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Original article

Postharvest preservation of citrus fruits (Kinnow) by gamma irradiation and its impact on physicochemical characteristics

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Summary. Citrus fruit (Kinnow) has several beneficial health and nutritive properties. Several techniques have been used to preserve postharvest quality of citrus fruits. Exposing foods to gamma radiation delays spoilage and increases protection by eliminating or lowering pathogenic bacteria. In the present study Kinnow fruit were exposed to radiation dose of 0.0, 0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 kGy. Physicochemical and microbial analysis was performed on control and irradiated samples stored at both ambient and refrigerated temperature on weekly intervals. No significant change was observed in physicochemical properties of Kinnow at optimum dose, epiphytic microbial flora reduced in irradiated samples than control samples. The radiation dose of 1.5 kGy along with refrigerated storage extended the shelf-life of Kinnow for 1week without affecting sensory and physicochemical properties.

Key words: kinnow, physicochemical properties, nutritional quality

Introduction

Citrus fruits are among the highly consumed fruits not only for their sweet and energizing properties but also for their nutritional and medicinal importance. Citrus juices and citrus fruits have several nutritive properties which are very beneficial for health (1). Globally Pakistan stands in top 10 citrus producing countries (2). However, more than 95% of citrus fruit are produced in province Punjab and almost 70% of them are Kinnow (3). Citrus business of Pakistan is dominated by Kinnow (4) which is a prominent variety and contributes a lot more than 70% of citrus production in the country (5). Citrus species are vulnerable to several diseases caused by different kinds of pathogens like bacteria, fungi, viroids, viruses, nematodes, spiroplasmas and phytoplasmas (6). Postharvest decay is commonly the main issue limiting extended storage (7) and specifically fungal diseases are significant reason for decay and losses in harvested fresh fruit. Significant postharvest fungal diseases of citrus are blue molds, green molds, grey mold, sour rot, brown rot and *Alternaria* rot (8). Food and Agriculture organization of United Nations determined that 25% of all food product are wasted after harvest worldwide (9). The degree of post-harvest losses in vegetables and fruits vary from 35 -45% in Islamic Republic of Pakistan (10). Post-harvest treatment by using artificial chemicals is comparatively cheap method, it's simple to use and have therapeutic action against pre-existent or ongoing infections (11). However use of these chemicals typically leave chemical residue on fruit skin that can affect the health of human (12). There is a strong and increasing requisite to search and implement control techniques alternative to ancient antifungal agents for the control of post-harvest losses due to blue and green molds of citrus fruits. On the basis of their nature, three different decay management strategies are often used chemical, biological and physical. The major advantage of using physical treatments against fungus control is the complete absence of any deposit on treated products as well as insignificant impacts on environment

(13). Radiation has been utilized as a treatment to prevent fungal decay of fresh fruits (14). Gamma rays are electromagnetic waves that have high penetrating power they pass through materials without leaving any residue, an advantage comparing to other disinfection treatment. The impact of gamma rays on the activity of microbes has attracted considerable deal of attention (15). Joint expert committee on food irradiation after the assessment of nutritional, physical, chemical and toxicological characteristics of foods confirmed that food irradiated up to 10kGy is safe and nutritionally acceptable (16). Under smart working practices and ensuring correct handling of the products, irradiation eradicates harmful microorganisms that may cause fatal illness and food decay (17).

Materials and methods

Collection preparation and gamma irradiation of samples

Kinnows of nearly uniform size and shape were collected from local market of Lahore. The selected samples were divided in two categories control and experimental. The fruit samples were weighed and packed in labeled polythene bags. The experimental group was subjected to different gamma radiation doses at Pakistan Radiation Services (PARAS) Lahore, Pakistan using Cobalt-60 as the source at the dose rate of 60 Gy/hr. Harwell Amber 3042 dosimeter was used for dose measurement. After irradiation first day packet was examined and the remaining packs were put on storage at ambient and refrigerated temperature. Periodic evaluations were carried out on day 1, 7, 14, 21 and 28. Controls were also run parallel.

Sensory analysis

Color and texture of both control and irradiated of fruits were determined visually (18).

Determination of Microbial flora: For the determination of microbial flora serial dilution method was used. Each fruit sample was washed with 100 ml of 0.9% sterilized saline water. Different dilution (10^{4}–10^{7}) were made from this stock. 100µl of each dilution was transferred to different petriplates containing Nutrient agar (for non-fastidious bacterial isolation), MaCconkey agar (for gram negative bacterial isolation), Salmonella Shigella agar (for *Salmonella* and *Shigella* sp. isolation) and Potato dextrose agar (for fungi). Plates were then incubated at 37°C for 24 hour and at 30°C for 3-4 days for bacterial and fungal growth respectively (19). Total viable count, coliform count and fungal count was determined according to following formula (20).

$$\text{Colony forming unit / gram} = \frac{\text{No. of colonies}}{\text{Dilution factor} \times \text{amount plated}}$$

Colony morphology characteristics i.e. color, shape, texture, elevation, margins and optical characteristics (opaque/translucent) was noted. Gram and endospore staining, as well as motility and catalase test were also performed. The API-20E test kit was used for the identification of enteric bacteria. Fungal species were identified on the basis of micro and macroscopic characteristics (21).

Physicochemical analysis

Physiological loss in weight

Fruits were weighed periodically and percentage weight loss was calculated (22)

$$\text{Percentage physiological loss in weight} = \frac{Wi - Ws}{Wi} \times 100$$

Where Wi=Initial weight; Ws = Weight at sampling period

Moisture and Ash content: Moisture and ash content of both control and irradiated samples were estimated (23).

