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Effect of Ethrel and 1-methylcyclopropene (1-MCP) on antioxidants in mango (*Mangifera indica* var. Dashehari) during fruit ripening

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ABSTRACT

Ripening affects the quality and nutritional contents of fleshy fruits. Mango, a climacteric fruit, is very susceptible to post-harvest losses, due to fast softening. In the present paper we report the effect of 1-methylcyclopropene (1-MCP) and Ethrel on antioxidant levels in mango fruit during ripening. Use of 1-MCP is applied commercially to delay ripening while Ethrel is used to accelerate ripening of climacteric fruits. 1-MCP treatment led to decreased levels of H_2O_2 and lipid peroxidation, concomitant with increased activities and isozymes of catalase (CAT) and superoxide dismutase (SOD), as compared to respective controls. On the other hand, Ethrel treatment led to an increase in H_2O_2 and lipid peroxidation, concomitant with a decrease in the activities and isozymes of catalase and SOD. Guaiacol peroxidase (GPX) could not be detected in the control or in treated fruits. Activity of ascorbate peroxidase (APX) was found to drastically increase in the presence of Ethrel while 1-MCP treatment led to only a marginal increase in APX.

1. Introduction

Fruit ripening has been described as an oxidative phenomenon that requires a turnover of active oxygen species, such as H_2O_2 and superoxide anion (Jimenez et al, 2002). The role of dietary antioxidants is to help fight excessive reactive oxygen species (ROS) in our bodies. By doing so, antioxidants are sacrificed to protect biomolecules from being oxidised and thus the antioxidant has fulfilled its function. Fruits play a significant role in the human diet by providing protection against cellular damage, caused by exposure to high levels of free radicals, while also aiding in digestion. This protection can be explained by the capacity of antioxidants in the fruits to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins, and nucleic acids (Halliwell & Gutteridge, 1984). Ripening of fleshy fruits affects their quality and nutritional contents. Climacteric fruits such as banana, tomato, pear, papaya and mango exhibit these changes very quickly. Thus, such fruits are more susceptible to post-harvest losses, due to fast ripening caused by the ripening trigger ethylene, which in turn, could come from any climacteric ripe fruit stored in the same environment with any climacteric green fruit. Therefore, to minimise these effects, fruits must be stored away from ethylene sources or the hormone level in the atmosphere should be decreased by oxidation with potassium permanganate or ultra-violet light, although these approaches have limited commercial application. Recently, 1-methylcyclopropene (1-MCP) has been employed in attempts to increase the shelf life of some climacteric fruits. By binding to the ethylene receptor, 1-MCP acts as an efficient ethylene antagonist and its effects can persist for a long time (Sisler, Alwan, Goren, Serek, & Apelbaum, 2003). Commercialisation of 1-MCP has mostly occurred for apple and banana fruits. These fruits responded extremely well to 1-MCP, showing inhibition of ethylene production, respiration rates, maintenance of firmness and other quality aspects both during and after storage (Itai, Tanabe, Tamura, & Tanaka, 2000; Watkins, Nock, & Whitaker, 2000). Ethylene released by the breakdown of Ethrel® is the cause of softening of fruit and hastens the onset of ripening of several fruits, including mango, as reported in our previous study (Rupinder, Poorinima, Pathak, Singh, & Dwivedi, 2007).

The antioxidant system includes catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD, EC 1.15.1.1), guaiacol peroxidase (GPX, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) (Miyake & Asada, 1996). The function of natural antioxidants in foods and biological systems has received much attention. Mango (*Mangifera indica*) var. Dashehari is a fruit of prime economic importance to India. This paper reports modulation of H_2O_2 levels and lipid peroxidation and activities, as well as the isozymic profile of several enzymatic antioxidants namely, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) in response to 1-MCP and Ethrel-treated fruits. A complete series of biochemical methods was carried out in this study and it is anticipated that results arising from this study will help to clarify the possible

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inter-relationships between chemicals in regulating the antioxidant activity. There are still considerable lacunae in our knowledge regarding the fate of antioxidants during the process of ripening, which requires further studies to explore their significance.

2. Materials and methods

2.1. Plant material

Mango (*M. Indica*) var. Dashehari fruits were collected from the Central Institute of Subtropical Horticulture (CISH) orchard, Lucknow, India. Mature unripe fruits free from disease were selected, washed with distilled water, and air dried; pulp and peels of the samples were taken for analysis. Three independent experiments were carried out for each chemical treatment and from a single batch of each treatment three aliquots were taken for analysis. Furthermore, 10 individual fruits were employed for each set of chemical treatments. Starting with day 0 (i.e. immediately after treatment) till day 11 (at specified time periods, as indicated in figures), one fruit from each of the control and treatments were taken and various analyses were performed.

2.2. Chemical treatments

Concentrations of 1-MCP (2 mg kg^{-1}) and Ethrel Ethephon, plant regulator, (2-chloroethyl phosphoric acid; 750 mg kg^{-1}), used in the present study, were standardised previously (Rupinder et al., 2007).

