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Full Paper

Green Tea Incorporated Edible Coating Extends the Postharvest Life of Strawberry Fruits (*Fragaria ananassa*)

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Abstract

In this study, the effects of green tea incorporated edible coating (EC) on the postharvest quality of strawberries were evaluated. EC was prepared by solubilizing carboxymethyl cellulose (0.75 % w/v), glycerol (2% v/v), and green tea concentrate (14% v/v) in distilled water at 80°C. Coated strawberries were packed in perforated polyethylene terephthalate boxes (10 fruits per box) and kept in refrigerated conditions (6±1°C). Uncoated strawberries were used as the control. Significant differences were observed in weight loss, visual quality, titratable acidity, and total soluble solids between the coated and uncoated fruits. The EC significantly reduced the total aerobic plate count and yeast and mold count of the stored fruits ($p<0.05$). EC exhibited decent in vitro inhibitory activity against *Botrytis cinerea*. The sensory properties of the coated fruits were comparable with fresh strawberries even on the 15th day of storage. In conclusion, green tea incorporated edible coating can be introduced as an effective coating material for the extension of the postharvest life of strawberry fruits.

Keywords: Extended shelf-life, fresh produce, physicochemical properties

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Introduction

Maintaining the quality of fresh produce is a huge challenge throughout the postharvest handling chain. Appearance or visual quality, physicochemical properties, nutritional value, and microbiological safety are the most important parameters which determine the quality of perishables like fruits and vegetables [1]. Deterioration of quality mainly takes place between the time of harvesting and the consumption of fresh produce. Postharvest practices such as handling, transportation, and storage need a considerable amount of time. Therefore, significant weight loss, nutrition loss, and loss of visual quality can occur when fresh produce reaches the consumer's table. Most of the time the damage cannot be immediately identified and this leads to high postharvest losses.

Strawberries are non-climacteric fruits that do not ripen after harvest. Therefore, the composition of strawberries changes before harvesting, and it is accepted that they should be harvested when it is fully matured and ready for consumption. At the same time, strawberry fruits are highly perishable due to their delicate nature with a very short postharvest life which means the fruits are at their best quality for a very short period [2]. Water loss and fungal decay are the most important factors which cause a reduction in the quality of the strawberries until they reach consumers [1]. Botrytis rot also called grey mold, caused by *Botrytis cinerea* is one of the major postharvest diseases of strawberry fruits. The disease mainly attacks

ripening fruits, but flower buds, petals, stems, green fruit, crowns, and seedlings also can be infected [1].
During the past few decades, various techniques have been developed to preserve the fruit quality and extend the shelf life of fresh produce such as low temperature and high relative humidity and controlled and modified atmosphere packaging [3]. An edible coating incorporated with natural antimicrobial agents is one of the innovative approaches to extending the shelf life of fresh produce. Edible coatings are a method used to extend fresh fruit's postharvest life. An edible coating is a suspension or emulsion of edible materials which is directly applied to the food surface and later becomes transformed into a film [4-6]. There are many advantages of using edible coatings compared to other techniques due to their moisture and gas barrier properties and ability to preserve and extend the shelf life of the fresh product [7]. It is evident that the postharvest quality of strawberries can be improved by coating techniques combined with refrigeration [8].

The incorporation of green tea into edible coatings as a natural antifungal agent possibly will improve the functionality of the coating solution. Green tea extract is comprised of different types of secondary metabolites which include catechin, caffeine, theanin, and saponin [9]. Besides contributing to numerous health benefits of green tea, these compounds are reported to have *in vitro* antibacterial [10], antifungal [11], and antiviral [12] activities. However, to the best of our knowledge, this is the first report in the existing literature on the evaluation of the postharvest quality of strawberry fruits treated with a green tea-incorporated edible coating. Taken together, the objective of the present study is to examine the effect of green tea-incorporated edible coating on the postharvest quality of strawberry fruits stored under low-temperature conditions. The postharvest quality of strawberries was evaluated based on the selected physicochemical properties of the fruits. In addition, the antifungal efficacy of green tea-incorporated edible coating solution was tested against *B. cinerea*.

