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Effect of Hot Water Dipping and Polyamines on Activity of Ripening Enhancer Enzymes during Storage of Ber

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A B S T R A C T

Effect of hot water dipping and polyamines on activity of ripening enhancer enzymes during storage of ber was carried out in the Department of Horticulture, Rajasthan College of Agriculture, Udaipur. Experiment was conducted from January 2013 to April 2013. Experiment consisted of 9 treatment combinations of water dipping at 20, 35 and 45ºC and polyamine treatments (Spm, Spd and Put) at 1mM L^{-1} concentrations. The uniform sized fully matured but unripe fruits of ber cv. 'Gola' at color turning stage were used for treatments and treated fruits stored at 6ºC temperature. These treatment combinations were evaluated under factorial completely randomized design with three replications. The stored fruit examined at 7 days interval up to 35 days for various change in ripening associate enzyme activity and chilling injury index. The activities of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5), polyphenol oxidase (PPO, EC 1.10.3.1) and polygalacturonase (PG, EC 3.2.1.15) were analyzed during the storage. It was observed that 45°C hot water + putrescine (1 mM L^{-1}) treatment combination was found to be better in maintaining desirable enzymatic activity than other treatment combinations. The chilling injury was also minimum in the treatment combination 45°C hot water + putrescine (1 mM L^{-1}). In dipping for 5 min at 45°C hot water with putrescine 1 mM L^{-1}, the maximum or minimum activity of PPO, PAL and PG were found in Gola ber fruit to be better than other treatment combinations during the storage at 6°C. During the storage, the activity of PPO, PAL and PG were increased during storage at 6°C for 35 day. Finally this study indicates that ber fruit can be stored at 6ºC for 35 days with using W_3 P_3 (45°C hot water + putrescine 1 mM L^{-1}) treatment combination by maintaining ripening associated enzymatic activity and minimum chilling injury index.

Introduction

Ber (*Ziziphus mauritiana* Lamk.) is one of the important minor fruits of India. It belongs to the family Rhamnaceae and is native to central Asia (Morton, 1987). It is being considered as poor man's apple due to high nutritive value. Rajasthan is one of the leading state of India in ber production with 28,800 tonnes of fruits from the acreage of 3,200 hectares (Anon., 2009). However, in an estimate *Z. mauritiana, Z. nummularia* and *Z. rotundifolia* covers an area of 20,000 hectare in Rajasthan (Pareek *et al.*, 2009a). Nutritionally, ber fruit is widely acclaimed for its rich source of ascorbic acid (70-165 mg 100 g^{-1}) (Bal and Mann, 1978). Apart from this, it is a good source of essential minerals

like Ca, P and Fe (Pareek, 1983). Pulp contains 12.8-13.6 per cent carbohydrates (Jawanda *et al.,* 1981). 'Gola' ber contains 20.10 per cent TSS, 0.34 per cent acidity, 160.56 mg 100 g^{-1} ascorbic acid and 6.97 per cent total sugars (Pareek *et al.,* 2002). Extensive studies have been carried out to prepare various processed products from ber fruit such as candy, preserve, dehydrated products including osmo-dehydrated products, jam, jelly, juice, squash and pickle (Pareek and Yahia, 2013). The previous study carried out in this laboratory on storage temperatures of 'Gola' ber fruit showed that shelf life can be increased at lower temperatures of 6^0C, but chilling injury (CI) limits the quality of fruit (Jat *et al.,* 2013).

Polyamines are organic cations containing amino groups that are present in all eukaryotic cells and intimately involved in, and required for, distinct biological functions. An increasing body of evidence indicates that the regulation of the cellular PAs is a central convergence point for the multiple signaling pathways driving various cellular functions. Over the last decade, considerable progress has been made in understanding the molecular functions of cellular PAs (Wang and Casero, 2006). In plant organs, PAs are positively implicated in plant growth and differentiation as well as in stress responses. In plant tissues, the PAs are putrescine (1, 4-diaminobutane), spermidine (*N*-3-aminopropyl-1, 4-diaminobutane) and spermine [bis (N-3-aminopropyl-1, 4-diaminobutane)]. The three PAs are present ubiquitously as polycationic compounds and are found in significant amounts in cell types to support a wide variety of cellular functions (Wang and Casero, 2006). PAs are potent inhibitors of many senescence-related processes in a variety of plant species and their relative effectiveness as antisenescent agents corresponds to the number of positive charges per molecule; Spm (triamine), which is more effective than Put (diamine) (Galston and Kaur-Sawhney, 1987). Much of this antisenescent activity may be membrane related (Ballas *et al.,* 1983) and this interaction serves to stabilize the bilayer surface and may thus retard membrane deterioration. PAs also have free-radical-scavenging properties (Drolet *et al.,* 1986). Protection of membranes from peroxidation by PAs could involve both their ability to interact with phospholipids and their antioxidant activity. Given the relationship between PAs and membrane protection, and between CI and membrane damage, the possible connection between PAs and CI is of great interest.

