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Postharvest Physicochemical Properties and Fungal Populations of Treated Cucumber with Sodium Tripolyphosphate/Titanium Dioxide Nanoparticles during Storage

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Abstract: Cucumbers have a limited shelf-life, from 10 to 14 days at commercial temperatures with relative humidity (80%). The aim of the work was to evaluate the postharvest physicochemical properties and fungal populations of cucumber treated with sodium tripolyphosphate/titanium dioxide nanoparticles during storage at 10 °C to prolong the shelf-life to 21 days. Cucumber samples treated with chitosan/sodium tripolyphosphate/titanium dioxide nanoparticles (Cu-CHS-TDN-ST) and (Cu-CHS-TDN-ST) were found to be in a well-hydrated state and with a green-colored appearance upon day 21, with excellent quality for consumption. Chitosan coating (Cu-CHS) alone prolonged the cucumber shelf-life for 14 days of storage. The Cu-CHS coating was less evident in reducing the respiration rate of cucumbers on day 14 than both nanocoatings, which were reported at the end of the storage period to be 5.09 and 5.38 mg·kg^{−1} h^{−1} for Cu-CHS-TDN-ST and Cu-CHS-TDN, respectively. The Cu-CHS-TDN-ST treatment reduced the loss of ascorbic acid content to 13.17 mg/100 g, delayed chilling injury, and had the highest chlorophyll contents during the whole storage period. The presence of sodium tripolyphosphate with the nanocoating delayed tissue damage. Peroxidase enzyme activity reached the maximum of 54.65 U·g^{−1} for Cu-CHS-TDN-ST on day 21, followed by Cu-CHS-TDN 50.1 U·g^{−1}. On day 21, the fungal populations of Cu-CHS-TDN samples (3.77 log CFU/g) were more than that of Cu-CHS-TDN-ST (3.15 log CFU/g) against Cu-Control (100 % spoiled). It was noted that the Cu-CHS-TDN-ST and CHS-TDN-ST coating treatments were capable of preserving the cucumber samples' quality during storage. The addition of sodium tripolyphosphate as a crosslinker for stabilizing the nanoparticle polymers in the coating treatments prolonged the shelf-life and achieved excellent quality for the cucumbers.

Keywords: cucumber; coating; sodium tripolyphosphate; chitosan; nanoparticles; shelf-life

1. Introduction

Cucumber (*Cucumissativus* L.) is a low-calorie fruit, rich in iron, magnesium, and potassium with a high moisture content. Cucumber quality is based on several factors such as shape, color, texture, size, absence of any signs of physical or microbial changes [1]. During the postharvest process, the cucumber tends to have some physicochemical changes, microbial populations, and desiccation, which influence the appearance and lower the nutrients. The moisture loss resulting from the transpiration process leads to wilting,

shrinkage, softening, and accelerates senescence. The microbial population is commonly caused by bacteria such as (*Xanthomonas* and *Erwinia* spp.), and by fungi such as (*Rhizopus* and *Alternaria* spp.) [2]. A storage condition of 7 °C is unfavorable as they are chill sensitive [3]. A chilled condition of 10 °C was applied to solve those problems which became more disposed to the quality loss after 2 weeks [4]. Novel techniques are needed for developing a sufficient moisture barrier and the antimicrobial support to prolong the cucumbers' shelf-life by waxing which have several disadvantages [5]. Treating with semi-permeable films is another technique used to delay the ripening processes [6–9]. Chitosan is effective against antifungal activities as it elicits the fungal cell wall degrading enzymes such as chitinase, phytoalexins 0-1, and 3-glucanase [10,11]. Recently, nanotechnology materials have appeared in all areas of life, due to their extremely small size [12]. Titanium dioxide nanoparticles (TiO₂) are manufactured worldwide in high quantities, due to their many uses in cosmetics, plastics, and paint, and even in foods [13,14]. The American Food and Drug Administration (AFDA) reported that TiO₂ nanoparticles are non-toxic and safe to be used in human food. They are used for their white coloring, photo-protective, coloring of TiO₂ photoprotective, and photocatalytic properties. Furthermore, consideration for plant protection extends the shelf-life of the active ingredients [15,16]. Sodium tripolyphosphate is an economical chemical component that can act as a crosslinker for stabilizing the nanoparticle polymers in nanoparticle formations [17].

