributed to the molecules of the polymer wall-forming anionic polymer (Eudragit L 100-55) that imparts a negative charge to the obtained NPs due to their methacrylic acid groups. The zeta potential of the NPs depends mainly on the chemical nature of the polymer, in addition to the chemical nature of the stabilizing agent. Therefore, when NPs are prepared for methacrylate-derived polymers using nonionic stabilizing agents, negative zeta potential values are obtained due to the presence of terminal carboxylic groups of the polymer[25]. Therefore, it follows that Eudragit effectively formed an envelope that constitutes the outer wall of the nanoparticles. The core will correspond to the nano-encapsulated EUG. Similarly, the zeta potential can be considered as an indicator of the stability of the NP dispersions. Although it is considered as a general rule that absolute values around 30 mV provide good stability. But when using surfactants (i.e. PVA), which act mainly by steric stabilization, values below 20 mV or much lower can provide sufficient stabilization of the dispersions[24,26]. This negative potential is also important because it could facilitate the interaction of NP-EUG with the membrane of plant pathogenic microorganisms which would ensure that the interaction of EUG is directly from the NP to the microorganism, thus improving, therefore, its antimicrobial effectiveness[27].

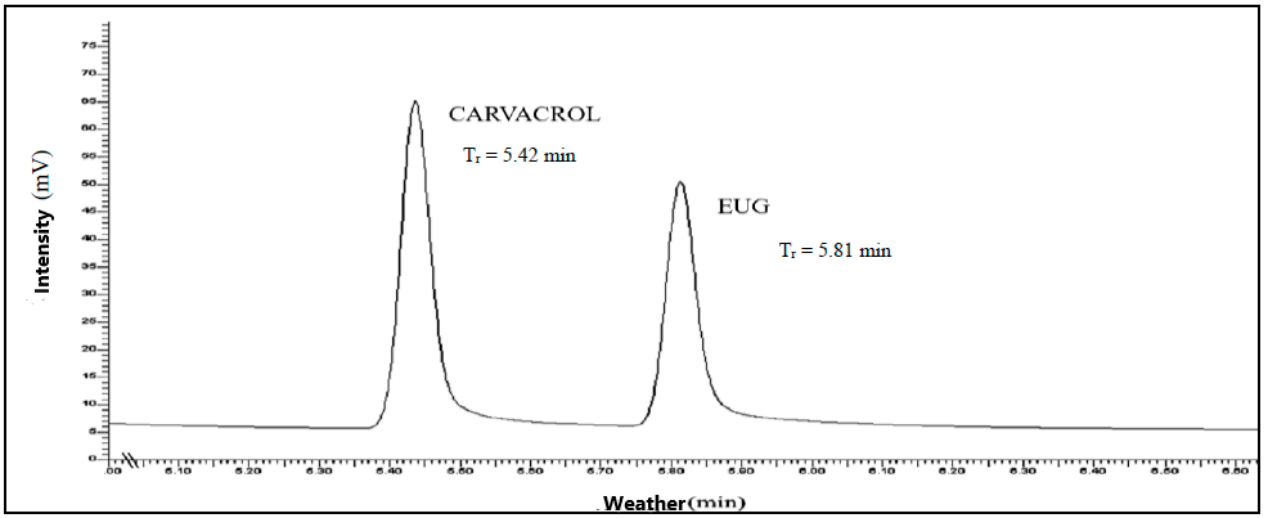

Figure 2. Chromatogram of EUG (40 μg·mL^{-1}) and carvacrol (10 μg·mL^{-1}) with CAR/PDMS coating (75 μm) by GC-FID.

To complete the physicochemical characterization of the NP-EUG, EUG was extracted from the NPs using the HS-SPME technique and quantified by a GC-FID analytical method to determine the encapsulation efficiency (%EE) (**Table 1**) using equation (1). Regarding the HS-SPME technique, a 75 μm CAR/PDMS fiber was used because EUG is a partially polar component (Log Po/w = 2.7) of low molecular weight (164.20 g·mol^{-1}) that has affinity for this type of fibers. **Figure 2** shows the chromatogram of EUG extracted by the HS-SPME technique and the internal standard (carvacrol) used

to determine the %EE by the GC-FID analytical method (**Figure 2**).

