




Article

Active Bionanocomposite Coating Quality Assessments of Some Cucumber Properties with Some Diverse Applications during Storage Condition by Chitosan, Nano Titanium Oxide Crystals, and Sodium Tripolyphosphate

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Abstract: Cucumbers have a short shelf-life of about 14 days, they are perishable due to the high moisture content. This study aimed to study the effects of nano-coating material such as titanium nanoparticles and chitosan with the addition of sodium tripolyphosphate as a crosslinker to enhance cucumber quality during storage. Some essential physical, chemical, and biological parameters were determined. CH-Nano-ST (chitosan/nano titanium oxide crystals/sodium tripolyphosphate) retained the maximum greenness, -7.99 , compared to CH-Nano samples, which recorded -7.31 . CH-Nano (chitosan/nano titanium oxide crystals) remained the lightest, 44.38 , and CH-Nano-ST was a little darker (43.73) compared to the others treatments. The discoloration was extra severe with control (22.30), which started to spoil after the end of the first week. After 21 days of the storage period at $10\text{ }^{\circ}\text{C}$, the reducing sugars content reduced to reach $-0.64\text{ g}/100\text{ g}$ and $-0.21\text{ g}/100\text{ g}$ for CH-Nano and CH-Nano-ST treatments, respectively. The CH-Nano-ST treatment presented a lower value of toughness, followed by CH-Nano at the end of the storage period. Moreover, the highest crispness index was detected for CH-Nano (5.12%), while CH-Nano-ST treatment had a slight decline to reach 4.92% . The biological results indicated that CH-Nano-ST treatment can be applied to delay the microbial contamination of *Salmonella* spp. in cucumbers as it reached 0.94 log CFU/g , while the CH-Nano treatment reached 1.09 log CFU/g , at the end of the storage period. In summary, nano-coating treatments with the addition of sodium tripolyphosphate can be applied to regulator postharvest quality measurements of the biological activities in cucumbers during storage at $10\text{ }^{\circ}\text{C}$ until 21 days.

Keywords: active bionanocomposite; application; coating; cucumber; quality; shelf-life; storage

1. Introduction

Cucumber (*Cucumis sativus* L.) is one of the essential seasonal vegetable crops globally and a favourite commodity export for markets and local consumptions. It represents one of the greatest vital and financial vegetables in many countries and especially Saudi Arabia [1]. Cucumber belongs to the *Cucurbitaceae* family and has great nutritional and medicinal functions, especially in diabetic patients, due to the useful ingredients such as 95% moisture, 3.6% carbohydrate, 0.65% proteins, 1.3 mg kg⁻¹ magnesium, as well as vitamins B5 (0.026 mg kg⁻¹) and ascorbic acid (0.28 mg kg⁻¹) [2]. Cucumber is cultivated and consumed as a fresh vegetable, sliced in salads during all seasons, and as pickles before the ripening phase [3]. Cucumbers are consumed worldwide due to their brittle flavor, nutrients, and health benefits. They have a short shelf-life of about 14 days, being perishable due to the high moisture content [4]. Cucumbers are influenced by respiration, moisture loss, yellowing, firmness, barrier, optical, mechanical changes, physiological injuries, shrivelling, and microbial growth during handling and storage processes [5]. The color can depend on the level of maturity [6] and chlorophyll degradation [7] as a result of the metabolic processes [8]. The customer judges the quality according to several sensorial factors such as firmness, appearance [9], and other related chemical properties such as vitamin C content and total soluble solids [10]. Storage can increase some biological activities such as growth of the bacteria, molds, and yeasts [11]. Moreover, several studies reported that stored cucumbers have been associated with some microbial contaminations such as *Salmonella*, being contaminated during post-harvest processes [12,13]. Recent techniques have included mild heat treatments, cold-shock temperature, essential oils, and atmospheric conditions; conversely, they lead to decline several physiological and sensorial characteristics [14].

Chitosan and nano chitosan are seafood by-products purchased from crab, shrimp, and crawfish, and can act as a barrier to the migration of moisture and gases, by reducing the oxidation of nutrients, delaying the senescence symptoms, and improving the visual and tactile features [15]. Moreover, coatings with high *aloe vera* gel, methoxy pectins, cassava starch, xanthan gum, glycerol, edible films, and nano-coatings are applied for several products in the food industry [16].

