International Journal of Horticultural Science and Technology
Journal homepage: http://ijhst.ut.ac.ir

Grape Seed and Skin Extracts as Natural Preserving Agents on Strawberry Fruit

Parvaneh Mohammadi-Benaruiyeh[1] and Gholam Reza Sharifi-Sirchi[2*]

1. M. Sc. Horticultural Science Department, Agriculture and Natural Resources College, University of Hormozgan, Bandar Abbas, Iran
2. Department of Biotechnology Engineering, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

ARTICLE INFO

Article history:

Received: 19 October 2020
Received in revised form: 21 January 2021
Accepted: 27 March 2021

Article type:

Research paper

Keywords:

Grape seed extract,
Grape skin extract,
Strawberry, Storage life,
Safe control.

ABSTRACT

Concerning highly restricted application of chemicals in postharvest technology of horticultural crops, it is necessary to introduce the safe methods for preserving food or methods of food preservation. This study aimed to improve quality and prolong storage life of strawberry fruit by application of grape seed and fruit skin extracts and to compare them with calcium chloride as a chemical. In this study, strawberry fruit was individually immersed in the 1% and 2% $CaCl_2$ solutions, seed and skin extracts (1 and 2 mg L^{-1}) and then placed in polyethylene packaging for 24 d at 5 ± 1 ˚C. Measurements of firmness, titrable acid, pH, weight loss, total antioxidant capacity, total phenolic, anthocyanin, vitamin C, enzymes' activity including catalase, peroxidase, and polyphenol oxidase and decay were carried out at 0, 6, 12, 18, and 24 d of storage. All applied treatments caused a significant effect on measured parameters including weight loss, titratable acidity, decay percentage and firmness, maintenance of anthocyanin and vitamin C contents, total phenolic, and antioxidant capacity. However, grape skin extract and grape seed extract showed the best results. Therefore, it can be concluded that Shiraz dark grape seed and skin extracts have the potential to control the decay incidence, prolong the storage life and preserve of postharvest valuable attributes of strawberry.

Introduction

According to massive growth in the human population until 2050, food supplies would need to be increased by 60% than the 2005 food supply level (Alexandratos et al., 2012). Limited agricultural land, water, and climate change are serious challenges in the food production cascade. Therefore, prohibition and reduction of postharvest losses is a critical component of food security in the future. According to prediction of the Food and Agriculture Organization of U.N., almost 1.3 billion tons of food (about 30-50% of total production) is lost worldwide annually (FAO, 2019). It means loss of 1.47-1.96 global hectares (Gha) of arable land, 0.75-1.25 trillion m^3 of water and 1-1.5% of global energy (A.O.A.C., 2000; Canuto et al., 2016).

Strawberry (*Fragaria ananasa*, Duchesne) is predominantly known for its characteristic aroma, sweetness edible fruit, and high amount of antioxidants, vitamin C, folate, and potassium. Strawberry contains polyphenols including ellagitannin agrimoniin,

* Corresponding Author's Email: sharifi-sirchi@uk.ac.ir

anthocyanins, flavonols, hydroxycinnamic acid, fisetin, and hydroxybenzoic acid (Skrovankova, 2015; Canuto et al., 2016). Strawberry phytochemical has shown several therapeutic effects including anti-inflammatory property and lowering blood LDL cholesterol levels in laboratory studies (Yao, 2005). Strawberry fruits are high corruptible due to their extreme frangibility, high level of respiration and susceptibility to mechanical damage and fungal or bacterial infections (Delmas, 2013). Strawberry fruits have short postharvest life and cannot be stored for long time.

Several postharvest technologies have been applied to prevent and reduce strawberry fruit decay and extend their shelf life including control atmospheres, hot water treatment, heat treatment, U.V light treatment, biologically active components, and chemical control. However, some of them have adverse effects on strawberry fruit aroma, color, texture, flavor and their amount of antioxidants, and vitamins. Moreover, chemical fungicide application is associated with a potential human health and environmental pollution risk. Essential oil and secondary metabolites are natural bioactive components from aromatic plants. In nature, these components play several important roles in the protection of plants against biotic and abiotic stress. As a safe treatment, essential oils are effective for quality maintenance and improvement of post-harvest longevity (Martinez-Romero et al., 2005, 2007; Serrano et al., 2005; Valero et al., 2006; Tzortzakis, 2007). Skin and seed essential oils of black grape are abundant sources of simple polyphenols include flavonoids stilbenes, proanthocyanidins and melatonin which have a vital role in the promotion of health (Munin and Edwards-Lévy, 2011; Delmas, 2013; Pezzuto et al., 2013; Fiume et al., 2014). Present study aimed to characterize the effect of skin and seed essential oils of dark red grape cultivar on some quality characteristics of Paros strawberry fruit during storage at 5 ± 1 ˚C and 70% relative humidity.

Materials and Methods

Fruit material

Strawberry fruit (*Fragaria* × *ananassa* Duch, cv. Paros) was harvested at the ripening stage (when more than 80% of fruit surface were red) in the first week of February from a greenhouse located in Jiroft city of Kerman province, Iran. Then, they were immediately transferred to the postharvest laboratory of Hormozgan University at the pre-cooled condition. The healthy, uniform sized and red color fruits were cleaned and selected for the present study. Grape fruit (*Vitis vinifera* cv. Shiraz dark grape) was harvested at the ripening stage in maturity stage, 10-20º Brix from vineyards of Bavanat city of Fars province, Iran.

Preparation of grape extracts

Grapes seeds and skins were powdered (by coffee blender Krups vintage, Germany) after being washed in distilled water and oven-dried for 72 h at 60 ˚C. Then, 1:10 ratio poured in methanol and was kept for 72 h on a shaker. Finally, they were condensed using rotary device under vacuum at 60 ˚C. Extracts were maintained for about 48 h in the open air for complete drying.

