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Effect of ^{60}Co gamma radiation on microbial flora and shelf life of *Malus domestica* Borkh

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Abstract

Apples are known worldwide for their pleasant taste. Apples, being perishable and susceptible to microbial spoilage, have a short shelf-life. The current study was carried out to investigate the effect of gamma irradiation treatment for maintaining storage quality and extending the shelf life of apples. Mature Kala kulu apples were collected from local fruit market of Lahore and packed in zip-locked bags. Apples were irradiated with dose range of 1, 1.5 and 2.0 kGy under refrigerated (4°C) conditions. Microbiological evaluations were carried out on 7th, 14th and 21st day of experiment. Sensory evaluations were done after every 7th day and data obtained from irradiated apples was compared with the observations obtained from un-radiated (control) samples. Studies revealed that total bacterial, yeast and mold counts significantly ($p \leq 0.05$) decreased in irradiated apples stored at 4°C. Maximum reduction of pathogenic bacteria and fungi at 2 kGy was 74% and 77%, respectively. Sensory evaluation revealed that irradiation treatment of 1kGy significantly maintained the storage quality of the fruit. While with 1.5 and 2 kGy dose treatment, fruit showed reduced firmness early during storage. The dose of 1kGy, proved most effective in enhancing the shelf life of apples by maintaining the fruit quality and hence improving the shelf life. Conclusively, irradiation has positive influence on decreasing microbial flora and increasing shelf life of apples, however appropriate dose must be applied. In the present study our results revealed that there is a great potential for the use of radiation in extending the storage life of apples.

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Introduction

Apple (*Malus domestica),* a premier table fruit of the world, is highly nutritious and delicious (Sabir *et al.*, 2004). Common varieties of apple in Pakistan are Red delicious, Golden Delicious, Kala kulu, Amri, Kashmiri and Kaja (Mukhtar *et al.*, 2010). Pakistan is 20th largest producer of apples but ranks at 57th position in apple exporting countries (FAOSTAT, 2012).Spoilage bacteria, molds and yeasts, dominate the micro flora on raw fruits and vegetables, the occasional presence of pathogenic parasites and bacteria are capable of causing human infections (Mukhtar *et al.*, 2010). Microbial analysis revealed the presence of *Klebsiella spp. Shigella spp. Sallmonella ssp., S.epidermides, S.aureus, Bacillus subtilis, E.coli, Pseudomaonasaeuroginosa, Aeromonas spp., Proteus spp., Aerococcus*and *Enterococcus* on apple surface (Oranusi and Wesley, 2012).

Despite the production of large quantities of fruits, 20-40% post-harvest losses occur during handling, packaging, and transportation of the produce (Hussain *et al.*, 2011). Due to post-harvest losses, substandard sterilization techniques and low shelf life, very less quantity of fruit is exported from Pakistan (PHDEB, 2007). To overcome quarantine barriers, it is required that exporting fruits should be free of microbial attack and are required to have greater storage life (Zaman *et al.*, 2013).Different treatments can be used to protect fruits from decay. However, gamma radiations have shown profound effects than other treatments. It is reported by the joint committee of FAO/IAEA/WHO that irradiation can be used for food preservation. Through food irradiation, the nutritive values of foods are maintained (PHDEB, 2007).

Judging from the available literature, there are many and very different technologies that are presently used to reduce loss of quality and increase safety of apples but gamma irradiation is an efficient method to maintain the quality and shelf-life of apples.

Material and methods

Sampling site and sampling

Fresh Kala kulu apple samples were collected from local market in Lahore city. Apples were randomly picked and placed in sealed bags.

Irradiation

Each polythene bag was labeled with the different gamma dose (1.0 kGy, 1.5kGy and 2.0 kGy) and sent to PARAS (Pakistan Radiation Services) for irradiation from ^{60}Co gamma source.

Microbial analysis

Enumeration of bacteria was done on nutrient agar, MacConkey Agar and *Salmonella-Shigella* agar while Yeast and fungal count on PDA. Serial dilutions were prepared of 10^{-4} for nutrient agar and 10^{-1} for MacConkey, *Sallmonella-Shigella*and PDA agar according to (Oranusi and Wesley, 2012). Under sterilized conditions an apple was removed from sealed bags and was rinsed with 100ml of sterilized distilled water in sterile beaker for 10 minutes. Colonies were counted and presented as cfu/ml (Leff and Fierer, 2013).

