

Extending Postharvest Longevity and Improving Quality of Strawberry (*Fragaria Ananasa* Duch Cv. 'Gaviota') Fruit by Postharvest Salicylic Acid Treatment

Mahsa Geransayeh (Corresponding author)

Department of Horticulture, College of Agriculture and Natural Resources

Science and Research Branch, Islamic Azad University Tehran, Iran

E-mail: m.geransayeh@yahoo.com

Sadegh Sepahvand, Vahid Abdossi

Department of Horticulture, College of Agriculture and Natural Resources

Science and Research Branch, Islamic Azad University Tehran, Iran

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Abstract

Strawberries are an extremely perishable fruit mainly due to their soft texture and sensitivity to fungal infection. Postharvest application of conventional fungicides to fruits is prohibited. As an alternative to fungicides, salicylic acid has been found to enhance disease resistance of horticultural crops. In order to study the effect of salicylic acid as a phenolic compound on the postharvest durability and quality characteristics of strawberry fruit. 'Gaviota' strawberries were treated with SA at different concentrations (0, 25, 50 and 100 μL^{-1}), then stored for 12 days at 4 °C and 75 % RH in darkness. Two different methods were applied (spray SA on fruits and paper disk method). Quality attributes such as weight loss, pH, TA, TSS, vitamin C, anthocyanin, calcium, pectin, CAT, POD, PG activity, decay percentage and sensory analyses evaluated every 3 days during storage. Results showed that, treated fruits with SA had lower weight loss, pH, TSS, POD, PG, decay and higher TA, vitamin C, anthocyanin, calcium, pectin, CAT and fruit quality compared with controls. Between two methods of treatment, paper disk method had higher effect on fruit decay and quality compared to spray method and as a general result, caused longer storability.

Keywords: Postharvest durability, Salicylic acid, Enzyme activity, Fruit quality.

1. Introduction

Strawberry fruit quality decrease rapidly after harvesting, due to the high metabolic activity (Lolaei *et al.*, 2012). Because of the harmful effects of chemical fungicides on human health and the environment, developing safe and nonchemical compounds are highly needed (Mandal *et al.*, 2009). Salicylic acid or ortho-hydroxybenzoic acid is one of the effective natural phenolic compounds has been extensively used for quality improvement in a number of crops and as a plant growth regulator can enhance disease resistance of plants (Pen and Jiang, 2006). SA is considered as generally recognized as safe (GRAS) (Hooper and Cassidy, 2006). Lolaei *et al.* (2012) were studied the effect of SA as postharvest treatment on the strawberry fruit quality. Treated fruits had higher TA, vitamin C and redness and less weight loss, decay than the control and delays the ripening of strawberry fruit. Application of SA at nontoxic concentrations to susceptible fruits and vegetables could enhance resistance to pathogens and control postharvest decay of crops (Asghari *et al.*, 2009; Asghari *et al.*, 2007; Babalar *et al.*, 2007). Methyl salicylate (MeSA) vapor was significantly affected postharvest decay of Hayward kiwifruit during storage period (Soleimani Aghdam *et al.*, 2009). Treatment of pear fruit in 1 mmol L⁻¹ SA solution effectively controlled fruit decay during 5 months of storage (Asghari *et al.*, 2007). Postharvest treatment of strawberry fruits with 1 and 2mmolL⁻¹ significantly controlled fruit decay and increased shelf life of fruit (Babalar *et al.*, 2007). Treatment of table grapes with SA before coating with chitosan effectively enhanced the efficacy of coating and decreased fruit total decay (Asghari *et al.*, 2009). Postharvest treatment of grapes with SA had a positive effect on hardness, appearance of fruit and controlled fungal infections compared with control (Duan *et al.*, 2007). The application of 2 mμ SA effectively increased antioxidant compounds, ascorbic acid content and TSS and prevented fungal infection of strawberries (Amborabe *et al.*, 2002) and softening of bananas and kiwifruits at maturity stage (Srivastava and Dwivedi, 2000; Wang and Zheng, 2001). SA significantly reduced the quality loss in peaches (Wang *et al.*, 2006), tomato (Ding *et al.*, 2001), sweet peppers (Fung *et al.*, 2004), and loquat fruits (Cai *et al.*, 2005). The aim of the present study was to evaluate the effects of postharvest salicylic acid application on quality parameters during cold storage and storage life of strawberry fruit.

2. Materials and Methods

2.1 Plant Material

strawberries (*Fragaria ananassa* L.), cv. 'Gaviota' were harvested in the morning randomly from a commercial greenhouse located in Hashtgerd, Karaj, Iran at commercial maturity stage and transported to the laboratory where undamaged fruits at the same ripening stage (80% of the skin red) were selected. Samples were taken every 3 days up to the end of experiment (12 days) and fruits were evaluated at each sampling time after keeping them a day at room temperature.

2.2 SA Treatment

Treatments with SA were performed by dissolving the requisite amounts of SA (0, 25, 50 and

100 μL^{-1}) in ethanol. Two different methods were applied (spray SA on fruits and paper disk method). SA sprayed on fruits and spotted onto filter paper at the final concentration of control, 25, 50 and 100 μL^{-1} then air dried. Each treatment was replicated three times with 150 g fruits per replicate. All packages were stored at 4 °C and 75 % RH in darkness for 12 days. Measurements were made at room temperature every 3 days. Quality attributes such as weight loss, pH, TA, TSS, vitamin C, anthocyanin, calcium, pectin, CAT, POD, PG activity, decay percentage and sensory analyses evaluated during storage.

2.3 Weight Loss Percentage

The effect of SA exposure on fruit weight loss was also investigated. Weight of individual fruits was recorded at the beginning of harvest and different sampling times and expressed as percentage of original weight (Saini *et al.*, 2006).

2.4 pH

pH was measured using a pH meter Metrohm Lab 827 (Saini *et al.*, 2006).

2.5 Titratable Acidity (TA)

Titration acidity was measured using titration method. To do that, 5 mL fruit juice was added to 25 mL distilled water plus two drops of phenolphthalein and titrated with 0.1N NaOH up to pH 8.1. The results were expressed as gram of citric acid per 100 g fresh weight (AOAC, 1990).

2.6 Total Soluble Solids (TSS)

TSS was determined using ATAGO-ATC-20E (Japan) refractometer at 20 °C and expressed as Brix.

2.7 Vitamin C Assay

The content of vitamin C was determined using indophenol procedure. 10 ml of samples were filtrated and titrated against sodium 2, 6-dichlorophenol indophenol dye to a faint pink color which persisted for 5-10 seconds. It was expressed as mg vitamin C/100g fruit weight (Titer \times dye equiv. \times dilution \times 100/ Wt. of sample) (Saini *et al.*, 2006).

