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Quality characteristics of strawberry fruit following a combined treatment of laser sterilization and guava leaf-based chitosan nanoparticle coating

Liela Mohamed Ali[1*], Abd El-Rahman Abd El-Raouf Ahmed[1], Helmy El-Sayed Hasan[2], Ahmed El-Raie Emam Suliman[3] and Sherif Saied Saleh[4]

Abstract

Background: Strawberry fruit is a rich source of antioxidants that are beneficial for human health. However, the rapid decline of strawberries dramatically reduces the shelf life and raises postharvest losses. To develop an efficient and ecological approach for maintaining the quality, strawberries (*Fragaria* x *ananassa*, cv. Festival) were treated with 0.5% chitosan coating (0.5% Ch), guava leaf-based chitosan nanoparticles coating (Gl-ChNps), and a combination treatment of 1.3 mW/cm^2 laser light followed by Gl-ChNps coating (combined treatment), then stored for 12 days at 10 °C and 85–90% RH. The untreated fruit served as a control.

Results: Semi-spherical particles with an average size of 21.92 nm, a monodisperse nature, and high solution stability were formed. The findings revealed that the combined treatment completely suppressed fungal decay compared to 50% decay in control, and significantly reduced weight loss percentage to 4.68% compared to 27.35% in control. In accordance, the combined treatment had the maximum anthocyanin content and vitamin C, at 42 and 81.1 mg/100 g, respectively. The results showed that treated strawberries had less change in color, total soluble solids, titratable acidity, and pH during storage than untreated strawberries, which exhibited higher chemical changes.

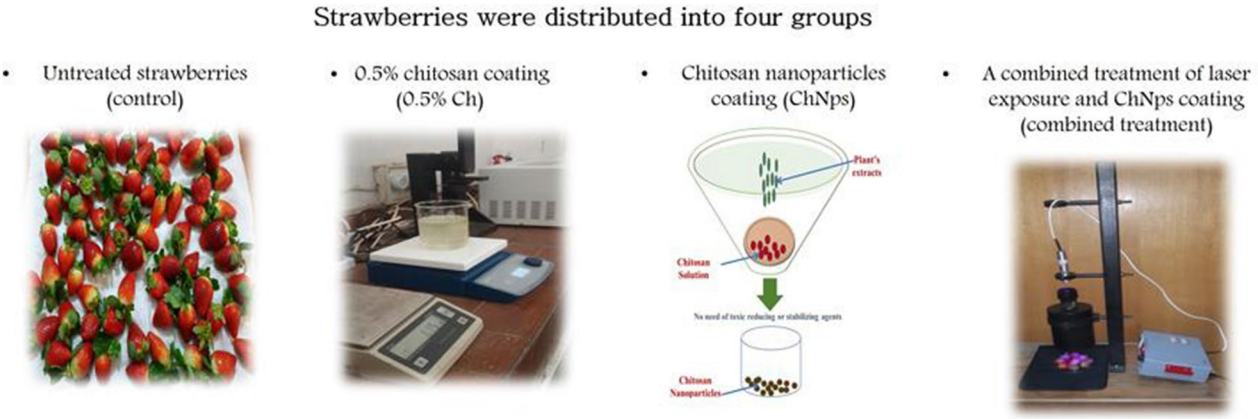
Conclusions: The edible film of chitosan nanoparticles acted as a semi-permeable barrier that modified and restricted gas exchange, reduced water loss, and delayed fruit senescence. In addition, the combination of laser light with chitosan nanoparticles has been shown to control the pathogens and retain the freshness of strawberries.

Keywords: Strawberry fruit, Biological synthesis, Chitosan nanoparticles, Diode laser

*Correspondence: Liela.ali@arc.sci.eg

[1] Agricultural Engineering Research Institute, Agricultural Research Center, Dokki, P.O. Box 256, Giza 12611, Egypt
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

The strawberry fruit (*Fragaria* × *ananassa* Duch.) is high in vitamins, minerals, and antioxidants, all of which positively impact human health. Unfortunately, the rapid decline of strawberries dramatically reduces the shelf life and raises postharvest losses. Gray mold caused by *Botrytis cinerea Pers*. and *Rhizopus stolonifer* rot are the most common causes of postharvest losses that influence the strawberry quality and appearance [1, 2].

Many studies have been carried out to prevent rotting and losses associated with poor handling and storage of strawberries, including cold storage [3, 4], modified and controlled atmosphere storage [5–7], and radiation [8–10]. Studies have shown the effectiveness of these approaches in extending shelf life and inhibiting microbial growth; nonetheless, some adverse effects on flavor, anthocyanin content, organic acid, and vitamin C have been reported [11–13].

Surface coating with edible biopolymer (polysaccharides, lipids, and proteins) film is one of the most well-known techniques to maintain quality fruits and vegetables [14–16]. The edible film is a thin layer of material that acts as a semi-permeable physical barrier to control gas (CO_2/O_2) exchange, reduce respiration rate, delay dryness, slow decline, and protect fruit skin from mechanical injuries and deterioration. Such a technique is cost-effective, simple, and environmental friendly [17, 18], Nonetheless, consumer concerns must be considered, as the edible film's composition should be organic, non-toxic, and chemical-free.

