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Minimization of residential microbial flora on spinach leaves using cobalt 60 to enhance its export potential

Roheena Abdullah[1*], Tayyaba Rafique[1], Afshan Kaleem[1], MehwishIqtedar[1], Mahwish Aftab[1], Tehreema Iftikhar[2], Faiza Saleem[1], ShaguftaNaz[1]

[1]*Department of Biotechnology Lahore College for Women University, Lahore, Pakistan*

[2]*Department of Botany Lahore College for Women University, Lahore, Pakistan*

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Abstract

A leafy vegetable particularly spinach locally known as palak, has been part of human diet for many years. Pakistan stands among top ten spinach producing countries in the world and contributes considerably to the income. However, lack of proper storage facilities and microbial attack result in post-harvest losses. Food irradiation reduced storage losses, extended shelf life and improved microbiological and parasitological safety of foods. In present study, flat leaf variety of spinach was exposed to various gamma radiation doses (0.5, 0.75 and 1.0 kGy). The microbial flora of spinach before and after irradiation was analyzed to determine significant changes in the population of spoilage microorganisms. In the present study 0.75 kGy proved to be the most beneficial in reducing the microbial flora without affecting the sensory properties of flat leaf spinach and extended the shelf life up to 4days. So, this study can be useful in shelf life enhancement for export purpose.

*** Corresponding Author:** Roheena Abdullah ✉ roheena_abdullah@yahoo.com

Introduction

Leafy vegetables have been part of human diet for so many years across the world. Leafy vegetables particularly spinach has acquired greater importance in diets of both rural and urban inhabitants. Spinach has become a cardinal part of human diet due to its high nutritive value and a very low calorie count (Akhter *et al*., 2013). The increased interest in the consumption of leafy vegetables is not only because of their refreshing properties but also as a result of their medicinal and nutritive properties. Spinach (*Spinaceaoleracea* L.) which is locally called as palak is an annual dioeceous edible flowering plant in the family of Amaranthaceae (Waseem and Nadeem, 2001). It is found in central and southwestern Asia .

Spinach is a highly invigorative vegetable, being enriched with both core nutrients and phytochemicals. Calcium, iron, potassium, folate, vitamin A, vitamin C and keratin constitute the major proportions of micronutrients in spinach. Antioxidant compounds containingphenolics and carotenoids account for a high content in spinach. Excessive oxidative stress is believed to cause a number of chronic diseases and health issues associated with ageing, so antioxidant activity is quite crucial in this regard. Lutein and zeaxanthin being the two major compounds in spinach are best known for their role in protecting against eye diseases such as macular degeneration (gradual loss of central vision, associated with old age). There is known to be an inverse relationship between spinach and cardiovascular diseases and different types of cataracts (Pereson*et al.,* 2006).

There are three main types of fresh spinach being produced all over the world, categorized on the basis of their leaf types. Savoy spinach is the one that has slightly curled leaves. Semi savoy has crinkled leaves but its leaves are not as rigid as those of savoy type. Third type is the flat or smooth leaf spinach, as the name indicates it has leaves that are flat in shape and smooth in their texture, this type is most commonly used for cooking purposes (Akhter *et al.*, 2013).

Pakistan stands among the top ten spinach producing countries of the world but almost 25% of total production is lost because of microbial spoilage that limits its export potential. *E. coli* and *Listeria monocytogens* are the main pathogens causing the most devastation in fresh produce (Barkai-Golan, 2001). Some important fungal diseases of spinach include downy mildew, white rust, leaf spot diseases and the causative organisms include *Aspergillusniger*, *Aspergillusflavus* and *Fusariumoxysporum* (Correll *et al.*, 1994). The magnitude of postharvest losses in fruits and vegetables vary from 35-45% in Pakistan. Globally, postharvest diseases are one of the major concerns of the consumers. Although there are various techniques to minimize postharvest losses, consumers look for agricultural products free of chemicals. It is therefore important to reduce environmental risks and raise consumer's confidence (Temur and Tiryaki, 2013).

