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The Effect of Beeswax and Aloe Vera Gel Combined with Clove (*Syzygium aromaticum*) Nanoemulsion and Salicylic Acid on the Shelf Life and Quality Characteristics of Strawberry Fruit (*Fragaria* × *ananassa*)

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ABSTRACT

Strawberry, as a highly popular yet perishable fruit, requires safe and natural methods to enhance its postharvest shelf life. This study was conducted as a factorial experiment in a completely randomized design with two factors (edible coatings and different storage durations) in three replications. The treated fruits were stored in a dark cold room at 4±1°C with 85-90% relative humidity under regular ventilation. Results demonstrated that untreated control fruits exhibited the lowest fruit juice pH after 15 days of storage compared to other treatments. The combined treatment of 30% aloe vera gel + 10% beeswax + 1 mM salicylic acid proved most effective, forming a protective layer that prevented cellular degradation and maintained fruit quality. This treatment doubled ascorbic acid content compared to the control and achieved 9.03% total soluble solids by day 15. It also showed minimal weight loss (9.1%) and the least changes in titratable acidity (18.64% reduction) on day 15. Additionally, the treatment containing 2‰ clove nanoemulsion significantly extended the strawberry's shelf life. The findings indicate that these natural compounds can serve as suitable alternatives to synthetic chemicals in postharvest applications, particularly for sensitive fruits like strawberries, demonstrating their potential for commercial use in food preservation technologies.

1.Introduction

Strawberry (*Fragaria × ananassa*), from the Rosaceae family, possesses high antioxidant activity due to its content of ascorbic acid and anthocyanins, and has therapeutic properties such as strengthening the immune system and reducing the risk of cancer. However, this fruit is highly perishable because of its high respiration rate, high water content, and intense metabolic activity [1]. The reduction in the quality and quantity of strawberries mainly occurs between harvest and consumption. Postharvest changes in gas balance lead to increased respiration, reduced metabolism, decay, and accelerated fruit aging [2].
Salicylic acid, as an endogenous growth regulator, regulates physiological processes such as growth, photosynthesis, ethylene production, and stress resistance [3]. Nanoemulsions, due to their high kinetic stability, optical clarity, and enhanced bioavailability, are superior to conventional emulsions. These systems are also used as drug carriers and have attracted attention because of their environmental compatibility, biodegradability, and thermodynamic stability. Medicinal clove (*Syzygium aromaticum*), with eugenol as its main component, possesses antibacterial, antifungal, and antioxidant properties, and is used as a natural food preservative [4].
Aloe vera is also widely used in the food and pharmaceutical industries due to its therapeutic and medicinal properties. Aloe vera gel contains polysaccharides, soluble sugars, proteins, and vitamins, and is used as a protective coating for fruits [3]. This gel, by forming a protective layer against oxygen and moisture, prevents microbial spoilage [5]. In addition, lipid-based compounds such as beeswax are used in fruit coatings due to their delaying effect. These coatings extend the shelf life of fruits by reducing gas exchange and transpiration [6]. Beeswax contains compounds such as flavonoids and terpenoids, which have antimicrobial and antioxidant properties. A study involving strawberries treated with a beeswax nanoparticle coating for 21 days showed that the coating reduced respiration, maintained tissue firmness, preserved color, and decreased microbial activity [7]. Additionally, beeswax coatings at 1% and 1.5% concentrations applied to mangoes extended their shelf life up to two months [8]. In another study, a 60% aloe vera gel coating combined with Shirazi thyme essential oil was applied to Golab apples. The results showed that this treatment reduced weight loss and ripening index by 3.8% and 31.4%, respectively, compared to the control. Additionally, the coating improved the antioxidant capacity and tissue firmness of the fruit [9].
Edible nanoemulsion coatings are effective in preserving fruit quality due to their reduced particle size and enhanced efficiency. These coatings, owing to their antimicrobial and antioxidant properties, contribute to extending the shelf life of fruits [10, 11]. Other studies have examined the effects of plant essential oils such as thyme, garlic, and cumin on various fruits. For example, Shirazi thyme essential oil at a concentration of 400 µL/L was more effective on mangoes [12], while garlic essential oil at a concentration of 0.1% showed stronger antifungal activity on bananas [13]. In addition, lavender essential oil was found to prevent browning in bananas [14].
Ultimately, edible coatings play a key role in preserving fruit quality after harvest by controlling gas exchange, reducing transpiration, and preventing physical damage [15]. These methods not only extend the shelf life of products but are also more environmentally friendly.
This study aimed to investigate the effect of essential oil and nanoemulsion of the medicinal plant clove, aloe vera gel, beeswax, and salicylic acid on the quality traits, shelf-life extension, and freshness preservation of strawberries during different storage periods.

2- Materials and Methods

This study was conducted during the fall of 2023 at the Razi Laboratory Complex of the Islamic Azad University, Science and Research Branch, Tehran, over a three-month period. The samples used were strawberry fruits cv. Albion, which were obtained on the first day of harvest from a commercial strawberry production greenhouse located in Damavand. The fruits were immediately transferred to covered plastic containers and transported to the laboratory (the location where the experiments were conducted). The treatment materials included beeswax (sourced from a herbal store in Tehran), aloe vera gel (sourced from an aloe vera plant in the greenhouse of the Islamic Azad University, Science and Research Branch), salicylic acid (from the commercial brand Merck, Germany), nanoemulsion, and clove essential oil. The treatments included:

1. Control (no coating),
2. Aloe vera gel,
3. Beeswax,
4. 30% aloe vera gel + 10% beeswax,
5. 30% aloe vera gel + 10% beeswax + 1‰ clove nanoemulsion,
6. 30% aloe vera gel + 10% beeswax + 2‰ clove nanoemulsion,
7. 30% aloe vera gel + 10% beeswax + 1% clove essential oil,
8. 30% aloe vera gel + 10% beeswax + 2% clove essential oil,
9. 30% aloe vera gel + 10% beeswax + 1 mM salicylic acid.

Preparation of Treatments

Beeswax was placed in a 2-liter container and melted at 70°C, then continuously heated until it reached a temperature of 80-90°C. Oleic acid (12.8 ml) was added to the melted beeswax, followed by the addition of 25.6 ml of triethanolamine (TEA). With continuous stirring, distilled water (previously heated to the same temperature of 80-90°C) was slowly added for 5 min and then allowed to dry in the air [16]. The resulting emulsion was cooled before use and stored in a closed container at room temperature. To prepare a 10% concentration of beeswax, its alcoholic (methanolic) extract was first prepared. For this, beeswax was mixed with 80% methanol in a 1:10 ratio and shaken at 150,000 rpm for 24 h. The resulting extract was then filtered using filter paper. After the extract was dried, the resulting extract powder was obtained. This powder was dissolved in 80% methanol at a 1:10 ratio and used as the stock solution. To prepare a 10% beeswax solution, the desired amount of the extract was diluted to a final volume of 100 ml and used for treatment by spraying the solution onto the fruit surface [17]. Clove essential oil was prepared using 25 g of dried clove flowers through water distillation with a Clevenger apparatus (Chemi Azma Gostar, Iran) for 3 h. The obtained essential oil was dehydrated using sodium sulfate and stored in a dark glass bottle at 4°C in the refrigerator until use [18].

To prepare the clove essential oil nanoemulsion, Tween 80 and Span 80 were used. The nanoemulsion contained 2% (W) surfactant, 96% (W) water, and 2% (W) essential oil. First, Tween, Span, and essential oil were weighed separately and placed in separate glass vials. Then, water was added to the Tween, Span, and essential oil mixture. Afterward, the essential oil and Span mixture was added to the water and Tween, and the resulting mixture was stirred for one min. It was then subjected to ultrasonic waves for 15 min using a laboratory sonicator (model UP200HT, Hielscher, Germany) with a power of 400 W and a frequency of 20.5 kHz. A titanium cylindrical sonotrode with a diameter of 8 mm, which is submerged up to a depth of 1 cm below the liquid surface, was used to transfer the ultrasonic waves from the transducer to the sample [19]. After separating the epidermis from the upper leaf of the Aloe vera plant, the gel was extracted using an electric stirrer. The Aloe vera gel edible coating was prepared by diluting it to a 30% concentration with distilled water at a 30% (W) ratio.