Pre-storage hot water dipping (HWD) of fruit has been investigated as a way of enhancing fruit resistance to CI (Lurie, 1998). A 38^oC postharvest heat treatment can inhibit ripening of tomato fruit (Lurie *et al.,* 1996) by inhibiting the synthesis of the enzymes involved in the ripening processes, including those involved in ethylene synthesis and fruit softening. This inhibition is removed once the temperature is lowered. In addition, a postharvest heat treatment applied prior to low temperature storage can reduce the incidence of CI in cold sensitive fruits, such as mango (McCollum *et al.,* 1993) and persimmon (Lay-Yee *et al.,* 1997).

Post-harvest application of PAs, by vacuum or immersion infiltration, has been reported to delay fruit ripening and extend shelf life in fruits. Keeping these in view the present experiment was carried out.

Materials and Methods

The experiment was conducted from January 2013 to April 2013. The uniform sized fully matured but unripe fruits of ber cv. 'Gola' at colour turning stage were obtained from Instructional Farm of Krishi Vigyan Kendra,

SK Rajasthan Agricultural University, Beechwal, Bikaner and brought to the Post Harvest Technology Laboratory of the Department on the next day. Ber fruits were inspected thoroughly for any damage and spoilage. The immature, over mature, spotted and off type fruits were discarded. The selected fruits were thoroughly washed with tap water to remove dirt and dust particles adhering to the surface of fruits. Then fruits are again washed with chlorinated water and allowed to shade dry.

The nine treatment combinations of hot water and polyamines concentration were used to treat the fruits. The treatment applied were: (1) Dipped at 20ºC hot water + Spermidine (1mM L^{-1}) for 5 minutes (W_1P_1); (2) Dipped at 20ºC hot water + Spermine (1mM L^{-1}) for 5 minutes (W_1P_2); (3) Dipped at 20ºC hot water + Putrescine (1mM L^{-1}) for 5 minutes (W_1P_3); (4) Dipped at 35ºC hot water + Spermidine (1mM L^{-1}) for 5 minutes (W_2P_1); (5) Dipped at 35ºC hot water + Spermine (1mM L^{-1}) for 5 minutes (W_2P_2); (6) Dipped at 35ºC hot water + Putrescine (1mM L^{-1}) for 5 minutes (W_2P_3); (7) Dipped at 45ºC hot water + Spermidine (1mM L^{-1}) for 5 minutes (W_3P_1); (8) Dipped at 45ºC hot water + Spermine (1mM L^{-1}) for 5 minutes (W_3P_2); (9) Dipped at 45ºC hot water + Putrescine (1mM L^{-1}) for 5 minutes (W_3P_3). Therefore, total 9 treatment combinations were used in this experiment. The treated fruits were stored at 6ºC temperature in cold storage.

Methodology used for observations

After applying treatments, the subsequent observations on enzymatic activity and chilling injury index were recorded at 7 days interval. The following observations were recorded during the course of investigation. Polygalacturonase (PG) (EC. 3.2.1.15) was extracted by the method of Zainon and Brady (1982) with slight modifications, Extraction and assay of Polyphenol oxidase (PPO) (EC. 1.14.18.1) was carried out as described by Matta and Dimond (1963), Extraction and assay of *Phenylalanine ammonia lyase* (PAL) (EC. 4.3.1.5) was carried out as described by Rao and Towers (1970) and Chilling injury index was determined with a five point hedonic scale based on the surface area of fruit affected by water soaked lesions, pitting and skin discolouration (Gonzalez-Aguilar *et al.,* 1997).

