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Exogenous application of salicylic acid regulates antioxidant enzyme activities and quality attributes of Umran ber (*Ziziphus mauritiana*) fruits under cold storage

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ABSTRACT

The present study was carried out to observe the influence of pre-harvest application of salicylic acid (1, 2 and 3 mM) on shelf life and quality attributes of ber fruits cv. Umran (*Ziziphus mauritiana* Lamk.) under cold storage conditions (7.5±1^{0}C and 90–95% RH). The cold-stored fruits were assessed for various physico-chemical attributes and enzymatic activities at 7 days interval up to 28 days of storage. Above study revealed that fruits treated with salicylic acid @3 mM resulted in reduced physiological loss in fruit weight, spoilage and increased firmness, soluble solids content, antioxidant activity and carotenoids. Furthermore, it suppressed activities of cell wall degrading enzymes like PG, PPO, PME and increased the activity of superoxide dismutase peroxidase and catalase enzymes. It was concluded that pre-harvest spray of salicylic acid @3 mM was more effective in regulating enzymatic activities, thereby improving quality attributes and shelf life of ber fruits up to 21 days at cold storage.

Keywords: Enzyme activity, Quality attributes, Salicylic acid, Shelf-life

Ber (*Ziziphus mauritiana* Lamk.) is most popular fruit crop of subtropical and arid regions of India, which belongs to family Rhamnaceae and is indigenous to India, Pakistan, Malaysia and parts of the Gulf countries (Shobha and Barathi 2007). Ber can be grown on peripheral lands, where other crops cannot be grown commercially. It is enriched with proteins, calcium, phosphorus and carotenoids (Bakhshi and Singh 1974). It is also a rich source of vitamin C and B_1 (thiamine), B_2 (riboflavin) and Vitamin P (bioflavonoid) content (Yang *et al.* 2021). Various factors like; maturity stages, fruit composition, storage conditions, storage type and packaging material affects quality and storage life of ber fruits (Kudachikar *et al.* 2000). Due to its perishable nature, it can be stored only for 4–5 days at ambient temperature.

Numerous investigations have demonstrated that salicylic acid is a phytohormone, which antagonistically affects ethylene biosynthesis and ethylene activity which also help in fruit ripening (Zhang *et al.* 2003). It is the signal molecule present in plant SAR (systemic acquired resistance) essential to ameliorate the plant protection system, which helps to extend shelf life (Bons and Sharma 2023) as well as modifies the antioxidative system of the fruit during cold storage (Wang *et al.* 2006).

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MATERIALS AND METHODS

The present study was carried out at the fruit research farm of Punjab Agricultural University, Ludhiana, Punjab (75.84°E, 30.90°N and 262.0 m amsl) during two consecutive years 2021 and 2022 on a 15-year-old Umran cultivar planted at 7.5×7.5 m spacing. The pre-harvest spray of salicylic acid (1, 2 and 3 mM) was applied twice prior to harvest. Different salicylic acid concentrations were prepared by dissolving in small amounts of ethanol and making the final volume of salicylic acid to 15 litres with water. Prepared concentrations were applied on the fruit trees at pit hardening and colour break stage. Ber fruits were harvested at full physiological mature stage, precooled, washed with clean water and dried at room temperature. Then, randomly selected fruits with uniform size and colour were wrapped and placed in corrugated fibre boxes having 5% perforation and stored under cold storage (7.5±1^{0}C and 90–95% RH). An experiment was carried out in CRD with 4 replications and periodical observations were recorded for physio-chemical attributes and enzymatic activities at 0th,7th, 14th, 21th and 28th days under cold storage conditions.

