Pre-storage putrescine application suppresses ethylene biosynthesis and retards fruit softening during low temperature storage in 'Angelino' Plum

Ahmad S. Khan [a], Zora Singh [a, *] and Nadeem A. Abbasi [a, b]

[a] *Curtin Horticulture Research Laboratory, Muresk Institute, Division of Science and Engineering, Curtin University of Technology, GPO Box U 1987, Perth 6845, Western Australia, Australia*

[b] *Present address: Department of Horticulture, University of Arid Agriculture, Murree Road, Rawalpindi, Pakistan*

***Corresponding author:**

Zora Singh

Curtin University of Technology, GPO Box U 1987, Perth 6845, Western Australia, Australia

Phone: +61 8 9266 3138

Fax: +61 8 9266 3063

Email: z.singh@curtin.edu.au

Abstract

To investigate the role of putrescine (PUT) in ethylene biosynthesis and fruit softening of plum (*Prunus salicina* Lindl. cv. Angelino), fruit on trees were sprayed one week before anticipated commercial harvest or after harvest dipped in an aqueous solution containing different concentrations of PUT (0.0, 0.1, 1.0 and 2.0 mM), and 'Tween 20' (0.01%) as a surfactant. Following PUT treatments fruit were stored at 0 ± 1^{o}C and 90 ± 5% RH for 0, 3 and 6 weeks. Ethylene production, activities of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) enzymes, and 1-aminocyclopropane-1-carboxylic acid (ACC) content, fruit firmness and fruit softening enzymes including exo-polygalacturonase (exo-PG), endo-polygalacturonase (endo-PG), pectin esterase (PE) and endo-1,4-β-D-glucanase were estimated after 0, 3 and 6 week of storage. Pre and postharvest PUT application reduced the ethylene production in 'Angelino' plum after 3 and 6 weeks of storage as compared to untreated fruit. Preharvest spray application of higher PUT concentrations substantially reduced ethylene production as compared to lower PUT concentrations and postharvest PUT treatments. Activities of ACS enzymes and ACC contents during storage decreased with increased concentration of PUT applied irrespective of method of its application both in skin and pulp tissues. While, preharvest PUT-sprayed fruit exhibited lower ACO activities than postharvest PUT-treated skin and pulp tissues. The preharvest spray application of higher concentrations of PUT (2.0 and 1.0) significantly reduced the activities of fruit softening enzyme (exo-PG, endo-PG, PE and EGase) in skin and pulp tissues during storage. In conclusions, pre-storage application of PUT retarded plum fruit softening during low temperature storage through suppressed ethylene biosynthesis and reduced

activities of fruit softening enzymes such as PE, EGase, exo and endo-PG in skin and pulp tissues.

Keywords: ACS; ACO; ACC; exo-PG; endo-PG; PE; EGase; enzymes

1. Introduction

Polyamines (PAs) are a class of positively charged small aliphatic amines that are ubiquitous in living organisms. Putrescine (PUT), spermidine and spermine are the major forms of PAs found in plants and have been reported to affect fruit ripening and softening (Cohen, 1998; Pandey et al., 2000; Perez-Vicente et al., 2002; Malik and Singh, 2004; Malik and Singh, 2005).

PAs inhibit ethylene biosynthesis (Smith, 1985) by competing with ethylene for common precursor S-adenosyl methionine (SAM) (Pandey et al., 2000). Ethylene and PAs have been found to exhibit opposite effects in fruit ripening and senescence. Reduced level of PAs have been correlated with increased ethylene production (Kumar et al., 1996; Walden et al., 1997). Exogenous application of PAs inhibit ethylene production (Bregoli et al., 2002; Perez-Vicente et al., 2002; Serrano et al., 2003) and inhibition of polyamine biosynthesis enhanced ethylene production (Paksasorn et al., 1995). A possible explanation of the competitive relationship between PAs and ethylene could be the competitive demand for limited pool of their common precursor SAM and feed back inhibition of enzyme action system. During climacteric fruit ripening a burst in ethylene production is concomitant with increased activities of ACS and ACO enzymes (Lelievre et al., 1997).

The ripening process in fruit is associated with enzymatic changes leading to fruit softening (Huber, 1983). Polygalacturonase (PG) has been reported to play

central role in fruit softening during fruit ripening process (Mithcham et al., 1991), while other enzymes involved in fruit softening include pectin methyl esterase (PME) (Zhou et al., 2000) and cellulases (Abu-Goukh and Bashir, 2003). Changes in the activities of cell wall softening enzymes have been investigated in some fruits during fruit ripening including apple (Knee, 1978), avocado (Jeong et al., 2002), cherry (Barrett and Gonzalez, 1994), papaya (Paull and Chen, 1983), pear (Hiwasa et al., 2003) and tomato (Lashbrook et al., 1994). PG is a key enzyme involved in the hydrolytic cleavage of α-(1-4) galacturonan linkage (Fischer and Bennett, 1991) and is responsible for pectin disassembly during fruit ripening (Sitrit and Bennett, 1998).

PAs have been reported to bind with negatively charged phospholipids component or other anionic sites on membrane and thus affect membrane fluidity and indirectly modulate the activities of membrane associated enzymes (Slocum et al., 1984). PAs have been claimed as anti-senescent agent and their application reduced the softening with delayed senescence in several fruits (Kramer et al., 1991). PAs retard the senescence by stabilizing cell membrane (Borrell et al., 1997). Application of spermidine (0.1, 1.0 and 5.0 mM), spermine (2.0 mM) and PUT (10 mM) significantly delayed the fruit softening in peach fruit (Bregoli et al., 2002). Apparently no information is available on the role PUT in regulating the activities of ethylene biosynthesis and fruit softening enzymes in plum during low temperature storage and warrants further investigations. This study investigate the role of pre- and postharvest application of PUT on the activities of ethylene biosynthetic enzymes such as ACS, ACO and ACC content, as well as fruit softening enzymes including exo-PG, endo-PG, PE and EGase in skin and pulp tissues of ‘Angelino’ plum during low temperature storage.

2 Materials and methods

2.1 Plant materials

The experiment was conducted on 15-year old plum (*Prunus salicina* Lindl. cv. Angelino) trees at Casuarina Valley Orchard (lat. 34°15′S; long. 116°09′E), Manjimup, South Western region of Western Australia. Trees were grafted on myrobalan (*Prunus cerasifera* Ehrh.) rootstock with row distances of 4.25 m x 4.25 m and plant distances of 2 m x 2 m, and were trained as palmette training system. Uniform trees free from pests and diseases were selected and all the experimental trees received similar cultural practices except the experimental treatments.

2.2 Pre- and postharvest application of putrescine (PUT)

Whole trees including fruit and leaves were sprayed with an aqueous solution containing different concentrations of PUT (0.0, 0.1, 1.0 and 2.0 mM) and 'Tween 20' (0.01%) as a surfactant till run-off, on March 11, 2004 about one week before anticipated commercial harvest. Five trees represented an experimental unit and were replicated three times. At a random total 288 fruit (24 per experimental unit) were harvested on March 17, 2004, at commercial maturity (TSS 13.85 ± 1.1% and firmness 36.82 ± 2.2N). Fruit free from visual symptoms of any disease or blemishes were harvested and immediately transported to the laboratory using an air-conditioned car.

For postharvest PUT treatments, 288 fruit (24 per experimental unit) were harvested randomly from 60 unsprayed trees at commercial maturity (as explained above) were divided into four lots. Each lot of fruit was dipped in an aqueous solution containing different concentrations of PUT (0.0, 0.1, 1.0 and 2.0 mM) and surfactant

'Tween 20' (0.01%) for 6 minutes. Following pre- and postharvest PUT treatments fruit were stored for 0, 3 and 6 weeks at 0 ± 1°C and 90 ± 5% RH. Ethylene production and fruit firmness were recorded after 0, 3 and 6 weeks of storage. Activities of ethylene biosynthesis enzymes including 1-aminocyclopropane-1-carboxylic acid synthase (ACS), 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) and of 1-aminocyclopropane-1-carboxylic acid (ACC) content; fruit softening enzymes exo-polygalacturonase (exo-PG), endo-polygalacturonase (endo-PG), pectin esterase (PE) and endo-1,4-*β*-D-glucanase (EGase), were determined in fruit skin (1 mm thick) and pulp tissues at 0, 3, and 6 weeks of storage.

2.3 Determination of ethylene production

Ethylene production from plum fruit was determined by using Gas Chromatograph (Agilent Technologies, 6890 N Network GC system, Palo Alto, California, USA) fitted with a 2 m long stainless steel Supelco column (Porapack-Q 1/8", mesh size 80/100) and a flame ionization detector (FID). A detailed method for estimation of ethylene form plum fruit has been reported earlier by Khan and Singh (2007). Ethylene concentration was expressed as pmol kg^{-1} h^{-1}.

