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EXOGENOUS ASCORBIC ACID AND PROCYANIDIN APPLICATION AFFECT QUALITY OF 'SENSATION' MANGOES DURING RIPENING

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ABSTRACT

Mango fruit undergoes rapid metabolic changes following harvest that decrease quality, limit shelf life and marketability. In a completely randomized design experiment, the effect of 2% ascorbic acid (AA) and 1% procyanidin (PN) postharvest dipping either alone or in combination on quality changes of 'Sensation' mangoes were evaluated during ripening at ambient (23±1 ºC and 60–70% RH) for 10 days. AA and PN treated fruit retained higher green color, firmness, titratable acidity (TA) and membrane stability index (MSI) but lower total soluble solids (TSS), TSS/TA ratio and weight loss than control during ripening. Total phenol (TPC) and total flavonoid (TFC) contents in peel and pulp showed different changes pattern during ripening but were higher in treated fruit than control. DPPH radical scavenging capacity (RSC) in peel increased with fluctuations and was higher in treated fruit than control after 3 and 6 days. In pulp, RSC increased during ripening and was higher in treated fruit than control after 10 days. Vitamin C decreased during ripening and was not affected by treatments. Changes in degradative enzymes (polygalacturonase (PG), xylanase and α-amylase) and oxidative enzymes (Polyphenoloxidase (PPO) and peroxidase (POD)) activities in peel and pulp were evaluated in relation to fruit quality. It is concluded that postharvest dipping in 2% AA or 1% PN delayed ripening and retained quality of 'Sensation' mangoes via inhibiting degradative enzymes and enhancing fruit antioxidant system.

Keywords: *Mangifera indica* L., Postharvest, Antioxidant, Enzymes, Shelf life

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INTRODUCTION

As a climacteric type of fruit, mango is characterized by rapid softening during ripening that limits transportation and marketing (Sivakumar *et al*. 2011). Thus, modulating fruit ripening and minimizing postharvest losses is critically required (Sivakumar *et al*. 2011, Al-Qurashi and Awad 2018). In Saudi Arabia, considerable postharvest loss occurs in mangoes including 'Sensation', one of the most commercially grown cultivars, due to inappropriate postharvest handling that favors rapid softening during ripening (Al-Qurashi and Awad 2018). AA, a naturally occurring organic acid, is widely used at concentrations ranging from 0.5 to 4% (w/v) to retain quality of intact or minimally processed fresh fruit due to its antioxidant and anti-browning activities (Singh and Mirza 2018; Ling *et al.* 2007). Postharvest AA treatment at 150-200 ppm reduced lenticel browning and improved skin appeal of four different mangoes cultivars during ripening (Prasad *et al*. 2016). Procyanidin (condensed tannins) is secondary plant metabolites derived from the condensation of flavan-3-ol and possess antioxidant properties (Santos-Buelga and Scalbert 2000). Postharvest 1% procyanidin treatment retarded ripening via reduction of ethylene production and respiration rates and protection of membranes against oxidative damage in banana fruit (Chen *et al.* 2019). However, no available information on the effect of PN or its combination with AA on postharvest quality and ripening of mangoes. The aim of this study was to assess the response of 'Sensation' mangoes to postharvest treatment with 2% AA and 1% PN either alone or in combination as an attempt to slow-down biochemical changes and retain quality during ripening at ambient conditions and thus enhance fruit transportation and marketing.

MATERIALS AND METHODS

Mango fruits (cv. 'Sensation') were harvested at physiological maturity stage (mature green-hard) from commercial orchard in Jizan region, Saudi Arabia during

the fruiting season 2019-20. Five random replicates (40 fruit of each) were prepared for each treatment and fruits of each treatment were drenched in either water (control), 2% ascorbic acid or 1% procyanidin solutions either alone or in combination for 10 min. The PN concentration of 1% was selected based on an optimum in preliminary trail using 0, 0.5, 1 and 2% PN. All treatments solutions contained 1ml/l Tween 20 as a wetting agent. All treatments/replicates were air drayed for 1 h at 23±1ºC, weighed and stored in perforated cardboard cartons at 23±1ºC and 60–70% (RH) for 10 days. A separate five replicates (5 fruits of each)/treatment were stored at the same conditions and periodically weighed (at 0, 3, 6 and 10 days) for loss in weight calculation. After 0, 3, 6 and 10 days of natural ripening, random samples (5 fruit) per replicate were collected for quality measurements as indicated below. Following peel color and pulp firmness measurements, samples of both pulp and peel were stored at –80 ºC for enzymes, TPC, TFC and RSC determinations. Additional portion of fruit pulp were directly used for TA, TSS and vitamin C measurements.

Fruit quality parameters and ions leakage measurements: Fruit peel color was measured by a Minolta Chroma Meter CR-410 (Minolta Camera Co. Ltd., Osaka, Japan) as detailed in Al-Qurashi and Awad (2018). Firmness of flesh was measured by a digital basic force gauge, model BFG 50N (Mecmesin, Sterling, Virginia, USA) supplemented with a probe of 11 mm diameter. TSS, TA and pH were quantified in composite juice samples collected from the five fruit as explained by Al-Qurashi and Awad (2018). Vitamin C was measured by the method of Ranganna (2000) and ions leakage of peel was determined according to Al-Qurashi and Awad (2018) and was calculated as percentage of stability (MSI %).

Total phenol and flavonoid content and RSC estimation: The methanolic extract preparation for both peel and pulp of the fruit samples were carried out following the procedure described by Al-Qurashi and Awad (2018). This filtrated methanol extract was used for TPC, TFC and RSC estimations. TPC was calorimetrically estimated as previously explored (Hoff and Singleton, 1977) while TFC was also calorimetrically estimated (Zhishen*et al.* (1999) as detailed in Al-Qurashi and Awad (2018). RSC was estimated by the method of 1,1-diphenyl-2-picrylhydrazyl (DPPH) as previously declared (Al-Qurashi and Awad 2018).

