EFFECT OF Co^{60} GAMMA RADIATION ON SHELF LIFE OF PAKISTANI MANGOES EVALUATED THROUGH SENSORY AND MICROBIAL ANALYSIS

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Abstract

The study was carried out to investigate the effect of gamma irradiation on storage quality and shelf life of mangoes. Mature green mangoes (local variety, *Sindhi*) were taken from fruit market and irradiated with Co^{60} gamma doses of 0.5, 1.0 and 1.5kGy. Samples were stored in refrigerator (4^oC) temperature up to three weeks and microbial analysis of both irradiated and non-radiated mangoes was performed weekly by plate enumeration method. The study revealed the presence of *Salmonella sonnie*, *Eschnichia.coli* and *Pseudomonas species* dominating on the fruit surface. Percentage reduction up to 95% was observed at 1kGy γ-radiation dose whereas maximum fungal reduction up to 40% was observed at 1.5kGy. For shelf life extension, 1kGy dose was optimum as it increased the shelf life of mango up to 2 weeks by delaying ripening without causing any damage to fruit as compared to control.

Introduction

Soil and climatic conditions of Pakistan favor the production of good quality mangoes. Pakistani mangoes, being tasty and delicious are in high demand in international market (Vásquez-Caicedo *et al.*, 2002). In 1970's, Pakistan was the world second largest producer of the mango but now its position has dropped to 5th (Usman *et al*., 2003). Besides, mango export has declined up to 20% in 2008 than 2007, due to the poor quality, making Pakistan 7th largest exporter of mango (Akhtar *et al*., 2009). Substandard sterilization techniques, post-harvest losses due to microbial attack and low shelf life of fruits are the main causes of low export of fruit from Pakistan. Microbial attack leads to annual loss of 25.2 % produced mangoes in Pakistan (Iram and Ahmad, 2013). Keeping in view the problem associated with mango export from Pakistan, there is a serious need to deal with microbial diseases of mangoes in order to increase its export and demand in international market.

To meet the quarantine regulations, it is required that exporting fruits should be free of pest and pathogen, free from damages caused by pathogens. Different treatments can be used to protect fruits from decay. Synthetic fungicides are frequently used. UV-C, ozone and heat treatments are beneficial in controlling microbial growth but have less penetration power. Chemical control is not preferred because of environmental and toxicological hazards (Hemmaty *et al*., 2007). However, gamma radiations have shown positive effects than other treatments. A major characteristic of *γ*-radiation is its high penetrating power. Low irradiation dose can also delay ripening of certain fruits. For delaying ripening and insect disinfestations FDA approved maximum gamma radiation dose of 1kGy for fruits and vegetables (Ganguly *et al.*, 2012).

It is a scientific fact that irradiation do not produce any toxic or radiolytic substances. It is observed that irradiation enhances food safety and quality standards by causing the destruction of pathogenic microorganisms such as *E. coli* O157:H7, *Campylobacter*, and *Salmonella* from food (Jha *et al.*, 2010). The present study was conducted to determine effectiveness of gamma irradiation for maintaining keeping qualities and increasing shelf-life of mangoes.

Materials and Methods

Sample collection: Mature green mangoes (variety: *sindhi*) were collected from local market in Lahore. The fruits were fresh, firm having uniform shape and size.

Irradiation treatment: Randomly selected two mangoes were packed in polythene bags and labeled with doses to be given and sent to PARAS for irradiation. Sample bags were stored in refrigerator (4°C) until further analysis.

Enumeration of microbial load: Nutrient agar was used for the enumeration of total bacterial load; MacConkey agar was used for enumeration of gram negative bacteria belonging to family *Enterobacteriacae* whereas *Salmonella-Shigella* agar was used for determination of gram negative lactose non-fermenters. For yeast and fungal count potato dextrose agar (PDA) was used. For microbial analysis randomly selected mango was rinsed in 100 mL sterilized distilled water. Mango was constantly shaken for 5 minutes and serial diluted

according to ISO standards were made (Jha *et al.*, 2010). Aliquots of 0.1mL from each diluted tube were spread on media plates. The microbial count was calculated as colony forming unit/mL.
cfu/mL = No. of colonies/Volume plated× dilution factor

Identification of microbial isolates: Microbes were identified both by colony morphology and cell morphology i.e. Gram staining, endospore staining, motility determination. Lactophenol blue staining were used for studying morphology and characteristics of fungi. Gram negative bacterial isolates were purely cultured on selective media and identified by using API 20E strips (bioMerieux, Inc.). Yeast was observed by slide techniques to observe their cellular morphology.

Statistical analysis: Results were statistically analyzed by using software program SPSS version 8^{th}. One Way ANOVA, Duncan Multiple Range test was used at $p \leq 0.05$.

