



RESEARCH PAPER

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Postharvest treatment of ethanol maintains quality and extends shelf life of table grape (*Vitis vinifera* L.) cv. perlette during cold storage

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Abstract

Present work was carried out in order to find the best concentration of ethanol as alternate to SO₂ for maintaining postharvest quality of table grapes (*Vitis vinifera* L.) cv. Perlette during 28 days of cold storage. Grape bunches were manually harvested at commercial maturity and were dipped in different concentrations (30%, 35%, 40%, 45% and 50%) of ethanol for 5 min and stored at 2-3°C, and 90-95% RH. Analysis of various physical and chemical parameters were evaluated at 0 day (before treatment) and at 7, 14, 21 and 28 days of storage. 50% Ethanol significantly reduced weight loss, berry shatter, decay rate followed by 45% ethanol treatment. These two treatments also retarded the degradation of TSS, TA, maintained higher ascorbic acid and rated 'very good' in organoleptic properties when compared with control. Fruit treated with 30%, 35% and 40% also extended shelf life of bunches and maintained postharvest quality during 28 days of cold storage. The postharvest dip of grapes in 50% ethanol for 5 min could be an effective technique for extending shelf-life of 'Perlette' grapes.

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Introduction

Table grape (*Vitis vinifera* L.) is one of the most important produce consumed in the world. It has relatively low physiological activity among category of non climacteric fruits. Although, factors like weight loss, fungal attack, rachis browning, color degradation, berry shatter and softening make it perishable in nature (Champa *et al.*, 2014). The standard method for maintaining postharvest quality of table grape is use of SO₂. However, SO₂ has already been excluded from United States Food and Drug Administration's 'generally recognize as safe' category in 1986 because of its safety hazards (Zahavi *et al.*, 2000). These hazards may include berry damage that is manifested as bleaching and cracks, poor fruit taste and hypersensitivity in some consumers (Lichter *et al.*, 2005). Since then, many attempts have been made in search of alternative safe treatments to maintain postharvest quality of table grapes.

Ethanol is a naturally occurring GRAS (Generally Recognize as Safe) chemical, which can be safely used with food (Anonymous, 2003). It is common food additive with highly potent antimicrobial characteristics (Larson and Morton, 1991). There are number of evidences to prove efficiency of ethanol as potent antifungal agent to maintain postharvest life of many horticultural produces like Peaches and Nectarine (Margosan *et al.*, 1997), Strawberry (Ayala-Zavala *et al.*, 2005; Vardar *et al.*, 2012), Broccoli (Asoda *et al.*, 2009; Mori *et al.*, 2009; Fukasawa *et al.*, 2010), Sweet cherries (Bei *et al.*, 2011), Mango (Gutierrez-Martinez *et al.*, 2012), Mandarins (Abd Elwahab and Rashid, 2013), Sweet melon (Liu *et al.*, 2012; Jin *et al.*, 2013), Asparagus (Herppich *et al.*, 2014), Lime (Opio *et al.*, 2015, 2017), Loquat (Wang *et al.*, 2015) and Chinese Bayberry (Mu *et al.*, 2017). Several studies have been documented on use of ethanol as SO₂ alternative to overcome fungal attack and to maintain storage quality of table grapes (Lichter *et al.*, 2002, 2005; Karabulut *et al.*, 2003, 2004, 2005; Gabler *et al.*, 2004, 2005; Chervin *et al.*, 2005, 2009; Pinto *et al.*, 2006; Lurie *et al.*, 2006; Romanazzi *et al.*, 2007; Sabir *et al.* 2010; Candir *et al.*, 2012; Elwahab *et al.*, 2014). Researchers used different concentrations of ethanol ranging 10-50% for grapes

dip prior to storage. Immersion of grape bunches in 30% ethanol solution successfully maintained postharvest quality by arresting conidia of *Botrytis cinerea* during cold storage (Elwahab *et al.*, 2014). Treatment of 50% ethanol significantly improved shelf life of grapes by reducing population of *Escheria coli* (Pinto *et al.*, 2006) while 35% ethanol killed conidia of *Botrytis cinerea* and significantly enhanced shelf life of grapes (Gabler *et al.*, 2005). Sabir *et al.* (2010) suggested that combination of 30% ethanol with modified atmosphere packaging significantly extended storage life of grapes. Del Nobile *et al.* (2008) found that grape dip in 50% ethanol for 5 min before storage as best treatment among chlorinated water and hot water treatments for extending postharvest shelf life of grape berries. As per hazards concern, Lichter *et al.* (2002) stated that use of less than 60% of ethanol concentration is completely safe.

