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Aloe vera coatings maintain antioxidants of fig (*Ficus carica* L.) fruit during storage

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Abstract: Demand for fig fruit is increasing because of its high antioxidant capacity and health benefit for human. So, interest in maintaining bioactive components of fruit has been increased. However, antioxidant capacity of fruits decreases during storage. Fresh fruits of the well-known fig (*Ficus carica* L.) cultivar 'Siyah' were grown in Fars Province, Iran, treated with different concentrations of Aloe vera gel prior to being placed into cold storage. We examined fruit for firmness, soluble solids contents, anthocyanin concentrations, total phenolic compounds, flavonoid concentrations, ascorbic acid content, antioxidant activity, phenylalanine ammonia-lyase and superoxide dismutase activities. Figs coated with *Aloe vera* gel maintained higher firmness, anthocyanin concentrations, total phenolic compounds, flavonoid concentrations, ascorbic acid content, and antioxidant capacity than the control. Phenylalanine ammonia-lyase and superoxide dismutase activities were enhanced with Aloe vera gel treatments. The results showed that Aloe vera gel treatments could be an alternative to the current chemical protocols for preserving nutraceutical traits of fig fruit by maintaining antioxidant capacity during storage.

1. Introduction

Fig fruit is an important part of the Mediterranean food and is rich in fibers and antioxidant compounds (Arvaniti *et al.,* 2019). Antioxidant compounds are rich in fruits like fig and can prevent free radical formation. Foods which are rich in phytochemicals decrease incidence of disease and maintain body healthful (Singh, 2016). For this reason, it is important to use these phytochemicals in our diet (Singh, 2016). It has been detected that anthocyanin intake can prevent heart diseases and promote anti-carcinogenic and hypoglycemic activities. Thus, high content of anthocyanin has made fig attractive for consumers. Also, figs con-

tain high level of flavonoid which reduces oxidative stress (Reyes-Avalos *et al.,* 2016). Delicate epidermal tissue, climacteric behavior and extensive softening make fig fruit susceptible to the wounding and spoilage which limit shelf life of fruit (Bahar and Lichter, 2018). Also, it has been confirmed that antioxidant activity of fruits decreases during storage (Madani *et al.,* 2016). Therefore, maintenance of antioxidants of fig fruit during storage is important, because of their health benefits for human.

Several strategies have been implemented to maintain bioactive compounds of fruit during storage. Among them, coatings are popular because of their biodegradable and non-toxic materials (Reyes-Avalos *et al.,* 2016; Allegra *et al.*, 2017). They provide a barrier against gas transfer, which delays ripening and improves taste and texture (Khaliq *et al.,* 2019). The Aloe vera gel compounds are mainly polysaccharides, minerals, sugars, vitamins, and antioxidant agents like phenolic compounds (Rasouli *et al.,* 2019). Application of Aloe vera gel has received increased attention by the food industry due to its effectiveness for increasing shelf-life of fresh products and increasing antioxidant activity (Sogvar *et al.,* 2016). Moreover, Aloe vera gel maintained antioxidant activity of button mushrooms (Mirshekari *et al.,* 2019) and sapota fruit (Khaliq *et al.,* 2019). However, few studies have addressed the effects of edible coatings on quality of fig under cold storage conditions. Application of alginate-chitosan decreased fungal contamination and increased firmness of fig fruit under cold storage (Reyes-Avalos *et al.,* 2016).

Thus, the objective of this study was to determine the effects of Aloe vera gel application at different concentrations for maintaining phytochemicals of fig fruit during cold storage. We examined several beneficial phytochemicals (anthocyanin, total phenolic compound, total flavonoid concentration, and ascorbic acid), antioxidant activity, and phenylalanine ammonia-lyase and superoxide dismutase activities.

