Postharvest putrescine and ultrasound treatments to improve quality and postharvest life of table grapes (*Vitis vinifera* L.) cv. Michele Palieri

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Abstract

The main objective of this study was to assess the effectiveness of putrescine (Put) (1 and 2 mM for 10 min) and ultrasound treatments (32 kHz for 10 min) alone or in combination on changing biochemical compounds and extending postharvest life of grape. After treatments, clusters were packed in boxes with modified atmosphere packaging and stored at 1-2 °C with 90-95% relative humidity for 60 days. The weight loss, total soluble solids, titratable acidity, total anthocyanins, total phenolic content, antioxidant capacity, stem browning, decay rate and visual appearance at 0, 20, 40 and 60 days after harvest were recorded. Statistically significant differences were observed between different treatments in all measured parameters except for weight loss and total soluble solids. The data showed that individual Put or ultrasound treatment had a positive response in maintaining grape quality during storage, but conjugation of Put with ultrasound treatments showed better effects. Combination treatments maintained higher levels of anthocyanins, total phenolic content, antioxidant capacity and reduced the loss of sensory acceptability and decay incidence compared to control. At the end of the storage, control grapes markedly lost their quality, reaching below the critical marketable level while all the treatments preserved better the visual quality. These results demonstrated that the combined treatments of Put and ultrasound could be a promising approach to maintain postharvest storage quality of grapes.

Keywords: biochemical compound, grape, polyamine, postharvest quality, ultrasonic

Introduction

Grapes are highly perishable commodities and thus length of storage is limited. Major factors limiting grape storage and shelf life and causing important economic losses to the industry are cluster dehydration (berry water loss and rachis browning), skin colour changes, accelerated softening and microbial spoilage, especially gray mould decay caused by the pathogen *Botrytis cinerea* (Soylemezoglu, 2001). Appropriate environmental conditions during storage or transportation and the use of sulphur dioxide (SO_2) technologies have successfully alleviated these problems. Despite its efficacy, the SO_2 technology may compromise fruit taste and can cause damage to the grape which is manifested as cracks and bleaching. In addition, hypersensitivity reaction was reported in humans justifying the search for alternative technologies (Lichter et al., 2006).

Several preservation technologies have been suggested to enhance the postharvest life of fresh horticultural produce. Recently, biologically active natural products have started to become an effective alternative to synthetic fungicides in maintaining fruit quality during storage (Tripathi and Dubey, 2004).

Polyamines (PA) are small aliphatic amines, having low molecular weight that are ubiquitous in living organisms and have been implicated in a wide range of biological processes, including plant growth, development and response to stress (Smith, 1985). In plants, they have been implicated in a wide range of biological processes, including growth, development and abiotic stress responses. The most common polyamines are Put, spermidine (Spd) and spermine (Spm) found in every plant cell (Asrey and Barman, 2015). Reddy et al. (2008) reported that Put was the major polyamine followed by spermidine and spermine in grapes.

Postharvest application of polyamines has been demonstrated to influence the shelf life and quality of various fruits of both climacteric and nonclimacteric nature. The polyamines concentrations commonly used vary between 0.01 and 2 mM (Champa, 2015).

Postharvest application of Put, by immersion or vacuum infiltration, has been reported to delay fruit ripening and extend shelf life in some climacteric or nonclimacteric fruits such as lemon (Valero et al., 1998), apricot (Martinez-Romero et al., 2001), mango (Malik and Singh, 2005), strawberry (Khosroshahi et al., 2007), sweet cherry (Bal, 2012), plum (Serrano et al., 2003; Davarynejad et al., 2013) and peach (Bal, 2013). Champa et al. (2014) reported that prestorage dip treatment of 0.5 mM Put or 0.5 mM Spd or 1.0 mM Spm for 5 min maintained the quality and extended the shelf life of grape cv. Flame Seedless for up to 60 days in cold storage. Further, postharvest Put-treated berries (1 and 2 mM) exhibited higher total phenolic content, catechin, total quercetin and antioxidant activity (Shiri et al., 2012).

Ultrasound (sonication) treatment, which is an emerging technology that is considered to be inexpensive, simple, reliable and environmentally friendly, has been studied for use in several applications including fruit processing (Lagnika et al., 2017). Frequency of 20 kHz to 100 MHz, which is beyond the audible range of human hearing Ultrasound effects on liquid systems are mainly related to the cavitation phenomenon (Kate et al., 2016).

Ultrasound is also one of the newest nonthermal methods to extend shelf life of fresh fruits during storage (Bal, 2013). Due to the elimination of microorganisms and enzymes without destroying nutrients of foods, ultrasound can be used as an alternative method (Ercan and Soysal, 2013). Although many studies have been done by applying ultrasound during food processing and preservation, there are few published reports on the effect of ultrasound treatments on postharvest horticultural physiology. In the studies, postharvest ultrasound treatments have been shown to extend shelf life and maintain quality in strawberries (Cao et al., 2010), litchis (Chen et al., 2012), plums (Chen and Zhu, 2011; Bal, 2016) and peach (Yang et al., 2011; Bal, 2013). But, effectiveness of postharvest ultrasound application on fruit quality has not been studied in table grapes.