Materials and Methods

Materials

Strawberry fruits (*Fragaria ananassa*) of variety *Chandler* were purchased from a commercial orchard in Nuwara Eliya, Sri Lanka. The fruits were selected for the absence of mechanical damage and diseases. The selected fruits were uniform in the stage of maturity, size, shape, and color. Then the fruits were field-packed in perforated transparent polyethylene terephthalate (PET) boxes and carefully transported to the laboratory using a rigifoam box within the shortest possible time. The temperature was maintained at 8±2°C by using gel ice.

All the ingredients used in the preparation of edible coating were of food grade and all the other chemicals used during physicochemical and microbial analysis were of standard analytical grade.

Preparation of Green Tea Concentrate

The dried green tea leaves were grounded in a commercial blender for 5 min. The ground leaves were then separated by using a stainless steel sieve of 100 μm mesh to get uniform particle size. Extraction conditions were fixed at 80°C for 30 min at a water-to-tea ratio of 20:1 (mg L−1) and extractions were carried out under magnetic stirring at 120 rpm. The extract was then centrifuged (Beckman CoulterTM centrifuge) at 8000 rpm for 20 min and the supernatant was filtered through a filter paper (Whatman, No.1).

Preparation of the Edible Coating (EC)

The edible coating solution was prepared by solubilizing carboxymethyl cellulose (CMC) powder (0.75 % w/v) in distilled water at 80°C. The mixture was stirred by using a magnetic stirrer to ensure uniform mixing and then glycerol (2% v/v) was added as a plasticizer. Finally, green tea concentrate (14% v/v) was added and the mixture was stirred for another 10 minutes under the same conditions [13].

Application of Edible Coating and Storage of the Treated Strawberries

Application of the formulated coating and storage of the coated and uncoated fruits were done according to a previously described method with slight modifications [14]. The selected strawberry fruits were sprayed homogeneously with formulated EC by using a hand-held sprayer and dried at room temperature for one hour. Then coated strawberry fruits were packed in perforated PET boxes (10 fruits per box) and kept in refrigerated condition (6±1°C) for up to 15 days. The fruits were analyzed for their physicochemical properties immediately after the treatment and at 3-day intervals during the storage period. Uncoated strawberry fruits were used as the control.

Determination of Percentage Weight Loss

Weight loss of strawberries was expressed as differences between the original weight and the weight recorded after 3-day intervals during the storage period. The original weight was measured right after applying the treatment using top loading digital balance (Apollo GX-A/GF-A, USA). The results were expressed as the percentage of weight loss on a fresh weight basis [15]. The percentage weight loss was calculated by using the equation 1:

$$\text{Weight loss (\%)} = \frac{m0-m}{m0} \times 100 \qquad \text{Equation (1)}$$

Where m0: weight of the sample before storage (g) and m: weight of the sample after storage intervals (g).

Determination of Visual Quality Rating (VQR)

The visual quality of the fruits was determined by using the previously described index and expressed as a visual quality rating (VQR). Accordingly, the visual quality of fruits was recorded at 3-day intervals during the storage period using a numerical scale (1-9) where 1=not edible and 9=excellent [16].

Determination of Physicochemical Properties (pH, Titratable Acidity, and Total Soluble Solids)

Selected physicochemical properties of the strawberry fruits were determined according to the previously described methods with slight modifications [17,18]. In brief, the fruits were homogenized into a pulp and filtered through a muslin cloth to get a strawberry puree. pH of the samples was determined using a previously calibrated (standard solutions: pH 4, 7, and 10) digital pH meter (Model - DPH-2, China). Titratable acidity (TA) was determined by titrating the fruit puree against 0.1N NaOH at the presence of Phenolphthalein indicator to an end-point and TA was expressed as a percentage of citric acid. Total soluble solids (TSS) were determined using a hand-held refractometer (RX- 5000, Atago Co., LTD., Japan) and results were expressed as percentage TSS (% TSS). All the determinations were carried out at room temperature and in triplicate for each of the parameters.

Microbiological Analysis

The microbiological analyses were carried out by standard plate counts. Total aerobic plate count was assessed according to ISO 4833-1 [19]. Briefly, 10±0.1 g of strawberry was weighed into a sterile blender jar and blended with 90 mL of peptone water at low speed (approximately 8000 rpm) for 2 minutes in order to get the 10^{-1} dilution. Then the appropriate serial dilutions were made and 1 mL of the sample was plated by following standard microbial pour plate and spread plate techniques. The yeasts and mold counts were obtained according to the procedures described by American Public Health Association (APHA) [20] with slight modifications. All the microbiological analyses were performed in triplicates. Microbial data were log transferred (log CFU g−1) before statistical analysis.