The physiological loss in weight was calculated on an initial weight basis and expressed in per cent. Fruit firmness was determined by using a penetrometer at each storage interval. Spoilage in ber fruits was recorded by counting numbers of rotten fruits at particular intervals. Fruit colour was determined by using a portable colorimeter (CR-400,

Konica Minolta, Japan). The biochemical attributes such as soluble solids content, ascorbic acid content, total phenols, antioxidant activity were recorded as methods given in (AOAC 2005). Total carotenoid was recorded using acetone (80%) as solvent as proposed by Kirk and Allen (1965). For the extraction of enzymes, i.e. Peroxidase (POD), Super dismutase (SOD), Polyphenol oxidase (PPO), and catalase, fruit pulp (100 mg) was taken from 4–5 fruits and homogenized with chilled phosphate buffer (*p*H 7.5) centrifuged at (11,000 x g) for 30 min at 4°C. Then, activity of enzymes was recorded as suggested by Zhang *et al.* (2010) by using the supernatant and expressed as U/g of FW. Polygalactouronase (PG) enzyme activity was assayed after blending ber pulp for 30 min in 0.5 Tris -HCl buffer containing 1 mM EDTA (5% PVP) after centrifugation (10,000 x g, 30 min) at 4°C. The activity of the enzyme was determined by the lucid supernatant and expressed as ug of D-galactose/g FW/min. Pectin methyl estrase (PME) was recorded by Mahadevan and Sridhar (1982). For it, 20 g ber pulp was taken and mixed with NaCl solution (80 mL) and centrifuged at (448 x g) for 30 min at 4°C. Supernatant extract was used for recording data on PME activity, expressed as µmol of methyl ester/g FW/min.

Pooled data of both years was examined by using one and two way analysis of variance and mean values were differentiated using the LSD test. Pearson's correlation was significantly analyzed among various physicochemical attributes. The regression equation between the two factors was analyzed and the P-value of ≤ 0.05 was found statistically significant for all the given treatments by using the SAS software (Version 9.3). The experimental data was displayed as mean ± standard deviation.

RESULTS AND DISCUSSION

Impact of pre-harvest application of salicylic acid on fruit firmness, sensory quality, physiological loss in weight and spoilage in ber fruits cv. Umran is presented in Table 1. Fruit firmness of ber fruits maintained during the storage period in both treated and untreated fruits. However, fruits treated with salicylic acid maintained (P≤0.05) fruit firmness significantly as compared to untreated. At the end of the storage period, fruits sprayed with salicylic acid @3 mM showed 18.16% higher fruit firmness as compared to the control fruits. A negative correlation and linear regression (-0.946, $R^2 = 0.865$) was observed between physiological loss in fruit weight and firmness. Fruit firmness is directly connected with the pectin content of fruits. The fruit firmness was inversely correlated with activity of PME enzyme during cold storage. The PME and firmness were found to have a significant (P≤0.05) Pearson correlation coefficient (0.312) during the storage period. Our results indicate that salicylic acid improved the fruit firmness by inhibiting 1-aminocycloproane-1-carborcylate oxidase (ACO) activity and suppression of ethylene production during storage. Fruits sprayed with salicylic acid @2 mM showed higher sensory scores up to 21 days of storage after that declined, whereas untreated fruits showed good sensory quality only up to 7 days of storage. The sensory quality improved due to reduction in softening of fruits and maintaining the integrity of the cell wall (Jain *et al.* 2017). Physiological loss in fruit weight declined irrespective of treatments with the advancement of storage (Table 1). Fruits treated with salicylic acid showed minimum weight loss. After 28th days of storage, salicylic acid treated (2 mM) fruits showed 29.11% lesser weight loss as compared to control. Salicylic acid can decrease the respiration process by inhibiting the synthesis of ethylene and closing stomata (Srivastava and Dwivedi 2000).

During storage, fruit spoilage increased up to 28th day. Fruits treated with salicylic acid showed no signs of spoilage up to 21 days of cold storage (Table 1), whereas untreated fruits showed 2.56% spoilage. Fruits spoilage after 28th days of storage, untreated fruits recorded maximum (37.45%) spoilage whereas, salicylic acid @2 mM treated fruits showed minimum spoilage (25.38%) during cold storage. A negative correlation (-0.930) was shown between fruit spoilage and fruit firmness during cold storage. This indicates that decrease in fruit firmness has made the fruit more susceptible to fruit spoilage. Higher firmness in treated fruits might be attributed to the reduced hydrolysis of soluble starch in ber fruits. So, salicylic acid treated fruits delayed the ripening process and reduced hydrolysis of soluble starch which was concentration-dependent. Similar results for fruit spoilage were recorded by Jain *et al.* (2017) in ber and in guava (Amanullah *et al.* 2017). The softening of fruits due to metabolic changes in fruits during storage can be retarded to some extent under cold storage conditions (Kaur and Bal 2014).

