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Targeting the Residential Microflora to Enhance the Mean Life of Raw *Lycopersicum esculentum* by Gamma Irradiation

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The effects of gamma irradiation on the microbial content and sensory attributes of tomato (*Lycopersicum esculentum*) were analyzed. Fresh fruits harbor high contaminating microflora influencing its quality and mean life. Irradiation (1.0, 1.5, 2.0 kGy) as cold pasteurization followed by storage under refrigerated conditions have been employed to ensure the microbial safety and extend the shelf life of tomatoes. In this study radiation dose of 2.0 kGy worked remarkably well to reduce both the bacterial as well as mold and yeast counts while preserving the sensory qualities for the longest duration of time when compared to other treatments. The shelf life of samples exposed to 2.0 kGy was extended to 21 days at 4° C against only 14 days for control samples.

Key words: Microflora, Tomato, Radiation, Shelf Life.

Tomatoes are the world's second largest vegetable crop, with more than 80 million tons grown each year[1] It is immensely cultivated throughout the world with its utility growing day by day. It is famous for its color, flavor & nutritional value. Tomatoes contribute to a healthy and proportionate diet. They are rich source of minerals, vitamins, vital amino acids, sugars and dietary fibers. Tomatoes harbors high amount of lycopene (71.6%), vitamin C (12.0%), pro vitamin A carotenoids (14.6%) vitamin E (6.0)[2]**.** Lycopene which is primarily accountable for the distinctive deep red color of ripe tomato fruits and tomato products has gained a lot of attention owing to its positive effects in the healing many diseases[3] Extensive scientific studies have found that this pigment appears to have strong antioxidant capabilities reducing the risk of certain human cancers related to prostate, lung and stomach[4] and chronic ailments such as cardiovascular disease[5].

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It is evident that vegetables have short mean life and consequently sustain maximum post-harvest losses[6] Factors accounting for low yield of tomato include inappropriate agronomic practices, continuous mono cropping, use of non-certified seed and a number of biotic and abiotic stresses. However, postharvest wastage is still a significant contributor towards low return per unit area and time. According to rough estimates about 30%-40% of vegetables/fruits are wasted due to carelessness and deficient processing facilities[7] The post harvest decay can be attributed to certain bacteria, fungi and nematodes etc. Microbial studies carried on various vegetables indicated a predominance of bacterial soft rot causing *Erwinia*, *Pseudomonas*, water and soil pathogens, yeast and moulds. Gram negative bacteria are the major cause of spoilage in fruits thriving and multiplying faster under ambient temperatures and high humidity surroundings[8]. Four bacterial pathogens of tomato are prevalent worldwide namely *Ralstonia solanacearum*, *Clavibacter michiganensis*, *Xanthomonas campestris* and *Pseudomonas syringe*. *Ralstonia solanacearum* is the causal agent of bacterial wilt[9]

An effort to lengthen the shelf life by plummeting the epiphytic microflora from vegetables and fruits by means of appropriate technologies is worthwhile. Such technology will intend to serve an incredible economic part and enhance the accessibility of vegetables to the consumers round the year. Conventional technologies are being used for postharvest disease control and preservation. Among previous techniques of preservation are drying, refrigeration, and fermentation. Latest methods include canning, pasteurization, freezing, irradiation, addition of chemicals and wax coatings[10] In 1976, the joint committee of FAO/IAEA/WHO recognized that irradiation can be used for food preservation. Literature has prescribed the Ionizing Radiations (Gamma radiations, e beam and X rays) as a promising technique for expanding the shelf life of food supplies by elimination of pathogenic bacteria, disinfestation of fresh fruits and vegetables as a postharvest quarantine treatment and microbial reduction[11]. Gamma radiations have been established to be energetically more potent than the x-rays[12]. This technique is therefore superior to other as gamma radiation has effective and homogeneous penetration power in tissues, needs a reasonably short radiation time and does not elevate the temperature of the specimen[13] Gamma radiation doses ranging between 1 and 3 kGy may eliminate molds. Most fruits and vegetables endure irradiation at a nominal dose of 0.25 kGy, without experiencing a change in quality. 2.25 kGy is usually the optimal dose that fruits and vegetables may tolerate while keeping the quality intact (change in flavor /taste, loss of firmness, hastened ripening, physiological breakage)[14].