2. Materials and Methods

Plant materials and treatments

Mature harvested figs (*Ficus carica* L.), cv. Siyah (black, firm with 13% soluble solids content) grown in Fars Province, Iran, were transported to the Faculty of Agriculture of Yasouj University. Mature leaves of greenhouse-grown Aloe vera plants were excised. The leaf matrix was detached from the outer cortex, and then the hydroparenchyma was mixed in a blender. The mixture was filtered through cheesecloth to remove the fibers, and the filtrate constituted fresh Aloe vera gel (Sogvar *et al.,* 2016). Defect-free fruits with uniform size and color were divided into four groups, each group received one of four treatments: 1) control (0%), 2) Aloe vera gel 1:3 (25%), 3) 1:1 (50%), and 4) 3:1 (75%). After that, all fruits were placed into plastic trays (285 x 125 x 65 mm) over-wrapped with plastic films [0.02 mm-thick polyvinyl chloride (PVC)]. Trays with fruit were stored at 2±1°C and 85-90% RH for 15 days (cold storage). Analyses were performed after 0, 3, 6, 9, 12, and 15 days of cold storage.

Firmness, soluble solids (SSC) and ascorbic acid content

A digital fruit hardness tester (STEP Systems GmbH, Germany) with a 5-mm diameter probe was used to determine fruit firmness (expressed in Newtons (N). Five g of the homogenate of a composite sample using a kitchen blender (Nu-777, Nautiunl, Japan) with 40 mL of distilled water was used for analyses of chemical parameters (Ranganna, 1986). Then samples filtered through cotton wool. SSC was measured with a handheld refractometer (Atago-Pal1, Tokyo, Japan) and expressed as percent. Ascorbic acid content was determined using the 2, 6-dichlorophenolindophenol dye titration method described by Mirshekari *et al.* (2017). Five g of fruit tissues homogenized in 90 ml of 3.0% metaphosphoric acid solution. An aliquot of the sample (10 ml) was titrated against 2, 6-dichlorophenolindophenol dye until a pink color persisted for 15 s and results expressed as mg ascorbic acid per 100 g of fresh weight (FW).

Anthocyanin and flavonoid concentration (FC)

Anthocyanin content in fruit samples was measured by the pH differential method described by Hassanpour (2015). Two g of samples was added to the 20 ml of methanol containing HCl (1%). The mixture was centrifuged at 17,000 g for 15 min at 4 °C. Absorbance of supernatant was measured in a spectrophotometer (Shimadzu, USA) at 530 and 700 nm in buffers at pH 1.0 and 4.5, using following formula:

$$A = [(A_{530}-A_{700})\ pH_{1.0} - (A_{530}-A_{700})\ pH_{4.5}]$$

Results were expressed as mg of cyanidin-3-O-glucoside equivalents per 100 g of FW.

FC was measured according to the method described by Saba and Sogvar (2016). Extraction from four g of the sample was done using 50 ml methanol. One mL aliquot of catechin standard solution (0-100

mg L^{-1}) or samples were added to 10 mL volumetric flasks containing 4 mL water. Initially 0.3 mL of 5% $NaNO_2$ was added to the flask, following 0.3 mL of 10% $AlCl_3$ was added after 5 min, and then 2 mL of 1 M NaOH was added to the mixture. Immediately, the solution was diluted to a final volume of 10 mL with water and mixed thoroughly. The absorbance was measured at 510 nm, using a spectrophotometer (UV/Vis Perkin Elmer, Lambda EZ201, USA) and result was expressed as mg catechin equivalents per 100 g of FW.

Total phenolic compounds (TPC) and antioxidant activity

Fruit extract was prepared using the method described by Ong *et al.* (2013). Fruit tissue (2 g) was homogenized in a glass tube with 10mL of methanol (80%). The mixture was then incubated at 45°C for 1 h. For TPC measurement 0.1 mL of the crude extract solution was placed in a test tube and 0.1 mL distilled water in a test tube served as the control (blank). Then six millilitres of water was added to the sample and blank. After that, 0.5 mL undiluted Folin-Ciocalteu reagent was added to the mixtures. Between 30 s and 8 min later, 1.5 mL saturated sodium carbonate was added. Then 1.9 mL water was added to the solutions to give a final volume of 10 mL and the mixture vortexes and incubated for 2 h at 35°C. The absorption of TPC was determined at 765 nm using a spectrophotometer. A calibration standard curve was established using gallic acid. TPC was determined against the standard gallic acid calibration curve and the absorbance value was converted to mg of gallic acid equivalents (GAE) per 100 gram of fresh weight (mg GAE 100 g^{-1} FW) (Ong *et al.,* 2013).