Several research papers [17,18] have indicated that coatings are significant in preventing cross-contaminations. Most uncoated cucumber samples in retail markets have lost moisture, appearance, and other sensorial evaluations during storage [19]. Therefore, long-term preservation methods are required to avoid spoilage so that cucumber might be consumed in off-seasons as well [20–22]. The titanium dioxide nanoparticles–chitosan composite has excellent photocatalytic activity against the organic pollutants, while chitosan has been reported to have antibacterial activities. In addition, the combination of nano titanium oxide crystals and chitosan has enhanced the mechanical properties of the formed films [23]. Sodium tripolyphosphate can be used as a crosslinker to stabilize the nanoparticle polymers in the nanoparticle formations [24].

The present research work aimed to determine active bionanocomposite coating quality assessments (using chitosan, nano titanium oxide crystals, and sodium tripolyphosphate) of some cucumber properties, such as color index profile, skin toughness, crispness index, reducing sugars, and total sugars, with the addition of the antimicrobial contamination against *Salmonella* spp., with some diverse applications during storage during the chilling condition for 21 days at 10 °C.

2. Materials and Methods

2.1. Materials

Fresh cucumber samples in the winter season were transferred from an orchard (Al-hada, Taif, Saudi Arabia) in 2020/2021. The substances were chitosan, derived from the 85%

deacetylation of chitin, which can be found in some fungi and seashells, nano TiO₂ crystals (VK-T25Q; TiO₂-30 nm with purity more than 99 wt %), and sodium triphosphate, Formula: Na₅P₃O₁₀ with ISO Purity of 90~99%), all obtained from Chengdu Dova, The New Material Co., Shanghai, China.

2.2. Preparation of Coating Solutions

Chitosan (CH) solution with 1% concentration was prepared by dissolving in acetic acid solution after adjusting the pH to reach (5.0) with buffer solution, and then the solutions were made to be (1.5 L) with sonicating overnight at >20 kHz. Chitosan–nano titanium oxide crystals (CH-Nano) solution was prepared by dispersing with 1% concentration of TiO₂ nanoparticles in 0.5 L of CH solution with constant stirring for 6 h. (CH-Nano-ST) solution was prepared by dispersing 2% of sodium triphosphate in 0.5 L of CH solution with stirring as described in the previous research by Khojah et al. [24].

2.3. Coating Processes

Cucumber samples were sorted for uniform size and no sign of any physical or mechanical damage or even fungus infection. Cucumber samples were washed with tap water to remove any loose soil, then with calcium hypochlorite (2%) for 15 min, and fan-dried at room temperature.

The cucumber fruits were randomly distributed into four groups:

- The first group (Control) was coated with distilled water as untreated samples (control).
- The second group (CH) was coated with chitosan.
- The third group (CH-Nano) was coated with a combination of chitosan and nano titanium oxide crystals.
- The fourth group (CH-Nano-ST) was coated with a combination of chitosan, nano titanium oxide crystals, and sodium triphosphate as a crosslinker among nanoparticle polymers. Figure 1 presents the formulations, coating application on cucumber samples, and quality evaluation and shelf-life extension.