Percentage juice, Total soluble solid, Titratable acidity and Ascorbic acid content

Juice content: Fruits were cut into equal half and squeezed to extract all the juice by using manual juice presser. The extracted juice was filtered through strainer. The percentage juice content was calculated (24)

$$\text{Juice percentage} = \frac{\text{Total weight of juice (g)} - \text{Beaker weight (g)}}{\text{Total weight of fruit}} \times 100$$

Total soluble solid content

Total soluble solid was calculated by using Digital Brix Refractometer (Model: Atago PAL-3). Small amount of juice was placed on lens of refractometer and reading was noted (24).

Titratable acidity

Equal volume of juice and distilled water was taken. 2 - 3 drops of 1% phenolphthalein were added to observe the end point as indicated by change in color. The sample was titrated by using 0.1 N sodium hydroxide solution. Results were recoded as percent citric acid (24).

$$\text{Acidity \%} = \frac{\text{NaOH used} \times 0.0064}{\text{Volume or weight of sample used}} \times 100$$

Ascorbic acid (Vitamin C)

10 ml of freshly squeezed juice was poured in 250 ml measuring flask, 0.4% oxalic acid solution was added up to the mark. After filtration an aliquot about 5ml was taken in flask and titration was carried out using 2, 6-dichlorophenoindophenol dye until light pink color appeared which persisted for only few seconds (24).

Vitamin C content was calculated as:

$$\text{Vitamin C (mg/100ml juice)} = \frac{1 \times R_1 \times V}{R \times W \times V_1} \times 100$$

Where

R1 = dye used in titration of aliquot

R = dye used in titration of standard ascorbic acid solution

V1 = volume of juice used

V = volume of aliquot made by addition of 0.4% oxalic acid

W = volume of aliquot used for titration

Statistical analysis: The results obtained were analyzed by Costat version 6.4 using completely randomized block design and mean values were compared using Duncan's New Multiple Range test at $p \leq 0.05$ with five replicates. The mean square error of replicates from mean value was also calculated.

Results

Sensory evaluation: In the present study Kinnow fruit were irradiated at different doses (0.25-2.0 kGy). Radiations had no adverse effect on texture of Kinnow, except at higher doses (Table 1). No difference in color was observed between radiated and control fruits. Visual defects were only found in control and irradiated fruits at higher doses. No rind disorder was seen up to 1.5 kGy. Changes in color, texture and appearance was observed at 2.0 kGy. When irradiated at 2.0 kGy pitting was observed at the 14th day of storage. The firmness also reduced at 2.0kGy. In citrus fruits irradiation at higher doses causes peel injury the intensity of which is directly proportional to the radiation dose applied.

Microbial analysis: In present study Kinnow samples were exposed to gamma radiation doses of 0.25-2.0 kGy and effect of these radiation doses was evaluated on total viable, coliform and fungal count along with storage of refrigerated and ambient temperature (Table 2a and b, 3 and b, 4a and b, 5a and b). On refrigerated temperature microbial flora on Kinnow decreased at all applied doses. The control and irradiated samples decayed completely till 28th day except samples treated at 1.5 kGy. However, reduction in microflora was observed at 1.5 kGy. Microflora on fruit surface of untreated samples increased as the storage time increased when stored at both ambient and refrigerated temperature. Bacterial count was minimized in all irradiated samples as compared to control samples maximum reduction was observed at a radiation dose of 1.5 in Kinnow. In the current study irradiation also reduced total fungal count at all applied doses. The percentage mold development was less in irradiated samples of citrus fruits as compared to untreated samples in both samples kept at ambient and refrigerated temperature. Irradiation up to 2kGy could not completely inhibit the growth of yeast and mold during the entire storage period; however it was still lower than fungal count on control samples.

Analysis of Physicochemical properties

Impact of gamma radiations on physiological weight loss in Kinnow: Figure 1a and b depicted that weight of control and irradiated samples decreased as the storage time increased. Weight loss increased in irradiated samples than control samples. More weight loss was observed at higher doses.

Influence of gamma radiations on moisture content of Kinnow: In the present study effect of gamma radiations on the moisture contents of Kinnow (Fig. 2a and b) stored at refrigerated and ambient temperature was evaluated. The moisture content of control sample decreased as the storage time increased at

Table 1. Impact of gamma radiations on sensory properties of Kinnow

Radiation Doses (kGy)	**Storage period (Days)**				
	1	7	14	21	28
Texture					
Control	Smooth	Smooth	Smooth	Smooth	Decay
0.25	Smooth	Smooth	Smooth	Smooth	Decay
0.50	Smooth	Smooth	Smooth	Smooth	Decay
0.75	Smooth	Smooth	Smooth	Smooth	Decay
1.0	Smooth	Smooth	Smooth	Smooth	Decay
1.5	Smooth	Smooth	Smooth	Smooth	Smooth
2.0	Smooth	Rough	Rough	Rough	Decay
Color					
Control	Dark orange	No change	No change	No change	Decay
0.25	No change	No change	No change	No change	Decay
0.50	No change	No change	No change	No change	Decay
0.75	No change	No change	No change	No change	Decay
1.0	No change	No change	No change	No change	Decay
1.5	No change	No change	No change	No change	No change
2.0	No change	No change	No change	No change	Decay
Visual Defects					
Control	No defect	Light Scurfs	Light scurfs, shrunk	Dark scurfs, shrunk	Decay
0.25	No defect	Light silver scurfs	Light scurfs, shrunk	Dark scurfs, shrunk	Decay
0.50	No defect	Light silver scurfs	light scurfs, shrunk	Dark scurfs, shrunk	Decay
0.75	No defect	Light silver scurfs	Light scurfs, Shrunk	Light scurfs, shrunk	Decay
1.0	No defect	Light silver Scurfs	Light silver scurfs	Light scurfs, pitting	Decay
1.5	No defect	Light silver scurfs	Light silver scurfs	Light scurfs	Light silver scurfs
2.0	No defect	Light silver scurfs	Pitting, shrunk	Pitting, shrunk	Decay