2.2.1. 1-MCP treatment

Ten fruits were placed in 20-l containers and exposed to 1-MCP (2 mg kg^{-1}) for 12 h at room temperature (RT) and 85% relative humidity (RH). Immediately following 1-MCP treatment, fruit was removed from the chambers, and placed in cardboard boxes with holes. Control fruit was maintained in identical containers without 1-MCP at room temperature.

2.2.2. Ethrel treatment

Fruits were dipped uniformly 1.8 ml l^{-1} Ethrel in hot water at 52 ± 2 °C for 5 min. Fruits were air dried and placed in cardboard boxes with holes.

2.3. Lipid peroxide measurement

About 200 mg fresh tissue was grounded in 5 ml of 10% trichloroacetic acid (TCA) containing 0.25% 2-thiobarbituric acid (TBA) and heated at 95 °C for 25 min. The mixture was quickly cooled in an ice bath and after centrifugation at 10,000*g* at 4 °C for 10 min, the absorbance of supernatant was read at 532 nm and corrected for unspecific turbidity by subtracting the absorbance of the same at 600 nm. The blank was 0.25% TBA in 10% TCA (Heath & Packer, 1968). Thiobarbituric acid-reactive substances (TBARS) were used as an index of lipid peroxidation. An extinction coefficient of 155 mM^{-1} cm^{-1} was used to quantify the concentration of lipid peroxides together with the oxidatively-modified proteins and expressed as nmol g^{-1} fresh weight.

2.4. Hydrogen peroxide (H_2O_2) measurement

About 150 mg of mango pulp samples were extracted using 3 ml of 50 mM sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 10,000*g* at 4 °C for 15 min. To determine H_2O_2 levels, 3 ml of extracted solution were mixed with 1 ml of 0.1% titanium sulphate in 20% (w/v) H_2SO_4 and mixture was then centrifuged at 6000*g* for 15 min. The intensity of the yellow colour of the supernatant was measured at 410 nm (Singh & Choudhuri, 1990). H_2O_2 level was calculated using the extinction coefficient 0.28 mM^{-1} cm^{-1} and was expressed as nmol $^{-1}$ g^{-1} fresh weight.

2.5. Enzyme assays

2.5.1. Superoxide dismutase

For the extraction of superoxide dismutase (SOD) about 200 mg of mango pulp was homogenised using prechilled mortar and pestle in 5 ml of 100 mM potassium phosphate buffer (pH 7.5) containing 1.0 mM EDTA, 0.1 mM Triton X-100 and 2% polyvinyl-pyrrolidone (PVP). Contents were centrifuged at 12,000*g* at 4 °C for 10 min and supernatant was collected. The assay mixture contained 50 mM sodium carbonate–bicarbonate buffer (pH 9.8), containing 0.1 mM EDTA, 0.6 mM epinephrine and 50 μl enzyme in a total volume of 3 ml. Epinephrine was the last component to be added (Misra & Fridovich, 1972). The adrenochrome formation over the next 3 min was recorded at 475 nm in a UV–Vis spectrophotometer (Genesys). One unit of SOD activity is expressed as the amount of enzyme required to cause 50% inhibition of epinephrine oxidation under the experimental conditions.

2.5.2. Catalase

The catalase activity was assayed by Aebi (1983) with slight modifications. Two hundred milligrammes of mango pulp were homogenised using a prechilled mortar and pestle in 5 ml of 50 mM Tris-HCl (pH 8.0), containing 1.0 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM Triton X-100 and 2% PVP. Contents were centrifuged at 12,000*g* at 4 °C for 10 min. The assayed medium consisted of sodium phosphate buffer (50 mM, pH 7.0), H_2O_2 (20 mM) and 100 μl enzyme in a total volume of 3.0 ml. The degradation of H_2O_2 was measured by the decrease in absorbance at 240 nm using a Genesys (Thermo Fisher Scientific, Waltham, MA) spectrophotometer. The molar extinction coefficient of H_2O_2 at 240 nm was taken as 0.036 mM^{-1} cm^{-1}. One unit of enzyme activity was defined as amount of enzyme catalysing the decomposition of 1 μmol H_2O_2 per minute at 30 °C.

2.5.3. Ascorbate peroxidase

About 200 mg of fruit pulp was extracted in 5 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM ascorbic acid, 1 mM EDTA and 2% PVP added fresh just prior to use. The assay mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.2 mM ascorbic acid, 0.2 mM EDTA, 20 μM H_2O_2 and 50 μl enzyme extract in a total volume of 3 ml. H_2O_2 was the last component to be added and the change in absorbance was measured at 290 nm (extinction coefficient of 2.8 mM^{-1} cm^{-1}). Enzyme specific activity was expressed as μmol ascorbate oxidised mg^{-1} of protein min^{-1} (Nakano & Asada, 1987).