In Vitro Antifungal Activity of Edible Coating Solution Against Botrytis Cinerea

Antifungal activity of EC against *B. cinerea* was carried out by using the well diffusion method [21]. Spore suspension of *B. cinerea* was inoculated on Rose Bengal Chloramphenicol Agar plates. EC solution (20 µl) was then applied into 6 mm wells and plates were incubated for 48 hours at 28°C. Distilled water was used in the control. The results were expressed as the mean diameter (mm) of the inhibition zone.

Sensory Evaluation

The sensory quality of strawberries was evaluated by using a 9-point hedonic scale, where 9=like extremely and 1=dislike extremely. Thirty un-trained panelists (15 males and 15 females, ages 20–40 years) with no problem with taste, odour, and flavor ability were chosen. Five examined attributes for the descriptive rating test were color, appearance, texture, taste, and overall acceptability. The test was conducted on day15 of the treated fruits. Before conducting the sensory evaluation, strawberries were taken out from the refrigerator and kept for one hour to equilibrate to room temperature. Fruits were chosen uniformly in size and shape to minimize any bias caused by the presentation. Fresh strawberries were used to compare the tested sensory attributes with the coated fruits. The panelists arranged the individual booth and the test was performed under white light. Each panelist was provided 5 strawberry fruits and asked to evaluate them according to the given hedonic scale. Between evaluations, water was given to rinse the palate and a break in 30 s was enforced [1].

Statistical Analysis

All experiments in this study were conducted in triplicate. One-way analysis of variance (ANOVA) was utilized to evaluate any significant differences among the data from different groups with the aid of SPSS Statistics 21.0 (SPSS, IBM, NY). The differences were considered significant at $P<0.05$. Sensory data were analyzed by using the non -parametric test, Friedman test with a 95% confidence level (5% significance level) using Minitab 17.

Results and Discussion

Weight Loss

The percentage of weight loss of both coated and uncoated fruits were shown in Figure 1. According to the results, the weight loss of coated fruits was significantly lower than that of uncoated fruits throughout the storage period. At the end of the storage, untreated strawberries showed 29.63±1.3% loss in weight, whereas the weight loss of coated fruits was 10.01±1.2%. Thus, almost three times lower weight loss was observed in coated fruits compared to uncoated fruits.

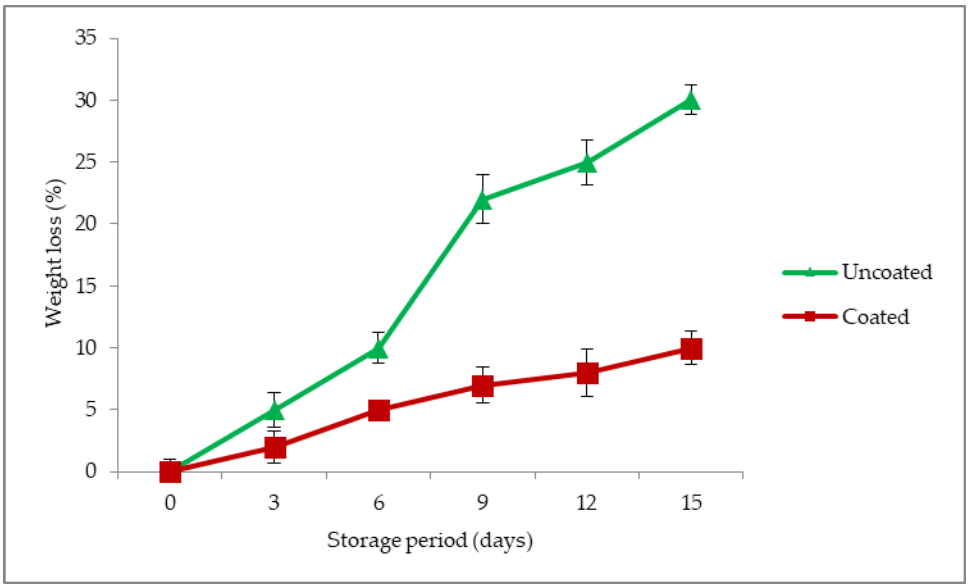


Figure 1. Effect of edible coating on weight loss of strawberries stored at 6±1°C for 15 days. The error bars represent standard deviations of triplicate assays with the confidence interval of 95%.