2-1- Experimental Procedure

The strawberries were washed by immersion in distilled water and then placed in room air to dry. After that, the strawberry fruits were coated by immersing them for 3 min in each treatment, including essential oil, nanoemulsion, or salicylic acid. Beeswax was sprayed onto the fruits. The fruits were then air-dried at room temperature and placed in polyethylene packages for storage and evaluation of the studied traits. They were stored at 4 °C with a relative humidity of 75 ± 5%. Each replicate contained 250 g of strawberries (15 fresh fruits). Evaluations of various traits were conducted on days 0, 5, 10, and 15.

2-2- Studied Traits

Fruit Weight Loss Percentage: The fruits were weighed before and after packaging at the end of each treatment period using a digital scale with an accuracy of 0.001 g, and the percentage of fruit weight loss was calculated using the following formula [20].

Weight Loss (%) = [(Initial Weight − Final Weight) / Initial Weight] × 100

Titratable Acidity: Five milliliters of fruit juice extract were mixed with 45 ml of distilled water and titrated with 0.1 N sodium hydroxide until the pH reached approximately 8.2. The results were expressed as a percentage of citric acid [20].

Soluble Solids Content: The fruits were juiced to measure this parameter, and the soluble solids content was determined using an ATG digital refractometer. The results were expressed in degrees Brix (°Brix) [20].

Fruit Firmness: A 1 cm slice was taken from the fruit at three different points using a knife, and the firmness of the fruit flesh was measured using a penetrometer (manual type). The results were recorded in kilograms per square centimeter (kg/cm^2).

Antioxidant Capacity: The free radical DPPH (2,2-Diphenyl-Picryl-Hydrazyl) was used to determine antioxidant capacity. First, plant extracts were prepared in various concentrations ranging from 5×10^{-2} mg/100 to 5×10^{-6} mg/100 in pure methanol. Then, a 1:1 mixture of DPPH solution (8 mg/100 ml) and the plant extracts at different concentrations was prepared. After 30 min at room temperature, the absorbance of the samples was measured at 517 nm using a Visible/UV-45 Lambda spectrophotometer. The percentage of DPPH radical scavenging activity of the samples was calculated using the following formula [21].

$R\%=AD–AS/AD\times100$

R%: Radical Scavenging Percentage, AD: Absorbance of DPPH at 517 nm, AS: Absorbance of the samples at 517 nm

To compare the activity of the extracts, the IC_{50} parameter was used (IC_{50} is the concentration of the extract that inhibits 50% of free radicals).

Fruit Anthocyanin: 10 g of fruit were ground using a methanol and 1 N hydrochloric acid extraction solution (acidic methanol) to extract the fruit anthocyanin. The resulting samples were placed in test tubes and incubated for 24 h at 4°C. Then, the samples were centrifuged at 5000 rpm for 5 min. Finally, the extracted sample was analyzed using a Visible/UV-45 Lambda spectrophotometer at wavelengths of 530 and 657 nm. The anthocyanin content in the fruits was calculated using the following formula and expressed in milligrams per gram of fresh tissue [22].

Fruit Anthocyanin = $A_{530} – 1.4\ A_{657}$

A: Absorbance

Ascorbic Acid (Vitamin C): The ascorbic acid content of strawberry fruit was measured using a two-step oxidation-reduction titration method. First, 5 g of fruit were extracted in a methanol solution using a Soxhlet extractor for 4 h. The resulting extract was filtered through filter paper and then concentrated using a rotary evaporator at 40°C. Fifty milligrams of the obtained extract were extracted with 50 ml of 1% metaphosphoric acid for 45 min. The extract was then filtered through filter paper (No. 4), and one milliliter of the filtered extract was mixed with 9 ml of the prepared solution. After 30 min, the absorbance of the samples was read at a wavelength of 530 nm using a Visible/UV-45 Lambda spectrophotometer. The standard curve was plotted using different concentrations of ascorbic acid, and the ascorbic acid concentration of the samples was expressed in milligrams of ascorbic acid per 100 g of sample weight [23].

Fruit taste index (TSS/TA): The fruit taste index was expressed as the ratio of total soluble solids to titratable acidity [24].

Fruit juice acidity (pH): First, two strawberries were sliced, and the fruit juice was extracted using a juice extractor. To clarify and clear the juice, a portion of it was placed in test tubes designed for the centrifuge and centrifuged for 5 min. After this process, a clear fruit juice solution was obtained. To measure the pH, the pH meter was first calibrated using buffer solutions of pH 4 and 7. Then, a portion of the filtered fruit extract was poured into a small beaker, the electrodes of the device were immersed in the beaker, and the pH of the extract was read and recorded [20].

Determination of Total Phenolic Content: After methanolic extraction of the strawberry fruit, the resulting extracts were filtered and concentrated using a rotary evaporator at 40 °C. The total phenolic compounds in the fruit extract were then measured colorimetrically using the Folin–Ciocalteu method [25]. A 0.5 ml aliquot of the extract was mixed thoroughly with 5 ml of Folin–Ciocalteu reagent (diluted tenfold with distilled water) [26] and 4 ml of 1 M sodium carbonate solution. The mixture was kept at room temperature for 15 min. The absorbance of the solution was measured at 765 nm using a Visible/UV-45 Lambda spectrophotometer [27]. For this purpose, the colorimetric method (Folin–Ciocalteu) was also applied to standard solutions of gallic acid at different concentrations. A standard curve was plotted based on the absorbance of gallic acid ($Y = 0.00114X + 0.01062$, where Y is the absorbance and X is the concentration in ppm). To determine the phenolic content of the samples, the absorbance values obtained from the spectrophotometer were substituted into the standard curve equation (Y), and the concentration of phenolic compounds in the samples was calculated in ppm (X). Finally, the total phenolic content of the extract was expressed as milligrams of gallic acid per gram of extract using the standard curve.

Polyphenol oxidase (PPO): To measure the activity of the PPO enzyme, 1.5 ml of phosphate buffer was added to 0.5 ml of fruit extract, and the mixture was centrifuged at 4°C. Then, 100 µl of the clarified supernatant was mixed with 2.5 ml of 100 mM phosphate buffer (pH = 6.4) and 50 mM catechol. The absorbance change curve was measured using a Visible/UV-45 Lambda spectrophotometer at a wavelength of 420 nm for a duration of 3 min [28].

PPO Activity = (ΔAbsorbance × 1000) / (0.001 × 25)

Shelf life determination: The number of days after harvest during which no signs of decay or softening (observed visually throughout the study) were detected on the strawberry fruit was recorded.

Statistical analysis: After data distribution and homogeneity of experimental error variances were checked, analysis of variance (ANOVA) was performed using a completely randomized design with SAS statistical software version 9.4. The means of the studied traits were then compared using Duncan's multiple range test at a 5% significance level. The graphs and statistical tables were created using Excel and Word Office 2007 software.

3- Results and Discussion

Weight loss of fruit: Based on the results of the analysis of variance, the weight loss of strawberry fruit was significantly affected by the main effect of edible coating and storage time at the 1% significance level. However, the interaction between these two treatments on the weight loss of strawberry fruit was not significant (Table 1).