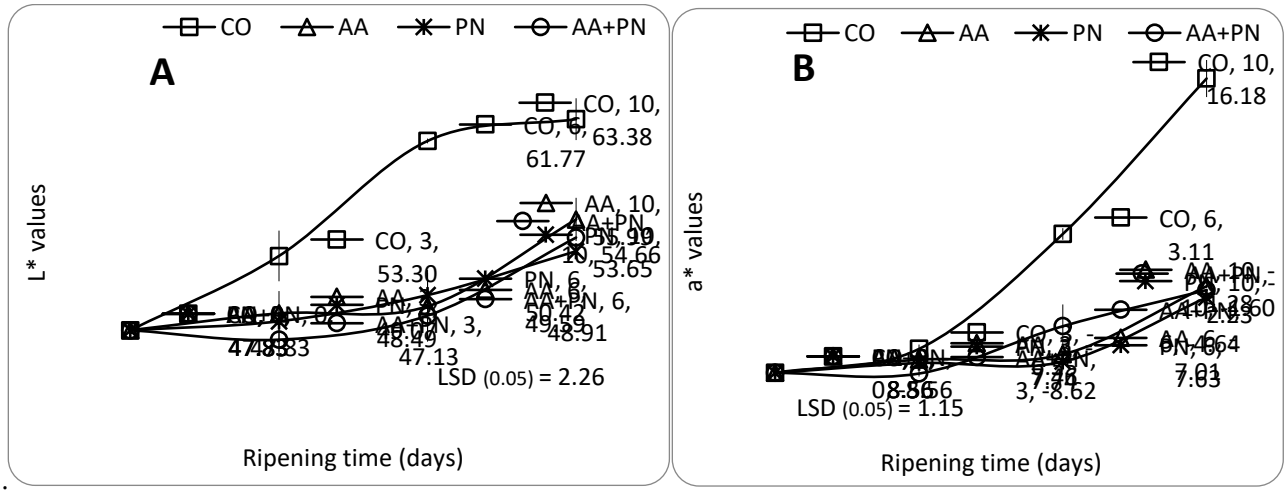
Enzymes assay: PPO (EC 1.14.18.1) activity was spectrophotometrically estimated as previously declared (Jiang *et al*. 2002) while, the method of Miranda *et al.* (1995) was applied for POD (EC 1.11.1.7) activity estimation as previously explored (Al-Qurashi and Awad 2018). The activities of PG (EC 3.2.1.15), xylanase (EC 3.2.1.8) as well as α-amylase (EC 3.2.1.1) were measured according to Miller (1959) as previously explored (Al-Qurashi and Awad 2018).

Statistical analysis: The experiment was laid in a completely randomized design with four treatments and five replications. Analysis of variance (two way-ANOVA) was done using treatment and ripening time as sources of variation by the statistical package software SAS (SAS Institute Inc., 2000, Cary, NC., USA). Comparisons between means were made by the Least Significant Difference (LSD) at $P \leq 5\%$. The entire experiment was repeated twice, in order to confirm the reproducibility of the results.

RESULTS

There were significant interaction effects between treatments and ripening period on all of the measured parameters, thus we passed over the main effects and focused instead on the interactions. Fruit color of fruit peel showed that the values of L* increased and were lower in treated fruit after 6 and 10 days than the control (Fig 1a). While, the values of a* increased and were lower in treated fruit after 6 and 10 days than control (Fig 1b). The values of b* and chroma increased during ripening and were lower in treated fruit than the control (Fig 1c,d). There were no significant differences among treated fruit in b* and chroma values during ripening. The loss in fruit weight increased during ripening reaching 4.48% after 10 days and was lower in treated fruit than the control after 6 and 10 days, except for PN treatment after 6 days (Fig 2a). The lowest loss in fruit weight (3.91%) was recorded in AA combined with PN treatment. Firmness sharply decreased during ripening and was higher in all treatments than the control after 3 and 6 days with no differences after 10 days (Fig 2b). MSI of fruit peel greatly decreased during ripening and was higher in treated fruit than the control (Fig 2c). TSS gradually increased and was lower in all treatments than the control, especially after 6 and 10 days (Fig 2d). TA content decreased and was higher in treated fruit, especially after 6 and 10 days, than the control except for AA combined with PN treatment after 10 days that was similar to control (Fig 2e). TSS/TA ratio increased and was lower in treated fruit after 6 and 10 days than the control (Fig 2f). TPC in peel of untreated fruit remain constant except after 6 days that was higher than initial (Fig 3a). While in treated fruit, TPC increased after 3 days with no significant changes after 6 and 10 days, except for AA treatment that gradually decreased thereafter. However, AA and PN treatments retained higher TPC than the control. TPC in pulp decreased after 3 and 6 days in all treatments, and was higher in treated fruit than the control. However, after 10 days, TPC increased in all treatments and remained higher in treated

