



International Journal of Agricultural and
Environmental Research
Available online at www.ijaaer.com
ISSN 2414-8245 (Online)
ISSN 2518-6116 (Print)



Free
and
open
Access

ROLE OF PECTIN-ESTERASE TO IMPROVE THE STORAGE LIFE OF KINNOW FRUIT DURING COLD STORAGE

Ayesha Maryam^{a*}, Adil Khan^a, Saeed Ahmad^a, Muhammad Jaffar Jaskani^a and Nazir Javed^b.

^a Institute of Horticulture Sciences, University of Agriculture, Faisalabad Pakistan.

^b Department of Plant pathology, University of Agriculture, Faisalabad Pakistan.

Corresponding author*: maryam.ayesha_22@rocketmail.com

Abstract

Kinnow mandarin (*Citrus nobilis* × *Citrus deliciosa*) is the leading commercial citrus cultivar of Pakistan. After harvesting some internal changes are started and deteriorate the fruit quality so its shelf life is less. Hence, to overcome these problems pectin-esterase is used to improve the cosmetic value and shelf life of Kinnow fruit during storage. For this purpose, Mature, healthy and disease free Kinnow mandarin fruits were harvested from the experimental orchard Square No. 9, Institute of Horticulture Sciences, University of Agriculture, Faisalabad. Fruits were treated with different concentration of pectin-esterase (0, 2, 3, 4, 5, and 6 %) of each three replications then stored at 5°C with relative humidity 90-95%. Data regarding different quality parameters were analysed at different interval during storage (0, 15, 45, and 60 days) such as physical parameters (Firmness, Chilling injury and Decay %), Biochemical parameters (Vitamin-C, TSS, TA) and phytochemical parameters (Total antioxidant). The results showed that treatment 2% pectin-esterase (PE) showed 10% decay after 60 days of storage. Minimum chilling injury (2.33%) was also observed in fruits of same fruits treatment. 2% PE treatment significantly maintained the higher fruit firmness (2.413 N) throughout the storage compared to other treatments. However, TSS (12.5 °Brix) and TA (12.5) were highest in 2% PE after 60-days storage than control fruit. PE treatment with 2% exhibited significantly increased in AO (31.193%) on 60-days storage. The Kinnow stored under these condition retained the acceptable quality above 60 days.

Key Words: Kinnow mandarin, Pectin-esterase, Cold storage, Fruit firmness.

1. INTRODUCTION

Kinnow has a shorter duration crop (January to April) due to short harvesting period thus increase the threat of high postharvest losses. The mass of post-harvest losses are significantly higher and range up to 35-40 % (Iqbal, 1996; PHDBE, 2004). Primary factor of post-harvest losses in citrus during storage is water loss due to higher respiration rate. The vastness of post-

harvest losses can be reduced by waxing of the fruit. So, waxing is mostly used to protect the fruit and minimize the activities of the fruit and lower the respiration rate (Henriod et al., 2005). After harvest, if the fruit is cooled rapidly, shelf life of the fruit can be extended. Respiration rates of the fruit and decay of fruit by organism is directly related to temperature (Elwahab and Rashid, 2013). Pectin is found abundantly 50% in cell wall. For accountable of wall loosening and mortification pectin naturally endure solubilisation and depolymerisation when fruit moderating starts (Fischer and Bennett, 1991). In plant, pectin present in the form of polysaccharides (Aspinall, 1970). Citrus peel and apple are the main sources of pectin. Pectin is widely used by the food processors for transformation of low-grade fruits into quality products like jam, jelly, marmalade and candies (Srirangarajan and Shrikhande, 1979; Jain *et al.*, 1984). Softening of many ripening fruits is due to modification of cell wall structure and middle lamella (Seymour and Gross, 1996). These modifications are occurred due to the hydrolysis pectin present in the cell wall which is responsible enzymes pectin-esterase and β -galactosidase. (Huber, 1983). Due to the enzyme-mediated modification in the structure and anatomy of the cell wall are the source of structural alternation of fruit softening (Prasanna et al., 2007). Firmness losses are reduced by a number of practices for example infusion of Ca, treatment of vitamin-C, treatment of PME and exposure to pulsed light (Charles et al., 2013). Among other study mixture of calcium and PME enzyme display good effects about the maintenance the structure of fruit (Draye et al., 2008). The hypothesis was developed to investigate the effects of pectin esterase to maintain the quality of fruit during cold storage.

2. MATERIAL AND METHOD

2.1 Collection of Fruit samples: For this experiment, three hundred and fifty mature, uniform and healthy Kinnow fruits were selected and harvested on Feb, 2015 from Experimental Fruit Orchard Square No. 9 (31 °25' N; 73 °09' E), Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The collected fruits were transferred to Post-Harvest Research Centre, Ayub Agriculture Research Institute, Faisalabad.

2.2 Preparation of wax solution: The wax solution was prepared by dissolving the pectin esterase (2, 3, 4, 5, and 6 g, depends upon the concentration) into 100ml water phase. This formulation produces very fine emulsion.

2.3 Pre-treatment and Treatment: Kinnow mandarin was washed by scrubbing gently the fruit surface in tap water. Fruits were air dried and ready to be used for the experiment. Fruits were divided into 6 groups (60 fruits/group). Each group was treated by different treatments. Coating was performed by dip method and fruits were then air dried. The fruit was dipped in the following concentrations of pectin-esterase solution (0, 2, 3, 4, 5, and 6) for one mint. All lots of fruits were packed according to the experimental layout and stored at 5°C with relative humidity of 90-95%. Fruit juice analysis was carried out after 15, 45, and 60 days interval and it was compared with 0 day storage. Different parameters like physical (decay % and chilling injury) chemical (Vitamin-C, TSS and TA) and phytochemical (total phenolic contents) analysis was carried out in Post-Graduate Pomology Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. This experiment was conducted under CRD factorial arrangement with three replications.