Table 1 Analysis of variance (ANOVA) results for the effects of edible coating treatment and storage time on weight loss, titratable acidity, soluble solids content, and fruit firmness of strawberry

Source of variation	df	Weight loss	Titratable acidity	Soluble solids	Fruit firmness
Edible coating	8	20.46**	0.02**	2.33**	0.19**
Storage time	3	970.09**	0.44**	18.41**	69.77**
Coating × Time	24	2.56ns	0.003ns	0.42**	0.28**
Error	72	2.96	0.002	0.02	0.05
CV (%)	-	24.90	7.54	1.98	3.90

** and ns indicate significant differences at 1% probability level and non-significant differences, respectively

Based on the results of the mean comparison for the main effect of edible coating, the highest weight loss of fruit (9.10%) occurred under the control treatment (without coating), while the lowest weight loss (5.19%) was observed under the treatment with 30% aloe vera gel + 10% beeswax + 1 mM salicylic acid. This combination was identified as the most effective treatment for extending the shelf life of strawberries (Figure 1).

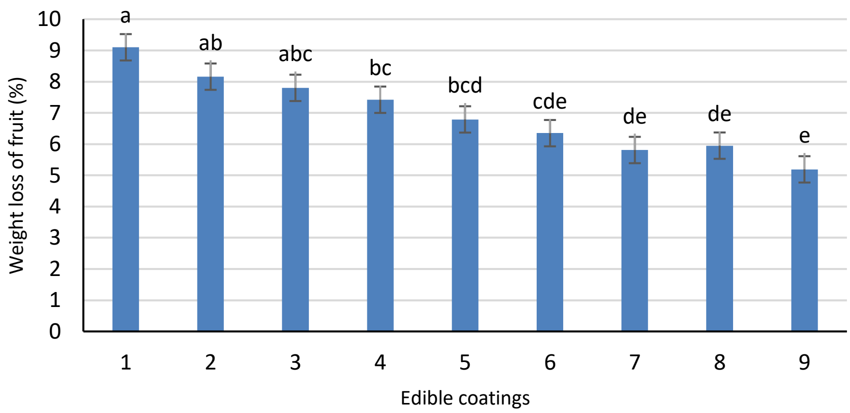

Fig 1 Comparison of the mean main effect of edible coating treatments on weight loss percentage of strawberry fruit; (1) control (uncoated), (2) Aloe vera gel, (3) Beeswax, (4) Aloe vera gel combined with beeswax, (5) Aloe vera gel (30%) + beeswax (10%) + clove nanoemulsion (1‰), (6) Aloe vera gel (30%) + beeswax (10%) + clove nanoemulsion (2‰), (7) Aloe vera gel (30%) + beeswax (10%) +

clove essential oil (1%), (8) Aloe vera gel (30%) + beeswax (10%) + clove essential oil (2%), and (9) Aloe vera gel (30%) + beeswax (10%) + salicylic acid (1 mM). The results demonstrate the varying efficacy of these coatings in reducing postharvest weight loss in strawberries

The highest weight loss of strawberry fruit (14.20%) was recorded on the 15th day of storage, while the lowest weight loss (0.30%) occurred on the first day of storage. As the storage period increased, the moisture loss of the fruit—and consequently its weight loss—also increased (Figure 2).

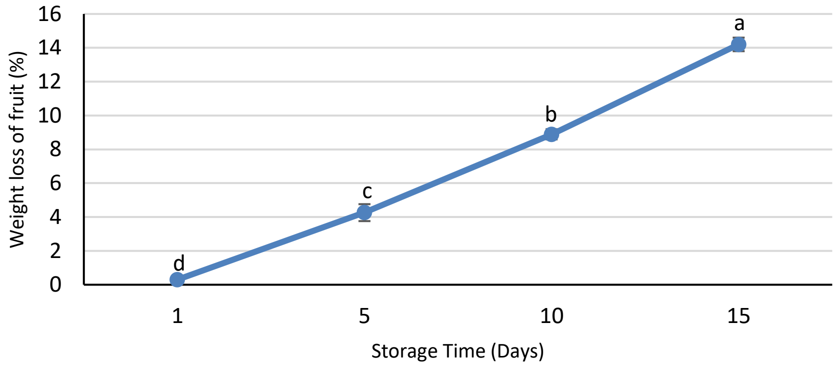

Fig 2 Comparison of the mean main effect of storage duration on weight loss percentage of strawberry fruit

An increase in respiration rate accelerates the ripening and senescence processes in fruits, leading to a higher consumption of internal nutrients and, consequently, weight loss [29]. This weight loss, along with the decline in internal fruit quality, is considered one of the main limiting factors for shelf life and is also economically significant. The use of edible coatings containing natural compounds such as plant extracts and essential oils has been introduced as an effective strategy for controlling postharvest decay and delaying senescence in plant tissues [30].

One of the main causes of fruit weight loss during storage is increased evaporation and transpiration from the fruit surface. Edible coatings help maintain fruit firmness and tissue integrity by reducing the activity of cell wall-degrading enzymes, thereby limiting gas exchange [31]. These coatings—such as wax and plant essential oils—form a protective layer between the fruit and the external environment, preventing dehydration and reducing moisture loss [6].

Titratable Acidity: The titratable acidity of strawberry fruit showed a significant difference under the main effects of edible coating and storage time at the 1% probability level. However, the interaction between the treatments did not show a significant effect on the titratable acidity of the strawberry (Table 1). Based on the results of the mean comparison for the main effect of edible coating, the highest titratable acidity (0.70%) was observed in the treatment of Aloe vera gel 30% + beeswax 10% + salicylic acid 1 mM, while the lowest (0.59%) was found in the control treatment (no edible coating) (Figure 3).

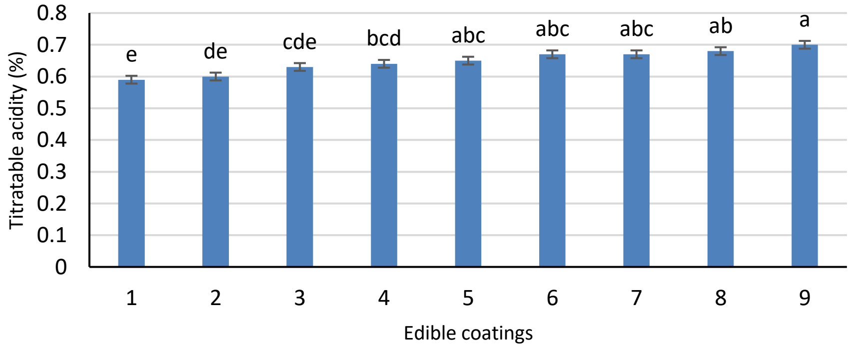

Fig 3 Comparison of the mean main effect of edible coating treatments on the titratable acidity of strawberry fruit; (1) control (uncoated), (2) Aloe vera gel, (3) Beeswax, (4) Aloe vera gel combined

with beeswax, (5) Aloe vera gel (30%) + beeswax (10%) + clove nanoemulsion (1‰), (6) Aloe vera gel (30%) + beeswax (10%) + clove nanoemulsion (2‰), (7) Aloe vera gel (30%) + beeswax (10%) + clove essential oil (1%), (8) Aloe vera gel (30%) + beeswax (10%) + clove essential oil (2%), and (9) Aloe vera gel (30%) + beeswax (10%) + salicylic acid (1 mM). The results demonstrate the varying efficacy of these coatings in reducing postharvest weight loss in strawberries

Based on the results of the mean comparison, the highest titratable acidity of strawberry fruit (0.80%) was recorded on the first day of storage, while the lowest titratable acidity (0.49%) was recorded on the 15th day of storage (Figure 4).

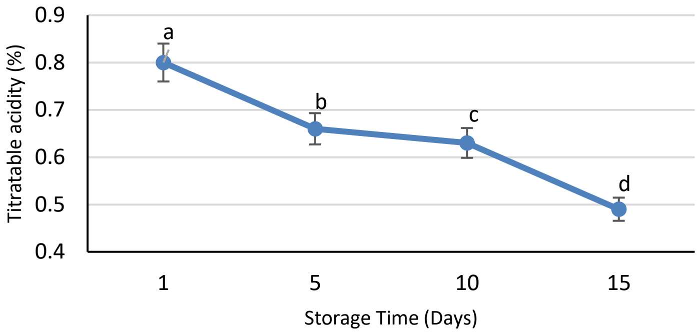

Fig 4 Comparison of the mean main effect of storage duration on the titratable acidity of strawberry fruit

Titratable acidity (TA) is directly related to the concentration of organic acids within the fruit. During respiration and metabolic changes, organic acids are converted to sugars, resulting in a decrease in TA over the storage period [3]. Studies on strawberries have also shown that acidity decreases during the storage period, but the use of edible coatings can slow down this process [32]. Organic acids are consumed as substrates in respiration, so it is natural for acidity to decrease and pH to increase during the post-harvest period [33]. Any factor that slows down the metabolism and senescence of the fruit can prevent the rapid decline of TA. Since organic acids decrease as the fruit ripens, their quantification can serve as an indicator for determining the degree of ripeness and the quality of the fruit [34].