The use of irradiation, with doses less than 1 kGy for fruits and vegetables slows down the ripening of fruits, inhibits germination, and eliminates insect infestation[15]. Commercial food radiation practice is on increase in Asia[16]. Many countries have started taking steps for the implementing this technology. Implementing the postharvest management practices will assist in reducing high postharvest wastage, raising production surplus by expanding the mean life of fresh produce. This will aid in stabilizing the rates in domestic markets along with significantly increased export opportunities to vastly profitable International markets.

MATERIALS AND METHODS

Sample collection

Fresh Roma tomatoes were collected from local market of Lahore. The fruits were apparently of good quality and without any physical injury.

Radiation Treatment

The tomatoes were sealed into polythene bags and treated with the gamma radiations (source Cobalt 60) at 1.0, 1.5, 2.0 kGy in radiation unit of Pakistan Radiation Services (PARAS). The dose rate during each treatment was 60 Gy/hr. Harwell Amber 3042 dosimeter was used for dose measurement. After radiation treatments, both the experimental (1.0, 1.5, 2.0 kGy) and control groups of tomatoes were stored under refrigerated conditions. Tomatoes were observed after every 7 days for epiphytic microbial load and sensory attributes till 21 days.

Microbial Analysis

Isolation of microorganisms was performed using serial dilution technique. The tomato was shaken methodically in 0.9% saline water to make full strength stock. The nutrient media used for microbial studies include Nutrient agar (for non fastidious bacterial isolation), MacConkey agar (for Gram-negative enteric bacilli isolation) Salmonella-Shigella agar (for *Salmonella* sp. and *Shigella* sp. isolation) and Potato Dextrose agar (for fungi isolation). The inoculated media plates were placed in the incubator at 37°C for 24 hours and at 30°C for 72 hours for bacterial and fungal growth respectively. Number of colony-forming units (CFU) was calculated according to Yousef et al[17]. Gram and Endospore staining of the bacteria was carried out. Enteric bacteria were identified with the API-20E test kit. Fungal species were determined on the basis of micro and macroscopic characteristics using Methylene Blue as staining dye.

Sensory Evaluation

For carrying out the sensory evaluation the tomatoes from the experimental and control group of fruits were cleansed under tap water and each one was divided into eight pieces using pre sterilized knife. The tomato juice was obtained by squeezing the pieces in a commercial juicer (National juicer blender, Model MJ-130N). The juice from Irradiated and non-irradiated fruit samples was given to panelists following radiation

treatment for sensory assessment. The practice undertaken for this assessment was related to that reported by Min et al[18]. Twenty five panelists belonging to the Department of Biotechnology, LCWU, Lahore took part in the sensory investigations. 15 ml of each sample was provided into 20 ml polypropylene bottles with polyethylene screw-cap labeled with 3 digits arbitrarily numbered. In addition, a glass of drinkable water and a piece of non-salted cracker were given to panelists for removing the remaining flavor amid tests. The panelists were requested to score the preference of odor, color, taste and overall acceptability in a hedonic scale ranging from 0 to 9 where (9= like extremely, 8= like very much, 7= like fairly, 6= like vaguely, 5= neither like nor dislike, 4= dislike vaguely, 3= dislike fairly, 2= dislike very much, 1= dislike extremely). A score of 4 or below was considered as objectionable and taken to specify the end of shelf-life.

Statistical Analysis

The data was statistically analyzed to evaluate the credibility and usefulness of information by using Costat program (version 6.4.). Duncan Multiple Range Test was carried out and difference at $p \leq 0.05$ was taken to be statistically significant.

