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Postharvest application of gamma irradiation affects fruit quality and antioxidant enzymes activities of 'kinnow' mandarin fruits during cold storage

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Abstract

Food safety with maintained fruit quality is an important challenge for postharvest industry of citrus fruits. The present study was designed to check the effects of gamma irradiation on postharvest fruit quality of 'Kinnow' mandarin stored at 5 ± 1 °C and 90 ± 5% Relative Humidity. In the first experiment, fruits were exposed to different doses of gamma radiation (100-500 Gy), while after analyzing the results of first experiment, in the second experiment fruits were treated with 500, 600, 800 and 1000 Gy of gamma radiation. It was observed that higher doses of gamma irradiation exhibited lower weight loss and disease incidence as well as inhibiting the increase of soluble solid content (SSC) and retained higher titratable acidity (TA) and lower SSC: TA ratio. After 90 days of storage, maximum levels ascorbic acid, total phenolic contents and total antioxidants along with catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activities were recorded higher in fruits treated with 800 Gy. These fruit also exhibited higher score of taste, aroma and texture during storage. Overall results suggest that pre-storage treatment of 800 Gy is the most effective treatment and have potential application for quality management of 'Kinnow' mandarin under long term cold storage.

Keywords: citrus; food irradiation, total phenolic contents; catalase; peroxidase antioxidant activity.

Prcatical Application: Application of gamma irradiation maintain quality attributes, antioxidant enzymes and shelf life of Kinnow mandarin during cold storage.

1 Introduction

'Kinnow' mandarin (*Citrus nobilis* Lour. × *Citrus deliciosa* Tenora.) is the most important citrus cultivar of Pakistan in terms of production and export. The country exported 370,000 tons of citrus during 2017-18 with a valuable foreign exchange of more than $220 million (The News International, 2019).

According to FAO reports, almost one third of the world's food supply is lost after harvesting which is estimated about 1.3 billion ton per year. With the increasing population and changing climate, most of the developing countries are experiencing serious food shortage. Recently many countries are further included in food shortage threat list by United Nations (Food and Agriculture Organization, 2022; World Food Program, 2022).

Due to improper postharvest facilities, Pakistan has to face higher postharvest losses of Kinnow mandarin, as in some production areas they are reported up to 45% of total production which cause higher economic losses (Food and Agriculture Organization, 2018; Ahmed et al., 2015).

Fruit deterioration occurs in citrus due to different internal metabolisms which continue in fruit even after harvest and during storage. Numerous postharvest technologies including food irradiation have been recognized worldwide to control fruit diseases and disorders. These technologies work to maintain fruit quality up to maximum levels, minimize postharvest losses and maintaining freshness during storage (Bajwa & Anjum, 2007). Some postharvest treatments increase the storage life but may adversely affect phytochemical attributes of fruit, which have radical scavenging activities and increase immunity against diseases in human body (Khan et al., 2021; Moreira et al., 2022). After irradiation, the defense system within the fruit activates due to oxidative stress which is produced due to irradiation and storage at low temperature. Sometimes, radiations may also result in the loss of nutrients and other bioactive contents present in citrus fruits (Oufedjikh et al., 2000; Patil et al., 2004).

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The use of gamma rays was mainly carried out to control green and blue molds (fungal diseases); higher doses are effective to minimize the incidence of fungal diseases in fruits but may harm fruit skin and its biochemical composition (Smilanick et al., 2008; Miller et al., 2000). The ionizing radiation is utilized to kill the microorganisms of food without affecting external and internal quality parameters and without any major changes in natural composition of food products (Zeuthen & Bøgh-Sørensen, 2003). Practical applications of food irradiation can be divided based on the radiation doses into three categories: low dose applications (up to 1 kGy), medium dose applications (1 kGy to 10 kGy) and high dose applications (above 10 kGy). Low doses are mostly used for sprout inhibition in bulbs and tubers; 0.25-1.0 kGy for delay in fruit ripening and 0.1-1.0 kGy for insect disinfestations and destruction of food borne parasites. Significant losses of vegetable crops like potatoes, onions, garlic, shallots, yam and ginger occur due to sprouting which may controlled by irradiation. A very low radiation dose of 0.2 kGy or less inhibits sprouting, leaves no residues and allows storage at higher temperatures. Radiation can also be used to delay ripening and prevention of microbes in the stored fruits which ultimately results in increasing the shelf life of different fruits and vegetables (Mostafvi et al., 2010; Singh & Singh, 2020).

Although there are many advantages of food irradiation; however, gamma irradiation may cause changes in the phenolic composition and flavonoids in leaves, fruit peel and pulp of citrus (Kim et al., 2012; Patil et al., 2004). Irradiation has been observed to encourage the induction of phenyl ammonia lyase (PAL) enzyme activity in fruits (Moussaid et al., 2000; Oufedjikh et al., 2000), and also induces synthesis of phenolic and flavonoids (Patil et al., 2004; Vanamala et al., 2005; Vanamala et al., 2007; Kim et al., 2012). Therefore, the present study was carried out to evaluate the effectiveness of gamma radiation on quality traits, antioxidative responses and shelf life of 'Kinnow' mandarin fruits during storage.

