

# Edible coatings from plant-derived gums and clove essential oil improve postharvest strawberry (*Fragaria × ananassa*) shelf life and quality

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## Abstract

Strawberries are difficult to store for a long period due to their soft texture and susceptibility to pathogens. The present study aimed to assess the effect of edible coatings composed of different combinations of guar gum, gum acacia and clove essential oil to extend the postharvest shelf life and preserve the quality of strawberry stored at  $6 \pm 2$  °C. Quality assessment included evaluation of weight loss, decay, pH, total acidity, soluble solid content, sugars, ascorbic acids, pigments, phenolic content, cell wall degrading enzymes, antioxidant enzymes and phenol oxidizing enzyme, recorded at regular intervals. There were significant differences observed between coated fruits compared to control fruits. The shelf life of treated strawberries was extended by 9 days, which is valuable for such a fleshy and soft fruit. The weight loss and decay of fruits were reduced in all treated fruits as compared to the control. The level of ascorbic acid was well preserved in guar gum 1% and guar gum 1% + clove essential oil 0.5% treated fruits during storage. Also, the amount of total phenolics was increased in fruits treated with guar gum 1% + clove essential oil 0.5%. Polyphenol oxidase enzyme activity was lower in all treatments of strawberry. Thus, it indicates that the postharvest application of polysaccharide-based edible coating combined with clove essential oil induces antioxidant potential, reduces decay and extends shelf life and nutritional quality of strawberries.

**Key words:** clove essential oil, gum acacia, guar gum, quality, shelf life, strawberry.

**Abbreviations:** CAT, catalase; CEO, clove essential oil; GA, gum acacia; GG, guar gum; NRS, non-reducing sugars; PG, polygalacturonase; POD, peroxidase; PPO, polyphenol oxidase; ROS, reactive oxygen species; RS, reducing sugars; SOD, superoxide dismutase; SSC, soluble solid concentration; TA, titratable acidity.

## Introduction

Strawberries are a popular summer fruit with a characteristic appearance, unique taste and flavour, and excellent nutritional value. This temperate fruit is a good source of vitamins, minerals, amino acids, natural antioxidants and polyphenols (Campaniello et al. 2008). However, the short life span of strawberry, a non-climacteric fruit, has highly perishable nature due to susceptibility to mechanical injury, desiccation, decay and microbial spoilage (Tournas, Katsoudas 2005). Microbial spoilage of the fruit occurs due to two main diseases: rhizopus rot caused by *Rhizopus stolonifer* and grey mold caused by *Botrytis cinerea* (Maas 1998). Several postharvest techniques have been used to improve strawberry shelf life and quality, including controlled or modified atmospheres, heat treatment and refrigerated storage (Harker et al. 2000). However, these techniques raise the fruit cost, which is too expensive for people of developing countries. Thus, there is a strong need to find a cost-effective alternative to extend the shelf life of strawberry.

Antimicrobial and antioxidant-enriched edible coatings are an alternate and effective way to preserve the quality

of many fruits (Sánchez-González et al. 2015). Edible coatings are thin layers of non-hazardous, biodegradable materials such as hydrocolloid (polysaccharides and proteins) and lipids of natural origins. An edible coating can act as a selective barrier against moisture loss, suppress respiration by controlling the transport of oxygen and carbon dioxide gases, maintain flesh firmness, and prevent microbial growth and evaporation of volatile compounds (Vargas et al. 2008). There is an increasing interest in developing environmentally friendly edible coatings based on biopolymers produced from agricultural waste, which can reduce the requirement of synthetic packaging (Maqbool et al. 2011). The public concerns over the use of hazardous synthetic agrochemicals have drawn interest towards the use of plant-derived essential oils in edible coating formulations as an alternative in the postharvest preservation in fruits and vegetables. In addition, essential oils have been in use as a food preservative that improves the antimicrobial efficacy of edible coatings (Guerreiro et al. 2015).

Gum acacia (GA) and guar gum (GG) are well-known polysaccharides for their ability to form rigid and stable gels (Rao et al. 2010). Gum acacia is air-dried gummy

exudate from stem and branches of some *Acacia* species such as *Acacia senegal* and *Acacia seyal* trees (Leguminosae; Maqbool et al. 2011). It is a natural composite polysaccharide of galactose, glucuronic acid, arabinose, rhamnose and protein (Maqbool et al. 2011). GA is easily soluble in water and forms a mildly viscous gel because of its emulsification, encapsulation and film-forming properties, which make it a popular coating that is used widely in the industrial sector. Moreover, the Joint Expert Committee of the FAO/WHO declared GA the most toxicologically safe component for consumption (Motlagh et al. 2006). There are many uses of GA including for the thickening of candies, jellies, chewing gums, and glazes; it is also used to retard sugar crystallization in confectionaries, which consumes more than 50% of the global supply (Fogarty 1988). Moreover, heating leads to its aggregation and increase in molecular weight and subsequent formation of hydrogels with the enhanced binding capability to water and the gum's mechanical characteristics (Ali et al. 2010).

Guar gum (GG) is extracted from *Cyamopsis tetragonoloba*, an annual plant in the Leguminosae family that is commonly known as Indian cluster bean (Dea, Morrison 1975). It is a type of galactomannan and composed of the sugars mannose and galactose. The molecular structure of GG includes linear chains of mannose backbone [i.e. (1-4)-linked  $\beta$ -D-mannopyranose] with attached short side branches of galactose [(1-6)-linked  $\alpha$ -D-galactopyranose] residues (Dea, Morrison 1975). The human diet includes galactomannans in different edible forms and a varied range of physicochemical properties makes them a versatile material for several applications. GG is an excellent stiffener with zero toxicity, which means it can be used in the biomedical, textile, cosmetics, pharmaceutical and food industries (Srivastava, Kapoor 2005). Galactomannan, like other hydrocolloids, is insoluble in organic solvents, but galactose molecules make GG soluble in cold water. Galactomannan is used as an edible coating in food packaging because of its high-water binding capacity and ability to form a viscous gel at low concentrations; it also exhibits significant emulsification activity and reduces the surface and interfacial tension of edible films (Garti et al. 1997). The effect of GG coatings in combination with other natural polysaccharides such as carrageenan, xanthan gum, agar, and starch are well documented (Cui et al. 2006).

Dried aromatic flower buds of *Syzygium aromaticum* (Myrtaceae), commonly known as clove, is a source of essential oil. Clove essential oil has been recognized for its antioxidant, antibacterial, antifungal and antiviral activities and is widely used in agriculture and food preservation (Chaieb et al. 2007; Bakkali et al. 2008). The United States Food and Drug Administration approves the consumption of clove oil as Generally Regarded as Safe up to 2.5 mg per kg of body weight (Kildea et al. 2004). Several bioactive compounds such as eugenol (76.8%),  $\beta$ -caryophyllene

(17.4%),  $\alpha$ -humulene (2.1%) and eugenyl acetate (1.2%) are present in clove oil (Chaieb et al. 2007). Clove oil has a strong antimicrobial activity against *Botrytis cinerea*, *Penicillium italicum* and *Penicillium digitatum* (Shao et al. 2015). Moreover, eugenol is the prime constituent of clove oil and found most effective against several pathogens (Chaieb et al. 2007). Essential oils diffuse slowly from edible coatings to the fruit surface and maintain a higher concentration of active molecules, which reduces the growth of microorganisms on the fruit surface (Chaieb et al. 2007).

