jpht
www.jpht.info

Journal of Postharvest Technology
2019, **07**(1): 1-8
http://www.jpht.info

RESEARCH ARTICLE

# Antioxidant properties of red delicious apples as influenced by harvest dates, pre-treatments and storage conditions

**Shaiq Ahmad Ganai[1], Hafiza Ahsan[2], Aamir Hussain Dar[1], Farhana Allali[1], and S. M. Wani[3]**

[1]Department of Food Technology, Islamic University of Science and Technology, Kashmir, India
[2]Division of Post Harvest Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India
[3]Department of Food Science and Technology, University of Kashmir, India

Received: 15.11.2018 Accepted: 10.12.2018

## ABSTRACT

The objective of this study was to investigate the effect of harvest dates and postharvest treatments (precooling, calcium chloride and wax coating) on physical properties of apple cv Red delicious during storage. Fruits from three harvest dates ($H_1$, $H_2$ and $H_3$) were subjected to various treatments include T1 (shade cooling), T2 (Hydrocooling), T3 (Hydrocooling+ calcium chloride), T4 (Hydrocooling + wax) and T5 (Hydrocooling + calcium chloride + wax) and were stored under ambient and refrigerated conditions for 100 days. All the treatments showed significant effect on anthocyanin, ascorbic acid and phenolic content. The harvest date $H_3$ showed the maximum anthocyanin (33.30 mg/100g) and ascorbic content (12.50 mg/100g), Whereas polyphenolic content observed maximum in $H_1$ (2.80 mg/100g). Among the treatments T5 showed the highest anthocyanin, ascorbic acid and phenolic content of 34.90 mg/100g, 12.50 mg/100g and 2.66 mg/100g, respectively. However, the anthocyanin content, ascorbic content and phenolic content were more decreased during ambient as compared to refrigerated storage.

**Key words**: Apple; anthocyanin; ascorbic acid; polyphenols; quality

**Citation:** Ganai, S.A., Ahsan, H., Dar, A.H., Allali, F., and Wani, S.M. 2019. Antioxidant properties of red delicious apples as influenced by harvest dates, pre-treatments and storage conditions. *Journal of Postharvest Technology*, 7(1): 1-8.

## INTRODUCTION

Apples are a widely consumed fruit worldwide, and they are one of the best sources of phenolics, anthocyanins and ascorbic acid (Eberhardt et al., 2000). Phenolic content are consistently higher in the skin than in the flesh, and peels have the highest antioxidant activity and anti-proliferation activity (Wolfe et al., 2003). In general, the total phenolic concentrations remain relatively stable during storage, but individual components may vary (Awad and de Jager, 2000; Awad and de Jager, 2003) and total phenolics and total antioxidant activity may increase in peel of cultivars stored in air or controlled atmospheric conditions (Leja et al., 2003; Napolitano et al., 2004).

The focus of current research in the nutrition literature for apples is mostly towards phenolics. Although ascorbic acid may contribute to the health benefits of apples, the compound represents a minor part of the antioxidant activity of the fruit (Lee et

* *For correspondence:* S. A. Ganai (shaiqafzal@gmail.com )

**ISSN: 2348-4330**

al., 2003; Boyer et al., 2004). Nevertheless, ascorbic acid has critical enzymatic and non-enzymatic functions in tissues and is involved in detoxification of damaging oxygen radicals and their derivatives, the reactive oxygen species (Davey et al., 2000). Many chronic diseases such as cancer, cardiovascular disease, asthma, diabetes occur due to oxidative damage to various molecules like lipids, proteins and nucleic acids by free radicals. Epidemiological studies have shown correlations between the consumption of apples and reduced risks of cardiovascular disease, asthma, diabetes and some cancers (Biedrzycka & Amarowicz, 2008). Phenolic compounds are the primary molecules responsible for the antioxidant capacity of fruits (Sun et al., 2002). Anthocyanins are responsible for the red pigmentation in apple peel and account for 1–3% of total phenolics (Vrhovsek et al., 2004) and they usually occur as glycosides monoside (Golding et al., 2001).