2 Materials and methods

2.1 Fruit materials and gamma treatment

Physiologically mature and healthy 'Kinnow' mandarin (*Citrus nobilis* Lour. × *Citrus deliciosa* Tenora.) fruits were harvested from the Experimental Fruit Orchard, Square No. 9, Institute of Horticultural Sciences, University of Agriculture, Faisalabad (31° 25' 0" North, 73° 5' 0" East) during 2015-16. The fruits were treated with gamma radiation of 0, 100, 200, 300, 400, 500, 600, 800 and 1000 Gy, respectively at PARAS (Pakistan Radiation Services) Foods, Lahore. The Co-60 was used as a source of gamma radiation for a specific time period to meet required dose. The study comprised of two experiments, in the first experiment fruits were exposed to lower doses of gamma radiation (100-500 Gy) and on the basis of these results second experiment was planned, in which the fruits were treated with relatively higher doses (600, 800 and 1000 Gy) in comparison of best treatment (500 Gy) selected from previous experiment. After treating with radiation, fruits were stored at 5 ± 1 °C and 90± 5% Relative Humidity (RH) for 90 days. Fruit analyses for different quality parameters were carried out at harvest day and after every 15 days till 90 days of cold storage. Each treatment comprised of four replications and five fruits from each replication were used for juice extraction and further analyses.

2.2 Disease incidence (%) and weight loss (%)

Disease incidence in treated fruits was recorded during the three months of storage and all the diseased or injured fruits were discarded immediately (Hafiz et al., 2018). Disease incidence was expressed in percentages. Fruit weight of all the samples was measured before, during and after storage using a digital balance (MJ-W176P, Panasonic Japan) and five fruits from each replication were selected and separated in a net bag and data regarding their weight were recorded after every 15 days and represented in percentage.

2.3 Soluble solid contents (SSC), titratable acidity (TA) and SSC: TA ratio

The Kinnow juice (taken from 5 fruits of each replication) was extracted and filtered through Whatman® filter paper No.1 and a digital refractometer (RX 5000, Atago, Japan) was used for the soluble solid contents (SSC) estimation at room temperature. The prism of refractometer was washed with distilled water after taking each reading. The TA of juice samples was determined by the method described by Hortwitz (1960). SSC:TA ratio of each sample was calculated by dividing the values of SSC with the corresponding TA.

2.4 Determination of sugars and ascorbic acid content

The method of Hortwitz (1960) with some modifications was used to estimate sugar contents in juice of treated fruits. Sample of fruit juice (10 mL) was taken, and 100 mL distilled water was used to dilute lead acetate (25 mL; 25%) and potassium oxalate (10 mL; 20%) solutions were added into fruit juice samples. The filtrate was used for determination of different types of sugar contents and expressed as percent. Ascorbic acid (mg 100 g^{-1}) in the juice was measured according to the method of AOAC (Association of Official Analytical Chemists, 1990).

2.5 Total phenolic contents (TPC) and total antioxidants

Total phenolic acid was determined according to the method Ainsworth & Gillespie (2007). The filtrate was homogenized by adding 5 mL solution of methanol:acetone:HCL (90:8:2), centrifuged (13000 x *g*) for 3 min and supernatant was used to measure the absorbance (at 765 nm and 517 nm). The results are expressed as mg Gallic Acid Equivalent/ 100 g. 2-diphenyl-1-picrylhydrazyl radical (DPPH) assay of Brand-Williams et al. (1995) was used to determine total antioxidants in the juice of 'Kinnow' fruit. The absorbance was measured at 517 nm by using spectrophotometer and expressed as percent inhibition.

2.6 Antioxidant enzymes

The extracted juice samples were cryopreserved with liquid nitrogen and stored at -80 °C. To determine antioxidant activity, then samples were homogenized in potassium phosphate buffer (2 mL with 7.2 pH) and centrifuged (at 10,000 x *g*) for 5 min at 4 °C. The supernatant was used to assess different antioxidant enzyme activities such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) assay by using the method of Liu et al. (2009). Absorbance of enzyme extract mixed with reaction mixtures was recorded at specific wavelength for each enzyme and was expressed as U mg^{-1} protein.

2.7 Sensory evaluation

The fruits were evaluated for different organoleptic characteristics and five fruits from each replication were placed to determine fruit colour, taste, texture, taste and aroma/ flavor using an arbitrary scale ranging from 1-9. A panel of 10 judges was asked to perform a sensory evaluation using the Hedonic scales (Stone et al., 2012).

2.8 Statistical analysis

The experiments were carried out under completely randomized design (CRD) with factorial arrangements. The recorded data were analyzed using analysis of variance (ANOVA) technique with the help of software Statistix ver. 8.1. The least significant difference (LSD) test was used to compare the treatment means (Steel et al., 1997).

