

EFFECT OF CHITOSAN, THYME ESSENTIAL OIL, AND PROPOLIS BASED NANOCOATINGS, ON TOMATO QUALITY DURING STORAGE AT CONTROLLED AND AMBIENT TEMPERATURES

**Zormy Nacary Correa Pacheco^{1*}, María Luisa Corona Rangel¹,
Silvia Bautista Baños¹, Laura Leticia Barrera Necha¹,
Rosa Isela Ventura Aguilar², Mónica Hernández López¹**

¹Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, Carr. Yautepec-Jojutla Km. 8.5, San Isidro Yautepec, Morelos, 62731, Mexico.

²CONACYT-Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, Carr. Yautepec-Jojutla Km. 8.5, San Isidro Yautepec, Morelos, 62731, Mexico.

zcorreap@ipn.mx

Correa Pacheco, Z. N., Corona Rangel, M. L., Bautista Baños, S., Barrera Necha, L. L., Ventura Aguilar, R. I., & Hernández López, M. (2023). Effect of chitosan, thyme essential oil, and propolis based nanocoatings, on tomato quality during storage at controlled and ambient temperatures. In E. San Martín-Martínez (Ed.). *Research advances in nanosciences, micro and nanotechnologies. Volume IV* (pp. 123-140). Barcelona, Spain: Omniascience.

Abstract

Nowadays, new alternatives for avoiding contamination due to the use of non-biodegradable packing and pesticides for fruit and vegetable preservation must be sought. In this work, three coatings based on chitosan nanoparticles, chitosan-thyme essential oil or chitosan-propolis nanoparticles were tested on inoculated and non-inoculated tomato with *Alternaria alternata*. The fruit was stored at 11 ± 2 °C for 14 days and at ambient temperature at 27 ± 2 °C for 7 days. The TEM and DLS showed a particle size in the range of 3.8 to 9 nm. The postharvest quality of tomato fruit was evaluated as firmness, weight loss, color and total soluble solids (TSS). It was obtained that the firmness decreased, the weight loss increased; and color and TSS were maintained over the storage days at both temperatures. The loss of firmness for the coated tomato fruit stored at 11 °C was less than 1 N. On the other hand, the weight loss was less than 3 % and 8 % for the controlled and ambient temperature stored fruit, respectively, on day 7. It was concluded that the chitosan nanoparticles coating maintained a better postharvest quality of tomato, representing a natural alternative for the preservation of this important horticultural commodity.

Keywords: *Alternaria alternata*, *Lycopersicon esculentum* L., nanoparticles, weight loss, firmness, color, postharvest preservation.

1. Introduction

Tomato (*Lycopersicon esculentum* L.) is the second important crop in Mexico with high nutritional value and uses in traditional cooking [1, 2]. According to data from the Food and Agriculture Organization of the United Nations Statistics Division (FAOSTAT), tomato represented 7.77 % of the export of the country by 2021, with a production of 4,149,240.67 ton [3, 4]. However, during storage, it is susceptible to various diseases caused by different microorganisms, among them, fungi such as *Alternaria alternata* that responsible for the black rot disease and bacteria deteriorating its postharvest quality [5].

Nowadays, nanotechnology has allowed the elaboration of coatings, in which the use of nanoparticles, enhances the fruit protection effect due to the highest surface area to volume ratio of the nanostructures, improving their activity [6]. Chitosan is a biocompatible and biodegradable polymer with a wide range of applications used for the elaboration of nanostructured coatings [7]. On this subject, Gutiérrez-Molina *et al.* [8] reported a nanostructured coating with 30 % of chitosan nanoparticles to preserve refrigerated tomatoes at 10 ± 1 °C for 19 days. A weight loss of less than 13 % and a color change of less than 5 % were observed compared to the control (uncoated fruit). Firmness did not change with the storage days. On the other hand, chitosan nanoparticles (NPQs) and chitosan added with thyme essential oil (QS+NPTs) coatings for different concentration of nanoparticles (15, 30 and 45 %) were evaluated for controlling the bacterium *Pectobacterium carotovorum* in tomatoes, stored for 7 days at 10 °C having a relative humidity of c.a. 70 %. In this study, there were differences in the variable color in fruit coated with QS+NPTs 30 %, especially in the luminosity and chroma values on the inoculated tomatoes. Regarding the hue value, the highest variation was for the fruit coated with QS+NPQs 45 %. For firmness, the lowest loss was for tomatoes treated with QS+NPQs 15 %. The values of TSS presented a difference between 3 and 5 % and of weight loss between 0.7 and 1.2 % [9]. The aim of this study was to assess the effect of three nanostructured coatings based on chitosan, chitosan-thyme essential oil and chitosan-propolis on the storage behavior of tomato at controlled and ambient temperature measuring the quality variables of weight loss, firmness, TSS, and color.

2. Materials and methods

2.1. Materials

The reagents used for nanoparticles' elaboration (NPs) were medium molecular weight chitosan with a deacetylation degree of 75-85 % (Sigma-Aldrich Corp., St. Louis, MO, United States) which was dissolved in acetic acid (Fermont,

Monterrey, N.L., Mexico) and distilled water (0.05 %). For the incorporation of the thyme essential oil (Essential Oils-Essencefleur, Mexico City, Mexico) or the aqueous extract of propolis (10 %) (REDSA, Cuernavaca, Morelos, Mexico) to the nanoparticles, methanol (JT Backer, Phillipsburg, NJ, United States) and Tween 20 (Hycel, Zapopan, Jal., Mexico) were used. For the nanocoatings elaboration, high molecular weight chitosan (América Alimentos, Zapopan, Jal., México) and deacetylation degree of 91 % (1 %) was used as matrix.

