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Chitosan-Based Edible Coating Delays Fungal Decay and Maintains Quality of Strawberries during Storage

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Abstract

Strawberry is a scrumptious but highly perishable fruit. Rapid softening and fungal decay are major challenges in extending storage quality and reducing postharvest losses of this delicate fruit. Chitosan is an environment-friendly hydrocolloidal polysaccharide with well-known antifungal properties. In this study, efficacy of edible coatings of chitosan to maintain fruit quality and storability of strawberries (*Fragaria* × *ananassa* Duch., cv. *Chandler*) was assessed. Healthy, uniformly sized and red ripe strawberry fruits were coated with 0, 0.5, 1 or 1.5% chitosan and stored at 5°C. Changes in physical, biochemical and organoleptic properties of the fruits were assessed every alternate day. Compared to control fruits, chitosan-coated strawberries remained 92% marketable for 8 more days due to significant decrease in fruit decay and sustained structural integrity, fresh weight, total soluble solids, total sugars and organoleptic attributes. Strawberries coated with 0.5% chitosan exhibited relatively less fruit decay and better fresh weight, total soluble solids and sensorial features compared to fruits coated with higher concentrations of chitosan. Interestingly, decline in total titratable acids and ascorbic acid contents in chitosan-coated fruits was steeper than decline in control fruits which suggested that chitosan coating accelerated degradation of organic acids in strawberries. Overall, findings of this study suggest that pre-storage coating of strawberries with 0.5% chitosan may help maintain fruit physical attributes whereas 1.0% chitosan coating helps strawberries retain biochemical attributes followed by 1.5% chitosan coating.

Keywords: Postharvest; Small fruits; Chandler; Cold storage; Marketable fruits; Titratable acids; Soluble solids; Ascorbic acid; Sugars; Sensorial evaluation

Introduction

Strawberry is believed to be originated from Northeast, Europe and North America but now cultivated all over the world in hilly tropics and temperate regions. It is not only relished for its desirable flavour and taste but also for being rich in vitamin C and E, phenolics, anthocyanins and *β*-carotene (Velde *et al*. 2013; Kårlund *et al*. 2014). Being an extremely perishable commodity, it possesses short shelf life under ambient conditions which is mainly due to textural softening, moisture loss and fungal decay (Goulas and Manganaris 2011). High respiration rate of strawberries generates water and heat which further accelerates its moisture loss (Vargas *et al*. 2006). Storage of strawberries under low temperature conditions, *i.e*. 4–5°C and >90% relative humidity, lowers respiration rate and extends its market life for as long as 10 days (Campaniello *et al*. 2008; Mishra and Kar 2014). It is also damaged by microbial and fungal infection and mechanical injury which results in deterioration of fruit quality and marketability (Vu *et al*. 2011). This generally results in 20–40% loss at postharvest level (Anwar 2016). Thus, proper postharvest management is imperative to overcome extensive losses in strawberries.

Several storage methods have been established and designed to enhance storage life of fresh horticultural commodities and expand market access. Controlled atmosphere storage, modified atmosphere packaging, refrigeration and application of fungicide and preservatives are among commonly used techniques. Some of these techniques have been shown to negatively impact fruit quality of strawberries (Holcroft and Kader 1999; Sallato *et al*. 2007). On the other hand, improper use of fungicide to reduce postharvest disease may negatively influence human health (Gullino and Kuijpers 1994). So, substitute

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technologies with ability to reduce moisture loss and fungal decay are required to maintain fruit quality during storage to distant markets.