Detail of treatments are given below

T₁= Control (without treatment), T₂= Pectin-esterase @ 2% , T₃= Pectin-esterase @ 3%

T₄ = Pectin-esterase @ 4%, T₅ = Pectin-esterase @ 5% , T₆= Pectin-esterase @ 6%

2.4 Procedures: Fruit firmness of Kinnow mandarin was determined by Penetrometer by using following scale by Anwar et al. (2008).

1= very firm, 2= firm, 3= slightly soft, 4= soft, 5= very soft.

Decay percentage was recorded after 7 days interval by visual observation. Spoiled fruit were discarded during storage from each treatment.

Decay%= Spoiled fruits/total fruits ×100.

Chilling injury was scored manually according to the following scale by Amin (2012)

1 = Nil; 2 = < 10% affected area; 3 = 10-25% affected area; 4 = 25-50% affected area; 5 = >50% affected area.

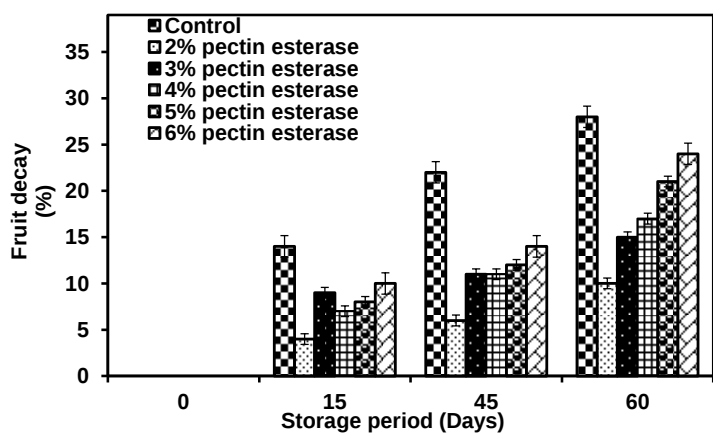
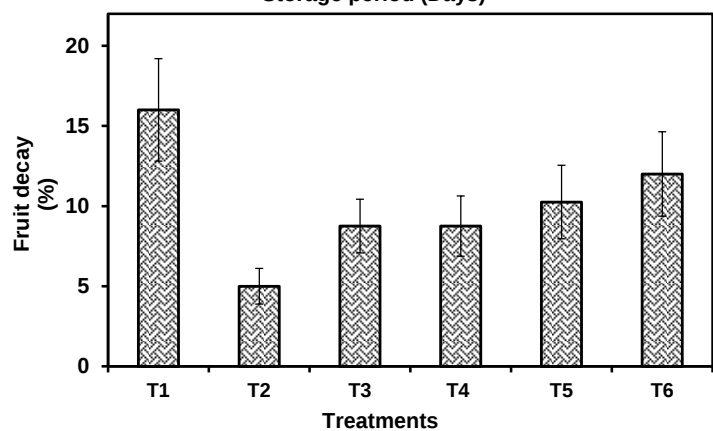
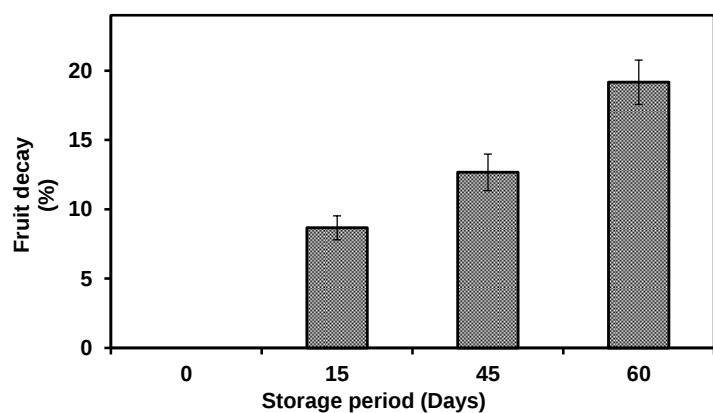
The TA of fruit juice was determined by method given by Hortwitz (1960). Total soluble solids were determined according to AOAC (1990) using hand Refrectro-meter at room temperature. The extracted juice from each lot was shaken well and the representative samples were placed on dry.

Refrectrometer prism and readings were taken directly. Ascorbic acid was determined by the indophenol's Titration method used by Rusk, (1963).Antioxidant were determined according to the method of described by (Zaharah and sign, 2011).

2.5 Statistical analysis: The experiment was carried out under completely randomized design. The data recorded were analysed using analysis of variance techniques with the help of computer run statistical program 8.1 and the least significant difference test ($P \leq 0.05$) was used to compare the differences among treatment means (Steel et al., 1997).

3. Results and discussion

3.1 Fruit Decay (%): There was significant fruit decay in the stored kinnow fruit with the progression in storage period (Fig. 4.1). Maximum fruit weight was observed on 60 day (19.16%) followed by 45-days cold storage (12.66 %) and the minimum fruit weight was observed at zero day followed by 30-days of storage. As far as the treatments are concerned, highest fruit decay was seen in control than PE treated fruits (Fig. 4.1). Therefore, the fruit decay was considerably reduced in those fruits treated with PE that was about 31% more, than control. Among the treatments applied, 2% PE-treated fruit retained minimum fruit decay at the end of storage period followed by fruits treated with 4 and 3% treatments. On the other hand, the interaction between the treatment and storage period markedly influenced the fruit decay during the prolonged cold-storage period (Fig. 4.1). In the different treatments used, 2% pectin esterase treatment showed least fruit decay (10%) after 60 days storage. In contrast, the highest fruit decay was perceived in control (28%) after 60-days cold storage. The water loss from the surface of fruit is probably the subsequent cause of cracks development that provides the site for the activity of microorganisms (Jiang and Fu, 1998). However, the reduction in fruit decay in the PE treated fruit might be due to the reduced transpiration from the peel surface of Kinnow during storage that to be brief decrease the rate of fruit spoilage during the period of storage. Similar results were also obtained by the Rodov et al. (1997).