Extractions were prepared by pouring methanol into a bottle containing 0.05 kg of seed or skin powder at a ratio of 1:10 (V/W) and kept at room temperature (RT) for 72 h on a shaker with 0.01 kg. Then, extracts were filtered and evaporated using a rotary evaporator (Strike 102, Italia) at 50 ˚C. The extracts were poured into the vials and weighed after drying. The grape seed and skin extractive values were calculated by the following formula (Fiume et al., 2014):

$$\text{Extractive value}(\%) = \frac{\text{weight of dry extract}}{\text{weight tacken for extraction}} \times 100$$

Treatments

The strawberry fruit was surface-disinfected by immersing in 0.03% (v/v) sodium hypochlorite solution for 15 min, washed with

tap water and air-dried at RT for 1 h. The strawberry fruit were immersed for 4 min in a various treatments including grape seed extract (1 mg L^{-1}) (T1), grape seed extract (2 mg L^{-1}) (T2), grape skin extract (1 mg L^{-1}) (T3), grape skin extract (2 mg L^{-1} (T4), calcium chloride 1% (T5) and calcium chloride 2% (T6) and distilled water as control (C). The calcium chloride treatments were considered as chemical treatment. Then, air-dried at RT for 1 h. Measurements of traits were accomplished at 0, 6, 12, 18, and 24 d of storage at 5 ± 1°C. The experiment was carried out with 4 replicates and the number of samples per replicate was 10 samples.

Weight loss

Fruit weight was measured just after harvesting and then every 6 d. The weight loss was expressed as the percentage of weight loss relative to the initial value (Soto-Zamora et al., 2005) and calculated by the following formula.

$$\frac{a-b}{b} \times 100$$

where a and b are initial fruit weight and the fruit weight after a special storage period, respectively.

Firmness

Fruit firmness was measured using a digital penetrometer, which fitted with a 5 mm diameter stainless flat probe, and the average value was expressed in Newton (N) (Kumah et al., 2011). Firmness was measured on two opposite sides of fruit per storage time per treatment.

Decay percentage

Fungal decay was visually inspected daily during the storage period. Strawberry fruit showing surface mycelia development was considered to be decayed. The decayed percentage was calculated by the following formula: $, \frac{a}{b} \times 100$ where a and b are the number of decayed fruit and the total number of fruit, respectively (Hernandez-Munoz et al., 2008).

pH

The pH of the fruit samples was assessed using a digital pH meter (Model:WinLab® Data Line pH-meter profi box Set 1) with a precision of ± 0.002 pH units according to the standard method described in AOAC (2005).

Titratable acidity (TA)

The TA was determined according to the method of Mazumdar and Majumder (2003) by titrating 5 mL of each juice sample with 0.1 N NaOH solution using phenolphthalein as an indicator. The volume of used alkali was noted and titratable acidity percentage was calculated by the following formula:

$$\text{Titratable acidity}(\%) = \frac{1 \times \text{Eq. Wt of acid} \times \text{normality of NaoH} \times \text{titer}}{10 \times \text{weight of sample}(\text{g})} \times 100$$

where Eq.Wt is equivalent weight of acid which is 0.064 for citric acid.

Ascorbic acid

Ascorbic acid content was determined by the colorimetric method described in A.O.A.C (2000) based on the reduction of 2,6-dichlorophenolindophenol-sodium (DCIP), standardized with ascorbic acid. The fruit ascorbic acid extracts was titrated with DCIP solution until a light rose pink hue persisted for 30 s. The amount of used DCIP solution in the titration stage were determined and used for the calculation of vitamin C (mg mL^{-1}) content.

Total anthocyanins

For anthocyanin determination, 10 mL of a solvent containing methanol/hydrochloric acid (99:1) was added to 0.0001 kg of each sample. The mixture was kept overnight at 4 °C. Then, the solution was strained on Watman filter paper No.1 and centrifuged at 11200 g for 15 min. Subsequently, the absorbance of the solutions was measured by spectrophotometer (model Spectro UV-Vis 0216 USA) at 550 nm and the pigments concentrations were calculated according Wagner's method (Wagner, 1979).

Antioxidant activity

Antioxidant content (AC) was determined using the DPPH assay based on the Brand-Williams procedure (Brand-Williams et al., 1995). Inhibition % = $\frac{\text{Abs t0} - \text{Abs t30}}{\text{Abs t0}} * 100$ Where, Abs *t*0 is the absorbance of DPPH at time 0 and Abs *t*30 is the absorbance of DPPH after 30 min of incubation.

Total phenols

The determination of the total phenolic content of strawberry fruit extracts was performed according to the Folin–Ciocalteu procedure (Slinkard and Singleton, 1977). The results were expressed as mg gallic acid equivalent per kg of fresh weight.

Enzyme extraction

The fruit flesh samples (0.0002 kg) were homogenized in ice-cold 0.1 M sodium phosphate buffer, pH 7.0, at 4 ˚C. After centrifugation at 11200 g for 10 min at 4 ˚C, the clear supernatant used for enzyme assay.

Catalase (CAT) activity

For the CAT activity assay, the final 3 mL reaction mix containing: 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2 and 0.1 mL enzyme extract was used. The reaction was initiated by adding the enzyme extract to a hydrogen peroxide solution. The decrease in absorbance of the reaction solution at 240 nm was recorded per second for 3 min. Blank cuvette contained phosphate buffer and enzyme extract. Enzyme activity was calculated by the following formula. Units/mL enzyme = (3.45) df/ (time) 0.1. Where 3.45 = decomposition of 3.45 μmoles of hydrogen peroxide in a 3.0 mL reaction mixture producing a decrease in the A_{240} from 0.45 to 0.40. df = dilution factor, time = min required for the A_{240} to decrease from 0.45 to 0.40, 1 = milliliter of enzyme solution added to the cuvette (Chance and Maehly, 1995).

