

Improving the quality and shelf-life of strawberries as coated with nano-edible films during storage

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ABSTRACT

Strawberry is a non-climacteric fruit but exhibits very limited postharvest life due to its rapid softening and decay. Therefore, the current study was carried out to evaluate the effect of edible coatings using chitosan (CH) and chitosan nanoparticles (CHNPs) to improve the quality and extending the shelf life of strawberries during storage. Chitosan nanoparticles (CHNPs) were prepared by an ionic gelation method. The size and shape of the synthesized CHNPs were observed by transmission electron microscopy (TEM) technique. The strawberry samples were stored (after coating) at ambient temperature ($25\pm 3^\circ\text{C}$) and cold temperature ($6\pm 2^\circ\text{C}$) then, we evaluated the effectiveness of edible coating for physicochemical and microbiological properties, as well as the shelf-life during storage. The results of the TEM showed that the average particle size was 18.8-21.8 nm. At the different temperatures of storage, the samples coated with CHNPs had lower values of weight loss, decay, total soluble solids, and malondialdehyde concentrations. The same treatment had higher levels of total acidity and anthocyanin compared to the CH and uncoated treatments, that lead to preserving their quality properties and extending the shelf-life. Also, counts of total aerobic, mold, and yeast for strawberry samples treated by CHNPs were lower than those for CH and control treatment during the storage. In conclusion, the antimicrobial edible coating by CHNPs improved the qualitative characteristics and increased the shelf-life from 2 to 6 days for fruits stored at $25\pm 3^\circ\text{C}$ and from 8 to 16 days for samples stored at $6\pm 2^\circ\text{C}$.

Keywords: Strawberry, edible coatings, chitosan nanoparticles, TEM, shelf-life.

INTRODUCTION

Supermarkets face challenges to keep the freshness of fruits and vegetables for longer times. During postharvest transportation and storage, fresh fruits undergo quality deteriorations due to the various physiology reactions and processes. These physiological changes are directly influenced by the surrounding environment, which leads to loss of water, texture, color and nutrients (Gong *et al.*, 2018). One major contributor to these losses is represented by the food spoilage, which was induced by a wide range of microorganisms (Tesfaye, 2019). In developing countries, the percentages of product losses are quite high due to a lack of appropriate technologies for postharvest storage of fruits and vegetables (Kumar *et al.*, 2019).

Strawberry fruits are one of the most consumed berries in worldwide (Rahimi *et al.*, 2018). Principally, it is consumed as fresh products for its sensory features, such as color, texture, aroma and flavor. In addition, it is a vegetable crop rich in vitamins (A, B and C) and minerals (K, Ca, Mg, Fe and S) that possess functional properties (Abozaid and Eldeeb, 2019).

Egypt is the fourth-largest producer of strawberry worldwide after the United States, Turkey, and Spain with production 479 thousand tons in 2018. Also, strawberry is one of the most important crops of exported vegetables. Egyptian Export statistics indicate that Egyptian strawberry exports to be about 139 thousand tons in 2018 (CAPMS, 2019). Fresh strawberries are highly perishable, a non-climacteric fruit. However, postharvest handling and storage of fresh strawberries are difficult mostly due to their high susceptibility to mechanical injury, water loss, microbial decay, physiological deterioration and high respiration rate (Liu *et al.*, 2018a).

The food industry is making great efforts to minimize the losses and extend the shelf life. Therefore, there is a constant search for new technologies that can help in achieving these goals. Nanotechnology is one of the major innovations which have already been applied in this area (Zorraquín-Peña *et al.*, 2020). In the last several years, the edible coatings have been widely studied for preserving fruits and vegetables. Previous studies related to chitosan indicated its potential to prolong storage period and control decay of many fruits. Chitosan (b-(1, 4)-2-amino-2-deoxy-d-glucose) is a natural polymer known by its biological properties, such as non-toxicity, biocompatibility and

biodegradability with excellent film forming properties (Younes and Rinaudo 2015). Chitosan nanoparticles (CHNPs) have characteristic features of chitosan biopolymer and nanoparticles such as quantum size effects, and they possess wide applications in the antimicrobial agents (Kalaivani *et al.*, 2020). The unique physical and chemical characteristics of CHNPs are consequences of reduction in particle size and increasing the surface to particle size ratio in nanoparticles (Roy *et al.*, 2019).

Many studies have focused on the efficacy of chitosan and nano-chitosan, and its application as a natural preservative in foods. In the study by Melo *et al.* (2018) CHNPs were responsible for delaying the ripening process of the grapes resulting in decreased weight loss, soluble solids and reducing sugar content and increased moisture retention and preservation of the titratable acidity values. Moreover, CHNPs exhibited an inhibitory effect against pathogenic foodborne bacteria.

Also, Esyanti *et al.* (2019) studied the effect of CHNPs as a good coat on the Cavendish bananas ripening process. The observations of physical characteristics of bananas ripening showed that fruits coated with CHNPs 0.2% have a slower skin discoloration by 2-3 days compared to control treatment.

Recently, Hasheminejad and Khodaiyan (2020) investigated the effect of coating on shelf-life and quality of minimally processed pomegranate arils during storage at 5 °C. The CHNPs were the most effective coating for extending shelf-life and controlling undesirable microbial, physicochemical and sensory alterations of pomegranate arils.

This study aims to evaluate formulations of chitosan and chitosan nanoparticle as antimicrobial to extend the strawberry storage at different temperatures. Also, we aimed to study the characteristics of physicochemical, microbiological and overall acceptability for coated fruits with each formulation and to compare them with uncoated sample (control) during the storage periods.

MATERIALS AND METHODS

Chemicals

Chitosan powder (assay 99%, degree of deacetylation 80%) was purchased from Suvchem, Mumbai, India. Acetic acid, sodium tripolyphosphate (TPP) and sodium hydroxide, glycerin, trichloroacetic acid and thiobarbituric acid were purchased from El-Nasr

Pharmaceutical Chemicals Company, Cairo, Egypt.