The effect of salicylic acid on carotenoid content of ber fruits under cold storage is shown in Table 2. During the storage period, a significant increase in carotenoid content (4.02 mg) was found in untreated fruits after 28 days and minimum (3.03 mg) was found in salicylic acid treated fruits at initial day of storage. With the progression of storage intervals, soluble solids content continued to increase (12.98%) up to 14 days in salicylic acid (3mM) treated fruits, while after 14 days of storage it declined. Maximum SSC was noticed in control fruits which continued to persist upto 14th days of storage. Fruits treated with salicylic acid showed slower reduction in soluble solids content than untreated fruits and also negative correlation coefficients was shown in firmness and spoilage (-0.719 and -0.730), decrease in firmness resulted in enhanced soluble solids content (SSC) (Table 3). Similar pattern of soluble solids was obtained by Singh *et al.* (2013), Jawandha *et al.* (2009) and Saran *et al.* (2004) in ber and Bhooriya *et al.* (2018) in guava fruits during storage. The increase in total soluble solids may be due to the hydrolysis of polysaccharides and dehydration of fruits. Thereafter, soluble solids content showed declined rate with the storage which might be due to an increase in senescence and high respiration rate (Kaur and Bal 2014).

The ascorbic acid declined with an increment of cold storage as shown in Table 2. However, fruits treated with salicylic acid showed lesser decrease in ascorbic acid as

Table 1 Effect of different concentrations of salicylic acid on physiological loss in weight, sensory quality, spoilage and fruit firmness of ber fruits cv. Umran under low-temperature conditions.

Parameter	Salicylic acid treatment (mM)	Storage period (days)				
		0	7	14	21	28
Fruit firmness (N)	1	13.64±0.06^{a}	13.46±0.08^{a}	12.91±0.01^{a}	11.04±0.04^{a}	9.86±0.06^{a}
	2	13.34±0.04^{b}	13.00±0.07^{d}	12.70±0.03^{b}	10.78±0.03^{c}	9.77±0.03^{b}
	3	13.56±0.04^{c}	13.25±0.04^{b}	12.90±0.04^{a}	10.83±0.05^{b}	9.80±0.01^{a}
	Control	13.36±0.03^{b}	13.11±0.05^{c}	11.90±0.05^{c}	10.06±0.06^{d}	8.02±0.03^{c}
Sensory quality (Hedonic score 1-9)	1	8.63±0.03^{a}	8.02±0.04^{b}	7.46±0.03^{a}	6.62±0.03^{b}	3.81±0.02^{a}
	2	8.65±0.04^{c}	7.78±0.06^{c}	7.36±0.07^{a}	7.11±0.04^{a}	4.13±0.03^{a}
	3	8.53±0.05^{b}	7.62±0.07^{d}	7.23±0.05^{c}	7.00±0.01^{a}	3.67±0.05^{b}
	Control	8.50±0.02^{b}	8.26±0.03^{a}	7.30±0.04^{b}	6.43±0.02^{c}	3.03±0.09^{c}
PLW (%)	1	-	1.68±0.02^{b}	1.87±0.03^{b}	2.80±0.02^{b}	4.78±0.03^{b}
	2	-	1.46±0.01^{c}	1.76±0.05^{c}	2.74±0.04^{c}	4.70±0.06^{c}
	3	-	1.65±0.03^{b}	1.83±0.02^{b}	2.80±0.05^{b}	4.75±0.01^{b}
	Control	-	2.60±0.02^{a}	3.93±0.01^{a}	4.25±0.03^{a}	6.63±0.04^{a}
Spoilage (%)	1	-	-	0.00±0.00^{b}	3.23±0.02^{b}	27.64±0.02^{b}
	2	-	-	0.00±0.00^{b}	2.98±0.04^{d}	25.38±0.04^{d}
	3	-	-	0.00±0.00^{b}	3.18±0.05^{c}	26.54±0.05^{c}
	Control	-	-	2.56±0.02^{a}	9.14±0.25^{a}	37.45±0.01^{a}