2.2. Methods

2.2.1. Nanoparticles elaboration

The nanoparticles were elaborated using the nanoprecipitation method [10]. Two phases were prepared, one aqueous and one organic. The aqueous phase consisted of a solution of medium molecular weight chitosan (0.05 % w/v) in a solution of glacial acetic acid and distilled water (1 % v/v). For the organic phase, Tween 20 (1 %) was dissolved in 40 mL of ethanol. The concentration of both the essential oil of thyme and the aqueous extract of propolis added to the organic phase was 5 %. Then, by using a peristaltic pump (MasterFlex, Vernon Hills, IL, USA), 2.5 mL of the aqueous phase was added dropwise to the organic phase, stirring for 10 min. Next, a rotary evaporator (model 300, Büchi, Flawil, St. Gallen, Switzerland) was used to concentrate the NPs solution at 40 °C and 40 rpm. Final volume was 2 mL, being stored at 4 °C.

2.2.2. Nanocoatings elaboration and application

Based on 33 % chitosan nanoparticles (NPQ), chitosan-thyme nanoparticles (NPT) or chitosan-propolis nanoparticles (NPP), 66.7 % of chitosan (1 %) and 0.3 % of glycerol (JT Backer, Phillipsburg, NJ, United States), three formulations were elaborated and two controls were considered: inoculated tomato fruit with *A. alternata* C(+) and non-inoculated tomato fruit C(-). The coatings were elaborated following the methodology of Gutiérrez-Molina *et al.* [10]. The order of components addition was as follows: glycerol was added to chitosan (1 %) and mixed using a homogenizer (Virtis, model 45, Los Angeles, CA, United States) for 5 min at 10,000 rpm. Next, the NPs were added and stirred for another 5 min [11].

The tomatoes were purchased at a local market in Cuautla, Morelos. First, the fruit were washed with distilled water and disinfected with a sodium hypochlorite solution (3 %) for 3 min. They were rinsed again with water and the formulations

were sprayed on the fruit and allowed to dry. Then, disks of 5 mm of diameter containing the fungus were placed inside 4 holes of 2 mm in diameter. The *A. alternata* isolate was obtained from infected bell pepper and previously cultivated in potato dextrose agar culture medium (PDA) (BD Bioxon, State of Mexico, Mexico) for 7 days. The effect of the nanocoating was evaluated on fruit stored at 11 ± 2 °C for 14 days and at 27 ± 2 °C for 7 days.

2.3. Nanoparticles characterization by transmission electron microscopy (TEM) and dynamic light scattering (DLS)

NPs morphology was observed using a transmission electron microscope (JEOL-JEM 2010, Tokyo, Japan) at an acceleration voltage of 200 kV placing a drop of nanoparticles solution on a copper grid. IMAGEJ software (National Institutes of Health, Bethesda, MD, USA) was used to calculate the average particle size. For the particle size distribution measurement, a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK) was employed, placing 3 mL of the NPs solution in a quartz cuvette. Before measurement, the NPs solution was sonicated for 10 min.

2.4. Effect of nanostructured coating on tomato quality essays

2.4.1. Weight loss

To determine the tomato fruit weight loss, a portable digital scale (OHAUS, Parsippany, NJ, U. S. A.) was used. The difference between the initial weight and the final weight of the tomato fruit was calculated and expressed as percentage.

2.4.2. Firmness

The firmness was measured on both sides of the tomato fruit using a penetrometer (Lutron FR-5120, Coopersburg, PA, USA) and results were reported in Newtons (N).

2.4.3. Total soluble solids (TSS)

For TSS determination, a drop of tomato fruit juice was placed in a refractometer (Atago N-1E, Fukaya-shi, Saitama, Japan). The results were expressed as percentage.

2.4.4. Color

The color was measured in two opposite sides of the tomato fruit using a colorimeter (Konica Minolta Sensing® 9992–995 BC 10, Chiyoda, Tokyo, Japan). The results were expressed as hue angle ($\text{Hue}=\tan^{-1} b^*/a^*$), chromaticity ($C^*=\sqrt{(a^*)^2+(b^*)^2}$) and lightness (L^*) [11].

2.5. Statistical analysis

The measurements for all variables were made in 5 fruit for treatment. The data obtained from the experimentation were analyzed using the InfoStat software (InfoStat, Sacramento, CA, 2018) with a double analysis of variance (ANOVA) and Tukey's mean comparison test ($p<0.05$).

3. Results and discussion

3.1. Nanoparticles characterization by transmission electron microscopy (TEM) and dynamic light scattering (DLS)

In Figure 1, the morphology of the nanoparticles can be seen. For the NPQ a more homogeneous distribution is observed (Figure 1a). On the other hand, the NPQ and NPP showed different sizes or possible agglomerates as seen in Figures. 1b) and 1c), respectively. The nanoparticles range was between 3.8 and 6 nm as calculated by Image J.