fruit than the control (Fig 3b). TFC in peel decreased with fluctuations with no significant variations among treatments after 3 and 6 days, except for AA combined with PN treatment that showed higher level and PN that showed lower level. However, treated fruit retained higher TFC than the control after 10 days (Fig 3c). However, TFC in pulp increased up to 6 days then decreased thereafter (Fig 3d). TFC was higher in treated fruit than the control, except for PN treatment after 10 days. Vitamin C content decreased and was not significantly affected by the treatments (Fig 3e). RSC of fruit peel decreased (higher DPPH IC_{50} values) after 3 days and then increased (lower DPPH IC_{50} values) to similar level to initial after 6 and 10 days, except for control after 6 days that was lower than initial (Fig4a). RSC in peel was higher in treated fruit than the control, especially after 3 and 6 days. However, after 10 days, only AA combined with PN treatment exhibited higher RSC in peel than the control. RSC of pulp increased (lower DPPH IC_{50} values) during ripening than initial, and was higher in treated fruit than the control after 10 days (Fig 4b). However, PN treatment after 3 days and AA combined with PN treatment after 6 days showed higher RSC in pulp than the control. PPO activity of fruit peel greatly increased during ripening in all treatments after 3 and 6 days, and was lower in treated fruit than the control (Fig 5a). However, after 10 days, PPO in peel remained constant in the control but decreased in treated fruit. In pulp, PPO activity greatly increased during ripening, except for PN treatment that fluctuated, and was lower in treated fruit than the control (Fig 5b). PN treatment gave the lowest PPO activity after 6 and 10 days of ripening. POD activity of fruit peel increased after 3 days in all treatments, and was higher in treated fruit than the control, except for AA treatment after 3 days that was similar to control (Fig 5c). However, after 10 days, PN and AA combined with PN treatments maintained higher POD activity in peel than initial. While, POD activity in pulp increased with fluctuation in all treatments, except for the control where POD activity