The %EE obtained for the NP-EUG was 31.85 ± 12.77 (**Table 1**) indicating that more than 30% of the EUG added during formulation was encapsulated in the NP using the nanoprecipitation technique, therefore, it is likely that this active compound is gradually released from the NP to the fruit surface.

3.2 Evaluation of the preservative effect of nanoformulation in combination with an edible coating on the shelf-life parameters of tomato (*Solanum lycopersicum*)

Firmness. Figure 3 shows the results of firmness tests performed on tomatoes after 16 days of storage at room temperature. The firmness of the tomatoes was 1.5 N at the beginning of the experiment. This parameter decreased for all groups of tomatoes tested. A 42.7% decrease in firmness was observed in tomatoes without any treatment (CTRL) (0.86 N). Tomatoes treated with ALG (0.68 N) and ALG-EUG (0.71 N) showed a loss of 54.7 and 52.7%, respectively, while tomatoes treated with NP-BCO (0.87 N) had a firmness value similar to the initial one. No significant difference was found between the ALG, ALG-EUG and NP-BCO groups with respect to the CTRL group.

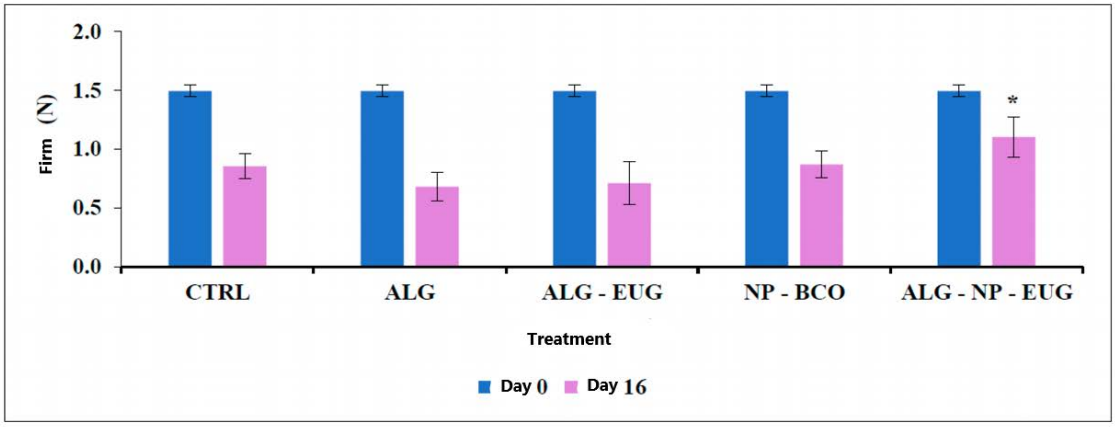

Figure 3. Firmness, day 0 and day 16, of tomatoes after storage for 16 days at 25 °C. CTRL = no treatment, ALG = alginate, ALG-EUG = alginate + free eugenol, NP-BCO = NP without active, ALG-NP-EUG = alginate + NP with eugenol (n = 3; $\bar{x} \pm DS$).
* Significant difference with respect to the CTRL group.

Tomatoes treated with NP-EUG in combination with RC (ALG-NP-EUG) showed higher firmness (1.10 N) compared to the four previous treatments, representing only 22% loss compared to untreated fruit (CTRL). This difference was statistically significant. Firmness of fruits and vegetables is related to cell wall structure, which depends on the turgor, cohesion, shape and size of the cells that make up the cell wall. Water loss is closely related to the loss of turgor of mesocarp cells, decreasing fruit firmness[28]. In this work, the presence of the RC ALG-NP-EUG could interfere with the decrease in fruit transpiration rate, resulting in a lower loss of firmness of the treated tomatoes. Similarly, the loss of firmness is related to an increase in the activity of hydrolytic enzymes (i.e. polygalacturonase) that act on cell wall pectins, resulting in tissue changes, which in turn cause fruit softening[29]. This enzyme activity is low during the first stage of fruit development, and then increases and reaches a maximum in the climacteric stage of the ripening process[30]. Similar results were reported by Fagundes[31] who obtained firmness results similar to the initial ones in tomatoes treated with hydroxypropyl methylcellulose and beeswax coatings after 15 days of storage. The authors attribute this firmness retention in coated tomatoes to the reduction in enzyme activities caused by the modification of the internal atmosphere of the fruit. That is, to a lower respiration rate. Similarly, in our study, the AL of the RC ALG-NP-EUG had the ability to act as a barrier that interfered with gas exchange, which led to a reduction in the respiration rate of the tomatoes and prevented water loss. Furthermore, it is possible that the biological activity of the nanoencapsulated EUG decreased the en-

zymatic activity of the fruit, resulting in slower ripening.