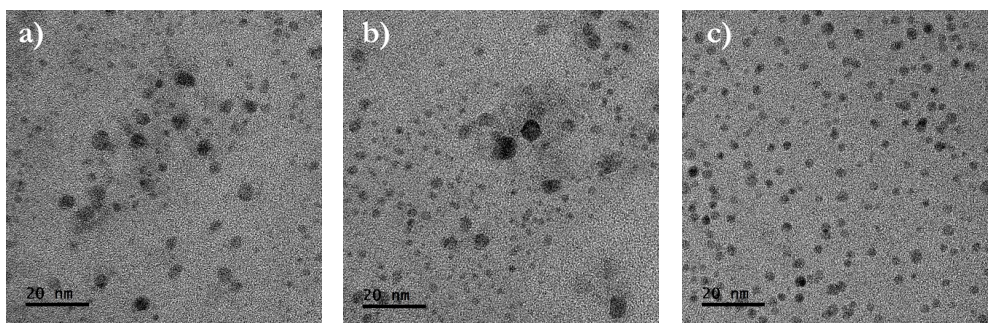


Figure 1. TEM of a) NPQ, b) NPT, and c) NPP. NPQ=chitosan nanoparticles, NPT=chitosan-thyme nanoparticles, NPP=chitosan-propolis nanoparticles.

Figure 2 shows the results of the DLS for the NPQ, NPT and NPP. A bimodal distribution is observed for the NPQ (Figure 2a) with the smallest particles in the range of 3.8 to 9 nm and the largest between 60 and 120 nm. Similarly,

the NPT presented a bimodal distribution with two populations. The first was between 8 and 15 nm and the second between 300 and 600 nm. Finally, the NPP showed a larger particle size with a unimodal distribution between 220 and 2000 nm. According to Sreekumar *et al.* [12] the presence of the second peak with the largest particle size may be related to aggregates. These results agreed with those previously published by our research group [13 – 16]. A possible reason for the formation of aggregates in the NPP coating could be related to the effect of propolis loading. It has been found that non-encapsulated propolis attached to the NPQ surface, increases the hydrophobicity and the interaction between NPQ and propolis with their subsequent aggregation [17].

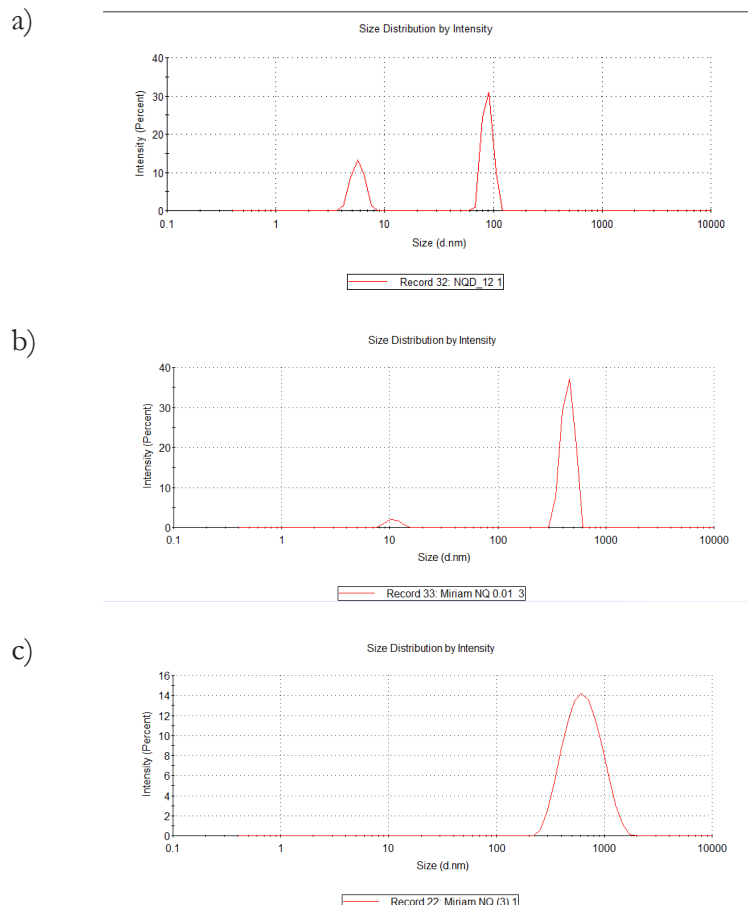


Figure 2. Particle size distribution (d=diameter) for: a) NPQ, b) NPT, and c) NPP. NPQ=chitosan nanoparticles, NPT=chitosan-thyme nanoparticles, NPP=chitosan-propolis nanoparticles.

3.2. Effect of nanostructured coating on tomato quality

3.2.1. Weight loss

In Figure 3, the weight loss of the tomato fruit refrigerated (Figure 3a) and stored at ambient temperature (Figure 3b) are shown. In general, weight loss is increased over time.

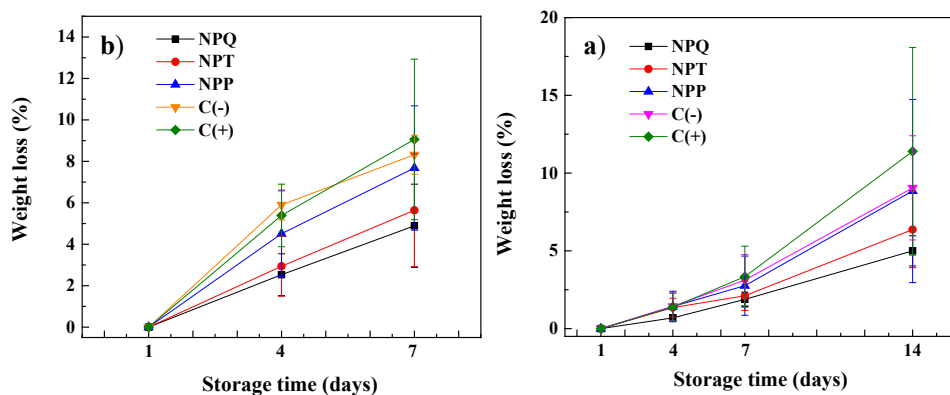


Figure 3. Weight loss of tomatoes coated with NPQ, NPT, and NPP and two controls, and stored at a) 11 ± 2 °C for 14 days and b) 27 ± 2 °C for 7 days. Error bars show the standard error of the mean. NPQ=chitosan nanoparticles; NPT=chitosan nanoparticles encapsulating thyme essential oil; NPP=chitosan nanoparticles encapsulating aqueous extract of propolis; C(-)=fruit not inoculated; C(+)=inoculated fruit.

