Impact of Hot Water Soaking on Antioxidant Enzyme Activities and Some Qualities of Storage Tomato Fruits

Pornanan Boonkorn[1]*
[1]Science Faculty, Lampang Rajabhat University, Lampang, 52100, Thailand
*Corresponding author. Tel.: +66 81 7066982 ; fax: +66 54 241052
Email: pornananb@yahoo.com

Abstract

Mature-green tomato fruits were soaked in hot water for 10 minutes at 35, 40, 45 or 50°C and then stored at 13°C for 20 days. Untreated fruits were soaking with tap water (22°C). The study showed that exposing fruit to hot water influenced peroxidase (POD) and catalase (CAT) activities. Activity of the two enzymes increased with the increasing of soaking temperature, except for the 50°C treatment. Decay of tomato fruits reduced concurrently with increasing of POD and CAT activities. Polyphenol oxidase (PPO) activity in all hot water treatments decreased when compared to control and slightly increased at the end of storage period. Hot water treatment did not alter qualities of tomato fruits in terms of total soluble solids, titratable acidity and ascorbic acid. From the study, hot water treatment of tomato fruits at 40°C for 10 minutes before storage at 13°C was the most effective in activating antioxidant enzymes and reducing fruits decay.

Keywords: Hot water soaking, Antioxidant enzyme, Tomato

Introduction

Tomatoes (*Lycopersicon esculentum* Mill.) are one of the important crops in Northern Thailand. Some fresh tomatoes are sold to local and export markets. Fungal decay is the main cause of tomatoes postharvest loss. Although rotting may be reduced with chemical disinfection, recently many people have been concerned about the effect on food safety. So alternatives to chemical disease control techniques are needed. Hot water treatment for decay control in fruits and vegetables has been reported for many years (Lurie, 1998). Generally, the higher the temperature, the shorter the period of exposure required. The temperatures most commonly employed are 38 to 50°C (Aborisade and Akomolafe, 2007). Zoran *et al*. (2001) tested the effectiveness of hot water treatment and reported that rinsing fresh harvested tomato at 52°C for 15 s while brushing significantly reduced decay development after storage and marketing simulation. Tohamy *et al.* (2004) found that dipping tomato fruits in hot water at 55°C for 7 min prevented decay development in tomato fruits up to 15 days when stored at 20°C. However, heat may induce oxidative stress in plant tissues, which produces reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide. Plants contain oxidative enzymes, such as peroxidase, polyphenol oxidase and catalase that can remove these radicals (Kangasiärvi *et al*., 1994). Increased synthesis of antioxidant enzymes can improve tolerance of oxidative stress and acclimatize plants to subsequent stressors, such as chilling temperature or pathogenic attack. Ummarat *et al*. (2011) reported that hot water treatment at 50°C for 10 minutes has led to an induction of antioxidants in banana fruits as indicated by an increase of antioxidants and a decrease of H_2O_2 during ripening, and all of which result in a delayed ripening of banana fruit. It was reported that heating Fortune mandarins at 37°C for 3 days induced 2.5-, 1.4-, and 1.2-fold increases in the activities of catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX), respectively, and that the differences in the activities were maintained during cold storage. They suggested that the effectiveness of conditioning treatments is related to induction and subsequent maintenance of antioxidant status of tissues. (Sala and Lafuente, 1999).

The objectives of this work were to examine the effects of hot water treatment on the activity of some antioxidant enzymes (CAT, PPO and POD) and some quality parameters

such as total soluble solids, titratable acidity and ascorbic acid content in the tomato fruits stored at 13°C.

