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Hukam Raj Saini
M.Sc Student Department of Horticulture, Rajasthan College of Agriculture MPUA&T, Udaipur, Rajasthan, India

Sunil Pareek
Senior Scientist, Department of Horticulture, Rajasthan College of Agriculture, MPUA&T, Udaipur, Rajasthan, India

DK Sarolia
Senior Scientist, Department of Horticulture, Rajasthan College of Agriculture MPUA&T, Udaipur, Rajasthan, India

Mukesh Nagar
SRF, Department of Horticulture, Rajasthan College of Agriculture MPUA&T, Udaipur, Rajasthan, India

Correspondence
Hukam Raj Saini
M.Sc Student Department of Horticulture, Rajasthan College of Agriculture MPUA&T, Udaipur, Rajasthan, India

Hot water and polyamines treatment effect on ripening decrease enzymes of ber fruit during storage

Hukam Raj Saini, Sunil Pareek, DK Sarolia and Mukesh Nagar

Abstract
Effect of hot water dipping and polyamines on activity of ripening decrease 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 superoxide dismutase (SOD, EC 1.15.1.1), β-galactosidase (β-Gal, EC. 3.2.1.22), ά-amylase (EC. 3.2.1.1) and pectin-methylesterase (PME, EC 3.1.1.11) 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 PME, β-Gal, SOD and α-amylase 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 PME, β-Gal, SOD and α-amylase were decreased 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_3P_3 (45°C hot water + putrescine 1 mM L^{-1}) treatment combination by maintaining ripening associated enzymatic activity and minimum chilling injury index.

Keywords: Superoxide dismutase, pectin methyl esterase, β-galactosidase, α-amylase and chilling injury

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) [35]. 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) [2]. However, in an estimate *Z. mauritiana*, *Z. nummularia* and *Z. rotundifolia* covers an area of 20,000 hectare in Rajasthan (Pareek *et al*., 2009) [39]. Nutritionally, ber fruit is widely acclaimed for its rich source of ascorbic acid (70-165 mg 100 g^{-1}) (Bal and Mann, 1978) [4]. Apart from this, it is a good source of essential minerals like Ca, P and Fe (Pareek, 1983) [36]. Pulp contains 12.8-13.6 per cent carbohydrates (Jawanda *et al*., 1981) [22]. '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) [38]. 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) [37]. 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) [21].

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) [43].

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) [43]. 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) [15]. Much of this antisenescent activity may be membrane related (Ballas *et al*., 1983) [5] 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) [12]. 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.

Prestorage hot water dipping (HWD) of fruit has been investigated as a way of enhancing fruit resistance to CI (Lurie, 1998) [24, 28]. A 38ºC postharvest heat treatment can inhibit ripening of tomato fruit (Lurie *et al*., 1996) [29] 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) [32] 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 Agricultrual 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. Pectin methyl esterase (PME) (EC. 3.1.1.11) was carried out as described by Hagerman and Austin (1986), Extraction and assay of β-galactosidase (β- Gal) (EC. 3.2.1.22) was carried out as described by Biswas (1985), Extraction and assay of Superoxide dismutase (SOD) (EC. 1.15.1.1) was carried out as described by (McCord and Fridovich, 1969) [33], Extraction and assay of ά-amylase (EC. 3.2.1.1) was carried out as described by Bernfeld (1955) [7] with some modification 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) [18].

Results and Discussion

Pectin Methyl Esterase (PME)

The PME activity of ber fruits decreased during storage and their values were significantly lowered by 45ºC hot water, putrecsine and their treatment combinations. PME activity decreased irrespective of treatments in the storage duration (Table 1). Hot water treatments decreased the PME activity with the increase in temperature. The minimum PME activity in 45ºC hot water treatment was recorded while it was maximum in fruits of W_1 treatment (20ºC). In general, softening of many ripening fruit is associated with solubilization of pectic substances in the cell wall (Brummell and Labavitch, 1997) [9]. The change of solubility of pectic substances involves the action of cell wall hydrolysis enzymes such as PME and PG (Fischer and Bennett, 1991) [14]. These enzymes may have profound effects on the cohesiveness of the wall during ripening (Carrington *et al.,* 1993) [10]. PME activity decreased slowly. A negative correlation was observed between the PME activity and firmness of mei fruit (Luo, 2006) [27]. PME activity of hot water treated fruit was almost completely suppressed (Luo, 2006) [27]. This suppression in PME activity by heat trearment has also been reported in mango (Ketsa *et al.*, 1998) [24]. This suppression in PME activity may be associated with mRNA synthesis and stability, or protein synthesis and degradation (Paull and Chen, 2000) [40].

