



EFFECT OF STORAGE TEMPERATURES ON ANTIOXIDANT CAPACITY AND BIOACTIVE COMPOUNDS IN RASPBERRY FRUIT.

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ABSTRACT: Antioxidants from fruits and vegetables are considered an important protection factor against oxidative stress and its deleterious consequences to human health. We evaluated quality and bioactive compounds and antioxidant characteristics of raspberries grown in Iran. The antioxidant capacity (FRAP), total anthocyanins, total phenolics and postharvest quality of raspberry (*Rubuscaesius L.*) kept at 0°C, 5°C and 10°C were investigated. Raspberry fruit stored at 10°C or 5° showed higher antioxidant capacity, total phenolics, and anthocyanins than those stored at 0°C. However, the postharvest life based on overall quality was longer at 0°C than at 5°C or 10°C. Titratable acidity and pH remained nearly unchanged during the storage period. In conclusion, Storage temperature at 10°C positively enhanced antioxidant capacity and bioactive compounds.

Key words: Storage temperature, Antioxidant capacity, bioactive compounds, Raspberries

INTRODUCTION

Raspberries and blackberries are excellent source of phytochemicals that are believed to have significant biological activity [17, 18]. During the last decade, much interest has been focused on raspberries due to their high levels of anthocyanins and antioxidant capacity. Many studies [4, 12, 14] evaluated the phenolic content of raspberries and found significant differences in the anthocyanins, phenolics and antioxidant capacity of phenolic content among the different species. Anthocyanins have been associated with the antioxidant properties of many common small fruit crops and have been characterized as having significant beneficial effects on various diseases. Anthocyanins had the greatest antiproliferation effect with an inhibition of greater than 50% as opposed to the phenolic acids, flavonols and tannins [19]. Phenolic compound from raspberry extracts have been reported to exhibit a broad range of protective health benefits that may reduced the risk factors associated with certain types of cancer, cardiovascular disease as well as other degenerative diseases [3]. Previous studies [22, 23] have been show that raspberries have high oxygen radical absorbance activity against peroxy radicals (ROO[•]), superoxide radicals (O₂^{•-}), hydrogen peroxide (H₂O₂), hydroxyl radicals (OH[•]), and single oxygen (¹O₂); and antioxidant activity were different among varieties [22]. There is a positive correlation between antioxidant activity and total phenolic or anthocyanin content [22, 16]. Interest in the role of antioxidants in human health has promoted research in the field of horticulture and food science to evaluate fruit and vegetable antioxidants and to determine how their content and activity can be maintained or even improved through crop breeding, cultural practices, and postharvest storage and processing. Preharvest factors, such as genetic background and cultural practices, have the potential to influence antioxidant capacity in crops. Postharvest storage can also affect anthocyanin, phenolic compound levels and antioxidant capacity in fruits and vegetables. It has also been reported that the freezing process decreased both the total phenolic content and free radical scavenging capacity by 4-20% in four cultivars of raspberries [2]. As antioxidant content is becoming an increasingly important parameter with respect to fruit and vegetable quality, it is of great interest to evaluate changes in antioxidant status during postharvest storage of horticultural crops. However, little information is available regarding the effects of storage conditions, such as temperature, on the changes of anthocyanins, phenolic compounds, and antioxidant capacity in raspberry fruit.

This study was undertaken to investigate the effects of different temperatures on total phenolics, total anthocyanins, and antioxidant capacity as well as the main fruit quality in raspberry fruit during postharvest storage.

MATERIALS AND METHODS

Plant material

Raspberry fruit (*Rubuscaesius L.*) grown at Kivi in Ardebil (Iran), were hand-harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and color. Berries were placed in trials (300 berries per trial) and stored in cold rooms at 0°C, 5°C and 10°C. Antioxidant capacity, total anthocyanins, phenolic compounds, and quality were evaluated on days 0, 2, 4, 6, and 8 after harvest.

Overall quality

Fifty fruit per treatment were used for each quality evaluation. Samples from each treatment were evaluated subjectively on the initial day and on days 2, 4, 6, and 8 during storage. Overall quality was evaluated on a 1-5 scale according to the percentage of surface area decayed, where 1= unacceptable (>50% surface affected), 2=bad (20-50% surface affected), 3= acceptable (5 to 20% surface affected), 4= good (up to 50% surface affected), and 5= excellent. Results were expressed as an overall quality index.

