

# Effects of Chitosan Coating with Putrescine on Bioactive Compounds and Quality of Strawberry cv. San Andreas During Cold Storage

Erdinç Bal<sup>1</sup> · Bahtiyar Aydın Ürün<sup>1</sup>

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# Abstract

The effect of postharvest treatment of Putrescine (PUT) alone or in combination with chitosan on maintaining quality of strawberry cv. San Andreas during cold storage was investigated. The treated strawberry fruits were examined for weight loss, soluble solids content, titratable acidity, ascorbic acid contents, total anthocyanins, total phenolic contents, antioxidant content, decay incidence and sensory analysis throughout the 15 storage days at 1 °C and 90–95% relative humidity. Fruit coated with chitosan and chitosan+PUT coatings showed significant delays in the change of weight loss and decay percentage as compared to uncoated fruit. Although alone PUT application was effective in the early days of storage, it was not as effective as coating treatments towards the end of storage. The addition of Putrescine into the chitosan coating solution had a positive effect on maintaining higher concentrations of total anthocyanins, total phenolic and antioxidant content, which decreased in control fruit due to over-ripening and senescence processes. Likewise, sensory analysis results also showed the effectiveness of chitosan+PUT treatment by retaining the quality of strawberry. These findings suggest that the chitosan+PUT coatings are useful for extending the shelf-life and maintaining quality of strawberry fruit at 1 °C.

Keywords Strawberry  $\cdot$  Edible coating  $\cdot$  Polyamine  $\cdot$  Quality  $\cdot$  Preservation

# Einfluss einer Chitosan-Umhüllung mit Putrescin auf bioaktive Inhaltsstoffe und Qualität von Erdbeeren der Sorte "San Andreas" während der Kühllagerung

Schlüsselwörter Erdbeere · Essbare Hülle · Polyamin · Qualität · Konservierung

# Introduction

Strawberries are among the most popular berries consumed worldwide, mainly because of rich content of essential nutrients and phytochemicals. However, they are extremely perishable and have a very demanding postharvest handling requirement. At room temperature the storage life of fresh strawberry is limited to 1–2 days (Zhang et al. 2006). Their short postharvest life is mainly due to their susceptibility towards mechanical injury, physiological deterioration, water loss and microbial decay. Loss of the fruit quality starts with the loss of membrane integrity that generally becomes the leading event in the senescence (Paliyath and Subramanian 2008). Therefore, strawberries must be cooled immediately to their lowest safe temperature  $(0-1 \,^{\circ}C)$  to prevent over ripening and decay.

The prolonging storability of perishable crops can be achieved by the application of edible coatings (Dhall 2013). Edible coating is a healthy and environment friendly approach. It is directly consumable and does not leave any residues. This technology not only retains the quality of fresh fruits but also extends their overall shelf-life. Edible coating alters various physical, physiological and biochemical aspects of fruit growth, to carry out its effect. They maintain the quality of fruits and vegetables by forming an edible film over the produce. The film acts as a partial barrier to different gases like O<sub>2</sub> and CO<sub>2</sub>, water vapor and other chemical compounds, which creates a modified atmosphere around the fruits and vegetables, thus decreasing the respiration rate and the water loss, and preserving its

Erdinç Bal ebal@nku.edu.tr

<sup>&</sup>lt;sup>1</sup> Department of Horticulture, Faculty of Agriculture, Tekirdag Namık Kemal University, Tekirdag, Turkey

texture and flavor. The main advantage of edible films over traditional synthetics is that they can be consumed with the packaged products (Prasad et al. 2018).

Edible coatings based on chitosan have proved to be effective to control decay and maintain quality of cold-stored strawberries (Han et al. 2004; Vargas et al. 2006; Riberio et al. 2007; Hernandez-Munoz et al. 2008; Gol et al. 2013; Wang and Gao 2013; Nasrin et al. 2017). Due to the fact that different strawberry cultivars have different physicochemical and disease resistance properties, it is important to examine the effectiveness of chitosan coatings on different cultivars (Han et al. 2004; Vargas et al. 2006).