The present study was conducted to investigate the potential utilization of Put alone or in combination with ultrasound as a postharvest tool to maintain quality and extend the postharvest life of grapes cv. Michelle Palieri in cold storage (1-2 °C and 90-95% RH) conditions.

Materials and methods

Table grape (*V. vinifera* L.) cultivar Michelle Palieri was harvested at ripe stage (TSS≥16 Brix) in a commercial vineyard in Tekirdag, Turkey. Clusters were selected for size and color uniformity. Fruits with physiological disorders or fungal infections were discarded. After preparation, clusters were weighed to about 2 kg and then randomly distributed into six groups before treatment.

Treatments

Ultrasound treatment was applied in a water bath (20 °C) in the ultrasonic chamber. Clusters were treated with 32 kHz ultrasound 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. Put concentrations of 1 and 2 mM were prepared by dissolving Put powder (Sigma Aldrich Co., USA) in hot distilled water. Fruits were divided into six groups. Treatments and abbreviations can be summarized as follows:

1. Control: Clusters were immersed in distilled water at 20 °C for 10 min.
2. Put 1 treatment: Clusters was immersed in solution at 1 mM Put at 20 °C for 10 min.
3. Put 2 treatment: Clusters was immersed in solution at 2 mM Put at 20 °C for 10 min.
4. Ultrasound treatment: Clusters was immersed (distilled water) in the ultrasonic chamber at 20 °C for 10 min.
5. Put 1 + ultrasound: Clusters was immersed in solution at 1 mM Put at 20 °C for 10 min in the ultrasonic chamber.
6. Put 2 + ultrasound: Clusters was immersed in solution at 2 mM Put at 20 °C for 10 min in the ultrasonic chamber.

After dipping treatment, the clusters were placed on kraft paper and allowed to dry. Grape samples of 2 kg each were packaged in polypropylene (PP) trays (260 mm * 170 mm * 32 mm), sealed in air conditions with modified atmosphere packaging (MAP) and stored at 1-2 °C and 90% RH for 60 days. Weight loss, total soluble solids (TSS), titratable acids (TA), antioxidant capacity, total phenolics content, total anthocyanin content, stem browning, decay rate and visual appearance were evaluated after 0, 20, 40 and 60 days of storage.

Weight loss

Clusters were weighed at the beginning of the experiment just after treatments, and also every 20 days interval during the storage period. Weight loss was expressed as the percentage loss of the initial total weight.

Total soluble solids and titratable acidity

The total soluble solids (TSS) content in the juice was determined with a digital refractometer and titratable acidity (TA) was measured by titration with 0.1 N NaOH to pH 8.1, the results expressed as g*100 mL^{-1} fruit juice.

Stem browning

The stem browning of the clusters was assessed visually using a 0-5 scale: the scores were 0, 1, 2, 3, 4, and 5 for stem browning being <10%, 10–30%, 30–50%, 50–70%, 70–90%, and >90%, respectively (Chervin et al., 2005).

Decay rate

Berry decay was evaluated by scoring the number of contaminated berries by fungi per cluster, i.e. 1- no decay, 2- up to 5 decayed berries per bunch, 3- up to 10 decayed berries per bunch, 4- up to 20 decayed berries per bunch, and 5- over 20 decayed berries per bunch (Lurie et al., 2006).

Visual appearance

Visual appearance of grapes was evaluated on a nine-point scale (1: extremely poor; 3: poor; 5: moderate and limit of marketability; 7: good; 9: excellent).

Total anthocyanin

The total anthocyanins content of grape extract was determined using pH differential method. (Giusti and Wrolstad, 2001). Absorbencies were read at 510 and 700 nm. The values were expressed as mg cyanidin-3-glucoside (c3g) equivalents per 100 g fresh weight (fw) using a molar extinction coefficient of 27.900.

Total phenolic content

Total phenolic content in extracts were determined (Slinkard and Singleton, 1977), using the Folin-Ciocalteu reagent and gallic acid in methanol as a standard. Briefly, 1 mL of approximately diluted samples and a standard solution of gallic acid were added to a 25 mL volumetric flask containing 9 ml of distilled water. A reagent blank using distilled water was prepared. 1 mL of Folin–Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of a 7% Na_2CO_3 solution was added with mixing and then allowed to stand for 2 h. The absorbance was measured at 760 nm. Total phenolic content was calculated as gallic acid equivalents (GAE)*100 g^{-1} fw.

Antioxidant capacity

The antioxidant capacity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method as described by Brand-Williams et al. (1995) with some modifications. Briefly, 4 mg of DPPH were dissolved in 100 mL methanol, and then 50 mL of grape extracts was added to 950 mL of DPPH radical and mixed by vortex and allowed to stand at room temperature in darkness. The absorbance of the samples was measured at 515 nm after 15 min using the spectrophotometer. A dose-response curve was generated, using Trolox as a standard, and the antioxidant capacity was expressed as µmol Trolox equivalent (TE)*g^{-1} fw.

