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[Research]

Effect of Methyl jasmonate treatment on antioxidant capacity, internal quality and postharvest life of raspberry fruit

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ABSTRACT
Native population of raspberry fruits (Rubus spp) were treated with Methyl Jasmonate (MJ) fumigation and were assayed for the antioxidant capacity, total anthocyanins and postharvest quality after 7 days storage at 4°C.
The result of experiment revealed that berries treated with methyl jasmonate (MJ) showed higher antioxidant capacity and total anthocyanins compared to the controled ones (non-treated). Decay incidence was reduced in fumigated fruits during storage at 4°C, which resulted to long postharvest life (as indicated by fugal decay) compared to the controled ones. Total soluble solid, titratable acidity and pH were influenced by storage periods. Therefore, MJ treatments could not suppress the declining, which happened during storage time. In conclusion, although raspberry fruits treated with MJ maintained higher levels of antioxidant capacity, total anthocyanins compared to untreated fruits during storage, but because of high perishable, MJ treated fruits could not maintain significant changes in internal quality.

Keywords: Raspberry, Methyl Jasmonate, Antioxidant, Anthocyanin, Decay and Fruit quality.

INTRODUCTION

Native raspberry (*Rubus spp*) constitutes a good source of natural antioxidant substances. Berry fruit including raspberries have been reported to contain high phenolic and anthocyanin content (Heinonen *et al.*, 1998). Thus, various antioxidants found in berry fruit provide significant health benefits. Although it is very valuable nutritionally, but because of high perishables fruits, the storage time was limited to just a very short period and just consumes in local markets. The storage life was limited by rots (*Botrytis cinerea*), loss of firmness and darkening of the attractive red colour. To obtain optimum flavor, shape and color the berries should be harvested for fresh consumption at a mature stage (Haffner *et al.*, 2002).

Several natural volatile compounds have been reported to possess antimicrobial activity. The methyl jasmonate (MJ) has been studied for their effectiveness in maintaining the quality of fresh fruits and vegetables. It has been reported that MJ treatment can reduce the development of chilling injury symptoms in zucchini (Wang and Buta, 1994) and mango (Gonzalez-Aguilar *et al.*, 2000). In addition, MJ has been shown to suppress the fungal growth in grapefruit (Droby *et al.*, 1999), reduce decay and maintain the postharvest quality of papayas (Gonz´alez-Aguilar *et al.*, 2003), and inhibit the microbial contamination of fresh-cut celery and peppe-rs (Buta and Moline, 1998). In raspberry, it is demonstrated that MJ increases the resistance of tissues against decay by enhancing their antioxidant system and their free radical scavenging capability. There is a positive correlation between antioxidant activity and total phenolic or anthocyanin content (Wang *et al.*, 1996 and Wang and lins, 2000). Therefore, antioxidant activity in different berries and berry wine rich in vitamin C, anthocyanins and other phenolic compounds has been described (Heinonen *et al*., 1998 and Tamura and Yamagami, 1994).

The postharvest life of fruits and vegetables has been traditionally defined in terms of visual appearance (freshness, color, and absence of decay or physiological disorders) and texture (firmness, juiciness and crispness) (Heffner *et al*., 2002). Although this concept involves aesthetic appeal and mechanical properties associated with quality, it disregards flavor and nutritional quality. 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, postharvest storage and processing (Heinonen, *et. al*., 1998). Postharvest storage can affect anthocyanin, phenolic compound levels and antioxidant capacity in fruits and vegetables Heinonen, *et al*., 1998). However, little information is available regarding the effects of storage conditions such as exposure to natural antimicrobial compounds, on the changes of anthocyanins, phenolic compounds and antioxidant capacity in raspberry.

Anthocyanins, which are responsible for color in most berries, easily degrade following various reaction mechanisms acted by oxygen, ascorbic acid, pH and temperature among other variables (Abers, J, E and Wrolstad, R. E. 1985). From the view point of quality, the color of raspberry fruit is one of the most important characteristics to be considered to preserve the fresh-like appearance of the raspberry products and this is usually related to their anthocyanin composition.

The purpose of present study is to determine the changes in the activities of antioxidant capacity in raspberries treated with natural volatile compounds, and how can it been probably effected by suppressing fruit decay to resulted from high storage life.