Polyamines are environment friendly in nature and are easily biodegradable without showing any negative externality on the environment. There are three polyamines, viz., Putrescine (PUT), spermine, and spermidine, which are prominently applied at a particular concentration to extend the shelf life of fruits. They play a vital role in prolonging the shelf life of perishable horticultural crops (Pareek et al. 2018). Polyamines have been reported as anti-senescence agents, the main effects in fruits being retarded colour changes, increased fruit firmness, delayed ethylene and respiration rate emissions, induced mechanical resistance and reduced chilling symptoms (Valero et al. 1999). Moreover, in several studies Putrescine applied exogenously have been reported to increase storage life and quality attributes of mango (Razzaq et al. 2014), apricot (Davarynejad et al. 2013), strawberry (Khosroshahi et al. 2007; Mortazavi et al. 2014), plum (Serrano et al. 2003) and grapes (Bal et al. 2017).

Incorporating essential oils, polyamines or antimicrobial compounds into edible coatings provides a novel way to improve the quality and postharvest life of fruit. Postharvest treatments with Putrescine dips alone or in combination with chitosan coating treatments have resulted in improved grape (Shiri et al. 2013) and banana (Hosseini et al. 2018) shelf-life. However, no information is available on the effect of chitosan edible coating enriched with PUT on quality of strawberries. This study was carried out to study the effect of PUT alone or in combination with chitosan on biochemical properties, fungal decay and sensory quality of 'San Andreas' strawberries during cold storage.

# **Materials and Methods**

# **Sample Preparation and Treatments**

Strawberries (*Fragaria* × *ananassa* cv. San Andreas) at commercial ripeness (>75% of the surface showing red colour) were harvested from local commercial farm in Turkey. After harvesting, fruits were sorted to eliminate damaged fruit and selected for uniform size and color. Fruits

in polypropylene baskets were immediately transferred to the laboratory at the University of Tekirdag Namik Kemal and were divided into four groups (750 fruits per group and 3 replications/treatment). Treatments and abbreviations can be summarized as follows:

1. Control:

Fruits was immersed in distilled water at 20 °C for 3 min, and then dried for 2 h at ambient temperature.

2. PUT treatment:

Fruits was immersed in distilled water with 1 mM PUT (Merck, Germany) at 20 °C for 3 min, and then dried for 2h at ambient temperature.

3. Chitosan coating treatment:

Chitosan solution was prepared according to Nasrin et al. (2017). For preparing 100 ml of 2% chitosan solution, 2g of chitosan (Sigma Chemical Co. U.S.A.), respectively, was dissolved in 75 ml of distilled water and 2 ml of glacial acetic acid was added. The mixture was heated with continuous stirring ( $55 \,^{\circ}$ C) for proper dissolution of chitosan. The final pH of the solution was adjusted to 5.6 with 2N NaOH and the volume was made up to 100 ml with sterilized distilled water. Strawberry fruits were submerged in this solution, respectively, for 3 min and allowed to drain for 2h at ambient temperature.

4. Chitosan-PUT edible coating treatment:

For preparing the chitosan emulsion loaded with PUT, 1 mM PUT was added to chitosan coating (2%) solution, then the mixture was heated with continuous stirring. Fruits were submerged in this solution, respectively, for 3 min and allowed to drain for 2h at ambient temperature.

The treated strawberry fruits were sealed in polypropylene baskets (0.250 kg) and stored at 1 °C and 90–95% relative humidity (RH) for 15 days. Measurements of all parameters started at the beginning of the storage and then continued until the day of 15 with 3 days intervals.

### **Analysis of Quality Attributes**

### **Determination of Weight Loss**

Strawberries were weighed at the beginning of the experiment just after treatment and air-drying, and thereafter every 3 days during the storage period. Weight loss was expressed as the percentage loss of the initial total weight.

# Determination of Total Soluble Solids, Titratable Acidity and Ascorbic Acid Contents

For the analysis of total soluble solids (TSS) content and titratable acidity (TA) of each sample, tissue sap was squeezed out from fresh fruit materials with a press. In the juice, SSC were determined with a hand refractometer (%). TA content was determined by titration method and calculating the result as grams of citric acid per 100g fresh weight. Ascorbic acid content was measured using 2,5-6 dicholorophenol indophenols' method described by A.O.A.C (1990) and expressed as mg  $100 \text{ g}^{-1}$ .

# Determination of Total Anthocyanins and Total Phenolic Contents

The anthocyanin was extracted and estimated by the method of Lees and Francis (1972). Briefly, 1 g of fruit pulp was blended with 20 ml of extracting solvent (95% ethanol: 1.5 N HCl, 85: 15) and allowed to stand overnight at 4 °C. The samples were filtered into a volumetric flask. The remained residue was washed with an extractor solution to completely remove the pigments. The filtrate was pooled to a total volume of 100 ml with the same solvent and absorbance was recorded at 535 nm to quantify the total anthocyanin content (mg 100 g<sup>-1</sup>) (Gol et al. 2013).

