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Original article

Efficacy of chitosan nanoparticles and wax coatings on maintaining post-harvest quality of "*Murcott*" mandarins

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ABSTRACT

Because of its high degree of biodegradability, chitosan is widely used as a component in food packaging. However, its poor physical properties, such as permeability, limit its applicability. Consequently, applying nano chitosan is regarded as the most effective solution to this issue. In the current study, we studied the effect of using different materials in the coating process on the quality of "*Murcott*" mandarin during cold storage. We used different concentrations of nano chitosan (50 and 100 ppm) without wax and 100 ppm nano chitosan with wax. We investigated the impact of these compounds on the chemical composition and quality of fruits. The most successful treatment for preventing weight loss from discarded fresh fruit was a combination of wax and 100 ppm nano chitosan. This combination also prevented the deterioration of vitamin C, maintained the fruit pulp, and preserved the fruit's superior taste during cold storage and shelf life. It also maintains a better total soluble solids and total acidity level than other treatments. In addition, the activity of antioxidant enzymes and the total number of antioxidants indicates no degradation of plant tissues compared to those not coated with nano chitosan. It also reduces the microbial load on the coated fruits. Consequently, this coating combination could suggest prolonging post-harvest life and increasing the marketing period of mandarin fruits.

1. Introduction

Mandarin (*Citrus reticulata* Blanco) is considered the most critical citrus fruit grown in Egypt and belongs to family Rutaceae. Moreover, mandarin fruits are the second variety for exportation after orange fruits in Egypt (Elasraag, 2023). Among the most often-used varieties of Mandarin in Egypt is "Murcott," which is characterized by a short shelf life (SL) and low marketability due to exposure to various post-harvest diseases and losses during storage. Furthermore, one of the primary quality variables that directly affects the price of fruit is its appearance (Kaur, 2022).

Citrus fruits experience a variety of physiological changes during storage, as citrus is a perishable fruit that is especially susceptible to quality loss after harvest due to decay and water loss through transpiration and respiration (Zhang et al., 2022a; Zhang et al., 2022b). The prevalence of diseases and sensitivity to chilling injury also contribute to substantial post-harvest and marketing string wastage, which in turn shortens the storage period (Carrillo-Lopez et al., 2000; Hoa et al., 2002; Sivakumar et al., 2011). In addition, when citrus fruits are washed, the wax that covers the peel is removed and needs to be restored to keep the fruit from losing moisture and drying out (Ahmed et al., 2007; Ridoutt et al., 2010).

Improving the storage behavior and minimizing decay during storage and transportation are the major issues for the citrus industry. Thus, attention must be paid to these factors to improve fruit quality and post-harvest losses and increase fruit storage and marketability. Citrus fruits are given an industrial wax coating at Egypt's packing facilities to increase gloss, stop water loss, and postpone shrinkage (Devi et al., 2023).

A useful method for providing additional preservation against physiological problems after harvest and chilling harm is fruit covering

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materials, as well as improving fruit appearance and quality, avoiding dehydration, reducing the incidence of peel spots and weight loss, and prolonging post-harvest life (Mohamed, 2001; Hoa et al., 2002; Dou, 2004; Nair et al., 2018; Fernández et al., 2021). Additionally, the mandarin covering showed preserved post-harvest fruit quality (Arnon et al., 2015). Fruit weight loss and respiration rate both increased while they were being stored. This increment was negligible in coated fruits compared to uncoated fruits (Hmmam et al., 2021).

The wax coating fundamentally maintains fruit quality, and adding imazalil (IMZ) effectively controls deterioration while protecting fruit against green mold (Njombolwana et al., 2013). Additionally, Samra et al. (2014) showed that coating with carboxy methyl cellulose could successfully preserve "Murcott" fruits for two months under cold storage (CS) conditions and six days in outdoor weather conditions after CS with outstanding quality (Samra et al., 2014).

Chitosan application is a tactic that has not received much research attention. This substance is edible, non-toxic, and safe for humans (Hirano et al., 1990). The fruit's quality is preserved, and its gas exchange is enhanced by the chitosan coating, which also reduces transpiration losses and delays senescence and maturation (Jiang and Li, 2001; Khalil et al., 2022; Wantat et al., 2022).

In addition, chitosan reduced the softness of the peel by delaying peel senescence by a number of days without impacting the internal fruit characteristics (Fornes et al., 2005). Furthermore, chitosan had high resistance against fungal attacks (Taghinezhad & Sharabiani, 2018).

Compared to the control, fruit weight and decay losses were reduced at the highest concentration of nano chitosan (8%) (Gardesh et al., 2016; Hosseini-Farahi et al., 2016; Gad & Ibrahim, 2018; Adiletta et al., 2019). In addition, nano chitosan coating significantly slowed the softening process, maintaining fruit pulp firmness (Gardesh et al., 2016; Gad & Ibrahim, 2018; El-Gioushy et al., 2022). The edible chitosan nanoparticle coatings were in charge of delaying the ripening phase of the grapes, which kept their sensory attributes and efficiently delayed vitamin C oxidation (Melo et al., 2018; Xiao et al., 2019). Interestingly, the coating combination with the carnauba emulsion and chitosan reduced fresh weight loss (Nascimento et al., 2016).

Using nano chitosan at various concentrations in addition to wax and mixing them to coat Murcott mandarin fruits will result in the fruits preserving their quality even after being stored for a longer period, making the marketing method easier. Accordingly, this study aims to use natural edible materials in nano form in the process of wrapping fruits before packing them at polyethylene to increase the storage period and SL.

2. Materials and methods

2.1. Fruit material

The samples were taken from a private farm located in the area in Wadi EL-Molak, Ismailia governorate, Egypt on mature yellow mandarin (*Citrus reticulata* L.Osbeck) fruits cv. Murcott throughout two seasons (2022 and 2023). The trees, which were six years old and had Volkamarina lemon rootstock, were planted five meters apart in sandy, loamy soil and underwent the customary horticultural care utilized in the region. Mandarin (600 fruits, 150 fruits/treatment, and 10 fruits/replicate) from both seasons were used on March 1st.

The fruits chosen were as consistent, healthy, and free of pathological and physiological aberrations as possible. The fruits were transferred to the laboratory for post-harvest transactions at the Faculty of Agriculture, Zagazig University; Zagazig, Egypt. The fruits were kept for 1 h at room temperature, washed well with soap and water, rinsed well from soap residues, and the fruits were then soaked into a 0.5% solution of sodium ortho-phenylacetate at pH 11.8–12.1 and 32°C. Fruits were air dried before making transactions.

The treatments included control (imazalil 1000 ppm and packaging in perforated 0.005% polyethylene (PPE), wax and 0.005% PPE, nano chitosan (50 ppm) and 0.005% PPE, nano chitosan (100 ppm) and 0.005% PPE, and wax + nano chitosan (100 ppm) and 0.005% PPE. The air-coated fruits were dried and covered with 0.005% perforated polyethylene (PPE). The fruits were stored at a temperature of 5°C and humidity of 90–95% for 4 months. Random samples were taken from each treatment for measurements during the CS period and the SL (six days) at room temperature.