Radiation has been used as a treatment to inhibit bacterial and fungal decay of fruits. Radiation is the emission of any rays or particle from a source .The effects of gamma radiation on the viability of microorganisms have attracted a lot of attention. Gamma rays are electromagnetic waves that have high penetrating power. They can pass through substances without leaving any adverse effects, an advantage comparing to other disinfection treatment (Adamo *et al.*, 2001). Irradiation represents an additional stress on the cells, which tends to disturb their organization (Geweely and Nawar, 2006).

Microbial potential has been observed to reduce by the application of Gamma radiation on microbial flora (Jones *et al.*, 2004). The joint FAO, IAEA, WHO export committee for food irradiation concluded that food irradiation up to 10 kGy are safe and non-toxic (WHO, 1981). The purpose of current study is to figure out the influence of different gamma radiation doses on microbial content of the vegetable leaves. Irradiation of a living cell at one gray induces 1000 single strand and 40 double strand breaks. The present study investigated the supremacy of gamma radiation to reduce the microbial bio burden associated with spoilage and in turn increase the shelf life of flat leaf spinach which results enhancing the export potential.

Materials and methods

Sample collection

Flat leaf variety of spinach (*Spinachiaoleraceae L.*)of good quality that showed uniformity in size, shape and colour and did not have any visual defects or mechanical injury were collected from the local market.

Physical evaluation prior to irradiation

Physical examination of spinach leaves for their colour, odour and texture was performed before getting them irradiated. The weight of sample was also recorded before irradiation so as to compare it with the weight after treatment(Gomes *et al.*, 2009).

Irradiation

20-30 g of intact leaves of uniform size and shape were packed in perforated paper bags. Each bag was labeled with the name of the product and the radiation dose it received. The samples were irradiated at different doses (0.5, 0.75 and 1 KGy) from ^{60}Cosource, located at the PARAS, Pakistan radiation services food Ltd. Lahore, Pakistan. Both treated and untreated samples were stored at 12°C for 8 days.The sample after irradiation was observed every alternate day for its physical properties, sensory attributes and microbial flora till 8 days.

Physiological Weight loss

To determine any significant change in the weight loss of spinach leaves during storage weight in grams of each sample was determined and weight loss percentage difference was calculated according toAkhter *et al.* (2013).

$$\% \text{ weight loss} = \frac{W1 - W2}{W1} \times 100$$

Decay Assessment

The percentage decay of the spinach leaves during storage was calculatedbased on visual inspection of each leaf for infection. Percent decay was calculated according to Gihan(2010).

$$\text{Decay percent} = \frac{Decayed\ leaves}{Total\ leaves} \times 100$$

0 = superficial fleck (no decay)
1 = 1- 20 % of the leaves decayed
2 = 21-45 % of the leaves decayed
3 = 46-74 % of the leaves decayed
4 = 75% or more of the leaves decayed.

Acceptability Test

Acceptability of both irradiated and control samples were tested using the 9- point hedonic scale. Attributes involving colour, texture, flavour and overall liking were assigned degrees using this scale to determine how much a sample was liked. Following are the nine categories of the hedonic scale. 1. Like Extremely, 2. Like Very Much, 3. Like Moderately, 4. Like Slightly, 5. Neither Like nor Dislike, 6. Dislike Slightly, 7. Dislike Moderately, 8. Dislike Very Much, 9. Dislike Extremely(Stone *et al.*, 2012).

Microbiological studies

Isolation of microorganisms

Serial dilution method was used for the isolation of microorganisms. 3 leaves were taken and were thoroughly washed for 20 min in 500 ml of sterile saline water. Serial dilutions were made out of it. 100µl of each dilution was transferred on to sterilized petri plates containing nutrient agar (for non-fastidious bacterial isolation), MacConkey agar (for gram negative enteric bacilli isolation), and Potato dextrose agar (for fungi isolation). The plates were incubated at 37°C for 24 hours and at 30°C for 72 hours for bacterial and fungal growth respectively. Total viable count of bacteria and fungi were calculated according to Lal*et al.* (2013).