Edible coatings make a biofilm on fruit surface and hinder gas and moisture movement, reduce microbial growth and thus enhance postharvest life of commodity (Rojas-Graü *et al*. 2010; Zhang *et al.* 2019). As safer choice, these coatings are also biodegradable, eco-friendly and non-toxic (Sánchez-González *et al*. 2011). Chitosan is a natural polysaccharide which consists of linear high-molecular weight polymer made of β-l, 4 linked glucosamine (GlcN) along with residues of N-acetylated GlcN (Shahidi *et al*. 1999). Being an anti-pathogenic but edible coating, chitosan has been implicated in developing resistance against pathogens and retain postharvest quality attributes in strawberries (Wang and Gao 2013; Zhang *et al*. 2014; Petriccione *et al*. 2015; Trevino-Garza *et al*. 2015; Perdones *et al*. 2016; Petriccione *et al*. 2017). Keeping in view the diversity in commercial cultivars and variation in supply chain systems of different areas, a concerted effort is needed to optimize and introduce chitosan-based coating for improving market life of strawberries. Therefore, with an objective to increase postharvest life of strawberries, effect of chitosan coatings on fruit quality of strawberries was evaluated during their cold storage at 5°C. In a recent study where efficacy of 2% chitosan, sodium alginate and *Aloe vera* gel was tested on refrigerated strawberries cv. Chandler, edible coating of *Aloe vera* gel has been shown to perform better than other edible coatings (Qamar *et al*. 2018). In this study, effect of chitosan on fruit decay and storage life of strawberries has been tested in relatively lower dose range (0.5, 1 and 1.5%) with an objective to investigate best concentrations of chitosan which would extend storage life of strawberries without compromising the edible and nutritional quality of strawberries cv. Chandler.

Materials and Methods

Strawberry fruits of variety Chandler (*Fragaria* × *ananassa* Duch.) were harvested from a commercial farm (31°31'13.4"N 74°11'36.0"E) near Sharaqpur, Sheikhupura district, Punjab, Pakistan. Runners of strawberry cv. Chandler had been sourced from a commercial nursery in Matta, Swat district, Khyber Pakhtunkhwa, Pakistan and transplanted on 18-inch high ridges mulched with black polythene. Strawberry production followed standard cultural practices including drip irrigation, mineral nutrition, weed management and insect/pest and disease control. This field had been under strawberry cultivation followed by bottle gourd (*Lagenaria siceraria*) or zucchini (*Cucurbita pepo*) production for the last five years.

Healthy and uniform-sized fruits weighing 20–25 g with ~75% red surface colour and free from visual signs of disease incidence, insect damage or mechanical injury were selected and immediately coated with chitosan. Chitosan coating solution was prepared as earlier described by Vargas *et al*. (2006). Briefly, 1% glacial acetic acid solution (v/v) was prepared and heated at 40°C before dissolving required amount of chitosan (0.5, 1 and 1.5%; w/v) under continues stirring. Wettability of solution was improved by adding 0.1% Tween-80 (v/v). After submerging fruits in solutions with or without chitosan (control) for 15 sec, fruits were taken out and air-dried under shade for 60 min. Fruits were gently packed in 500 g plastic box and transported under cool condition (13±2°C) to storage facility. At destination, fruits were stored at 5°C and 95% relative humidity for maximum period of 14 days. To determine change in postharvest quality of treated and untreated fruits, five boxes (one box per replication) of each treatment were analysed for physical and biochemical attributes before storage and then every alternate day after storage. Before every analysis, cold stored fruits were first taken out of storage and kept under ambient conditions for 30 min.