Weight loss of fruits is mainly occurred due to high moisture loss during the postharvest period. As fruit weight is one of the most important parameters, which determines the quality, this reduction results in huge economic loss. Since strawberries are highly perishable and delicate fruits with the thin outer skin, moisture evaporates more rapidly causing shrinkage and shriveling of the fruits. In addition, postharvest loss of strawberry fruits is further increased due to postharvest diseases. Generally, the rate of moisture evaporation depends on factors such as the water pressure gradient between the fruit surface and the external environment, storage temperature, and relative humidity. Edible coatings act as moisture barriers for coated fruits as they can cover the stomata on the fruit surface, which regulate fruit transpiration [22]. Also based on some previous findings, CMC-based coatings have exhibited barrier properties to oxygen, oil, and moisture transfer when applied to some fruits and vegetables [13,23]. According to the findings of the current study, green tea incorporated EC significantly reduced the percentage weight loss of strawberries. Thus, the results demonstrated the greater barrier properties of the tested EC against moisture loss during the storage period.

Visual Quality Rating (VQR)

As shown in Figure 2. VQR of both coated and uncoated fruits were comparable to each other up to 3 days of storage. However, VQR values of coated fruits were significantly higher beyond 3 days of storage compared to uncoated fruits. Coated fruits showed superior visual quality even on the 15th day of the storage period. The visual quality of fruits is one of the main external attributes of fruits and interferes with consumers' decisions to purchase. The reduction of VQR in uncoated strawberries after day 3 is mainly due to the fungal decay and shrinkage of fruits. Excessive moisture loss of uncoated fruits leads to shrinkage and further reduces the visual appeal of the fruits. Therefore, there is a direct relationship between moisture loss and VQR as well. On the other hand, the highest VQR values obtained by coated strawberries can correlate with the glossiness imparted by the coating, low moisture loss, low darkening of the fruits, and absence of fungal decay.