2.2. Preparation of nano chitosan particles

Biological nano chitosan particles was prepared using *Penicillium oxalicum*. The fungus was cultivated for three days in the Czepak Dox broth (Lab M Limited, Lancashire, UK) as recommended by Saad et al. (2022). Proteins were extracted from *P. oxalicum* by saturating the medium with 80% (w/v) $(NH_4)_2SO_4$. Five milligrams of proteins were applied to a 30 × 2.5 cm column of cellulose to obtain the protein (Sathiyabama and Balasubramanian, 2000). The unbound proteins eluted from the column were used to produce the nano chitosan particles.

The pH was adjusted to 4.8 after dissolving 0.5% (w/v) of shrimp chitosan in 1% (v/v) acetic acid. After 30 min of magnetic stirring, 15 mL of chitosan solution was added to 180 g/mL proteins. The combination was kept at 25°C for the remainder of the day. Centrifugation for 10 min at 10,000 × g was used to produce nano chitosan, which was then washed three times with sterilized distilled water. The produced chitosan nanoparticles were used in the coating procedure.

2.3. Identification of nano chitosan particles

The maximum UV absorption was identified at 290 nm (Fig. 1**A**). It has been noted that the average diameter of the nano chitosan particles obtained by TEM was 20–56 nm, indicating that these proteins can convert chitosan particles into the nano form (Fig. 1**B**). XRD results also showed that the biosynthetic nano chitosan particles are spherical. Furthermore, the zeta potential findings showed that the average size of the nano chitosan particles was 20 nm (Fig. 1**C**), where the zeta potential was −31 mV (Fig. 1**D**).

2.4. Physicochemical analysis of nano chitosan particles

2.4.1. Determination of discarded fruit (DF) percentage

The proportion of abandoned fruits to all fruits was measured as the DF percentage (Gemail et al., 2023).

2.4.2. Determination of fruit weight loss (FWL) percentage

FWL percentage was calculated by taking the weight of the fruit before and after storage and the SL of each replicate (Shah and Hashmi, 2020, Gemail et al., 2023). Using the following equation (1), FWL was determined as a percentage of the starting weight.

$$FWL\% = ((Wi - Ws)/Wi))*100 \quad (1)$$

Where Wi = weight of the fruit at the beginning.
Ws = Fruit weight at the time of sample.

2.4.3. Determination of fruit pulp firmness (FPF)

Fruits from each replicate were individually peeled, and the pulp firmness (PF) was measured as g/cm^2 utilizing a push–pull dynamometer (Liu et al., 2020, Gemail et al., 2023).

2.4.4. Determination of panel test index (PTI)

Five individuals evaluated a random fruit sample of each replicate and assigned a PTI score based on the following:

0 = unacceptable fruits; 1 = acceptable; 2 = good; 3 = very good and 4 = xxcellent taste.

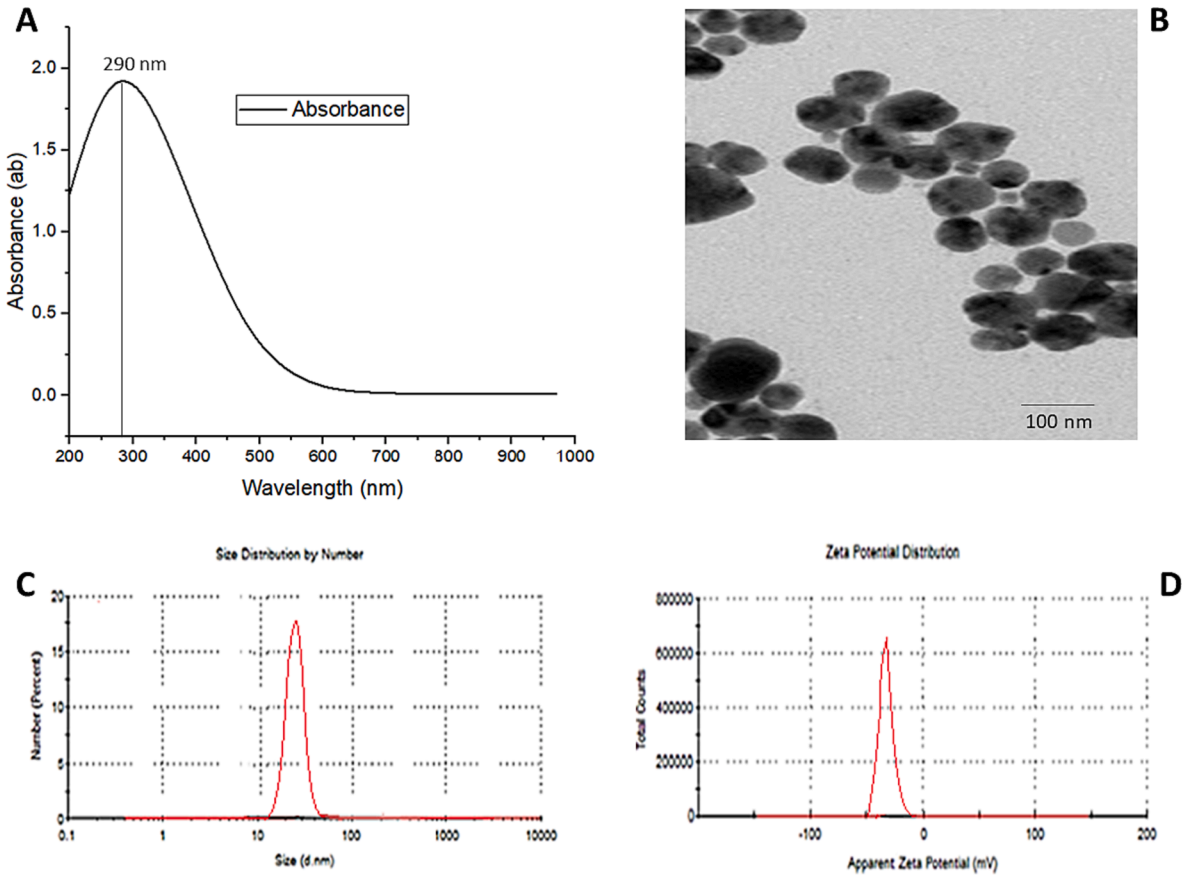

Fig. 1. Characterization of green chitosan nanoparticles. (A) UV absorbance at 290 nm, (B) Transmission electron micrograph image with a size of 20–56 nm, (C) zeta sizer calculates the size of nanoparticles 20 nm, and (D) zeta potential calculates the charge on nanoparticles of −31 mV.

2.4.5. Determination of chemical properties of juice

By titrating with 2,6-dichlorophenol-indophenol dye, the ascorbic acid level was calculated and defined as mg/100 mL of juice (Ayesha et al., 2014). The percntage of the total soluble solids (TSS) was determined using a hand refractometer as Brix̊. Citric acid (Juice total acidity TA) percentage was determined as grams per 100 mL of fruit juice following the Association of Official Analytical Chemists (AOAC) (Fletcher, 1980). The TA was titrated with 0.1 N NaOH in the presence of the total indicator phenolphthalein.

2.5. Identification of enzymes and antioxidants

2.5.1. Extraction

The mandarin peels were powdered after being dried in a vacuum oven at 45°C. The powder (10 g) was dissolved in 100 mL of 50% ethanol and stirred for several hours at a temperature of 25°C. The supernatant was collected by filtering the samples and the solvent was then discharged using the rotary evaporator (Saad et al., 2021).