2.8 Anthocyanin Assay

Total anthocyanin content of strawberry extract was measured using the pH differential method. Absorbance was measured at 510 and 700 nm, respectively, in different buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{\text{pH} 1.0} - (A_{510} - A_{700})_{\text{pH} 4.5}]$ with a molar extinction coefficient for cyanidin-3-glucoside of 29600. Results were expressed as milligrams of cyanidin-3-glucoside (C3G) equivalents per 100 g of fresh weight (Cheng & Breen, 1991).

2.9 Calcium Content

Calcium was precipitated as calcium oxalate. The precipitate was dissolved in hot dilute sulfuric acid and titrated with standard potassium permanganate. $1\text{ml} \cdot 0.1\text{N} \text{KMnO}_4 = 0.002 \text{ gm. Calcium}$ (Ruck, 1969).

2.10 Pectin Content

Pectin was precipitated as calcium pectate from an acid solution by the addition of calcium chloride. The calcium pectate precipitate was washed with water until chloride-free, then dried and weighed. Ca pectate (%) = wt. of Ca pectate \times 100 / wt. of sample (Ruck, 1969).

2.11 Peroxidase Activity

POD activity was assayed Spectrophotometrically with guaiacol by measuring an increase in absorbance at 470 nm ($\epsilon = 26.6 \text{ mM}^{-1}\text{cm}^{-1}$) according to Maehly and Chance (1954). The mixture of 0.5 cm³ of the enzyme extract, 0.5 cm³ of 50 mM acetate buffer (pH 5.6), 0.5 cm³ of 20 Mm guaiacol and 0.5 cm³ of 60 mM H₂O₂ was used. The enzyme activity was expressed in units (mmol tetraguaiacol min⁻¹) per g fresh weight.

2.12 Catalase Activity

CAT activity was determined at 25°C according to Aebi (Aebi, 1984). The reaction mixture contained 40 mM phosphate buffer pH 7.0 and 0.1 ml pure enzyme in a total volume of 3ml. CAT activity was estimated by decreased in absorbance of H₂O₂ at 240nm.

2.13 Polygalacturonase Activity

PG activity was determined by measuring reducing groups released from sodium polypectate, using D-galacturonic acid as the standard. The assay medium reagents were 0.2 M acetate buffer, pH 4.5 to the amount 0.2 ml, and 1% polygalacturonic acid in 0.05 M acetate buffer solution pH 4.5 to the amount 0.3 ml. One ml of enzyme solution and distilled water was added. The reaction started by adding the enzyme, and it was then left for 30 min at 37 °C, after which the reaction was stopped by adding 3, 5 – dinitrosalicylic acid (DNS). The solution was then boiled in water for 5 min, after which it was diluted and absorbance measured at a wavelength of 520 nm, using galacturonic acid (0–1mg/ml) as the standard solution (Miller, 1959). One unit of polygalacturonase activity (U/g) was defined as the amount of enzyme which released one mol of galacturonic acid per minute per gram of substrate.

2.14 Decay Percentage

Percent of decay was scored on a 1-5 scale, where: 1= intact fruit, 2= more than 5 % Decay, 3= between 5-20 % decay, 4= between 20-50 % decay, 5= more than 50% decay (Ayala-Zavala *et al.*, 2005).

2.15 Sensory Evaluation

Sensory analyses to compare the quality of treated and control fruits were carried out by a 10 trained adults aged 25-40 years. It was about aroma, taste, firmness, appearance and texture. Panelists scored fruits between 1-10. Ten being the best total quality and 1 being the worst (Hernandez-Munoz *et al.*, 2008). Samples were scored for overall quality by using an interval hedonic scale. Assessments were continued until fruits condition were considered unacceptable.

2.16 Statistical Analysis

Statistical analysis of the data obtained in the present study was carried out using split factorial method in a completely randomized design layout with 3 replications. Data obtained were subjected to analysis of variance (ANOVA).

3. Results and Discussion

3.1 Weight Loss

No significant differences in weight loss were observed among all of the SA treatment concentrations. SA treatment decreased weight loss of strawberries during storage for 12 days compared with controls. Between two methods of treatments fruits that treated with paper disk method (Figure 1) had more weight loss than spray method (Figure 2). SA can also decrease the respiration rate and fruit weight loss (Zheng and Zhang, 2004). Lolaei *et al.* (2012) reported that strawberry fruits dipped by SA had less weight loss and the greatest fruit weight loss was calculated in plants treated with 7mM. Besides, our results are in agreement with Garcia *et al.* (1995) who found similar results in strawberry fruits cv. ‘Tudla’ and Soleimani Aghdam *et al.* (2011) who stated that weight loss of the kiwifruit was significantly decreased when they were treated by methyl salicylate (MeSA). Weight loss in fruit decreased with increasing of MeSA concentration. Decrease in fruit metabolic activities results to decrease in fruit water content, weight loss, carbohydrate depletion rate and delay fruit senescence process (Wills *et al.*, 1998). Kazemi *et al.* (2011) reported that maximum weight loss occurred in control apples while lowest loss was recorded in 3 mM SA treatment. SA also decreases in respiration rate and fruit weight losses by closing stoma (Zheng & Zhang, 2004).

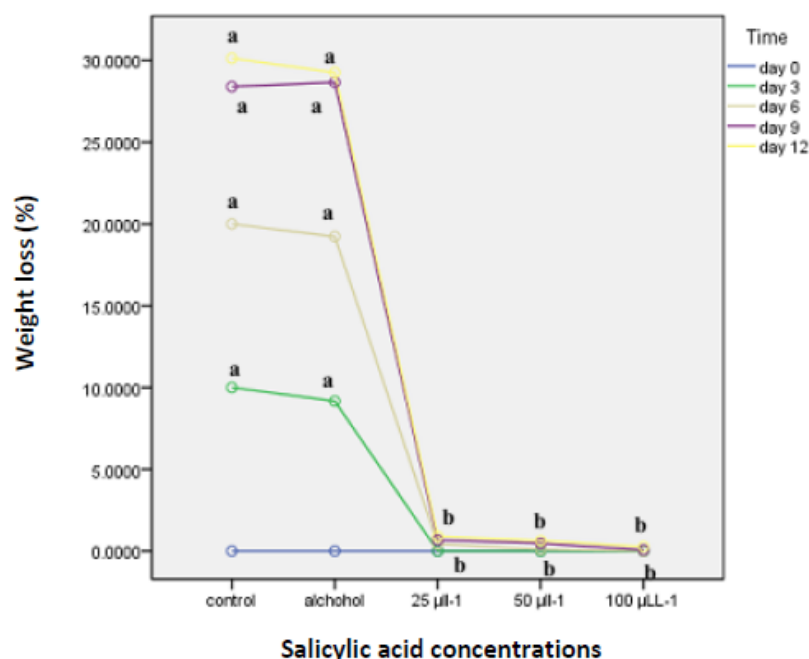


Figure 1. The effect of salicylic acid treatment on weight loss of strawberry fruit in paper disk method.

