

Changes in phenolic compounds and antioxidant capacity of fresh-cut table grape (*Vitis vinifera*) cultivar 'Shahaneh' as influence by fruit preparation methods and packagings

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Abstract

The changes in fresh cut grape quality were evaluated over 14 days storage at 5 °C that harvested by two different methods (1-berry and 4-berries cutting), packaged in polyethylene terephthalate (PET) and polyvinylchloride (PVC) bags. The results showed that 4-berries cutting, which packaged in PVC bags showed the lowest weight loss, decay incidence, berry shattering, with the highest acceptability as compared to the other treatments. Total soluble solids (TSS) and TSS/TA were increased during storage in all treatments. The phenolic content in 1-berry cutting increased over the storage time, but its content declined in 4-berries cutting. At almost all of treatments, the flavenoids compositions including catechin, quercetin 3-galactoside and total quercetin decreased significantly at the end of storage. Antioxidant capacity increased up to seven days storage and thereafter decreased at the end of storage. Results showed that fruits packaged in PVC bags had higher total phenolic content, phenolic compositions such as catechin, quercetin 3-galactoside and total quercetin and antioxidant capacity than PET ones.

Keywords: Berry quality; Catechin; minimal process; Polyethylene terephthalate; Polyvinylchloride; Quercetin 3-galactoside.

Abbreviations: PET-polyethylene terephthalate; PVC-polyvinylchloride; TSS-Total soluble solids; TA-Titratable acidity; FW-Fresh weight; HPLC- High-performance liquid chromatography.

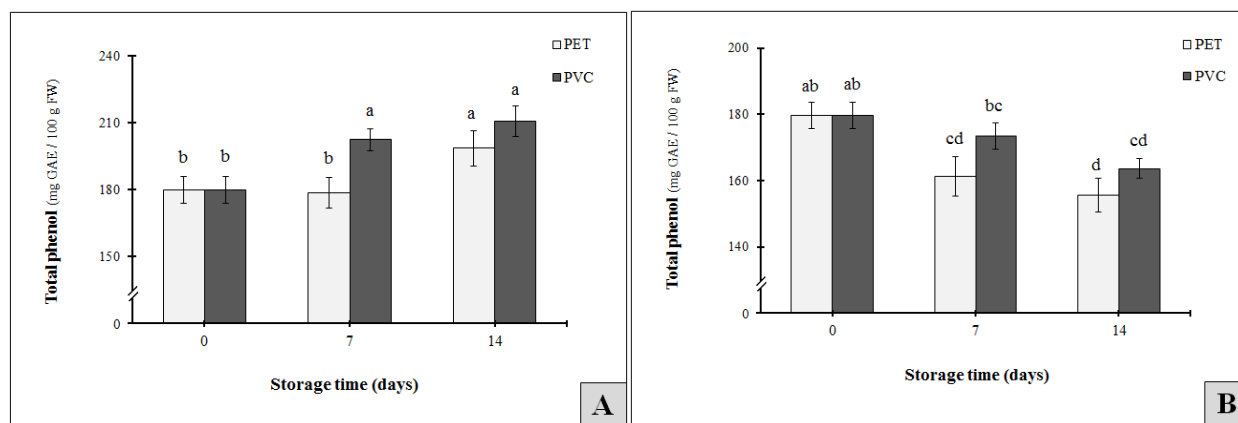
Introduction

Demand to ready-to-eat fruits with fresh quality is rapidly growing in recent years. It is well-known that minimally processed fruits and vegetables are generally more perishable than the original raw materials (Pittia et al., 1999). Physical damages during the peeling and cutting processes cause to increase in respiration rate, biochemical changes and microbial spoilage, which often result in degradation of color, texture (flaccidity due to loss of water) and flavour of the product (Saltveit, 1997). Nutrient losses may also be accelerated when plant tissues are wounded (Klein, 1987). Little information is available concerning the changes of phenolic compounds and antioxidant capacity in fresh-cut fruits during cold storage. Biochemical changes in vitamin C, sugars and phenolic compounds during storage of fresh-cut fruits are very important, because they are used as primary quantitative parameters of quality (Gorny, 2001). Packaged fresh-cut fruits and vegetables are increasingly becoming popular, because they are convenient and nutritious snack alternatives (Conte et al., 2009). However, fresh-cut products have limited shelf stability due to rapid quality deterioration (Jacxsens et al., 2002). The main limiting factor of packaged fresh-cut grapes is decaying (Holz et al., 2003). Current commercial practices for preparation of fresh-cut grapes are removal of cap stems, but decay incidence and quality loss are primarily caused by tissue injury during stem removal (Kou et al., 2007). Physical damages often stimulate respir-