Fungal decay index

Fungal decay was visually inspected during the course of the experiment. Raspberry fruits showing surface mycelia developments were considered decayed. Fungal decay was evaluated on a 1-5 scale, where 1=normal, 2=trace (up to 5% surface affected), 3= slight (5-20% surface affected), 4= moderate (20-50% surface affected), 5=severe (>50% surface affected). Results were expressed as overall decay index.

Total soluble solids (TSSs), total titratable acidity (TA), and pH determinations

Thirty fruits from each replicate were wrapped in cheesecloth and squeezed with a hand press, and the juice was analysed for TSSs, pH, and TA. TSSs were determined at 20°C on Atago DBX-55 refractometer (Atago Co. Ltd, Tokyo, Japan). pH was measured with a pH meter. TA was determined by diluting each 5 mL aliquot of raspberry juice in 95mL of distilled water and then titrating to pH 8.2 using 0.1 mol/L NaOH.

Total phenolic compound analysis

Total soluble phenolics in the fruit juice extract were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton [20] using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalent per 100 g of fresh weight.

Extraction and measurement of total anthocyanins

Some of frozen tissue was ground to a fine powder under liquid nitrogen by cold mortar and pestle and 1g of the resultant powder was added to 10 ml of methanol containing HCl (1%, v/v) and held at 0°C for 10 min [7]. The slurry was centrifuged at 17,000× g for 15 min at 4 °C and then the supernatant was used. Total anthocyanins content was measured with the pH differential absorbance method, as described by Cheng and Breen [6]. Briefly, absorbance of the extracts were measured at 510 and 700 nm in buffers at pH 1.0 (hydrochloric acid-potassium chloride, 0.2 M) and 4.5 (acetate acid- sodium acetate, 1 M). Anthocyanin content was calculated using a molar extinction coefficient of 29,600 (cyaniding-3- glucoside).

$$\text{Absorbance (A)} = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}$$

Results were expressed as mg cyaniding 3-glucoside equivalent per 100g of fresh weight.

Determination of the antioxidant capacity by FRAP assay

The FRAP assay [5] was conducted using three aqueous stock solutions containing 0.1 mol⁻¹ acetate buffer (pH 3.6), 10 mmol⁻¹ TPTZ [2, 4, 6-tris (2-pyridyl)-1, 3, 5-triazine] acidified with concentrated hydrochloric acid, and 20 mmol⁻¹ ferric chloride. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1, v/v/v) to form the FRAP reagent just prior to analysis. For each assay laboratory duplicate, 2.97 ml of FRAP reagent and 30µl of sample extract were mixed. After 10 min, the absorbance of the reaction mixture was determined at 593 nm on a spectrophotometer. The result was compared with the standard curve obtained by using different concentrations of FeSO₄. 7H₂O.

Statistical analysis

Experiments were performed according to a completely randomized design. Analysis of variance (ANOVA) of data was performed for this experiment using SPSS. The effect of temperature storage and time storage on fruit quality (decay, TSS, TA and pH) and the values of phenolics, anthocyanins, and their antioxidant capacity were evaluated by the Fischer test. Differences between means of data were compared by least significant differences (LSD). Differences at p < 0.05 were considered to be significant.

RESULTS

Raspberry fruit has a very short postharvest life, mostly due to their relatively high water content and high metabolic activity, and the incidence of microbial molds and rots. Fig. 1 shows the overall quality index of raspberries stored at 0°C, 5°C, and 10°C. Overall quality loss increased continuously at a higher rate in raspberries stored at 10°C than in those stored at 5°C and 0°C. Fungal decay increased rapidly in berries stored at 10°C especially after 4 days of storage (Fig 2). Berries stored at 5°C showed slight fungal decay during 8 days of storage. Fig. 3 shows the changes of TSSs in raspberry stored at 0°C, 5°C, and 10°C. Soluble solids content decreased in all treatments during storage. Raspberries stored at 10°C had the lowest soluble solids content after 8 days of storage. No differences in pH among temperature treatments were found (Table 1). Different storage temperatures also did not affect the total TA. Total anthocyanin content was significantly affected by the temperature and storage period ($p < 0.05$). As observed in Fig. 5, anthocyanin content decreased in raspberry fruit stored at 0°C and 5°C during the first 4 days. Meanwhile, anthocyanin content in fruit stored at 10°C increased gradually during the storage period and reached its highest values near the end of storage period. Fig. 4 shows the effect of storage temperatures on total phenolic compounds on raspberry fruit. Total phenolic compounds increased continuously in berries stored at 10°C and 5°C. However, raspberry fruit stored at 0°C maintained a constant value of total phenolic compounds during the storage period. Both temperature and storage time had a significant effect ($p < 0.05$) on total phenolic compounds of raspberry fruits. In this study, we found that storage temperatures significantly affected the FRAP of raspberry fruit (Fig. 6). The FRAP values in raspberries changed very little during storage at 0°C. However, significant increase of FRAP values were found in raspberries stored at 5°C and 10°C. The higher the storage temperature, the greater the increase. One explanation for this difference could be related to different total phenolic and anthocyanin contents.

