

## Combined Treatment of Modified Atmosphere Packaging and Salicylic Acid Improves Postharvest Quality of Nectarine (*Prunus persica* L.) Fruit

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### ABSTRACT

The main objective of this study was to assess the effectiveness of individual application and combination effect of salicylic acid treatments (0, 0.5, 1 mM) in both unpacked and Modified Atmosphere Packaging (MAP) on changing biochemical compounds and extending postharvest life of nectarine. Fruits were stored at 0°C with 90% RH for 40 days. Experimental fruits were analyzed for weight loss, soluble solids, titratable acidity, fruit firmness, total phenolic compounds, flavonoids, antioxidant capacity and overall quality during the storage period at 10 day intervals. The results showed that total phenolic, flavonoid concentrations and antioxidant activity fluctuated progressively until the 30 days of storage, and then decreased until the end of the experiment in all applied treatments. Salicylic acid treatment especially at 1 mM salicylic acid concentration with MAP had a positive effect on biochemical compounds. Furthermore, the combined treatment of MAP had a clear advantage over the other treatments in reducing weight loss, retarding softening, increasing shelf life, and maintaining higher overall fruit quality. The results indicated that salicylic acid with MAP can be used as a safe alternative chemical to keep the quality and for storage of nectarine.

**Keywords:** Biochemical compounds, Postharvest, *Prunus persica*, Quality, Salicylic acid.

### INTRODUCTION

Stone fruits are characteristically soft fleshed and highly perishable and they have a limited market life potential (Crisosto and Mitchell, 2011). Therefore, cold storage of peaches and nectarines after harvest is necessary to minimize excessive softening, quality loss and decay and to prolong time for marketing (Buescher and Griffith, 1976). The storage life of nectarines under ideal conditions of 0°C and high relative humidity (90-95%) is limited to 2-4 weeks (Karen, 1991). Lurie and Crisosto (2005) reported that maximum storage life for nectarines can be achieved near or below 0°C, depending on the soluble solids content of the fruit.

Various postharvest methods have been developed to delay the onset of disorders, thereby enabling an extension of the storage period. Modified Atmosphere Packaging (MAP) storage has been successfully applied in order to prolong the shelf-life of nectarines (Akbulak and Eris, 2004). Studies on MAP of peaches and nectarines have showed that MAP slowed down the respiration rate of fruits and retarded the decrease in titratable acidity values, maintained the fruit sugar and soluble solids content, flesh firmness, vitamin C and juice content, and slowed deterioration through decreasing fruit injury and browning development (Lurie and Crisosto, 2005; Santana *et al.*, 2010; Bal, 2012). However, traditional MAP is not enough to ensure

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quality and safety preservation to fulfill consumer demand. Therefore, MAP storage must be combined with other new treatments. Fungicides are the primary means to control postharvest losses. However, postharvest application of some fungicides is prohibited in the European Union (Karabulut and Baykal, 2004), so alternative methods are investigated. Recently, natural products have started to be an effective alternative to synthetic chemicals in maintaining fruit quality during storage.

Salicylic Acid (SA), an endogenous plant growth regulator, has been found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development. SA as a natural and safe phenolic compound exhibits a high potential in controlling post-harvest losses of horticultural crops (Asghari and Aghdam, 2010). Postharvest application of SA has been shown to be effective in retarding ripening and softening of banana (Srivastava and Dwivedi, 2000) and kiwifruit (Bal and Celik, 2010); reducing chilling injury of loquat (Cai *et al.*, 2006), tomato (Ding *et al.*, 2007), peach (Wang *et al.*, 2006), and sweet peppers (Fung *et al.*, 2004); and improving quality characteristics of strawberry (Babalar *et al.*, 2007; Shafiee *et al.*, 2010) and sweet cherry (Bal, 2012). Leslie and Romani (1988) reported that SA inhibits ethylene biosynthesis by the inhibition of conversion of 1-AminoCyclopropane-1-Carboxylic acid (ACC) into ethylene. Besides, it has been discovered that exogenous application of SA could enhance resistance to pathogens and control postharvest decay in peach (Wang *et al.*, 2006), sweet cherry (Xu and Tian, 2008), strawberry (Shafiee *et al.*, 2010) and persimmon fruits (Khademi *et al.*, 2012). Thus, salicylic acid has remarkable ability to maintain the fruit quality during postharvest storage life of fruits.

