EFFECTS OF LOW Co^{60} GAMMA RADIATION ON MICROBIAL FLORA AND SHELF LIFE OF PEANUTS

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Abstract

The present study aimed at reducing the microbial content thereby enhancing the shelf life of peanuts through Co^{60} gamma irradiation dose optimization. Peanut pods were obtained from local fruit market of Lahore and were shelled under hygienic conditions that were later subjected to irradiation at 0.3, 0.75, 0.9kGy of doses. Effect of radiation treatment on microbial quality and insect infestation of samples kept at room temperature was analysed after every 30 days of interval for up to 60 days. Total bacterial count obtained for all samples ranged from 10^5 to 10^6 whereas no log reduction was observed in treated samples as compared to control. No gram negative Enterobactereace were observed either in control or treated samples. All doses were able to reduce fungal growth to 10^4 that remained persistent throughout the experimental period. Fungal species identified were *Aspergillus niger, Aspergillus flavus, Fusarrium spp., Penicillum spp. and Rhizophus spp.* Conclusively, low Cobalt-60 gamma irradiation treatment could prove effective if combined with physical or chemical treatments before packaging.

Introduction

Peanut, *Arachis hypogea,* is a rich source of edible oil and protein. It is consumed as food by humans as well as animals because of high protein content. More than two third of peanut is crushed for its oil while remaining is used as food or fodder for animals (Hammons, 1994). Peanuts are well placed to be incorporated into the human diet because of their nutritional importance as reported many times in scientific literature. Health benefits of peanut include prevention of type II Diabetes, decrease the risk of cardio vascular disease, and assist in lowering cholesterol and weight loss, via hunger suppression and protection against Alzheimer disease, peanuts also have great industrial importance (Kris-Etherton *et al* 1999; Higgs and Blade, 2005). It has variety of industrial end uses.

According to FAO approximately 25% of the world's food is lost due to infestation caused by insects, bacteria, fungus and enzymes that degrade or contaminate foods as they are removed from the ground or during subsequent harvest, drying and storage. Garren (1966) identified six fungal taxa associated with peanut in his study which include *Aspergillus, Rhizophus, Mucor, Curvularia, Fusarium and Penicillium* all of which except *Curvularia* were accompanying and accountable for rotting of about one-half of putrefied peanut.

Fungal infections mainly of *Aspergillus flavus* produces aflatoxins which are highly toxic. Various physical and chemical methods that have been used for the detoxification of aflatoxins include heat ozonization, ammonization and irradiation using electron beam, X-rays, gamma rays etc. However various preservation techniques and principles are already applied to minimize the microbial load and to maintain the chemical integrity and quality of food. Food irradiation is one among many of accessible technologies that promote to enhance the food's safety.

Food irradiation is a physical method of food processing in which the food is exposed to ionizing energy, such as γ photons emitted by Co^{60} (or infrequently by 137Cs) radioisotopes, X-rays ,UV light or electron beam and is packed either in polythene bags or is wrapped (Moh´acsi-Farkas and Farkas 2011). Various methods have been used for the irradiation of food and gamma irradiation using Co^{60} is presently the preferred method because of its deeper penetration capacity (Prado, 2005; Prado *et al.,* 2006). But gamma irradiation using Co^{60} is preferred one instead of cesium Cs and is more commonly practiced (Satin, 1996).

The present study has been undertaken to investigate whether low Co^{60} dose of gamma irradiation is effective in enhancing the shelf life of peanuts at room temperature along with meeting the requirements of food safety and export standards.

Materials and Methods

Sample collection: Roasted peanuts of Punjab origin were collected from the local market of Lahore, Pakistan.

Sampling: Before packing peanut pods were shelled under hygienic laboratory conditions. Peanut seeds (4-5 g) were packed in air tight polythene sealed bags. Each bag was labeled according to the Co 60 gamma radiation

dose that will be applied and sent to Pakistan radiation service (PARAS). For this peanut were treated with 0.3kGy, 0.75kGy and 0.9 kGy. Both treated and untreated (control) samples were kept at room temperature and were quantified for total microbial flora, insect infestation and sensory attributes for up to 60 days.

Sensory evaluation of peanut: Sensory evaluation was conducted on treated and control samples. The quality attributes, including texture, color, appearance and overall acceptability was evaluated. 9-point hedonic scale was used for the evaluation of qualities attributes like appearance acceptance, texture acceptance, taste and aroma of the peanut sample. Each group was also analyzed for insect infestation and surface defects. Infestation was checked by opening peanut to check presence of insect larvae or internal damage.