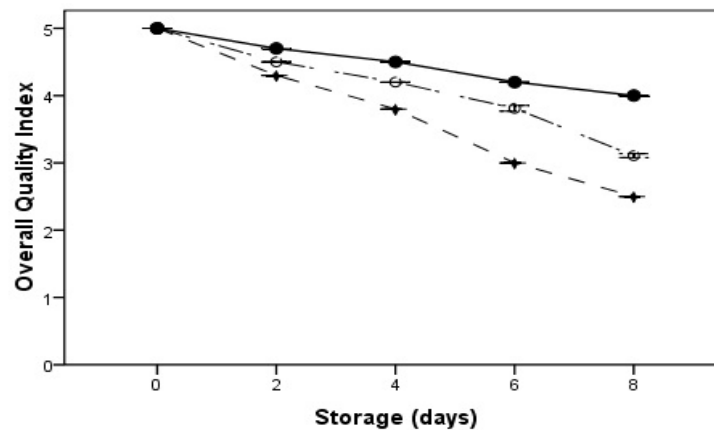


Fig.1. Overall quality index of raspberries (*Rubuscaesius L.*) stored at 0°C (●), 5°C (○), and 10°C (◆). Data points are means of three replicates.

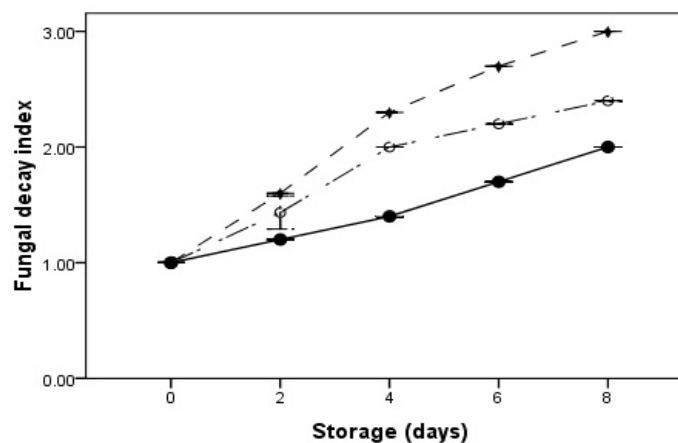


Fig. 2. Fungal decay index of raspberries (*Rubus caesius L.*) stored at 0°C (●), 5°C (○), and 10°C (◆). Data points are means of three replicates.

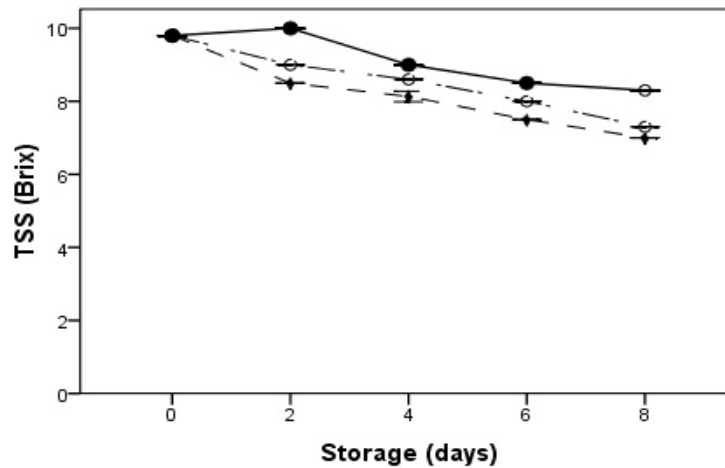


Fig. 3. Effect of different storage temperatures on soluble solids content in raspberries (*Rubuscaesius L.*) stored at 0°C (●), 5°C (○), and 10°C (◆). Data points are means of three replicates.