2.5.2. Determination of DPPH radical scavenging activity

Total antioxidants were determined in the extract obtained from all treatments (500 g/mL) (Bhakya et al., 2016). Ethanolic DPPH (1 µL) was mixed with 100 µL of each extract and incubated for 30 min in the dark. The absorbance was measured at 517 nm using a microtiter plate reader according to the following equation (2):

$$Radical\ scavenging\ activity\ (\%) = \frac{(Abs.\ control - Abs.\ sample)}{(Abs.\ control)} \times 100 \qquad (2)$$

2.5.3. Determination of catalase and superoxide dismutase activities

Catalase (CAT) activity was measured using the technique recommended by Aebi et al. (1972) and Fossati et al. (1980). The magnitude of catalase activity is inversely related to the amount of chromophore adsorption generated in the extract (Rup et al., 2006). Briefly, 0.05 mL of the extract was mixed in 0.5 mL of phosphate buffer (pH = 7), and 0.1 mL of chromogen inhibitor at 25°C for 1 min. Distilled water (0.5 mL) and 0.20 mL of chromogen inhibitor was added. The mixture was incubated for 10 min at 37°C, and the absorbance was measured at 510 nm.

The reaction mixture for measuring SOD activity contained 0.01 mol/L methionine, 33 µmol/L nitro blue tetrazolium (NBT), and 0.006 mol/L EDTA, 0.3 mL of crude enzyme extract, 0.003 mmol/L riboflavin, and 50 mmol/L phosphate buffer (pH 7.8). The production of blue formazan was determined by the absorbance at 560 nm. One unit (U) of SOD activity was defined as the amount of enzyme that reduced NBT by 50%. Specific activity was represented as U/mg FW (Beauchamp and Fridovich, 1971).

2.6. Enumeration of microbial population

After four months, the total number of mesophilic bacteria, molds, and yeasts was enumerated during CS periods during the second season on mandarin fruits (Gutiérrez-Pacheco et al., 2020). In 90 ml of peptone water, 10 g of mandarin fruit peels were homogenized for 1 min. A volume of each extract (1 mL) was placed onto the surface of several agar media. The groups of organisms selected for enumeration and the media used were as follows: (i) total aerobic bacteria on nutrient agar medium (Product Code: LAB008) (Lab M); (ii) *Salmonella* spp. on xylose lysine decarboxylase agar (XLD agar) (Product Code: LAB032) (Lab M Limited), (iii) *Escherichia coli* on eosin methylene blue (EMB agar) (Product Code: LAB061) (Lab M Limited); and (iv) total yeasts and

molds count on Sabouraud dextrose agar (Product Code: MH063) (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Samples were incubated at 37°C for 24 h for mesophilic bacteria and at 25°C for five days for molds and yeasts.

2.7. Statistical analysis

Pretests were carried out before a one-way analysis of variance (ANOVA) was performed. The Levene test with a P-value of 0.01598 was used to determine the homogeneity and to evaluate the normality assumption on sample distributions.

In the present study, Costat program was used version 6.4 (Costat 2008). One way ANOVA was used to compare the means of the three data sets with a 95% confidence level (Wallenstein et al., 1980). Equation (2) was used to determine the sample size. $n = \left(\frac{ZSD}{E}\right)^2$(2).

3. Results

3.1. Determination of percentage of DF

The data displayed in (Table 1) showed the influence of post-harvest applied treatments, regardless of CS periods, on DF percentages of "Murcott" mandarin during two seasons. The results showed that the control treatment gave the highest DF percentage relative to other used treatments in both seasons. On the other side, the lowest DF percentage was found by the treatment of coating with wax + 100 ppm nano chitosan particles . Moreover, coating with nano chitosan at either 50 or 100 ppm was equally effective in lowering the percentage of DF relative to wax treatment, which gave a high value (Table 1).

The results revealed that the best treatments used were the mixture of wax plus 100 ppm nano chitosan, which led to a lower percentage of DF than the other treatments. Furthermore, using the concentration of 100 ppm nano chitosan was better than 50 ppm in reducing DF percentage. The increase in the DF percentage in the control treatments was constant in both seasons compared to the other treatments, and the increase in the CS period and the increase in SL led to a stable increase in the DF percentage in both seasons (Table 1).

In addition, there was no effect of the interaction between treatments and CS periods on DF after one month of storage in all packaging treatments. Therefore, there were no DF after one month of storage. There were also no losses in the stored fruits after three months when using concentrations of 50 and 100 ppm nano chitosan. After four months of CS of the samples, the highest percentage of fruit loss was obtained in the control samples.

In contrast, during the two studied seasons, it was found that coating with wax + 100 ppm nano-chitosan had the lowest percentage of DF. After the last month of CS, it could be noticed that coating with 50 or 100 ppm nano chitosan was equally effective in reducing DF percentage relative to coating with wax, which recorded a high value. When the interaction between the post-harvest factor and the SL durations was followed, it could be found that all applied treatments, except for the control, succeeded in preventing the incidence of DF during the first and the second durations of SL. The control treatments showed the highest DF percentage after the third and fourth SL duration. On the contrary, the lowest DF percentage was recorded by coating with wax plus 100 ppm nano chitosan. Coating with nano chitosan reduced DF percentage after the last SL duration relative to coating with wax throughout the study.

3.2. Determination of percentage of FWL

The response of "Murcott" mandarins to different post-harvest treatments was reported regardless of the duration of the CS (Table 2). The results showed that using wax and 100 ppm nano chitosan for coating resulted in the most minor FWL during the two seasons. In both seasons, the control treatment consistently dropped the FW when compared to the other treatments. Regarding the impact of post-harvest applied treatments, the results shown in (Table 2) demonstrated that coating with 100 ppm nano chitosan alone or in combination with wax was similarly efficient in preventing FWL compared to other treatments. This was true regardless of the SL durations. On the other hand, the control treatment had the most significant rise in the FWL percentage. Throughout the investigation's two seasons, this trend remained consistent.

There was a significant increase in the FWL percentages with the progress of CS and SL periods during both seasons. Furthermore, the findings of the interaction between the storage time and the treatments utilized revealed that the control samples had the most substantial decrease in FW during the final month of storage. Wax, nano chitosan at 50 or 100 ppm, and wax mixed with 100 ppm nano chitosan were shown to have minor FWL after the first month of CS. These therapies have a comparable effect on recent FWL. This pattern of results was seen when the interaction between post-harvest used treatments and SL lengths component was monitored.

3.3. Determination of FPF

The results indicated that coating with 100 ppm nano chitosan alone or combined with wax was capable of maintaining the highest firmness of "Murcott" mandarin pulp when compared to other applied treatments (Table 3). This was true regardless of the storage period factor. Contrarily, during both studied seasons, the pulp hardness produced by the control treatment was the lowest. Notwithstanding the SL duration factor, the findings on the response of "Murcott" FPF to post-harvest

Table 1
Impact of wax and nano chitosan on discarded fruit percentage (DFP%) of "Murcott" mandarin fruits in both seasons.