RESULTS AND DISCUSSION

Sensory analysis

Sensory characteristics are fundamental for acceptance of food quality. The current study aimed at increasing the shelf life of tomatoes. The accomplishment of such an aim would rely on the acceptability profile of treated and stored samples. Preservation of sensory qualities owing to treatment and storage for longer duration of time can be regarded as a productive attempt. On the contrary an undesirable decline in acceptance may pinpoint towards a possibility for advancement in the practices or management or storage environments so as to efficiently enhance the shelf life without a great deal of modifications in the sensory attributes of stored tomatoes. In the current study sensory analysis of treated and untreated tomatoes stored at refrigerated temperatures were evaluated. A contrast was made to determine the alterations in sensory qualities of stored tomatoes previously exposed to different doses of gamma irradiation.

The mean life behavior of tomatoes based on sensory qualities such as color, odor, taste and overall acceptability was evaluated (Fig 1 and 2). It is indicated that gamma irradiation at doses of 1.0

Table 1. Impact of gamma irradiation on sensory qualities of tomato juice during storage at 4°C

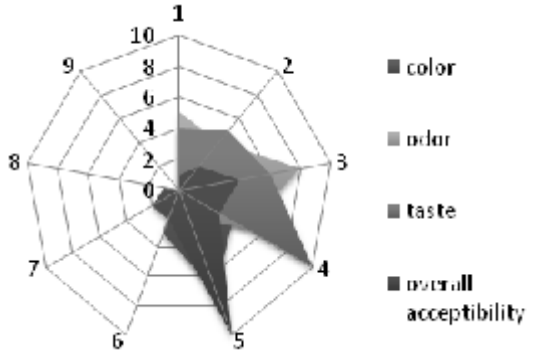
Sensory Parameters	No of Days Post Treatment	Gamma Radiation Treatment (kGy)			
		0.0	1.0	1.5	2.0
Color	0	8.7±0.05	8.6 ± 0.05	8.6 ± 0.1	8.5 ± 0.05
	7	7.4±0.05	7.9 ± 0.10	7.9 ± 0.05	8.0 ±0.05
	14	3.6 ± 0.05	6.7± 0.25	7.1 ± 0.25	7.3 ±0.05
	21	——	4.0 ± 0.20	6.2 ± 0.15	6.5 ± 0.05
Odor	0	8.8 ± 0.05	8.7 ± 0.05	8.7 ± 0.03	8.0 ± 0.15
	7	7.5 ± 0.05	8.3 ±0.05	8.4 ± 0.05	7.9 ±0.25
	14	4.0 ± 0.03	6.7 ±0.15	7.1 ± 0.15	7.4 ± 0.05
	21	——	5.1 ± 0.25	6.6 ± 0.03	7.1 ± 0.05
Taste	0	9.0 ± 0.05	8.8 ± 0.05	8.7 ± 0.10	8.2 ± 0.05
	7	7.7 ± 0.20	7.9 ±0.10	7.7 ± 0.20	7.1 ± 0.15
	14	——	6.7 ±0.40	7.2 ± 0.25	7.2 ± 0.15
	21	——	3.5 ±0.25	4.0 ± 0.05	7.1 ± 0.25
Overall Acceptability	0	8.8 ± 0.20	8.7 ± 0.10	8.7 ± 0.20	8.1 ± 0.15
	7	7.7 ± 0.25	7.9 ± 0.40	7.7 ± 0.40	7.5 ±0.10
	14	——	6.7 ± 0.20	7.2 ± 0.25	7.2 ±0.10
	21	——	3.6 ± 0.10	3.9 ± 0.15	7.2 ± 0.05

