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Effect of gibberellic acid and Vapor Gard on ripening, amylase and peroxidase activities and quality of mango fruits during storage

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SUMMARY

Post harvest application of gibberellic acid at 200 mg l^{-1}, Vapor Gard (di-1-p-menthene) at 2.5% and their combination was studied on 'Mallika' mangoes (*Mangifera indica* L.) stored at ambient temperature (37 ± 2° maximum and 34 ± 2°C minimum) and at 15°C. Significant delay in the ripening of mango fruits was observed when gibberellic acid was applied with or without Vapor Gard. Gibberellic acid significantly retarded the degradation of ascorbic acid and chlorophyll in the peel, and reduced α-amylase and peroxidase activities during storage. Loss of weight decreased following treatment with Vapor Gard either alone or with gibberellic acid during storage at both ambient temperature and at 15°C. A pronounced retardation of ripening was observed when fruits were treated with gibberellic acid and Vapor Gard and stored at 15°C. The study thus suggests that mango fruits can be successfully stored for 20 d by application of gibberellic acid (200 mg l^{-1}) in combination with Vapor Gard (2.5%) and stored at 15°C.

INDIA is a major producer of mangoes, with 62% of world production, a crop which has great export potential (Chadha, 1988). Mangoes are climacteric fruits with a short shelf-life of 6–8 d at ambient conditions (Kalra and Tandon, 1983) and are highly susceptible to chilling injury when stored below 8°C (Subramanyam *et al.*, 1975). Development of a method to delay ripening would therefore be of immense value in prolonging the shelf life and increasing the international marketing potential. Earlier, treatment with gibberellic acid (200 mg l^{-1}) as a post-harvest dip was reported to extend the shelf life by 2–3 d at ambient storage conditions (Khader *et al.*, 1988). Wax emulsions have also been reported to extend the shelf life of fruit of many cultivars of mango both at ambient and 14°C storage (Passam, 1982). Vapor Gard as postharvest treatment has been found useful in extending the vase life of leather leaf fern (Nell *et al.*, 1985). The present study investigates the effect of GA alone and with Vapor Gard (a water emulsifiable organic concentrate which acts as an antitranspirant) on ripening, activity of α-amylase and peroxidase and quality attributes during storage at ambient or 15°C.

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MATERIALS AND METHODS

Fruits of cv. Mallika hand picked at the mature, hard green stage from the experimental farm, Central Institute of Horticulture for Northern Plains, Lucknow, India during July 1988 and 1989 were divided into five lots to represent 0, 4, 8, 12 and 16 days after storage. Each lot was further divided into approximately 2 kg portions to receive four treatments, each replicated four times. The treatments included: gibberellic acid (Hi Media Laboratories, India) at 200 mg l^{-1}, Vapor Gard (VG), an antitranspirant, film forming organic compound (di-1-p-menthene) from Miller Chemical and Fertilizer Corporation, USA at 2.5%, GA at 200 mg l^{-1} in 2.5% VG, as well as an untreated control. Teepol at 0.1% was used as a surfactant in all treatments. Within two hours of harvest, treatments were given uniformly for 5 min by dipping fruit in the chemical solutions. The control lot was dipped in water with 0.1%

teepol only. The fruits were air-dried, wrapped in paper and kept in wooden boxes at ambient storage temperature of 37 ± 2°C maximum and 34 ± 2°C minimum with 50 ± 10% r.h. Fruit subjected to a similar set of treatments with six storage durations (0, 4, 8, 12, 16 and 20 d) was kept in a cold room at 15°C ± 1°C with 85 ± 5% r.h. During the second year (1989), the sample size was increased to 4 kg of fruits per treatment per replication with the same storage conditions as previously. Biochemical analysis were carried out at intervals during storage in 1988. Each lot was evaluated after 4, 8, 12, 16 and 20 d of storage to assess their ripening behaviour using the scoring technique of Ranganna (1977). The following four-point ripening index was used:

1 = Over-ripe with off-flavour;
2 = Yellow and soft with pleasant aroma;
3 = Yellow green and hard with mild aroma;
4 = Hard, green and unripe.