Results and Discussion

Polygalacturonase (PG)

Put at 1.0 mM concentrations was the most effective among all three polyamines treatments. PAs are able to bind negatively charged molecules like pectic polysaccharides (Bagni and Pistochi, 1990). The formation of pectin–PAs complexes in the cell wall would make pectin less accessible to PG attack. PAs also inhibits biosynthesis of ethylene in plants (Flores *et al.,* 1990) and the activation of transcription of the PG gene occurs after ethylene synthesis (Seymur *et al.,* 1993).

Delayed fruit softening in Put-treated fruit may be ascribed to the reduction in the activities of fruit softening enzymes such as PG. It has been reported that PG enzyme is primarily responsible for ripening associated pectin degradation and fruit softening (Brady, 1987). PME catalyses the softening process through desertification of pectin followed by pectin depolymerisation, catalysed by PG (Roe and Bruemmer, 1981). The lowest PG activity in treatment combinations was recorded in hot water at 45ºC for 5 minutes + putrecsine (1 mM L^{-1}) and maximum in water treatment at 20^0C water for 5 minutes + spermidine (1 mM L^{-1}) (Table 1). The PG activity increased apparently during storage period from 1 to 35 days in all the treatment studied.

Polyphenol oxidase (PPO)

The PPO activity of ber fruits increased during storage and their values were significantly lowered by 45ºC hot water and putrecsine treatment and their combination (Table 2). Hot water treatment at 45ºC was found to be most effective over other hot water treatments to prevent the increase in PPO activity. Hot water treatment for 10 min at 35°C markedly lowered the PPO activity of jujube fruit (Promyou *et al.*, 2012). The heat treatment effectively reduced the membrane damage and skin darkening of jujube fruit under chilling storage. Promyou *et al.*, (2008) reported that the application of hot water treatment reduced the activity of PPO in 'Gros Michel' and 'Namwa' banana and a reduction in gene encoding of PPO was detected in the hot-water-treated fruit.

PAs treatments alone and combined with hot water treatments was significantly inhibited the activity of PPO.

Table.1 Interaction effect of hot water and polyamine treatments on polygalacturonase activity (μg g^{-1}) during storage

Treatment combination	Storage day				
	7	**14**	**21**	**28**	**35**
W_1P_1	192.60	193.30	197.90	202.40	225.20
W_1P_2	187.30	187.50	189.50	189.70	198.20
W_1P_3	138.40	180.60	183.10	187.10	192.66
W_2P_1	137.20	164.15	179.86	180.40	180.96
W_2P_2	129.60	135.70	159.30	167.80	177.60
W_2P_3	106.60	128.80	135.60	150.30	151.30
W_3P_1	85.90	96.60	97.40	125.70	145.40
W_3P_2	84.90	89.40	95.40	102.30	110.60
W_3P_3	76.50	79.90	82.40	87.60	89.70
SEm ±	1.87	2.10	2.27	2.50	2.63
CD ($P \leq 0.05$)	5.56	6.25	6.73	7.42	7.82

Table.2 Interaction effect of hot water and polyamine treatments on polyphenol oxidase (PPO) activity (OD at 400 nm) during storage

Treatment combination	Storage day				
	7	**14**	**21**	**28**	**35**
W_1P_1	0.206	0.215	0.219	0.226	0.245
W_1P_2	0.164	0.175	0.208	0.210	0.211
W_1P_3	0.125	0.134	0.157	0.167	0.186
W_2P_1	0.093	0.098	0.154	0.159	0.172
W_2P_2	0.092	0.093	0.145	0.158	0.163
W_2P_3	0.081	0.083	0.113	0.152	0.156
W_3P_1	0.069	0.070	0.112	0.113	0.128
W_3P_2	0.065	0.067	0.109	0.114	0.126
W_3P_3	0.064	0.066	0.072	0.105	0.124
SEm ±	0.002	0.003	0.002	0.003	0.002
CD ($P \leq 0.05$)	0.005	0.008	0.007	0.009	0.007

Table.3 Interaction effect of hot water and polyamine treatments on *phenylalanine ammonia lyase (PAL)* activity (µ Moles) during storage