increased up to 3 days and then remains constant (Fig 5d). After 3 days, POD activity in pulp was higher in AA combined with PN treatment than other treatments, but after 6 and 10 days, all treated fruit retained higher POD activity than the control, except for PN after 6 days that was similar to the control. PG activity in peel showed higher level than initial in all treatments after 3 days but decreased thereafter and was lower in treated fruit than the control after 3 and 6 days (Fig 6a). PG activity in pulp exhibited higher level than initial in all treatments after 3 days, except for AA combined with PN treatment (Fig 6b). While, after 6 and 10 days, PG activity in treated fruit decreased to a close level to initial. The treated fruit exhibited lower PG activity in pulp than the control. Xylanase activity in fruit peel showed higher level than initial after 3 days, except for AA combined with PN treatment that remains constant (Fig 6c). However, after 6 and 10 days, xylanase activity in peel was much lower than initial in all treatments, except for the control after 6 days that remains higher than initial. Treated fruit showed lower xylanase activity in peel, especially after 3 and 6 days than the control with no significant variations among treatments after 10 days. Xylanase activity in fruit pulp showed higher level than initial after 3 and 6 days, except for PN alone or in combination with AA that remain constant (Fig 6d). However, after 10 days, xylanase activity in pulp sharply decreased in all treatments, but was lower in treated fruit than the control. α-amylase activity of peel increased in the control until day 6 but sharply decreased thereafter. In treated fruit, α-amylase activity was lower after 6 and 10 days than initial and was lower than control (Fig 6e). AA combined with PN treatment showed the lowest α-amylase activity in peel after 6 days. However, α-amylase activity in pulp showed lower level than initial, except for the control that was higher than initial after 3 and 6 days (Fig 6f). The treated fruit exhibited lower α-amylase activity in pulp than the control.



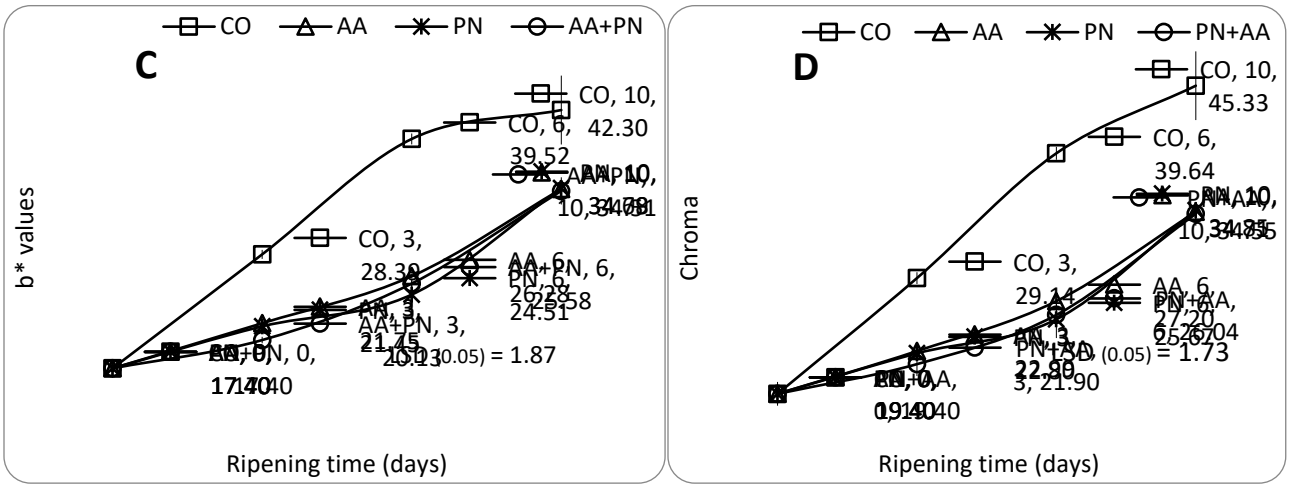
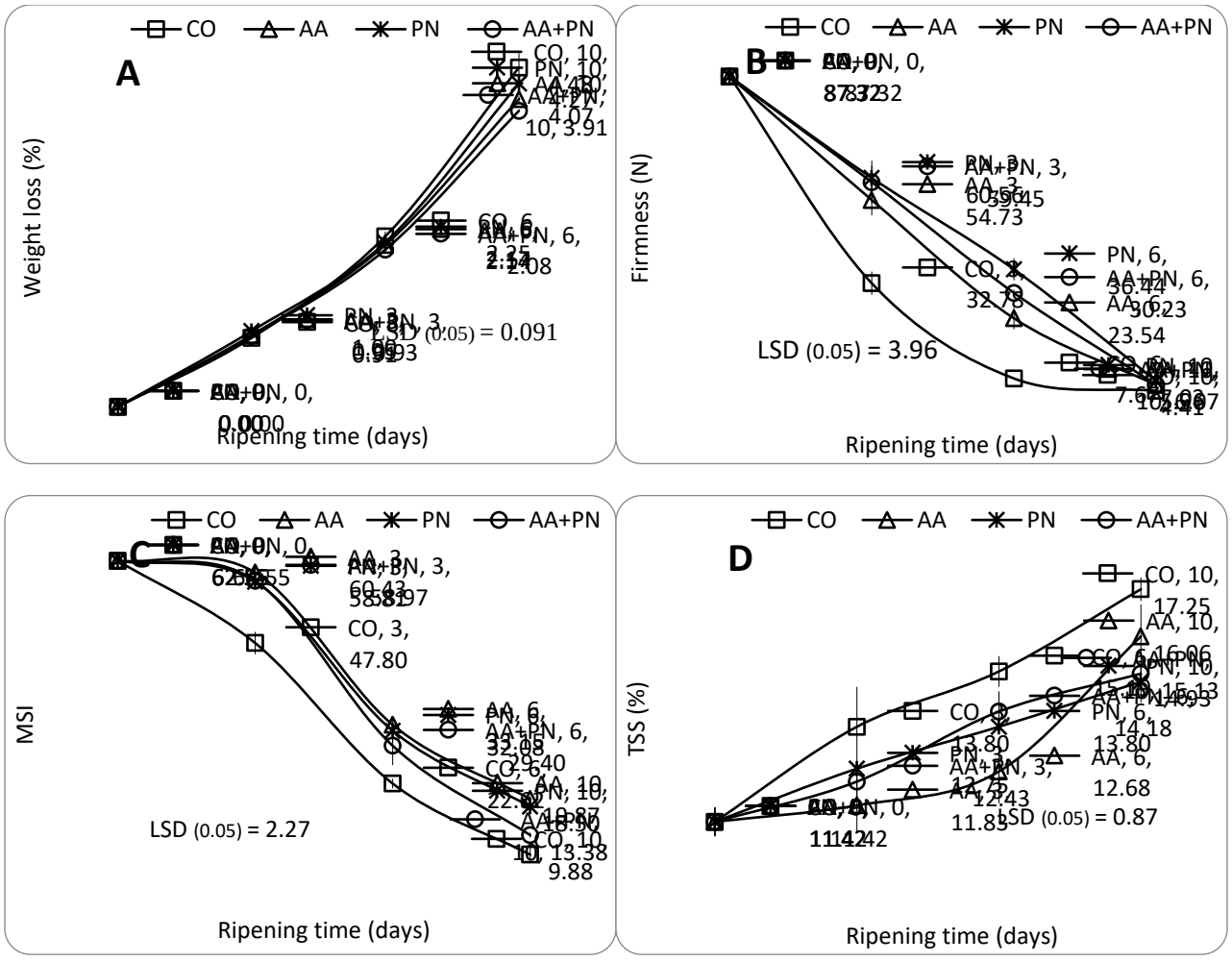