2.5.4. Guaiacol peroxidase

The guaiacol peroxidase (GPX) activity was assayed by the method of Pütter (1974) with slight modifications, using guaiacol as substrate. The assay mixture contained sodium phosphate buffer (50 mM: pH 7.0), H_2O_2 (0.067%), guaiacol (3.33 mM) and 50 μl enzyme in a final volume of 3 ml. Tetraguaiacol formation was monitored spectrophotometrically by measuring the increase in absorbance at 470 nm. The molar extinction coefficient of tetraguaiacol was taken as 6.39 cm^2/μmol. One unit of enzyme activity was defined as the amount of enzyme catalysing the production of one μmol of tetraguaiacol formed per min.

2.6. Isozyme analysis

Native polyacrylamide gel electrophoresis (PAGE) was performed, as described by Davis (1964), with separating gel (10%)

overlaid with a stacking gel (3%). Equal quantity of protein was loaded in all the wells of the gel (0.75 mm thick) and the electrophoresis was performed using Tris glycine buffer system (pH 8.0) at 4 °C at 30 mA. In gel activity staining for different enzymes was done as described below.

2.6.1. Superoxide dismutase

In-gel activity staining for SOD was carried out, as described by Fridovich (1989). Gel was rinsed in distilled water followed by a 30 min incubation in 2.5 mM nitroblue tetrazolium (NBT). Gels were then immersed in 1.17 μm riboflavin for 20 min and later removed to a petri dish for irradiation with a fluorescent lamp. Light exposure led to the development of the purple colour of insoluble formazan throughout the gel, except for the locations where SOD was localised by negative staining.

2.6.2. Catalase

For catalase, in-gel activity staining was carried out according to the method of Wayne and Diaz (1986). After electrophoresis, the gel was washed with water and incubated in H_2O_2 (0.003%) for 2 min. After incubation the gel was washed again with cold water and stained with ferric chloride (0.4%) and potassium ferricyanide (0.4%), until the gel turned blue. Catalase activity bands were visualised as clear zones on a blue background (negative staining).

2.6.3. Ascorbate peroxidase

In-gel activity staining for ascorbate peroxidase was done as described by Nakano and Asada (1987). After electrophoresis, gels were rinsed in distilled water and incubated for 15 min at room temperature in 0.1 M potassium phosphate buffer (pH 6.4) containing 4 mM ascorbate and 4 mM H_2O_2. Gels were then rinsed with water and stained in a solution of 0.125 N HCl containing 0.1% potassium ferricyanide and 0.1% ferrichloride (w/v). Isozymes of APX were visualised as colourless bands on a greenish background (negative staining).

2.6.4. Guaiacol peroxidase

In-gel activity staining for guaiacol peroxidase was performed as described by McDougall (1991). The gel was incubated in sodium phosphate buffer (100 mM, pH 7.0). The peroxidases bands were visualised by incubating the gel in a solution containing guaiacol (10 mM) and H_2O_2 (0.2%). Peroxidase isozymes were visualised as brick-red bands.

2.7. Protein estimation

Soluble protein concentration was measured according to the method of Bradford (1976), using bovine serum albumin as standard.

2.8. Statistical analysis

Statistical analysis of data was performed by ANOVA using Prism software (GraphPad Software Inc., San Diego, CA).

3. Results

Mango is a climacteric fruit. In our earlier studies we had demonstrated that mango ripening is associated with a respiratory burst on day 3 (Rupinder et al., 2007). Accordingly, we have divided the ripening process into preclimacteric (0–3 days) and postclimacteric (3–11 days). The results of the present investigation have been described in this context.

3.1. Effect of Ethrel and 1-MCP on lipid peroxidation during mango fruit ripening

Lipid peroxidation during mango fruit ripening, in the absence and presence of Ethrel and 1-MCP, was investigated and results are shown in Fig. 1. Lipid peroxidation in control mango fruit was found to increase gradually during ripening under both preclimacteric and postclimacteric periods. Ethrel treatment, on the other hand, led to inhibition of lipid peroxidation during the preclimacteric period while a rapid increase in lipid peroxidation during the postclimacteric period, as compared to the control, was observed during mango fruit ripening. Thus, there was a 1.39-fold increase in LPO during the postclimacteric period in Ethrel-treated mango. In the case of 1-MCP treatment, only a marginal increase in LPO was observed throughout ripening, though the net LPO was much lower than that of the control.

3.2. Effect of Ethrel and 1-MCP on H_2O_2 levels during mango fruit ripening

H_2O_2 production during mango fruit ripening was measured in the absence and presence of Ethrel and 1-MCP. Data are shown in Fig. 2. H_2O_2 production was found to increase gradually throughout mango fruit ripening. However, Ethrel treatment led to several-fold increase in H_2O_2 production during mango fruit ripening, while 1-MCP treatment led to a minor increase in H_2O_2 production, as compared to that of the control. Thus, a 2.18-fold increase in H_2O_2 production was observed in Ethrel-treated fruit as compared to the control in the postclimacteric period.