PME activity decreased gradually during storage (Table 1). PAs treatments such as (Put, Spm and Spd) could induce a decrease in PME activity, but Put treatment was more effective in decreasing PME activity than Spm and Spd treatments. In apricot, PAs applications retarded the senescence by stabilising the cell membrane and inhibiting the activities of PG and PME involved in the softening of enzymes (Martinez-Romero *et al.,* 2002) [31]. The minimum PME activity in treatment combination W_3P_3 (hot water at 45º C for 5 minutes + putrecsine) and maximum in W_1P_1 (water at 20^0 C for 5 minutes + Spermidine) was recorded (Table 4.4). The PME activity was decreased apparently during storage period from 1 to 35 days in all the treatments studied. The cell wall softening enzymes play key role in cell wall degradation and fruit softening during fruit ripening. Exogenous

applications of PAs have also been reported to maintain the fruit firmness during ripening and at low temperature storage in 'Frior', 'Black Star' and 'Santa Rosa' plum cultivars (Abu-Kpawoh *et al.,* 2002) [1].

Table 1: Interaction effect of hot water and polyamine treatments on pectin methyl esterase (PME) activity (OD at 620 nm) during storage

Treatment combination	Storage day				
	7	14	21	28	35
W_1P_1	0.470	0.290	0.265	0.231	0.220
W_1P_2	0.392	0.281	0.245	0.225	0.215
W_1P_3	0.357	0.268	0.223	0.219	0.193
W_2P_1	0.349	0.267	0.208	0.193	0.165
W_2P_2	0.319	0.262	0.192	0.171	0.143
W_2P_3	0.260	0.189	0.175	0.155	0.136
W_3P_1	0.217	0.189	0.173	0.125	0.112
W_3P_2	0.215	0.182	0.162	0.122	0.102
W_3P_3	0.186	0.165	0.095	0.094	0.092
SEm ±	0.005	0.004	0.003	0.003	0.002
CD ($P \leq 0.05$)	0.014	0.011	0.008	0.008	0.007

β-galactosidase (β-Gal)

The β-Gal activity of ber fruits decreased during cold storage and their activity were significantly induced to reduce by both hot water and polyamine treatments and their combinations (Table 2). In case of water treatments, 45ºC hot water was found to be more effective to reduce the activity of β-Gal enzyme than other treatments. Among polyamines, Put treatment (1 mM L^{-1}) was more effective than Spd and Spm treatments and also in interaction, 45ºC hot water and Put treatment combination was more effective over other treatment combinations. The β-Gal activity decreased irrespective of treatments in the storage duration. β-Gal has been studied in strawberry fruit (Martínez and Civello, 2008). It has been characterized in association with the removal of galactosyl residues from cell wall polymers during fruit softening (Gross and Sams, 1984) [19]. Indeed, de-galactosidation in response to β-Gal action has been proposed as an important process in cell wall modification during ripening (Smith and Gross, 2000) [42]. Therefore, such modifications might involve glycosidases, such as β-Gal, acting in cooperation with pectolytic enzymes (e.g., PG) in pectin metabolism during fruit ripening (Giovannoni, 2001) [16].

Table 2: Interaction effect of hot water and polyamine treatments on β-galactosidase activity (µg g^{-1}) during storage

Treatment Combination	Storage day				
	7	14	21	28	35
W_1P_1	863	843	835	816	812
W_1P_2	853	788	630	561	466
W_1P_3	813	770	598	461	375
W_2P_1	812	751	595	429	362
W_2P_2	747	670	567	416	355
W_2P_3	679	622	562	415	345
W_3P_1	667	615	476	410	335
W_3P_2	654	516	468	390	315
W_3P_3	569	449	397	354	308
SEm ±	10.81	9.86	8.15	6.29	5.39
CD ($P \leq 0.05$)	32.13	29.31	24.21	18.68	16.02

