Postharvest treatment of antioxidant reduces lenticel browning and improves cosmetic appeal of mango (*Mangifera indica* L.) fruits without impairing quality

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Abstract The present study reports the usefulness of ascorbic acid for reducing lenticels browning (LB) in mango, which impairs the cosmetic appeal and thereby the export. Postharvest dip treatment of ascorbic acid at different concentrations (100, 150 and 200 ppm) was given to four varieties of mango (Indian—‘Dashehari’, ‘Langra’; Exotic—‘Sensation’, ‘Eldon’). After treatment, fruits were stored at ambient conditions (35 ± 4 °C and 65 ± 5 % RH) for 10 days. At the end of storage period, observations were recorded on several parameters. Our results revealed that fruits of ‘Langra’ mango exhibited 100 % LB, followed by ‘Dashehari’ (52.8 %), ‘Sensation’ (42.9 %) and ‘Eldon’ (38.3 %). All concentrations of ascorbic acid reduced the LB to greater extent and improved the cosmetic appeal of fruits of all the varieties significantly over untreated fruits but the best results were obtained with 200 ppm concentration for LB (4.8, 1.6, 3.7, 3.2 %, respectively) and other attributes, non-significantly followed by 150 ppm concentration. All concentrations of ascorbic also reduced the activities of polyphenol oxidase, peroxidase and lipoxigenase enzymes, and also reduced respiration rate and ethylene evolution rate but without impairing eating quality attributes (soluble solids concentrates and total carotenoids). It can be concluded that

postharvest dip application of ascorbic acid at 150 ppm reduced LB and improve face value without impairing fruit quality.

Keywords Lenticel browning · Cosmetic appeal · Ascorbic acid · Fruit quality · Enzymes

Introduction

Mango (*Mangifera indica* L.), commonly called as the ‘King of fruits’ in India, is being cultivated on an area of over 2.5 million ha in India with a production of about 18 million tonnes annually (Anon 2014). Indian mango production accounts for over 56 % of the global mango production. Although mango export is more than Rs. 210 crores annually from India, but postharvest losses of fruits are still very high. All the mango producing countries of the world wanted to reduce the postharvest losses significantly and to increase the export of fresh mangoes (Chatopadhyay 2014) but several inherent problems affect fruit quality and thereby the export. Of the several such factors, one which has limited the export to a great extent and which affects the appearance of mango fruits greatly, is the problem of lenticels browning (LB) or lenticels discolouration (LD) (Rymbai et al. 2012). LB has now become as one of the main reasons of quality loss in mango cultivars grown in India and abroad. LB not only decreases the shelf-life of the mango fruits but also affects the appearance of fruits, which has become a point of hindrance in export of mango to other countries (Rymbai et al. 2012). Several efforts have been made in the past to reduce factors, which affect the mango fruit quality including appearance and ultimately the export.

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Lenticels are macroscopic openings that are present on the surface of mango fruits. They can be seen on fruit surface and other parts of fruits such as apple, mango, avocado etc. (Dietz et al. 1988; Ersoy et al. 2008; Everett et al. 2008; Wenneker and Kohl 2014). These openings play significant role in transpiration and exchange of gases. Although lenticels act as a necessary evil as they are required for several physiological functions in the plant but their discoloration leads to the loss in face value and quality, and thus it is considered as one of the main problems in postharvest management of mango (Rymbai et al. 2012). The mechanism of LB is not fully understood, however, reasons for the development LB have been investigated from various horticultural and postharvest management points of view (O'Hare and Prasad 1992; Du-Plooy et al. 2002). For instance, Kangatharalingam et al. (2002) reported that the red discolouration in mango is linked to either anthocyanin or flavonoid (Dixon and Paiva 1995) production and is suspected to be a mechanism of self-protection. Grassmann et al. (2002) proposed that LB is primarily a cosmetic condition, and probably due to a physiological stress response by fruit tissue surrounding the lenticels entrance. Later, Du-Plooy et al. (2006) did in-depth studies on LB and changes in the profiles of phenolics in discoloured versus unaffected lenticels and the corresponding cytological changes, and reported that accumulation of cell-bound phenolics does not result from structural damage such as vacuolar collapse or membrane disintegration. Further, accumulation of phenolic compounds in a distinct zone of affected tissue around the lenticels creates a barrier between the atmosphere and the rest of the mesophyll. They further reported that although LB is an inherent self-defence mechanism supported by continued metabolic activity, it is a superficial, cosmetic defect, and confirmed that the compounds responsible for the discolouration of lenticels were simpler flavonones rather than more complex anthocyanidins (Du-Plooy et al. 2009). Since, LB affects the cosmetic appeal of the fruits; it has a long bearing on the consumer's preference. LB affected mango fruits fetch low rates in the market. Thus, there is an urgent need to standardize techniques for reducing LB in mango to improve the appearance of fruits and to increase export of such an important commodity.