Materials and methods

Tomato plants ("Seeda" variety) were field grown in a commercial orchard in Chiang Mai, Thailand. The seeds were sowed in greenhouse in December, 2013, transplanted to outdoor fields in January, 2014 and hand harvested at mature-green stage in March, 2014. Before any commercial postharvest treatment was applied, the fruits were selected by hand and transported to the laboratory at Lampang Rajabhat University within 3 hours. The surface of the fruits was washed with tap water and dried for 3 hours at room temperature. A water bath filled with ten liters of tap water was used to submerge 40 fruits at a time. The temperature was monitored automatically. Fruits were then soaked in hot water for 10 minutes at 35, 40, 45 or 50°C. Water was changed for each treatment to avoid any possible contamination. Fruits were then stored at 13°C for 20 days. Untreated fruits were soaking with tap water (22°C).

For enzymatic analysis, tissue samples of tomato pericarp (0.5 g) were homogenized with 0.1 M sodium phosphate buffer (pH 7.0) containing 3 mM EDTA and 0.1 g of polyvinylpyrrolidone. The homogenate was centrifuged at 12,000 xg for 20 min at 4 °C. The supernatant was analyzed for activity of PPO, POD and CAT immediately. PPO activity was assayed spectrophotometrically at 420 nm using cathecol as a substrate. The reaction mixture contained 2.45 mL of substrate buffer (containing 0.2 M cathecol in 0.10 M sodium phosphate buffer) and 0.05 mL of enzyme extract. The blank sample contained the same mixture solution without the enzyme extract. Changes in the absorbance at 420 nm were measured after 5 min at 25°C. One unit of enzyme activity was defined as the amount causing a change of 0.01 in absorbance capacity per minute (Flurkey and Jen, 1978). POD activity was determined spectrophotometrically at 470 nm according to a little modified method of Yahia *et al.* (2007). The reaction medium contained 2.855 mL sodium phosphate buffer (0.10 M, pH 7.0), 45 µL guaiacol (1%), 40 µL H_2O_2 (0.3%), and 60 µL enzyme extract. The absorbance was recorded at 470 nm and one unit of enzyme activity was defined as the amount causing a change of 0.01 in absorbance capacity per min. CAT activity was determined at 25^{o}C according to Aebi (1984). The reaction mixture contained 40 mM H_2O_2 in a 50 mM phosphate buffer pH 7.0, and 0.1 mL of crude enzyme in a total volume of 3 mL. The activity was estimated by decreased in absorbance of H_2O_2 at 240 nm. The specific activity of enzyme was expressed as units mg protein^{-1}, soluble protein content was determined according to the method of Lowry *et al.* (1951).

The fruits were inspected every four days for disease incidence. Tomato fruits showing soft, watery, decolorized spots on the peels were considered as infected fruits. Fruit disease incidence was expressed as percentage of infected fruits. Fruit firmness was determined at middle positions of fruit by an Effigi pressure tester with a 0.79 cm diameter puncture head. The puncture head was inserted to a depth of 0.5 cm on opposite side of each fruit. Firmness of fruit was calculated and the value was expressed as Kg cm^{-2}. For total soluble solids (TSS) and titratable acidity, a juice sample was taken from each of three fruits of the three replications in each treatment. TSS was measured by a digital refractometer (ATAGO PAL-1, Japan) and expressed as °Brix. Titratable acidity (TA) was determined by the titrimetric method, involving the titration of fruit juice with 0.1M sodium hydroxide, and the formation of pink precipitate was monitored using phenolphthalein. The results were calculated as equivalents of malic acid, which is the main organic acid in tomato fruits. Ascorbic acid content was determined by the 2,6-dichloroindolephenol titrimetric method. Ascorbic acid reduced indicator dye, 2,6-dichloroindophenol, to a colorless solution. At the end point, excess unreduced dye was rose pink in acid solution.

Statistical analysis

Statistical analyses were performed with The Statistical Package for the Social Science (SPSS) software. The data were analyzed by one-way analysis of variance (ANOVA), and the means were compared by the least significant difference (LSD) test at a significance level of 0.05.