For the total phenolics assay, the ethanol extract of fruit was added to 2.0 ml of 1 mol  $1^{-1}$  Folin-Ciocalteau reagent. After mixing thoroughly for 5 min, 1.5 ml of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was added into the mixture. The mixtures were then incubated for 1 h at room temperature in the dark and the absorbance was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalent per 100 g of fresh weight (Liu et al. 2018).

#### **Antioxidant Analyze**

2,2-Diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity was determined according to the Brand-Williams et al. (1995). Briefly, an aliquot (0.1 ml) of the extract was added to 3.9 ml of a DPPH methanolic solution. After 30 min of incubation at room temperature in the dark, the absorbance was measured at 515 nm. The results were expressed as mmol trolox equivalent kg<sup>-1</sup> fw.

### **Decay Incidence**

Decay incidence of strawberry fruit was the number of fruit showing decay symptoms (rot, lesions or visible fungal growth) relative to the total number of fruit and expressed in percentage (%).

# **Sensory Analysis**

Sensory evaluation, based on general visual appeal, colour and visible structural integrity, was conducted using a 7point hedonic scale by a six members panel. The scores were: like extremely (7); like very much (6); like moderately (5); neither like nor dislike, marketable (4), dislike moderately (3); dislike very much (2); and dislike extremely (1). Fruit scored above 4 was considered acceptable (Hernandez-Munoz et al. 2008).

# **Statistical Analysis**

The experiment was of a completely randomized factorial design of three replications per treatment. Analysis of Variance (ANOVA) was the means for analyzing the difference between means and while LSD test being applied for mean separation at p < 0.05. All the analyses were carried out through SPSS as statistical software. Results are reflected as the mean  $\pm$  SE.

# **Results and Discussion**

#### Weight Loss

Epidermis of strawberries consists of large cells and thin cell walls contribute to their high level of susceptibility to weight loss (Szczesniak and Smith 1969). Therefore, it is necessary to reduce the water loss in strawberries. In the study, all fruit showed a progressive loss of weight during storage and the weight loss of uncoated fruit was significantly greater than that of coated fruit (Table 1). At the end of storage, untreated strawberries showed 15.2% loss in weight followed by PUT treated strawberries (13.8%), whereas the weight losses of samples coated with chitosan and chitosan riched PUT were 6.9% and 6.1%, respectively. The results were consistent with previous studies showing a reduction in weight loss was due to the effects of chitosan coating, which served as semipermeable barrier against gases and moisture (Han et al. 2004; Riberio et al. 2007; Prasad et al. 2018). On the other hand, PUT addition, as a polyamine, could contribute to reduce the weight loss by delaying the ripening process and slowing the respiration (Valero et al. 1999; Serrano et al. 2003; Shiri et al. 2013).

# **Total Soluble Solids and Titratable Acidity**

TSS and TA of fruits is a major quality parameter, which is correlated to the texture and composition. The results regarding the effect of PUT and chitosan coating treatments on TSS and TA in strawberries are presented in Table 1. It can be seen from this table that TSS values of samples showed a decreased according to harvest value (with values between 8.9 and 7.5%) and uncoated fruits exhibited higher TSS compared to other chitosan-coated fruits due to high water loss at the end of cold storage. Similarly, TA also showed a decrease throughout cold storage in strawberry fruits, with lower values in uncoated fruit compared

Treatments	Storage times (day)	Weight loss (%)	TSS (%)	TA (%)
3	2.5 c	8.9 a	0.92 a	
6	4.4 de	8.2 a	0.88 a	
9	7.4 h	7.8 a	0.84 a	
12	10.6 1	7.5 a	0.79 a	
15	15.2 k	8.3 a	0.72 a	
PUT	At harvest	_	8.5 a	0.95 a
	3	2.5 c	8.7 a	0.96 a
	6	4.3 de	8.8 a	0.91 a
	9	7 gh	8 a	0.85 a
	12	9.6 1	7.5 a	0.81 a
	15	13.8 j	8 a	0.74 a
Chitosan	At harvest	-	8.5 a	0.95 a
	3	1.2 ab	8.9 a	0.93 a
	6	2.1 bc	8.5 a	0.90 a
	9	3.7 d	8.2 a	0.85 a
	12	5.7 f	8 a	0.83 a
	15	6.9 gh	7.5 a	0.81 a
Chitosan+ PUT	At harvest	-	8.5 a	0.95 a
	3	1.1 a	9 a	0.97 a
	6	1.9 abc	8.8 a	0.93 a
	9	3.5 d	8.3 a	0.89 a
	12	5.2 ef	7.9 a	0.86 a
	15	6.1 fg	7.6 a	0.79 a

