American Journal of Plant Physiology

ISSN 1557-4539

Academic Journals Inc.

www.academicjournals.com

American Journal of Plant Physiology 6 (2): 113-119, 2011
ISSN 1557-4539 / DOI: 10.3923/ajpp.2011.113.119

Effect of Salicylic Acid Treatments on Quality Characteristics of Apple Fruits During Storage

[1]M. Kazemi, [2]M. Aran and [1]S. Zamani

[1]Department of Horticulture Sciences, Faculty of Agriculture and Natural Resources, Islamic Azad University, Karaj Branch, Young Researchers Club, Karaj, Iran

[2]Department of Landscape, College of Agriculture, University of Zabol, Zabol, Iran

Corresponding Author: M. Kazemi, Department of Horticulture Sciences, Faculty of Agriculture and Natural Resources, Islamic Azad University, Karaj Branch, Rajaee St., Eivan, Ilam, P.O. Box 69417-47611, Iran Tel: +98 842 3230192

ABSTRACT

Apple texture can deteriorate during cold storage, resulting in softness and mealiness. The purpose of this work was to estimate shelf-life and to study the behavior of 'Jonagold' apples kept at 5°C in a normal atmosphere. The experiment was started in season 2010-2011 and Fruit Weight Losses, Fruit Firmness, Total soluble solids, Titratable acidity, Peroxidase activity, ascorbic acid content (Vitamin C) and Superoxide dismutase activity were measured at 15, 30 and 60th days of postharvest life. In this research, fruits were immersed in salicylic acid solution (0, 1.5, 3 mM) for 5 min, stored at 5°C up to 60 days. The results showed that fruit weight loss significantly decreased in all SA concentrations in comparison to control. Also, the results showed that fruits treated in SA solution for 5minutes had higher firmness, TA, Peroxidase activity, Superoxide dismutase activity and lower TSS than fruits that treated in control. Furthermore, significant changes were observed in browning index and relative electrical conductivity during storage in all treatments. The results showed that SA application was influenced on vitamin C value in comparison to control. In general, this experiment showed that post-harvest SA treatment prevented fruit softening and decreased weight losses. This treatment can be easily used to improve of apple fruits during.

Key words: Postharvest life, salicylic acid weight loss, firmness, titratable acidity

INTRODUCTION

Apples are the natural source of dietary mineral salts, vitamins, antioxidants, fibre, organic acids and sugars. The highest concentration of bioactive substances, including antioxidants, is found in or near the peel, so it is recommended as a dietary supplement (Wolfe *et al*., 2003; Wolfe and Liu, 2003). Apple is a climacteric fruit with a long post-harvest life in cool storage. Losses in fruit quality are mostly due to its relatively high metabolic activity during storage (Fattahi *et al*., 2010). Cool storage is widely used to reduce respiration rate, Ethylene production and extend the shelf-life of fruits (Fattahi *et al*., 2010). Ethylene is a gaseous plant hormone that at very low concentrations plays a major role in the regulation of the metabolism of harvested horticultural crops (Saltveit, 1999). The responses of harvested fruits, vegetables and ornamental crops to endogenously produced and exogenously applied ethylene are numerous and varied and they can be beneficial or detrimental depending on each case (Saltveit, 1999). In general, ethylene can influence the postharvest life of both climacteric and nonclimacteric fruit by affecting their quality