Results and discussion

Effects of hot water soaking on antioxidant enzyme activities

Exposing fruit to hot water influenced POD and CAT activities (Fig.1 and 2). The effectiveness of enzyme activation was related with the temperature of water soaking; as temperature got higher, enzyme activities increased, except for the 50°C treatment. For POD, the activity in 50°C treatment decreased significantly after soaking for 10 minutes. Rapid decreased in activity in high temperature might be due to the denatured of disulfide bond in the active site or in three dimensional conformation of the enzyme (Saeidian and Ghasemifar, 2013). POD activity in hot water treatment fruits, especially in the 35 and 40 °C treatments, showed a statistically increase at the beginning of the storage, reaching the highest level after 4 days of storage period and then decreased thereon rapidly. Lurie *et al*. (1997) also found that during the heat treatment (38°C for 3 days), peroxidase activity was higher than in tomato fruits held at 20°C, and new peroxidase isoforms appeared. These isoforms disappeared when the tomato fruits were removed to 20°C and the fruits became sensitive to fungal infection. The finding indicated that peroxidases may be involved in the resistance of heated tomatoes to pathogen infection. CAT reached the maximum activity on day 8. The activity of CAT in 35 and 40°C treatments maintain statistically higher level than other treatments throughout storage periods. Sala and Lafuente (1999) conditioned Fortune mandarins at 37°C for 3 days also found the induction of catalase (CAT) activities about 2.5 fold increases during cold storage. CAT plays a key role in maintaining H_2O_2 homeostasis in plant cells. The enzyme is important in the removal of H_2O_2 generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration and purine catabolism (Gill and Tuteja, 2010). The ability of fruits to maintain high CAT activity may be an important role in the defense mechanism of the fruit against stressors. Magbanua *et al.* (2007) found a correlation between resistance of maize to the *Aspergillus flavus* infection and the level of CAT activity. From the study, PPO activity in all hot water treatments tended to decreased when compared to control and slightly increased at the end of storage period (Fig. 3). PPO is a heat labile enzyme (Kayani *et al.*, 2011). A study of temperature effect on PPO activity of litchi pericarp pointed out that PPO activity was highest at 20ºC and then decreased gradually at 40 and 50ºC and was fully inactivated at 60ºC. (Mizobutsi *et al*., 2010)

Effects of hot water soaking on fruit decay and some quality parameters

Hot water soaking at 40°C for 10 minutes significantly delayed the decay percentage in tomato fruits stored at 13°C (Table 1). Twenty days after storage, the fruits exposed to tap water (22°C) or hot water at 35, 40, 45 or 50 °C had disease incidence of 85.5, 60.3, 45.2, 59.6 or 100%, respectively. Fallik *et al*. (1993) also found that holding inoculated tomato fruits for 3 days at 38°C completely inhibited decay caused by *Botrytis cinerea*, one of the main postharvest pathogens of tomato fruits in Israel. Oladele and Olayinka (2011) reported the effectiveness of hot water treatment at 45°C for 3 minutes in reducing decay and maintaining the firmness of tomato fruits stored at room temperature.

Throughout 20 days of storage, fruit weight loss in almost treatments was less than 5%, except for the 50°C treatment which had 8.6 % weight loss (Table 1). Firmness of the fruits in 45 and 50°C treatment decreased rapidly after 12 days of the storage (data not shown), implied that the fruits had a slight heat injury. The mold attack soft areas of the peel

and appeared as a soft, watery, decolorized spot on the rind causing the fruit to collapse eventually. Total soluble solids, titratable acidity and ascorbic acid content in fruits treated by hot water were not statistically different among treatments. In accordance with Paull and Chen (2000) whom reviewed that heat treatments, 38-48°C for 1 h to 3 days, have no effect on soluble solids or acidity in tomato fruits. However, after storage for longer time periods, it was found that the content of titratable acidity and ascorbic acid decreased. The observed decrease may be due to the fact that the acids are used as substrate for respiration (Nagar, 1994) and as an antioxidant to scavenge free radicals that occurred from various activities of the cells (Dalton, 1995).