Mean values followed by the same superscript within a column are significantly at par ($P \leq 0.05$), n = 4 ± S.E. PLW, Physiological loss in weight

compared to the control. Maximum ascorbic acid (115.11 mg) was retained in fruit treated with salicylic acid @2 mM during the initial day of storage. In salicylic treated fruits, the activities of oxidizing enzymes must have decreased resulting in higher ascorbic acid during cold storage (Singh *et al.* 2010). Similar results were noticed by Singh *et al.* (2019) in guava, Saran *et al.* (2004) and Mani *et al.* (2018) in ber fruits.

The data regarding the peel colour of ber during different storage intervals is presented in Supplementary Table 1. After 28th day of cold storage, the minimum L*and b* valves (31.11 and 18.19) were noticed in salicylic acid @1 and 2 mM respectively, during cold storage. Salicylic acid delayed ripening, degradation of chlorophyll, more carotenoid accumulation and retarded ethylene production thereby ultimately delaying colour change in fruits.

The level of antioxidant activity and phenols was significantly higher in salicylic acid treated fruits (Fig 1). During storage, total phenols showed a decreasing trend.

Table 2 Effect of different concentrations of salicylic acid on vitamin C, soluble solids content and carotenoids of ber fruits cv. Umran under low-temperature conditions.

Parameter	Salicylic acid treatment (mM)	Storage period (days)				
		0	7	14	21	28
Carotenoids	1	3.04±0.05^{c}	3.12±0.02^{d}	3.34±0.04^{c}	3.44±0.02^{c}	3.57±0.03^{c}
	2	3.03±0.02^{c}	3.18±0.01^{c}	3.43±0.05^{b}	3.53±0.05^{b}	3.65±0.03^{b}
	3	3.13±0.01^{b}	3.22±0.03^{b}	3.37±0.02^{c}	3.46±0.04^{c}	3.55±0.04^{c}
	Control	3.49±0.03^{a}	3.65±0.02^{a}	3.84±0.02^{a}	3.92±0.07^{a}	4.02±0.05^{a}
Soluble solids content	1	11.05±0.04^{d}	11.57±0.04^{c}	12.48±0.03^{b}	12.84±0.03^{b}	11.89±0.03^{b}
	2	11.13±0.03^{c}	11.51±0.04^{d}	12.35±0.07^{c}	12.93±0.03^{a}	12.01±0.04^{a}
	3	11.25±0.02^{b}	12.63±0.09^{b}	12.88±0.02^{a}	12.44±0.02^{c}	11.41±0.06^{c}
	Control	11.39±0.06^{a}	12.83±0.07^{a}	12.85±0.05^{ab}	11.11±0.03^{d}	11.05±0.07^{d}
Ascorbic acid	1	111.12±1.14^{b}	102.56±0.88^{b}	88.29±0.82^{b}	64.15±0.94^{b}	36.95±0.81^{b}
	2	115.11±0.98^{a}	105.04±0.74^{a}	92.16±0.72^{a}	76.77±0.73^{a}	46.08±0.84^{a}
	3	111.39±0.82^{b}	101.52±0.84^{c}	87.90±0.76^{bc}	63.10±0.63^{c}	36.15±0.93^{b}
	Control	103.05±0.92^{c}	94.94±0.97^{d}	87.31±0.84^{c}	56.15±0.66^{d}	31.61±0.88^{c}

Mean values depicted by same superscript within a column are significantly at par ($P \leq 0.05$)

After the 28th day of storage, maximum phenol content (97.74 mg) was retained in salicylic acid @2 mM whereas antioxidant activity was 15.72% higher in salicylic acid @3 mM than control fruits. Reduction of phenol content and antioxidant activity of treated fruits may be due to reduced polyphenol oxidase activity. These antioxidative chemicals scavenge reactive oxygen species preventing oxidation of polyphenols (Tomas-Barberan *et al.* 1997). Similar results were also noticed by Thurzo *et al.* (2010) in sweet cherry, Jawandha *et al.* (2009) in ber and Tareen *et al.* (2012) and Razavi *et al.* (2014) in peaches under cold storage conditions.

