

Increasing Shelf Life of Pakistani Peaches Using Gamma Irradiation to Overcome Quarantine Barriers in Fruit Exports From Pakistan

Mehwish Iqtedar^{1*}, Uzma Sarwar¹, Roheena Abdullah¹,
Afshan Kaleem¹, Mahwish Aftab¹, Shagufta Naz², Fakhar-un Nisa²,
Saima Sharif², Saima Aftab³ and Shagufta Naz¹

¹Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan.

²Department of Zoology, Lahore College for Women University, Lahore, Pakistan.

³Department of Environmental Science, Lahore College for Women University, Lahore, Pakistan.

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The present study was carried out to determine the effect of gamma irradiation on the microbiological and organoleptic properties of peaches to improve the quality and increase in shelf-life of indigenous fruit variety of Pakistani peaches. The matured peaches (variety no. 4) were collected from local market of Lahore and samples were irradiated with Gamma radiation doses of 0.25, 0.5, and 0.75kGy. Study period comprised of three weeks during which samples were kept at refrigerated temperature (4°C). Microbial evaluation of peaches revealed the presence of diverse aerobic mesophilic bacteria, yeast and molds. The microbial count decreased significantly with increasing dose level. *Salmonella sonnie* were detected only in control samples. Peaches exposed to 0.75kGy dose showed highest reduction in bacterial count i.e. upto 59.8% on nutrient agar whereas fungal load was reduced up to 77.4% on PDA. Gamma-irradiation dose of 0.75kGy was found to be more effective in reducing microbial load. Sensory evaluation of the fruits revealed that gamma irradiation preserved the texture and appearance of fruit and also delayed the fruit decay by 7 days. Hence, gamma irradiations can be used as an effective treatment for improving the fruit quality and increasing marketable shelf life of peaches.

Key words: Gamma irradiation, microbial load on Peaches, Quarantine barriers, food borne pathogens on peaches.

Peach (*Prunus persica*) is one of the important stone fruits. Peaches belong to plant family *Rosaceae* and sub-family *Amygdaloidae*.¹ They are an excellent source of Vitamin A, B and C. Peaches are comprised of 10-14 % sugars and 2 % proteins. ²More than 42 varieties of peach are grown in Pakistan. ³China is the largest producer of peaches with annual production of 12,027,600 tons while production in Pakistan was 56,000 tons in 2012. Although Pakistan yields (mostly from

Khabar Pakhtun Khuwan KPK) a large amount of fruit and ranks 25th in world.² The reason for this low export is the post-harvest losses due to substandard handling and storage procedures that effect low shelf life of fruits⁴.

Only 2 % peaches were exported to international market in 2011-12. On average, post-harvest loss ranges from 18%-31% of which 77% loss occurs in peach picking stage while 23% during transportation. ⁵More precise studies showed a post-harvest loss of 31 %, 22%, 24%, 18% and 22% in Variety 4, Variety 5, Variety 6, Variety 7 and Variety 8, respectively.

Peach fruit is difficult to store for a long period of

* To whom all correspondence should be addressed.
E-mail: miqtedar@gmail.com

time due to microbial growth and the hot and humid climatic conditions found in KPK. ⁶For the export of peach to the global markets, it is required to have greater storage life and should be protected from the pathogens for a long period of time. ⁷Microbiological analysis of peaches revealed the presence of *Monilinia fructicola*, *Penicillium expansum*, *Mucor piriformis*, *Botrytis cinerea*, *Geotrichum candidum*, *Alternaria alternata*, and *Rhizopus stolonifer* on fruit surface. ⁸Bacterial pathogens of peaches are found to be member of *Microbacteriaceae*, *Enterobacteriaceae*, *Rhodobacteriaceae*, *Flavobacteriaceae*, *Oxalobacteriaceae* and *Bacillaceae* families.

To meet the World Trade Organization (WTO) and quarantine regulations, pest and pathogen-free horticultural products are essentially required. ⁹Minimum requirement for peach export according to is that it must be practically free from pests, free from damages caused by pathogens. Gamma radiations, although being highly penetrative are considered safe are an effective option for sterilization of food. ¹⁰These irradiations kills the disease causing pathogens, without effecting the quality of fruit thus enhances the shelf life and can helps to overcome quarantine barriers for the peach export from Pakistan. ¹¹Hazard Analysis and Critical Control Points (HACCP) also declared food irradiation as safe to use. The present study was conducted with the aim of determining the effect of gamma irradiation on microbiological and organoleptic properties of Pakistani peaches.