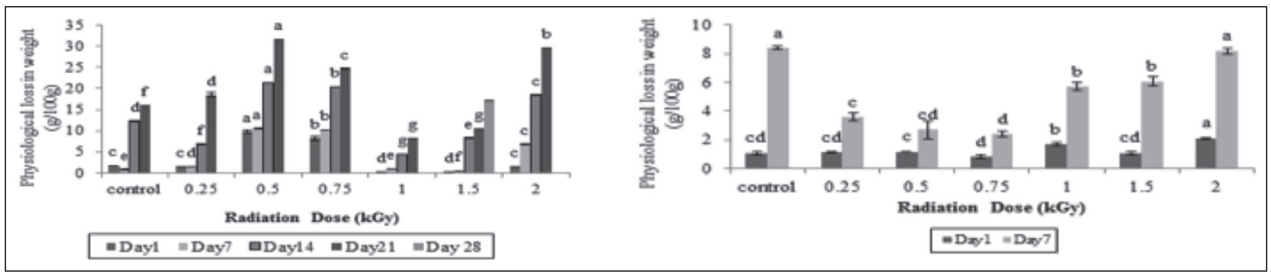

Figure 1. a) Effect of gamma radiations on physiological loss in weight of Kinnow stored at refrigerated temperature; b) Effect of gamma radiations on physiological loss in weight of Kinnow stored at ambient temperature

Table 2a. Effect of gamma radiations on total viable count of bacteria on Kinnow kept at refrigerated temperature using nutrient agar

Radiation Doses (kGy)	Nutrient agar (cfu/ml)				
	Storage Period (Days)				
	1	7	14	21	28
Control	$2.75\times10^{5}\pm0.022^{a}$	$2.82\times10^{5}\pm0.016^{a}$	$2.90\times10^{5}\pm0.024^{b}$	$2.96\times10^{5}\pm0.030^{c}$	Decayed
0.25	$2.63\times10^{5}\pm0.016^{b}$	$2.60\times10^{5}\pm0.018^{b}$	$1.59\times10^{5}\pm0.022^{d}$	$1.20\times10^{5}\pm0.025^{d}$	Decayed
0.50	$2.41\times10^{5}\pm0.016^{c}$	$2.39\times10^{5}\pm0.032^{c}$	$1.52\times10^{5}\pm0.030^{de}$	$1.12\times10^{5}\pm0.018^{d}$	Decayed
0.75	$2.04\times10^{5}\pm0.030^{d}$	$2.05\times10^{5}\pm0.028^{d}$	$1.98\times10^{5}\pm0.028^{c}$	$1.15\times10^{5}\pm0.024^{d}$	Decayed
1.0	$1.85\times10^{5}\pm0.020^{e}$	$1.97\times10^{5}\pm0.014^{e}$	$1.09\times10^{5}\pm0.023^{f}$	$1.05\times10^{5}\pm0.012^{d}$	Decayed
1.5	$1.65\times10^{5}\pm0.041^{f}$	$1.83\times10^{5}\pm0.018^{f}$	$1.43\times10^{5}\pm0.020^{e}$	$9.80\times10^{4}\pm0.282^{a}$	$1.60\times10^{4}\pm0.203$
2.0	$1.3\times10^{5}\pm0.023^{g}$	$1.62\times10^{5}\pm0.015^{g}$	$7.20\times10^{4}\pm0.089^{a}$	$6.3\times10^{4}\pm0.189^{b}$	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates.
Different superscript in the same column indicates that the mean difference is significant at p≤0.05.

Table 2b. Effect of gamma radiations on total viable count of bacteria on Kinnow kept at ambient temperature using nutrient agar

Radiation Doses (kGy)	Nutrient agar (cfu/ml)				
	Storage Period (Days)				
	1	7	14	21	28
Control	$2.91\times10^{5}\pm0.069^{a}$	$3.00\times10^{5}\pm0.325^{a}$	Decayed	Decayed	Decayed
0.25	$2.68\times10^{5}\pm0.078^{b}$	$2.78\times10^{5}\pm0.056^{a}$	Decayed	Decayed	Decayed
0.50	$2.52\times10^{5}\pm0.051^{c}$	$2.38\times10^{5}\pm0.038^{b}$	Decayed	Decayed	Decayed
0.75	$2.48\times10^{5}\pm0.039^{c}$	$2.39\times10^{5}\pm0.052^{b}$	Decayed	Decayed	Decayed
1.0	$2.28\times10^{5}\pm0.032^{d}$	$1.88\times10^{5}\pm0.028^{c}$	Decayed	Decayed	Decayed
1.5	$1.96\times10^{5}\pm0.032^{c}$	$1.89\times10^{5}\pm0.031^{c}$	Decayed	Decayed	Decayed
2.0	$1.64\times10^{5}\pm0.028^{f}$	$1.59\times10^{5}\pm0.041^{c}$	Decayed	Decayed	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts indicate that the mean difference is significant at p≤0.05.

