



EFFECT OF UV-C RADIATION ON ANTIOXIDANT ENZYMES IN STRAWBERRY FRUIT (*Fragaria x ananassa* cv. Camarosa)

Nasrin Mohammadi¹, Soraya Mohammadi², Vahid Abdossi¹ and Masoud Mashhadi Akbar Boojar³

¹Department of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran

²Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

³Department of Biology, Science and Research Branch, Tarbiat Moalem University, Tehran, Iran

E-Mail: Mohamadi.soraya@ymail.com

ABSTRACT

The changes in antioxidant enzyme activity and oxidative enzyme, polyphenol oxidase enzyme (PPO) in strawberry fruit (*Fragaria x ananassa* cv. Camarosa) illuminated with different UV-C dosages were investigated. Three UV-C illumination dosages 0.25, 0.5 and 0.75 kJ/m² tested, promoted antioxidant enzyme activity and reduced oxidative enzyme, polyphenol oxidase, activity at 10°C compared to the control. UV-C radiation for 0.5 and 0.75 kJ/m² showed the best results for enhancing antioxidant enzyme activity including superoxide dismutase (SOD) and catalase (CAT), also decreasing polyphenol oxidase activity. All UV-C dosages enhanced antioxidant enzyme activity and reduced, polyphenol oxidase activity but 0.75 kJ/m² UV illumination gave the best results.

Keywords: strawberry, antioxidant enzymes, polyphenol oxidase, UV-C.

1. INTRODUCTION

Free oxygen radicals play an important role in etiology of several diseases like arthritis, cancer, atherosclerosis etc. In plants and animals, these free radicals are deactivated by antioxidants. These antioxidants act as an inhibitor of the process of oxidation even at relatively small concentration and thus have diver's physiological role in the body. Antioxidant concentrations of plant material act as radical scavengers and helps the converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources liked fruits, vegetables and tea, etc (Sulekha mandal *et al.*, 2009). Berry fruits including raspberries (*Rubus ideaus*) (Chanjirakul *et al.*, 2006), deerberries (*Vaccinium stamineum* L.) (Wang and Ballinton, 2007), blackberries (*Fubus fruticosus*) (Chanjirakul *et al.*, 2007), blueberries (*Vaccinium corymbosum*) (wang *et al.*, 2008) and strawberries (*Fragaria x ananassa*) (Erkan *et al.*, 2008, Wang *et al.*, 2007) have been reported to contain high antioxidant content. Nutrient-derived antioxidants like ascorbic acid (Vitamin C), tocopherol and tocotrienols (vitamin E), carotenoids, antioxidant enzymes include superoxide dismutase (SOD), glutathione peroxidase (GSH-POD), glutathione reductase (GR), metal binding proteins such as ferritin, lactoferrin (Sulekha mandal *et al.*, 2009). The main antioxidants in strawberries are phenolics, antocyanin, tannins, carotenoids, vitamin C and antioxidant enzymes (Szajdek and Boroska, 2008, paredes-Lopez *et al.*, 2010).

Polyphenol oxidase (PPO) catalyzed browning reactions in fruits and vegetables that cause deterioration and loss of quality. This enzyme oxidated phenolic compounds to quinones and their polymerization to melanin pigments (Yourk and Marshall, 2003).

Low dosages of UV-C radiation referred to 'hormesis', induced number of changes in fruits and vegetables (Shama *et al.*, 2005). Postharvest use of UV-C delayed scencece in broccol florets (Lorenza costa *et al.*,

2006), reduced botrytis storage rot of table grapes (Nigro *et al.*, 1998), retarded development of decay in strawberry (Nigro *et al.*, 2000). Exposure to UV-C light enhanced antioxidant activity in strawberry (Erkan *et al.*, 2008) and deerberry (Wang and Ballinton, 2007). UV-C treatment, increased ascorbic acid and total phenolic contents and delayed senescence in tomatoes (Jagadeesh *et al.*, 2009, Maharaj *et al.*, 2010) in tomatoes. It was useful way of reducing decay and maintaining bell pepper fruit quality and also, chilling injury incidence and severity reduced by short UV-C treatment (Ariel *et al.*, 2005).

The aim of this study was investigated the effect of different dosages of UV-C radiation on the activity of antioxidant enzymes and polyphenol oxidase enzyme.