Peroxidase (POD) activity

For POD activity assay, the final 3 mL reaction mix containing: 50 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 40 mM H_2O_2 and 0.1 mL enzyme extract was used. The reaction was initiated by adding the enzyme extract. An increase in absorbance of the reaction solution at 470 nm was recorded after every second for 3 min. One-unit POD activity was defined as an absorbance change of 0.01 unit min^{-1} (Chance and Maehly, 1995).

Polyphenol oxidase (PPO) activity

The activity of polyphenoloxidase was determined with some modification (Kar and Mishra, 1976). The 3 mL reaction mixture contained 25 mM phosphate buffer (pH 7), 0.1 mM pyrogallol, 0.1 mL enzyme extract, and blank without pyrogallol. The absorbance of the purpurogallin formed was recorded at 420 nm and activity was calculated using the extinction coefficient for purpurogallin.

Statistical analysis

Statistical analysis was performed by SAS software (SAS 9.4) according to the factorial experiment basis on a completely randomized design. The first and second factors were the time and postharvest treatments. Significant differences between means were compared by Duncan's multiple ranges at a *95% confidence* interval. The experiment was carried out with 4 replicates. Each biochemical assay was estimated in triplicate and mean of them used for analysis.

Result

Weight loss

Weight loss occurred by all treatments on strawberry fruit throughout the storage time ($P \leq 0.05$). The highest weight loss (0.73%) was related to the control fruit and the lowest weight loss (0.31% and 0.29%) after 6 days of storage was belonged to the fruit which treated with grape skin extract (1 mg L^{-1}) and grape seed extract (1 mg L^{-1}), respectively. These results were replicated on the 12th day of storage and these treatments showed the highest (0.83%) and the lowest (0.44 and

0.40) weight loss. After 18 days of storage, the weight loss values reached 2.49% in the control fruit, 0.71% and 0.61% in the fruit treated with grape skin extract (1 mg L^{-1}) and grape seed extract (1 mg L^{-1}), respectively. On the 24th day of storage, the control fruit had 3.66% weight loss, whereas, the fruit treated with calcium chloride (1%) and grape skin extract (1 mg L^{-1}) possessed 1.73 and 1.62% weight loss, respectively (Table 1).

Firmness

The treatments significantly maintained the firmness of strawberry fruit throughout the storage time ($P \leq 0.05$). The firmness was 4.25 at harvest time. After 6 days of storage, the lowest (3.51) and the highest (4 and 3.75) fruit firmness were related to the control and the fruit which were treated with grape seed extract (2 mg L^{-1}) and grape skin extract (1 mg L^{-1}), respectively. On the 12th day of storage, the fruit firmness was 3.35 in the control, 3.70 in calcium chloride 1% treated fruit and 3.73 in grape skin extract (1 mg L^{-1}) treated fruit. The firmness values reached 3.21 in control fruit, 3.64 in grape seed extract (2 mg L^{-1}) treated fruit and 3.66 in grape skin extract (1 mg L^{-1}) treated fruit after 18 days of storage. On the 24th day of the storage, the treated fruit with calcium chloride 2% and the control had the least firmness (2.93 and 3.16 respectively), whereas, calcium chloride 1% and grape seed extract (2 mg L^{-1}) treated fruit possessed 3.49 and 3.52 firmness, respectively (Table 1).

Decay percentage

Statistical analysis showed that the treatments × storage time interaction was significant ($P \leq 0.05$) on the decay percentage. After 6 days of storage, the lowest (1.5%) and the highest (4.75%) amount of decay rates were sequentially related to the grape seed extract (1 mg L^{-1} and 2 mg L^{-1}) treated fruit and the control. On the 12th day of storage, the fruit treated by calcium chloride (1%) and grape seed extract (2 mg L^{-1}) had the lowest decay rate (2.75% and 3% respectively) and the control fruit showed the highest decay (10.75%). The fruit decay rate was 33% in the control (the highest), 8% (the lowest) in the treated fruit with grape seed extract 1 mg L^{-1} and 10.75% in the treated fruit with grape seed extract (2 mg L^{-1}) after 18 days of storage. At the end of the storage period, the lowest and the highest decay rates were related to the fruit which treated with grape seed extracts of (2 mg L^{-1}) (16%) and (1 mg L^{-1}) (16.75%) and the control (68.75%), respectively (Table 1).

pH

The treatment × storage time interaction effect had a significant difference ($P < 0.05$) on the pH parameter. After 6 days of storage, the lowest pH values (2.24) were sequentially related to the grape seed extract 1 mg L^{-1} and calcium chloride 1% treated fruit and the highest pH value (2.27%) was belonged to the control fruit. On the 12th day of storage, the fruit treated by grape seed extract (1 and 2 mg L^{-1}) and grape skin extract (2 mg L^{-1}) had the lowest pH values of 2.25 and 2.26, respectively and the control fruit showed the highest pH value (2.33). The pH values reached to 2.74 (the highest) in the control fruit, 2.30 in the grape seed and skin extract (2 mg L^{-1}) treated fruit and 2.27 (the lowest) in grape skin extract (1 mg L^{-1}) treated fruit on the 18th day of storage. On 24th day of storage, the grape seed extract (2 mg L^{-1}) and grape skin extract (1 mg L^{-1}) treated fruit possessed 3.28 and 3.27 (the lowest) pH values, whereas the control fruit had 3.49 pH value (the highest) (Table 1).