There are some researches that performed publications on the effects of either SA treatment or MAP individually on fruits; however, there are no published data yet on

the effects of SA treatment and MAP combinations on nectarines. Therefore, the aim of our study was to investigate the efficacy of SA treatments alone and in combination with MAP on postharvest quality of 'Fantasia' nectarines during storage at 0°C and 90% RH.

## MATERIAL AND METHODS

Nectarine (*Prunus persica* var. nectarine, cv. Fantasia) fruits were harvested at commercial maturity based on changes in skin ground color and flesh firmness from a commercial orchard in Turkey. The fruits were selected for uniform size, color and absence of mechanical damage. Fruits were divided into six groups and treatments were applied as follows;

Control: Fruits were immersed into distilled water at 20°C for 10 minutes;

0.5 mM SA: Fruits were immersed into solution at 0.5 mM SA (Sigma Chemical Co.) at 20°C for 10 minutes;

1 mM SA: Fruits were immersed into solution at 1 mM SA at 20°C for 10 minutes;

MAP: Fruits were packed into modified atmosphere bags;

MAP plus 0.5 mM SA (MAP-SA 0.5),

MAP plus 1 mM SA (MAP-SA 1).

SA treated fruits were left to air-dry at room temperature for one hour before storage.

The treated fruits were placed in polypropylene baskets (1 kg) and stored at 0-1°C with 90% RH for 40 days.

Samples were taken initially and at 10 day intervals during storage for analysis such as weight loss (%), Total Soluble Solids (TSS) content (%), Titratable Acidity (as malic acid, TA) (%), fruit firmness (N), total phenolic compounds [mg GAE 100 g<sup>-1</sup> fresh weight (fw)], flavonoids (mg RE 100 g<sup>-1</sup> fw), antioxidant capacity (mg AEAC g<sup>-1</sup> fw) and overall quality (1-5 scale). Fruit samples were weighed at the start of experiment and at the end of each storage interval. The difference between initial and final fruit weight was considered as total weight loss

during that storage interval. Total soluble solids of fruit juice were measured using a hand refractometer and expressed as percentage. Titratable acidity of the fruit was determined through titration method. Flesh firmness was measured on both cheeks of each fruit with an Effegi penetrometer equipped with 8.9 mm diameter plunger and expressed as Newton (N). The analysis of total phenolic compounds was performed in accordance with the Folin-Ciocalteu spectrophotometric method (Slinkard and Singleton, 1977) and was expressed as mg Gallic Acid Equivalents (GAE) per 100 g fresh weight (fw). The total flavonoid contents were measured by a colorimetric assay (Zhishen *et al.*, 1999) and the results were expressed as mg Rutin Equivalent (RE) per 100 gram fresh weight. The antioxidant capacity with the 2,2-DiPhenyl-1-PicrylHydrazyl radical (DPPH) was performed as reported by Brand-Williams *et al.* (1995). Ascorbic acid was used as a standard and DPPH radical scavenging activity was expressed in mg of ascorbic Acid Equivalents Antioxidant Capacity (AEAC) per gram fresh weight. Overall quality (percentage of fruit surface area decayed, shrunken and adversely affected) was evaluated by 5 trained panelists using a 1-5 scale, where 1= Unacceptable (> 50% surface affected); 2= Bad (20-50% surface affected); 3= Acceptable (5-20% surface affected); 4= Good (up to 5% surface affected), and 5= Excellent (no decay, shrinkage or any other adverse effects on fruit surface were seen). Results were expressed as an overall quality index.

### Statistical Analysis

Factorial with completed randomized design and three replications were used in the experiment. Statistical analysis was carried out using the Analysis Of Variances (ANOVA) performed in SPSS software program. The treatment means were separated using the Least Significant Difference (LSD) method at a significance

level of  $P \leq 0.05$ . Data are shown as mean  $\pm$  Standard Error (SE).