Microbiological analysis: Microbial analysis right after radiation treatment and after every 30 days of interval for up to 60 days was done. For the enumeration and identification of bacteria and fungi associated with peanut four growth media were used. Nutrient agar for total bacterial count, MacConkey agar for Gram negative lactose non fermenting enterobactereacae, PDA for fungi species and Salmonella Shigella agar for Gram negative lactose fermenting enterobactereacae species.

Total bacterial count: Total bacterial count was obtained by making original dilution i.e. by shaking 1g peanut sample in 100mL sterilized distilled water for 5 minutes. Serial dilutions were prepared as according to the method of Banson and Harold (2002). About 0.1mL of each dilution was spread on to prepared nutrient agar Petri plates. Inoculated plates were incubated at 37°C overnight. After 24h of incubation bacterial growth was measured by counting colonies and calculating colony forming unit (cfu/g).

Enumeration of Gram Negative Enterobactereacae lactose non-fermenters: Serial dilutions mentioned above were also spread (0.1mL) on to MaConkey agar for obtaining Gram Negative enterobactereacae lactose non-fermenters count. Inoculated plates were incubated at 37°C overnight. After 24h of incubation bacterial growth was measured by counting colonies and calculating colony forming unit (cfu/g).

Enumeration of Gram Negative Enterobactereacae lactose fermenters: Calculating the Gram negative Enterobactereacae (lactose fermenters) count serial dilutions were spread on to Salmonella Shigella agar plates. Inoculated plates were incubated at 37°C overnight. After 24h of incubation bacterial growth was measured by counting colonies and calculating colony forming unit (cfu/g).

Enumeration of Fungal count: Fungal count in treated and untreated samples were obtained by spreading 0.1ml of serial dilution on to potato dextrose agar. Inoculated plates were incubated at 28-30°C for 72h. After the incubation period fungal growth was measured by counting colonies and calculating colony forming unit (cfu/g).

Bacterial Identification

Colony morphology: Colonial morphology of obtained microbial growth on nutrient agar, *Salmonella* and *Shigella* and MacConkey agar was observed. For which colony size, shape, color, texture, margins and elevations were recorded as according to Banson and Harold (2002).

Cell Morphology: Cell morphological characteristics were determined for bacterial and fungal growth by Gram's staining technique and methylene blue method, respectively (Banson and Harold, 2002). Cell morphology was observed at 40x and 100x oil immersion objective.

Statistical Analysis: The data was statistically evaluated for its credibility and usefulness of information by using Costat 6.4 program. Multiple analysis of variance (ANOVA) was used with significant level $p \leq 0.05$.

Insect infestation frequency: Each peanut sample was analyzed throughout the study period for any sort of insect infestation both qualitatively and quantitatively. Presence of eggs, larva or adult stage was the criteria set for qualitative study.

Results and Discussion

Outbreaks related to consumption of raw dry nuts are reported quiet often (Miksch *et al*., 2013). Many studies have reported the presence of pathogenic bacteria, fungus and aflatoxins on raw peanuts (Miksch *et al*., 2013; de Camargoa *et al*., 2015). Peanuts rich in edible oil and protein content are prone to damage by microorganisms mainly fungi. Due to increase in demand of peanut production it is the need of hour to increase

its shelf life to reduce postharvest loses. Due to inadequate storage conditions a large portion of peanut yield is lost which is known as postharvest losses. Various physical and chemical methods are being used to reduce microorganism and insect infestations during storage and prolonging the shelf life of peanuts. Among the methods used gamma irradiation using Co^{60} is a promising technology that is safe and is used widely in reducing microorganisms during storage period without effecting the nutritional quality of food (Chiou *et al,* 1991; de Camargoa *et al*., 2015).

Sensory evaluation: Texture, colour, appearance and consumer acceptance for the radiated samples were slightly more as compared to non-radiated samples. Sensory attributes of radiated samples remained acceptable for up to 8 months under room temperature unlike control sample where change in colour was observed (Figure 3, Table 1, 2). Other studies have reported no deteriorating effect of low gamma radiation on sensory attributes of peanuts in stored conditions (Chiou *et al*, 1991). Similarly, Mexis and Kontominas (2009) in their study reported no harmful effect of gamma radiation on the sensory quality of raw peanut up to 7kGy.

Total bacterial count: Of all the doses studied no log reduction was seen in treated samples as compared to control ones although slight decline in count was observed. Radiation dose of 0.9kGy was effective in reducing and maintaining the microbial count to 10^5 for up to 60 days at room temperature (Figure 1). Low doses of Co^{60} gamma radiation had slight effect on total viable count. Microbial load on fruits depicts its microbiological quality. In the present study control sample and irradiated sample demonstrate countless number of colonies on zero day. However the number of colonies reduces with the increasing doses to 0.9kGy reduces the microbial load into acceptable range among all the radiation doses. Gamma-irradiation at 5.0 kGy decreased the microbiological count of the product reported in studies by de Camargoa *et al.* (2015).