Chitosan is a biodegradable, biocompatible natural polysaccharide polymer with immunological, antibacterial, and wound healing characteristics [19, 20]. According to Radhakrishnan et al. [21], the edible film of chitosan was found to delay water loss, suppress microbial growth, and preserve the color of papaya, mango, and strawberries. Furthermore, chitosan film retarded enzymatic browning and discoloration in cut pieces of apple [22] and mushroom [23]. However, the low solubility of chitosan in the aqueous solution limits its application as an antifungal agent [24], which promotes the use of nanoparticles form to improve chitosan's antifungal activity and wettability. In this regard, Ramezani et al. and Sathiyabama & Parthasarathy [25, 26] found that chitosan nanoparticles exhibited higher antimicrobial activity than chitosan in bulk form, possibly due to the nanoparticle's larger surface area, higher mechanical properties and more robust linking with bacteria cells. Furthermore, when compared to chemical synthesis using sodium tripolyphosphate, biological synthesis of chitosan nanoparticles using plant extract as a reducing and capping agent is an eco-friendly, simple, and rapid process with smaller particle size and more stable results [27, 28].

Guava leaves' antimicrobial and antioxidant properties have been linked to bioactive components, such as flavonoids, polyphenol, and ascorbic acid [29]. Because of its recognized medical characteristics and availability, it has been used as a reducing and capping agent in several investigations for green nanoparticle production [30, 31].

On the other hand, laser light has been shown to have a bio-stimulation impact on microorganism phytochromes, altering their vitality and growth [32–34]. The exceptional characteristics of laser light, such as monochromaticity, collimation, and coherence [35, 36], enable laser application for efficient surface disinfection of plants. However, few studies have examined the effectiveness of laser exposure in maintaining the quality of fruits and vegetables [37, 38]. This investigation aims to evaluate the effect of a combined treatment of laser sterilization and edible chitosan nanoparticles coating on

the quality attributes of strawberry fruit when used as a refrigeration complement.

Materials and methods

In this experiment, strawberries were distributed into four groups: untreated strawberries (control), 0.5% chitosan coating (0.5% Ch, positive control), guava leaf-based chitosan nanoparticles coating (Gl-ChNps), and a combined treatment of laser exposure and Gl-ChNps coating (combined treatment).

Preparation of chitosan (0.5% Ch) and chitosan nanoparticles (Gl-ChNps) coatings

Chitosan solution was obtained by adding 0.5% (w/v) chitosan (deacetylation of 93% and molecular weight of 161.16 kDa) to 1% (w/v) ascorbic acid under stirring for 90 min at room temperature until the chitosan was completely dissolved [39].

The chitosan functionalities were enhanced by crosslinking chitosan with guava leaves (*Psidium guajava* L.) extract at room temperature to produce its bio-nanostructure as a cost-effective and eco-friendly green route. Chitosan nanoparticles (Gl-ChNps) were prepared based on ionic gelation interaction between positive charges of chitosan and negative charges of guava leaf function groups. Nanoparticles were optimized following the approach documented in a prior study published by our group [40] that detailed the preparation and characterization procedures of ChNps based on guava leaf extract.

In brief, Gl-ChNps were formed using a 1:1 mixture of chitosan solution (5 mg/ml) adjusted at 5 pH with 1 N NaOH and dried guava leaves extract 10% (w/v). The mixture was stirred at 110 rpm for 30 min. For the obtained solution, dynamic light scattering (DLS), transmission electron microscope (TEM), and zeta potential analyses were measured and discussed in detail in our previous study [40]. Chitosan and chitosan nanoparticles coatings were prepared and used fresh.

Laser irradiation

A continuous wave (CW) diode laser at a wavelength of 450 ± 10 nm, 100 mW output power, and a beam diameter of 2 mm was employed in this study. A beam expander of an expansion power of 50-fold was placed in front of the laser light to enlarge the collimated beam. The optimum exposure time of laser light was selected after preliminary experiments that were discussed in depth in a previous study published by our group [38].

Strawberry preparation

Fresh strawberries (*Fragaria* × *ananassa* cv. Festival) with red color on more than 75% of the surface and uniform in size were purchased from a local market. On the previous day, fruits were picked and transported from Qalyubia, Egypt, in a refrigerated truck. Strawberries were carefully immersed in distilled water for 30 s to remove dust, then dried on a soft cloth for 30 min.

For coating treatments, strawberries were immersed in the prepared chitosan (0.5% Ch), or guava leaf-based chitosan nanoparticles (Gl-ChNps) solutions for 2 min, then raised and left to dry on a clean soft cloth at room temperature for 2 h [41].

For the combined treatment, the fruit sample was exposed to a diode laser (450 nm) at fluence of 234 mJ/cm^2. The sample was 20 cm away from the radiation source; strawberries were turned upside down and exposed to the same laser duration of 3 min to guarantee a thorough exposure on the whole surface of fruit. After laser exposure, the strawberries were coated with Gl-ChNps solution by dipping for 2 min, then raised and left to dry at room temperature for 2 h.