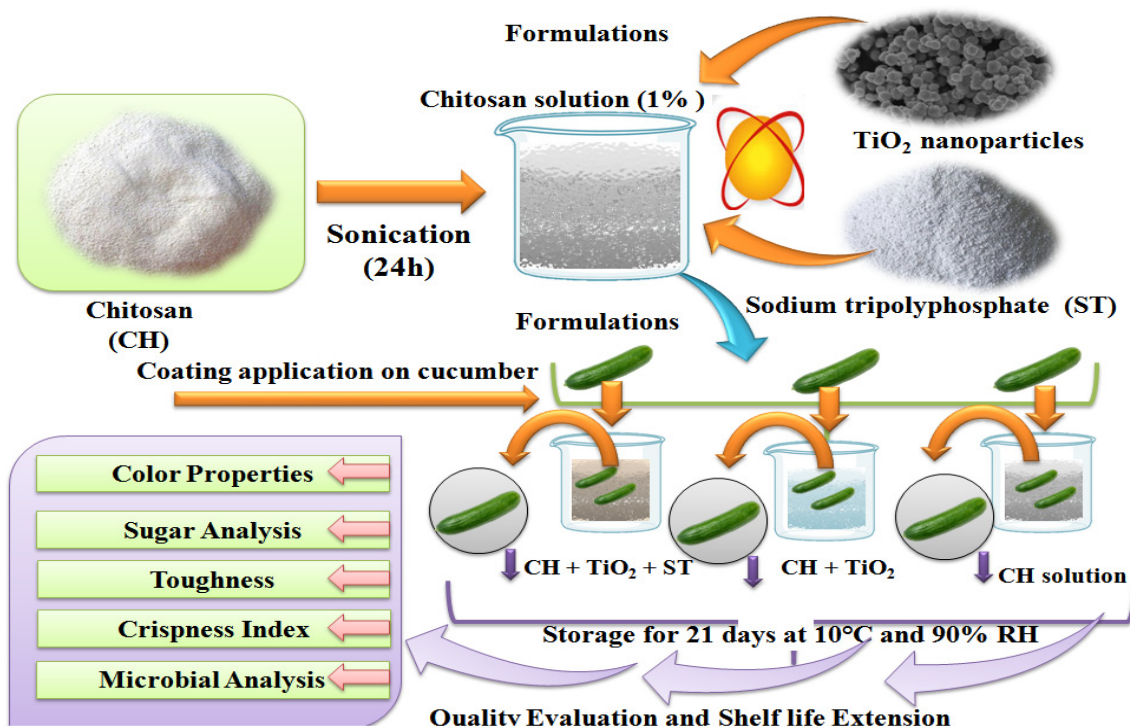


Figure 1. Formulations, coating application on cucumber samples, quality evaluation, and shelf-life extension.

2.4. Coating Applications

Cucumber samples were dipped in the distilled water and coating solutions for 10 s, the excess solutions were drained, and (coated and control) cucumber samples were air-dried. After drying, they were chilled in plastic trays with perforations at 10 °C and 90% relative humidity. At regular intervals of seven days, the cucumber samples were removed and evaluated for quality parameters until 21 days of the storage period. Three replicates were applied for each treatment, while each replicate included five cucumber fruits [24].

2.5. Color Index Profile

The color parameters of cucumber samples were evaluated by using the Miniscan XE Plus (Hunter & Associates Laboratory, Hunter Lab, Reston, VA, USA) spectrophotometer that provided changes in L^* , a^* , and b^* color space values [25,26]. The reflectance spectra were carried out in triplicate for three equatorial opposite sides of the cucumber surfaces ($n = 9$), then the final average of L^* , a^* , and b^* values were obtained for each treatment every 7 days.

2.6. Reducing Sugar and Total Sugar Analysis

The reducing and total sugars from cucumber samples of each group were triplicated and evaluated using a UV-Vis spectrophotometer (Max M2, San Jose, CA, USA) [27–29]. An aliquot of freeze-dried samples in liquid N_2 was refluxed with 100 mL alkaline solution of copper salt, boiling for 30 min [30]. Next, the titration method was applied with a protein-free solution in the presence of methylene blue for reducing sugar analysis, while total sugar analysis was evaluated after the inversion with concentrated hydrochloric acid.

2.7. Toughness, Crispness Indexes Evaluation

The physical attributes of cucumber texture such as toughness and crispness indexes were evaluated at regular intervals of seven days until 21 days of storage period [31]. The toughness of every treatment was evaluated using a texture profile unit (TPU-2S, Yamaden Co., Tokyo, Japan) with a 3 mm plunger moving at 150 mm min^{-1} . The crispness index was achieved from the force distance curves for the samples [32].

2.8. Evaluation of Antimicrobial Contamination

Antimicrobial contamination analyses were achieved *in vivo* according to Jung and Schaffner [13]. Approximately 2 mL of 20X PBS (phosphate-buffered saline), pH 7.4, was added to lab blender bags (Seward Stomacher 400, Seward Stomacher, West Sussex, UK), each containing 10 g cucumber. Serial dilutions were made (10^{-1} , 10^{-2} , and 10^{-3}) in PBS, and 0.1 mL of each dilution was plated on the surface of the selective media Hektoen enteric agar (Difco, BD, Fisher Scientific, Hampton, NH, USA) with 50 $\mu g/mL$ nalidixic acid. The plates were incubated at (± 37 °C) for 24 h for *Salmonella* spp. growth. Results were enumerated as log CFU/g (colony-forming unit)/grams of cucumbers) [33].