At every sampling time, percentage of marketable fruits was calculated by counting number of healthy fruits (without any visual sign of tissue softening or disease infection) in each box. Fruits were visually scored for fungal decay according to the five-point scale used by Babalar *et al*. (2007) and presented as fungal decay index. Scoring method was based on the percentage of surface area showing signs of fungal decay where; 1 = no decay on fruit surface, 2 = <5% of fruit surface decayed, 3 = 5–20% of fruit surface decayed, 4 = 20–50% of fruit surface decayed, and 5 = >50% of fruit surface decayed (Babalar *et al*., 2007). Electrolyte leakage was measured as earlier described by Huan *et al*. (2016). Fresh fruit weight was calculated by randomly selecting ten fruits per replication whereas fruit dry matter content, as percentage of fresh pulp weight, was calculated by drying 70 g of fresh tissue at 70°C until constant tissue weight was attained (Kamperidou and Vasilakakis 2006). For biochemical analyses, homogenous pulp of strawberry fruits was prepared and centrifuged at 5,000 rpm for 15 min at 4°C. The pH was determined with pre-calibrated pH/Temp meter (Milwaukee, MW 102, Romania) whereas total soluble solids (TSS, %) in supernatant were determined at 20°C with refractometer (ATAGO, RS-5000, Atago, Japan). Total titratable acidity (TTA) was determined by titrimetric method (Jouquand *et al*. 2008) where supernatant was titrated against 0.1 N NaOH to pH 8.1 end-point. Ascorbic acid was determined by titration with 2,6-dichlorophenolindophenol (Sogvar *et al*. 2016). Briefly, 5 mL of the filtered aliquot from homogenized solution of supernatant juice portion and 0.4% oxalic acid (1/10, v/v) was titrated against freshly prepared 2,6-dichlorophenol indophenol dye until light pink colour end-point was retained for 15 sec. Ascorbic acid content in fruit juice (mg 100 g^{-1} fresh weight) was measured using different concentrations of ascorbic acid for standard curve. Sugars in centrifuged supernatant were determined as reducing sugars, non-reducing sugars and total sugars by copper titration

method (Khan *et al*. 2009) and were expressed as percentage. For sensory analysis, strawberry fruits were divided into four pieces using sharp knife and placed in unmarked clean plates. Samples were placed in randomized sequence for organoleptic evaluation by a panel of 15 persons. Each sample was evaluated for external appearance, flavour, texture and aroma using a nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) whereas sweetness and sourness of strawberries were scored according to five-point just-right scale (1 = not sweet/sour enough, 3 = just right, 5 = too sweet/sour) (Jouquand *et al*. 2008; Resende *et al*. 2008; Schwieterman *et al*. 2014).

Storage and analyses were conducted under Completely Randomized Design (CRD) with five replications (30 fruits per replication) per treatment. Analysis of Variance (ANOVA) technique was employed to test significance among treatments. Treatment means were further analysed with Duncan's multiple range test with significance level at 5%. Polynomial trendlines were used to describe general pattern of change in physical or biochemical parameter. All data analyses were carried out with analytical software package 'Statistix 8.1'.

Results

Effect of chitosan coating on marketability of strawberries is presented in Fig. 1. Control fruits started exhibiting visual quality defects after four days in cold storage and 10% strawberries lost the marketable share in control category. This loss remained consistently persistent until 12 days of storage when 87% strawberries were found marketable. Overall, chitosan-coated strawberries maintained better quality and remained >92% marketable until 12 days of storage. Percentage of marketable fruit declined with increase in chitosan concentration. Edible coating of 0.5% chitosan was more effective in preserving visual quality of fruits compared to 1 and 1.5% chitosan coatings. Strawberry fruits were also observed for visual signs of fungal infection (Fig. 1). Control fruits could be seen with signs of mycelial growth after 8 days of storage whereas chitosan-coated strawberries remained visually fungus-free for 2 more days and started showing signs of fungal infection after 12 days of storage. Though, slightly different but all concentrations of chitosan were statistically equal in effectively resisting fungal spread and extending storage life of strawberries cv. Chandler. Structural integrity of fruit tissues was measured as electrolyte leakage percentage (Fig. 1). Electric conductivity test revealed that electrolyte leakage from control fruit gradually increased after 6 days until termination of study whereas chitosan-coated fruits exhibited relatively stable electrolyte leakage percentage. Since, strawberry fruits showed steep decline in marketability after 12 days, so further study of fruit quality parameter was limited to only first 12 days.