To our knowledge, there are no reports available on the use of guar gum and gum acacia edible coating incorporated with clove essential oil in postharvest maintenance of strawberries. The objective of our present study was to develop an antimicrobial, edible composite coating using guar gum and gum acacia alone and in combination with clove essential oil and determine the effectiveness of these coatings to extend shelf life and postharvest quality characteristics of fresh strawberries.

## Materials and methods

### Plant material

Strawberry (*Fragaria × ananassa*) cv. Camarosa fruits were harvested at their commercial maturity stage from an agricultural field in the Saputara region of the Dang district, Gujarat, India. The selected fruits were free from visual defects and carefully selected according to uniform color, shape, size and physical integrity for this experiment. GA, GG and CEO were procured from the HiMedia Laboratories Pvt. Ltd. (Mumbai, India).

### Edible coating preparation and application

Different edible coating treatments tested in the study included guar gum 1% (T1), guar gum 1% + clove oil 0.5% (T2), gum acacia 1% (T3), and gum acacia 1% + clove oil 0.5% (T4). To prepare the 1% guar gum, 1 g guar gum was dissolved in 100 mL cooled deionized water. Likewise, 1% gum acacia was prepared by heating and stirring 1 g gum acacia powder into 100 mL deionized water for 1 h. The coating solutions were filtered through muslin cloth to remove impurities. Clove oil (0.5 mL) was added separately to the 100 mL of guar gum and gum acacia solutions to formulate treatments T2 and T4, respectively. Glycerol was added to each treatment to improve the physical properties of the edible coatings.

The selected strawberries were surface disinfected with 2% sodium hypochlorite for 3 min followed by rinsing with distilled water and air dried at ambient temperature and later, divided into five different groups. Fruits were dipped in the different edible coating solutions for 3 min. Control samples were treated by dipping them in deionized water. All the treated and control fruits were air dried at ambient temperature and then stored in plastic containers at  $6 \pm$

2 °C. Each edible coating treatment group contained five replicates, 10 fruits in each. The quality parameters were analyzed at 0, 3, 6 and 9 days, starting from the day of storage. All of the physiochemical estimations were carried out in triplicate.

#### *Weight loss percentage*

Strawberry fruits were initially weighed at day 0 and then at three-day intervals. The standard method of Association of Analytical Communities (AOAC 1994) was followed for the determination of the weight loss percentage:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100.$$

#### *Decay evaluation*

Decay rate of coated and uncoated fruits was calculated following formula described by El-Anany et al. (2009).

$$\text{Decay rate (\%)} = \frac{\text{No. of decayed fruits}}{\text{Initial No. of fruits}} \times 100.$$

#### *Shelf-life determination*

shelf life of strawberry fruit was determined by calculating the number of days after harvest to the last stage of ripening (Mondal 2000).

#### *pH, titratable acidity, total soluble solids*

The strawberry tissue (4 g) was homogenized and centrifuged to obtain aqueous extracts. The pH was recorded using a pH meter (Eutech Instruments, Singapore) from the clear supernatant of the extracts. A method described by Mazumdar and Majumder (2003) was followed to determine titratable acidity (TA). The total soluble solids (TSS) content of the fruit was estimated using a digital refractometer (Pal-1, Atago Co., Tokyo, Japan).

#### *Reducing and non-reducing sugar concentration*

Reducing and non-reducing sugars were extracted and estimated using 3,5-dinitrosalicylic acid according to the method previously described by Thimmaiah (1999).

#### *Ascorbic acid concentration*

Ascorbic acid concentration in strawberry fruits was estimated by the method in Roe (1954), using 2,4-dinitrophenylhydrazine.

#### *Total phenolic concentration*

Total concentration of phenolics in strawberry fruits was evaluated following the method described by Bray and Thorpe (1954) using Folin-Ciocalteu reagent.

#### *Chlorophyll, lycopene and carotenoid concentration*

Concentration of lycopene and carotenoids was estimated using the method of Wang et al. (2005). A fruit sample (1 g fresh weight) was ground in a pre-cooled mortar with 10 mL hexane and acetone (60:40). The upper organic layer

was collected and incubated overnight at 4 °C. Samples were filtered through Whatman No. 1 filter paper and the clear supernatant phase was collected for further analysis. Approximately 1 mL of the total volume of the organic extract was used for measuring the optical density at 450 nm, 502 nm, 643 nm and 663 nm on a spectrophotometer. We calculated the amount of each pigment in 1 mL of sample with the following equations:

$$\begin{aligned} \text{Carotenoids } (\mu\text{g mL}^{-1}) &= 4 \times \text{OD}_{450}; \\ \text{Lycopene } (\mu\text{g mL}^{-1}) &= 3.12 \times \text{OD}_{502}; \\ \text{Chlorophyll } (\mu\text{g mL}^{-1}) &= (20.2 \times \text{OD}_{645}) + (8.2 \times \text{OD}_{663}). \end{aligned}$$

#### *Cell wall degrading enzymes assay*

Extraction and assay procedures to determine the enzyme activity of polygalacturonase and cellulase enzymes were performed by the method explained in Srivastava and Dwivedi (2000). Strawberry pulp (1 g) was blended with 10 mL 20 mM sodium phosphate buffer (20mM cysteine-HCl, 20 mM EDTA and 0.05% Triton X-100; pH 7.0). This semi-liquid mixture was centrifuged at 4 °C and 15 000 ×g for 30 min and the clear supernatant was collected (Eppendorf 5430 R).

Polygalacturonase activity was assayed by incubating 1 mL reaction mixture containing 0.1 mL fruit extract, 0.2 mL 200 mM sodium acetate buffer (pH 4.5), 0.1 mL 200 mM sodium chloride, and 0.3 mL 1% polygalacturonic acid (PGA; adjusted to pH 4.5 at 37 °C for 1 h). To this reaction mixture, 3 mL DNS reagent was added before the mixture was placed in a boiling water bath. At the end of 5 min incubation, PGA was added in the reaction mixture of the blank tube to avoid any reaction and the absorbance was read at 540 nm. D-galacturonic acid was used as a standard for the calculation of the reducing groups formed. One enzyme unit was regarded as the amount of enzyme required to produce 1 μM of reducing group per min at 37°C. The enzyme activity was expressed as U mg<sup>-1</sup> protein.

To assay cellulase (EC 3.2.1.4) activity, 1 mL reaction mixture was made by mixing 0.25 mL 100 mM sodium acetate buffer (pH 5.0), 0.5 mL 1.5% (v/w) carboxymethylcellulose and 0.1 mL fruit extract. The reaction mixture was incubated for 16 h at 37 °C, which was followed by the addition of 3 mL DNS reagent and heating in a boiling water bath for 5 min. Then, the substrate was added to a control sample and the colour was read at 540 nm. Glucose was used as a standard to prepare a calibration curve and the amount of reducing groups released from carboxymethylcellulose was calculated. One unit of the enzyme was defined as the amount of enzyme required to produce 1 μM of reducing group in one min at 37 °C. The enzyme activity was expressed as U mg<sup>-1</sup> protein.

To determine activity of invertase (EC 3.2.1.26), 1 mL reaction mixture was made by combining 0.3 mL 100 mM sodium acetate buffer (pH 4.5), 0.4 mL 100 mM sucrose and 0.1 mL enzyme extract. The mixtures were incubated for 1 h at 37 °C. The substrate was then added to the control

tubes and invertase was assayed using DNS. The formation of reducing sugars was estimated from the standard curve prepared using sucrose. One unit of invertase was regarded as the amount of enzyme capable of producing 1  $\mu\text{M}$  of reducing groups in one min at 37 °C. The enzyme activity was expressed as U  $\text{mg}^{-1}$  protein.