Morphological evaluation of bacterial colonies obtained after incubation was also performed. The attributes included color, texture, margins, elevation, surface characteristics (rough, dry, glistening) and optical properties (opaque or translucent) were recorded. Gram and Endospore staining of the bacterial cultures was also carried out(Benson, 2001).

The bacteria were identified by using API strips(Holmes *et al.*, 1978), while fungi were identified on the basis of their micro and macroscopic characteristics.

Statistical analysis

The validity and usefulness of the data was analyzed by Costat 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 standard deviation and mean square error of replicates from mean value were also found out.

Results and discussion

Decay assessment

The decay percent analysis of both control and irradiated leaves in recent study showed (Table1) that the irradiated ones depicted less decay percent compared to the non-irradiated and therefore remained healthy for longer period of time. No signs of decay were observed on control sample till 2nd day of storage. However, by 4th day more than 75% of the surface of leaves was decayed. Among the irradiated samples, 0.5 kGy treated spinach remained healthy for up to 4 days while by the 8th day it was completely spoiled. Dose of 1.0 kGy damaged the texture of the leaves and caused extreme tissue browning immediately after irradiation However, Dose of 0.75 kGy was the most influential in keeping leaves safe from decay throughout the storage period. This reduction in decay percent with increase in radiation dose might be due to the fact that increased radiation dose is more effective in targeting more of the spoilage microorganisms(Farkas, 1998).

Table 1. Influence of various gamma radiation doses on decay percent of spinach leaves stored at refrigerated temperature.

DAYS OF STORAGE	IRRADIATION DOSES (kGy)			
	0	0.5	0.75	1.0
1	0	0	0	Decayed
2	2	0	0	Decayed
3	3	0	0	Decayed
4	4	0	0	Decayed
5	Decayed	0	0	Decayed
6	Decayed	1	0	Decayed
7	Decayed	3	0	Decayed
8	Decayed	3	0	Decayed

Influence of different gamma radiation doses on percentage weight loss of spinach leaves

In present study a decrease in the percentage weight loss of irradiated leaves was observed as compared to the non-irradiated with both samples stored under the similar conditions (Table2). Weight loss might be associated with the loss of water which reduces firmness, loss in nutritional quality and brings undesirable changes in color. Water loss might be caused by the water transpiration and respiration(Gutiérrez-Rodríguez *et al.*, 2013). Both of these factors were controlled by irradiation so weight loss in irradiated samples was considerably low as compared to the control. As weight loss might be the consequence of transpiration, leaves lost more water as the storage time progressed.

Table 2. Influence of Irradiation on physiological weight loss of spinach.

Percentage weight loss					
Gamma Radiation doses (kGy)	Storage period				
Control	6.66^{a}	12.2^{a}	Decayed	Decayed	Decayed
0.5	2.66^{b}	3.33^{b}	4^{bc}	4.66^{b}	5.33^{ab}
0.75	1.33^{c}	1.95^{c}	2.46^{bc}	3.15^{bc}	3.87^{c}
1.0	Decayed	Decayed	Decayed	Decayed	Decayed

Minimum weight loss throughout the storage period was noticed for samples treated at 0.75 kGy following the 0.5 kGy treated samples and control. Maximum weight loss was observed for control samples as complete wilting of leaves was observed at 4^{th} day of storage. So, the dose of 0.75 kGy was proved to be most promising to preserve the water constituents and maintain the optimum respiration rates of spinach.

