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Effect of oxalic acid on ripening attributes of 'Gola' ber (*Ziziphus mauritiana* Lamk.) fruit during storage

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Abstract
The effect of exogenous oxalic acid treatment on ripening attributes of ber fruit cv. 'Gola' during storage was investigated. Prior to storage fruits were subjected to 10 min dipping in aqueous solution of oxalic acid at different concentrations (2 mM, 4 mM, 6 mM, 8 mM and 10 mM). Fruits under control were survived only for 5 days and with 2 mM oxalic acid for 7 days. Whereas, shelf life was extended up to 9 days with other concentrations of oxalic acid. In dipping treatment for 10 min at ambient temperature with 10 mM oxalic acid the Physiological loss in weight (PLW) and Ripening were increased during storage at ambient temperature for 9 days. Total carotenoids showed an increasing trend throughout the storage period. Furthermore, fruit treated with oxalic acid @ 10 mM concentrations were found to be best in maintaining enzymatic activity of Phenylalanine ammonia lyase (PAL), Malondialdehyde (MDA) and Superoxide dismutase (SOD), their minimum values were observed with this treatment. The overall acceptability was registered with significantly highest scores in fruits treated with 10 mM OA. Therefore, it can be concluded that 10 mM oxalic acid is beneficial in extending the shelf life up to 9 days at room temperature, and maintaining quality parameters by maintaining physiology and enzymatic activity of ber fruits.

Keywords: ber fruit, oxalic acid, ripening, hue angle, enzymatic activity

1. Introduction
Ber fruit is very perishable during storage at room temperature due to rapid ripening and high susceptibility. Fresh ber fruits deteriorate fast and cannot be kept for more than 5 days under ambient conditions without serious deterioration (Kadzere *et al.*, 2004) [12], even though some improved cultivars in India are known to store for up to 15 days without loss of organoleptic quality (Pareek, 2001) [20]. Ber has been traditionally considered to be functional fruit with a very high marketing value in India, because of its nutrition value, special components and delicious taste. However, ber fruit is highly perishable, extremely susceptible to decay and easily loses its commercial value after harvest (Lin *et al*. 2004) [14]. The storage life of ber fruits is extremely short and the rapid perishability of the fruits is a problem. Rates of respiration and ethylene production increased after 1 week of storage at ambient temperature, while peaks were observed after 2 weeks at 12 and 6°C. At ambient temperature a shelf-life of 2–4 days is common. Due to the surplus of fruits in the local markets during peak season, a substantial quantity goes waste, resulting in heavy postharvest losses. Profits could be enhanced if efforts to increase production are supplemented with efforts to minimise postharvest losses and enhance shelf-life (Gupta *et al*., 1992) [8].

The application of oxalic acid (OA) to harvested fruit has received much attention. As an organic acid present in various organisms (Kostman *et al*., 2001; Munir *et al*., 2001) [13, 17], oxalic acid has shown some antioxidant activities (Zheng *et al*., 2007) [4] and, thus, could play an important role in systemic resistance, programmed cell death, redox homeostasis in plants, and an anti-senescence effect in harvested fruits (Ding *et al*., 2007; Wu *et al*., 2011) [30]. Zheng *et al*. (2011) [36] reported that postharvest oxalic acid treatment could be a promising method to suppress quality deterioration and extend the storage shelf-life of mango fruit. In addition, the organic acid can delay jujube fruit senescence by reducing the ethylene production rate, repressing fruit reddening, and decreasing alcohol content (Wang *et al*., 2009) [29].

The objective of the present study was to investigate the role of oxalic acid in postharvest ripening of ber fruit and to examine its effects on physiological activity and antioxidant

antioxidant enzyme activity systems during storage. The study should be helpful for further development of commercial postharvest technology for quality maintenance and shelf life extension of ber fruit.