#### *Superoxide dismutase, catalase and peroxidase assays*

The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were extracted and their activity in strawberries was assayed following the procedure described by Xing et al. (2011). Crude enzyme extract was obtained by extracting 1 g strawberry fruit tissue with 10 mL extraction buffer (25 mM PBS, pH 7.8, 20  $\mu\text{M}$  polyvinylpyrrolidone and 1 mM EDTA). This semi-liquid was then centrifuged at 4 °C and 12 000  $\times g$  for 20 min to collect the supernatant for further use. Extraction procedures for the enzymes was conducted at 4°C.

To determine SOD (EC 1.15.1.1) enzyme activity, the reaction mixture (3 mL) that included 50 mM sodium phosphate buffer (pH 7.8), 75  $\mu\text{M}$  nitroblue tetrazolium, 13 mM methionine, 10  $\mu\text{M}$  EDTA, 2  $\mu\text{M}$  riboflavin and 0.1 mL fruit extract was illuminated for 10 min under constant light (60  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and the color was read at 560 nm and 25 °C. Identical solutions placed in dark conditions were used as the blank. One enzyme unit was regarded as the volume of enzyme that corresponded to 50% inhibition of nitroblue tetrazolium reduction at 560 nm. The activity was expressed as U  $\text{mg}^{-1}$  protein.

For CAT (EC 1.11.1.6) determination, the reaction mixture included 2 mL 50 mM sodium phosphate buffer (pH 7.0), 0.5 mL 40 mM  $\text{H}_2\text{O}_2$  and 500  $\mu\text{L}$  fruit extract. The decomposition of  $\text{H}_2\text{O}_2$  was measured by the decline in absorbance at 240 nm and 25 °C. The specific activity of CAT enzyme was expressed as U  $\text{mg}^{-1}$  protein, where U corresponds to 0.1  $\Delta A_{240} \text{min}^{-1}$ .

For POD (EC 1.11.1.7) measurement, 500  $\mu\text{L}$  of fruit extract was added in a reaction mixture that contained 2 mL 100 mM sodium phosphate buffer (pH 6.4) and 8 mM guaiacol. This reaction mixture was incubated at 30 °C for 5 min and the increase in the absorbance at every 30 s was measured for a total of 120 s at 460 nm after adding 1 mL 24 mM  $\text{H}_2\text{O}_2$ . POD activity was expressed as U  $\text{mg}^{-1}$  protein, where  $U = 0.01 \Delta A_{460} \text{min}^{-1}$ .

#### *Polyphenol oxidase enzyme assay*

Polyphenol oxidase (PPO) activity was determined according to the method of Wang et al. (2004). The crude enzyme extract for the assay was obtained by homogenizing 1 g fruit tissue from each treatment with 10 mL 100 mM sodium phosphate buffer (pH 7.8) and 7.5  $\mu\text{M}$  polyvinylpyrrolidone. The homogenate was centrifuged at 20 000  $\times g$  and 4 °C for 30 min and the clear supernatant was used in the enzyme assay.

PPO enzyme assay was conducted at 25 °C by monitoring

the release of quinones from the catechol substrate. For the determination of PPO activity, 2.5 mL 100 mM sodium phosphate buffer (pH 6.4), 50 mM catechol and 0.5 mL of fruit extract were used. Changes in absorbance maximum were recorded at 398 nm every 30 s for 3 min. The specific activity of the enzyme was expressed as U  $\text{mg}^{-1}$  protein,  $U = \Delta A_{398} \text{min}^{-1} \text{mg}^{-1} \text{protein}$ .

#### *Total proteins*

Concentration of proteins of all extracted enzyme samples was determined following the method Lowry et al., (1951), using bovine serum albumin as the standard.

#### *Statistical analysis*

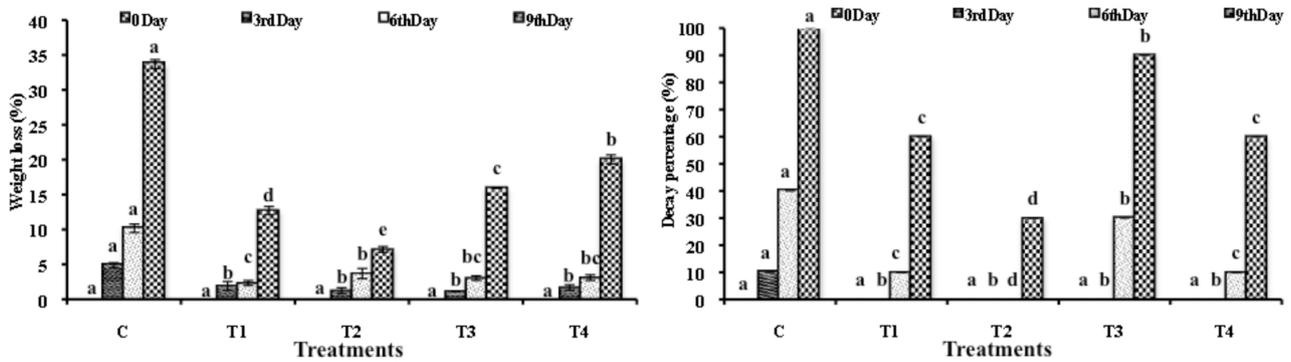
Statistical analysis was performed by following a complete randomized design. All the quality assessments were carried out in three replicates and the standard deviation of the mean was calculated. The statistical significance was evaluated by analyzing the data using one-way analysis of variance (ANOVA) with SPSS commercial software (Version. 22), SPSS Inc., Chicago, IL. Multiple comparisons between the treatments were conducted using the least significant difference (LSD) and Duncan test at  $P \leq 0.05$  (Bico et al. 2009).

## **Results and discussion**

#### *Weight loss*

In the present study, freshly harvested strawberry fruits were treated with different edible coating treatments such as guar gum 1% (T1), guar gum 1% + clove oil 0.5% (T2), gum acacia 1% (T3) and gum acacia 1% + clove oil 0.5% (T4). The control and treated fruits were analyzed for quality assurance at regular intervals.

Weight loss due to gaseous exchange and moisture evaporation is a primary parameter used to measure the quality of a fruit. All the fruits in the edible coating treatments displayed significantly smaller weight loss as compared to control fruits. The weight loss in the control fruits was 33.68%, while fruits in the T2 and T1 treatment groups lost only 7.14 and 12.7%, respectively, at the end of storage. We observed that the edible coating treatments T2 and T4 greatly reduced the weight loss (Fig. 1). The basic mechanism of weight loss in fresh fruits and vegetables is through the lenticels by vapour pressure, resulting in increased metabolic rates that can cause softening of fruit, faster ripening, and senescence (Bai et al. 2003), which results in the release of carbon (Pan, Bhowmilk 1992). It was suggested that the lower weight loss in fruits may be due to the formation of a high relative humidity atmosphere around the fruits, reducing the transmission of water vapour and resultant respiration rate (Bal 2013). These results are consistent with the finding that the coatings lower the respiration, metabolic activities, oxidation reaction and water loss of fruits by forming a semi-permeable barrier



**Fig. 1.** Effect of edible coating treatments on weight loss percentage (A) and decay percentage (B) of strawberry fruit during storage at  $6 \pm 2$  °C. C, control; T1, guar gum 1%; T2, guar gum 1% + clove oil 0.5%; T3, gum acacia 1%; T4, gum acacia 1% + clove oil 0.5%. Different letters over the bars represent significant differences between the treatments on the same storage day at  $P \leq 0.05$  according to Duncan's Multiple Range Test

that reduces the loss of respiratory gases (Baldwin et al. 1999). The prevention of weight loss in tomatoes using alginate coating (Zapata et al. 2008) and gum arabic (Ali et al. 2010) has also been reported.

