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DEVELOPMENT OF GAMMA-IRRADIATED LOW MICROBIAL VEGETABLE SALADS FOR IMMUNOCOMPROMISED PATIENTS

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Abstract
Immunocompromised peoples cannot eat raw, uncooked or undercooked foods because of the associated high risk of infection. One way to overcome this situation is the use of ionizing radiation applied to food. This work covers the research and development of some salads, which due to the normal risk of adverse microbiological contamination, are not usually served to immunocompromised hospital patients. Cucumber, tomato, carrot, green leaf lettuce and green capsicum were treated with 1, 2, 2.5 and 3 kGy radiation from a ^{60}Co gamma irradiator. Changes of the "native" microflora, and some specific nutritional and physical-chemical properties of irradiated salad vegetables were analysed. It was observed that generally 1 kGy irradiated samples had less nutritional loss and better sensory score than the samples irradiated with higher doses. But the initial microflora of the samples were so high that minimum doses required to meet the sanitary microbiological levels suggested for foods intended for immunocompromised people and other potential target groups were 2, 2.5, 2.5 and 2 kGy for cucumber, tomato, carrot and green capsicum respectively. In case of green leaf lettuce the criteria were not met even at above radiation doses. The initial microflora of the samples will have to be reduced before irradiation to meet microbiological sanitary criteria at low dose treatment before safely recommend irradiated salads for hospitalized immunocompromised peoples and other target groups.

Keywords: Microbiological sanitary criteria, irradiation, sensory, biochemical, salads

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1.INTRODUCTION

Fruits and vegetables are important items of a healthy diet. So, there is an international trend to increase their consumption (FAO/WHO, 2004). There is an increasing trend in many countries to centrally prepare and process fresh fruits and vegetables for distribution and marketing. Since vegetables are often grown, processed or packed in areas that may be exposed to microbial pathogen contamination, there is an increasing concern that fresh, pre-cut vegetables may harbor microbial pathogens (IAEA, 2006). Fresh vegetables at harvest have a natural epiphytic microflora much of which is non-pathogenic. During any of the steps- growth, harvest, processing, packaging, transportation, handling, retail etc. further microbial contamination can occur from a variety of sources, e.g. environmental, animal or human. There is a risk that this may include pathogens (FAO/WHO, 2008).

Prepared salads are generally considered safe to eat by consumers (FSA, 2007). Salad preparation often involves handling of pre-cooked or ready-to-eat ingredients with little or no further cooking steps to reduce the microbial risk. Two main pathways to contaminate the final product are- improper handling of ingredients and contamination after processing. Fresh vegetables have been implicated as vehicles for the transmission of microbial food-borne disease worldwide (Beuchat, 2006). Problems linked with pathogens in fresh produce, including the associated public health and trade implications,

have been reported in a number of countries worldwide (FAO/WHO, 2008). In recent years the importance of prepared salads as potential vehicles of infection has been highlighted by several large outbreaks both nationally and across international boundaries (Little and Gillespie, 2008).

Although everyone is susceptible to food-borne diseases, certain segments of the population are particularly at risk of contracting a food-borne illness, namely the immunocompromised, infants, young children, the elderly, pregnant women, travelers (WHO, 2000), astronauts, post-operative patients (IAEA, 2010) etc. Immunocompromised hospital patients are estimated to be 20% of the total world population. People with immune-compromised systems cannot eat several types of food because of the associated high risk of infection. Among these foods are fruits, raw vegetables, raw eggs and food made with them, raw fish, unpackaged and undercooked meats, unpasteurized creams and cheeses, ice-cream, uncooked nuts, and dried fruits (IAEA, 2010). The immunocompromised are not only more susceptible to infections, but suffer more serious sequelae as a result of infection. Infections of healthy adults with food-borne pathogens usually result in self-limiting gastroentritis that does not require antibiotic therapy. However, the immunocompromised persons are at increased risk of complications (septicemia, arthritis, meningitis, pneumonia) and death, even if the infecting dose is low (Trevejo *et al.*, 2005). So, ensuring food safety is especially important for people who have impaired immune systems (IAEA, 2010).

Sanitary microbiological levels given by the 1st Research Coordination Meeting (CRP 15052/RO) of International Atomic Energy Agency (IAEA) suggested for foods intended for immunocompromised people and other potential target groups are shown in Table 1**.** These criteria have been derived from Brazilian guidelines, the International Commission on Microbiological Specifications for Foods (IAEA, 2010), information in a scientific paper by Pizzo *et al*. (1982), European Regulations on food hygiene and criteria recommended by Ju-Woon Lee that were certificated for use in space flight conditions by the Russian Institute for Biomedical Problems (IAEA, 2010).

Food irradiation is the treatment of food by a certain type of energy (ICGFI, 1999). Food irradiation could be beneficial to society in general and in particular to immunocompromised patients who require high sanitary standards and whose diets are currently restricted to heat treated foods. The application of irradiation in combination with other preservative technologies can contribute to addressing the pressing need for low microbial diets in a hospital environment for immunocompromised patients and other target groups (IAEA, 2010).

Table 1. Sanitary microbiological levels suggested for foods intended for immunocompromised people and other potential target groups

Criterion	**Microbiological quality colony-forming unit (cfu) per gram unless specified**
Aerobic Plate Counts	< 500
Listeria spp	not detected in 25 g
Salmonella spp	not detected in 25 g
Yeast and Mould	< 10
Total Coliform	< 10
Staphylococcus aureus	< 10
Aerobic spore count	< 10
Anaerobic spore count	< 10

Reference: IAEA, 2010

Recent research undertaken under a CRP (2002-2006) (IAEA, 2009) on the use of irradiation to ensure the safety and quality of prepared meals established that ionizing radiation, in combination with good manufacturing practices and refrigeration, greatly reduces the risk of food-borne diseases in a wide variety of foods, and results in both nutritional and psychological benefits for immunocompromised patients (IAEA, 2010).
The main objectives of the study were: microbiological quality assessment of fresh-cut cucumbers, tomatoes, carrots, green leaf lettuce and green capsicum; use of different irradiation doses for shelf-life extension of the vegetable salads and for inactivation of pathogenic microbial contaminants; evaluation of some biochemical and nutritional changes in the irradiated products; evaluation the sensorial and physical changes in the irradiated products during refrigerated storage; and identify the optimum irradiation dose for each of the product that reduce microbial load and inactivate pathogens with minimum changes in sensory and physical quality attributes.