Results

Enumeration of microbes on Nutrient Agar: Microbial load was reduced in all radiated sample as compared to control in three weeks as shown in Table 1. In first week microbial load on control, 0.5 and 1.5 kGy was 294×10^4 cfu/mL, 150×10^4 cfu/mL and 69×10^4 cfu/mL, respectively. Least microbial load was obtained on 1kGy dose (60×10^4 cfu/mL) that was significantly lower than that of control and all other doses. Whereas in second week average microbial load on control was 228×10^4 cfu/mL while at 0.5, 1.0 and 1.5 kGy bacterial load was 175×10^4 cfu/mL, 72×10^4 cfu/mL and 74×10^4 cfu/mL. Again maximum reduction occurred at 1kGy on second week. Similarly microbial load when observed for third week showed lowest count for 1kGy dose (90×10^4 cfu/mL). The optimum dose with respect to maximum reduction was 1kGy.

Enumeration of Enterobactereacae lactose fermenters: Enterbacteracae count for first week on control, 0.5kGy, 1kGy and 1.5 kGy was 14×10^4 cfu/mL, 7×10^4 cfu/mL, 2×10^4 cfu/ml and 9×10^4 cfu/mL, respectively. After second week microbial load of control was 18×10^4 cfu/mL while none for radiated samples. By comparing the viable count of all doses and control in all three weeks it is concluded that 1 kGy dose was better in all three weeks which significantly ($p\leq0.05$) reduced microbial load as compared to other treatments (Table 1.). *E.coli* and *Pseudomonas species* were identified using API strips.

Enumeration of Enterobactereacae non-lactose fermenters: Microbial load at 0.5, 1.0 and 1.5kGy doses after first week interval was 5×10^4cfu/mL, 0×10^4cfu/mL and 3×10^4cfu/mL, respectively. In second and third week no microbial load was observed at either 1kGy or 1.5kGy. Mangoes irradiated with dose 1kGy showed no lactose non fermenter bacteria. Upon identification by using API strips, *E.coli* and *Shigella sonnie* were identified.

Enumeration of fungal load: Microflora on control and irradiated samples (0.5, 1.0 and 1.5kGy) was 91×10^4 cfu/mL, 90×10^4 cfu/mL, 83×10^4 cfu/mL and 54×10^4 cfu/mL, respectively after first week. After second week, microbial count on control, 0.5 and 1.0kGy was 325×10^4 cfu/mL, 310×10^4 cfu/mL and 176×10^4 cfu/mL, respectively. Microflora on 1.5kGy was 286×10^4cfu/mL. After third analysis control sample has load of 310×10^4cfu/mL while for 0.5, 1.0 and 1.5kGy, microbial load was 298×10^4cfu/mL, 190×10^4 cfu/mL and 100×10^4 cfu/mL, respectively. Keeping in view the result of all the three week it is concluded that 1.5kGy dose was best in first and third week with significantly reduced ($p\leq 0.05$) fungal load as compared to other irradiated and control samples. However in second week dose 1kGy showed minimum fungal load. Various molds were identified on microscopic basis including *Aspergillus*, *Alterneria* and *Rhizopus spp.*

Ripening evaluation: Ripening evaluation of irradiated and control samples of mangoes during three weeks is shown in Table 2. In first week there was no difference in texture of control and treated sample (0.5kGy, 1kGy and 1.5kGy). All sample had green peel color. After second week control mangoes started to ripe and became soft indicated by yellowing of the peel color. While all treated sample had green peel color but mangoes irradiated with dose 0.5kGy became soft. No changes were observed in mangoes irradiated with 1kGy and 1.5kGy. After third week, non-radiated sample became very soft and peel was of yellow green color. Sample irradiated with dose 0.5kGy showed blackening of peel and soft texture. While peel of sample irradiated with 1.5kGy was of yellow green color while size of the mangoes was reduced (shrink) at this dose. But at dose 1kGy there was no any sign of ripening and fruit was firm.

Table 1. Total microbial count of control and irradiated mangoes (0.5kGy, 1kGy and 1.5kGy) stored at 4°C on different media for three weeks.