Antioxidant capacity (ferric reducing antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC) assays were determined spectrophotometrically, according to Benzie and Strain (1996) and Kerem *et al.* (2006), respectively. The results were expressed as Fe^{2+} equivalents mM kg^{-1} for FRAP and µM trolox equivalents for TEAC in 100 g of FW.

Phenylalanine ammonia-lyase (PAL) and superoxide dismutase (SOD) activities

Fig samples were frozen in liquid nitrogen and then stored at -80°C until analysis. Each frozen sample (10 g) was ground with a mortar and pestle and used to determine PAL and SOD activity. Two g of samples was homogenized in a 4 mL solution containing 0.05 mol L^{-1} Tris-HCl buffer (pH =7.5), 3 mmol L^{-1} $MgCl_2$ and 1 mmol L^{-1} EDTA at 4°C. The homogenate was then centrifuged at 25,000 g for 20 min at 4°C and the supernatant was used as the crude extract for SOD and PAL assays (Maghoumi *et al.,* 2013). Measurement of PAL activity was performed at 290 nm, according to the method described by Aghdam *et al.* (2012). PAL activity was evaluated as nM cinnamic acid h^{-1} mg^{-1} protein. The procedure for assay of SOD was performed using methods described by (Maghoumi *et al.,* 2013). SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium. The absorbance by the reaction mixture was read at 560 nm. Concentration of protein of the extracts was determined according to Bradford (1976) with bovine serum albumin as a standard. Enzyme activity was expressed as unit mg^{-1} protein.

Sensory evaluation

Texture, taste and overall quality of samples analyzed by ten trained panelist after 15 days at cold storage (2±1°C) and transferring fruits to the room temperature for sensory analysis. The sensory were evaluated using a hedonic scale 1-5, where 1= very poor, 2= poor, 3= fair, 4= good and 5= excellent.

Experimental design and statistical analysis

All experiments were conducted within a completely randomized design (CRD). Data were pooled before analysis and the whole experiment was repeated three times. In each biological and technical experiment 328 fruit were used. There were four replicates per treatment in each experiment. Data were subjected to analysis of variance using the Statistical Analysis System (SAS, ANOVA procedure) version 8.2 (SAS Institute Inc., Cary, NC, USA). The means were compared with the Duncan's Multiple Range Test (DMRT) at significance level of 0.05.

3. Results and Discussion

Firmness, SSC and ascorbic acid content

Firmness is the key factor of quality and which changes during ripening (Madani *et al.,* 2014). Fruit firmness was reduced during storage irrespective of treatment, but Aloe vera gel treated fruits softened more slowly (Fig. 1A). Short postharvest life of fig fruit is because of softening and epidermal cracking (Villalobos *et al.,* 2016). Aloe vera film acts as a barrier which prevents O_2 uptake on fruit, thereby decreases softening and ripening processes (Hassanpour, 2015). Moreover, polygalacturonase and pectin methylesterase activity increases during ripening, and this causes fruit softening (Madani *et*

al., 2014). The results are comparable with Reyes-Avalos *et al.* (2016) who indicated that alginate-chitosan coating could maintain firmness of fig fruit during storage. Aloe vera gel might decrease polygalacturonase and pectin methylesterase activity and thereby maintain firmness of fig fruit during cold storage.

There were significant differences in the SSC among treatments. SSC of control fruits increased during storage (Fig. 1B). However, Aloe vera gel treated fruits had the lowest SSC compared to the control during cold storage. The increase in SSC might be related to the solubilization of polyuronides and hemicelluloses of fruit cell walls and hydrolysis of insoluble polysaccharide into simple sugars (Tanada-Palmu and Grosso, 2005). These results are comparable with Rasouli *et al.* (2019) and (Martínez-Romero *et al.,* 2017) who mentioned that Aloe vera gel reduced the SSC of orange and plum fruit, respectively. Aloe vera gel might decrease respiration rate and SSC in fig fruit.