First Season								
Storage treatments	**Cold storage (CS) period (months)**				**Shelf-life (SL) (days)**			
	1	**2**	**3**	**4**	**1**	**2**	**3**	4
Control	0.00 a	3.32 a	6.72 a	10.18 a	0.00 a	6.71 a	10.05 a	10.21 a
Wax and PPE	0.00 a	0.00 b	3.40 b	6.67 b	0.00 a	0.00 b	6.43 b	6.84 b
Nano chitosan (50 ppm) and PPE	0.00 a	0.00 b	0.00 c	3.34 c	0.00 a	0.00 b	0.00 d	5.10 cd
Nano chitosan (100 ppm) and PPE	0.00 a	0.00 b	0.00 c	3.39 c	0.00 a	0.00 b	3.35c	5.64 c
Wax + nano chitosan (100 ppm) and PPE	0.00 a	0.00 b	0.00 c	0.00 d	0.00 a	0.00 b	0.00 d	3.40 d
Second season								
Control	0.00 a	3.17 a	6.85 a	10.16 a	0.00 a	4.44 a	10.03 a	10.23 a
Wax and PPE	0.00 a	0.00 b	3.44 b	6.63 b	0.00 a	0.00 b	6.64 b	6.97b
Nano chitosan (50 ppm) and PPE	0.00 a	0.00 b	0.00 c	3.48 c	0.00 a	0.00 b	1.11 d	5.02 cd
Nano chitosan (100 ppm) and PPE	0.00 a	0.00 b	0.00 c	3.53 c	0.00 a	0.00 b	3.38 c	5.37c
Wax + nano chitosan (100 ppm) and PPE	0.00 a	0.00 b	0.00 c	1.11 d	0.00 a	0.00 b	0.00 e	3.38 d

PPE, perforated 0.005% polyethylene; control (imazalil and packaging in 0.005% PPE). Values with the same letter in a column are not significantly ($P > 0.05$) different according to the least significant difference (LSD) test for each season.

Table 2
Impact of wax and nano chitosan on **fruit weight loss** (FWL) % of "Murcott" mandarin fruits in both seasons.

First season								
Storage treatments	**Cold storage (CS) period (months)**				**Shelf-life (SL) (days)**			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Control	1.80 a	2.17 a	2.40 a	2.90 a	2.03 a	2.30 ac	2.60 a	3.10 a
Wax and PPE	0.72 d	1.17 c	1.80 c	2.23 b	1.15 b	1.63 b	2.00 c	2.50 b
Nano chitosan (50 ppm) and PPE	0.83 c	1.27 b	2.10 b	2.10 c	1.09c	1.57 c	2.20b	2.43 bc
Nano chitosan (100 ppm) and PPE	0.92 b	1.27 b	2.03 b	2.13 c	1.03 cd	1.47 d	2.10 bc	2.37 c
Wax + nano chitosan (100 ppm) and PPE	0.80 cd	1.03 d	1.73 d	2.10 c	0.98 d	1.47 d	1.97 c	2.37 c
Second season								
Control	1.73 a	2.33 a	2.50 a	2.90 a	2.07 a	2.53 a	2.23 a	3.03 a
Wax and PPE	0.80 c	1.30 b	1.77 c	2.30 b	1.08 b	1.63 b	2.03 c	2.47 b
Nano chitosan (50 ppm) and PPE	0.81 c	1.23b	2.13 b	2.17 bc	1.06 b	1.50 c	2.23 a	2.40 b
Nano chitosan (100 ppm) and PPE	0.92 b	1.10 c	2.00 bc	2.00 c	1.07 b	1.43 d	2.13 b	2.30 c
Wax + nano chitosan (100 ppm) and PPE	0.86 c	1.13c	1.77 c	2.03 c	1.06 b	1.50 c	1.93 d	2.30 c

PPE, perforated 0.005% polyethylene; control (imazalil and packaging in 0.005% PPE). Values with the same letter in a column are not significantly ($P > 0.05$) different according to the least significant difference (LSD) test for each season.

Table 3
Effect of wax and nano chitosan on "Murcott" mandarin fruit pulp firmness (FPF g/cm^2) during cold storage, and shelf-life spans over two seasons.

First Season								
Storage treatments	**Cold storage (CS) period (months)**				**Shelf-life (SL) (days)**			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Control	180.00 c	165.00 b	141.67 c	115.00 d	165.00 b	145.00 d	137.33 c	106.67 d
Wax and PPE	190.00 a	173.00 ab	145.00 b	125.00 b	170.00 ab	167.33 a	144.33 b	113.33 c
Nano chitosan (50 ppm) and PPE	188.67 ab	177.67 a	147.67 b	120.00 c	170.00 ab	151.67c	142.67 b	120.00 b
Nano chitosan (100 ppm) and PPE	189.33 a	175.67 a	158.33 a	120.00 c	172.67 a	163.00 b	147.00 ab	111.67 c
Wax + nano chitosan (100 ppm) and PPE	185.67 b	176.00 a	152.33 ab	144.33 a	170.00 ab	167.00 a	148.67 a	135.00 a
Second season								
Control	180.00 c	160.00 c	141.00 c	113.33 c	160.00 c	141.67 d	137.67 c	103.33 c
Wax and PPE	195.00 a	173.00 b	147.67 b	128.33 b	175.00 a	165.67 ab	145.33 b	115.00 b
Nano chitosan (50 ppm) and PPE	188.67 b	178.33 ab	146.67 b	125.0 b	170.00 b	153.33 c	144.33 b	116.67 b
Nano chitosan (100 ppm) and PPE	192.67 ab	179.33 a	158.33 a	126.67 b	171.67 ab	162.33 b	146.67 ab	110.00 c
Wax + nano chitosan (100 ppm) and PPE	186.67 b	177.00 ab	157.67 ab	147.33 a	172.33 ab	167.33 a	149.00 a	133.33 a

PPE, perforated 0.005% polyethylene; control (imazalil and packaging in 0.005% PPE). Values with the same letter in a column are not significantly ($P > 0.05$) different according to the least significant difference (LSD) test for each season.

treatments also showed that wax plus 100 ppm nan chitosan coating treatment had the maximum FPF when compared to other treatments (Table 3). Additionally, compared to the control, which produced the lowest value of pulp hardness, the wax, and 50 or 100 ppm nano chitosan treatments generated higher FPF values (Table 3).

The information on CS and SL durations showed a detectable decline in FPF as both seasons' CS and SL durations advanced (Table 3). Following the relationship between post-harvest treatments and storage durations, variations in the FPF value of "Murcott" mandarin fruits were recorded (Table 3). After the first month of CS, it was clear that the wax and 100 ppm nano chitosan treatments had exceptionally maintained the highest FPF (Table 3). On the other hand, the control treatments in both seasons led to the lowest value of FPF following the last term of CS (Table 3). The interplay of post-harvest treatments and SL durations showed that all applied treatments successfully maintained the greatest FPF values compared to the control after the first SL duration (Table 3). Compared to the coating treatment with wax plus 100 ppm nano chitosan, which consistently had the highest FPF values throughout the two

Table 4
Impact of wax and nano chitosan on panel test index of "Murcott" mandarin fruits in two seasons.