## RESULTS AND DISCUSSION

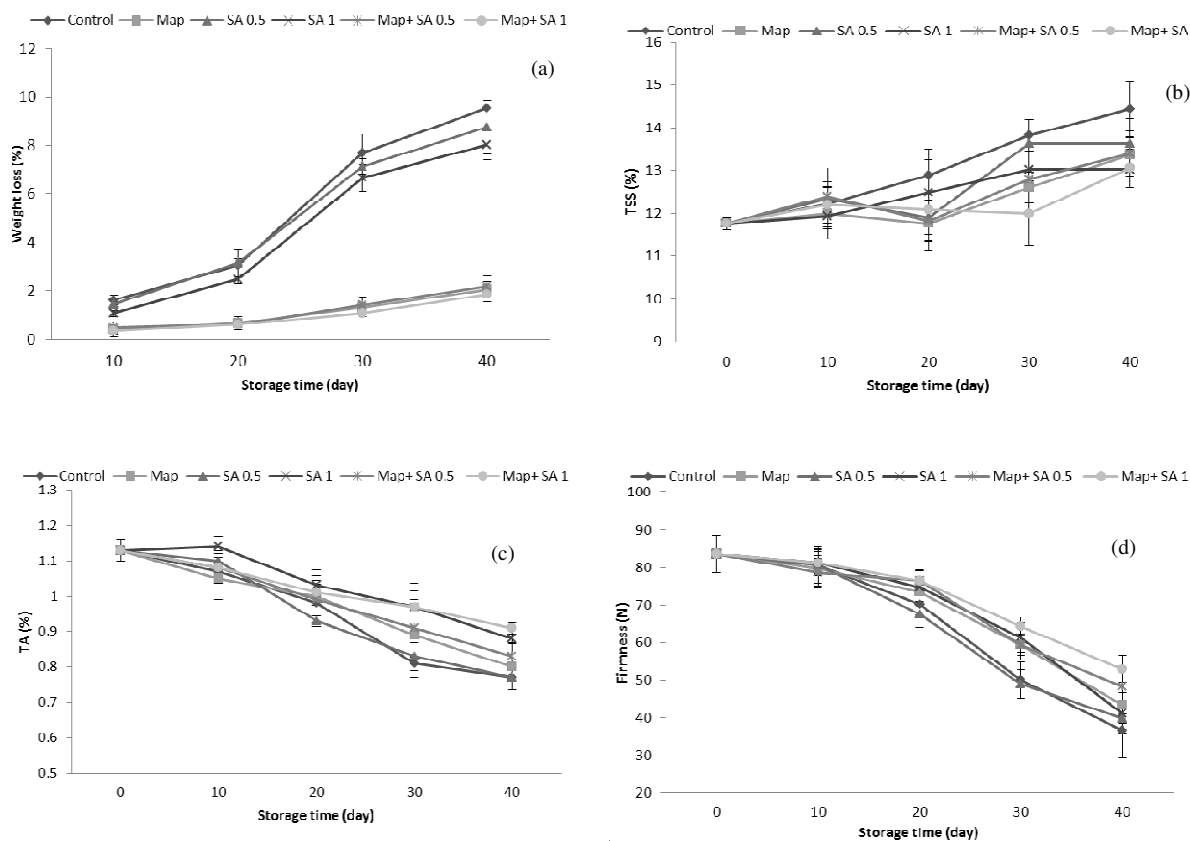
### Weight Loss

Fruit shriveling occurs when fruits lose approximately 5-8% of their water content. This loss is sufficient to cause visual shrivel in peaches and nectarines (Ceponis *et al.*, 1987).