Enumeration of Gram negative enterobactereacae lactose non fermenters: No growth was observed on MaConkey and Salmonella Shigella agar indicating samples were free from enteric pathogens.

Enumeration of fungal count: In control samples fungal count was observed throughout the study period having log value more than 10^5 which is unacceptable. Of all the doses studied radiation dose of 0.9kGy proved effective in the reduction of fungal count to an acceptable level i.e. 10^4 (Figure 2). Humidity and the temperature are most important factor that influence the growth of the fungi. At zero day analysis the temperature and relative humidity of the environment was 36^oC and 41%. Both of these factors favour the growth of fungi on zero day. After 30 days of interval temperature was 39^oC and the relative humidity was 23% which seemed to be did not favour growth in irradiated samples. After 60 days interval when temperature was 41^oC less fungal growth was observed. According to Woodroof (1984) storage facilities that encountered the moisture content of 8% or more than this is suitable for the growth of fungi in both unshelled and shelled groundnuts. According to Horn (2005) highest growth of *Aspergillus flavus* is encouraged at 22 °C–37 °C. Chiou *et al*., (1991) has reported the presence of fungal growth in stored peanuts when treated with 2.5KGy. However in a study by de Camargo *et al*. (2012) dose of 5.2 kGy was effective in preventing Mycotoxin producing fungal growth.

Identification of fungi: Based upon colony morphology and microscopic studies following fungal taxa were identified *Aspergillus niger, Aspergillus flavus* and *Penecillium spp.* these were the most frequent fungi and were found in all the sample from the first observation. Control samples in addition to previous ones had *Candida albicans*. No growth was found in irradiated samples on 30^{th} day of analysis but control group had countless colonies. On 60^{th} day *Rhizophus spp.* was present in samples treated with 0.3kGy. While sample treated with 0.75kGy showed presence of *Candida albicans.* According to Chiou *et al*. (1991) study dose of 5.2kGy was effective in the disinfection of fungi of peanut i.e. at this dose fungi are completely eliminated.

Insect Infestation frequency: No infestation was observed on both control and treated samples (0.3kGy, 0.75kGy and 0.9kGy) for up to 8 months at room temperature. According to Nesci *et al.* (2011), a strongly positive correlation was observed between peanut samples contaminated with insects and insects contaminated with *Aspergillus* section Flavi. Low doses of gamma radiation (0.2–0.8 kGy) are also efficient for killing and sterilizing insects (disinfestation of food) (Farkas, 2006).

Conclusion

It can be concluded that low doses are not effective in reducing the microbial contamination however are quite effective in inhibiting insect infestation for a prolong period. It can be suggested for future study that low gamma doses in combination with physical or chemical treatment can prove productive in enhancing the shelf life and quality of peanuts under stored conditions.

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Table 1. Sensory evaluation of peanut samples upon exposure to different Co^{60} gamma radiation doses while stored at room temperature.

	Gamma Radiation Doses			
Sensory attributes	**Control**	**0.3 kGy**	**0.75 kGy**	**0.9 kGy**
Zero day				
Taste	No change	No change	No change	No change
Aroma	No change	No change	No change	No change
Texture	Crunchy	crunchy	Crunchy	crunchy
Color	No difference	No difference	No difference	No difference
After 30 days				
Taste	Slight change	No change	No change	No change
Aroma	No change	No change	No change	No change
Texture	Crunchy	crunchy	Crunchy	crunchy
Color	No difference	No difference	No difference	No difference
After 60 days				
Taste	Slight change	Slight change	No change	No change
Aroma	No change	No change	No change	No change
Texture	Crunchy	crunchy	Crunchy	crunchy
Color	No difference	No difference	No difference	No difference

Sample means ± standard error and significance for consumer acceptance n=1

Table 2. Sensory evaluation of peanuts using 9-point Hedonic scale.