Superoxide dismutase (SOD)

The SOD activity of ber fruits decreased during cold storage and their values were significantly induced to decrease by hot water and polyamines and their treatment combinations. As shown in (Table 3), SOD activity was significantly lowers ($p \leq 0.05$) in treated fruit with 45ºC hot water and Put 1 mM L^{-1} in comparison to other treated fruits on the 7th to 35th day of cold storage. However, there was significant difference in SOD activity among the treated samples. Hot water treatment, as a temperature stress, could induce a defense reaction which might protect the plant tissue from other stresses, such as cold (Fallik, 2004) [13]. SOD is an important antioxidant enzyme, which scavenges reactive oxygen species there by maintaining membranes of plant tissue (Lamb and Dixon, 1997) [25]. The damage of membranes caused by cold stress is thought to be main cause of chilling injury (Wang, 1990). This result showed that hot water combined with PAs (Put, Spd and Spm) treatment induced to decrease the activity of SOD, which would have a membrane protecting function via enhancing the antioxidant enzymes and pathogenesis related proteins (Ding *et al.,* 2002) [11]. Thus, the maintenance of intact membranes of ber tissue by hot water and PAs treatments, as well as their combination, might be another mechanism of alleviating internal browning. The decrease of SOD activity was more significantly reduced in Spd-treated fruit compared with those in untreated stressed fruits. The drought-induced increase in superoxide radical levels was reduced in Spd-treated fruits. It was suggested that PAs were implicated in direct scavenging of free radicals, thereby reducing oxidative stress (Roberts *et al.,* 1986) [41].

Table 3: Interaction effect of hot water and polyamine treatments on superoxide dismutase (SOD) activity (Unit for 50% inhibition) during storage

Treatment Combination	Storage day				
	7	14	21	28	35
W_1P_1	1700	1670	1650	1640	1620
W_1P_2	1670	1620	1611	1550	1520
W_1P_3	1650	1600	1576	1540	1500
W_2P_1	1640	1580	1520	1500	1470
W_2P_2	1610	1500	1490	1480	1430
W_2P_3	1500	1490	1400	1390	1380
W_3P_1	1480	1380	1340	1290	1270
W_3P_2	1370	1340	1340	1280	1245
W_3P_3	1320	1310	1300	1230	1200
SEm ±	17.09	16.21	15.98	14.60	14.19
CD ($P \leq 0.05$)	50.77	48.2	46.37	43.37	41.25

α-amylase

In the present investigation, the α-amylase activity of ber fruits decreased during cold storage and their values were significantly decreased by hot water and polyamines treatment combinations (Table 4). The α-amylase activity decreased progressively in all the treatments in storage. Similarly other workers (Kadam *et al.,* 1993; Anuradha and Siddiqui, 1999) [23, 3] have also reported to decrease amylase activity with advancement of ber fruit ripening.

Table 4: Interaction effect of hot water and polyamine treatments on α-amylase activity (µg maltose / 5 min) during storage

Treatment Combination	Storage Day				
	7	14	21	28	35
W_1P_1	85.13	65.30	51.23	38.42	26.23
W_1P_2	83.21	65.21	45.32	32.55	25.63
W_1P_3	81.21	62.91	43.77	28.77	17.77
W_2P_1	79.14	62.74	41.22	27.42	15.22
W_2P_2	76.19	60.89	38.64	21.85	12.64
W_2P_3	75.25	60.14	35.16	14.21	11.43
W_3P_1	72.29	59.28	33.46	13.89	10.79
W_3P_2	67.49	55.17	32.43	11.82	10.32
W_3P_3	62.47	52.14	30.67	11.29	9.41
SEm ±	0.82	0.66	0.42	0.42	0.21
CD ($P \leq 0.05$)	2.45	1.96	1.24	1.25	0.62

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 5). 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) [24, 28]. In addition, heat treatments have been reported as a potential method to reduce chilling injury in persimmon (Lay-Yee *et al*., 1997) [26] and pomegranate (Mirdehghan and Rahemi, 2005) [34]. González- Aguilar *et al*. (2000) [17] 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) [6] and pomegranate (Mirdehghan and Rahemi, 2005) [34].

Table 5: 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

Conclusion

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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