3.3. Effect of Ethrel and 1-MCP on SOD activity and its isozymes during mango fruit ripening

Investigation of SOD activity during mango fruit ripening, in the absence and presence of Ethrel and 1-MCP, revealed that in the control, as well as the Ethrel-treated mango, the SOD activity decreased gradually throughout ripening. Though the decrease in SOD activity in the presence of Ethrel was more pronounced (5.71-fold) compared to the control (2.78), as shown in Fig. 3. 1-MCP had the opposite effect to that of Ethrel as, in the presence of 1-MCP, SOD activity increased throughout ripening with a 1.36-fold increase. SOD activity was inhibited by cyanide but not by chloroform-ethanol, indicating a cupro-zinc enzyme. Isozyme

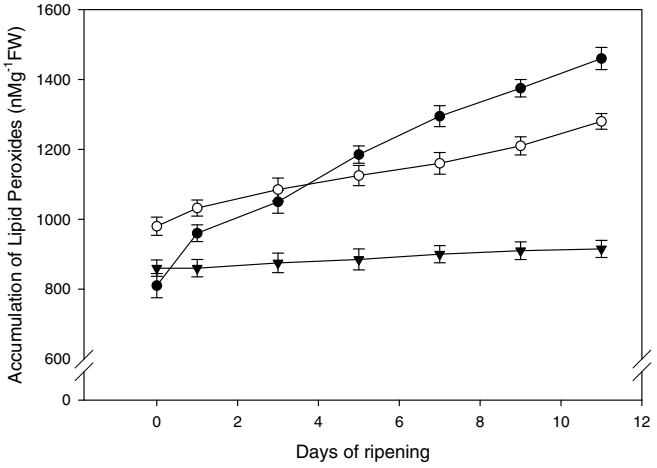

Fig. 1. Lipid peroxidation during ripening of mango fruit in the absence (○) and presence of Ethrel (●) and 1-MCP (▼) at room temperature. Each value represents a means ± SD of three independent experiments of each in triplicate. The data were analysed by Newman–Keuls multiple method and found to be significant ($p < 0.05$).

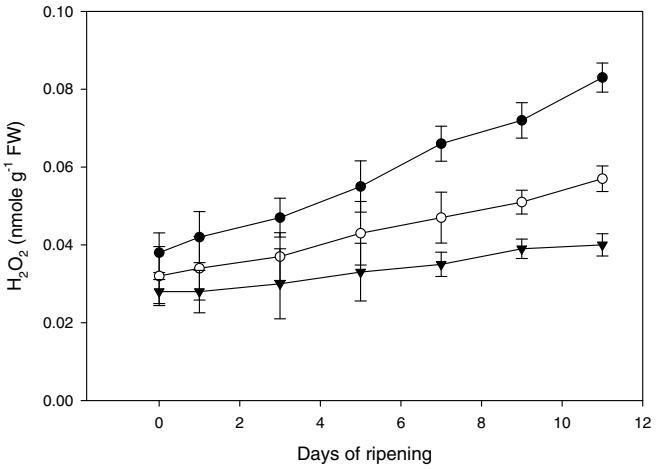

Fig. 2. H_2O_2 production during ripening of mango fruit at room temperature in the absence (○), and presence of Ethrel (●) and 1-MCP (▼). Each value represents a means ± SD of three independent experiments of each in triplicate. The data were analysed by Newman–Keuls multiple method and found to be significant ($p < 0.05$).

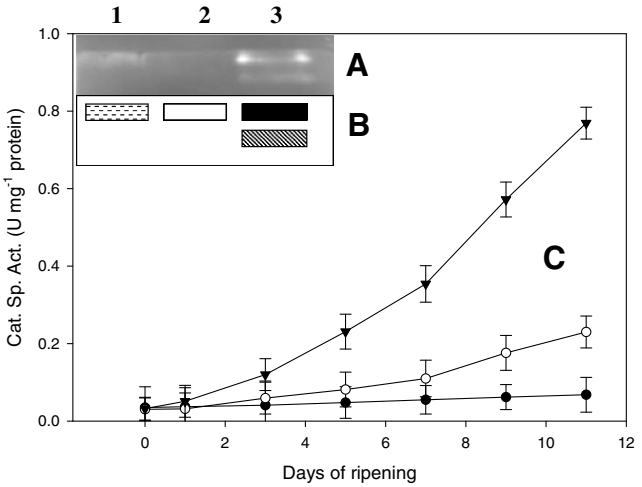

Fig. 4. Inset (panels A and B): Isozyme analysis of catalase on day 11 of mango fruit ripening in the absence (lane 1), and presence of Ethrel (lane 2) and 1-MCP (lane 3). PAGE and in-gel activity staining were performed as described in Section 2. Figure in panel A depicts the photograph of the gel while panel B depicts the zymogram for the same (▨ ▩ □ ■) represent the relative intensities of the bands as +4, +3, +2, +1, respectively). Panel C: Catalase activity during ripening of mango fruit at room temperature in the absence (○), and presence of Ethrel (●) and 1-MCP (▼). Each value represents a means ± SD of three independent experiments of each in triplicate. The data were analysed by Newman–Keuls multiple method and found to be significant ($p < 0.05$).

analysis of SOD revealed 3 isozymes, namely (I, III and IV) in the control fruit. Ethrel treatment led to the disappearance of isozyme (I) and the appearance of isozyme (II) while other isozymes (III and IV) persisted. 1-MCP led to the reappearance of isozyme (I), while other isozymes (II, III and IV) also persisted. Intensities of bands varied from treatment to treatment. Data on activity measurement and isozyme profiling are in agreement with each other.