Conclusion

The experiment indicated that storage life of tomato fruits could be prolonged by soaking in hot water (40°C for 10 minutes) before storage at 13°C. The increasing of peroxidase (POD) and catalase (CAT) activities in the fruits possibly due to the defense response of fruits against the effect of high temperature. Throughout the 20 days of the experiment, the qualities of tomato fruits in terms of total soluble solids, titratable acidity and ascorbic acid were not significantly affected by hot water treatment.

Acknowledgements

I would like to thank the Faculty of Science, Lampang Rajabhat University, Lampang, Thailand, 52100 for supporting grant of the study.

References

Aborisade, A.T. and Akomolafe, O.M. 2007. Effect of heat treatment of plantain (*Musa paradisiacal*) fruits on peel characteristics and control of decay by *Fusarium verticilioides*. Asian Journal of Plant Sciences 6: 523-527.

Aebi, H. 1984. Catalase *in vitro*. Methods in Enzymology 105: 121-126.

Dalton, D.A. 1995. Antioxidant defenses of plants and fungi. pp. 298-342. In: S. Ahmad (Ed.), Oxidative Stress and Antioxidant Defenses in Biology. Chapman&Hall, New York.

Fallik, E., Klein, J.D., Grinberg, S., Lomaniec, E., Lurie, S. and Lalazar, A., 1993. Effect of postharvest heat treatment of tomatoes on fruit ripening and decay caused by *Botrytis cinerea*. Plant Disease 77: 985 – 988.

Flurkey, W.H. and Jen, J.J. 1978. Peroxidase and polyphenol oxidase activities in developing peaches. Journal of Food Science 43: 1826-1831.

Gill, S.S. and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48: 909-930.

Kangasjärvi, J., Talvinen J., Utriainen, M. and Karjalainen, R.1994. Plant defence systems induced by ozone. Plant, Cell & Environment 17: 783-794.

Kayani, W.K., Majid, S.A., Mahmood, T., Naqvi, S.M.S. and Waheed, A. 2011. Effect of temperature stress on polyphenol oxidase activity in grains of some wheat cultivars. Pakistan Journal of Botany 43(2): 1011-1020.

Lowry, O.H., Rosebrough, N.J., Far, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193: 265-275.

Lurie, S., Fallik, E., Handros, A. and Shapira, R. 1997. The possible involvement of peroxidase in resistance to *Botrytis cinerea* in heat treated tomato fruit. Physiological and Molecular Plant Pathology 3: 141-149.

Lurie, S. 1998. Postharvest heat treatments of horticultural crops. Horticultural Reviews 22: 91-121.

Magbanua, Z.V., De Moraes, C.M., Brooks, T.D., Williams, W.P. and Luthe, D.S. 2007. Is catalase activity one of the factors associated with maize resistance to *Aspergillus flavus*?. Molecular Plant-Microbe Interactions 20: 697-706.

Mizobutsi, G.P., Finger F.L., Ribeiro R.A, Puschmann, R., Neves, L.L. and Mota W.F. 2010. Effect of pH and temperature on peroxidase and polyphenoloxidase activities of litchi pericarp. Scientia Agricola (Piracicaba, Brazil) 67(2): 213-217.

Nagar, P.K. 1994. Physiological and biochemical studies during fruit ripening in litchi (*Litchi chinensis* Sonn.). Postharvest Biology and Technology 4: 225-234.

Oladele, O.O. and Olayinka, J.O. 2011. Quality of ripe tomato (*Lycopersicon esculentum*) following hot water treatment. Annals of Science and Biotechnology 2: 36-39.

Paull, R.E. and Chen, N.J. 2000. Heat treatment and fruit ripening. Postharvest Biology and Technology 21: 21-37.

Saeidian, S and Ghasemifar, E. 2013. Effect of temperature on guaiacol peroxidase of *Pyrus communis*. International Letters of Natural Sciences 5: 46-51.