Fig 1 Peel color parameters L* (A), a* (B), b* (C) and chroma (D) of 'Sensation' mangoes treated with 2% ascorbic acid (AA) and/or 1% procyanidin (PN) during ripening at ambient (23±1 ºC and 60–70% RH). Means with differences within the LSD value are not significantly different $P \leq 0.05$.



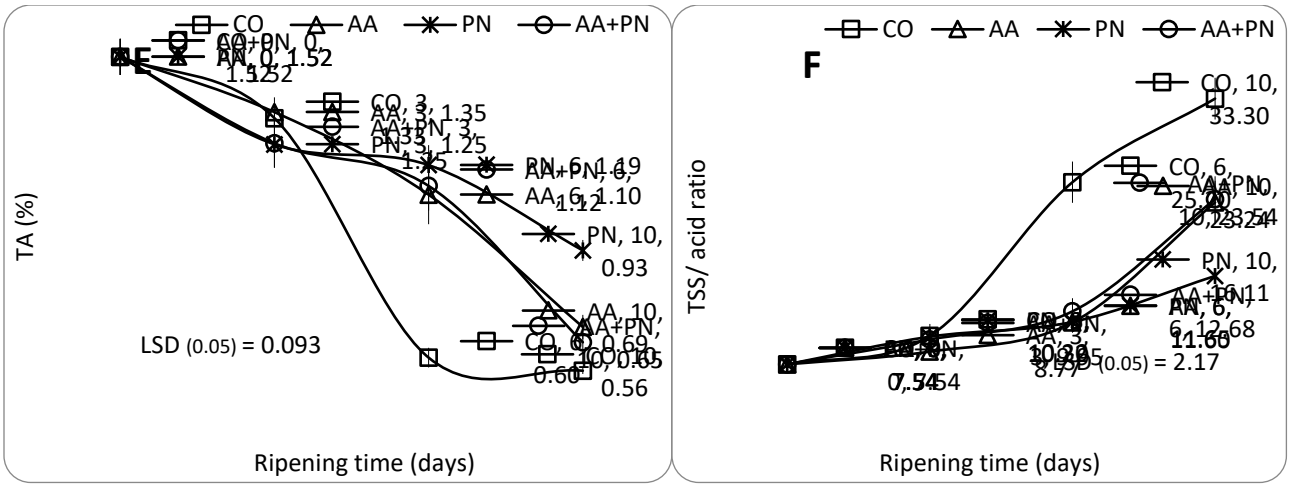
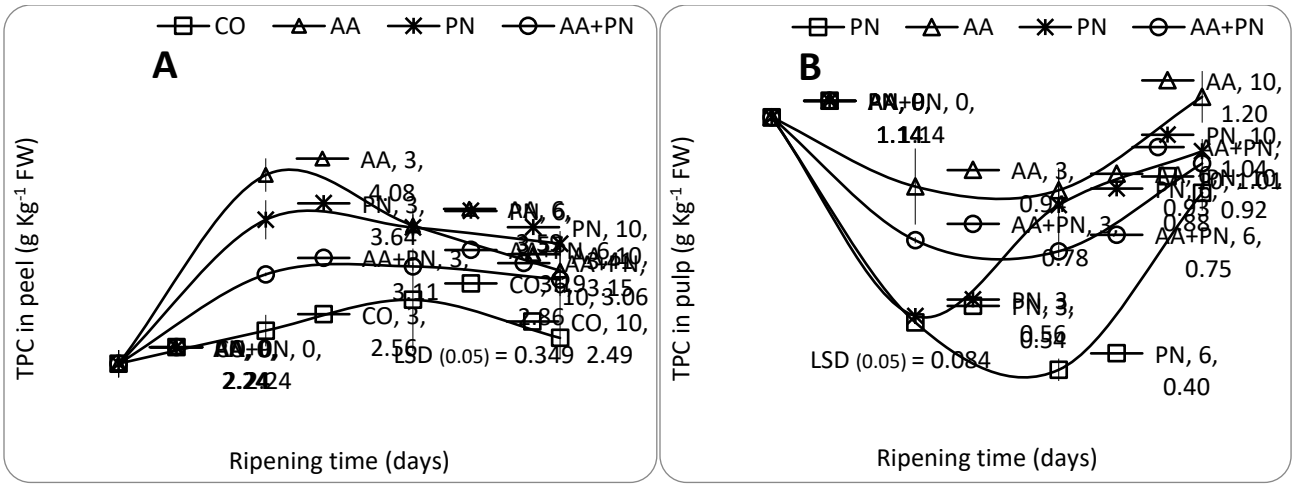
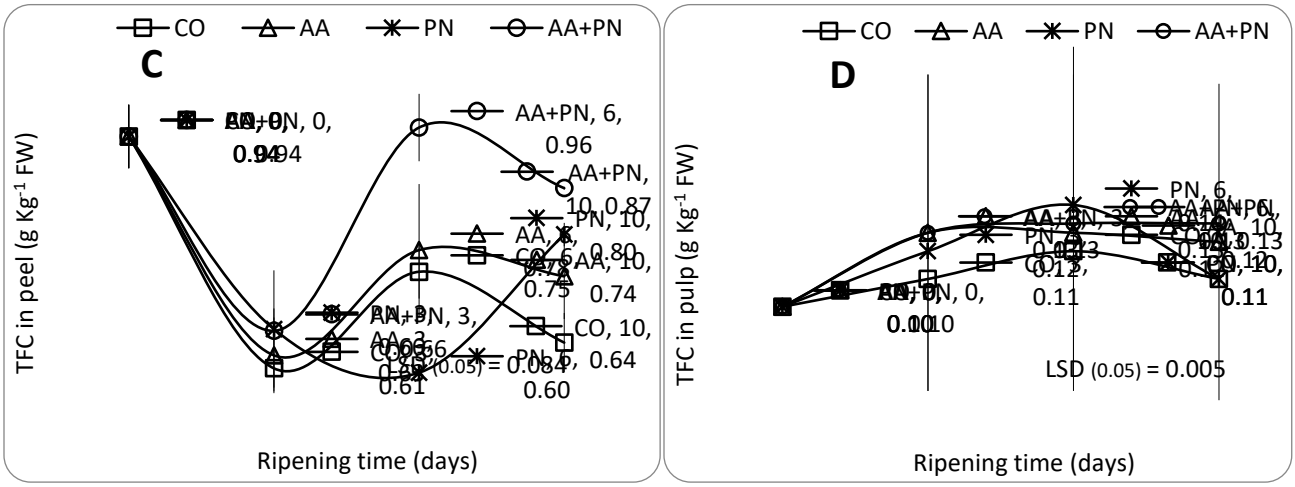


Fig 2 Weight loss (A), firmness (B), membrane stability index (MSI) (C), total soluble solids (TSS) (D), titratable acidity (TA) (E) and TSS acid ratio (F) of 'Sensation' mangoes treated with 2% ascorbic acid (AA) and/or 1% procyanidin (PN) during ripening at ambient (23±1 ºC and 60–70% RH). Means with differences within the LSD value are not significantly different $P \leq 0.05$.





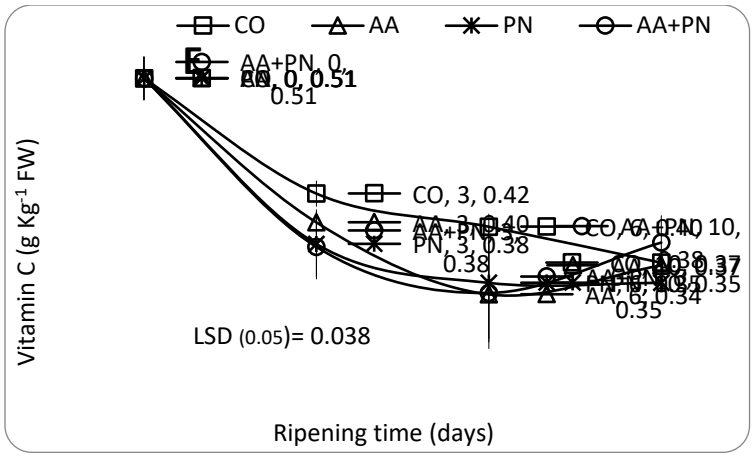


Fig 3 Total phenol (TPC) in peel (A) and pulp (B), total flavonoid (TFC) in peel (C), TFC in pulp (D) and vitamin C (E) contents in pulp of 'Sensation' mangoes treated with 2% ascorbic acid (AA) and/or 1% procyanidin (PN) during ripening at ambient (23±1 ºC and 60–70% RH). Means with differences within the LSD value are not significantly different $P \leq 0.05$.