2. MATERIALS AND METHODS

Samples collection

The study was conducted on five commonly consumed salad vegetables in Bangladesh: Cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.), carrot (*Daucus carota* L.), green leaf lettuce (*Lactuca sativa* L.), green capsicum (*Capsicum annuum* L.). The samples were purchased from one Kitchen market of Dhaka city during winter and spring, 2011 (January-May).

Samples preparation

The samples were washed into running tap water as we wash in home. Then cucumbers and carrots were first peeled with a sterile peeler then uniformly sliced with a sterile knife on a clean sterile chopping board. Sterilization was done by autoclaving. Tomatoes were only sliced. Lettuce and capsicum were chopped. Stems of all samples were removed. The samples were packed into sterilized (with 15 kGy radiation dose) food grade transparent low-density polythene (LDPE, 200 gauge) and then sealed with a sealer (Impulse Sealer, TEW Electronic Heating Equipment CO. Ltd., Taiwan). Packet size was different according to the amount of sample packed. For five different irradiation doses (0, 1, 2, 2.5, 3 kGy), there were five packets for microbiological analysis containing 30 g of sample each, five packets for biochemical analysis containing 50 g of sample each, five packets for sensory quality analysis each of which containing five small packets with five slices of sample. 0 means non-irradiated sample. All the procedures were done inside laminar hood.

Irradiation of samples

Doses were applied to the samples at room temperature from the Co-60 gamma irradiator source (Located at Atomic Energy Research Establishment, Institute of Food and Radiation Biology, Dhaka, Bangladesh) by calibrating with dose and time basis on central distance from source to sample where these were placed.

Microbiological analysis

The microbial contamination in the samples and the effect of irradiation treatment on the microorganisms was analysed by counting the microbial population on the day of irradiation. The microbiological procedures used to analyse were decimal dilution technique followed by pour plating (Gerard *et al.*, 2004). All the microbiological procedures were done inside a laminar hood. 5 g of sample (25 g for *Listeria* spp.) was homogenized by a autoclaved mortar and pestle and filtered through a sterile muslin cloth to a conical flask with 50 ml saline (0.9% NaCl) water (previously sterilized) to prepare the stock sample. For the enumeration of total aerobic spore these suspensions were heated at 80°C for 10 min in a water bath. 1 ml sample from conical flask was taken in a test tube containing 9 ml of previously sterilized saline water. Thus 10^{-1} dilution was got. This procedure was repeated where further dilution was required. With the help of micropipette, 1 ml of the sample from the test tube was poured

into Petri dishes then sterilized specific media was poured into Petri dishes and shaken horizontally to spread out the sample uniformly over the media. After solidification of the media the Petri dishes were covered with lids. Then the Petri dishes were placed in upturned position in incubator at 37°C (30°C for yeast and mold) for 24-48 hr.

The analyses were enumeration of total aerobic flora, total anaerobic bacteria, total aerobic spore, total yeast and mold, total coliform, *Listeria* spp. and *Staphylococcus aureus*. For microbiological purposes Nutrient Agar, Thioglycollate media, Potato Dextrose Agar, MacConkey Agar, Mannitol Salt Agar were purchased from Scharlau Chemie S.A. (Spain). *Listeria* Selective Agar Base (Oxford formulation) was purchased from Oxoid LTD (England).

For anaerobic bacteria Thioglycollate media was used. After spreading, plates were kept into an anaerobic jar. The lid of the jar was closed. After that a vacuum pump was attached to one port of the jar, and a nitrogen source was attached to another port of the jar. Then the air inside the jar was sucked out with vacuum pump and the jar was filled with nitrogen gas to maintain anaerobic condition inside the jar. Then the jar was put inside the incubator.

Biochemical and nutritional analysis

Determination of moisture content

The change of weight was estimated under certain temperature. The moisture content was determined by drying the sample at some elevated temperature and reporting the loss in weight as moisture (AOAC, 1975).

Determination of ash content

Ash content in the sample was determined according to Carpenter (1960). About 5-10 g of the macerated sample was weighted into a pre-weighted crucible. The crucible with the content was heated first over a low flame till all the material was completely charred. Then charred sample was kept in an Electric Muffle Furnace (Model no.L9/11/C6, Nabertherm, Germany) for 4-5 hours at about 600°C for ashing completely. To ensure the completion of ashing the crucible was again heated for half an hour, cooled and then weighed. The weighed residue was reported as ash.

Determination of ascorbic acid

The estimation of ascorbic acid content was carried out by the titration result of the sample extract with 2, 6-Dichlorophenol-Indophenol dye (BDH Chemicals Ltd., England). The dye which is blue in alkaline solution and red in acid solution is reduced by ascorbic acid to colourless form. The reaction is quantitative and practically specific for ascorbic acid in solutions between the pH ranges 1-3.5 (The association of vitamin chemists, 1966; Johnson, 1948).

Determination of total carotenoid and chlorophyll

Total carotenoid and chlorophyll was estimated by non-maceration method. Total carotenoid and chlorophyll was extracted in 80% acetone and the absorption at 663, 645 and 480 nm read in a spectrophotometer. Using the absorption coefficients, the amount of total carotenoid, chlorophyll “a”, chlorophyll “b” and total chlorophyll was calculated using formula (Hiscox and Isrealstam, 1979).