attributes and the development of physiological disorders and postharvest diseases (Palou *et al*., 2003). Respiration and Ethylene production causes a sharp increase in production of oxygen free radicals which is responsible for stress dependent peroxidation of membrane lipids. The effects of ethylene can be reduced by inhibitors of ethylene biosynthesis and increase enzyme antioxidant activity (Khan *et al*., 2003; El-Tayeb *et al*., 2006; Shi and Zhu, 2008; Joseph *et al*., 2010). Salicylic acid is known as a signal molecule in the induction defense mechanisms in plants. SA is a well known phenol that can prevent ACO activity that is the direct precursor of ethylene and decrease Reactive Oxygene Species (ROS) with increase enzyme antioxidant activity (Ansari and Misra, 2007; Mba *et al*., 2007; Mahdavian *et al*., 2007; Canakci, 2008). It delays the ripening of banana fruit (Srivastava and Dwivedi, 2000), inhibit ethylene production in kiwifruit (Fattahi *et al*., 2010) and carrot cell suspension cultures (Roustan *et al*., 1990). As Zhang *et al*. (2003) reported, application of SA on kiwifruit increased superoxide free radical and Lipoxygenase (LOX) activity. In that case, climacteric rise in ethylene production was retarded. So, fruit ripening and senescence were delayed (Zhang *et al*., 2003). Application of exogenous methyl salicylate (MeSA) vapor on kiwifruits led to prevent the softening process of fruit flesh, kept ascorbic acid content and firmness during 5 months storage (Solaimani *et al*., 2009). The aims of this study was to determine the effect of salicylic acid application on the quality and storage life of apple fruit during storage.

MATERIALS AND METHODS

The experiment was started in season 2010-2011 and Fruit Weight Losses, Fruit Firmness, total soluble solids, titratable acidity, peroxidase activity (POD), ascorbic acid content (Vitamin C) and superoxide dismutase (SOD) activity were measured at 15, 30 and 60th days of postharvest life. *Malus domestica var. Jonagold* were harvested at commercial maturity stage from an experiment orchard at the apple Research Institute of Iran (uromieh, Iran). Fruits were subsequently transferred to laboratory and sorted based on size and the absence of physical injuries or infections. Fruits were randomly divided into six groups, each group containing 100 fruits in four replicates and immersed into solution of (0, 1.5, 3 mM) SA and in distilled water as control for 5 min. Fruits were then dried for about 24 h and then stored at 5°C and 85-90% relative humidity for two months. After15, 30 and 60th days storage, 30 fruits per treatment were taken from cool storage for fruit quality assessment.

Experimental design and statistical analysis: Experiment was arranged in complete randomized design with four replications. Analysis of variance was performed on the data collected using the General Linear Model (GLM) procedure of the SPSS software) Version 16, IBM Inc.). The mean separation was conducted by tukey analysis in the same software ($p = 0.05$).

Weight loss: Weight loss was determined by using Tefera *et al*. (2007) method, by periodical weighing of apple fruits 15, 30 and 60 days after storage.

Fruit firmness: Firmness was determined by measuring compression using a hand-held Effegi penetrometer with a 7.9 mm probe after removal of skin to a vertical depth of 1 mm on two sides of the fruit. The firmness considered as an average peak force of 10 fruits and expressed as kg/7.9 mm^2.

Total soluble solid: Total Soluble Solids (TSS) were measured by the method described by Dong *et al.* (2001).

Titratable acidity: Titratable acidity was determined using 5 mL of fruit puree from five fruits mixed with 25 mL of distilled water with two drops of phenolphthalein (1%) as indicator, titrated with 0.1N NaOH to an endpoint pink (pH 8.2). The results were expressed as percent anhydrous citric acid since it is the dominant acid in apple.

Ascorbic acid content (Vitamin C): Ascorbic Acid (AA) content of apple was determined by the 2,6-dichlorophenolindophenol method (Tefera *et al.*, 2007). An aliquot of 10 mL apple fruit juice extract was diluted to 50 mL with 3% metaphosphoric acid in a 50 mL volumetric flask. The aliquot was filtered and titrated with the standard dye to a pink endpoint (persisting for 15 sec).

Browning index: Browning index was assessed by measuring the extent of browning area as described by Wang *et al.* (2005).

Relative electrical conductivity: Relative electrical conductivity was measured by the method described by Fan and Sokorai (2005).