2.4 Determination of activities of ethylene biosynthesis enzymes and ACC content in fruit skin and pulp tissues

Activities of ACS and ACO enzymes and ACC contents from fruit skin and pulp tissues were determined as detailed by Khan and Singh (2007) and were expressed as pmol ACC mg $protein^{-1}$ h^{-1}, nmol C_2H_4 mg $protein^{-1}$ h^{-1} and pmol g^{-1} FW respectively.

2.5 Fruit firmness

Fruit firmness was determined by using electronic pressure tester (model EPT-1 pressure tester, Lake City Technical Products Inc, Kelowna, BC, Canada) fitted with an 8 mm tip. After removing a thin slice of fruit skin, firmness was recorded from two sides of each fruit and means were expressed as newtons (N).

2.6 Determination of activities of fruit softening enzymes in fruit skin and pulp tissues

Activities of fruit softening enzymes including exo-PG, endo-PG, PE and EGase were determined from fruit skin and pulp as explained earlier by Khan and Singh (2007). The activities of exo-PG, endo-PG, PE and EGase enzymes were expressed as μg galacturonic acid mg protein^{-1} h^{-1}, viscosity changes mg protein^{-1} h^{-1}, mM NaOH mg protein^{-1} h^{-1} and viscosity changes mg protein^{-1} h^{-1} respectively.

2.7 Protein determination

Protein contents from fruit skin and pulp tissues were determined by using the method of Bradford (1976) and were expressed as mg g^{-1} FW.

2.8 Statistical Analysis

Data were subjected to analysis of variance (ANOVA), using Genstat 9 release (Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK). Experimental data were analysed by using three factor factorial ANOVA including PUT concentrations, method of application and storage time. The effects of various treatments were assessed within ANOVA and least significance differences (Fisher's LSD) were calculated following significant F test ($P \leq 0.05$). All the assumptions of analysis were checked to ensure validity of statistical analysis. Pearson correlations were calculated using SPSS package V.14.0 for windows, USA, to determine the

relationship between ethylene production and ethylene biosynthesis and fruit softening enzymes; fruit firmness and ethylene biosynthesis and fruit softening enzyme at $P \leq 0.05$.

3. Results

3.2 Ethylene production and activities of ethylene biosynthesis enzymes

Pre- and postharvest application of PUT reduced the ethylene production in plum fruit after 3 and 6 weeks of storage as compared to untreated fruit. Reduction in ethylene production was concentration dependent and progressively decreased with increased concentrations of PUT applied (Fig. 1A and 1B). Mean ethylene production in postharvest PUT-treated fruit was 1.4-fold and 1.7-fold higher than preharvest PUT treatments after 3 and 6 weeks of storage respectively (Fig. 1C).

Pre- and postharvest PUT treatments significantly ($P \leq 0.05$) affected the activities of ACS, ACO enzymes as well as ACC content in fruit skin and pulp tissues after 3 and 6 weeks of storage (Fig. 2A-F). During storage, methods of PUT application did not show any difference in the activities of ACS enzymes in the skin as well as in pulp tissues. All PUT treatments reduced the ACS activities in fruit skin and pulp tissues as compared to untreated fruit, whilst no significant difference was observed among the PUT concentrations. Pulp tissues exhibited lower mean ACS activities than skin tissues (Fig. 2C and 2F). PUT treatments also significantly reduced the activities of ACO enzyme during storage both in fruit skin and pulp tissues (Fig. 3A-F). Reduction in the ACO activities with exogenous PUT treatments were concentration dependant, higher PUT concentration (2.0 mM) significantly reduced ACO activities in skin and pulp tissues compared to other PUT concentrations. Preharvest PUT-sprayed fruit exhibited 1.2-fold lower mean ACO

activity before storage and maintained it even after 3 and 6 weeks storage in both skin and pulp tissues respectively (Fig. 3C and 3F). Method of PUT application did not affect the ACC contents in the skin and pulp tissues after 3 and 6 weeks of storage. Higher PUT concentrations (1.0 mM and 2.0 mM) reduced ACC contents during storage as compared to 0.5 mM PUT-treated and control fruit (Fig. 4A-F). Mean ACC contents were higher in skin tissue as compared to pulp tissues irrespective of method of PUT application. In pulp tissues mean ACC contents in preharvest PUT-treated fruit before storage were about 1-fold lower than postharvest PUT-treated fruit, after 6 weeks of storage both pre- and postharvest PUT treatments did not show any difference between their mean ACC contents in pulp tissues (Fig. 4F).

3.2.1 Fruit softening and fruit softening enzymes

Pre- and postharvest PUT treatments to plum fruit markedly retarded the fruit softening during 3 and 6 weeks of storage period at 0 ± 1^{o}C and 90 ± 5% RH (Table 1). The methods of PUT application did not show any significant ($P \leq 0.05$) effect on fruit firmness (Table 1). After 3 weeks of storage, the delay in fruit softening in preharvest PUT-treated fruit was concentration dependent and fruit treated with preharvest 2.0 mM PUT were 9.4% more firm as compared to other treatments, whilst after 6 weeks of storage no significant differences were observed among preharvest PUT concentrations (Table 1). Postharvest PUT (1.0 mM and 2.0 mM) treated fruit maintained higher fruit firmness (14.3% and 16.5%) than control fruit after 3 and 6 weeks of low temperature storage respectively.

Methods of PUT application and its concentrations significantly affected the exo-PG activities in fruit skin and pulp tissues during storage. Preharvest PUT-treated fruit skin tissue exhibited lower exo-PG activities than postharvest PUT treatments

after 0, 3 and 6 weeks of low temperature storage (Fig. 5A-C). Postharvest PUT-treated fruit skin tissue exhibited lower exo-PG activities than control fruit; however no significant differences were recorded among the PUT concentrations applied. Mean activities of exo-PG enzymes were lower in fruit pulp tissues than skin tissues during storage period. Pulp tissues of preharvest PUT-sprayed fruit before storage showed lower exo-PG activities, whilst after 3 and 6 weeks of storage no significant differences were recorded between control and preharvest PUT-sprayed fruit pulp tissues (Fig. 5D). Pulp tissues of postharvest PUT-treated fruit irrespective of PUT concentrations applied had significantly reduced the exo-PG activities compared to untreated fruit (Fig. 5E). Mean exo-PG activities in preharvest PUT-treated fruit skin tissues were lower than postharvest PUT application (Fig. 5C and 5F).

The endo-PG activities were reduced in the skin of preharvest PUT-sprayed fruit with increased storage period and concentration of PUT applied. Whilst, postharvest PUT dip treatments did not show any change in activities of endo-PG enzymes in fruit skin tissues up to 3 weeks of storage. Later on it decreased with increase in the concentrations of PUT applied (Fig. 6A-C). Similarly, higher concentrations of pre- and postharvest application of PUT reduced the activities of endo-PG enzyme in pulp tissues as compared to pulp tissues of control fruit (Fig. 6D-E). Mean endo-PG activities were higher in postharvest PUT treated fruit skin and pulp tissues than preharvest spray application (Fig. 6C and 6F).

Pre- and postharvest PUT treatments and storage time significantly affected the PE activities in plum fruit skin and pulp tissues (Fig. 7A-F). Fruit sprayed with higher PUT concentrations (1.0 mM and 2.0 mM) exhibited lower PE activities in fruit skin tissues after 3 and 6 weeks of storage than 0.1 mM PUT-treated and

untreated fruit (Fig. 7A). Postharvest PUT-treated fruit skin tissues resulted in reduced activities of PE after 3 and 6 weeks of storage as compared to control fruit (Fig. 7B). Activities of PE enzymes in postharvest PUT-treated fruit pulp tissues increased after 3 and 6 weeks of storage and fruit treated with higher PUT concentration (2.0 mM) exhibited 27.4% and 25.6% lower PE activities in pulp tissues after 3 and 6 weeks of storage as compared to untreated fruit respectively (Fig. 7E). Storage period did not affect the PE activities in preharvest PUT-sprayed fruit pulp tissue, whilst PE activities in control fruit pulp tissues increased with extended storage period (Fig. 7D). Postharvest PUT-treated fruit pulp tissues exhibited higher mean PE activity than preharvest PUT-sprayed fruit pulp tissues (Fig. 7F).

PUT treatments did not affect the EGase activities before storage in both pre- and postharvest PUT-treated fruit skin tissues (Fig. 8A and 8B). After 3 and 6 weeks of storage, pre- and postharvest PUT-treated fruit skin tissues exhibited reduced EGase activities with increased concentration of PUT applied as compared to control fruit (Fig. 8A and 8B). Mean EGase activity was higher in postharvest PUT-treated fruit skin tissue than preharvest PUT-sprayed fruit skin tissue (Fig. 8C). Similarly, pre- and postharvest PUT treatments reduced the EGase activities in pulp tissues during 3 and 6 weeks of storage compared to untreated fruit (Fig. 8D and 8E). However, postharvest PUT-treated fruit pulp tissues exhibited higher activities than preharvest PUT-treated pulp tissues (Fig. 8C).