Identification of microbial isolates

Cellular morphology of culture isolates was examined according to following microscopic techniques: Gram staining, endospore staining and motility determination. Isolates of yeast and molds were identified on the basis of colony morphology and microscopy. Colony characteristics i.e. branching hyphae; type of branching; and the color, separation, and thickness of hyphae in fungi species were observed (Gupta *et al.*, 2013).

Sensory evaluation

Sensory evaluation was conducted on irradiated and control samples. The quality attributes, including texture, firmness and overall acceptability was evaluated. Effects on surface defects were done for each group.

Statistical analysis

Each experiment was performed in five replications. Statistical analysis was performed on microbial growth obtained for control and irradiated samples using Co-stat 6.4 program.

Results and discussion

Enumeration of total bacterial count

The effect of gamma irradiation on total viable count was determined for both control and treated samples (1kGy, 1.5kGy and 2kGy) and is given in the table1, fig.1a. Each analysis (after 7 days) showed that microbial load is relatively lower for irradiated samples (1kGy, 1.5kGy, 2kGy) as compared to control. After 21 days (3 weeks), maximum microbial reduction was observed on apples irradiated with 2kGy dose. These results are in accordance to (Bibi *et al.*, 2006) who stated that 2.5 and 3.0 kGy dose reduced total bacterial count to minimum level and hence it proved the efficiency of gamma irradiation in controlling microbial load.

Table 1. Mean colony forming unit (cfu/ml) of microflora on different agar of control and irradiated (1kGy, 1.5kGy and 2kGy) apples.

Gamma irradiation (kGy)	Microbial load on Nutrient agar (cfu/ml) Analysis period (days)		
	7 d	14 d	21 d
Control	$3.9\times10^{5}\pm1.02^{aC}$	$5.4\times10^{5}\pm0.28^{aB}$	$8.3\times10^{5}\pm1.4^{aA}$
1kGy	$2.4\times10^{5}\pm1.64^{bC}$	$4.6\times10^{5}\pm0.57^{bB}$	$7.6\times10^{5}\pm1.2^{bA}$
1.5kGy	$1.4\times10^{5}\pm0.894^{cC}$	$2.5\times10^{5}\pm0.84^{cB}$	$3.3\times10^{5}\pm1.5^{cA}$
2kGy	$1.3\times10^{5}\pm1.17^{cB}$	$1.6\times10^{5}\pm1.3^{cB}$	$3.2\times10^{5}\pm0.6^{cA}$
Microbial load on MacConkey agar (cfu/ml)			
Control	$7.8\times10^{3}\pm0.28^{aB}$	$6.1\times10^{3}\pm1.02^{aC}$	$1.15\times10^{4}\pm1.41^{aA}$
1kGy	$2.3\times10^{3}\pm0.56^{bC}$	$3.8\times10^{3}\pm1.65^{bB}$	$6.0\times10^{3}\pm1.2^{bA}$
1.5kGy	$2.1\times10^{3}\pm0.84^{bB}$	$2.4\times10^{3}\pm0.89^{cB}$	$4.2\times10^{3}\pm1.5^{cA}$
2kGy	$3\times10^{2}\pm0.63^{cC}$	$2.3\times10^{3}\pm1.17^{cB}$	$3.0\times10^{3}\pm1.17^{dA}$
Microbial load on *Salmonella-Shigella* agar (cfu/ml)			
Control	$0\times10^{2}\pm0^{aB}$	$1\times10^{2}\pm0.28^{aB}$	$6.9\times10^{3}\pm0.28^{aA}$
1kGy	$2\times10^{2}\pm0.28^{aB}$	$0\times10^{2}\pm0^{bB}$	$2.2\times10^{3}\pm0.56^{bA}$
1.5kGy	$0\times10^{2}\pm0^{bB}$	$0\times10^{2}\pm0^{bB}$	$5\times10^{2}\pm0.84^{cA}$
2kGy	$2\times10^{2}\pm0.57^{bB}$	$0\times10^{2}\pm0^{bC}$	$5\times10^{2}\pm1.31^{cA}$
Microbial load on Potato Dextrose agar (cfu/ml)			
Control	$5.0\times10^{3}\pm1.01^{aC}$	$9.6\times10^{3}\pm1.65^{aB}$	$1.59\times10^{4}\pm0.89^{aA}$
1kGy	$3.8\times10^{3}\pm1.17^{bB}$	$5.7\times10^{3}\pm0.28^{bA}$	$5.9\times10^{3}\pm0.56^{bA}$
1.5kGy	$2.2\times10^{3}\pm0.84^{cB}$	$4.5\times10^{3}\pm1.31^{cA}$	$5.0\times10^{3}\pm1.41^{bA}$
2kGy	$8\times10^{2}\pm1.2^{dC}$	$3.0\times10^{3}\pm1.5^{dB}$	$4.8\times10^{3}\pm0.63^{cA}$