All above mentioned studies on ethanol necessitated a study to fig. out exact concentration of ethanol as postharvest treatment for table grapes to maintain storage quality. So, the objective of our work was to find the best concentration of ethanol which can be used as SO₂ alternative for extending postharvest life of table grapes.

Materials and methods

Plant material and treatments

Mature bunches of Table grapes (*Vitis venifira* L. cv. Perlette) were harvested manually from commercial vineyard "Rawat fruit farm" and transported to postharvest laboratory of PMAS Arid Agriculture University immediately with proper handling. In order to remove field contamination, grapes were washed with distilled water. Properly formed bunches without any visible defect were selected for experiment, and 500 g of fruit were used as day 0 analysis. Bunches were divided into 6 experimental units containing 6kg each. 5 units were dipped in ethanol concentration of 30%, 35%, 40%, 45% and 50% for 5 min respectively except 1 unit which was kept as control. Dipped bunches were air-dried at room temperature for 12-15 min and then packed in cartons for cold storage (2-3°C, RH 90-95%) along with control sample for four weeks.

Analyses were carried out on 1.5 kg of fruit from each unit further dividing into 3 replications (500g each) and data was recorded after every week interval.

Weight loss, berry shatter, decay incidence and color

Weight loss was calculated by following formula:

Weight loss (%) =

$$\frac{[\text{Fruit weight at harvest (day 0)} - \text{Fruit weight at storage interval}]}{\text{Fruit weight at harvest (day 0)}} \times 100$$

Fruit weight at harvest (day 0)

Bunches were shaken manually in paper kraft bag for 1 min constantly to count number of shattered berries. Decay incidence parameter was expressed in number of rotten berries per sample which were separated from bunches. Color of grape berries was measured using Konica Minolta Chromameter (CR-300). The values of L^* a^* b^* were recorded as assessment of bunches was taken from opposite sites at equatorial region.

Chemical analysis

50g of fruit from each replication was taken to extract juice for chemical analysis. These berries were squeezed and juice was filtered through a cheese cloth. TSS was assessed with the help of digital refractometer (Atago PAL-1, model 3810, Japan) and expressed in percentage. TA was determined by the method described by Candir *et al.* (2011). Ascorbic acid (Vitamin C) was measured using Spectrophotometer (sp-3000 plus) in which absorbance of supernatant was measured at 243nm. Remaining procedure was followed as described by Hans (1992).

Sensory analysis

Postharvest sensory quality of fruit was judged in term of appearance, sweetness, crispiness, taste, aroma and flavor. These analyses were evaluated by a panel consisting of 10 well trained people. Panel was asked to observe, chew and taste the random berries from each sample and grade every parameter according to grading scale 1-5 (1=excellent, 2=very good, 3=good, 4=bad, 5=very bad).

Statistical analysis

Data of experiment was analyzed as factorial in a completely randomized design by analysis of variance (ANOVA) using Statistix 8.1 (Analytical software, 2005).

Each treatment was repeated three times using 500g of fruit per replication. Means were compared using LSD at $p < 0.005$ level of significance as recommended by Chase and Brown (1997).

Results

All ethanol treatments significantly maintained weight loss as compared to control samples although it increased with passage of time (fig. 1). Fruit treated with 50% ethanol concentration proved more potent in reducing weight loss in comparison to all other treatments. By the end of 4 weeks of cold storage, control berries measured with 10.23% weight loss, a rate much higher than grapes treated with 50% ethanol (7.17%) and 45% ethanol (7.43%) treatments. After total storage period, cumulative weight loss was 7.88%, 8.67 and 9.02 in berries treated with 40%, 35% and 30% concentration of ethanol, respectively.