Gamma irradiation (kGy)	Microbial load on Nutrient agar (cfu/mL) Analysis period (weeks)		
	Week 1	Week 2	Week 3
Control	$2.94\times10^{6}\pm1.01^{aA}$	$2.28\times10^{6}\pm0.82^{aC}$	$2.76\times10^{6}\pm1.41^{aB}$
0.5kGy	$1.50\times10^{6}\pm1.6^{bB}$	$1.75\times10^{6}\pm0.56^{bA}$	$1.15\times10^{6}\pm1.2^{bC}$
1kGy	$6.0\times10^{5}\pm0.89^{dC}$	$7.2\times10^{5}\pm0.84^{cB}$	$9.0\times10^{5}\pm1.5^{dA}$
1.5kGy	$6.9\times10^{5}\pm1.16^{cC}$	$7.4\times10^{5}\pm1.31^{cB}$	$9.8\times10^{5}\pm0.632^{cA}$
Enterobactereacae lactose fermenters count (cfu/mL)			
Control	$1.4\times10^{5}\pm1.01^{aA}$	$1.8\times10^{5}\pm1.6^{aA}$	$1.7\times10^{5}\pm0.89^{aA}$
0.5kGy	$7\times10^{4}\pm1.16^{bA}$	0 ± 0^{bC}	$3\times10^{4}\pm0.632^{bB}$
1kGy	$2\times10^{4}\pm0.28^{cA}$	0 ± 0^{bB}	0 ± 0^{cB}
1.5kGy	$9\times10^{4}\pm0.56^{bA}$	0 ± 0^{bB}	0 ± 0^{cB}
Enterobactereacae non-lactose fermenters count (cfu/mL)			
Control	$8\times10^{4}\pm1.16^{aA}$	$3\times10^{4}\pm0.56^{aB}$	$2\times10^{4}\pm0.6^{aB}$
0.5kGy	$5\times10^{4}\pm0.89^{bA}$	0 ± 0^{bB}	$1\times10^{4}\pm0.48^{abB}$
1kGy	0 ± 0^{cA}	0 ± 0^{bA}	0 ± 0^{bA}
1.5kGy	$3\times10^{4}\pm0.28^{bA}$	0 ± 0^{bB}	0 ± 0^{bB}
Total fungal count (cfu/mL)			
Control	$91\times10^{4}\pm0.632^{aC}$	$325\times10^{4}\pm1.16^{aA}$	$310\times10^{4}\pm1.31^{aB}$
0.5kGy	$90\times10^{4}\pm1.64^{aC}$	$310\times10^{4}\pm0.89^{bA}$	$298\times10^{4}\pm0.84^{bB}$
1kGy	$83\times10^{4}\pm1.2^{bC}$	$176\times10^{4}\pm1.5^{dB}$	$190\times10^{4}\pm0.56^{cA}$
1.5kGy	$54\times10^{4}\pm1.41^{cC}$	$286\times10^{4}\pm1.01^{cA}$	$100\times10^{4}\pm0.28^{dB}$

Results represented as Mean of cfu/mL ± SE. Superscript a,b,c,d shows significant ($p \leq 0.05$) difference between cfu/mL values of doses given whereas superscript A.B,C,D shows significant ($p \leq 0.05$) difference between cfu/mL values of three weeks. Significant difference ($p \leq 0.05$) determined by Duncan's New Multiple Range Test.

Table 2. Ripening and sensory evaluation of control and irradiated mangoes (0.5kGy, 1kGy and 1.5kGy) stored at 4°C for three weeks.

Gamma irradiation (kGy)	Ripening evaluation					
	Week 1		Week 2		Week 3	
	Texture	Color	Texture	Color	Texture	Color
Control	Firm	Green	Soft	Slightly Yellow	Too soft	Yellow
0.5	Firm	Green	Less firm	Green	Too soft	Yellow with black spots
1.0	Firm	Green	Firm	Green	Soft	Green
1.5	Firm	Green	Firm	Green	Slightly Firm	Slightly yellow with black spots

Shelf life extension: By keeping in view the results of all three week it is concluded that irradiation extend the shelf life of mangoes as compared to control sample. Doses of 0.5kGy and 1.5kGy showed unfavorable

symptoms on fruit on third week. Whereas mangoes irradiated with dose 1kGy delayed ripening for 21 days without causing harm to fruit therefore 1kGy is appropriate dose for extending the shelf life of mango up to 14 days by delaying ripening.

Discussion

In this study, gamma irradiation doses used were 0.5 ,1.0 and 1.5kGy and their effect was studied on microbial load and shelflife of mangoes upto three weeks at 4^{o}C temperature. Result showed that fungal count reduced by increasing dose. Dose 1.5kGy was approperiate in reducing the fungus and molds. Studies have shown that at 1.0 and 1.5 kGy dose, incidence anthracnose and stem end rot diseases decreases in mangoes (Wisutiamonkul and Suwanagul, 2010). It is also observed that doses in the range 0.75-1.5 kGy were needed to control the fungal pathogen *Colletotrichum gleosporoides* and *Diplodia natalensi* (Thomas and Moy, 1986).
For total viable count and enterobacterace, 1kGy dose was optimum in reducing the microbial flora throughout the study period. Irradiation is a feasible process because doses necessary to ensure good microbiological quality do not change the overall quality of the fruits tested. Irradiation reduces the fungal, *Pseudomonas* and Lactic acid bacterial count as compared to control. It was reported in a study that high radiation up to 2kGy was sufficient in reducing the fungal load to acceptable level (Landgraf *et al.*, 2006).