#### Decay percentage and shelf life

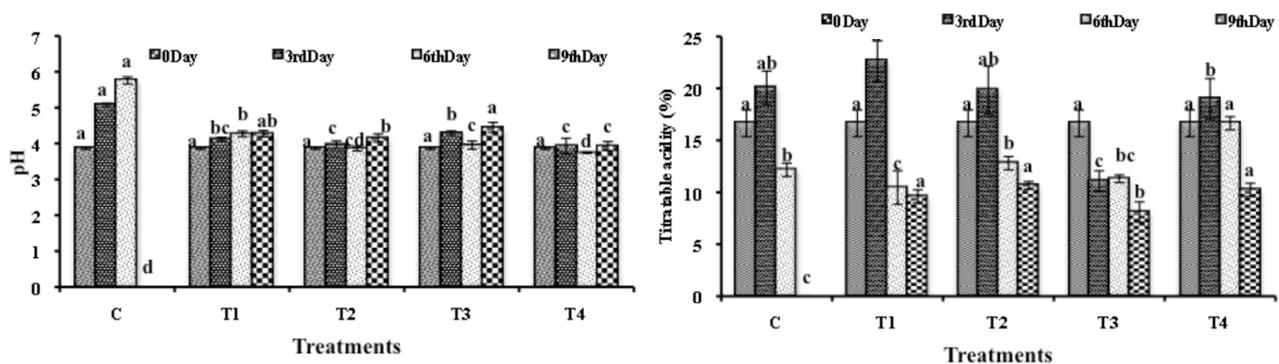
The improvement in the shelf life is directly dependent on the decay rate of that fruit. There was no visible evidence of decay in coated and control strawberry fruits on the third day of storage. Decay percentage in all treatments increased with storage time, but edible coating treatments reduced the decay rate of treated fruits compared to the untreated control, which were infected and decayed by the sixth day. Fruits in the T2 treatment group were fresh and disease free on the sixth day. The minimum decay was observed in treatment T2 (30%; Fig. 1). In the present study, the T2 coating was better than the other treatments and could be correlated with the positive effect of edible coatings on decay reduction through the protection of fruits from pathogens and delay of senescence that reduces cell or tissue integrity (Tanada-Palmu, Grosso, 2005). Clove oil is a potent antimicrobial agent against several foodborne pathogens and also a strong antioxidant agent (Gülçin et al.

2012). Similarly, the guar gum and ginseng extract reduced the decay in treated sweet cherry fruits during storage (Dong, Wang 2018).

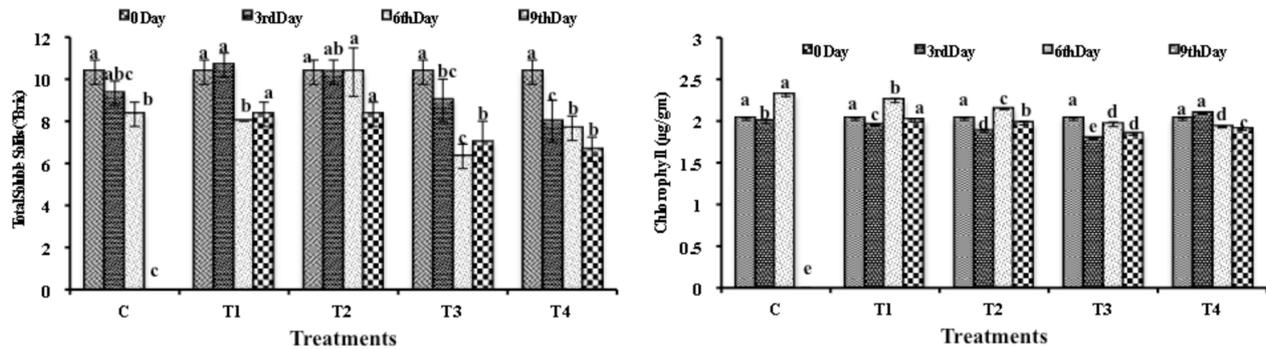
#### Changes in pH, titratable acidity, soluble solid content

The pH of control fruits was significantly higher compared to the fruits treated with edible coatings. The pH of the control fruits increased steadily over time compared to coated fruits (Fig. 2). Edible coating treatments composed of GG-CEO (T2) and GA-CEO (T4) had a significant effect on maintaining lower pH in strawberries. A lower pH found in unripe fruits is attributed to a higher level of organic acids responsible for the major contribution of hydrogen ions (Hernandez-Munoz et al. 2008). Therefore, the increase in the pH of fruits could be due to the loss of organic acids and also due to various biochemical activities in the fruit. The edible coating-treated fruits in our study showed less change in pH, which can be associated to the maintained level of organic acids due to the reduction in biochemical activities.

Titratable acidity (TA) in strawberries increased in the initial stage of storage and declined at the end in control and treated fruits. (Fig. 2). The lowest TA was noted in



**Fig. 2.** Effect of edible coating treatment on pH (A) and titratable acidity (B) of the strawberry fruit during storage at  $6 \pm 2$  °C. C, control; T1, guar gum 1%; T2, guar gum 1% + clove oil 0.5%; T3, gum acacia 1%; T4, gum acacia 1% + clove oil 0.5%. Different letters over the bars represent significant differences between the treatments on the same storage day at  $P \leq 0.05$  according to Duncan's Multiple Range Test..



**Fig. 3.** Effect of edible coating treatments on total soluble solids (A) and total chlorophyll concentration (B) in strawberry fruit during storage at  $6 \pm 2$  °C. C, control; T1, guar gum 1%; T2, guar gum 1% + clove oil 0.5%; T3, gum acacia 1%; T4, gum acacia 1% + clove oil 0.5%. Different letters over the bars represent significant differences between the treatments on the same storage day at  $P \leq 0.05$  according to Duncan's Multiple Range Test.

T2 (10.66%) on the sixth day of storage compared to the control (12.16%). At the end of storage, T1 (9.60%) and T2 (8.11%) showed the least TA content, which could be due to the protective layer around fruit that acted as an  $O_2$  barrier or reduced  $O_2$  supply and thus, decreased the rate of respiration. It was suggested that the reduction in the acidity of fruit may be because of the reduced conversion of organic acid in the sugars and their further utilization into the metabolic activity of the fruit (Srinivasa et al. 2002). The results of this study are consistent with other studies that water loss is reduced due to less availability of organic acid content for enzymatic reactions in edible coating-preserved strawberry fruit (Debeaufort 1998). Acidity of cold-stored strawberry fruit treated with chitosan and starch-based coatings decreased with time, but the amount of TA remained high compared to control fruits (Zhang, Quantick 1998).

The soluble solid content (SSC) of strawberries in different treatments decreased up to two-fold in comparison with the control (Fig. 3). The SSC of the control was 8.33 °Brix, which was higher than in T3 (6.33 °Brix) on the sixth day. At the end of storage, the lowest SSC content was recorded in T4 (6.67 °Brix) among the treatments. Accumulation of SSC increases during ripening due to the activity of hydrolyzing enzyme that release sugars from insoluble polysaccharides. The lower accumulation of SSC in GA coated fruits during storage reduced the activity of hydrolyzing enzymes, metabolic activity, respiration rate and limited gas exchange, which could delay the nutrient decomposition (Dong, Wang 2018). Similarly, in carambola fruit, an alginate-based coating composite with olive oil coating helped to reduce accumulation of total SSC (Baraiya et al. 2014).