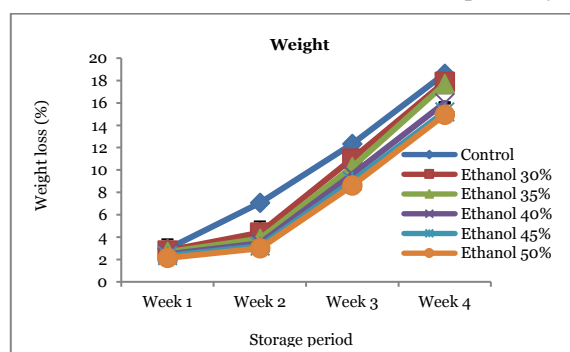


Fig. 1. Effect of ethanol treatments on weight loss (%) of Perlette grapes during 4 weeks of cold storage.

The significant effect of ethanol on decay development can be clearly seen in fig. 2. There was variation found in number of rotten berries in ethanol treatments and control sample after 28 days of storage. No significant difference was found among ethanol treatments although increase in ethanol concentration suppressed more decay development (Table 1). Maximum numbers of diseased affected berries were 20.70 in untreated samples and minimum were found in grapes treated with 50% and 45% ethanol (17.42 each) at the end of storage period. Berry shatter increased with storage time but it was significantly lower in all ethanol treatments (fig. 3). 45% of ethanol concentration was found most efficient in reducing number of berry shatter with 12.46 berries as compared to untreated grapes with

16.93 berries after 4 weeks of storage. There was no significant difference found among ethanol treatments, but significant data was found when compared to control. Color of berries measured at storage time ($L^* = 49.50$, $a^* = -8.92$ and $b^* 23.39$) changed throughout 28 days of storage period in all treated and untreated samples. All treated berries slightly changed their brightness towards darkness (lowering L^* value) except untreated berries with

mean L^* value of 49.63 during 4 weeks of storage (Table 1). All samples slightly lost greenish color (higher a^* value) and maximum reading was recorded in controls while minimum in grapes treated with 50% ethanol. No significant difference was found among all ethanol treated fruit in terms of yellow color of berries, although untreated berries exhibited more yellowness during cold storage (Table 1).

Table 1. Effect of ethanol treatments on different postharvest quality parameters of table grapes cv. “Perlette” during cold storage.

Treatments	Weight loss (%)	Diseased berries	Berry Drop	Titratable acidity (%)	TSS ($^{\circ}$ Brix)	Ascorbic Acid (mg/100g FW)	Color		
							L^*	a^*	b^*
Control	10.23 A	20.70 A	16.93 A	0.720 C	16.46 A	1.86 C	49.63 A	7.44 C	23.65 A
Ethanol (30%)	9.02 BC	17.92 B	13.70 B	0.847 AB	15.47 D	2.58 B	46.07 B	7.06 B	21.66 B
Ethanol (35%)	8.67 C	17.83 BC	13.07 B	0.862 AB	15.46 D	2.61 B	46.12 B	6.91 AB	21.60 B
Ethanol (40%)	7.88 D	17.50 BC	12.60 BC	0.900 A	16.13 B	2.66 B	46.19 B	7.00 B	21.54 B
Ethanol (45%)	7.43 DE	17.42 BC	12.47 BC	0.856 AB	15.60 CD	2.86 AB	45.92 B	7.06 B	21.55 B
Ethanol (50%)	7.17 E	17.42 BC	12.53 BC	0.860 AB	15.53 CD	3.00 A	45.53 BC	6.87 A	21.66 B

Means within a column not sharing same letter are significantly different by the LSD test at $p \leq 0.05$.

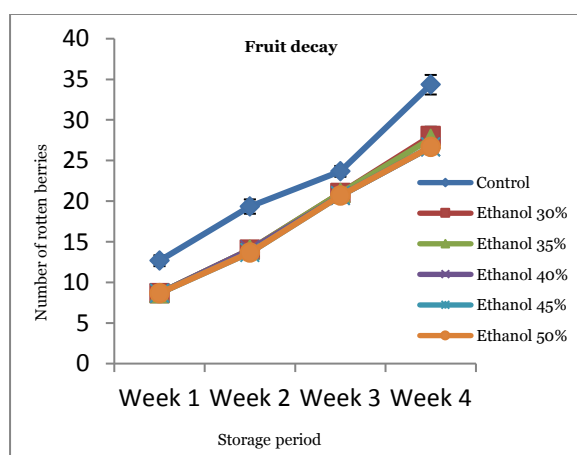


Fig. 2. Effect of ethanol treatments on fruit decay of Perlette grapes during 4 weeks of cold storage.