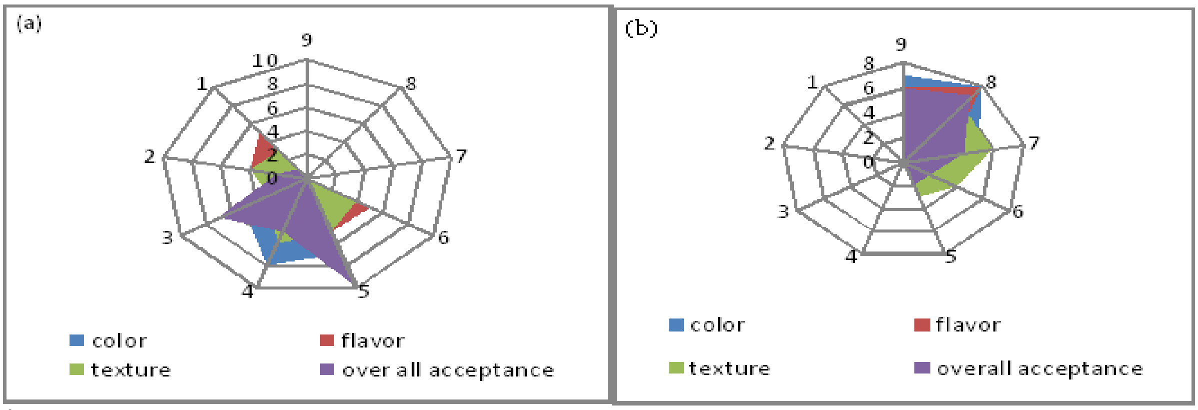

Fig. 1. Sensory evaluation of color, flavor, texture and overall acceptance using 9 point hedonic scale a) Control b) irradiated sample.

Sensory evaluation

In present study sensory evaluation of both control and irradiated samples was carried out using 9-point hedonic scale. Attributes including color, flavor, texture and overall acceptability were studied (Fig1). Irradiated samples showed superiority in liking behavior of all the attributes compared to control. Among irradiated groups 0.5 kGy treated spinach showed loss of green color particularly on the lower parts of the leaves by the 4^{th} day which might be due to the decrease in chlorophyll content or a consequence of fungal attack(Oladele, 2011). However, irradiated samples were observed to be slightly off-flavor which might be the consequence of auto-oxidation of fats, breakdown of proteins having sulphur containing amino acid, both of which give rise to rancid off-flavors. Softening of surface was noticed for sample treated at 1.0 kGy that might be because of the breakdown of high molecular weight carbohydrates into smaller ones which is a process responsible for softening of fresh vegetables by breakdown of cell wall materials e.g. pectin. Whereas, 0.75 kGy treated samples were ranked high on the scale in terms of likeness of all the attributes being judged.

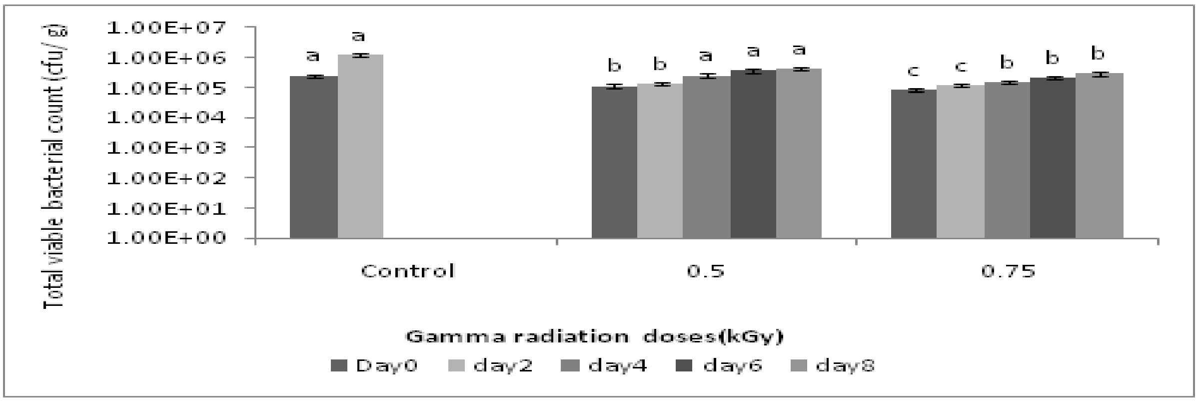

Fig. 2. Influence of different gamma radiation doses on total viable bacterial count of spinach using nutrient agar Each value is the mean of five replicates. The error bars represent standard error from mean value. Superscripts show that mean difference is significant at the level of $p \leq 0.05$ by Duncan's New Multiple Range Test.