#### Reducing sugars and non-reducing sugars

Reducing sugars had accumulated more in the control than in coated fruits. On the sixth day, the reducing sugar content was significantly higher in the control (16.43 mg  $g^{-1}$ ) than in the other coating treatments: T1 (11.48 mg

$g^{-1}$ ), T2 (10.75 mg  $g^{-1}$ ), T3 (11.16 mg  $g^{-1}$ ) and T4 (11.04 mg  $g^{-1}$ ) with a significant difference. However, on the ninth day, the sugar content was lower compared to the sixth day in all treated fruits. Non-reducing sugars had highest concentration in the control (29.48 mg  $g^{-1}$ ) than in treated fruits on the sixth day of storage (Table 1). Similarly, the non-reducing sugar content also reduced on the ninth day in all edible coating treatments. As compared to the control group, lower sugar content was found in fruits coated with GG, GG-CEO and GA-CEO, which could be because the edible coatings created a semi-permeable barrier against  $O_2$  and helped reduce respiration and metabolic activity in strawberry fruits, resulting in a slower breakdown of complex polysaccharides (Baldwin et al. 1999). Similar results were observed in banana coated with chitosan and wax (Gol et al. 2011).

#### Effect of edible coating on ascorbic acid

Ascorbic acid is an antioxidant that scavenges free reactive oxygen species and subsequently generates the lipid-soluble antioxidant product,  $\alpha$ -tocopherol (Davey et al. 2000). Ascorbic acid content significantly increased 0.5 to 0.7-fold in all the treatments initially and later decreased (Table 2). On the sixth day, the ascorbic acid content of control fruits was 133.06  $\mu g g^{-1}$ , while it was 142.04  $\mu g g^{-1}$  in T1 and 149.38  $\mu g g^{-1}$  in T2. At the end of storage, the highest amount of ascorbic acid was observed in T1 (59.59  $\mu g g^{-1}$ ) and T2 (71.02  $\mu g g^{-1}$ ) compared to other treatments. Generally, the ascorbic acid in fruits starts declining after fruit reach full maturity and start ripening (AOAC 1994). In our study, the ascorbic acid decreased at the end of storage, which is in agreement with studies showing that the decline in the ascorbic acid content may be caused by the loss of water after harvest from the increase in the deteriorative oxidation reaction (Nunes et al. 1998). However, edible coating treatments T1 and T2 in our study reduced the loss of ascorbic acid in strawberries because it controlled  $O_2$  and  $CO_2$  permeability and increased antioxidation potential (Togrul et al., 2004). These results are similar

**Table 1.** Changes in reducing and non-reducing sugars, activity of polygalacturonase, cellulase and invertase in strawberry fruits during storage at  $6 \pm 2$  °C. C, control; T1, guar gum 1%; T2, guar gum 1% + clove oil 0.5%; T3, gum acacia 1%; T4, gum acacia 1% + clove oil 0.5%. Different letters in the column indicate significant differences at  $P \leq 0.05$  according to Duncan's Multiple Range Test

Treatments	Day 0	Day 3	Day 6	Day 9
Reducing sugars ( $\text{mg g}^{-1}$ )				
C	$8.64 \pm 0.37$ a	$12.68 \pm 0.38$ a	$16.43 \pm 0.37$ a	-
T1	$8.64 \pm 0.37$ a	$10.68 \pm 0.48$ b	$11.48 \pm 0.80$ b	$9.44 \pm 0.27$ a
T2	$8.64 \pm 0.37$ a	$10.36 \pm 0.44$ b	$10.76 \pm 0.33$ b	$8.64 \pm 0.28$ b
T3	$8.64 \pm 0.37$ a	$9.08 \pm 0.59$ c	$11.16 \pm 0.64$ b	$7.68 \pm 0.23$ c
T4	$8.64 \pm 0.37$ a	$9.28 \pm 0.83$ c	$11.04 \pm 0.67$ b	$6.93 \pm 0.33$ d
Non-reducing sugars ( $\text{mg g}^{-1}$ )				
C	$13.19 \pm 0.46$ a	$31.50 \pm 0.73$ a	$29.48 \pm 0.56$ a	-
T1	$13.19 \pm 0.46$ a	$19.90 \pm 0.64$ c	$08.05 \pm 0.24$ d	$4.66 \pm 0.50$ c
T2	$13.19 \pm 0.46$ a	$15.73 \pm 0.81$ d	$20.21 \pm 0.33$ b	$6.79 \pm 0.43$ a
T3	$13.19 \pm 0.46$ a	$22.60 \pm 0.35$ b	$18.28 \pm 0.48$ c	$5.42 \pm 0.50$ b
T4	$13.19 \pm 0.46$ a	$12.66 \pm 0.29$ e	$18.90 \pm 0.62$ c	$6.01 \pm 0.22$ b
Polygalacturonase activity ( $\text{U mg}^{-1}$ protein)				
C	$0.0056 \pm 0.0005$ a	$0.0155 \pm 0.0009$ a	$0.0111 \pm 0.0009$ a	-
T1	$0.0056 \pm 0.0005$ a	$0.0061 \pm 0.0010$ c	$0.0079 \pm 0.0009$ b	$0.0170 \pm 0.0017$ a
T2	$0.0056 \pm 0.0005$ a	$0.0077 \pm 0.0006$ bc	$0.0081 \pm 0.0011$ b	$0.0071 \pm 0.0006$ b
T3	$0.0056 \pm 0.0005$ a	$0.0090 \pm 0.0002$ b	$0.0077 \pm 0.0003$ b	$0.0029 \pm 0.0003$ c
T4	$0.0056 \pm 0.0005$ a	$0.0090 \pm 0.0017$ b	$0.0073 \pm 0.0004$ b	$0.0075 \pm 0.0010$ b
Cellulase activity ( $\text{U mg}^{-1}$ protein)				
C	$0.0058 \pm 0.0005$ a	$0.0094 \pm 0.0002$ a	$0.0072 \pm 0.0000$ a	-
T1	$0.0058 \pm 0.0005$ a	$0.0069 \pm 0.0008$ b c	$0.0048 \pm 0.0004$ b	$0.0066 \pm 0.0002$ a
T2	$0.0058 \pm 0.0005$ a	$0.0063 \pm 0.0001$ c	$0.0070 \pm 0.0014$ a	$0.0043 \pm 0.0006$ b
T3	$0.0058 \pm 0.0005$ a	$0.0067 \pm 0.0009$ c	$0.0049 \pm 0.0018$ b	$0.0044 \pm 0.0003$ b
T4	$0.0058 \pm 0.0005$ a	$0.0079 \pm 0.0005$ b	$0.0058 \pm 0.0002$ ab	$0.0039 \pm 0.0002$ b
Invertase ( $\text{U mg}^{-1}$ protein)				
C	$0.0064 \pm 0.0002$ a	$0.0100 \pm 0.0001$ a	$0.0047 \pm 0.0003$ b	-
T1	$0.0064 \pm 0.0002$ a	$0.0062 \pm 0.0002$ d	$0.0037 \pm 0.0004$ c	$0.0030 \pm 0.0000$ a
T2	$0.0064 \pm 0.0002$ a	$0.0073 \pm 0.0002$ c	$0.0058 \pm 0.0006$ a	$0.0043 \pm 0.0001$ a
T3	$0.0064 \pm 0.0002$ a	$0.0092 \pm 0.0008$ b	$0.0044 \pm 0.0001$ b	$0.0014 \pm 0.0003$ b
T4	$0.0064 \pm 0.0002$ a	$0.0089 \pm 0.0000$ b	$0.0059 \pm 0.0002$ a	$0.0033 \pm 0.0019$ a

to those reported for kiwi fruits, where ascorbic acid concentration initially increased and then declined with further storage (Bal, Celik 2010). It was found that edible coatings composed of different polysaccharides effectively reduced the loss of ascorbic acid in mango fruits (Zhu et al. 2008).