An activity of SOD enzyme increased initially followed by decrease up to 28th day (Fig 1). The SOD activity of control fruits was always lesser than other treatments throughout the storage. At the end of storage, the SOD activity of salicylic acid @1 mM treated fruits showed a consistently higher rate (37.59%) than control. Therefore, the correlation coefficients showed significant (P≤ 0.05) relation between SOD and spoilage (-0.922) during the cold storage (Table 3) which helped in preventing the ber fruits from spoilage. As SOD catalyzes the dismutation of O_2 to H_2O_2, however, the defensive action of SOD against O_2 changes with advancement of time (Droillard and Paulin 1990). The level of catalase enzyme activity in all treated and untreated fruits increased steadily up to 7 days of storage (Fig 1). In the beginning, the activity of the catalase enzyme increased up to 7 days in all treatments. After the 28th day, salicylic-treated fruits had 21.41% higher enzyme activity as compared to control. Spoilage of ber fruits was negatively correlated with catalase enzymes (-0.922, R2 =0.613) during the storage (Table 3 and Fig 2). The PPO activity of all the treatments increased progressively during cold storage (Fig 1). At the end of storage, salicylic acid @2 mM recorded 23.24% lower activity as compared to control. The results revealed that Pearson correlation coefficient had significant

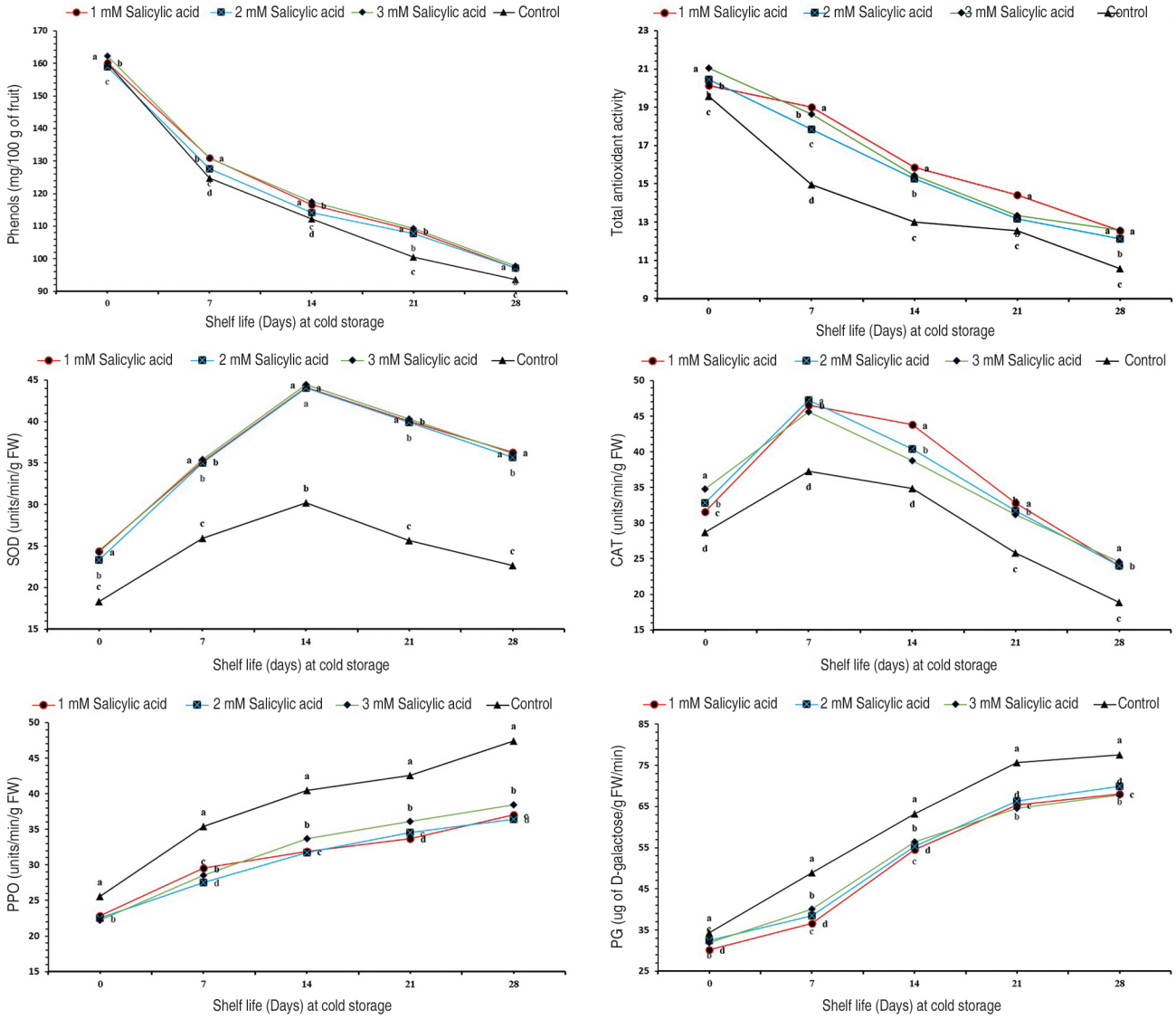