3.2.2 Role of PUT regulated ethylene biosynthesis and fruit softening enzymes in fruit softening

Pre- and postharvest applications of PUT to plum fruit reduced ethylene production and activities of ethylene biosynthesis enzymes (ACS and ACO) and as

well as fruit softening enzymes in skin and pulp tissues during low temperature storage (Fig. 1-8). The activities of exo-PG and PE enzymes in PUT-treated skin and pulp tissues were negatively correlated to changes in fruit firmness and positively correlated to ethylene production regulated with PUT application during low temperature storage (Table 2). Fruit firmness showed significant negative correlation ($P \leq 0.01$) with ACS activities in fruit skin (r = -0.590) and pulp (r = -0.457) tissues. ACO activities in fruit skin (r = -0.502) and pulp (r = -0.537) tissues exhibited significant ($P \leq 0.01$) negative correlation with fruit firmness. Fruit firmness showed a significant ($P \leq 0.01$) negative correlation with ACC content in fruit skin (r = -0.42) and pulp (r = -0.564) tissues, as regulated with pre- and postharvest PUT treatments.

Plum fruit firmness showed significant negative ($P \leq 0.01$ and $P \leq 0.05$) correlation with exo-PG activities in fruit skin (r = -0.67) and pulp (r = -0.726) tissues respectively, while ethylene production regulated with pre- and postharvest PUT treatments showed a significant ($P \leq 0.01$) positive correlation with exo-PG activities in fruit skin (r = 0.614) and pulp (r = 0.6) tissues (Table 2). PE activities in skin and pulp tissues of PUT-treated fruit showed significant ($P \leq 0.01$) negative correlations (r = -0.386 and -0.369) with fruit firmness respectively, while the activities of PE in the skin and pulp tissues of PUT-treated fruit showed significant ($P \leq 0.01$) positive correlations with ethylene production (r = 0.429 and 0.467) respectively. There were no significant correlations between ethylene and endo-PG activities in fruit skin as well as in pulp tissues. Similarly no significant correlations were found between fruit firmness and EGase activities in skin and pulp tissues, and between fruit firmness and endo-PG activities in pulp tissues.

4. Discussion

4.1 Ethylene production and activities of ethylene biosynthesis enzymes

Pre- and postharvest PUT treatments reduced the ethylene production during storage in 'Angelino' plum fruit. There was inverse relationship between concentrations of PUT applied and ethylene production. The reduction in ethylene production with PUT treatment may be attributed to competitive biosynthesis mechanism between ethylene and polyamines (Cohen, 1998) and may also be argued to the reduction of ACS and ACO activities as evident from our experimental data (Fig. 1-2) and also reported earlier (Apelbaum et al., 1981; Even-Chen et al., 1982; Ke and Romani, 1988; Kakkar and Rai, 1993; Lee et al., 1997). Similarly, reduction in ethylene production following PAs treatments has been reported in apricot (Martinez-Romero et al., 2001), kiwifruit (Petkou et al., 2004), Mango (Malik and Singh, 2005), peach (Bregoli et al., 2002), and some plum cultivars (Perez-Vicente et al., 2001; Serrano et al., 2003).

To the best of our knowledge it is the first time that effects of pre- and postharvest PUT treatments on the activities of ethylene biosynthesis enzymes (ACS and ACO) and ACC contents in plum have been investigated. Our experimental data show that exogenous PUT treatments reduced ACS and ACO activities in fruit skin and pulp tissues after 3 and 6 weeks of storage which resulted in suppressed ethylene production. Possibly PUT induced reduction in ACS activity seems to be associated with the competition of ethylene and PAs biosynthesis pathways for their common precursor SAM. The reduction in ACS activities resulted in the production of markedly reduced ACC contents and activities of ACO enzymes which consequently suppressed the endogenous ethylene production. PAs induced reduction of ACS activities have also been reported in pear and avocado (Kakkar and Rai, 1993). PAs

have been reported to inhibit conversion of SAM into ACC in orange (Even-Chen et al., 1982). Similarly exogenous application PAs has been claimed to reduce ethylene production in variety of plant tissues by reducing activity of ACS and ACO enzymes (Ke and Romani, 1988; Lee et al., 1997) and by changing the flux of SAM leading to PAs synthesis (Even-Chen et al., 1982; Lee et al., 1997). Thus it may be argued that PUT influences the ethylene production by competing for the common precursor SAM as well as suppressing the activities of ethylene biosynthesis enzymes.

4.2 Fruit softening and activities of fruit softening enzymes

Exogenous application of PUT either as preharvest spray or postharvest dip retarded fruit softening during storage (Table 1). The reduction in fruit softening with PUT treatments may be due to reduction in endogenous ethylene production as evident from a significant ($P \leq 0.01$) negative correlation between fruit firmness and the activities of ethylene biosynthesis enzymes and ACC contents in both fruit skin and pulp tissues (Table 2). Moreover exogenous application of 1-methylcyclopropene (1-MCP, ethylene inhibitor) has been found to reduce fruit softening in plum (Khan and Singh, 2004). Similarly, exogenous applications of PAs have also been reported to maintain the fruit firmness during ripening and at low temperature storage in 'Frior', 'Black Star' and 'Santa Rosa' plum cultivars (Abu-Kpawoh et al., 2002; Valero et al., 2002; Serrano et al., 2003).

The cell wall softening enzymes play key role in cell wall degradation and fruit softening during fruit ripening. Delayed fruit softening in PUT-treated fruit may be ascribed to the reduction in the activities of fruit softening enzymes such as exo-PG, endo-PG, PE and EGase. It has been reported that PG, PE and EGase enzymes are primarily responsible for ripening associated pectin degradation and fruit

softening (Huber, 1983; Brady, 1987). PE catalyses the softening process through de-esterification of pectin followed by pectin depolymerisation, catalysed by PG (Roe and Bruemmer, 1981). Our experimental data show that softening of 'Angelino' plum fruit during storage is correlated with activities fruit softening enzymes (Table 2). The activities of exo-PG, endo-PG, PE and EGase enzymes were higher in untreated fruit skin and pulp tissues as compared to PUT-treated fruit (Fig. 5-8). Similarly in apricot, PAs applications retarded the senescence by stabilising the cell membrane and inhibiting the activities of PG and PME involved in the softening of enzymes (Martinez-Romero et al., 2002).

The direct role of PUT in the reduction of activities of fruit softening enzymes is yet not well understood. This might be associated to the inhibitory effect of PUT on the endogenous ethylene production as the activities of exo-PG and PE enzymes in fruit skin and pulp tissues showed a significant ($P \leq 0.01$) positive correlation with endogenous ethylene production (Table 2). Recently, reduced fruit softening and reduction in the activities of exo-PG, end-PG, PE and Egase enzymes with exogenous application of ethylene inhibitor (1-MCP) has been reported in plum (Khan and Singh, 2007) further support this hypothesis. In 'Songold' plum, increased PG activity was found to be responsible for the increased rate of pectin degradation and fruit softening (Taylor et al., 1995). The cell wall softening enzymes were also reported to play key role in cell wall degradation in peach and nectarine (Ben-Arie and Sonego, 1980).

In conclusion preharvest spray and postharvest dip application of PUT retarded plum fruit softening during low temperature storage due to suppressed ethylene production, reduced activities of ethylene biosynthesis enzymes (ACS, ACO)

and ACC contents, and as well as fruit softening enzymes (PE, EGase, exo and endo-PG) in skin as well as pulp tissues.

Acknowledgements

Ahmad S. Khan acknowledges the financial support from Higher Education Commission (HEC), Pakistan for awarding partial support for PhD studies abroad, Curtin University of Technology for granting IPRS award, and Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan for granting him study leave to complete his PhD studies. We thank Dr. Susan Lurie, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel for her help in estimation of fruit softening enzymes. We are grateful to Casuarina Valley Orchards, Karrugullen Manjimup, Western Australia for providing the plum fruit for the research and Mrs. Alicia Pasznicki, Manager, Curtin Horticulture Research Laboratory at Curtin University of Technology for her technical assistance. Professor N. A. Abbasi also acknowledges HEC of Pakistan for awarding him Post Doctoral fellowship.

References

Abu-Goukh, A.A., Bashir, H.A., 2003. Changes in pectin enzymes and cellulase activity during guava fruit ripening. Food Chem. 83, 213-218.

Abu-Kpawoh, J.C., Xi, Y.F., Zhang, Y.Z., Jin, Y.F., 2002. Polyamine accumulation following hot-water dips influences chilling injury and decay in 'Friar' plum fruit. J. Food Sci. 67, 2649-2653.