Strawberry fruits

Fresh strawberry fruits (*Fragaria ananassa*) "Camarosa" were obtained from AL-Mollak Farm, Abo-Hamad City, Al-Sharqia Governorate, Egypt. The fruits were harvested on mid-April, 2019 when bright red color (3/4 of surface showing red) at the same ripening stage. The fruits were selected based on the uniform color, same size and no pests, as well as the absence of blemishes or disease, with no physical damage.

Methods

Preparation of chitosan nanoparticles

Chitosan nanoparticles (CHNPs) were prepared by ionic gelation method as described by Vimal *et al.* (2013). Chitosan solution was prepared by dissolving 1.0 g chitosan in 100 mL acetic acid (1%) and leaving it under stirring for until the solution was transparent. The pH was adjusted to pH 5.5 with 0.01N NaOH. Then, sodium tripolyphosphate solution (1%) was added to chitosan solution dropwise under magnetic stirring. Once the dropwise addition was completed, the resulting suspension was then left under stirring for 45 min.

Preparation of coated strawberry samples

The coating solutions were prepared by adding CH or CHNPs (at concentrations 0.5 and 1%) and glycerol as a plasticizer (1.5:100 v/v). The solution was stirred over a magnetic stirrer for 15 min at ambient temperature before use. The strawberries were washed with portable water and was left for airdrying. The strawberries were randomly distributed into five samples. Four samples were treated (coating with CHNPs or with CH at different concentrations) and the fifth sample was used as control (untreated). After that samples were dipped in the different coating solutions for 3 min and left to let it dried, packaged in polypropylene container, then were divided into two groups: the first was kept at ambient temperature ($25\pm 3^{\circ}\text{C}$) and the other sample kept at cold temperature ($6\pm 2^{\circ}\text{C}$). The samples were periodically analyzed every 2 days during the storage at $25\pm 3^{\circ}\text{C}$, and every 4 days during the storage at $6\pm 2^{\circ}\text{C}$ until the samples spoilage.

Analytical Methods

Characterization of synthesized chitosan nanoparticles

The morphological features of CHNPs were examined by High-Resolution Transmission

Electron Microscopy (TEM) which provides accurate information about the size and shape of the formed nanoparticles. TEM characterization is performed using (JEOL, JEM-1230, Japan) instrument with an acceleration voltage of 120 kV.

Determination of physiochemical quality criteria for coated fruits.

Weight loss (%)

The weight loss was calculated as the percentage loss of initial weight as reported by Qin *et al.* (2015).

Total soluble solids (%)

Total soluble solids (TSS) were determined by the refractometric method according to AOAC (2016) using a hand refractometer and the results were expressed as % TSS.

Total titratable acidity (%)

Total titratable acidity (TA %) was determined according to method of Hajji *et al.* (2018) based on content of citric acid per 100 g of fresh weight.

Malondialdehyde concentration

The malondialdehyde concentration (MDA) was measured according to method of Liu *et al.* (2018).

Measurement of anthocyanin content

Anthocyanin content was extracted by the method of Holzwarth *et al.* (2012). Then it is calculated according to Vargas *et al.* (2006).

Determination of microbiological quality criteria

Inspection of decay

At each time of analysis, visually decayed fruits were removed and the decay percentage was calculated as the number of decayed fruits according to the method of Li *et al.* (2019).

Total bacterial count

The total aerobic bacteria count was determined according to the procedures described by FAO/WHO (1995).

Yeast and mold counts

The yeast and mold counts were determined by the method described by APHA (1992).

Measurement of shelf-life

The shelf-life of strawberry samples was calculated by counting the days required for them when they remained still acceptable for marketing according to Mondal (2000).

Statistical analysis

The experiment was conducted in a completely randomized design with three replications. The statistical analysis was performed using the Cohort software CoStat (version 6.45). One-way ANOVA at the level of significance of $p < 0.05$, using the Duncan test was performed to compare the means among different treatments.

RESULTS AND DISCUSSION

Particle size and morphological analysis of chitosan nanoparticles

Transmission electron microscope (TEM) imaging showed the morphological properties and surface appearance of CHNPs, which had a nearly spherical shape, smooth surface with average particle size 18.8- 21.8 nm as illustrated in Fig (1).

Effect of coating with nanoparticles on quality criteria of strawberry fruits during storage

Weight losses (%)

Strawberry fruits are highly susceptible to rapid loss of water, resulting in very thin skin, which induced the shrinkage and weakening of tissues (Otoni *et al.*, 2017). The effect of coatings on weight losses for strawberry fruits treated by CH or CHNPs at different concentrations during storage was studied and results are presented in Table (1).

During a prolonged storage periods, a gradually increase ($p < 0.05$) in weight loss (%) was observed for all samples at different temperatures, which might be related to the continuous movement of water from the fruits to the surrounding environment (Duan *et al.*, 2011). These results are in similar with the findings of Nguyen *et al.* (2020) who reported that the weight loss (%) was gradually increased with increasing of storage periods. At different storage temperatures, coating significantly reduced ($p < 0.05$) weight loss percentage compared to uncoated strawberry throughout storage. Also, based on the mean comparison, it can be concluded that, the weight loss % at $25 \pm 3^\circ\text{C}$ in all treatments was more than those at $6 \pm 2^\circ\text{C}$.

During the storage at $25 \pm 3^\circ\text{C}$ after the six days of storage, the highest weight losses ($p < 0.05$) were noted for the untreated fruits (28%) followed by the sample treated with CH 0.5% (23%). While, the lowest losses were observed in the sample treated with CHNPs 1% (13.5%).

With regard to the effect of cold storage ($6 \pm 2^\circ\text{C}$), at the end of storage (16 days), surely

coating formulas significantly reduced ($p < 0.05$) weight loss in all coated samples compared to uncoated sample. The uncoated strawberry recorded weight loss of 13.65%. While, the rates of weight loss in treated samples ranged from 3.85 to 9.75% at the same storage period. It is worth noting that the lowest loss ($p < 0.05$) was observed in the sample treated by CHNPs 1% (3.85%).

Generally, the results are consistent with previous studies showing a reduction in weight loss was related to the effects of coatings. These effects served as semipermeable barriers against oxygen, carbon dioxide, and moisture, thus reducing respiration, water loss and oxidation reactions. Also, nanoparticles are responsible for creating a zigzag in the film structure, which would hinder the passage of permeates such as O_2 , CO_2 and water vapor as reported by Svagan *et al.* (2009).