Of the several postharvest treatments available in literature to reduce LB, we attempted the use of ascorbic acid with the objective to use an antioxidant for reducing oxidation of polyphenols, PPO and POD activity in mango lenticels, and thereby LB, which will be useful to improve the appearance of mango fruits.

Materials and methods

Experimental site and materials

The study was undertaken in the Division of Food Science and Postharvest Technology, Indian Agricultural Research Institute, New Delhi during 2014–2015. First, preliminary study was conducted on 20 Indian and ten exotic varieties of mango for their susceptibility to LB (data not shown) which indicated that among Indian varieties, 'Langra' was the most susceptible (100 % LB), followed by 'Dashahari' (52.8 % LB). Among exotic varieties, 'Sensation' was most susceptible to LB (42.9 %), followed by 'Eldon' (38.3 %). Based on this preliminary study, two most susceptible Indian ('Langra' and 'Dashehari') and two exotic ('Sensation' and 'Eldon') varieties of mango were selected for the present study. The fruits of these mango varieties were procured from the experimental orchard of the Division of Fruits and Horticultural Technology, IARI, New Delhi-110 012. Fully-mature fruits (120 days old) were harvested from the trees at full maturity, de-sapped in the field and transferred to laboratory for ripening at a temperature of 22° C and 75–80 % relative humidity (RH). The fruits were then subjected to different concentrations of ascorbic acid as described below.

Details of treatments

After harvesting fruits at fully maturity, these were de-sapped by keeping the fruits upside down in trays specially designed for this activity. After de-sapping, 50-fruits of each of the selected mango varieties were subjected to postharvest dip treatment with different concentrations of ascorbic acid viz. 100, 150 and 200 ppm which were replicated thrice. The control fruits were subjected to tap-water dip. After treatment, the fruits were stored at ambient conditions (35 ± 4 °C and 65 ± 5 % RH) for 10 days.

Determination of the incidence of LB

The incidence of lenticels browning was estimated by counting the number of lenticels browned on the fruit peel with respect to total lenticels present on the fruit and represented as percentage (%). Randomly selected ten fruits were used for determination of LB incidence.

Cosmetic appeal of mango fruits

The cosmetic appeal of untreated as well as treated mango fruits was determined getting the data/feedback from a panel of ten semi-technical experts on hedonic scale,

ranging from 0 to 9, in which 9 indicated extremely appealing, 8—very much appealing, 7—moderately appealing, 6—slightly appealing, 5—neither appealing nor non-appealing, 4—slightly non-appealing, 3—moderately non-appealing, 2—very much non-appealing, and 1—indicated extremely non-appealing. The semi-technical experts (scientist and technical personnel) were asked to give score to the fruits on the basis of parameters such colour, bruising, browning, appearance, acceptability and overall appeal of fruit. The average values were included for assessing the overall appeal of mango fruits consumers.