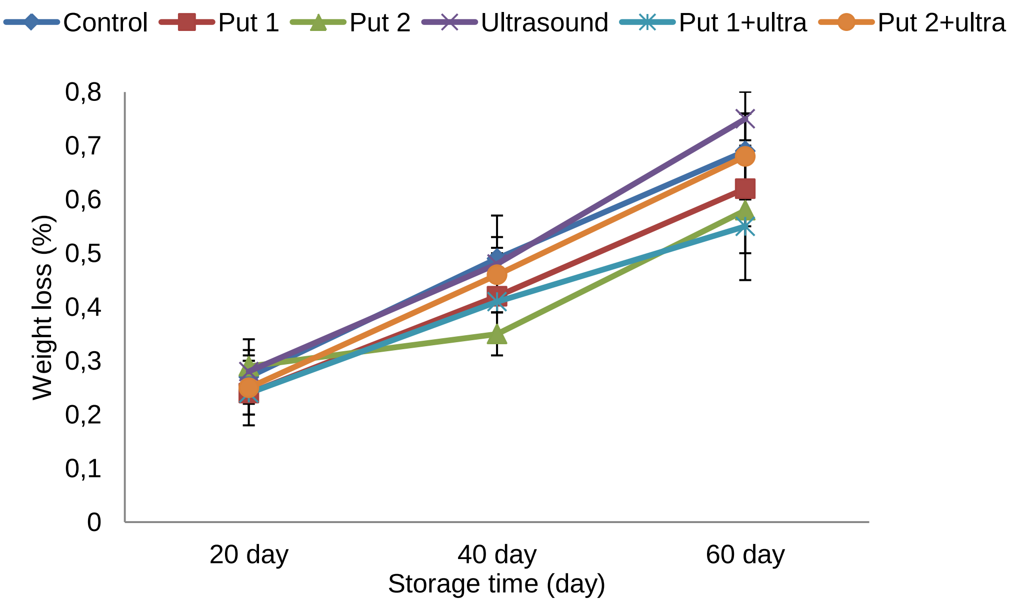
Statistical analysis

The data was analyzed statistically using completely randomized design experiment. Three replicates of samples were tested per treatment and mean ± standard deviation values were reported. Differences among samples were determined by least significant differences (LSD) using Minitab, and were considered to be significant when $P<0.05$.

Results and discussion

Weight loss

Weight loss is one of the most critical quality attributes of postharvest life and quality of grape during storage. Effects of Put and ultrasound treatment on grapes are shown in Figure 1. Weight loss of grapes increased slightly during storage period and was at low levels in all treatments at the end of the storage. This result is in agreement with previously found weight loss in grapes increased with prolonging storage period (Soylemezoglu, 2001). However, there were no significant differences ($P<0.05$) between treatments in terms of their effects on weight loss content during storage. At the end of the storage period, the lowest weight loss was determined in Put 1+ultrasound treated fruits (0.55%) and followed by Put 2 treatment (0.58%). The highest weight loss was observed in Put 2+ultrasound-treated fruits (0.68%) and followed by control fruits (0.69%). These results were consistent with previous studies with peaches and sweet cherry, polyamine and salicylic acid treatments with ultrasound treatments had no effect on weight loss (Yang et al., 2011; Bal, 2013). The most important reason for weight loss being low might be due to MAP application. It’s known that MAP is proven as an effective tool for reducing weight loss due to its inhibition of water vapour diffusion (Kader, 2002).

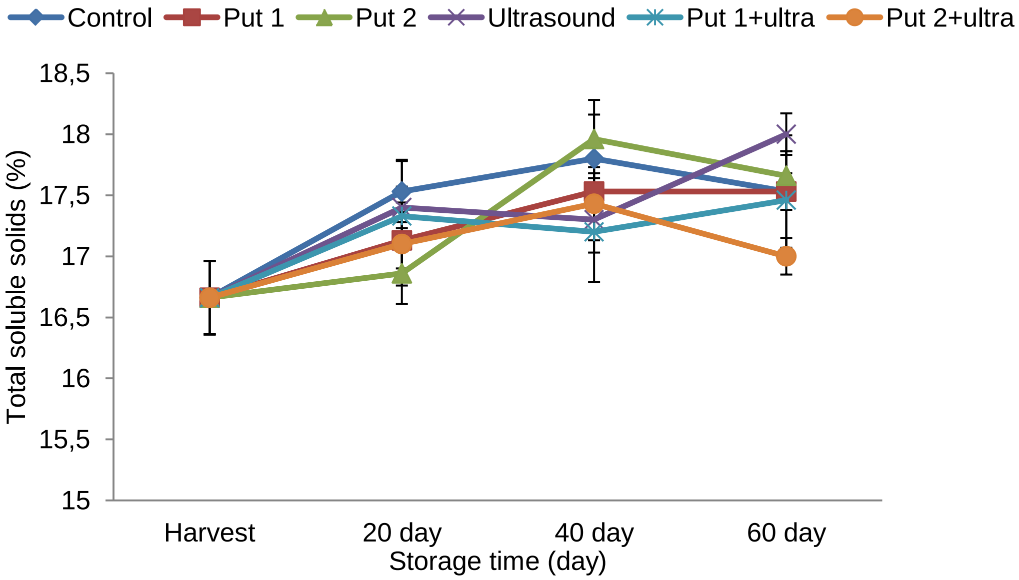


Vertical bars represent standard deviations of means (n=3)

Figure 1. Effects of Put and ultrasound treatment on weight loss of grapes

Total soluble solids

The changes in TSS content of grapes are presented in Figure 2. The treatments were not found to be significant (P<0.05). However, the storage period was found to be significant with regard to the changes in the TSS. Although fluctuations occurred in TSS content in the form of increases and decreases, increases occurred in all applications at the end of the storage time. The increases in soluble solids over the storage period could be due to weight loss and, therefore, fruit juice concentration. The initial TSS content of grapes was 16.6%. After two-month storage, the ultrasound group showed the highest TSS (18%), while Put 2+ultrasound group showed lowest TSS (17%). These results are in agreement with previously found that TSS content of grape increased slightly towards the end of storage (Bal and Kok, 2007).