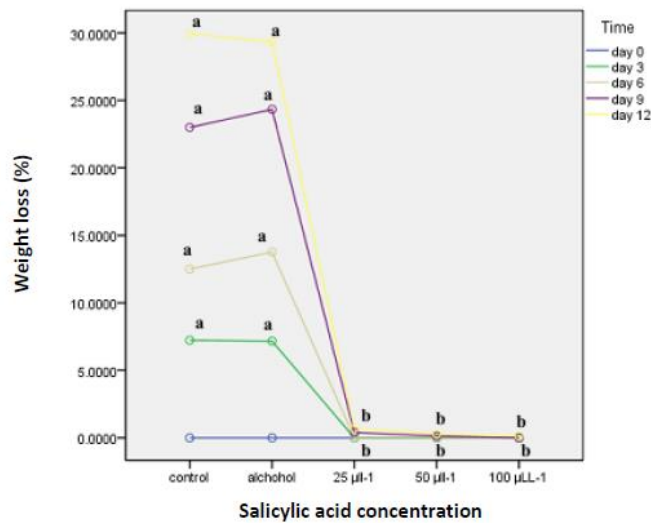


Figure 2. The effect of salicylic acid treatment on weight loss of strawberry fruit in spray method.

3.2 pH

The pH value of strawberry fruit increased slightly, corresponding to a decrease in TA during storage. Little difference in pH value was observed among all of the treatments. Control fruits had the most pH value and 100 µL⁻¹ concentration of SA had the least pH value (Figure 3). No significant differences were observed between methods of treatments application. Our results are in agreement with Soleimani Aghdam *et al.* (2011) who found that MeSA treatment significantly affected pH and pH of kiwifruit fruit juice increased after 3 months of storage but then decreased to end of storage. The fluctuations of pH might be due to the variations in TA or temperature of storage and the decline of acidity is attributed due to increased activity of citric acid content may be due to their conversion into sugars and further utilization in metabolic process during storage (Rathore *et al.*, 2007).

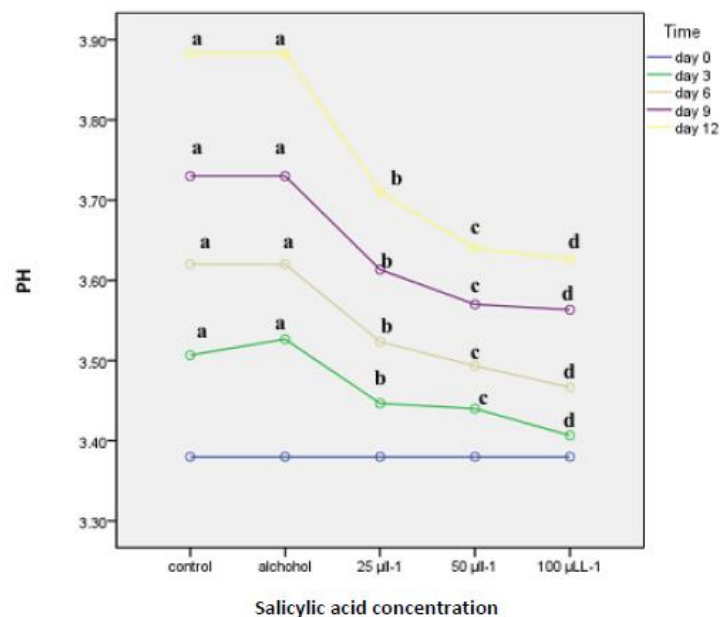


Figure 3. The effect of salicylic acid treatment on pH of strawberry fruit.

3.3 TA and TSS Content

SA treatment increased TA content and decreased TSS content in strawberries compared to controls. TA decreased gradually during storage. TA of 100 μL^{-1} concentration of SA was the highest among all SA concentrations (Figures 4 and 5). TSS of fruits increased during storage. Among all concentrations of SA 25 μL^{-1} had the lowest TSS amount (Figure 6). Fruits that treated with spray method had more TA content compared with paper disk method (Figures 4 and 5). Lolaei et al. (2012) found that postharvest SA treatments induced higher TA values than the control that was in agreement with our results. Lu et al. (2011) reported that postharvest treatment of strawberry with SA resulted in an increased TA of fruit. Also, Bal and Celik (2010) stated that TA content was lower in controls than other treated kiwifruits. TA is directly related to the concentration of organic acid present in the fruit which are an important parameter in maintaining the quality of fruits (Kazemi et al., 2011). Study of Lu et al. (2011) on pineapple fruit showed that the application of SA as postharvest treatment resulted in a decreased TSS. Shafiee et al. (2010) reported that postharvest treatment of strawberry with ASA resulted in a lower TSS than the control. Asghari (2006) reported that SA decreased TSS in strawberry cv. Selva and Lolaei et al. (2012) showed that treatment of kiwifruits with 32 mL^{-1} MeSA maintained a lower TSS content than the control fruits at the end of cold storage. These results are in agreement with our results. TSS content of fruits during storage is considered an index of fruit ripening and an increase in TSS of control fruits corresponds to a conversion of starch to soluble sugars.

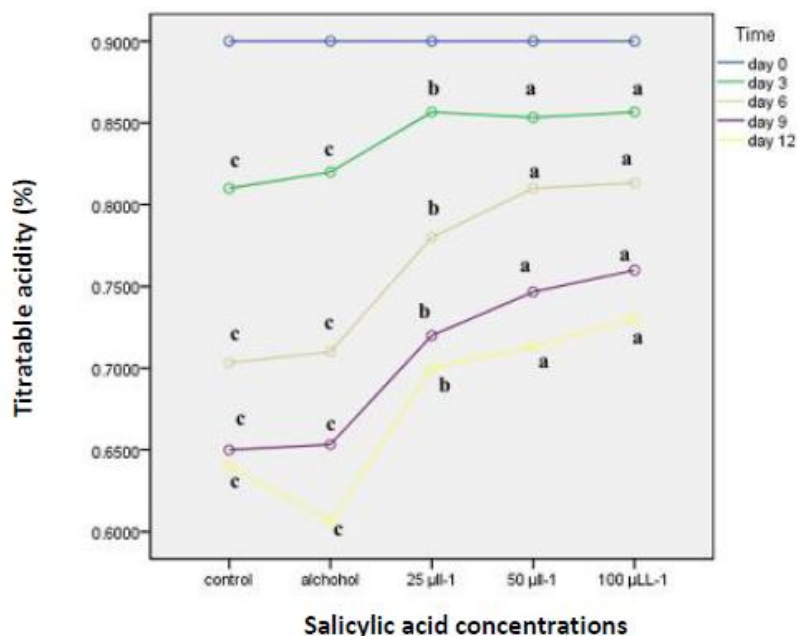


Figure 4. The effect of salicylic acid treatment on titratable acidity of strawberry fruit in paper disk method.