First Season								
Storage treatments	**Cold storage (CS) period (months)**				**Shelf-life (SL) (days)**			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Control	4.33 c	3.00 b	2.33 c	1.33 c	3.67 c	3.00 c	2.00 c	1.00 c
Wax and PPE	4.67 b	4.00 a	3.33 b	3.00 b	4.00 b	3.33 b	3.00 b	2.67 b
Nano chitosan (50 ppm) and PPE	5.00 a	4.00 a	3.00 bc	3.00 b	4.00 b	3.33 b	3.00 b	3.00 a
Nano chitosan (100 ppm) and PPE	5.00 a	4.00 a	3.33 b	3.00 b	4.33 a	3.67 a	3.33 a	3.00 a
Wax + nano chitosan (100 ppm) and PPE	4.67 b	4.00 a	4.00 a	3.33 a	4.33 a	3.33 b	3.00 b	3.00 a
Second season								
Control	4.00 c	3.33 c	2.33 d	1.33 c	4.00 c	3.00 c	2.00 c	1.00 c
Wax and PPE	4.67 b	4.00 b	3.67 b	3.00 b	4.33 b	3.67 a	3.00 b	2.67 b
Nano chitosan (50 ppm) and PPE	5.00 a	4.00 b	3.67 b	3.00 b	4.33 b	3.67 a	3.00 b	3.00 a
Nano chitosan (100 ppm) and PPE	5.00 a	4.33 a	3.33 c	3.33 a	4.33 b	3.33 b	3.33 a	3.00 a
Wax + nano chitosan (100 ppm) and PPE	5.00 a	4.33 a	4.00 a	3.33 a	4.67 a	3.67 a	3.33 a	3.00 a

PPE, perforated 0.005% polyethylene; control (imazalil and packaging in 0.005% PPE). Values with the same letter in a column are not significantly ($P > 0.05$) different according to the least significant difference (LSD) test for each season.

seasons, "Murcott" mandarin fruits from the control had the lowest value of FPF at the end of SL (Table 3).

3.4. Determination of PTI

All applied treatments had equally great PTI relative to the control during the two seasons when it came to the influence of some post-harvest treatments independent of the factor of CS periods, (Table 4). Independent of the CS period factor, the influence of post-harvest applied treatments (regardless of SL lengths) on PTI followed the same trend as post-harvest treatment data (Table 4). Throughout the two seasons, this pattern of results remained constant. It was observed that the PTI after the first month of CS was outstanding, and as the CS time went on, it tended to decline gradually (Table 4). As the SL periods increased, a similar pattern was observed (Table 4).

The PTI demonstrated that all employed treatments had a more favorable PTI after the first month of CS during both seasons compared with the control, except the second season (Table 4). The interaction between post-harvest applied treatments and CS periods influenced the PTI. All treatments, except the control, continued to taste fine after the second month of CS (Table 4). Compared to the control, which provided "Murcott" mandarin fruits with a tolerable flavor PTI, all applied treatments maintained a very good PTI by the end of CS after 4 months (Table 4). However, the differences among them were not great enough to be significant. Throughout the two seasons, this pattern of results remained constant (Table 4).

Murcott mandarin fruits of all applied treatments had the same PTI in both seasons after the first SL duration (Table 4). All treatments showed a change in this data pattern as the SL times were extended. At the end of the SL, the control showed a more severe decline in the flavor PTI (Table 4). On the other hand, the changes in the PTI for all applied treatments as the SL times increased were negligible (Table 4). Furthermore, at the end of the SL, all applied treatments had an equivalent impact on the PTI of "Murcott" mandarin fruits, compared to the other treatments, which retained their acceptable flavor after the final SL period (Table 4).

3.5. Determination of vitamin C content

The effects of the applied treatments on the vitamin C content of "Murcott" mandarin juice were shown in (Fig. 2). The findings showed that, in comparison to other applied treatments, coating with either 50 ppm nano chitosan or wax plus 100 ppm nano chitosan resulted in the highest concentration of vitamin C (Fig. 2). Compared to the control treatment, which produced the lowest quantity of vitamin C, coating with wax and coating with 100 ppm nano chitosan both boosted vitamin C content (Fig. 2). Despite the CS period issue, this tendency persisted for two seasons (Fig. 2).

Regardless of the SL duration factor, the impact of different treatments on the vitamin C of "Murcott" mandarin juice was also displayed

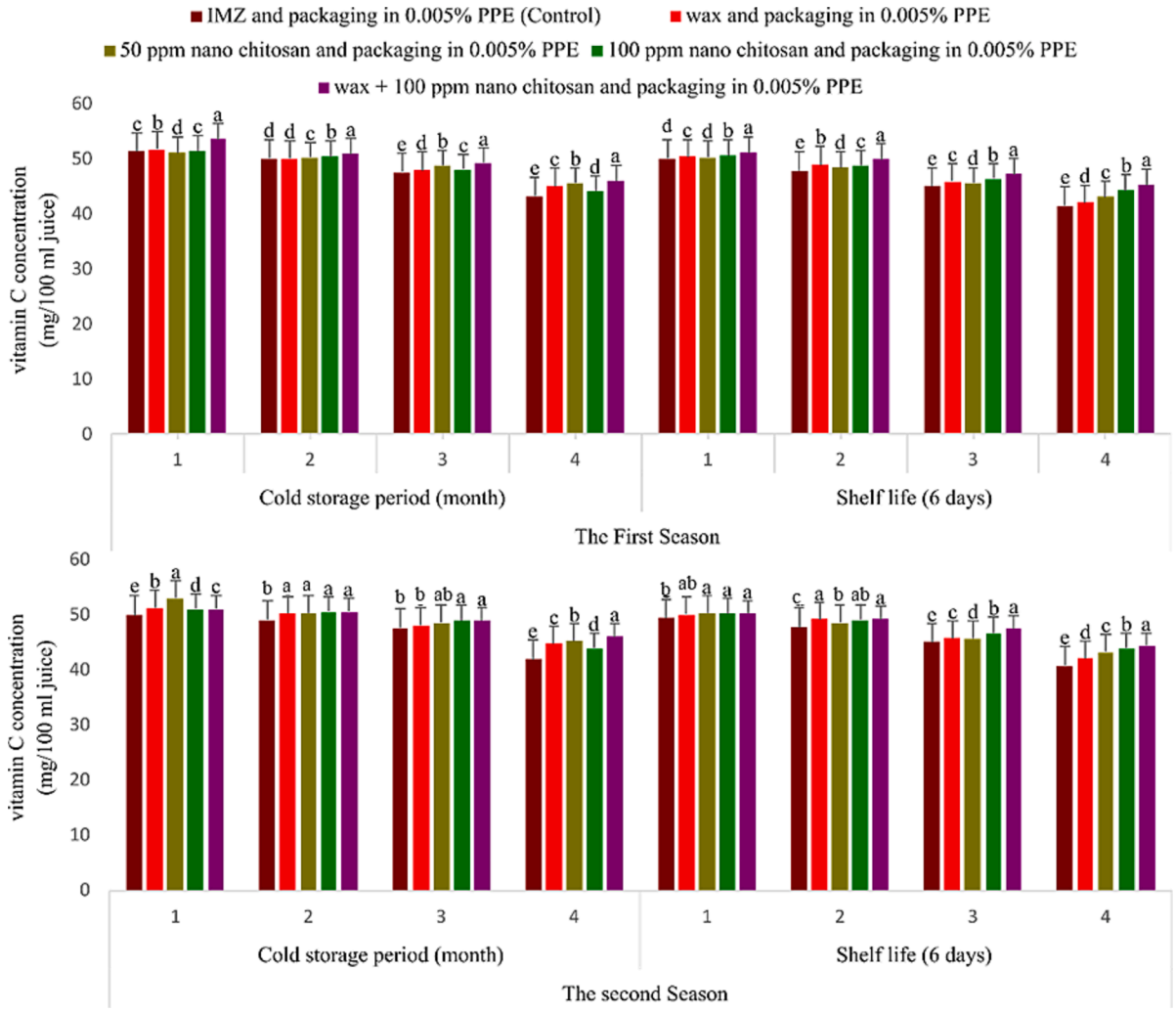

Fig. 2. Impact of wax and nano chitosan on vitamin C content (mg/100 ml juice) of "Murcott" mandarin fruits during cold storage and after shelf-life periods during the two seasons. Values with the same letter in a column are not significantly ($P > 0.05$) different according to the least significant difference (LSD) test.