Determination of polyphenol oxidase (PPO), peroxidase (POD) and lipoxygenase (LOX) enzyme activity

The PPO activity was estimated by using method of Sharma et al. (2000) and expressed as $\Delta A_{410} \text{ O.D min}^{-1} \text{ g}^{-1} \text{ FW}$. The POD activity was determined as per method of Sharma and Singh (2002) and expressed as $\Delta A_{470} \text{ O.D.min}^{-1} \text{ mg}^{-1}$. The LOX activity in the mango fruits was determined as per the method of Feys et al. (1980) with slight modifications (Sharma et al. 2012) and expressed as $\mu\text{moles min}^{-1} \text{ g}^{-1}$ fresh weight.

Determination of respiration and ethylene evolution rates

Auto gas analyzer (Model: Checkmate 9900 O_2/CO_2 , Dansensor PBI, Denmark) was used for measuring respiration rate of mango fruits, which was expressed as $\text{ml CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (Jhalegar et al. 2015). For this, randomly ten fruits were randomly selected and their respiration rate was determined as per method suggested by Sharma et al. (2015). For determination of ethylene evolution rate, Hewlett Packard (H.P.) gas chromatograph (model 5890 Series II) was used. Randomly selected ten fruits were weighted and then used for determination of ethylene evolution rate as per method suggested by Sharma et al. (2014). Ethylene evolution rate was expressed as $\mu\text{l kg}^{-1} \text{ h}^{-1}$ of evolved ethylene.

Fruit quality attributes

The soluble solids concentrates (SSC) of mango fruit pulp samples were estimated at room temperature by using FISHER Hand Refractometer, having 0–50 range and results were expressed in $^\circ\text{B}$ (Ranganna 2000). Total carotenoids (TC) were determined by using Roy (1973) method and expressed as $\text{mg } 100 \text{ g}^{-1} \text{ FW}$ (fresh weight).

Statistical design and analysis of data

The experiments were laid out in factorial CRD design with each treatment consisting of three replications. The data obtained from the experiments were analyzed

following standard procedures (Panse and Sukhatme, 1984). Differences between means were evaluated using Duncan's Multiple Range Test at $p \leq 0.05$.

Results and discussion

Lenticel browning (LB)

In the recent years, LB has appeared as one of the most alarming postharvest problems, which affects the export of mango from one country to other. In this study, we have observed that the variable incidence of lenticels browning was exhibited by different varieties of mango, being cent percent (100 %) in untreated fruits of 'Langra'. In general, exotic varieties ('Sensation' and 'Eldon') exhibited lower incidence of LB than Indian varieties ('Dashehari' and 'Langra') (Table 1). Differences in LB among varieties may be attributed to genetic variation among the varieties. Further, all concentrations of ascorbic acid have reduced the incidence of LB to a variable extent in all mango varieties. However, ascorbic acid @ 200 ppm was the most effective concentration in reducing LB in all the varieties, non-significantly followed by the fruits which were treated with 150 ppm of ascorbic acid (Table 1). The reduction in LB incidence by ascorbic acid can be explained by the concept of inhibition of browning reactions by the formation of ascorbyl, as ascorbic acid directly scavenges the damaging radicals and reduces the o-quinones, produced by polyphenol oxidase enzyme (Abbasi et al. 2013). Progressive reduction in LB by increasing concentration of ascorbic acid may be due to increased inhibition of PPO due to the concentration-dependent effect of ascorbic acid, which may vary from commodity-to-commodity, primarily due to the chelating property of ascorbic acid at very high concentration as explained by Kambol and Upadhyay (2012).

Cosmetic appeal

The cosmetic appeal of fruits has a great influence on consumer attraction and thereby acceptability (Oosthuysen 2002). Fruits having attractive colour, free from defects, bruising, browning etc., fetch good price in the market than those having bruises and other defects (Du-Plooy et al. 2006). In this study, we have observed a highly significant effect of ascorbic acid treatments on cosmetic appeal of fruits of different mango varieties (Table 1). The values for cosmetic appeal of untreated fruits of all the varieties was quite lower than those treated with ascorbic acid, indicating that there was significant improvement in overall appearance of mango fruits treated with ascorbic acid. This improvement in cosmetic appeal of mango fruits may be

