



Maintenance of Physicochemical Qualities of Nectarine Fruits During Cold Storage Using Ultrasonic Treatment with Salicylic Acid

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ABSTRACT

This study was carried out to retain the high quality of nectarine cv. 'Venus' fruits during the cold storage. In the study, fruits were treated with ultrasonic and salicylic acid (1 and 2 mM) separately or in combination as well as water in control and then, they were stored at 0°C and 90±5% relative humidity for 60 days. Fruits were examined for weight loss, fruit firmness, soluble solids content, titratable acidity, ascorbic acid, total flavonoids, total phenolics, antioxidant content and chilling injury at 15 days intervals. The results showed that no significant differences were observed among control and ultrasonic alone treatments in the experiment. However, salicylic acid combined with ultrasonic treatment has more potential than salicylic acid alone in regulation of nectarine fruit ripening. Moreover, combination treatments, in comparison to the control, led to better preservation of firmness, ascorbic acid, total phenols, flavonoids and antioxidant contents, more weight loss control, alleviating the chilling injury symptoms. Synergistic effects between salicylic acid and ultrasonic treatment were observed and the most effective treatment for preserving the quality of nectarine fruits was the combination of 2 mM salicylic acid with ultrasonic treatment. These results demonstrated that the combined treatments of salicylic acid and ultrasonic could provide a useful means of extending nectarine postharvest life during cold storage.

1. Introduction

Peaches and nectarines are similar genetically and horticulturally, but for commercial purposes are regarded as two different fruits. The nectarine is essentially a fuzzless peach (Brown et al 1983). Nectarine is high functional fruits as a consequence of their bioactive compounds and deteriorate rapidly at ambient temperature. Therefore, cold storage of nectarines after harvest is necessary to minimize excessive softening, quality loss and decay and to prolong time for marketing (Celik et al 2006). Nectarines as climacteric stone fruits have a limited post-harvest life and they remain fresh only for 2-6 weeks stored at 0°C and 90-95% relative humidity depending on cultivar (Karen 1991).

After harvest, the nutritional and organoleptic quality of fresh produce start to decline as a result of altered plant metabolism. Quality deterioration of fruits is the result of produce transpiration, senescence, ripening associated processes and development of postharvest disorders (Kader 2001). However, the most important factor that limits the post-harvest life of nectarine is chilling injury (CI) or internal breakdown (Candır et al 2009). Fruits stored between 2.2°C and 7.6°C are

more prone to chilling injury than those stored at 0°C or lower (Crisosto et al 1999). Therefore, it is very important to prevent the postharvest losses of nectarine fruit.

Salicylic acid (SA), the plant natural organic compounds which is found in a wide range of plant species, has been reported to play a vital role in regulating plant growth and development. Moreover, this compound have been proven to be photochemical inducing bioactive compounds such as antioxidants and antioxidant enzymes and have been use for maintaining postharvest quality of perishable commodities (Supapvanich&Promyou 2013).

SA induces hydrogen peroxide (H₂O₂) accumulation at high temperatures while reducing H₂O₂ at lower temperatures. During chilling stress, the activities of antioxidant enzymes are decreased, which leads H₂O₂, and other reactive oxygen species. SA is involved in chilling tolerance through H₂O₂ metabolism mediation. (Kang et al 2003). SA has also been reported to reduce spoilage in peach fruit by controlling cell membrane electrolyte leakage, decreasing respiration and ethylene production, maintaining flesh firmness, and increasing antioxidant enzymes activities (Han et al 2003). In recent years, exogenous application of SA has been reported to improve storage life and storage quality

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attributes in many fruit like peach (Tareen et al 2012; Awad 2013), plum (Sabir 2017), apricot (Ezzat et al 2017).