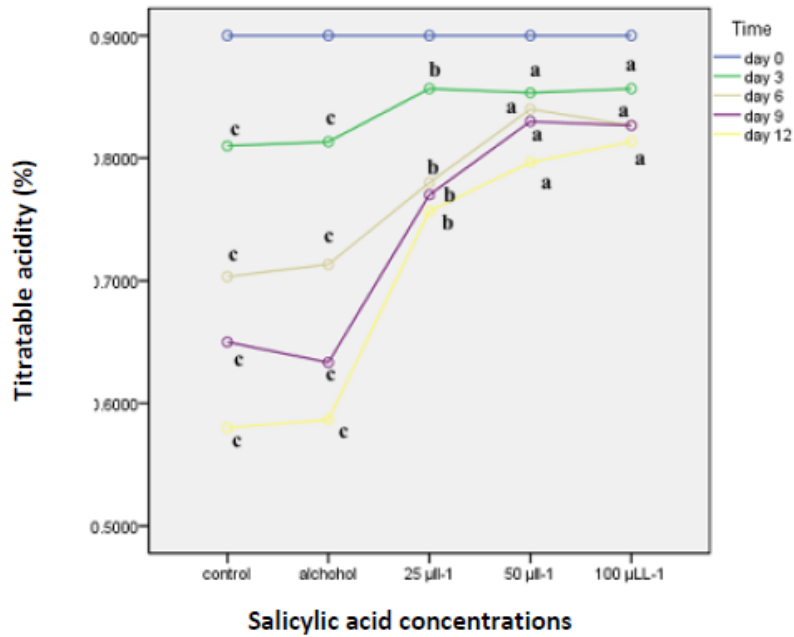


Figure 5. The effect of salicylic acid treatment on titratable acidity of strawberry fruit in spray method.

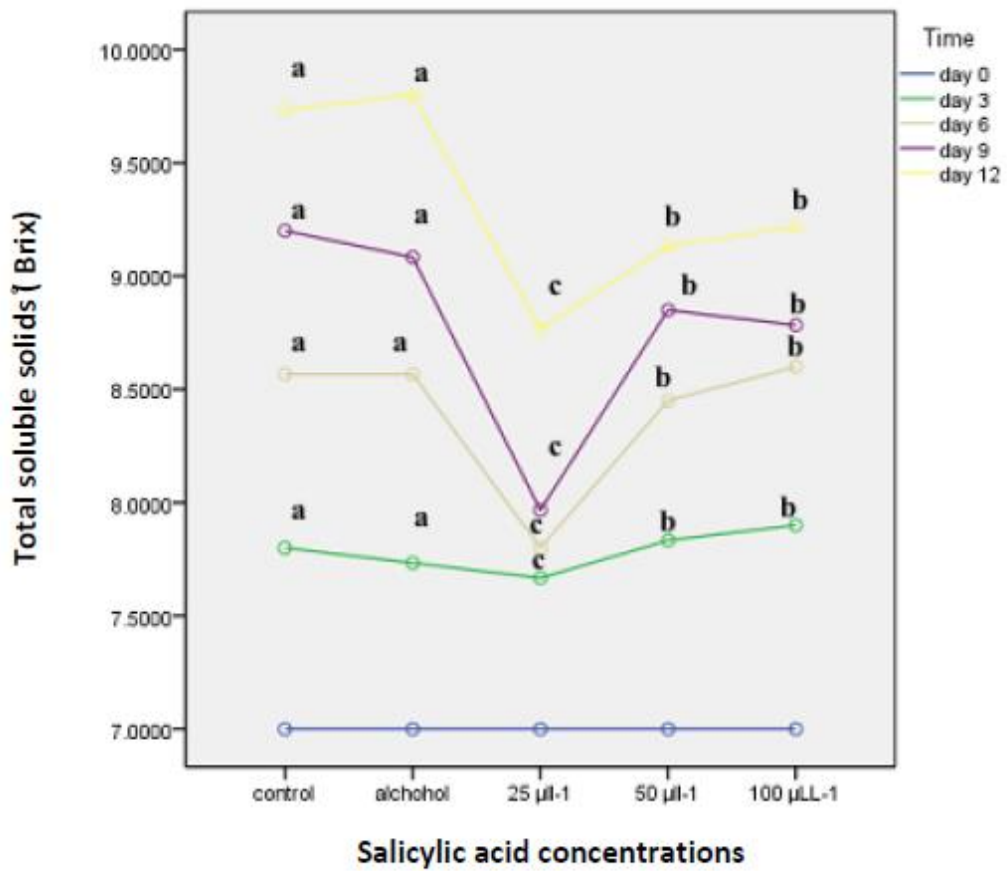


Figure 6. The effect of salicylic acid treatment on total soluble solids of strawberry fruit.

3.4 Vitamin C

Vitamin C content of fruits was markedly affected by SA treatment, whereas high concentrations of SA (50 or 100 μL^{-1}) had the highest amount of vitamin C (Figure 7). The levels of vitamin C were higher in SA-treated fruits than in control samples. The content of strawberry vitamin C did not show changes in response to SA (Lolaei et al., 2012). The application of SA can increase vitamin C content and then decrease antioxidant in strawberry fruit (Jing-Hua et al., 2008). Soleimani Aghdam et al. (2011) found that MeSA treatment maintained significantly ascorbic acid content of the kiwifruit during storage and there was a positive correlation between MeSA concentration and fruit ascorbic acid content that are in agreement with our results. Hung et al. (2007) suggested that high ascorbate (AA) contents in the pulp of pretreated fruit with SA may result from an acceleration of biosynthetic pathways or a decrease in catabolism through an accumulation of dehydroascorbate (DHAA).