Change in fresh and dry fruit weight of cold stored

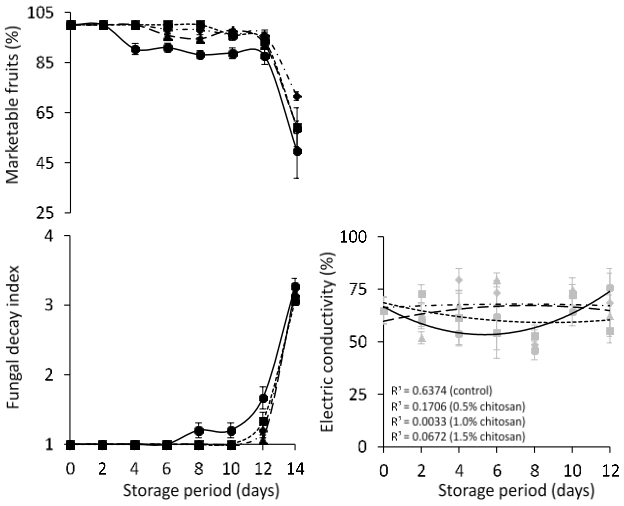

Fig. 1: Changes in percentage of marketable fruits, disease incidence and tissue integrity (electrolyte leakage) of strawberry fruit cv. Chandler coated with chitosan and stored at 5°C. Markers in scatter plots represent average values (control – ●; 0.5% chitosan – ■; 1.0% chitosan – ◆; 1.5% chitosan – ▲) and lines (only in right-side figure) indicate second order polynomial trendline for each treatment (control, ——— ; 0.5% chitosan, ---------1.0% chitosan, -·-·-·-; 1.5% chitosan, — — —). Error bars represent ±SD (n = 5, 30 fruits per replication)

strawberries was also recorded. Though, fresh weight of both control and chitosan-coated fruits declined gradually but chitosan-coated fruits retained slightly better fruit weight during later period of storage (Fig. 2). Decline in fresh weight in both coated and control fruit categories was around 25% of initial fresh weight during first week of storage whereas, in second week, control fruits showed further 5% decline in fresh weight whereas chitosan-coated strawberries stayed fresh without further loss in fresh weight. Strawberries coated with 0.5% chitosan exhibited relatively better fresh weight compared to fruits coated with higher concentrations of chitosan. Strawberries coated with 0.5 and 1% chitosan also exhibited consistent decline in dry weight (Fig. 2).

Control fruits did not exhibit any change in pH (~3.2) during storage whereas chitosan-coated fruits, regardless of chitosan concentration, also showed consistently similar but relatively higher levels of pH (~3.4) during storage (Fig. 2). Total soluble solids in control fruits did not change much and remained around 15% during first 8 days of storage after which TSS level declined sharply to 11.4% and then 9.5% on 10th and 12th day, respectively (Fig. 2). In chitosan-coated fruits, TSS contents slowly but gradually declined below TSS level in control fruits during first 8 days in storage but, in contrast to further drop in control fruits, TSS levels remained unchanged during next four days. Though, TTA contents of both control and chitosan-coated fruits declined during storage but decline in chitosan-coated fruits was steeper than decline in control fruits (Fig. 2). Overall,

sugar:acid ratio increased with increase in chitosan concentration in fruit coating (Fig. 2).