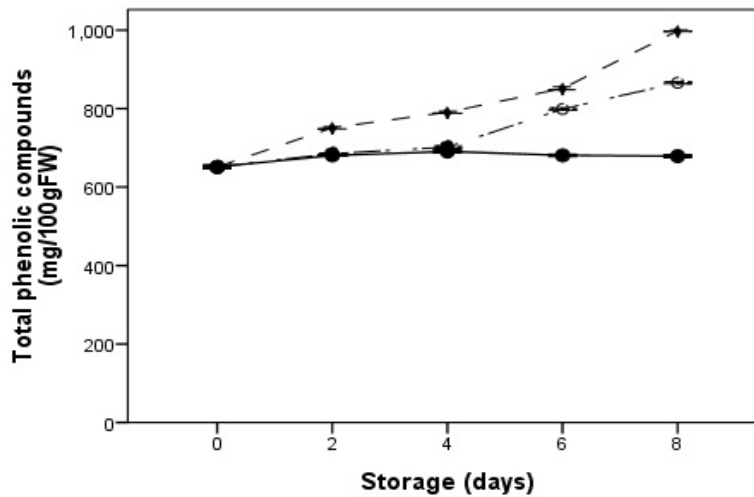


Fig. 4. Total phenolic compounds in raspberries (*Rubuscaesius L.*) stored at 0°C (●), 5°C (○), and 10°C (◆). Data points are means of three replicates.

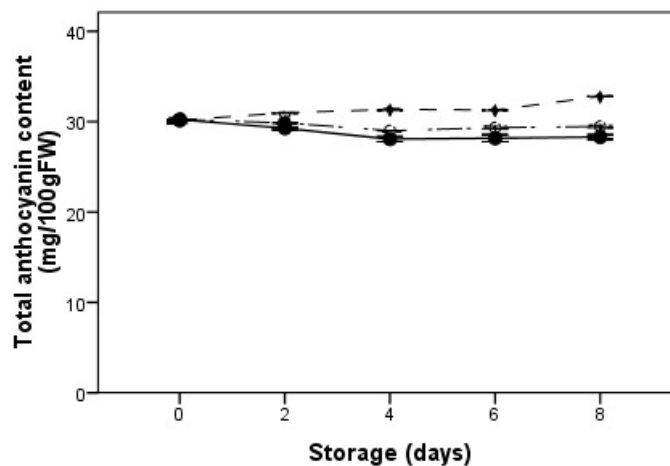


Fig. 5. Total anthocyanin compounds in raspberries (*Rubuscaesius L.*) stored at 0°C (●), 5°C (○), and 10°C (◆). Data points are means of three replicates.

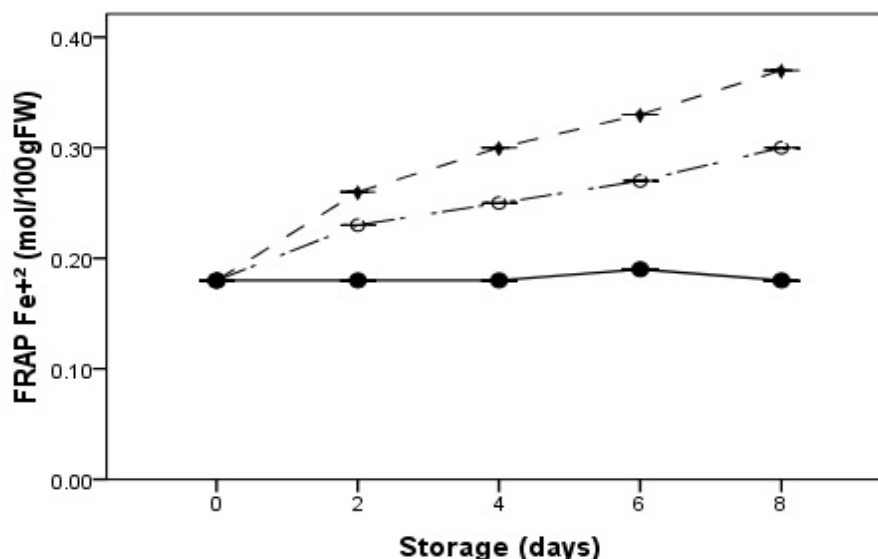


Fig.6. Antioxidant capacity in raspberries (*Rubuscaesius L.*) stored at 0°C (●), 5°C (◻), and 10°C (◄). Data points are means of three replicates.