Table 1 Influence of postharvest ascorbic acid treatment on lenticel browning and cosmetic appeal of mango fruits, stored at ambient conditions (35 ± 4 °C and 65 ± 5 % RH) for 10 days

Treatment	Lenticel browning (%)				Cosmetic appeal (0–9 scale)			
	Indigenous variety		Exotic variety		Indigenous variety		Exotic variety	
	Dashehari	Langra	Sensation	Eldon	Dashehari	Langra	Sensation	Eldon
Control	52.8 ^a	100 ^a	42.9 ^a	38.3 ^a	4.6 ^a	2.5 ^a	7.4 ^a	7.2 ^a
Ascorbic acid @ 100 ppm	12.2 ^b	18.3 ^b	12.6 ^b	6.0 ^b	7.4 ^b	5.6 ^b	8.8 ^b	8.4 ^b
Ascorbic acid @ 150 ppm	2.0 ^c	5.6 ^c	4.0 ^c	4.3 ^c	7.9 ^b	5.9 ^b	9.0 ^b	8.6 ^b
Ascorbic acid @ 200 ppm	1.6 ^c	4.8 ^c	3.7 ^c	3.2 ^c	7.9 ^b	5.7 ^b	9.0 ^b	8.7 ^b

Means within the column with the same letter are not significantly different by Duncan's Multiple Range Test at $p \leq 0.05$

attributed to decreased incidence of LB, which is directly involved in reducing the face value of fruits (Rymbai et al. 2012). Further, cosmetic values were high for exotic varieties ('Eldon' and 'Sensation') primarily because these varieties develop attractive red colour than Indian varieties, and also they had lower incidence of LB than Indian varieties ('Dashehari' and 'Langra').

Polyphenol oxidase and peroxidase activity

PPO and POD are known for browning reactions in plants and their products. The activities of PPO and POD enzymes were significantly higher in untreated fruits of all varieties of mango than those treated with different concentrations of ascorbic acid (Table 2). Lower activities of PPO in ascorbic acid treated mango fruits may be due to lower incidence of LB. PPO is known to bring discolouration in products after action on phenolics in presence of elemental oxygen (Sharma et al. 2000). Present results corroborate with the work of Zhang et al. (2009) who reported the same trend of PPO activity using different concentrations of ascorbic acid in kiwifruit. The increase in PPO activity at higher concentrations of ascorbic acid may be due to increased browning as Sanchez et al. (2005) reported the increase in PPO activity with lenticels browning in 'Haden' and 'Tommy Atkins' mango cultivars. It has been reported that LD in 'Keitt' and 'Tommy Atkins' may be due to endomembrane collapse and the liberation of PPO enzyme (Du-Plooy et al. 2004) by the fruit tissues surrounding the lenticels (Grassmann et al. 2002). In a similar study, Robinson et al. (1993) have also reported higher PPO activity in browned lenticels of mango than normal cells. In this study, POD enzyme activity exhibited a pattern similar to PPO enzyme. Among varieties, fruits of 'Langra' have shown the maximum POD activity and that of 'Eldon' exhibited the lowest (Table 2). Higher POD activity in untreated mango fruits of different varieties may be due to higher incidence of LB, resulted by oxidation of polyphenols (Sharma et al. 2000). Our results are in line with the findings of Mikani et al. (2011) who

reported the gradual increase in POD enzyme activity up to a threshold concentration, which is primarily due to the increase in disease incidence (Mikani et al. 2011).

Lipoxygenase (LOX) activity

Lipoxygenase (LOX) (EC1.13.11.12) catalyzes the hydroperoxidation of polyunsaturated fatty acids, and plays significant role in senescence of plant tissues (Sharma and Singh 2010). Ascorbic acid concentration showed significant effect on LOX activity in mango fruits of different varieties, being quite lower in treated than untreated ones (Table 2). Among the varieties, the LOX activity was significantly higher in 'Langra' and minimum in 'Eldon' variety (Table 2). Variation in LOX activity among varieties might be due to genetic differences among the varieties, and higher LOX activity in untreated mango fruits might be due to higher incidence of LB, which might have made such fruits more senescent than those treated with ascorbic acid. Our finding corroborates to the work done by Zhang et al. (2009) who reported that ascorbic acid affects LOX activity, which was quite higher in diseased/affected fruits.