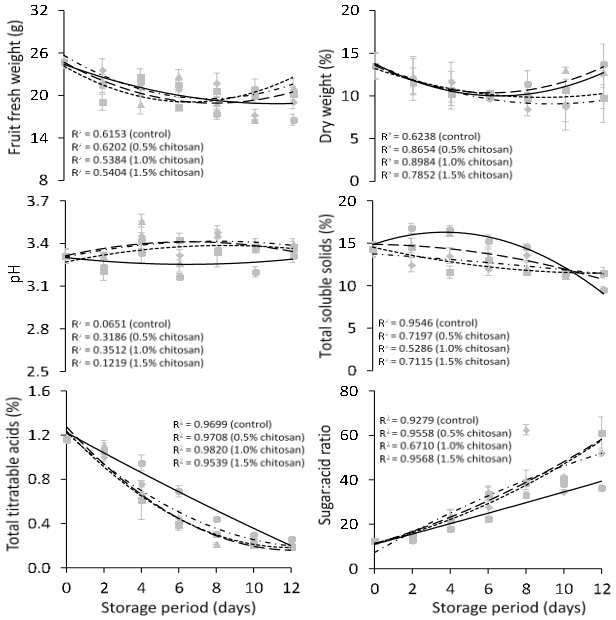

Fig. 2: Changes in fresh fruit weight, dry weight percentage, pH, total soluble solids (TSS), total titratable acidity as percent citric acid and TSS/TTA ratio of strawberry fruit cv. Chandler coated with chitosan and stored at 5°C. Markers in scatter plot represent average values (control – ●; 0.5% chitosan – ■; 1.0% chitosan – ◆; 1.5% chitosan – ▲) and lines indicate second order polynomial trendline for each treatment (control, ———; 0.5% chitosan, ---------; 1.0% chitosan, -·-·-·-; 1.5% chitosan, – – – -). Error bars represent ±SD (n = 5, 30 fruits per replication)

Ascorbic acid level in pre-treated strawberry fruits was 81.59 mg/100 g FW before storage (Fig. 3). Un-coated fruits (control) did not exhibit any change in ascorbic acid contents until 6th day in storage and after which ascorbic acid content gradually dropped to 36.48 mg/100 g FW on 12th day in storage. Chitosan-coated fruits also exhibited an early decline in ascorbic acid and the decline was noticeable even on 2nd day in storage which was much earlier than decline observed in control fruits. Magnitude of this trend was dependent on percentage of chitosan coating. Control fruits did not exhibit significant change in reducing sugars whereas chitosan-coated fruits exhibited slight increase in first half and then decreased back to initial levels in second half of storage (Fig. 3). Magnitude of increase was inversely proportional to concentration of chitosan in fruit coating as strawberry fruits coated with 0.5% chitosan exhibited highest increase followed by fruits coated with 1% and then 1.5% chitosan. Non-reducing sugar percentage in control fruits reduced during storage whereas its trend in chitosan-coated fruits was opposite to that in reducing sugars (Fig. 3). During first half of storage, reduction in non-reducing sugars was least in 0.5% chitosan-coated fruits whereas the trend was reverse in second half of storage. Pattern of change in total sugars seems to be more influenced by non-reducing sugars (Fig. 3). Control fruits exhibited 2.6% decline in total sugars after 12 days in storage.

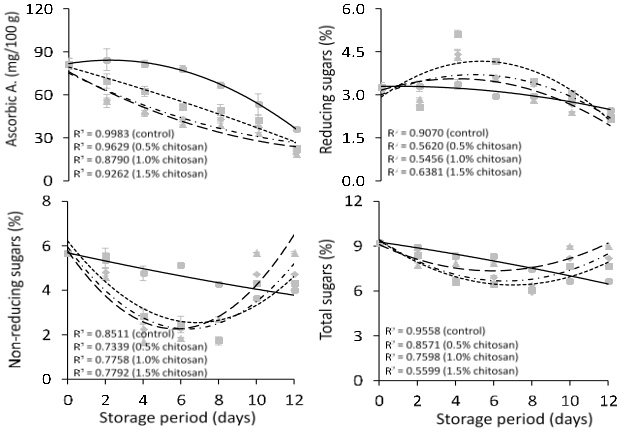

Fig. 3: Changes in ascorbic acid and sugars (reducing, non-reducing and total constituents) in strawberry fruit cv. Chandler coated with chitosan and stored at 5°C. Markers in scatter plot represent average values (control – ●; 0.5% chitosan – ■; 1.0% chitosan – ◆; 1.5% chitosan – ▲) and lines indicate second order polynomial trendline for each treatment (control, ———; 0.5% chitosan, ---------; 1.0% chitosan, -·-·-·-; 1.5% chitosan, – – – -). Error bars represent ±SD (n = 5, 30 fruits per replication)