Total soluble solids (TSS)

Total soluble solids (TSS) play an important role in affecting fruit quality and consumer acceptability. TSS content of strawberry samples as affected by coatings with CH or it is nanoparticles during storage at room and cold temperatures for different periods are presented in Table (2). Data proved that strawberry fruits stored on room temperature recorded higher values in Brix percentage than those stored on fridge conditions. TSS content of samples ranged from 5.6 to 5.8%. Also, it could be observed that TSS content of all samples was increased during storage periods. These results are in agreement with Zhang *et al.* (2018) who indicated that TSS of fruits increased during storage due to breakdown of starch into soluble sugars or the hydrolysis of cell walls.

During the storage at room temperature, there was no considerable difference in changes in TSS content of coated samples. In contrast, there was a considerable difference ($p < 0.05$) in changes in TSS content between these samples and uncoated fruit sample (control). Also, it could be observed that TSS content of all samples was increased during storage periods. At the 2nd day of storage, the control sample showed the highest content of TSS (6.58%) compared to the coated samples which ranged from 5.83 to 6.00%. At the 4th day of storage, TSS contents reached to 6.12, 6.05, 6.22 and 6.18% for strawberry sample treated by CH (at 0.5 and 1%) and CHNPs (at 0.5 and 1%), respectively. While, at the end of storage all tested samples were spoiled, except sample treated by CHNPs at 1%. On the other side, at cold storage, TSS

content for all samples showed a slight decrease at the end of storage period. On the 8th days, TSS content of uncoated strawberry and treated with CH 0.5% was decreased to 5.65 and 5.75%, respectively, while, other coated samples had a continuous increase and recorded 5.81, 6.03 and 5.79 for CH 1% and CHNPs (at 0.5 and 1%), respectively. Similarly, at the 12th days, TSS content of samples treated with CH (at 0.5 and 1%) was decreased to 5.66 and 5.74%, while, the other coated samples had a continuous increase and recorded 6.19 and 5.97 for CHNPs (at 0.5 and 1%).

At the end of storage (the 16th day), TSS content was decreased in the all treated samples and recorded 5.56, 6.1 and 5.95 for CH 1% and CHNPs (at 0.5 and 1%), respectively. These results are in line with those reported by Nguyen *et al.* (2020), who found that TSS of strawberry fruits were increased and then were slowly declined due to hydrolysis until the end of the storage time.

Titrateable acidity content

The organic non-volatile acids are among the most important components and represent the second most important component of strawberry flavor, after soluble sugars (Khalifa *et al.*, 2016). The effect of coatings by CH and CHNPs on titrateable acidity content for strawberry fruits is presented in Table (3). The result indicates that no significant differences were recorded in titrateable acidity content between uncoated and all coated samples. The titrateable acidity contents were found to be 0.48 to 0.53g citric acid/100g for samples at initial storage.

During the storage at room temperature, TA contents didn't show significant changes from effect of storage periods in strawberry samples treated by CHNPs. On the contrary, significant changes ($p < 0.05$) in TA contents were observed between uncoated (control) and strawberries treated by CH. This observation of TA variation was similar to those observations of Thakur *et al.* (2018). TA content in strawberries reduced from 0.48, 0.52, 0.51, 0.53 and 0.52% at initial storage to 0.37, 0.45, 0.47, 0.48 and 0.49% after the 2nd day of storage for control and samples coated with CH (at 0.5 and 1%) and CHNPs (at 0.5 and 1%), respectively. These values reached to 0.34, 0.36, 0.43 and 0.43% for the same samples (without control) at the 4th day. At the end of storage (6 days), all samples were spoiled, except the sample treated with CHNPs 1% recorded TA content (40%).

With regard to the storage of strawberry samples on a cold temperature, no considerable

changes were observed in TA contents between the samples during different storage periods. On the 8th day, the highest reduction in TA content was recorded in uncoated sample (10%). While, the lowest reduction was observed in the sample treated by CHNPs 1% (4%) compared to the other samples that ranged from 5 to 6% as portrayed in Table (3). On the 12th day of storage, TA contents were reduced to 0.44, 0.44, 0.45 and 0.45% for CH (at 0.5 and 1%) and CHNPs (at 0.5 and 1%), respectively. At the end of cold storage (16 days), the minimum reduction in TA for strawberry samples was noted in sample treated with CHNPs 1% (8%). Nano-membrane could inhibit the respiration of samples and slow down the consumption of acid in the physiological metabolic activities of fruits. Therefore, the nano-membrane can effectively slow down the downward trend of titratable acid and extending the shelf life of the fruits (Zhang *et al.*, 2018).

Anthocyanin content

Colour is the most important factor which describes the fruit general quality. The effect of coating on anthocyanin content for strawberry fruits treated by CH or CHNPs at different levels was studied and the results are presented in Table (4). The the results indicated that no significant differences were recorded in anthocyanin content between uncoated sample and all coated samples at initial storage, but it significantly differed ($p < 0.05$) during different storage periods. In general, it is noticed that the anthocyanin content in strawberry fruits was increased with the progress in storage periods and then was decreased at the end of storage periods. These results were in line with the previous study of Nguyen *et al.* (2020).

During storage at room temperature, the uncoated sample showed a higher significantly increase (52.76 mg /100g) than the coated samples ($p < 0.05$), which ranged from 46.58 to 48.88 mg /100g at the 2nd day of storage. It could be explained as a natural process during fruit ripening, besides the effect of high weight loss that could contribute to the concentration of pigments, according to Badawy *et al.* (2017). Also, these results are in agreement with those obtained by Rahimi *et al.* (2018), who found that uncoated strawberry had a greater anthocyanin content than coated fruits. On the 4th day, the anthocyanin contents recorded 44.60, 47.80, 50.96 and 54.70 mg/100g for CH (at 0.5 and 1%) and CHNPs (at 0.5 and 1%), respectively. At the end of storage (the 6th day), all samples were spoiled, except the sample treated with CHNPs 1% which recorded 58.25 mg/100g.