A slice of mesocarp was cut from each fruit of a treatment in each replication, homogenized in a blender, and stored at 4°C for biochemical determinations. Total soluble solids were measured using a hand refractometer, while total acidity and ascorbic acid content were estimated titrimetrically (Ranganna, 1977). The method of Smith and Benitiz (1955) was followed for estimating total chlorophyll content of the peel after extracting the chlorophyll in 80% acetone. Total carotenoid was estimated according to the method of Ranganna (1977), with some modifications. Five grams of pulp were ground in acetone (15 ml) and filtered through a cotton plug. A final washing was given in petroleum ether. The filtrate was clarified by transferring to a separating funnel containing 2% aqueous NaCl. The pigment layer was finally made up to 50 ml with 3% acetone in petroleum spirit. Absorbance was read at 452 nm using a Spectronic-21 (Milton Roy Company) using pure β-carotene (Sigma Chemicals, USA) as standard.

α-amylase activity was assayed by the modified procedure of Bernfeld (1955). Crude enzyme extract was prepared by homogenizing 5 g of pulp with 1 g of polyvinyle pyrrolidone and a pinch of potassium metabisulphite in 0.02 M phosphate buffer (pH 6.9) at 4°C, and filtered through four layers of muslin cloth. The final volume was made up to 50 ml. One ml of this crude enzyme extract was added to the reaction mixture containing 0.5 ml of 1.0% starch in 0.02 M phosphate buffer (pH 6.9) with 0.007 M NaCl, and incubated for 30 min at 37°C. The reaction was stopped by adding 1 ml of 3, 5-dinitrosalycilic acid dye followed by holding the tubes in boiling water for 5 min. The final volume was made to 10 ml and read at 546 nm. α-amylase activity was expressed as μ moles of maltose formed per gram of fresh weight per 30 min.

Peroxidase was assayed by the method of Luck (1963). The reaction mixture contained 5 ml phosphate buffer at pH 6.0, 1 ml of 0.1% H_2O_2 and 1 ml of 0.5% p-phenylenediamine dihydrochloride dye. The reaction was initiated by adding 1 ml of crude enzyme extract from 100 mg tissue, continued for 5 min and then arrested with 1 ml 5N H_2SO_4. The corresponding blank for each sample was run simultaneously for which 1 ml of 5N H_2SO_4 was added to the reaction mixture followed by addition of 1 ml crude enzyme extract. The absorbance was measured at 430 nm and enzyme activity was expressed as the difference in optical density between the sample and the corresponding blank for 100 mg tissue.

Analysis of variance was used on the data with mean separation at $P = 0.05$ using least significant difference (Panse and Sukhatme, 1961).

RESULTS

Ripening behaviour of fruits stored at ambient conditions

Ripening, as indicated by colour and aroma, was significantly delayed on days 8 and 12 of storage in treated fruits compared with control ones. The loss in weight of untreated fruits on day 8 was significantly higher than that of the treated fruits; GA_3 + VG treatment producing least weight loss. By day 12, untreated fruits had lost more weight, had a bright yellow colour and good aroma and by day 16 the fruits were in an unmarketable condition. Fruits treated with VG showed significantly delayed ripening up to day 12, but by day 16 these were comparable with the untreated fruits. GA_3 treated fruits maintained higher values of ripening index and weight loss and were superior to both control and VG treated fruits on day 12. When GA_3 was combined with VG,

TABLE I

Effect of GA_3 and VG on ripening index (R.I.) and weight loss of mango fruits stored for 8–16 days at ambient conditions and 8–20 days at 15°C

Treatment	Days of storage at ambient: R.I.			Weight loss (%)	Days of storage at 15°C: R.I				Weight loss (%)
	8	12	16	8	8	12	16	20	16
Control	2.14[a]	1.12[a]	1.00[a]	12.2[d]	3.00[a]	2.28[a]	1.70[a]	1.11[a]	12.0[c]
GA_3	2.47[b]	1.64[b]	1.12[a]	9.6[c]	3.69[b]	3.27[c]	2.30[c]	1.61[c]	8.9[b]
VG	2.46[b]	1.65[b]	1.00[a]	8.3[b]	3.48[b]	3.03[b]	1.96[b]	1.44[b]	7.5[a]
GA_3 + VG	2.46[b]	1.77[c]	1.41[b]	7.7[a]	3.72[b]	3.21[c]	2.46[c]	2.08[d]	7.4[a]

Values within columns followed by the same superscript are not significantly different at $P = 0.05$.

the effect was more pronounced (Table I), from the start of storage.