2. Materials and Methods

2.1 Fruit materials and treatments

The experiment was carried out at the Department of Horticulture, Rajasthan College of Agriculture, Udaipur. The fruit were selected for uniformity of size and appearance, and blemished and diseased fruit were discarded. Fruits were sorted as per maturity and external colour. Approximate 1.5 kg fruits were taken for per treatment per replication and taken five liter distilled water in five containers and added oxalic acid at a concentration of T_2 (2 mM), T_3 (4 mM), T_4 (6 mM), T_5 (8 mM), T_6 (10 mM) and then dipped the ber fruit in solution at 20ºC for 10 minutes. The fruits of each treatment were replicated four times. After dip treatments, fruit were dried at room temperature using portable fan. The treated fruits were stored at ambient temperature for 9 days in the room storage and sampled periodically to analyse various physiological and biochemical characteristics, enzymatic activity. Observations on phsico-chemical properties were carried out at every 48 hours interval for PLW, Ripening index, Total Carotenoids, PAL, MDA, SOD, hue angle, overall organoleptic score.

2.2 Determination of Fruit Physiological loss in weight

Physiological loss in weight (PLW) of fruits was calculated by considering the differences between initial weight and final weight on the day of observation divided by their initial weight.

$$PLW\ (\%) = \frac{\text{Initial weight (g) - final weight (g)}}{\text{Initial weight (g)}} \times 100$$

2.3 Measurements of ripening index

Postharvest ripeness of the ber fruits was quantitatively described by a ripening index (RPI_{KS}) calculated according to equation, where the dimensionless fruit firmness (SF_{KS} = $|SF_{KS,i}|$) refers to the absolute value of the specific maximum load at the ripening time RT_i (in days) and TSS/TA specifies the sugar/acid ratio of the corresponding ber mesocarp sample that was calculated from TSS and TA (Vasquez-Caicedo, 2005).

$$RPI_{KS} = \ln (100 . SF_{KS} . TSS . TA)$$

2.4 Measurements of total carotenoids

Flesh was cut into small pieces longitudinally and mixed with 80 per cent aqueous acetone for 2 hours at 50º C using an orbital shaker. Then it was filtered through Whatman paper. Filtrate was kept at -20º C prior to analysis. Five ml of sample extract was mixed with 5 ml distilled water and 1 ml of mix (hexane/acetone/methanol) (50/25/25 v/v). The sample was kept at centrifuge @ 3000 rpm for 10 min. The absorbance of upper layer was measured at 450 nm. Total carotenoids of the sample were calculated as µg $100g^{-1}$ (Velioglu *et al.*, 1998) [27].

$$\text{Total carotenoids (µg } 100g^{-1}) = \frac{A \times \text{Volume of extract}}{A^x \times \text{Sample weight}}$$

(A = Absorbance, A^x = Absorbance coefficient)

2.5 Measurement of phenylalanine ammonia lyase

Extraction and assay of PAL was carried out as described by Rao and Towers (1970). Fruit tissues (3 g) were homogenized in extraction buffer containing 2.6 ml of sodium borate buffer plus 2- mercaptoethenol (0.8 ml L^{-1}). Extract was filtered through cheese cloth 5.5 pH of filtrate was carried out by addition of 1M acetic acid. Protamine sulfate solution was added to filtrate and vortex for 10 min and centrifuge at 7000g for 10 min and supernatant was used as enzyme source. One ml of 0.05 M Tris-HCL buffer (pH 8.8), 0.5 ml of 0.01M L^{-1} phenylalanine and 0.4 ml of water was incubated at 30º C for 5 min. The reactions initiate by addition of 0.1 ml of enzyme and incubated for 60 min at 30º C. Blank was prepared without phenylalanine. The reaction terminated by addition of 0.5 ml of 1 N HCl. The mixture was extracted two times with 3.5 ml of ether. Ether phase was removed and dried under a stream of air. The residue dissolved in 3 ml of 0.05 N NaOH. Absorbance was read at 268 nm and standard curve prepared by cinemas acid similarly. Enzyme activity expressed measured as µ-moles of cinnamic acid produced min^{-1}.