The sample was cut into small pieces (or thin slices). About 1 g of sample was taken and grinded to a fine pulp in mortar and pestle with about 10 ml of 80% acetone (MERK, Germany). The pulp was centrifuged (Laboratory centrifuge, Model 800, China) at 1790 x g for 5 min. The supernatant was filtered to a 25 ml volumetric flask. The sediment in the centrifuge tube was scraped and ground it again in the same mortar and pestle with a small amount of 80% acetone. The mixture was centrifuged as done earlier and the extract was filtered in the 25 ml volumetric flask (containing the previous supernatant). The homogenate was washed out three to four times with 5 ml of 80% acetone each time. The final volume was made to 25 ml with 80% acetone. The extract was kept in refrigerator for 10 min to lower the temperature. The absorbance of the extract was

taken in spectrophotometer (SYSTRONICS, UV-Vis spectrophotometer, 118, Sr No. 1064, India) at 663, 645 and 480 nm using 80% acetone as the blank.

The absorbance of the carotenoids at 480 nm was determined using the equations provided by Kirk and Allen (1965). This equation compensates for interference at this from chlorophyll. To quantify the carotenoids, Price and Hendry (1991) and Venkatarayappa *et al*. (1984) followed the following equation:

Total carotenoid (mg/g) = A_{480} + (0.114 × A_{663}) – (0.638 × A_{645}) × V/1000 × W

Where, A= Absorbance at given wavelength
V= Final volume of 80% acetone in ml
W= Weight of the sample in grams

The amount of chlorophyll "a", "b" and total are determined using the following formulas given by Arnon (1949) based on the work of Mackinney (1941) who provided the values of extraction coefficients.

Chlorophyll "a" (mg/g) =
(12.7 × A_{663}) – (2.69 × A_{645}) × V/1000 × W
Chlorophyll "b" (mg/g) =
(22.9 × A_{645}) – (4.68 × A_{663}) × V/1000 × W
Total chlorophyll "a"+ "b" (mg/g) =
(8.02 × A_{663}) + (20.2 × A_{645}) × V/1000 × W

Where, A= Absorbance at given wavelength
V= Final volume of 80% acetone in ml
W= Weight of the sample in grams

Sensory analysis

Method developed by Peryam and Pilgrim (1975) was used for sensory evaluation. Following nine points of hedonic scale was used for sensory evaluation by five judges (Miyauchi *et al*., 1964):

9= Like extremely
8= Like very much
7= Like
6= Like slightly
5= Neither like nor dislike
4=Dislike slightly
3= Dislike
2=Dislike very much
1=Dislike extremely

Average sensory score 5 (neither like nor dislike) is usually acceptable in organoleptic evaluation. But because we were intended to supply salads for hospitalized immunocompromised patients and other potential target groups, we had to make sure that they will like the food. So, the acceptability threshold we considered was around 7, which means "like" in hedonic scale. Sensory quality attributes including colour, flavour, taste and texture of minimally processed cucumber, tomato, carrot, green leaf lettuce and green capsicum were evaluated immediately after irradiation and during refrigeration (4 ± 1°C) storage. Our intention was to supply foods as early as possible after irradiation. So, two days sensory scores were observed.

Statistical analysis

Results were expressed as mean ± SD (Standard deviation of mean). One way ANOVA was performed for data analysis. ANOVA was followed by Fisher's Protected Least Square Differences (PLSD) for post hoc comparisons. The statistical program used was StatView®5.0 (MindVision Software, Abacus Concepts, Inc., Berkeley, CA, USA). $p<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSIONS

Microbiological analysis

The extent of contamination by microorganisms in cucumber, tomato, carrot, green leaf lettuce and green capsicum and the effect of different doses of gamma radiation treatment on the contaminated microorganism level on the day of analysis were determined.

Effect of irradiation on total aerobic plate count

Initial total aerobic plate counts were around 5.244, 3.505, 6.352, 6.350, 5.185 log cfu/g in cucumber, tomato, carrot, green leaf lettuce and green capsicum respectively. Khan *et al*. (1992) were collected fresh samples of cucumber, carrot and lettuce from different markets in Dhaka metropolitan city, Bangladesh. Bacterial loads were found to be $7{\cdot}1x10^4$ to $6{\cdot}34x10^8$ cfu/100 g. From our study

around $3.2x10^5$ to $2.25x10^8$ cfu/100 g of aerobic bacterial load was observed initially after washing. Significant differences ($p<0.05$) were detected comparing the control with the results obtained for the irradiated samples (Table 2). At 1 kGy radiation dose, cucumber, tomato, carrot, green leaf lettuce and green capsicum showed approximately 2.943, 0.391, 2.882, 2.511 and 5.185 log cfu/g decrease respectively. At 2 kGy, cucumber, tomato, carrot, green leaf lettuce showed approximately 5.244, 0.903, 6.352, 2.8 log cfu/g decrease respectively. At 2.5 kGy tomato and green leaf lettuce showed approximately 3.505 and 3.095 log cfu/g decrease respectively. Microbiological criteria for immunocompromised people and other potential target groups (Table 1) were met at radiation dose 1, 2.5, 2 and 1 kGy for cucumber, tomato, carrot and green capsicum respectively. In green leaf lettuce the criteria were not met at even 2.5 kGy dose.

Effect of irradiation on total anaerobic plate count

Initial total anaerobic plate counts were around 4.349, 6.114, 4.916, 5.267 log cfu/g in cucumber, carrot, green leaf lettuce and green capsicum respectively. No colonies were detected on tomato. At 1 kGy radiation dose total reduction of anaerobic bacteria were observed in every sample (Table 3).