Edible coatings such as wax, resin, or polymeric compounds form a protective layer that prevents moisture loss and the penetration of oxygen and microorganisms. By limiting oxygen availability, these coatings reduce the breakdown of organic acids and thus help maintain higher levels of titratable acidity in the fruit [35]. Fruit acidity, which is directly related to the concentration of dominant organic acids, is considered one of the key parameters in preserving the quality of horticultural products.

Soluble Solids Content: Based on the results of variance analysis, the soluble solids content of strawberry fruit was significantly affected (at the 1% probability level) by the main effects of edible coating and storage time, as well as their interaction (Table 1).

Based on the results of mean comparison for the interaction between edible coating treatments and storage time, the highest soluble solids content in strawberry fruit was observed in the treatments without edible coating (control) and with Aloe vera gel on the 15th day of storage. In contrast, the lowest soluble solids content was recorded in the control treatment on the first day of storage and in the aloe vera gel treatment on the 15th day (Figure 5).

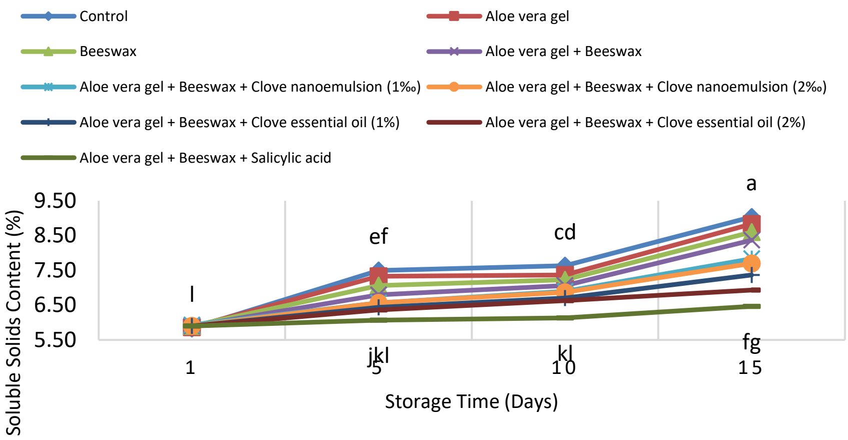

Fig 5 Comparison of the mean interaction effects of edible coating × storage time (days) on soluble solids content of strawberry fruit

Studies have shown that the total soluble solids (TSS) content in fruits tends to increase during storage. This increase is primarily attributed to the breakdown of carbohydrates and pectic substances, the hydrolysis of proteins, and the cleavage of glycosides into smaller units as part of the respiration process [2]. Typically, the highest TSS values are observed toward the end of the storage period, particularly in fruits treated with higher concentrations of edible coatings.

Edible coatings, by forming a thin protective layer on the fruit surface, help reduce the respiration rate and prevent the rapid breakdown of carbohydrates. This contributes to the relative preservation of total soluble solids (TSS) compared to uncoated fruits [36]. Moreover, the increase in TSS during the later stages of storage may result from the degradation of cell wall components into simple sugars, which is indicative of fruit tissue senescence and deterioration [1, 31].

In addition to reducing moisture loss, edible coatings also serve as a barrier against environmental factors such as oxygen, light, and heat [37]. However, under conditions like high storage humidity, fruits may absorb additional moisture, potentially affecting the concentration of total soluble solids (TSS) [38]. Overall, by regulating gas exchange and slowing metabolic rates, edible coatings play a crucial role in maintaining fruit quality and preventing undesirable increases in TSS [4].

Tissue Firmness: The results of variance analysis showed that the tissue firmness of strawberry fruit was significantly affected (at the 1% probability level) by the main effects of edible coating and storage time, as well as by their interaction (Table 1).

Based on the results of mean comparisons for the interaction effects between edible coating treatments and storage duration, the highest fruit firmness (indicating the best treatment) was recorded on the first day of storage with the application of aloe vera gel + beeswax, which fell into the same statistical group as all other treatments on day one. The lowest firmness (3.20 N) was observed in the untreated control on day 15 of storage. Prolonged storage led to a decline in fruit firmness; however, the use of edible coatings improved and helped preserve fruit firmness (Figure 6).

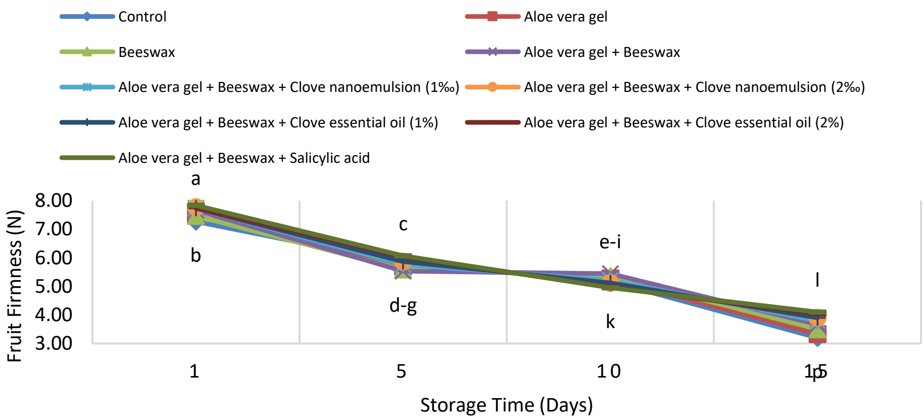

Fig 6 Comparison of the mean interaction effects of edible coating × storage time (days) on strawberry fruit firmness

Fruit firmness, as one of the most important quality indicators, plays a crucial role in marketability and consumer acceptance [39]. Studies have shown that edible coatings help maintain fruit quality and delay the softening process through various mechanisms, including reducing the activity of cell wall-degrading enzymes such as polygalacturonase and galactosidase [40], controlling gas exchange and reducing respiration rate [41], and preventing moisture loss [37]. These coatings, by forming a semi-permeable layer on the fruit surface, inhibit the degradation of structural cell wall polysaccharides such as hemicellulose and pectin, thereby slowing the conversion of insoluble protopectin into soluble pectin and pectic acid [7, 42]. The use of essential oil-derived compounds significantly preserved the firmness of strawberry fruit compared to the untreated controls [43]. Additionally, the phenolic compounds present in essential oils, due to their antioxidant and antimicrobial properties, not only help preserve tissue firmness but also prevent undesirable phenomena such as enzymatic browning [39, 41]. Although a gradual reduction in firmness is inevitable over time, the use of composite edible coatings with synergistic effects can significantly slow this process and extend the product's shelf life. This effect is particularly notable in highly perishable fruits like strawberries, which have a high metabolic rate, and can play a crucial role in reducing postharvest losses and maintaining fruit quality.

Antioxidant Capacity: Based on the results of the analysis of variance, the antioxidant capacity of strawberry fruit was significantly affected by the main effects of edible coating and storage duration, as well as by their interaction, at the 1% probability level (Table 2).