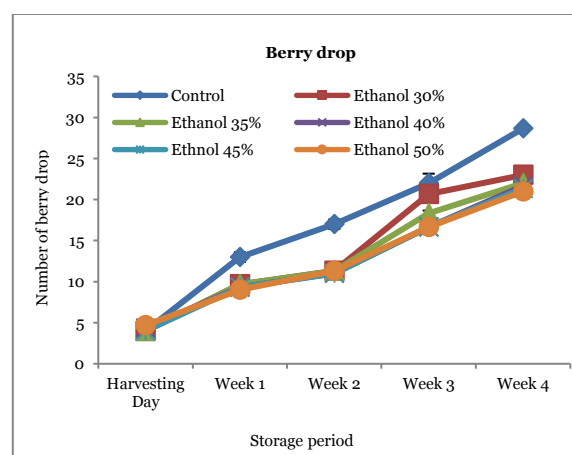


Fig. 3. Effect of ethanol treatments on berry drop of Perlette grapes during 4 weeks of cold storage.

TA decreased up to 2 weeks of storage and then sudden increase was observed in all samples (fig. 4). Maximum percentage of TA was found in fruit treated with 40% ethanol (0.9%) followed by 35% ethanol (0.82%) and 50% ethanol after complete storage period. Control samples measured with significantly minimum TA (0.72%) when compared to all ethanol treatments. All treatments of ethanol significantly affected grapes during cold storage (Table 1).

There was increase in TSS content found with storage period (fig. 5) and maximum TSS was measured in control berries (16.46°Brix). Minimum TSS content was recorded in 35% ethanol (15.46°Brix) and 30% ethanol (15.47°Brix) treatments followed by 50% ethanol (15.53°Brix), 45% ethanol (15.60°Brix) and 40% ethanol (16.13°Brix) respectively by the end of 28 days of storage. Ethanol treatments were found significantly effective in maintaining ascorbic acid

contents of berries during cold storage although ascorbic acid decreased in all treatments with time (fig. 6). Minimum ascorbic acid measured was 1.86mg/100g FW in untreated grapes while maximum (3mg/100mg FW) was measured in 50% ethanol treated grapes.

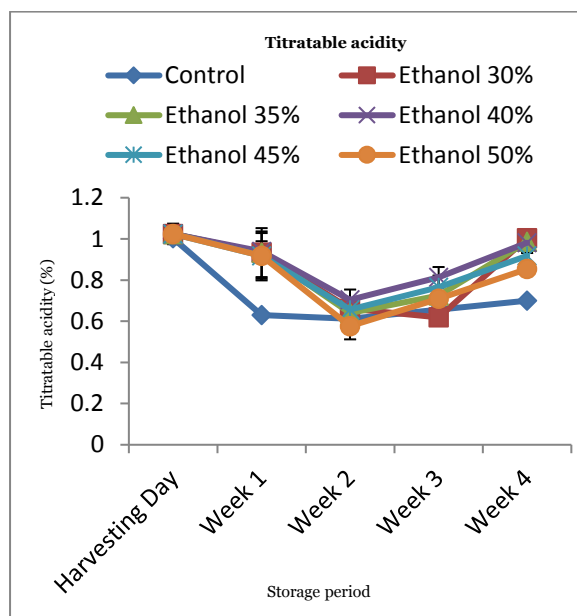


Fig. 4. Effect of ethanol treatments on titratable acidity of Perlette grapes during 4 weeks of cold storage.

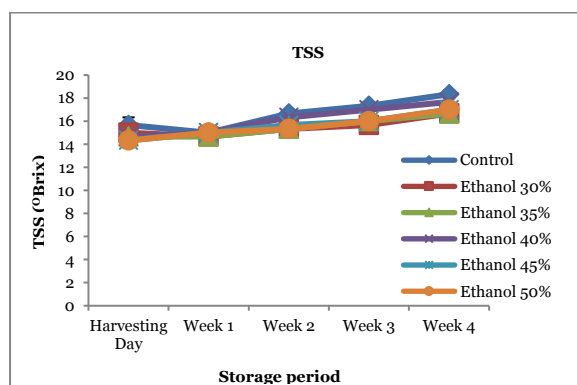


Fig. 5. Effect of ethanol treatments on TSS of Perlette grapes during 4 weeks of cold storage.