2. MATERIALS AND METHODS

2.1. Plant material

Strawberries (*Fragaria x ananassa* cv. Camarosa) used in this study were grown at farms in Mazandaran, Iran and were hand-harvested at commercially mature stage stored to eliminated damagesd, shrivele and unripe fruit, and selected for uniform size and color. Selected strawberries were randomized and used for experiment.

2.2. UV-C radiation treatments

For UV-C treatment fruits were exposed to germicidal UV-C lamp (output 2W, Philips) with peak emmission 245nm and irradiated at 15cm from the lamp for different duration to obtain the desired UV dosages. Each fruit were rotated manuall in 2 different positions to ensure total exposure to UV-C light. Three UV-C intensities (0.25, 0.5 and 0.75 kJ/m²) were applied to strawberry fruits. Not-illuminated strawberries were considered as the control. After the treatment fruits were placed in plastic packets and stored at 10°C. Samples were taken initially and on 5, 10, and 15 days intervals during storage.



Samples were stored at -80°C until they were assayed for activity of antioxidant enzymes (superoxid dismutase SOD, glutathione peroxidase GSH-POD, catalase CAT) and polyphenol oxidase (PPO).

2.3. Antioxidant enzyme measurements

2.3.1. Glutathione peroxidase (GSH-POD)

Four grams of fruit tissue, fresh weight, were homogenized in 4 ml 0.1 mol L^{-1} Tris-HCl buffer (PH=7.8) containing 2 mmol L^{-1} EDTA-Na, 2 mmol L^{-1} dithiothreitol (DTT). The homogenate was centrifuged at $20,000 \times g$ for 30 min at 4°C , and the supernatant was used for enzymes assays.

GSH-POD activity was determined using the method of Tappel (1978) with slight modification. The reaction mixture contained 0.1 mol L^{-1} Tris-HCl buffer (PH=8.0), 0.4 mmol L^{-1} EDTA, 1.0 mmol L^{-1} NaN_3 , 1.0 mmol L^{-1} H_2O_2 , 1.0 mmol L^{-1} glutathione (GSH), 0.15 mmol L^{-1} NADPH, 1 unit of glutathione reductase and $100 \mu\text{l}$ enzyme extract. The total reaction volume was 1.0 ml. The reaction was started by adding H_2O_2 . GSH-POD activity was determined by the rate of NADPH oxidation at 340 nm via spectrophotometer.

2.3.2 Superoxide dismutase (SOD)

SOD activity was determined by the protocol of Beyer and Fridovich (1972). The reaction mixture contained Tris-HCl buffer (disodic phosphate, 1.3 mmol L^{-1} EDTA, 0.1 mmol L^{-1} monosodic carbonate), 0.25 mmol L^{-1} epinephrine as substrate. Total reaction volume was 1.0 ml. The reaction was started by adding epinephrine. SOD activity was determined by the value of epinephrine oxidation at 480 nm .

2.3.3. Catalase (CAT)

The reaction was carried in a reaction mixture containing 25 mmol L^{-1} (pH=6.5) phosphate buffer, 20 mmol L^{-1} H_2O_2 and $50 \mu\text{l}$ extract. Total reaction volume was 1.0 ml. CAT activity was determined by the rate of reduction of H_2O_2 at 240 nm (Luck, 1974).

2.4. Polyphenol oxidase (PPO)

Fruit tissue (5g) homogenized in 50 ml K-phosphate buffer (0.1 mol , pH=7.2) containing triton X-100. The homogenate centrifuged at $5000 \times g$ for 15 min (2°C) (Coseteng and Lee., 1987). The supernatante was used for the PPO assay by using the method described by Gonzalez *et al.* (1999). The reaction mixture was contained 25 ml K-phosphate buffer (pH=6), 0.3 ml catechol as substrate and then 0.2 ml extract was added. Total reaction volume was 3 ml and it was incubated at 37°C for 1 min. The rate of change in absorbance at 420 nm was measured, and the level of enzyme activity was expressed as the difference in absorbance.

2.5. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using the SPSS Statistical Analysis. Enzymes

activities were evaluated by the Duncan's test. Differences at $p \leq 0.05$ were considered significant.