Determination of Acc-oxidase activity: For the measurement of ACC oxidase activity, flesh slices of 1 mm thickness (approximately 1 g) were put into 40 mL Erlenmeyer flasks containing 2 mL of incubation buffer consisting of 1 mM ACC, 0.4 M mannitol and 0.1 M Tricine (pH 7.5). ACC oxidase activity was determined both in the absence and in the presence of 30 mM sodium ascorbate, 0.1 mM $FeSO_4$ and 20 mM $NaHCO_3$ according to the method described by Moya-Leon and John (1994). The flasks were incubated at 30°C for 1 h and the ethylene formed was determined as described above. The activity was expressed as ethylene (in nanomoles) produced per gram fresh weight per hour.

Superoxide dismutase (SOD) activity: A 1.0 g aliquot of frozen powder was added to 10 mL of cold ethanol absolute for 30 min, then centrifuged at 0°C and 10,000×g for 10 min and the supernatant discarded. The ethanol extraction was repeated twice. The pellet was then resuspended in 5.0 mL of cold 100 Mm sodium-potassium phosphate buffer (NaKPi), pH 7.0, 0.1% (w/v) polyvinylpolypyrrolidone (PVPP), prepared and stored at 4°C the day before and, after 30 min, centrifuged at 4°C and 10,000x g for 30 min. The supernatant was recovered and used for the enzyme activity assay. Total SOD activity was measured after Madamanchi *et al.* (1994). For each sample assayed, six tubes were set up containing 10, 20, 40, 60, 80 and 500 μL of the enzyme extract. The reaction mixture contained 2 μM riboflavine, 10 μM l-methionine, 50 μM Nitro Blue Tetrazolium (NBT), 20 μM KCN, 6.6 M Na2EDTA, 10-500 μL of the enzyme extract and 65 μM NaKPi, pH 7.8, to give a total volume of 3.0 mL. SOD activity was assayed by measuring the capacity of the enzyme extract to inhibit the photochemical reduction of NBT to blue formazan. Glass tubes were thermostated at 25°C for 10 min in absence of direct light. The reaction was started by exposing the mixture to four white fluorescent lamps (Leuci, 15 WTS preheat, daylight 6500°K) in a box (80×50×50 cm) with aluminium-foil-coated walls. Blanks were obtained with non-illuminated duplicates. The blue colour developed in the reaction was spectrophotometrically measured at 560 nm and the corresponding non-exposed samples were used as blank. The volume of sample causing 50% inhibition in colour development was taken as one unit of SOD activity.

Peroxidase activity (POD): A 1.0 g aliquot of frozen powder was added to 10.0 mL of cold 200 mM NaPi, pH 7.0, 5 mM Na2EDTA, 0.1% (w/v) PVPP, 3 mM dithiothreitol, 15 mM-mercaptoethanol, 10 mM sodium metabisulfite, prepared and stored at 4°C the day before and after 30 min, centrifuged at 15,000x g for 30 min. The supernatant was recovered and used for the enzyme activity assay. The reaction mixture (3.0 mL final volume) consisted of 50 μL of 10 mM guaiacol, 2.9 mL of 10 mM NaPi, pH 7.0, 10 μL of 40 mM H_2O_2. A 40 μL aliquot of the crude enzyme extract was then added to start the reaction. The activity of the mixture was determined spectrophotometrically at 470 nm after 10 min at 20°C.

RESULTS AND DISCUSSION

Effect salicylic acid on weight loss: The results indicate that treatment with 1.5 mM SA solution slightly reduced the weight loss (Table 1). maximum weight loss occurred in control while lowest loss was recorded in 3 mM SA($p = 0.05$) (Table 1). Weight loss was highest during the eighth weeks. Overall highest weight loss occurred in control during the Fifth week ($p = 0.05$) (Table 1). It's thought that SA can decrease respiration through inhibition of ethylene biosynthesis or action (Srivastava and Dwivedi, 2000). Salicylic acid also caused decrease in respiration rate and fruit weight losses by closing stoma (Zheng and Zhang, 2004).