Increasing public demands for improved safety and quality of fruits and vegetables in the fresh market, awoken a growing interest for novel technologies for the preservation of postharvest fruits and vegetables before storage. Ultrasonic technology provides one of the methods that with better treating time, enhanced products quality, reduced chemical hazards, low consumption of energy, and is environmentally friendly (Yuting et al 2013). Ultrasonic is composed of mechanical sound waves that originate from molecular movements that oscillate in a propagation medium (Gallo et al 2018). Postharvest ultrasonic treatments (UT) have been shown to extend shelf life and maintain quality in strawberries (Aday&Caner 2014), litchis (Chen et al 2012), pears (Zhao et al 2007), and plums (Chen&Zhu 2011). In addition, there are various reports indicating that combination of ultrasonic and other chemicals effectively increased postharvest life of horticultural crops (Chen&Zhu 2011; Yang et al 2011, Bal 2013; Bal 2016; Bal et al 2017, Khademi et al 2019). However, there is no study on the combined effects of ultrasonic with SA treatment on quality controlling in postharvest nectarine fruit. Thus the purpose of this study was to investigate the ability of ultrasonic treatment with SA to maintain physicochemical qualities and alleviate CI of nectarine fruits during cold storage.

2. Materials and Methods

Nectarine (*Prunus persica* var. *nectarina*) cv. 'Venus' fruits were harvested manually at firm-ripe stage (firmness was about 75 N; SSC was about 12.7%) from a commercial orchard in Turkey and immediately transported to the postharvest physiology laboratory. Fruits were selected for similar size, uniform maturity and appearance and freedom from defects.

Treatments and Storage Conditions

Ultrasonic treatment was applied in sonicator bath with water (20°C) in the ultrasonic chamber. Fruits were treated with 32 kHz ultrasonic at powers of 60 W*L⁻¹ for 10 min in 10 L distilled water. A surfactant Tween 20[®] at 1 g*L⁻¹ was also added to enhance infiltration. SA concentrations of 1 mM and 2 mM were prepared by dissolving SA powder (Sigma Aldrich Co.) in hot distilled water. Fruits were divided into four groups. Treatments and abbreviations can be summarized as follows:

1. Control: Fruits was immersed in distilled water at 20°C for 10 min
2. Ultrasonic treatment (UT): Fruits was immersed (distilled water) in sonicator bath at 20°C for 10 min
3. SA1 treatment: Fruits was immersed in sonicator bath at 1 mM SA and 20°C for 10 min
4. SA2 treatment: Fruits was immersed in sonicator bath at 2 mM SA and 20°C for 10 min

5. Ultrasonic treatment with SA1: Fruits was immersed in sonicator bath at 1 mM SA and 20°C for 10 min

6. Ultrasonic treatment with SA2: Fruits was immersed in sonicator bath at 2 mM SA and 20°C for 10 min

After dipping treatments, fruit placed on craft paper were allowed to dry at room temperature for approximately 60 min. Dried nectarines were placed in plastic boxes and stored at 0°C and 90±5% relative humidity for 60 days. During storage, 10 fruit of each replicate were analyzed at 15 days intervals. Other group of 20 fruit was used for initial analyses.

Analysis of Quality Attributes

Weight loss of nectarines was expressed as the percentage of loss of weight with respect to the initial weight (%). Firmness was determined using a hand penetrometer with an 8 mm long measuring plunger and was expressed as Newton (N).

For the analysis of soluble solids content (SSC) and titratable acidity (TA) of each sample, tissue sap was squeezed out from fresh fruit materials with a press. In this juice, SSC were determined with a hand refractometer (%). TA content was determined by titrating method and calculating the result as grams of malic acid per 100 g fresh weight (%).

Ascorbic acid content of the samples was determined according to the recommended method of A.O.A.C. (2000) using 2,6-dichlorophenol indophenol and expressed as mg kg⁻¹.

The total flavonoid contents were measured by a colorimetric assay (Zhishen et al 1999) and the results were expressed as mg (rutin equivalent) 100 g⁻¹. Total phenolics of the nectarine extract were quantified spectrophotometrically using Folin-Ciocalteu reagent based on the method (Slinkard&Singleton 1977). Results were expressed as mg (gallic acid equivalent) 100 g⁻¹.

Total antioxidants was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method as described by Brand-Williams et al (1995) and was expressed as µmol (trolox equivalent) g⁻¹.

For evaluation of CI, nectarine fruits were longitudinally cut into halves for the evaluation of the occurrence of CI according to the severity of exocarp browning and flesh translucency (Khan et al 2011). CI was estimated visually as the percentage of the affected area compared with the total surface area of each section on a scale where: 0 = no change; 1 = less than 10%; 2 = 10-25%; 3 = 25-50%; 4 = 50-75%; and 5 = more than 75%.