3. RESULTS

3.1. Superoxide dismutase (SOD)

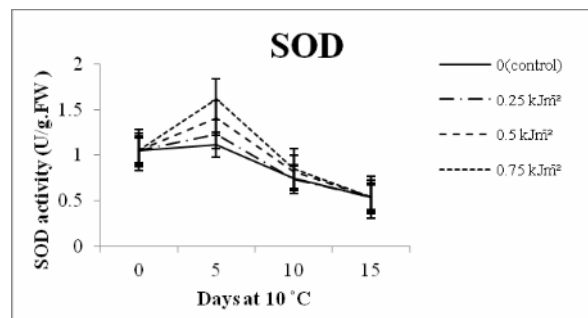


Figure-1. Superoxide dismutase (SOD) activity values in strawberry fruit illuminated with different UV-C dosages stored at 10°C . Vertical bars represent standard error.

Strawberry extracts taken after 5 days storage had higher SOD activities than those taken after 10 and 15 days of storage as shown in Figure-1. SOD activity of strawberry illuminated by different dosage of UV-C, increased during the first 5 days of storage and then decreased during the rest of storage at 10°C . After 15 days of storage, strawberry extracts from all UV-C treated fruits had higher SOD activities than those from the control fruits.

3.2. Glutathion peroxidase (GSH-POD)

GSH-POD activities of strawberry extract stored for 15 days were varied among treatments and storage durations as shown in Figure-2. After 5 days of storage, strawberry extract from 0.75 and 0.5 kJ/m^2 UV-C illuminated treatments had highest activities. Extracts from control and 0.25 kJ/m^2 UV-C illuminations had lower activities compared with those from 0.75 and 0.5 kJ/m^2 UV-C illuminations. After 15 days storage, samples from the control treatment had the lowest activities for GSH-POD.

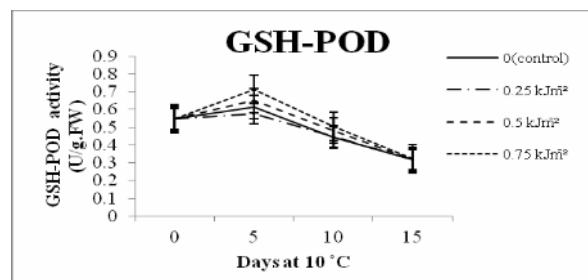


Figure-2. Glutathion peroxidase (GSH-POD) activity values in strawberry fruit illuminated with different UV-C dosages, stored at 10°C . Vertical bars represent standard error.



3.3. Catalase (CAT)

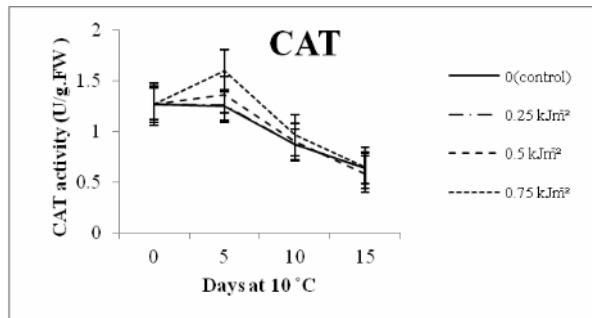


Figure-3. Catalase (CAT) activity values in strawberry fruit illuminated with different UV-C dosages, stored at 10°C. Vertical bars represent standard error.

UV-C radiation treatments increased catalase activity in strawberry treated fruits (Figure-3). Catalase activity was higher at 5 days after storage in all UV-C radiation treatments and then decreased during 10 and 15 days after storage. Control treatment had lower catalase activity during 15 days of strawberry fruit storage at 10°C. Strawberry treated with 0.75 kJ/m² UV-C illumination had highest CAT activity after 5 days, than the other treatments.

3.4. Polyphenol oxidase (PPO)

UV-C illumination in different dosages decreased Polyphenol oxidase activity of strawberry extracts during 15 days storage at 10°C (Figure-4). In all treatments PPO activity was lower than control. The lowest activity of PPO was caused by 0.75 kJ/m² UV-C radiations followed by 0.5 and 0.25 kJ/m² UV-C radiations. After 5 days of storage PPO activity increased during 10 and 15 days of storage but was lower than control.

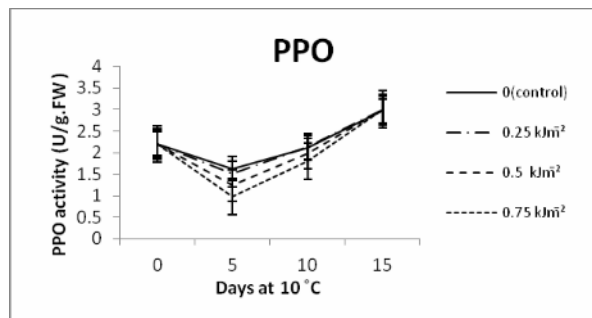


Figure-4. Polyphenol oxidase (PPO) activity values in strawberry fruit illuminated with different UV-C dosages, stored at 10°C. Vertical bars represent standard error.