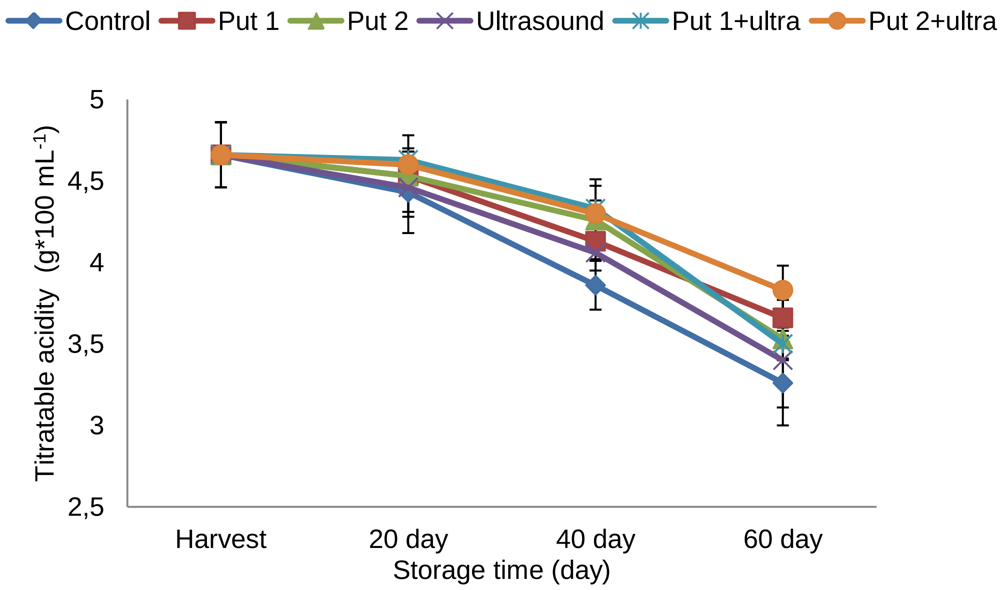


Vertical bars represent standard deviations of means (n=3)

Figure 2. Effects of Put and ultrasound treatment on TSS of grapes

Titratable acidity

The organic acids play an important role in flavor perception for consumers and fruit acidity usually tends to decrease due to using of organic acids as substrates for respiratory metabolism during storage period (Valero and Serrano, 2010). As described in Figure 3, with progress of storage, TA content in grapes decreased significantly ($P<0.05$) when compared with the initial value (4.66 g*100 mL^{-1}). In the study, reduction of TA content was followed by decline in visual quality. After the end of 60 days of storage, TA contents of all the treatments were significantly higher than non-treated control grape (3.26 g*100 mL^{-1}). On the 60^{th} day, the highest TA content was determined in Put 2+ultrasound treated fruits (3.83 g*100 mL^{-1}), followed by Put 1 treated fruits (3.66 g*100 mL^{-1}). Put treatment or Put treatment combined with ultrasound treatment would induce a lower physiological maturation in table grapes, since both sugars and organic acids are substrates of the fruit respiration. However, Liu et al. (2006) reported that the reasons remain unclear why PAs modify soluble solutions and titratable acids. Cao et al. (2010) and Bal (2016) also informed that ultrasound treatment had no significant effect on TA.