On the other hand, during the storage on cold temperature, a slow increase was recorded in the anthocyanin content to 44.68, 39.62, 37.00, 36.92 and 34.57 mg/100g at the 4th day for control and samples coated with CH (at 0.5 and 1%) and CHNPs (at 0.5 and 1%), respectively. At the 8th day of storage, a decrease in anthocyanin content was occurred for uncoated sample (38.36 mg/100g), while the other treated samples had a continuous increase and the values ranged from 38 to 43.8 mg/100g. At the end of storage (the 16th day), anthocyanin contents were observed in all remaining samples and reached to 40.17, 47.25 and 45.56, mg/100g for samples treated with CH 1% and CHNPs (at 0.5 and 1%), respectively. These results in agreement with the results obtained by Qamar *et al.* (2018), who showed less change in anthocyanin content in fruits of raspberry and table grapes coated with chitosan.

Malondialdehyde concentration

The malondialdehyde (MDA) is a secondary end final product of membrane lipid oxidation. It is usually considered as an indicator to assess the progress of fruit ripening which represents the structural integrity of the membranes of plants (Gao *et al.*, 2016).

The effect of coatings with CH or CHNPs during storage on MDA concentration for strawberry fruits is presented in Tables (5). The finding indicated that no differences were observed in MDA concentrations between uncoated and all coated samples at the initial storage of MDA concentration in the all tested strawberry samples was found to be 2.45 mmol/g, but it differed significantly ($p < 0.05$) during the different storage periods. In general, during prolonged storage periods, a gradual increase ($p < 0.05$) was detected in MDA concentrations for all coated and uncoated samples. These results are in agreement with a previous study which reported that MDA concentration was gradually increased with prolonged storage periods for strawberry fruits (Nguyen *et al.*, 2020). Also, the uncoated sample recorded a higher MDA concentration ($p < 0.05$) than those for coated samples at different storage temperatures. To compare the effect of storage temperatures on the MDA concentration, this study indicated that the storage at room conditions led to higher MDA concentration than those for the samples stored at fridge conditions.

During the storage at room temperature on the 2nd day, the uncoated sample showed the highest value of MDA (3.95 mmol/g) compared to the coated samples which reached to 3.5, 3.38,

3.39 and 3.28 for CH (at 0.5 and 1%) and CHNPs (at 0.5 and 1%), respectively. At the 4th day, the uncoated sample was spoiled and MDA concentration in coated samples reached to 4.65, 4.38, 4.35 and 4.21 mmol/g. At the end of the storage period (the 6th day), all the samples were spoiled, except the sample coated by CHNPs at 1% which had 5.04 mmol/g of MDA concentration.

At cold a temperature, due to the decrease in respiration trend, the chemical changes in fruit tissue occur slowly. So, the changes of MDA were slower than those changes at the room temperature. The MDA values of all strawberry samples were clearly increased ($p < 0.05$) during the storage period and the MDA value of uncoated sample was increased to 4.29 mmol/g after 8 days then it was spoiled on the 12th day. While, the rate of MDA increasing was lower in the coated samples. At the end storage (the 16th day), the MDA values reached to 5.53, 5.62 and 5.18 mmol/g for coated samples by CH 1% and CHNPs (at 0.5 and 1%), respectively.

According to the previous results, CHNPs coatings could be effective in maintaining lower MDA concentration during the storage at room or fridge temperatures. These results suggest that, the nano-coatings are a promising tool for preventing postharvest oxidative damage during storage.

Visual decay percentage

Decay is one of the key factors which responsible for the postharvest losses in fruits and vegetables. Fruits are directly exposed to the contaminating microorganisms through soil, dust, water and postharvest processing equipment (Xi *et al.*, 2017). The effect of coatings on decay percentage for strawberry fruits treated by CH or CHNPs is presented in Table (6). There is a marked increase in the number of infected strawberries during prolonged storage periods for all the coated and uncoated samples. However, the coating treatments reduced the decay percentage in all strawberry fruits during the storage periods. These results are in line with those reported by Bose *et al.* (2019) who showed that chitosan coatings are effective in reducing fungal decay at postharvest stages of strawberry fruits.

During the storage at room temperature, visual decay started quickly on the control fruits with 10% of fruits exhibited the sign of infection after 2 days of storage. The infection rates were increased with increasing storage periods and the infection percentage recorded 80 and 100% on the 4th day and at the end storage period (after 6 days). On the other hand,

the coated strawberry fruits started to exhibit the first infection signs of decay at the 4th day of storage and the samples showed 40, 30, 20 and 10% infection for treated by CH (at 0.5 and 1%) and CHNPs (at 0.5 and 1%), respectively. At the end storage period (the 6th day), the infection rate reached to 100, 75, 70 and 50% for the same fruits.

Regarding the effect of cold storage on strawberry fruits, the uncoated strawberry fruits showed signs of decay at the 4th day (15%). On the 8th day, 45% of uncoated fruits were infected. In contrast, no sign of visible infestation in the coated strawberry fruits was detected up to the 8th day of storage, except the strawberry sample coated with 0.5% CH which recorded 10% of the infection rate. On the 12th day of cold storage, the infection rates were increased in uncoated strawberry and recorded 100%. On the other side, the other coated strawberry samples started to exhibit first signs infection of decay and showed 60, 20, 25 and 10 % infection for the samples treated with CH (at 0.5 and 1%) and CHNPs (at 0.5 and 1%), respectively. At the end storage, (the 16th day) 100% of fruits treated with CH 0.5% were infected. While, the infection of the other coated strawberry fruits was 70, 60 and 40% for samples treated with CH 1% and CHNPs (at 0.5 and 1%), respectively.

Microbial load

The control of the microbial load has a great important role in maintaining high-quality attributes, especially the hygienic quality of the final product (Rani *et al.*, 2017). The changes of TBC, mold and yeast counts during storage for uncoated and coated strawberry fruits are exhibited in Table (7). The microbial load of all strawberry samples was influenced by coating treatments during storage. The TBC and mold and yeast were linearly increased with progressing the storage periods of all samples, especially the control sample which was rapidly increased than the other coated samples. Moreover, all coating treatments had slower rates for the growth of TBC, mold and yeast counts during the storage periods.