Table 2 Analysis of variance (ANOVA) results for the effects of edible coating treatment and storage time on antioxidant capacity, anthocyanin content, ascorbic acid, and TSS/TA ratio of strawberry fruit

Source of variation	df	Antioxidant capacity	Anthocyanin (mg/100g FW)	Ascorbic acid (mg/100g DW)	TSS/TA
Edible coating	8	352.62**	167.32**	287.93**	33.13**
Storage time	3	4655.68**	5159.48**	4031.07**	397.20**
Coating × Time	24	28.32**	13.04ns	31.16**	7.67**
Error	72	4.59	16.82	7.04	0.93
CV (%)	-	3.64	2.32	3.59	8.50

** and ns indicate significant differences at 1% probability level and non-significant differences, respectively

The highest antioxidant capacity of strawberry fruit (89.67%) was recorded in the treatment involving 30% aloe vera gel + 10% beeswax + 1 mM salicylic acid on the 15th day of storage (the most effective treatment), while the lowest antioxidant capacity (41.33%) was observed in the untreated control on the first day of storage (Figure 7).

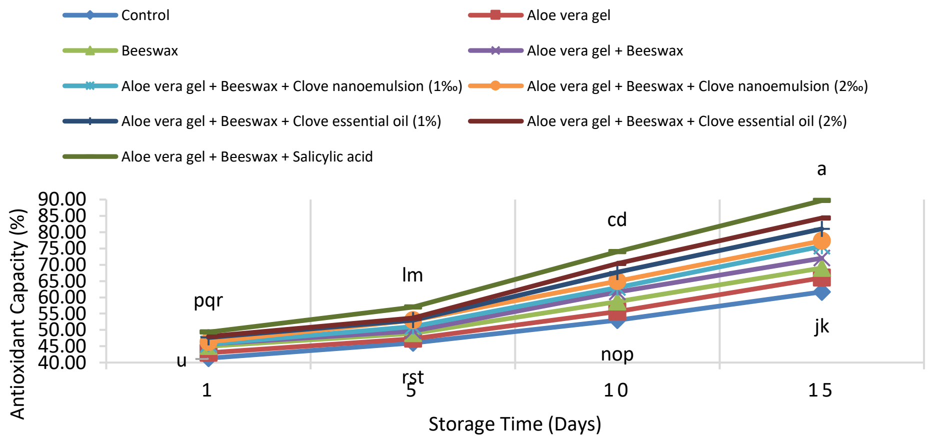

Fig 7 Comparison of the mean interaction effects of edible coating × storage time (days) on antioxidant capacity of strawberry fruit

The antioxidant system protects fruits against oxidative damage by detoxifying reactive oxygen species, thereby enhancing both the nutritional and visual quality of the product.

Studies have shown that edible coatings contribute significantly to postharvest quality maintenance of horticultural products through multiple mechanisms, including activation of plant cell defense systems, reduction of ethylene production, inhibition of degradative enzymes, and enhancement of antioxidant potential [44]. These findings are consistent with research conducted on various fruits [45], vegetables [46], carrots [47], and strawberries [48].

Edible coatings form a protective barrier on the fruit surface, preventing direct exposure of the product to harmful environmental factors such as oxygen, light, and moisture. This feature helps reduce oxidative damage and better preserve internal antioxidant compounds [48]. This is particularly important for strawberries, which are rich in phenolic compounds and anthocyanins that are highly susceptible to oxidation. The application of protective coatings can therefore play a significant role in maintaining antioxidant activity and the nutritional value of the fruit during storage.

Anthocyanin Content: Analysis of variance revealed that the anthocyanin content of strawberry fruit was significantly affected by the main effects of edible coating and storage duration at the 1% probability level. However, their interaction did not have a statistically significant effect on anthocyanin concentration (Table 2).

Based on the results of the mean comparison for the main effect of the edible coating, the highest anthocyanin content in strawberry fruit (183.00 mg per 100 g fresh weight) was obtained in the treatment of 30% Aloe vera gel + 10% beeswax + 1 mM salicylic acid, while the lowest anthocyanin content (171.08 mg per 100 g fresh weight) was observed in the control treatment without any coating. The use of edible coatings resulted in improved coloration of strawberry fruit. In strawberries, color is one of the key quality attributes that influences marketability, and with the application of edible coatings, the anthocyanin content and coloration of the fruit were enhanced (Figure 8).

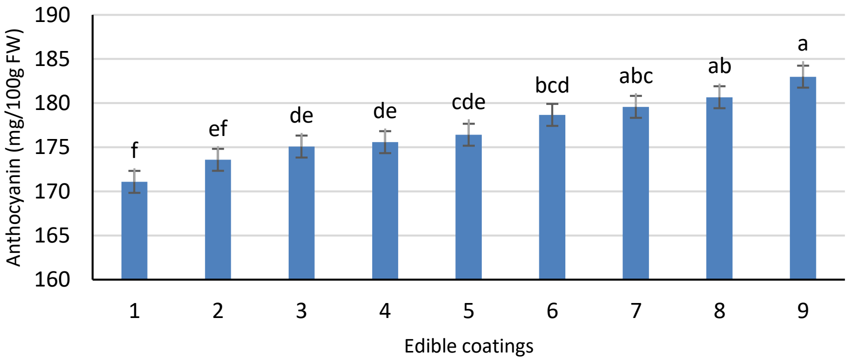

Fig 8 Comparison of the mean main effect of edible coating treatments on anthocyanin content of strawberry fruit; (1) control (uncoated), (2) Aloe vera gel, (3) beeswax, (4) Aloe vera gel combined with beeswax, (5) Aloe vera gel (30%) + beeswax (10%) + clove nanoemulsion (1‰), (6) Aloe vera gel (30%) + beeswax (10%) + clove nanoemulsion (2‰), (7) Aloe vera gel (30%) + beeswax (10%) + clove essential oil (1%), (8) Aloe vera gel (30%) + beeswax (10%) + clove essential oil (2%), and (9) Aloe vera gel (30%) + beeswax (10%) + salicylic acid (1 mM). The results demonstrate the varying efficacy of these coatings in reducing postharvest weight loss in strawberries

According to the results of the mean comparison for the main effect of storage duration, the highest anthocyanin content in strawberry fruit (193.00 mg per 100 g fresh weight) was observed on the first day of storage, while the lowest anthocyanin content (161.78 mg per 100 g fresh weight) was found on the 15th day of storage. Thus, with the increase in storage duration, the anthocyanin content of the fruit decreased (Figure 9).

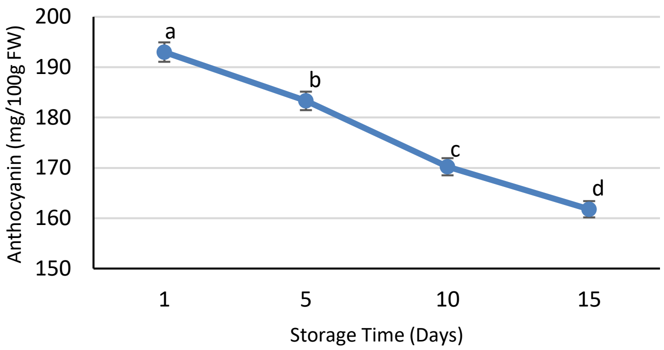

Fig 9 Comparison of the mean main effect of storage duration on anthocyanin content of strawberry fruit

Numerous studies have demonstrated that edible coatings play a significant role in preserving the bioactive compounds in fruits. For instance, coated pomegranate arils exhibited higher antioxidant activity and greater levels of phenolic compounds and anthocyanins compared to the uncoated control samples [49]. These findings are consistent with other research conducted on raspberries coated with lemon verbena essential oil and chitosan nanoemulsion. In these studies, it was observed that coating not only increased the levels of phenolic compounds and anthocyanins but also enhanced the activity of phenylalanine ammonia-lyase (PAL), an enzyme that plays a key role in the biosynthesis of phenolic compounds [42].

Edible coatings such as wax, plant essential oils, and aloe vera gel form a protective barrier against harmful environmental factors such as oxygen, moisture, light, and temperature, thereby preventing the degradation of antioxidant compounds and undesirable changes in the fruit [10]. By reducing the rate of oxidation and inhibiting free radical activity, these coatings create optimal conditions for the long-term storage of fruits [15].