Table 1. Means comparisons of storage time at 4 °C and weight loss, firmness, decay percentage and pH factors interaction effects according to Duncan's Multiple-Range Test (SSR test) at a $p \leq 0.5$

Days of recording at 4° C storage	Treatment	Weight loss (%)	Firmness (N)	Decay percentage (%)	pH
1st	Control	0	4.25 a	0	2.24 e
	GSEE 1 mg L^{-1}	0	4.25 a	0	2.24 e
	GSEE 2 mg L^{-1}	0	4.25 a	0	2.24 e
	GSKE 1 mg L^{-1}	0	4.25 a	0	2.24 e
	GSKE 2 mg L^{-1}	0	4.25 a	0	2.24 e
	$CaCl_2$ 1%	0	4.25 a	0	2.24 e
	$CaCl_2$ 2%	0	4.25 a	0	2.24 e
6th	Control	0.73 ghijk	3.51 cdefgh	4.75 kl	2.27 e
	GSEE 1 mg L^{-1}	0.29 mn	3.65 cdef	1.5 lm	2.24 e
	GSEE 2 mg L^{-1}	0.48 jklm	4 ab	1.5 lm	2.25 e
	GSKE 1 mg L^{-1}	0.31 lmn	3.75 bc	1.75 lm	2.25 e
	GSKE 2 mg L^{-1}	0.49 jklm	3.68 cde	2 lm	2.24 e
	$CaCl_2$ 1%	0.51 jklm	3.51 cdefgh	1.75 lm	2.26 e
	$CaCl_2$ 2%	0.39 klm	3.70 cde	2.75 lm	2.26 e
12th	Control	0.83 ghij	3.35 fghi	10.75 hij	2.33 e
	GSEE 1 mg L^{-1}	0.40 klm	3.59 cdefg	4.25 l	2.25 e
	GSEE 2 mg L^{-1}	0.65 ghijkl	3.64 cdef	3 lm	2.26 e
	GSKE 1 mg L^{-1}	0.44 klm	3.73 cd	3.25 lm	2.30 e
	GSKE 2 mg L^{-1}	0.68 ghijk	3.50 cdefgh	3.25 lm	2.26 e
	$CaCl_2$ 1%	0.61 hijklm	3.70 cde	2.75 lm	2.30 e
	$CaCl_2$ 2%	0.48 jklm	3.63 cdef	8.75 ij	2.32 e
18th	Control	2.49 c	3.21 hi	33 bc	2.74 c
	GSEE 1 mg L^{-1}	0.61 hijklm	3.54 cdefg	8 jk	2.48 d
	GSEE 2 mg L^{-1}	1.30 f	3.64 cdef	10.75 hij	2.30 e
	GSKE 1 mg L^{-1}	0.71 ghijk	3.66 cdef	11.5 hij	2.29 e
	GSKE 2 mg L^{-1}	0.98 g	3.45 cdefghi	12.25 hi	2.30 e
	$CaCl_2$ 1%	0.90 gh	3.49 cdefgh	13 gh	2.57 d
	$CaCl_2$ 2%	0.84 ghi	3.18 ij	18 ef	3.33 b
24th	Control	3.66 a	3.16 ij	68.75 a	3.49 a
	GSEE 1 mg L^{-1}	1.77 de	3.41 defghi	16.75 f	3.29 b
	GSEE 2 mg L^{-1}	2.02 d	3.52 cdefgh	16 fg	3.28 b
	GSKE 1 mg L^{-1}	1.62 e	3.31 fghi	21.75 d	3.27 b
	GSKE 2 mg L^{-1}	2.03 d	3.40 efghi	30 c	3.34 b
	$CaCl_2$ 1%	1.73 de	3.49 cdefgh	21 de	3.34 b
	$CaCl_2$ 2%	2.86 b	2.93 j	34.5 b	2.52 d

Similar letters within columns are not significantly different. GSEE; grape seed extract. GSKE; grape skin extract.

TA

The treatment × storage time interaction effect was shown significant difference ($P < 0.05$) on the TA parameter. After 6 days of storage, the lowest TA value (0.24) was related to control fruit and the highest TA values (0.29 and 0.28) were sequentially belonged to the grape skin extracts (2 and 1 mg L^{-1}) treated fruit. On the 12th day of storage, the fruit treated by calcium chloride 1% and grape skin extracts (2 mg L^{-1}) were shown the lowest reduction with TA values of 0.28 and 0.29, respectively. The TA values reached 0.25 (the highest) in the fruit treated with grape seed extracts (2 mg L^{-1} and 1 mg L^{-1}) and 0.21 (the lowest) in the control fruit on 18th day of storage. On 24th day of storage, the grape skin extracts (2 mg L^{-1}) treated fruit possessed 0.17 (the highest) TA value, whereas the control fruit had 0.13 TA value (the lowest) (Table 2).

Maturity index

Statistical analysis revealed that the treatment had no significant effect on the maturity index. The only main effect of the storage period was significant on the maturity index. The maturity index of all strawberries increased during storage. The results showed that the lowest maturity index value (19.86) on the first day of storage, while the highest maturity index value (40.77) was recorded on the last day of storage.

Ascorbic acid

The treatments and storage time factors and their interaction had statistically significant differences ($P < 0.05$) in ascorbic acid content. In general, the ascorbic acid content of all the strawberry fruit decreased as the storage time was prolonged. The initial ascorbic acid content of the strawberry fruit was 7.95 mg kg^{-1}. The mean comparison analysis on ascorbic acid content of the strawberry fruit showed that only the fruit treated with grape seed extract (2 mg L^{-1}) treatment had a significant difference. The treated fruit with this treatment had the lowest ascorbic acid reduction during storage time especially on 12^{th} and 18^{th} days of storage (Table 2).

Anthocyanin content

The treatments × storage time interaction effect caused a significant difference ($P < 0.05$) on the anthocyanin content of strawberry fruits. Generally, the anthocyanin content increased during storage period. The initial anthocyanin content of the strawberry fruit was 19.635 mg kg^{-1} fresh weight. On the 6^{th}, 12^{th} and 18^{th} days of storage, the control fruit showed the highest significant anthocyanin content and the treated fruit with grape seed and skin extracts had the lowest anthocyanin contents. On the first and 24^{th} day of storage, there was no significant difference between treatments on anthocyanin content (Table 2).