In this study, the loss of weight progressively increased with storage time and was linear for all treatments. All treated fruits significantly maintained more fruit weight in comparison with control (Figure 1-a). After 40 days of storage, the highest weight loss was observed in control fruits (9.5%) while the least weight losses were observed in MAP (2.0%) and MAP-SA 1 treatments (1.8%). The MAP and the combined MAP-SA treatments dramatically inhibited weight loss compared to the control and SA treatments without MAP. Modified atmosphere packaging has been known to reduce weight losses in nectarine (Akbulak and Eris, 2004; Santana *et al.*, 2010; Bal, 2012) mainly by maintaining moisture levels inside the packages thus preventing weight loss. SA treatments became effective on preventing weight loss in MAP through retarding ripening process. These results agreed with those reported by Zheng and Zhang (2004), Srivastava and Dwivedi (2000), and Shafiee *et al.* (2010) that SA caused a decrease in respiration and fruit weight losses during storage period.

### Total Soluble Solids

High consumer acceptance in nectarines is attained on fruit with high soluble solid content (Crisosto and Kader, 2000). Brady (1993) reported that soluble solids in mature nectarines should be more than 12% for acceptable quality.



**Figure 1.** Changes in weight loss (a) Changes in total soluble solids (b) Changes in titratable acidity (c) Changes in firmness (d) of nectarine fruits treated with SA and MAP during cold storage. Data are mean±SE. SA= Salicylic Acid, MAP= Modified Atmosphere Packing.

In the study, TSS changes in nectarines were not significant during storage period and no regular trend was observed (Figure 1-b). But, TSS for all treatments increased towards the end of the storage. These increases in soluble solids over the storage period could be due to moisture loss, hydrolysis of polysaccharides and concentration of juice as a result of degradation. At harvest, TSS content of nectarines was 11.7%. At the end of storage, the highest TSS content in the trials was shown in the control application (14.4%), while the lowest TSS values were determined in SA 1 and MAP-SA 1 (13.0%). These results showed a clear effect of MAP and SA on decreasing fruit metabolism, including respiration rate, leading to maintenance of respiration substrates and in

turn to a delay of the postharvest ripening process.

**Titrateable Acidity**

Depending upon the treatments, a gradual decrease for TA was found in all treatments with extended storage. However, TA of all the groups of nectarine was not significantly different at a level of  $P < 0.05$  (Figure 1-c). It is well known that the decrease in acidity during storage could be attributed to the use of organic acids as a respiratory substrate during storage (Ulrich, 1974). Similar results were found on nectarines (Bahar and Dundar, 2003; Celik *et al.*, 2006; Bal, 2012). After 40 days of storage, the highest TA was determined in MAP-SA 1 treatments

(0.91%), while the least TA was observed in control (0.77%) and SA 0.5 treatments (0.77%). Some of the reports show that SA treatments had no significant effect on TA (Karlidag *et al.*, 2009; Sayyari *et al.*, 2009) while Srivastava and Dwivedi (2000) indicated that application of SA was effective in maintaining TA concentration as well as in increasing TSS in ripening bananas.

### Fruit Firmness

Firmness is an important attribute of nectarine for the prediction of harvest time and fresh market. There were significant differences in fruit firmness (Figure 1-d). In general firmness decreased in fruits of all treatments during storage. The softening of flesh during storage could be due to the degradation of soluble pectin by high activity of endopolygalacturonase enzyme in fruits (Martin-Cabrejas *et al.*, 1994). At harvest, firmness of nectarines was 83.6 N. The combination of SA treatment and MAP has retained the firmness of nectarine. The lowest fruit firmness in the trials was determined in the control (36.5 N) and SA 0.5 application (39.8 N) at the end of storage, while the highest TSS values were shown in MAP-SA 1 (52.9 N) and MAP-SA 0.5 (48.3 N) treatments. These results are in accordance with the findings of Yan *et al.* (1998), Li and Han (1999) and Tareen *et al.* (2012) on peach fruits who reported that optimal concentration of SA increased flesh firmness. Similar results have also been found by Srivastava and Dwivedi (2000) in banana, and Bal and Celik (2010) in kiwifruit. The effect of MAP on fruit firmness could be attributed to the beneficial effects of atmospheres with low O<sub>2</sub> and/or high CO<sub>2</sub> content on reducing softening.