3 Results

3.1 Disease incidence and Weight loss (%)

In the first experiment, 500 Gy dose significantly ($P \leq 0.05$) minimized the disease incidence during storage, whereas maximum disease incidence (27%) was observed in control treatment while minimum (11%) was noted in the fruits treated with 500 Gy (Figure 1a). In the second experiment, maximum disease incidence (25%) was recorded in control fruits and lower (9%) disease incidence in 1000 Gy treatment ($P \leq 0.05$; Figure 1c). In both experiments, gamma radiation doses significantly ($P \leq 0.05$) affected weight loss percentage in fruits for 90 days of storage. It was observed that higher weight loss (28%) was observed in control treatment than fruits treated with 500 Gy (15%) and 800 Gy (11%) and 1000 Gy (10%) in both experiments (Figure 1b & d).

3.2 SSC, TA and SSC: TA ratio

Irrespective to treatments, SSC contents were increased with prolonged storage time (Figure 2a & d). In the first experiment, minimum changes were noted ($P \leq 0.05$) in the fruits treated with 500 Gy of radiation compared to lower doses and control treatment (Figure 2a). Fruits treated with 500 Gy had maximum TA (1.1%) at the end of 90 storage while, minimum (0.61%) was found in control treatment (Figure 2b). After 90 days, maximum SSC: TA ratio (13.23%) was observed in control while it was minimum (12.33%) in the fruits treated with 500 Gy (Figure 2c). In the second experiment, control treatment had maximum SSC (12.8%) and SSC: TA ratio (14.59%) after 90 days of storage; however, minimum values (SSC; 11% and SSC: TA ratio 8.21%), and maximum TA (1.3%) were noted in

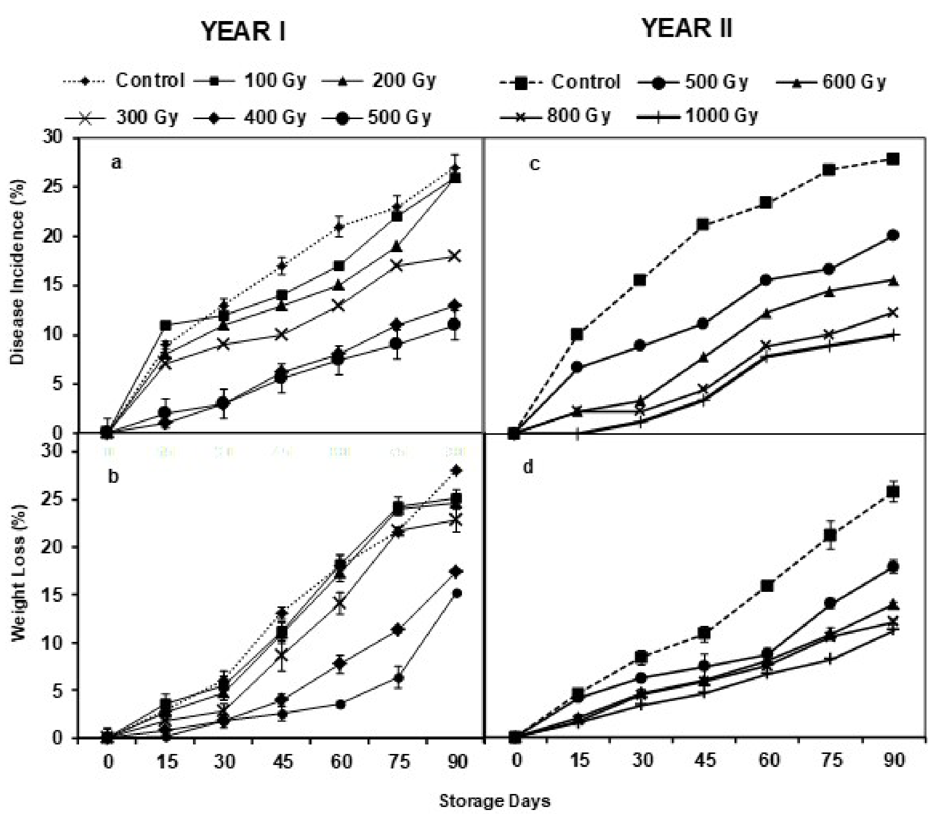

Figure 1. Effects of different doses of gamma radiation on disease incidence (a, c) and fruit weight loss % (b, d) of 'Kinnow' mandarin during cold storage.