4. DISCUSSIONS

There epidemiological evidence correlating higher intake of components foods with antioxidant abilities to lower incidence of various human morbidities or mortalities. Current research reveals the different potential application or control of disease (Devasagayam

et al., 2004). The importance of diet in relation to human health has increased the intensity of consumers on nutritious rich foods and especially on fruits and vegetables. Berries such as strawberry are rich sources of a wide variety of antioxidants and usually consumed in fresh or processed forms in human diet (Parades-Lopez., 2010). In our study antioxidant enzymes activity increased in strawberry extract treated with different UV-C illumination dosages. Strawberries stored at 10°C for 15 days had a higher antioxidant enzymes activity than the control. Super oxide dismutase (SOD) is an enzyme that removes the superoxide radical, repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body (Chakraborty *et al.*, 2009). SODs a class of metal-containing proteins catalyze the dismutation of superoxide radical anions in to H₂O₂ and molecular oxygen (Scandalion, 1993). There are three different types of SOD categorized by their metal cofactor: Cu/Zn (Cu/Zn-SOD), Mn (Mn-SOD), and Fe (Fe-SOD) (Mckersie *et al.*, 1993). However in this study we determined only the total SOD activity in strawberry treated with different dosages of UV-C radiation. The result showed that SOD activity decreased in all treatments during storage at 10°C, but after 15 days of storage treated strawberries had been more than control. This enzyme glutathione peroxidase utilizes reduced glutathione to eliminate hydrogen peroxide and convert it to harmless water. The activity of glutathione peroxidase is dependent on the availability of the reduced ascorbate and glutathione that are maintained by enzymes, such as GR, DHAR and MDHAR using NADPH as an electron donor (Roxas *et al.*, 2000). In our study UV-C illumination at enhanced GSH-POD activity during the storage period, 0.75 kJ/m² UV-C radiation had the highest GSH-POD activity. Catalase is common enzyme found in living organisms. Its functions include catalyzing the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover rates of all enzymes. One molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (Chakraborty *et al.*, 2009). CAT activity altered after treatment by different UV-C dosages, whereas highest change observed after 5 days of storage by 0.75 kJ/m² UV-C illumination. PPO is typically present in the majority of plant tissues (Haruta *et al.*, 1999). Enzymatic browning occurs as a result of the oxidation by PPO, of phenolic compounds to quinons and their eventual (nonenzyme-catalyzed) polymerization to melanin pigments (Whitaker, 1995). In this study we determined that PPO activity in strawberry extracts affected by UV-C treatment. PPO activity decreased compare with the control and lowest activity observed after 5 days of storage by 0.75 kJ/m² UV-C illuminations.

5. CONCLUSIONS

In conclusion, this study showed that strawberries illuminated with UV-C consistently had higher antioxidant enzyme activity and less PPO activity than control fruits. These results suggest that UV-C treatment may be an



effective non-chemical way of increasing strawberry fruit quality and extending their antioxidant nutritional fact. Further investigation is needed to elucidate the underlying relationship between UV-C illumination and antioxidant capacity and quality properties in strawberries.