2.9. Statistical Analysis

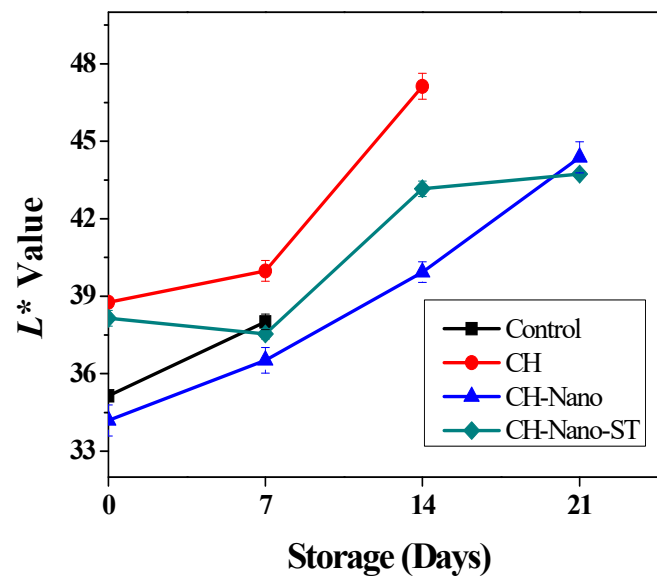
The experimental data and results were analyzed by a multifactor analysis of variance (ANOVA), while the significance of differences between treatments was carried out using Tukey's range test at $p < 0.01$.

3. Results

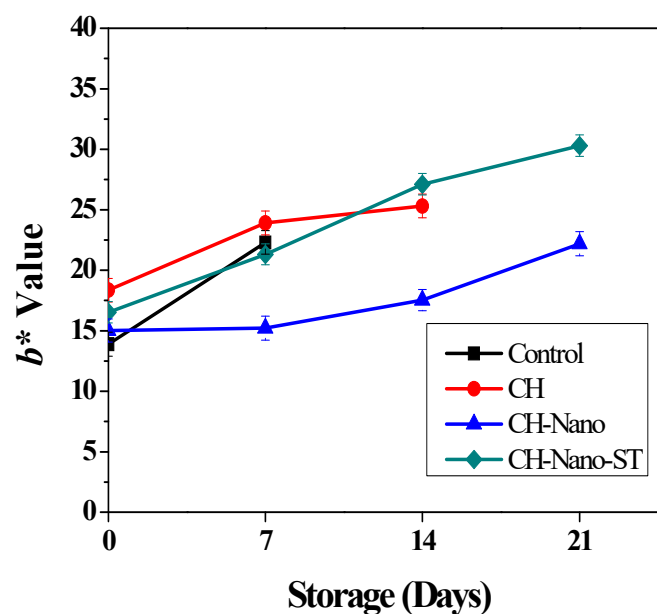
3.1. Color Properties

Color is one of the significant signs of cucumber quality and influences customer acceptance during storage [34]. On the day of preparation, the lightest treatment was CH followed by CH-Nano-ST and CH-Nano, while at the end of 21 days, CH-Nano remained the lightest with 44.38 and CH-Nano-ST was a little darker (43.73) than the others, which had some signs of spoilage and aging on cucumbers; Figure 2a. As presented in Figure 2b, discoloration was detected in all treatments due to the transformation of chlorophyll into carotenoids. The discoloration was extra severe with control 22.3, which started to spoil

after the end of the first week. After the 14th day, in CH samples, a little higher value of b^* (value 25.31) was detected, then it had some signs of spoilage, demonstrating a color difference, compared with those coated with nano-materials. Chitosan coatings have been applied by several researchers to delay color change [35,36]. CH-Nano and CH-Nano-ST treatments had the lowest values after 21 days of the storage period, 22.19 and 30.3, respectively. In terms of greenness a^* value, CH-Nano-ST retained the maximum greenness (-7.99) compared to CH-Nano samples, which recorded -7.31 ; Figure 2c. Overall, coating with nano-materials promoted the preservative properties and inhibited yellowing in the peel of cucumber samples. That may be due to the ability of chitosan and nano titanium oxide crystals to retard the degradation of chlorophyll by slowing metabolism in the cucumber peel [24]. Nano titanium oxide crystals reduced the conversion of chlorophyll to yellow-olive-colored pheophytin [37].



(a)



(b)

Figure 2. Cont.