MATERIALS AND METHODS

Sample collection

Peaches (Variety No.4) were collected from local market in Lahore and were divided into four random groups (one control group and three groups were specified for a particular dose). Weight of each group was determined. The samples were then packed in the polythene bags.

Irradiation

Each polythene bag was labeled with the different gamma dose (0.25kGy, 0.5kGy and 0.75kGy) and sent to PARAS(Pakistan Radiation Services) for irradiation.

Microbiological analysis

Samples for the microbiological tests were processed under sterilized conditions. Each sample

was rinsed in 100ml of sterilized distilled water in 100 ml capacity sterilized beaker. ¹²The sample was constantly shaken for 5 minutes and then serial dilutions were prepared according to ISO standard 7218:2007 recommendations. Aliquots of 0.1ml from each dilution were spread onto prepared media plates.

Bacteriological analysis

Total microbial load on irradiated and un-irradiated samples were carried out weekly for 3 weeks. ¹³For the enumeration of bacteria, three growth media were selected. Nutrient agar was used for non-fastidious bacterial load analysis, MacConkey agar a deferential media was used for the isolation of Gram-negative enteric bacteria whereas, Salmonella-Shigella Agar was used for *Salmonella* spp. and *Shigella* spp. Enumeration.

Yeast and Mold count

Yeast¹³ and molds associated with peaches were enumerated using Potato dextrose agar and prepared according to manufacturer's instruction.

Identification of bacterial isolates

Bacterial isolates were pure cultured through streaking on respective media. Grams staining and endospore staining of isolates were performed for determination of cell morphology. ¹⁴Gram negative bacterial isolates were identified on biochemical basis using API 20 E strips (bioMerieux, Inc.) as described in Bergey's manual of determinative bacteriology.

Identification of Molds

Molds were identified from the macroscopic morphology of the colony grown on PDA plates and the microscopic morphology of the fungal isolates using methylene blue staining. ¹⁵Yeast isolates were observed by simple slide technique according to their cell morphology and arrangement at 40 and 100x.

Weight loss determination

Percentage¹⁵ weight loss was determined by weighing samples periodically after each week. Weight loss was calculated from initial weight using the formula:

$$\text{Weight loss (\%)} = (W_i - W_s / W_i) \times 100$$

Where,

W_i = initial weight; W_s =weight at sampling period. Decay percentage determination:

¹⁶Determination of decay percentage was done visually from known number of fruits. Any fruit

with fungal growth, extreme softness and brownish appearance was considered as decayed. Decay percentage was calculated as:

$$\text{Decay percentage} = (\text{No of decayed fruits} / \text{Total number of fruits}) \times 100$$

Sensory Evaluation of Peaches

Sensory evaluation was conducted on irradiated and control samples. ¹⁷Quality attributes, including texture, color, microbial infestation and overall acceptability was evaluated.

Statistical analysis¹⁸

One way ANOVA was used for statistical analysis of data using Co-stat 6.4 program.

RESULTS AND DISCUSSION

Total viable count

Ten different bacterial colonies representing different species appeared on the nutrient agar during three weeks analysis. Most of them were Gram negative and were rod shaped and non-endospore former. Effect of gamma irradiation on total viable count of irradiated (0.25, 0.5, 0.75 kGy) and unirradiated samples is shown in Table 1. Keeping in view the viable count obtained for irradiated (0.25, 0.5, 0.75kGy) and control samples during storage for three weeks at

Table 1. Impact of gamma irradiation on total bacterial, Gram negative Enterobacteriaceae, Salmonella-Shigella, Fungal count and percent decay of control and irradiated peaches kept at 4°C for three weeks