Another effective treatment in maintaining ascorbic acid content was 45% ethanol with 2.86mg/100mg FW ascorbic acid followed by 40% ethanol (2.66mg/100mg FW), 35% ethanol (2.61mg/100mg FW) and 30% ethanol (2.58mg/ 100mg FW) during 28 days of storage. Table grapes treated with 50% ethanol were marked as excellent in sweetness followed by 'very good' in crispiness and appearance after 28 days of

storage. Samples treated with 45% ethanol treatment, were ranked as 'very good' in all aspects of analysis except flavor, which was marked as 'good'. There was no significant difference in terms of appearance, sweetness, crispiness and taste among 30%, 35% and 40% ethanol treated berries although they were marked 'bad' in flavor. Untreated samples marked as 'bad' in appearance and taste and 'very bad' in sweetness and crispiness after 4 weeks of storage.

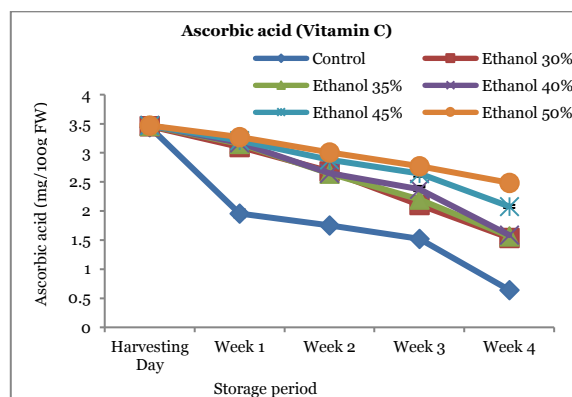


Fig. 6. Effect of ethanol treatments on ascorbic acid contents of Perlette grapes during 4 weeks of cold storage.

Discussion

Weight loss occurred in all treated and untreated grapes and it increased with storage time. The rate of weight loss was clearer up to second week and was significantly higher in untreated samples as compared to ethanol treated bunches. After second week of storage, magnitude of water loss tended to slow down, which might be related with water driving forces arise during long period of storage (Sabir *et al.*, 2013). In present study, rate of weight loss decreased with increase in ethanol concentration (Table 1). Our findings are in accordance with some previous studies, in which higher concentration of ethanol showed better results in lowering weight loss during weeks of cold storage (Karabulut *et al.*, 2004; Elwahab *et al.*, 2014). Mainly, it is believed that weight loss is due to water loss through intercellular evaporation (Wills *et al.*, 1998) leads to fruit softening, ripening and senescence by many metabolic reactions (Bai *et al.*, 2003). Another concept behind weight loss is the phenomenon of water gradient between internal and external environment of berry (Sanzhez-Gonzalez *et al.*, 2011).

Ethanol minimized decay rate hence controlled weight loss and effectively maintained postharvest quality of table grapes. Al-Qurashi and Awad (2013) also found that ethanol treatments on table grapes significantly reduced weight loss by controlling fungal attack. Efficiency of postharvest ethanol treatments in maintaining fruit firmness and delaying ripening is already well documented for many fruits like mandarin, avocado, nectarine and grapes (Pesis, 2005) which are the main factors for weight loss during storage.

In our study, ethanol treatments found significantly effective in reducing decay rate when compared with control. There are many studies that prove that ethanol has great potential for controlling postharvest diseases of table grapes (Lichter *et al.*, 2002, 2005; Karabulut *et al.*, 2003, 2004, 2005; Gabler *et al.*, 2004, 2005; Chervin *et al.*, 2005, 2009; Pinto *et al.*, 2006; Lurie *et al.*, 2006; Romanazzi *et al.*, 2007; Sabir *et al.* 2010; Candir *et al.*, 2012; Elwahab *et al.*, 2014). According to Elwahab *et al.* (201), ethanol works as protective layer against fungi and is very effective in surface sterilization; hence, controls decay rate during storage. The main mode of action of ethanol is direct that adheres to the fruit skin. Another way of ethanol to protect berry is its wash off effect of organic debris and dust. This organic matter may contain insect remains or feces which may create foci of bacterial or fungal development (Pinto *et al.*, 2006). In this study, decay rate was found minimum in fruits treated with higher concentration of ethanol while it was significantly highest in untreated berries (Table 1). Romanazzi *et al.* (2012) described similar findings stating that grapes treated with 20% ethanol controlled more decay as compared to grapes treated with 16% ethanol. We applied ethanol concentration more than 30% because Lichter *et al.* (2003) already stated that ethanol concentration must be at least 30% or above to control germination of spores of *Botrytis cinerea*, and results were excellent. Another study suggested that more than 30% of ethanol concentration is required to kill spores of *Rhizopus stolonifer* and *Aspergillus niger* (Gabler *et al.*, 2004). Pinto *et al.* (2006) found 50% ethanol treatment of grapes completely inhibited growth of fungi during