#### Total chlorophyll, lycopene and carotenoid concentration

Changes in colour of most of the fruits during ripening is due to the degradation of chlorophyll. The concentration of chlorophyll in the strawberry fruit at the beginning of the storage period was  $2.023 \mu\text{g g}^{-1}$  (day 0). On the third day, the highest concentration of total chlorophyll was observed in T4 ( $2.09 \mu\text{g g}^{-1}$ ) while treatment T3 ( $1.79 \mu\text{g g}^{-1}$ ) showed the least amount of chlorophyll. Treatment T1 showed the highest amount of total chlorophyll ( $2.01 \mu\text{g g}^{-1}$ ) whereas, the least concentration was noted in T3 ( $1.84 \mu\text{g g}^{-1}$ ) at the end of the storage period (Fig. 3). The maintenance of total chlorophyll concentration might be due to the effect of

edible coating in reducing the respiration rate by creating a physical barrier that lowers chlorophyllase activity (Baraiya et al. 2014).

Lycopene concentration (Table 2) increased initially in all the coated and uncoated strawberry fruits, then declined toward the end of the storage period. On the third day, the amount of lycopene pigments was highest in T3 and T4 treatments ( $2.57$  and  $2.28 \mu\text{g g}^{-1}$ , respectively) and eventually decreased towards the end of storage period. Uncoated fruits ( $2.50 \mu\text{g g}^{-1}$ ) and treatment T1 ( $2.50 \mu\text{g g}^{-1}$ ) and T2 ( $2.42 \mu\text{g g}^{-1}$ ) had high lycopene concentration on the sixth day, which then decreased on the ninth day. Uncoated fruits had the maximum amount of lycopene on the sixth day of storage, which indicated a higher rate of ripening. Faster ripening might be the reason for the increase in lycopene concentration at early stages of storage. The lycopene content in fruits changes with the stage of ripening (Javanmardi, Kubota 2006). Similarly, significant difference in the concentration of lycopene was seen during

**Table 2.** Changes in the concentration of ascorbic acid, carotenoids and lycopene of strawberry fruits during storage at  $6 \pm 2$  °C. C, control; T1, guar gum 1%; T2, guar gum 1% + clove oil 0.5%; T3, gum acacia 1%; T4, gum acacia 1% + clove oil 0.5%. Different letters in the column indicate significant differences at  $P \leq 0.05$  according to Duncan's Multiple Range Test

Treatments	Day 0	Day 3	Day 6	Day 9
Ascorbic acid ( $\mu\text{g g}^{-1}$ )				
C	137.96 $\pm$ 3.74 a	175.51 $\pm$ 19.9 ab	133.06 $\pm$ 29.2 a	–
T1	137.96 $\pm$ 3.74 a	179.59 $\pm$ 26.2 ab	142.04 $\pm$ 21.8 a	59.59 $\pm$ 12.6 a
T2	137.96 $\pm$ 3.74 a	159.18 $\pm$ 2.4 ab	149.38 $\pm$ 15.3 a	71.02 $\pm$ 11.2 a
T3	137.96 $\pm$ 3.74 a	145.30 $\pm$ 21.4 b	96.32 $\pm$ 8.6 b	41.63 $\pm$ 02.4 b
T4	137.96 $\pm$ 3.74 a	190.20 $\pm$ 12.6 a	93.87 $\pm$ 18.5 b	44.08 $\pm$ 04.9 b
Carotenoids ( $\mu\text{g g}^{-1}$ )				
C	3.15 $\pm$ 0.02 a	2.89 $\pm$ 0.02 e	4.79 $\pm$ 0.02 a	–
T1	3.15 $\pm$ 0.02 a	3.25 $\pm$ 0.02 c	3.63 $\pm$ 0.02 b	3.09 $\pm$ 0.02 a
T2	3.15 $\pm$ 0.02 a	3.11 $\pm$ 0.02 d	3.57 $\pm$ 0.02 c	3.00 $\pm$ 0.04 b
T3	3.15 $\pm$ 0.02 a	6.44 $\pm$ 0.04 a	3.45 $\pm$ 0.02 d	3.04 $\pm$ 0.04 ab
T4	3.15 $\pm$ 0.02 a	3.75 $\pm$ 0.02 b	3.00 $\pm$ 0.04 e	2.92 $\pm$ 0.04 c
Lycopene ( $\mu\text{g g}^{-1}$ )				
C	2.09 $\pm$ 0.05 a	2.069 $\pm$ 0.02 c	2.506 $\pm$ 0.02 a	–
T1	2.09 $\pm$ 0.05 a	2.101 $\pm$ 0.02 c	2.506 $\pm$ 0.02 a	2.174 $\pm$ 0.02 a
T2	2.09 $\pm$ 0.05 a	1.914 $\pm$ 0.02 d	2.423 $\pm$ 0.02 b	2.090 $\pm$ 0.03 b
T3	2.09 $\pm$ 0.05 a	2.569 $\pm$ 0.02 a	2.132 $\pm$ 0.02 c	1.997 $\pm$ 0.03 c
T4	2.09 $\pm$ 0.05 a	2.278 $\pm$ 0.03 b	2.069 $\pm$ 0.02 d	1.955 $\pm$ 0.02 d

the storage of tomato fruits coated with a polysaccharide-based edible coating (Ali et al. 2013). Formation of lycopene pigments in fruits was affected by the temperature and the rate of respiration (Javanmardi, Kubota 2006).

Carotenoid pigment concentration increased significantly from day 0 to the sixth day in coated and uncoated strawberry fruits during the storage period (Table 2). The carotenoid concentration of coated and uncoated fruits was  $3.14 \mu\text{g g}^{-1}$  on 0 day; however, the carotenoid pigments increased and were highest in treatment T3 ( $6.44 \text{ mg g}^{-1}$ ) and T4 ( $3.75 \text{ mg g}^{-1}$ ) on the third day. The carotenoid pigments increased the least in both T1 and T2 treatments ( $3.63$  and  $3.57 \text{ mg g}^{-1}$ , respectively) compared to the control fruits ( $4.78 \mu\text{g g}^{-1}$ ) on the sixth day of storage. On the ninth day, the carotenoid pigments decreased to  $3.04 \text{ mg g}^{-1}$  (T3) and  $2.92 \text{ mg g}^{-1}$  (T4). An early increase in total carotenoid concentration in control and treatments T3 and T4 indicated that these fruits ripened earlier compared to fruit treated with 1% GG (T1) and 1% GG + 0.5% CEO (T2). Total carotenoid content increased during storage in tomatoes treated with heat at  $34$  °C and stored at  $20$  °C for four weeks (Yahia et al. 2007). Control of the increase in the carotenoid content in treated strawberry fruits in the present study might be because the coating slows down the breakdown of chlorophyll and/or biosynthesis of carotenoids by delaying ripening and senescence processes.