From our study it is concluded that irradiation extend the shelf life of mangoes. Doses of 0.5kGy and 1.5kGy were not effective in reducing the microflora. Mangoes irradiated with dose 1kGy delayed ripening for 21 days without causing harm to fruit therefore 1kGy is appropriate dose for extending the shelf life of mango by delaying ripening. In an another study low-dose gamma irradiation of mango fruits at 100Gy extended the shelf life by 5 or 6 days at ambient temperature (28–32^{o}C) while irradiation at 200Gy cause a maximum extension of approximately 8–10 days (Janave and Sharma, 2005). It was also reported that when mangoes were exposed to 1.2 kGy and stored subsequently under ambient conditions (23 to 39°C) increased the storage life up to 24 days as compared to 16 days for non-radiated fruits (Ramos *et al.*, 1991).

Conclusion

Mangoes irradiated at different gamma radiation doses of 0.5, 1 and 1.5 kGy differentially extended the shelf life of mangoes but the most effective was 1.0 kGy which enhanced the shelf life to 14 days by delaying ripening and without causing harm to fruit. Dose 1kGy was also effective in controlling total microbial load on mangoes. Gamma irradiation has enhanced mango quality and shelf life.

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References

Akhtar, S., Mahmood, S., Naz, S., Nasir, M. and Saultan, M. T. (2009). Sensory evaluation of mangoes (Mangifera Indica L.) grown in different regions of Pakistan. *Pak. J. Bot*. 41(6): 2821-2829.

Ganguly, S., Mukhopadhayay, S. K. and Biswas, S. (2012). Preservation of food items by irradiation process. *Int. J. Chem. Biochem. Sci*. 1(2012): 11-13.

Hemmaty, S., Moallemi, N. and Naseri, L. (2007). Effect of UV-C radiation and hot water on the calcium content and postharvest quality of apples. *Span. J. Agric. Res.* 5(4): 559-568.

Iram, S. and Ahmad, H. M. I. (2013). Major post harvest diseases of Mango and their management. *Intl. J. Agron. Plant. Prod.* 4(12): 3470-3848.

Janave, M. and Sharma, A. (2005). Extended storage of gamma-irradiated mango at tropical ambient temperature by film wrap packaging. *J. Food sci. Technol*. 42(3): 230-233.

Jha, S. N., Jaiswal, P., Narsaiah, K., Bhardwaj, R., Sharma, R., Kumar, R. and Basediya, A. L. (2010). Post-harvest micro-flora on major cultivars of Indian mangoes. *Sci. Hortic*. 125(4): 617-621.

Landgraf, M., Goularte, L., Martins, C., Cestari, A. J. R., Nunes, T., Aragonalegro, L., Destro, M., Behrens, J., Vizeu, D. and Hutzler, B. (2006).Use of irradiation to improve the microbiological safety of minimally processed fruits and vegetables. *Use of Irradiation to Ensure the Hygienic Quality of Fresh, Pre-Cut Fruits and Vegetables and Other Minimally Processed Food of Plant Origin*. 41-59

Ramos, L. J., Lara, S. P., McMillan, R. T. and Narayanan, K. R. (1991). Tip dieback of mango (*Mangifera indica*) caused by *Botryodiplodia ribis*. *Plant disease*. 75(3): 315-318.

Thomas, P. and Moy, J. H. (1986). Radiation preservation of foods of plant origin. III. Tropical fruits: Bananas, mangoes, and papayas. *Crit. Rev. Food Sci. Nutr*. 23(2): 147-205.

Usman, M., Fatima, B., Khan, M. M. and Chaudhry, M. I. (2003). Mango In Pakistan: A Chronological Review. *Pak. J. Agri. Sci*. 40(2-3): 151-154.

Vásquez-Caicedo, A., Neidhart, S., Pathomrungsiyounggul, P., Wiriyacharee, P., Chattrakul, A., Sruamsiri, P., Manochai, P., Bangerth, F. and Carle, R. (2002). Physical, Chemical, and Sensory Properties of Nine Thai Mango Cultivars and Evaluation of their Technological and Nutritional Potential. *International Symposium: Sustaining Food Security and Managing Natural Resources in Southeast Asia Challenges for the 21st Century*. 1-8.

Wisutiamonkul, A. and Suwanagul, A. (2010). Effect of gamma irradiation on quality of mango fruit cv. Nam Dok Mai Si Thong. *Thai. J. Agri. Sci.* 41: 99-102.