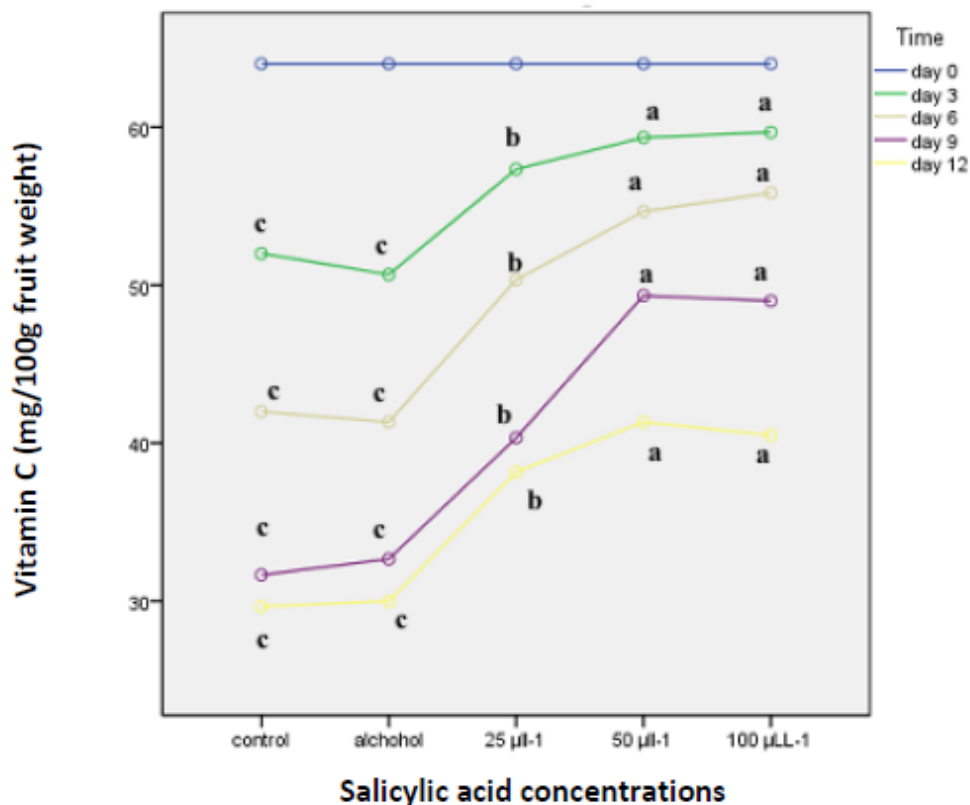


Figure 7. The effect of salicylic acid treatment on vitamin C of strawberry fruit.

3.5 Anthocyanin content

Anthocyanin content decreased during storage period. As shown in Figure 8, the levels of anthocyanin were higher in SA-treated fruits than in control samples; whereas the higher concentration of SA (100 and 50 μL^{-1}) treated fruits had the most amount of anthocyanin. There were not significant differences between two methods of SA application. Anthocyanins are a group of phenolic compounds responsible for the red-blue color of many fruits. Javaheri

et al. (2012) stated that application of SA increased the fruit lycopene content also they showed that SA activated the synthesis of carotenoids and xanthophylls. In accordance with our results Tareen et al. (2012) reported that SA treatments significantly affected skin color of peach fruits during 5 weeks of storage period.

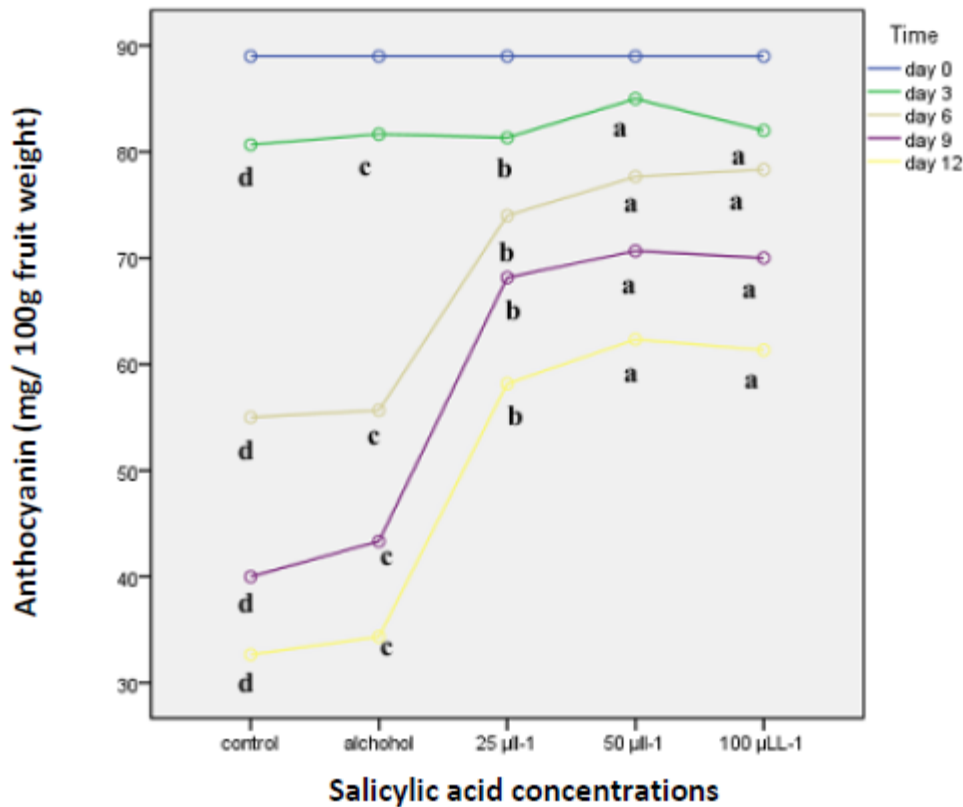


Figure 8. The effect of salicylic acid treatment on anthocyanin of strawberry fruit.

3.6 Calcium and Pectin Contents

Calcium and pectin contents decreased gradually during storage period. SA-treated fruits had more calcium and pectin content compared to controls. There was no significant difference in pectin and calcium between methods of SA application. The effect of SA treatment on fruit calcium and pectin significant varied with the concentrations applied. As shown in Figure 9 and 10 treatment with 50 µL⁻¹ had the highest amount of calcium and pectin and the lowest concentration of SA (25 µL⁻¹) had the lowest amount of calcium and pectin. Pectins are likely to be the key substances involved in the mechanical strength of the primary cell wall which are important to the physical structure of the plant (Sirisomboon *et al.*, 2000). Ca⁺² appears to be necessary because it induces the cross-linking of polygalacturonan chains into a structure that can be recognized by its isoperoxidase (Penel *et al.*, 1999). Coway *et al.* (1987) stated that the loss of firmness due to call wall carbohydrate metabolism during storage has been associated with increased susceptibility to infection by fungal pathogens.