All samples, both untreated and treated, were packed in perforated plastic boxes, each holding approximately 350 g and ~ 13 strawberries; the initial weight of each box was recorded after packaging, and the boxes were stored at 10 °C and 85–90% relative humidity for 12 days.

Quality attributes

Fungal decay

The percentage of infected strawberries was used to calculate the fungal decline. Any fruit with signs of contamination, brown spots, or softening areas was considered rotten and counted. According to [13], fungal decay (%) = (The number of decayed fruits/ Total number of fruits) × 100.

Weight loss percentage

The decayed fruits were discarded, and the final weight was measured. The percentage of weight loss was calculated as follows: weight loss % = (Initial weight–Final weight)/ Initial weight × 100.

Firmness

The fruit firmness was measured using a digital penetrometer with a 10 mm diameter of flat end plunger (ST308—made in Italy) and expressed in kg/cm^2. Fruit firmness was assessed at three distinct points in the equatorial region, and the average was recorded [42]. The firmness loss was calculated as a percentage of the initial value.

Surface color

The surface color of both sides of each strawberry was measured on the equatorial zone using a chromameter (CR-400, Konica Minolta, Japan) according to the

Commission International de I'Eclairage (CIE) LAB color parameters: L^* (luminance), a^* (redness–greenness) and parameter b^* (yellowness–blueness). Parameters a^* and b^* were used to calculate chroma: $C^* = [a^{*2} + b^{*2}]^{1/2}$ and hue: h° = arctangent[b^*/a^*] [43, 44].

Titratable acidity and pH

Titratable acidity was measured in strawberry puree using the titration method [45] and expressed as the percentage of citric acid per 100 g of fresh weight. In addition, the value of the pH of strawberry juice was measured using a pH meter (Model Lutron pH-224-Taiwan) at 20 °C [46].

Total soluble solids

The total soluble solids (TSS) of the strawberries puree were measured via a digital refractometer (BEB53, Boeco, Germany) at 20 °C and expressed as a percentage [47].

Ascorbic acid

Spectrophotometric determination of ascorbic acid was performed as described by Bajaj and Kaur [48] and expressed as mg/100 g of fresh weight.

Anthocyanin content

Anthocyanin content was measured using the pH-differential method [49]. 4 g of strawberry puree was extracted with 40 mL of solvent ethanol: 0.1 M HCl (85:15%, v/v) and sonicated for 10 min. After centrifugation, one mL of sample extract was mixed with 9 mL of each of the buffer solutions potassium chloride (0.025 M, pH 1.0) and sodium acetate (0.4 M, pH 4.5), and the absorbance (A) was measured using a spectrophotometer at 520 and 700 nm, respectively. The total anthocyanins content was expressed as mg of pelargonidine-3-monoglucosid per 100 g of fresh weight according to the following formula: $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$.

DPPH radical-scavenging activity

The antioxidant activities were evaluated using the DPPH method described by Brand-Williams et al. [50]. Five grams of each sample were prepared in 50 mL methanol. An aliquot of the extract was added to a methanolic DPPH solution (100 µL, 0.2 mM). The mixture was stirred and left in the dark for 15 min. The absorbance was then measured against a blank at 517 nm. Percentage scavenging effect was calculated as: $[(A_0 - A_1) / A_0] \times 100$, where: A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample.

The statistical analysis

All treatments were carried out in triplicate. One-way analysis of variance (ANOVA) was used to analyze the data using the Excel program (Microsoft Office Professional Plus 2010), assuming a 95% confidence level ($P < 0.05$). The means of data were separated using Tukey's honest significance test (HSD).

Results and discussion

Chitosan nanoparticles characterization

The crosslinking of protonated ammonium groups of chitosan with anionic groups of guava leaf extract resulted in semi-spherical nanoparticles with an average size of 21.92 nm. The nanoparticles have a monodisperse nature, as measured by the polydispersity index (PDI) of 0.471, and good stability, as evaluated by the zeta potential of -27.1 mV, which aid in preventing agglomeration.

Quality attributes of strawberry

Fungal decay of strawberry

The findings did not show any deterioration in any of the treatments until the fourth day of the storage, Table 1 and Fig. 1. Furthermore, chitosan nanoparticles coating (Gl-ChNps) inhibited decay more effectively than chitosan in bulk ($P < 0.05$, $HSD = 5.99$), which can be attributed to the uniformity of nanoparticles coating, that improved adhesion, cohesion, and durability. According to Eshghi et al. [41], the antimicrobial activity of chitosan is related to the biopolymer's ability to induce severe damage in mold cell structure, which could explain the lower decay observed in coated strawberries compared to the control. This coating contributes to a decrease in respiration rate and physical damage in strawberries.

On the sixth day, the infected uncoated strawberries reached 20.23%, and progressively increased to 50.25% by the end of the storage period, while the strawberries coated with Gl-ChNps had only a 11.12% decline. Eshghi et al. [41] found that on the twelfth day of storage at 4 ± 1 °C and 70% RH, 20% of ChNps coated fruit displayed visible fungal rot. This increased degradation percentage compared to the present results suggests that guava leaf extract could improve the antimicrobial activity of chitosan nanoparticles.