Therefore, the current study aims to determine some of the postharvest physico-chemical properties and microbial population effect of cucumber treated with sodium tripolyphosphate/titanium dioxide nanoparticles during storage.

2. Materials and Methods

2.1. Coating Materials

The materials used for the coating solutions were sodium tripolyphosphate, titanium dioxide nanoparticles (particle size: 30 nm), chitosan (deacetylation medium of 85% molecular weight), and acetic acid (98%) as a preservative. All the used materials in the current study were obtained from (Benchmark, St. Louis, MO, USA).

2.2. Plant Materials and Coating Processes

Aark green, cucumber (*Cucumissativus* L.) cultivar was obtained from a private orchard at Taif City, Saudi Arabia. The harvested cucumber fruits were selected for the color, maturity and lack of any physiological and pathological disorders. Fruits were divided into four sets in three replicate and each one included 20 uniform cucumber samples. Control cucumber set (Cu-Control) was immersed for 10 s in distilled water instead of coating solutions then air-dried before storage. Chitosan (1%) solution was prepared with 0.1 M citric acid and sonicating overnight. The second set of cucumbers (Cu-CHS) were dipped in a coating solution of chitosan for 10 s, while the third set (Cu-CHS-TDN) was coated with chitosan/titanium dioxide nanoparticles 1%. The fourth coating set was (Cu-CHS-TDN-ST) with the addition of sodium tripolyphosphate 2% as a crosslinker for stabilizing the nanoparticles. The main factor was the chilled condition periods at 10 °C and the quality parameters were recorded at 0, 7, 14, and 21 days of the storage period.

2.3. Chilling Injury

Chilling injury degree is a physiological disorder that results in surface pitting, decay and intercellular movement [7,18]. The chilling injury was determined every seven days after cucumbers were transferred from the refrigerator to the ambient temperature. Chilling injury degree ranged (from 0 to 1) according to the Equation (1) [19]:

$$\text{Chilling injury (CI)} = \frac{\sum[(CI_{\text{scale}}) \times (\text{number of cucumbers at CI})]}{4 \times \text{total number of cucumbers per treatment}} \quad (1)$$

where 0 = no signs of pitting, 1, 2, 3, and 4 represent <25%, 25% to 50%, 51% to 75%, and >75%, respectively.

2.4. CO₂ Respiration Rate

The respiration rate for the treated cucumbers was evaluated during storage by placing in an airtight chest, a CO₂ sensor (Testo AG-435-2, Co. KGaA, Testo, Germany) to monitor the concentration at 1-min intervals for 60 min, and expressed as mg·kg⁻¹ h⁻¹ according to the Equation (2) [11,20].

$$\text{Respiration rate (mg·kg}^{-1}\text{h}^{-1}) = \frac{(\Delta y_{\text{CO}_2} \times V)}{(100 \times W \times \Delta t)} \quad (2)$$

where Δy_{CO_2} = concentration (%), V (mL) = free volume, W (kg) = weight, and Δt (s) = testing time in seconds.

2.5. Chlorophyll Contents

The chlorophyll contents were evaluated by mixing 5 g of various samples with 20 mL of 80% acetone for 30 s. The mixtures were centrifuged, measured at 654, 663 nm, and expressed in mg/g according to the Equations (3) and (4) [21].

$$\text{Chlorophyll } a = [12.7(A_{663}) - 2.69(A_{645})] \times \frac{V}{1000 \times W \times a} \text{ (mg/g)} \quad (3)$$

$$\text{Chlorophyll } b = [22.9(A_{645}) - 4.68(A_{663})] \times \frac{V}{1000 \times W \times a} \text{ (mg/g)} \quad (4)$$

where A = absorbance, W = weight in (g), and a = path length of light (1 cm)