Table 1. Effect of storage temperatures on pH and titratable acidity (9 mg of citric acid /100g F.W.) of raspberries (*Rubuscaesius L.*)

Storage time (days)	Temperature					
	0°C		5°C		10°C	
	pH	TA	pH	TA	pH	TA
0	3.24 ± 0.01	0.58 ± 0.01	3.24 ± 0.01	0.58 ± 0.01	3.24 ± 0.01	0.58 ± 0.01
2	3.18 ± 0.02	0.69 ± 0.01	3.16 ± 0.02	0.75 ± 0.01	3.12 ± 0.01	0.72 ± 0.01
4	3.14 ± 0.01	0.71 ± 0.01	3.22 ± 0.02	0.77 ± 0.02	3.18 ± 0.01	0.76 ± 0.01
6	3.21 ± 0.01	0.68 ± 0.01	3.18 ± 0.01	0.67 ± 0.01	3.23 ± 0.02	0.69 ± 0.01
8	3.24 ± 0.01	0.64 ± 0.01	3.26 ± 0.01	0.68 ± 0.03	3.28 ± 0.02	0.72 ± 0.01

Data expressed as mean ± SEM.

DISCUSSION

Storage temperature of 0°C was the most effective in maintaining the highest overall quality of raspberry fruit during the storage period. Raspberries at 5 °C maintained an acceptable quality up to 4 days. Temperature is the most important factor in the postharvest life of fresh produce because of its dramatic effects on rates of biological reactions and microbial growth [16]. Water loss during storage is a major cause of fruit deterioration. Reduction in turgidity as a result of water loss causes shriveling and faster depletion of nutrients. Storage temperature of 0°C was very effective in suppressing fungal decay of raspberries. Therefore, temperature is an important factor that significantly affects the fungal decay of raspberries. Disease caused by *Botrytis cinerea* is the most serious disease of raspberry [9]. *Botrytis* fruit rot, also known as gray mold, is widespread in the environment. It can infect raspberry flowers and cause flowers to rot or it can become dormant. Dormant infections resume activity on the berries later in the season anytime before or after harvest when sugars increase and conditions become favorable to disease development. Soluble solids content in fruit stored at 0°C and 5°C also declined but to a lesser extent. Storage time and temperature treatments showed significant effects ($p < 0.05$) on soluble solids content of raspberries. High sugar and relatively high acid content are required for good raspberry flavor [10]. Although not all raspberries with high TSSs are high quality, the absence of high TSSs makes good quality unlikely. Ali et al [1] reported that Fructose and glucose were found to be the two major sugars in strawberry fruit comprising more than 65% of the TSSs. Anthocyanins occur almost universally, and they are largely responsible for the red color of ripe raspberries. The four main raspberry anthocyanins detected in raspberry are cyaniding-3-sophoroside, cyaniding-3-glucoside, cyaniding-3-glucosylrutinoside and cyaniding-3-rutinoside. Cyanidin-3-sophoroside is the most abundant and characteristic of red raspberry [21, 15].

The observed increase of total anthocyanins during storage is in agreement with Kalt et al [11], who observed a substantial increase of total anthocyanins and phenolics in raspberry when stored at 10°C and 20°C and increasing storage time up to 8 day while at 0°C only small effects were observed. Haffner et al [8] reported an increased anthocyanin level even after at 7 day storage at 1.7°C. In contrast, Mullen et al [15] who stored their raspberries cv. Glen Ample (3day at 4°C and 3day at 4°C followed by 1 day at 18°C, respectively) did not find any storage effects on total anthocyanin content and a slight but significant increase in phenolics. Kruger et al [13] reported different storage conditions strongly influenced the content of total anthocyanin with the levels after 1 day (24h at 20°C) and 3 day and 1 day (3d at 2-4°C followed by 1d at 20°C) being higher than those in fresh berries. Anthocyanins as red pigment are synthesized during the last stage of maturity when fruit become red. The accumulation of anthocyanins and total phenolics in raspberry and strawberry fruit during postharvest was explained by Kalt et al., [11] by the decrease of organic acids which provide carbon skeletons for the synthesis of phenolics including anthocyanins. However, since in our study the level of titratable acidity was not altered during the different storage conditions. Raspberry stored at 10°C resulted in significantly increased total phenolic and anthocyanin content. However, even though antioxidant activity was the highest at 10°C, this elevated temperature may not be optimal for obtaining the best quality of raspberry fruit [13].

CONCLUSION

The data presented in this paper indicate that storage temperature significantly affect raspberry antioxidant capacity, anthocyanin, phenolic compounds and overall quality. New detailed information is presented on the effect of storage temperature on raspberry bioactive compounds and antioxidant capacity which suggests that even though overall quality was better maintained at 0°C, storage temperature at 10°C positively enhanced antioxidant capacity and the production of bioactive compounds.

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