On the other hand, the combined treatment showed no signs of deterioration after 12 days of storage at 10 °C. In a previous study by Wang and Gao [51], the same result was achieved with 1.5% Ch coated fruit; however, strawberries were stored at 5 °C.

In this regard, the role of laser exposure before Gl-ChNps film in the combined treatment is evident in the inhibition of microbial development.

Table 1 Combined treatment delayed decay and maintained the appearance of strawberry fruit after12 days of cold storage at 10 °C and 85–90% RH

Storage durations	Treatments			
	Control	0.5% Ch coating	Gl-ChNps coating	Combined treatment
4 days				
8 days				

Table 1 (contined)

Storage durations	Treatments			
	Control	0.5% Ch coating	Gl-ChNps coating	Combined treatment
12 days				

Treatments: untreated strawberries (control), 0.5% chitosan coating (0.5% Ch), guava leaf-based chitosan nanoparticles coating (Gl-ChNps), and a combination treatment of 1.3 mW/cm^2 laser light followed by Gl-ChNps coating (combined treatment)

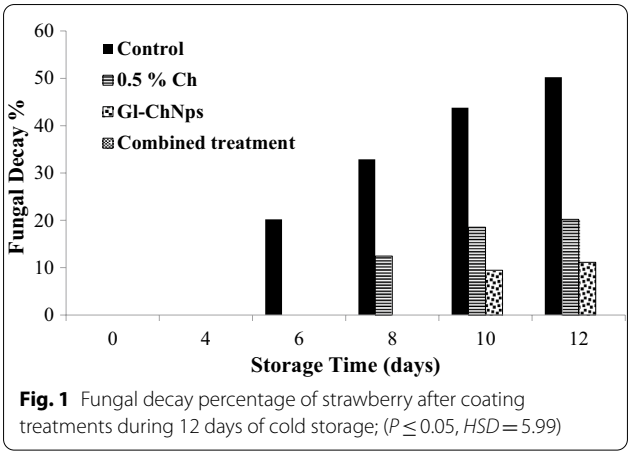


Fig. 1 Fungal decay percentage of strawberry after coating treatments during 12 days of cold storage; ($P \leq 0.05$, $HSD = 5.99$)

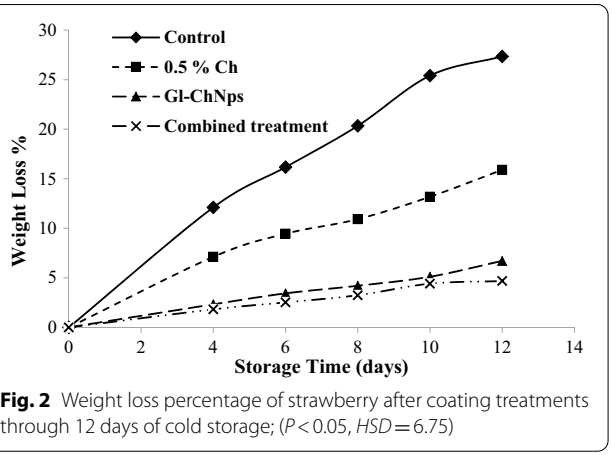


Fig. 2 Weight loss percentage of strawberry after coating treatments through 12 days of cold storage; ($P < 0.05$, $HSD = 6.75$)

According to Braga et al. [52], exposure to light activates the photosensitizer in microorganisms, which damages the cell's biomolecular structure by producing reactive oxygen species (ROS), such as singlet oxygen and hydroxyl radicals, effectively damaging the cell membrane, intracellular enzymes, and nucleic acids [53]with little to no negative effects on the host. Zhang et al. [54] reported that, a combination of blue light and salicylic acid reduced the incidence and severity of strawberries decay compared to control. In addition, for 10 days of cold storage, pulsed light of 11.9 and 23.9 J/cm^2 delayed and reduced the incidence of strawberries inoculated with *Botrytis cinerea* by 16–20% compared to control [55].

Weight loss percentage

As shown in Fig. 2, untreated strawberries lost 27.35% of their weight after 12 days of storage, whereas 15.9 and 6.71% of weight loss were found in the 0.5% Ch and Gl-ChNps treated fruit, respectively, ($P < 0.05$, HSD = 6.75). The 0.5% Ch coating was more effective in delaying weight loss and significantly decreased weight loss by 41.86% compared to the control. As the skin of strawberries is very thin, it is prone to rapid water loss, resulting in a weight loss and shriveling. On the other hand, the edible coating provides a physical barrier to CO_2, O_2, and ethylene, which reduces gas exchange and water loss. Lee et al. [56] observed that control strawberries showed a higher respiration rate than multi-polysaccharide coated fruit during storage. According to Hernández-Muñoz et al. [57], 1.5% Ch reduced weight loss by 48.92% compared to control, suggesting that a thicker layer created by a coating with a higher concentration of chitosan prevented excessive moisture loss. Furthermore, strawberries treated with polysaccharide edible coating solutions comprising oregano essential oil, sodium alginate, chitosan nanofibers, and cellulose nanocrystals lost 10.8% of their weight after 9 days of storage as opposed to 37% of untreated fruit [56]. This is due to the thin layer coating, which reduces moisture loss and inhibits microbial infection.