Effect salicylic acid on firmness: The results indicate that maximum firmness was recorded in 3 mM SA as compared to control and 1.5 mM SA. Maximum firmness was recorded in 3 mM SA during 60 day ($p = 0.05$) (Table 1). This result was in agreement with the report of Solaimani *et al.* (2009) that suggested postharvest application of kiwifruit by MeSA decreased softening and kept firmness during storage. Zhang *et al.* (2003) reported that rate of fruit ripening related to internal SA concentration. Our results suggested that firmness caused by SA associated with ACO activity inhibitory. This suppression may mostly due to inhibitory effect of SA on ACC conversion to ethylene (Li *et al.*, 1992).

Effect salicylic acid on total soluble solids, titratable acidity: The results in Table 1 show that the storage period has a significant effect on TSS% and TA of fruits ($p = 0.05$). The results indicate that minimum TSS was observed in 3 mM salicylic acid, the highest TSS was recorded in control. Total soluble solids content of fruits during storage is considered an index of fruit ripening and an increase in TSS corresponds to a conversion of starch to soluble sugars. Also, The results indicate that maximum TA was observed in 3 mM salicylic acid and lowest TA was recorded in control. Titratable acidity is directly related to the concentration of organic acids present in the fruit which are an important parameter in maintaining the quality of fruits. Titratable acidity increased gradually in all treatments except control (Table 1) and did seem to be influenced by the postharvest SA. Delay in fruit ripening and extended shelf-life after SA treatment also reported in banana fruit by Srivastava and Dwivedi (2000). Similarly, Zhang *et al.* (2003) found that the rate of softening in kiwifruit treated by SA reduced because had remained relatively high levels of SA concentrations.

Effect salicylic acid on ascorbic acid (Vit C) content: The results indicate that the values of Vitamin C significantly increased with increasing SOD and POD activity and decreased ACO activity in the storage duration. All treatments had significant effect on the values of vitamin C

Table 1: Mean comparison of fruit weight loss, Firmness, TA, Ascorbic acid, Browning index, REC, SOD, POD, ACC oxidase activity in different concentration SA solution during 60 days storage at 5°C

Time storage (day)	Treatment ---------- Sa (nM)	Weight loss (%)	Firmness (kgf)	[1]TSS (brix %)	[2]TA (%)	Ascorbic acid mg 100 g^{-1} FW	Browning index (%)	[3]REC (%)	[4]SOD (U g^{-1} Protein)	ACC Oxidase Activity (nmol/gFW/h)	[5]POD acticity (units/g.fresh weight)
15	0	1.75A	1.48C	13.11A	0.62AC	20.11AC	18.11B	51.17C	54.7AC	89.14C	67AC
	1.5	1.14B	2B	10B	1AB	27.14AB	14.31C	45.5AB	69.7AB	50AB	87.14AB
	3	0.33C	3.1A	8C	2.1B	30.47C	10.09AB	22D	179.5B	13.47D	211.07C
30	0	2.55A	1.68C	14A	0.55AC	22.14AC	30.14A	60.54B	60.14AC	90.14B	60.7AC
	1.5	1.6B	2.07B	11.2B	0.97AB	30.78C	14C	40.78AB	77.58AB	56.89AB	90.06AB
	3	0.3C	2.89A	8.79C	1.79C	46B	9.74AB	25AC	160C	16.9AC	227.14B
60	0	2.12A	1C	13.45A	0.22AC	17.53D	34.17A	67.17A	50D	100.3A	50D
	1.5	1.89B	2.01B	9.89B	1AB	31.14C	17.25B	48AB	70.58AB	48.32AB	89.6AB
	3	0.4C	3A	8C	2.89A	50A	10.74AB	23.14D	186.36A	12.09AD	254.11A
F-test probabilities	SA	0.001	0.001	0.004	0.01	0.003	0.001	0.02	0.001	0.04	0.001