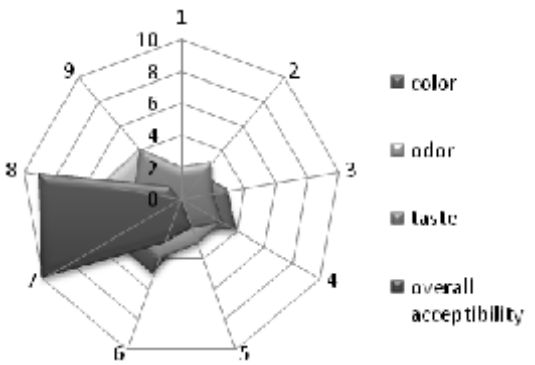
kGy and 1.5kGy had no noteworthy changes in the colour, odor, taste and overall acceptability. However, although the highest dose of 2.0kGy did not affect the color of the tomatoes it did considerably affect the odor, taste (Table 1) and overall acceptability of samples where the panelists gave these samples the minimum but still acceptable scores. Table 1 also specified that as the days progress the sensory qualities scores for irradiated and non irradiated samples declined but at different rates. This decrease was more profound in the non irradiated samples as compared to the irradiated ones. The sensory assessment of irradiated tomatoes showed that superior dosages of gamma irradiation effected textural properties, flavor and aroma and with escalating levels of irradiation, panelists observed a reduction in fresh tomato aroma and flavor and a rise in ripe tomato aroma[19]. The control sample was primarily discarded after the first week of storage due to offensive aroma and color changes. Therefore these samples could not be evaluated for flavor on the 14th day of storage. Panelists objected to the qualities of samples irradiated at 1.0 and 1.5kGy after 21 days of storage at 4°C. In general the sensory scores of the experimental and control group of samples were not considerably different immediately after irradiation. Though, the sensory attributes of the non-irradiated carrot and kale juice reduced with time, and it was the worst at the 3rd day storage as reported by Song et al[20].

Microbial Analysis

Despite of supplying food safety measures to a high level, microbiological hazards occurs. The principle causes for postharvest losses of tomatoes are decay, microbial spoilage, external damages incurred during harvest and handling, and harvest at an improper maturity stage all over the world [6]. Microbiota analysis indicated that the control tomatoes harbored a large amount of bioburden owing to the high contaminating microflora of the raw tomatoes acquired from the soil, water or handling. In the recent years, the use of gamma irradiation as sterilization technique has gained much of attention [16] so, gamma radiation from cobalt-60 source was used to target the micro-organisms present on the surface of tomatoes so that they can be stored for long period of time. Different doses of gamma radiation i.e. 1 kGy, 1.5 kGy and 2.0 kGy were given to tomatoes and then tomatoes were kept at refrigerated temperature.

Four types of media were used for the identification of micro-organisms present on the surface of tomatoes. Nutrient agar was used for the isolation of non-fastidious bacteria. MacConkey agar was used for the isolation of gram negative bacteria and salmonella shigella agar is a differential media which was used for the isolation of *Salmonella* and *Shigella* species. Potato dextrose agar was used for isolation of fungi present on the surface of tomatoes. The quantitative analysis of bacterial population grown on nutrient, MacConkey and Salmonella Shigella agar also showed that total viable count was found maximum on control group and it decreased with increasing radiation doses and minimum bacterial





Sensory evaluation for qualities like color, odor, taste and overall acceptance on the scale of 1 to 9 where 9= extremely like, 1= extremely dislike. (By trained panel members, n=25)

Fig. 1. Sensory evaluation for the quality of irradiated samples

Fig. 2. Sensory evaluation for the quality of un-irradiated samples

population was observed at 2 kGy. These results are in line with those of Farkas *et al*.[21] who concluded that the treatment of gamma irradiation at all does significantly reduced the load of yeast, mold and bacteria.

Enumeration of bacteria present on nutrient agar

Initially the number of bacterial colonies on nutrient agar were 1.75×10^5 cfu/ml for control samples and 1.15×10^5 cfu/ml (1 kGy), 7.4×10^4cfu/ml (1.5 kGy) and 6.0×10^4 cfu/ml (2kGy) for irradiated ones indicating that irradiated samples harbored much less amount of bacteria than the non irradiated ones (Figure 3). During the second week of analysis increase in the number of bacterial colonies were observed both in control and irradiated samples. The second week results revealed that the bacterial population was significantly lower for tomatoes irradiated at 2 kGy dose when compared with bacterial counts for other doses. Bacterial colonies isolated in the third week showed a progressive increase in the bacterial growth on both controls and irradiated tomatoes but followed the same trend as that of the second week. The radiation dose of 2 kGy was the superior one to effectively control the bacterial content among all other doses.