Overall, coating of fruits with chitosan enhanced consumer acceptance for strawberries during long-term cold storage (Fig. 4). After six days of storage, control fruits started losing high scores for appearance, flavour, texture and aroma. After 12 days in storage, control fruit category received least acceptance for appearance (5.3 score), flavour (4.0 score), texture (5.0 score) and aroma (4.7 score). Chitosan-coated fruits maintained these sensory descriptors at negligible loss of score until 10 days in storage. Changes in appearance, flavour and aroma of chitosan-coated fruits were not affected by the concentration of chitosan in coating solution. Overall, fruits coated with 0.5% chitosan were found better in maintaining fruit texture (8.0 score) than other coatings. In contrast, control fruits gained sweetness and lost sourness score during storage whereas chitosan-coated fruits, especially treated with 0.5% chitosan, maintained sweetness score during the study period.

Discussion

Strawberry is a delicate fruit with very short shelf life. Increase in demand for scrumptious strawberries in consumer-driven markets has invoked researchers to develop technologies that may help to retain its textural delicacies and unique nutritional attributes in diverse value chains. Though, low-temperature storage has been proven successful in slowing down nutritional decline (Kårlund *et al*. 2014; Barbieri *et al*. 2015) and is being widely used to

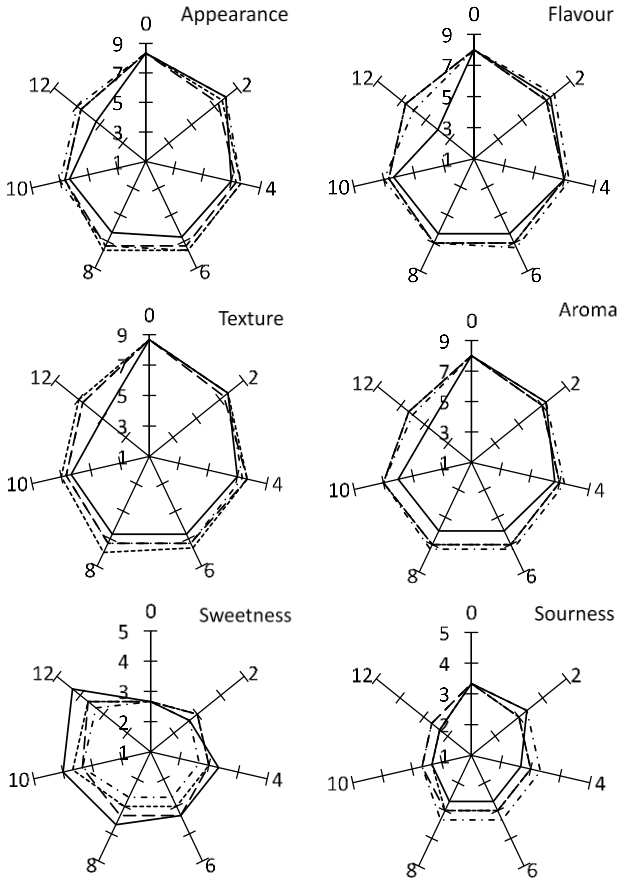

Fig. 4: Effect of chitosan coating on sensory attribute of strawberries cv. Chandler during cold storage at 5°C. Clockwise axis values represent storage period (0, 2, 4, 6, 8, 10 and 12 days). Values on vertical axis represent average organoleptic score assigned to each treatment (control, ———; 0.5% chitosan, ---------; 1.0% chitosan, -·-·-·-; 1.5% chitosan, — — —) on a given day. Fruit slices from each treatment were evaluated by fifteen panellists