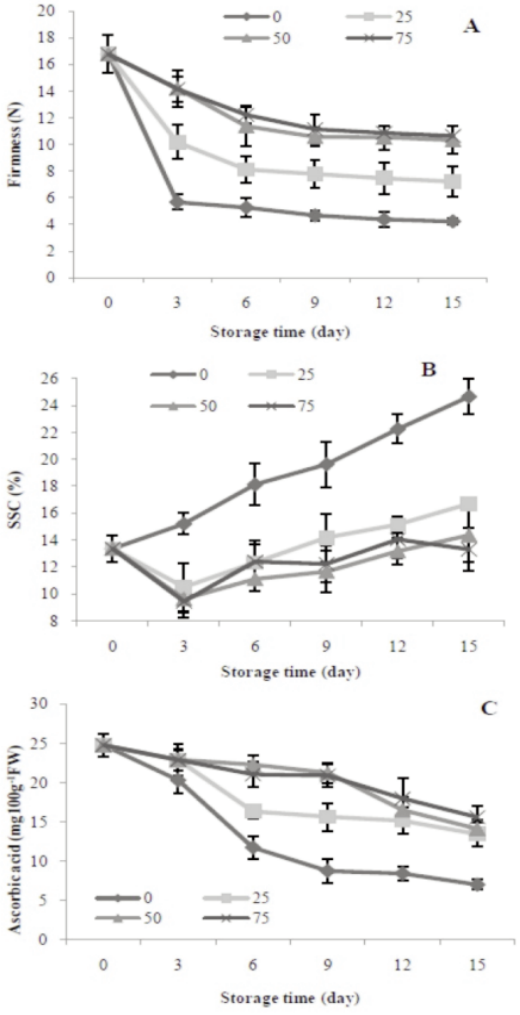

Fig. 1 - Firmness (A), soluble solid concentration (SSC) (B) and ascorbic acid content (C) values in figs (*Ficus carica* L.) 'Siyah' fruit treated with Aloe vera gel (0, 25, 50 and 75%). Fruit were stored at 2°C. Vertical bars represent standard error of means of three experiments with four replicates per experiment.

During cold storage, ascorbic acid content of control fruit decreased from 24.75 mg per 100 g of FW to 7 mg per 100 g of FW (Fig. 1C). However, Aloe vera gel treated fruits maintained ascorbic acid content relative to the control. Ascorbic acid is the most important antioxidant which decreases the damage of ROS (Rasouli *et al.,* 2019). Autoxidation causes ascorbic acid losses during storage when combines with oxygen in the air (Baraiya *et al.,* 2015). Aloe vera gel might causes a barrier layer for gas and decreases oxidation of ascorbic acid which caused by ascorbate oxidase enzyme in the presence of oxygen (Sogvar *et al.,* 2016). Since ascorbic acid has beneficial effect on human health, the positive effects of Aloe vera gel on maintaining ascorbic acid content of fig fruit can be interested for nutraceutical purposes.

Anthocyanin, FC, TPC and antioxidant capacity

Fig fruit is rich in phenolic compounds, which are responsible for antioxidant activity (Ercisli *et al.,* 2012). Anthocyanin of non-treated fruits at harvest and after 15 days of cold storage was 11.12 and 21.45 of mg cyanidin-3-O-glucoside equivalents per 100 g FW, respectively. (Fig. 2A). Aloe vera gel 50% and 75% treatments increased anthocyanin after 15 days at cold storage relative to the control. (Fig. 2A). Moreover, FC of Aloe vera gel treatments was significantly higher than control during cold storage (Fig. 2B). TPC decreased during cold storage regardless of treatments; but TPC of treated fruits were significantly higher than that of non-treated fruits (Fig. 2C). From a biological and nutritional perspective, the antioxidant capacity of anthocyanin is important (Wang *et al.,* 1996); therefore, maintaining anthocyanin is potentially beneficial. It has been also reported that strawberries, and bush blueberry treated with chitosan maintained higher levels of anthocyanin (Wang and Gao, 2013; Chiabrando and Giacalone, 2015).