Quantitative analysis of total bacterial count

Impact of radiation on total viable bacterial count using nutrient agar

Figure 2 shows the effect of different gamma radiation doses on the bacterial load present on spinach stored at refrigerated temperature. The initial bacterial count on the control group of spinach was 1.24×10^5cfu/g. This count reached up to 5.1×10^5cfu/g at the third day of storage. While complete spoilage of control sample was seen at the fourth day. Initial colony count on irradiated leaves at 0.5 and 0.75 kGy was 1.1×10^5 and 8.2×10^4cfu/g respectively. However, total bacterial colony counts on 8th day of storage were 4.3×10^5 and 2.8×10^5 cfu/g at 0.5 and 0.75 kGy respectively which were far less than those of controls. Various gamma radiation doses affected the total viable bacterial counts differently. The highest dose of 0.75 kGy reduced the maximum number of bacteria (2.8×10^5 cfu/g) on nutrient agar throughout the storage period.

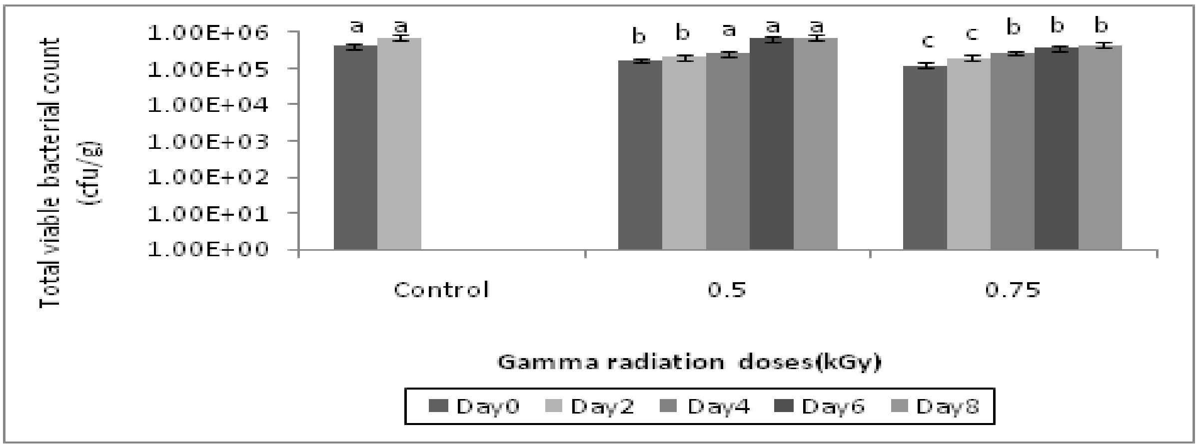

Fig. 3. Influence of different gamma radiation doses on total viable bacterial count of spinach using MacConkey agar. Each value is the mean of five replicates. The error bars represent standard error from mean value. Superscripts show that mean difference is significant at the level of $p \leq 0.05$ by Duncan's New Multiple Range Test.

Influence of radiation on total viable bacterial count using MacConkey agar

Figure 3 represents total viable bacterial count of the irradiated and control spinach observed on MacConkey agar. Initially, total viable bacterial count of control was 4.1×10^5 cfu/g. The bacterial load increased as the storage period progressed. Control sample was completely spoiled by the 4th day of storage. Initial bacterial count of the irradiated spinach at 0.5 and 0.75 kGy were 2.0×10^5 and 1.3×10^5 respectively. Reduction in total viable bacterial count in the irradiated group of spinach was more evident with increasing radiation dose. Bacterial counts were lowest at 0.75 kGythroughout the storage period (1.3×10^5, 2.7×10^5, 3.7×10^5, 4.5×10^5) at 2nd, 4th, 6th and 8th day respectively. The identified epiphytic bacteria present on spinach were included *E.coli* and *Salmonella sp*. The dose of 0.75 kGy almost eliminated all the pathogenic bacteria that rendered spinach safe for human consumption. This reduction in microbial count with increase in radiation dose might be because of the reaction of high energy photons from an isotope source Co-60 with water surrounding the cell leading to the formation of free radicals that diffuses far enough to reach and damage the DNA. These photons induced changes at the molecular level cause the death of the organism or render the organism incapable of reproduction