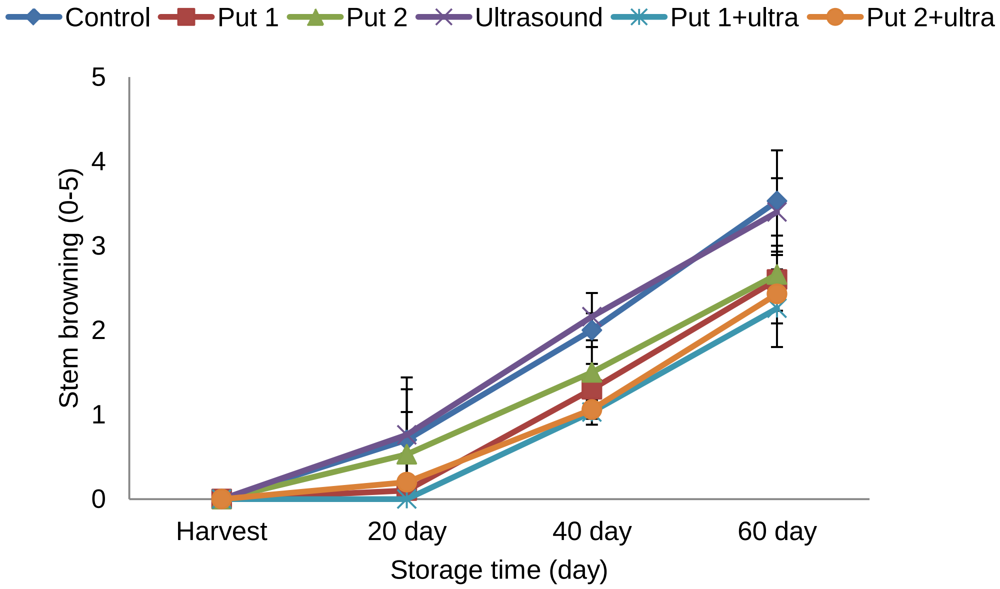


Vertical bars represent standard deviations of means (n=3)

Figure 3. Effects of Put and ultrasound treatment on TA of grapes

Stem browning

Stem browning is an important storage problem of grapes, which greatly affects consumer preference and fruit price (Lichter et al., 2006). Stem browning in cold storage has been commonly associated with water loss and oxidation processes (Crisosto et al., 2001). In the study, there were significant differences (P<0.05) between treatments in terms of their effects on stem browning during storage (Figure 4). During the storage period, except only ultrasound treatment all tested treatments maintained better stem colour than control. Ultrasound treated grapes and control grapes reached to the highest browning value (3.4 and 3.5; 50-70% browning) at the end of 60 days of storage. On the other hand, 60th day of storage, all of other treatments had score of 2.0 for stem browning revealing lower level (30-50%) of stem browning in the bunch. The obtained results indicated that both doses of Put played a very effective role in maintaining the green color of the stem. Moreover, in combination treatments, ultrasound treatment also increased the effectiveness of Put treatment. These results are in accordance with the finding of Champa et al. (2014) on grapes who reported that Putrescine treatments effectively retarded the degradation of stem colour during storage period. Similarly, Valero et al. (1998) and Martinez-Romero et al. (2001) also reported that postharvest polyamine applications delayed colour changes in lemon and apricot during storage.

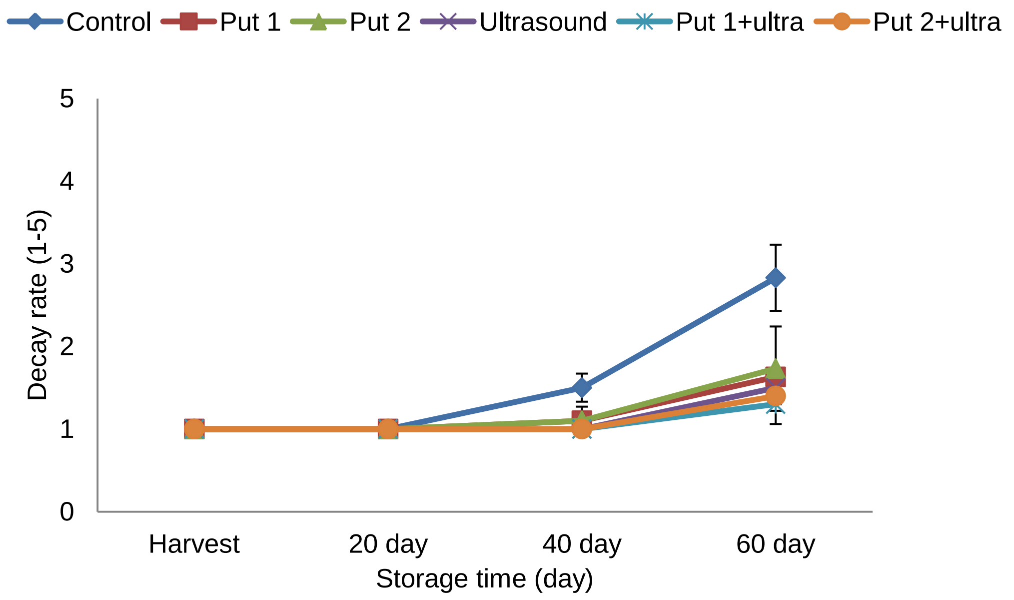


Vertical bars represent standard deviations of means (n=3)

Figure 4. Effects of Put and ultrasound treatment on stem browning of grapes

Decay

Put, ultrasound and the combination of both decreased a significantly decay rate in clusters during storage (Figure 5). Control and all treated grapes did not show any visible infection during the first 20th days of storage. On 40th day, decayed berries were determined only in control group (1.5), Put 1 treatment (1.1) and Put 2 treatment (1.1). At the end of the storage, the score of decayed berries in control was as high as 2.8 point, whereas the treatments markedly delayed the decay incidence with the scores of 1.3, 1.4, 1.5 and 1.7 for Put 1+ultrasound, Put 2+ultrasound, ultrasound and Put 1, respectively. The results revealed that Put treatments combined with ultrasound treatment was more effective in inhibiting fungal decay during storage than Put treatments alone. This might be a result of direct inhibition of microbial growth by ultrasound treatment or activating defense responses in fruit and thus contributed to alleviate and reduce tissue colonization by pathogen. Among the work reported in the literature, most of them focused on the use of ultrasound to ensure microbial safety of produce. When used alone or combined with sanitizers, ultrasonication enhanced the destruction or removal of bacteria, molds, and yeasts on the surfaces of produce such as strawberries (Cao et al., 2010), peach (Yang et al., 2011) and plums (Chen and Zhu, 2011). Moreover, effectiveness of Put treatments could result from having an antipathogenic effect. These results are in agreement with the results obtained by Khosroshahi et al. (2007) and Bal (2012), who found that Put treatments decreased decay incidence in fruits.