Enumeration of bacteria present on MacConkey agar

The number of colonies of gram negative bacteria observed was 1.3×10^4cfu/ml and

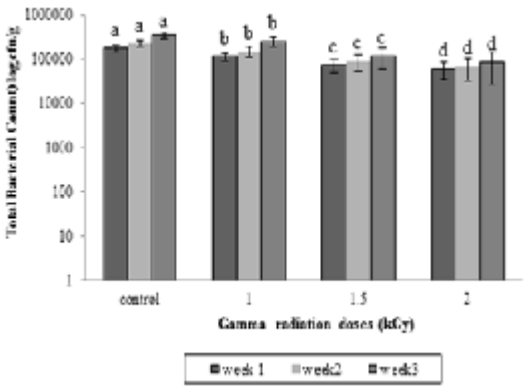


Fig. 3. Impact of different gamma radiation doses on bacteria present on the tomato using nutrient agar as testing media

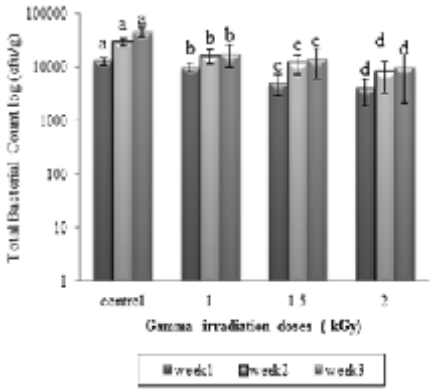


Fig. 4. Impact of different gamma radiation doses on bacteria present on the tomato using MacConkey agar as testing media

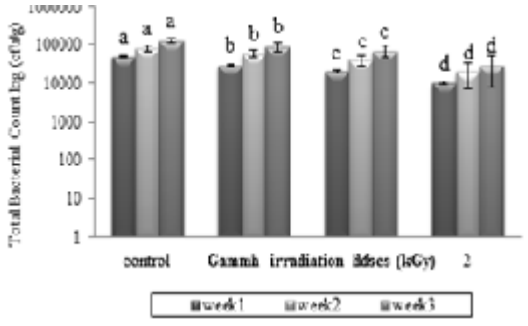


Fig. 5. Impact of different gamma radiation doses on bacteria present on the tomato using salmonella shigella agar as testing media

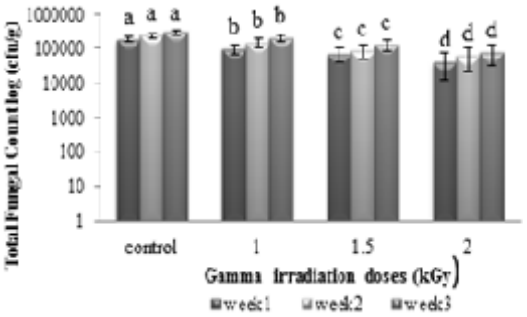


Fig. 6. Impact of different gamma radiation doses on fungi present on the tomato using potato dextrose agar as testing media

Each bar is the means of five parallel replicates. The error bars indicate the standard deviation from the mean value. Letter (a, b, c and d) signifies that the means vary significantly at $p< 0.05$ according to Duncan New Multiple Range test

2.0×10^4cfu/ml for control sample during first and second week of analysis respectively (Figure 4). The number of colonies of bacteria was reduced to a level of 1.0×10^4cfu/ml (first week) and 1.6×10^4cfu/ml (second week) after irradiation at 1 kGy. However, when tomatoes were exposed to gamma radiation dose of 1.5 kGy, the number of colonies of bacteria reduced to 5×10^3cfu/ml (first week) and 7×10^3cfu/ml (second week) and for 2 kGy, the number of colonies of bacteria further reduces to 2×10^3 cfu/ml (first week) and 4×10^3cfu/ml (second week) respectively. The microbial analysis for the third week revealed the fact that gamma irradiation has the potential to control the number of colonies of bacteria present on MacConkey agar. During third week, infinite number of colonies was observed on control sample i.e 5.7×10^4 cfu/ml while the tomatoes irradiated at 1 kGy, 1.5kGy and 2 kGy doses were having significantly ($p \leq 0.05$) lower number of gram negative bacteria having cfu/ml value of 1.8×10^4, 9×10^3 and 5.8×10^3 respectively. Gram negative bacteria identified on MacConkey agar were *Salmonella choleraesuis* and non-fermenter sp.