Treatment combination	Storage day				
	7	14	21	28	35
W_1P_1	12.46	14.60	14.89	14.96	15.67
W_1P_2	10.43	10.68	12.34	12.63	13.66
W_1P_3	9.92	10.23	12.23	12.60	13.00
W_2P_1	9.43	10.13	12.07	12.55	12.99
W_2P_2	9.26	10.12	10.37	11.99	12.30
W_2P_3	8.86	9.97	10.36	11.25	12.12
W_3P_1	8.79	9.85	10.36	10.95	11.97
W_3P_2	8.74	9.77	10.30	10.80	11.38
W_3P_3	8.62	9.40	9.66	10.13	11.08
SEm ±	0.15	0.17	0.19	0.19	0.21
CD ($P \leq 0.05$)	0.45	0.51	0.56	0.57	0.61

Table.4 Interaction effect of hot water and polyamine treatments on chilling injury index (five point hedonic scale) during storage

Treatment combination	Storage day				
	7	14	21	28	35
W_1P_1	--	--	0.43	1.07	1.34
W_1P_2	--	--	0.40	0.98	1.28
W_1P_3	--	--	0.00	0.73	1.00
W_2P_1	--	--	0.00	0.70	0.95
W_2P_2	--	--	0.00	0.51	0.82
W_2P_3	--	--	0.00	0.43	0.60
W_3P_1	--	--	0.00	0.40	0.54
W_3P_2	--	--	0.00	0.31	0.44
W_3P_3	--	--	0.00	0.08	0.10
SEm ±	--	--	0.02	0.01	0.01
CD ($P \leq 0.05$)	--	--	0.06	0.02	0.04

However, at heat treatment conditions of 60ºC, 60 min, there was a significant decrease in PPO activity (Ciou *et al.*, 2011). Dogan and Dogan (2004) reported that optimum temperature for PPO was within the range of 25–45ºC, respectively, depending upon the substrate and pH-value.

Dogan and Dogan (2004) reported that high temperatures (70ºC) and long heating times with various substrates significantly decreased PPO activity.

Phenylalanine Ammonia Lyase (PAL)

PAL activity increased irrespective of treatments in the storage duration. As shown in (Table 3), PAL activity was significantly lowers ($p < 0.05$) in fruits treated with 45ºC hot water and Put 1 mM L^{-1} than other water treatments (20ºC and 35ºC) and PAs (Spd and Spm) treatment combinations on all the storage days. In this study, PAL activity was lower in both hot water and PAs treatments. This suggests that the activity of PAL might

be associated with chilling tolerance in ber fruits. There are few reports on the relationship between PAL activity and heat induced chilling tolerance in harvested fruit (Martinez–Tellez and Lafuente, 1997). PAL activity can be induced by various stresses, such as chilling (Lafuente *et al.,* 2003), wounding (Campos-Vargas *et al.,* 2005) and plant harmone including ethylene, jasmonic acid and salicylic acid (Campos-Vargas and Saltveit, 2002).

Chilling Injury Index (CII)

Chilling injury symptoms were seen in 35 and 45ºC hot water treated fruits after 28 days of storage whereas it was appeared slightly earlier in fruits dipped in 20ºC hot water *i.e.*, on 21st day of storage (Table 4). The symptoms characterized by the purplish colour on fruit skin, water soaked lesions, pitting in advance stage and uneven ripening. Chilling injury index (CII) increased sharply after 21 days of storage. Pre storage heat treatments (both hot air and hot water treatments) are widely accepted as effective in the control of decay and insect activity in fresh commodities (Lurie, 1998). In addition, heat treatments have been reported as a potential method to reduce chilling injury in persimmon (Lay-Yee *et al.,* 1997) and pomegranate (Mirdehghan and Rahemi, 2005). González- Aguilar *et al.,* (2000) reported that the reduction in chilling injury of hot-water-treated pepper fruit was clearly related to the high levels of polyamines. Moreover, the application of heat treatments to reduce CI in fruit has been reported for 'Valencia' oranges (Bassal and El-Hamahmy, 2011) and pomegranate (Mirdehghan and Rahemi, 2005).

This study indicates that 'Gola' ber fruit can be stored at 6ºC for 35 days with using W_3 P_3 (45°C hot water + putrescine 1 mM L^{-1}) treatment combination (dipping of fruits for 5 min) and maintains ripening associated enzymatic activity with minimum chilling injury index. This standardized storage technology has promising future for technology utilization by small, medium and large scale processors and entrepreneurs.

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