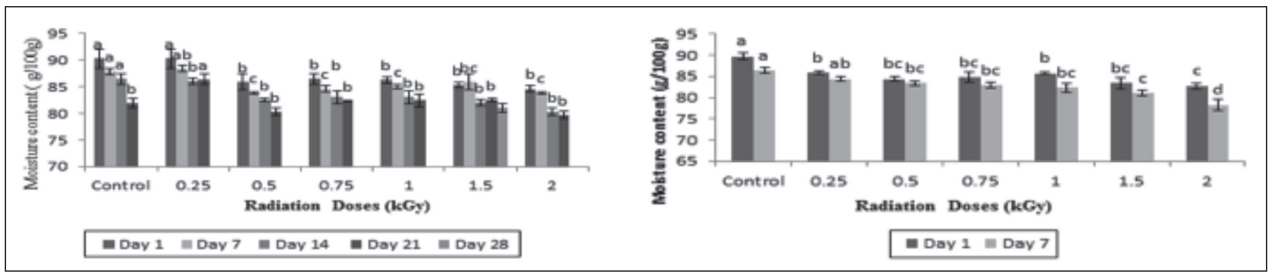

Figure 2. a) Impact of gamma radiations on moisture content of Kinnow stored at refrigerated temperature; b) Impact of gamma radiations on moisture content of Kinnow stored at ambient temperature

Table 3a. Effect of gamma radiations on total coliform count of Kinnow kept at refrigerated temperature using MacConkey agar

Radiation Doses (kGy)	MacConkey agar (cfu/ml)				
	Storage period (Days)				
	1	7	14	21	28
Control	$2.75\times10^{5}\pm0.022^{a}$	$2.59\times10^{5}\pm0.030^{a}$	$2.38\times10^{5}\pm0.034^{a}$	$2.37\times10^{5}\pm0.028^{a}$	Decayed
0.25	$2.37\times10^{5}\pm0.020^{b}$	$2.28\times10^{5}\pm0.022^{b}$	$1.58\times10^{5}\pm0.024^{b}$	$1.94\times10^{5}\pm0.020^{b}$	Decayed
0.50	$2.20\times10^{5}\pm0.015^{c}$	$1.32\times10^{5}\pm0.016^{d}$	$1.13\times10^{5}\pm0.016^{c}$	$1.39\times10^{5}\pm0.017^{c}$	Decayed
0.75	$1.75\times10^{5}\pm0.026^{d}$	$1.68\times10^{5}\pm0.034^{c}$	$1.09\times10^{5}\pm0.016^{c}$	$1.15\times10^{5}\pm0.015^{d}$	Decayed
1.0	$1.50\times10^{5}\pm0.023^{e}$	$9.90\times10^{4}\pm0.340^{e}$	$8.70\times10^{4}\pm0.357^{d}$	$3.90\times10^{4}\pm0.252^{e}$	Decayed
1.5	$1.50\times10^{5}\pm0.012^{e}$	$9.30\times10^{4}\pm0.291^{e}$	$6.40\times10^{4}\pm0.203^{e}$	$2.10\times10^{4}\pm0.089^{e}$	$9.00\times10^{3}\pm0.894$
2.0	$5.00\times10^{4}\pm0.172^{f}$	$4.00\times10^{4}\pm0.266^{f}$	$3.00\times10^{4}\pm0.152^{f}$	$3.50\times10^{4}\pm0.22^{ce}$	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts in the same column indicate that the mean difference is significant at p≤0.05.

Table 3b. Effect of gamma radiations on total coliform count of Kinnow kept at ambient temperature MacConkey agar

Radiation Doses (kGy)	MacConkey agar (cfu/ml)				
	Storage Period (Days)				
	1	7	14	21	28
Control	$2.68\times10^{5}\pm0.046^{a}$	$2.84\times10^{5}\pm0.038^{a}$	Decayed	Decayed	Decayed
0.25	$2.57\times10^{5}\pm0.045^{b}$	$2.60\times10^{5}\pm0.028^{b}$	Decayed	Decayed	Decayed
0.50	$2.43\times10^{5}\pm0.023^{c}$	$2.42\times10^{5}\pm0.037^{c}$	Decayed	Decayed	Decayed
0.75	$2.39\times10^{5}\pm0.022^{c}$	$2.21\times10^{5}\pm0.029^{d}$	Decayed	Decayed	Decayed
1.0	$2.08\times10^{5}\pm0.040^{d}$	$1.98\times10^{5}\pm0.049^{e}$	Decayed	Decayed	Decayed
1.5	$1.78\times10^{5}\pm0.017^{e}$	$1.61\times10^{5}\pm0.037^{f}$	Decayed	Decayed	Decayed
2.0	$1.59\times10^{5}\pm0.049^{f}$	$1.42\times10^{5}\pm0.018^{g}$	Decayed	Decayed	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts indicate that the mean difference is significant at p≤0.05.

both refrigerated and ambient temperature. Moisture content followed the same pattern in irradiated samples. The maximum reduction in moisture content was observed at dose 2.0 kGy. Highest value for moisture content was observed for control as compared to irradiated fruits kept at both ambient and refrigerated temperature. In irradiated samples kept at refrigerated temperature the lowest value of moisture contents obtained was79.67 g/100g at dose 2.0 kGy. Whereas in irradiated samples kept at room temperature the lowest value of moisture content obtained was 78.23 g/100g at 2.0 kGy. Irradiated samples were slightly lower in moisture content than control samples. However, decrease in moisture content was only significant at higher doses showing that by increasing the radiation dose moisture content decreases.