Flavonoids are water soluble polyphenolic molecules which have health promoting effects like antioxidants, radical scavengers, anti-mutagenic, anti-inflammatory, anti-carcinogen, and anti-depressant (Singh, 2016). Moreover, with their antioxidant activity, they increase shelf life of fruits and vegetables (Ververidis *et al.,* 2007). Nair *et al.* (2018) showed higher FC in guava fruit treated with chitosan coatings. Also, (Khaliq *et al.,* 2019) indicated that

Aloe vera gel treated sapodilla fruits had higher FC.

Phenols are one of the most important antioxidant compounds of fruits and vegetables. The application of coatings like Aloe vera gel might delay senescence and decrease TPC loss during storage (Rasouli *et al.,* 2019). Increase in anthocyanin, FC and TPC of fruit treated with Aloe vera gel is comparable to those reported previously by Hassanpour (2015) for raspberry fruit treated with aloe vera gel. This may be related to the persistent biosynthesis of anthocyanin, flavonoids and TPC after harvesting. Also, enzymes which are involved in biosynthesis process of TPC, flavonoids and anthocyanin like PAL might be up regulated with Aloe vera gel treatment. The higher nutraceutical compounds detected in loquat fruits coated with chitosan could be related to the lower ROS due to the long-time physiological stress of storage (Petriccione *et al.,* 2015). Therefore, Aloe vera treated fruit might have a protective effect on nutraceutical compounds in delaying their oxidative processes and bio-transformation during storage.

Figs treated with Aloe vera gel had higher antioxidant activity during cold storage, which indicated by TEAC and FRAP. However, antioxidant activity of control fruit decreased during storage (Fig. 3A-B). Serrano *et al.* (2006) observed that Aloe vera gel treatment increased antioxidant activity in grape. It was supposed that antioxidant activity of Aloe vera gel is related to aloe-emodin, a hydroxyanthraquinone present in Aloe vera gel leaves and extracts (Serrano *et al.,* 2006). Therefore, higher antioxidant capacity observed in Aloe vera gel treated fig fruit could be related to the biochemical components of Aloe vera extract. Association was stated between the bioactive compounds and antioxidant activity, with the coated samples recording higher antioxidant activity (Anraku *et al.,* 2011).

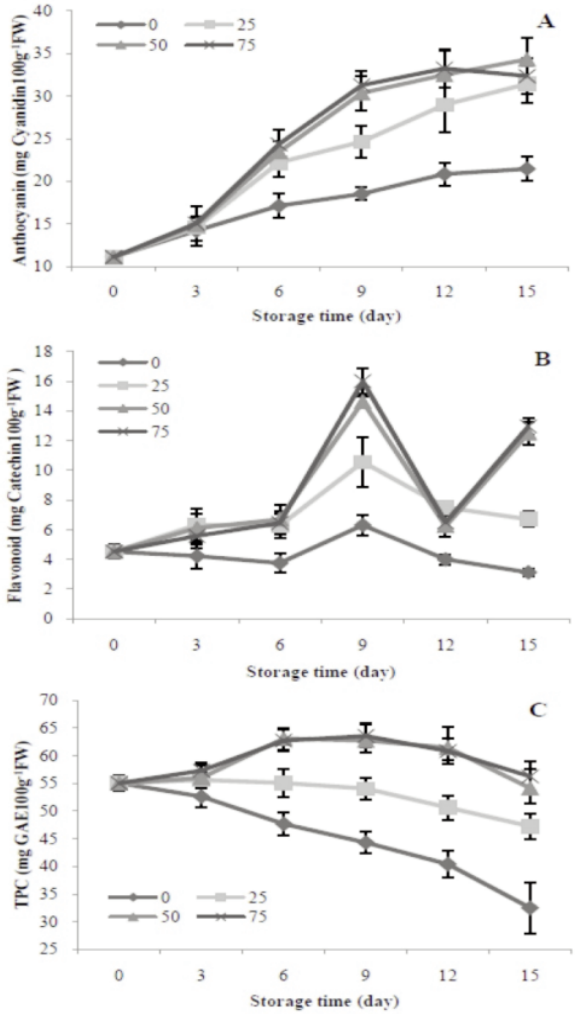

Fig. 2 - Anthocyanin (A), flavonoid (B) and total phenolic concentrations (TPC) (C) values in figs (*Ficus carica* L.) 'Siyah' fruit treated with Aloe vera gel (0, 25, 50 and 75%). Fruit were stored at 2°C. Vertical bars represent standard error of means of three experiments with four replicates per experiment.