Effect of irradiation on total aerobic spore count

No spores were detected in any sample except green leaf lettuce (Table 4). Initial total aerobic spore count was around 2.845 log cfu/g in green leaf lettuce. At dose 1 kGy, around 0.845 log cfu/g reduction was observed. At dose 2 kGy, no colonies were detected.

Table 2. Effect of irradiation treatment on total aerobic plate count

Dose / Samples	$\times10^3$ cfu/g				
	0 kGy	**1 kGy**	**2 kGy**	**2.5 kGy**	**3 kGy**
Cucumber	175.5 ± 51.619^a	0.2 ± 0.283^b	-	-	-
Tomato	3.2 ± 1.697^a	1.3 ± 1.697^a	0.4 ± 0.283^b	-	-
Carrot	2250 ± 282.843^a	$2.95\pm.778^b$	-	-	-
Green leaf lettuce	2240 ± 212.132^a	6.9 ± 1.131^b	3.55 ± 0.495^b	1.8 ± 0.283^b	2.15 ± 0.212^b
Green capsicum	153 ± 9.899^a	-	-	-	-

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at $p<0.05$ [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]. cfu/g: Colony forming unit per gram, -: No colony detected (Detection limit ≥10 cfu/g)

Table 3. Effect of irradiation treatment on total anaerobic plate count

Dose / Samples	$\times10^3$ cfu/g				
	0 kGy	**1 kGy**	**2 kGy**	**2.5 kGy**	**3 kGy**
Cucumber	22.35 ± 1.061^a	-	-	-	-
Tomato	-	-	-	-	-
Carrot	1300 ± 28.28^a	-	-	-	-
Green leaf lettuce	82.5 ± 14.849^a	-	-	-	-
Green capsicum	185 ± 7.071^a	-	-	-	-

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at $p<0.05$ [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]. cfu/g: Colony forming unit per gram, -: No colony detected (Detection limit ≥10 cfu/g)

Table 4. Effect of irradiation treatment on total aerobic spore count

Dose / Samples	×10³ cfu/g				
	0 kGy	**1 kGy**	**2 kGy**	**2.5 kGy**	**3 kGy**
Cucumber	-	-	-	-	-
Tomato	-	-	-	-	-
Carrot	-	-	-	-	-
Green leaf lettuce	0.7±.283[a]	0.1±0.0[b]	-	-	-
Green capsicum	-	-	-	-	-

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at $p<0.05$ [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]. cfu/g: Colony forming unit per gram, -: No colony detected (Detection limit ≥10 cfu/g)

Table 5. Effect of irradiation treatment on total yeast and mould count

Dose / Samples	×10³ cfu/g				
	0 kGy	**1 kGy**	**2 kGy**	**2.5 kGy**	**3 kGy**
Cucumber	-	-	-	-	-
Tomato	-	-	-	-	-
Carrot	-	-	-	-	-
Green leaf lettuce	0.6±0.283[a]	-	-	-	-
Green capsicum	-	-	-	-	-

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at $p<0.05$ [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]. cfu/g: Colony forming unit per gram, -: No colony detected (Detection limit ≥10 cfu/g)

Table 6. Effect of irradiation treatment on total coliform count

Dose / Samples	×10³ cfu/g				
	0 kGy	**1 kGy**	**2 kGy**	**2.5 kGy**	**3 kGy**
Cucumber	19.0±2.970[a]	-	-	-	-
Tomato	0.15±0.071[a]	-	-	-	-
Carrot	1975±176.777[a]	0.2±0.141[b]	0.1±0.0[b]	-	-
Green leaf lettuce	127.5±7.778[a]	0.15±0.212[b]	-	-	-
Green capsicum	235±77.782[a]	-	-	-	-

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at $p<0.05$ [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]. cfu/g: Colony forming unit per gram, -: No colony detected (Detection limit ≥10 cfu/g)

Effect of irradiation on total yeast and mould count

No yeast and mould was detected in any sample except green leaf lettuce (Table 5). Initial total yeast and mould count was around 2.778 log cfu/g in green leaf lettuce. At dose 1 kGy, total reduction was observed. Basbayraktar *et al*. (2006) observed that the fungal count of control sample was 5.76 log cfu/g initially and the fungal count of 1 kGy irradiated sample was 3.70 log cfu/g. Our study also showed around 2 log cfu/g decrease of fungal count at 1 kGy. Bibi *et al*., (2006) observed that a dose of 2 kGy in tomato samples was effective in lowering fungal colony to safe limits even after 14 days of storage.

Effect of irradiation on total coliform count

Significant differences ($p<0.05$) were detected comparing the control with the results obtained for the irradiated samples (Table 6). Initial total coliform counts were approximately 4.279, 2.176, 6.296, 5.106, 5.371 log cfu/g in cucumber, tomato, carrot, green leaf lettuce and green capsicum respectively. At 1 kGy radiation dose, cucumber, tomato and green capsicum showed total reduction of coliform whereas carrot and green leaf lettuce showed around 3.995 and 2.93 log cfu/g decrease respectively. At 2 kGy, carrot showed approximately 4.296 log cfu/g reduction. No coliform was detected in green leaf lettuce at 2 kGy. At 2.5 kGy, carrot showed total reduction of coliform. Microbiological criteria (Table 1) were met at radiation dose 1 kGy in cucumber, tomato and green capsicum. In green leaf lettuce and carrot, the criteria were met at 2 kGy and 2.5 kGy respectively. Basbayraktar *et al*. (2006) observed that the dose of 1.0 kGy resulted in the 3 log-cycle reduction of *E. coli* in different minimally processed fruits and vegetables. Hammad *et al*. (2006) predicted that, the 5 log reduction of *E. coli* in fresh produce could be achieved by about 0.55 – 1.55 kGy. We also observed approximately 3 to 5 log-cycle reduction of coliform at 1 kGy.