Total fungal count

Influence of radiation on total fungal count of spinach using potato dextrose agar

Figure 4 represents the total viable fungal count on control and irradiated group of spinach. Fungal colonies decreased with increase in gamma radiation dose. Control sample harbored the maximum fungal count of 1.3 × 10^5 cfu/g. Spinach samples irradiated at 0.5 and 0.75 kGy showed a considerable decrease in total viable fungal count from control samples. Initially 4.8 × 10^4 cfu/gof fungi was observed onsamples irradiated at 0.5 kGy. Whereas spinach sample irradiated at 0.75 kGy showed the minimum fungal counts of 2.0 × 10^4cfu/g. So, the most effective dose in reducing the viable fungi was 0.75 kGy. The count for the above doses increased to 2.9 × 10^5 and 2.2 × 10^5 respectively till the 8^{th} day of storage. The fungi isolated and identified on the basis of macroscopic and microscopic characteristics were *Aspergillusniger*, *Aspergillusflavus*, *Penicillium*sp ,*Fusariumoxysporium* and yeast species. Fungal attack was reduced at 0.75 kGy but not completely eliminated because fungal species require high radiation doses to be completely removed(Geweely and Nawar, 2006). However, control and 0.5 kGy treated samples depicted more spoilage as compared to 0.75 kGy. So, irradiation at 0.75 kGy was proved to be an effective method of reducing the microbial load and hence enhancing the shelf life of fresh flat leaf spinach. The present investigations and results are in line with the findings of Akhter *et al.* (2013) who reported irradiation of flat leaf spinach at low doses as a potential method to eliminate all the pathogenic bacteria and reduce disease causing fungal species.

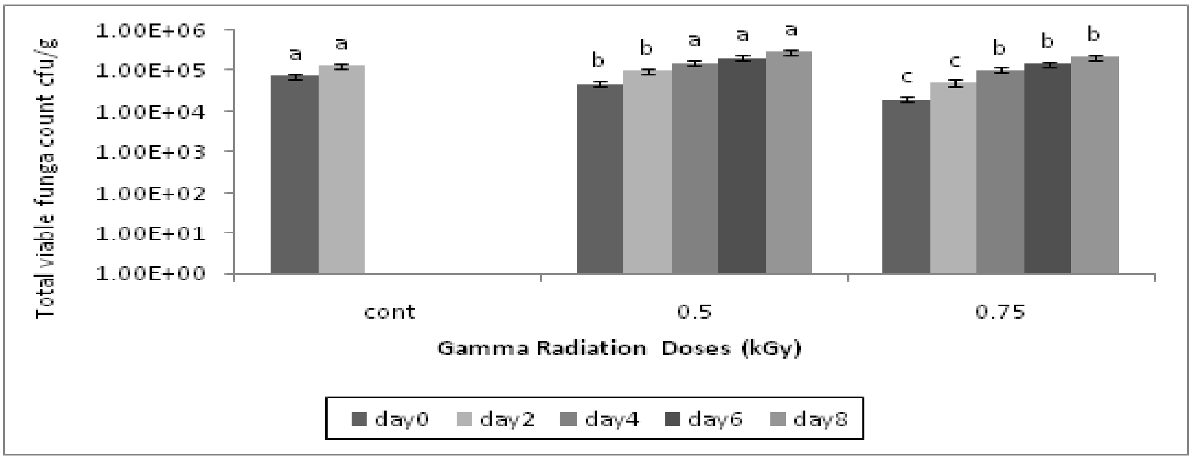

Fig. 4. Influence of different gamma radiation doses on total viable fungal count of spinach using Potato Dextrose agar. Each value is the mean of five replicates. The error bars represent standard error from mean value. Superscripts show that mean difference is significant at the level of $p \leq 0.05$ by Duncan's New Multiple Range Test.

Conclusion

Among all the radiation doses tested, 0.75 kGy proved to be the most efficient in reducing epiphytic microflora and maintaining the quality of flat leaf spinach that resulted in extension of shelf life hence increasing export potential.

