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Effect of exogenous putrescine on postharvest life of sweet cherry (*Prunus avium*) fruit, cultivar "Surati-e Hamedan"

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Abstract

The purpose of this study was to investigate the effect of exogenous putrescine on postharvest life and quality of sweet cherry fruit, cultivar "Surati-e Hamedan" at 2˚C. Fruits were treated with 0.5, 1, 2, 3 and 4 mM putrescine as well as distilled water (Control) for 10 minutes, then transferred into the fridge (2˚C). The rate of ethylene production, weight loss, tissue firmness, soluble solids content, titratable acidity and pH of fruits were determined 5, 10, 15, 20 and 25 d after the beginning of storage. Parameters associated with ripening processes, including softening and loss of titratable acidity, significantly decreased by application of putrescine. Soluble solids content of cherries also increased by the putrescine treatment. In addition, cherries treated with higher concentrations of putrescine showed lower rate of ethylene production. Weight loss of the fruits was affected by putrescine in a concentration dependent manner, while putrescine did not affect pH of fruit juice.

Key words: Sweet cherry, *P. avium,* Surati-e-Hamedan, putrescine, postharvest life

Introduction

Sweet cherries (*Prunus avium*) are very perishable commodity. Optimum extension of the postharvest life of fleshy fruits is critically dependent on the reduction and/or retardation in the physiological process of maturation and senescence. An important approach to minimize or eliminate this problem is to apply fruit ripening retardants (*i.e.* polyamines).

Polyamines (PAs) are low molecular weight polycationic organic compounds existing in all living organisms (Liu *et al.*, 2006). The major forms of PAs including diamine putrescine, triamine spermidine and tetraamine spermine are found in every plant cell. Less common PAs (*i.e.* 1,3-diaminopropane and homospermidine) differ from the major forms of these compounds in terms of the number of methylenic moieties between their amine groups. The association of the uncommon PAs with the capacity of some biological systems to grow or function under extreme conditions has provided opportunities for new investigations into their potential functions (Pandey *et al.*, 2000). Polyamines are implicated in various plant growth and developmental processes. These include stimulation of cell division, DNA and protein synthesis, dormancy breaking of tubers and germination of seeds, response to environmental stresses and regulation of rhizogenesis, embryogenesis, senescence, floral development, and fruit ripening (Kakkar and Sawhney, 2002; Tassoni *et al.*, 2003). Polyamines may mediate the action of hormones as a part of their signal response, and are thus suggested as hormonal second-messengers (Kakkar and Sawhney, 2002). Polyamines also serve as precursors for secondary metabolites such as nicotine, and can be conjugated with phenolic acids to produce plant defense-related compounds (Martin-Tanguy, 1997). The biological activity of PAs is attributed to the cationic nature of these molecules. Polyamines occur in plants in free form, bound electrostatically to negatively charged molecules, and conjugated to small molecules (*i.e.* phenolic acids) and proteins (Walters, 2000). Interactions of PAs with phosphate groups of DNA and RNA, anionic components of phospholipids and cell wall components (such as pectic polysaccharides) have been reported (Kakkar and Sawhney, 2002). PAs also bind to the negatively charged phospholipids or other anionic sites on membranes, resulting in altering the stability characteristics of such membranes. PA binding is also known to affect membrane fluidity. Therefore, PAs may indirectly modulate the activities of membrane-associated enzymes (Slocum *et al.*, 1984). Binding of PAs to proteins in *Petunia* protoplast (Mizrahi *et al.*, 1989) also suggests the direct interaction between the PAs and the membranes. PAs protect the damage of DNA by neutralizing charge and/or conformational changes of DNA (Kakkar and Sawhney, 2002).

Polyamines are reported to be effective anti-senescence agents and found to retard chlorophyll loss, membrane deterioration and to increase in RNAse and protease activities, all of which help to slow down the senescence process (Evans and Malmberg, 1989). Exogenously applied polyamines are potent inhibitors of senescence in oat leaf protoplasts and leaves and storage tissues of several plants (Kaur-Sawhney *et al.*, 1982).