Enumeration of total Gram negative Enterobacteriacae count

Results of total Gram negative *Enterobacteriacae* count are shown in table1, fig.1b API tests confirmed the identity of the isolates *Pseudomonas aeuroginosa* and *E.coli* that were isolated from the apple surface. Studies performed by (Oranusi and Wesley, 2012) reported the presence of *Klebsiella* spp., *E. coli* and *Pseudomaonas aeuroginosa* on apple fruit. Present study showed that viable count (cfu/ml) for control sample was significantly higher (1.15×10^{4}cfu/ml) than samples treated with 2.0 kGy dose (3.0×10^{3}cfu/ml) upon storage for 21 days.

Enumeration of Salmonella-Shigella count

Microbial load obtained on *Salmonella-Shigella* agar during 21 days of storage is given in table1, fig.1c. *Shigella sonnei, Salmonella epidermidis* were isolated from fruit surface.

These microbes were also reported by (Mukhtar *et al*., 2010) according to which that bacteria residing on apple fruit surface are capable of causing infections. Certain *Shigella spp*. and *Sallmonella ssp*. are also reported by (Oranusi and Wesley, 2012) as infecting agents of apple.Radiation treatment progressively decreased the gram negative count. After 21days, *Salmonella-Shigella* count observed on control sample was 6.9×10^3cfu/ml. While minimum load was detected at sample exposed to 2.0 kGy radiation dose i.e. 5×10^2cfu/ml. Therefore 2kGy dose proofed effective in reducing the Gram negative microflora on apple surface during storage period.