After the 2nd day of storage at room temperature, the initial values of TBC, mold and yeast were raised to 4.37 and 4.02 log CFU/g for uncoated strawberry compared to 3.95, 3.62, 3.69 and 3.45 log CFU/g of TBC in the samples coated with CH (at 0.5 and 1%) and CHNPs (at 0.5 and 1%), respectively; and to 3.90, 3.83, 3.85 and 3.42 log CFU/g of mold and yeast for the same samples. At the 4th day of storage, TBC values of coated strawberry

samples were ranged from 4.62 to 5.63 log CFU/g, compared to uncoated sample, which was 5.97 log CFU/g. Also, all coated samples were lower in mold and yeast counts than the uncoated samples. Where, mold and yeast counts ranged from 4.58 to 5.60 log CFU/g against 5.85 CFU/g for uncoated strawberry sample.

At the 6th day of storage, the uncoated strawberry sample and coated sample with CH 0.5% were spoiled. The other samples recoded TBC 6.15, 6.1 and 5.68 log CFU/g for samples coated with CH 1% and CHNPs (at 0.5 and 1%), respectively. While, mold and yeast counts for the same samples were 5.92, 5.83 and 5.51 log CFU/g. These results are in line with the results obtained by Khan *et al.* (2019) who showed that the strawberries coated with chitosan as edible coatings supplemented with carotenoproteins possess a lower number of molds and yeast growth compared to the uncoated fruits.

With regard to the effect of storage on cold temperature, Table (7) indicates that TBC, mold and yeast counts were rapidly increased in uncoated strawberry than those in coated samples, starting from the fourth day. At the 8th day of cold storage, the initial values of TBC, mold and yeast counts were raised to 6.65 and 5.82 log CFU/g for uncoated strawberry compared to 4.58, 4.01, 3.88 and 3.56 log CFU/g for samples coated with CH (at 0.5 and 1%) and CHNPs (at 0.5 and 1%), respectively. While, mold and yeast counts reached to 5.1, 4.78, 4.73 and 4.45 log CFU/g for the same samples.

Finally, the results indicated that, the strawberry fruits coated with CH had a lower microbial load than uncoated, while it had a higher microbial load than nano-coated samples throughout the storage. This may be because the CHNPs can effectively inhibit the growth of fruit microorganisms. These results are consistent with findings of Fan *et al.* (2017) who found that nano active film is better for food preservation.

Shelf-life of strawberry fruits

The most important advantage of any antimicrobial technology is the ability to extend the shelf-life of food system. Shelf-life is based on the physical appearance as judged by the retention of color, glossy appearance and pathogenic decay (Moradinezhad and Jahani 2019). In general, the shelf-life qualities of strawberry fruits stored at refrigerator conditions (6±2°C) were better than those stored at room conditions (25±3°C). Data presented in Fig (2) reveals that different strawberries treatments can modify their

spoilage profile and extend the shelf-life. For instance, the uncoated fruits were spoiled at the 4th day of storage on room temperature and after the 8th days for storage at cold conditions. Thus, the samples coated with CH 0.5% had a shelf-life of 4 days for the storage at room temperature, while the same sample stored at fridge conditions had a shelf-life of 12 day. Whereas, the samples coated with CH 1% and CHNPs 0.5% had a shelf-life of 4 and 16 days at room and cold temperatures, respectively. On the other hand, the sample coated with CHNPs 1% had a shelf-life of 6 and 16 days at the room and cold temperatures, respectively. This indicates a significant economic and very promising effects for strawberry fruits producers.

CONCLUSION

Coatings represent one of the most important methods which used for preserving the quality of the fruits and vegetables. The application of CHNPs appears to be highly promising in the field of food processing for extending the shelf-life of strawberries during storage. Based on comprehensive comparison and evaluation, strawberries coated by CHNPs could be kept in good quality for 16 days at 6±2°C and for 6 days at 25±3°C.

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Table 1. Effect of coating with CH or CHNPs on % weight loss of strawberry fruits during storage at different temperatures.

| Treatment | Storage period (day) at 25±3°C | | | | Storage period (day) at 6±2°C | | | | | |
|-----------|-----------------------------------|--------------------|---------------------|---------------------|----------------------------------|--------------------|--------------------|--------------------|---------------------|--------------------|
| | 0 | 2 | 4 | 6 | 0 | 4 | 8 | 12 | 16 | |
| Control | 0.0 | 5.80 ^{Ca} | 11.50 ^{Ba} | 28.00 ^{Aa} | 0.0 | 2.35 ^{Da} | 4.35 ^{Ca} | 8.35 ^{Ba} | 13.65 ^{Aa} | |
| CH | 0.5% | 0.0 | 4.50 ^{Cb} | 8.85 ^{Bb} | 23.00 ^{Ab} | 0.0 | 2.20 ^{Da} | 2.95 ^{Cb} | 4.90 ^{Bb} | 9.75 ^{Ab} |
| | 1% | 0.0 | 4.30 ^{Cb} | 7.80 ^{Bc} | 18.20 ^{Ac} | 0.0 | 2.00 ^{Da} | 2.60 ^{Cb} | 3.49 ^{Bc} | 5.80 ^{Ac} |
| CHNPs | 0.5% | 0.0 | 4.20 ^{Cb} | 7.41 ^{Bc} | 16.00 ^{Ad} | 0.0 | 2.15 ^{Da} | 2.70 ^{Cb} | 3.35 ^{Bc} | 5.50 ^{Ac} |
| | 1% | 0.0 | 4.10 ^{Cb} | 6.50 ^{Bd} | 13.50 ^{Ae} | 0.0 | 1.95 ^{Da} | 2.55 ^{Cb} | 3.10 ^{Bc} | 3.85 ^{Ad} |

Means values in the same row (as a capital letter) or column (as a small letter) with different letters are significantly different ($p \leq 0.05$). CH: chitosan and CHNPs: chitosan nanoparticles.