Anthocyanins, as a major group of phytochemical compounds, are not only responsible for the color and flavor of fruits but also play a significant role in promoting human health due to their strong antioxidant properties. However, these compounds are sensitive to environmental factors such as oxygen, light, and moisture, which can lead to anthocyanin degradation and reduced fruit quality during storage [50]. Edible coatings help prevent the degradation of anthocyanins by mitigating the effects of harmful environmental factors. As a result, they not only preserve the sensory and nutritional attributes of the fruit but also enhance its antioxidant activity [42]. These findings highlight the significance of edible coatings as a natural and effective postharvest strategy for maintaining the quality and nutritional value of horticultural products.

Ascorbic Acid: The analysis of variance indicated that the ascorbic acid content of strawberry fruit was significantly affected by the main effects of edible coating and storage duration, as well as by their interaction, at the 1% probability level (Table 2).

Based on the results of mean comparison for the interaction effects of edible coating × storage duration, the highest ascorbic acid content in strawberry fruit (89.67 mg ascorbic acid per 100 g dry weight) was observed in the treatment with 30% Aloe vera gel + 10% beeswax + 1 mM salicylic acid on the first day of storage. The lowest ascorbic acid content was recorded in the treatments without edible coating (control) and with aloe vera gel on the fifteenth day of storage (Figure 10).

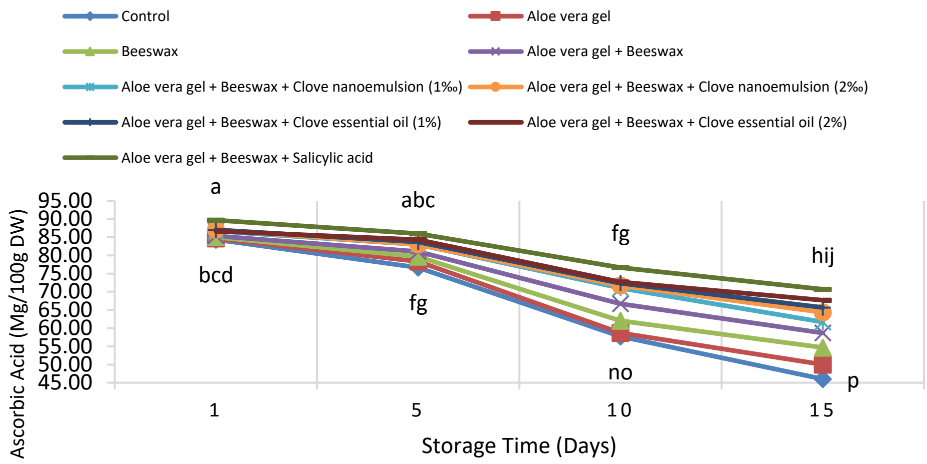

Fig 10 Comparison of the mean interaction effects of edible coating × storage time (days) on ascorbic acid content of strawberry fruit

Studies show that the vitamin C (ascorbic acid) content in fruits—especially strawberries—significantly decreases during the storage period, with the highest levels observed on the first day and the lowest at the end of storage. This decline is influenced by various factors, including the fruit cultivar, soil conditions, ripening stage, and climatic conditions. Temperature and storage duration are among the most critical factors affecting the retention of ascorbic acid [51]. These findings are consistent with results from studies on strawberries [48], apples [52], and pineapples [53; 54].

The use of edible coatings can help prevent the oxidation and degradation of this vitamin by forming a protective barrier against harmful environmental factors such as oxygen, light, and moisture [55]. These coatings, by limiting gas exchange and reducing oxygen permeability, not only protect ascorbic acid from degradation but also help maintain cellular integrity, creating optimal conditions for preserving this valuable compound [56]. Therefore, the combined use of edible coatings and precise control of storage conditions can serve as an effective strategy for retaining this essential nutrient in fruits.

Flavor Index (TSS/TA): Analysis of variance showed that the TSS/TA ratio of strawberry fruit was significantly affected by the main effects of edible coating and storage duration, as well as by their interaction, at the 1% probability level (Table 2).

Based on the mean comparison results of the interaction effects, the highest TSS/TA ratio of strawberry fruit was observed in all edible coating treatments on the 15th day of storage, while the lowest TSS/TA ratio (7.20) was recorded on the first day of storage (Figure 11).

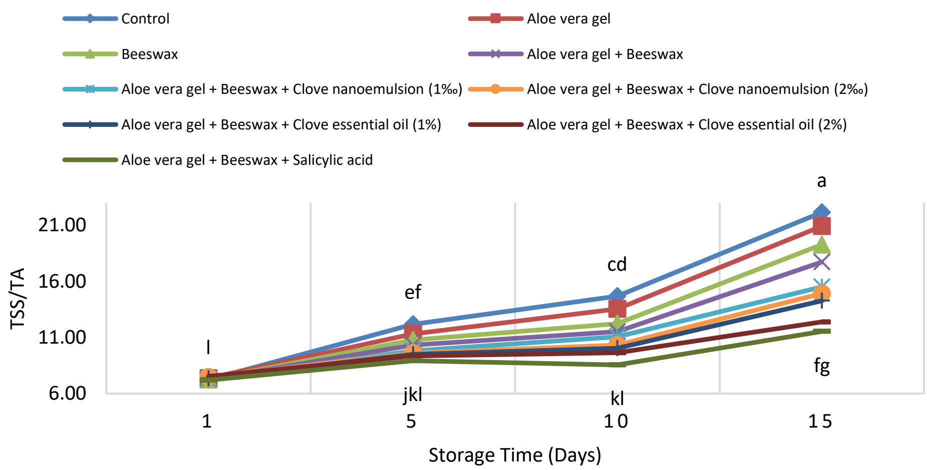

Fig 11 Comparison of the mean interaction effects of edible coating × storage time (days) on TSS/TA ratio of strawberry fruit

The total soluble solids (TSS) content is considered an important indicator of fruit maturity, typically increasing as the ripening process progresses. The TSS/TA ratio (total soluble solids to titratable acidity) is recognized as a key metric for evaluating fruit flavor quality, playing a crucial role in consumer acceptance [2]. In this study, the application of various edible coatings significantly improved the flavor index, which can be attributed to their ability to preserve sugar compounds and control the reduction of acidity. Edible coatings contribute to enhanced sensory quality by slowing down respiration and metabolism, preventing the breakdown of carbohydrates to retain sugars, minimizing the loss of organic acids (as respiration substrates), and creating favorable conditions for maintaining a balance between sugars and acids. These findings demonstrate that edible coatings can effectively preserve and enhance fruit flavor quality during storage by maintaining an optimal TSS/TA ratio.

Juice pH: Analysis of variance showed that the pH of strawberry juice was significantly affected by the main effects of edible coating and storage duration, as well as their interaction, at the 1% probability level (Table 3).

Table 3 Analysis of variance (ANOVA) results for the effects of edible coating treatment and storage time on juice acidity, total phenols, and polyphenol oxidase activity of strawberry fruit

Source of variation	df	pH	Total phenols (mg GAE/g DW)	Polyphenol oxidase (µmol/kg·s)
Edible coating	8	0.12**	238.99*	62998.15**
Storage time	3	0.31**	3367.45**	849714.51**
Coating × Time	24	0.02**	17.77**	9847.84**
Error	72	0.007	3.30	1326.85
CV (%)	-	13.13	3.70	3.55

** and ns indicate significant differences at 1% probability level and non-significant differences, respectively

Based on the results of the interaction mean comparison between edible coating treatments and storage duration, the highest pH of strawberry juice was observed in the control (no edible coating) on day one of storage, while the lowest pH (0.30) was found in the control (no edible coating) on day 15 of storage (Figure 12).