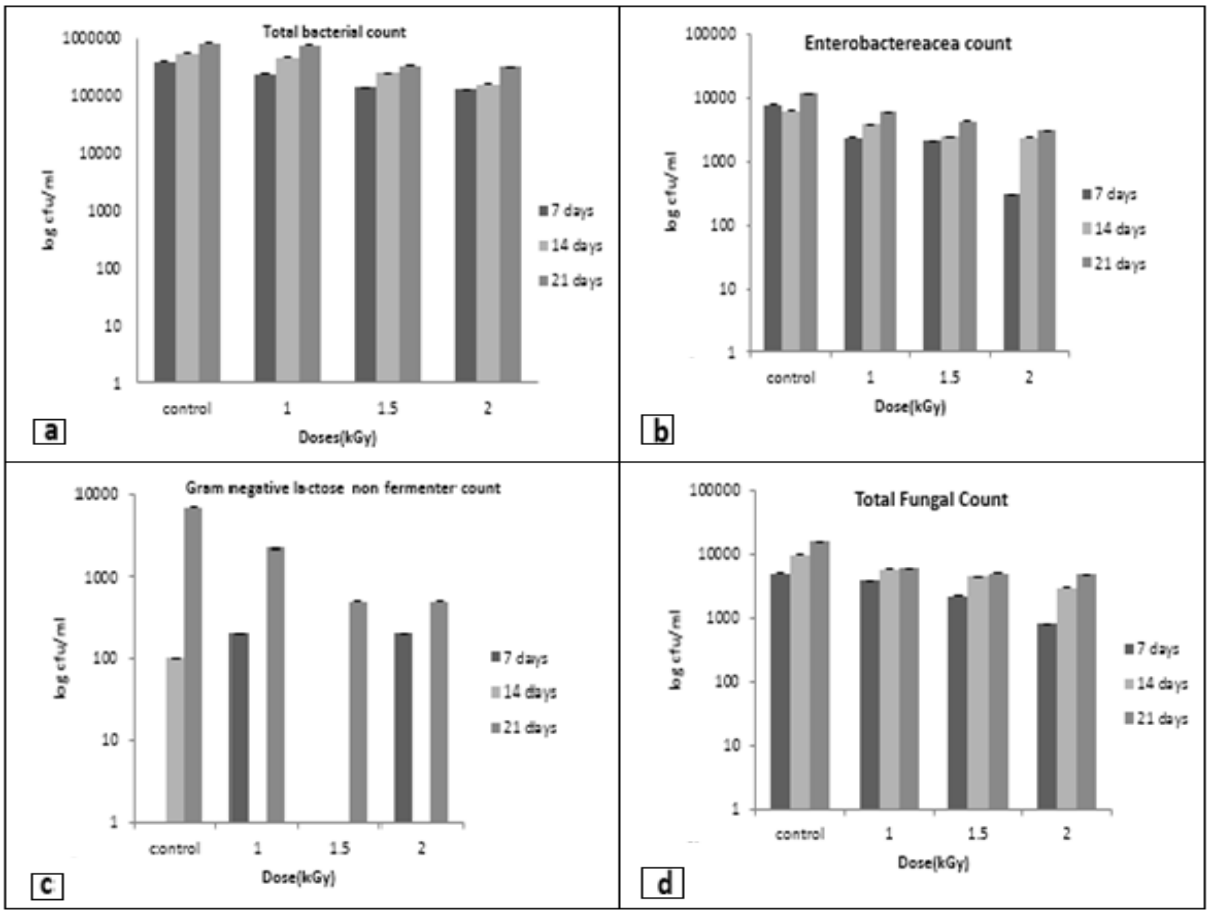

Fig. 1. Colony forming unit per ml obtained after 60 Co gamma irradiation of apples during21 days of storage period at 4 ^oCa) Total bacterial count b) Enterobacteriacae count c) gram negative lactose non fermentating bacterial count d) yeast and fungal count.

The results represented as mean ± SEM followed by superscripts that indicate statistical significance within groups at $p\leq 0.05$, determined by Duncan's Multiple Range Test. Values with different letters within a row (a-d) and a column (A-C) differ significantly ($p<0.05$).

Enumeration of yeast and mold count

Microbial load on Potato Dextrose agar during 21 days of storage is given in table1, fig. 1d.

Saccharomyces cerevisiae, Aspergillus niger, Aspergillus flavus and *Rhizopus stoloniferwer* dominant colonies and were identified using colony morphology and microscopic analysis. These microbes have also been identified by(Mukhtar *et al*., 2010; Juhnevica *et al*., 2011; Oranusi and Wesley, 2012) as affecting agents of apple. Gamma irradiation treatment has proved to be highly effective technique for delaying the yeast and mold proliferation in irradiated samples during storage.

Radiation dose of 2 kGy decreased the fungal count to 8×10^2cfu/ml while on control samples it was observed to be4.8×10^3 cfu/ml during 21 days of storage at refrigerated temperature. Fungal count reduction upto 3 log was also observed by (Oranusi and Wesley, 2012).

Effect of gamma irradiation on sensory properties of apples

Sensory attributes for control and treated (1kGy,1.5kGy and 2kGy) samples were observed for up to 42 days (Fig. 2). The color, texture and firmess of control samples remained intact for up to 21 days however they started to decay after that fig. 2. Irradiation dose of 1kGy didn't effected the texture, color and appreance of apples and hence fruit remained intact for 42 days.Irradiation dose of 1.5kGy didn't effected the color and visual appearance of apples, however, after 35 days fruit firmness started to reduce.It was studied by (Drake *et al.*, 1999) that dose of 1kGy. 1.5kGy and 2kGy didn't affect the external appearance of apples. While according to (Bibi *et al.*, 2006) samples irradiated at 3.0 kGy and stored for 14 days (5◦C) showed better sensory qualities than non-irradiated samples. In the present study, irradiation dose of 2kGy maintained the texture colour and visual apperance for only up to14 days (4°C). However on 21st day blistering started to appear on fruits andwere softer then control samples. It was also determined by (Farkas 1998) that hardness of apples decreased with increasing dose levels as well as over storage. In present study, control and treated (1kGy) remains intact for 42 days while samples treated at 1.5 and 2kGy reduced firmness during storage period. It was also described by (Mostafavi *et al.*, 2012) that irradiation dose of 0.9kGy to 1.2kGy decreases firmness of fruit with increase in storage time.