3.4. Effect of Ethrel and 1-MCP on catalase activity and its isozymes during mango fruit ripening

Investigation of the catalase activity during mango fruit ripening in absence and presence of Ethrel and 1-MCP revealed that catalase activity increased gradually throughout mango fruit ripening in the control, with a 7.66-fold increase from days 0 to 11 (Fig. 4). Ethrel treatment led to a decrease in catalase activity that remained almost constant throughout ripening, as compared to control. 1-MCP treatment, on the other hand, led to a rapid increase in catalase activity throughout ripening of mango. Thus, there was a 24-fold increase in catalase activity with 1-MCP treatment between days 0 and 11. Our data on the catalase activity was further corroborated by the data on isozyme analysis of catalase. Thus, in control mango only one isozyme (I) of catalase was observed. Ethrel treatment led to a slight decrease in the activity of this isozyme (I) as evident by a relatively less intense, as compared to that of control. On the other hand 1-MCP treatment led to the appearance of one additional isozyme of catalase (II), with increased activity of isozyme (I), as compared to control (Fig. 4).

3.5. Effect of Ethrel and 1-MCP on ascorbate peroxidase activity and its isozymes during mango fruit ripening

The APX activity of mango fruit increased gradually during ripening in both treated and untreated controls. In 1-MCP-treated

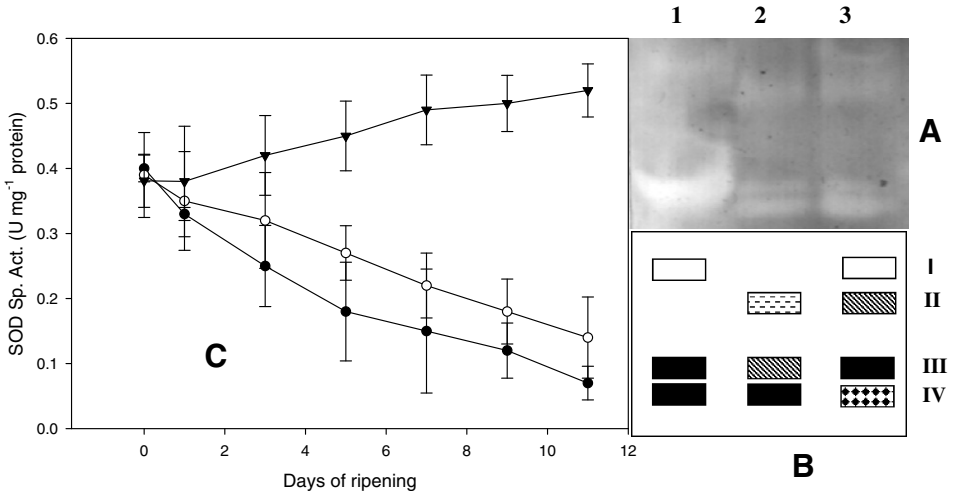

Fig. 3. Inset (panels A and B): Isozyme analysis of SOD on day 11 of mango fruit ripening in the absence (lane 1), and presence of Ethrel (lane 2) and 1-MCP (lane 3). PAGE and in-gel activity staining were performed as described in Section 2. Figure in panel A depicts the photograph of the gel while panel B depicts the zymogram for the same (▦ ■ ▨ ▩ □) represent the relative intensities of the bands as +5, +4, +3, +2, +1, respectively). Panel C: SOD activity during ripening of mango fruit at room temperature in the absence (○), and presence of Ethrel (●) and 1-MCP (▼). Each value represents a means ± SD of three independent experiments of each in triplicate. The data were analysed by Newman–Keuls multiple method and found to be significant ($p < 0.05$).