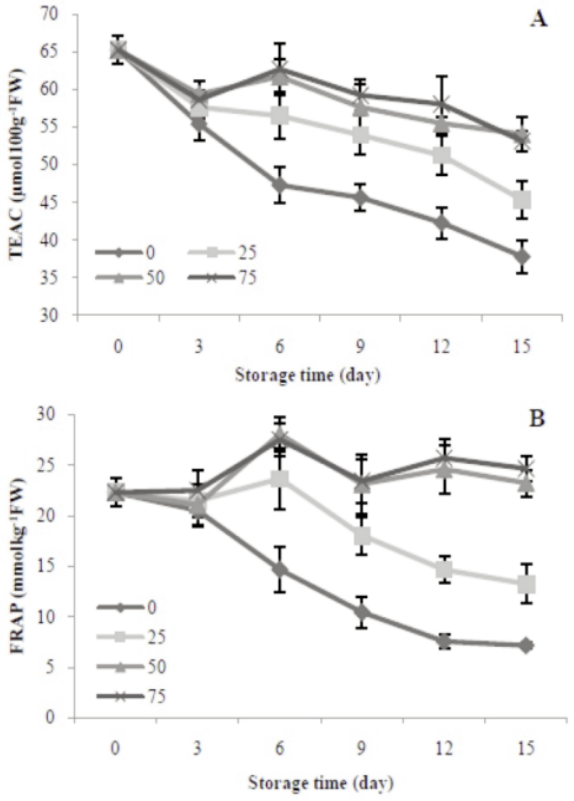

Fig. 3 - Antioxidant capacity with trolox equivalent antioxidant capacity (TEAC) (A) and ferric reducing antioxidant power (FRAP) (B) in figs (*Ficus carica* L.) 'Siyah' fruit treated with Aloe vera gel (0, 25, 50 and 75%) during cold storage at 2°C. Vertical bars represent standard error of means of three experiments with four replicates per experiment.

PAL and SOD

PAL activity in treated fruits increased during storage when compared with the control (Fig. 4). This indicates that Aloe vera gel treatment activated enzymes that are important in biosynthetic pathways of secondary metabolites of fruit. PAL is the first enzyme in phenylpropanoid pathway which catalyzes conversion of phenylalanine to trans-cinnamic acid and plays an important role in phenolic compounds biosynthesis (Hassanpour, 2015). PAL connects primary metabolism (shikimic acid pathway) to secondary metabolism (phenylpropanoid pathway) (Razavi and Hajilou, 2016). The results are comparable with Hassanpour (2015) who demonstrated that PAL activity in raspberry fruit increased when treated with Aloe vera gel. We suggest that Aloe vera gel treatment might be an efficient strategy for maintaining phenolic content in fig fruit via activation of PAL.

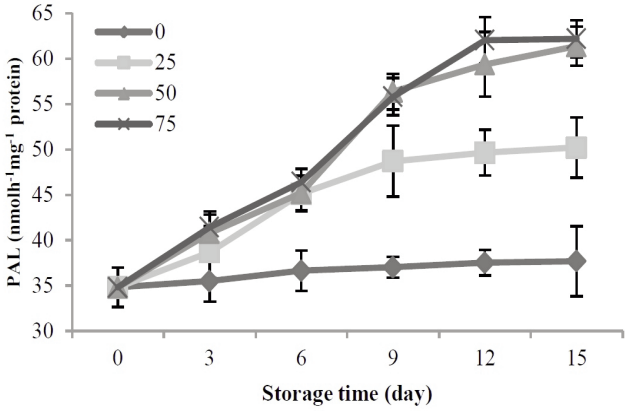

Fig. 4 - Phenylalanine ammonia-lyase (PAL) activity in figs (*Ficus carica* L.) 'Siyah' fruit treated with Aloe vera gel (0, 25, 50 and 75%) during cold storage at 2°C. Vertical bars represent standard error of means of three experiments with four replicates determinations per experiment.