Means in each column followed by similar letters are not significantly different at 5% level. [1]Total soluble solids, [2]Titratable acidity, [3]Relative electrical conductivity, [4]Peroxidase activity, [5]Superoxide dismutase activity

except control (p = 0.05). The results indicate that maximum SOD and POD activity was observed in 3 mM salicylic acid (p = 0.05) also, the results indicate that Acc-oxidase activity decreased with increasing SOD and POD activity in the storage duration. Lamikanra and Watson (2001, 2002) indicated the ascorbate dependency of peroxidase (POD) enzymes in a number of commonly fresh-cut processed fruits whose activities appear to be related to the level of oxidative stress in cut fruit. Ascorbic acid level decreased gradually during the ten weeks storage period. Ascorbic acid is an important nutrient quality parameter and is very sensitive to degradation due to its oxidation compared to other nutrients during food processing and storage. These results showed that SA treatments had a significant effect on retaining ascorbic acid content in apple fruit. As Zhang *et al.* (2003) reported, application of SA on kiwifruit increased superoxide free radical and enzyme antioxidant activity. In that case, climacteric rise in ethylene production was retarded. So, fruit ripening and senescence were delayed (Zhang *et al.*, 2003). Our result showed that higher concentrations of SA delayed the rapid oxidation of ascorbic acid with increasing SOD and POD activity and decreased Acc-oxidase activity in the storage duration.

Effect salicylic acid on Browning Index (BI) and Relative Electrical Conductivity (REC): The BI and REC decreased with increasing SOD and POD activity and decreased Acc-oxidase activity in the storage duration. salicylic acid (3 mM SA) had a significantly influence in reducing the BI and REC in fruits compared to control in the storage duration (p = 0.05). The results indicate that maximum BI and REC were recorded in control as compared to other treatment. Oxidative membrane injury allows the mixing of the normally separated enzyme (PPO) and oxidizable substrates (polyphenols) which lead to browning. Respiration and Ethylene production causes a sharp increase in production of oxygen free radicals which is responsible for stress dependent peroxidation of membrane lipids. As Zhang *et al.* (2003) reported application of SA on kiwifruit increased superoxide free radical and Lipoxygenase (LOX) activity. In that case, climacteric rise in ethylene production was retarded. So, fruit ripening, REC, BI and senescence were delayed (Zhang *et al.*, 2003).

CONCLUSION

From the results of the present study, it can be concluded that Salicylic acid treatments significantly retained maximum firmness, total soluble solids, ascorbic acid content, Peroxidase activity(POD), Superoxide dismutase (SOD) activity and reduced Browning Index (BI), Relative Electrical Conductivity (REC) and weight loss compared to the control.

REFERENCES

Ansari, M.S. and N. Misra, 2007. Miraculous role of salicylic acid in plant and animal system. Am. J. Plant Physiol., 2: 51-58.

Canakci, S., 2008. Effects of salicylic acid on fresh weight change, chlorophyll and protein amounts of radish (*Raphanus sativus* L.) seedlings. J. Biol. Sci., 8: 431-435.

Dong, L., H.W. Zhou, L. Sonego, A. Lers and S. Lurie, 2001. Ripening of Red Rosa plums: Effect of ethylene and 1-methylcyclopropene. Aust. J. Plant Physiol., 28: 1039-1045.

El-Tayeb, M.A., A.E. El-Enany and N.I. Ahmed, 2006. Salicylic acid-induced adaptive response to copper stress in sunflower (*Helianthus annuus* L.) Int. J. Bot., 2: 372-379.

Fan, X. and K.J.B. Sokorai, 2005. Assessment of radiation sensitivity of fresh-cut vegetables using electrolyte leakage measurement. Postharvest Biol. Technol., 36: 191-197.

Fattahi, J., R. Fifall and M. Babri, 2010. Postharvest quality of kiwifruit (*Actinidia deliciosa* cv. Hayward) affected by pre-storage application of salicylic acid. S. Western J. Hortic. Biol. Environ., 1: 175-186.

Joseph, B., D. Jini and S. Sujatha, 2010. Insight into the role of exogenous salicylic acid on plants grown under salt environment. Asian J. Crop Sci., 2: 226-235.

Khan, W., B. Prithiviraj and D.L. Smith, 2003. Photosynthetic response of corn and soybean to foliar application of salicylates. J. Plant Physiol., 160: 485-492.