Ethylene evolution rate

Ethylene evolution is a major deterrent for the postharvest life of fruits (Jhalegar et al. 2014). It was observed that the ethylene evolution rate was significantly influenced by both varieties and treatments. The ethylene evolution rate was significantly higher in untreated fruits of mango varieties and quite lower in ascorbic acid treated fruits (Table 3). Ethylene evolution rate was lowest in fruits treated with ascorbic acid at 200 ppm, non-significantly followed by those treated with 150 ppm. Variation in ethylene evolution rate among varieties might be due to the differences in the genetic makeup of the varieties. Furthermore, variable response of mango fruits to ethylene evolution rate may be due to dose-dependent effect of ascorbic acid. Our findings are in line with the work of

Table 2 Polyphenol oxidase (PPO), peroxidase (POD) and lipoxygenase (LOX) activity in mango fruits, stored at ambient conditions (35 ± 4 °C and 65 ± 5 % RH) for 10 days as influenced by postharvest ascorbic acid dip treatment

Treatment	Polyphenol oxidase activity (ΔA_{410} O.D. $\text{min}^{-1}\text{g}^{-1}$)				Peroxidase activity ΔA_{470} O.D. $\text{min}^{-1}\text{mg}^{-1}$)				Lipoxygenase activity $\mu\text{moles g}^{-1}\text{fresh wt}^{-1}\text{min}^{-1}$)			
	Dashehari	Langra	Sensation	Eldon	Dashehari	Langra	Sensation	Eldon	Dashehari	Langra	Sensation	Eldon
Control	0.816	1.267	0.608	0.476	0.134	0.197	0.093	0.055	6.143	7.146	3.982	7.483
Ascorbic acid @ 100 ppm	0.453	0.890	0.288	0.268	0.078	0.111	0.042	0.022	3.348	4.867	2.859	4.256
Ascorbic acid @ 150 ppm	0.327	0.682	0.218	0.196	0.055	0.093	0.032	0.016	2.915	3.654	2.158	3.254
Ascorbic acid @ 200 ppm	0.309	0.601	0.189	0.187	0.045	0.082	0.025	0.011	2.885	3.522	2.142	3.125
C.D. at $p \leq 0.05$	Variety (V) = 0.004; Treatment (T) = 0.004; V x T = 0.009				Variety (V) = 0.001; Treatment (T) = 0.001; V x T = 0.003				Variety (V) = 0.006; Treatment (T) = 0.006; V x T = 0.011			

Table 3 Impact of ascorbic acid treatment on ethylene evolution and respiration rates of mango fruit, stored at ambient conditions (35 ± 4 °C and 65 ± 5 % RH) for 10 days

Treatment	Ethylene evolution rate ($\mu\text{l kg}^{-1}\text{h}^{-1}$)				Respiration rate ($\text{ml CO}_2 \text{ kg}^{-1}\text{h}^{-1}$)			
	Dashehari	Langra	Sensation	Eldon	Dashehari	Langra	Sensation	Eldon
Control	0.916 ^a	1.412 ^a	0.806 ^a	0.789 ^a	35.23 ^a	79.56 ^a	24.30 ^a	52.43 ^a
Ascorbic acid @ 100 ppm	0.452 ^b	0.755 ^b	0.481 ^b	0.413 ^b	31.23 ^b	70.55 ^b	20.18 ^b	43.25 ^b
Ascorbic acid @ 150 ppm	0.393 ^c	0.614 ^c	0.326 ^c	0.364 ^c	29.24 ^c	68.24 ^c	18.31 ^c	39.14 ^c
Ascorbic acid @ 200 ppm	0.387 ^c	0.598 ^c	0.306 ^c	0.353 ^c	29.07 ^c	67.92 ^c	17.96 ^c	38.76 ^c

Means within the column with the same letter are not significantly different by Duncan's Multiple Range Test at $p \leq 0.05$

Cooper et al. (1968) who reported the decrease in ethylene evolution with the treatment of ascorbic acid in apple, pear and citrus. Further, an increase in ethylene evolution may be due to the increased decay of fruits that had occurred at higher concentration of ascorbic acid (Fallik et al. 1993).