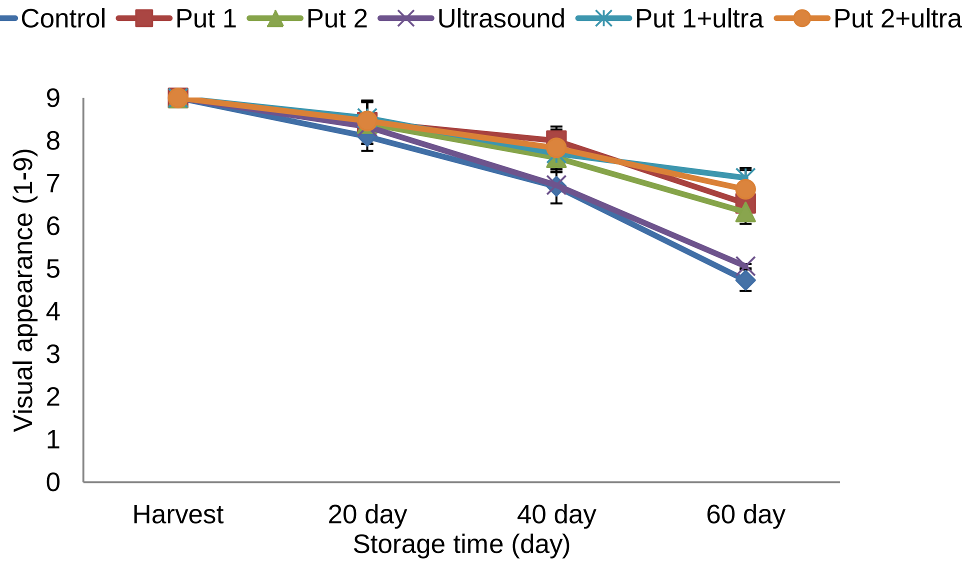


Vertical bars represent standard deviations of means (n=3)

Figure 5. Effects of Put and ultrasound treatment on decay rate of grapes

Visual appearance

High consumer acceptance in visual appearance of grapes are lack of defects such as decay, cracked berries, stem browning, shriveling, sunburned, dried berries, and insect damage (Crisosto and Smilanick, 2007). As shown in Figure 6, during storage period, for all treatments slight decreased in visual appearance were observed, with differences among treatments. On the 20th and 40th day of storage the scores were between 7 (good) and 9 (excellent) in all treatments. At the end of the storage, the highest value on the visual quality was obtained from Put 1+ultrasound (7.1) and Put 2+ultrasound (6.8), followed by Put 1 (6.5) and Put 1 (6.3). Ultrasound treated grapes had scores just at the limit of marketability (5.0). However, control grapes markedly lost their quality, reaching below the critical marketable level (4.7) on the 60th day while all the treatments preserved better the visual quality. These inferior scores given to controls were due to decay development and stem browning. Numerous reports have demonstrated the beneficial effects of Put treatment or ultrasound treatment on fruit or vegetables sensorial quality (Khosroshahi et al., 2007; Champa et al., 2014; Pinheiro et al., 2015; Onursal et al., 2015; Yu et al., 2016).

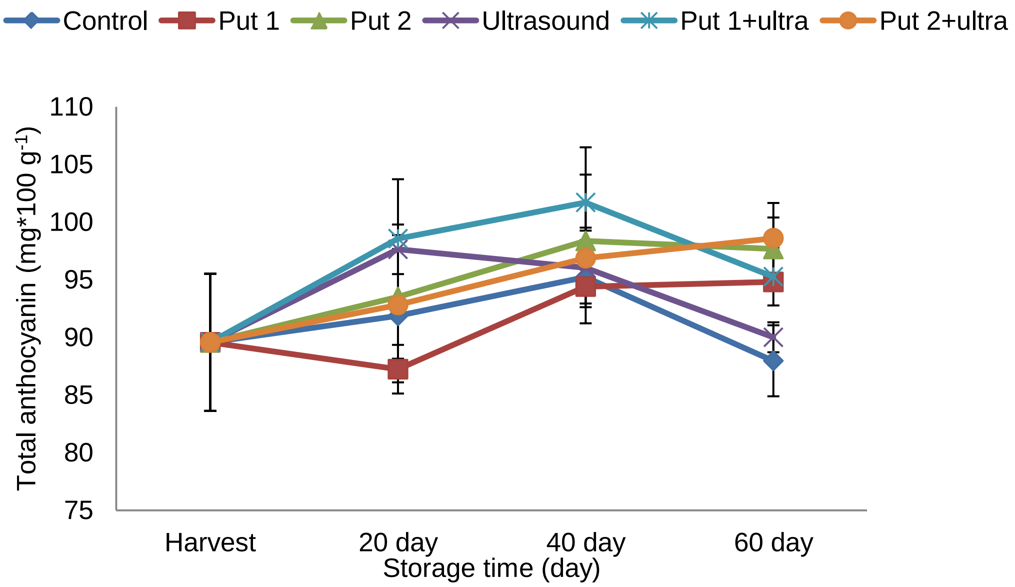


Vertical bars represent standard deviations of means (n=3)