3.2 Chilling injury (Score): The susceptibility to the chilling injury was remarkable during the period of storage (Fig. 4.2). Minimum CI was exhibited after 15-days storage (1.16), while maximum was observed after 60-days (3.88). As much as the treatments are concerned, highest degree of CI was observed in control kinnow fruit during the entire storage period (Fig. 4.2). Therefore, the fruit quality was expressively increased in the control fruit than pectin esterase treated fruits with the progress in storage period that was about 18% less, than control. Among the treatments applied, 2% PE-treated fruit showed lowest chilling injury at the end of storage period followed by fruits treated with 3 and 5% treatments, respectively. CI was prominently influenced by the interaction between the treatment and storage period (Fig. 4.2). In the different treatments used, minimum chilling injury was observed in those fruits treated with 2% PE treatment (2.33) after 60 days storage. In comparison, the highest chilling injury was perceived in control after 60-days cold-storage. The membrane leakage plays an important role in the fruits under cold-storage for prolonged storage. The membrane leakage by the process of moisture loss is the remarkable sign of fruit senescence (Wang et al., 2004). The reduced chilling stress in the fruit with PE coating may be due to the reduced membrane leakage and preserved peel weight of 'Kinnow'.

3.3 Fruit firmness (N): During cold storage period, the firmness of kinnow fruit presented continuous and linear drop in all the fruits (Fig. 4.3). This decreasing tendency is more significant after 60-days cold storage. Maximum firmness was observed on zero-day that was 2.42 N (Fig. 4.3). But, the minimum fruit firmness was noted at 60-day followed by 45-days of storage with 1.76 N and 2.09 N, respectively. Statistical analysis shows that highest mean fruit firmness was found in 2% pectin esterase treated fruit as compared to other treatments during cold-storage (Fig. 4.3). However, the least firmness was observed in 6% PE (2.04 N) treatment during period of storage (Fig. 4.3). In comparison to control, 2% pectin esterase treatment significantly maintained the higher fruit firmness (2.413 N) throughout the storage, than control (1.6 N). The least firmness was noted in 6% pectin esterase treatment that was 1.45 N after 60 days storage (Fig. 4.3). In the current study, the firmness of the kinnow fruit reduced with the advancement of the storage period due to the incidence of higher transpiration from the peel of the fruit that ultimately reduced the thickness and strength of peel tissues. According to Volz et al. (2004), the firmness of fruit decreases with the proceeding in storage period. The other reason is the reduced supply of O₂ and CO₂ that lower the softness of the fruits (Maftoonazad and Ramaswamy, 2005;Ladaniya, 2008). In the present study the decreased firmness in all the fruits is directly correlated with the fruit weight loss. The higher firmness in PE treatments could be due to reduced weight loss and fruit decay.

3.4 Total soluble solids (°Brix): The TSS of Kinnow fruit stored showed increasing trend up to 45-days but decreased on 60-days (Fig. 4.4). This falling tendency was more substantial after 60-days cold storage. Maximum TSS was observed on 45-day (12.15 °Brix) followed by 15-days cold storage (12.13 °Brix). However, the minimum TSS was perceived at 0-day followed by 60-days of storage with 10.16- and 11.64 °Brix, respectively. Highest TSS was detected in those fruits treated with 2% PE during the entire storage period (Fig. 4.4). Therefore, the TSS was expressively lessened in the control than PE-treated fruits with the expansion in storage period that was about 17% more, than control. However, 2% PE-treated fruit sustained greatest TSS among all the treatments applied. The relationship between the storage period and

treatment noticeably exaggerated the TSS in the prolonged cold-storage period (Fig. 4.4). In the different treatments used, 2% PE treatment showed highest mean TSS (13.333 °Brix) on 45-days storage. Similarly, after 60-days storage the highest TSS was 12.5 °Brix in those fruits treated with 2% PE. In contrast, the minimum TSS was observed in 5% PE (9.767 °Brix) on 0-days storage. TSS is essential considerations for the determination of 'Kinnow' fruit quality. SSC declines because senescence in fruit with the continuing of storage period. Similarly, Alkimuzzaman et al. (2011) reported that the decrease in TSS might be due consumption of sugars for inhalation with the improvement of storage period. Moreover, weight loss also raises the reduction in TSS (Nager, 1994). Higher TSS PE treated fruits could be due preserved cell integrity, reduced weight loss and less senescence.

3.5 Ascorbic acid content (mL/100 mL): During cold storage period, the ascorbic acid content of kinnow fruit continuously declined from 0- to 60-days (Fig. 4.6). This reducing trend was more considerable after 60-days cold storage. Maximum ascorbic acid content was seen on 0-day that was 61.193 mL/100 mL of juice. But, the minimum ascorbic acid content was distinguished at 60-day followed by 45-days of storage with 43.34- and 48.43 mL/100 mL juice, respectively. Statistical analysis shows that highest mean ascorbic acid content in 2% pectin esterase treated fruit as compared to other treatments during cold-storage (Fig. 4.6). However, the least ascorbic acid content was observed in control (38.69 mL/100 mL juice) kinnow fruit at the end of storage period. In comparison to removal and treatments, the interaction between storage period and treatment significantly exaggerated the ascorbic acid content as compared to control (Fig. 4.6). In contrast to control, 2% pectin esterase treatment considerably preserved the higher fruit ascorbic acid content throughout the storage, than control. The least ascorbic acid contents were exhibited in control that was 29.567 mL/100 mL of juice at the end of storage period. It has been observed that there is decrease in ascorbic acid content during storage possibly due to the utilization of the ascorbic acid contents during the senescence of the fruits (Marti et al., 2009). However, the higher ascorbic acid content in the coated fruit could be due to the higher membrane integrity and reduced senescence along with fruit decay (Cohen et al., 1990).