Sala J.M. and Lafuente M.T. 1999 Catalase in the heat-induced chilling tolerance of cold-stored hybrid Fortune mandarin fruits. Journal of Agricultural and Food Chemistry 47(6): 2410-2414.

Tohamy, M.R.A., Helal, G.A., Ibrahim, K.I. and Shadia A.A. 2004. Control of postharvest tomato fruit rots: II Using heat treatments. Egyptian Journal of Phytopathology 32: 129-138.

Ummarat, N., Matsumotoc, T.K., Wall, M.M. and Seraypheap, K. 2011. Changes in antioxidants and fruit quality in hot water-treated 'Hom Thong' banana fruit during storage. Scientia Horticulturae 130: 801–807.

Yahia, E.M., Soto-Zamora, G., Brecht, J.K. and Gardea, K. 2007. Postharvest hot air treatment effect on the antioxidant system in stored mature-green tomatoes. Postharvest Biology and Technology 44: 107-115.

Zoran, I., Yulia, P., Sharon, A., Azica, C. and Elazar, F. 2001. A short prestorage hot water rinse and brushing reduces decay development in tomato, while maintaining its quality. Tropical Agricultural Research and Extension 4: 1-5.

Table 1 Fruits decay and some quality parameters of tomatoes after soaking in various temperatures of hot water and then stored at 13 °C for 20 days.

Hot water soaking (°C)	Fruit decay (%)	Weight loss (%)	Firmness (Kg. cm^{-2})	Total soluble solids (°Brix)	Titratable acidity (% malic acid)	Ascorbic acid (mg. 100 ml^{-1})
Tap water (22°C)	85.5^{b}	4.7^{b}	1.24^{b}	4.2^{a}	11.0^{a}	24.2^{a}
35	60.3^{c}	3.8^{b}	1.83^{a}	4.0^{a}	10.4^{a}	23.8^{a}
40	45.2^{d}	4.5^{b}	1.65^{a}	4.0^{a}	9.7^{a}	25.6^{a}
45	59.6^{c}	4.3^{b}	1.05^{b}	4.3^{a}	9.0^{a}	24.8^{a}
50	100.0^{a}	8.6^{a}	0.91^{b}	4.0^{a}	10.7^{a}	23.6^{a}

Means with difference letters within the same column represent significant differences at $P<0.05$