Regarding the refrigerated tomatoes (Figure 3a) on day 7, the weight loss was between 2-3 %, less than for the tomatoes stored at ambient temperature. No significant statistical differences ($p < 0.05$) were found between formulations (not shown). Moreover, the weight loss for coated tomatoes was lower than for the controls. On the other hand, on day 14, the weight loss values were between 5-11 %, being 9 % less than the coated fruit. Similar behavior to fruit stored at ambient temperature.

For the samples stored at ambient temperature for 7 days (Figure 2b), there was a weight loss of less than 8 % for the coated fruit. The weight loss of the coated tomato fruit was slightly lower than the controls, being higher for the inoculated tomato fruit, although no significant statistical differences were observed ($p < 0.05$). Among the coated fruit, the highest weight loss was for NPP, then for NPT and finally for NPQ, However, the values were similar. Therefore, there was no significant weight loss of the fruit during the storage period.

Refrigeration of fruit and vegetable is a technique that has been used over the years to preserve the horticultural products for an extended time [18]. Kibar *et al.* (2018) [19] used 0.5, 1.0 and 2.0 % chitosan solutions to extend the postharvest quality of tomatoes stored at two different temperatures (at 5 °C with relative humidity of c.a. 90 % and at 21 °C with relative humidity of c.a. 65 %). There was a reduction in weight loss for the chitosan-coated tomato fruit with a more evident effect for the solution with a concentration of 1 % having a weight loss value of less than 2 % after 10 days of refrigerated storage and with a loss of less than 4 % after 7 days of storage at ambient temperature. Also, a higher percentage of weight loss was observed for the non-coated fruit. This is related to the barrier property of the coating on the fruit that prevents further dehydration and therefore the weight loss [20]. Azmai *et al.* (2019) [21] found weight loss values for tomatoes stored at ambient temperature for 12 days at concentrations of 0.5, 0.75 and 1 % of chitosan of 7.5, 6.7, and 7.2 %, respectively, and of 6.5 % for the untreated tomatoes. These values were lower than those obtained in this present research for a longer storage time perhaps because of the degree of ripening (just changing color from green to red) of the tomato used by cited authors. For tomatoes refrigerated at 6 °C and coated with chitosan solutions at different concentrations, Sucharitha *et al.* [22] reported tomato weight loss of about 0.54, 1.3 and 3.9 % with concentrations of 0.25 %, 0.5 % and the control, respectively, after 6 days of storage. Similar to this study, values of 2-3 % for the coated fruit and higher than 3 % for controls were obtained with a lower value of weight loss of the coated fruit compared to the control. On the other hand, Mustafa *et al.* [23] reported a weight loss of 7 % for fruit stored at 15 °C with a R.H. of c.a. 80 % using a coating based on chitosan particles of 400, 600, and 800 nm, after 20 days of storage. A similar value obtained for the 14 days of storage of this work.

Compared with our previous research where nanostructured coatings were used, for tomatoes stored for 7 days at 10 °C, a weight loss of less than 2 % was obtained for NPQ coated fruit at different concentrations of nanoparticles in the formulation (15 %, 30 %, and 45 %). For the NPT coated fruit and the same concentrations, the weight loss was also less than 2 % [14]. On the other hand, for tomatoes refrigerated at 10 °C, the weight loss value for coated fruit with NPQ (30 %) was less than 3 % on day 8 of storage [8].

Firmness results are shown in Figure 4. For the tomato fruit stored at controlled temperature (Figure 4a) and ambient temperature (Figure 4b) there was a decrease in firmness over time. The decrease in firmness with ripening in tomato

is attributed to moisture loss due to transpiration and enzymatic changes. These changes cause cell wall deterioration in tomato tissues [24].

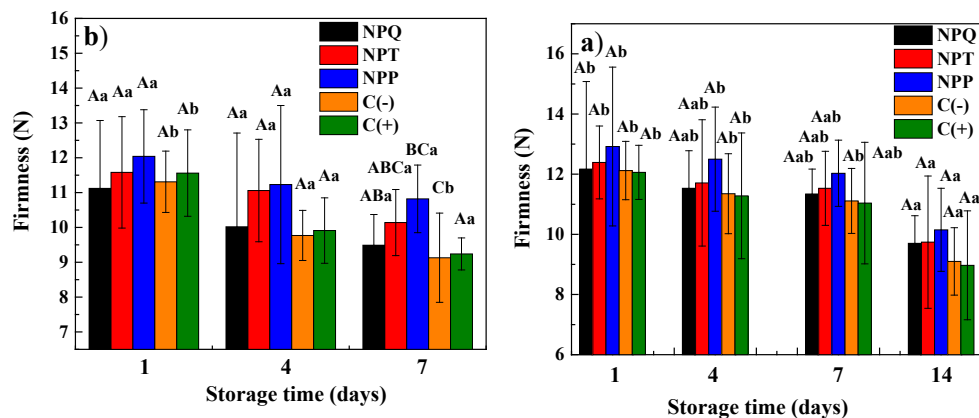


Figure 4. Firmness of tomatoes coated with NPQ, NPT, and NPP and two controls stored at: a) 11 ± 2 °C for 14 days and b) 27 ± 2 °C for 7 days. Different letters show statistical differences between formulations (upper case letters) and between days (lower case letters) calculated by ANOVA and Tukey's test ($p < 0.05$). Initial firmness value = 11.7 ± 0.8 N. Error bars show the standard error of the mean. NPQ=chitosan nanoparticles; NPT=chitosan nanoparticles encapsulating thyme essential oil; NPP=chitosan nanoparticles encapsulating aqueous extract of propolis; C(-)=fruit not inoculated; C(+)=inoculated fruit.