increase market life of strawberry but emerging postharvest technologies seem promising in further extending the storage period by preserving quality attributes. In this perspective, chitosan has been found to be an ideal preservative coating material for fresh berries due to its antifungal and film-forming properties (Han *et al*. 2005; Rahman *et al*. 2014). Since, cultivars vary significantly in terms of ripening physiology and postharvest metabolic activities related to nutritional quality (Sturm *et al*. 2003; Capocasa *et al*. 2008; Goulas and Manganaris 2011; Kårlund *et al*. 2014; Mishra and Kar 2014; Nankali *et al*. 2014), there was a need to optimize concentration of chitosan in coating solution with respect to its effect on the mycelial growth on fruit surface and various physical, biochemical and sensorial attributes of strawberry cv. Chandler. In a recent study, efficacy of 2% chitosan alone or in combination with sodium alginate and *Aloe vera* gel was tested on refrigerated strawberries cv. Chandler which showed edible coating of *Aloe vera* gel performing better than 2% chitosan alone (Qamar *et al*. 2018). So, this study was initiated to determine effect of chitosan in relatively lower dose range (0.5, 1 and 1.5%) and find out best concentrations of chitosan which would extend storage life of strawberries without compromising the edible and nutritional quality of strawberries cv. Chandler.

Chitosan coatings delayed fungal decay and prolonged marketable life of strawberries (Fig. 1). These results support the antifungal properties of chitosan. Han *et al*. (2004) also reported 33 and 40% decrease in mold growth on strawberries cvs. Driscoll's and Puget Reliance, respectively when coated with chitosan and stored at 2°C and 88% relative humidity for 14 days. Chito-oligosaccharide, an enzymatically produced chitosan, has not only been shown to significantly inhibit growth of *Botrytis cinerea* but has also been shown to synergistically enhance antifungal activity of synthetic fungicides even when used in low doses (Rahman *et al*. 2014). Recently, use of 2% chitosan as an edible coating on strawberries cv. Chandler has been reported to extend storage life of refrigerated strawberries by four more days partly due to inhibition in decay incidence (Qamar *et al*. 2018). Chitosan coating elicits expression of defence-associated proteins in strawberry (Petriccione *et al*. 2017). In vitro studies have shown that application of 5 mg/L chitosan upregulates expression of pathogenesis-related proteins and antioxidant genes in tomato which results in inhibition of radial mycelial growth by 54.8% (Chun and Chandrasekaran 2019). Decrease in percentage of electrolyte leakage from chitosan-coated strawberries gives a scientific justification of resistance in these strawberries against fungal infection (Fig. 1). Chitosan-coated fruits remained structurally stable for longer period than control fruits. Increased leakage of cellular electrolyte indicates loss in structural integrity which might have favoured mycelial growth leading to early fungal infection on control fruits whereas film-coating properties of chitosan maintained structural integrity and resisted electrolyte leakage and thereby delayed fungal infection in concentration-dependent manner (Fig. 1). Overall results suggest that application of chitosan in relatively lower concentration *i.e*. 0.5% is more effective in maintaining membrane integrity and inhibiting fungal decay thereby increasing market life of strawberries.

Strawberries coated with 0.5 and 1% chitosan also exhibited relatively better fresh weight but lower dry weight under long-term cold storage (Fig. 2) which suggests that carbon metabolism in these fruit tissues may be relatively more active to uphold respiration and other cellular activities whereas lower concentration of chitosan may have acted as low-density polymer coating allowing reasonable exchange of water and respiratory gases. Further research is required to explore and validate such chitosan-mediated regulations. Total soluble solids, total titratable acids and sugar:acid ratio (TSS/TTA) are the basic fruit quality