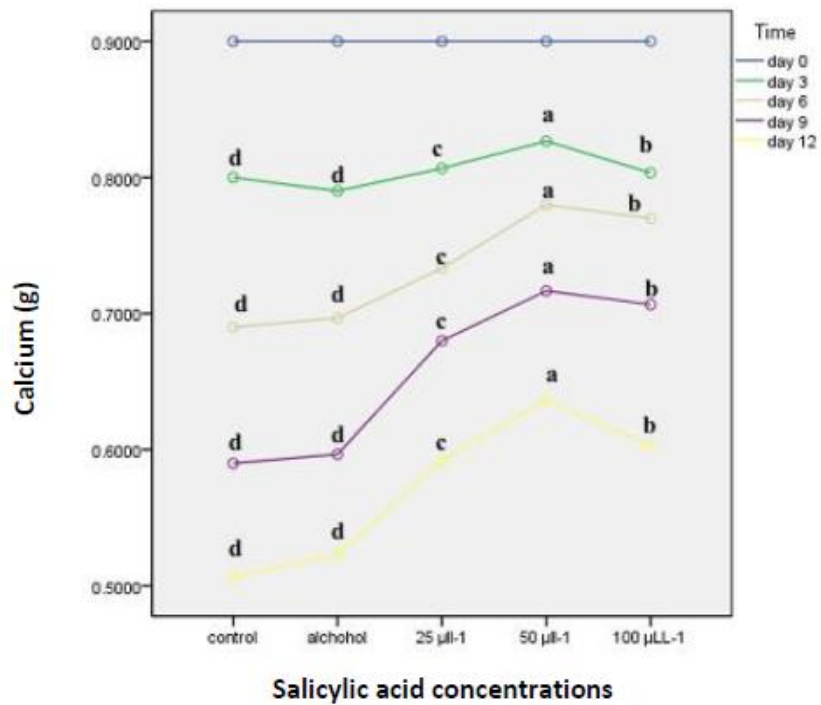


Figure 9. The effect of salicylic acid treatment on calcium of strawberry fruit.

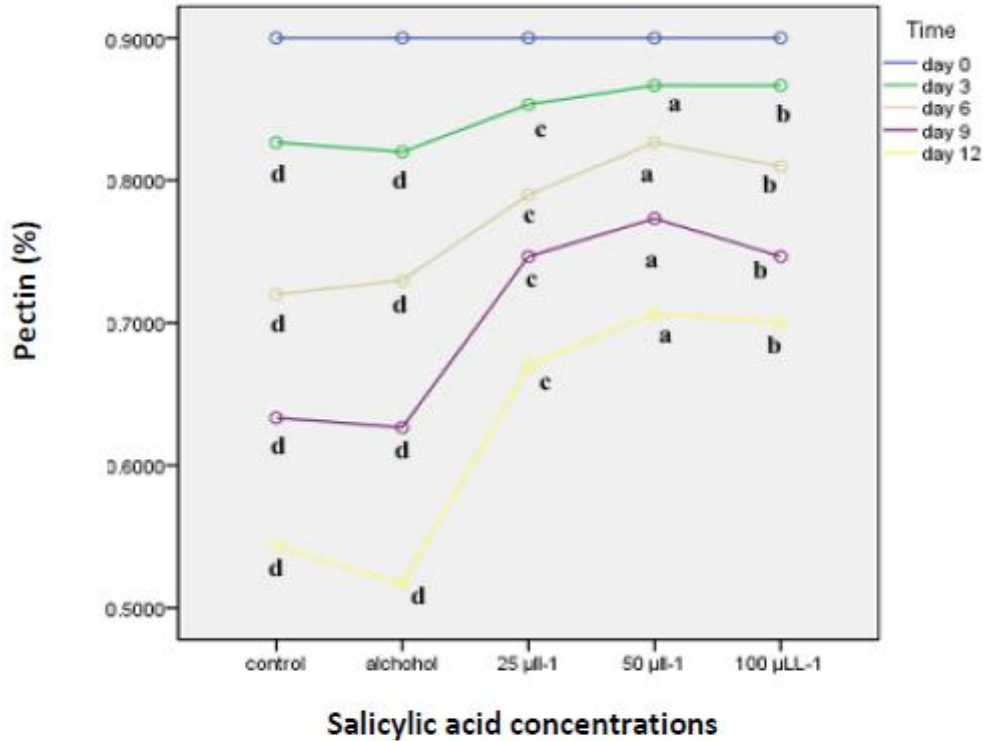


Figure10. The effect of salicylic acid treatment on pectin of strawberry fruit.

3.7 CAT Activity

Result showed that the catalase activity decreased at the end of the storage period. The samples which were subjected to SA with 50 and 100 μL^{-1} concentrations had the highest catalase activity (Figure 11). Catalase eliminates H_2O_2 by breaking it down directly to form water and oxygen. In consistent of our results, Soleimani Aghdam *et al.* (2011) stated that the lowest CAT activity observed when 32 μL^{-1} MeSA applied at all determination times, while the highest CAT activity was related to control fruits. SA interaction with CAT leads to high levels of H_2O_2 accumulation in cells, which induces fruit resistance against pathogens via activating protective enzymes and pathogenesis related (PR) proteins (Klessig and Malamy, 1994; Malamy and Klessig, 1992).

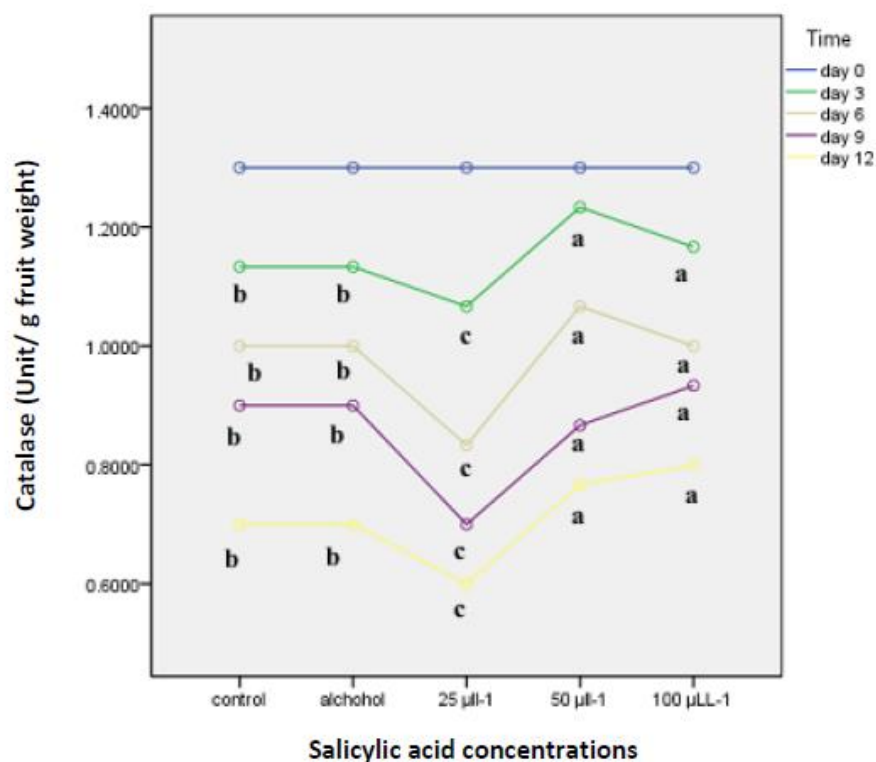


Figure 11. The effect of salicylic acid treatment on catalase activity of strawberry fruit.