Figure 6. Effects of Put and ultrasound treatment on visual appearance of grapes

Total anthocyanin

The pigment responsible for red colorations of grapes is anthocyanins and changes of anthocyanin content strictly depend on the cultivar, growing conditions, maturity and postharvest conditions (Shiraishi and Watanabe, 1994). In the study, statistical analyses showed that total anthocyanin content of grapes were significantly ($P^{<}$ 0.05) affected by treatments (Figure 7). Total anthocyanin content of grapes was 89.56 mg*100 g^{-1} at the time of initial storage. In general, anthocyanin content of grapes tended to increase during storage even though there were slight fluctuations in anthocyanin content. After 60 days in storage, the lowest anthocyanin content was found in the control treatment with 87.96 mg*100 g^{-1}, while Put 2+ultrasound had the highest value with 98.6 mg*100 g^{-1}. The results showed that, Put and the combination of Put and ultrasound treatments maintained anthocyanin in comparison with the untreated control. When combined with power ultrasound, the combinational treatments could show a synergetic effect. This is in agreement with Yuting et al. (2013) who reported that ultrasound could facilitate PA penetration into the tissue cells of fruits; a quicker and stronger resistance is induced. Champa et al. (2014) reported that prestorage dip treatment of polyamines effectively reduced the rate of grape berry softening, impaired degradation of peel colour, stabilized anthocyanins and phenolic compounds and suppressed the activity of pectin methylesterase while reducing the rate of electrolyte leakage. The results are consistent with Serrano et al. (2003) and Malik and Singh (2005) who reported that polyamine treatments delayed fruit skin color degradation.

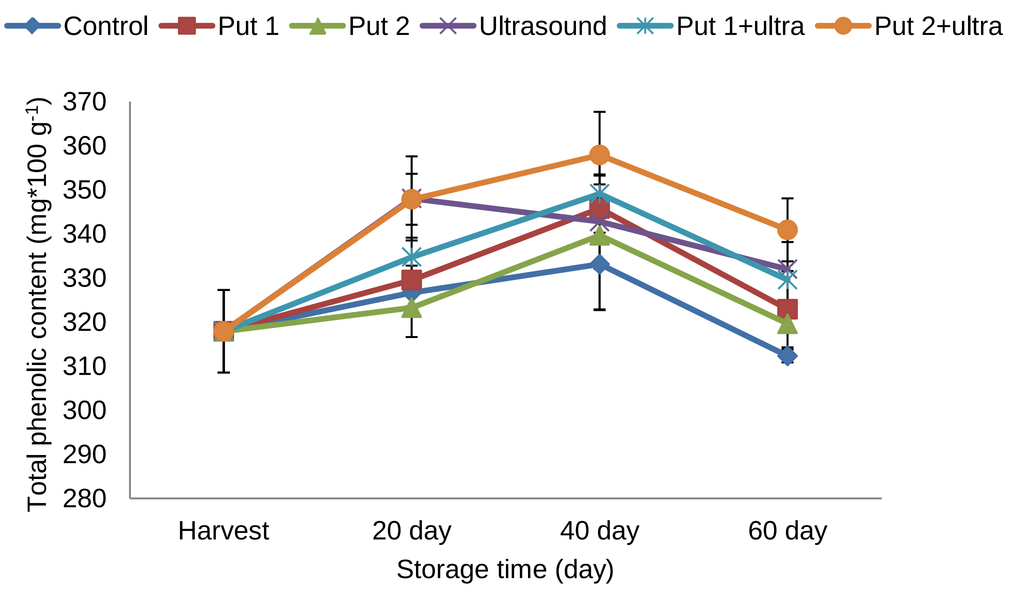


Vertical bars represent standard deviations of means (n=3)

Figure 7. Effects of Put and ultrasound treatment on total anthocyanin of grapes

Total phenolic content

Phenolic compounds are an important group of secondary metabolites in grape and strongly influence the berry quality such as color, flavor, bitterness, and astringency. Phenolics are highly unstable and undergo various changes throughout storage (Sharma et al., 2008). Total phenolic content of grapes as significantly affected by Put and ultrasound treatments are presented in Figure 8. In the study, the levels of total phenolic in grapes at harvest were found to be 317.9 mg*100 g^{-1}. Total phenolic content of grapes initially showed an increasing trend up to 20 days of storage and decreased towards end of storage period. The relatively higher content of phenolic in ultrasound treated alone and combined with Put was observed. Similarly, previous studies showed that Put treatments had significant positive effect on phenolic compound of fruits (Shiri et al., 2012; Davarynejad et al., 2013). At the end of the storage, the highest total phenolic content was found in Put 2+ultrasound treatment (340.9 mg*100 g^{-1}) and ultrasound treatment (332 mg*100 g^{-1}) while least amount was detected in control (312.3 mg*100 g^{-1}) and Put 2 treatment (319.6 mg*100 g^{-1}). The results showed that sonicated samples showed better retention or preservation of phenolic compounds when compared to other treatments. This result is in agreement with these previous studies that ultrasound treatment maintained higher total phenolic content of peach (Yang et al., 2011), litchi (Chen et al., 2012) and plum (Bal, 2016). Yeoh and Ali (2017) also reported that hormetic dosage of ultrasound treatment can enhance the activity of PAL and total phenolic content and hence the total antioxidant capacity to encounter with oxidative stress.