Table 2. Effect of coating with CH or CHNPs on total soluble solids (%) of strawberry during storage at different temperatures.

| Treatment | Storage period (day) at 25±3°C | | | | Storage period (day) at 6±2°C | | | | | |
|-----------|-----------------------------------|--------------------|---------------------|---------------------|----------------------------------|--------------------|---------------------|----------------------|---------------------|---------------------|
| | 0 | 2 | 4 | 6 | 0 | 4 | 8 | 12 | 16 | |
| Control | 5.70 ^{Ba} | 6.58 ^{Aa} | ND | ND | 5.70 ^{Aa} | 5.87 ^{Aa} | 5.65 ^{Ab} | ND | ND | |
| CH | 0.5% | 5.70 ^{Ba} | 6.00 ^{ABb} | 6.12 ^{Aa} | ND | 5.70 ^{Aa} | 5.81 ^{Aa} | 5.75 ^{Aab} | 5.66 ^{Ac} | ND |
| | 1% | 5.60 ^{Ba} | 5.90 ^{ABb} | 6.05 ^{Aa} | ND | 5.60 ^{Aa} | 5.72 ^{Aa} | 5.81 ^{Aab} | 5.74 ^{Abc} | 5.56 ^{Ab} |
| CHNPs | 0.5% | 5.80 ^{Ba} | 6.00 ^{ABb} | 6.22 ^{Aa} | ND | 5.80 ^{Ba} | 5.93 ^{ABa} | 6.03 ^{ABa} | 6.19 ^{Aa} | 6.10 ^{ABa} |
| | 1% | 5.70 ^{Ca} | 5.83 ^{BCb} | 6.18 ^{ABa} | 6.23 ^A | 5.70 ^{Ba} | 5.71 ^{Ba} | 5.79 ^{ABab} | 5.97 ^{Aab} | 5.95 ^{Aa} |

Means values in the same row (as a capital letter) or column (as a small letter) with different letters are significantly different ($p \leq 0.05$). CH: chitosan and CHNPs: chitosan nanoparticles, and (ND): not determined.

Table 3. Effect of coating with CH or CHNPs on total acidity of strawberry during storage at different temperatures.

| Treatment | Storage period (day) at 25±3°C | | | | Storage period (day) at 6±2°C | | | | | |
|-----------|-----------------------------------|--------------------|---------------------|--------------------|----------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | 0 | 2 | 4 | 6 | 0 | 4 | 8 | 12 | 16 | |
| Control | 0.48 ^{Aa} | 0.37 ^{Ba} | ND | ND | 0.48 ^{Aa} | 0.41 ^{Aa} | 0.38 ^{Aa} | ND | ND | |
| CH | 0.5% | 0.52 ^{Aa} | 0.45 ^{ABa} | 0.34 ^{Ba} | ND | 0.52 ^{Aa} | 0.48 ^{Aa} | 0.46 ^{Aa} | 0.44 ^{Aa} | ND |
| | 1% | 0.51 ^{Aa} | 0.47 ^{ABa} | 0.36 ^{Ba} | ND | 0.51 ^{Aa} | 0.47 ^{Aa} | 0.46 ^{Aa} | 0.44 ^{Aa} | 0.42 ^{Aa} |
| CHNPs | 0.5% | 0.53 ^{Aa} | 0.48 ^{Aa} | 0.43 ^{Aa} | ND | 0.53 ^{Aa} | 0.49 ^{Aa} | 0.47 ^{Aa} | 0.45 ^{Aa} | 0.43 ^{Aa} |
| | 1% | 0.52 ^{Aa} | 0.49 ^{Aa} | 0.43 ^{Aa} | 0.40 ^A | 0.52 ^{Aa} | 0.50 ^{Aa} | 0.48 ^{Aa} | 0.45 ^{Aa} | 0.44 ^{Aa} |

Means values in the same row (as a capital letter) or column (as a small letter) with different letters are significantly different ($p \leq 0.05$). CH: chitosan and CHNPs: chitosan nanoparticles, and (ND): not determined.

Table 4. Effect of coating with CH or CHNPs on the anthocyanin content (mg/100g) during storage at different temperatures.

| Treatment | Storage period (day) at 25±3°C | | | | Storage period (day) at 6±2°C | | | | | |
|-----------|-----------------------------------|---------------------|----------------------|---------------------|----------------------------------|---------------------|---------------------|----------------------|---------------------|---------------------|
| | 0 | 2 | 4 | 6 | 0 | 4 | 8 | 12 | 16 | |
| Control | 32.52 ^{Ba} | 52.76 ^{Aa} | ND | ND | 32.52 ^{Ca} | 44.68 ^{Aa} | 38.36 ^{Bc} | ND | ND | |
| CH | 0.5% | 32.73 ^{Ca} | 48.88 ^{Ab} | 44.60 ^{Bd} | ND | 32.73 ^{Ca} | 39.62 ^{Bb} | 43.80 ^{Aa} | 33.50 ^{Cc} | ND |
| | 1% | 32.13 ^{Ba} | 47.40 ^{Abc} | 47.80 ^{Ac} | ND | 32.13 ^{Da} | 37.00 ^{Cc} | 41.25 ^{Bb} | 48.20 ^{Aa} | 40.17 ^{Bb} |
| CHNPs | 0.5% | 32.52 ^{Ca} | 47.00 ^{Bbc} | 50.96 ^{Ab} | ND | 32.52 ^{Da} | 36.92 ^{Cc} | 41.98 ^{Bab} | 49.35 ^{Aa} | 47.25 ^{Aa} |
| | 1% | 32.69 ^{Da} | 46.58 ^{Cc} | 54.70 ^{Ba} | 58.25 ^{Ac} | 32.69 ^{Ca} | 34.57 ^{Cd} | 38.00 ^{Bc} | 45.75 ^{Ab} | 45.56 ^{Aa} |

Means values in the same row (as a capital letter) or column (as a small letter) with different letters are significantly different ($p \leq 0.05$). CH: chitosan and CHNPs: chitosan nanoparticles, and (ND): not determined.