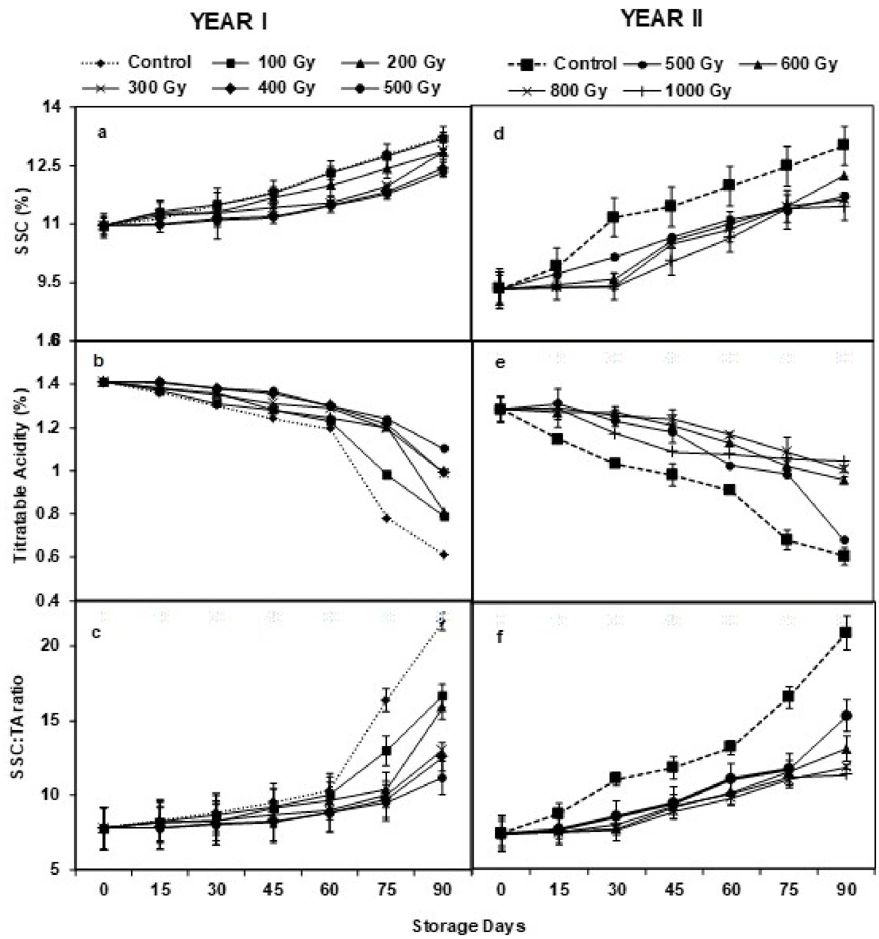

Figure 2. Effects of different doses of gamma radiation on SSC (a, d) TA percentage (b, e) and SSC: TA ratio (c, f) of 'Kinnow' mandarin during storage.

fruits treated with 1000 Gy of gamma radiation (Figure 2d-f) as compared to control treatment (Figure 2d-f).

3.3 Total, reducing and non-reducing sugars

In both experiments, the values of total sugars were increased continuously till the end of 90 days storage (Figure 3a & d). In both experiments, control fruit exhibited maximum total sugars (19.23%) and reducing sugars (13.24%) while the minimum total sugars was recorded in the fruits exposed to 500 Gy (15.49%) and lower reducing sugars (8.07%) in 1000 Gy treated fruits throughout the storage period (Figure 3a, b & d, e). However, higher doses (800 and 1000 Gy) had minimum changes in total sugars of 'Kinnow' mandarin fruit during storage. However, non-reducing sugars were decreased and the maximum value of 8.23% was found in the fruits treated with 800 Gy of gamma radiation as compared to other treatments after 90 days of storage (Figure 3c & f).

3.4 Ascorbic acid contents, total phenolic contents and total antioxidant activity

Irrespective to radiation doses, all treated fruits showed increase in ascorbic acid contents during initial 45 to 60 days of storage followed by a gradual decrease till 90 days (Figure 4a & d). Maximum ascorbic acid contents (49.75 g kg^{-1}) were found in 500 Gy-treated fruits, and 600 Gy irradiation treatment (47.01 g kg^{-1}) as compared to control during storage (Figure 4a & d). It was observed that total antioxidant activity was found higher in the fruit treated with 500 Gy (43.01%) and 800 Gy treatment (51.98%) as compared to untreated fruits after 90 days of storage period (Figure 4b & e). The results revealed that maximum total phenolic contents were observed in the fruits treated with 400 Gy (176.72 g kg^{-1}) and 600 Gy (147.45 g kg^{-1}) than untreated control fruits at the end of storage period (Figure 4c & f). It was interesting to observe that total phenolics were maintained by all radiation doses up to 60 days of storage, but 1000 Gy exhibited more decline in total phenolic contents as compared to 600 and 800 Gy.

3.5 Antioxidative enzymes

In the first experiment, fruits treated with 500 Gy significantly exhibited higher activities of CAT (12.58 U mg^{-1} protein), POD (0.76 U mg^{-1} protein) and SOD (116.78 U mg^{-1} protein) enzymes as compared to untreated control fruits at the end of storage