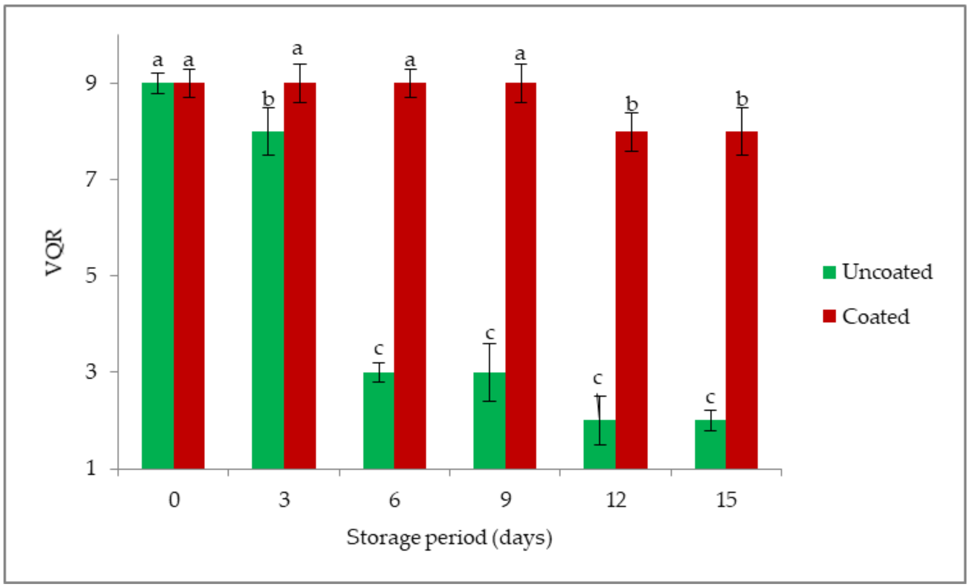


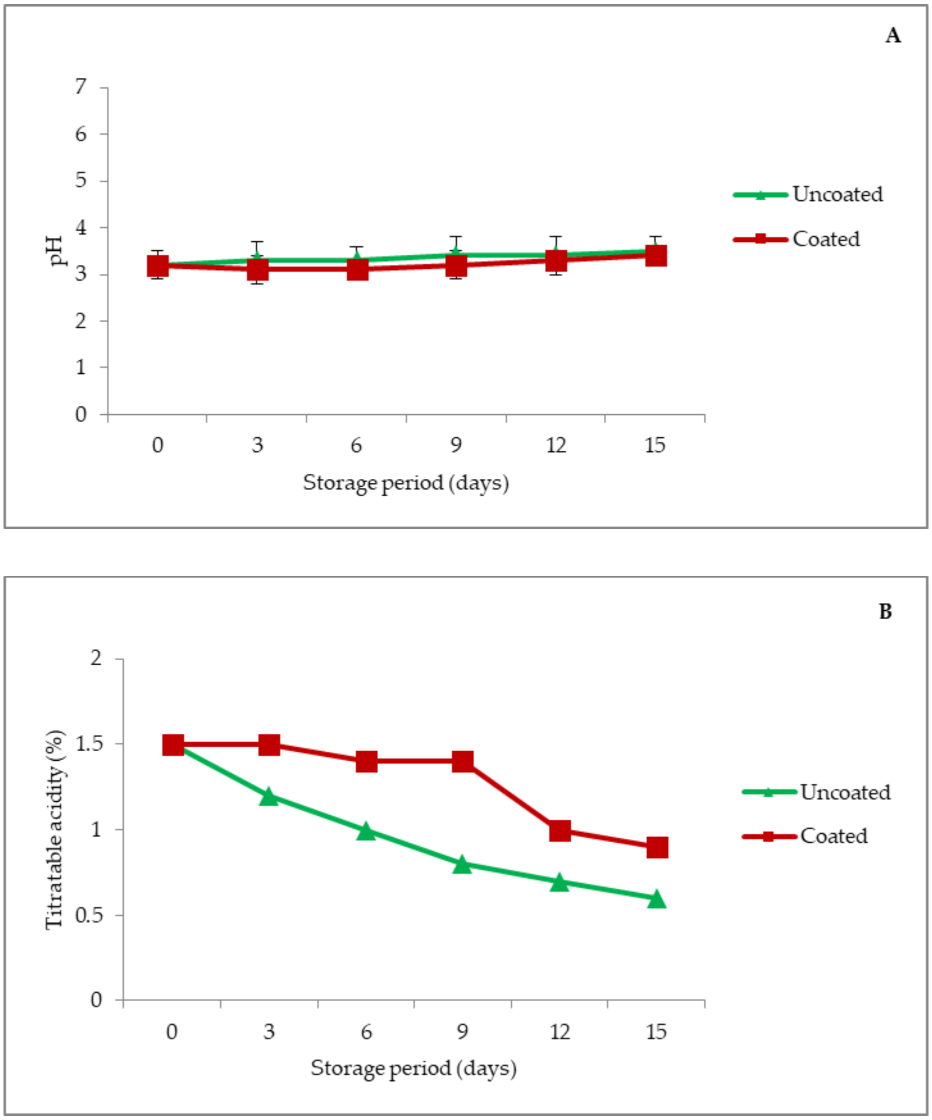
Figure 2. Effect of edible coating on visual quality rating (VQR) of strawberries stored at 6±1°C for 15 days. The error bars represent standard deviations of triplicate assays with the confidence interval of 95 %.

Physicochemical Properties

Results of the tested physicochemical properties i.e. pH, TA, and TSS of both coated and uncoated strawberries were shown in Figure 3. No significant differences were observed in the pH of coated and uncoated fruits throughout the storage period. The mean fruit pH varied from 3.2±0.2 to 3.4±0.1 in coated fruits while the mean fruit pH varied from 3.2±0.2 to 3.5±0.2 in uncoated fruits (Figure 3A).

In this study, 1.5±0.2% was the highest recorded TA for both coated and uncoated fruits which were observed at the begging of the storage period. However, a significant reduction of TA of both coated and uncoated strawberries was recorded towards the end of the storage period. Moreover, 0.9±0.1% and 0.6±0.1% were the lowest recorded TA values for coated and uncoated fruits respectively. In addition, slightly higher TA values were obtained for coated fruits when compared to uncoated fruits during the storage and coated strawberries were used to retain more acidity compared to uncoated fruits at the 15th day of storage (Figure 3B).