Color change. In the food industry, to measure color change in a product such as tomato, coordinates expressed in numerical values have been established to correlate color with maturity. In this study, color changes were determined by the CIEL a* b* color scale, where a* is the red/green coordinate (+a* indicates more red and −a* indicates more green), while b* is the yellow/blue coordinate (+b* indicates more yellow and −b* indicates more blue). **Figure 4** shows the analysis of the 3 coordinate values obtained, presented as the color change (ΔE), using equation (2), produced in each of the groups treated and stored for 16 days. A ΔE value of 219.75 was obtained for CTRL, 255.71 for ALG and 210.80 for ALG-EUG, 167.76 for NP-BCO and 190.47 for RC ALG-NP-EUG. Less color change is interpreted as preservation of tomato quality. Although there was no significant difference between the groups, it can be observed that the presence of the NP in combination with the ALG-NP-EUG RC on the fruit surface could have an effect on color preservation. It is important to mention that tomato, upon reaching commercial maturity, undergoes minimal changes in color, which is the characteristic of climacteric fruits. Different authors have mentioned that the application of coatings can delay color changes in tomatoes during storage by creating a modified atmosphere in the fruit[23,31].

Total soluble solids and titratable acidity. TSS of fruits tended to increase during ripening. The TSS value (**Figure 5**) for tomatoes at the beginning of the experiment was 3.64 °Brix. After 16 days of storage, this parameter increased for control tomatoes (4.83 °Brix), as well as for tomatoes treated with ALG-EUG (4.74 °Brix), ALG (5.21 °Brix), NP-CO (5.08 °Brix) and RC ALG-NP-EUG (4.31 °Brix).

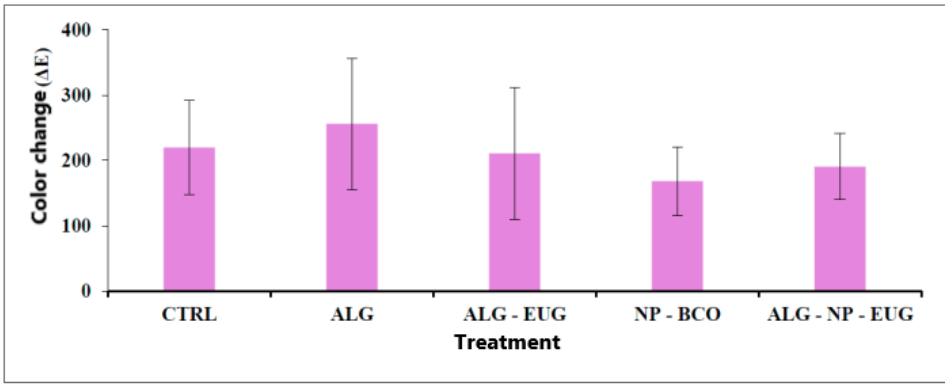

Figure 4. Color change of tomatoes after storage for 16 days at 25 °C. CTRL = no treatment, ALG = alginate, ALG-EUG = alginate + free eugenol, NP-BCO = NP without active, ALG-NP-EUG = alginate + NP with eugenol (n = 3; $\bar{x} \pm DS$).