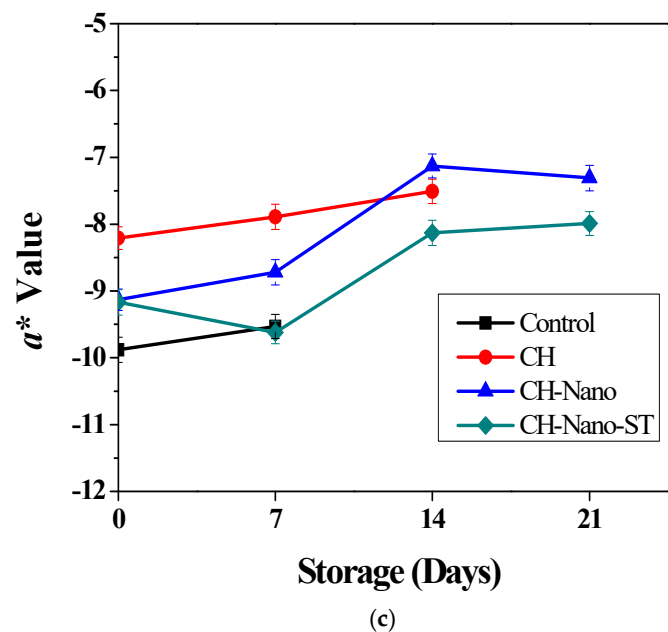


Figure 2. Effect of coating treatments of cucumber samples on color index profile; (a) L^* value, (b) a^* value, and (c) b^* value.

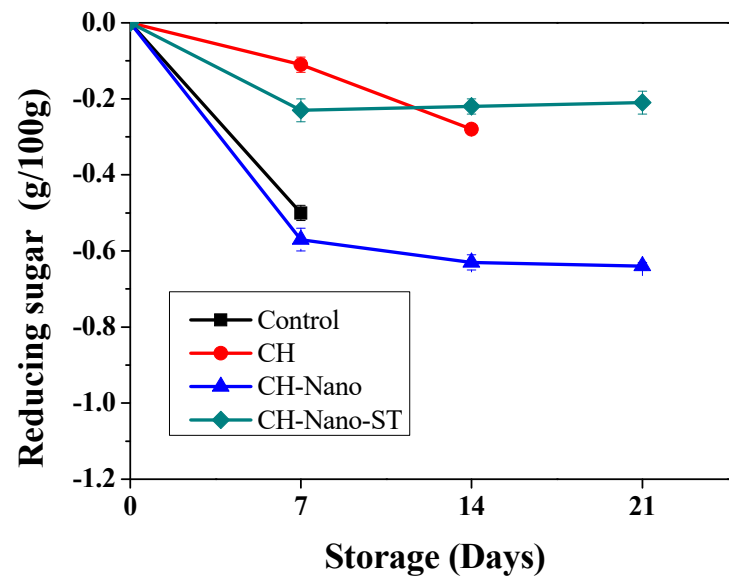
3.2. Sugar Analysis

The rate of change of reducing sugars and total sugars of cucumber samples stored for three weeks at 10 °C is presented in Figure 3a,b. Control and CH samples were spoiled after the 7th and the 21st days of the whole storage, respectively. After 21 days of the storage period, the reducing sugar content reduced to reach -0.64 g/100g and -0.21 g/100 g for CH-Nano and CH-Nano-ST treatments, respectively. Parallel changes were detected in total sugar contents, which reached -0.66 g/100 g and -0.26 g/100 g for CH-Nano and CH-Nano-ST treatments, respectively. The presence of sugar in the stored cucumbers is a vital index due to its benefit for health [24,38].