Apelbaum, A., Burgoon, A.C., Anderson, J.D., Lieberman, M., Ben-Arie, R., Mattoo, A.K., 1981. Polyamines inhibit biosynthesis of ethylene in higher plant tissue and fruit protoplasts. Plant Physiol. 68, 453-456.

Barrett, M.D., Gonzalez, C., 1994. Activity of softening enzymes during cherry maturation. J. Food Sci. 59, 574-577.

Ben-Arie, R., Sonego, L., 1980. Pectolytic enzyme activity involved in woolly breakdown of stored peaches. Phytochem. 19, 2553-2555.

Borrell, A., Carbonell, L., Farras, R., Puig-Parellaba, P., Tiburcio, A.F., 1997. Polyamines inhibit lipid oxidation in senescing oat leaves. Physiol. Plant. 99, 385-390.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Anal. Biochem. 72, 248-254.

Brady, C.J., 1987. Fruit Ripening. Ann. Rev. Plant Physiol. 38, 155-178.

Bregoli, A.M., Scaramagli, S., Costa, G., Sabatini, E., Ziosi, V., Bionsim, S., Torrigiani, P., 2002. Peach (*Prunus persica*) fruit ripening: aminoethoxyvinylglycine (AVG) and exogenous polyamines affect ethylene emission and flesh firmness. Physiol. Plant. 114, 472-481.

Cohen, S.S. 1998. *A guide to polyamine.* Oxforad University Press, New York, pp. 1-959

Even-Chen, Z., Mattoo, A.K., Goren, R., 1982. Inhibition of ethylene biosynthesis by aminoethoxyvinylglycine and polyamines shunts label from 3,4-(^{14}C)methyonine into spermidine in aged orange peel discs. Plant Physiol. 69, 385-388.

Fischer, R.L., Bennett, A.B., 1991. Role of cell wall hydrolysis in fruit ripening. Ann. Rev. Plant Physiol. Plant Mol. Biol. 42, 675-703.

Hiwasa, K., Kinugasa, Y., Amano, S., Hashimoto, A., Nakano, R., Inaba, A., Kubo, Y., 2003. Ethylene is required for both the initiation and progression of softening in pear (*Pyrus communis* L.) fruit. J. Expt. Bot. 54, 771-779.

Huber, D.J., 1983. The role of cell wall hydrolyses in fruit softening. Hort. Rev. 5, 169-219.

Jeong, J., Huber, D.J., Sargent, S.A., 2002. Influence of 1-methylcyclopropene (1-MCP) on ripening and cell-wall matrix polysaccharides of avocado (*Persea americana*) fruit. Postharvest Biol. Technol. 25, 241-256.

Kakkar, R.K., Rai, V., K., 1993. Plant polyamines in flowering and fruit ripening. Phytochem. 33, 1281-1288.

Ke, D., Romani, R.J., 1988. Effects of spermidine on ethylene production and the senescence of suspension-cultured pear fruit cells. Plant Physiol. Biochem. 26, 109-116.

Khan, A.S., Singh, Z., 2004. Postharvest application of 1-MCP affects ethylene biosynthesis and firmness of `Tegan Blue' plum. Acta Hort. 687, 409-410.

Khan, A.S., Singh, Z., 2007. 1-MCP regulates ethylene biosynthesis and fruit softening during ripening of 'Tegan Blue' plum. Postharvest Biol. Technol. 43, 298-306.

Knee, M., 1978. Metabolism of polygalacturonase in apple fruit cortical tissue during ripening. Phytochem. 17, 1085-1087.

Kramer, G.F., Wang, C.Y., Conway, W.S., 1991. Inhibition of softening by polyamine application in 'Golden Delicious' and 'McIntosh' apples. J. Am. Soc. Hort. Sci. 116, 813-817.

Kumar, A., Taylor, M.A., Mad Arif, S.A., Davies, H.V., 1996. Antisense and sense expression of S-adenosylmethionine decarboxylase cDNA in transgenic potato plant and its effects on polyamines and ethylene biosynthesis. Plant J. 9, 147-158.

Lashbrook, C.C., Gonzalez-Bosch, C., Bennett, A.B., 1994. Two divergent endo-1,4-*ß*-D-glucanase genes exhibit overlapping expression in ripening fruit and abscising flowers. The Plant Cell 6, 1485-1493.

Lee, M.M., Lee, S.H., Park, K.Y., 1997. Effect of spermine on ethylene biosynthesis in carnation (*Dianthus caryophyllus* L.) flowers during senescence. J. Plant Physiol. 151, 68-73.

Lelievre, J.M., Latche, A., Jones, B., Bouzayen, M., Pech, J.C., 1997. Ethylene and fruit ripening. Physiol. Plant. 101, 727-739.

Malik, A.U., Singh, Z., 2004. Endogenous free polyamines of mangos in relation to development and ripening. J. Am. Soc. Hort. Sci. 129, 280-286.

Malik, A.U., Singh, Z., 2005. Pre-storage application of polyamines improves shelf-life and fruit quality in mango. J. Hort. Sci. Biotechnol. 80, 363-369.

Martinez-Romero, D., Serrano, M., Carbonell, A., Brugos, L., Riquelme, F., Valero, D., 2002. Effects of postharvest putrescine treatment on extending shelf life and reducing mechanical damage in apricot. J. Food Sci. 67, 1706 -1712.

Martinez-Romero, D., Valero, D., Riquelme, F., Zuzunaga, M., Serrano, M., Burlo, F., Carbonell, A., 2001. Infiltration of putrescine into apricots helps handling and storage. Acta Hort. 553, 189-192.

Mithcham, E.J., Gross, K.C., Ng, T.J., 1991. Ripening and cell wall synthesis in normal and mutant tomato fruit. Phytochem. 30, 1777-1780.

Paksasorn, A., Hayasaka, T., Matsui, H., Ohara, H., Hirata, N., 1995. Relationship of polyamine content to ACC content and ethylene evolution in Japanese apricot fruit. J. Jpn. Soc. Hort. Sci. 63, 761-766.

Pandey, S., Ranade, S.A., Nagar, P.K., Kumar, N., 2000. Role of polyamines and ethylene as modulators of plant senescence. J. Biosci. 25, 291-299.

Paull, R.E., Chen, R.J., 1983. Post harvest variation in cell wall degrading enzymes of papaya during fruit ripening. Plant Physiol. 72, 382-385.

Perez-Vicente, A., Martinez-Romero, D., Carbonell, A., Burlo, F., Serrano, M., Valero, D., Riquelme, F., Zuzunaga, M., 2001. Role of exogenous putrescine on the metabolism of conjugated polyamines in mechanically damaged plum during storage. Acta Hort. 553, 193-194.

Perez-Vicente, A., Martinez-Romero, D., Carbonell, A., Serrano, M., Riquelme, F., Guillen, F., Valero, D., 2002. Role of polyamines in extending shelf life and the reduction of mechanical damage during plum (*Prunus salicina* Lindl.) storage. Postharvest Biol. Technol. 25, 25-32.

Petkou, I.T., Pritsa, T.S., Sfakiotakis, E.M., 2004. Effects of polyamines on ethylene production, respiration and ripening of kiwifruit. J. Hort. Sci. Biotechnol. 79, 977-980.

Roe, B., Bruemmer, J.H., 1981. Changes in pectin substances and enzyme during ripening and storage of 'Keitt' mango. J. Food Sci. 54, 186-189.

Serrano, M., Martinez-Romero, D., Guillen, F., Valero, D., 2003. Effect of exogenous putrescine on improving shelf life of four plum cultivars. Postharvest Biol. Technol. 30, 259-271.

Sitrit, Y., Bennett, A.B., 1998. Regulation of tomato fruit polygalacturonase mRNA accumulation by ethylene: A re-examination. Plant Physiol. 116, 1145-1150.

Slocum, R.D., Kaur-Sawhney, R., Galston, A.W., 1984. The physiology and biochemistry of polyamines in plants. Arc. Biochem. Biophys. 235, 283-303.

Smith, T.A., 1985. Polyamines. Annu. Rev. Plant Physiol. 36, 117-143.

Taylor, M.A., Rabe, E., Jacobs, G., Dodd, M.C., 1995. Effect of harvest maturity on pectic substances, internal conductivity, soluble solids and gel breakdown in cold stored 'Songold' plums. Postharvest Biol. Technol. 5, 285-294.

Valero, D., Perez-Vicente, A., Martinez-Romero, D., Castillo, S., Guillen, G., Serrano, M., 2002. Plum storability improved after calcium and heat postharvest treatments: Role of polyamines. J. Food Sci. 67, 2571-2575.