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References

Adamo M, Brizzi M, Magaudda G, Martinelli G, Plossi-Zappala M, Rochetti F, Savagnone F. 2001. Gamma radiation treatment of paper in different environmental conditions: chemical, physical and microbiological analysis. Restaurator **22,** 107-131.

Akhter F, Islam M, Khatun A, Munshi M, Hossain M, Hoque M, Huque R. 2013. Biochemical composition and effects of radiation on sensory, biochemical and physiological quality of fresh spinach (*Spinacia oleracea L.*). International Journal of Biosciences **3,** 25-34.

Barkai-Golan R. 2001. Postharvest diseases of fruits and vegetables: development and control, ed.Elsvier. 267-279 P.

Benson H. 2001. Microbiological applications lab manual. The McGraw Hill Companies, 265-301 P.

Bon VI, Jacobson A, Monday SR, Feng PC. 2008. Microbiological quality of bagged cut spinach and lettuce mixes. Applied and Environmental Microbiology **74,** 1240-1242.

Correll JC, Morelock TE, Black MC, Koike ST, Brandenberge LP, Dainello FJ. 1994. Economically important diseases of spinach. Plant Disease **78,** 653-660.

Farkas J. 1998. Irradiation as a method for decontaminating food: A review. International Journal of Food Microbiology **44,** 189-204.

Geweely NS, Nawar LS. 2006. Sensitivity to gamma irradiation of post-harvest pathogens of pear. International Journal of Agriculture Biology **6** ,710-716.

Gihan GA. 2010. Effect of gamma radiation on microbial load and chemical constituents of banana fruits stored under different temperatures. Research Journal of Agriculture and Biological Sciences **6(4),** 431-442.

Gomes CLFDL. 2010. Radiosensitization strategies for enhanced e-beam irradiation treatment of fresh produce. In: (Ed.), Texas A&M University 120-135 P.

Gutierrez-Rodriguez E, Lieth H. J, Jernstedt JA, Labavitch JM, Suslow TV, Cantwell MI. 2013. Texture, composition and anatomy of spinach leaves in relation to nitrogen fertilization. Journal of Science Food and Agriculture **93,** 227-237.

Holmes B, Willcox W, Lapage S. 1978. Identification of enterobacteriaceae by the ApI 20E system. Journal of Clinical Pathology **31,** 22-30.

Jones HE, West HM, Chamberlain PM, Parekh NR, Beresford NA, Crout NM. 2004. Effects of gamma irradiattion on *Holcus lanatus* and associated microorganisms. Journal of Environmental Radioactivity **74,** 57-71.

Lal S, Singh R, Kumar R. 2013. Heavy metal concentration and bacterial load on spinach (*Spinacia oleracea L.*) phyllosphere under different regions in lucknow, uttar pradesh. International Journal of Pharmacy and Life Sciences **4,** 2823-2827.

Oladele OO. 2011. Microorganisms associated with the deterioration of fresh leafy indian spinach in storage. Journal of Plant Pathology and Microbiology **2,** 2.
http://dx.doi.org/10.4172/2157-7471.1000110

Pereson J J, Beecher GR, Bhagwat SA, Dwyer JT, Gebhardt SE, Haytowtiz DB, Holden JM. 2006.Flavanones in grapefruit, lemons, and limes: A compilation and review of the data from analytical literature. Journal of food consumption and analysis **19,** 74-80.

Stone H, Bleibaum R, Thomas HA. 2012. *Sensory evaluation practices*. Academic press, City. 28-86.

Temur C, Tiryaki O. 2013. Irradiation alone or combined with other alternative treatments to control postharvest diseases.African Journal of Agriculture Research **8,** 421-434.

Waseem K, Nadeem MA. 2001. Enhancement of production by varying sowing dates, row spacing and frequency of cuttings. Online Journal of Biological Sciences **1,** 902-904.

WHO. 1981. Wholesomeness of irradiated food, World health organization technical report series 659 on line available at.
http://apps.who.int/iris/handle/10665/41508