Gamma irradiation (kGy)	Total viable count (cfu/ml) Period of Analysis (weeks)		
	Week 1	Week 2	Week 3
Control	3.05×10 ⁵ ±1.01 ^{ab}	3.10×10 ⁵ ±0.63 ^{aA}	3.10×10 ⁵ ±0.63 ^{aA}
0.25	1.91×10 ⁵ ±0.66 ^{bc}	3.0×10 ⁵ ±0.89 ^{bb}	3.07×10 ⁵ ±0.63 ^{bA}
0.5	8.4×10 ⁴ ±0.63 ^{cC}	2.77×10 ⁵ ±0.63 ^{cB}	3.0×10 ⁵ ±0.89 ^{cA}
0.75	4.5×10 ⁴ ±0.63 ^{dC}	1.91×10 ⁵ ±0.66 ^{dB}	2.84×10 ⁵ ±0.63 ^{dA}
Gram negative Enterobacteriaceae(cfu/ml)			
Control	1.8×10 ⁴ ±0.63 ^{aC}	2.63×10 ⁵ ±0.89 ^{ab}	3.16×10 ⁵ ±0.63 ^{aA}
0.25	6.0×10 ³ ±0.63 ^{bB}	1.0 ⁴ ±0.28 ^{bC}	1.97×10 ⁵ ±1.01 ^{bA}
0.5	ND ^{cB}	ND ^{bB}	9.7×10 ⁴ ±0.89 ^{cA}
0.75	ND ^{cB}	ND ^{bB}	7.0×10 ³ ±0.63 ^{dA}
Salmonella-Shigella count (cfu/ml)			
Control	ND ^B	2.67×10 ⁵ ±0.89 ^{ab}	2.98×10 ⁵ ±0.63 ^{aA}
0.25	ND ^B	1.0 ⁴ ±0.28 ^{bB}	2.50×10 ⁵ ±1.01 ^{bA}
0.5	ND ^B	ND ^{bB}	1.21×10 ⁴ ±0.89 ^{cA}
0.75	ND ^B	ND ^{bB}	7.0×10 ³ ±0.63 ^{dA}
Fungal count (cfu/ml)			
Control	2.99×10 ⁵ ±0.89 ^{ab}	3.0×10 ⁵ ±0.63 ^{ab}	3.12×10 ⁵ ±0.89 ^{aA}
0.25	1.4×10 ⁴ ±0.63 ^{bC}	1.26 ⁴ ±0.28 ^{bB}	1.37×10 ⁵ ±0.89 ^{bA}
0.5	9.0×10 ³ ±1.01 ^{cC}	6.1×10 ⁴ ±0.63 ^{cB}	9.0×10 ⁴ ±0.89 ^{cA}
0.75	5.0×10 ³ ±0.63 ^{dC}	2.1×10 ⁴ ±1.26 ^{dB}	7.5×10 ⁴ ±0.63 ^{dA}
Decay percentage (%)			
Control	16±0.63 ^{aC}	50±0.63 ^{a,B}	FD ^{a,A}
0.25	ND ^{b,C}	66±1.01 ^{b,B}	83±0.89 ^{bA}
0.5	ND ^{b,C}	33±0.63 ^{cB}	66±0.28 ^{cA}
0.75	ND ^{b,C}	16±0.84 ^{d,B}	33±0.56 ^{dA}

The results represented as mean ± SEM followed by superscripts that indicate statistical significance within groups at p≤ 0.05, determined by Duncan’s Multiple Range Test. Values with different letters within a column (a-d) and a row (A-C) differ significantly (p<0.05) where ND = Not decayed ; FD = Fully decayed

refrigerated temperature (4°C), it is concluded that peaches irradiated with higher dose (0.75kGy) showed significantly low viable count.¹⁸ It was reported that peaches stored at ambient temperature for 6 days had more bacterial count that was 4.68 logs CFU/g. At dose of 1.5 kGy, the bacterial and yeast count was not detected for a week.

Total gram negative *Enterobacteriaceae* count

The effect of gamma irradiation on the total viable count of irradiated (0.25, 0.5, 0.75 kGy) and unirradiated samples is shown in Table 1. Three Types of bacteria were isolated on the MacConkey agar. *Escherichia coli* and *Pseudomonas spp.* were isolated and identified on peach surface.⁸ A study showed the occurrence of *Enterobacteriaceae* and *Bacillaceae* bacteria on peach fruit.

The data obtained from the microbial analysis of irradiated and control samples during storage for three weeks (4°C) represented that no enteric count (cfu/ml) was observed on irradiated peaches (0.5kGy and 0.75kGy) for the first and the second week while count was detected after analysis of third week. However, count was significantly lowest in irradiated (0.75kGy) peaches.

Total gram negative lactose non-fermenting bacterial count

Two types of bacterial colonies were found on Salmonella-Shigella agar, *Escherichia coli* and *Shigella sonnie* were identified using API strips. Microbial analysis for the third week showed that control samples contain total lactose non-fermenting count of $2.98 \times 10^5 \pm 0.63$ cfu/ml (Table 1). However, it was significantly ($p \leq 0.05$) reduced to $2.50 \times 10^5 \pm 1.01$ cfu/ml for irradiated (0.25kGy) and $1.21 \times 10^5 \pm 0.89$ cfu/ml (0.5kGy) dose. Lactose non-fermenting bacterial count was significantly lowest ($7.0 \times 10^3 \pm 0.63$ cfu/ml) of treated sample (0.75kGy).