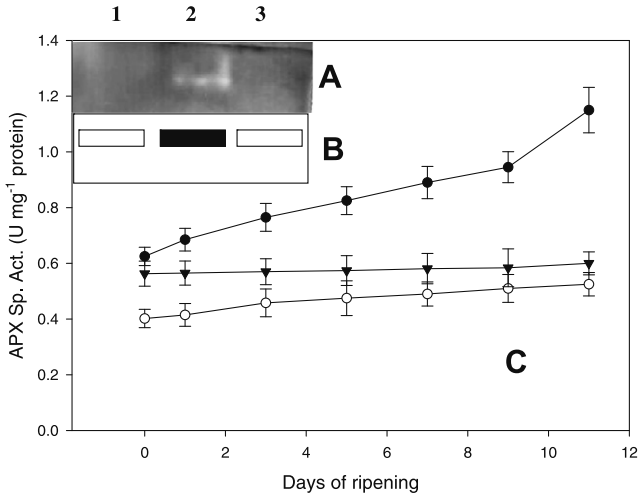

Fig. 5. Inset (panels A and B): Isozyme analysis of APX on day 11 of mango fruit ripening in the absence (lane 1), and presence of Ethrel (lane 2) and 1-MCP (lane 3). PAGE and in-gel activity staining were performed, as described in Section 2. Figure in panel A depicts the photograph of the gel while panel B depicts the zymogram for the same (▬ ▭) represent the relative intensities of the bands as +4, +1, respectively. Panel C: APX activity during ripening of mango fruit at room temperature in the absence (○), and presence of Ethrel (●) and 1-MCP (▼). The data were analysed by Newman–Keuls multiple method and found to be significant ($p < 0.05$).

fruits, there was only marginal increase in APX activity during ripening, as compared to control, while in Ethrel-treated fruit, a rapid increase in APX activity, as compared to that of the control, was observed (Fig. 5). Thus, 1-MCP-treated fruit showed only a 1.06-fold increase in APX activity, while Ethrel treated fruit showed a 1.84-fold increase in APX, as compared to the control. APX isozyme analysis revealed one isozyme in the control and treated mango fruit during ripening. Although there was no change in the isozyme numbers a more intense isozyme band in Ethrel-treated fruit was observed, in agreement with our data on activity measurement of APX.

3.6. Effect of Ethrel and 1-MCP on guaiacol peroxidase activity and its isozymes during mango fruit ripening

A noteworthy result of this study was that guaiacol peroxidase activity was observed neither in control nor in treatments. The apparent absence of the enzyme is puzzling in light of all other peroxidases being present in considerable amounts.

4. Discussion

The primary objective of this study was to assess antioxidant levels, with reference to commercially-used chemical treatments for post-harvest management in mango, as these have been scarcely investigated until now. The antioxidant enzyme activity of cells is determined by inherent characteristics of the cells, their metabolic specialisation and environmental factors to which the cells are exposed, such as the level of oxygenation and the presence of metabolites. Antioxidant enzymes exhibit synergistic interactions by protecting each other from specific free radical attacks. SOD protects catalase from inactivation by superoxide radicals, whereas catalase and glutathione peroxidase protect SOD from inactivation by hydroperoxides (Blum & Fridovich, 1985). However, there could be other factors that play a role in oxidative stress-related enzymatic activities, because in a biological system the observed effect of an enzyme would thus be the net effect all synergistic and antagonistic effects of other enzymes and compounds present in the cellular environment.

In the present study, the following two general conclusions were drawn: (a) the antioxidant enzyme activity of mango varied considerably, according to the treatment, hence, this aspect of quality could be improved by treatment selection; (b) a considerable loss of antioxidant fraction occurs during ripening. The results obtained in the present study suggest that not only are these antioxidants the main components of the fruit mesocarp, but they seem to be also major constituents of the proteins of the mesocarp (Zamora, Alaiz, & Hidalgo, 2001). Recent years have witnessed a plethora of reports correlating increases in one or more of the antioxidant enzymes under stress conditions. This interest is based on the early observations noted in apples that chilling injury, superficial scald, CO_2 injury along with colour change, softening, activities of polyphenoloxidase and lipid peroxidation were lower in diphenylamine (DPA) treated fruits than in untreated ones (Lurie, Klein, & Ben-Arie, 1989).

Changes in oxidative stress during fruit development can be assessed by the extent of lipid peroxidation. Accumulation of lipid peroxidation products is indicative of increasing oxidative stress during the ripening phase of fruit development. TBARS are produced from the spontaneous decomposition of lipid hydroperoxides and are regarded as sensitive markers of peroxidative damage. Previous work on lipid peroxidation and expression of one member of the lipoxygenase gene family in kiwi fruit, suggests that lipid peroxidation and expression increase with ethylene-induced fruit ripening (Zhang et al., 2006). Interestingly, this effect was also observed in our study, where ethylene released by decomposition of Ethrel had resulted in a significant increase in lipid peroxidation. Superoxide free radicals and hydroperoxides produced *via* the lipoxygenase pathway not only take part in peroxidation of cell membrane lipids, but also might be involved in other aspects of cell degradation. Lipid peroxidation has been shown to increase in conjunction with ripening processes such as loss of firmness in kiwi fruit (Zhang et al., 2006).

Hydrogen peroxide present in aerobic cells acts as a metabolite at low concentrations, generated by non-enzymatic and superoxide dismutase-catalyzed dismutation reactions. It is of interest that application of Ethrel leads to formation of peroxides. Conversely, H_2O_2 generated from it may stimulate ethylene synthesis, which in turn leads to autocatalytic synthesis of ethylene during fruit ripening (Elstner, 1991). Our results showed that a rapid increase and accumulation of H_2O_2 occurred to damaging levels at full ripeness in Ethrel-treated sample. On the other hand, 1-MCP treatment showed negligible accumulation of H_2O_2, which may be due to high catalase activity, which decomposes H_2O_2 to water and molecular oxygen without consuming reductants and, thus, may provide plant cells with an energy-efficient mechanism to remove H_2O_2 (Scandalios, Guan, & Polidoros, 1997).