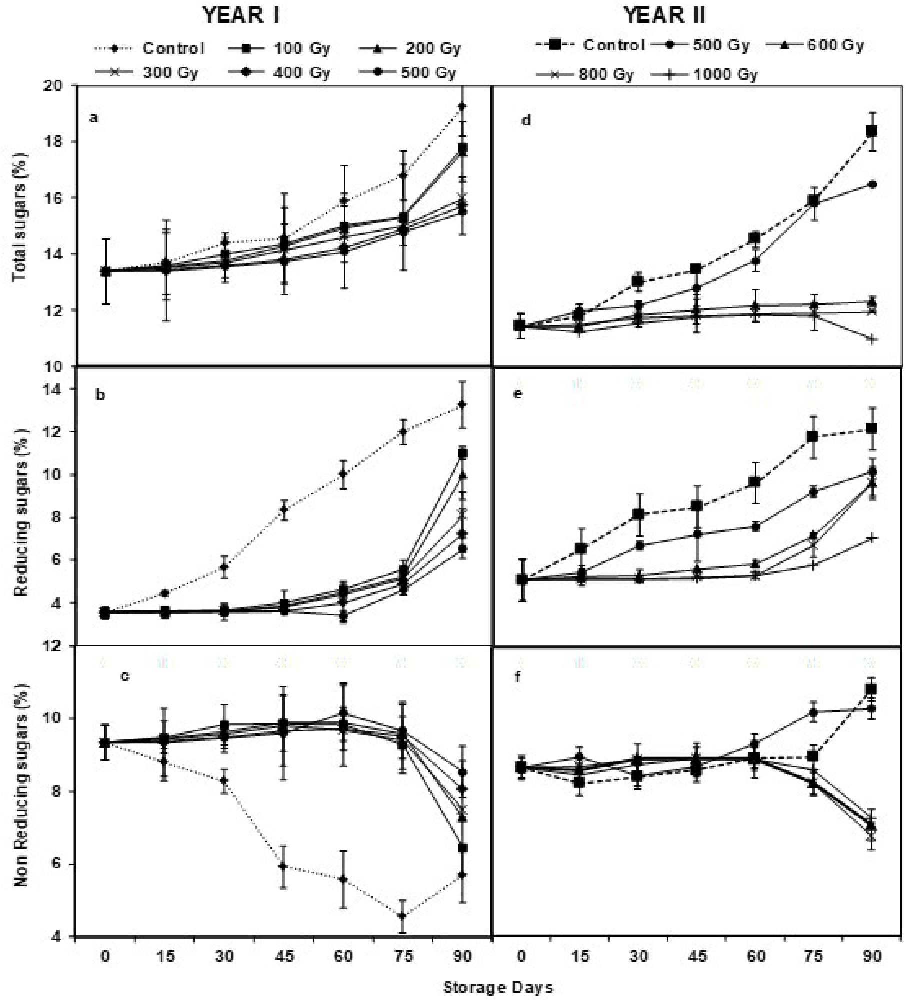

Figure 3. Effects of different doses of gamma radiation on total sugars (a, d), reducing sugars (b, e) and non-reducing sugars (c, f) in 'Kinnow' mandarin during storage.

(($P \leq 0.05$; (Figure 5a & c). In the second experiment, 600 Gy treated fruits retained higher CAT activity (22.63 U mg^{-1} protein), whereas fruits subjected to 800 Gy irradiation displayed higher POD (0.67 U mg^{-1} protein) and SOD (112.1 U mg^{-1} protein) activity than control fruits (Figure 5d-f).

3.6 Sensory attributes

Results regarding organoleptic evaluation indicated that radiation doses above 300 Gy significantly maintained fruit taste, aroma and texture. In both experiment, control fruits had minimum score of sensory parameters at the end of 90 days storage (Figure 6a-f). In the first experiment, fruits treated with 500 Gy maintained maximum score while in the second experiment 800 and 1000 Gy were more effective to maintain fruit taste, aroma and texture during storage.

4 Discussion

During postharvest storage, respiration in citrus fruit continues even after harvest and in this process, oxygen is absorbed, and CO_2 released and burning of sugars which may contribute to deteriorate the fruit quality (Taiz & Zeiger 2003; Amin et al 2020; Le et al., 2022). In this study, minimum disease incidence was recorded in higher doses of radiation (500 Gy, 800 Gy and 1000 Gy) during 90 days of cold storage (Figure 1a & c). The energy of gamma rays penetrates into fruit and microbes tissues and inhibits their reproduction ability (Amin et al., 2020). In present study, control fruit exhibited more weight loss than treated ones which was probably due to higher moisture loss, respiration, and transpiration rate (Figure 1b & c). Mostly, higher doses of respiration reduce respiration rate and metabolic activities which lead to minimize the weight loss of fruits (Tariq et al., 2004; Boynton et al., 2006; Azam et al., 2020).