Antioxidant activity

Statistical analysis showed that the main effects of treatment and storage period had significant differences ($P \leq 0.05$) on the antioxidant activity. The interaction between treatments and storage periods was not significant. In general, the antioxidant activity of all strawberry fruit decreased during storage (Table 3). The decrease was greater in the untreated fruit than the treated fruit. The statistical analysis showed that the strawberry fruit treated with the skin and seed extracts had significantly the highest antioxidant activity (Fig. 1).

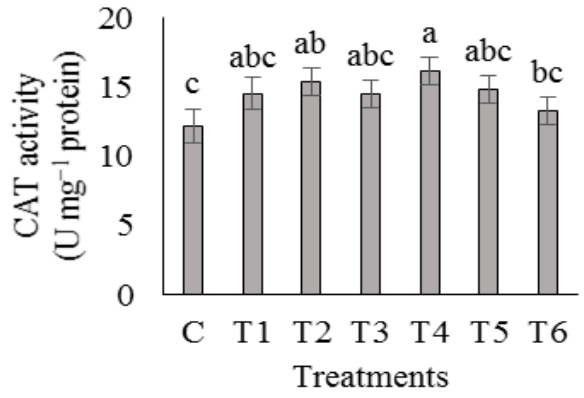

Fig. 1. Mean comparison of the treated strawberry fruit's antioxidant content (U mg^{-1} protein) in shelf-life condition at 4° C. Different letters show significant differences according to Duncan's Multiple-Range Test (SSR test) at a $p \leq 0.5$. Standard error (SE) bars are presented for each sample. C; Control. T1; Grape seed extract (1 mg mL^{-1}). T2; Grape seed extract (2 mg mL^{-1}). T3; Grape skin extract (1 mg mL^{-1}). T4; Grape skin extract (2 mg mL^{-1}). T5; $CaCl_2$ 1%. T6; $CaCl_2$ 2%.

Total phenol

The treatments and storage time factors and their interactions had significant differences ($P \leq 0.05$) in the total phenol content parameter. In general, the phenol content of treated strawberries decreased during storage. The treated fruit had higher phenol content than the untreated fruit. After 6 days of storage, the lowest phenol content (0.672) was related to the control fruit and the highest phenol content (0.812 and 0.850) was sequentially belonged to the calcium chloride 1% and grape seed extracts (1 mg L^{-1}) treated fruit. On the 12th day of storage, fruit treated by calcium chloride 1% and grape seed extracts (2 mg L^{-1}) were shown the lowest reduction with phenol content, 0.705 and 0.719, respectively. The phenol content reached to 0.634 and 0. 665 (the highest) in fruit treated with the grape seed extracts (1 mg L^{-1}) and grape skin extracts (2 mg L^{-1}) and 0.407 (the lowest) in the control fruit on 18th day of storage. On 24th day of storage, the grape skin extracts (2 mg L^{-1}) treated fruit possessed 0.547 phenol content (the highest), whereas the control fruit had 0.364 phenol content (the lowest) (Table 2).

Table 2. Results for means comparisons of storage time (at 4°C), TA, ascorbic acid, anthocyanin and phenol factors interaction effects according to Duncan's Multiple-Range Test (SSR test) at a ($p \leq 0.05$).