Walden, A., Cordeirro, A., Tiburcio, A.F., 1997. Polyamines: small molecules triggering pathways in plant growth and development. Plant Physiol. 113, 1009-1013.

Zhou, H.W., Ben, A.R., Lurie, S., 2000. Pectin esterase, polygalacturonase and gel formation in peach pectin fractions. Phytochem. 55, 191-195.

Table 1

Effects of different concentrations of putrescine (PUT) applied as preharvest spray and postharvest dip application on fruit firmness in 'Angelino' plum.

PUT concentrations (mM)	Method	Storage period (weeks)			Mean
		0	3	6	
0.0	Preharvest	37.2	33.2	29.9	33.4
0.1	Preharvest	36.8	34.4	30.9	34.0
1.0	Preharvest	36.4	35.1	30.8	34.1
2.0	Preharvest	37.2	36.3	30.7	34.7
Mean		36.8	34.7	30.5	
0.0	Postharvest	36.7	30.85	28.3	31.9
0.1	Postharvest	34.7	33.13	31.1	33.0
1.0	Postharvest	36.3	35.2	32.4	34.6
2.0	Postharvest	36.6	36.0	33.0	35.2
Mean		36.9	33.8	31.2	
LSD $P \leq 0.05$					
PUT concentrations		0.65	0.64	0.66	0.65
Method		NS	NS	NS	NS
Storage period		0.56	0.56	0.54	0.55
PUT concentrations x storage period		1.11	1.12	1.10	1.09
PUT concentrations x method		0.91	0.90	0.92	0.91
PUT concentrations x storage period x method		NS	NS	NS	NS

n = 24 (8 fruit x 3 replications). NS = not significant.

Table 2

Relationships between ethylene production, ethylene biosynthesis and fruit softening enzymes; and fruit firmness, ethylene biosynthesis and fruit softening enzymes in skin and pulp tissues of 'Angelino' plum treated with different concentrations of PUT as preharvest spray and postharvest dip applications.

Variable compared	Pearson's correlation	
	Skin	Pulp
Ethylene *vs* ACS	0.433**	0.341**
Ethylene *vs* ACO	0.491**	0.504**
Ethylene *vs* ACC	0.288*	0.386**
Ethylene *vs* exo-PG	0.614**	0.600**
Ethylene *vs* endo-PG	NS	NS
Ethylene *vs* PE	0.429**	0.467**
Ethylene *vs* EGase	NS	NS
Firmness *vs* ACS	-0.590**	-0.457**
Firmness *vs* ACO	-0.502**	-0.537**
Firmness *vs* ACC	-0.420**	-0.564**
Firmness *vs* exo-PG	-0.670**	-0.726*
Firmness *vs* endo-PG	-0.290*	NS
Firmness *vs* PE	-0.386**	-0.369**
Firmness *vs* EGase	NS	NS

NS, *, ** = not significant or significant at $P \leq 0.05$ or 0.01 respectively

Fig. 1.

Effects of different concentrations of PUT (T) applied as preharvest spray and postharvest dip applications on ethylene production in 'Angelino' plum stored for 0, 3 and 6 weeks (SP). (A = preharvest, B = postharvest and C = mean ethylene production). n = 3 replicates. Vertical bars represent S.E. of means. LSD ($P \leq 0.05$) for preharvest: T = 0.47, SP = 0.41, T x SP = 0.81. Postharvest: T = 0.46, SP = 0.4, T x SP = 0.81. Mean: T = 0.46, SP = 0.41, T x SP = 0.82.

Fig. 2.

Effects of different concentrations of PUT (T) applied as preharvest spray and postharvest dip applications on the activities of ACS enzymes in skin and pulp tissues of 'Angelino' plum stored for 0, 3 and 6 weeks (SP).(A and D = preharvest, B and E = postharvest and C and F = mean ACS activities). n = 3 replicates. Vertical bars represent S.E. of means. LSD ($P \leq 0.05$) for skin, preharvest: T = 0.062, SP = 0.05, T x SP = 0.11. Postharvest: T = 0.06, SP = 0.05, T x SP = 1.11. Mean: T = 0.06, SP = 0.05, T x SP = 0.11. Pulp, preharvest: T = 0.06, SP = 0.05, T x SP = 0.09. Postharvest: T = 0.06, SP = 0.05, T x SP = 0.1. Mean: T = 0.06, SP = .0.05, T x SP = 0.1.

Fig. 3.

Effects of different concentrations of PUT (T) applied as preharvest spray and postharvest dip applications on the activities of ACO enzymes in skin and pulp tissues of 'Angelino' plum stored for 0, 3 and 6 weeks (SP). (A and D = preharvest, B and E = postharvest and C and F = mean ACO activities). n = 3 replicates. Vertical bars represent S.E. of means. LSD ($P \leq 0.05$) for skin, preharvest: T = 0.6, SP = 0.52, T x SP = NS. Postharvest: T = 0.61, SP = 0.52, T x SP = NS. Mean: T = 0.61, SP = 0.53, T x SP = NS. Pulp, preharvest: T = 0.53, SP = 0.46, T x SP = NS. Postharvest: T = 0.52, SP = 0.46, T x SP = NS. Mean: T = 0.53, SP = 0.45, T x SP = NS. NS = not significant.

Fig. 4.

Effects of different concentrations of PUT (T) applied as preharvest spray and postharvest dip applications on the ACC content in skin and pulp tissues of 'Angelino' plum stored for 0, 3 and 6 weeks (SP). (A and D = preharvest, B and E = postharvest and C and F = mean ACC contents). n = 3 replicates. Vertical bars represent S.E. of means. LSD ($P \leq 0.05$) for skin, preharvest: T = 0.09, SP = 0.08, T x SP = NS. Postharvest: T = 0.093, SP = 0.08, T x SP = NS. Mean: T = 0.09, SP = 0.08, T x SP = NS. Pulp, preharvest: T = 0.06, SP = 0.05, T x SP = NS. Postharvest: T = 0.06, SP = 0.05, T x SP = NS. Mean: T = 0.06, SP = 0.053, T x SP = NS. NS = not significant.

Fig. 5.

Effects of different concentrations of PUT (T) applied as preharvest spray and postharvest dip applications on the activities of exo-PG enzymes in skin and pulp tissues of 'Angelino' plum stored for 0, 3 and 6 weeks (SP). (A and D = preharvest, B and E = postharvest and C and F = mean exo-PG activities). n = 3 replicates. Vertical bars represent S.E. of means. LSD ($P \leq 0.05$) for skin, preharvest: T = 1.46, SP = 1.27, T x SP = 2.53. Postharvest: T = 1.45, SP = 1.25, T x SP = 2.52. Mean: T = 1.47, SP = 1.26, T x SP = 2.53. Pulp, preharvest: T = 1.53, SP = 1.33, T x SP = NS. Postharvest: T = 1.54, SP = 1.34, T x SP = NS. Mean: T = 1.54, SP = 1.34, T x SP = NS. NS = not significant.

Fig. 6.

Effects of different concentrations of PUT (T) applied as preharvest spray and postharvest dip applications on the activities of endo-PG enzymes in skin and pulp tissues of 'Angelino' plum stored for 0, 3 and 6 weeks (SP). (A and D = preharvest, B and E = postharvest and C and F = mean endo-PG activities). n = 3 replicates. Vertical bars represent S.E. of means. LSD ($P \leq 0.05$) for skin, preharvest: T = 1.35, SP = 1.17, T x SP = NS. Postharvest: T = 1.35, SP = 1.16, T x SP = NS. Mean: T = 1.35, SP = 1.17, T x SP = NS. Pulp, preharvest: T = 1.31, SP = 1.14, T x SP = 2.23. Postharvest: T = 1.32, SP = 1.15, T x SP = 2.28. Mean: T = 1.32, SP = 1.14, T x SP = 2.28. NS = not significant.

Fig. 7.

Effects of different concentrations of PUT (T) applied as preharvest spray and postharvest dip applications on the activities of PE enzymes in skin and pulp tissues of 'Angelino' plum stored for 0, 3 and 6 weeks (SP). (A and D = preharvest, B and E = postharvest and C and F = mean PE activities). n = 3 replicates. Vertical bars represent S.E. of means. LSD ($P \leq 0.05$) for skin, preharvest: T = 0.003, SP = 0.002, T x SP = NS. Postharvest: T = 0.003, SP = 0.002, T x SP = NS. Mean: T = 0.002, SP = .003, T x SP = NS. Pulp, preharvest: T = 0.003, SP = 0.002, T x SP = 0.005. Postharvest: T = 0.002, SP = 0.003, T x SP = 0.005. Mean: T = 0.002, SP = 0.003, T x SP = 0.005.NS = not significant.

Fig. 8.