Ripening behaviour of fruits stored at 15°C

Fruits stored at 15°C maintained their hardness and green colour even on day 8 and showed higher ripening index values than those stored at ambient temperature. However, by day 12, untreated fruits started ripening. The external appearance and the ripening index of the untreated fruits on day 8 at ambient temperature was similar to that of fruits on day 12 at 15°C, suggesting that the lower temperature delayed ripening by at least four days. Fruits treated with GA_3, either alone or with VG behaved similarly up to day 16; the latter treatment maintained a better ripening index than all other treatments until the end of storage. Untreated fruits became over-ripe by day 20 while other fruits were still marketable. The weight loss on day 16 was significantly more in the control fruit than in those given GA_3 + VG and VG treatments.

Quality of fruits stored at ambient conditions

Total soluble solids and carotenoids increased, while total acidity, ascorbic acid and total chlorophyll decreased steadily with increasing duration of storage irrespective of treatment (Table II). Total soluble solids were significantly lower in fruit of all treatments than those of the control up to day 12, the lowest value following GA_3 + VG treatment. Values of total acidity were similar in both control and VG treated fruits while GA_3 + VG treated fruits maintained higher acidity during storage. Fruits treated with GA_3 either alone or with VG retained significantly more ascorbic acid during storage than did untreated fruits. Carotenoid development was faster in control fruits; treatments effects were significant from day 12 onwards. Fruits treated with GA_3 and GA_3 + VG had lower carotenoid concentrations than those treated with VG alone untreated. A similar trend was also observed in total chlorophyll content. As fruits were fully ripe by day 16, chlorophyll in the peel was not detectable in control fruits. In contrast, fruits treated with GA_3 and GA_3 + VG retained significantly more chlorophyll during storage, with detectable amounts even on day 16.

Quality of fruits stored at 15°C

Although quality followed the same trends as seen in fruit stored at ambient conditions, the rate of change was slower; total soluble solids on day 8 was 13% compared with 19% in control fruits at ambient conditions (Table II). Effects of GA_3 and GA_3 + VG were similar to other treatments until day 12. Thereafter the control and VG treated fruits had significantly more total soluble solids until the end of storage. Total acidity was higher during the initial stages particularly in the GA_3 + VG treated fruits. By the end of storage (day 20), VG and control fruits contained less acidity while fruits treated with GA_3 + VG had significantly more. Ascorbic acid was significantly influenced by GA_3 treatment either alone or in combination with VG throughout storage. Carotenoid concentration increased steadily in untreated fruits but was slower in treated fruits except that in VG treated fruits full carotenoid development was attained by day 16. By day 20, control and VG treated fruits had comparable level of carotenoids, significantly higher than those of the GA_3 and GA_3 + VG treated fruits. Chlorophyll content in the peel gradually declined as carotenoid developed during storage. GA_3 and GA_3 + VG treated fruits retained more chlo-

TABLE II
Effect of GA_3 and VG on quality of mango fruits during storage at ambient and 15°C