The experiment was set up according to the factorial randomized design with 3 replications (10 fruit per replication). Analysis of Variance 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. Data were expressed as the mean ± SE for all parameters.

3. Results and Discussion

Weight loss

Weight loss is a major factor reflecting the quality of fruit. Weight loss of nectarine fruit constantly increased during whole storage duration due especially to respiration and transpiration process, regardless of the treatments (Figure 1). Ultrasonic treatment alone did not affect weight loss of fruit. However, combined treatment with ultrasonic and SA showed significantly reduced loss of weight, than control. UT + SA1 and UT + SA2 treatments reduced weight loss of fruits. At the end of the storage, the highest weight loss was determined in ultrasonic treated fruits (6.9%) and control fruits (6.6%), while the lowest weight loss was determined in UT + SA2 treatment (5.2%) followed by UT + SA1 treatment (5.3%). The anti-senescent action and maintenance of cellular integrity by SA in the present study might be the reason in lowering weight loss of nectarine (Bal 2016; Ezzat et al 2017). These findings for SA were supported on different fruit crops (Srivastava&Dwivedi 2000; Zheng&Zhang, 2004). Moreover, salicylic acid as an electron donor produces free radicals which prevents normal respiration (Wolucka et al 2005) and can also decrease respiration rate and fruit weight loss by stoma closing (Zheng&Zhang, 2004).

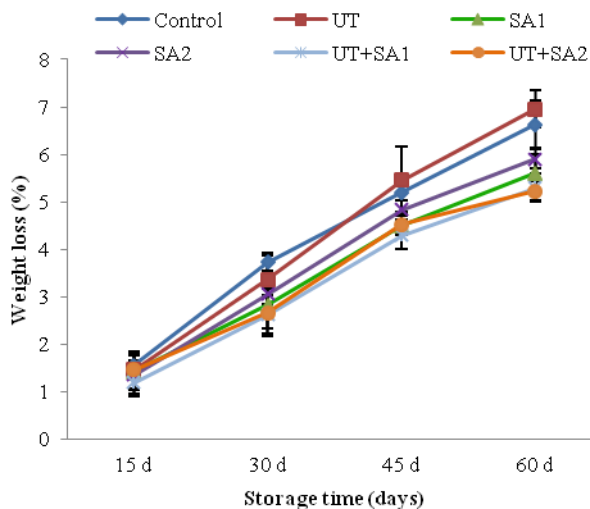


Figure 1
Effect of ultrasonic and salicylic acid treatments on weight loss of nectarine fruit during storage
SSC and TA

SSC and TA were assessed as indicators of the metabolic activity and ripening stage of the fruit. In the study, While TA decreased gradually during storage with no significant differences between the treatments, a significant increase in SSC was observed (Figure 2,3). A similar increase in SSC during storage of nectarine fruit has been previously reported (Ozdemir et al 2006; Bal 2018). Increases in SSC usually accompany with ripening of climacteric fruits. At the end of storage, the highest SSC value was determined in control fruits (15%), while the lowest SSC value was determined in UT + SA2 treatment (13.4%). Comparing with

the control fruit, UT + SA2 treatment retarded SSC increase of nectarine fruit, effectively maintaining the initial quality. These results are in accordance with those obtained by Erbas et al (2015) and Sabır (2017) who showed that SSC increased slightly with SA treatments during storage.

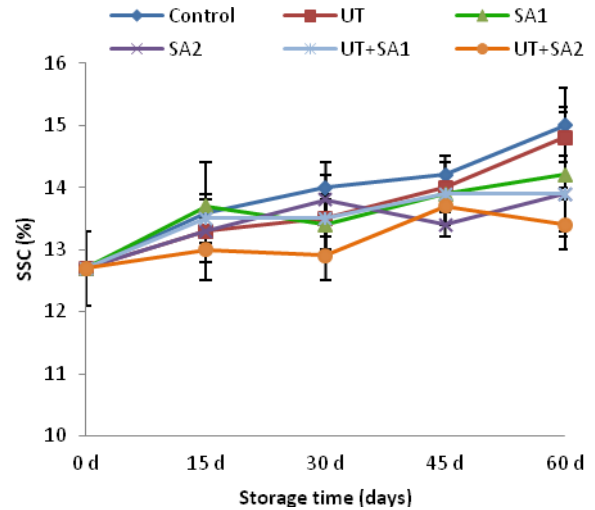


Figure 2
Effect of ultrasonic and salicylic acid treatments on SSC and TA of nectarine fruit during storage