Polyamines, especially spermidine and spermine compete with ethylene for a common substrate, S-adenosylmethionine (SAM) and make it plausible to modulate postharvest fruit development. Enormous works have demonstrated that exogenously applied polyamines affect fruit quality, through some change in fruit firmness, weight loss, ethylene evolution, soluble solutions and titratable acids. Spermidine and spermine treatment retards softening of apple (Kramer *et al.*, 1991) and strawberry fruits (Ponappa *et al.*, 1993). Exogenously applied putrescine results in the reduction of mechanical damage and increasing firmness of lemon (Martinez-Romero *et al.*, 1999), apricot (Martinez-Romero *et al.*, 2002) and plum (Perez-Vicente *et al.*, 2002). Application of polyamines has also reduced or delayed browning, peroxide level and ethylene production, coupled with elevated levels of

polyamines in litchi fruits stored at 5°C (Jiang and Chen, 1995). Plum fruits treated with 1 mM putrescine have shown a delay and/or reduction in ethylene production, together with an increase in fruit firmness and a decrease in soluble solutions, titratable acids and weight loss and also a delay in color change, leading to extended storage life (Valero *et al.*, 2002). Apricot fruits treated with putrescine have significantly shown lower weight loss compared with the untreated fruits (Martinez-Romero *et al.*, 2002). In kiwifruit, putrescine treatment reduced or slowed color change, ethylene emission and respiration (Petkou *et al.*, 2004). Similarly, application of putrescine by vacuum infiltration has notably increased lemon fruit firmness and delayed the color change compared with the control (Valero *et al.*, 1998). It is known that, fruit softening is primarily due to the breakdown of the cell wall. The above-mentioned effects of polyamines on fruit texture (firmness and softening) could be ascribed as a part of their properties such as inhibiting enzymes degrading pectic acids (Martinez-Romero *et al.*, 2002) and/or their ability to bind the cell wall and membrane (Ponappa *et al.*, 1993), leading to rigidification of cell wall and stabilization of membrane. Inhibited ethylene production might also account for the enhanced firmness and delayed softening. The reason why polyamines modify soluble solutions and titratable acids has remained unclear. The objective of this study was to investigate the effect of exogenous putrescine treatment on improvement of the characterstics related to the postharvest life of sweet cherry cv. "Surati-e Hamedan".

Materials and methods

Sample preparation: Thirty kg of flawless cherries, cv. "Surati-e Hamedan" were harvested by hand at the commercial maturity stage (as soon as their shine colour turned into pink) from a local orchard in Hamedan, put in the wooden boxes and transported immediately to the laboratory for the experiments. Experiment was conducted in a completely randomized design including 6 treatments (0.5, 1, 2, 3 and 4 mM putrescine and distilled water "control") and three replications. Two kg of fruits was used for each replicate. Putrescine (98%) was purchased from Merck Chemical Co. Fruits were immerged in putrescine solutions (5 L, 20°C) as well as distilled water (control) and left for 10 min, transferred into the baskets for 20 min to dry. Putrescine treated fruits together with controls were then transferred into 2 L plastic containers and kept into the fridge (2°C). Observations were carried out 5, 10, 15, 20 and 25 d after the beginning of storage.

Ethylene determinations: Ethylene production was measured by placing five fruits in a 1 L glass jar tightly fitted with a rubber cap for 1 h. One mL of the holder atmosphere was withdrawn using a gas syringe, and the ethylene was quantified by a Gas Chromatograph (Shimadzu, C-R 4A, Japan) apparatus. Results were expressed in nL of ethylene released per g of fruit tissue per h (nL kg^{-1} h^{-1}). Tissue firmness determination was carried out using a penetrometer (Wagner, Model FDK 32, Italy) apparatus through measurement of the force required for a 3 mm probe to penetrate fruit tissue. Titratable acidity was determined by titration with 0.1 N NaOH up to pH 8.1 and expressed as g of malic acid per 100 g fresh weight. Soluble solids content was determined using a portable refractometer (Atago N1, Japan) at 20°C, and expressed as °Brix. pH of fruit juice was measured using a Jenway 3320 pH meter calibrated by pH 4 and 6.4 buffer solutions. To determine the weight loss, ten fruits for each treatment were weighed at the beginning of the experiment and during storage. Results were expressed as percentage of weight loss toward the initial value.

Statistical analysis: The data was analyzed using MSTATC statistical software and the means were compared by Duncan's Multiple Range Test.

Results and discussion

Fruit tissue firmness: Putrescine application had a significant effect on fruit firmness. Tissue firmness was higher in putrescine-treated cherries as compared with controls. There was also a significant difference between the various concentrations of putrescine in terms of their effects on tissue firmness ($P<0.05$). The highest tissue firmness was observed when 4 mM putrescine applied at all determination times (5, 10, 15, 20 and 25 days after the beginning of storage), while the lowest rate of firmness was related to distilled water treatment (Fig. 1). The inhibitory role of exogenous putrescine on fruit softening has been reported in apples (Kramer *et al.*, 1991), strawberry (Ponappa *et al.*, 1993), tomato (Law *et al.*, 1991), lemon (Valero *et al.*, 1998), peach (Bregoli *et al.*, 2002) and plum (Serrano *et al.*, 2003). The effect of putrescine on increasing fruit firmness and reducing softening could be attributed to cross-linking properties of this compound to pectic substances which result in cell wall rigidification. This binding also blocks the access of degradative enzymes to the cell wall, which reduces the rate of tissue softening during storage (Valero *et al.*, 1998). Putrescine can also inhibit the activity of pectin degrading enzymes such as polygalacturonase through binding to pectic acid (Kramer *et al.*, 1991).