Treatments	Days after storage at ambient condition				Days after storage at 15°C				
	0	8	12	16	0	8	12	16	20
Total soluble solids (%)									
Control	7.9[a]	19.4[a]	20.3[d]	20.1[b]	8.0[a]	14.3[c]	17.0[b]	20.0[d]	20.6[d]
GA	8.0[a]	17.3[b]	18.5[b]	19.1[a]	7.9[a]	12.4[a]	14.7[a]	17.3[b]	19.6[b]
VG	7.8[a]	17.6[b]	19.3[c]	19.6[ab]	7.9[a]	13.0[b]	17.0[b]	18.2[c]	19.9[c]
GA + VG	8.0[a]	16.1[a]	17.1[a]	19.2[a]	8.0[a]	12.4[a]	14.4[a]	16.0[a]	19.0[a]
Total acidity (as % citric acid)									
Control	2.00[a]	1.13[a]	0.50[a]	0.19[b]	1.96[a]	1.48[a]	1.06[a]	0.53[a]	0.21[a]
GA	2.08[a]	1.29[b]	0.71[b]	0.21[c]	2.00[a]	1.62[b]	1.19[b]	0.67[b]	0.32[b]
VG	1.92[a]	1.19[a]	0.56[a]	0.17[a]	1.92[a]	1.58[b]	1.33[c]	0.56[a]	0.26[a]
GA + VG	1.96[a]	1.32[b]	0.81[c]	0 24[d]	1.96[a]	1.72[c]	1.25[d]	0 69[b]	0.43[c]
Ascorbic acid (mg 100 g^{-1})									
Control	48.3[a]	38.0[a]	22.3[a]	14.8[a]	52.5[a]	40 9[a]	26.7[a]	20.3[a]	16.1[a]
GA	52.0[a]	41.8[b]	27.5[c]	19.2[b]	49.5[a]	46.4[b]	32.2[c]	25.5[c]	21.4[c]
VG	49.5[a]	41.1[b]	25.2[b]	18.8[b]	53.3[b]	43.2[a]	30.2[b]	22.1[b]	17.7[b]
GA + VG	52.1[a]	43.3[c]	27.7[c]	20 1[c]	52.7[a]	47.1[b]	33.0[d]	29.2[d]	25.3[d]
Carotenoids (mg 100 g^{-1})									
Control	0.34[a]	2.18[c]	4.12[b]	4.00[b]	0.31[a]	1.65[d]	3.29[b]	3.69[b]	4.00[b]
GA	0.39[a]	2.10[b]	3.28[a]	3.75[a]	0.35[a]	1.36[b]	3.11[bc]	3.39[a]	3.75[ab]
VG	0.35[a]	2.06[b]	4.18[b]	3.97[b]	0.36[a]	1.50[c]	2.72[a]	4.02[c]	3.97[b]
GA + VG	0.37[a]	1.76[a]	3.26[a]	3.55[a]	0.35[a]	1.23[a]	2.90[b]	3.56[a]	3.55[a]
Total chlorophyll (mg l^{-1})									
Control	0.247[a]	0.203[a]	0.079[a]	–	0.256[a]	0.224[a]	0.202[a]	0.087[a]	–
GA	0.262[a]	0.228[b]	0.108[b]	0.030	0.258[a]	0.250[b]	0 220[b]	1.110[b]	0.024
VG	0.258[a]	0.207[a]	0.083[a]	–	0.257[a]	0.224[a]	0.192[a]	0.082[a]	0.020
GA + VG	0.256[a]	0.249[c]	0.121[c]	0.047	0.264[a]	0.264[c]	0.264[c]	0 127[c]	0.039

Values within columns followed by the same superscript are not significantly different at $P = 0.05$.

rophyll in the peel at all stages of storage with traceable amounts even on day 20, in contrast to control and VG treated fruits.

α-amylase and peroxidase activities of fruits stored at ambient conditions

α-amylase and peroxidase activities in all treatments attained peak values on day 12, then declined (Table III). The activity of these two enzymes was low in GA_3 and GA_3 + VG treated fruits. By day 16, the values were higher than in the control fruit in these treatments when fruits were ripe.

α-amylase and peroxidase activities of fruits stored at 15°C

Although enzyme activity was at its maximum by day 12 as with fruits stored at ambient conditions, the decrease in activity thereafter was slower (Table III). GA_3 and GA_3 + VG treated fruits had lower enzyme activities until day 16, when values were comparable with

TABLE III
Effect of GA_3 and VG on α-amylase and peroxidase activity during storage of mango fruits at ambient conditions and 15°C