The loss of firmness was lower for the refrigerated fruit (Figure 4a). On day 7 of storage the same trend was observed, with similar values. The highest loss of firmness was for the NPP coated tomato fruit ($\Delta = 0.89$ N), followed by the NPT-coated one ($\Delta = 0.86$ N), and finally for the coated NPQ tomato fruit ($\Delta = 0.83$ N). The uncoated tomato fruit showed a higher loss of firmness, with a variation of approximately 1 N for both controls. On day 14 the same trend was observed as for day 7. However, there was a higher difference in loss of firmness, almost approximately 3 N with higher values than the non-refrigerated fruit, although no significant statistical differences were found ($p < 0.05$). For the tomatoes stored at ambient temperature (Figure 4b), the highest loss of firmness was for NPP coated fruit with values decreasing from 11.1 N to 9.4 N ($\Delta = 1.63$ N), followed by NPT coated fruit with values from 11.5 N to 10.1 N ($\Delta = 1.44$ N), and finally, for those coated with NPQ from 12.0 N to 10.8 ($\Delta = 1.22$ N). The controls showed a higher loss of firmness, being this value higher for the inoculated fruit with a variation of $\Delta = 2.18$ N compared to the non-inoculated fruit ($\Delta = 2.32$ N). Firmness values were higher than those for the coated tomato. For both storage

conditions, no significant statistical differences were observed ($p < 0.05$) among coatings and during storage the days.

In a previous work [14], after 7 days at 10 °C, firmness values between 17 and 24 N were obtained in fruit refrigerated for different NPQ and NPT concentrations (15, 30, and 45 %) in the coating. For the controls, values were 18.8 N for C(-) and 28.5 N for C(+). Similarly, in other study, for tomatoes stored at 10 °C, on day 8, firmness values of 22 N were obtained for coated fruit (30 % of nanoparticles) compared to the initial value of 20 N [8]. A slight change in the value of firmness was observed like the values obtained in this research for tomatoes stored at controlled temperature. The difference between those values and the values obtained in this work is related the ripening grade of the fruit. In another study, for coated tomatoes with chitosan (0.5, 1, and 2 %) Kibar *et al.* [19] reported an initial firmness of 20 N for fruit stored at 5 °C and at 21 °C. For fruits stored at ambient temperature on day 7, the values of firmness were between 8 and 11 N for the coated fruit and of 12 N for the control fruit. Final values between 13 and 17 N were obtained for the coated refrigerated fruit and of 16 N for the control. The loss of firmness was lower for refrigerated fruit like the reported in this research. However, the variation of firmness with storage was higher. However, the. On the other hand, Azmai *et al.* [21] reported variations of firmness values of $\Delta = 1.8$ N for tomatoes stored at ambient temperature for 12 days treated with 0.5 % chitosan coating and of $\Delta = 1.1$ N for chitosan concentrations of 0.75 and 1 %, with a progressive decrease in firmness with storage time. For the control, the firmness variation was much higher ($\Delta = 0.5$ N). Except for the control, the results of the coated fruit were similar to those obtained in this work.

In Figure 5, the TSS data is shown. In both cases, no differences between treatments and storage days were observed as well as statistical differences ($p < 0.05$). For the refrigerated fruit (Figure 5a), the values were between 4.0 % and 4.2 % for the coated tomatoes with a value of 4.2 % for C(-) and 4.0 oBrix for C (+) on day 7. On day 14, the values were between 4.0 % and 4.1 % for the coated tomato fruit and 4.0 % for C(-) and 4.1 % for C (+) on day 14. At ambient temperature (Figure 5b), the values for the coated tomato fruit ranged between 3.9 % and 4.0 % and for the controls they were 3.8 % for C(-) and 4.0 % for C(+). for day 7.

In previous work carried out on NPQ coated tomatoes and stored for 7 days at 10 °C, the TSS value reported for NPs concentrations of 15, 30, and 45 % were between 3 % and 4 % and for fruit with NPT coatings a value of 4 % was found, like those obtained in this work [14]. For fruit stored at ambient

temperature for 12 days with chitosan coating of 0.5 %, 0.75 %, and 1 %, Azmai *et al.* [20] obtained values between 3.2 % and 3.8 % for the coated fruit and of 3.3 % and 3.4 % for the controls without significant variations of these values with storage time. Sree *et al.* [25] obtained values between 4-4.5 % after 7 days of storage for fruit coated with chitosan at 0.5 %, 1 %, 2 %, and 2.5 % and 4.2 % for the control, although there was a tendency to increase this value over storage time. In this work, values similar to those reported by these authors were obtained, without significant variations during storage time.