SOD activity of non-treated figs was reduced during storage. However, SOD activity increased from day 3 to 6 in cold storage and decreased afterward when treated with Aloe vera gel (Fig. 5). Superoxide dismutases (SODs) which are metalloenzymes, are believed to play a crucial role in antioxidant defense because they catalyze the dismutation of O_2^- to H_2O_2. However, defensive action of SOD against O_2^-shows age-related changes. Higher SOD activity of Aloe vera coating treatments have been associated with cold storage stress tolerance fruit because it neutralizes the reactivity of the superoxide radical, which is over produced under stress (Bowler *et al.,* 1992). These results are comparable with Sun *et al*. (2010) who reported that SOD activity in litchi fruit treated with chitosan was higher than control fruit. These results have suggested that Aloe vera gel treatment might maintain TPC, FC and anthocyanin of fig fruit by increasing activity PAL and SOD enzymes.

Sensory evaluation

According to the judges, postharvest Aloe vera application did not have any negative effect on texture, taste and overall quality of fig (Fig. 6). The positive effect has an important role for consumers to buy the fruit. These results are in agreement with Song *et al.,* 2013 in which Aloe vera gel coated fresh cut apple had higher score than un-coated fruits.

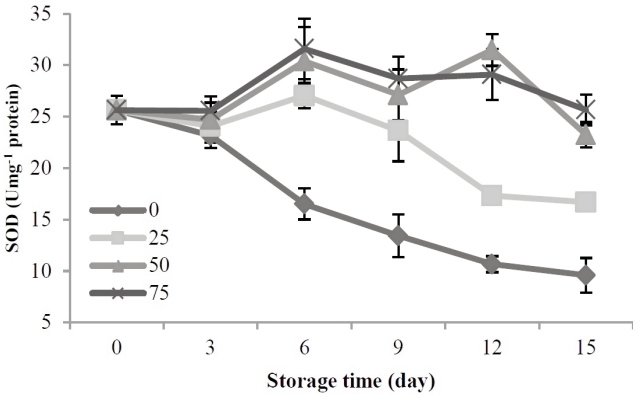

Fig. 5 - Superoxide dismutase (SOD) activity in figs (*Ficus carica* L.) 'Siyah' fruit treated with Aloe vera gel (0, 25, 50 and 75%) during cold storage at 2°C. Vertical bars represent standard error of means of three experiments with four replicates determinations per experiment.

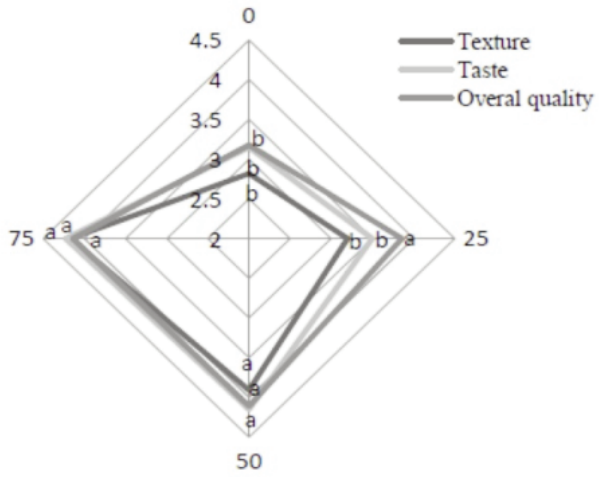

Fig. 6 - Sensory evaluation in figs (*Ficus carica* L.) 'Siyah' fruit treated with Aloe vera gel (0, 25, 50 and 75%) after transferring from 15 days in cold storage to the ambient temperature.

Thus, these results revealed that Aloe vera coting could decrease the loss of the sensory characteristics of fruit.

4. Conclusions

This research indicated that Aloe vera gel plays a positive role in maintaining TPC, anthocyanins, FC and antioxidant than control fruit. We conclude that Aloe vera gel treatment could be a useful alternative to the current chemical protocols for preserving nutraceutical traits of fig fruit by maintaining antioxidant capacity during storage.

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