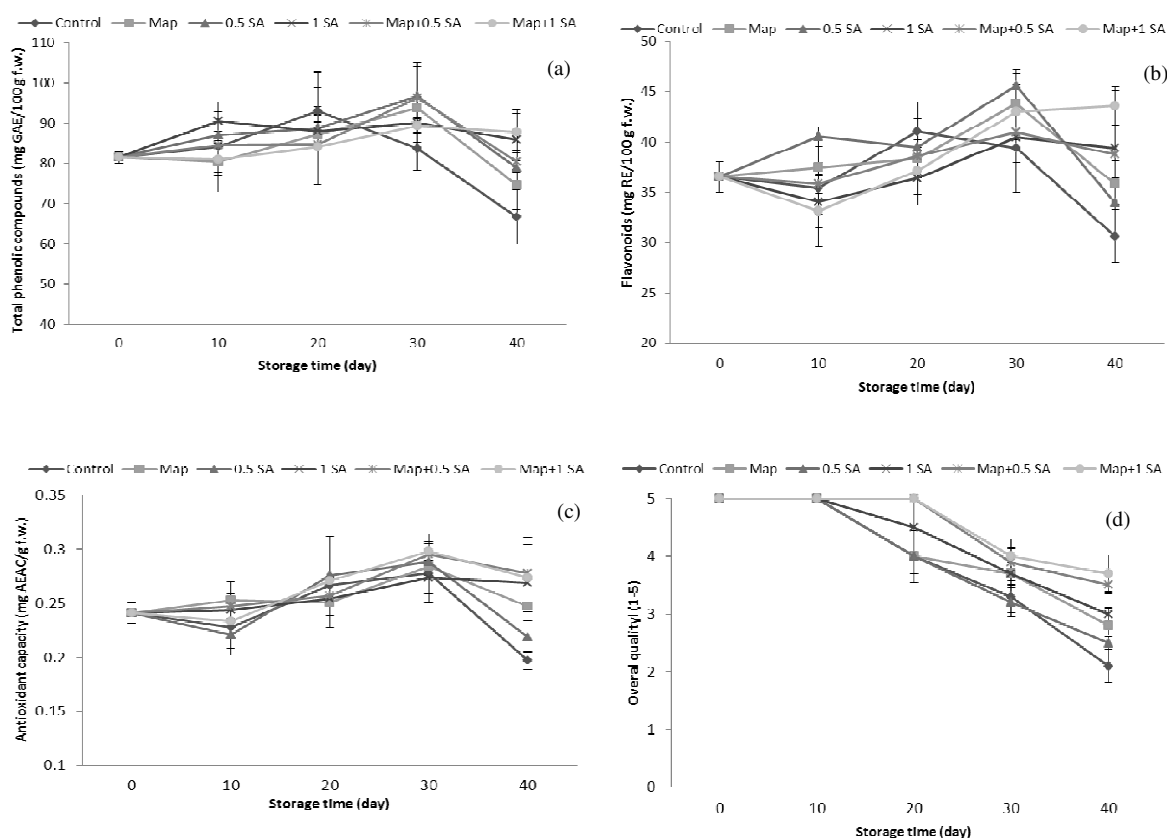
### Total Phenolic Compounds

The fruit phenolics are highly unstable and they undergo various changes

throughout storage depending on the ripeness, cultivars, storage conditions etc. (Sharma *et al.*, 2008). In the present study, total polyphenol compounds at harvest were 81.5 mg GAE 100 g<sup>-1</sup> and the treated fruits exhibited a significantly higher phenolic content than the control fruits at the end of the storage (Figure 2-a). In general, total phenol compounds of nectarines fluctuated progressively until the first 30 days of storage, and then decreased until the end of the experiment in all the treatments. Similar fluctuating behavior has been observed during storage of peach (Khademi and Ershadi 2013; Razavi *et al.*, 2014). The decrease in total phenolic compounds of nectarines at the later stage of storage might also be due to senescence of tissues and oxidation by common polyphenol oxidase. Several studies have shown that polyphenolic compounds generally decrease in climacteric fruits, such as mangos, persimmon, and plum during ripening (Kim *et al.*, 2007, Del Bubba *et al.*, 2009; Singh and Singh, 2012). At the end of the storage, the highest total phenol compounds was determined in MAP-SA 1 (87.8 mg GAE 100 g<sup>-1</sup>) and SA 1 (85.9 mg GAE 100 g<sup>-1</sup>) treatments, while the lowest total phenol compounds was determined in control fruits (66.6 mg GAE 100 g<sup>-1</sup>). The increases in total phenolic compounds were delayed by the use of MAP and MAP with SA. These findings are in agreement with those reported by Chen *et al.* (2006), Bal (2012), and Pila *et al.* (2010). Asghari and Aghdam (2010) also reported that SA, as a natural and safe phenolic compound, exhibits a high potential in decreasing production and delays the ripening process of horticultural crops.