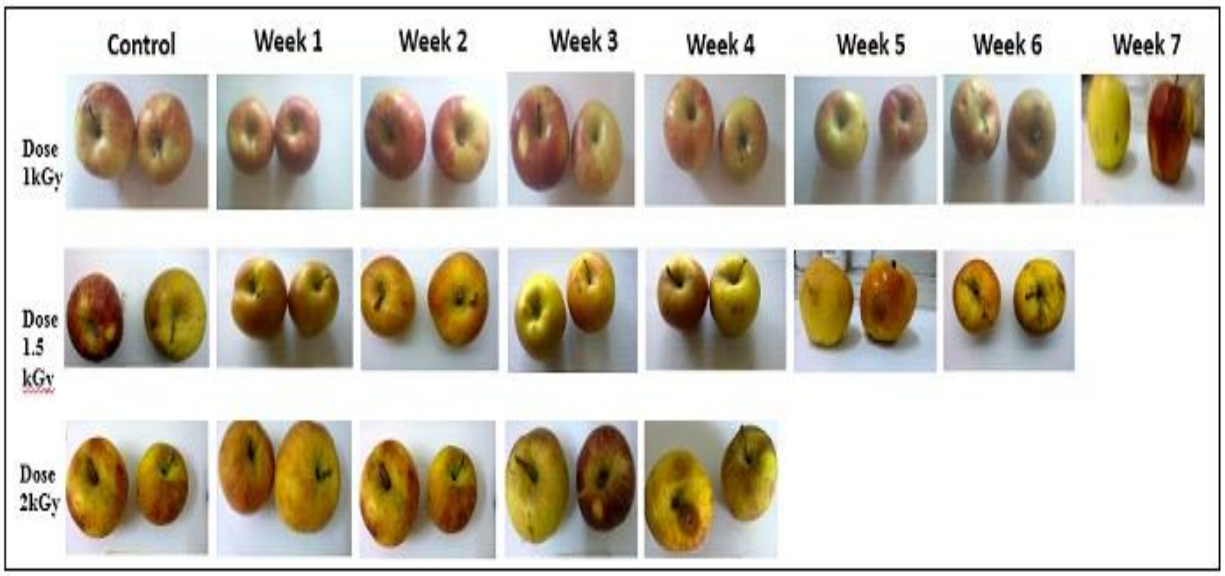

Fig. 2. Appearance of control and irradiated apple samples at 1kGy and 2kGy after 1, 2, 3, 4. 5, 6, 7th week of interval.

Effect of different gamma radiation doses on shelf life of apples

The rotting of non-irradiated apples occurred after 21 days at 4°C but samples irradiated at 1.5kGy extended the shelf life to 14 days whereas maximum 21 days was extended by 1kGy dose as compared to control.

These results are in accordance to (Hussain *et al.*, 2011) in which he reported that irradiation extended the shelf life of apples by 20-25 days at refrigerated temperature (4°C). Effect of gamma irradiation on marketable life of apple was also determined by (Hussain *et al.*, 2011) and it was concluded that shelf life extended upto 90 days under refrigerated temperature.

The difference between results is due to variety difference and apples used in the current study were not taken immediately after harvesting.

Conclusion

In conclusion apples irradiated at different gamma radiation doses of 1kGy, 1.5kGy and 2.0 kGy at storage conditions of 4°C extended the shelf life for different days but the most effective was 1.0 kGy which enhanced the shelf life up to 21 days by reducing the decay, maintaining fruit quality and inhibiting the epiphytic microbial population of fruit.

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