 Table 1
 Effect of PUTrescine application and chitosan coating on weight loss, TSS and TA of strawberry during storage

Data are means of 3 replicates

Values followed by the same letter within a column are not significantly different at P < 0.05

to chitosan-coated fruit (Table 1). However, these decreases in TSS and TA content were not statistically significant (p<0.05). These findings are in agreement with the reports by Valenzuela et al. (2013) and Khosroshahi et al. (2007), who studied the treatment of chitosan-based edible coating or PUT on strawberries, did not observe significant changes on the TSS and TA of coated samples during the storage time.

### **Ascorbic Acid Contents**

Among the berry species, fresh strawberries are considered to be one with the highest content of ascorbic acid. As it is known, there is a gradual decrease in ascorbic acid content as the storage temperature or duration increases (Kalt et al. 1999; Koyuncu and Dilmacunal 2010). In the present study, the initial content of ascorbic acid was  $54.3 \text{ mg } 100 \text{ g}^{-1}$  of fresh fruit. The results illustrated in (Fig. 1) revealed that there was a significant decrease in ascorbic acid values of fruits along with the storage period. The rate of decrease in vitamin C was significantly higher in untreated control fruits as compared with other treated fruits. While the addition of Putrescine into the coating solution did not contribute significantly, PUT alone treatment was slightly more effective than control for delaying degradation of ascorbic acid. At the end of 15 days cold storage, the lowest ascorbic acid value was determined in control fruits (27.7 mg  $100 g^{-1}$ ) followed by PUT treated fruits (30.9 mg  $100 g^{-1}$ ), while the highest ascorbic acid value was determined in chitosan treated fruits (38.6 mg 100 g-1) followed by chitosan + PUT treated fruits  $(36.4 \text{ mg } 100 \text{ g}^{-1})$ . The reduction of ascorbic acid content during the storage of strawberries has been reported by Nasrin et al. (2017). In the study, water loss from strawberries might accelerate the loss of ascorbic acid due to increased oxidation (Nunes et al. 1998). The lowering of vitamin C loss of fruit with coatings can be attributed to the gases permeability of these coatings inhibit ascorbic acid oxidation. The results are consistent with Gol et al. (2013) and Eshghi et al. (2014), who found that the ascorbic acid content was higher in chitosan coated strawberry than the control.

### **Total Anthocyanins Content**

The trend of total anthocyanin content of strawberry during storage shows different results based on storage conditions, postharvest treatments, as well as variety (Eshghi et al. 2014). The effect of coatings and PUT on the total anthocyanin content of strawberries is revealed in Fig. 2. The initial total anthocyanin content of strawberry was 40.1 mg 100 g<sup>-1</sup> and fluctuations occurred in anthocyanin content in the form of increases and decreases in all applications during storage period. In the study, the highest anthocyanin content were obtained by control (58.1 mg 100 g-1) and PUT treatment (53.1 mg 100 g<sup>-1</sup>) on 9th day. The greater anthocyanin content presented by uncoated samples can be explained by the higher respiration rate. After 9th day, a significant decrease in total anthocyanin content was recorded in control and PUT treated fruits as the storage period advanced and over-ripe. Combination of chitosan coating and PUT treatment proved significantly effective in inhibiting the decrease in total anthocyanin content towards the end of the storage. This result indicated that combinational treatment could show a synergetic effect. The positive effect of polyamines in maintenance of anthocyanin content was also reported for strawberry (Mortazavi et al. 2014) and grape (Bal et al. 2017). At the end of storage time, the control samples showed the lowest total anthocyanin content (39.1 mg 100 g<sup>-1</sup>) while chitosan + PUT treated samples showed the highest amount (52.2 mg 100 g<sup>-1</sup>). Similar results were also found by Wang and Gao (2013) and Hussain et al. (2012), who reported that the strawberry fruit immersed in chitosan solutions and stored significantly main-