### Flavonoids

Nectarine and peach contain ascorbic acid, flavonoid, and phenolic compounds, which are considered prime sources for antioxidants (Tomas-Barberan *et al.*, 2001).



**Figure 2.** Changes in total phenolic compounds (a) Changes in flavonoids (b) Changes in antioxidant capacity (c) Changes in overall quality (d) of nectarine fruits treated with SA and MAP during cold storage. Data are mean $\pm$ SE. SA= Salicylic Acid, MAP= Modified Atmosphere Packing.

Flavonoids followed a pattern very similar to that of phenolics. The experimental result showed that the initial flavonoid content of nectarine was 36.5 mg RE 100 g<sup>-1</sup> and there were significant changes with increasing storage periods (Figure 2-b). In the present study, although fluctuations occurred in flavonoid content (fluctuation implies increase and decrease), reductions occurred in all applications except for MAP-SA 1 (43.6 mg RE 100 g<sup>-1</sup>) at the end of the storage time. Similarly, Tsantili *et al.* (2010) and Zhang *et al.* (2014) reported that total phenolic and flavonoid concentration in peaches fruits decreased towards the end of storage. Chaparzadeh and Yavari (2013) also reported that flavonoids content of apples fruits decreased significantly with increasing cold storage up

to 135 days. The highest flavonoid content in the trials was obtained in SA 0.5 application (45.5 mg RE 100 g<sup>-1</sup>) in the 30<sup>th</sup> day of storage, while the lowest flavonoid content was determined in control fruits (30.6 mg RE 100 g<sup>-1</sup>) at the end of the storage period. In the study, the decreases in flavonoid content were delayed by the use of MAP-SA 1. This could be mainly due to activating the metabolic pathway for the synthesis of flavonoid compounds with exogenous application of salicylic acid. Similar to our study, Razavi *et al.* (2014) reported that salicylic acid treatment enhanced postharvest quality of peach fruit by maintaining firmness, inducing bioactive compounds such as total flavonoids contents.

### Antioxidant Capacity

Antioxidant activity cannot be related to a single compound but to synergistic and additive effects between different phytochemicals (Gardner *et al.*, 2000). As shown in Figure 2-c, treatments significantly changed the antioxidant capacity of nectarine fruits after 40 days of storage. In the study, as total phenol compounds, antioxidant capacity of nectarines also fluctuated progressively during the first 30 days of storage, and then decreased until the end of the experiment in the treatments.

Reduction of antioxidant capacity of fruits in the long-term storage could be attributed to the reduction in phenolic and other biochemical compounds. This result agreed with Ali *et al.* (2007) and Tsantili *et al.* (2010) who reported that higher phenolic compound levels could change antioxidant activity and also showed a linear correlation between phenolic compounds and antioxidant activity. At the end of the storage, while the highest antioxidant activity was detected in MAP-SA 1 (0.274 mg AEAC g<sup>-1</sup>) and MAP-SA 0.5 treatments (0.278 mg AEAC g<sup>-1</sup>), the lowest antioxidant activity was detected in control fruits (0.197 mg AEAC g<sup>-1</sup>). This result showed that the use of MAP together with SA significantly maintained the antioxidant activity of nectarine. This is in agreement with Wang *et al.* (2006) who reported that SA treatment maintained greater firmness and antioxidant systems in peach fruit during cold storage. Sayyari *et al.* (2011) and Barman and Asrey (2014) also reported that SA treatment maintained higher antioxidant capacity in pomegranate and mango.

