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Comparative Effect of Gamma Irradiation, UV-C and Hot Water on Antioxidant Potential of Mango (*Mangifera indica* L.) Fruit

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Abstract: Use of gamma irradiation and UV-C was compared over conventional used hot water treatment on mango pulp and peel. The storage study was also carried out to explore the potential of these techniques for the retention of total polyphenolic substances and antioxidants activity. Results indicated that polyphenolic substances decreased during storage. This decline in polyphenolic substances can be controlled by using gamma irradiation and UV-C treatment in mango peel and pulp. Lower doses (0.5 KGY) is more effective in controlling these losses. Comparing the varietal differences, white chaunsa showed better phenolic and antioxidant retention potential as compared to black chaunsa.

Key words: Gamma irradiation, UV-C, hot water treatment, total phenols, antioxidant activity

INTRODUCTION

Mango is an important tropical fruit in Asia, found abundantly in South East Asian region. In this region, Pakistan is renowned for various varieties of mangoes and overall ranked at 6th position in worlds mango production. The Total production of mango in Pakistan was 1950000 metric tons in 2012 (FAO, 2012). Pakistani mangoes varieties possess strong aroma and intense peel coloration. In Pakistan, the major varieties of mango are: Langra, Neelum, Dussehri, black Chunsa, white Chaunsa. Anwar Ratole, Sindhri and Gulab Khas.

Apart from taste, flavor and nutrition of the Pakistani mango varieties, these are rich source of polyphenolic substances having antioxidative properties that is associated with several health benefits most important is anticarcinogenic activity (Fresco *et al.*, 2006). With these antioxidants and phenolic substances, mango fruit is rich source of carotenoids, anthocyanins, ascorbic acid and tocopherols (Naczk and Shahidi, 2006; Leontowicz *et al.*, 2003).

To determine antioxidant activity various techniques are in use. The most renownend are the DPPH radical scavenging method, ferric ion reducing antioxidant power (FRAP) assay, total peroxyl radical trapping antioxidant parameter (TRAP) or trolox equivalent antioxidant capacity assay (TEAC) assay, oxygen absorbance capacity (ORAC) assay.

A lot of techniques and preservation techniques are in use to prevent the losses of nutrients in fruits and vegetables., this may include hot water treatment, low temperature treatment and others. Amoungest these, Irradiation and UV light treatment and hot water treatment have a greater scope to extend the shelf life with minimum losses. However these effects are not compared in case of mango fruit. The present research project was planned to compare the effect of gamma radiation, UV-C and hot water treatment on the antioxidants of mangoes to have an idea how we can use the preservation techniques for maximum retention of antioxidants in mango fruit.

MATERIALS AND METHODS

Raw material: Fresh and mature green mango fruits (cv. White and Black Chuansa) were purchased (at the same day of harvest) from the farm located in Multan city of Pakistan. After proper cleaning, mango fruits were transported at 11±1°C in reefer container to Post Harvest Research Center, Ayub Agriculutre Research Institute, Faisalabad, Pakistan. The mango fruits were divided into different groups randomly for application of the post harvest treatments and were transferred to cold storage room at 11±1°C. One group of mango fruits were irradiated at various levels of gamma irradiation separately (0.5, 1, or 1.5 kGy) using ^{137}Cs-generated γ-rays through a Gamma-cell Elan 3000 (Elitemodel D, Nordion International, Inc., Ottawa, Canada). The fruits belong to ultraviolet group were exposed to radiations (UV-C<280 nm) for 30 and 60 min period. Mango fruits were subjected to hot water treatment in cotton bags at 55°C for 5 min and immediately cooled by dipping in cold water at 20°C and air dried. For analysis of phenolic substances in peel and pulp separately, peel and pulp of both mango varieties (black Chaunsa and white chaunsa) were oven dried at 45°C for 48 h. The dried peels and kernels were crush to fine powder to pass through a 30 mesh sieve using a small laboratory grinder. Finally prepared powder was stored for further use.

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Preparation of material for total phenolics and antioxidant activity: The mango peel and pulp were dried in oven at 60°C for 1 h and grinded into powder form. The respective powders (10 g each) were extracted for one hour with 50 mL each of ethanol and water on mechanical shaker at room temperature and centrifuged for 15 min at 7000 rpm. Extracted samples were centrifuged. The contents from each sample was filtered through Whatman filter paper. The solvent from the supernatant was separated at 50°C in a rotary vacuum evaporator (EYELA, N-N series, Japan). The extract of each sample was weighted to determine the yield of soluble constituents and stored at 4°C until use.