Enumeration of bacteria present on Salmonella Shigella agar

Effect of radiation on gram negative lactose non-fermenting bacteria is shown in Figure 5. Initially the number of colonies of bacteria on control sample was observed to be 5×10^4cfu/ml whereas the number of colonies of bacteria at dose 1 kGy was found to be 3×10^3cfu/ml while at dose 1.5 kGy and 2 kGy, the number of colonies of bacteria was reduced to level of 2×10^3cfu/ml and 1×10^3cfu/ml respectively. It was observed that in second week, the number of colonies of bacteria was reduced to 6×10^3 cfu/ml,4×10^3 cfu/ml and 2×10^3 cfu/ml at doses 1 kGy, 1.5kGy and 2 kGy that was significantly lower than the number of colonies of bacteria on the control (8×10^4cfu/ml). During the third week, lower number of colonies of bacteria 3×10^3 cfu/ml was observed at dose of 2 kGy as compared to 9×10^3 cfu/ml at dose 1 kGy and 7×10^3cfu/ml at 1.5 kGy.

Different staining techniques were performed like Gram staining, Endospore staining and motility test. 20 E API strips were used for the identification of gram negative bacteria present on the surface of tomatoes. Three types of species that were identified include *Salmonella choleraesuis, Shigella sonnei* and non-fermenter specie.

Enumeration of fungi on potato dextrose agar

Most of the spoilage of tomatoes is caused by fungal pathogens[22]. It is therefore of utmost importance to control these pathogenic fungi for the prevention of post harvestlosses. The effect of radiation on fungi is given in Figure 6. Control sample showed higher number of colonies of fungi in the first week of analysis i.e 1.90×10^5 cfu/ml. However, tomatoes exposed to gamma irradiation, showed much lower number of colonies 1.0×10^5 cfu/ml, 7.3×10^4cfu/ml and 4.4×10^4 cfu/ml during first week of analysis at doses 1, 1.5 and 2 kGy. During second week, the number of colonies of fungi increases to (2.47×10^5 cfu/ml) in the control sample but it was significantly ($p\leq0.05$) lower than the irradiated samples. The number of colonies of fungi in irradiated samples was observed to be 1.5×10^5cfu/ml at 1 kGy, 9.0×10^4 cfu/ml at 1.5 kGy and 6.2×10^4cfu/ml at dose of 2 kGy. During third week the results showed the same trend with the dose of 2 kGy being most efficient in reducing the microfora from 2.62×10^5cfu/ml to 8.1×10^4 cfu/ml. So gamma irradiation has proved to be highly important technique for delaying the fungi proliferation in irradiated samples. Based on the macroscopic and microscopic characteristics, the dominant fungi to be identified were *Aspergillus niger, Aspergillus flavus, Alternaria alternate* and *Fusarium oxysporum.*

CONCLUSION

It was concluded that tomatoes showed increase in shelf life upto 21 days and 2 kGy was acceptable radiation dose for reducing the bioburden on the surface of tomatoes having no significant effect on sensory properties, firmness and antioxidant vitamins.