storage. These results are well similar to our findings in which 45% and 50% ethanol significantly reduced decay rate compared to control. Decay rate was more in grapes treated with low concentration of ethanol as compared to high concentration. Possible reason behind this phenomenon could be because survival of fungi followed by ethanol effect is strain dependent and some ethanol producing yeast can survive at low concentration of ethanol treatment (Lichter *et al.*, 2002).

Berry drop was significantly reduced in all ethanol treated berries as compared to untreated and it was measured minimum in grapes treated with higher concentrations of ethanol. Our results corroborate few previous studies in which ethanol treated grapes kept rachis fresh (Karabulut *et al.*, 2004) and reduced berry shatter during cold storage (Elwahab *et al.*, 2014). Ethanol works as protector of fungal attack and therefore stopped up fruit damage and so minimized the berry shattering (Elwahab *et al.*, 2014). Grapes treated with 50% ethanol lost less greenish color as compared to those treated with lower ethanol concentrations and control. In terms of brightness, all ethanol treated samples showed some darkness. These results of color measurements are well evident from previous study by Gabler *et al.* (2005). This change in color could be due to degradation of anthocyanins due to increase in polyphenol oxidase activity (Underhill and Critchley, 1993). Delay in increase of TSS content and TA during storage was observed in fruit treated with 50% ethanol followed by 45% ethanol (Table 1). The decrease in TSS is because of slower change of sugars from carbohydrates (43). This might be because of slowing down respiration, metabolic activity and delaying in ripening and senescence. Ascorbic acid was maintained significantly by 50% ethanol treated berries followed by berries treated with 45% and 40% concentration of ethanol during 4 weeks of cold storage. The slower rise in TA and maintaining ascorbic acid could be due to reduction in metabolic changes of organic acids into carbon dioxide and water as a result of reducing respiration rate and therefore maintain higher rate of acids. As per results declared by penal, grapes treated with 50% ethanol marked as excellent in sweetness followed by 'very

good' in crispiness and appearance after 28 days of storage. 45% ethanol treatment was found as another best treatment in which bunches were ranked as 'very good' in all aspects of analysis except flavor, which was marked as 'good'.

Untreated samples marked as 'bad' in appearance and taste and 'very bad' in sweetness and crispiness after 4 weeks of storage. Panel did not find alcoholic flavor in all ethanol treated grapes similarly reported in previous studies (Chervin *et al.*, 2005; Lichter *et al.*, 2005; Lurie *et al.*, 2006). Furthermore, some bunches treated with ethanol were marked as 'bad' in flavor after complete storage period. Deng *et al.* (2006) suggested that this off-flavor in ethanol treated berries might be due to the high TSS content, thereby masking the detection of ethanol by taste panelists.

Conclusions

Postharvest treatment of 50% Ethanol exhibited higher efficiency in extending shelf-life and maintaining berry quality of table grape cv. Perlette during cold storage (2-3°C and 90-95% RH) of 28 days. Grapes treated with higher concentrations (especially 50% and 45%) of ethanol reduced weight loss, decay rate, berry drop; delayed the increase in TSS and TA; maintained higher ascorbic acid and found very good in organoleptic analysis. Other treatments of ethanol also significantly maintained postharvest quality of table grapes but their efficacy was lower than of higher concentrations of ethanol. On the whole, we conclude that 50% ethanol has proved to be an effective treatment for extending shelf-life and maintaining postharvest quality of table grapes cv. Perlette.

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