REFERENCES

- Beyer WF and Fridovich I. 1987. Assaying of superoxide dismutase acting: Anal Biochem. 161: 559-566.
- Coseteng. M.Y and Lee C.Y. 1987. Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. Journal of Food Science. 52: 985-989.
- Chakraborty P., Kumar S., Dutta D. and Gupta V. 2009. Role of antioxidants in common health diseases. Research Journal Pharm. and Tech. 2(2):238-244.
- Chanjirakul K., Wang S. Y, Wang C. Y. and Siriphanich J. 2007. Natural volatile treatments increase free-radical scavenging capacity of strawberries and blackberries. Journal of the Science of Food and Agriculture.
- Devasagayam T.P.A., Tilak J.C., Boloor K.K., Sane K.S., Ghaskadbi S.S. and Lele R.D. 2004. Free radicals and antioxidants in human health: Current status and future prospects. JAPI. 52: 794.
- Erkan M., Wang S.Y. and Wang C.Y. 2008. Effect of UV treatment on antioxidant capacity, antioxidant enzyme activity and decay in strawberry fruit. Postharvest Biology and Technology. 48: 163-171.
- Gonzalez E. M, de An os B and Cano M. P. 1999. Partial Characterization of polyphenol oxidase activity in raspberry fruits. Journal of Agricultural and Food Chemistry. 47: 4068-4072.
- Haruta M., Murata M., Kadokura H. and Homma S. 1999. Immunological and molecular comparison of polyphenol oxidase in Rosaceae fruit trees. Phytochemistry. 50: 1021-1025.
- Jagadeesh S. L., Charles M. T., Garipey Y., Goyette B., Raghava G. S. V. and Vigneault C. 2009. Influence of postharvest UV-C hormesis on the bioactive components of tomato during post-treatment handling. Food Bioprocess Technology.
- Luck H. 1974. Catalase in: Bregmeyer, H.U. (Ed). , Methods of Enzymatic analysis. Vol. 2. Academic press, New York, USA. p. 885.
- Maharaj R., Arul J. and Nadeau P. 2010. UV-C irradiation of tomato and its effects on color and pigments. Advances in Environmental Biology. 4(2): 308-315.
- Mckersie B.D., Chen Y., de Beus M., Bowley S.R., Bowler C., Inze D., D'Hallnin K. and Botterman J. 1993. Super oxide dismutase enhances tolerance of freezing in transgenic alfalfa (*Medicago sativa* L.). Plant physiology. 103: 1155-1163.
- Nigro F., Ippolito A., Lattanzio V. and Lima Giuseppe. 1998. Use of UV-C light to reduce Botrytis cinerea storage rot of Table grapes. Postharvest Biology and Technology. 13: 171-181.
- Nigro F., Ippolito A., Lattanzio V., Vener D. Di and Salemo M. 2000. Effect of ultraviolet - C light on postharvest decay of strawberry. Journal of Plant Pathology. 82(1): 29-37.
- Paredes-Lopez O. and Cervantes-Ceja M. L. 2010. Berries: Improving human health in healthy aging, an promotin quality life - A review. Plant Foods Hum Nutr. 65: 299-308.
- Roxas V.P., Lochi S.A., Garrett K.D., Mohan J.R. and Allen R.D. 2000. Stress tolerance in transgenic tobacco seedling that over express glutathion S-transferase/glutathion-peroxidase. Plant Cell Physiology. 41: 1229-1234.
- Scandalios J.G. 1993. Oxygen stress and superoxide dismutase. Plant Physiology. 101: 7-12.
- Shama G. and Alderson P. 2005. UV hormesis in fruits: a concept ripe for commercialization - Review. Trends in Food Science and Technology. 16: 128-136.
- Sulekha M., Satish Y., Sanita Y. and Rajesh Kumar N. 2009. Antioxidant: A Review. Journal of Chemical and Pharmaceutical Research. 1(1): 102-104.
- Szajdek A. and Borowsska E.J. 2008. Bioactive compounds and health-promoting properties of berry fruits: A Review.
- Tapple A.L. 1978. Glutathione peroxidase and hydroperoxidase. Methods Enzymol. 52c: 506-513.
- Vicente A. R., Pineda C., Lemoine L., Civello P. M., Martinez G. A. and Chaves A. R. 2005. UV-C treatments reduce decay, retain quality and alleviate chilling injury in pepper. Postharvest Biology and Technology. 35: 69-78.
- Wang C.Y., Wang S.Y. and Chen C. 2008. Increasing Antioxidant Activity and Reducing Decay of Blueberries by Essential Oils. J. Agric. Food Chem. 56: 3587-3592.
- Wang C.Y., Wang S.Y., Yin J. and Parry L.L. Yu. 2007. Enhancing antioxidant, Antiproliferation, and Free Radical Scavenging Activities in Strawberries with Essential Oils. J. Agric. Food Chem. 55: 6527-6532.



Wang S. Y. and Ballington J. R. 2007. Free radical scavenging capacity and antioxidant enzyme activity in deerberry (*Vaccinium stamineum* L.). LWT. 40: 1352-1361.

Whitaker J.R. 1995. Polyphenol oxidase. In: Food Enzymes Structure and Mechanism. D.W.S. Wong. (Ed.). Chapman and Hall, New York, USA. pp. 271-307.

Yourk R. and Marshall M. 2003. Physicochemical properties and function of plant polyphenol oxidase: A review. Journal of Food Biochemistry. 27: 361-422.