2.6 Measurement of Malondialdehyde

MDA measurement was carried out by the method of Heath and Packer (1968) [9], with a slight modification. Fresh fruit pulp was homogenized in 10 ml of 10 per cent trichloroacetic acid and centrifuged at 10,000g for 20 minutes at 4ºC. The supernatant was collected, and 1 ml of supernatant was mixed with 3 ml of 0.5 per cent thiobarbituric acid. The mixture was boiled for 15 min, then quickly cooled in an ice bath and centrifuged at 12,000g for 15 min and the supernatant used for the assay. The supernatant was collected and used to measure absorbance at 450, 532 and 600 nm. The concentration of MDA on a fresh weight basis was calculated in mmol kg^{-1}. The MDA concentration was calculated according to the formula:

$$6.453\times (A532-A600) - 0.563\times A450$$

2.7 Assay for Superoxide dismutase activity

One gram of fresh tissue was homogenized with 3.0 ml of cold 0.1 mM Tris- HCl buffer (pH 7.5) containing 1 mM EDTA, 3 per cent polyvinyl pyrrolidone and 1mM $CaCl_2$ in a pre-chilled pestle and mortar. The homogenate was filtered through four layers of cheese cloth and the filtrate centrifuged at 10,000g for 20 min in a refrigerated centrifuge at 4º C. The supernatant so obtained was referred to as crude enzyme extract. SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) adopting the method of Beauchamp and Fridovich (1971). The reaction mixture (3.0 ml) contained 50 mM Tris – HCl (pH 7.8), 14 mM L-methionine, 60 µM NBT, 3 µM riboflavin, 0.1 mM EDTA and 0.1 ml of enzyme extract. Riboflavin was added at the end. The tubes were properly shaken. After terminating the reaction, tubes were covered with black cloth to protect them from light. A non–irradiated reaction mixture that did not develop colour served as the control. The reaction mixture developed maximum colour without enzyme extract and its absorbance decreased with the addition of enzyme. The absorbance was recorded at 560 nm. Per cent inhibition was calculated as per formula given by Asada *et al.* (1974) [1].

Per cent inhibition = V-v/V ×100

(V= Rate of assay reaction in absence of SOD, v= Rate of assay reaction in presence of SOD) One unit (U) of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the reduction of NBT as monitored at 560 nm.

2.8 Evaluation of Organoleptic score

The organoleptic evaluation of ber fruits was judged by visual method and on the basis of palatability, scored from 1 to 9 on Hedonic Rating Test Scale. For this purpose, a panel of five judges, who examined the skin colour, pulp color, sweetness and overall acceptance of fruits. The organoleptic evaluation of ber fruits was examined at alternate day of storage (Rangana, 1978) [21].

$$\text{Acceptance (\%)} = \frac{\text{Number of fruits per each degree of liking}}{\text{Total number of fruit in each treatment}} \times 100$$

3. Results and Discussion

3.1 Effect of oxalic acid on fruit physiological loss in weight

The PLW increased irrespective of treatments in the storage duration. Fruits under control were not survived on day 7 whereas fruits in T_2 treatment not survived on day 9. Minimum per cent loss in PLW from day 1 to end of the storage was recorded in T_6 treatment *i.e.*, 9.80 per cent and highest per cent loss was recorded in T_3 treatment *i.e.*, 11.79 per cent (Table 3.1). Weight loss is mainly related to respiration through skin, transpiration and metabolic process in fruit. The reduced metabolic activity produces a decrease in respiration rate, which in turn, results in lower rates of weight loss (Alves *et al*., 2004) and moisture evaporation through the skin. Lower weight losses in OA treated ber might be due to slower metabolic process resulting in moisture retention and the highest weight loss (16.25%), which might be due to a reduction in fruit firmness, indicating structural damage to the cross-linkages in the cell wall (Saengnil *et al.*, 2006) [22].