Effects of different concentrations of PUT (T) applied as preharvest spray and postharvest dip applications on the activities of EGase enzymes in skin and pulp tissues of 'Angelino' plum stored for 0, 3 and 6 weeks (SP). (A and D = preharvest, B and E = postharvest and C and F = mean EGase activities). n = 3 replicates. Vertical bars represent S.E. of means. LSD ($P \leq 0.05$) for skin, preharvest: T = 2.26, SP = 1.96, T x SP = 3.93. Postharvest: T = 2.27, SP = 1.97, T x SP = 3.94. Mean: T = 2.26, SP = 1.67, T x SP = 3.95. Pulp, preharvest: T = 2.45, SP = 2.12, T x SP = 4.25. Postharvest: T = 2.46, SP = 2.13, T x SP = 4.26. Mean: T = 2.46, SP = 2.13, T x SP = 4.26.

Figure 1 Khan et al.,

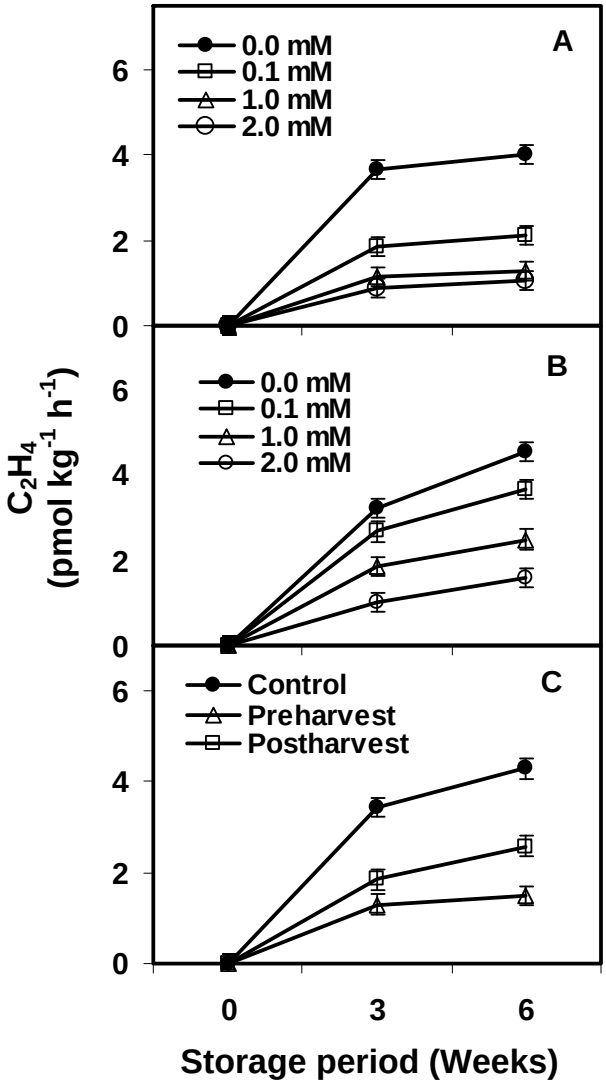

Figure 2 Khan et al.,