Influence of gamma radiations on ash content of Kinnow

Ash content of control samples of Kinnow was 0.64g/100g at first day of analysis. The ash content of control sample decreased as the storage time increased when kept at refrigerated and room temperature. Ash content followed the same pattern in irradiated sam-

Table 4a. Effect of gamma radiations on bacterial count of Kinnow kept at refrigerated temperature using Salmonella Shigella agar

Radiation Doses (kGy)	**Salmonella Shigella agar (cfu/ml)**				
	Storage period (Days)				
	1	7	14	21	28
Control	$6.9\times10^{4}\pm0.322^{a}$	$5.7\times10^{4}\pm0.200^{a}$	$3.4\times10^{4}\pm0.181^{a}$	$2.1\times10^{4}\pm0.152^{a}$	Decayed
0.25	$6.4\times10^{4}\pm0.141^{a}$	$4.3\times10^{4}\pm0.172^{b}$	$3.2\times10^{4}\pm0.241^{a}$	$2.0\times10^{4}\pm0.205^{a}$	Decayed
0.50	$5.3\times10^{4}\pm0.282^{b}$	$3.7\times10^{4}\pm0.144^{bc}$	$2.9\times10^{4}\pm0.228^{ab}$	$1.8\times10^{4}\pm0.164^{a}$	Decayed
0.75	$4.5\times10^{4}\pm0.278^{b}$	$3.1\times10^{4}\pm0.278^{cd}$	$2.5\times10^{4}\pm0.203^{bc}$	$1.3\times10^{4}\pm0.101^{b}$	Decayed
1.0	No Growth	$2.9\times10^{4}\pm0.200^{d}$	$2.0\times10^{4}\pm0.266^{c}$	$2.0\times10^{3}\pm0.282^{c}$	Decayed
1.5	No Growth	$2.0\times10^{4}\pm0.215^{e}$	$1.3\times10^{4}\pm0.101^{d}$	$1.2\times10^{4}\pm0.101^{b}$	No Growth
2.0	$2.2\times10^{4}\pm0.178^{c}$	$1.8\times10^{4}\pm0.200^{e}$	$1.0\times10^{4}\pm0.063^{d}$	$8.0\times10^{3}\pm0.894^{c}$	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts in the same column indicate that the mean difference is significant at p≤0.05.

Table 4a. Effect of gamma radiations on bacterial count of Kinnow kept at ambient temperature using Salmonella Shigella agar

Radiation Doses (kGy)	**Salmonella Shigella agar (cfu/ml)**				
	Storage Period (Days)				
	1	7	14	21	28
Control	$7.8\times10^{4}\pm0.382^{a}$	$7.9\times10^{4}\pm0.325^{a}$	Decayed	Decayed	Decayed
0.25	$6.7\times10^{4}\pm0.282^{b}$	$5.2\times10^{4}\pm0.272^{b}$	Decayed	Decayed	Decayed
0.50	$6.5\times10^{4}\pm0.272^{b}$	$4.9\times10^{4}\pm0.368^{b}$	Decayed	Decayed	Decayed
0.75	$5.3\times10^{4}\pm0.200^{c}$	$4.6\times10^{4}\pm0.340^{b}$	Decayed	Decayed	Decayed
1.0	$3.1\times10^{4}\pm0.342^{d}$	$2.8\times10^{4}\pm0.230^{c}$	Decayed	Decayed	Decayed
1.5	$2.6\times10^{4}\pm0.291^{d}$	$2.2\times10^{4}\pm0.170^{cd}$	Decayed	Decayed	Decayed
2.0	$2.4\times10^{4}\pm0.282^{d}$	$1.5\times10^{4}\pm0.202^{d}$	Decayed	Decayed	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts in the same column indicate that the mean difference is significant at p≤0.05.

ples. In irradiated samples the lowest value obtained was 0.39 g/100g at dose 2.0 kGy (Fig 3a) whereas at ambient temperature the lowest value obtained was 0.51 g/100g (Fig. 3b) irradiated at 2.0 kGy.

Impact of gamma radiations on juice content of Kinnow

The juice content of samples kept at ambient and refrigerated temperature was decreased as storage time and radiation dose increased. At day one the juice content of non-irradiated Kinnow was 26.70 g/100ml at refrigerated temperature whereas at the end of storage it was 21.23 g/100ml (Fig. 4a). For control samples stored at ambient temperature the juice content at day one was recorded to be 28.13 and 21.98 g/100ml at last (7th day) of storage period. In irradiated-refrigerated samples minimum amount of juice content on 28th day was 15.65 g/100ml at 2.0 kGy. Whereas at 7th day Kinnow irradiated and stored on ambient temperature had juice content of 18.31 g/ml at 2.0 kGy (Fig. 4b). Juice content in irradiated and control fruits decreased throughout the storage period at ambient and refrigerated temperature.

Table 5a. Effect of gamma radiations on fungal count of Kinnow kept at ambient temperature using potato dextrose agar

Radiation Doses (kGy)	**Potato Dextrose agar (cfu/ml)**				
	Storage period (Days)				
	1	7	14	21	28
Control	$1.9\times10^{4}\pm0.360^{a}$	$2.8\times10^{4}\pm0.170^{a}$	Decayed	Decayed	Decayed
0.25	$1.6\times10^{4}\pm0.130^{a}$	$2.6\times10^{4}\pm0.165^{ab}$	Decayed	Decayed	Decayed
0.50	$1.5\times10^{4}\pm0.228^{ab}$	$2.5\times10^{4}\pm0.223^{ab}$	Decayed	Decayed	Decayed
0.75	$1.3\times10^{4}\pm0.170^{abc}$	$2.5\times10^{4}\pm0.228^{ab}$	Decayed	Decayed	Decayed
1.0	$1.6\times10^{4}\pm0.212^{a}$	$2.1\times10^{4}\pm0.212^{b}$	Decayed	Decayed	Decayed
1.5	$9.0\times10^{3}\pm0.141^{bc}$	$1.1\times10^{4}\pm0.070^{c}$	Decayed	Decayed	Decayed
2.0	$8.0\times10^{3}\pm0.200^{c}$	$12.0\times10^{3}\pm0.013^{d}$	Decayed	Decayed	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts in the same column indicate that the mean difference is significant at p≤0.05.