MATERIALS AND METHODS

Plant materials

Native populations of raspberry (*Rubus spp)* grown near the Caspian sea, Iran were hand-harvested at a commercially mature stage and sorted to eliminate damaged and unriped fruits. Fruits in the same size and color were selected. A total of 180 fruits were divided into three lots. Sixty berries were placed in 0.5 jars containing 8, 16, and 24 $\mu l l^{-1}$ Methyl Jasmonate (MJ) or water (for the control) spotted onto filter paper for a day at 25ºC in darkness. Three replicates of each treatment were used for analyses. After treatment, the glass jars were ventilated in a fume hood, covered with perforated polyethylene film and stored in refrigerator at 4ºC for 7 days. The characteristics such as fruit decay, antioxidant capacity, total anthocyanins and internal qualities were evaluated after 0, 3, 5, and 7 interval days during storage time.

Fungal decay incidence

Fungal decay was visually inspected during storage (4ºC) at days third, fifth and seventh. Raspberry fruits showing surface mycelial developing symptoms were considered as decayed fruits. Fungal decay incidence was estimated by the mean proportion (percentage) of fruit that showed any fungal decay (i.e. a level of >0).

Chemical analysis

Twenty fruits from each replicate were wrapped in cheesecloth and squeezed with a hand press, then the fruit juice was analyzed by triplicate for total soluble solids (TSS) and titratable acidity (TA). TSS was determined by refractometer (Digital ABBE, U.S.A) and TA by diluting each 2 ml aliquot of raspberry juice in 20 ml of distilled water and then titrated to pH 8.2 using 0.1 molar sodium hydroxide. PH value was indicated by pH meter (HBJ-260). Analysis of total anthocyanin content in fruit juice was determined using the pH differential method (Wrolstad, R. E., 1976). Absorbance was measured in a Shimadzu spectrophotometer at 530 and 700 nm, in buffers at pH 1.0 and 4.5, using $A = [(A_{530} - A_{700})\ pH_{1.0} - (A_{530} - A_{700})\ pH_{4.5}]$ formula by the molar extinction coefficient of cyanidin 3-glucoside (30200) for raspberry fruit juice. Results were expressed as the milligrams of cyanidin 3-glucoside equivalent in 100 g of fresh weight. The antioxidant activity of the raspberry juice was evaluated by DPPH free radical-scavenging according to modified method of Elmastasa, *et al*., (2007). 100 µl of the various concentrations of the extracts in methanol was added to 2.900(µl) of 0.1 mM DPPH methanolic solution. The mixture was shaken immediately after adding DPPH solution and allowed to stand at room temperature in darkness. The decrease in absorbance at 517 nm was measured after 25 min until the reaction reach to a plateau. These experiments were run in duplicate. The inhibitory percentage of DPPH was calculated using the formula: Scavenging effect (%) = [(*A*o − (*A* − *A*b)/*A*o] × 100; where *A*o = *A*517 of DPPH without sample (control), A = *A*517 of sample and DPPH, and Ab =*A*517 of sample without DPPH (blank). All tests were performed in triplicate at 25°C.

Statistical analysis

Experiments were performed according to a completely randomized design.To analysis of the variance (ANOVA) of the data was performed in this experiment using Statistical Analysis System (NCSS, 2000). Duncan multiple range test (p = 0.01) calculated for the comparison of treatment means. SPSS analysis was carried to correlate between characteristics.

RESULTE AND DISCUSSION

Fungal decay percent

Raspberry fruit is high perishable, mostly due to their relatively high water content, high metabolic activity, and the susceptibility to microbial molds and rots. Fungal decay increased continuously during storage which resulted in fruit deterioration up to 40 percent after 7 days storage at 4°C (Table 1). All of the fumigated fruits reduced the decay incidence during storage at 4°C compared to control ones. No difference was found among different MJ levels (Table 2). Overall, the higher rate of fruit decay was in untreated raspberry compared to those treated with Methyl Jasmonate (MJ). Methyl jasmonate has been shown to induce the synthesis and expression of some stress proteins such as heat-shock proteins and pathogenesis-related proteins, which lead to the increased resistance of the pathogens and the decreased incidence of the decay (Ding *et al.*, 2001 and Ding *et al.*, 2002). Jasmonic acid and its derivatives such as MJ have been described as signaling compounds that stimulated the expression of wound-inducible and defense related genes, as well as being involved in many developmental processes in plants (Deng, 1993). In strawberry fruits, it is observed that the suppression of fungus decay maybe induces the chemical defense mechanisms of plant tissues by low concentration jasmonate (Fernando Ayala-Zavala, 2005).