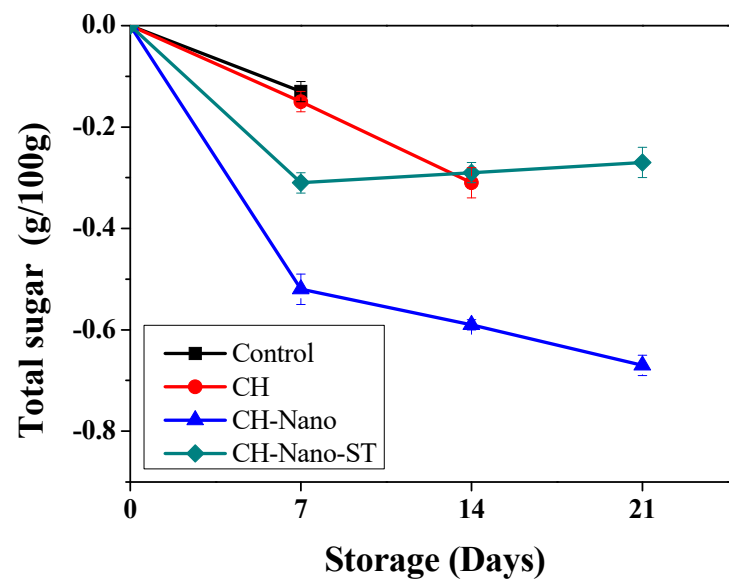
The sugar analysis for cucumbers was affected by distilled water, CH, CH-Nano, and CH-Nano-ST interactions, which decreased with increasing the storage period. However, providing nano-coating with the addition of sodium tripolyphosphate may preserve the cucumbers' sugar analysis, alleviate the adverse effects, and lead to a desirable quality of cucumber samples during storage. Sugar is utilized as a substrate and energy source for respiratory metabolism. Zapotoczn and Markowski [32] reported that a higher sugar content meant a better quality was preserved of cucumber samples during storage.

3.3. Texture Properties

The texture properties such as toughness and crispness indexes of treated cucumber samples during storage at 10 °C are presented in Figure 4a,b. The CH-Nano-ST treatment presented a lower value of 1.05 for toughness, followed by CH-Nano (1.13%) at the end of the storage period. Cucumbers with CH treatment had the toughest skin (0.93%) after the 14th day, followed by CH-Nano (0.32%); Figure 4a. Control samples showed the highest toughness value on the seventh day of storage, with signs of ageing and spoilage from then until the end of the storage period. Generally, CH-Nano-ST treatment positively affected the cucumber texture, which had softer and extra tender skin.



(a)

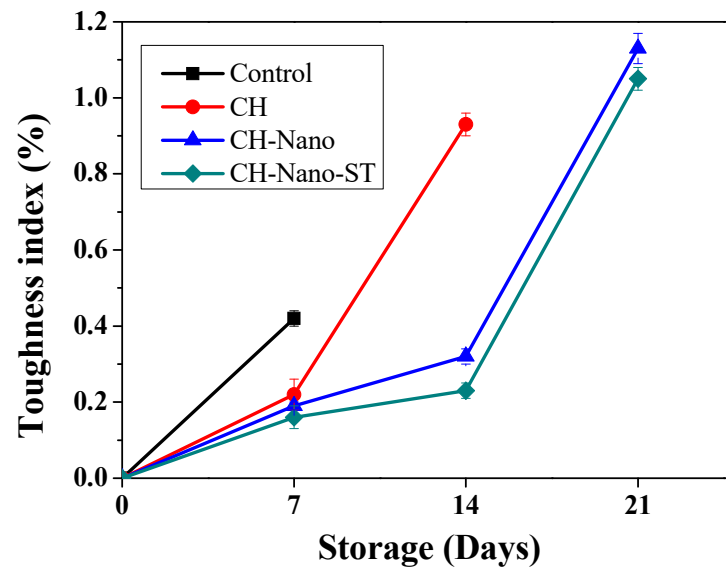


(b)

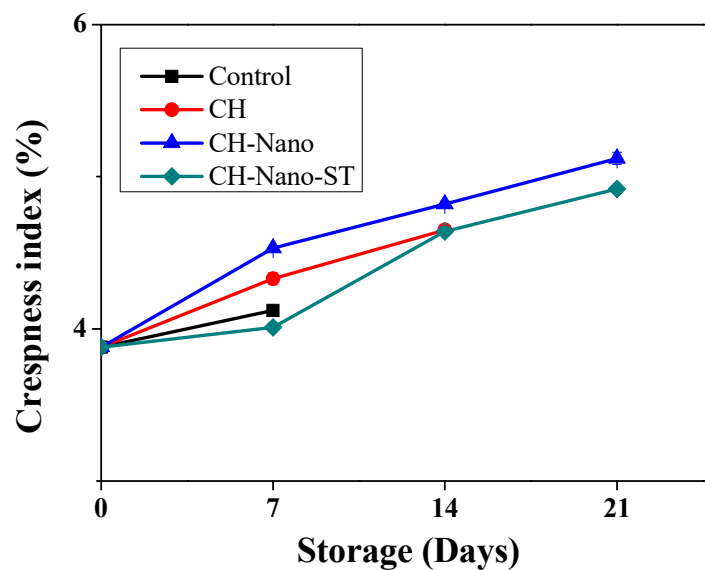
Figure 3. Effect of coating treatments of cucumber samples on reducing sugar (a), and total sugar contents (b).