2.6. Ascorbic Acid Content

The amount of ascorbic acid content was detected by the titrimetric method using 2,6 Dichloro-phenol- indophenol dye as described in the following Equation (5) [11,22]:

$$\text{Ascorbic acid content (\%)} = \frac{\text{titration volume} \times 111 \times \text{dilution factor} \times 100}{\text{sample volume} \times 1000} \quad (5)$$

2.7. Total Phenolic Content

Determination of total phenolic contents reacting with phosphomolybdic acid by Folin–Ciocalteu reagent in alkaline medium, produced blue colored complex after incubation at 45 °C and detected at 765 nm [10,23]. A standard curve was plotted by Gallic acid and the concentrations were expressed as mg/100 g.

2.8. Antioxidant Activity

The antioxidant activity was evaluated by DPPH (2,2-diphenyl-2-picrylhydrazyl) and FRAP (Ferric reducing antioxidant power) scavenging activity methods according to the following Equation (6) [24,25]:

$$\text{Antioxidant activity (\%)} = \frac{A_{br} - A_{ar}}{A_{br}} \times 10 \quad (6)$$

where A_{br} = absorbance before reaction and A_{ar} = absorbance after the reaction. Antioxidant activity values can be achieved by comparing the absorption changes.

2.9. Peroxidase Enzyme Activity

Peroxidase enzyme activity (POD) was evaluated with minor modifications [6,26]. Approximately 1 g of treated cucumbers homogenized in 0.1 mol/L pyrocatechol solution (3 mL) in a chilled mortar and centrifuged 12,000 × g for 10 min. The supernatants were taken and evaluated at 470 nm [26].

2.10. Fungal Populations

The measurements of fungal populations (yeast and mold counts) were carried out during the storage period every seven days until 21 days. A potato dextrose agar (PDA; Becton Dickinson, Franklin Lakes, NJ, USA) was applied as the medium for treated cucumbers. Approximately 10 g was used aseptically to prepare 1:10 dilutions, then added to a stomacher bag with 90 mL of 0.1% peptone water. The incubation temperature was (20–25°C) and the plates were inspected after 48 h [27,28].

2.11. Statistical Analysis

All extractions were applied in triplicate, and the data obtained were expressed based on fresh weight. The data were subjected to Duncan's multiple range tests followed by SPSS statistical software (17.00). Significant differences were accepted at $p \leq 0.05$.

3. Results and Discussion

3.1. Chilling Injury

Chilling injury is a severe problem that affects a number of vegetables and fruits during chilled conditions. Symptoms of chilling injury appear as severe shriveling, surface pitting, sunken spots, water-soaked areas, and decay which could be due to ethylene, amino cyclopropane carboxylic acid, and putrescine increase [18,29]. The results of chilling injury of the treated cucumbers stored at 10 °C for 21 days are presented in Figure 1. On the 7th day, the chilling injury of 0.58 was severe in Cu-Control and slight to moderate was reported in Cu-CHS, 0.39, while chilling injury was not detected in any nanocoated samples. A chilling injury index higher than 0.4 was considered unacceptable for customers. At the end of the 14th day of storage, Cu-Control was spoiled, while Cu-CHS was suffering severe chilling injury. On day 21, the Cu-CHS samples were not in marketable condition, the Cu-CHS-TDN-ST samples were in a more acceptable condition than the Cu-CHS-TDN cucumbers. Therefore, the onset of chilling injury symptoms in the CHS-TDN-ST samples was the most delayed. Mohamed et al. [30] reported that coating materials could reduce catalase activity and improve flavonoid, phenolic, and alternative oxidase that involved modulating enzymes and could enhance visual quality and chemical composition during storage.

Figure 1. Effect of coating treatments on chilling injury.