(Fig. 2). The data showed that the coating treatment with wax and 100 ppm nano chitosan produced the highest vitamin C content compared to other treatments. However, the control treatment produced the lowest vitamin C content throughout the two studied seasons. The findings regarding the impact of CS and SL periods revealed that as these factors were extended, the vitamin C content during the two seasons significantly decreased (Fig. 2).

The largest vitamin C content was obtained by coating with wax or wax + 100 ppm nano chitosan after the first month, notably in the first season, according to the relationship between the post-harvest CS durations and applied treatments (Fig. 2). Conversely, control fruits had the lowest vitamin C content after the final month of CS throughout the two studied seasons (Fig. 2). Compared to other applied treatments, coating treatment in the second season gave the highest vitamin C content after the first month (Fig. 2).

Coating with wax alone and coating with wax plus 100 ppm nano chitosan after the first duration of SL produced the most increased vitamin C content (Fig. 2). This was in reference to the impact of the relationship between post-harvest treatments utilized and SL durations. On the other hand, the control treatment after the final SL duration produced the lowest vitamin C content in "Murcott" mandarin juice (Fig. 2).

3.6. Determination of percentage of TA

The overall TA percentage decreased steadily and significantly with increasing CS duration in both studied seasons (Table 5). Thus, the lowest percentage was reported after four months in the two seasons. Additionally, in all seasons, all coating treatments with nano chitosan maintained much greater TA percentages than the control, with the coating with wax and 100 ppm nano chitosan treatment only outperforming the others (Table 5).

Additionally, there was a substantial interaction between the coating treatments and the CS time throughout the two studied seasons (Table 5). After one month of CS, fruits coated with 50 ppm nano chitosan maintained the highest TA percentage. After four months in both seasons, the control treatment displayed the lowest values. The results also indicated that the TA percentage reduced as the SL duration increased (Table 5). Therefore, after four months during the two seasons, the percentage during SL was at its lowest (Table 5).

Additionally, coating with nano chitosan treatments maintained a much greater TA percentage in the two studied seasons than control. Both studied seasons strongly interacted with the tested treatments and SL duration. As a consequence, after four months of storage, all treatments had the lowest TA values during the SL (Table 5).

3.7. Determination of TSS

Throughout the CS period the percentage of TSS gradually increased as the CS period progressed in the two seasons (Table 6). As a result, in both studied seasons, the highest (10.40 and 10.42) and lowest (9.24 and 9.37) percentage of TSS were reported after four and one month of storage, respectively (Table 6). There were also negligible changes in the TSS percentages between other coatings in both seasons. The wax coating and 100 ppm nano chitosan treatment showed the lowest TSS percentage in the two studied seasons (Table 6).

Coating treatments and the length of CS had a significant interaction throughout both seasons, supporting the effects of each factor on TSS. The data also showed that TSS percentages gradually increased throughout the two seasons following the SL of CS (Table 6). The examined treatments' impact on juice TSS during the SL period followed the same pattern as their impact during CS (Table 6). There was a strong correlation between the SL duration and coating treatments in the two studied seasons.

3.8. Determination of enzymatic activities

The change of the CAT enzyme activity in Murcot mandarin to various post-harvest treatments is shown (Fig. 3A) regardless of the CS period. The results showed that the control (imazalil) and 50 ppm nano chitosan had the highest levels of CAT activity compared to the other treatments (Fig. 3 A). After four months of CS, the 100 ppm nano chitosan treatment showed the least CAT activity. Furthermore, the wax treatment with 100 ppm nano chitosan was the best since it demonstrated that the SOD enzyme activity decreased in all instances when compared to the control (Fig. 3B).

3.9. Determination of antioxidant activities

The findings in Fig. 4 demonstrated how post-harvest treatments influenced total antioxidant levels. After two months of CS, the treatments coated with 50 ppm nano chitosan and imazalil indicated the greatest levels of total oxidants in the extract. However, after one month in CS, the wax and imazalil treatments showed the highest total antioxidant levels (Fig. 4).

3.10. Determination of microbial population

After four months of CS, the total amount of bacteria, *Salmonella*, *E. coli* and total molds, and yeasts on fruit peels was shown in Table 7. A gradual decrease in the microbial population was observed according to the type of coverage compared to the control, where using a mixture of

Table 5
Effect of coating "Murcott" mandarin fruits with various concentrations of nano chitosan and wax on total acidity percentage during cold storage and following shelf-life periods throughout two seasons.

First Season								
Storage treatments	**Cold storage (CS) period (months)**				**Shelf-life (SL) (days)**			
	1	**2**	**3**	**4**	**1**	**2**	3	4
Control	0.900 c	0.650 d	0.600 d	0.495 d	0.750 c	0.657 c	0.507 d	0.467 d
Wax and PPE	0.947 c	0.733 c	0.650 c	0.573 c	0.900 b	0.687 c	0.565 c	0.503 c
Nano chitosan (50 ppm) and PPE	1.200 a	0.870 b	0.670 c	0.617 b	1.030 a	0.750 b	0.580 c	0.530 b
Nano chitosan (100 ppm) and PPE	1.030 b	0.970 a	0.733 b	0.653 ab	0.897 b	0.650 c	0.633 b	0.501c
Wax + nano chitosan (100 ppm) and PPE	1.060 b	0.950 a	0.800 a	0.680 a	0.980 ab	0.850 a	0.763 a	0.583 a
Second season								
Control	0.980 b	0.633 d	0.603 c	0.493 d	0.750 d	0.603 d	0.503 d	0.477 d
Wax and PPE	1.100 ab	0.710 c	0.680 b	0.573 c	0.907 c	0.667 c	0.570 c	0.500 c
Nano chitosan (50 ppm) and PPE	1.133 a	0.940 b	0.677 b	0.607 b	0.995 b	0.743 b	0.593 c	0.540 b
Nano chitosan (100 ppm) and PPE	0.998 b	0.953 ab	0.740 ab	0.643 a	1.027 a	0.713 bc	0.617 b	0.513 c
Wax + nano chitosan (100 ppm) and PPE	1.100 ab	0.963 a	0.797 a	0.647 a	0.983 b	0.813 a	0.787 a	0.580 a

PPE, perforated 0.005% polyethylene; control (imazalil and packaging in 0.005% PPE). Values with the same letter in a column are not significantly ($P > 0.05$) different according to the least significant difference (LSD) test for each season.