During storage, the abundance of both the total and individual phenolic compounds have been reported to remain relatively stable in some studies, while other studies have detected minor fluctuations in phenolic concentrations (Awad & de Jager, 2003; Golding et al., 2001). The inhibition of biochemical processes, which cause the aging of apples and shortening of their storage, may be achieved with the help of natural and artificially made chemical substances, which are use d for after harvest treatment for fruits (Alleyne and Hagenmaier, 2000; Bai et al., 2002). Due to inappropriate postharvest management practices, lack of proper scientific storage and transportation facilities post harvest losses of 20-40 % results during harvesting, handling, packaging, transportation, marketing and storage (Ghani *et al.,* 2003). Present study was carried out to assess the combined influence of harvest dates and postharvest treatments on antioxidant properties of apple cv. “Red Delicious” during storage.

## MATERIALS AND METHODS

### Materials

Apple cv. “Red Delicious” of uniform shape, size and firm texture was procured locally from an apple orchard of Pulwama, Kashmir. Apple fruits were harvested at three different dates with an interval of seven days designated as $H_1$, $H_2$ and $H_3$ at around 6.00 pm with $H_2$ being the optimum harvest time. After harvest, these were manually sorted by discarding deformed, bruised, punctured and stemless fruits. One lot of fruits was separated and kept under shade for 12 hours for cooling which served as control $T_1$ (shade-cooling). The remaining fruits were pre-cooled by spraying cold tape water for 10 minutes with occasional turning $T_2$ (hydrocooling). Next day hydrocooled fruits were divided into two more lots, one lot was sprayed with 3 % calcium chloride which served as $T_3$ (hydrocooling + $CaCl_2$). Then these fruits were packed in plastic crates and brought to laboratory. Next day a portion from both hydrocooled fruits ($T_2$) and (hydrocooled + $CaCl_2$) $T_3$ were waxed by 6 % paraffin wax which served as $T_4$ (hydrocooling + 6% paraffin wax) and $T_5$ (hydrocooling + 3% $CaCl_2$ + 6% paraffin wax), respectively. Samples of all treatments were taken out from cold stores (2±1ºC, RH 85±5 %) after 20 days of storage interval and kept at ambient temperature (18±2ºC, RH 75±5%) for some time and were evaluated for each parameter.

### Analytical Methods

#### Polyphenols (mg/100 g)

Polyphenol content of fruit was estimated through a procedure developed by Elizabeth and Kelly (2007). Five gram of sample was boiled for 30 minutes in 400ml distilled water, diluted to mark with distilled water in a 500ml volumetric flask and filtered. In order to estimate the phenolic content, 0.1 ml of filtrate was taken in a test tube and after adding 0.2 ml of 10 % Folin-ciocalteau reagent, the mixture was vortexed thoroughly. After vortexing 0.8 ml of sodium carbonate was added in to each tube and was incubated at room temperature. After incubation absorbance was measured at 765 nm and poly phenolic

content was calculated using standard curve of gallic acid through following equation:

$$\text{Polyphenolic content (mg/100 g)} = \frac{\text{mg of Gallic acid from standard curve} \times \text{dilution}}{\text{ml of sample taken for colour development} \times \text{Weight of solids in sample}} \times 100$$