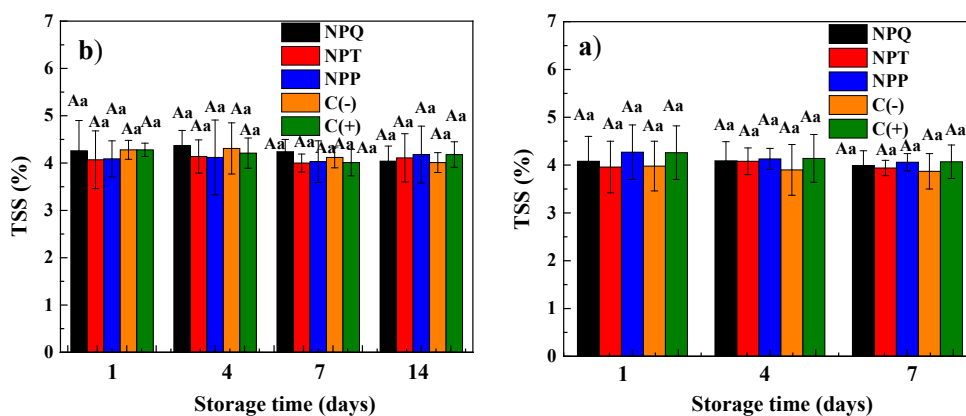


Figure 5. Tomatoes TSS coated with NPQ, NPT, and NPP and two controls, and stored at: a) 11 ± 2 °C for 14 days and b) 27 ± 2 °C for 7 days. Different letters represent statistical differences between formulations (upper case letters) and between days (lower case letters) calculated by ANOVA and Tukey's test ($p < 0.05$). Initial TSS value=4.0 oBrix. Error bars show the standard error of the mean. NPQ=chitosan nanoparticles; NPT=chitosan nanoparticles encapsulating thyme essential oil; NPP=chitosan nanoparticles encapsulating aqueous extract of propolis; C(-)=fruit not inoculated; C(+)=inoculated fruit.

In Figure 6, the results for chroma, hue, and luminosity color parameters for tomatoes stored controlled temperature (Figures 5a, b, c) and ambient temperatures (Figures 6d, 6e, 6f) can be seen. No significant statistical differences ($p < 0.05$) were observed between treatments or for the storage days; therefore, the color variation in the fruit was not affected by the application of the coating. This could be associated with the harvesting ripening stage of the tomato (full red) [26, 27]. Compared to previous works published by our group, for tomatoes stored at 10 °C for 7 days, the values between 29-31 for chroma, 48-54 for hue and 37-39 for luminosity were for tomatoes with NPQ coatings (15, 30, and

45 %) and between 26-29 for chroma, 47 for hue and between 37-38 for luminosity for tomatoes coated with NPT (15, 30, and 45 %) [14].

On the other hand, Gutiérrez-Molina *et al.* [8] reported values of luminosity between 39-45, similar to those reported in this present research. Also, Sucharitha *et al.* [22] found no differences with the storage days in coated tomatoes with 0.25 % and 0.5 % of chitosan solutions and stored for 30 days at 5 °C, similar data to that obtained in this research.

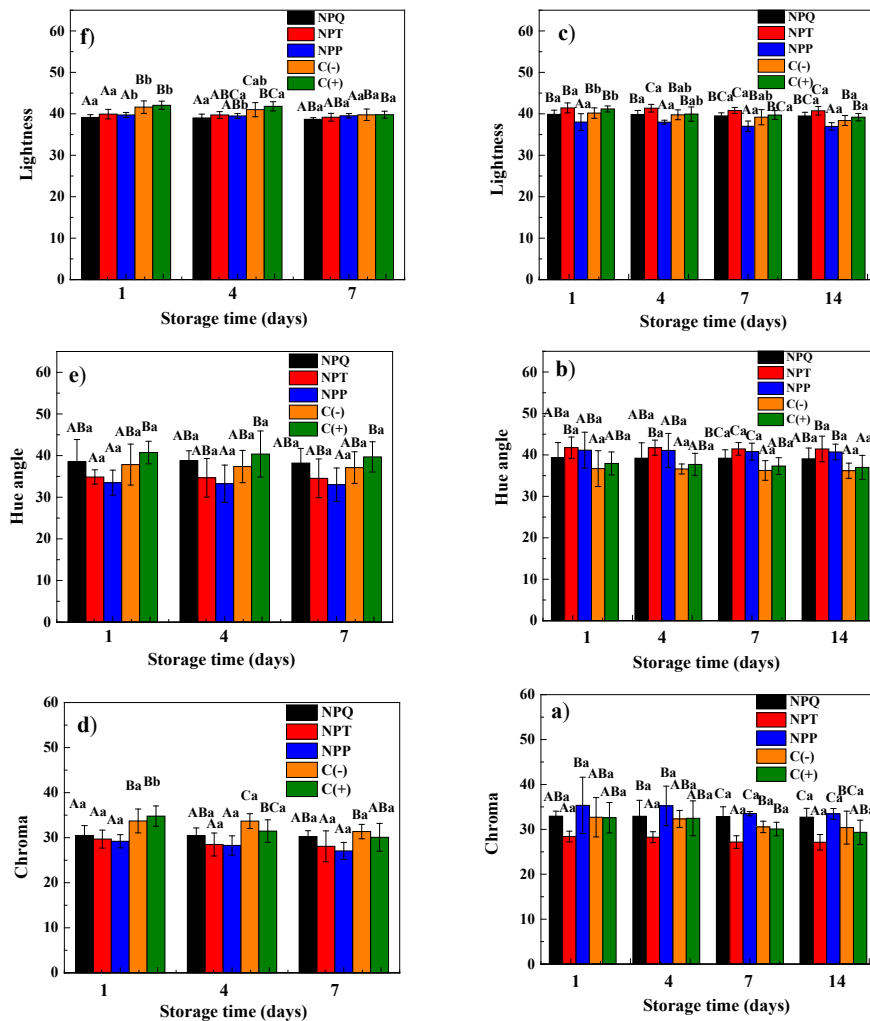


Figure 6. Change of tomato color coated with NPQ, NPT, and NPP and two controls C (+) and C(-), stored at 11 °C±2 for 14 days: a) chroma C*, b) hue angle h, and c) lightness L* and at 27 °C±2 for 7 days: d) chroma C*, e) hue angle h and f) lightness L*.