3.6 Total antioxidant (%): Total antioxidants of kinnow fruit presented a constant decrease with the development in storage period. This reducing pattern was more significant after 60-days cold storage (Fig.4.7). Maximum antioxidant was perceived on 0-day (44.76 %) subsequent by 15-days cold storage (42.135 %). Nevertheless, the minimum antioxidant was noticed at 60-day followed by 45-days of storage with 33.36 and 29.68%, respectively. On an average, the least amount of antioxidant was observed in control fruit during the entire storage period, than other treatments. Among the treatments applied, 2% PA-treated fruit retained highest antioxidant level. As far as the interaction is concerned, among the treatments and storage period expressively declined the antioxidants during the extended cold-storage period (Fig. 4.7). In the altered treatments, 2% PE treatment exhibited significantly increased mean antioxidants (31.19 %) on 60-days storage. However, minimum antioxidants were seen in 6% PE-treated fruit (29.01) on 60-days. Kinnow fruit contain many antioxidants that include phenolic, ascorbic acid, glutathione, procyanidin B2, procyanidin B4 and epicatechin (Sarni-Manchado et al., 2000). The level of antioxidants decreased during cold storage because of

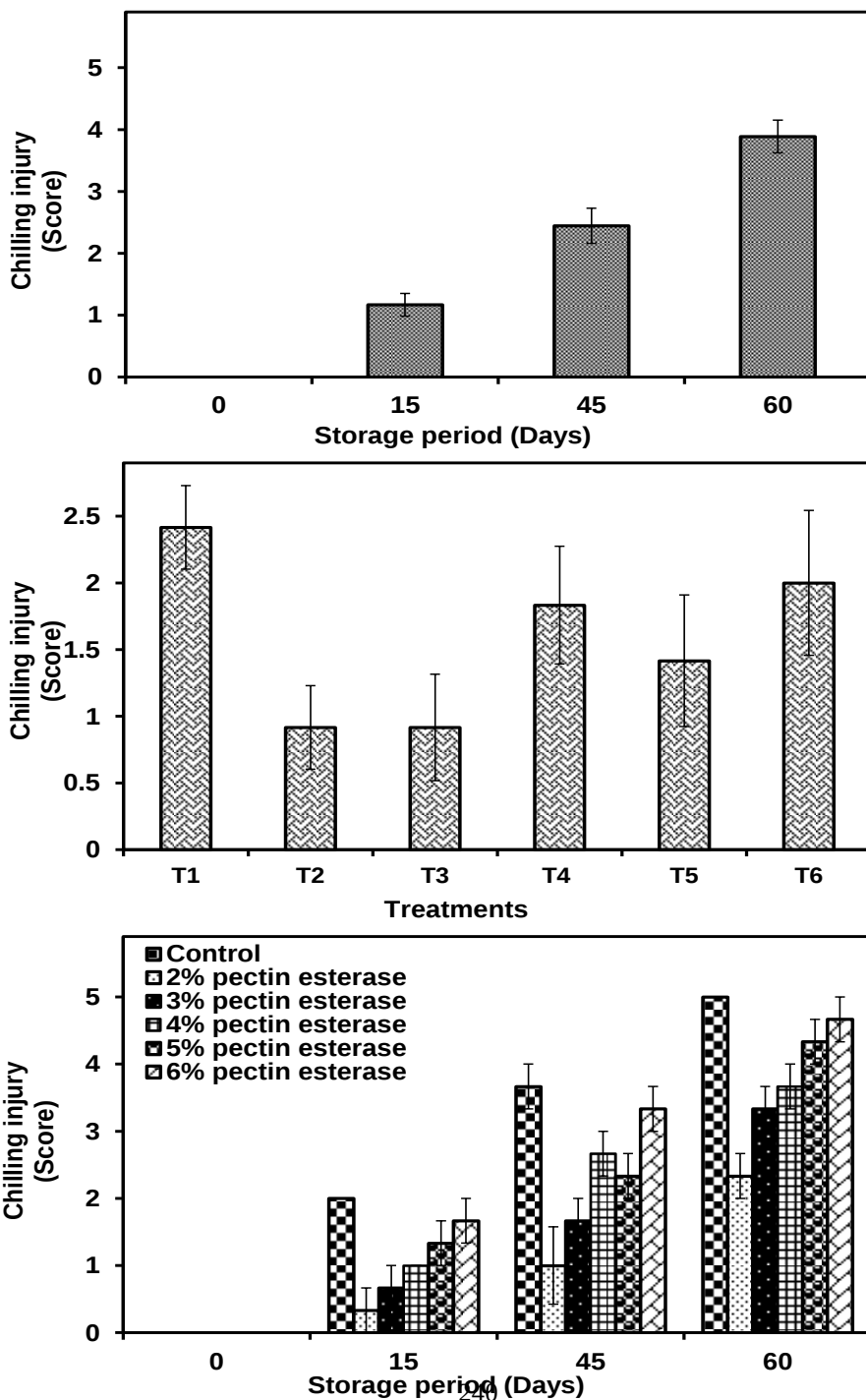


Fig. 4.3 Effect of pectin esterase coating on the chilling injury of Kinnow fruits during cold-storage.