ation rate (Taiz and Zeiger, 1991), microbial growth, product decay and quality deterioration (Kou et al., 2007). Modified atmosphere packaging (MAP) is the most common manner to preserve the initial color of fresh-cut fruits (Del Nobile et al., 2006). Modified atmosphere (MA) conditions can be done via packaging, which is a passive system, by balancing produced respiration and gas exchange through package materials. There has been an increase in the use of plastic film packaging, such as low density polyethylene (LDPE), polyvinylchloride (PVC) and polypropylene (PP) for fresh fruits and vegetables (Lee et al., 1996). These materials are generally transparent, provide a barrier to water vapor transmission, and are very selective in gas permeability to CO₂ versus O₂ (Lee et al., 1996). Recently, rigid containers offer several advantages over flexible pouches, including serving convenience, protection from mechanical damage and stacking capability. Hence, they are used increasingly in the fresh-cut industry (Chonhenchob et al., 2007). There are only a few publications available regarding to the optimal conditions for maintaining the quality of fresh-cut table grapes. Therefore, the main objectives of the present study were to evaluate the effects of different preparation methods and packaging bags on the quality maintenance and phenolic compounds of fresh-cut table grape.

Table 1. Quality parameters and methods employed to evaluate table grape quality after storage for 14 days at 5 °C

Quality parameters	Methods of evaluation and units
Berry appearance	Visual index of clusters: excellent, 1; good, 2; slightly dull, 3; <50% brownish and soft berries, 4; >50% brownish and soft berries, 5
Shatter	Number of shattered berries/kg
Acceptability	9=excellent, no defects, 7=Very good, minor defects, 5=fair, moderate defects, 3=poor, major defects, 1=unusable
Decay	1=none, 2=slight (up to 5% surface affected), 3=moderate (5–20% surface affected), 4=moderately severe (20–50% surface affected) and 5=extreme (>50% surface affected)
Flavor	Flavor acceptability, using a five-point scale: excellent, 1; good, 2; acceptable, 3; poor, 4; unacceptable, 5

**Fig 1.** Changes in total phenolics of 1-berry cutting (A) and 4-berries cutting (B) grape fresh-cut packaged with PET and PVC during storage at 5 °C. Vertical bars indicate standard error (n=3).

Results and discussion

General quality

The general quality of fresh-cut berries was decreased during storage (Table 2), because of weight loss, color changes, decay incidence and browning (Crisosto et al., 2002). The grapes without stem (1-berry cutting) indicated more quality declining than 4-berries cutting. Physical damages were occurred during the grape removal (tearing out the stems) and the openings which created after stem removal, have made the grape berries more susceptible to decrease the quality. In addition, berries which packaged in PVC bags could maintain quality and reduce shattering and decay percentage during 14 days storage. The extended shelf-life was observed at 4-berries cutting which packaged in PVC bags may be due to the lower physical damage and respiration rate.

Berries weight loss

All fruits showed a progressively weight loss during the storage period (Table 2). The weight loss is mainly related to respiration rate and moisture evaporation from the fruits. The results showed that 1-berry cutting had higher weight loss than 4-berries cutting both in PET and PVC bags. It may be due to the higher respiration rate of 1-berry, consequence of the damage sustained by the grape tissue when the cap stem is pulled out (Taiz and Zeiger, 1991). This can be explained that packaging of fruits in PVC may cause the better control atmosphere conditions and resulted in the lower respiration rate and weight loss.

TSS and TA

The results showed that TSS was increased significantly throughout entire storage of both 1 and 4-berries cutting (Table 2). Increase in TSS may be due to the higher water loss (Tanada-Palmu and Grosso, 2005). In contrast, TA content was decreased slightly during the storage life (Table 2), which was consistent with fruit quality decline, but there was no significant different between both cutting and packaging treatments. TA is directly related to the concentration of organic acids present in the fruits. The decreasing acidity during storage might be due to the metabolic changes in fruits or due to the use of organic acid in respiratory process which is in agreement with Echeverria and Valich (1989). TSS/TA significantly increased during 14 days storage at 5 °C that was attributed to the decreasing in TA content rather than to an increase in TSS (Table 2).