**Ascorbic acid content (mg/100 g)**

Ascorbic acid was determined by the standard method as reported in AOAC (1990). Dye solution for Ascorbic acid determination: Fifty mg of 2, 6 dichlorophenol indophenols dye and 42 mg of sodium bicarbonate was weighed, dissolved in hot distilled water and volume was made up to 250 ml. Fifty mg of standard ascorbic acid was taken in 50ml volumetric flask and the volume was made up 0.4 % oxalic acid. This standard ascorbic acid was titrated against dye. Titration of the sample Ten ml of sample was taken in 100 ml volumetric flask and volume was made by adding 0.4 % oxalic acid .Then 10 ml of prepared sample was taken in the flask and was titrated against dye until light pink colour appeared, which persisted for 15 seconds. Three consecutive readings were taken for each sample. The ascorbic acid was calculated by using the following formula;

$$\text{Ascorbic acid} = \frac{F \times T \times 10}{D \times S} \times 100$$

F = Factor from standardisation = (ml of Ascorbic acid)/ml of dye

T = ml of dye used for sample

S = ml of diluted sample taken for titration

D = ml of sample taken for dilution

**Anthocyanin content**

The anthocyanin content was estimated by blending a known weight of fruit with a known volume of ethanolic HCl (95% ethanol : 1.5 N HCl) in a blender and stored overnight under refrigeration at 4ºC. The mixture was filtered and residue was washed repeatedly till a known volume was obtained. A small aliquot was diluted with ethanolic HCl to yield optical density (OD) checked at 530 nm measurements within the optical range of spectrophotometer.

**Statistical analysis**

The data was statistically analysed through R-Software using Completely Randomized Design (CRD) in factorial experiment (Gomez and Gomez, 1984).

**RESULTS AND DISCUSSION**

**Polyphenolic content**

Harvest dates significantly effects the total polyphenol (Table 1). Early harvested fruits $H_1$ showed maximum total polyphenol content (2.80 mg/100g) while mid harvested apples ($H_2$) showed minimum total polyphenol content (2.65 mg/100g). After the 100 days of storage period early harvested apples ($H_1$) showed the maximum polyphenol content than $H_2$ and $H_3$. The reason behind the higher level of polyphenol in early harvested apple (H1) might be due to the reduced size, less break down and less ROS (Reactive oxygen species) production due to stress. These results are in accordance with Malina et al. (2005).

Among the treatments $T_1$ (shade cooling) recorded the minimum total phenol content while as $T_5$ (hydrocooling + $CaCl_2$ + wax) recorded the maximum concentration of total phenol. The main reason behind the higher polyphenol in $T_5$ (hydrocooling + $CaCl_2$ + wax) might be the protective effect of calcium chloride and wax coating an oxidative stress, hence less production of free radicals and less break down of amino acids. These results are in accordance with Suzy et al. (1998).

It is evident from the (**Table 1**) that there was continuous decline in total polyphenol content throughout the storage period irrespective of harvest and treatments. This might be due to the more production of ROS during storage. Decrease in polyphenols was more pronounced in ambient storage than in refrigerated storage.