Respiration rate

Among several processes, respiration rate is a major metabolic process of a fruit or vegetable, which is directly related to storage life of a commodity (Jhalegar et al. 2014). We observed that in this study, the pattern of respiration rate was similar to ethylene evolution rate. Among varieties, 'Langra' fruits have exhibited the highest respiration rate and 'Sensation' the least (Table 3). This may be due to the genetic variation and fruit physiological differences among the varieties. Similarly, among treatments, lowest respiration rate was recorded in the fruits treated with ascorbic acid treatment @150 ppm, whereas, the untreated (control) fruits have exhibited the highest respiration rate (Table 3). Our results are in line with the work of Petri et al. (2008) who reported the decrease in respiration rate by the use of preservatives including ascorbic acid. A slight increase in respiration rate at higher

concentration of ascorbic acid may be directly correlated with the extent of disease incidence.

Soluble solid contents (°B)

It is evident from the data presented in Table 4 that there was significant difference in soluble solids content (SSC) of varieties, whereas, differences among the treatments were non-significant except for ascorbic acid @100 ppm treatment. Such variations in TSS among varieties may be attributed to genetic differences existing among them. Similarly, non-significant effect of ascorbic acid on TSS of mango may be due to fact TSS in mango is mainly due to dissolved sugars, and LB does not have any impact on sugar content. Moreover, ascorbic acid and browning are unrelated to TSS content; hence TSS remained unaffected by ascorbic acid treatment.

Total carotenoids

There was no significant effect of any concentration of ascorbic acid on total carotenoids in different mango varieties (Table 4). Although, varieties different significantly for total carotenoids, which might be due to genetic differences among them.

Table 4 Influence of postharvest ascorbic acid treatment on soluble solid contents and total carotenoids in mango fruits, stored at ambient conditions (35 ± 4 °C and 65 ± 5 % RH) for 10 days

Treatment	Soluble solids concentrates (°B)				Total carotenoids (mg/100 g pulp)			
	Dashehari	Langra	Sensation	Eldon	Dashehari	Langra	Sensation	Eldon
Control	20.4 ^a	18.2 ^a	17.6 ^a	16.3 ^a	5.25 ^a	3.62 ^a	2.92 ^a	4.16 ^a
Ascorbic acid @ 100 ppm	21.6 ^b	19.9 ^b	18.7 ^b	17.2 ^b	5.82 ^b	3.83 ^a	3.72 ^b	4.65 ^b
Ascorbic acid @ 150 ppm	21.2 ^b	19.5 ^b	18.2 ^b	17.0 ^b	5.62 ^b	3.96 ^a	3.84 ^b	4.62 ^b
Ascorbic acid @ 200 ppm	20.9 ^a	19.4 ^b	18.0 ^a	16.6 ^a	4.93 ^a	3.95 ^a	3.96 ^b	4.12 ^a

Means within the column with the same letter are not significantly different by Duncan's Multiple Range Test at $p \leq 0.05$

Conclusion

Postharvest dip treatment with different concentrations of ascorbic reduced the LB and improved the cosmetic appeal of all mango ('Dashehari', 'Langra', 'Sensation' and 'Eldon') varieties evaluated. Ascorbic acid @ 150 ppm was most effective in reducing LB and improving cosmetic appeal of the fruits. This treatment also delayed the onset of respiratory climacteric and inhibited the activities of PPO, POD and LOX enzymes without affecting the fruit quality. Hence, this treatment may be used for the management of LB and improving cosmetic appeal of mangoes.

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