Effect of irradiation on total *Listeria* spp. count

Initial total *Listeria* spp. counts were around 5.207, 7.122, 6.051, 5.65 log cfu/25 g in cucumber, carrot, green leaf lettuce and green capsicum respectively. At 1 kGy radiation dose, cucumber and green capsicum showed total reduction of *Listeria* spp. (Table 7). Carrot and green leaf lettuce showed approximately 3.724 and 1.612 log cfu/g decrease respectively. At 2 kGy, carrot showed total reduction. At 2 kGy, 2.5 kGy and 3 kGy, green leaf lettuce showed around 2.176, 2.653 and 2.953 log cfu/g reduction respectively. Basbayraktar *et al*. (2006) observed that the dose of 1.0 kGy resulted in the 3 log-cycle reduction of *L. monocytogenes* count. Hammad *et al*. (2006) predicted that, 2.6–3.3 kGy should inactivate 5 log cycles of *Listeria monocytogenes*. We also observed around 1-5 log reduction of *Listeria* sp. at 1-3 kGy. Microbiological criteria (Table 1) were met at radiation dose of 1 kGy, in cucumber and green capsicum. In carrot, at 2 kGy the criteria were met but in green leaf lettuce the criteria were not met at our given radiation doses.

Effect of irradiation on *Staphylococcus aureus* count

Initial total *Staphylococcus aureus* counts were approximately 4.19, 3.161, 2.602, 4.602 and 3.161 log cfu/g in cucumber, tomato, carrot, green leaf lettuce and green capsicum respectively. Significant differences were detected comparing the control with the results obtained for the irradiated samples (Table 8). At 1 kGy radiation dose, cucumber showed total reduction; tomato, carrot, green leaf lettuce and green capsicum showed around 0.86, 0.602, 1.824, 0.763 log cfu/g reduction respectively. At 2 kGy, tomato, carrot and green capsicum showed total reduction. At 2, 2.5 and 3 kGy green leaf lettuce showed 2.204, 2.602 and 2.903 log cfu/g reduction respectively. Basbayraktar *et al*. (2006) and Hammad *et al*. (2006) predicted that, the 5 log reduction of *Staphylococcus aureus* could be achieved by about 2.1 – 2.7 kGy in different minimally processed fruits and vegetables.

Table 7. Effect of irradiation treatment on total *Listeria* spp. count

Samples \ Dose	×10^4 cfu/25g				
	0 kGy	**1 kGy**	**2 kGy**	**2.5 kGy**	**3 kGy**
Cucumber	16.125±0.53^a	-	-	-	-
Tomato	-	-	-	-	-
Carrot	1325±318.198^a	0.25±0.0^b	-	-	-
Green leaf lettuce	112.5±21.213^a	2.75±2.475^b	0.75±0.353^b	0.25±0.353^b	0.125±0.178^b
Green capsicum	44.625±19.623^a	-	-	-	-

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at p<0.05 [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]. cfu/25g: Colony forming unit per 25 gram, -: No colony detected (Detection limit ≥2 cfu/g)

Table 8. Effect of irradiation treatment on *Staphylococcus aureus* count

Samples \ Dose	×10^3 cfu/g				
	0 kGy	**1 kGy**	**2 kGy**	**2.5 kGy**	**3 kGy**
Cucumber	15.5±2.121^a	-	-	-	-
Tomato	1.45±0.778^a	0.2±0.283^b	-	-	-
Carrot	0.4±0.283^a	0.1±0.0^a	-	-	-
Green leaf lettuce	40±0.0^a	0.6±0.141^b	0.25±0.071^c	0.1±0.141^c	0.05±0.071^c
Green capsicum	1.45±0.212^a	0.25±0.354^b	-	-	-

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at p<0.05 [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]. cfu/g: Colony forming unit per gram, -: No colony detected (Detection limit ≥10 cfu/g)

Table 9. Effect of irradiation treatment on moisture content

Samples \ Dose	%				
	0 kGy	**1 kGy**	**2 kGy**	**2.5 kGy**	**3 kGy**
Cucumber	98.05±0.17^a	97.84±0.72^a	97.39±0.34^a	97.66±0.24^a	97.34±0.05^a
Tomato	95.89±0.32^a	95.58±0.06^a	95.64±1.06^a	95.14±0.03^a	94.88±0.16^a
Carrot	92.26±0.16^a	91.29±0.29^a	91.57±0.62^a	90.76±0.11^a	92.14±0.55^a
Green leaf lettuce	96.2±0.14^a	95.67±0.41^a	95.5±0.09^a	96.32±0.1^a	95.73±0.58^a
Green capsicum	94.7±0.13^a	94.78±0.02^a	94.83±0.07^a	94.67±0.06^a	94.56±0.3^a

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at p<0.05 [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]

Table 10. Effect of irradiation treatment on total ash content

Samples \ Dose	**g/100g of fresh weight of edible food**				
	0 kGy	**1 kGy**	**2 kGy**	**2.5 kGy**	**3 kGy**
Cucumber	0.310±0.031^a	0.272±0.013^{ab}	0.229±0.021^b	0.275±0.016^{ab}	0.256±0.012^b
Tomato	0.568±0.011^a	0.524±0.057^a	0.526±0.004^a	0.532±0.008^a	0.538±0.040^a
Carrot	0.475±0.010^a	0.460±0.006^a	0.459±0.023^a	0.475±0.004^a	0.480±0.001^a
Green leaf lettuce	0.461±0.161^a	0.260±0.013^b	0.251±0.002^b	0.273±0.017^{ab}	0.266±0.023^b
Green capsicum	0.203±0.012^a	0.268±0.021^b	0.268±0.004^b	0.283±0.004^b	0.292±0.039^b

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at p<0.05 [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]