3.2 Effect of oxalic acid on ripening index

Lowest increase in ripening index from day 1st to 9th was recorded in T_6 treatment *i.e.*, (22.69%) and highest per cent increase in ripening index from day1st to 9th was recorded in T_3 treatment *i.e.*, 59.36 per cent (Table 3.2). Ripening is the composite of the processes that occur from the later stages of growth and development through the early stages of senescence, and which result in characteristic aesthetic or food quality, as evidenced by changes in composition, colour, texture or other sensory attributes (Kader, 1999) [11]. Some studies have shown that the retards of ripening in climacteric fruit by postharvest treatments were associated with the decrease of ethylene production (Luo *et al*., 2010) [15]. Treatment with OA at 5 mM slowed the respiration of peach fruit, increased activity of antioxidant enzymes, maintained membrane integrity and delayed the fruit ripening process (Zheng *et al*., 2007a) [34]. Zheng *et al*. (2007b) [35] reported that the OA delayed mango fruit ripening was accompanied by considerable inhibition of ethylene production during storage.

3.3 Effect of oxalic acid on Total carotenoids

The lowest per cent increase in total carotenoids from 1st to 9th day were recorded in T_6 treatment *i.e.*, 17.19 per cent and highest per cent increase in T_3 treatment *i.e.*, 24.09 per cent. The carotenoids are responsible for the bright yellow colour of the fruits and are important lipophilic radical scavengers found in many fruits and vegetables. Although, somewhat stable within an intact plant cell, carotenoid concentrations may be altered during postharvest handling or processing. The inhibition of carotenoids accumulation by OA treatment is consistent with the retard of fruit reddening. PAL is the key enzyme involved in the biosynthesis of carotenoids in fruits (Flores *et al*., 2005) [6]. In some climacteric and non-climacteric fruits, such as apple (Faraghet and Brohier, 1984) [5] and strawberry (Villarreal *et al*., 2010) [28], PAL activities were considered to be responsible for carotenoids accumulation and reddening of fruits. In present study the PAL activity inhibited by OA treatment ((Table 3.3) was consistent with the retard of carotenoids in ber fruits.

3.4 Effect of oxalic acid on Phenylalanine ammonia lyase

Lowest per cent increase in *PAL* activity from 1st to 9th day was recorded in T_3 (19.46%) treatment and highest per cent increase in PAL activity (26.14%) was recorded in T_6 treatment (Table 3.4). There are few reports on the PAL activity tended to decrease; while OA treated plum fruit had much lower PAL activities than control fruits (Wu *et al*., 2011) [30]. PAL activity can be induced by various stresses, such as wounding (Campos-Vargas *et al.*, 2005) [3] and plant harmone including ethylene, jasmonic acid and salicylic acid (Campos-Vargas *et al.*, 2005) [3]. In present study, inhibition of PAL activity was found in OA treatment fruit under ambient storage compared with control fruits. This previous report, as total phenols in peel of mango fruit treated with oxalate were significantly elevated during most of the storage time, without stimulation of PAL enzyme during storage (Zheng *et al*., 2012) [32]. The previous study was found inhibition of PAL activity by OA in plum fruit at ambient storage, which might result in lower anthocyanins and the retard of fruit reddening (Wu *et al*., 2011) [30].

3.5 Effect of oxalic acid on Malondialdehyde

MDA activity increased in all OA treatments in the storage duration. At the end of storage (9 d), lowest MDA activity (3.56 mmole) was observed in T_3 treatment while it was maximum (5.00 mmole) in T_6 treatment (Table 3.5). MDA content, in the OA treated litchi fruit, was significantly lower than that in the control after 4th day of storage (Zheng and Tian, 2006) [36]. Pepper leaves were sprayed with OA for 3rd day before heat stress, the damage to the membrane was alleviated, and the production of MDA and hydrogen peroxide was decreased (Zhang *et al*., 2001) [31]. The content of MDA is often used as an indicator of lipid peroxidation, resulting from oxidative stress (Smirnoff, 1995) [25]. Results showed that OA could affect MDA activity in ber fruit and might play a protective role in membrane integrity in ber because of the declining oxidation during storage. A similar study was found in banana (Huang *et al*., 2013b) [10].