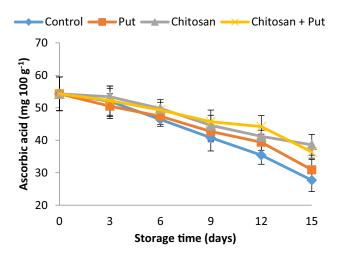


Fig. 1 Effect of PUT and chitosan coating on ascorbic acid of strawberries during storage

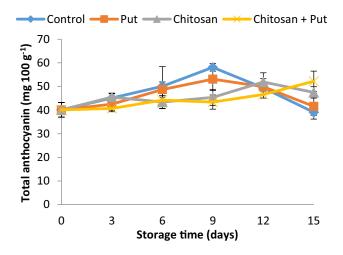


Fig. 2 Effect of PUT and chitosan coating on total anthocyanin of strawberries during storage

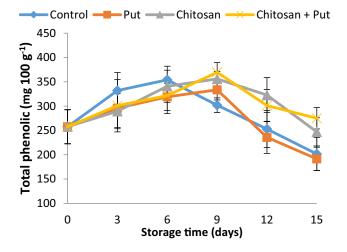


Fig. 3 Effect of PUT and chitosan coating on total phenolic content of strawberries during storage

tained higher anthocyanins than the control samples at the end of storage.

### **Total Phenolic Contents**

Strawberry constitutes one of the major sources of polyphenols among berries (Aaby et al. 2007). In the study, phenolic compounds of strawberries are presented in Fig. 3 and were 257.6 mg 100 g<sup>-1</sup> at harvest. Total phenolic content significantly increased during 9 days of storage except for control and subsequently decreased over the storage time in all treatments, with a slow decrease recorded with coated fruits. During storage, process of senescence, solubilisation of cell wall pectic substances and microbial infestation result in subcellular decompartmentation, disruption of membrane integrity and oxygen penetration, thereby leading to enhanced activity of polyphenol oxidase (PPO) responsible for oxidation of phenols (Hussain et al. 2012). Decreases in the activity of PPO and enhances the phytochemical content after chitosan treatment has been reported for strawberry (Eshghi et al. 2014; Petriccione et al. 2015). Nevertheless, the decrease in phenolic content in longer storage period might be due to break down of cell structure released phenolics to be exposure to enzymatic oxidation (Khalifa et al. 2016). At the end of the storage, the lowest total phenolic content was determined in PUT (191.5 mg 100 g<sup>-1</sup>) followed by control fruits (201.5 mg 100 g<sup>-1</sup>), while the highest total phenolic content was determined in chitosan + PUT treatment (275 mg 100 g<sup>-1</sup>) followed by chitosan treatment (247 mg 100 g<sup>-1</sup>). The result demonstrated that combined treatment was beneficial to maintain phenolic content. As a result of increased weight loss and excessive maturation at the end of storage, the amount of phenolic content in control and alone PUT treated strawberries sharply reduced. Similar results have been mentioned that fruits showed a decrease in phenolic compounds due to excessive maturation (Nunes et al. 2005; Gol et al. 2013).

### **Antioxidant Content**

Antioxidant content was significantly affected by PUT and chitosan coating treatments (Fig. 4). In general, antioxidant contents increased and then decreased during storage in according with phenolic compound behavior except for chitosan + PUT treatment. Previous studies indicate a linear correlation between phenolic content and antioxidant capacity of fruits (Razzaq et al. 2014; Hosseini et al. 2018). Wang and Gao (2013) reported a positive effect of chitosan-based edible coating on antioxidant activity of strawberry fruit. High values of antioxidant content observed in the berries treated with chitosan + PUT could be attributed to the high level of phenolic compounds. At the end of the storage, chitosan + PUT coated strawberries had higher an-

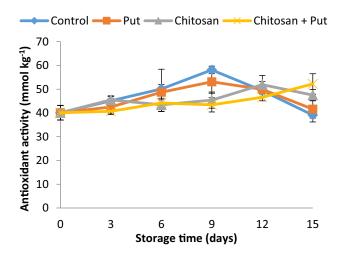


Fig. 4 Effect of PUT and chitosan coating on antioxidant activity of strawberries during storage

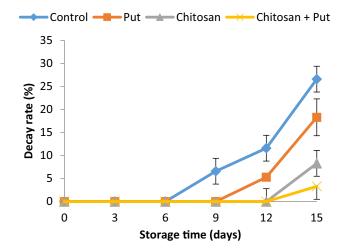


Fig. 5 Effect of PUT and chitosan coating on decay rate of strawberries during storage