3.2. Respiration Rate

Respiration rate is one of the main factors which affects postharvest losses of cucumbers due to O_2 consumption and CO_2 subsequent production [11,20]. The respiration rate during storage is plotted in Figure 2. Results showed a high production of CO_2 in the initial storage period for the untreated cucumbers. There was a minor decrease in respiration rate in the Cu-CHS-TDN and Cu-CHS-TDN-ST coated cucumbers in comparison with Cu-Control which reported $8.38 \text{ mg}\cdot\text{kg}^{-1}\text{h}^{-1}$ on day 7. The Cu-CHS coating was less evident in reducing the respiration rate of cucumbers on day 14 than both nanocoatings which were reported at the end of the storage period 5.09 and $5.38 \text{ mg}\cdot\text{kg}^{-1}\text{h}^{-1}$ for Cu-CHS-TDN-ST and Cu-CHS-TDN, respectively. Sodium tripolyphosphate can act as a crosslinker for stabilizing the nanoparticle polymers in nanoparticle formations and delay oxidation processes [11,17]. Ghidelli et al. [31] reported a lower respiration rate by chitosan coating, due to the gaseous barrier properties. The results of the current research suggest that the addition of sodium tripolyphosphate to nanocoating could have affected the metabolic reactions and delayed the senescence course of cucumbers during the storage period.

Figure 2. Effect of coating treatments on respiration rate.

3.3. Chlorophyll Contents

Chlorophyll is responsible for the green pigment of the cucumber peel, when chlorophyll is decomposed, the yellowness reduces the sensory evaluation and the marketing value [32,33]. Table 1 shows the changes in chlorophyll contents of cucumbers during 21 days of storage at 10°C . The chlorophyll degradation was initiated directly during storage which was at slower rates in coated cucumbers as compared with Cu-Control. The chlorophyll “a” loss was 0.20 mg/g in Cu-CHS on day 14, while the nanocoated cucumbers Cu-CHS-TDN and Cu-CHS-TDN-ST were similar at the end of the storage time, with 0.24 and 0.25 mg/g , respectively, while Cu-Control samples reached 0.17 mg/g loss on day 7 then were completely spoiled by the end of the storage period. The changes in chlorophyll “b” contents were comparable to chlorophyll “a” contents with lower values under the same conditions. Hosam and Aly [34] observed a similar trend in lower chlorophyll contents after treating cucumber samples with chitosan, cassava starch, and gelatin. Throughout storage, the Cu-CHS-TDN-ST samples had the highest chlorophyll content of all the treatments, which could be due to the combination of sodium tripolyphosphate and the nanocoating to reduce the conversion of chlorophyll to yellow-olive-colored pheophytin.

Table 1. Effect of coating treatments on chlorophyll contents.

Days	Cu-Control	Cu-CHS	Cu-CHS-TDN	Cu-CHS-TDN-ST
Chlorophyll <i>a</i> (mg/g)				
0	0.23 ± 0.01 ^c	0.24 ± 0.03 ^c	0.32 ± 0.02 ^b	0.34 ± 0.01 ^a
7	0.17 ± 0.02 ^d	0.22 ± 0.02 ^c	0.26 ± 0.03 ^b	0.28 ± 0.03 ^a
14	-	0.20 ± 0.01 ^b	0.27 ± 0.02 ^a	0.27 ± 0.02 ^a
21	-	-	0.24 ± 0.01 ^a	0.25 ± 0.03 ^a
Chlorophyll <i>b</i> (mg/g)				
0	0.10 ± 0.03 ^d	0.12 ± 0.01 ^c	0.16 ± 0.02 ^b	0.21 ± 0.01 ^a
7	0.09 ± 0.02 ^d	0.11 ± 0.02 ^c	0.14 ± 0.03 ^b	0.17 ± 0.02 ^a
14	-	0.08 ± 0.03 ^c	0.12 ± 0.01 ^b	0.14 ± 0.02 ^a
21	-	-	0.11 ± 0.02 ^b	0.13 ± 0.03 ^a

Each value presents as mean ± standard deviation. Different letters indicate a significant difference ($p \leq 0.05$).