Table 1. Effect of storage time (at harvest, 3, 5, and 7 days) at 4°C on decays incidence and internal fruit quality of raspberry (*Rubus spp*).

Time (days)	% Decay	% TSS	% TA	TSS/TA	pH
At harvest	0.0d	5.80a	1.56a	5.38a	3.35c
3	7.5c	4.86b	1.06b	3.75b	3.39c
5	14.58b	3.67c	0.94b	3.71b	3.72 b
7	37.69a	3.36c	0.91b	3.60b	3.93a

Values within column followed by the same letter are not significantly different at P = 0.01 (Duncan's Multiple Range test).

Table 2. Effect of different Methyl Jasmonate (MJ) fumigation levels on decays incidence and internal quality of raspberry (*Rubus spp*) after 7 days storage at 4°C.

Treatments	% Decay	% TSS	% TA	TSS/TA	pH
Control	23.75a	4.65a	1.16a	4.27a	3.69a
MJ_1	14.57b	4.36a	1.14a	4.23a	3.58a
MJ_2	10.74b	4.35a	1.11a	4.08a	3.56a
MJ_3	10.71b	4.33a	1.06a	3.85a	3.56a

Values within column followed by the same letter are not significantly different at P = 0.01 (Duncan's Multiple Range test).

Total soluble solids and total titratable acidity

Total soluble solids of berries significantly (P>0.01) decreased during short term storage (Table 1). MJ treated fruits showed similar level of TSS like untreated fruits. The depletion of TSS in fruits could be explained by a high metabolism of fruits and senescence processes. Ayala-Zavala *et al.*, (2005) demonstrated that strawberry fruits treated with MJ were maintained higher level of TSS. But in present study, fumigation treatments with this natural compound could not maintain TSS level during storage (Table 2). The organic acid composition of raspberry cultivars grown in Spain showed in HPLC-analysis that over 90% of the organic acids are citric acid (Ancos *et al.*, 1999). It was implicated in the color quality of berry fruits (Wrolstad *et al.*, 1970). At harvest time, fruit had the highest titratable acidity, but its content gradually decreased during storage. Declining both TSS and TA resulted in decreasing TSS to TA ratio, although no differences were found after 3 days storage at 4°C (Table 1). The titratable acidity of berries treated with MJ was similar to untreated fruits after 7 days of storage (Table 1). Thus, at the end of storage, no significant differences were observed among the treatments. This agreed with the results of Ayala-Zavala *et al.*, (2005). MJ treated fruits also could not maintain pH value. Therefore, its content increased during 7 days storage like control fruits (Table 1 and 2). As a result, the pH values showed the same tendency of TA. The treatments with higher content of acids had lower pH values.

Total anthocyanin content

Anthocyanins are a group of phenolic compounds responsible for the red-blue color of many fruit and vegetables, and provide beneficial effects to human health (Garcıa-Alonso *et al.*, 2004). The higher anthocyanin content in most raspberry cultivars expressed as cyanidin-3-glucoside (Ancos *et al.*, 1999). The antioxidant capacity of anthocyanidin may be one of their most significant biological properties (Wang *et al.*, 1996). Therefore, it is important to maintain the higher levels of these compounds during storage and shelf life period. Total anthocyanin content was significantly affected by MJ treatment and storage period. As observed in Fig. 1, at the end of the storage time, the treatment of berries with 24µl.l-1 maintained the highest level of anthocyanin as compared with other fumigated treatments and control fruits. Ayala-Zavala *et al.*, (2005) revealed that strawberries treated with combination MJ showed the highest antocyanin pigment at the end of storage period. MJ probably enhanced the production of total phenolic compound and anthocyanin content in our study and promoted the defense mechanism in raspberries.

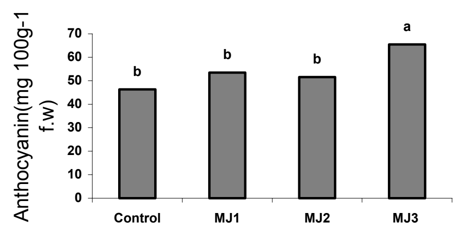

Fig1. Total anthocyanin content in raspberry fruit treated with various Methyle Jasmonate compound levels (0, 8, 16 and 24 µll-1) and stored at 4°C for 7 days. Values with the same letter are not significantly different at P = 0.01 (Duncan's Multiple Range test).