Days	Control	0.3 kGy	0.75 kGy	0.9 kGy	Control	0.3 kGy	0.75 kGy	0.9 kGy
	Texture				**Appearance**			
0	9	9	9	9	9	9	9	9
30	7	8	8	8	8	9	9	9
60	6	8	8	8	8	9	9	9

*__9-point Hendonic scale:__ **9**=like extremely, **8**=like very much, **7**=like moderately, **6**=like slightly, **5**=neither like nor dislike, **4**=dislike slightly, **3**=dislike moderately, 2=dislike very much, 1= dislike extremely.
*Attributes are significance at $p \leq 0.0$

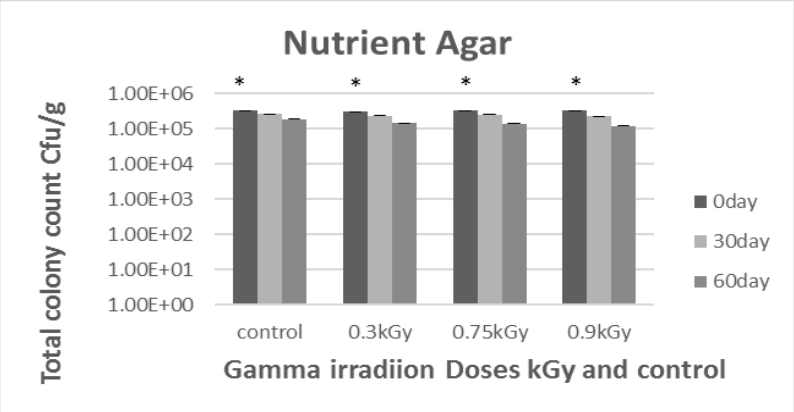

Fig. 1. Effect of low gamma radiation doses on peanut total bacterial count at different time interval during storage period at room temperature. The error bar indicate standard deviation from the mean value. The values vary significantly at ($p \leq 0.05$). *Shows countless number of colonies.

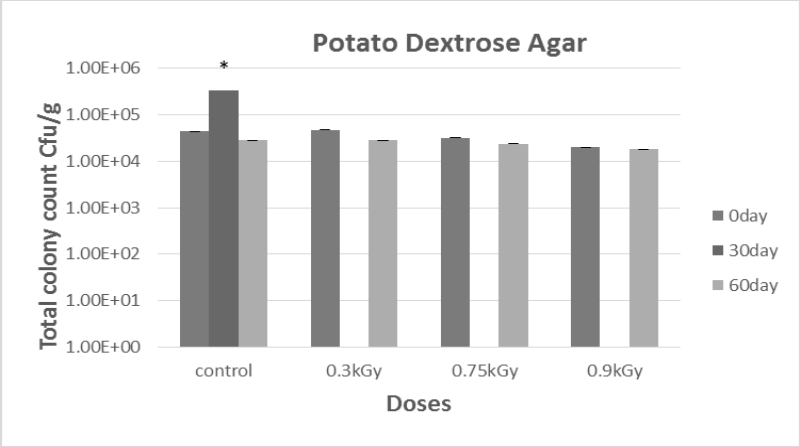

Fig.2. Effect of low gamma radiation doses on peanut total fungal count at different time interval during storage period at room temperature The error bars indicate standard deviation from the mean value. The values vary significantly at ($p \leq 0.05$). *Shows countless number of colonies.

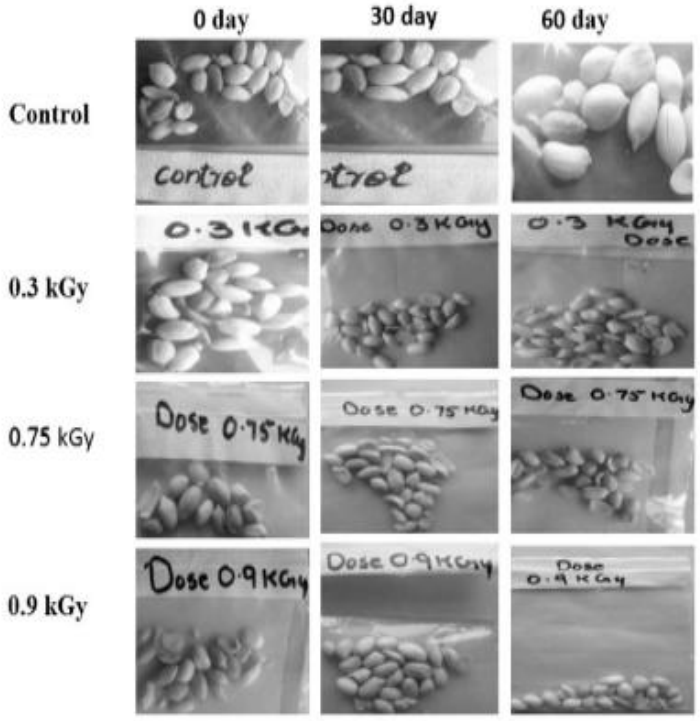

Figure 3: Sensory evaluation of gamma irradiated and control peanut samples from 0 to 60 days of storage at room temperature.

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