Table 5a. Effect of gamma radiations on fungal count of Kinnow kept at refrigerated temperature using potato dextrose agar

Radiation Doses (kGy)	**Potato Dextrose agar (cfu/ml)**				
	Storage period (Days)				
	1	7	14	21	28
Control	$6.0\times10^{3}\pm0.894^{ab}$	$5.0\times10^{4}\pm1.264^{a}$	$3.1\times10^{4}\pm0.164^{ab}$	$5.3\times10^{4}\pm0.141^{a}$	Decayed
0.25	$5.0\times10^{3}\pm0.894^{bc}$	$2.4\times10^{4}\pm0.215^{b}$	$2.9\times10^{4}\pm0.322^{b}$	$3.8\times10^{4}\pm0.152^{b}$	Decayed
0.50	$2.0\times10^{3}\pm0.400^{c}$	$2.5\times10^{4}\pm0.203^{b}$	$3.0\times10^{4}\pm0.116^{ab}$	$3.2\times10^{4}\pm0.178^{b}$	Decayed
0.75	$4.0\times10^{3}\pm0.894^{bc}$	$2.0\times10^{4}\pm0.291^{b}$	$3.7\times10^{4}\pm0.189^{a}$	$3.8\times10^{4}\pm0.233^{b}$	Decayed
1.0	$7.0\times10^{3}\pm1.264^{ab}$	$1.8\times10^{4}\pm0.203^{b}$	$3.4\times10^{4}\pm0.268^{ab}$	$3.5\times10^{4}\pm0.291^{b}$	Decayed
1.5	No Growth	$2.7\times10^{4}\pm0.205^{b}$	$1.3\times10^{4}\pm0.101^{c}$	$2.1\times10^{4}\pm0.228^{c}$	$2.0\times10^{4}\pm0.266$
2.0	$9.0\times10^{3}\pm1.166^{a}$	$1.3\times10^{4}\pm0.089^{b}$	$1.8\times10^{4}\pm0.205^{c}$	$2.0\times10^{4}\pm0.189^{c}$	Decayed

Each value is the mean obtained from five parallel replicates. ± indicates standard error among the replicates. Different superscripts indicate that the mean difference is significant at p≤0.05.

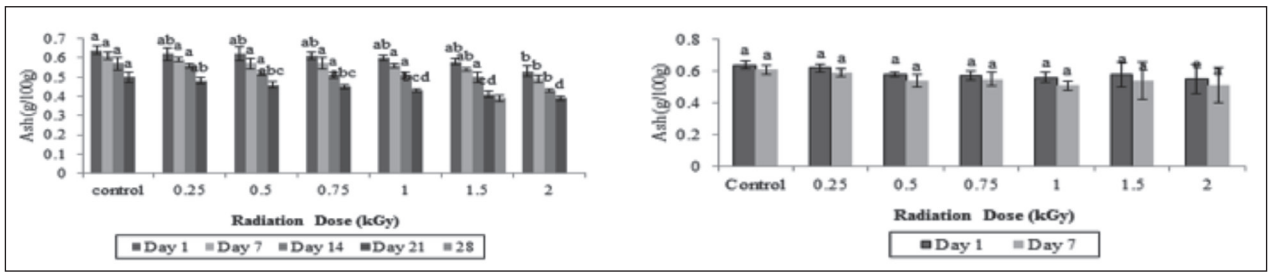

Figure 3. a) Influence of gamma radiations on ash content of Kinnow stored at refrigerated temperature; b) Influence of gamma radiations on ash content of Kinnow stored at ambient temperature

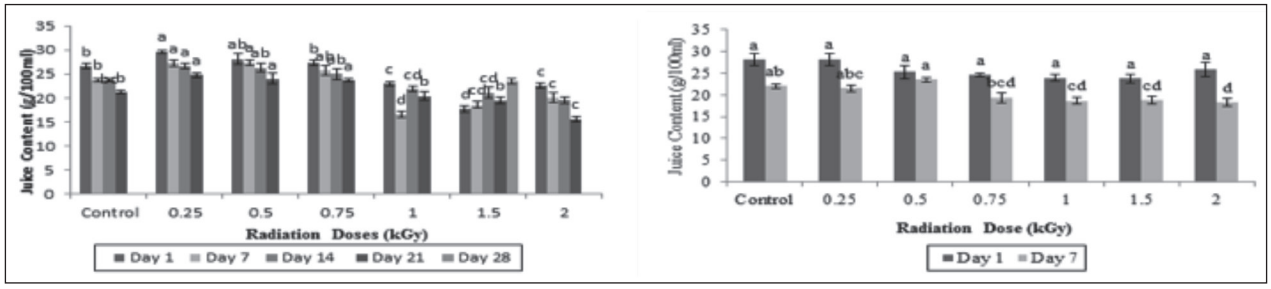

Figure 4. a) Effect of gamma radiations on juice content of Kinnow stored at refrigerated temperature; b) Effect of gamma radiations on juice content of Kinnow stored at ambient temperature

Influence of gamma radiations on total soluble solid (TSS) content of Kinnow

In the present study, effect of gamma radiations on total soluble solid of Kinnow stored at ambient and refrigerated temperature was evaluated (Fig 5a and b). As the storage period of control samples stored at ambient and refrigerator temperature was increased an increase in TSS was observed. Same pattern was followed in irradiated samples at all doses. At 28th day on refrigerated temperature TSS was 14.7Brix° for sample irradiate at 2.0 kGy. Whereas at ambient temperature the maximum value obtained was 13.2 Brix°for sample irradiated at 2.0 kGy.