Fruits which undergone MJ fumigation treatments maintained anthocyanin level higher during storage period (Fig. 2), but control fruit sharply decreased antho-cyanin content, although they had finally the same content. Anthocyanins are regarded as important antioxidants in berry fruits (Mullen *et al.*, 2002). A significant negative correlation (r= -0.67*) was determined between antocyanin content and decay incidence.

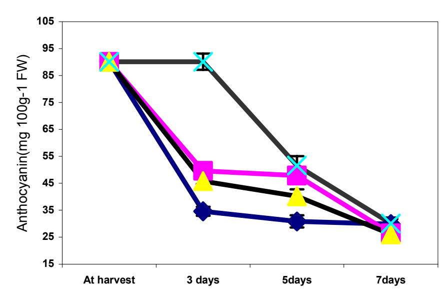

Fig 2. Effect of storage periods and MJ treatments on Anthocyanin content berries.
Each value is expressed as mean standard error (n = 3).
♦: Control, ▲: MJ1, ■: MJ2, ×: MJ3

Antioxidant capacity

Several previous studies have been shown that berries are a good source of natural antioxidants (Wang *et al*., 1996, Heinonen *et al*., 1998). These natural plant antioxidants include phenolic compounds and anthocyanins (Wang and Lin, 2000). In our study, antioxidant capacity was variable in raspberry extracts treated with the different concentrations of MJ. The highest antioxidant capacity was found in berries that treated with 16 and 24 µl.l^{-1}, followed by 8 µl.l^{-1} and control (Fig 3). During storage time, the highest antioxidant activity was recorded in berries which treated with 24 µl.l^{-1}. Chanjirakul *et al*., (2006) have demonstrated that MJ may increase the resistance of tissues against decay through enhancing their antioxidant systems and their free radical scavenging capabilities. The present study also demonstrated that all of fumigated fruits reduced the decay incidence during storage at 4°C compared to controlled ones which is correlated with enhancing antioxidant power in treated fruits (Table 2). In current study the strong antioxidant activity of MJ treated fruits were possibly also responsible for low percentage of decay in raspberries. Storing raspberry fruits at 4°C for 7 days decreased antioxidant capacity in MJ treatments and controlled ones (fig 4). At three first days, antioxidant activity slightly increased and after that it decreased significantly (P>0.01) through the end of the storage periods in all treatments. The rate was strongly dependent on prestorage treatments (Fig 4). Fruit treated with MJ fumigation showed slight declining in antioxidant activity at the end of the storage. A significant negative correlation was determined between scavenging effect, decay incidence and anthocyanin content with scavenging effect (r= -0.85* and r=0.78*, respectively).

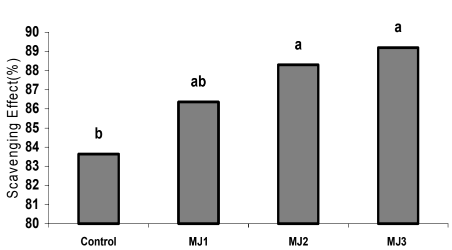

Fig 3. Scavenging effect percent in raspberry fruit treated with various Methyle Jasmonate compound levels (0, 8, 16 and 24 µL.L^{-1}) and stored at 4°C for 7 days.
Values with the same letter are not significantly different at P = 0.01 (Duncan's Multiple Range test).

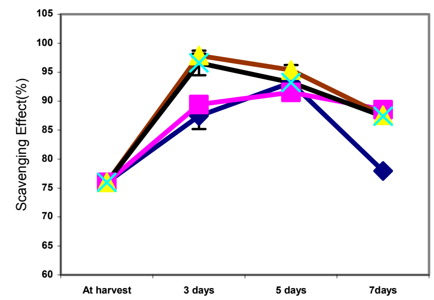

Fig 4. Effect of storage periods and MJ treatments on Anthocyanin content berries.
Each value is expressed as mean standard error (n = 3).
♦: Control, ▲: MJ1, ■: MJ2, ×: MJ3

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