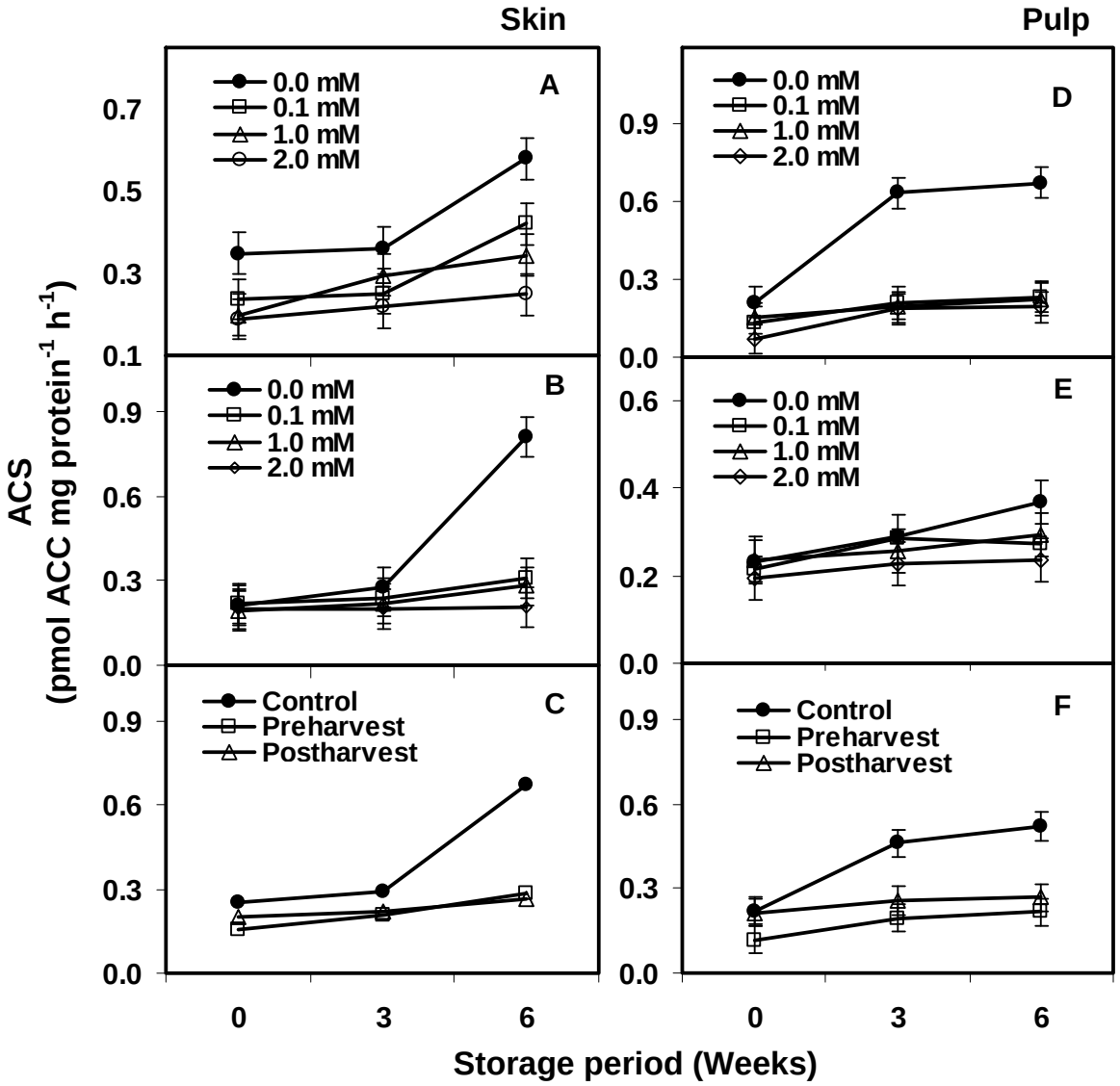

Figure 3 Khan et al.,

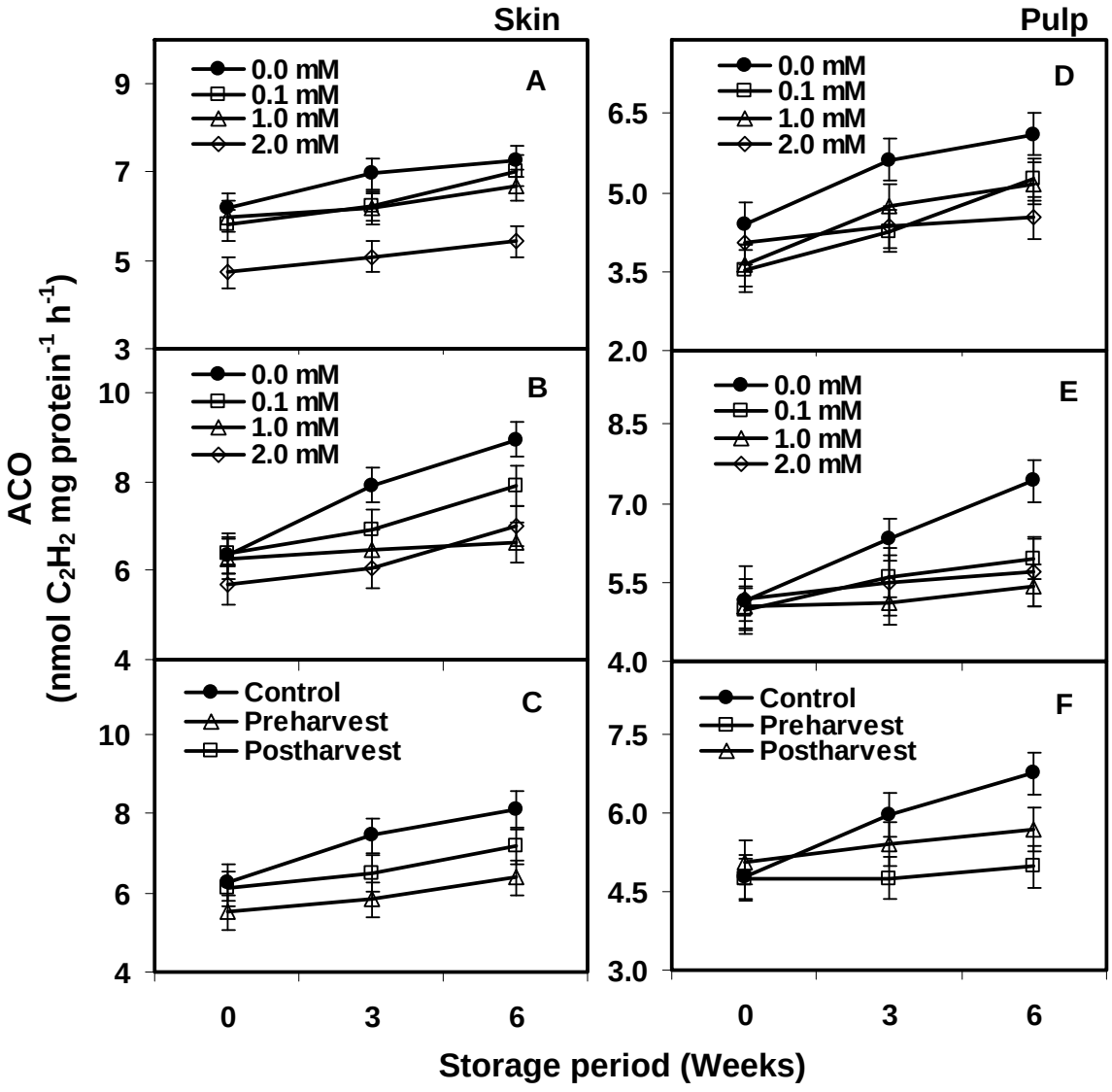

Figure 4 Khan et al.,

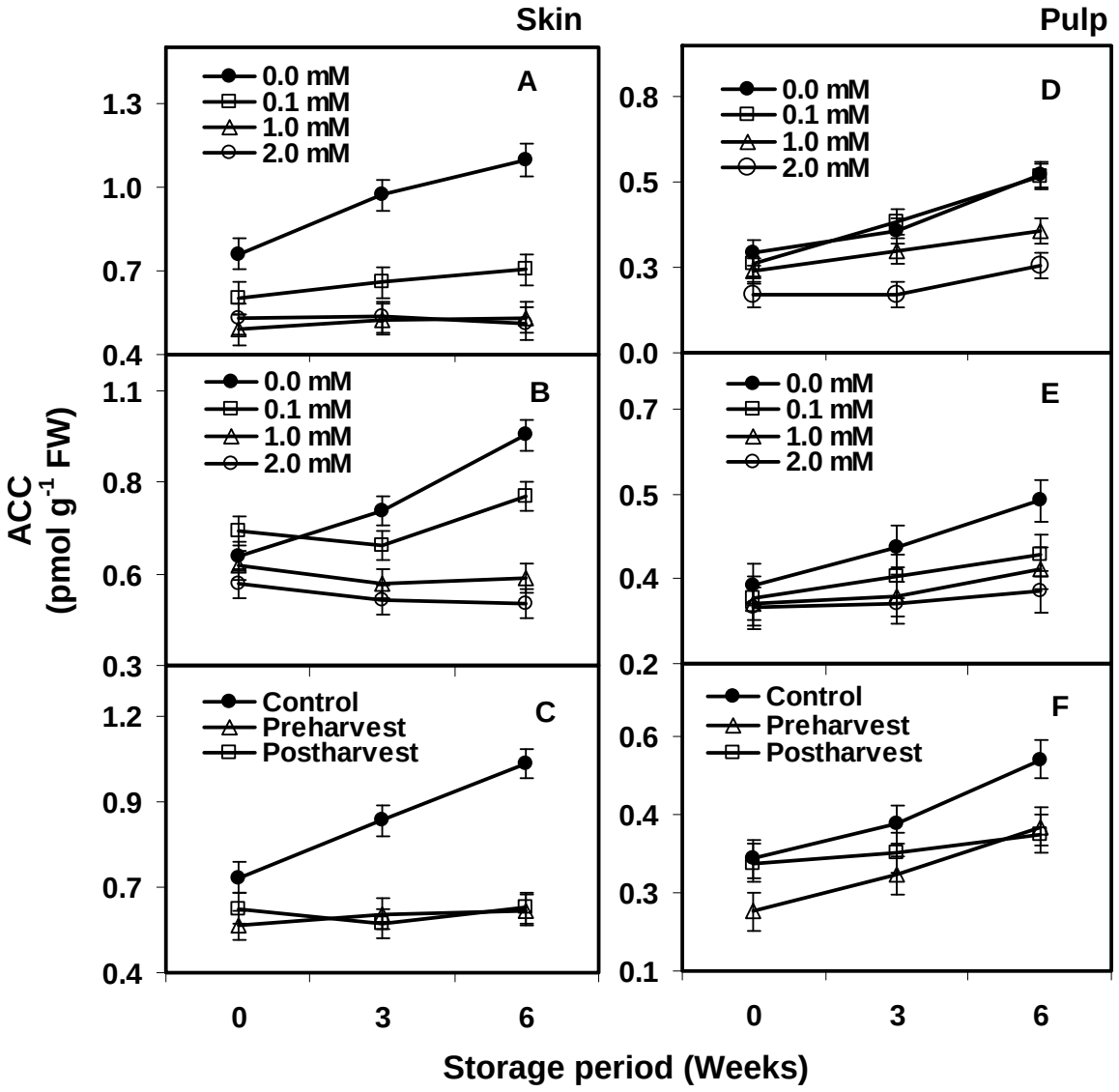

Figure 5 Khan et al.,

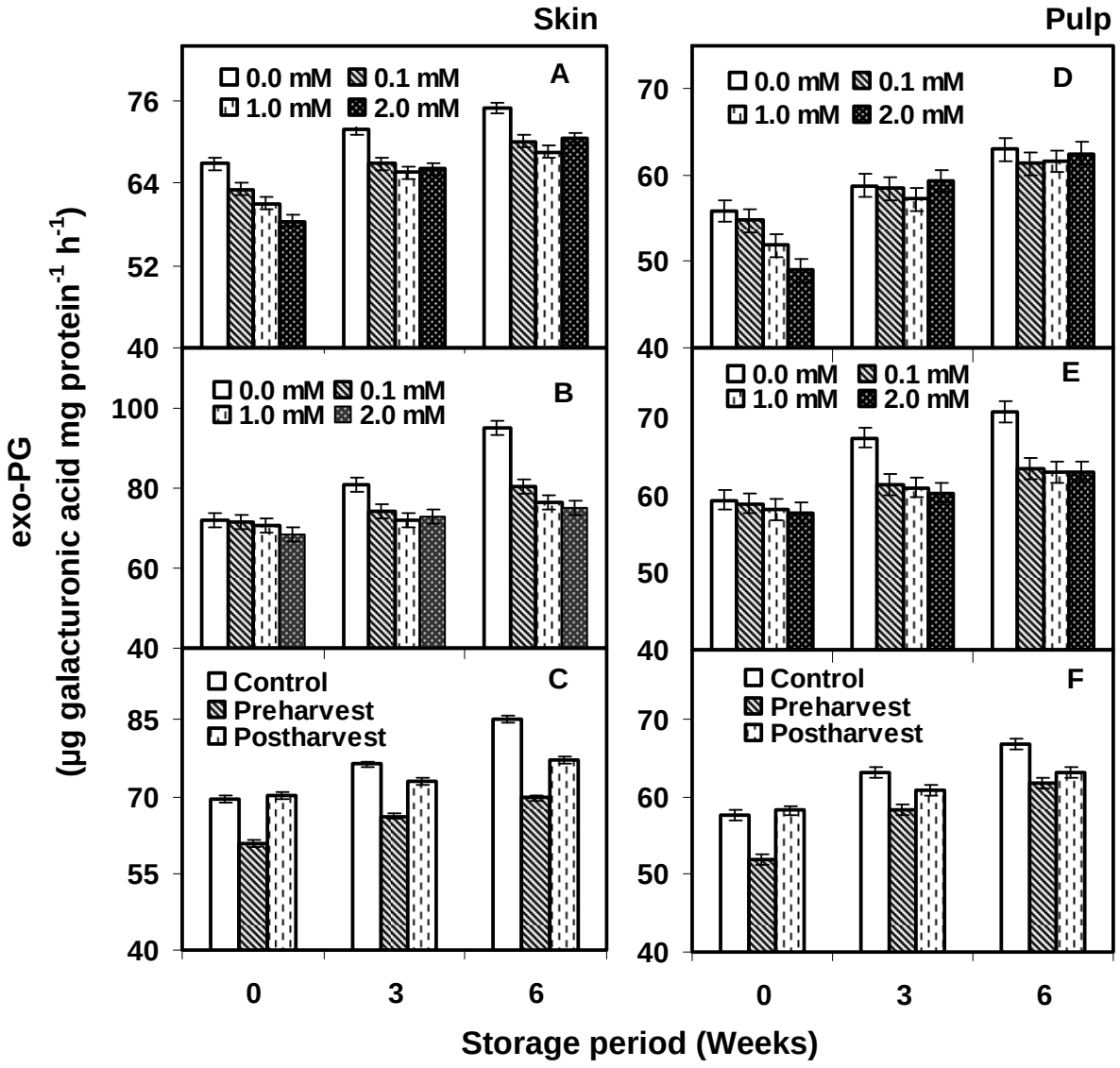

Figure 6 Khan et al.,

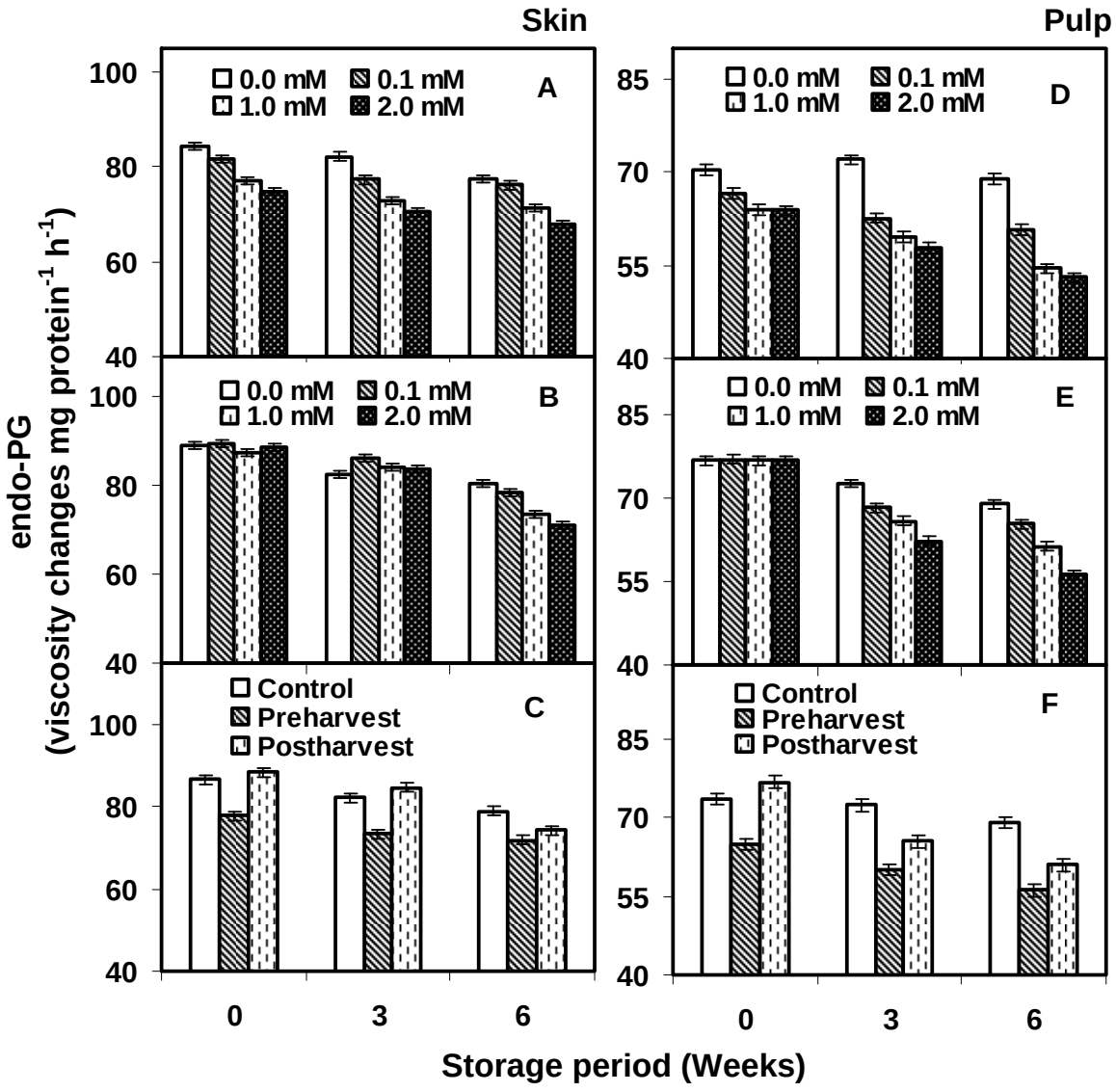

Figure 7 Khan et al.,

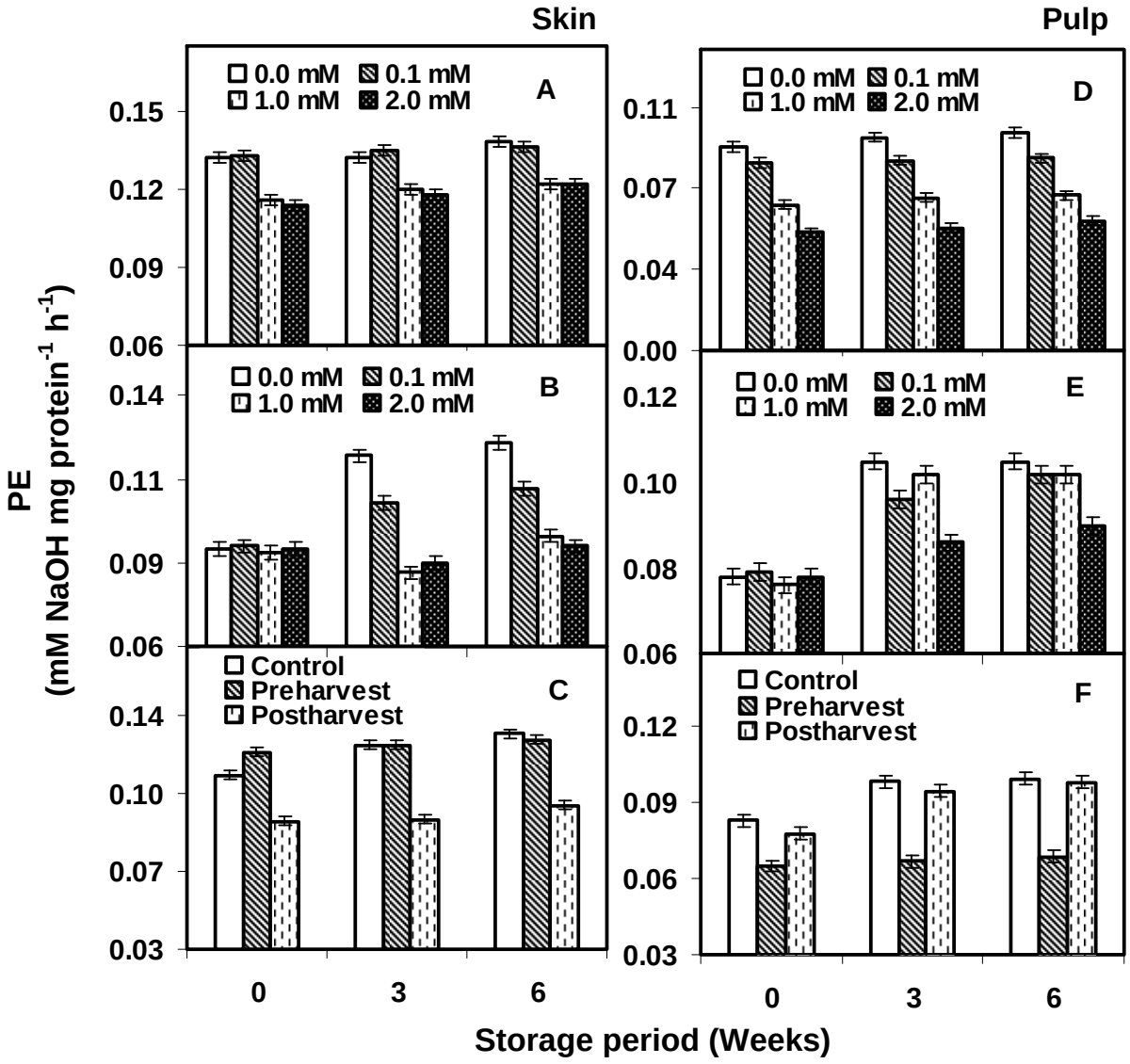

Figure 8 Khan et al.,