Table 5. Effect of coating with CH or CHNPs on the malondialdehyde concentration (mmol/g, FW) during storage at different temperatures.

| Treatment | Storage period (day) at 25±3°C | | | | Storage period (day) at 6±2°C | | | | | |
|-----------|-----------------------------------|--------------------|--------------------|---------------------|----------------------------------|--------------------|---------------------|---------------------|--------------------|--------------------|
| | 0 | 2 | 4 | 6 | 0 | 4 | 8 | 12 | 16 | |
| Control | 2.45 ^{Aa} | 3.95 ^{Aa} | ND | ND | 2.45 ^{Ca} | 3.00 ^{Ba} | 4.29 ^{Aa} | ND | ND | |
| CH | 0.5% | 2.45 ^{Ca} | 3.50 ^{Bb} | 4.65 ^{Aa} | ND | 2.45 ^{Da} | 2.82 ^{Cab} | 3.45 ^{Bb} | 4.53 ^{Aa} | ND |
| | 1% | 2.45 ^{Ca} | 3.38 ^{Bb} | 4.38 ^{Aab} | ND | 2.45 ^{Ea} | 2.73 ^{Dbc} | 3.26 ^{Cbc} | 3.88 ^{Bb} | 5.53 ^{Aa} |
| CHNPs | 0.5% | 2.45 ^{Ca} | 3.39 ^{Bb} | 4.35 ^{Aab} | ND | 2.45 ^{Da} | 2.69 ^{Dbc} | 3.18 ^{Ccd} | 3.90 ^{Bb} | 5.62 ^{Aa} |
| | 1% | 2.45 ^{Da} | 3.28 ^{Cb} | 4.21 ^{Bb} | 5.04 ^A | 2.45 ^{Da} | 2.58 ^{Dc} | 3.00 ^{Cd} | 3.75 ^{Bb} | 5.18 ^{Ab} |

Means values in the same row (as a capital letter) or column (as a small letter) with different letters are significantly different ($p \leq 0.05$). CH: chitosan and CHNPs: chitosan nanoparticles, and (ND): not determined.

Table 6. Effect of coating with CH or CHNPs on visual decay percentage during storage at different temperatures.

| Treatment | Storage period (day) at 25±3°C | | | Storage period (day) at 6±2°C | | | | |
|-----------|-----------------------------------|----|-----|----------------------------------|----|-----|-----|-----|
| | 2 | 4 | 6 | 4 | 8 | 12 | 16 | |
| Control | 10 | 80 | 100 | 15 | 45 | 100 | 100 | |
| CH | 0.5% | 0 | 40 | 100 | 0 | 10 | 60 | 100 |
| | 1% | 0 | 30 | 75 | 0 | 0 | 20 | 70 |
| CHNPs | 0.5% | 0 | 20 | 70 | 0 | 0 | 25 | 60 |
| | 1% | 0 | 10 | 50 | 0 | 0 | 10 | 40 |

CH: chitosan and CHNPs: chitosan nanoparticles.

Table 7. Effect of coating with CH or CHNPs on total bacterial count (log CFU/g) of strawberry fruits during storage at different temperatures.

| Treatment | | Total bacterial count (log CFU/g) | | | | | | | | | |
|-----------|------|-----------------------------------|------|------|------|------|----------------------------------|------|------|------|--|
| | | Storage period (day) at 25±3°C | | | | | Storage period (day) at 6±2°C | | | | |
| | | 0 | 2 | 4 | 6 | 0 | 4 | 8 | 12 | 16 | |
| Control | | 2.65 | 4.37 | 5.97 | ND | 2.65 | 4.96 | 6.65 | ND | ND | |
| CH | 0.5% | 2.63 | 3.95 | 5.63 | ND | 2.63 | 3.31 | 4.58 | 6.23 | ND | |
| | 1% | 2.60 | 3.62 | 5.04 | 6.15 | 2.60 | 3.30 | 4.01 | 5.05 | 6.20 | |
| CHNPs | 0.5% | 2.62 | 3.69 | 5.00 | 6.10 | 2.62 | 3.25 | 3.88 | 5.18 | 6.23 | |
| | 1% | 2.59 | 3.45 | 4.62 | 5.68 | 2.59 | 3.21 | 3.56 | 4.70 | 5.58 | |
| Treatment | | Mold and yeast count (log CFU/g) | | | | | | | | | |
| | | Storage period (day) at 25±3°C | | | | | Storage period (day) at 6±2°C | | | | |
| | | 0 | 2 | 4 | 6 | 0 | 4 | 8 | 12 | 16 | |
| Control | | 2.28 | 4.02 | 5.85 | ND | 2.28 | 3.89 | 5.82 | ND | ND | |
| CH | 0.5% | 2.25 | 3.90 | 5.60 | ND | 2.25 | 3.65 | 5.10 | 6.15 | ND | |
| | 1% | 2.22 | 3.83 | 5.15 | 5.92 | 2.22 | 3.47 | 4.78 | 5.43 | 6.09 | |
| CHNPs | 0.5% | 2.23 | 3.85 | 5.05 | 5.83 | 2.23 | 3.55 | 4.73 | 5.30 | 5.91 | |
| | 1% | 2.20 | 3.42 | 4.58 | 5.51 | 2.20 | 3.19 | 4.45 | 5.19 | 5.68 | |