In our pervious study [38], strawberries that had been exposed to 3 min of laser light lost weight by 4.86% compared to 21.53% of control after 7 days of storage. According to Romero Bernal et al. [55], additional stress factor(s) would be required to increase light action and achieve a higher level of inactivation of fungal contamination while retaining the quality of the fruit. This was demonstrated by the combination of laser light and Gl-ChNps coating, which completely prevented the decay and had the smallest weight loss of 4.68% after 12 days of storage.

Firmness

Strawberry is a soft fruit that loses its firmness rapidly over the validity period, which has a substantial impact on the consumer's acceptability [2]. During storage, the firmness of all coated strawberries was significantly higher than that of uncoated strawberries (P<0.05, $HSD = 0.36$), as shown in Fig. 3. On the fourth day of storage, uncoated fruit lost around 71% of their flesh firmness, and 82% by the end of the storage period, compared to 68.21, 24.86 and 10.4% for 0.5% Ch coating, Gl-ChNps coating, and combined treatment, respectively. The results suggested that combined treatment coating had a positive effect on maintenance of strawberry firmness.

According to Del-Valle et al. [58], fruit texture properties are affected by the structure, degradation of polysaccharides in the cell wall, and loss of water due to the cell breakdown, which explains why the combined treated strawberries had the least significant firmness loss, as their water content and the cell turgidity pressure remained unchanged. Contrarily, Zhang et al. [53] found

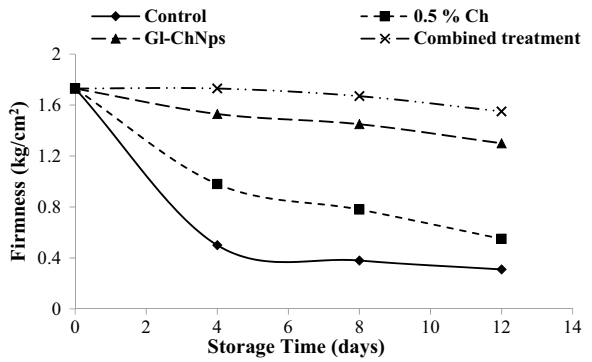


Fig. 3 Firmness of strawberry after coating treatments through 12 days of cold storage; ($P< 0.05$, $HSD = 0.36$)

that a combination of UV-A light and chitosan–gallic acid coating caused a decrease in firmness compared to the control, and that the high temperature generated by the UV-A bulb caused increased water loss, which accelerated the deterioration of texture and color. This suggests that the low power laser light has no heat effect on the surface of fruit.

It was found that the firmness of 0.5% Ch treated fruit was significantly higher than that of uncoated fruit up to 8 days of storage, but the difference faded to insignificance after that. This finding is consistent with Lee et al. [56], who found that after 9 days of storage at 6 °C and 25% RH, coated strawberries had retained more than 40% of their firmness. Conversely, the uncoated strawberries had lost nearly 90% of their firmness. Eshghi et al. [41] also reported that after 8 days of storage at 4 ± 1 °C, the loss of firmness in uncoated fruit was around 45% compared to 27% in fruit coated with chitosan nanoparticles.

As reported by Tanada-Palmu and Grosso [59], the creation of a sufficient internal atmosphere as a result of the edible film coating may explain the delay in softening and senescence.

Surface color

The results showed that all the samples darkened during the storage time, Fig. 4a. However, after the fourth day, the uncoated sample was significantly darker (more ripening) than the coated fruit samples ($P<0.05$, $HSD = 6.03$). By the end of the storage period, the loss percentage of the L^* parameter was 46.15, 23.89, 18.96, and 17.48% for uncoated, 0.5% Ch coating, Gl-ChNps coating, and combined treatment, respectively, which is consistent with Perdones et al. [60], who found that the coated fruit showed the highest luminosity values at the end of storage. This could be attributed to the coating layer's control of moisture loss, which decelerates the ripening process