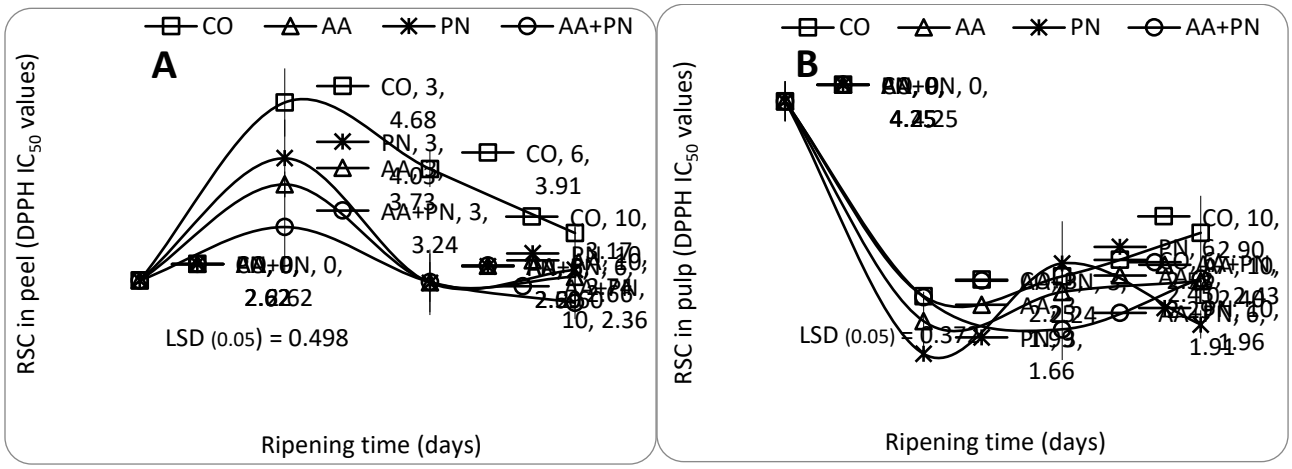
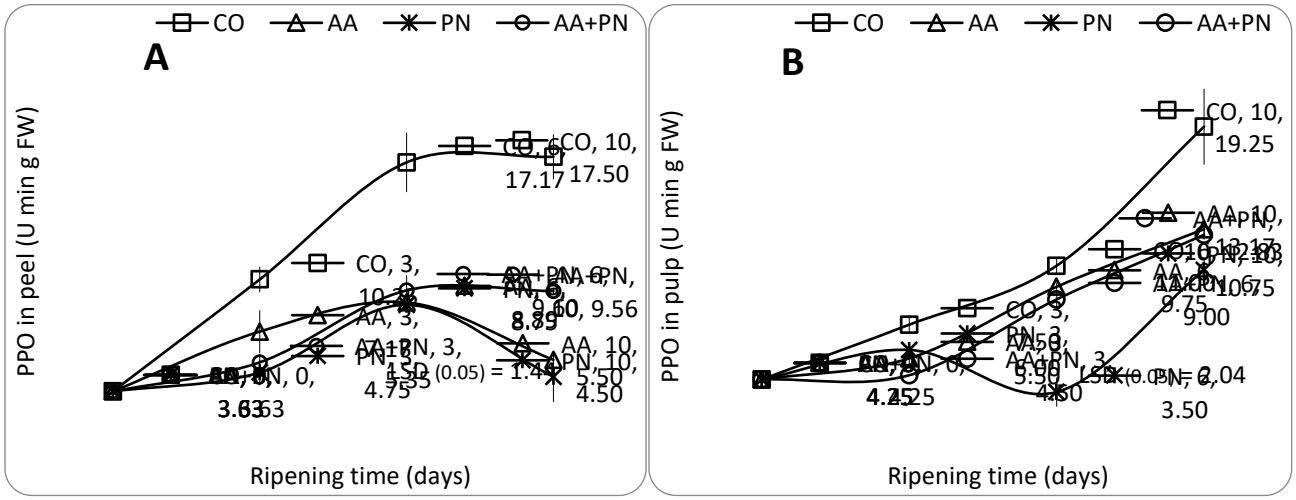


Fig 4 Radical scavenging capacity (RSC) in peel (A) and pulp (B) of 'Sensation' mangoes treated with 2% ascorbic acid (AA) and/or 1% procyanidin (PN) during ripening at ambient (23±1 ºC and 60–70% RH). Means with differences within the LSD value are not significantly different $P \leq 0.05$.