3.4. Ascorbic Acid Content

The maintenance of ascorbic acid content in cucumber samples under various coating treatments and storage period was extremely significant. The values ranged from 16.02 to 12.77 mg/100 g until the end of the storage period. The ascorbic acid content of cucumbers, 16.02 mg/100 g was detected on the day of preparation. The ascorbic acid content was lower in Cu-Control as 12.98 mg/100 g on day 7. At the end of the 14th day, the Cu-CHS samples reached a reduced 11.27 mg/100 g, Figure 3. The Cu-CHS-TDN-ST treatment reduced the loss of ascorbic acid content during the whole storage period. Vitamin C concentrations are highly sensitive to light, thus coating can enhance the ascorbic acid content during the storage period [35,36]. Similar effects were reported after coating treatments for several fruits and vegetables [4,7]. It was noted that the Cu-CHS-TDN-ST coating treatment was capable of preserving the ascorbic acid content in cucumber during storage.

Figure 3. Effect of coating treatments on the ascorbic acid content.

3.5. Total Phenolic Content

Polyphenols are responsible for sensory evaluation and have an essential role in vivo and in vitro oxidation [11,37]. The total phenolic content in the cucumbers on the day of preparation was 232, 322, 343, and 375 mg/100 g, respectively, Figure 4. The coating treatments preserved the total phenolic contents compared to Cu-Control which was spoiled at the end of the 7th day. The Cu-CHS samples reached 167 mg/100 g at the end of the 14th day, while the nanocoated cucumbers retained a high total phenolic content until

the end of the storage period. The Cu-CHS-TDN-ST and Cu-CHS-TDN treatments reported 213 and 192 mg/100 g, respectively. Vallverdu-Queralt et al. [38] reported a decrease in the total phenolic contents of tomato during storage. Camargo and Dunoyer [39] suggested that chilling may help in increasing the total phenolic contents by changing the metabolism of the phenolic components.

Figure 4. Effect of coating treatments on total phenolic content.

3.6. Antioxidant Activity

The effect of various coating treatments on antioxidant activities such as DPPH and FRAP are presented in Figure 5. The initial DPPH scavenging activity value in cucumbers was 55.14%. The Cu-Control samples reported 39.55% on the 7th day, while Cu-CHS reported 29.02% on the 14th day, Figure 5a. At the end of the storage period, the Cu-CHS-TDN reported a high DPPH scavenging activity value of 27.73%, while the Cu-Control and Cu-CHS samples were spoiled. The Cu-CHS-TDN-ST samples reported the highest DPPH scavenging activity value at the end of the storage period compared to the other samples. It was noted that the chilled conditions, combinations of sodium tripolyphosphate, nanocoating, and chitosan coatings could decrease the antioxidant compound losses and enhance the postharvest quality of cucumbers. The decrease in antioxidant activity could be related to a decrease in the total phenolic, ascorbic acid contents, and redox status [40–43].

Figure 5b shows the FRAP scavenging activity of cucumbers subjected to coating treatments and the storage period. The initial FRAP scavenging activity value of cucumbers was 0.79%. A decrease in the FRAP scavenging activity was noticed in all treatments during the whole storage, ranging from 0.39% to 1.12%. Cu-CHS samples reported 0.39% on day 14 then spoiled upon continuous storage. Cu-CHS-TDN-ST 1.12% achieved the highest increase in FRAP scavenging activity value on day 21 followed by Cu-CHS-TDN 0.99%. The presence of sodium tripolyphosphate and nano-coating enhanced the FRAP scavenging activity and delayed the tissue damage [20,44]. Results were linked with Zhang et al. [45] who reported higher antioxidant activities after coating cucumbers with chitosan-gallic acid, while the reduction of the antioxidant activity of the uncoated cucumbers could be due to the decay and senescence.

Figure 5. Effect of coating treatments on antioxidant activities; (a) DPPH and (b) FRAP scavenging activities.