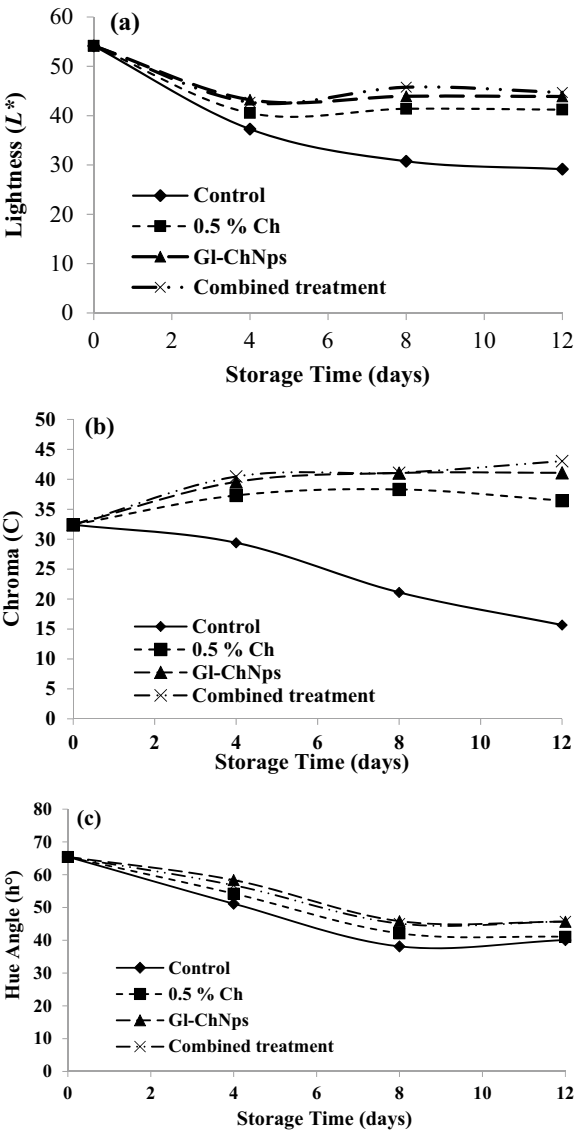


Fig. 4 Effect of coating treatments on (**a**) lightness ($P< 0.05$, $HSD = 6.03$), (**b**) chroma ($P<0.05$, $HSD = 9.36$), and (**c**) hue ($P > 0.05$) of strawberry during 12 days of cold storage

and helps to minimize the external color changes in the fully ripe strawberry.

On the other hand, the coated sample showed a slightly increased in chroma, as an indication of maturation over the storage time. At the end of the storage period, chroma increased to 36.45, 41.09, and 43.04 of strawberries treated with 0.5% Ch coating, Gl-ChNps coating, and combined treatment, respectively, without a significant difference among treatments, Fig. 4b. Meanwhile, the uncoated sample was less deeply red, and chroma dropped by 51.66% ($P < 0.05$, $HSD = 9.36$), which can be attributed to the greater water loss and

surface drying of the uncoated ripe strawberries Nunes et al. [61]. In contrast, Hernández-Muñoz et al. [57] found that both coated and uncoated fruit developed a less vivid coloration, as shown by lower chroma values; however, chroma was reduced by roughly 10% for coated fruit and 30% for control.

The fruit was obtained with a red color surface covering 75% of the surface, with a hue angle in the orange–red range of 65.45°. As a result of ripening over time, the external color developed to red and deep red with a decrease in the hue angle.

In accordance with Lee et al. [56], the red surface color of the coated strawberries turned fewer darker than that of the control. The findings showed that all treatments reduced the hue angle without significant difference ($P > 0.05$) and by the end of the storage period, hue angle decreased to 40.09°, 41.08°, 45.68°, and 45.74° for uncoated fruit, 0.5% Ch coating, Gl-ChNps coating, and combined treatments, respectively. This implies that the coating minimized the surface color change by reducing moisture loss and cell wall degradation.

Titratable acidity (TA) and pH

Titratable acidity is directly related to the amount of organic acids in the fruit, and a reduction in acidity may be expected as a result of metabolic changes in fruit or due to the use of organic acids in the respiratory process [62]. The initial TA of strawberry, measured as a percentage of citric acid per wet weight, was 0.77%, and it increased slightly for all treatments, possibly because of water loss through storage. However, the coating treatments had a negligible effect on the acidity percentage of strawberries compared to the uncoated fruit ($P > 0.05$, data not shown), implying that the organic acids have not yet been metabolized [15]. This result is consistent with Vargas et al. [63], who found that acidity did not increase significantly during storage and was not affected by coating application. Conversely, Lee et al. [56] and Yan et al. [64] found a slight decrease in acidity of all strawberries; however, the effect of coating on the acidity was negligible.

The results showed a slight decrease of the initial pH value of 3.36 through the storage without a significant difference between treatments ($P > 0.05$, data not shown). According to Perdones et al. [60], samples coated with chitosan containing lemon essential oil had significantly lower pH values at the end of storage ($p < 0.05$), indicating that essential oil components may affect fruit metabolic activity. Other research found that the pH of strawberries increased slightly during storage without significant differences between coated and uncoated fruit [65].

Total soluble solids (TSS)

Total soluble solids of strawberries mainly contain sugars and organic acid, which are important contributors to the flavor. TSS are expected to increase throughout the storage period in line with the progress of the ripening process and water loss [60]. On the fourth day of the storage, TSS value decreased from the initial value at 6.1% to 5.9, 5.9, and 5.8% after 0.5% Ch coating, Gl-ChNps coating, and combined treatment, respectively, due to fruit metabolic activity and respiration, Fig. 5. While TSS value of the control sample significantly increased to 6.8% and then reached 7.7% by the end of the storage ($P^{<}0.05$, $HSD = 0.83$). This could be caused by excess water loss and degradation in the cell wall of control sample [57].

By the end of the storage, Gl-ChNps coating and combined treatment maintained TSS value at 6.3 and 6%, respectively, in agreement with Vargas et al. [63] and Yan et al. [64], who found that soluble solids did not change significantly during storage and were not affected by coating application. The reduced TSS accumulation in coated fruit is probably due to a decrease in respiration and a delay in the ripening process [15]. TSS results show that the untreated strawberry fruit exhibited a more active metabolism than treated strawberries.