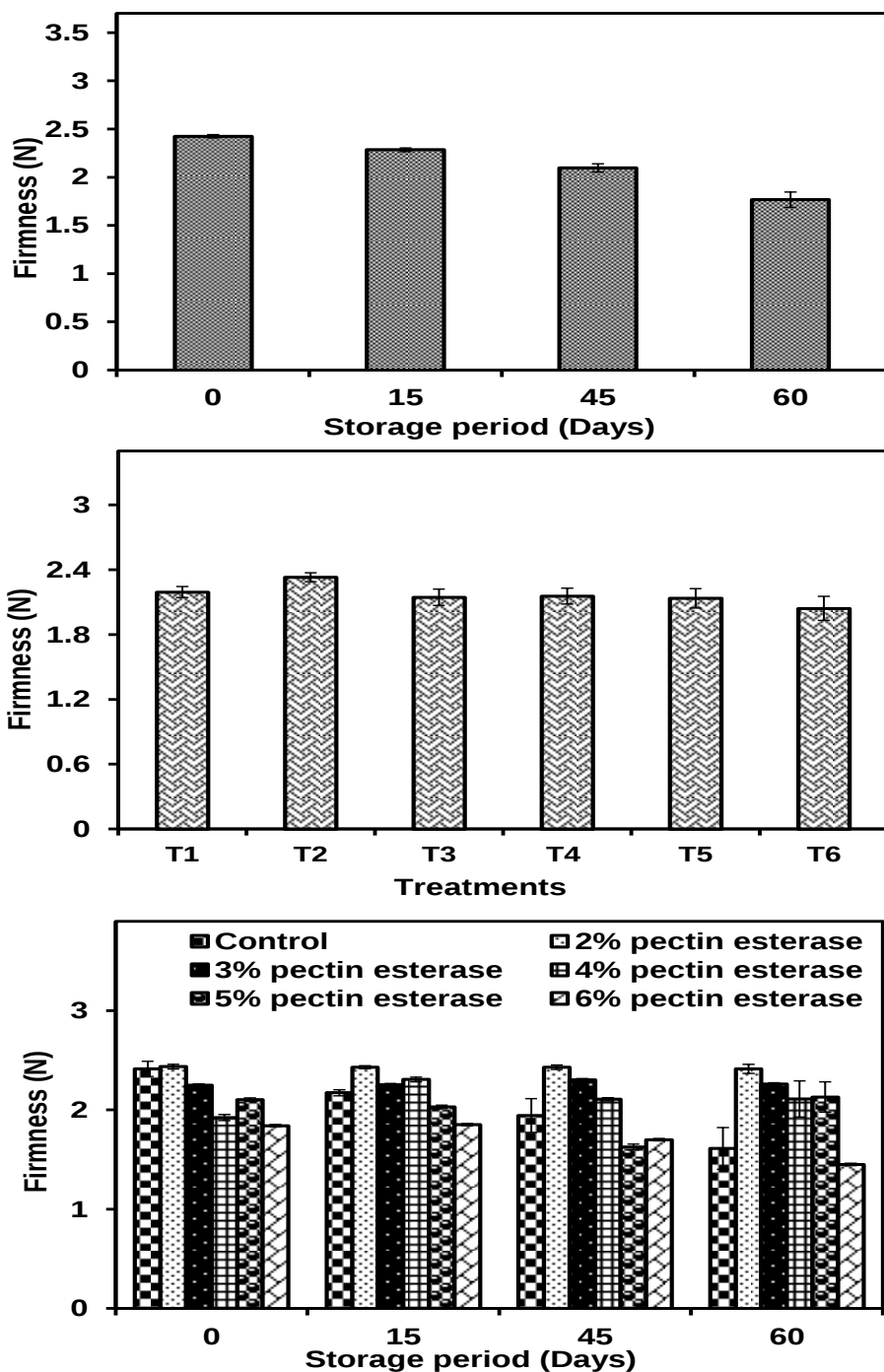


Fig 4.3 Effect of pectin esterase coating on the firmness of Kinnow fruits during cold-storage.