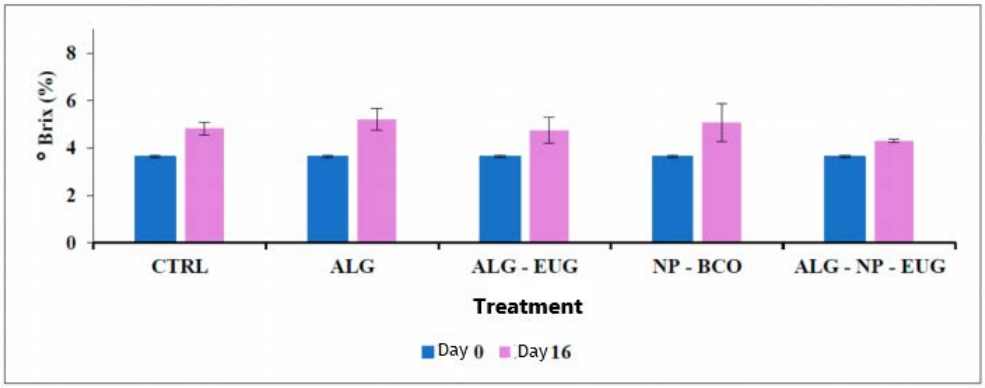

Figure 5. Total soluble solids, day 0 and day 16, of tomatoes after storage for 16 days at 25 °C. CTRL = no treatment, ALG = alginate, ALG-EUG = alginate + free eugenol, NP-BCO = NP without active, ALG-NP-EUG = alginate + NP with eugenol (n = 3; $\bar{x} \pm DS$).

The increase in TSS during storage is due to respiration. During the ripening process, sugar accumulation will depend on the degradation of starch, the main energy storage compound in green tomato. In addition, metabolic activity continues as a result of fruit ripening, leading to the conversion of carbohydrates and organic acids into sugars to be used in various metabolic processes[10]. On the other hand, compared to the control group, the coating in combination with NP-EUG (ALG-NP-EUG), showed the lowest TSS values. This behavior could be attributed to the presence of AL CR and a synergy with NP-EUG, which causes a more effective deceleration of respiration and metabolic activity, delaying the fruit ripening process. These results are similar to that reported by Sucharitha, Beulah and Ravikiran[33] who treated tomatoes with chitosan coatings and reported a significant difference at the end of the storage period (15 days) between samples coated and not coated with chitosan. In our study, a difference in TSS was observed between RC ALG-NP-EUG (4.31 °Brix) and ALG alone (5.21 °Brix). This could be due to the presence of the NPs with encapsulated EUG on the fruit surface for a longer time and, in addition to the barrier properties of the coating, the sustained release of EUG from the NPs could have extended the biological activity of EUG (e.g. antioxidant) on the surface of the tomato, which contributed to better fruit preservation.

Finally, as shown in **Figure 6**, the TA of tomatoes was 1.6 g 100 mL^{-1} at the beginning of the experiment and after 16 days of storage, TA values decreased for all groups. This reduction in TA values is associated with the metabolism of organic acids in the fruits during the ripening process. Organic acids are responsible for fruit acidity which is expressed as TA. During the ripening of tomatoes, the amount of organic acids decreases, and this is due to the fact that organic acids are metabolized mainly to ensure the additional supply of carbon for obtaining sucrose, glucose and fructose in the fruit[34]. As for the groups of tomatoes with treatments, the TA results had the same trend as those obtained for TSS. After 16 days of storage, tomatoes in the control group had the most significant decrease in TA, while those in the ALG-NP-EUG had the lowest decrease in TA. This means that the coating in combination with the NP-EUG was the most effective treatment in delaying ripening, which can be attributed to the presence of the AL coating that acted as a barrier and decreased the respiration rate (metabolism) of the tomatoes.