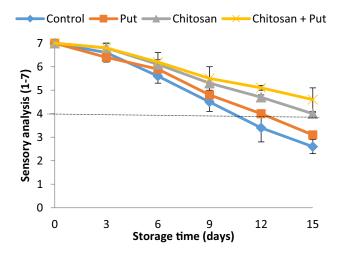


Fig. 6 Effect of PUT and chitosan coating sensory analysis of strawberries during storage

tioxidant content than fruits coated with single chitosan, which indicated that adding PUT into chitosan coating also showed synergistic effect on improving antioxidant systems. These results are in accordance with the findings of Razzaq et al. (2014) and Davarynejad et al. (2013) who showed the beneficial effects of Putrescine in maintaining antioxidant content stored mango and apricot. At 15th day of storage, the highest antioxidant content was determined in chitosan+PUT treatment (52.2 mmol kg<sup>-1</sup>), while the lowest antioxidant content was determined in control fruits (39.1 mmol kg<sup>-1</sup>). This result suggests that breakdown of cell structure due to increase in decay rate could lead to a fast decrease in antioxidant content of control fruits. Wang et al. (2007) also reported that high antioxidant activity is important in scavenging reactive oxygen species and protecting cellular constituents from oxidative damage, thereby strengthening the defensive system in the tissues against microbial invasion and reducing the spoilage of fruit.

# **Decay Incidence**

Although physicochemical characteristics play a large role in the consumer acceptance and quality evaluation of strawberry, decay is a major concern for supply chain operators and the main cause of postharvest losses (Alceo 2016). In the study, decay incidence of strawberries coated with chitosan and chitosan+PUT was lower than that control and PUT treated fruits during storage (Fig. 5). The capacity of chitosan coatings to inhibit the growth of several fungi has been shown for an extensive variety of harvested fruits and vegetables (Han et al. 2004; Hernandez-Munoz et al. 2008; Bal 2013; Eshghi et al. 2014). El Ghaouth et al. (1991) also suggested that chitosan induces chitinase, as defense enzyme catalyzes the hydrolysis of chitin, preventing or delayed fungi growth on fruit surface. Results showed that decay was observed for the first time after 9d of storage in control fruits, after that, decay incidence increased as storage time progressed. At the end of the storage, control fruits had the highest decay rate (26.6%) followed by PUT treatment (18.3%), while strawberry treated with chitosan + PUT (3.3%) had the lowest decay rate followed by chitosan treatment (8.3%). Fungal infection of Putrescine-treated fruits was also less than control treatments indicating the role of Putrescine in controlling fungal infection. Indeed, its main effectiveness was observed in combination application. This suggests that Putrescine could enhance the antimicrobial properties of chitosan. Khosroshahi et al. (2007) and Bal et al. (2017) also reported that the Putrescine treatment suppressed decay development significantly in strawberries and grapes during storage.

# **Sensory Analysis**

Strawberries are highly perishable products that lose sensory quality shortly after harvest. In general, sensory quality decreased during storage according to Fig. 6. The loss of strawberry quality was due to weight loss, color changes and high incidence of decay, which all lead to shelf-life reduction. The sensory attributes evaluated for strawberries were acceptable (over 4 point) in all applications until day 9 of storage. On the 12th day of storage, only control strawberries fell below the limit of acceptability. At the end of the storage, only chitosan + PUT and chitosan treated fruits were marketable. These treatments dramatically improved the sensory quality of fruits due to the greater protection of visual appeal, colour and visible structural integrity. However, the addition of PUT in chitosan coating was more beneficial in maintaining higher overall acceptability of treated strawberries compared other treatments during storage. Similar results were observed in a study by Shiri et al. (2013) in which grape treated with composite coatings of Putrescine and chitosan were superior in overall acceptability.

# Conclusion

The results of the present study indicate that chitosan coating alone and chitosan coating with PUT treatments have a significant beneficial impact on reducing weight loss, delaying fungal growth and maintaining higher overall acceptability. Chitosan edible coating enriched with PUT treatment is a more effective way to retard postharvest strawberries ripening process demonstrated by reducing physiological changes and maintaining higher phytochemicals. In conclusion, chitosan coating treatment combined with PUT showed promising results for maintaining 'San Andreas' strawberries quality and extending storage life at 1 °C for 15 days.

**Conflict of interest** E. Bal and B.A. Ürün declare that they have no competing interests.

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