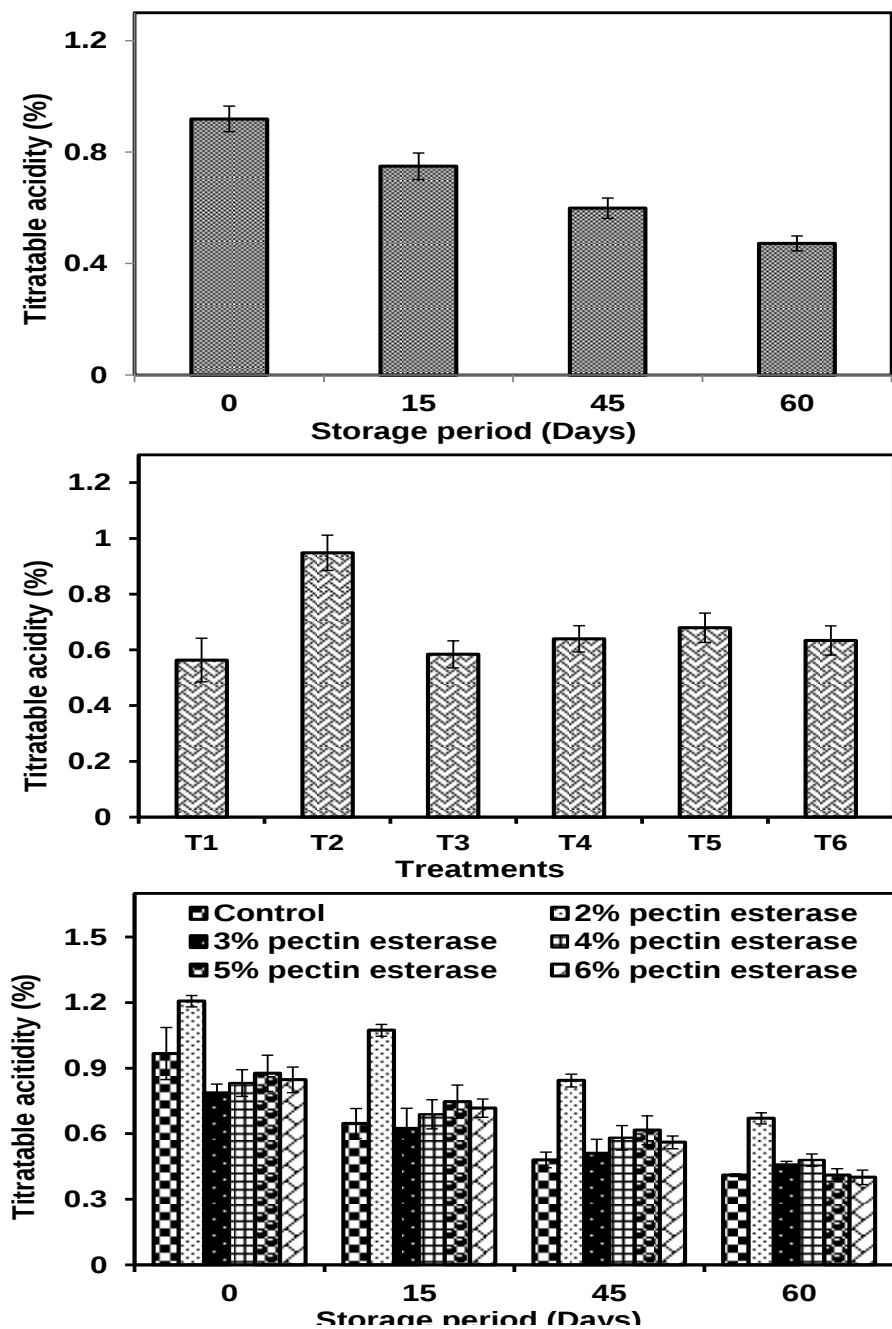


Fig 4.5 Effect of pectin esterase coating on the titratable acidity of Kinnow fruit during cold-storage.

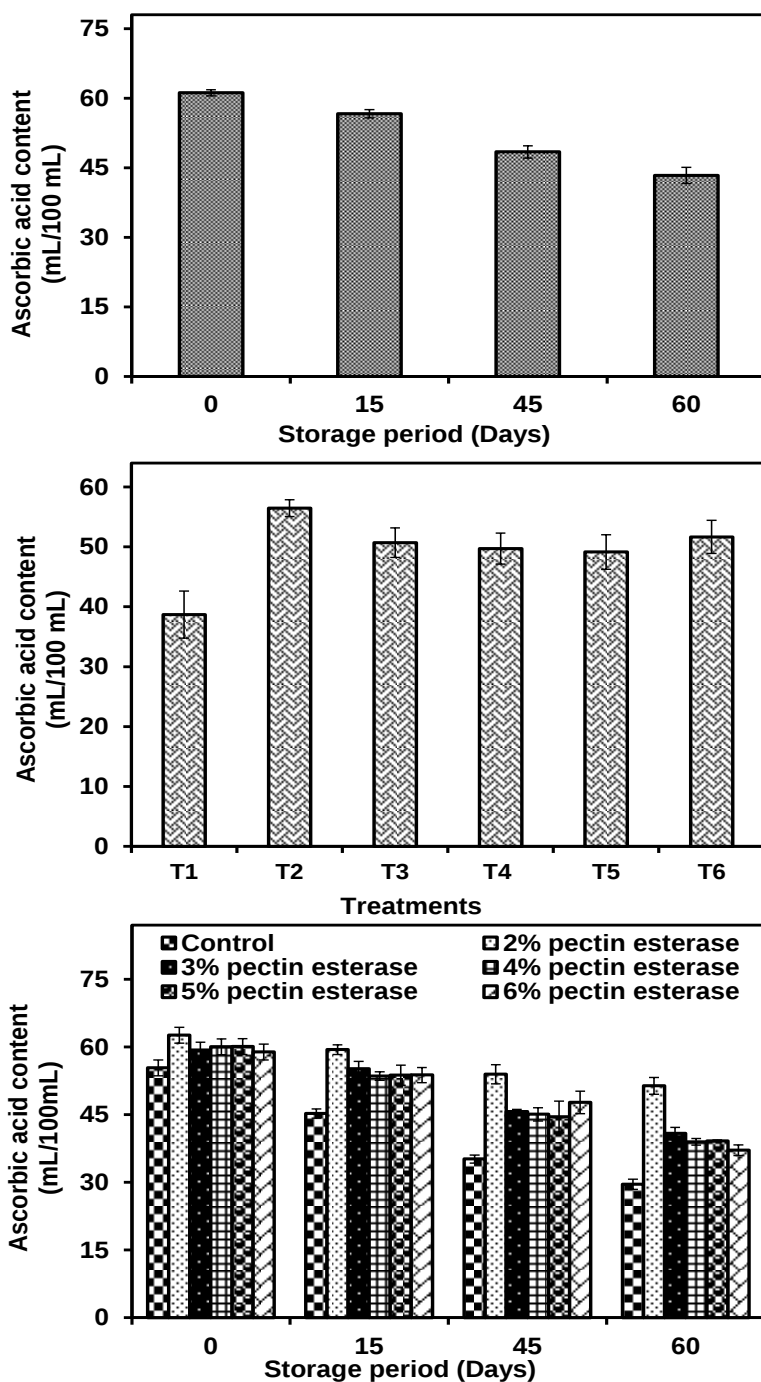


Fig 4.6 Effect of pectin esterase on the ascorbic acid contents of Kinnow fruits during cold-storage.