Extract analysis: Extracts were analyzed for antioxidant activity, through free radical scavenging activity (DPPH assay).

Total phenolic contents: The total phenolic compounds were determined by the Folin-Ciocalteu method (Sun *et al.*, 2006). For the calibration curve 1 mL aliquots of 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mg/mL Catechol solutions in ethanol were mixed with 5 mL of Folin-Ciocalteu reagent (diluted ten-fold) and 4 ml of sodium carbonate solution (20 g/100 mL). The absorbance was checked after 30 min at 20°C at 765 nm and the calibration curve was plotted using the absorbance against the, respective standards (Fig. 1).
One mL of ethanolic mango extract (10 g/100 mL) was mixed with the similar reagents as described above. After 1 h the absorbance was measured for the total plant phenolics determination. In triplicate total contents of phenolic compounds in plant ethanol extracts in gallic acid equivalents (GAE) was calculated through the following formula:

$$C = c \times \frac{V}{m}$$

Here:

C = Total phenolic compounds (mg/g) of plant extract, in CAE
c = Concentration (mg/mL) of Catechol calculated from the calibration curve
V = Volume (mL) of extract
m = Weight (g) of plant ethanolic extract

The results were determined by using a standard curve previously developed with six different concentrations ranging 0.0 and 150 ug/mL (Fig. 1) and expressed as mg gallic acid/100 g of fresh fruits. From the standard curve, x is the gallic acid concentration and y is the absorbance. Thus:

$$Y = 0.00959524+0.00692238\ x$$

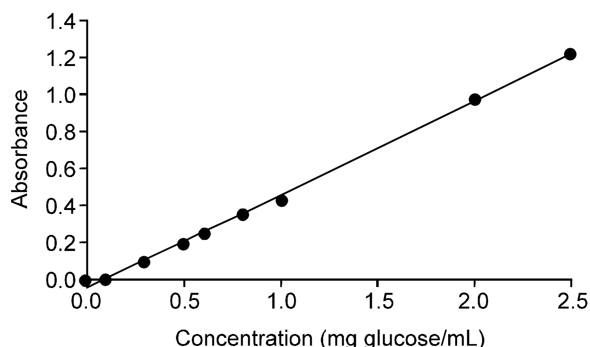

Fig. 1: Standard curve of total phenolics (ug gallic acid/mL)

Total phenolics concentration was calculated as follows:

$$x(\text{mg}/100\ \text{g}) = \frac{[y+0.00956]}{0.00692\ \text{mL}} \frac{\mu\text{g x}50\text{mL}}{\text{W sample (g)}} \frac{\text{x}0.001\ \text{mg}}{1\ \mu\text{g}} \text{x}100$$

y-axis absorbance (wt of sample in g)
x-axis gallic acid concentration (mg/mL)

Antioxidant activity: Most commonly used methods for the determination of antioxidant activity of plant extracts is 2,2-di(4-ter-octaphenyl)-1-picrylhydrazyl (DPPH).

Free radical scavenging activity (DPPH assay): DPPH radical scavenging activity of extracts was estimated by following the procedure of Brigita *et al.* (2005) with small modification. For the preparation of extract solutions, 0.025 g of dry extract was dissolving in 10 ml of ethanol. A fresh solution of DPPH in ethanol (6×10^5 M) was prepared before measurements. Three milliliter of that solution were mixed with 77 µL extract solution in 1 cm path length disposable microcuvettes (final mass ratio of extracts to DPPH was about 3:1, 1.5:1 and 0.75:1). The samples were placed in the dark place for 15 min at room temperature and then the decrease in absorbance was measured at 515 nm on a UV/visible light spectrophotometer (CESIL CE7200). Absorbance of blank sample containing the same amount of ethanol and DPPH solution was also measured in the same fashion:

$$\text{Reduction of absorbance (\%)} = [(AB-AA)/AB] \times 100$$

where:
AB: absorbance of blank sample (t = 0 min)
AA: absorbance of tested extract solution (t = 15 min)

Statistical analysis: The data was interpreted by analysis of Variance (ANOVA) using M-Stat C software package as described by Steel *et al.* (1997). ANOVA analysis tested the significance of the differences between samples at 5% level of significance. The

Duncan's Multiple Range (DMR) test was used to estimate the level of significance that existed between the mean values.