Catalase, a porphyrin-containing enzyme, is a typical enzyme of peroxisomes that may also exhibit pseudoperoxidative activity under certain conditions (Elstner, 1991) and is considered to be one of the most important enzymes in protecting oxidatively challenged tissues. The results obtained in the present study revealed that CAT activities were highest in mature green fruit (preclimacteric), but declined as fruit developed to the fully ripe stage (postclimacteric). CAT activities (protein basis) were higher in mature green *Amelanchier alnifolia* fruit but declined as fruit developed to the fully ripe stages (Rogiers, Kumar, & Knowles, 1998), which was similar to our results. It is believed that increasing oxidative stress, which probably results from lower activity of catalase, is evidently needed to facilitate many of the metabolic changes associated with maturation and ripening of mango fruit. The results presented here together with others already published (Fu, Cao, Li, & Lin, 2007) have shown that the activity of CAT is higher in 1-MCP-treated pear fruits than in the control fruits. The beneficial effect of 1-MCP on improving post-harvest quality and reducing physiological

disorders might be due to its ability to increase the antioxidant potential as well as to delay fruit ripening along with senescence.

Similar to that of catalase, SOD activity was found to decrease during the fruit ripening process. Renhua, Xia, Hu, Lu, and Wang (2007) also observed a decrease in SOD and catalase with ripening and maturation of sweet orange. The above observations, together with the results presented here in our study, suggest that ripening leads to a decrease in activities of SOD, as well as catalase activities, which accelerated in the case of Ethrel-treated fruits, while 1-MCP-treated fruit showed high specific activity. Superoxide anions generated either by auto-oxidation processes or by enzymes, produce other kinds of cell-damaging free radicals and oxidising agents. Due to a substantial increase in respiration of this climacteric fruit, oxy free radical production probably increases with ripening. The concomitant decline in SOD and CAT activities would thus contribute to accumulation of O_2^- and H_2O_2, affecting increased oxidative stress with ripening. Fleshy cortex tissue has a higher water concentration, which could also be a factor leading to lowering of SOD in few cases (Gong, Toivonen, Lau, & Wiersma, 2001).

Another antioxidant, APX, is critical for the disposal of H_2O_2 in mango fruit. Our data support the previously characterised pea APX activity in demonstrating its inability in the absence of ascorbate, the high specificity for ascorbate, and the narrow pH optimum for its activity (Jablonski & Anderson, 1982). Recent investigations by Jiménez, Gómez, Navarro, and Sevilla (2002) have shown higher APX specific activities in mitochondria from red fruits of *Capsicum annuum*, as compared to green fruits, during ripening. A similar trend in behaviour of APX activity was observed in our work, with sharp increase of activity during ripening, in Ethrel-treated ripe sample, as compared to the control. Higher APX specific activities of fruit might play a role in avoiding the accumulation of any activated oxygen species generated and suggest an active role for these enzymes during ripening. In the absence of guaiacol peroxidase or at least below the detection level, the role of APX becomes more important in Dashehari mango ripening.

It is well known that *M. indica* var. Dashehari is a highly perishable fruit, and in some cases, does not reach processors and consumers at optimal quality after transportation. To ensure that these fruits reach consumers with the maximum organoleptic, nutritional, and functional quality attributes, the role of biochemicals turns out to be of prime importance. It is premature to draw conclusions about the biological applicability of these chemicals, but it is tempting to speculate on some possible avenues for further study, as the finding of our research could turn out to be significant to the juice industry.

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References

Aebi, H. (1983). *Methods of enzymatic analysis 3*. Weinheim, Germany: Verlag Chemie. 273-277.

Blum, J., & Fridovich, I. (1985). Inactivation of glutathione peroxidase by superoxide radical. *Archives of Biochemistry Biophysics, 240*, 500–508.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Davis, B. J. (1964). Disc electrophoresis. *Annual New York Academic Sciences, 121*, 404–427.

Elstner, E. F. (1991). Mechanism of oxygen activation in different compartments of plant cell. In E. J. Pell, K. L. Steffan, & M. D. Rockville (Eds.), *Active oxygen/oxidative stress and plant metabolism* (pp. 13–25). American Society of plant physiology.

Fridovich, I. (1989). Superoxide dismutases. An adaptation to a paramagnetic gas. *Journal of Biological Chemistry, 264*, 7761–7764.

Fu, L., Cao, J., Li, Q., & Lin, L. (2007). Effect of 1-methylcyclopropene on fruit quality and physiological disorders in yali pear (*Pyrus bretschneideri*) during storage. *Food Science and Technology International, 13*, 49–54.