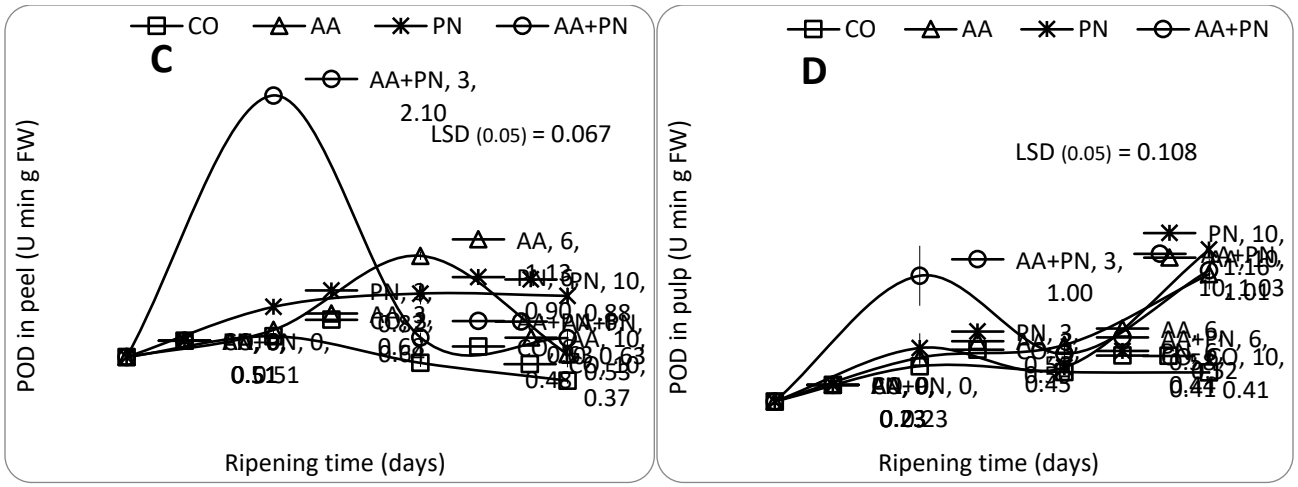
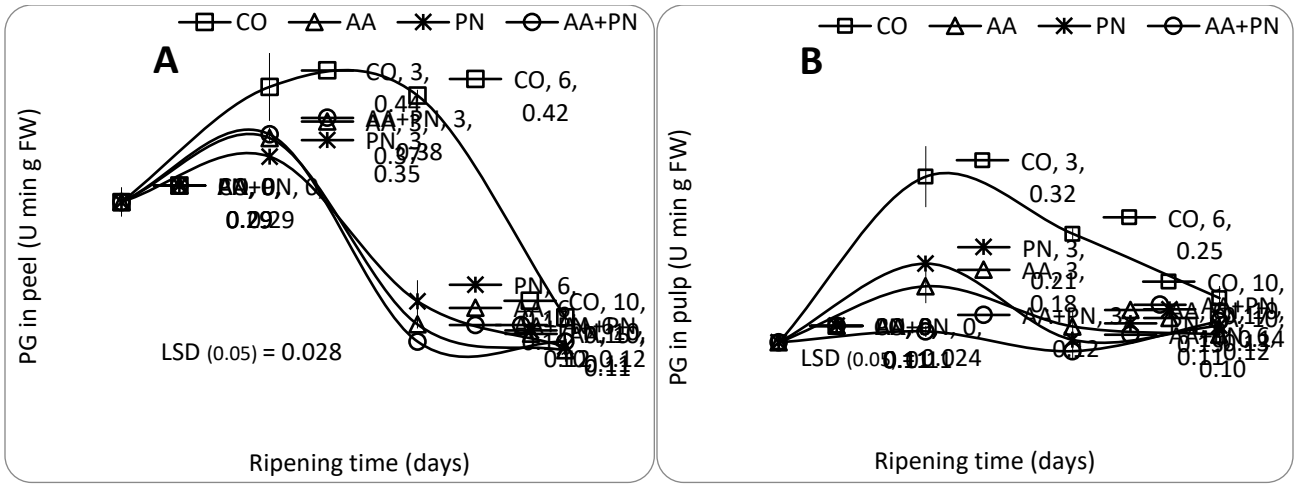
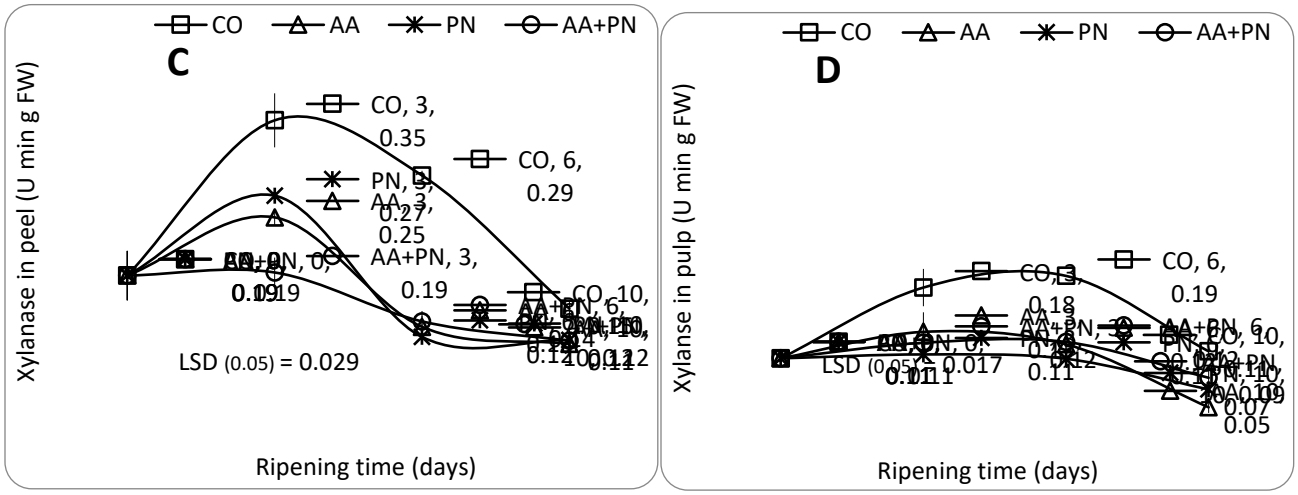


Fig 5 Activities of polyphenoloxidase (PPO) in peel (A) and pulp (B), and peroxidase (POD) in peel (C) and pulp (D) of 'Sensation' mangoes treated with 2% ascorbic acid (AA) and/or 1% procyanidin (PN) during ripening at ambient (23±1 ºC and 60–70% RH). Means with differences within the LSD value are not significantly different $P \leq 0.05$.