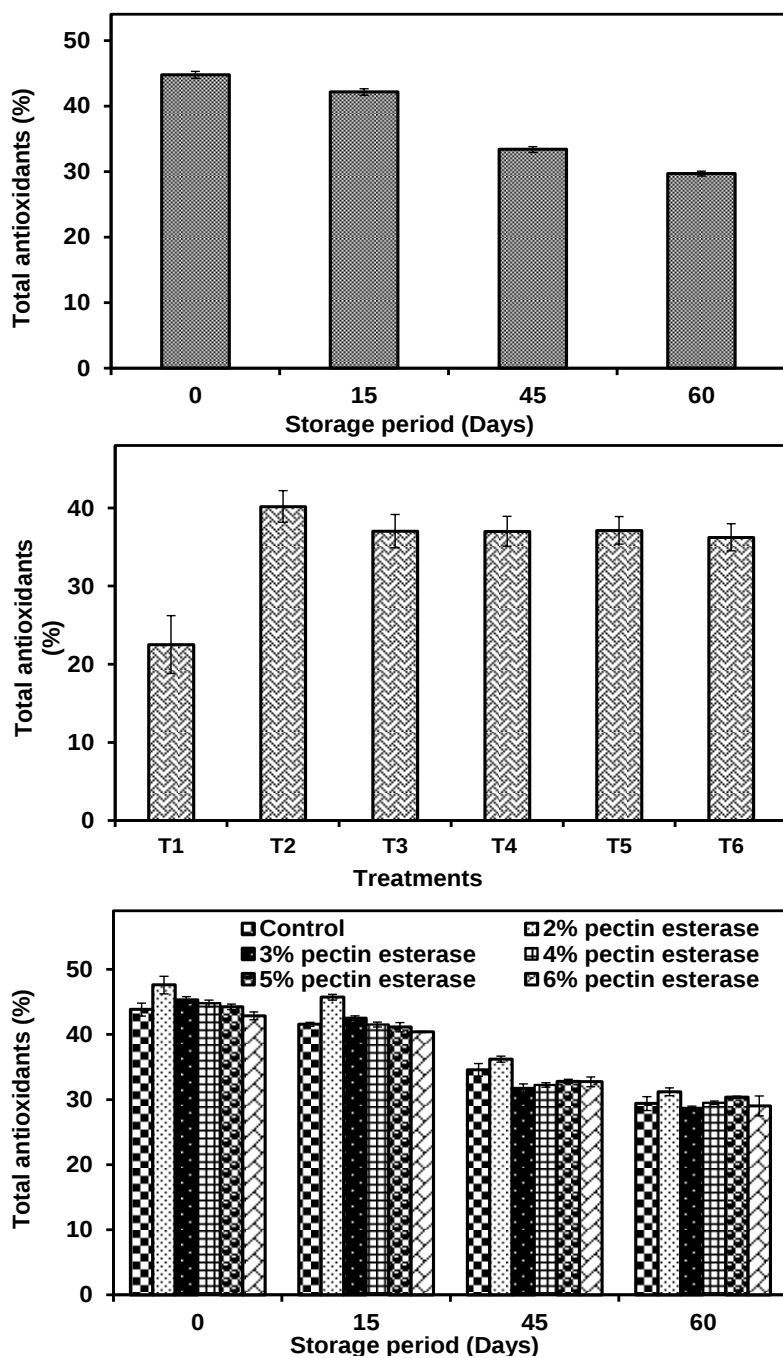


Fig 4.7 Effect of pectin esterase coating on the total antioxidants of Kinnow during cold storage

decrease in total phenolic contents and Vitamin-C with progression of storage. Moreover, phenolic compounds are also used to scavenge the reactive oxygen species and the activities of PPO and POD oxidizes the phenolic compounds. Phenolic and ascorbic acid contents decreased with the progression of storage period in sweet cherry, mango and pomegranate, respectively. However, this higher antioxidant level is mainly due to the reduced oxidation of phenolic is stated by Asrey et al. (2013). Comparative analysis on the effects of several concentration of pectin-esterase (PE) and storage condition on the quality and the shelf-life of Kinnow mandarin as observed in the present study, reveals that the combination of 2% pectin-esterase coating and storage at 5°C was the best treatment for maintaining the quality and extending the shelf-life of Kinnow mandarin over other treatments or control, which was exhibited by the least decay incidence, higher firmness, higher titratable acidity, lower total soluble solids, higher ascorbic acid, and also higher total phenolic contents over other treatment or control. On the basis of these results, it can be concluded that the concentration of 2% pectin-esterase and storage at 5°C for 60-days of storage was the most effective method in maintaining the quality and extending the shelf-life of Kinnow mandarin and it should be further tested by conducting systematic research studies for increasing the shelf life of Kinnow mandarin.

4. ACKNOWLEDGEMENT

I am thankful to my appreciating parents and Associate Professor Dr. Saeed Ahmad Institute of Horticultural Sciences, University of Agriculture, Faisalabad, for all their assistance to conduct this research.

5. CONCLUSIONS AND RECOMMENDATIONS

It was concluded that 2 % Pectin-esterase wax emulsion in combination with 5° C storage temperature proved to be best wax emulsion that were very effective in improving the overall quality and extending the storage life of Kinnow fruits. Our conclusion about pectin-esterase wax emulsion concentration should be further tested by conducting systematic research studies for increasing the shelf life of Kinnow fruit.

6. REFERENCES

- Aklimuzzaman, M.D., C. Goswami, J. Howlader, H.A. Kader and K. Hassan. 2011. Postharvest storage behavior of litchi. *J. Hort. Forest. Biotechnol.*, 15:1-8.
- Amin, M. 2012. Integrated approaches for improving fruit quality and shelf life of two commercial mango cultivars of Pakistan. Ph. D. Thesis, Inst. Hortic. Sci., Univ. Agric., Faisalabad, Pakistan.
- Aspinall, G.O. 1970. *Polysaccharides*. Oxford: pergamon press. USA. Canada, Deptt. of Agric. 1154.
- Charles, F., V. Vidal, F. Olive, H. Filgueiras and H. Sallanon. 2013. Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. In. *Food Sci. Emer. Tech.*, 18:190-195.