RESULTS AND DISCUSSION

Unrestrained oxidation can spoil both food commodities and human health. It has sword of double action, as it deteriorate the quality of food material and secondly intake of such items results in health hazards. The Process of auto-oxidation can be restricted with the help of antioxidant supplementation and a variety of synthetic agents are in steady use.

Mango variety White Chaunsa (WC) have higher total phenolics contents in pulp as compared to the Black Chaunsa (BC). Total phenolics of both the varieties in pulp decreased as a function of storage in all treated samples. However, treatment T_2 consisting of 0.5 KGy level of irradiation was most effective in retention of phenolic substances over a period of 28 days in both varieties. Comparing this level of irradiation in both varieties, white chaunsa performed better as compared to black chaunsa for phenolics contents. Overall, irradiation at different levels showed better result for retention of total phenolics as compared to UV-C and hot water treatment. Hot water treatment was less effective even as compared to control in retention of phenolics substances at all storage intervals (Fig. 1). Similarly, the average phenolic content in pulp of mango WC variety was higher as compared to Black Chaunsa (BC). Mangoes peels treated with gamma irradiation (dose 0.5 KGy/45 min) had higher total phenolics values as compared to other treated samples. Comparing the other methods of preservation, irradiation at all levels appeared the best method for retention of phenolic substances in peel (Fig. 2). This appeared as less destructive process for the phenolic substances. Use of UV-C also appeared as better method of preservation in case of phenolic substances in peel. All methods showed some deterioration and loss of phenolic substances in case of mango peel. Phenolics and polyphenolic compounds make up the main class of natural antioxidants and may contribute directly to