Significant differences were observed in TSS between coated and uncoated fruits during the storage (Figure 3C). According to the results obtained, significantly higher TSS were recorded in coated fruits compared to uncoated fruits. The TSS of the coated fruits ranges from 10.2±0.2% to 7.0±0.2% while the TSS of the uncoated fruits ranges from 7.0±0.2% to 3.2±0.4%.



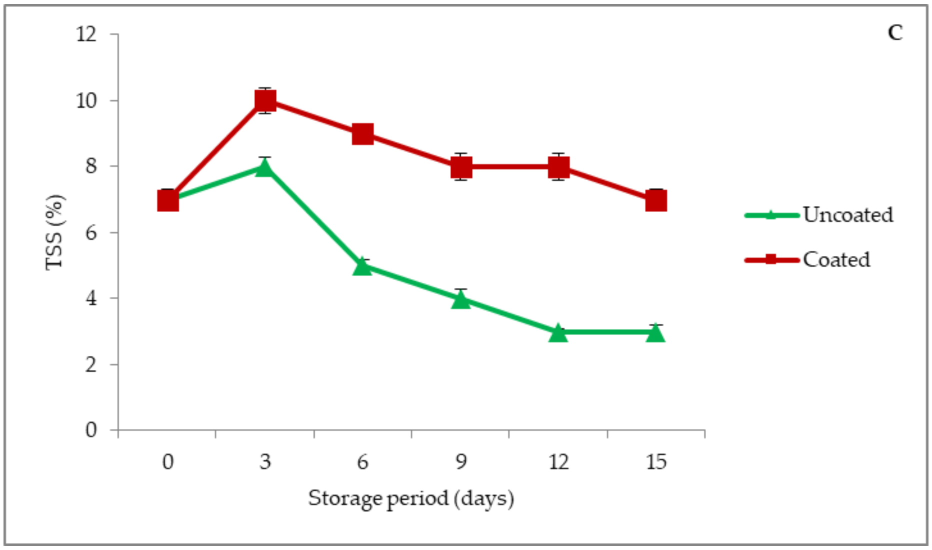


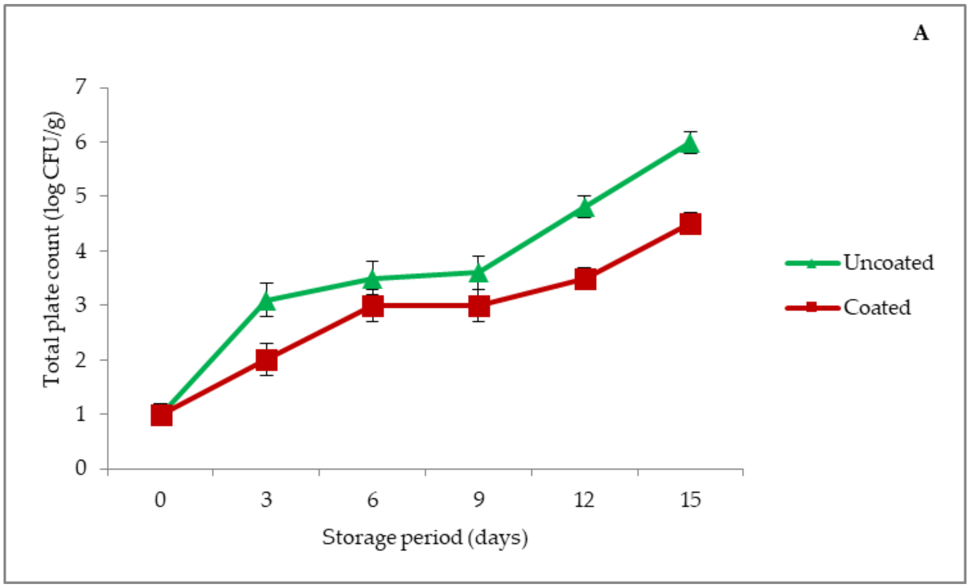
Figure 3. Effect of edible coating on (A) pH, (B) Titratable acidity (TA) and (C) Total soluble solids (TSS) of strawberries stored at 6±1°C for 15 days. The error bars represent standard deviations of triplicate assays with the confidence interval of 95%.