Treatments	Days after storage at ambient				Days after storage at 15°C				
	0	8	12	16	0	8	12	16	20
α-amylase (μ moles maltose g^{-1} 30 min^{-1})									
Control	88[a]	204[d]	374[d]	84[a]	87[a]	115[b]	274[c]	235[d]	120[a]
GA	82[a]	165[c]	337[b]	98[a]	88[a]	107[a]	238[b]	190[b]	165[c]
VG	87[a]	144[a]	352[c]	86[a]	94[a]	113[b]	256[c]	214[c]	130[b]
GA + VG	83[a]	158[b]	317[a]	139[b]	89[a]	104[a]	216[a]	171[a]	166[c]
Peroxidase (Δ OD 100 mg^{-1})									
Control	0.021[a]	0.043[bc]	0 092[bc]	0.049[a]	0.023[a]	0.042[b]	0.125[c]	0.084[a]	0.074[b]
GA	0.023[a]	0.034[ab]	0.085[b]	0.060[b]	0.026[a]	0.038[b]	0.092[a]	0 092[b]	0.082[c]
VG	0.028[a]	0.038[b]	0.100[c]	0.050[a]	0.022[a]	0.041[b]	0.113[b]	0.080[a]	0.065[a]
GA + VG	0 025[a]	0 028[a]	0.072[a]	0 064[b]	0.023[a]	0.025[a]	0.100[d]	0.079[a]	0.069[a]

Values within columns followed by the same superscript are not significantly different at $P = 0.05$.

those of day 12 in similar treatments at ambient storage conditions.

DISCUSSION

Irrespective of treatment, the findings in general showed that total soluble solids, carotenoid accumulation and α-amylase and peroxidase activities tended to increase, while ascorbic acid, acidity and chlorophyll content in the peel decreased during storage in conformity with previous reports (Subramanyam *et al.*, 1975; Kalra and Tandon, 1984). Ripening was influenced by temperature with untreated fruits attaining edible ripeness in eight days under ambient condition, compared with 12 days at 15°C. Low temperature may also reduce respiration, which in turn suppresses fruit metabolic activity (Subramanyam *et al.*, 1975). Amylase and peroxidase activities were found to peak on day 12 during storage at both ambient and 15°C, although the decline in activity was rapid in the former and more gradual in the latter.

GA_3 treated fruits exhibited a significant delay in ripening as evidenced by colour and aroma and also their biochemical constituents during storage. This agrees with an earlier report that a GA_3 dip at 200 ppm extended the shelf life of mango fruit (Khader *et al.*, 1988) by retarding ascorbic acid decrease and chlorophyll degradation and decreasing enzyme activities.

It has been also reported that the storage life of fruits can be extended by post-harvest application of wax and oil emulsions in gooseberry (Gupta and Mukherjee, 1982) banana (Banks, 1984) and mango (Passam, 1982). In the present investigation, Vapor Gard retarded biochemical changes and subsequently retarded ripening until day 12 in fruits stored at ambient and day 16 at 15°C. Passam (1982) observed that the film coating by Vapor Gard on fruit peel possibly suppresses metabolic processes associated with ripening, by impeding the diffusion of gases. He also observed that wax at a concentration of 5% solids or higher applied to mango fruits reduced the rate of weight loss and retarded ripening. Such treatments, however, affect flavour and quality severely during storage. Probably a higher dose of wax restricts gaseous exchange and induces anaerobic fermentation. Similar effects of low O_2 or high CO_2 concentration have also been reported during storage of mangoes under modified atmospheric storage (Laxminarayanan and Subramanyam, 1970). Preliminary results in the present study suggest that the optimum concentration of Vapor Gard is 2.5%, and that doses at 5% and above result in early fruit senescence with off flavours.

The present and previous findings (Khader *et al.*, 1988) indicate that GA_3 delays ripening of mango fruits by retarding degradation of chlorophyll and ascorbic acid, slowing carotenoid accumulation, and reducing the activities of α-amylase and peroxidase. GA_3 treated fruits also show less weight loss than control fruits, probably by impeding ethylene biosynthesis, which triggers ripening (Burg and Burg, 1962). Vapor Gard treatment on the other hand physically restricts inward diffusion of oxygen during the development of climateric, when oxygen demand of tissues increases (Banks, 1984). The present study suggests that combinations of GA_3 and Vapor Gard are more effective in delaying ripening than either chemical alone. Vapor Gard as a film on fruits, in addition to affecting ripening as discussed above, might increase the effectiveness of GA_3 by retaining it for longer. The finding that storage at 15°C after treatment with GA_3 + Vapor Gard would prolong the storage life of mango fruits is worth large scale investigation for its commercial feasibility.

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