Days at 4° C	Treatment	TA	Ascorbic acid (mg kg^{-1})	Anthocyanin (mg kg^{-1})	Phenol (mg kg^{-1})
1st	Control	0.32 ^{a}	7.95 ^{a}	19.635 ^{h}	1.488 ^{a}
	GSEE 1 mg L^{-1}	0.32 ^{a}	7.95 ^{a}	19.635 ^{h}	1.488 ^{a}
	GSEE 2 mg L^{-1}	0.32 ^{a}	7.95 ^{a}	19.635 ^{h}	1.488 ^{a}
	GSKE 1 mg L^{-1}	0.32 ^{a}	7.95 ^{a}	19.635 ^{h}	1.488 ^{a}
	GSKE 2 mg L^{-1}	0.32 ^{a}	7.95 ^{a}	19.635 ^{h}	1.488 ^{a}
	$CaCl_2$ 1%	0.32 ^{a}	7.95 ^{a}	19.635 ^{h}	1.488 ^{a}
	$CaCl_2$ 2%	0.32 ^{a}	7.95 ^{a}	19.635 ^{h}	1.488 ^{a}
6th	Control	0.24 ^{defg}	7.4 ^{cdef}	33.075 ^{f}	0.672 ^{ghij}
	GSEE 1 mg L^{-1}	0.27 ^{bcde}	7.7 ^{abcd}	29.453 ^{fg}	0.850 ^{b}
	GSEE 2 mg L^{-1}	0.27 ^{bcd}	7.875 ^{a}	27.803 ^{fg}	0.754 ^{de}
	GSKE 1 mg L^{-1}	0.28 ^{b}	7.775 ^{ab}	26.528 ^{fgh}	0.807 ^{bc}
	GSKE 2 mg L^{-1}	0.29 ^{b}	7.8 ^{a}	25.718 ^{gh}	0.785 ^{cd}
	$CaCl_2$ 1%	0.28 ^{bc}	7.8 ^{a}	30.633 ^{fg}	0.812 ^{bc}
	$CaCl_2$ 2%	0.25 ^{defg}	7.85 ^{a}	32.588 ^{fg}	0.738 ^{ef}
12th	Control	0.23 ^{fgh}	7.15 ^{fgh}	47.810 ^{e}	0.623 ^{jk}
	GSEE 1 mg L^{-1}	0.27 ^{bcde}	7.675 ^{abcde}	30.938 ^{fg}	0.698 ^{fgh}
	GSEE 2 mg L^{-1}	0.27 ^{bcde}	7.8 ^{a}	29.535 ^{fg}	0.719 ^{efg}
	GSKE 1 mg L^{-1}	0.23 ^{fgh}	7.675 ^{abcde}	47.170 ^{e}	0.625 ^{jk}
	GSKE 2 mg L^{-1}	0.29 ^{ab}	7.65 ^{abcde}	46.763 ^{e}	0.681 ^{ghi}
	$CaCl_2$ 1%	0.28 ^{bc}	7.8 ^{a}	45.318 ^{e}	0.705 ^{fgh}
	$CaCl_2$ 2%	0.23 ^{fgh}	7.325 ^{ef}	45.318 ^{e}	0.626 ^{jk}
18th	Control	0.21 ^{h}	6.75 ^{ij}	62.463 ^{abc}	0.407 ^{p}
	GSEE 1 mg L^{-1}	0.25 ^{cdef}	7.675 ^{abcde}	54.508 ^{d}	0.634 ^{ijk}
	GSEE 2 mg L^{-1}	0.25 ^{cdef}	7.775 ^{ab}	58.493 ^{bcd}	0.640 ^{ijk}
	GSKE 1 mg L^{-1}	0.22 ^{gh}	7.275 ^{fg}	55.473 ^{d}	0.618 ^{k}
	GSKE 2 mg L^{-1}	0.24 ^{fgh}	7.425 ^{bcdef}	55.333 ^{fg}	0.066 ^{hijk}
	$CaCl_2$ 1%	0.22 ^{gh}	7.35 ^{def}	51.773 ^{cd}	0.562 ^{l}
	$CaCl_2$ 2%	0.18 ^{i}	6.75 ^{ij}	62.263 ^{abc}	0.474 ^{no}
24th	Control	0.13 ^{j}	6.475 ^{j}	63.195 ^{abc}	0.364 ^{p}
	GSEE 1 mg L^{-1}	0.15 ^{j}	6.975 ^{ghi}	65.008 ^{ab}	0.463 ^{o}
	GSEE 2 mg L^{-1}	0.14 ^{j}	7.75 ^{abc}	68.343 ^{a}	0.538 ^{lm}
	GSKE 1 mg L^{-1}	0.15 ^{j}	6.925 ^{hi}	63.813 ^{abc}	0.516 ^{lm}
	GSKE 2 mg L^{-1}	0.17 ^{i}	6.75 ^{ij}	63.9 ^{abc}	0.547 ^{lm}
	$CaCl_2$ 1%	0.13 ^{j}	6.975 ^{ghi}	63.838 ^{abc}	0.505 ^{mno}
	$CaCl_2$ 2%	0.13 ^{j}	6.675 ^{ij}	63.49 ^{abc}	0.4 ^{p}

Similar letters within columns are not significantly different. GSEE; grape seed extract. GSKE; grape skin extract.

Catalase activity

Statistical analysis showed that the main effects of treatment and storage period had significant differences ($P \leq 0.05$) in the catalase activity. In general, the catalase activity of all strawberry fruit decreased during storage (Table 3). The decrease being greater in untreated fruit than that of treated ones (Fig. 2). Mean comparison analysis showed that the treatments of grape seed extract (2 mg L^{-1}) and grape skin extract (2 mg L^{-1}) significantly inhibited decrease of catalase activity compared with the control fruit. The fruit treated with the grape skin extract (2 mg L^{-1}) had the lowest decrease in catalase activity (16.16), while the control fruit showed the highest decrease in catalase activity (12.20).

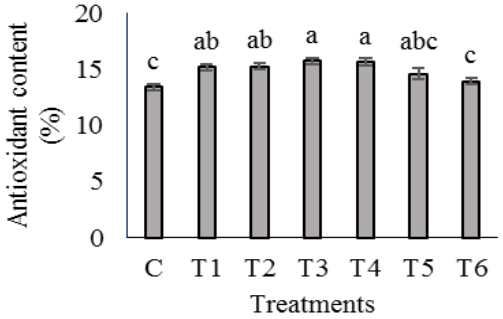

Fig. 2. Mean comparison of the treated strawberry fruit's CAT activity (%) in shelf-life condition at 4 °C. Different letters show significant differences according to Duncan's Multiple-Range Test (SSR test) at a $p \leq 0.05$. Standard error (SE) bars are presented for each sample. C; Control. T1; Grape seed extract (1 mg mL^{-1}). T2; Grape seed extract (2 mg mL^{-1}). T3; Grape skin extract (1 mg mL^{-1}). T4; Grape skin extract (2 mg mL^{-1}). T5; $CaCl_2$ 1%. T6; $CaCl_2$ 2%.

Peroxidases activity

Statistical analysis showed that the main effects of storage period and treatments had statistically significant differences ($P \leq 0.05$) in the peroxidase activity. In general, the peroxidase activity of all strawberries increased during storage period (Table 3).

The results showed that the lowest peroxidase activity (0.11) was related to the first day of storage, while the highest peroxidase activity (0.17) was recorded on the last day of storage. The treated fruit with grape seed and skin extracts had lower peroxidase activity than the control fruit (Fig. 3).