Table 6
Effect of varied nano chitosan and wax concentrations on total soluble solids (TSS) as (Brixo) of Murcott mandarin juice in both seasons.

First Season								
Storage treatments	**Cold storage (CS) period (month)**				**Shelf-life (SL) (days)**			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Control	9.50 a	10.07 a	10.50 a	10.70 a	9.80 a	10.33 a	10.40 b	10.90 a
Wax and PPE	9.07 b	9.50 c	10.30 ab	10.50 ab	9.53 bc	10.00 b	10.53 a	10.63 b
Nano chitosan (50 ppm) and PPE	9.33 ab	9.87 b	10.00 c	10.33 b	9.60 b	10.00 b	10.40 b	10.60 b
Nano chitosan (100 ppm) and PPE	9.30 ab	9.87 b	10.23 b	10.47 ab	9.77 ab	10.10 b	10.30 c	10.50 c
Wax + nano chitosan (100 ppm) and PPE	9.00 b	9.43 c	10.00 c	10.00 c	9.23 c	9.93 c	10.30 c	10.53 c
Second season								
Control	9.70 a	10.03 a	10.47 a	10.77 a	9.87 a	10.87 a	10.30 c	10.63 ab
Wax and PPE	9.50 ab	9.60 b	10.23 ab	10.47 b	9.67 b	10.00 b	10.47 a	10.67 a
Nano chitosan (50 ppm) and PPE	9.40 b	9.87 ab	10.03 c	10.37 c	9.83 a	10.10 b	10.40 b	10.53 b
Nano chitosan (100 ppm) and PPE	9.20 c	9.87 ab	10.10 b	10.40 b	9.63 b	10.00 b	10.33 c	10.47 c
Wax + nano chitosan (100 ppm) and PPE	9.03 d	9.53 b	9.93 c	10.10 d	9.30 c	9.87 c	10.33 c	10.53 b

PPE, perforated 0.005% polyethylene; control (imazalil and packaging in 0.005% PPE). Values with the same letter in a column are not significantly ($P > 0.05$) different according to the least significant difference (LSD) test for each season.

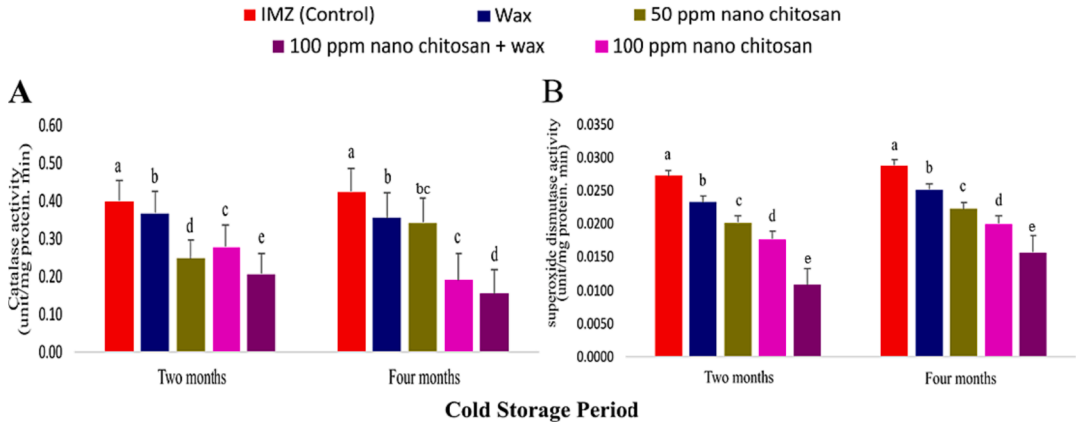

Fig. 3. The effect of various post-harvest treatments on (A) the catalase (CAT) and (B) superoxide dismutase (SOD) activity of Murcott mandarin fruit throughout the second and fourth months of cold storage. Values with the same letter in a column are not significantly ($P > 0.05$) different according to least the significant difference (LSD) test.

wax and nano chitosan was the best, as the lowest bacterial and fungal growth was recorded (Table 7).

4. Discussion

One of the most significant unfavorable signs in fruit preservation is water loss, which affects the fruit's quality, causes shrinkage, and makes marketing the preserved fruit more challenging (Grierson and Wardowski, 1978, Kawada and Albrigo, 1979). The obtained results may be attributable to the post-harvest handling technique, wax coating, wich showed that edible films and coatings changed the environments and reduced weight loss during transport and storage by limiting the permeability and gaseous exchange (Cuq et al., 1995a, Cuq et al., 1995b). Additionally, water is lost from the body through metabolic processes, including respiration and transpiration, and the stomata and epidermal layers typically regulate this water loss. The coating strategy slows down these processes by forming a film on top of the skin and serving as an additional barrier to moisture loss (Toğrul & Arslan, 2004).

To preserve quality criteria such as wasted fruits, FWL, FPF, and PTI of "Murcott" mandarin fruits during CS and SL, post-harvest coating treatments such as wax, nano chitosan, and the combination of wax with nano chitosan were utilized. Nano chitosan alone or mixed with wax could preserve fruit post-harvest quality, reducing discarded fruits, FWL, FPF and maintaining a better PTI. These outcomes agreed with those attained by (Samra et al., 2014, Nascimento et al., 2016, Gad and Ibrahim, 2018, Melo et al., 2018, Taghinezhad and Sharabiani, 2018, Adiletta et al., 2019, Xiao et al., 2019, Hmmam et al., 2021).

In addition, it retarded the degradation of vitamin C, where chemical and enzymatic oxidation can occur with ascorbic acid (McErlain et al., 2001). Thus, maintaining the fruits' fresh color, ascorbic acid is considered an antioxidant for brown phenolic substances resulting from oxidation. (Golan-Goldhirsh and Whitaker, 1984).

The acidity of the chitosan-treated fruits steadily was reduced towards the end of the storage period, and this drop was connected to a decline in the eating quality (El Ghaouth et al., 1992, Jiang and Li, 2001, Srinivasa et al., 2002). The second season was the only season in which these treatments outperformed coatings with wax and 100 ppm nano chitosan. In addition, all coating treatments with nano chitosan maintained considerably higher TA percentages than the control in both seasons. A possible cause of the lowering TA over the storage period is the loss of organic acids from oxidation and their usage in respiration activities inside fruit tissues. As the storage period wore on, it was reported that the fresh fruits' respiration rate increased (Hussein 1998).

Numerous studies showed that TA rose on products treated with chitosan (strawberries, tomatoes, and peaches) near the end of storage. In contrast, TA slowly dropped in other crops (mangoes, longans), with a corresponding decline in eating quality (El Ghaouth et al., 1992, Jiang and Li, 2001, Srinivasa et al., 2002). The significant effect of the different treatments on TSS was also observed, as the lowest number of TSS was recorded when using a mixture of wax and 100 ppm nano chitosan in both seasons. This positive effect may be due to the inhibition of biological processes in the fruits, such as respiration and transpiration, by these treatments (Khaliq et al., 2017).