Fig 1 Variation in phenol content, antioxidant activity, superoxide dismutase (SOD), catalase (CAT), polyphenol oxidase (PPO) and polygalacturonase (PG) of ber fruits cv. Umran under low-temperature conditions in relation to different salicylic acid concentrations.

Table 3 Pearson's correlation coefficients between various quality attributes of ber fruits cv. Umran

	PLW	Sensory quality	Spoil-age	Firm-ness	SSC	Acidity	AoA	SOD	POD	Catalase	PME	PPO	PG
PLW	1												
Sensory quality	-0.859	1											
Spoilage	0.999**	-0.842	1										
Firmness	-0.719	0.280	-0.730	1									
SSC	-0.946	0.858	-0.930	0.691	1								
Acidity	0.889	-0.560	0.892	-0.952*	-0.876	1							
AoA	-0.951*	0.841	-0.936	0.721	0.999**	-0.896	1						
SOD	-0.934	0.754	-0.922	0.810	0.983*	-0.945	0.989*	1					
POD	0.307	-0.751	0.279	0.416	-0.359	-0.116	-0.322	-0.181	1				
Catalase	-0.991**	0.903	-0.983*	0.665	0.974*	-0.861	0.974*	0.947	-0.396	1			
PME	0.246	-0.443	0.265	0.312	-0.006	-0.155	0.007	0.109	0.549	-0.213	1		
PPO	0.993**	-0.909	0.990**	-0.634	-0.937	0.831	-0.938	-0.903	0.410	-0.992**	0.328	1	
PG	0.978*	-0.894	0.966*	-0.674	-0.991**	0.868	-0.990**	-0.966*	0.392	-0.996**	0.133	0.975*	1

**Significant at P ≤ 0.01, *Significant at P ≤ 0.05. AoA, antioxidant activity; SOD, Super dismutase; POD, Peroxidase; PG, polygalacturonase; PLW, Physiological loss in weight; SSC, Soluble solids contents; PME, Pectin methyl estrase; PPO, Polyphenol oxidase.