Table 11. Effect of irradiation treatment on ascorbic acid content

Samples \ Dose	mg/100g fresh weight of edible food				
	0 kGy	1 kGy	2 kGy	2.5 kGy	3 kGy
Cucumber	6.486±0.374^{ab}	5.737±0.098^{bc}	5.196±0.565^{ce}	7.391±0.0^{ad}	4.553±0.643^{e}
Tomato	6.972±0.0^{a}	6.891±0.0^{a}	6.811±0.0^{a}	6.759±0.0^{a}	6.798±0.0^{a}
Carrot	4.643±0.219^{ab}	5.376±0.272^{b}	4.144±0.374^{ac}	4.184±0.296^{ac}	3.250±0.270^{d}
Green leaf lettuce	3.786±0.765^{a}	3.279±0.0^{a}	2.292±0.0^{b}	2.322±0.0^{b}	2.175±0.0^{b}
Green capsicum	4.982±0.641^{a}	3.906±0.0^{ab}	3.839±0.0^{ab}	3.378±0.682^{b}	3.241±0.655^{b}

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at p<0.05 [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]

Table 12. Effect of irradiation treatment on total carotenoid content

Samples \ Dose	µg/g fresh weight of edible food				
	0 kGy	1 kGy	2 kGy	2.5 kGy	3 kGy
Cucumber	0.328±0.057^{a}	0.249±0.023^{ab}	0.226±0.097^{ab}	0.143±0.032^{b}	0.181±0.017^{b}
Tomato	2.25±0.071^{a}	2±0.141^{a}	2.3±0.283^{a}	2.45±0.071^{a}	2.4±0.283^{a}
Carrot	6.869±2.49^{a}	6.665±0.615^{a}	5.043±0.603^{a}	6.056±2.65^{a}	6.321±0.538^{a}
Green leaf lettuce	24.5±0.707^{a}	24.5±0.707^{a}	18±4.243^{b}	17±2.828^{b}	18±0.0^{b}
Green capsicum	3.74±0.057^{ab}	3.014±0.339^{c}	3.265±0.205^{ac}	3.082±0.318^{c}	2.967±0.109^{c}

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at p<0.05 [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]

Table 13. Effect of irradiation treatment on chlorophyll "a", "b" and total chlorophyll content

Samples	Chl \ Dose	µg/g fresh weight of edible food				
		0 kGy	1 kGy	2 kGy	2.5 kGy	3 kGy
Cucumber	**a**	4.3±0.46^{a}	3.31±1.06^{ab}	3.52±1.09^{ab}	1.96±0.09^{b}	2.07±0.06^{b}
	b	7.76±1.27^{a}	4.39±1.21^{b}	4.44±1.24^{b}	2.91±0.77^{b}	2.55±0.11^{b}
	Total	12.06±0.81^{a}	7.70±2.27^{b}	7.96±2.33^{b}	4.86±0.68^{b}	4.69±0.06^{b}
Tomato	**a**	1.9±0.8^{a}	1.3±0.1^{ab}	0.7±0.1^{bc}	1.1±0^{ab}	0.6±0.1^{bc}
	b	1.7±0.5^{a}	1.3±0.1^{ab}	3.2±0.4^{ac}	3.8±1.1^{c}	4.5±1.1^{c}
	Total	3.50±1.27^{ab}	2.50±0.14^{b}	3.85±0.21^{ab}	4.85±1.06^{ac}	5.10±0.99^{ac}
Carrot	**a**	-	-	-	-	-
	b	-	-	-	-	-
	Total	-	-	-	-	-
Green leaf lettuce	**a**	282±57^{ab}	349±9^{b}	217±51^{ac}	212±30^{ac}	229±21^{ac}
	b	93±23^{ab}	104±1^{b}	71±11^{ac}	76±6^{ab}	77±8^{ab}
	Total	375.5±79.9^{ab}	452.5±10.61^{b}	288±62.23^{ac}	288±35.36^{ac}	306±28.28^{ac}
Green capsicum	**a**	42.5±0.71^{a}	37.55±1.48^{a}	31.1±8.63^{a}	35.5±6.08^{a}	39.2±0.42^{a}
	b	15.8±1.13^{a}	17.45±1.34^{ab}	20.5±2.12^{bc}	17.25±2.47^{ab}	16.83±0.18^{ab}
	Total	58.3±0.42^{a}	55±0.14^{a}	51.6±6.51^{a}	52.75±8.56^{a}	56.03±0.24^{a}

Results are expressed as mean ± SD (Standard deviation) [n=3 replicates of each sample for each radiation dose]. Values in the same row with different superscripts are significantly different at p<0.05 [One-way ANOVA followed by Fisher's PLSD for post hoc comparisons]. Chl: Chlorophyll, -: No chlorophyll detected

We also observed approximately 3-4 log reduction of *Staphylococcus aureus* at 2-3 kGy. Microbiological criteria (Table 1) were met at radiation dose 1 kGy in cucumber; at 2 kGy, in tomato, carrot and green capsicum. In green leaf lettuce the criteria were not met at our given radiation doses.

Effect of irradiation on biochemical and nutritional quality

Similar to other food processing techniques, irradiation can induce certain alterations that can modify the chemical composition and nutritive values of food. Studies have shown that the macronutrients such as proteins, carbohydrates and fat are quite stable to the doses up to 10 kGy. But some vitamins such as Thiamin (B1) and vitamins A, C and E are labile to irradiation (Crawford and Ruff, 1996; WHO, 1994; Wiendl, 1984; Kilcast, 1994; Giroux and Lacroix, 1998).

Effect of irradiation on moisture content

The moisture content is often an important aspect of various foodstuffs as excess moisture can promote microbial growth, which rapidly deteriorates the quality of food. From our study, we observed all the samples had moisture content of more than 90%. So, they were very favorable for microbial growth. No statistically significant changes ($p<0.05$) were observed in irradiated samples comparing to the non-irradiated samples (Table 9). Cucumber had the highest and carrot has the lowest moisture content.