3.6 Effect of oxalic acid on Superoxide dismutase activity

SOD activity decreased in all OA treatments in the storage duration. At the end of storage (9 d), lowest SOD activity (1005.25 Unit for 50% inhibition) was observed in T_6 treatment while it was maximum (1342.25 Unit for 50% inhibition) in T_3 treatment (Table 3.6). SOD has hallmark of first defense line during oxidative stresses to which fruits confront during storage period. SOD acts as a major defense against cellular membranes damage due to ROS because of oxidative damages to which organisms are exposed (Natvig *et*

al., 1996) [18]. The catalytic ability of SOD through which superoxide free radicals (O_2^-) is dismutated to hydrogen peroxide (H_2O_2) further to this catalase and POD convert H_2O_2 to H_2O and O_2 (Schantz *et al*., 1995 [24]; Mondal *et al*., 2004) [14]. Damage to cells can be avoided through SOD and CAT activity by the conversion of hazardous superoxide radicals and hydrogen peroxide to molecular O_2 and H_2O (Scandalios, 1993) [23]. Enzymes are localized in the compartments of subcellular level and entire aerobic organisms that go under oxidative stress (Blokhina *et al*., 2003) [2]. Higher SOD and CAT activities have great influence in delaying senescence.

3.7 Effect of oxalic acid on organoleptic score

On 5th day of storage the minimum overall organoleptic score was recorded in T_1 (5.1) and maximum in T_6 (8.3) and also found that T_4 and T_5 were at par with T_6 treatment. At the end of storage, highest overall organoleptic rating (6.2) was found in T_6 treatment while it was lowest in T_3 treatment (4.9) (Table 3.7). The general appearance and organoleptic qualities *i.e*., shape, size, colour, texture, flavour, aroma and taste of the fruits altogether determine the consumer's acceptability of the fruits. Organoleptic characters are very much influenced by the postharvest treatments of fruits. The overall organoleptic rating like color, texture, appearance and taste of the fruit of all treatments deteriorated on account of faster ripening, reduced TSS and consequent decline in acidity. Generally, all quality parameters contribute to overall acceptability of a commodity. In present study, overall acceptability is the average of organoleptic parameters such like texture, flavor or taste. Overall acceptability of ber fruits is greatly influenced by appearance, color, texture and flavor (Garg *et al*., 2005) [7]. The organoleptic rating of ber varieties (Naik and Rokhade, 1997) [19] revealed that varieties having medium to high vitamin-C content, TSS and total sugars scored higher while lower values for any these character resulted in lower score.

Table 3.1: Effect of oxalic acid on physiological loss in weight (%) during storage

Treatments	Physiological loss in weight				
	1st day	3rd day	5th day	7th day	9th day
T_1 (Control)	1.52	5.26	9.98	-	-
T_2 (2 mM)	1.30	3.49	9.72	14.74	-
T_3 (4 mM)	1.27	3.90	9.25	13.36	16.25
T_4 (6 mM)	1.30	4.39	9.18	12.25	15.40
T_5 (8 mM)	1.23	4.09	8.93	11.32	13.61
T_6 (10 mM)	1.14	4.20	8.18	10.32	12.40
SEm±	0.08	0.37	0.50	0.17	0.19
CD (P=0.05)	NS	NS	NS	0.50	0.59

'___' denotes fruits not survived, NS- Non significant

Table 3.2: Effect of oxalic acid on ripening index during storage

Treatments	Ripening index				
	1st day	3rd day	5th day	7th day	9th day
T_1 (Control)	5.30	6.00	7.23	-	-
T_2 (2 mM)	5.20	5.80	7.00	8.10	-
T_3 (4 mM)	5.02	5.60	6.70	7.70	8.00
T_4 (6 mM)	4.95	5.40	6.20	7.25	7.55
T_5 (8 mM)	4.90	5.20	5.80	6.56	6.75
T_6 (10 mM)	4.89	5.00	5.40	5.80	6.00
SEm±	0.10	0.24	0.11	0.11	0.12
CD (P=0.05)	NS	NS	0.34	0.34	0.37