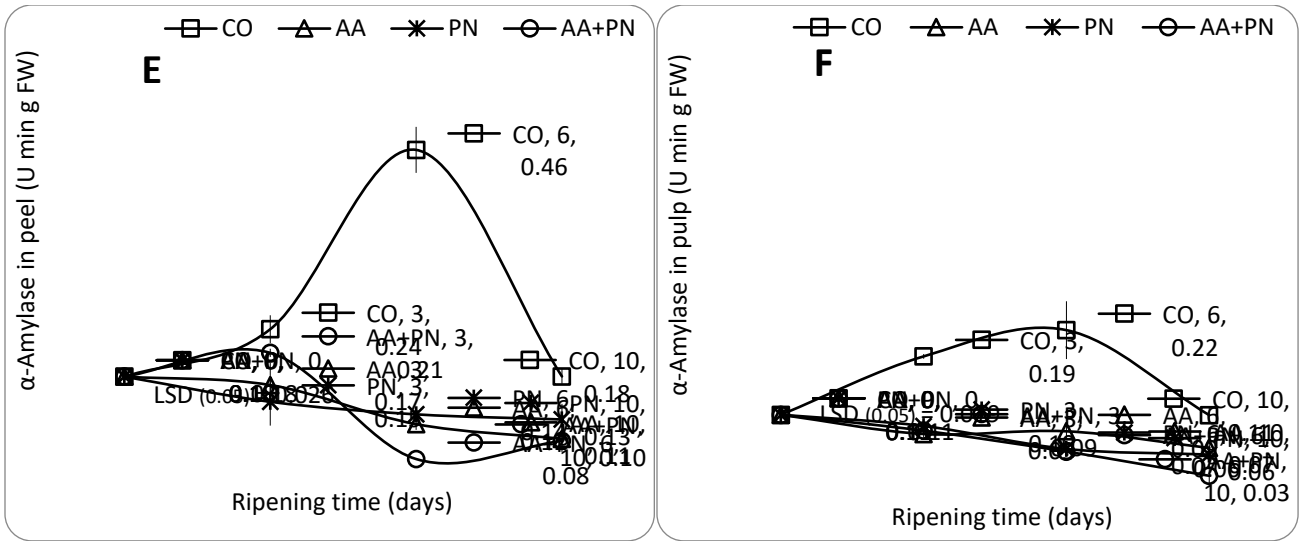


Fig 6 Activities of polygalacturornase (PG) in peel (A) and pulp (B), xylanase in peel (C) and pulp (D) and α-Amylase in peel (E) and pulp (F) of 'Sensation' mangoes treated with 2% ascorbic acid (AA) and/or 1% procyanidin (PN) during ripening at ambient (23±1 ºC and 60–70% RH). Means with differences within the LSD value are not significantly different $P \leq 0.05$.

DISCUSSION

As a climacteric fruit, mango express high metabolic activities leading to rapid softening associated with an increase in TSS and a decrease in TA during ripening at ambient conditions (Sivakumar *et al.* 2011, Al-Qurashi and Awad 2018). Both transpiration and respiration are the main physiological processes contributing to loss in fruit weight during ripening and storage (Narayana *et al.* 1996). AA and PN treatments reduced loss in fruit weight during ripening possibly by slow-down metabolic activities of fruit. Respiration and ethylene production rates were not measured in the current study, however, in a previous study, postharvest AA dipping decreased both of these parameters in four mango cultivars during ripening at ambient conditions (Prasad *et al*. 2016). Also, postharvest 1% PN dipping decreased respiration and ethylene production rates in 'Brazil' bananas during ripening at ambient conditions (Chen *et al.* 2019). In overall, both AA and PN treatments delayed ripening of 'Sensation' mangoes during 10 days, as indicated by greener peel color (lower a* values), firmness, MSI, and TA, but lower TSS and TSS/TA ratio than the control. Postharvest AA dipping delayed ripening of 'Hindi-Besennara' mangoes at ambient conditions (Al-Qurashi and Awad 2020), guavas during cold storage (Gill *et al*. 2014) and litchis at ambient conditions (Kumar *et al.* 2013). In other climacteric fruit, 1% procyanidin dipping retarded ripening of bananas via reduction of ethylene production and respiration rates and protection of membranes against ROS (Chen *et al*. 2019). Ripening retardation in AA and PN treated 'Sensation' mangoes might be attributed to their general properties as antioxidative molecules. The transition of fruit from maturation into ripening was associated with a rapid trend toward an oxidation status (Goulao and Oliveira 2008). AA has been found to maintain cell wall integrity and protect membranes against ROS that rise in fruit during ripening (Akram *et al*. 2017). Also, procyanidin dipping decreased ROS levels, malondialdehyde content, and electrolytes leakage in banana fruit peel, but enhanced superoxidedismutase, catalase, and POD activities and expressions during ripening (Chen *et al*. 2019). The higher pulp firmness and peel MSI in AA and PN treated fruit is possibly attributed to degradative enzymes inhibition and antioxidants enhancement (POD activity and TPC and TFC contents). Cell wall membrane stability was associated with degradative enzymes activity such as cellulase, PG, α-amylase and xylanase (Hurber 1983). AA protect fruit against oxidative damage via enhancing antioxidant defense system that retard softening and decay (Ling *et al*. 2007). AA and PN treated fruit exhibited higher POD and lower PPO in both peel and pulp than control during ripening. Similarly, postharvest 1% iso-ascorbic acid treatment decreased browning and PPO activity in litchi fruit during shelf life (Liu *et al*. 2006, Kumar *et al*. 2013). Procyanidin treatment suppressed ROS levels and enhanced enzymatic and non-enzymatic antioxidant system of bananas during ripening (Chen *et al*. 2019). TPC and TFC were higher in AA and PN treated fruit than control during ripening partially confirming those on 'Hindi-Besennara' mangoes (Al-Qurashi and Awad 2020), 'Yali' pears (Ling *et al*. 2007) and guavas (Gill *et al.* 2014). TPC and TFC changes pattern during ripening suggesting different metabolic activities in peel and pulp

and such changes were not associated with RSC. In partial confirmation, TPC in peel decreased during storage at 6 ℃ of 'Choke anan' mangoes in contrast to those stored at 12 ℃ that remained constant while, RSC increased at both storage temperatures (Kondo *et al.* 2005).

Conclusion: It could be concluded that postharvest dipping in 2% AA or 1% PN delayed ripening and improved quality of 'Sensation' mangoes at ambient conditions (23±1 ºC and 60–70% RH) as reflected by higher green color, firmness, TA and MSI but lower TSS, TSS/TA ratio and loss in fruit weight than the control. These effects were attributed to inhibiting degradative enzymes (PG, xylanase and α-amylase) activities and enhancing antioxidant system of fruit including POD enzyme and TPC and TFC contents. However, AA and PN combination treatment provided no additional positive effects on most quality parameters of fruit.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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