The changes of total phenolics and flavenoids

The changes of total phenolic content of fresh-cut grape during storage are shown in the Fig. 1a and 1b. As the results showed, the phenolic content gradually increased during storage in 1 berry cutting in both PET and PVC packaging. In contrast, 4-berries cutting showed a decline in phenolics over 14 days of storage. In both preparation methods, PVC packaging maintained higher phenolic content than PET bags. Phenolic compounds are generally synthesized by the shikimate pathway in which phenylalanine ammonialyase (PAL) is the key enzyme. The physical damage of plant tissue can increase PAL activity, which leads to an increase

Table 2. Effect of different packaging (PET and PVC) and fruits preparation methods (1-berry and 4-berry) on some characteristics of fresh-cut grapes during 14 days storage

Treatment	Storage time	Fruit quality								
		Berry appearance	Shattering	Flavor	Decay	Acceptability	Weight loss	TSS	TA	TSS/TA
1-berry										
PET	0	1 c	0.0 c	1 c	0.0 d	9 a	0.0 c	15.5 b	0.6 ns	28.1 b
	7	3 b	30.5 b	3 ab	13.3 c	3 ab	9.5 ab	19.4 a	0.5	38.9 ab
	14	5 a	50.9 a	4 a	36.7 a	1 b	12.5 a	19.9 a	0.4	46.4 a
PVC	0	1 c	0.0 c	1 c	0.0 d	9 a	0.0 c	15.5 b	0.6	28.1 b
	7	3 b	29.8 b	2 ab	11.7 c	3 ab	5.8 b	17.0 ab	0.5	32.1 ab
	14	4 ab	48.3 c	4 a	26.7 b	1 b	5.9 b	17.1 ab	0.4	40.0 ab
4-berries										
PET	0	1 b	0.0 c	1 ns	0.0 b	9 a	0.0 d	15.5 b	0.6 ns	28.1 b
	7	2 ab	16.8 b	1	3.3 b	7 ab	8.1 a	17.0 ab	0.5	30.3 b
	14	3 a	28.8 a	2	16.7 a	5 b	8.9 a	18.0 a	0.4	40.3 a
PVC	0	1 b	0.0 c	1	0.0 b	9 a	0.0 d	15.5 b	0.6	28.1 b
	7	1 b	14.5 b	1	1.7 b	9 a	3.1 c	16.0 b	0.5	32.2 b
	14	2 ab	28.0 a	1	15.0 a	7 ab	5.2 b	17.1 ab	0.5	35.4 ab

Means of three replicates followed by the same letters were not statistically significant different ($P \leq 0.01$). ns = non-significant. PET: polyethylene terephthalate; PVC-polyvinylchloride

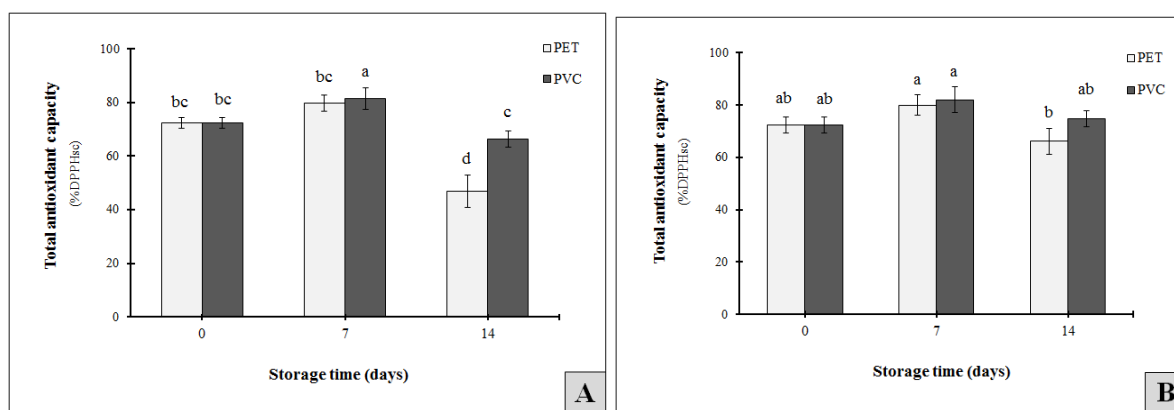


Fig 2. Changes in total antioxidant capacity of 1-berry cutting (A) and 4-berries cutting (B) grape fresh-cut packaged with PET and PVC during storage at 5°C. Vertical bars indicate standard error (n=3).