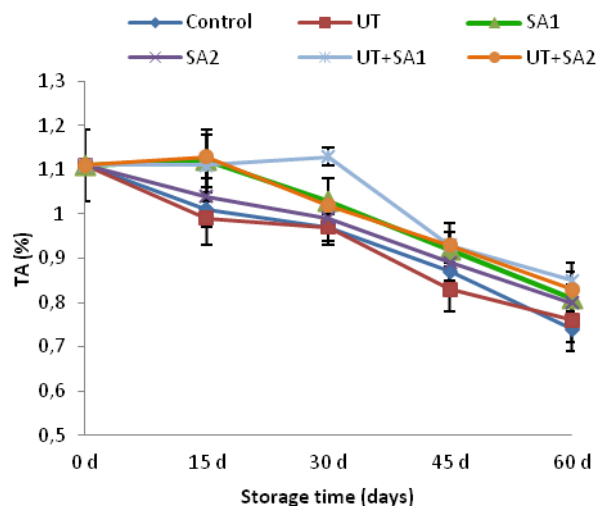


Figure 3
Effect of ultrasonic and salicylic acid treatments on SSC and TA of nectarine fruit during storage
Fruit firmness

Fruit softness occurs as a result of deterioration in cell wall structures and changes in cellulose and pectin components. In the study, nectarine firmness decreased during postharvest storage due to softness of fruit tissues via metabolic changes induced by enzymatic action and respiration (Figure 4). Firmness value of nectarine fruits at harvest time was 75.3 N. In control and ultrasonic treatment, fruit firmness value decreased to 45 N during 60 day cold storage. At the end of the storage, the highest firmness was determined in UT + SA2 treatment (58 N), followed by SA2 (54.6 N), UT + SA1 treatment (53.3 N) and SA1 treatment (50 N),

respectively. Ultrasonic alone had no influence, but when it was combined with SA, it resulted in greater retardation of firmness softening than SA alone. This is in agreement with Yuting et al (2013) and Bal et al (2017) who reported that ultrasonic could facilitate polyamine penetration into the tissue cells of fruits; a quicker and stronger resistance is induced. Moreover, delayed ripening process in SA treated fruits was concentration dependant and 2 mM SA dose maintained the firmness better than 1mM SA. Higher firmness in SA alone or combination treated fruits might be attributed to the reduced hydrolysis of soluble starch. Increased retention of firmness as the result of SA treatment has also been reported in several horticultural crops (Srivastava&Dwivedi 2000; Zhang et al 2003; Asghari&Aghdam 2010).

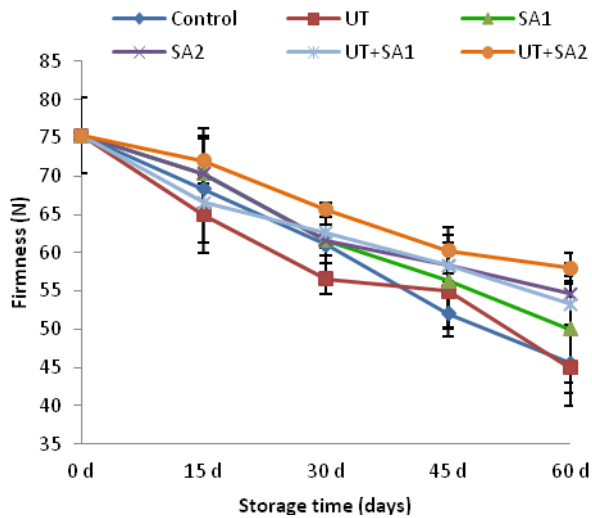


Figure 4
Effect of ultrasonic and salicylic acid treatments on firmness of nectarine fruit during storage