Ascorbic acid

Lee and Kader [66] suggested that storage temperature is the most important reason to maintain vitamin C in fruits and vegetables and the losses of vitamin C are accelerated during a long storage period at high storage temperature. Cordenunsi et al. [4] stated that ascorbic acid content (vitamin C) is affected by climatic conditions, postharvest management, and cultivar variety.

There was no significant difference between treatments until the fourth day of the storage period. Ascorbic acid

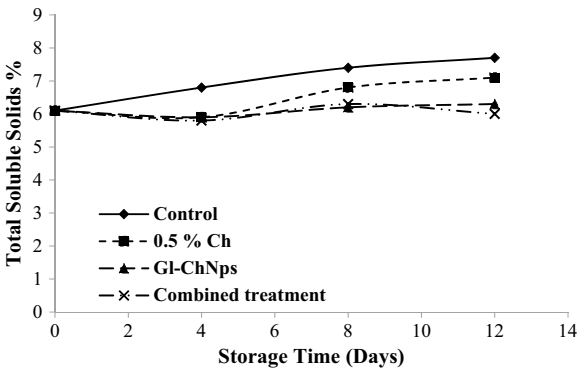


Fig. 5 Total soluble solids (TSS) change of strawberry after coating treatments during 12 days of cold storage; ($P < 0.05$, $HSD = 0.83$)

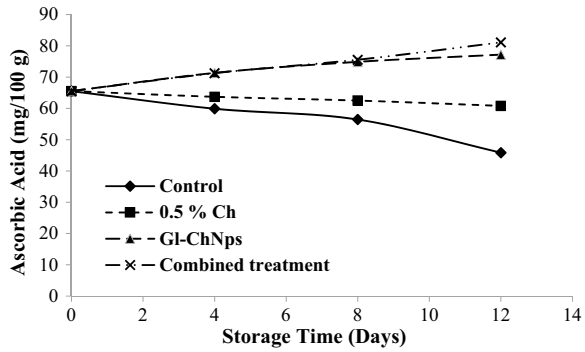


Fig. 6 Effect of coating treatments on ascorbic acid of strawberry during 12 days of cold storage; (P<0.05, HSD = 12.02)

content of strawberry fruit varied from its initial value of 65.52 mg/100 g, Fig. 6. By the end of the storage period, ascorbic acid significantly increased in Gl-ChNps coating and combined treated fruit by 17.79 and 23.78%, respectively, whereas it decreased in control and 0.5% Ch coated samples by 30 and 7.2%, respectively, (P<0.05, HSD = 12.02).

It has been reported that blue light exposure had no remarkable impact on ascorbic acid content [54], whereas exposure to low power of laser light increased the vitamin content [38]. This explains why the combined treated sample contains more ascorbic acid than Gl-ChNps treatment.

The findings indicated that 0.5% Ch coating significantly retarded the decrease of ascorbic acid compared to the uncoated sample. These findings are in agreement with Pagliarulo et al. [67], who found a greater loss in the ascorbic acid in the control sample compared to the initial concentration of 66.76 mg/100 g, while this value increased in the coating samples. Wang and Gao [51] reported that different concentration of Ch coating retarded the decrease of ascorbic acid compared to control; however, by the end of the storage at 5 and 10 °C, ascorbic acid in coated strawberries had significantly decreased.

In contrast to Eshghi et al. [41], a significant reduction in ascorbic acid content was observed for chitosan nanoparticles loaded with and without copper-coated fruit through storage at 4 ± 1 °C. The presence of copper ions in the coating formula accelerated the degradation of ascorbic acid content in strawberries compared to the uncoated sample, making the antimicrobial agents used in the coating solution a critical issue that could harm sensitive components, such as ascorbic acid.

Anthocyanin content

Figure 7 shows the changes in the anthocyanin content of coated fruit after storage for 12 days at 10 °C and 85–90% RH compared to the initial value of 23.9 mg/100 g. Untreated strawberries showed an increase in the anthocyanin content of 32 mg/100 g on the fourth day of storage, followed by a rapid reduction of 17 mg/100 g by the end of the storage period. In line with Shin et al. [68], anthocyanin concentration in red, ripe untreated fruit slowly decreased through storage, but a rapid reduction was observed in the fruit stored at 10 °C by the end of the storage time. A significant difference in anthocyanin content between control and treated samples was observed on the eighth day of the storage period (P < 0.05, HSD = 6.3).

Anthocyanin content of coated strawberry fruit increased gradually at a slow rate and did not decline at the end of the storage period, indicating that strawberries darkened with ageing, which is similar to Wang and Gao [51] who found that total anthocyanin increased at a slow rate in fruit treated with chitosan coating (0.5, 1.0, and 1.5 g/100 mL) and did not display a decline compared to the control sample at the end of storage at 5 and 10 °C.