Effect of irradiation on total ash content

Irradiation's effect on permeability and functionality of cell membranes can result in electrolyte leakage and loss of tissue integrity. These effects are limited at dose levels below 1 kGy, but at higher dose levels, electrolyte leakage may cause a soggy and wilted appearance. The increase in electrolyte leakage varies among vegetables. No statistically significant change ($p<0.05$) in ash content was observed in tomato and carrot (Table 10). In cucumber, significant decrease of ash content was observed at dose 2 and 3 kGy. In green leaf lettuce significant decrease of ash content was observed at dose 1, 2 and 3 kGy. In green capsicum statistically significant increase of ash content was observed in irradiated samples. Around 11.29-17.419% decrease of ash content was observed in cucumber due to irradiation. 43.601-45.553% decrease of ash content was observed in green leaf lettuce. And 32.02-43.842% increase of ash content was observed in green capsicum. The decrease in ash content may happen due to electrolyte leakage from the samples because of irradiation. Fan *et al*. (2006) saw that electrolyte leakage increased linearly with increasing radiation dose for all vegetables. All vegetables had radiation thresholds of at least 0.6 kGy. The dose thresholds for most of the fresh-cut vegetables were between 1 and 2 kGy. For green leaf lettuce 1.3 kGy and carrots 0.6 kGy. Green leaf lettuce at 3 kGy 4.1% and Carrots 9.1% electrolyte leakage was observed.

Effect of irradiation on ascorbic acid content

Statistically significant ($p<0.05$) decrease of ascorbic acid was observed at 2 and 3 kGy in cucumber (Table 11). No significant change was observed in tomato. Statistically significant decrease was observed at 3 kGy in carrot; at 2, 2.5 and 3 kGy in green leaf lettuce and at 2.5 and 3 kGy in green capsicum. In cucumber, ascorbic acid content was decreased 11.548-29.803% due to irradiation except dose 2.5 kGy. At 2.5 kGy, ascorbic acid was increased 13.953%. At 1 kGy, carrot showed approximately 15.787% increase then 9.886-30.002% decrease due to irradiation. 13.391-42.552% decrease of ascorbic acid was observed in green leaf lettuce due to irradiation. In green capsicum, 21.598-34.946% decrease was observed due to irradiation. Fan and Sokorai (2002) observed that at low dose levels (≤1 kGy), irradiation can reduce ascorbic acid (vitamin C) in some vegetables, but the decrease is generally insignificant, given the natural variation observed in fresh produce, and does not exceed the decrease seen during storage. Fan and

Sokorai (2005) showed that irradiation converts ascorbic acid to dehydroascorbic acid, both of which exhibit biological activity and are readily interconvertible.

Effect of irradiation on total carotenoid content

Carotenoids are a class of vegetal pigments, and some of them can be converted to vitamin A in human body. The most important precursor of vitamin A is β-carotene, a carotenoid with the highest pro-vitamin A activity (Oliveira and Marchini, 1998). . Bandekar *et al*. (2006) observed that there was no significant difference ($p<0.05$) in total carotenoids in the radiation processed (1-2 kGy) and control carrot and cucumber. Variation in the content carotenoids during storage also was not statistically significant from the control samples. We observed statistically significant ($p<0.05$) decrease of total carotenoid at 2.5 and 3 kGy in cucumber (Table 12). No significant change was observed in tomato and carrot. Green leaf lettuce was showed significant decrease of total carotenoid at 2, 2.5 and 3 kGy. Green capsicum showed statistically significant decrease of total carotenoid at 1, 2.5 and 3 kGy. In cucumber, total carotenoid content was decreased 24.085-56.402% due to irradiation. In green leaf lettuce, no change in total carotenoid content was observed at dose 1 kGy. Around 26.531-30.612% decrease in total carotenoid content of green leaf lettuce was observed due to radiation dose 2-3 kGy. In green capsicum, approximately 12.701-20.668% decrease of total carotenoid content was observed due to irradiation.

Effect of irradiation on chlorophyll content

Salunkhe (1956); Wishnetsky *et al*. (1959) showed chlorophylls are sensitive to irradiation. A linear decrease in the chlorophyll content of green beans and broccoli was directly related to the absorbed dose of 4.9 to 92.9 kGy (Wishnetsky *et al*., 1959). Our study showed no significant total chlorophyll reduction except cucumber at 1-3kGy. Irradiation temperatures, type of vegetables, and headspace atmosphere have an influence on degradation of carotenoids and chlorophylls (Franceschini *et al*., 1959). No chlorophyll was detected in carrot (Table 13). Irradiated tomato, green leaf lettuce and green capsicum didn't show statistically significant changes of total chlorophyll content compared to control.

Effect of irradiation on sensory quality

Historically, the high radiation doses used in attempts to produce a sterile or shelf-stable fruit or vegetable commodity have resulted in unpalatable products. Irradiation may induce the loss of firmness (softening) in some fruits (Gunes *et al*., 2000; Palekar *et al*., 2004). On low dose levels (1 kGy or less), most fresh-cut vegetables show little change in appearance, flavor, color, and texture, although some products can lose firmness. From the sensory quality analysis we observed the change in sensory quality mainly loss of firmness at higher irradiation dose than 1 kGy. Mohácsi-Farkas *et al*. (2006) observed 1 kGy was acceptable radiation dose for the treatment of pre-cut tomato, having no significant effect on sensory properties, firmness and antioxidant vitamins.

Basbayraktar *et al*. (2006) observed that at 1.0 kGy irradiation lack of adverse effects on sensory attributes. Bibi *et al*. (2006) observed that a dose of 2 kGy for carrots, 2.5 kGy for cucumber was sufficient to keep them microbiologically and sensorially acceptable for two weeks at refrigerated temperature. Tomato due to the soft texture and microbial spoilage cannot be stored up to two weeks, a dose of 2.5 kGy for tomato can be recommended for the refrigerated storage up to one week. No significant ($p<0.05$) radiation induced change in sensory quality of cucumber on the day of irradiation.