in phenolic compounds (Fan, 2005). Therefore, increasing in phenolic content at 1-berry cutting may be due to a stress in berries during grape removal from the caps. At 4-berries cutting phenolic content decreased during storage that can be related to the postharvest fruit metabolic processes, such as respiration, ethylene production and enzyme activity. Furthermore, the decrease of total phenol content is probably due to the oxidation by polyphenol oxidase (PPO) (Altunkaya and Gökmen, 2008). The changes of flavonoids, including catechin, quercetin 3-galactoside and total quercetin are shown in Table 3. At 1-berry cutting, catechin significantly increased after 14 days storage, but at 4-berry cutting, catechin content decreased during storage. As the results showed, at both 1 and 4-berries cutting, quercetin 3-galactoside and total quercetin content were decreased significantly during storage. This result is in agreement with those of Piretti et al. (1994), who reported that catechin, epicatechin and quercetin glycosides were decreased during storage in 'Granny Smith' apples fruit stored at 0°C. Flavonoids are phenolic derivatives and found in substantial amounts in grape. The decrease in flavonoid is associated with antioxidant capacity during storage (Table 4). Factors that may profoundly differentiate flavonol composition are

also those associated with ageing and storage conditions. The flavonoids such as quercetin and catechin are common PPO substrates (Nagai and Suzuki, 2001). It was also reported that quercetin and catechin were oxidized directly by PPO (Jiménez and García-Carmona, 1999). Therefore, the decrease of phenolic composition could be due to the oxidation by PPO (Yamaguchi et al., 2003).

Antioxidant capacity

Antioxidant capacity was increased up to seven days storage and thereafter decreased to end of storage in both berry preparation methods. Decrease in antioxidant capacity with prolonged storage may be due to the O₂-promoted oxidation of the constitutive phenolic compounds and vitamin C (Stewart et al., 1999). Thus, it could be found that 4-berries cutting maintained higher antioxidant capacity than 1-berry one. In addition, fruits which were packaged in PVC bags had higher antioxidant capacity than PET ones after 14 days of storage at 5 °C (Fig. 2A, B). It seems that both preparation methods could induce a stress in the samples, leading to the release of an additional amount of antioxidants, revealed in the increased antioxidant capacity at the early storage (Plaza

Table 3. Effect of different packaging (PET and PVC) and fruit preparation methods (1-berry and 4-berry) on catechin, quercetin 3-galactoside and total quercetin concentration of table grape fresh-cut after 14 days.

Treatment	Storage time	Phenolic composition		
		Catechin	Quercetin 3-galactoside	Total quercetin
1-berry				
PET	0	73.34 b	45.25 a	542.34 a
	14	76.33 b	33.34 c	464.54 c
PVC	0	73.34 b	45.25 a	542.34 a
	14	96.15 a	38.74 b	497.73 b
4-berries				
PET	0	73.34 a	45.25 a	542.34 a
	14	50.67 b	30.66 c	509.52 c
PVC	0	73.34 a	45.25 a	542.34 a
	14	68.10 a	39.48 b	528.42 b

Means of three replicates followed by the same letters were not statistically significant different ($P \leq 0.01$). ns = non-significant.

Table 4. Correlation coefficients between total phenolics, antioxidant capacity, catechin, quercetin and total quercetin in table grape fresh-cut

	Total phenolic content	Antioxidant capacity	Catechin	Quercetin 3-galactoside	Total quercetin
1-berry cutting					
Total phenolic content	1				
Antioxidant capacity	0.05 ns	1			
Catechin	0.81 **	0.04 ns	1		
Quercetin 3-galactoside	-0.68 **	0.87 **	-0.27 ns	1	
Total quercetin	-0.74 **	0.91 **	-0.36 ns	0.97 **	1
4-berries cutting					
Total phenolic content	1				
Antioxidant capacity	0.25 ns	1			
Catechin	0.73 **	0.60 *	1		
Quercetin 3-galactoside	0.65 *	0.55 *	0.95 **	1	
Total quercetin	0.61 *	0.45 ns	0.86 **	0.96 **	1

** and * Significant difference values $P \leq 0.01$ and $P \leq 0.05$. ns = non-significant.

et al., 2009). Interestingly, there was a significant correlation between antioxidant capacity and flavonoids (Table 4). Beninger and Hosfield (2003) were found a positive correlation between antioxidant activity and flavonoid compositions. Moreover, Pinelo et al. (2004) were reported that antioxidant capacity of flavonoid is in agreement with variations in their antiradical activity.