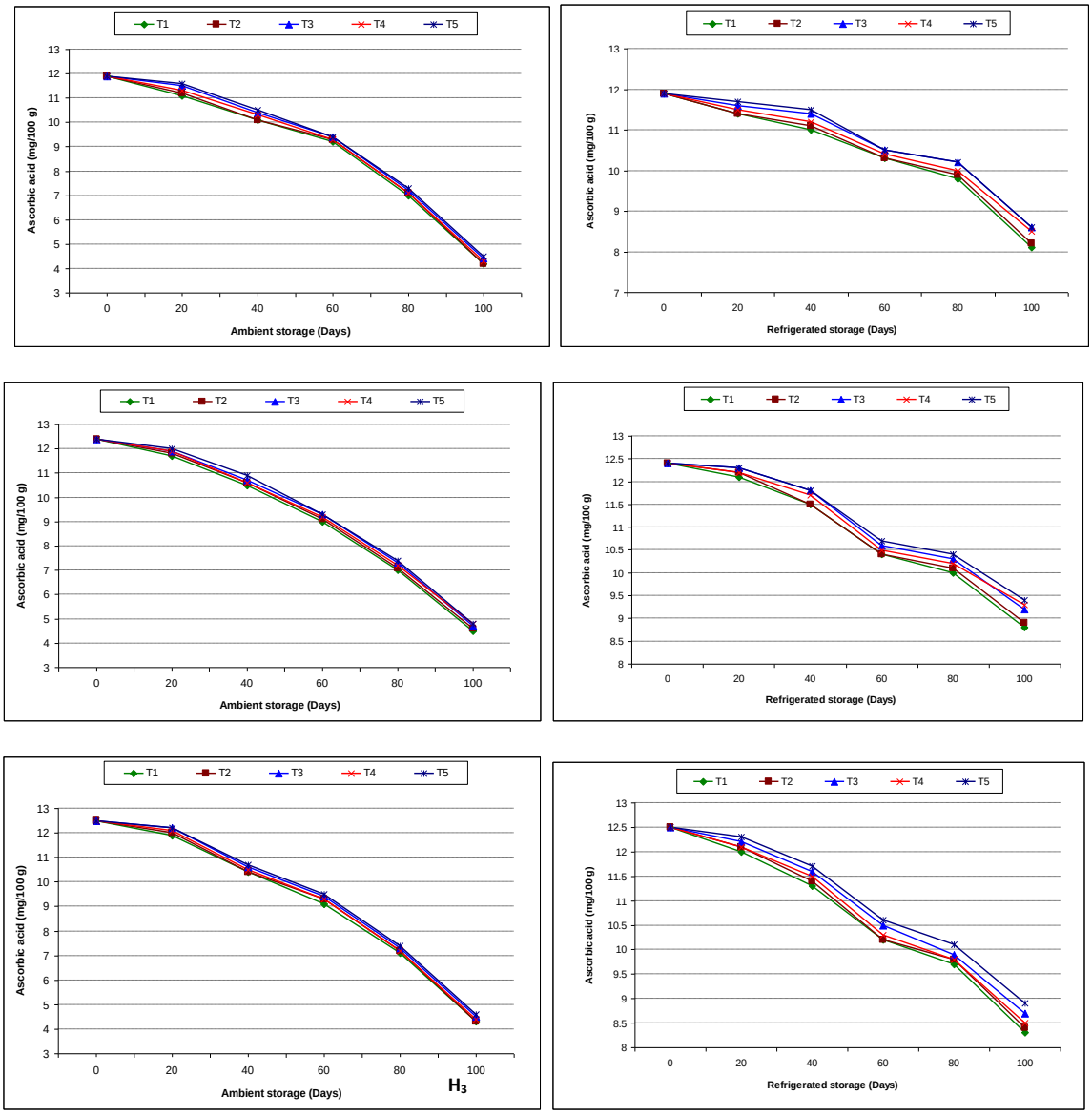


Fig. 1: Effect of harvest dates, post harvest treatments and storage conditions on ascorbic acid (mg/100 g) of apple