Different letters represent statistical differences between formulations (upper case letters) and between days (lower case letters) calculated by ANOVA and Tukey's test ($p < 0.05$). Initial chroma value = 8.76 ± 1.27 . Initial hue value = 40.0 ± 2.5 . Initial luminosity value = 40.8 ± 0.5 . Error bars show the standard error of the mean. NPQ = chitosan nanoparticles; NPT = chitosan nanoparticles encapsulating thyme essential oil; NPP = chitosan nanoparticles encapsulating aqueous extract of propolis; C(-) = fruit not inoculated; C(+) = inoculated fruit.

4. Conclusions

From DLS measurements the NPQ showed the most homogeneous distribution. On the overall, the nanoparticles were spherical in shape according to TEM observation. Considering the quality essays, the most effective coating for tomatoes preservation was the NPQ followed by the NPT and NPP due to lower weight loss and loss of firmness for the refrigerated and ambient temperature stored tomato. No significant changes were observed for color change or TSS. It would be useful to carry out microbiological tests considering variables such as severity and disease incidence to achieve a whole evaluation of the performance of nanocoatings. It will be the subject of further research.

Acknowledgement

The authors would like to thank Dr. Nicolás Cayetano from Nanoscience and Micro Center and Nanotechnologies-IPN and Dr. Eduardo San Martín from CICATA-Legaria for TEM and DLS measurements, respectively.

References

1. García-Estrada, R. S., Diaz-Lara, A., Aguilar-Molina, V. H., & Tovar-Pedraza, J. M. (2022). Viruses of economic impact on tomato crops in Mexico: From diagnosis to management: A review. *Viruses*, 14 (1251), 1-16.
<https://doi.org/10.3390/v14061251>
2. Valerino-Perea, S., Lara-Castor, L., Armstrong, M.E.G., & Papadaki, A. (2019). Definition of the traditional mexican diet and its role in health: A systematic review. *Nutrients*, 11(2803), 1-33.
<https://doi.org/10.3390/nu11112803>
3. Montaña, I., Valenzuela, I., & Villavicencio, K. (2021). Competitiveness of the Mexican red tomato in the international market: analysis 2003-2017. *Revista Mexicana de Ciencias Agrícolas*, 12(7), 1185-1197.
<https://doi.org/10.29312/remexca.v12i7.2531>
4. FAOSTAT, Mexican tomato production (2020).
<https://www.fao.org/faostat/en/#home>. Accessed 27 September 2020.
5. Blancard, D. (2012). *A Color Handbook. Tomato Diseases. Identification, Biology and Control*. Second edition. Academic Press, USA.
<https://doi.org/10.1201/b15145>
6. Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*, 12(7), 908-931.
<https://doi.org/10.1016/j.arabjc.2017.05.011>
7. Jiménez-Gómez, C. P., & Cecilia, J. A. (2020). Chitosan: A natural biopolymer with a wide and varied range of applications. *Molecules*, 25(3981), 1-43.
<https://doi.org/10.3390/molecules25173981>
8. Gutiérrez-Molina, J., Corona-Rangel, M. L., Ventura-Aguilar, R. I., Barrera-Necha, L. L., Bautista-Baños, S., & Correa-Pacheco, Z. N. (2021). Chitosan and *Byrsonima crassifolia*-based nanostructured coatings: Characterization and effect on tomato preservation during refrigerated storage. *Food Bioscience*, 42(101212), 1-9.
<https://doi.org/10.1016/j.fbio.2021.101212>
9. Correa-Pacheco, Z. N., García-Paniagua, K. D., Bautista-Baños, S., & Corona-Rangel, M. L. (2019). Efecto de nanorecubrimientos de quitosano-aceite esencial de tomillo sobre la calidad postcosecha en frutos de jitomate. *Revista Mexicana de Fitopatología*, 37(1), 29-36.
<https://doi.org/10.18781/R.MEX.FIT.1903-5>
10. Luque-Alcaraz, A., Lizardi-Mendoza, J., Goycoolea, F., Higuera-Ciapara, I., & Argüelles-Monal, W. (2016). Preparation of chitosan nanoparticles by nanoprecipitation and their ability as a drug nanocarrier. *RSC Advances*, 6, 59250-59256.
<https://doi.org/10.1039/C6RA06563E>