Titratable acidity: Titratable acidity of cherries stored at 2°C decreased during storage time. It was observed that the acidity increased with increasing putrescine concentrations. As seen in Fig. 2, the titratable acidity of cherries in all treatments was significantly different at $P<0.05$. However, titratable acidity was not significantly different at 5, 10 and 25 days of storage for cherries treated with 3 and 4 mM putrescine. Since organic acids are substrates for the enzymatic reactions of respiration, a reduction in the acidity and an increase in pH values are expected. No marked changes in pH value were observed during fruit storage in the present study. Putrescine application reduces the respiration rate (Perez-Vicente *et al.*, 2002) which causes a delay in utilization of organic acids.

Soluble solids content: Soluble solids content of cherries slightly decreased during storage time. However, these changes were significantly lower in putrescine-treated ($P<0.05$) than the control (Fig. 3). At all determination times, the soluble solids content was significantly higher in putrescine-treated cherries than those found in controls, but the difference between cherries treated with various concentrations of putrescine was significant only at 10th and 15th days. Moreover, the higher putrescine concentration significantly increased the soluble solids content of cherries at these two storage times. It is difficult to explain how in the putrescine-treated cherries soluble solids increase but it seems to be due to a reduction in the respiration of the fruits.

Weight loss: Fruit weight loss increased throughout storage period, although increase was significantly higher in control (Fig. 4). The weight loss was lower in putrescine-treated cherries as

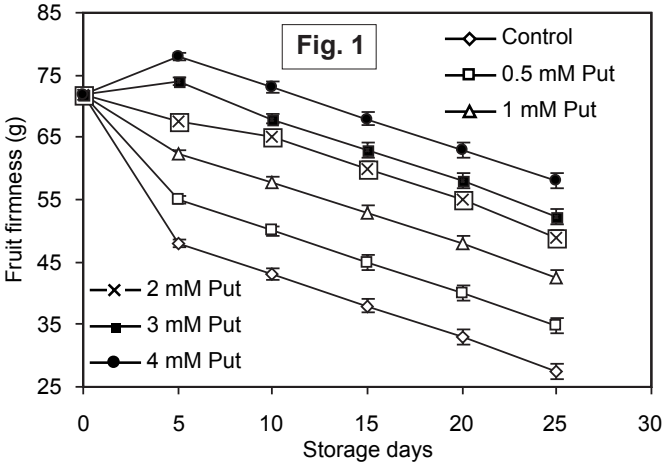

Fig. 1. Changes in fruit firmness of sweet cherry, cv. Surati-e Hamedan during storage at 2˚C.

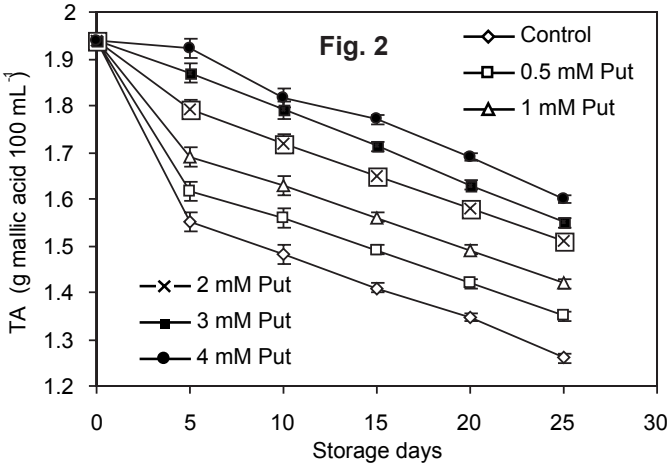

Fig. 2. Changes in titratable acidity of sweet cherry, cv. Surati-e Hamedan during storage at 2˚C.