#### Activity of polygalacturonase and cellulase enzymes

Textural changes in fruits are the main reason to limit storage life and transportation. The firmness of fruit is reduced as it ripens due to the degradation of cell wall

components by the increased activity of enzymes such as polygalacturonase, cellulase and pectin methylesterase. Pectins are de-esterified and depolymerized by enzymatic reactions because of its easy water solubility compared to other cell wall polysaccharides. Polygalacturonase is an important softening enzyme that catalyzes the hydrolysis of the  $\alpha$ -1,4-galacturonan linkage of demethylated pectin that is formed by pectin methyl esterase and converts it into the usable form of sugars, organic acids, etc. (Prasanna et al. 2007).

Activity of polygalacturonase (PG) increased in all the different treatments (Table 1), but was lowest in treated fruit in comparison to the control. The lower PG activity in coated strawberries suggests that the firmness of coated fruits could be due to the lower activity of pectin esterase and polygalacturonase enzymes (Zapata et al. 2008). Similar changes in the activity of PG were reported for banana fruit (Lohani et al. 2004). During studies of the role of PG in fruits it was noted that increased PG activity is responsible for the initiation of ripening in tomato during their climacteric stage (Poovaiah, Nukaya 1979). The firmness of the fruit and PG activity were significantly correlated. Chitosan-coated peach fruit successfully reduced the activity of PG during storage (Ruoyi et al. 2005).

Cellulase activity in all the treatments increased initially and then declined during storage (Table 1). The activity was lower in coated fruits compared to the uncoated control. At the end of the storage period, treatment T2, T3 and T4 showed the least cellulase activity compared to the T1 treatment. Inhibition of cellulase activity in pear fruit treated with an edible coating based on shellac and

**Table 3.** Changes in the concentration of total phenolics, activity of polyphenol oxidase, catalase, superoxide dismutase and peroxidase enzymes of strawberry fruits during storage at  $6 \pm 2$  °C. C, control; T1, guar gum 1%; T2, guar gum 1% + clove oil 0.5%; T3, gum acacia 1%; T4, gum acacia 1% + clove oil 0.5%. Different letters in the column indicate significant differences at  $P \leq 0.05$  according to Duncan's Multiple Range Test

Treatments	Day 0	Day 3	Day 6	Day 9
Total phenolics (mg g <sup>-1</sup> )				
C	0.56 ± 0.01 a	0.56 ± 0.04 b	0.83 ± 0.01 a	-
T1	0.56 ± 0.01 a	0.66 ± 0.05 a	0.59 ± 0.06 e	0.49 ± 0.06 c
T2	0.56 ± 0.01 a	0.63 ± 0.02 a	0.76 ± 0.02 b	0.95 ± 0.01 a
T3	0.56 ± 0.01 a	0.66 ± 0.03 a	0.68 ± 0.01 c	0.62 ± 0.01 b
T4	0.56 ± 0.01 a	0.64 ± 0.02 a	0.62 ± 0.01 d	0.44 ± 0.02 d
Polyphenol oxidase activity (U mg <sup>-1</sup> protein)				
C	0.0028 ± 0.0004 a	0.0031 ± 0.0001 b	0.1171 ± 0.0151 a	-
T1	0.0028 ± 0.0004 a	0.0037 ± 0.0001 ab	0.0380 ± 0.0046 c	0.0753 ± 0.0086 a
T2	0.0028 ± 0.0004 a	0.0033 ± 0.0004 b	0.0280 ± 0.0061 c	0.0349 ± 0.0051 b
T3	0.0028 ± 0.0004 a	0.0043 ± 0.0008 a	0.0594 ± 0.0176 b	0.0253 ± 0.0023 b
T4	0.0028 ± 0.0004 a	0.0021 ± 0.0005 c	0.0233 ± 0.0086 c	0.0289 ± 0.0099 b
Catalase activity (U mg <sup>-1</sup> protein)				
C	0.0706 ± 0.001 a	0.0734 ± 0.018 a	0.1828 ± 0.070 a	-
T1	0.0706 ± 0.004 a	0.0832 ± 0.007 a	0.1039 ± 0.025 a	0.0094 ± 0.004 a
T2	0.0706 ± 0.004 a	0.0890 ± 0.026 a	0.1513 ± 0.032 a	0.0726 ± 0.071 a
T3	0.0706 ± 0.004 a	0.0688 ± 0.010 a	0.1133 ± 0.093 a	0.0617 ± 0.031 a
T4	0.0706 ± 0.004 a	0.0548 ± 0.026 a	0.2285 ± 0.165 a	0.0881 ± 0.065 a
Superoxide dismutase activity (U mg <sup>-1</sup> protein)				
C	7.396 ± 0.25 a	10.34 ± 0.39 a	16.256 ± 1.98 b	-
T1	7.396 ± 0.25 a	8.610 ± 0.23 b	16.483 ± 2.41 b	11.378 ± 0.97 b
T2	7.396 ± 0.25 a	7.472 ± 0.68 c	14.651 ± 1.11 b	18.039 ± 2.47 a
T3	7.396 ± 0.25 a	10.24 ± 0.04 a	25.302 ± 3.92 a	09.413 ± 0.45 b
T4	7.396 ± 0.25 a	8.368 ± 0.40 b	16.890 ± 2.24 b	17.534 ± 1.48 a
Peroxidase activity (U mg <sup>-1</sup> protein)				
C	0.0046 ± 0.0007 a	0.0066 ± 0.0007 b	0.0125 ± 0.0018 b	-
T1	0.0046 ± 0.0007 a	0.0051 ± 0.0001 b	0.0109 ± 0.0045 b	0.0093 ± 0.0027 ab
T2	0.0046 ± 0.0007 a	0.0070 ± 0.0011 b	0.0127 ± 0.0013 b	0.0196 ± 0.0097 a
T3	0.0046 ± 0.0007 a	0.0100 ± 0.0001 a	0.0220 ± 0.0052 a	0.0087 ± 0.0010 ab
T4	0.0046 ± 0.0007 a	0.0096 ± 0.0019 a	0.0137 ± 0.0010 b	0.0198 ± 0.0091 a

semperfresh was reported (Zhou et al. 2011). Increase in cellulase activity during senescence was previously reported in several other fruits such as avocado, strawberry, peach, papaya and tomato (Hobson 1981).

#### Changes in invertase activity

Invertase activity in control fruits increased 1.6-fold during first three days and then decreased (Table 1). Amongst the treatments, T1 and T2 showed lesser invertase activity during entire storage, suggesting ability of the coatings to reduce enzyme activity by forming a barrier against O<sub>2</sub> and CO<sub>2</sub> exchange. Salicylic acid as an elicitor reduced invertase activity in banana fruit (Srivastava, Dwivedi 2000).