11. Martínez-González, M. C., Bautista-Baños, S., Correa-Pacheco, Z. N., Corona-Rangel, M. L., Ventura-Aguilar, R. I., Del Río-García, J. C. *et al.* (2020). Effect of nanostructured chitosan/propolis coatings on the quality and antioxidant capacity of strawberries during storage. *Coatings*, 10(90), 1-12.
<https://doi.org/10.3390/coatings10020090>
12. Sreekumar, S., Goycoolea, F., Moerschbacher, B., & Rivera-Rodriguez, G. (2018). Parameters influencing the size of chitosan-TPP nano- and microparticles. *Scientific Reports*, 8 (4695), 1-11.
<https://doi.org/10.1038/s41598-018-23064-4>
13. Sotelo-Boyás, M. E., Correa-Pacheco, Z. N., Bautista-Baños, S., & Corona-Rangel, M. L. (2017). Physicochemical characterization of chitosan nanoparticles and nanocapsules incorporated with lime essential oil and their antibacterial activity against food-borne pathogens. *LWT*, 77, 15-20.
<https://doi.org/10.1016/j.lwt.2016.11.022>
14. Correa-Pacheco, Z. N., Bautista-Baños, S., Marquina-Valle, M. A., & Hernandez-López, M. (2017). The effect of nanostructured chitosan and chitosan-thyme essential oil coatings on *Colletotrichum gloeosporioides* growth *in vitro* and on cv Hass Avocado and fruit quality. *Journal of Phytopathology*, 165(5), 297-305.
<https://doi.org/10.1111/jph.12562>
15. Barrera-Necha, L. L., Correa-Pacheco, Z. N., Bautista-Banos, S., Hernández-Lopez, M., Martínez-Jiménez, J. E., & Morán Mejía, A. F. (2018). Synthesis and characterization of chitosan nanoparticles loaded botanical extracts with antifungal activity on *Colletotrichum gloeosporioides* and *Alternaria* species. *Advances in Microbiology*, 8, 286-296.
<https://doi.org/10.4236/aim.2018.84019>
16. González-Saucedo, A., Barrera-Necha, L. L., Ventura-Aguilar, R. I., Correa-Pacheco, Z. N., Bautista-Baños, S., & Hernández-López, M. (2019). Extension of the postharvest quality of bell pepper by applying nanostructured coatings of chitosan with *Byrsonima crassifolia* extract (L.) Kunth. *Postharvest Biology and Technology*, 149, 74-82.
<https://doi.org/10.1016/j.postharvbio.2018.11.019>
17. Zhang, H., Fu, Y., Niu, F., Li, Z., Ba, C., Jin, B. *et al.* (2018). Enhanced antioxidant activity and *in vitro* release of propolis by acid-induced aggregation using heat-denatured zein and carboxymethyl chitosan. *Food Hydrocolloid*, 81, 104-112.
<https://doi.org/10.1016/j.foodhyd.2018.02.019>
18. Duan, Y., Wang, G., Fawole, O. A., Verboven, P., Zhang, X., Wu, D. *et al.* (2020). Post-harvest precooling of fruit and vegetables: A review. *Trends in Food Science & Technology*, 100, 278-291.
<https://doi.org/10.1016/j.tifs.2020.04.027>

19. Kibar, H. F., & Sabir, F. K. (2018). Chitosan coating for extending postharvest quality of tomatoes (*Lycopersicon esculentum* Mill.) maintained at different storage temperatures. *AIMS Agriculture and Food*, 3(2), 97-108.
<https://doi.org/10.3934/agrfood.2018.2.97>
20. Peralta-Ruiz, J., Grande-Tovar, C. D., Sinning-Mangonez, A., Coronell, E. A., Marino, M. F., & Chaves-Lopez, C. (2020). Reduction of postharvest quality loss and microbiological decay of tomato “chonto” (*Solanum lycopersicum* L.) using chitosan-E essential oil-based edible coatings under low-temperature storage. *Polymers*, 12(1822), 1-22.
<https://doi.org/10.3390/polym12081822>
21. Azmai, W. N. S. M., Latif, N. S. A., & Zain, N. M. (2019). Efficiency of edible coating chitosan and cinnamic acid to prolong the shelf life of tomatoes. *Journal of Tropical Resources and Sustainable Science*, 7(1), 47-52.
<https://doi.org/10.47253/jtrss.v7i1.509>
22. Sucharitha, K. V., Beulah, A. M., & Ravikiran, K. (2018). Effect of chitosan coating on storage stability of tomatoes (*Lycopersicon esculentum* Mill). *International Food Research Journal*, 25(1), 93-99.
23. Mustafa, M. A., Ali, A., & Manickam, S. (2013). Application of a chitosan based nanoparticle formulation as an edible coating for tomatoes (*Solanum Lycopersicum* L.). *Acta Horticulturae* 1012, 445-452.
<https://doi.org/10.17660/ActaHortic.2013.1012.57>
24. Al-Dairi, M., Pathare, P. B., & Al-Yahyai, R. (2021). Effect of Postharvest Transport and Storage on Color and Firmness Quality of Tomato. *Horticulturae*, 7(7), 163.
<https://doi.org/10.3390/horticulturae7070163>
25. Sree, K. P., Sree, M. S., & Samreen, P. S. (2020). Application of chitosan edible coating for preservation of tomato. *International Journal of Chemical Studies*, 8(4), 3281-3285.
<https://doi.org/10.22271/chemi.2020.v8.i4ao.10157>
26. Kasampalis, D. S., Tsouvaltzis, P., & Siomos, A. S. (2020). Chlorophyll fluorescence, non-photochemical quenching and light harvesting complex as alternatives to color measurement, in classifying tomato fruit according to their maturity stage at harvest and in monitoring postharvest ripening during storage. *Postharvest Biology and Technology*, 161(111036), 1-9.
<https://doi.org/10.1016/j.postharvbio.2019.111036>
27. Khairia, A. N., Falaha, M. A. F., Suyantohadia, A., Takahashib, N., & Nishinab, H. (2015). Effect of Storage Temperatures on Color of Tomato Fruit (*Solanum lycopersicum* Mill.) Cultivated under Moderate Water Stress Treatment. *Agriculture and Agricultural Science Procedia*, 3, 178-183.
<https://doi.org/10.1016/j.aaspro.2015.01.035>