Gong, Y., Toivonen, P. M. A., Lau, O. L., & Wiersma, P. A. (2001). Antioxidant system level in 'Braeburn' apple is related to its browning disorder. *Botanical Bulletin Academia Sinica, 42*, 259–264.

Halliwell, B., & Gutteridge, J. M. C. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal, 219*, 1–14.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archieves of Biochemistry and Biophysics, 125*, 189–198.

Itai, A., Tanabe, K., Tamura, F., & Tanaka, T. (2000). Isolation of cDNA clones corresponding to genes expressed during fruit ripening in Japanese pear (*Pyrus pyrifolia Nakai*): Involvement of the ethylene signal transduction pathway in their expression. *Journal of Experimental Botany, 51*, 1163–1166.

Jablonski, P. P., & Anderson, J. W. (1982). Light-dependent reduction of hydrogen peroxide by ruptured pea chloroplasts. *Plant Physiology, 69*, 1407–1413.

Jimenez, A., Cressen, G., Kular, B., Firmin, J., Robinson, S., Verhoeyen, M., et al. (2002). Changes in oxidative process and components of the antioxidant system during tomato fruit ripening. *Planta, 214*, 751–758.

Jiménez, A., Gómez, J. M., Navarro, E., & Sevilla, F. (2002). Changes in the antioxidative systems in mitochondria during ripening of pepper fruits. *Plant Physiology and Biochemistry, 40*, 515–520.

Lurie, S., Klein, J., & Ben-Arie, R. (1989). Physiological changes in diphenylamine-treated Granny Smith apples. *Israel Journal of Botany, 38*, 199–207.

McDougall, G. J. (1991). Cell wall associated peroxidases and lignification during growth of flax fibres. *Journal of Plant Physiology, 139*, 182–186.

Misra, H. P., & Fridovich, I. (1972). The purification and properties of superoxide dismutase from *Neurospora crassa*. *Journal of Biological Chemistry, 247*, 3410–3414.

Miyake, C., & Asada, K. (1996). Inactivation mechanism of ascorbate peroxidase at low concentrations of ascorbate: Hydrogen peroxide decomposes compound I of ascorbate peroxidase. *Plant Cell Physiology, 37*, 423–430.

Nakano, J., & Asada, K. (1987). Purification of ascorbate peroxidase in spinach chloroplasts: its inactivation in ascorbate depleted medium and reactivation by monodehydroascorbate radical. *Plant Cell Physiology, 28*, 131–140.

Pütter, J. (1974). Peroxidases. In H. U. Bergmeyer (Ed.), *Methods of enzymatic analysis*. New York: Verlag Chemie Weinheim Academic Press Inc..

Renhua, H., Xia, R., Hu, L., Lu, Y., & Wang, M. (2007). Antioxidant activity and oxygen-scavenging system in orange pulp during fruit ripening and maturation. *Scientia Horticulturae, 113*, 166–172.

Rogiers, S. Y., Kumar, G. N. M., & Knowles, N. R. (1998). Maturation and ripening of fruit of *Amelanchier alnifolia* are accompanied by increasing oxidative stress. *Annals of Botany, 81*, 203–211.

Rupinder, S., Poorinima, S., Pathak, N., Singh, V. K., & Dwivedi, U. N. (2007). *Modulation of mango ripening by chemicals: Physiological and biochemical aspects Plant Growth Regulation, 53*, 137–145.

Scandalios, J. G., Guan, L., & Polidoros, A. N. (1997). Catalases in plants: Gene structure, properties, regulation, and expression. In J. G. Scandalios (Ed.), *Oxidative stress and the molecular biology of antioxidant defenses* (pp. 343–406). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Singh, S., & Choudhuri, M. A. (1990). Effect of salinity (NaCl) on H_2O_2 metabolism in Vigna and Oryza seedlings. *Biochemical Physiology Pflanz, 186*, 69–74.

Sisler, E. C., Alwan, T., Goren, R., Serek, M., & Apelbaum, A. (2003). 1-Substituted cyclopropenes: Effective blocking agents for ethylene action on plants. *Plant Growth Regulation, 40*, 223–228.

Watkins, C. B., Nock, J. F., & Whitaker, B. D. (2000). Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biology and Technology, 19*, 17–32.

Wayne, L. G., & Diaz, G. A. (1986). A double staining method for differentiating between two classes of mycobacterial catalase in polyacrylamide electrophoresis gels. *Analytical Biochemistry, 157*, 89–92.

Zamora, R., Alaiz, M., & Hidalgo, F. J. (2001). Influence of cultivar and fruit ripening on olive (*Olea europaea*) fruit protein content, composition, and antioxidant activity. *Journal of Agricultural and Food Chemistry, 49*, 4267–4270.

Zhang, B., Chen, K., Bowen, J., Allan, A., Espley, R., Karunairetnam, S., et al. (2006). Differential expression within the LOX gene family in ripening kiwifruit. *Journal of Experimental Botany, 57*, 3825–3836.