Chitosan affects the production or activity of several enzymes in

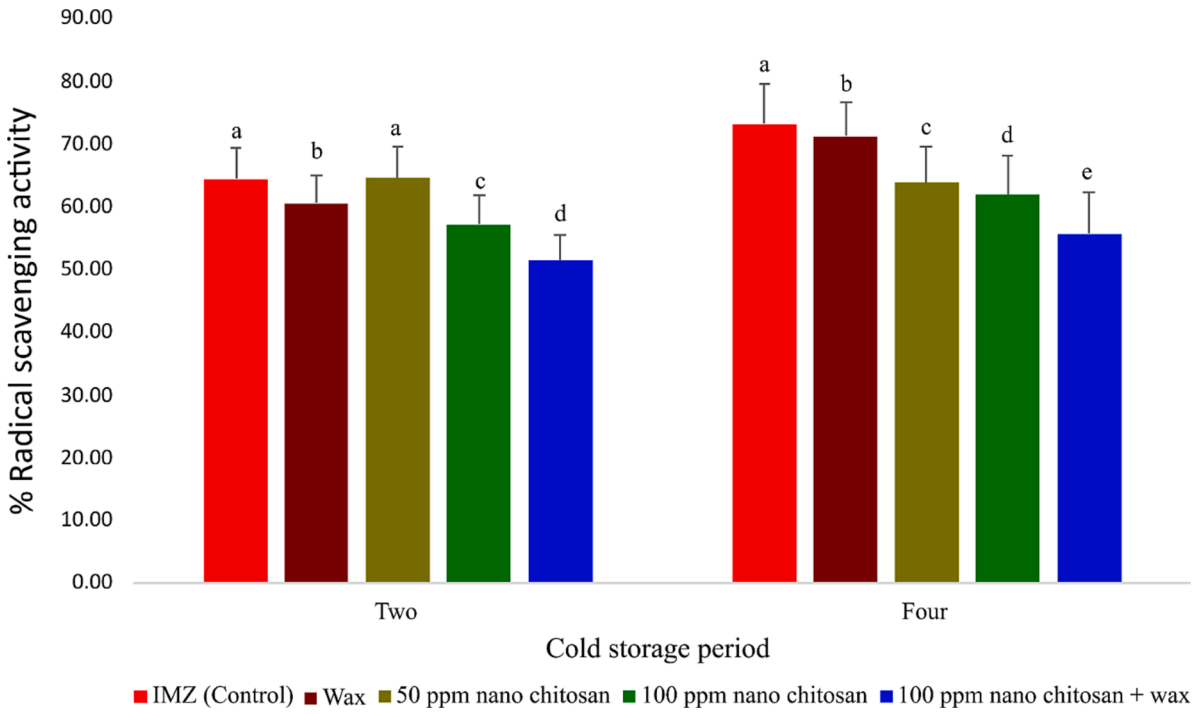

Fig. 4. The effect of several post-harvest treatments on the total antioxidant activity of Murcott mandarin fruit during the second and fourth months of cold storage. Values with the same letter in a column are not significantly ($P > 0.05$) different according to the least significant difference (LSD) test.

Table 7
Effect of coating with different concentrations of nano chitosan and wax on the microbial burden of Murcott mandarin peel during cold storage periods during the second season.

Microbial count Log $_{10}$ CFU/mL	Control	Wax and PPE	Nano chitosan and PPE		Wax + nano chitosan (100 ppm)
			50	**100**	
Total bacterial count	7.6 a	5.2 b	4.9 c	4.2 cd	3.9 d
Salmonella	0.6 a	–	–	–	–
Escherichia coli	3.6 a	3 b	2 c	1.5 d	1.4 d
Total molds and yeasts count	5.3 a	4.9 b	4.3 bc	4 c	3.8 d

Values with the same letter in a row are not significantly ($P > 0.05$) different according to the least significant difference (LSD) test.

various plant species, including pectate lyase, phenylalanine ammonia-lyase, chitinase, chitosanase, glucanase, SOD, cellulase, poly-galacturonase, and polyphenol oxidase (El Ghauth et al., 1994, Reddy et al., 2000, Jiang and Li, 2001, Li and Yu, 2001, Romanazzi et al., 2002), and the peroxidase in orange flavedo tissue (Fajardo et al., 1998). Antioxidant enzymes like CAT and SOD are crucial to prevent tissue damage because they eliminate dangerous elements like free radicals. Thus, the decrease in antioxidant enzymes in the tissues of the fruits coated with nano chitosan indicates that the tissues are not damaged compared to those not coated with it (Meng et al., 2012).

Imazalil (IMZ), when added to wax, provides effective protective control on green mold and suppresses sporulation, protecting fruit from *Penicillium digitatum*. Applying wax is essential for maintaining fruit quality (Njombolwana et al., 2013). It is important to note that chitosan has been used to coat fruits like peaches, Japanese pears, kiwis, and apples. Gas exchange is affected, post-harvest fruit quality is preserved, and transpiration losses are postponed (Du et al., 1997, Hu and Zou, 1998, Li and Yu, 2001). Furthermore, chitosan's antisenescence and antifungal capabilities may help to explain how it prevents peel problems. Fruit degradation is more likely to occur at this early stage of peel injury development because fungi more easily invade the tissues. Chitosan's antifungal properties have been demonstrated in citrus fruit (El-Ghaouth et al., 2000).

The antibacterial action of nano chitosan may be related to the presence of an amino group (Verlee et al., 2017). The effect of nano chitosan on microorganisms increases when they are in direct contact with the active site (Elsabee et al., 2016), and it is considered as a bactericidal (Moreira et al., 2011). According to previous studies, applying chitosan nanoparticles might maintain the quality of banana fruits and increase SL (Lustriane et al., 2018). One of the prospective solutions to guarantee the microbiological safety of food is chitosan edible coatings. However, the characteristics of these materials can be enhanced by adding nanostructures to films and coatings (Osheba et al., 2013, Bugnicourt et al., 2014, Pilon et al., 2015). Therefore, using nano chitosan with wax is considered an edible preservative intended to preserve the quality characteristics of the fruits over long storage periods.

5. Conclusion

The storage period of citrus fruits is the most important factor affecting the yield. As a result, research tended to employ a variety of materials used in fruit covering. These materials are selected as they can extend the SL of fruits and not be toxic, as well as being edible, such as plant extracts, in addition to nanomaterials. In the current study, we used several different coatings of wax and nano chitosan and a mixture of them to wrap the fruits. We examined the effect of these treatments on the fruit quality and chemical properties over the CS period (four months) and SL (six days). Using a combination of wax and nano chitosan (wax plus 100 ppm nano chitosan and 0.005% PPE) was determined as the most effective packaging method. As a result, we anticipate that the incorporation of nano chitosan into fruit coating formulations will be crucial, given that it prolongs the SL of high-quality fruits.

CRediT authorship contribution statement

Nourah A. Al Zahrani: Formal analysis, Investigation, Data curation, Visualization, Methodology. **Mohamed M. Gad:** Conceptualization, Investigation. **Ahmed M. Fikry:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Visualization, Methodology. **Ahmed Ezzat Ahmed:** Data curation, Writing – original draft, Visualization, Methodology. **Khaled A. El-Tarabily:** Conceptualization, Investigation, Data curation, Writing – original draft, Visualization, Methodology. **Hend A. Elakkad:** Conceptualization, Investigation, Data curation, Writing – original draft, Visualization, Methodology. **Ibrahim Eid Elesawi:** Writing – original draft, Data curation, Conceptualization, Formal analysis, Investigation, Visualization, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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