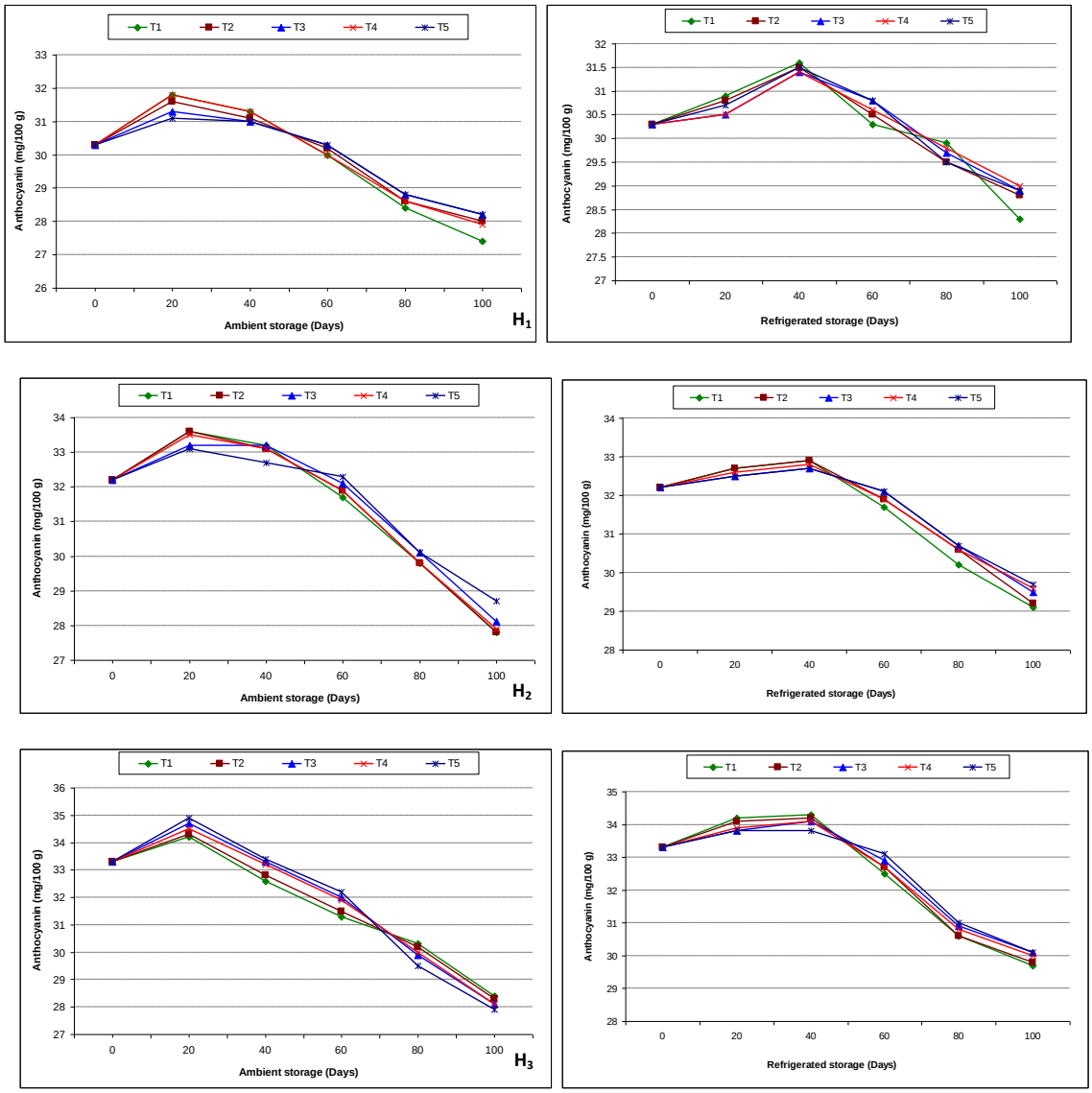


Fig. 2: Effect of harvest dates, post harvest treatments and storage conditions on anthocyanin (mg/100 g) of apple

**Ascorbic acid content**

Ascorbic acid is usually considered as an index of nutrient quality in apple fruit. Ascorbic acid is a bioactive compound having antioxidant properties (Lata, 2007). The ascorbic acid content changed according to harvest dates and differed significantly at different dates (Figure 1). Later harvested ($H_3$) fruits had higher ascorbic acid content (12.50 mg/100g) than $H_2$ (12.40 mg/100g) and $H_1$ (11.90 mg/100g). After 100 days of storage apples harvested at $H_3$ and among treatments T5 (hydrocooling + $CaCl_2$ + wax) were of best quality.