At the end of the storage period, the combined treated fruit had the highest anthocyanin content of 42 mg/100 g followed by 37.1 and 36.5 mg/100 g in Gl-ChNps and 0.5% Ch coated strawberries, respectively. According to Eshghi et al. [41], strawberries coated with copper-free nano chitosan had the highest anthocyanin concentration of 390 mg/kg after 12 days of storage at 4 ± 1 °C with 70% RH. Considering that the quantity of anthocyanin is important in assessing the attractiveness and maturity of strawberries, it has been shown that coating of chitosan can improve the fruit's appearance while preserving the health benefits of strawberry. According to our earlier findings [38], anthocyanin accumulation in strawberries

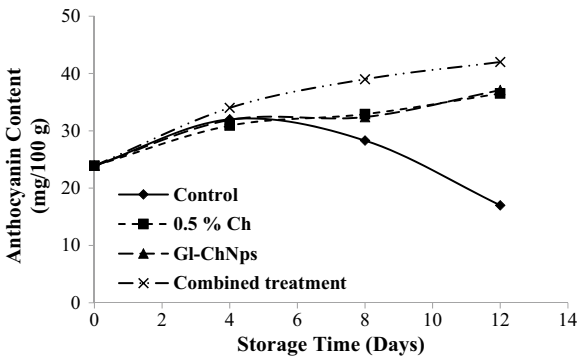


Fig. 7 Effect of coating treatments on anthocyanin content of strawberry after 12 days of cold storage; (P < 0.05, HSD = 6.3)

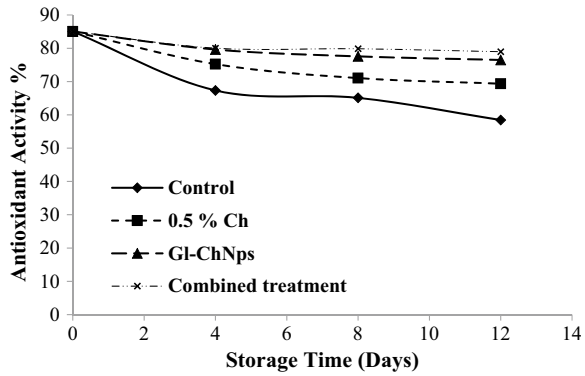


Fig. 8 Effect of coating treatments on antioxidant activity of strawberry after 12 days of cold storage; ($P < 0.05$, $HSD = 7.53$)

may be slightly influenced by laser light, while storage temperature had the main impact.

Antioxidant activity

The primary defensive function of fruit has been attributed to the antioxidants which can prevent chemical damage caused by free radicals. In addition to anthocyanins, other flavonoids, phenolic acids, and vitamins may also contribute to the protective effect against oxidative damage to cells. As shown in Fig. 8, antioxidant activity decreased over the storage period for all treatments ($P < 0.05$, $HSD = 7.53$), these findings were consistent with Cordenunsi et al. [4]. It's possible that the inverse relationship between antioxidant and anthocyanin concentration is due to the fact that antioxidant activity and anthocyanin have a complementary or superimposing impact in strawberries [69].

From the 8th to the 12th day of the storage, antioxidant activity was found to be relatively equal in coated sample, which can be attributed to the delay in the maturation and ageing of the coated samples.

At the end of the storage time, coated strawberries maintained the antioxidant activity compared to fresh fruit by reduction percentages of 18.44, 10.06, and 7.16% for 0.5% Ch coating, Gl-ChNps coating, and combined treatment, respectively, whereas the antioxidant activity reduction percentage of control reaches 31.27%, which may be due to senescence and deterioration in uncoated fruit, suggesting the ability of chitosan nanoparticles coating and combined treatment to retain higher antioxidant activity in strawberries after storage. These results are comparable with the findings reported by Pagliarulo et al. [67] who observed a decrease in antioxidant activity with a significant difference between coated and uncoated fruit through the storage.

Wang and Gao [51] reported that the elevated level of antioxidant activity in chitosan coated strawberries strengthened the mechanism of microbial defense and emphasized the resistance against fungal attacks.

Conclusions

A novel postharvest approach combining laser irradiation with guava leaf-based chitosan nanoparticles coating was developed to maintain the quality of strawberries. The antimicrobial action of chitosan nanoparticles formed by crosslinking chitosan and guava leaf extract was shown to be more efficient than chitosan in bulk, as the edible coating of chitosan in nano-size could exhibit markedly improved barrier properties at a lower concentration of chitosan. Moreover, the intracellular reactive oxygen species (ROS) released after exposure to laser light combined with chitosan nanoparticles effectively controlled pathogens, delayed senescence, and reduced water loss, which is reflected in the overall quality of strawberries.

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Author contributions
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Availability of data and materials
The data sets used and analyzed during the current study are available to readers as in the manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Agricultural Engineering Research Institute, Agricultural Research Center, Dokki, P.O. Box 256, Giza 12611, Egypt. [2]National Institute of Laser Enhanced Sciences (NILES), Cairo University, Giza, Egypt. [3]Agricultural Engineering Dept., Faculty of Agriculture, Cairo University, Giza, Egypt. [4]Medicinal and Aromatic Dept., Horticultural Research Institute, Agricultural Research Center, Giza, Egypt.

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