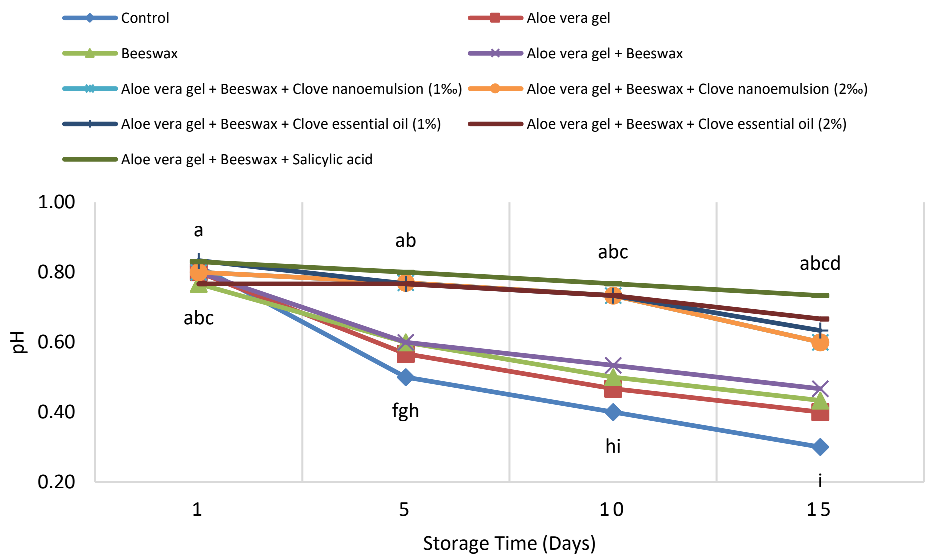

Fig 12 Comparison of the mean interaction effects of edible coating × storage time (days) on juice acidity of strawberry fruit

Studies have shown that during the storage period, the main respiratory substrates, including sugars and organic acids, gradually decrease, leading to significant changes in the pH of the fruits [57]. Over time, the pH of the fruits tends to increase, although the use of edible coatings at higher concentrations has been able to significantly prevent this increase [58]. It appears that these coatings, by delaying the synthesis of proteins and degradative enzymes, prevent the conversion of organic acids into sugars.

The mechanism behind these changes is that the gradual reduction of organic acids and sugars, which act as the main substrates for respiration, leads to fluctuations in pH levels [8]. This process varies across different fruits, with some fruits experiencing a noticeable increase in pH due to the reduction of acids, while in others, these changes are less pronounced [59]. In the case of strawberries, the observed changes in pH are primarily due to the breakdown of carbohydrates and pectic substances, hydrolysis of proteins, and the cleavage of glycosides into smaller building units during the respiration process [60].

Total Phenol Content of Fruit: According to the analysis of variance results, the total phenol content of strawberry fruit was significantly affected by the main effects of edible coating and storage duration, as well as their interaction, at the 1% probability level (Table 3).

Based on the results of the mean comparison of the interaction effects between edible coating treatments and storage duration, the highest total phenol content in strawberry fruit (74.67 mg gallic acid per gram of dry matter) was observed in the treatment with no edible coating (control) on the 15th day of storage. The lowest total phenol content in strawberry fruit (34.33 mg gallic acid per gram of dry matter) was found in the treatment with 30% aloe vera gel + 10% beeswax + 1 mM salicylic acid on the first day of storage (Figure 13).

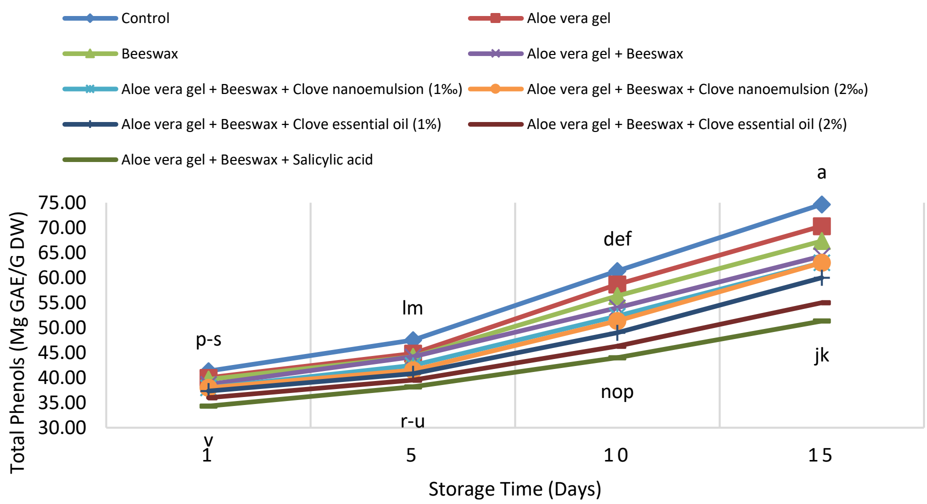

Fig 13 Comparison of the mean interaction effects of edible coating × storage time (days) on total phenolic content of strawberry fruit

PPO and phenylalanine ammonia-lyase (PAL) enzymes play a key role in the phenylpropanoid pathway, responsible for producing plant secondary metabolites with defensive properties [39; 61]. However, excessive activity of these enzymes can lead to the production of compounds such as quinones, which cause browning of the tissue and a decline in product quality [42]. In this study, edible coatings contributed to prolonged product quality by reducing PPO enzyme activity. It appears that these coatings help maintain the pH within an appropriate range—considering the high activity of PPO in alkaline conditions—thus preventing its excessive activation and consequently preserving phenolic compound levels at desirable levels [62].

Studies have shown that improper storage conditions can lead to fluctuations in the phenolic content of fruits. The increase in these compounds may result from deacetylation reactions triggered by stress or physiological damage [39]. In this context, edible coatings act as semi-permeable barriers that regulate gas exchange and, by reducing oxygen levels and increasing carbon dioxide concentration around the fruit, help prevent the excessive production of phenolic compounds [6]. This mechanism significantly contributes to maintaining both the visual and nutritional quality of the fruit during the storage period.

Polyphenol Oxidase (PPO): The analysis of variance showed that the PPO activity in strawberry fruit was significantly affected by the main effects of edible coating and storage duration, as well as by their interaction, at the 1% probability level (Table 3).

Based on the results of the mean comparison for the interaction between edible coating treatments and storage duration, the highest PPO activity in strawberry fruit was observed in the untreated control samples on the 10th and 15th days of storage. In contrast, the lowest PPO activity was recorded in the untreated control samples on the first day of storage (Figure 14).

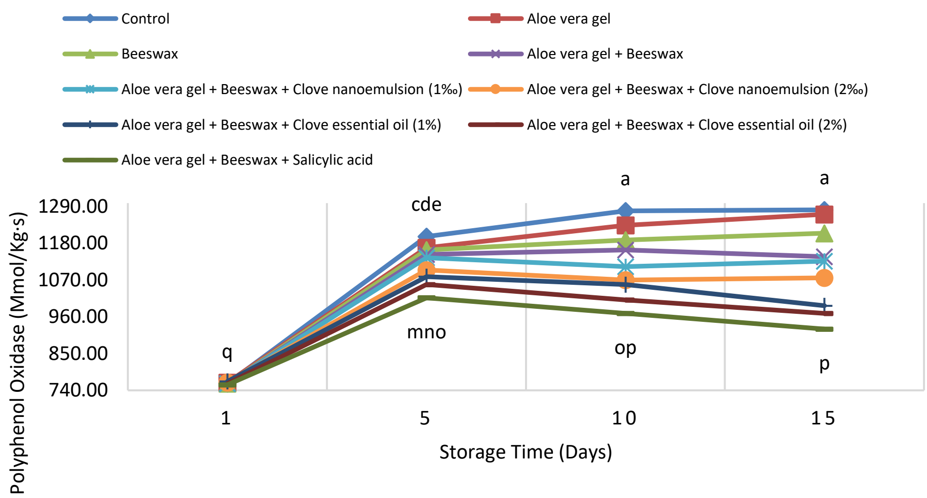

Fig 14 Comparison of the mean interaction effects of edible coating × storage time (days) on polyphenol oxidase activity of strawberry fruit

Peroxidase, with more than 1,000 distinct isoenzymes that differ structurally and functionally, exhibits varying responses to environmental factors and different treatments [40]. This isoenzyme diversity has led to variation in the peroxidase enzyme's response to the edible coating treatment in this study, depending on the enzyme source. Edible coatings, by creating a modified atmospheric environment through reducing available oxygen levels, slow down physiological processes and limit the access of PPO and peroxidase enzymes to their substrate (oxygen) [10; 11; 63]. This mechanism not only prevents the oxidation of phenolic compounds but also preserves these valuable compounds, thereby maintaining the nutritional quality of the fruit during storage.