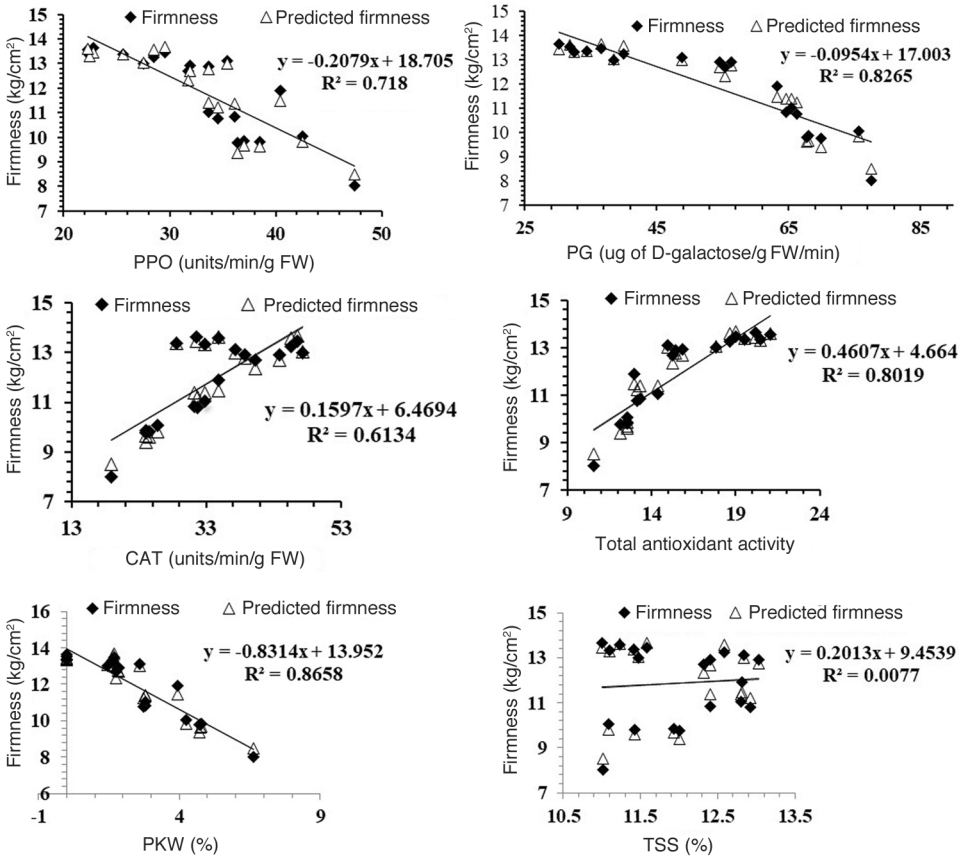

Fig 2 Regression relation between firmness and Polyphnol oxidase (PPO), Polygalacturonase (PG), Catalase (CAT), Antioxidant activity (AoA), Physiological loss in weight (PLW) and Soluble solids content (SSC) of ber fruits cv. Umran under low-temperature conditions in relation to different salicylic acid concentrations.

(P ≤ 0.05) correlation between PPO and SSC, (-0.937,0.007), PPO and firmness (-0.634, R2 = -0.718) during the storage period (Table 3 and Fig 2). The findings suggested that salicylic acid may be behind the delaying activity of polyphenol oxidase enzyme due to decreased respiratory activity of ber fruits. Similar results were also observed by Han *et al.* (2003) in peaches where salicylic acid immersions inhibited the activities of PPO. PG activity of all treatments gradually increased during the cold storage period (Fig 1). However, PG activity in untreated fruits was higher as compared to treated fruits. After 28th day of storage, untreated fruits showed 12.68% higher PG activity than salicylic @3 mM treated fruits (Fig 1) and recorded significant results in pearson correlation coefficient relation between PG and firmness (0.826) (Table 3). PG of

ber fruits destroyed demethylated pectin and maintained firmness during storage (Zhou *et al.* 2011).

This study concludes that, an application of salicylic acid @3 mM was found more effective in maintaining firmness, sensory quality and other quality attributes by suppressing activities of cell wall degrading enzymes like polygalacturonase, polyphenol oxidase, pectin methyl esterase and enhanced storage life up to 21 days under cold storage conditions.

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