Influence of gamma radiations on titratable acidity of Kinnow

As the storage period of control and irradiated samples kept at refrigerated and ambient temperature increased a decline in titratable acidity was observed (Fig 6a and b). At day one the titratable acidity of non-irradiated Kinnow was 0.42 g/100ml at refrigerated temperature whereas at 28th day it was 0.30 g/100ml. For control samples stored at ambient temperature the titratable acidity at day one was recorded to be 0.49 g/100ml and 0.39 g/100ml at the end of 28th day respectively. In irradiated refrigerated samples the lowest amount of titratable acidity at the end of storage was

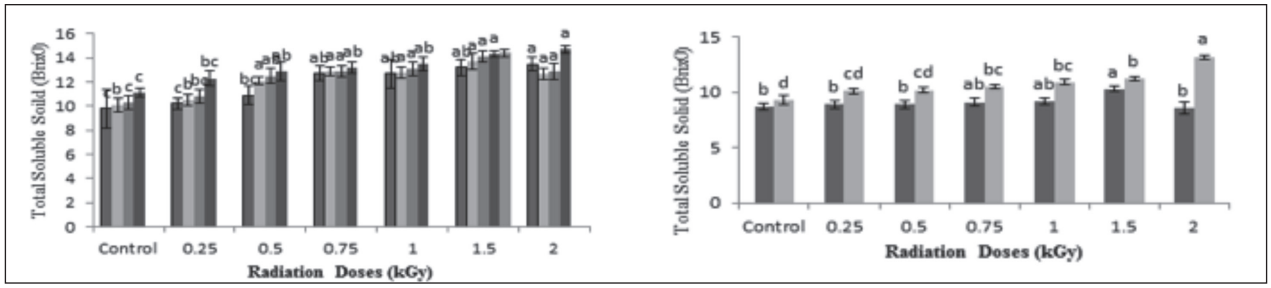

Figure 5. a) influence of gamma radiations on total soluble solid content of Kinnow stored at refrigerated temperature; b) Influence of gamma radiations on total soluble solid content of Kinnow stored at ambient temperature

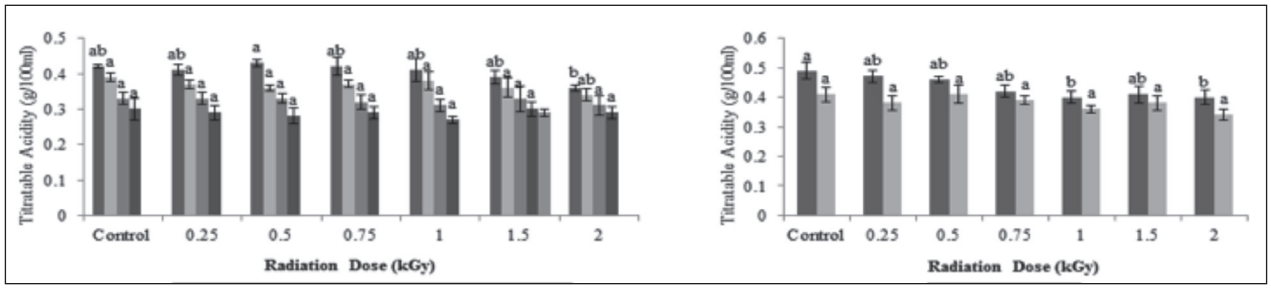

Figure 6. a) Effect of gamma radiations on titratable acidity of Kinnow stored at refrigerated temperature; b) Effect of gamma radiatens on titratable acidity of Kinnow stored at ambient temperature

0.29 g/100ml at 2.0 kGy. Whereas Kinnow irradiated and stored on ambient temperature had lowest titratable acidity of 0.34 g/100ml at 2.0 kGy.

Impact of gamma radiations on ascorbic acid of Kinnow

Decrease in ascorbic acid was observed as the storage time increased among untreated samples at both ambient and refrigerated temperature. Same pattern was followed in irradiated samples at all doses. However, maximum reduction was observed at higher doses. When stored under refrigerated temperature minimum ascorbic acid content was 26.8 mg/100ml at dose of 2.0 kGy (Fig.7 a). However, when stored at room temperature minimum ascorbic acid content was 33.9 mg/100ml at dose of 2.0kGy (Fig.7b).

Discussion

Exposing food to radiation treatment delays spoilage and enhance the safety of produce by eliminating or decreasing pathogenic microorganisms (25). In the present study Kinnows were irradiated at different doses (0.25-2.0 kGy). Radiations had no adverse effect on texture of Kinnow except at higher doses. Changes in color, texture and appearance was observed at 2.0 kGy. The change in color might be due to the decrease concentration of carotenoids in the peel (26). In citrus fruits irradiation at higher doses causes peel injury. This was probably due to the accumulation of substantial amounts of phenolic compounds in cells following irradiation which then lead to peel damage and cell necrosis (27). In this study no pre-treatment was given to fruits so the surface of untreated fruits was found to be highly contaminated with epiphytic microbial flora. This might be due to poor hygienic conditions in the local market and during transport, similar findings were observed by Gultie and Sahile (28). The predominant pathogens isolated from the surface of citrus fruits were *Staphylococcus aureus*, *Escherechia coli*, *pseudomonas aeroginosa* and *Shigella* sp. Presence of these coliforms on fruit surface indicated fecal contamination of fruit which can cause serious illness such as food poisoning and diarrhea so fresh fruit and vegetables can serve as a vehicle for the organism most likely to cause outbreaks. Gamma radiation worked remarkably to reduce the bacterial load. Bacterial count was minimized in all irradiated samples as compared to control samples and maximum reduction was observed at a radiation dose of 1.5 in Kinnow, perhaps it was due the reason that radiation breaks the bonds in the DNA molecules, of these microbes and cause defects in their genetic instructions or due to the biosynthesis of phenolic compounds following radiation treatment that extend the storage life of fruit and in some cases induce resistance against pathogens (29-30).