**Table 1: Effect of harvest dates, post harvest treatments and storage conditions on polyphenols (mg/100 g) of apple**

| Harvest dates | Storage / Treatment | Ambient storage (Days) | | | | | | | Refrigerated storage (Days) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 20 | 40 | 60 | 80 | 100 | Mean | 0 | 20 | 40 | 60 | 80 | 100 | Mean |
| $H_1$ | $T_1$ | 2.80 | 2.80 | 2.60 | 2.50 | 2.50 | 2.40 | 2.60 | 2.80 | 2.80 | 2.70 | 2.70 | 2.60 | 2.60 | 2.70 |
| | $T_2$ | 2.80 | 2.80 | 2.70 | 2.60 | 2.50 | 2.50 | 2.60 | 2.80 | 2.80 | 2.70 | 2.70 | 2.60 | 2.60 | 2.70 |
| | $T_3$ | 2.80 | 2.80 | 2.70 | 2.60 | 2.50 | 2.20 | 2.65 | 2.80 | 2.80 | 2.80 | 2.80 | 2.70 | 2.70 | 2.77 |
| | $T_4$ | 2.80 | 2.80 | 2.70 | 2.60 | 2.50 | 2.50 | 2.65 | 2.80 | 2.80 | 2.80 | 2.80 | 2.70 | 2.70 | 2.77 |
| | $T_5$ | 2.80 | 2.80 | 2.70 | 2.60 | 2.50 | 2.50 | 2.65 | 2.80 | 2.80 | 2.80 | 2.80 | 2.70 | 2.70 | 2.77 |
| | Sub Mean | 2.80 | 2.80 | 2.68 | 2.58 | 2.50 | 2.42 | 2.63 | 2.80 | 2.80 | 2.76 | 2.76 | 2.66 | 2.66 | 2.74 |
| $H_2$ | $T_1$ | 2.65 | 2.65 | 2.45 | 2.45 | 2.35 | 2.35 | 2.48 | 2.65 | 2.65 | 2.55 | 2.45 | 2.45 | 2.45 | 2.53 |
| | $T_2$ | 2.65 | 2.65 | 2.45 | 2.55 | 2.35 | 2.35 | 2.50 | 2.65 | 2.65 | 2.65 | 2.55 | 2.55 | 2.55 | 2.60 |
| | $T_3$ | 2.65 | 2.65 | 2.55 | 2.55 | 2.45 | 2.45 | 2.55 | 2.65 | 2.65 | 2.65 | 2.65 | 2.65 | 2.65 | 2.65 |
| | $T_4$ | 2.65 | 2.65 | 2.55 | 2.55 | 2.45 | 2.45 | 2.55 | 2.65 | 2.65 | 2.65 | 2.65 | 2.55 | 2.55 | 2.62 |
| | $T_5$ | 2.65 | 2.65 | 2.55 | 2.55 | 2.45 | 2.45 | 2.55 | 2.65 | 2.65 | 2.65 | 2.65 | 2.65 | 2.65 | 2.65 |
| | Sub Mean | 2.65 | 2.65 | 2.51 | 2.53 | 2.41 | 2.41 | 2.53 | 2.65 | 2.65 | 2.63 | 2.59 | 2.57 | 2.57 | 2.61 |
| $H_3$ | $T_1$ | 2.66 | 2.66 | 2.46 | 2.46 | 2.36 | 2.36 | 2.49 | 2.66 | 2.66 | 2.56 | 2.46 | 2.46 | 2.46 | 2.54 |
| | $T_2$ | 2.66 | 2.66 | 2.56 | 2.56 | 2.46 | 2.46 | 2.56 | 2.66 | 2.66 | 2.66 | 2.56 | 2.60 | 2.56 | 2.62 |
| | $T_3$ | 2.66 | 2.66 | 2.56 | 2.56 | 2.56 | 2.46 | 2.58 | 2.66 | 2.66 | 2.66 | 2.66 | 2.66 | 2.66 | 2.66 |
| | $T_4$ | 2.66 | 2.66 | 2.56 | 2.56 | 2.56 | 2.46 | 2.57 | 2.66 | 2.66 | 2.66 | 2.66 | 2.66 | 2.56 | 2.64 |
| | $T_5$ | 2.66 | 2.66 | 2.56 | 2.56 | 2.56 | 2.46 | 2.58 | 2.66 | 2.66 | 2.66 | 2.66 | 2.66 | 2.66 | 2.66 |
| | Sub Mean | 2.66 | 2.66 | 2.54 | 2.54 | 2.50 | 2.44 | 2.56 | 2.66 | 2.66 | 2.64 | 2.60 | 2.61 | 2.58 | 2.62 |
| Grand Mean | 2.70 | 2.70 | 2.58 | 2.55 | 2.47 | 2.42 | | 2.57 | 2.70 | 2.70 | 2.68 | 2.65 | 2.61 | 2.60 | 2.66 |