- Draye, M. and C.P. Van. 2008. Pectin methylesterases induce an abrupt increase of acidic pectin during strawberry fruit ripening. *J. Plant Physiol.*, 165: 1152-1160.
- Echeverria, E and J.K. Burns. 1989. Vacuolar acid hydrolysis as a physiological mechanism for
- Mohammed, A.A. 2004. Microflora contamination of Gum Arabic (Acacia Senegal Gum) from tree to store. M. Sc. Thesis, University of Khartoum.
- Osman, M.E. 1993. Industrial uses of Gum Arabic (Acacia Senegal) Ph.D. Thesis, Salford University, U.K. sucrose breakdown. *Plant Physiol.*, 90:530-533.
- Echeverria, E and J.K. Burns. 1989. Vacuolar acid hydrolysis as a physiological mechanism for sucrose breakdown. *Plant Physiol.*, 90:530-533.
- Elwahab, S.M.A. and I.A.S. Rashid. 2013. Effect of acetic acid fumigation and waxing on decay, storability and marketing of mandarin fruits. *J. Appl. Sci. Res.* 9:2146-2155.
- Fischer, R.L. and A.B. Bennett. 1991. Role of cell wall hydrolases in fruit ripening. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 42:675-703.
- Henriod, R.E., M.R. Gibbered and M.T. Treeby. 2005. Storage temperature effects on moisture loss and the development of chilling injury in lanes late and navel orange. *Aust. J. Exp. Agric.*, 45:453-458.
- Holcroft, D.M. and E.J. Mitcham. 1996. Postharvest physiology and handling of litchi (*Litchi chinensis* Sonn.). *Postharvest Biol. Technol.*, 9:265-281.
- Huber, D.J. 1983. The role of cell wall hydrolases in fruit softening. *Hort. Rev.* 5: 169-219.
- Iqbal, H.M. 1996. Type and extent of postharvest losses in horticulture commodities on pakistan. p. 33-42. In: U. Mustafa (ed.). *Proc. Nat .Conf. Postharvest Technol. Hort. Commod. Quetta, Pakistan*
- Jain, R.E., S.S. Chankrota and J.D. Agarwal. 1984. Isolation and standardization of pectin from apple pomace. *Indian Food Packer.*, 36:60-65.
- Jiang, Y.M. and J.R. Fu. 1998. Inhibition of polyphenol oxidase and the browning control of litchi fruit by glutathione and citric acid. *Food Chem.*, 62:49-52.
- Ladaniya, M.S. 2008. *Citrus fruit: Biology, technology and evaluation*. San Diego CA. Elsevier Academic Press.
- Ladaniya, M.S. 2008. *Citrus fruit: Biology, technology and evaluation*. San Diego CA. Elsevier Academic Press.
- Ladaniya, M.S. 2008. *Citrus fruit: Biology, technology and evaluation*. San Diego CA. Elsevier Academic Press.
- Maftoonazad, N. and H.S. Ramaswamy. 2005. Postharvest shelf-life extension of avocados using methyl cellulose-based coating. *Leb. Wis. Technol.*, 38:617-624.
- Nagar, R.K. 1994. Physiological and biochemical studies during fruit ripening in litchi (*Litchi chinensis* Sonn.). *Postharvest Biol. Technol.*, 4:225-234.
- Nagar, R.K. 1994. Physiological and biochemical studies during fruit ripening in litchi (*Litchi chinensis* Sonn.). *Postharvest Biol. Technol.*, 4:225-234.
- PHDEB. 2004. A Report on Kinnow Export Development Strategy. Pakistan Horticulture Development and Export Board, Ministry of Commerce, Government of Pakistan.
- Prasanna, V., T.N. Prabha and R.N. Tharanathan. 2007. Fruit ripening phenomena-an overview. *Crit. Rev. Food Sci. Nutr.*, 47:1-19.

- Ribeiro, S.M.R., J.H. Queiroz, M.E.L.R. De Queiroz, F.M. Campos and H.M.P. Santana. 2007. Antioxidant in mango (*Mangifera indica* l.) pulp. Plant Foods Hum. Nutr., 62:13-17.
- Rodov, V., S. Ben-Yeshoshua, R. Albagli and D.Q. Fang. 1995. Reducing behavior of litchi. J. Hort. Forest. Biotechnol., 15:1-8.
- Rusk, J.A. 1963. Chemical methods for analysis of fruits and vegetables. Res. State Summer Land, Res. Branch estrogen antagonists. Embo J., 23:2293-303.
- Sarni-Manchado, P., E. Le Roux, C. Le Guerneve, Y. Lozano and V. Cheynier. 2000. Phenolic composition of litchi fruit pericarp. J.A.F.C.A.U. 48:5995-6002.
- Seymour, G.B and K.C. Gross. 1996. Cell wall disassembly and fruit softening. Postharvest News Inform., 7:45-54.
- Srirangarajan, A.N. and A.J. Shrikhande. 1979. Comparative aspects of pectin extraction in from the peels of different varieties of mango. J. Food Tech., 14:539-540.
- Volz, T.J., S.J. Farnsworth, S.D. Rowley, G.R. Hanson and A.E. Fleckenstein. 2004. Age dependent differences in dopamine transporter and vesicular monoamine transporter-2 function and their implications for methamphetamine neurotoxicity. Synapse., 63:147-151.
- Zaharah, S.S. and Z.Singh. 2011. Postharvest nitric oxide fumigation alleviates chilling injury, delays fruit ripening and maintains quality in cold-storage 'Kensington Pride' mango postharvest Biol. Technol., 60:202-210.