3.8 POD Activity

Peroxidase activity increased along the storage. The samples treated with SA concentrations 50 and 100 μL^{-1} showed a significant decreased in POD rate (Figure 12). Peroxidase (POD) activity plays an important role in the oxidative degradation of phenolic compounds, which can lead to the production of brown polymers (Tomás-Barberán and Espín, 2001). Srivastava and Dwivedi (2000) stated that SA treatment decreased levels of POD, than their respective controls, in a concentration dependent manner, during the ripening of banana fruits. In consistent with our results Kazemi *et al.* (2011) indicated that maximum POD activity was observed in 3 mM SA in the storage duration.

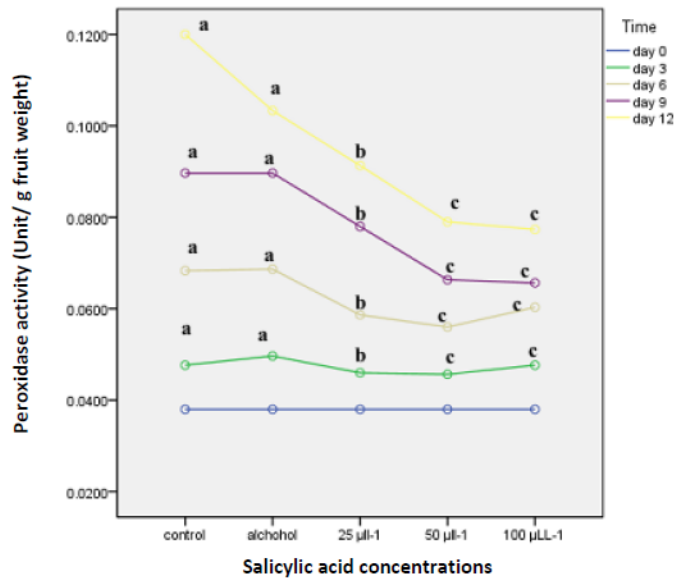


Figure 12. The effect of salicylic acid treatment on peroxidase activity of strawberry fruit.

3.9 PG Activity

PG content of fruits increased significantly along the storage period. The controls had the highest PG activity and the samples which were subjected to SA had less PG activity compared to controls (Figure 13). The samples which were treated with 100 µL⁻¹ SA had the lowest PG activity. Treatment of banana with SA resulted in decreased level of cell wall degrading enzyme in a concentration dependent manner, during the ripening of fruit (Srivastava and Dwivedi, 2000). PG is reported to be primarily responsible for ripening associated pectin degradation and fruit softening (Huber, 1983). Physical and chemical treatments which suppress ripening inhibit PG gene expression (Ogura et al., 1975; Picton et al., 1993).

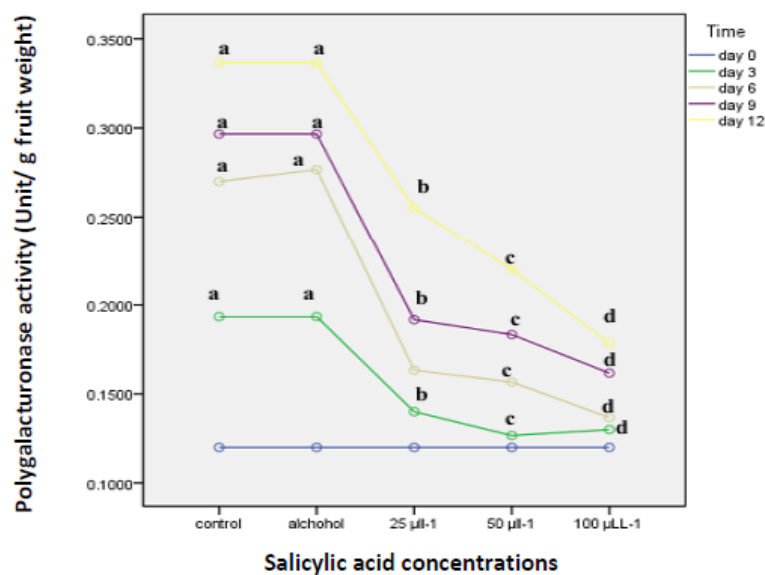


Figure 13. The effect of salicylic acid treatment on polygalacturonase of strawberry fruit.

3.10 Fruit Decay

The effect of SA treatment on fruit decay significant varied with the concentrations applied and storage time. Treatment with $100 \mu\text{L}^{-1}$ SA significantly inhibited fruit decay throughout the storage period, whereas control samples had the least effect. Paper disk method inhibited fruit decay more than spray method (Figures 14 and 15). SA is also involved in local and systemic resistance to fungal pathogens (Meena *et al.*, 2001). It is known that SA can enhance disease resistance of detached plant organs (Meena *et al.*, 2001; Qin *et al.*, 2003). SA can induced disease resistance by coordinate activation of a specific set of PR-genes many of which encode for proteins with antimicrobial activity (Durrant and Dong, 2004; Van Loon *et al.*, 2006). Duan *et al.* (2007) stated that SA treatment of grapes decreased fungal infections which showed significant differences with control. Soleimani Aghdam *et al.* (2011) showed that kiwifruit fungal decay was significantly affected by MeSA vapor in the end of shelf life period compared with control fruits. Application of SA in a concentration dependent manner from 1 to 2mmolL^{-1} effectively reduced fungal decay in ‘Selva’ strawberry fruit (Babalar *et al.*, 2007). Asghari *et al.* (2007) found that dipping of pear fruit in 1mmolL^{-1} SA solution effectively controlled fruit decay during cold storage period. These results are in agreement with our results.