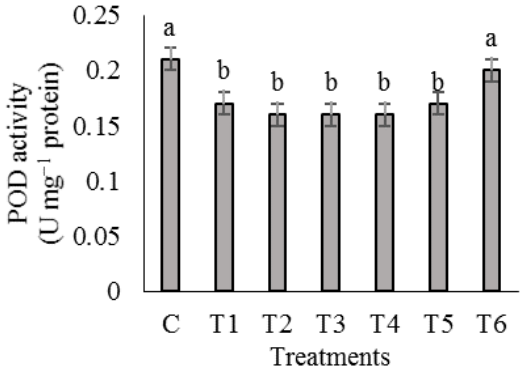

Fig. 3. Mean comparison of the treated strawberry fruit's POD activity (U mg^{-1} protein) in shelf-life condition at 4°C. Different letters show significant differences according to Duncan's Multiple-Range Test (SSR test) at a $p \leq 0.05$. Standard error (SE) bars are presented for each sample. C; Control. T1; Grape seed extract (1 mg mL^{-1}). T2; Grape seed extract (2 mg mL^{-1}). T3; Grape skin extract (1 mg mL^{-1}). T4; Grape skin extract (2 mg mL^{-1}). T5; $CaCl_2$ 1%. T6; $CaCl_2$

Polyphenol oxidase activity

Statistical analysis showed that the main effects of treatment and storage period had significant differences ($P \leq 0.05$) in the polyphenol oxidase activity. The interaction between treatments and storage period was not significant. In general, the polyphenol oxidase activity of all strawberries decreased during storage period (Table 3).

The highest polyphenol oxidase activity (3.23) was related to the first day of storage, while the lowest polyphenol oxidase activity (0.89) was recorded on the last day of storage. Mean comparison analysis showed that all treatments except calcium chloride 2% had lower polyphenol oxidase activity than the control fruit. The fruit treated with the grape seed extract (2 mg L^{-1}) had the lowest polyphenol oxidase activity (2.03), while the control fruit showed the highest polyphenol oxidase activity (2.36) (Fig. 4).

Table 3. Results from mean comparisons of storage time (at 4° C) main effect for Antioxidant, catalase (CAT), peroxidase (POD), and poly phenol oxidase (PPO) traits.

parameters	Storage time				
	1st	6th	12th	18th	24th
Antioxidant	21.63[a]	15.39[b]	14.05[c]	12.21[d]	10.88[e]
CAT	13.60b[c]	23.97[a]	15.21[b]	7.07[d]	12.25[c]
POD	0.11[e]	0.14[d]	0.24[a]	0.21[b]	0.17[c]
PPO	3.23[a]	2.37[b]	2.49[b]	1.69[c]	0.89[d]

Similar letters within rows are not significantly different according to Duncan's Multiple-Range Test (SSR test) at a $p \leq 0.05$.

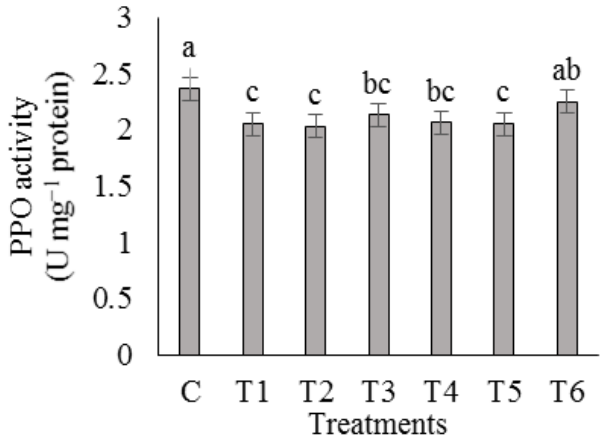

Fig. 4. Mean comparison of the treated strawberry fruit's PPO activity (U mg^{-1} protein) in shelf-life condition at 4° C. Different letters show significant differences according to Duncan's Multiple-Range Test (SSR test) at a $p \leq 0.05$. Standard error (SE) bars are presented for each sample. C; Control. T1; Grape seed extract (1 mg L^{-1}). T2; Grape seed extract (2 mg L^{-1}). T3; Grape skin extract (1 mg L^{-1}). T4; Grape skin extract (2 mg L^{-1}). T5; $CaCl_2$ 1%. T6; $CaCl_2$ 2%.

Sensory evaluation

Since quality is the most important factor which determines the marketability of fruit, the sensory data were considered as the marketability index. The sensory data for the strawberry fruit which stored for one week at 10 °C and 70 ± 5% RH are presented in Table 4. There were significant differences ($p \leq 0.05$) in aroma, taste and texture of the control and the treated fruit (Table 4). The fruit treated with grape seed extracts showed higher marketability than the control fruit and there was no significant difference between the two levels of seed extracts in retaining fruit

marketability index. The change in marketability index of the stored strawberry fruit might be due to the high decay incidence and the change in carbohydrates, proteins, amino acids, lipids, and phenolic compounds which can have influence in the aroma, texture, and taste of fresh fruit during the last days of storage period especially in the control fruit.

Table 4. Results of the strawberry fruit's marketability index (aroma, taste and texture) after one-week storage at 4°C.

parameter	days	c	T_1	T_2	T_3	T_4	T_5	T_6
Aroma	1	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}
	6	4.75^{ab}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}
	12	3.75^{def}	5^{a}	4.75^{ab}	5^{a}	5^{a}	5^{a}	4.5^{abc}
	18	2.75^{g}	4.75^{ab}	4.5^{abc}	4.25^{bcd}	4.5^{abc}	4.75^{ab}	3.5^{ef}
	24	1.5^{h}	4.25^{bcd}	4.25^{bcd}	4.25^{bcd}	4^{cde}	4^{cde}	3.25^{f}
Taste	1	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}
	6	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}
	12	4^{cde}	4.75^{ab}	5^{a}	5^{a}	4.75^{ab}	4.75^{ab}	4.25^{bcd}
	18	3.25^{f}	4.75^{ab}	4.75^{ab}	4.5^{abc}	4.25^{bcd}	4.5^{abc}	3.75^{def}
	24	1.25^{g}	4.5^{abc}	4.5^{abc}	4.25^{bcd}	4^{cde}	4.25^{bcd}	3.5^{ef}
Texture	1	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}
	6	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}	5^{a}
	12	3.75^{d}	5^{a}	5^{a}	5^{a}	4.75^{a}	5^{a}	4.5^{ab}
	18	3.75^{d}	5^{a}	5^{a}	5^{a}	4.75^{a}	5^{a}	4.5^{ab}
	24	1.25^{f}	4.25^{bc}	4.5^{ab}	4^{cd}	3.75^{d}	4.25^{bc}	3.25^{e}