parameters which are often employed to infer metabolic changes in fruit tissues and to indirectly measure consumer acceptability (Sturm *et al*. 2003; Rutkowski *et al*. 2006; Testoni *et al*. 2006; Testoni and Nuzzi 2006). Fruits coated with 0.5 or 1% chitosan had least TSS (2–3% lower than control) and TSS level slightly increased (1% higher) as concentration of chitosan in edible coating increased from 1 to 1.5%. Overall, chitosan-coated fruits maintained TSS for at least four days more than control fruits (Fig. 2). Moreover, steeper decline in TTA of chitosan-coated fruits compared to control fruits indicates that chitosan may have role in regulation of total titratable acid pools in strawberry fruits cv. Chandler and this effect is independent of the concentration range used in this study. Altogether, the proposed phenomenon may be involved in improving ripening index (sugar:acid ratio) of the chitosan-coated strawberries (Fig. 2). Secondly, concentration range of chitosan in coating solutions seems equally effective in altering these biochemical attributes (Fig. 2).

Ascorbic acid is one of the reactive compounds in fruits (Kårlund *et al*. 2014). Extent of its progressive loss during storage is positively correlated with storage temperature (Nunes *et al*. 1998). Chitosan coating reduced ascorbic acid levels in strawberries in percentage-dependent manner. Pattern of ascorbic acid profile in fruits coated with 0.5% chitosan was relatively closer to control fruits whereas higher percentage of chitosan enhanced ascorbic acid loss which resulted in low level of ascorbic acid in chitosan-coated fruits (Fig. 3). Mishra and Kar (2014) also reported decrease in total soluble solids, ascorbic acid and total sugars in Chandler strawberries with the advent of storage at 5°C for 9 days. Aligned with this study, decline in ascorbic acid and titratable acid has also been reported in other cultivars harvested at fully matured stage and stored at ambient temperature (Rahman *et al*. 2016). Though, chitosan-coated fruit exhibited slight dip in total sugars during mid-period of storage but, overall, total sugar percentage at the start and end of study remained almost same (Fig. 3).

Coating of fruits with chitosan enhanced consumer acceptance for strawberries for longer period (Fig. 4). Change in appearance, flavour and aroma of chitosan-coated fruits were not affected by the concentration of chitosan in coating solution whereas fruits coated with 0.5% chitosan were found better in maintaining fruit texture (8.0 score) than other coatings. Han *et al*. (2005) evaluated organoleptic changes in cold stored strawberries applied with 1% chitosan-based edible coatings and concluded that chitosan coatings increased consumer acceptance but did not change consumer acceptability for flavour, sweetness or firmness. In another study, chitosan coating has been reported to alter fruit aroma composition without effecting sensorial perception of strawberries (Perdones *et al*. 2016). These findings suggest that film-formation of chitosan over strawberries was helpful in maintaining organoleptic properties of fruits during storage.

Conclusion

Edible coating of chitosan can be a safer option to inhibit fungal decay and maintain overall fruit quality during cold storage. Pre-storage coating of strawberries with 0.5% chitosan may help maintain fruit physical attributes whereas 1.0% chitosan coating helps strawberries retain biochemical attributes followed by 1.5% chitosan treatment. The study was designed to assess impact of chitosan-based coatings on physical, biochemical and sensorial properties of strawberries mostly reflecting consumer preferences. Comprehensive nutritional profiling of strawberries coated with 0.5% chitosan, concentration optimized in this study, may further help to comprehend its effect on human health. Further biochemical, enzymatic and molecular studies may further delineate chitosan-mediated regulatory mechanisms that inhibit fungal decay and delay deterioration in quality attributes of strawberry fruit cv. Chandler. It would also be intriguing to investigate enhanced capabilities of chitosan when combined with essential oils, natural extracts and hormones.

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R. Anwar conceived the idea and J. Hassan conducted the research studies. All authors equally contributed in editing, commenting, revising and approving the manuscript without conflict of interest. Research study was partly funded by the Higher Education Commission, Pakistan (Project No. 21-668SRGP) to R. Anwar.

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