There were no significant changes in SSC contents and sugars of irradiated Kinnow fruits during storage (Figure 2a-f). During storage, hydrolysis of starch into sugars leads to increase the soluble solids within the fruit. Previous studies reported that shelf life of fruit is extended by radiation without any harmful effects on biochemical attributes (Boylston et al., 2002; Moy & Wong, 2002). Likewise, Vanamala et al. (2007) reported that

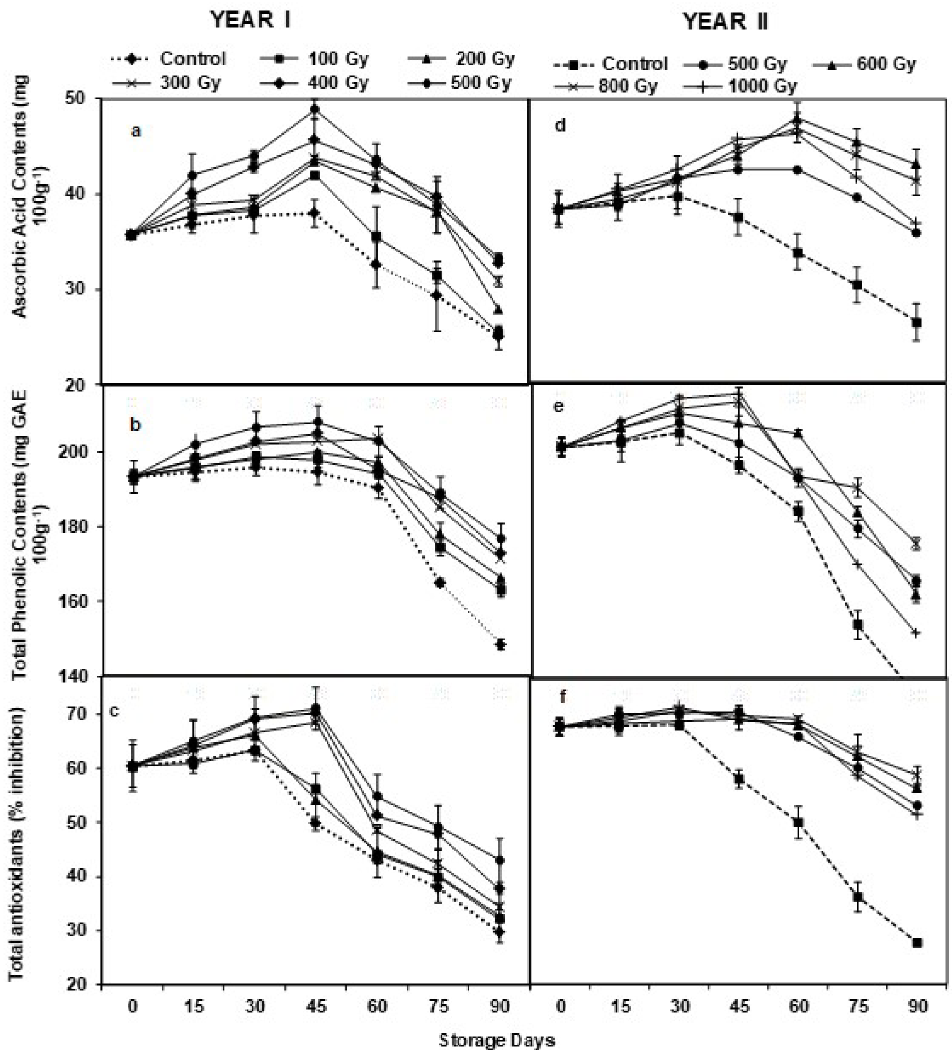

Figure 4. Effects of different doses of gamma radiation on ascorbic acid contents (a, d), total phenolics (b, e) and total antioxidants (c, f) of 'Kinnow' mandarin during storage.

grapefruit subjected to irradiation treatments displayed slight changes in SSC contents under cold storage. Gamma radiation treatments showed higher TA contents as compared with control fruits (Figure 2b & e). Similarly, during ripening process and storage, acid contents of fruits convert into sugars, which cause decrease in titratable acidity (Hassan et al., 2014; Azam et al., 2021; Liangjie et al., 2022). SSC: TA ratio is considered important to evaluate the ripening and maturity stages of fruits and vegetables. In the present study, gamma radiation maintained lower SSC: TA ratio than untreated control fruits during cold storage (Figure 2c & f). Taste of citrus fruits mostly depends on this parameter and mandarin fruits are liked among consumers due to their lower SSC: TA ratio (Arpaia & Kader, 2000).

During storage, ascorbic acid is proved to be very sensitive to oxidation and leaching of water into water-soluble media therefore considered as an important parameter to estimate nutrient retention and overall quality of food products (Davey et al., 2000). In our study, Irradiated fruits retained higher ascorbic acid contents during storage, however; after treating with gamma rays, an immediate but slight increase was observed during storage (Figure 4a & d). Increase in ascorbic acid contents of citrus may be due to its association with increased antioxidant activity in fruits. However, decline in ascorbic acid contents during storage may be due to low temperature and its effects on metabolism control (Silva et al., 2013; Ahmad et al., 2012).