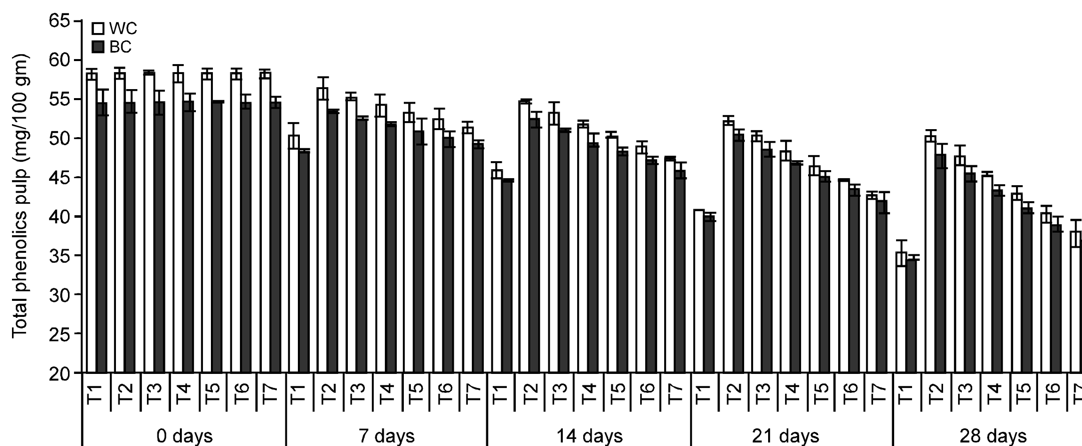

Fig. 2: Effect of treatments on total phenolics in pulp with respect to days

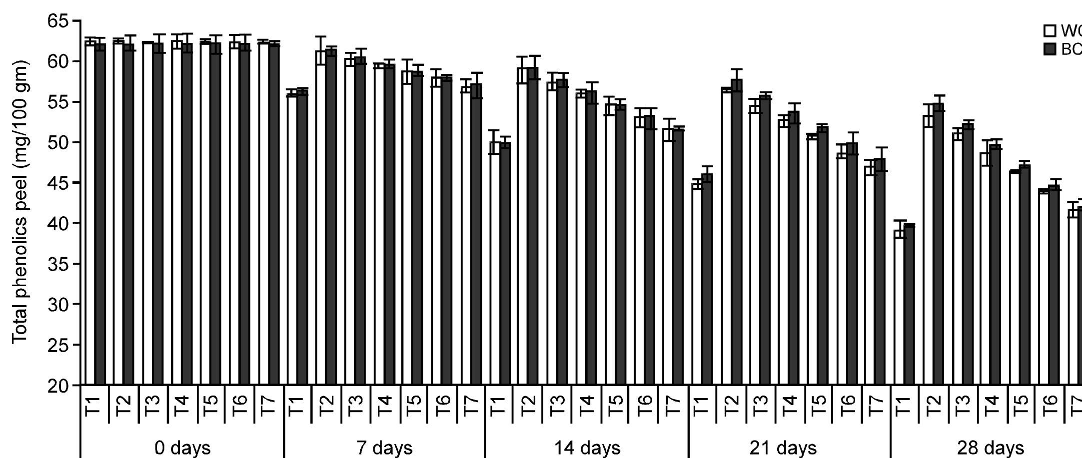

Fig. 3: Effect of treatments on total phenolics in peel with respect to days

antioxidative feat (Awika *et al.*, 2003).The loss in phenolic compounds may be originated to their property of free radical scavenging activity as well as metal chelation. The results were comparable to the findings of the Ajila *et al.* (2007) and Alasalvar *et al.* (2005) who studied total Phenolics contents in different tissues of Mango like pulp and peel which were ranging from 54.67 to 109.70 μg/mg. Another reason for variation in total phenolic contents of different mango cultivars is due to genotypic character of the cultivars.

Overall, total phenolics contents of mango peel in variety WC decreased from 62.32±0.17 to 46.26±1.10 mg/100 gm and variety BC decreased from 62.10±0.30 to 47.14±1.13 mg/100 gm at the end of 28 days of storage. Since variation of total phenolics value with storage time was the criteria for selection of variety to maintain quality. The result discussed in this study demonstrated that variety WC exhibited the higher phenolics value. The total phenolic contents in irradiated mangoes was higher than the control the sample treated with 0.5 kGy had the higher concentration but a significant ($p<0.05$) decrease was observed in samples irradiated at high dose with respect to storage days. The accumulation of phenolics compounds after irradiation is associated with different factors such as the increase in solubility due to the modifications in cell structures, increase in extractability and the variation between maturity levels among the samples, no loss was observed in induced mangoes by