Usually, the amount of organic acids in fruits decreases with fruit ripening. They are utilized in respiration or converted into sugars [1]. In this study, TA was decreased along with the storage in both coated and uncoated strawberries. However, coated fruits retained more organic acids than uncoated fruits throughout the storage period. A similar finding was reported by Tanada-Palmu & Grasso [8] in strawberry fruits coated with gluten film.

The TSS of a fruit which mainly includes sugars and organic acids directly influences the taste and flavour of the fruit. Usually, in non-climacteric fruits like strawberries reduces their TSS content during the postharvest period. As these fruits have few or no energy sources (starch) after harvesting, the sugars present in the fruit act as an energy source for respiration. This process leads to the reduction of TSS content during the postharvest period. However, one of the previous studies reported an increase in TSS content of strawberries during storage that were covered with gluten–based and chitosan–based edible coatings respectively [8]. This may be due to the high water loss which leads to the elevation of soluble solid concentration. As reported by Shin et al [24], coating treatment reduces the loss of TSS compared to control as the coating act as a gas barrier and hence reduces the respiration rate of the commodity.

Microbiological Analysis

Total aerobic plate counts and yeast and mold counts of both coated and uncoated fruits were increased towards the end of the storage period (Figure 4A and B). That is expected and obvious according to the previous studies as well. El-Mogy et al [25] reported significantly reduced molds and *Escherichia coli* counts with the application of the edible coating in fresh artichoke bottoms. However, significantly lower counts were recorded in coated fruits compared to uncoated fruits ($p<0.05$).



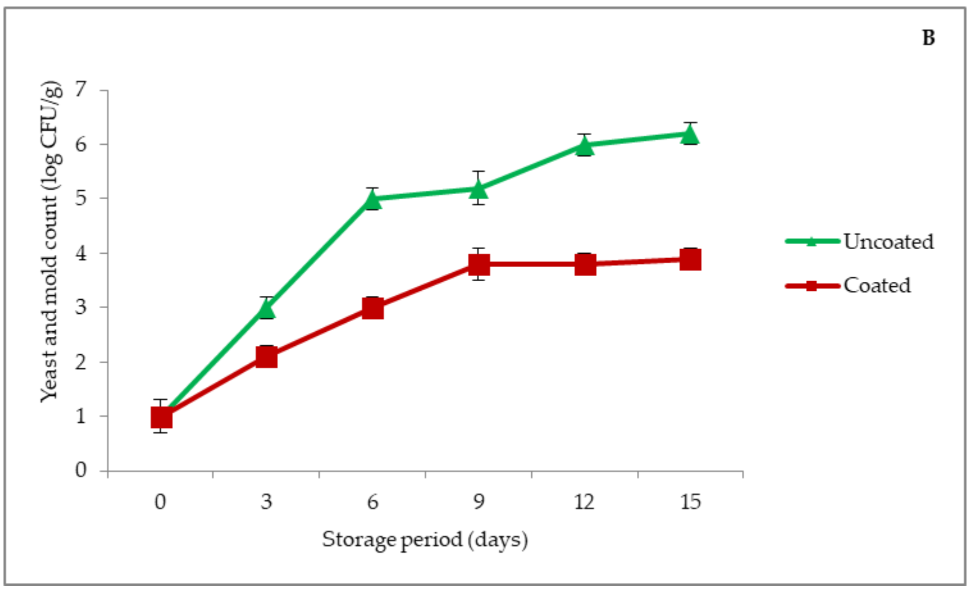


Figure 4. Effect of edible coating on (A) Total aerobic plate counts and (B) Yeast and mold counts of strawberries stored at 6±1°C for 15 days. The error bars represent standard deviations of triplicate assays with the confidence interval of 95%.

In Vitro Antifungal Activity Against Botrytis Cinerea

The antimicrobial activity of the coating solution against *B. cinerea* was tested using the good diffusion method. According to the results, the mean diameter of the inhibition zone was recorded as17±2 mm (Table 1).

