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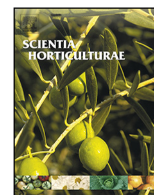

Interaction effects of salicylic acid and methyl jasmonate on total antioxidant content, catalase and peroxidase enzymes activity in “Sabrosa” strawberry fruit during storage

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ABSTRACT

Strawberry is very susceptible to postharvest losses and more than 50% of harvested fruit is lost in Iran. For many years synthetic chemicals have been used to increase fruit storage life but, because of food safety issues, the use of chemicals in postharvest technology is highly restricted. Extending the storage life of harvested products by enhancing natural resistance and antioxidative systems of the crop is an alternative strategy to chemicals in postharvest technology. Effect of postharvest treatment with salicylic acid (at 0, 1 and 2 mmol/L) and methyl jasmonate (at 0, 8 and 16 μmol/L) on antioxidant content and some defense enzymes activity of Sabrosa strawberry fruit during storage at 1 ± 0.5 °C with 90–95% RH. for 14 days followed by 24 h at 20 °C was studied. Both phytochemicals, separately or in combination, enhanced fruit total antioxidant content, catalase and peroxidase enzymes activity. Catalase enzyme activity was decreased after treatment with methyl jasmonate but the effect of methyl jasmonate in decreasing catalase activity was prevented by salicylic acid. It seems that in order to stablish a strong resistance network and extend fruit postharvest life a positive crosstalk between the two phytochemicals is necessary in “Sabrosa” strawberry fruit.

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1. Introduction

Similar to plant cells, the aging and senescence process in human cells is highly related to active oxygen species (AOS) and free radicals as oxidative agents. AOS and free radicals are the major players in aging and diseases like inflammation, arthritis, immune system impairment, different cancers and heart disease and much more focus has been given to the involvement of antioxidants in free radical scavenging and related senescence and diseases prevention (Cai et al., 2004; Kaefer and Milner, 2008; Huang et al., 2010). Several antioxidants are responsible for detoxifying the plant and human cells from oxidative agents. The antioxidant systems in plants and harvested crops include antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT) as well as nonenzymatic compounds with antioxidant activity such as ascorbic acid, glutathione, phenolic compounds, flavonoids, catkin, carotenoids, and α-tocopherols (Asghari and Soleimani Aghdam, 2010). Since natural antioxidants are more readily acceptable than synthetic ones, fruits containing high antioxidants are of more commercial importance. Colorful fruits such as strawberries are rich sources of antioxidants playing critical roles in preventing above-mentioned diseases and decreasing senescence rate. Strawberry fruit is an important source of natural antioxidants and phytochemicals, particularly anthocyanins, flavonoids, phenolic acids and ellagic acid, which have potent antioxidant and anti-inflammatory functions and also essential minerals making it as one of most commercial horticultural crops (Heinonen et al., 1998; Rice-Evans and Miller, 1996). It has been demonstrated that fresh juice of strawberries has high oxygen radical absorbance activity against peroxyl ($ROO^{\bullet}$), superoxide ($O^{\bullet}{}_2{}^{-}$) and hydroxyl radicals ($OH^{\bullet}$), singlet oxygen ($^{\bullet}O_2$) and hydrogen peroxide (H_2O_2) as the major oxidative agents. It is well demonstrated that the antioxidant activity is different among varieties and is highly influenced by postharvest treatments and conditions (Wang and Jiao, 2000).

Because of consumption during free radical scavenging process the antioxidant content of harvested crops is decreased during storage leading to accelerated fruit senescence and decreased nutritional quality. Strawberries are perishable fruits and more than 50% of produced strawberries is lost in Iran during postharvest handling and, because of using improper postharvest technologies, the remaining part received by the consumers are poor in antioxidants

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and other nutritional properties. Then the use of proper postharvest technologies is essential to decrease the adverse effects of stress conditions, rate of aging process, enhancing storage life and keeping nutritional quality of strawberries.

Recently, because of the food safety issues and environmental concerns, use of synthetic chemicals in postharvest technology of horticultural crops is highly restricted and much more researches have been focused on generally regarded as safe compounds (GRASC). Extending the storage life of harvested products by enhancing natural resistance and antioxidative systems of the crop has been considered as an alternative strategy to chemicals in postharvest technology. Different biotic and abiotic stresses such as pathogens and chilling injury (CI) are responsible for oxidative burst in the cells of harvested fruits leading to increased membrane damage and antioxidant consumption. By means of hormones and plant growth regulators, plant cells are capable of stablishing different resistance systems against such dangerous conditions (Asghari and Soleimani Agdam, 2010; Ladu et al., 2012). Salicylates and jasmonates are of main elicitors of natural resistance and antioxidant systems in plants and harvested crops. Both phytochemicals, known as plant hormones, have been shown to play key roles in several plant cell communication and signaling processes including ethylene production, defense responses against different biotic and abiotic stresses, anthocyanin and other phytochemicals synthesis, regulating stress responses, heat production or thermogenesis, photosynthesis, stomatal conductance, transpiration, ion uptake and transport, seed germination, sex polarization, crop yield and glycolysis (Asghari and Soleimani Aghdam, 2010; Dar et al., 2015). Exogenous jasmonates and salicylates, at non-toxic concentrations, have been shown to alleviate postharvest CI and oxidative stress in horticultural crops (Cao et al., 2009; Jin et al., 2009; Siboza et al., 2014; Valero et al., 2015; Aghdam et al., 2012a,b; Lu et al., 2011; Luo et al., 2012; Promyou et al., 2012). Treatment with salicylic acid (SA) and methyl jasmonate (MeJA) has been reported to activate some heat shock proteins (HSPs) in plants and harvested plant products which are preservative agents of plant cells against different stresses (Asghari and Soleimani Aghdam, 2010; Soleimani Aghdama et al., 2013). Decreased susceptibility to chilling injury and prevention of PPO activity has been reported as the result of treatment with MeJA in peach Fruit (Jin et al., 2009).