Moreover, the highest crispness index was detected for cucumbers with CH-Nano (5.12%) at the end of the storage period, while those treated with CH-Nano-ST treatment had a slight decline to reach 4.92%. The crispness index of cucumbers with CH treatment was clarified and increased with the storage period until 14 days to reach 4.65%, then they had some signs of spoilage. The crispness index value on control was distinctly observed and raised with the period of storage to reach 4.12% on the seventh day, becoming approximately spoiled at 14 days. Slight distinct influences on the physical properties of cucumber samples were detected under the CH-Nano-ST treatment. There were no clear differences for CH-Nano cucumbers compared with control and CH samples due to the limitation of samples that spoiled and had less desirable texture, juicy and even firm and crisp after 7 and 14 days, respectively. Nakamachi et al. [39] reported that loss can be due to some biological, chemical, or functional changes on tissues during storage. The

current study was in agreement with earlier studies, which showed a decrease in physical properties as the storage period proceeded [40].



(a)



(b)

Figure 4. Effect of coating treatments of cucumber samples on texture properties; toughness indexes (a), and crispness index (b).

3.4. Antimicrobial Contamination Analyses

As a result of the antimicrobial contamination analyses in cucumbers, *Salmonella* was detected as 0.79 log CFU/g on the preparation day. On the other hand, CH samples clarified 1.12 log CFU/g on the 14th day of the storage period (Table 1). The results were in agreement with the work of Hara-Kudo et al. [41], who detected that the *Salmonella* spp. growth in cucumbers during storage reached 0.1–0.2 log CFU/g, which can lead to infections in humans.

Table 1. Bacterial contamination of *Salmonella* spp. isolated from coated cucumber samples during storage at 10 °C.

| Storage (Days) | Control | CH | CH-Nano | CH-Nano-ST |
|----------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 0 | 0.79 ± 0.02 ^a | 0.79 ± 0.03 ^a | 0.79 ± 0.02 ^b | 0.79 ± 0.04 ^d |
| 7 | 0.88 ± 0.01 ^b | 0.93 ± 0.02 ^b | 0.81 ± 0.04 ^c | 0.80 ± 0.05 ^c |
| 14 | | 1.12 ± 0.04 ^c | 0.86 ± 0.01 ^c | 0.81 ± 0.03 ^b |
| 21 | | | 1.09 ± 0.05 ^a | 0.94 ± 0.08 ^a |

Notes: ^{a-d}: means within the same column with different superscript letters are significantly different at $p < 0.01$.

The results indicated that CH-Nano-ST treatment can be applied to delay the microbial contamination of *Salmonella* spp. in cucumbers as it reached 0.94 log CFU/g at the end of the storage period. Moreover, CH-Nano treatment also delayed the *Salmonella* spp. growth to reach 1.09 log CFU/g. In a word, *Salmonella* spp. was detected during the storage of cucumber samples; however, coating with nano-materials and sodium tripolyphosphate as a cross-linker delayed the bacterial generations. The pathogens may infect customers, possibly causing demanding conditions to treat the infection due to their antibiotic resistance [42,43]. Consequently, nano-coating treatments with the addition of sodium tripolyphosphate can be applied to regulate *Salmonella* spp. in cucumber and other vegetables. Sodium tripolyphosphate may act as a cross-linker for stabilizing the nanoparticle polymers in nanoparticle formations in coating treatments to prolong the shelf-life and achieve an excellent quality [44].

4. Conclusions

Chitosan/nano-titanium oxide crystals/sodium tripolyphosphate films can be used for the extension of shelf-life by maintaining some quality parameters such as color index profile, skin toughness, crispness index, reducing sugars, and total sugars, with the addition of the antimicrobial contamination against *Salmonella* spp. during the chilling condition for 21 days at 10 °C. Using sodium tripolyphosphate as a crosslinker, is needed for nanotechnology applications in the food packaging industry.

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