CH: chitosan and CHNPs: chitosan nanoparticles, and (ND): not determined

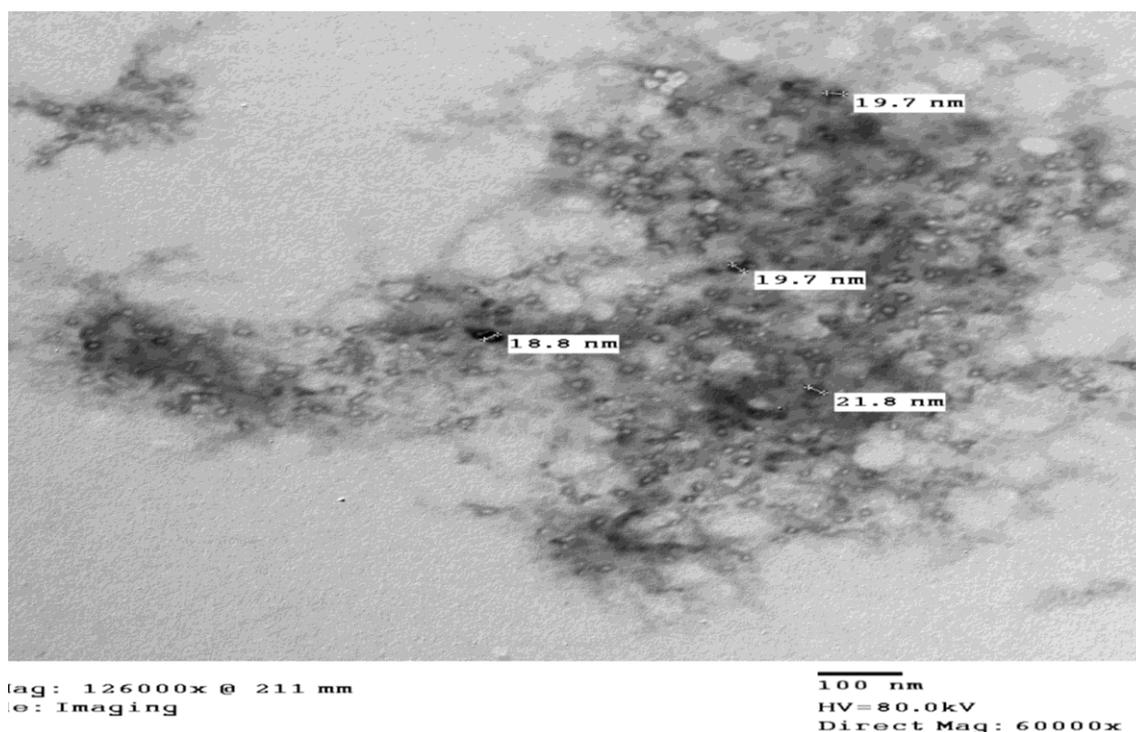


Figure 1. Transmission electron microscopy micrograph of chitosan nanoparticles

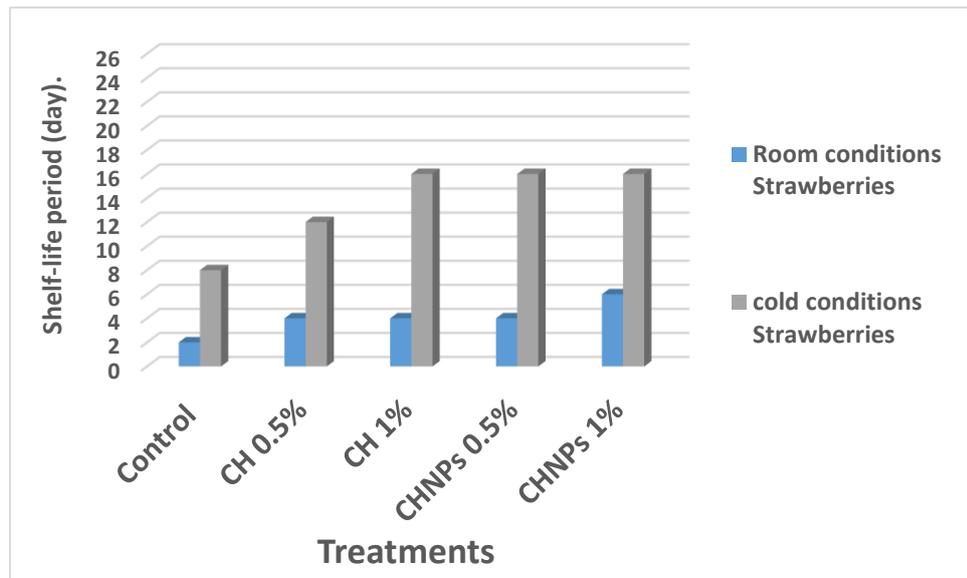


Figure 2. Shelf-life of strawberry fruits.

تحسين الجودة والعمر الافتراضي لثمار الفراولة المغطاة بالأغشية الصالحة للأكل المتناهية الصغر

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الملخص العربي

ثمار الفراولة ثمار هشة لها عمر افتراضي قصير بسبب النضج السريع والتحلل. ولقد أجريت الدراسة الحالية لتقييم تأثير الأغشية القابلة للأكل باستخدام جزيئات الشيتوزان وجزيئات الشيتوزان المتناهية الصغر لتحسين الجودة وإطالة العمر الافتراضي أثناء التخزين. تم تحضير جزيئات الشيتوزان النانوية باستخدام طريقة الجل الايونية. تم الكشف عن حجم وشكل الجزيئات النانوية باستخدام المجهر الإلكتروني. قسمت الثمار المعاملة إلى مجموعتين حفظت الأولى على $3 \pm 25^\circ\text{C}$ بينما حفظت الثانية على $2 \pm 6^\circ\text{C}$ بعد ذلك قيمت فعالية الطلاءات على الصفات الطبيعية والكيميائية والميكروبية والعمر الافتراضي للثمار. أظهرت صور المجهر الإلكتروني أن جسيمات الشيتوزان تقع في نطاق النانو بحجم يتراوح من 18.8 إلى 21.8 نانومتر. أثناء التخزين اظهرت الثمار المعاملة بجزيئات الشيتوزان النانوية انخفاضاً في قيم الفقد في الوزن والتعطب والمواد الصلبة الكلية الذائبة وتركيز المألونالدهيد واحتفظت بمستويات أعلى في قيم الحموضة الكلية ومحتوى الانثوسيانين مقارنة بالثمار المعاملة بالشيتوزان فقط والثمار غير المعاملة (الكنترول). فيما يتعلق بالخواص الميكروبيولوجية كانت قيم العدد الكلي للبكتريا والفطريات والحمائر للثمار المعاملة بالجزيئات النانوية أقل من تلك القيم للثمار المعاملة بالشيتوزان والثمار غير المعاملة. الخلاصة تم الحفاظ على صفات الجودة للثمار المعاملة والمحفظة لمدة 16 يوماً عند درجة حرارة 6°C ولمدة 6 أيام عند درجة حرارة 25°C مقارنةً بالعينة غير المعاملة التي فقدت قبولها بشكل حاد بسبب سرعة النضج والعدوى الفطرية وأظهرت جودة رديئة في اللون والملمس بعد 8 أيام على 6°C وبعد 2 يوم فقط على 25°C .

الكلمات الاسترشادية: جزيئات الشيتوزان النانوية، الفراولة، المجهر الإلكتروني، التوصيف، فترة الصلاحية.