3.7. Peroxidase Enzyme Activity

The cell walls of the plants are protected with antioxidant defense systems, such as enzymes, to support the cell walls [9,46]. Figure 6 shows that POD enzyme activities increased radically in all the cucumber samples during the whole storage period and reached the maximum 54.65 Ug^{-1} for Cu-CHS-TDN-ST on day 21 followed by Cu-CHS-TDN, at 50.1 Ug^{-1} . The Cu-CHS samples reached 43.18 Ug^{-1} on the 14th day then spoiled upon continuous storage, compared with Cu-Control which reached 28.34 Ug^{-1} , the lowest POD value on the 7th day, then completely spoiled upon continuous storage. Koh et al. [47] suggested that the maintenance of POD activity was related to the presence of consistent abiotic stress.

Figure 6. Effect of coating treatments on peroxidase enzyme activity.

3.8. Fungal Populations

Microbial safety is vital in evaluating the quality of the cucumbers. In this current research work, the effects of coating on fungal populations were examined in control and coated cucumbers. Table 2 presents the development of fungal populations of treated cucumber samples during the 21 days of storage at 10°C , expressed as log CFU/g. The

fungal populations increased progressively upon a continuous storage period in both control and coated cucumbers. The rate of fungal populations was the highest in the Cu-Control on the 7th day of storage and achieved a final fungal count of 3.91 log CFU/g, then it became completely spoiled before the 14th day as it was being largely contaminated by molds and yeasts. The Cu-CHS treatment efficiently reduced the fungal populations for the first 14 days of storage, reported a little sign of fungal decay, 3.85 log CFU/g, then it became completely spoiled before day 21. On the other hand, no sign of any visual populations was detected for either of the nanocoated cucumbers. The results were linked with Waewthongrak et al. [48] who used chitosan for inhibiting the growth of pathogenic fungi in citrus fruits. On day 21, the fungal populations of the Cu-CHS-TDN samples (3.77 log CFU/g) were more than that of Cu-CHS-TDN-ST (3.15 log CFU/g) against Cu-Control (100% spoiled). It was noted that the Cu-CHS-TDN-ST and CHS-TDN-ST coating treatments were capable of preserving the cucumber sample's quality during storage. Though, Cu-CHS-TDN-ST can be recommended as the most efficient antifungal treatment which kept cucumber samples in a well-hydrated state and with green-colored appearance upon day 21, with excellent quality for consumption. Sodium tripolyphosphate may act as a crosslinker for stabilizing the nanoparticle polymers in nanoparticle formations in coating treatments to prolong the shelf-life and achieve an excellent quality for cucumbers [17].

Table 2. Effect of coating treatments on fungal populations (log CFU/g).

Days	Cu-Control	Cu-CHS	Cu-CHS-TDN	Cu-CHS-TDN-ST
0	2.08 ± 0.21 ^a	2.08 ± 0.13 ^a	2.08 ± 0.12 ^a	2.08 ± 0.15 ^a
7	3.91 ± 0.11 ^a	2.31 ± 0.14 ^c	2.87 ± 0.18 ^b	2.17 ± 0.11 ^d
14	-	3.85 ± 0.11 ^a	3.15 ± 0.14 ^b	2.98 ± 0.13 ^c
21	-	-	3.77 ± 0.19 ^a	3.15 ± 0.17 ^b

Values in the same column with different superscripts a,b,c,d are significantly different $p \leq 0.05$.

4. Conclusions

Cucumber fruits require a satisfactory preservation method in order to retain quality during storage. The coating treatments used were chitosan/sodium tripolyphosphate/titanium dioxide nanoparticles. The results reported that chitosan coating alone can preserve cucumber quality up to 14 days in chilled conditions at 10 °C, while nanocoating treatments were capable of preserving the cucumber sample's quality during the whole storage period. POD enzyme activities reached the maximum of 54.65 U g⁻¹ for Cu-CHS-TDN-ST on day 21 followed by Cu-CHS-TDN 50.1 U g⁻¹. On day 21, the fungal populations of the Cu-CHS-TDN samples (3.77 log CFU/g) were more than that of Cu-CHS-TDN-ST (3.15 log CFU/g) against Cu-Control (100% spoiled). The addition of sodium tripolyphosphate as a crosslinker for stabilizing the nanoparticle polymers prolonged the cucumbers' shelf-life, achieved excellent postharvest quality and can be applied in the food industry.

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