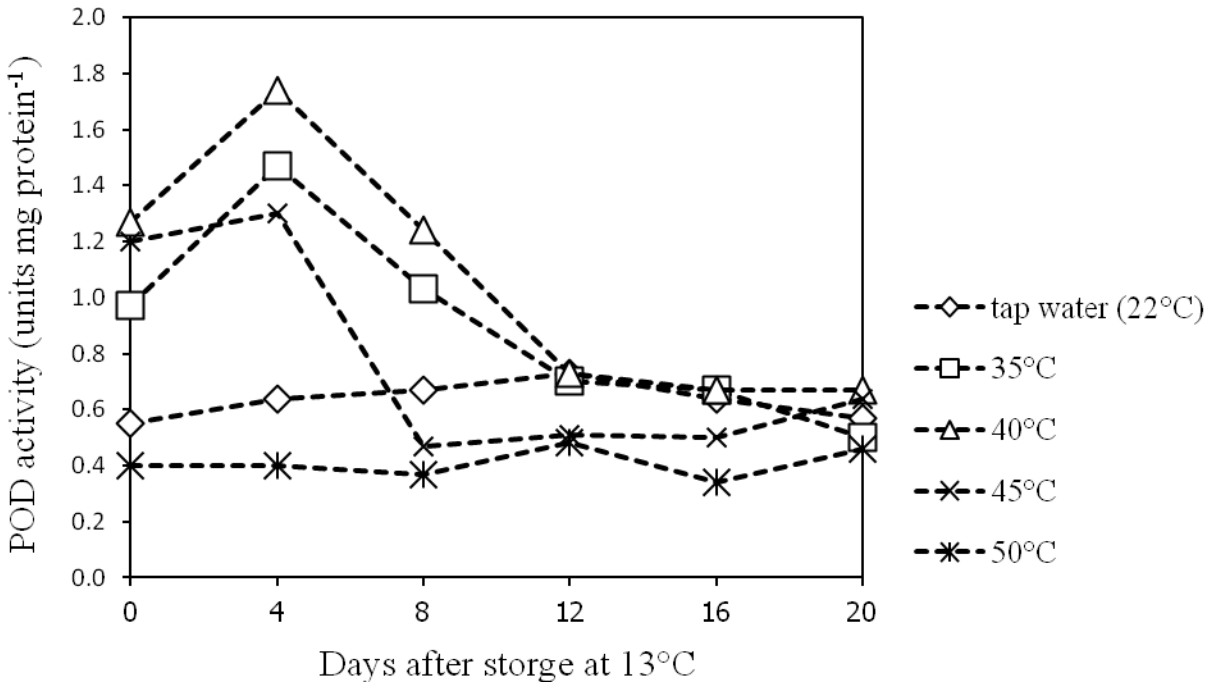

Figure 1 Peroxidase activity of tomatoes after soaking in various temperatures of hot water and then stored at 13 °C for 20 days (Note that the value on day 0 was measured after soaking treatment).