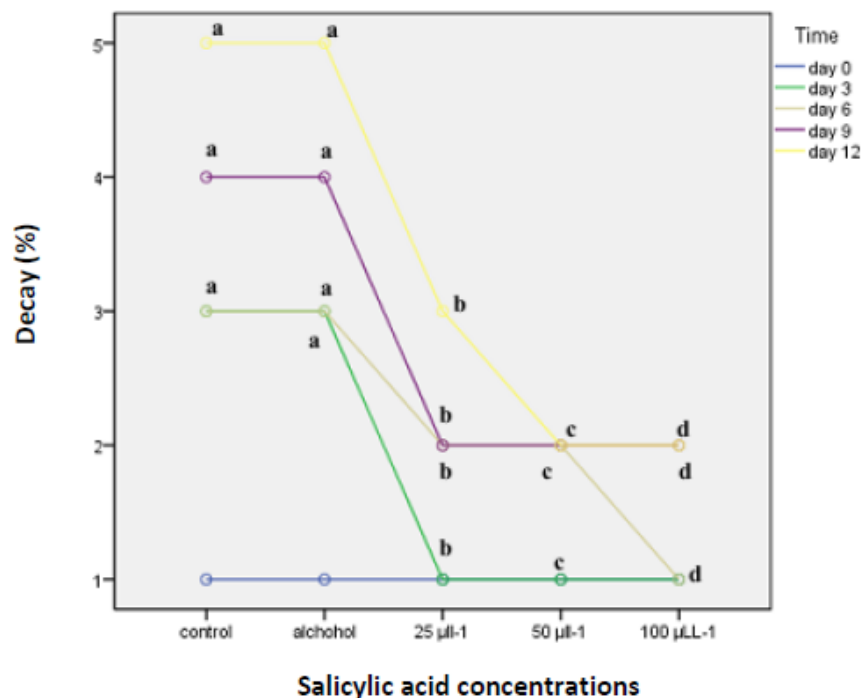


Figure 14. The effect of salicylic acid treatment on decay percentage of strawberry fruit in paper disk method.

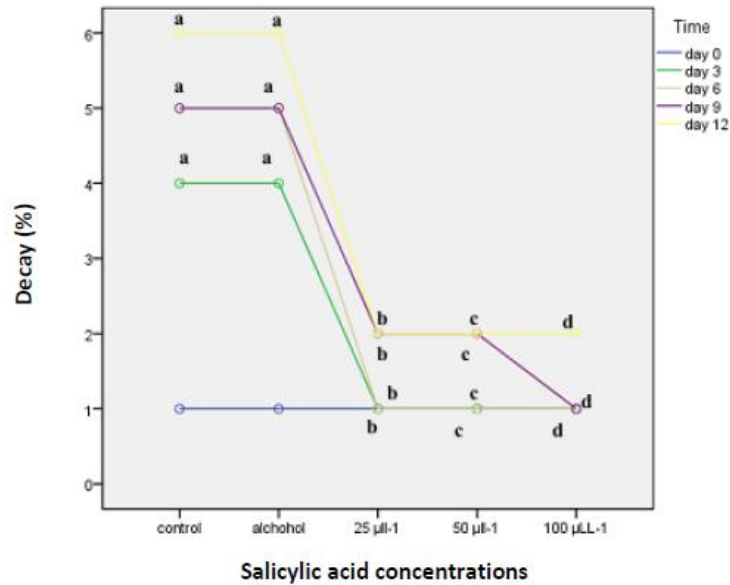


Figure 15. The effect of salicylic acid treatment on decay percentage of strawberry fruit in spray method.

3.11 Sensory analyses

Overall quality decreased continuously during storage at higher rate in untreated fruits compared with those treated with SA. Treatment with $100\mu\text{L}^{-1}$ SA had the highest effect on fruit quality among all of concentrations. Fruits that treated with paper disk method had better fruit quality compared with spray method (Figures 16 and 17). These results showed that SA treatment had a significant effect on retaining quality parameters in strawberry fruit. Our results are in accordance with Kazemi *et al.* (2011) about apple fruit.

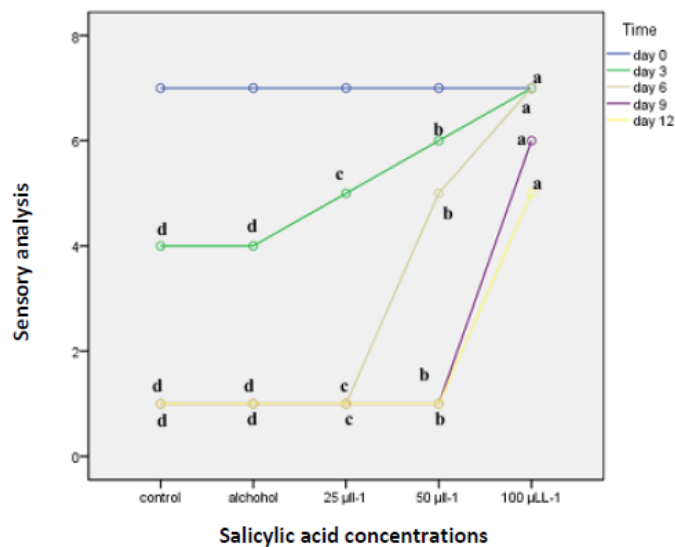


Figure 16. The effect of salicylic acid treatment on sensory analysis of strawberry fruit in paper disk method.

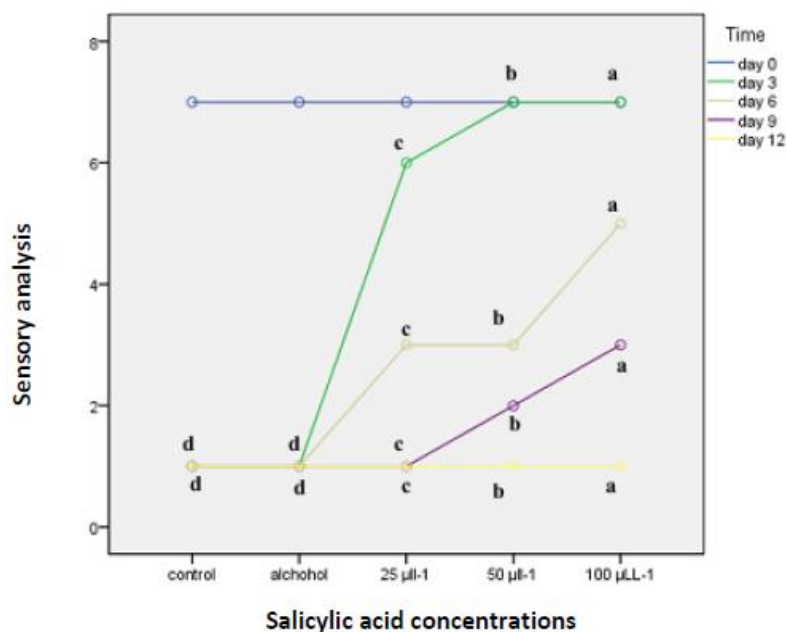


Figure 17. The effect of salicylic acid treatment on sensory analysis of strawberry fruit in spray method.

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