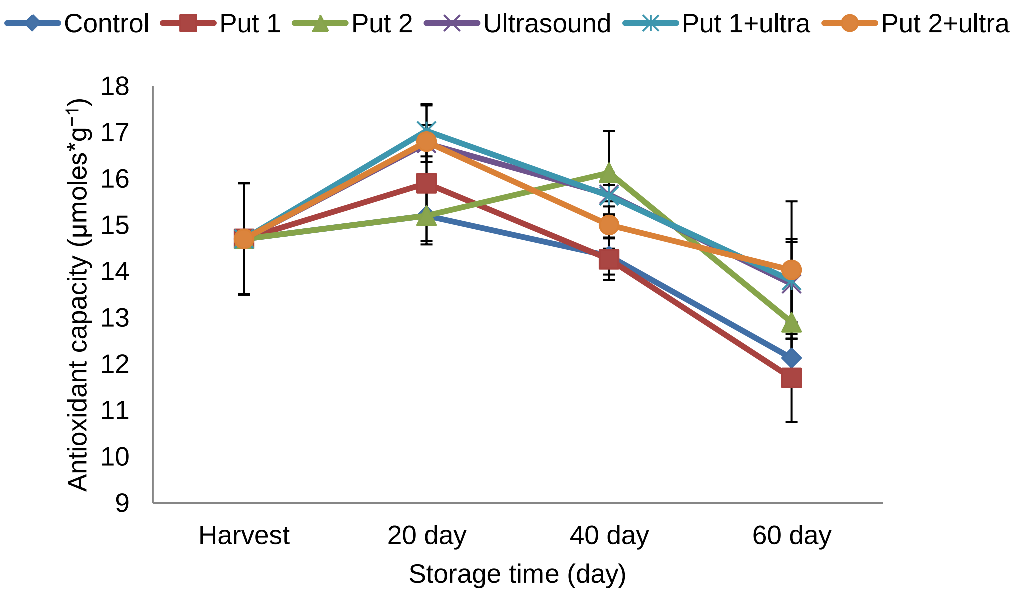


Vertical bars represent standard deviations of means (n=3)

Figure 8. Effects of Put and ultrasound treatment on total phenolic content of grapes

Antioxidant capacity

Antioxidant properties of fruits and vegetables mainly contributed to their polyphenols and vitamin content (Song, 2015). It was determined a positive relationship between antioxidant capacity and total phenolic contents, indicating an effect of polyphenol content on antioxidant capacity. There was no significant difference on antioxidant capacity among individual treatment of Put and ultrasound. However, the combined treatment of ultrasound plus Put maintained antioxidant capacity better than individual treatments. In the study, antioxidant capacity of grapes tended to rise in the early stage of storage (Figure 9). These increases were especially more observed in ultrasound treated grapes. After this initial increase, antioxidant capacity values then decreased gradually during the rest of the storage. During storage period, the highest antioxidant capacity was observed in Put 1+ultrasound treatment (17.03 μmol*g^{-1}) on 20th day. At the end of the storage, the highest antioxidant capacity detected in Put 2+ultrasound treatment (14.03 μmol*g^{-1}), the lowest antioxidant capacity detected in Put 1 treatment (11.7 μmol*g^{-1}) followed by control (12.1 μmol*g^{-1}). More increase in antioxidant capacity of ultrasound treated grapes may be due to enhancing secondary metabolites accumulation in fruits. Yu et al., (2016) reported that lettuce treated with ultrasound exhibited an increase in PAL activity after storage for 60 h, resulting in production of phenolic compounds as secondary metabolites and enhancement of antioxidant capacity. Similarly, some reports documented an increase of total phenolics and antioxidant capacity in sonicated produce samples (Potrebko and Resurreccion, 2009; Sales and Resurreccion, 2010).



Vertical bars represent standard deviations of means (n=3)

Figure 9. Effects of Put and ultrasound treatment on antioxidant capacity of grapes

Conclusions

The results of the above experiments indicate that, individual Put or ultrasound treatment had a positive response in maintaining grape quality during storage, but conjugation of Put with ultrasound treatments showed better effects. Ultrasound combined with Put treatments contributed to maintaining the quality of grapes by reducing decay rate and stem browning, delaying changes of visual quality as well as increasing antioxidant capacity and phenolic compound. It is considered that postharvest dip treatment of Put in the ultrasonic bath (20 °C) can be used commercially to extend the storage life at 1-2 °C of grape up to two months with minimum losses of fruit quality without any SO_2 treatment.

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