Similar letters within columns are not significantly different according to Duncan's Multiple-Range Test (SSR test) at a $p \leq 0.05$. C; Control. T1; Grape seed extract (1 mg L^{-1}). T2; Grape seed extract (2 mg L^{-1}). T3; Grape skin extract (1 mg L^{-1}). T4; Grape skin extract (2 mg L^{-1}). T5; $CaCl_2$ 1%. T6; $CaCl_2$ 2%.

Discussion

Fruit weight loss is mainly associated with respiration and moisture evaporation through the skin. The thin skin of strawberry fruit makes them susceptible to rapid water loss, resulting in shriveling and deterioration. The previous experiments using natural antifungal compounds (eugenol, thymol, and menthol vapors) revealed some benefits due to reduced weight loss percentage in cherries and grapes (Serrano et al., 2005; Rattanapitigorn, 2006). The retardation of weight loss from grape seed and skin extracts applications may be attributed to their additional protective layer on the surfaces of strawberry fruit openings, antioxidant and antifungal activities and also minimizing effects of the metabolic activities including, respiration and transpiration. The softening of strawberry fruit during ripening mainly occurs as a result of the cell wall and middle lamella degradation of cortical parenchyma cells (Perkins-Veazie, 1995) by hydrolytic enzymes activities. Inhibitory effect of grape seed and skin extracts on hydrolase enzyme activity and their antifungal characteristic might be the possible reason for maintenance of strawberry fruit firmness.

In the present study, we found that all treatments delayed fungal decay development on the strawberry fruit. These results can be related to the existence of phenolic components in extracts with inhibitory effects on pectinase and cellulase enzymes which produced by fruit-rotting fungi. Our results are in accordance with those observed by other authors (Barbosa et al., 2009; Gebel and Magurno, 2014). The integration of plantarum L. A7 with thyme and cumin essential oils is a potential biocontrol tool as a biofungicide in postharvest stage (Yu-Jie et al., 2015). The pH parameter is a good index of the fruit ripening and senescence (Han et al., 2004), indicating the degradation of organic acids into sugar during respiration (Martinez-Ferrer et al.,

2006). The lower pH value during storage time in treated strawberry fruit points to the possible effect of treatments on metabolic pathway of organic acid catabolism, delaying the senescence.

TA value is an important event among postharvest evaluation traits and according to Hong et al. (2012), the faster reduction in TA value, the faster senescence in fruit. The reduction of TA value in the strawberry fruit which is started on the 6th day of the storage time can be as a result of acid oxidation during the Krebs cycle.

The ascorbic acid as an antioxidant is more prone to decomposition during storage time and its reduction is attributed to the respiration rate of stored fruit. Treatments which that target on respiration rate prolong storage, showed lower ascorbic acid reduction.

The increase of anthocyanin may be due to the continued biosynthesis of phenolic compounds during storage time and is related to the ripening processes. Our results are in agreement with those of El Ghaouth et al. (1991) who reported that the chitosan coated strawberries synthesized anthocyanin at a slower rate. Also, the heat treatments inhibited phenylalanine ammonia lyase (PAL) activity and subsequently, anthocyanin synthesis in 'Selva' strawberries (Vicente et al., 2002).

Antioxidant property of fruit and vegetables is a momentous quality parameter for enhancing human health and ability to fight against chronic diseases. The decrease in antioxidant activity at the storage period might be due to senescence and decay. It is hypothesized that extracts could act as "signaling compounds" that triggers a signal for production of additional phenolic compounds, which increase the antioxidant activities (Sogvar et al., 2016; Wang and Gao, 2013). Total phenols as secondary metabolites in response to abiotic and biotic stresses are the major antioxidants in the fruit (Cisneros-Zevallos, 2006). The phenolic compounds may help to protect cells against the oxidative damages caused by scavenging free radicals (Chun et al., 2003; Wada and Ou, 2002). Similar results were also observed in peaches (Jin et al., 2009; Yao et al., 2005), cherries (Jin et al., 2009) and loquat fruits (Cao et al., 2008) which confirmed that the fruit treated with the extracts had higher level of catalase activity during storage period than the control. Catalase enzyme has been confirmed to play crucial roles in scavenging free radicals and able to delay, retard or prevent oxidation processes and senescence (Mittler, 2002; Ballester et al., 2006).

POD is critical oxyradical detoxification enzyme in the fruit (Zeng et al., 2010; Zamani-Zadeh et al., 2014). The higher POD activity found in the control strawberry fruit than in extracts-treated fruit reflect the progress of tissue damage during storage of the control fruit rather than the extracts-treated fruit. The investigation conducted by Xiao et al. (2011) also indicated that the pear samples treated by sodium chlorite and chitosan have low PPO activity. The similar results were also reported by Xing et al. (2010, 2011). Polyphenol oxidase activity is considered as a negative indicator of shelf-life of fruit as any rise in its activity will result in oxidation of polyphenols which can produce undesirable browning colors (Oms-Oliu et al., 2010). The present study indicated that the best results of biochemical evaluation on strawberry fruits were attained through the use of the grape seed extracts, which provided good preservation of the strawberry fruit by reducing respiration, preventing oxidation processing, delaying in senescence.

Acknowledgements

We would like to thank vice chancellor for research of University of Hormozgan, for funding this project.

Conflict of interest

The authors indicate no conflict of interest for this work.

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