$T_1$ = Shade cooling (Control); $T_2$ = Hydro cooling; $T_3$ = Hydro cooling + $CaCl_2$; $T_4$ = Hydro cooling + wax; $T_5$ = Hydro cooling + $CaCl_2$ + wax

Generally, the fruit harvested at early maturity had lower ascorbic acid indicating that the fruit may have still been synthesizing ascorbic acid when harvested at the early mature stages. Since the degradation of ascorbic acid is faster at higher than lower temperature (Pardio-Sedas *et al.*, 1994). The ascorbic acid loss during storage is known to be due to its antioxidant activity especially under postharvest storage conditions (Davey *et al.*, 2000). The ascorbic acid can be irreversible oxidized (Parviainen and Nyyssonen, 1992; Pardio-Sedas et al., 1994), thus decreased during storage (Jung and Watkins, 2008). The retention of relatively high ascorbic acid with the application of $CaCl_2$ and wax may due to the lower respiration rate and regulation of oxidative processes in the cytosol responsible for ascorbic acid degradation (Faust and Shear, 1972). Decrease in ascorbic acid content was more pronounced in ambient storage than in refrigerated storage condition.

**Anthocyanin content**

Anthocyanin changed according to harvest dates and varied significantly with each harvest date (Figure 2). Late harvested fruits $H_3$ showed highest anthocyanin (33.30 mg/100g) content while ($H_1$) early harvested showed minimum anthocyanin content (30.30 mg/100g). After the 100 days of storage ($H_3$) late harvested apple retained the maximum anthocyanin content then $H_1$ and $H_2$. The reason behind the highest anthocyanin in late harvested apples might be due to full colour development by associated enzymes (PAL) and the lowest anthocyanin in $H_1$ early harvested apple might be due to more chlorophyll than anthocyanin.

Among the treatments $T_5$ (hydrocooling + $CaCl_2$ + wax) proved best to retain the maximum anthocyanin than other treatments. The reason behind this is the protective effect of precooling, $CaCl_2$ and wax an overall degradation of fruit Wijewardane and Guleria (2009). The anthocyanin content were decreased in all harvest dates as well as treatments. This may be due to the progressive senescence of fruit tissue which involves the degradation of pigments (Wijewardane and Guleria, 2013). Decrease in anthocyanin content was more pronounced in ambient storage than in refrigerated storage.

## CONCLUSION

The Apple fruits were rich source of ascorbic acid, polyphenols and anthocyanin content. T5 (Hydrocooling + calcium chloride + wax) retained the maximum amount of anthocyanin, ascorbic acid and polyphenols. The refrigerated conditions were observed more suitable for retaining the antioxidant properties as compared to ambient conditions.

## ACKNOWLEDGEMENTS

The authors are thankful to the division of Post-harvest Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, for providing laboratory facilities.

## REFERENCES

Alleyne, V., & Hagenmaier, R.D. (2000). Candelilla-shellac-an alternatie formulation for coating apples (*Malus domestica* Borkh.). *Horticulture Science* 35, 691-693.

AOAC, (1990). Official Methods of Analysis. Analytical Chemist, 15th Edi. Washington DC, USA.

Awad, M.A., & De Jager, A. (2003). Influences of air and controlled atmosphere storage on the concentration of potentially healthful phenolics in apples and other fruits. *Postharvest Biology & Technology,* 27: 53-58.