Total yeast and mold count

Most of the colonies were smaller in size and white in color. Colorful colonies isolated on potato dextrose agar were yellow, pink and off-white in color. *Aspergillus niger*, *Alternaria spp.*, *Penicillium expansum* and *Rhizopus spp.* were identified on the basis of microscopic and macroscopic observation.⁷ These identifications were in accordance with (Palou *et al.*, 2009) who isolated revealed the presence of *Penicillium expansum*, *Alternaria alternata*, and *Rhizopus stolonifer* on fruit surface. After three weeks, count

Table 2. Impact of gamma irradiation on organoleptic properties of control and irradiated (0.25kGy, 0.5kGy and 0.75kGy) peaches kept at 4°C for three weeks

Organoleptic Gamma irradiation (kGy)	Evaluation											
	Week 1			Week 2			Week 3					
	Texture	Color	Spots	Microbial growth	Texture	Color	Spots	Microbial growth	Texture	Color	Spots	Microbial growth
Control	Too firm	Yellow	No	No	Firm	Reddish yellow	Yes	No	Too soft	Brown	Yes	Yes
0.25	Firm	Yellow	No	No	Less firm	Reddish	Yes	No	Too soft	Brown	Yes	Yes
0.5	Firm	Yellow	No	No	Firm	Reddish yellow	yes	no	Soft	Reddish brown	Yes	No
0.75	Firm	Yellow	No	No	Firm	yellow	no	no	Slightly Firm	Reddish yellow	Yes	No

was significantly ($p \leq 0.05$) lowest ($7.5 \times 10^4 \pm 0.63$ cfu/ml) in treated (0.75kGy) samples (Table 1). These results were close to the observations determined on peaches during storage for 6 days at $20 \pm 3^\circ\text{C}$ to inactivate the fungal pathogens. ¹⁸Decimal reduction doses were proved to be 0.4 -0.6 kGy for fungi.

Percentage decay assessment

The effect of gamma irradiation on the decay (%) of irradiated (0.25, 0.5, 0.75 kGy) and unirradiated samples is given in (Table 1). It is concluded that peaches irradiated with higher dose (0.75kGy) showed 33 % less decay as compared to the control sample, which got completely decayed after third week. ¹⁵Effect of gamma irradiation on the percentage decay of the peaches shows percent decay is delayed in the irradiated samples. Minimum weight loss was observed in peaches irradiated with 1.2kGy dose.

Percentage weight loss assessments

Weight loss evaluation for third week showed that weight loss in control sample was $10 \pm 0.63\%$. However, it was calculated to be $7.87 \pm 0.84\%$ for irradiated (0.25kGy) samples. For irradiated samples (0.5kGy) sample, it was evaluated to be almost half ($5.58 \pm 0.56\%$) as observed for control sample (10 ± 0.63). Significant difference was seen in weight loss ($3.11 \pm 1.01\%$) of peaches irradiated with 0.75 kGy dose (Table 1). Present studies done with gamma irradiation treatment revealed the fact that irradiation processing is highly useful in retarding the decay (%) and inhibiting the microbial load, thereby increasing the shelf life of the peaches. Decay (%) reduced to a level of 33 % as compared to the control sample, which was fully decayed in the third week. So, shelf life of peaches was increased up to a period of 7 days. ¹⁶Significant increase in shelf life of fruit due to gamma radiation has been reported from other studies too.

Sensory evaluation

Organoleptic evaluation after third week showed that control samples as well as irradiated (0.25kGy) samples were too much soft. The color changed from yellow to brown. However, control samples showed more microbial growth than peaches irradiated with 0.25kGy dose. It was observed that un-radiated samples were better in texture than irradiated (0.25kGy) samples of two weeks; while similar observations were obtained

after three weeks (Table 2). Irradiated (0.5kGy) samples were less soft than control sample and browning was less prominent in irradiated (0.5kGy) peaches. Control samples showed clear microbial growth while no microbial growth was observed in irradiated with 0.5kGy dose while no such growth was seen on peaches irradiated with 0.75kGy dose. Also the irradiated (0.75kGy) samples were firm in texture. However color was slightly reddish for peaches irradiated with 0.75kGy dose. ¹⁵It was reported by that fruit firmness was preserved in irradiated samples for up to two weeks while control samples had gone soft by then.

CONCLUSION

The study showed that gamma irradiation treatment is significantly ($p \leq 0.05$) effective in controlling the bacterial as well as yeast and mold count providing low storage temperature (4°C) conditions. Gamma dose of 0.75 kGy was considered optimum as microbial load was reduced to acceptable level while the texture and quality of fruit was not affected. Furthermore, at this dose, shelf life extension up to a period of 7 days was observed. Keeping in view the effectiveness of the process, it can be said that gamma irradiation of fruit is a safe method and ensures increase in shelf life that can eventually effect the export in a positive way.

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