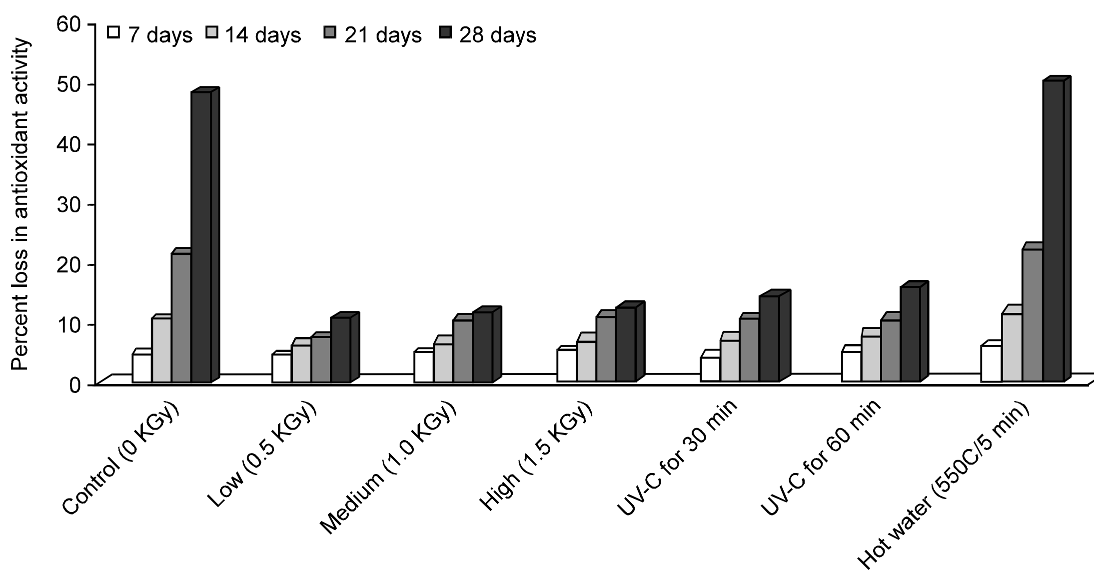

Fig. 4: Loss in antioxidant activity in mango peel during storage

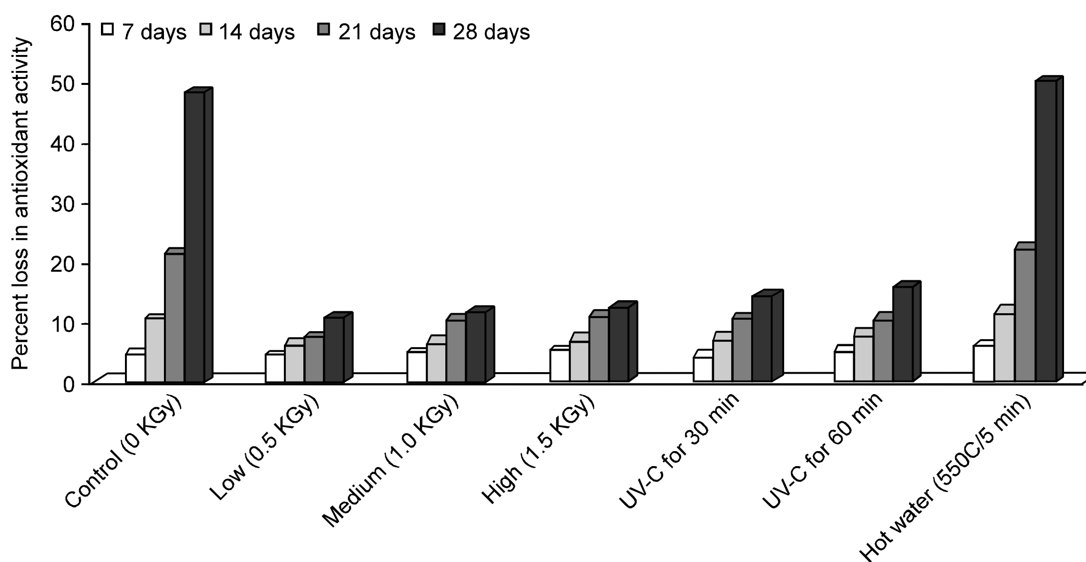

Fig. 5: Loss in antioxidant activity in mango pulp during storage

irradiation. In brief, it may be concluded that variety WC and treatment T2 (0.5 Kgy Irradiation) was the best for storage and shelf life of mangoes.

Antioxidant Activity of Mango pulp, peel: Antioxidants activity in mango pulp and peel was attributed to phenolic substances. It was observed that antioxidants lost in mango peel with the passage of time. As the time progresses more loss in antioxidant is evident, no matter what type of treatment was used. However, this loss in antioxidant was relatively slow in samples treated with irradiation followed by UV-C Treatment. Hot water treatment was not much suitable for restricting the antioxidants losses (Fig. 4). This loss in antioxidants in hot water treatment was due to the sensitive nature of phenolic substances present in peel. Small non significant difference was observed in loss of antioxidants when different levels of radiation was used (0.5, 1.0, 1.5 KGy). Similar non significant variation was observed at different level of UV-C treatment at different time intervals.

In mango pulp loss of antioxidant is evident in all treated and non treated (control) samples. In control samples initially the loss was low but as the storage time increase this loss in antioxiadants increased many fold in mango pulp. Treated sample show some resistant in the loss of antioxiadant activity. The role of gamma radiation and UV-C is equally important in controlling the antioxidant losses. Comparing both Gamma radiation and UV-C, Gamma radiation appeared better in controlling antioxidant activity (Fig. 5). At higher level 1.5 KGy) of gamma radiation more losses observed as compared to low doses of gamma radiation (0.5 KGY). So it can be concluded that Gamma radiation is better technique as compared to conventioinal used hot water treatment in retention of antioxidant activity in mango pulp. This technique have a great potential to be used on commercial scale. The results for mango peel and pulp were comparable to the findings of the Ajila *et al.* (2007) who investigated Free radical scavenging activity in different tissues of Mango which were ranging from 45.21 to 60.33% inhibition. In the similar work which was done by Ajila *et al.* (2010) who indicated that the DPPH values in peels from different varieties of mango fruits at different stages of maturity were altered. Ajila *et al.* (2008) found the similar results that mango peel contained 79.06% inhibition of DPPH.

The DPPH% for WC and BC variety is given as under:

	0 day	7 day	14 day	21 day	28 day
WC	58.22±1.44	60.22±2.4	61.35±0.17	50.49±4.4	53.33±1.2
BC	69.66±0.54	69.77±0.46	68.58±0.96	66.47±0.88	61.85±1.16

Conclusion: The present investigation suggests that Mango pulp, peel have a great antioxidant potential which must be utilized in different foods. But for useful applications the suitable treatment must be used during storage. This had made strong impact on maintaining a suitable level of antioxidant and phenolic substances in mango peel and pulp. The two mango cultivars black chaunsa and white chaunsa showed a possible source of natural antioxidants and the treatments like gamma irradiation, UV-C/30 min, UV-C60 min had great potential for retention of significant effects for the production of antioxidants.

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