Ascorbic acid

Ascorbic acid is an important nutrient quality parameter and is very sensitive to degradation due to its oxidation compared to other nutrients during food processing and storage (Veltman et al 2000). The variation on ascorbic acid content is indicated in Figure 5. The content of ascorbic acid dropped notably during the storage of 40 days in all the samples. These results are consistent with previous reports showing that the levels of ascorbic acid in peaches, nectarines and apricots increased soon after harvest and decreased during storage (Lee&Kader 2000; Zhao et al 2018). The application of UT + SA2 and SA2 treatment significantly slowed the falling tendency. After 60 days of storage, the lowest ascorbic acid value was determined in ultrasonic treated fruits ($161 \text{ mg } 100 \text{ g}^{-1}$) followed by control fruits ($175 \text{ mg } 100 \text{ g}^{-1}$), while the highest ascorbic acid value was determined in UT + SA2 treated fruits ($205 \text{ mg } 100 \text{ g}^{-1}$) followed by SA2 treated fruits ($195 \text{ mg } 100 \text{ g}^{-1}$). The ascorbic acid degrades during storage period due to oxidative reduction and activity of ascorbate oxidase. The markedly delayed ascorbic acid degradation in UT

+ SA2 and SA2 treated fruits could possibly be due to its restrained oxidation-induced breakdown and retarding ripening. Similar to these findings, Lu et al (2011) and Awad (2013) reported that SA delayed the decline of ascorbic acid content and prevented the destruction, so high contents of ascorbic acid in treated pineapple and peach fruits could improve the fruit quality.

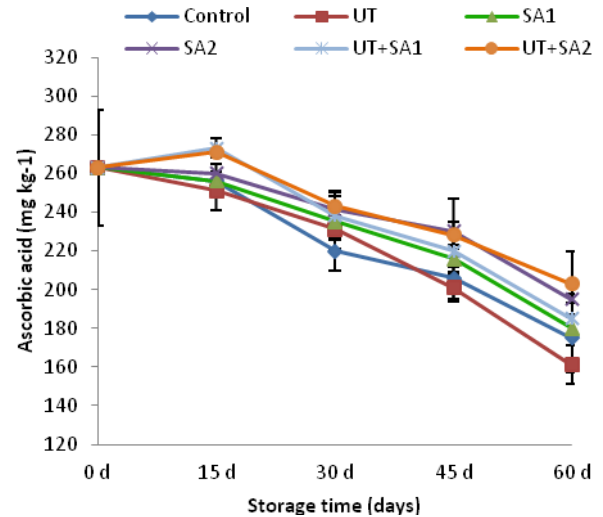


Figure 5
Effect of ultrasonic and salicylic acid treatments on ascorbic acid of nectarine fruit during storage

Total flavonoid and total phenolic content

In stone fruit the most abundant phenolics are flavonols and cinnamic acids, including chlorogenic and neochlorogenic acids (Ramina et al 2008). As shown in Figure 6 and Figure 7, at the beginning of the storage, the amounts of total flavonoid and phenolic contents was $93 \text{ mg } 100 \text{ g}^{-1}$ and $294 \text{ mg } 100 \text{ g}^{-1}$. In the study, flavonoids followed a pattern very similar to that of phenolics, as also reported by Bal (2016) in SA treated peaches, the contents of total phenols and flavonoids in nectarine fruits fluctuated until 45th day and then decreased in all treatment. Fruits treated with individual SA and combination of SA and UT had higher values of total flavonoid and total phenolic content than the untreated control fruits and UT alone. At the end of the storage, the highest both total flavonoid and total phenolic content were determined in UT + SA2 treated fruits ($108 \text{ mg } 100 \text{ g}^{-1}$ and $320 \text{ mg } 100 \text{ g}^{-1}$, respectively). The results showed that the contents of total phenols and flavonoids of the nectarine fruits treated with UT + SA2 were higher than those treated with UT + SA1, which suggested that the effects of SA on total phenols and flavonoids contents of the nectarine fruits were concentration dependent. According to the results obtained that synergistic effects between salicylic acid and ultrasonic treatment were observed and combined treatment was more effective on enhancing total phenols and flavonoids. Similarly, Chen & Zhu (2011) and Bal et al (2017) reported that ultrasonic treatment with other chemicals had synergistic effect that maintained the biochemical compound of fruits. Moreover, Perez-Balibrea et al (2011) and Razavi et al (2018) have

reported that the total phenols and flavonoids contents in broccoli sprouts and peaches were significantly increased by SA treatment.