Awad, M.A., & De Jager, A. (2000). Flavonoid and chlorogenic acid concentrations in skin of 'Jonagold' and 'Elstar' apples during and after regular and ultra low oxygen storage. *Postharvest Biology & Technology,* 20, 15–24.

Bai, J., Baldwin E.A., & Hagenmaier, R.D. (2002). Alternatives to shellac coatings provide comparable benefits in terms of gloss, internal gases modification, and quality for "Delicious" apple fruit. *Horticulture Science* 37: 559-563.

Boyer, J., Brown, D., & Liu, R.H. (2004). Uptake of quercetin and quercetin3-glucoside from whole onion and apple peel extracts by caco-2 cell monolayers. Journal of Agricultural & Food Chemistry, 52, 7172–7179.

Boyer, J., & Liu, R. (2004). Apple phytochemicals and their health benefits. Nutr. J. 3, 5.

Davey, M.W., Van Montagu V., Inzé D., Sanmartin M., Kanellis A., Smirnoff N., Benzie I.J.J., Strain J.J., Favell D., Fletcher J. (2000). Plant l-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agric. 80, 825–860.

Eberhardt, M.V., Lee, C.Y., Liu, R.H. (2000). Antioxidant activity of fresh apples. Nature, 405, 903–904.

Elizabeth, A.A., Kelly, M.G. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteau reagent. *Nature Protocols,* 2, 875-877

Faust, M., Shear, C.B. (1972). Fine structure of the fruit surface of three apple cultivars. Journal of the American Society for Horticultural Science, 97, 351-355.

Ghani, M.Y., Baigh, G.M. Mir, M.A. (2003). Incidence of pear spoilage in market of Srinagar, SKUAST-K. *Journal Reserach.* 5, 137-140.

Lee, K.W., Kim, Y.J., Kim, D.O., Lee, H.J., Lee, C.Y. (2003). Major phenolics in apple and their contribution to the total antioxidant capacity. Journal of Agricultural & Food Chemistry, 51, 6516–6520.

Leja, M., Mareczek, A., Ben, J. (2003). Antioxidant properties of two apple cultivars during long-term storage. Food Chemistry, 80, 303–307.

Malina, D.P. Recasens, I. (2005). Harvest maturity related changes and their influence on antioxidant potential in “Golden Smoothee” apples. *Acta Horticulture,* 682, 579-585.

Napolitano, A., Cascone, A., Graziani, G., Ferracane, R., Scalfi, L., DiVaio, C., Ritieni, A., Fogliano, V. (2004). Influence of variety and storage on the polyphenol composition of apple flesh. Journal of Agricultural & Food Chemistry, 52, 6525–6531.

Pardio-Sedas, V.T., Waliszewski-Kubiak, K.N., Garcia-Alvarado, M.A. (1994). Ascorbic acid loss and sensory changes in intermediate moisture pineapple during storage at 30-40°C. *International Journal of Food Science & Technology,. Tech.* pp. 551.

Parviainen, M.T., Nyyssonen, K. (1992). Ascorbic acid. In: *Modern chromatographic analysis of vitamins*. [Eds. A.P.D. Leenheer, W.E. Lambert and H. Nelis]. Marcel Dekker, New York.

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

Wijewardane, R.M.N.A., Guleria, S.P.S. (2009). Combined effects of pre-cooling, application of natural extracts and packaging on the storage quality of apple (*Malus domestica*) cv. Royal Delicious. *Journal of Tropical Agriculture,* 21, 10-20.

Wijewardane, R.M.N.A., Guleria, S.P.S. (2013). Effect of pre-cooling, fruit coating and packaging on postharvest quality of apple. Journal of Food Science & Technology, 50, 325-331..

Wolfe, K., Wu, X., Liu, R.H. (2003). Antioxidant activity of apple peels. Journal of Agricultural & Food Chemistry, 51, 609–614.