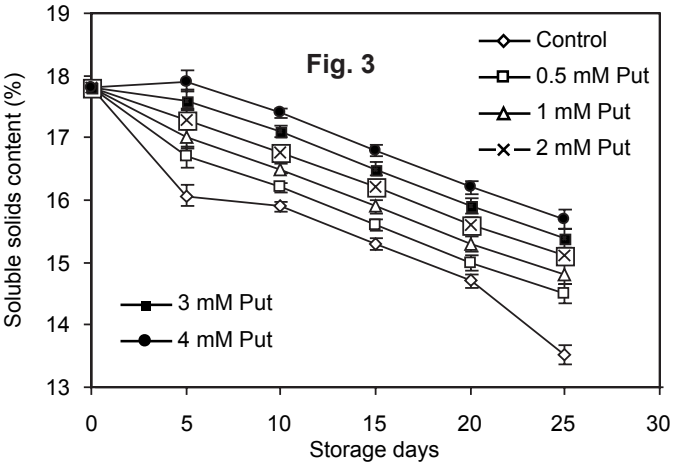

Fig. 3. Changes in soluble solids content of sweet cherry, cv. Surati-e Hamedan during storage at 2˚C.

compared with the controls at all determination times, but the difference between cherries treated with various concentrations of putrescine was significant only at 10th and 15th day. The loss of weight during storage of fruit is caused by water exchange between the internal and external atmosphere, the transpiration rate being accelerated by cellular breakdown. Putrescine treatment might have maintained membrane integrity and delayed the removal of epicuticular waxes which play an important role in water exchange through the skin, as has been reported in mandarin

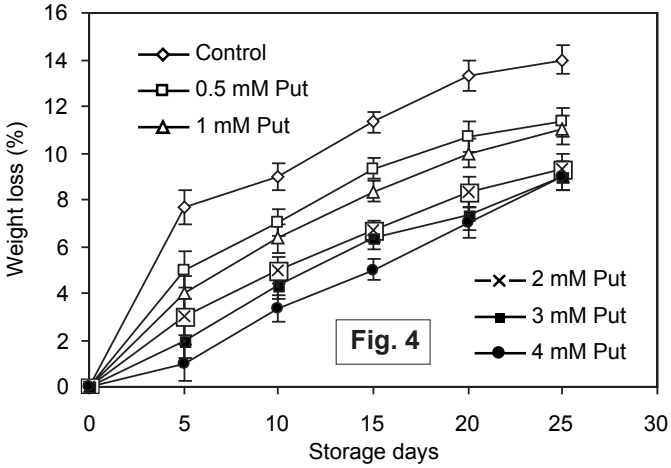

Fig. 4. Changes in weight loss of sweet cherry, cv. Surati-e Hamedan during storage at 2˚C.

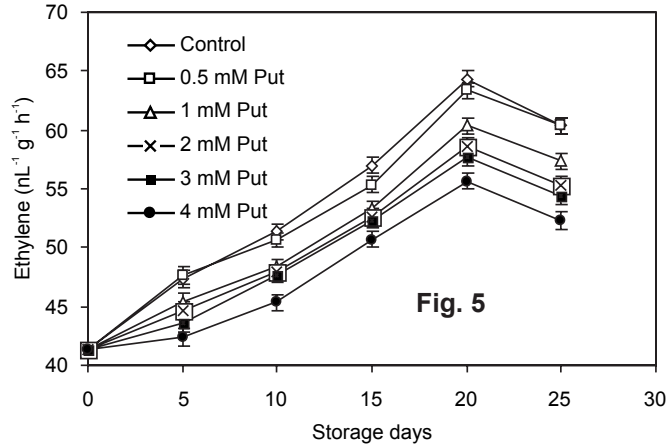

Fig. 5. Changes in ethylene production of sweet cherry, cv. Surati-e Hamedan during storage at 2˚C.

(Schirra and D'Hallewin, 1997), apricot (Martinez-Romero *et al.*, 2002) and plum (Serrano *et al.*, 2003).

Ethylene production: There were no significant difference between 0.5 mM putrescine-treated and untreated (Control) cherries for ethylene production, but the difference between controls and cherries treated with higher concentrations were significant ($P<0.05$). The highest and lowest rates of ethylene production occurred in control and 4 mM putrescine treatment, respectively at all sampling dates (Fig. 5), but there was no difference between various concentrations of putrescine in terms of ethylene production.

pH: The pH of the sweet cherry juice was not significantly altered by various concentrations of putrescine during storage time (data not shown).

By taking into account the parameters related to fruit quality including firmness, soluble solids content, titratable acidity, ethylene production and weight loss as well as the visual appearance of the fruits, the estimated postharvest life of the control cherries was 17 days. The postharvest life increased up to 19, 21, 23, 27 and 28 days for cherries treated with 0.5, 1, 2, 3 and 4 mM putrescine, respectively.

In conclusion, the exogenous application of putrescine is an effective method to prolong storability and increase shelf life of sweet cherry during storage at 2˚C.

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