Lamikanra, O. and M.A. Watson, 2001. Effects of ascorbic acid on peroxidase and polyphenoloxidase activities in fresh-cut cantaloupe melon. J. Food Sci., 66: 1283-1286.

Lamikanra, O. and M.A. Watson, 2002. Biochemical changes associated with fresh-cut fruit processing and storage. ACS Symp. Ser., 836: 52-68.

Li, N., B.L. Parsons, D. Liu and A.K. Mattoo, 1992. Accumulation of wound-inducible ACC synthase transcript in tomato fruit is inhibited by salicylic acid and polyamines. Plant Mol. Biol., 18: 477-487.

Madamanchi, N.R., J.L. Donahue, C.L. Cramer, R.G. Alscher and K. Pedersen, 1994. Differential response of Cu, Zn superoxide dismutases in two pea cultivars during a short-term exposure to sulphur dioxide. Plant Mol. Biol., 26: 95-103.

Mahdavian, K., K.M. Kalantari and M. Ghorbanli, 2007. The effect of different concentrations of salicylic acid on protective enzyme activities of pepper (*Capsicum annuum* L.) plants. Pak. J. Biol. Sci., 10: 3162-3165.

Mba, F.O., X. Zhi-Ting and Q. Hai-Jie, 2007. Salicylic acid alleviates the cadmium toxicity in Chinese cabbages (*Brassica chinensis*). Pak. J. Biol. Sci., 10: 3065-3071.

Moya-Leon, M.A. and P. John, 1994. Activity of 1-aminocyclopropane-1-carboxylate (ACC) oxidase (ethylene-forming enzyme) in the pulp and peel of ripening bananas. J. Horst. Sci., 69: 243-250.

Palou, L., C.H. Crisosto, D. Garner and L.M. Basinal, 2003. Effect of continuous exposure to exogenous ethylene during cold storage on postharvest decay development and quality attributes of stone fruits and table grapes. Postharvest Biol. Technol., 27: 243-254.

Roustan, J.P., A. Latche and J. Fallot, 1990. Inhibition of ethylene production and stimulation of carrot somatic embryogenesis by salicylic acid. Biol. Plant, 32: 273-276.

Saltveit, M.E., 1999. Effect of ethylene on quality of fresh fruits and vegetables, Postharvest Biol. Technol., 15: 279-292.

Shi, Q. and Z. Zhu, 2008. Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. Environ. Exp. Bot., 63: 317-326.

Solaimani, M., Y. Mostofi, A. Motallebiazar, F.J. Moghadam and M. Ghasemnezhad, 2009. Effects of MESA vapor treatment on the postharvest quality of Hayward kiwifruit. Proceedings of the 6th International Postharvest Symposium, Turkey.

Srivastava, M.K. and U.N. Dwivedi, 2000. Delayed ripening of banana fruit by salicylic acid. Plant Sci., 158: 87-96.

Tefera, A., T. Seyoum and K. Woldetsadik, 2007. Effect of disinfection, packaging and storage environment on the shelf life of mango. Biosyst. Eng., 97: 201-212.

Wang, Y.S., S.P. Tian and Y. Xu, 2005. Effects of high oxygen concentration on pro- and anti-oxidant enzymes in peach fruits during postharvest periods. Food Chem., 91: 99-104.

Wolfe, K. and R.H. Liu, 2003. Apple peels as a value-added food ingredient. J. Agric. Food Chem., 51: 1676-1683.

Wolfe, K., X. Wu and R.H. Liu, 2003. Antioxidant activity of apple peels. J. Agric. Food Chem., 51: 609-614.

Zhang, Y., K. Chen, S. Zhang and I. Ferguson, 2003. The role of salicylic acid in postharvest ripening of kiwifruit. Postharvest Biol. Technol., 28: 67-74.

Zheng, Y. and Q. Zhang, 2004. Effects of polyamines and salicylic acid postharvest storage of Ponkan mandarin. Acta Hortic., 632: 317-320.