### Overall Quality

Overall quality is the most important factor in fruit marketability assessment. Fruits lacking any kinds of decay and shrivels with high red color are considered as marketable (Babalar *et al.*, 2007). The

overall quality of investigated fruits is given in Figure 2-d and scores were found significantly different among all the treatments during storage period. At the end of the storage, nectarine fruits treated with MAP-SA 1 had maximum scores (3.7) of overall acceptability followed by MAP-SA 0.5 (3.5) and SA 1 (3). Fruits treated with SA 0.5 (2.5), MAP (2.8) and control (2.1) were not acceptable after 40 days of storage. These fruits presented increasing drastic softening, significant weight loss and decay incidence. SA 0.5 mM concentration was not effective in the overall quality. This may be due to lower SA concentrations that were not able to maintain fruit quality attributes. Similarly, Wang *et al.* (2006) reported that only low concentrations of SA were ineffective on softening of peach fruit belonging to the climacteric type.

In conclusion, salicylic acid and MAP play a very effective role in controlling the weight loss, decay and other compositional changes such as titratable acidity, total soluble solids, total phenols, flavonoids and antioxidant activity of nectarine during cold storage. Especially MAP-SA 1 treatment delayed the ripening process more effectively and with a minimum quality loss, as compared to the control samples which had greater compositional changes with maximum quality loss. Thus MAP-SA 1 treatment has the potential to prolong the storage life and preserve valuable attributes of post harvest nectarine. Note that in the present research the microbial test, eletrolytic leakage and respiration rate on the stored fruit were not measured, so further research on these characters increase the warranty of the potential of stored fruit under MAP and low temperature storage.

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## تیمارهای ترکیبی اسید سالیسیلیک و بسته بندی اتمسفر اصلاح شده در بهبود کیفیت شهد میوه شلیل (*Prunus persica* L.) پس از برداشت

### ۱. بال

### چکیده

هدف اصلی این تحقیق بررسی تأثیر گذاری استفاده ی ترکیبی تیمارهای اسید سالیسیلیک (۰، ۵، ۱ و ۱۰ mM) در هر دو حالت بسته بندی با اتمسفر اصلاح شده (MAP) و عدم بسته بندی، بر تغییر ترکیب بیوشیمیایی و بهبود کیفیت شهد شلیل پس از برداشت بوده است. میوه ها در دمای ۰ درجه سانتیگراد با ۹۰٪ RH به مدت ۴۰ روز نگهداری شدند. میوه های آزمایشی از نظر کاهش وزن، مواد جامد قابل حل، اسیدیته، سفتی و سختی میوه، ترکیبات فنولی، فلاوونوئیدها، ظرفیت آنتی اکسیدانی و کیفیت کلی در زمان نگهداری در دوره های ۱۰ روزه مورد بررسی و آنالیز قرار گرفتند. نتایج حاکی از این بود که در تمام تیمارها ترکیبات فنولی کل، غلظت فلاوونوئید و فعالیت های آنتی اکسیدانی تا ۳۰ روز پس از نگهداری نوسان تدریجی داشته و سپس تا انتهای آزمایش کاهش داشت. تیمار اسید سالیسیلیک با بسته بندی معمولی و بسته بندی تحت شرایط تغییر یافته (MAP) خصوصاً در غلظت ۱ mM تأثیر مثبتی بر ترکیبات بیوشیمیایی داشت. علاوه بر آن، تیمار ترکیبی MAP برتری واضحی بر دیگر تیمارها در کاهش کمبود وزن، به تعویق انداختن نرم شدگی، افزایش عمر مفید و حفظ کیفیت برتر کلی میوه. نتایج نشان داد که اسید سالیسیلیک با MAP میتواند به عنوان یک ماده شیمیایی جایگزین بی خطر برای حفظ کیفیت و برای نگهداری شلیل استفاده شود.