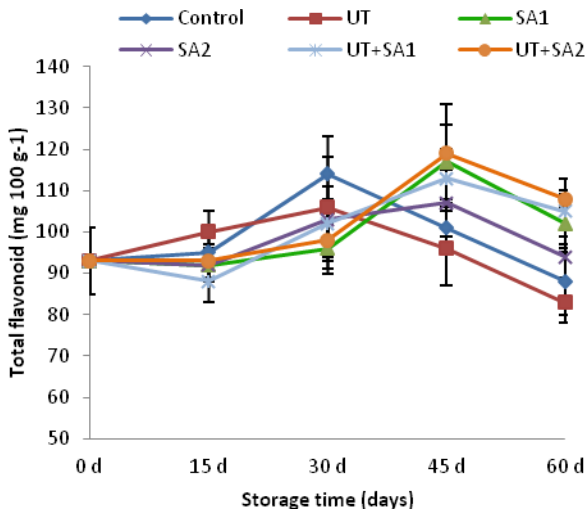


Figure 6
Effect of ultrasonic and salicylic acid treatments on total flavonoid of nectarine fruit during storage

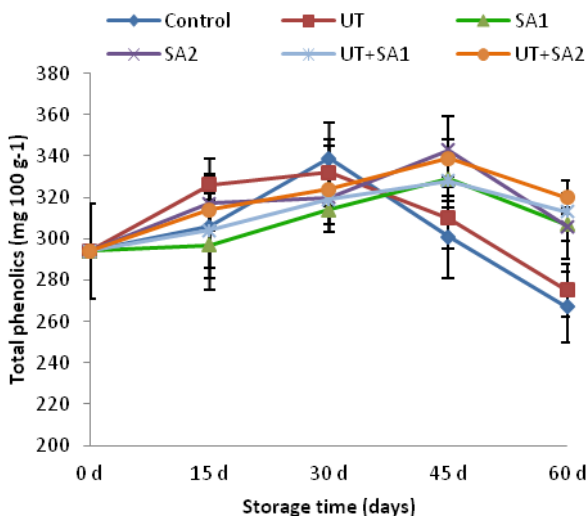


Figure 7
Effect of ultrasonic and salicylic acid treatments on total phenolics of nectarine fruit during storage

Antioxidant content

Nectarines are a good source of natural antioxidants, which provide protection against harmful reactive oxygen species and are associated with a lower incidence of chronic diseases (Shui&Leong 2006). The antioxidant contents showed an initial increase, followed by a decrease during cold storage (Figure 8); this is consistent with previous studies (Xi et al 2017; Zhao et al 2018). The results of antioxidant activity showed that fruits treated with SA and UT and combination of SA and UT had higher antioxidant activity than control fruits after 60 days of storage. At the end of the storage, control fruits had the lowest antioxidant content ($13 \mu\text{mol g}^{-1}$), while nectarine fruit treated with UT + SA2 ($16.7 \mu\text{mol g}^{-1}$) had the highest total phenolic content followed by UT + SA1 treatment ($16.5 \mu\text{mol g}^{-1}$). Ac-

cordingly, SA molecules could have had more opportunities to penetrate the fruit tissue by ultrasonic application. Moreover, SA has been reported to regulate antioxidants and maintain dietary value during storage (Huang et al 2008). The regulation of antioxidants as a result of SA application is not clear. It may be due to activation of antioxidant system in response to signaling SA which results in systemic acquired resistance in the cells (Tareen et al 2012). Taking into account the change in UT + SA2 treatment, it could be confirmed that total phenolics, total flavonoids and ascorbic acid are the main compounds contributing to the antioxidant capacity of the nectarine fruits, in agreement with previous reports (Sayyari et al 2011; Gimenez et al 2014; Davarynejad et al 2015).