In line with the findings of this study, previous research has also shown that edible coatings can effectively reduce the respiration rate of fruits and prevent the oxidation of phenolic compounds [48]. This protective effect results from the creation of a semi-permeable barrier that regulates gas exchange and provides optimal conditions for fruit storage. Therefore, it seems that edible coatings, due to their ability to create a modified atmospheric environment and control the activity of oxidative enzymes, can serve as an effective solution in post-harvest industries.

Shelf life: The results of variance analysis showed that the post-harvest shelf life of strawberry fruit was significantly affected by the main effect of edible coating at the 1% probability level (Table 4).

Table 4 Analysis of variance (ANOVA) of the effect of edible coating treatment on shelf life of strawberry fruit

Source of variation	df	Shelf life (days)
Edible coating	8	0.81**
Error	24	0.27
CV (%)	-	4.68

** indicates significant differences at 1% probability level.

According to the results of the mean comparison for the main effect of edible coating, the shortest post-harvest shelf life of strawberry fruit (an average of 7.75 days) was observed in the control treatment (no edible coating), while the longest shelf life (an average of 11.5 days) was achieved with the treatment of 30% Aloe vera gel + 10% beeswax + 1 mM salicylic acid (Figure 15).

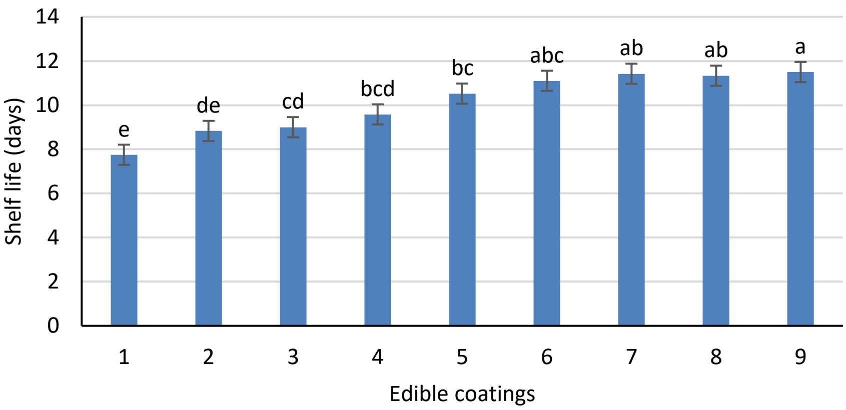

Fig 15 Comparison of the mean main effect of edible coating treatments on shelf life of strawberry fruit; (1) control (uncoated), (2) Aloe vera gel, (3) beeswax, (4) Aloe vera gel combined with beeswax, (5) Aloe vera gel (30%) + beeswax (10%) + clove nanoemulsion (1‰), (6) Aloe vera gel (30%) + beeswax (10%) + clove nanoemulsion (2‰), (7) Aloe vera gel (30%) + beeswax (10%) + clove essential oil (1%), (8) Aloe vera gel (30%) + beeswax (10%) + clove essential oil (2%), and (9) Aloe vera gel (30%) + beeswax (10%) + salicylic acid (1 mM). The results demonstrate the varying efficacy of these coatings in reducing postharvest weight loss in strawberries

Edible coatings containing minerals such as calcium chloride and potassium chloride inhibit enzymatic activity and reduce degradation processes through their interaction with fruit enzymes [63]. Among these, plant essential oils, such as clove oil, which contain active compounds like caryophyllene and eugenol, play a significant role in preventing agricultural product spoilage under storage conditions due to their strong antioxidant properties. These bioactive compounds not only extend the shelf life of products but also preserve their quality and nutritional value, meeting the growing market demand for fresh horticultural products.

4- Conclusion

The results of this study clearly showed that the application of combined edible coatings had a significant impact (at a 1% probability level) on preserving the quality and extending the shelf life of strawberry fruit. Analysis of variance of the data indicated that the main effect of edible coatings was significant for all the traits examined, including weight loss, pH, soluble solids content, fruit firmness, antioxidant capacity, anthocyanins, ascorbic acid, and shelf life. The combined treatment of 30% aloe vera gel + 10% beeswax + 1 mM salicylic acid was identified as the most effective treatment, reducing weight loss by 43% (from 9.10% to 5.19%), increasing titratable acidity by 18.6% (from 0.59% to 0.70%), improving anthocyanin content by 7% (from 171.08 to 183.00 mg per 100 g), and extending shelf life by 50% (from 7.75 days to 11.5 days). Additionally, this treatment significantly increased antioxidant capacity by up to 89.67% and better preserved the fruit's texture. Based on these results, it is recommended to use this coating combination as a natural, safe, and cost-effective method in post-harvest strawberry industries. This approach not only helps reduce post-harvest losses but also significantly improves the nutritional quality and marketability of the product. For optimal results, it is suggested that the application of this coating be evaluated in semi-industrial scale and real storage conditions.

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اثر موم زنبور عسل و ژل آلوئه‌ورا همراه با نانو امولسیون میخک (*Syzygium aromaticum*) و اسید سالیسیلیک بر عمر قفسه‌ای و خصوصیات کیفی میوه توت فرنگی (*Fragaria × ananassa*)

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چکیده

توت‌فرنگی به‌عنوان میوه‌ای با محبوبیت بالا و فسادپذیری سریع، نیازمند روش‌های ایمن و طبیعی برای افزایش ماندگاری پس از برداشت است. پژوهش حاضر به صورت فاکتوریل در قالب طرح کاملاً تصادفی با دو فاکتور پوشش‌های خوراکی و زمان مختلف انبارداری، در ۳ تکرار انجام گرفت. میوه‌ها پس از تیمار در داخل انبار تاریک با دمای ۱±۴ درجه سلسیوس و رطوبت نسبی ۹۰-۸۵ درصد که تهویه به صورت مرتب انجام می‌شد، نگهداری شدند. نتایج نشان داد میوه‌های تیمار نشده (شاهد) پس از ۱۵ روز نگهداری در انبار کمترین مقدار pH آب میوه را در مقایسه با سایر تیمارها داشتند، تیمار ترکیبی ژل آلوئه‌ورا ۳۰ درصد + موم زنبور عسل ۱۰ درصد + اسید سالیسیلیک ۱ میلی‌مولار مؤثرترین روش بود که با ایجاد لایه محافظ، از تخریب سلولی جلوگیری و کیفیت میوه را حفظ کرد. این تیمار میزان اسید آسکوربیک را نسبت به شاهد ۲ برابر افزایش داد و مواد جامد محلول را به ۹/۰۳ درصد در روز پانزدهم رساند. کمترین کاهش وزن (۹/۱ درصد) و کمترین تغییرات در اسیدهای قابل تیتراسیون (کاهش ۱۸/۶۴ درصدی) در روز پانزدهم، مربوط به این تیمار بود. همچنین تیمار حاوی نانوامولسیون میخک عمر انباری میوه را به‌طور چشمگیری افزایش داد. یافته‌ها نشان می‌دهد این ترکیبات طبیعی می‌توانند جایگزین مناسبی برای مواد شیمیایی در صنایع پس از برداشت باشند و برای میوه‌های حساس مانند توت‌فرنگی بسیار مناسب هستند.