Table 1. In vitro antifungal activity of edible coating solution against *Botrytis cinerea*

Treatment	Mean diameter of the inhibition zone (mm)
Edible coating solution	17±1.2
Control	0±0

Values are mean ± SD of three determinations

In this study, coated fruits showed significantly lower microbial load compared to uncoated fruits. This reduction may be mainly due to the inhibitory activity of green tea extract against microbes. Further, green tea incorporated edible coating solution showed good inhibitory activity against *B. cinerea*. Inhibition zones <5 mm are considered weak inhibitory activity and inhibition zones 5-10 mm and >10 mm are considered as moderate and good inhibitory activity respectively [26]. Therefore, with the findings of the present study, green tea incorporated edible coating solution exhibited good inhibitory activity against *B. cinerea*. Botrytis rot or grey mold caused by *B. cinerea* is the main postharvest disease that reduces the shelf life of strawberries [27]. Previous findings also suggested that tea polyphenols have a significant control effect against grey mold and that tea polyphenols are effective for the inhibition of spore germination and mycelium growth. [28,29]. The present work also proves that green tea concentrate which includes polyphenols and other important secondary metabolites has inhibitory activity against tested postharvest fungal disease. Further, a considerable number of previous studies provide supportive evidence for incorporating active ingredients and their efficacy in edible coatings [30].

Sensory Evaluation

There were no significant differences observed between fresh and coated strawberries with reference to all the tested sensory attributes except slight deviation in appearance and taste (Figure 5). Therefore, all the tested sensory attributes of coated fruits even on the 15th day of storage were comparable with fresh strawberries. The sensory properties of fruits play a vital role in consumers' perspectives. Any postharvest treatment should not alter the inherent sensory attributes of a particular commodity. In the present study, we found that the tested EC did not significantly change the intrinsic sensory characteristics of strawberries during the tested period. Therefore, the findings suggest that the formulated EC has a more promising possibility to be used in commercial applications.

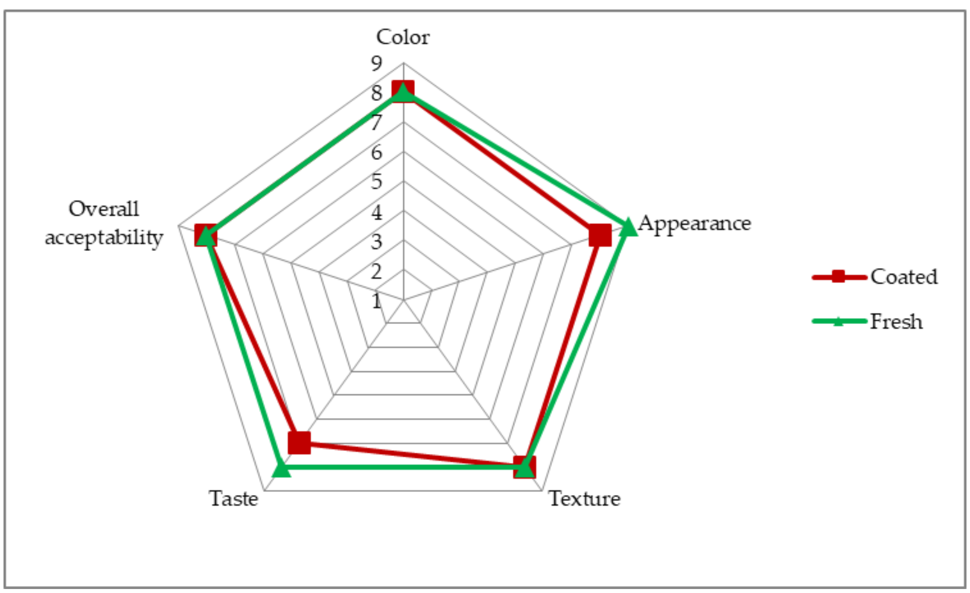


Figure 5. Descriptive rating scale of fresh strawberries and strawberries with edible coating at 15th day stored at 6±1 °C. Nine-point hedonic scale: 9 = like extremely, 7 = like moderately, 5 = neither like nor dislike, 3 = dislike moderately and 1 = dislike extremely. Data are expressed as means ± SD of scores given by thirty panelists.

Conclusion

Tested edible coating demonstrated good barrier properties against moisture loss and reduced the weight loss of strawberry fruits. In addition, it contributed towards retaining not only the visual quality of fruits but also the important chemical attributes in order to maintain the inherent sensory properties of strawberries. Moreover, green tea incorporated edible coating showed a beneficial antimicrobial effect against *B. cinerea*, one of the major postharvest disease-causing organisms of strawberry fruits. Hence, the green tea-incorporated edible coating can be identified as an effective and worthwhile postharvest treatment to extend the shelf life of strawberries while maintaining their quality.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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