'___' denotes fruits not survived, NS- Non significant

Table 3.3: Effect of oxalic acid on total carotenoids (%) during storage

Treatments	Total carotenoids				
	1st day	3rd day	5th day	7th day	9th day
T_1 (Control)	0.175	0.181	0.188	-	-
T_2 (2 mM)	0.171	0.175	0.181	0.189	-
T_3 (4 mM)	0.166	0.172	0.176	0.187	0.206
T_4 (6 mM)	0.165	0.170	0.172	0.180	0.198
T_5 (8 mM)	0.161	0.165	0.166	0.176	0.190
T_6 (10 mM)	0.157	0.159	0.161	0.168	0.184
SEm±	0.04	0.04	0.01	0.02	0.03
CD (P=0.05)	NS	NS	0.01	0.01	0.01

'___' denotes fruits not survived, NS- Non significant

Table 3.4: Effect of oxalic acid on phenylalanine ammonia lyase (PAL) activity (µ Moles) during storage

Treatments	Phenylalanine ammonia lyase				
	1st day	3rd day	5th day	7th day	9th day
T_1 (Control)	14.24	15.15	15.94	-	-
T_2 (2 mM)	13.68	13.90	14.10	14.60	-
T_3 (4 mM)	12.43	13.13	13.79	14.00	14.85
T_4 (6 mM)	11.84	12.00	12.50	13.05	13.79
T_5 (8 mM)	10.43	11.10	11.97	12.00	12.85
T_6 (10 mM)	9.18	10.24	10.84	11.03	11.58
SEm±	1.168	1.16	0.20	0.20	0.22
CD (P=0.05)	NS	NS	0.60	0.61	0.68

'___' denotes not survived, NS- Non significant

Table 3.5: Effect of oxalic acid on malondialdihyde (MDA) activity (mmol kg^{-1}) during storage

Treatments	Malondialdihyde				
	1st day	3rd day	5th day	7th day	9th day
T_1 (Control)	1.03	1.25	2.16	-	-
T_2 (2 mM)	1.15	1.43	2.56	3.00	-
T_3 (4 mM)	1.32	1.85	2.94	3.95	3.56
T_4 (6 mM)	1.63	2.08	3.42	4.97	4.15
T_5 (8 mM)	1.86	2.45	4.15	5.89	4.50
T_6 (10 mM)	2.00	3.02	5.35	6.97	5.00
SEm±	0.23	0.37	0.71	1.09	0.42
CD (P=0.05)	NS	NS	NS	NS	NS

'___' denotes fruits not survived, NS- Non significant

Table 3.6: Effect of oxalic acid on superoxide dismutase (SOD) activity (Unit for 50% inhibition) during storage

Treatments	Superoxide dismutase				
	1st day	3rd day	5th day	7th day	9th day
T_1 (Control)	1664.25	1615.00	1598.50	-	-
T_2 (2 mM)	1546.25	1496.00	1478.37	1460.50	-
T_3 (4 mM)	1436.50	1378.75.	1365.29	1351.20	1342.25
T_4 (6 mM)	1319.50	1263.25	1248.50	1236.20	1228.20
T_5 (8 mM)	1204.25	1153.49	1132.45	1125.50	1119.25
T_6 (10 mM)	1093.25	1035.00	1021.00	1016.00	1005.25
SEm±	21.45	137.59	173.09	20.34	13.36
CD (P=0.05)	NS	NS	NS	61.31	41.17

'___' denotes fruits not survived, NS- Non significant

Table 3.7: Effect of oxalic acid on overall organoleptic rating (out of 9 marks) during storage

Treatments	Overall organoleptic rating				
	1st day	3rd day	5th day	7th day	9th day
T_1 (Control)	5.1	6.4	5.1	-	-
T_2 (2 mM)	5.3	6.3	7.2	4.6	-
T_3 (4 mM)	5.7	6.9	7.4	5.1	4.9
T_4 (6 mM)	5.9	7.0	7.8	5.6	5.2
T_5 (8 mM)	6.1	7.2	8.1	6.2	5.6
T_6 (10 mM)	6.5	7.8	8.3	7.0	6.2
SEm±	0.32	0.34	0.20	0.08	0.08
CD (P=0.05)	NS	NS	0.58	0.24	0.25

'___' denotes fruits not survived, NS- Non significant

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