In the current study irradiation also reduced total fungal count at all applied doses. The percentage mold development was less in irradiated samples of citrus fruits as compared to untreated samples in both samples kept at ambient and refrigerated temperature. On the basis of macroscopic and microscopic analysis the dominant fungi identified on Kinnows samples were *Aspergillus flavus, Aspergillus niger, Alternaria Alternata, Penicillium*, *Fusarium, Mucor* and Yeast. Irradiation up to 2kGy could not completely inhibit the growth of yeast and mold during the entire storage period; however it was still lower than fungal count on control samples. It might be due to the reason that somewhat higher doses are required to have a fungicidal effect on these citrus damaging fungi (22-31).

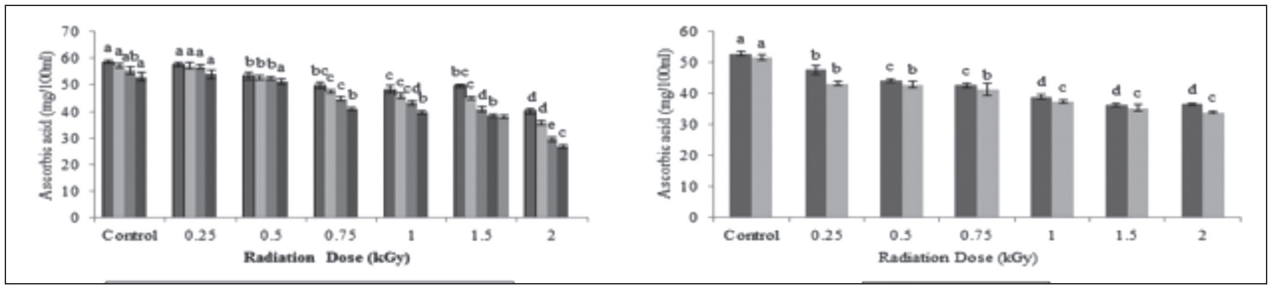

Figure 7. a) Effect of gamma radiations on scorbutic acid content of Kinnow at refrigerated temperature; b) Effect of gamma radiations on scorbutic acid content of minnow stored at ambient temperature

In the present study evaluation of physicochemical parameters of Kinnows stored at both ambient and refrigerated temperature showed that irradiation only at higher doses affect the nutritional aspects of fruit. Irradiation could not control the senescence of fruit, but along with refrigerated temperature, it caused the maximum retention of fruit quality attributes as it was also evident by the study made by Selles (32) and Srijaya*et al.* (22) who stated that irradiation at low doses along with refrigerated temperature did not cause any significant change in fruit quality characteristics. During the present work it was observed that weight of control and irradiated samples decreased as the storage time increased. More weight loss was observed in irradiated sample of Kinnow as compared to control samples with the passage of time. .Weight loss increased in irradiated samples than control samples of Kinnow. A more weight loss was observed at higher doses. Our result was in accordance to the result of Miller *et al.* (33). Decrease in fruit weight with the progression of storage period is also a natural phenomenon which might be due to transpiration occurring from fruit surface (34).

The moisture content of control and irradiated Kinnow was decreased as the storage time increased. Irradiated samples were slightly lower in moisture content than control samples. However, decrease in moisture content was only significant at higher doses showing that by increasing radiation dose moisture content decreases. These results were also similar to the results of Hajare *et al.* (35) who reported that increasing gamma radiation dose caused an increase in moisture loss of fruit. This was probably due to the ability of gamma radiation to target water molecules and breaking of hydrogen bonds, which ultimately lead to low moisture content in radiated fruits. Since high radiation dose caused increase moisture loss so the consequences were shrinkage and softening of the fruit at 2.0 kGy. The ash content is the amount of total mineral present in food. In this study the effect of gamma radiation on ash content showed that the ash content decreased following irradiation and ripening period of fruit at both refrigerated and ambient temperatures. Irradiated fruits had low ash content as compared to control. However, the effect was significant only at higher doses. The reason might be that the decrease was due the conversion of minerals into toxic substances with the time (36). Juice content in irradiated and control fruits decreased throughout the storage period at ambient and refrigerated temperature. This might be due to the reason that juice content increased towards maturity and then decreased once the maturity was over (18).

Total soluble solids content is an important parameter of fruit quality. In fruits total soluble salt consist of about 75-80% sucrose. In this study total soluble solids increased with the increase of storage period as well as radiation dose. Total soluble solids in irradiated samples were higher than control samples at both ambient and refrigerated temperature. Since, Total soluble solids consist of total dissolved solids and moisture content of the fruit, so the rise in TSS content may be due to low moisture content of the fruit (37).The effect of gamma radiations on Kinnow showed that increasing radiation dose caused decrease in titratable acidity of fruit at both ambient and refrigerated temperature. This reduction was also observed in control samples as the storage time increased. These observations were in line with observations made by Ahmad *et al.* (38) who stated decrease in titratable acidity of fruit occur with increasing radiation dose and storage period. The decrease in titratable acidity was might be due to the utilization of these constituent acids in the fruit respiratory process (34).Ascorbic acid content is an important constituent of citrus fruits. In the present study ascorbic acid content in Kinnow decreased with the increase in storage temperature and radiation dose. Same pattern was observed in control samples. These findings were similar to the findings made by Ahmad *et al.* (38) who reported reduction of ascorbic acid in oranges with the increase of storage temperature and radiation dose. The decrease in ascorbic acid content also might be due to the respiratory activity of fruit (39).

Conclusions

It was concluded that Kinnow irradiated at 1.5 kGy had no deleterious effects on quality and nutritional characteristics of fruit up to the storage period of 28 days along with refrigerated temperature. Gamma irradiation helped to improve the shelf life of Kinnow by 1week

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