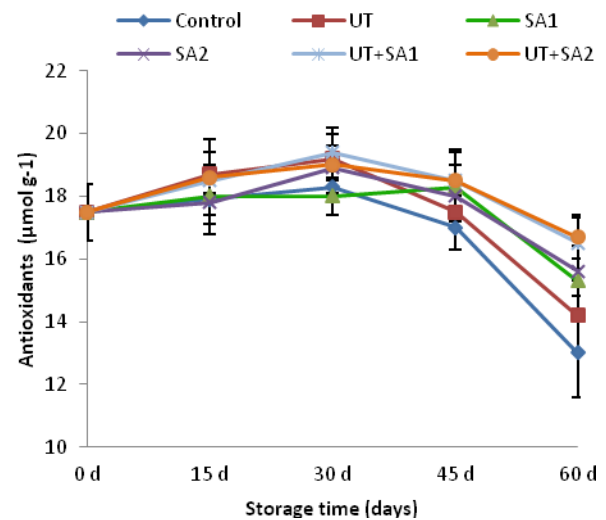


Figure 8
Effect of ultrasonic and salicylic acid treatments on antioxidants of nectarine fruit during storage

Chilling Injury

CI limits the storage life of peaches and nectarines under low temperature. It has been widely reported that the expression of CI symptoms, especially internal browning, develops faster and more intensely when susceptible fruit are stored at temperatures between 2.2 and 7.6°C than those stored at 0°C or below but above their freezing point (Lurie&Crisosto 2005). As shown in Figure 9, no visible symptoms of CI were observed in the fruit when stored at 0°C for 30 days. In the present study, it was found that salicylic acid and combined treatment with ultrasonic treatment could effectively reduce CI in nectarine fruit, and 2 mM was the most effective concentration. CI symptoms, characterized by exocarp browning and flesh translucency, were observed the highest rate (10-25%) in ultrasonic treated fruit and control fruit after 45 days of storage. At the end of the storage, the lowest CI rate was determined in UT + SA2 treated fruits (1.1) followed by UT + SA2 and SA2 treated fruits (1.5), while the CI rate was determined in UT treated fruits (3.5) followed by control fruits (3). The lower CI symptoms in nectarines treated with SA alone and combined ultrasonic may be due to slower metabolic rates and retention of various

bioactive compounds in fruits. Nowadays, it has been obvious that the CI symptoms are created due to the oxidative stress caused by overproduction of ROS and high values of antioxidant compounds inhibit ROS and contribute to reduce the CI symptoms (Yang et al 2011). Kang et al (2003) also reported that SA is involved in chilling tolerance through H_2O_2 metabolism mediation. Similar to previous studies (Cao et al 2010; Aghdam et al 2014; Khademi et al 2019), we found that SA treatment alleviated the CI symptoms of fruits.

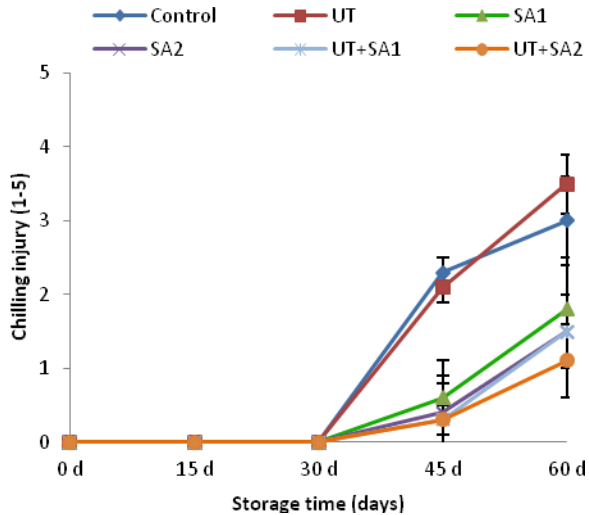


Figure 9
Effect of ultrasonic and salicylic acid treatments on chilling injury of nectarine fruit during storage

Conclusion

In conclusion, ultrasonic combined with SA treatment was more effective in alleviating CI and maintaining quality in nectarine fruit during the cold storage. However, ultrasonic treatment alone had similar effect to control treatment. Among the assayed doses (1 mM and 2 mM), the highest effect were found with SA at 2 mM. The combination of 2 mM SA with ultrasonic treatment was especially successful in preserving quality attributes with a higher nutraceutical value through ascorbic acid, phenolic, flavonoid and antioxidant content. These results suggested that 2 mM SA with ultrasonic treatment might be a powerful strategy to enhance antioxidant potential and quality of nectarine fruits. In further research, the potential benefit of using the combination of ultrasonic technology and other safe chemicals as commercial postharvest treatments to maintain quality in nectarine fruit should be explored.

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