In conclusion, 4-berries cutting followed by PVC bags packaging could extend storage life and maintain quality of table grape fresh-cuts during low storage temperature

Materials and methods

Plant materials and treatments

Fruits of the second main table grape (*Vitis vinifera*) cultivar in Iran which is called 'Shahaneh', were obtained from farms during the commercial harvest. Fruits were selected for uniformity in size and color. Blemished, damaged or diseased berries were discarded carefully. Grapes were prepared by two different methods as described by Kou et al. (2007). The grape stems were manually removed so that the grapes were completely stemless (1-berry cutting); and the rachis of the grapes was cut short with a sanitized scissors so that four-berries of each cluster were remained per stem (4-berries cutting). The grapes were then sanitized with 100 mg L⁻¹ chlorine solution (NaOCl), adjusted to pH 6.5 with HCl, for 1 min, followed by draining and air drying (Kou et al., 2007). Each 500 g sample of grapes, including 1-berry and 4-berries cutting were randomly packaged in two different ways: 1)

rigid polyethylene terephthalate (PET) container (19 × 12.5 × 4 cm) and 2) a polyvinylchloride (PVC) bags (10 × 13 × 3 cm³) with 48 μm thickness and sealed with a 29.2 pmol/s/m²/Pa oxygen transmission rate film. Finally, the fruits were stored at 5 °C for 14 days. Evaluation of berries quality was performed over 14 days with three replications consisting two bags in each replicate.

General appearance

General quality was examined by evaluation of berry appearance, incidence of shattered berries, decay incidence, acceptability and flavor by a panel of five trained judges according to table 1 as describe by Xu et al (2007).

Weight loss

Grapes were weighed at the beginning of the experiment, and thereafter every week during the storage time. Weight loss was expressed as the percentage loss of the initial total weight.

Total soluble solids (TSS) and titrable acidity (TA)

Percentage of TSS as an index of soluble sugar content in fruit was determined by a digital refractometer (CETI-Belgium) at 20 °C. Percentage of TA was determined by titration of 25 ml filtrated juice with adding of 0.1 N NaOH solution to the juice to reach the pH of 8.2.

Total phenolic content

Total phenolics were analyzed spectrophotometrically using the modified Folin–Ciocalteu colorimetric method with some modifications as described by Singleton et al. (1999). Each sample (1g) was extracted with 10 mL acidic methanol and then 250 µL of the methanolic extract were mixed with 250 µL of distilled (DI) water in a test tube followed by addition of 2.5 mL of 10 % Folin–Ciocalteu reagent and allowed to stand for 6 min. Then, 2 mL of 7.5 % sodium carbonate solution were added. Each sample was allowed to stand for 90 min at room temperature in darkness and the absorbance was measured at 760 nm using an UV/Vis spectrophotometer (model PG Instrument +80, Leicester, United Kingdom). Results are expressed as mg gallic acid / 100 g FW.

HPLC analysis of flavonoids

Flavonoids were determined using high-performance liquid chromatography (HPLC) as described by Bakhshi and Arakawa (2006). For polyphenols extraction, 2 mL of solvent (methanol/acetic acid, 85:15, v/v) was added to 1 g of berry powder. The samples were kept in a refrigerator for 24 h and centrifuged for 10 min at 10000 rpm. The supernatant of centrifuged samples were filtered by disposable 0.45µm syringe filter. Fifty microliters of the filtered sample were injected in HPLC (Waters, 1525, Milford, USA) equipped with a UV-Visible detector (Waters Dual λ Absorbance 2487), C18 column: Waters Symentery C18 5µm 4.6×150 mm (Waters, Dublin, Ireland), at 280 and 350 nm. The flavonoides were identified by comparing their UV spectra and retention times with those of the corresponding standards and by the spiking of samples with the appropriate standard. Catechin standard purchased from Sigma-Aldrich (Canada Ltd) and quercetin 3-galactoside from extrasynthase, France. Quercetin and catechin was determined immediately after treatments and the end of 14 days storage.

Antioxidant activity

The antioxidant activity was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method according to the procedure of Brand-Williams et al. (1995), with some modifications. Briefly, 1 mL of methanolic extract was added to 2 mL of 0.15 mM solution of DPPH in methanol by vortexing and allowing standing at room temperature in darkness. The absorbance of the samples was measured at 515 nm after 15 min using an UV/Vis spectrophotometer (PG Instrument +80, Leicester, United Kingdom). For each sample, three separate determinations were carried out. The antioxidant activity was expressed as the percentage of decline of the absorbance, relative to the control, corresponding to the percentage of DPPH that was scavenged. The percentage of DPPH, which was scavenged (%DPPHsc), was calculated using

$$\%DPPHsc = [(A_{cont} - A_{samp}) / A_{cont}] \times 100$$

Where A_{cont} is the absorbance of the control, and A_{samp} is the absorbance of the sample.

Statistical analysis

The experiment was conducted using a completely randomized design with three replications. Data were analyzed as a 2-factor linear model using the PROC GLM procedure (SAS software ver. 9.1 2002-2003) with package and storage time as the factors. Significance of the differences was defined as $P < 0.05$.

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