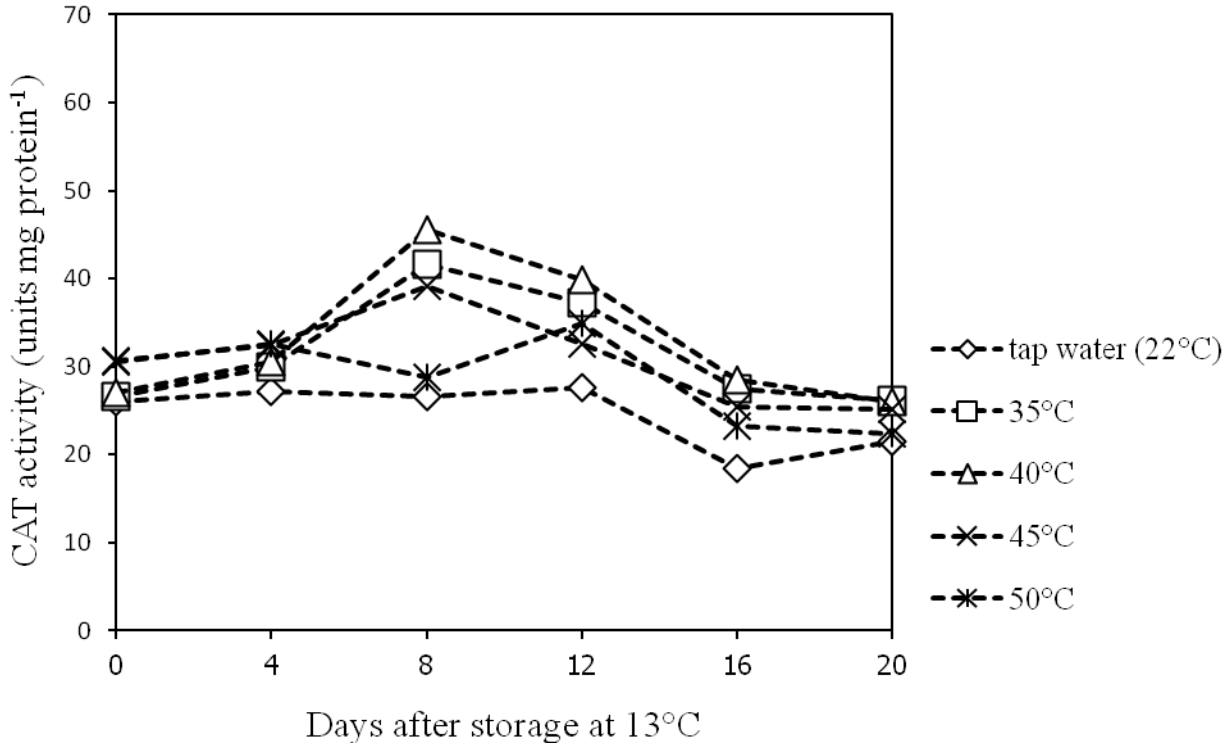

Figure 2 Catalase activity of tomatoes after soaking in various temperatures of hot water and then stored at 13 °C for 20 days (Note that the value on day 0 was measured after soaking treatment).

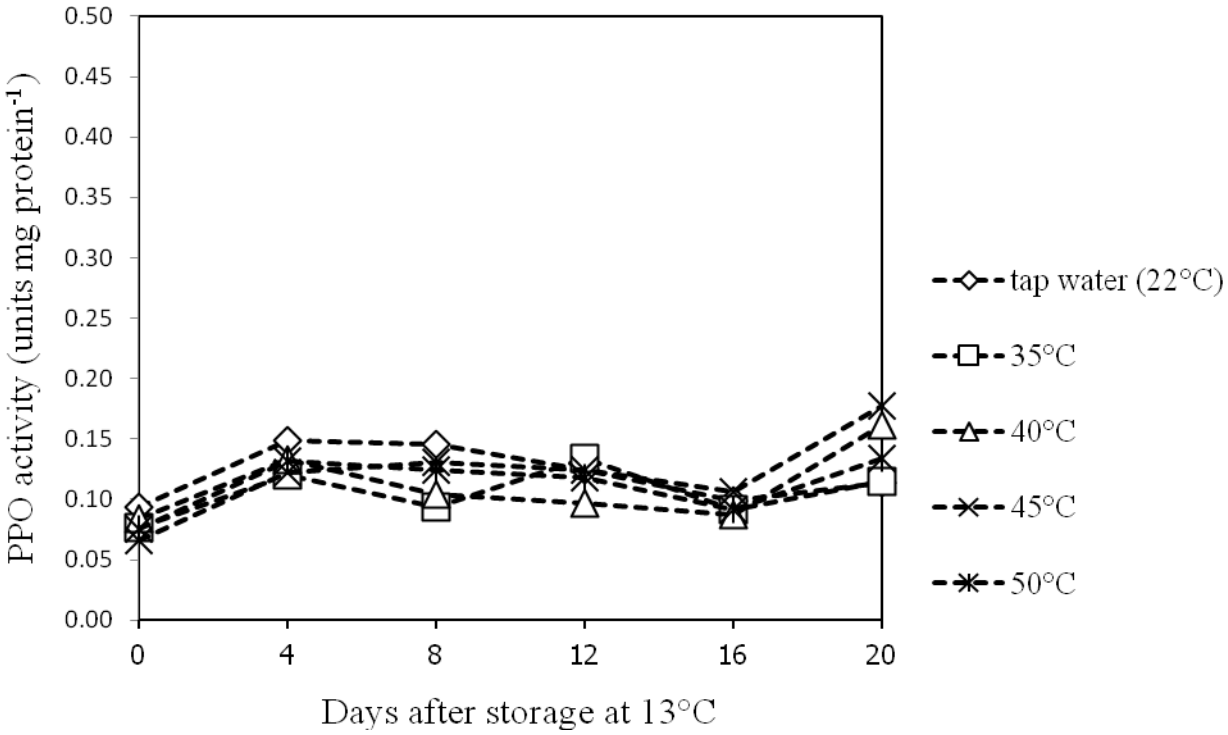

Figure 3 Polyphenol oxidase activity of tomatoes after soaking in various temperatures of hot water and then stored at 13 °C for 20 days (Note that the value on day 0 was measured after soaking treatment).