Since there was no enough evidence about the interaction between the two phytochemicals in harvested strawberries, this study was conducted to determine the interaction between SA and MeJA on total antioxidant content (TAC) and some defense enzymes in harvested "Sabrosa" Strawberry fruit.

2. Materials and methods

2.1. Sample preparation

"Sabrosa" strawberry fruits (*Fragaria* × *ananassa* Duch. cv. Sabrosa), at commercial ripeness (>75% of the surface showing red color), were harvested from a commercial production greenhouse in Urmia (Iran) and transported to postharvest laboratory at Urmia University. Fruit were selected for uniformity of color and size and any fruit with apparent injuries, disease or infections were removed.

2.2. Treatments with SA

SA was purchased from Sigma Co., (Sigma–Aldrich, Germany). Fruits were divided into three groups and dipped in SA solutions (0, 1 and 2 mM/L) for 5 min at 20 °C. Each treatment was conducted on 20 fruits and replicated five times. Control (0 mM/L) fruit were immersed in distilled water at 20 °C. Both control and treated fruit were put in 100 ml plastic jars and stored at 1 ± 0.5 °C with 90–95% RH. for 14 days followed by 24 h at 20 °C.

2.3. Treatments with MeJA

MeJA was purchased from Sigma Co., (Sigma–Aldrich, Germany). Fruits were placed in a 120 L sealed container and treated with different levels of MeJA (0, 8 and 16 μmol/L) at 20 °C. The appropriate volume of MeJA to reach the desired concentration (960 μL for 8 μmol/L and 480 μL for 16 μmol/L) was deposited on filter paper at the bottom of the container and then was immediately sealed. Duration of the treatment was 16 h, after which the fruits from each treatment were randomly divided into 5 replicates and putted in 100 mL plastic jars and transferred to cold storage. Each replication (plastic jar) was consisted of 20 fruits. Control fruit received no treatment. Both control and treated fruits were stored at 1 ± 0.5 °C with 90–95% RH. for 14 days followed by 24 h at 20 °C.

For combination treatments the fruits were first treated with SA and then were treated with MeJA at abovementioned concentrations.

Fruit TAC, CAT and POD enzymes activity were measured after 7 and 14 days of cold storage plus 24 h at 20 °C.

2.4. Determination of TAC

Fruit juice TAC was determined by ferric ions reducing antioxidant power assay (FRAP) according to Benzie and Strain (1996) with slight modifications. The stock solutions included 5 mL of a 10 mmol/L TPTZ (2,4,6-tripyridyl-*s*-triazine) with 40 mmol/L HCL plus 5.41 mL of $FeCl_3$ (20 mmol/L) and 50 mL of phosphate buffer, (0.3 mmol/L, pH 3.6) and was prepared freshly and warmed at 37 °C. Fruit extracts (150 mL) were allowed to react with 2.85 mL FRAP solution and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37 °C for 10 min. For construction of calibration curve five concentrations of $FeSO_4 7H_2O$ (1000, 750, 500, 250, 125 μmol/L) were used to obtain the calibration curves. The values were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L $FeSO_4$ ($y = 0.0009x - 0.0275$, $R^2 = 0.995$).

2.5. Determination of CAT enzyme activity

All enzyme extract procedures were conducted at 4 °C. CAT activity was measured according to the Beers and Sizer (1952) with slight modifications. The reaction mixture consisted of 2.5 mL sodium phosphate buffer (50 mmol/L, pH 7.0), 0.2 mL H_2O_2 (1%) and 0.3 mL enzyme. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm. The specific activity was expressed as U mg/protein, where one unit of catalase converts one μmol of H_2O_2 per min.

2.6. Determination of POD enzyme activity

In order to determine the POD activity, the reaction mixture contained 2.5 mL of 50 mM phosphate buffer (PH 6.1), mL of 1% hydrogen peroxide, 1 mL of 1% guaiacol and 0.1 mL of the enzyme extract. The increase in absorbance at 490 nm was followed for one minute and expressed as U/mg protein (Upadhayaya et al., 1985).

2.7. Statistical analysis

The experiment was designed as a completely randomized design with 3 (MeJA concentrations) × 3 (SA concentrations) × 2 (storage time) = 18 factors in 5 replicates (each replication was included 20 fruits). Analysis of variance (ANOVA) was performed

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