Many phytochemicals are produced by plants which contribute to activate plant defense system and protect the plants against stress conditions as well as pathogen attack (Zobel, 1997). In this experiment, it was concluded that higher doses of irradiation helped to maintain the maximum phenolic contents of fruits during storage up to 90 days of cold storage (Figure 4b & e). Gamma irradiation invigorates PAL enzyme activity which eventually leads to higher production of secondary metabolites including phenolic contents (Vanamala et al., 2007; Alothman et al., 2009; Bravo et al., 2013). Similar results have

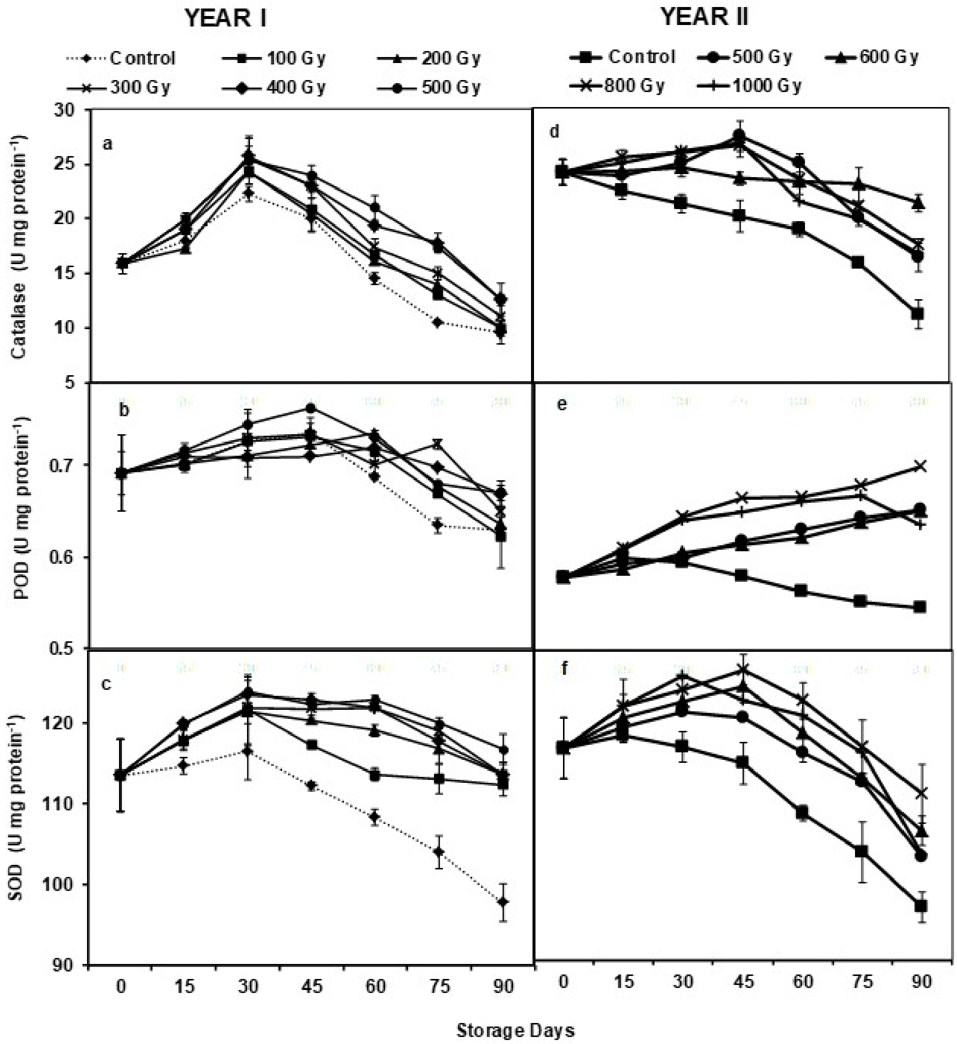

Figure 5. Effects of different doses of gamma radiation on catalase (a, d) peroxidase (b, e) and superoxide dismutase (c, f) activities of 'Kinnow' mandarin during storage.

been reported in different fruits and vegetables after exposing to gamma radiation under cold storage (Patil et al. 2004; Vanamala et al. 2007). Total antioxidant activity of 'Kinnow' mandarin peaked during first 30 days and in some higher doses up to 45 or 60 days and after a particular time frame, continued to decrease till the end of storage (Figure 4c & f). Decrease in phenolic contents may also reduce antioxidant activity of fruits (Alothman et al., 2009). The initial increase in antioxidant enzymatic activity treatments may be due to low temperature increases the level of reactive oxygen species which ultimately modifies the cellular homeostasis (Suzuki & Mittler, 2006). Moreover, antioxidant activity of fruits also affects fluctuation in ascorbic acid (Du et al., 2009). Gamma radiation was helpful to enhance health promoting compounds in early season grapefruit (Patil et al., 2004).