#### Total phenolic concentration and enzymatic activity of polyphenol oxidase

Browning of fruits occurs through an enzymatic reaction and is an important factor in determining the quality of

fruit and vegetables because it can have a negative impact on their organoleptic qualities. As a consequence of the reaction between polyphenol oxidase (PPO) enzyme and phenolic compounds, dark pigments responsible for browning accumulate (Holderbaum et al. 2010). Plant phenolic compounds are known for their antioxidant properties during stress conditions, which protect cells against oxidative deterioration by scavenging reactive oxygen species (Chun et al. 2003). Initially, strawberries contained 0.56 mg g<sup>-1</sup> of phenolic compounds during storage. The phenolic compounds found in the coated fruits had higher concentration compared to those of control. On the sixth storage day, the total phenolic concentration in uncoated fruits was higher compared to coated fruits (Table 3). The concentration of total phenolics was greater in treatment T2 fruits during the entire storage period. Fruits treated with edible coatings had a higher amount of total phenolics, indicating the positive effect of guar

gum and clove oil coating as antioxidants and inhibitors of phenolic degradation during storage (Glüçin et al. 2012). Moreover, the total phenolic concentration was observed to increase in strawberries stored at the temperature greater than 0 °C and the magnitude of the increase in phenolic content was related to the storage temperature (Kalt et al. 1999). A similar result was noted in zein-coated Jamun fruit (Baraiya et al. 2015). The level of total phenolics in the strawberries of T1, T3 and T4 group was lower at the end of the storage because senescence had started in the fruits (Macheix et al. 1990). Moreover, the coatings could have affected the concentration of total phenolic compounds by reducing PPO enzyme activity and their oxidative effect on phenolics (Table 3). In the present experiment, different edible coating treatments and control fruits had a significantly altered PPO activity profile. Activity of PPO in control fruits was higher than in the coated fruits, especially in T2 and T3. Initially, PPO activity was lower in fruits of all treatment groups (0.0028 U mg<sup>-1</sup> protein at day 0) and did not change significantly until the third day of storage. Thereafter, this activity increased in strawberries in all treatment and control groups. Control fruits displayed a higher rate of PPO activity on the sixth day (0.1171 U mg<sup>-1</sup> protein) compared to the T1 (0.0380 U mg<sup>-1</sup> protein), T2 (0.0280 U mg<sup>-1</sup> protein) and T4 (0.0233 U mg<sup>-1</sup> protein) treatment groups. Among various coating treatments, T1 (0.0753 U mg<sup>-1</sup> protein) had a higher PPO enzyme activity compared to other treatments. The strawberry fruits coated with 1% GG + 0.5% CEO and 1% GA + 0.5% CEO showed the least PPO enzyme activity during storage, which may be because the edible coating and potent antioxidant agent in clove oil induced antioxidant potential in coated fruits. A lower PPO enzyme activity was also reported previously in a chitosan treated pomegranate aril during storage and from their study, and it was suggested that this was indicative of inhibition of enzymatic browning by edible coating treatments (Ghasemnezhad et al. 2010). Increased accumulation of total phenolics in wax coated tomatoes was also noted by Dávila-Aviña et al. (2014).

#### *Effect of edible coating on superoxide dismutase, catalase and peroxidase enzyme activity*

Deterioration in postharvest fruit quality is due to the accumulation of reactive oxygen species (ROS) such as hydrogen peroxide, singlet oxygen, and hydroxyl and superoxide radicals (Mittler 2002). Fruits limit ROS damage through the production of antioxidants like SOD, POD, CAT, ascorbate peroxidase, ascorbic acid, phenolics and flavonols (Han et al. 2006). Accumulation of ROS is a consequence of changes in the balance between scavenging capacity and the production of ROS. Furthermore, the accumulation of ROS boosts the degradation process in freshly harvested fruits and reduces the quality and consumer acceptance (Hodges et al. 2004). SOD is responsible for the conversion of ROS into hydrogen peroxides, which are further processed

into water with the help of POD, CAT and APX enzyme (Mittler 2002). The activity of all three antioxidant enzymes (SOD, CAT, POD) increased in strawberry fruits during their storage period (Table 3). Activity of POD increased significantly in both coated and uncoated strawberry fruits during their storage. Activity of POD was higher in coated strawberry fruits compared to uncoated fruits. Activity of POD was 0.0046 U mg<sup>-1</sup> protein in coated and uncoated strawberry fruits on day 0. On the third day, T3 (0.0100 U mg<sup>-1</sup> protein) and T4 (0.0096 U mg<sup>-1</sup> protein) had higher POD activity compared to that of T1 (0.051 U mg<sup>-1</sup> protein), T2 (0.070 U mg<sup>-1</sup> protein) and uncoated fruits (0.066 U mg<sup>-1</sup> protein). Activity of POD increased in all strawberry fruit groups on the sixth day. The POD activity increased at the end of storage (ninth day) and reached its maximum in treatments T2 (0.0196 U mg<sup>-1</sup> protein) and T4 (0.0198 U mg<sup>-1</sup> protein). These results indicate that edible coating treatments of natural gum and clove oil effectively reduced ROS by enhancing the antioxidant activity of POD to maintain fruit quality. Similar findings in coated fruits of *Prunus persica* during refrigerated storage was reported (Ruoyi et al. 2005).

A gradual increase in SOD activity was noted in both control and coated strawberry fruits (Table 3). SOD activity was higher towards the end of the storage period. Initially, SOD activity was 7.396 U mg<sup>-1</sup> protein on the 0-day. Thereafter, SOD activity in the T3 treatment group increased to 10.244 U mg<sup>-1</sup> protein on the third day, 25.301 U mg<sup>-1</sup> protein on the sixth day and it was highest in T2 (18.039 U mg<sup>-1</sup> protein) and T4 (17.534 U mg<sup>-1</sup> protein) groups on the ninth day. These results indicated that the ROS produced in fruits were degraded by the increased SOD activity during storage, which was similar to the results in jujube treated with UV light and chitosan (Zhang et al. 2014).

Uncoated and coated fruits showed a progressive increase in CAT activity during the storage of strawberry fruits (Table 3) but it was not significantly different amongst the treatment and control groups. On the third day, CAT activity increased and then subsequently decreased towards the end of the storage period. It can be assumed from the results that CAT activity increased in response to ROS produced in fruits during their storage period. Normally, plants induce the activity of these antioxidant enzymes in stress conditions to control oxygen radicals (Jahnke et al. 1991).

Cinnamon essential oil is a rich source of antimutagenic, phenolic and antioxidant compounds (Jayaprakasha et al. 2007). Clove oil is a powerful antioxidant source that can be used as an antioxidant source to maintain quality and extend the shelf life of fresh agricultural and pharmaceutical products (Gülçin et al. 2012). The increase in antioxidant enzyme activity and extended shelf life of strawberry fruits reported in our study could be due to the positive antioxidant potential of clove oil, which helps to

reduce the microbial load on the fruit surface and induces the activity of antioxidant enzymes, thus decreasing ROS accumulation. Similarly, increase in the antioxidant potential of antioxidant enzymes and extended shelf life in sweet peppers coated with chitosan and cinnamon essential oil during storage was reported (Ribeiro et al. 2007).

## Conclusions

The guar gum and gum acacia-based edible coating incorporated with clove essential oil could extend the shelf life of strawberry fruit stored at  $6 \pm 2$  °C. The coating treatments also improved some of the quality attributes compared to untreated strawberry fruits. The polysaccharide-based coating incorporated with clove oil also enhanced the activity of antioxidant enzymes, induced the defense system of fruits that reduced the build-up of reactive oxygen species responsible for degradation of fruit tissue, and prevented the loss of enzymatic and non-enzymatic antioxidants. Incorporation of clove oil had a beneficial effect on preventing pathogen attack, which is the main cause of strawberry fruit degradation. Guar gum and gum acacia used as edible coating had an advantageous effect on the visual appearance of fruits and other physical attributes like weight loss and decay incidence. Therefore, polysaccharide gum based edible coatings with incorporated clove essential oil can be recommended for coating perishable fruits and vegetables to extend their shelf life and maintain their nutritional quality.

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