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REFERENCES

1. Kalyoncu, F., Tamer,A. U.,Oskay.M. Determination of fungi associated with tomatoes (*Lycopersicum esculentum* M.) and tomato pastes. *Plant Path*., 2005; **4**:146-149.
2. García-Closas, R., Berenguer,A., Tormo,M. J., Sánchez,M. J., Quiros,J. R. Navarro, C., Arnaud, M.R., Dorronsoro, Chirlaque,M. D.,Barricarte, A. Dietary sources of vitamin C, vitamin E and specific carotenoids in Spain. *Br J Nutr.*,2004; **91**: 1005-1011.
3. Shi, J., Maguer,M. L. Lycopene in tomatoes: chemical and physical properties affected by food processing. *Crit Rev Food Sci Nut* ., 2000; **40**: 1-42.
4. Giovannucci, E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J. Natl Cancer inst*., 1999; **91**: 317-331.
5. Sesso, H. D., Liu,S., Gaziano,J. M.,Buring, J. E. Dietary lycopene, tomato-based food products and cardiovascular disease in women. *J. Nutr.,* 2003; **133**: 2336-2341.
6. Saeed, A. F. U. H., Khan, S. N. Post harvest losses of tomato in markets of district Lahore. *Mycopath*., 2010; **8**: 97-99.
7. Zaman, W., Paul,D., Alam,K., Ibrahim,M., Hassan,P. Shelf life extension of banana (*Musa sapientum*) by gamma radiation. *J. Biol. Sci*., 2007; **15**: 47-53.
8. Tirado, M., Clarke,R., Jaykus,L., McQuatters-Gollop, A.,Frank,J. Climate change and food safety: A review. *Food Res Int*., 2010; **43**: 1745-1765.
9. Fujiwara, A., Kawasaki,T., Usami,S., Fujie, M., Yamada,T. Genomic characterization of *Ralstonia solanacearum* phage ÆRSA1 and its related prophage (ÆRSX) in strain GMI1000. *J. Bacteriol*.,2008; **190**: 143-156.
10. Shi, X., Namvar,A., Kostrzynska,M., Hora,R.,Warriner, K. Persistence and growth of different *Salmonella serovars* on pre-and postharvest tomatoes. *J. Food Protect*. 2007; **70**: 2725-2731.
11. Follett, P. A., Weinert,E. D. Comparative radiation dose mapping of single fruit type and mixed fruit boxes for export from Hawaii. *J. Food Process Preserv*.,2009; **33**: 231-244.
12. Fan, X., Sommers, C. H. Food irradiation research and technology. John Wiley & Sons, 2nd 2012; pp. 29-46.
13. Dionísio, A. P., Gomes,R. T. Oetterer,M., Ionizing radiation effects on food vitamins: a review. *Braz. Arch. Biol. Technol*.,2009; **52**: 1267-1278.
14. Lacroix, M.,Ouattara,B. Combined industrial processes with irradiation to assure innocuity and preservation of food products—a review. *Food Res. Int*.,2000; **33**: 719-724.
15. Sidrak, G. and A. Suess. Effects of low doses of gamma radiation on the growth and yield of two varieties of tomato. *Radiat. Bot*., 1973; **13**: 309-314.
16. Kume, T., Furuta,M., Todoriki,S.,Uenoyama,N., Kobayashi, Y. Status of food irradiation in the world. *Radiat. Phys. Chem*.,2009; **78**: 222-226.
17. Yousef, A. E.Carlstrom,C. Food microbiology: a laboratory manual. John Wiley & Sons., 2003.
18. Min, S., Jin, Z. T., Zhang,Q. H. Commercial scale pulsed electric field processing of tomato juice. *J. Agric. Food Chem*., 2003; **51**: 3338-3344.
19. Prakash, A., Manley,J., DeCosta,S., Caporaso,F.,Foley,D. The effects of gamma irradiation on the microbiological, physical and sensory qualities of diced tomatoes. *Radiat. Phys. Chem*.,2002 ; **63**: 387-390.
20. Song, H.-P., Byun,M.-W., Jo,C., Lee,C.-H.,Kim,K.S.,Kim,D. H. Effects of gamma irradiation on the microbiological, nutritional, and sensory properties of fresh vegetable juice. 2007. *Food Control*., **18**: 5-10.
21. Farkas, J., Saray,T., Mohacsi-Farkas,C., Horti, K.,Andrassy,E. Effects of low-dose gamma radiation on shelf-life and microbiological safety of pre-cut/prepared vegetables. *Adv. Food Sci*., 1997; **19**: 111-119.
22. Prakash, A., Chen,P.C., Pilling,R. L.,Johnson, N.,Foley,D. 1% calcium chloride treatment in combination with gamma irradiation improves microbial and physicochemical properties of diced tomatoes. *Foodborne Pathog Dis*., 2007; **4**: 89-98.