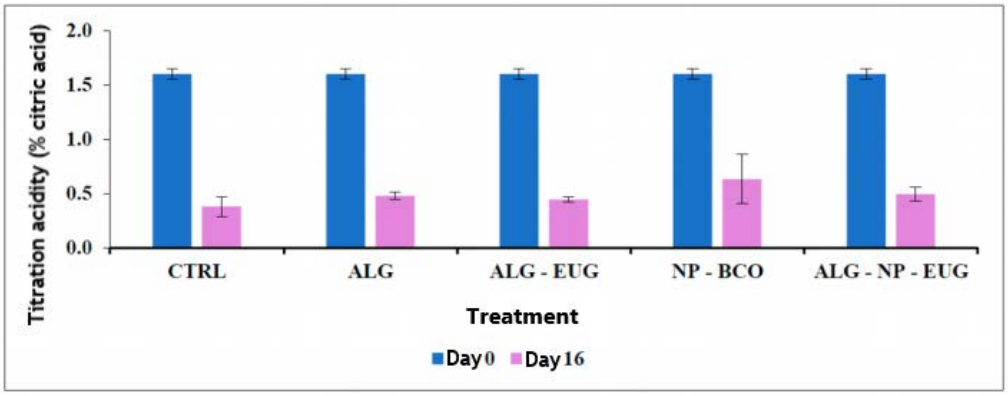

Figure 6. Titratable acidity, day 0 and day 16, of tomatoes after storage for 16 days at 25 °C. CTRL = no treatment, ALG = alginate, ALG-EUG = alginate + free eugenol, NP-BCO = NP without active, ALG-NP-EUG = alginate + NP with eugenol (n = 3; $\bar{x} \pm DS$).

3.3 Evaluation of the protective effect of nanoformulation in combination with edible coating on tomato fruits inoculated with the phytopathogen Colletotrichum gloesporoides

The tomato crop is affected by various postharvest diseases, many of which are caused by fungi. Among the main postharvest phytopathogenic agents of tomato are: Fusarium solani, Botritys cinerea, Alternaria alternata, Penicilium expansum and, particularly, Colletotrichum gloesporoides[35]. In the present study, tomatoes with each of the aforementioned treatments were inoculated by

puncture with the phytopathogen Colletotrichum gloesporoides and their development was observed for 5 days at room temperature in humidity chambers. The results are shown in **Figure 7**. After 5 days, the tomatoes treated with RC ALG-NP-EUG showed no fungal growth (**Figure 7E**), while the rest of the fruit showed characteristic colonies of the fungus Colletotrichum gloeosporioides. This protection is due to the antimicrobial capacity and effect on phytopathogens of EUG[36–38]. Hong[39] demonstrated that clove EO and eugenol exhibited significant inhibition of Colletotrichum gloeosporioides growth by reducing the diameter of a lesion in immature green bell pepper inoculated with this phytopathogen. In addition, the incorporation of EUG into the polymeric structure of the NP may cause an increase in residence time on the fruit surface by decreasing its rapid evaporation when applied freely.

It has been proposed that the mechanism of action of the antifungal activity of EO components appears to depend on their chemical structure and their ability to pass through the cell wall and penetrate between the fatty acid chains of the lipid bilayer, making the cell membrane much more permeable and, as a result, cause cell death or inhibition of sporulation and germination of fungi or other spoilage-causing microorganisms[40–42]. Taking into account this mechanism, the EUG incorporated in the NPs in combination with the RC helped to have a more intimate and prolonged contact between the active and the microorganisms on the fruit surface.

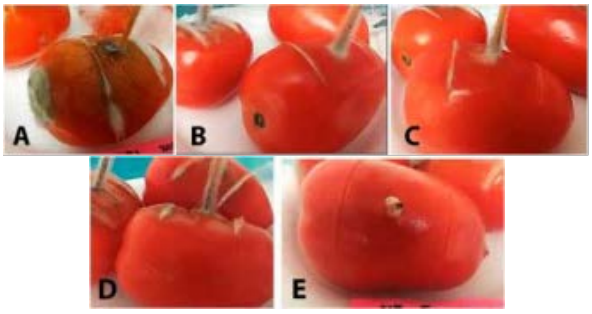

Figure 7. Pathogenicity test with C. gloesporoides after 5 days of storage of tomatoes with different treatments. **A**) = control, **B**) = alginate, **C**) = alginate + free eugenol, **D**) = white NP, **E**) ALG-NP-EUG. (n = 3; $\bar{x} \pm DS$).

4. Conclusions

The physicochemical properties of the individual components of the RC in combination with NP-EUG contributed positively to delay ripening of tomatoes and protect them from phytopathogens such as Colletotrichum gloesporoides. While AL RC acted as a barrier that reduced fruit transpiration and metabolism, EUG with antimicrobial activity prevented the growth of the phytopathogenic microorganism. In addition to this, due to its size and multiparticulate nature, the EUG-incorporated NPs were able to distribute more evenly on the fruit surface, releasing the EUG gradually and increasing its residence time in the fruit. This demonstrates that nanoencapsulated EUG in combination with RC are a favorable alternative to traditional preservation methods.

Conflict of interest

The authors declared no conflict of interest.

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