Enzymatic antioxidants include CAT, POD and SOD stimulate the defence system of plants against several biotic and a biotic stress. Fruit radiation may initiate free radical production, which triggers internal defence system of fruits by increasing the antioxidant enzyme activities (Erkan et al., 2008; Khan et al., 2021; Maharaj et al., 2015). Our results revealed that higher dose of gamma radiation displayed higher activities of CAT, POD and SOD enzymes in Kinnow fruits during storage (Figure 5a-f). It was observed that higher doses of gamma radiation help to maintain the fruit taste, aroma and texture of kinnow fruits during cold storage period (Figure 6a-f). Accumulation of H_2O_2 occurs after low temperature exposure which causes oxidative stress and increased activity of CAT and POD enzymes under cold storage. However, after a specific time period, decline in enzyme activities could be due to low temperature liability and light sensitivity (Hertwig et al., 1992; Masia, 1998). Previously, increased activities of antioxidant enzymes (CAT, POD, SOD) after radiation have also been reported in mango, banana and strawberry fruits during cold storage (Erkan et al., 2008; Mohammadi et al., 2012)

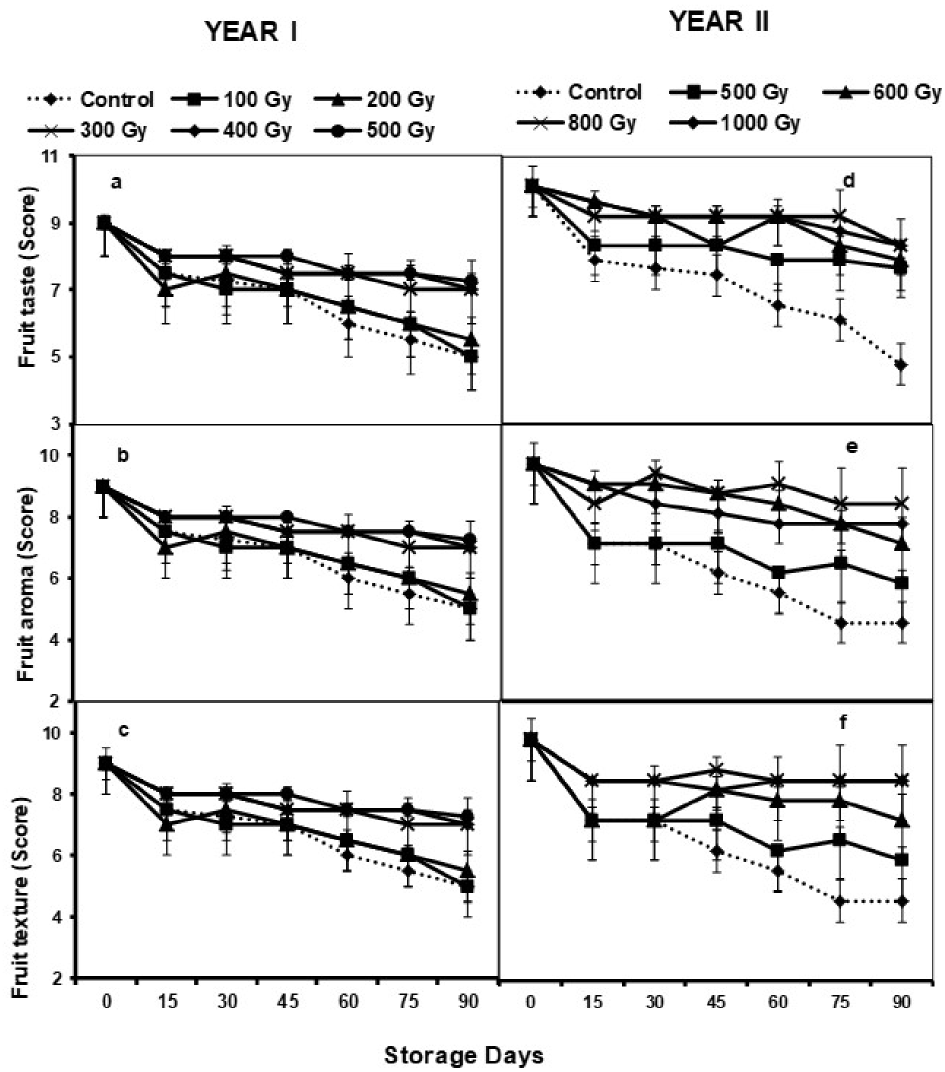

Figure 6. Effects of different doses of gamma radiation on fruit taste (a, d) aroma (b, e) and texture (c, f) of 'Kinnow mandarin (± SE) during storage.

5 Conclusion

In conclusion, food irradiation has potential to contribute in reducing the magnitude of such problems by minimizing postharvest losses of citrus fruit. gamma radiation doses above 300 Gy helped to control disease incidence however, 800 Gy was most effective to reduce disease incidence. Comparatively higher doses (600-1000 Gy) had a significant effect on fruit taste, however; physico-chemical parameters were slightly changed during long term cold storage. Bioactive components such as total phenolic contents and antioxidant activities were found higher in 800 Gy treated fruits. Most of the treatments maintained maximum overall fruit quality for 45-60 days but at the end of storage, maximum activities of bioactive juice components were recorded in the fruit treated with 800 Gy. Therefore, the radiation dose of 800 Gy is recommended to maintain the higher amount of health promoting substances along with maximum retained fruit quality of 'Kinnow' mandarin. This technology can benefit as an effective method for reducing postharvest food losses, as well as can be used as substitute for chemical fumigants applied during postharvest storage.

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