On day 1, all scores were over acceptability threshold (7). On day 2, 2 kGy and 2.5 kGy irradiated sample's texture score was below acceptability threshold (around 5). In tomato, softening of texture observed at dose 3 kGy on the day of irradiation. 3 kGy irradiated tomato texture score was below acceptability threshold on day 1.

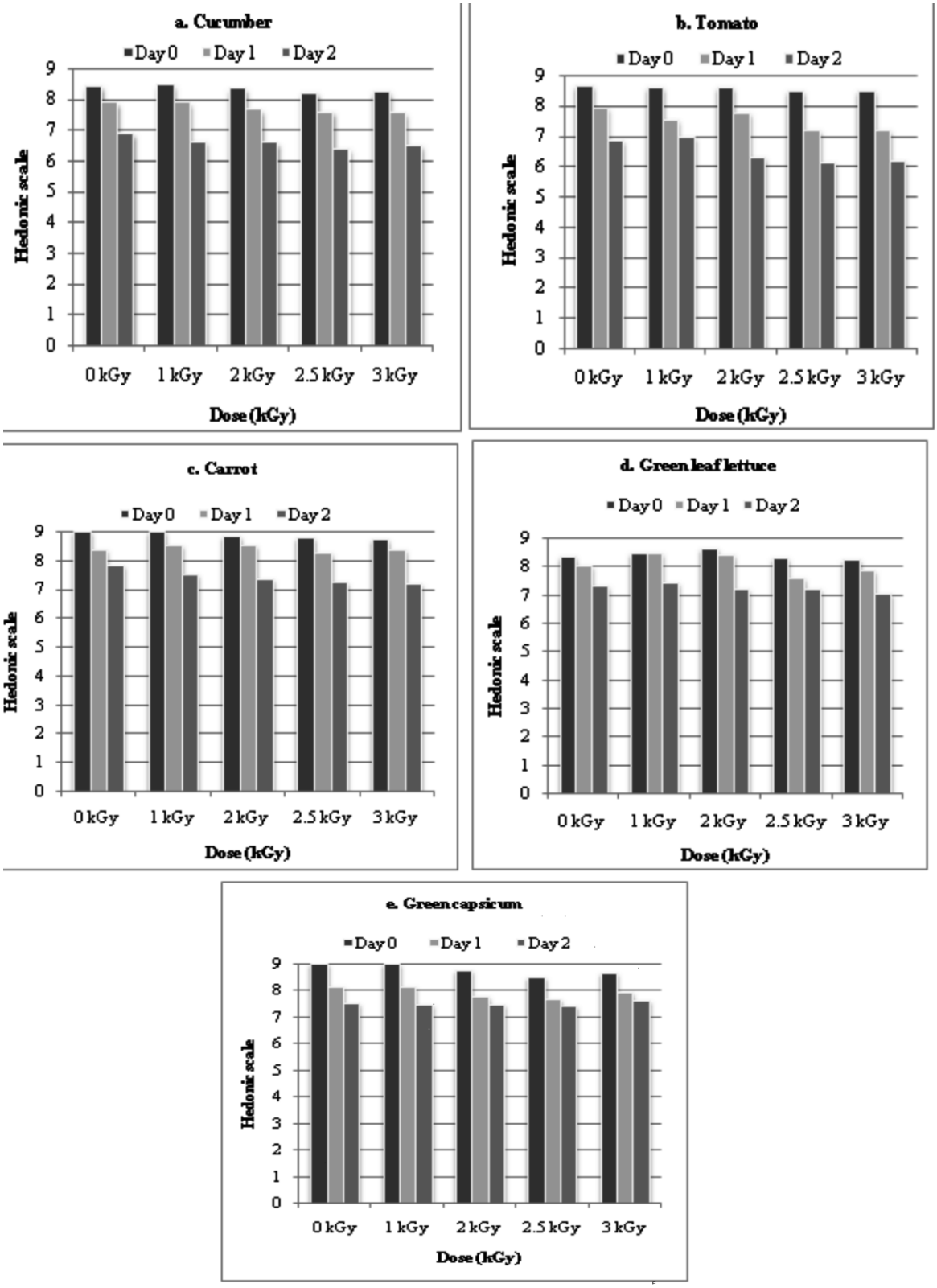

Figure 1. Changes of overall acceptance of cucumber (a), tomato (b), carrot (c), green leaf lettuce (d) and green capsicum (e) during two consecutive days of storage at 4°C. Results are expressed as average of sensory scores (colour, flavour, taste and texture). Sensory scores according to hedonic scale. 7= Acceptability threshold. With the progress of storage period, overall acceptance was decreased both in non-irradiated and irradiated samples. Carrot, green leaf lettuce and green capsicum showed better overall acceptance than cucumber and tomato in two days storage period.

On day 2, taste score of 2 and 2.5 kGy tomato and texture score of 2-3 kGy tomato were below acceptability threshold. In carrot and green capsicum, softening of texture was observed at dose 2-3 kGy on the day of irradiation. With the progress of storage day sensory scores decrease more in irradiated samples than control sample (see Figure 1). But all the sensory scores were over acceptability threshold in two days of storage. No significant radiation induced change in sensory quality of green leaf lettuce was observed on the day of irradiation. On day 2, significant change in flavour, taste and texture were observed at dose 1 and 2 kGy. But all the sensory scores were over acceptability threshold in two days of storage. On day 2, control, 1 and 2 kGy irradiated green capsicum's texture was below acceptability threshold. On day 2, overall acceptance of 2.5 and 3 kGy irradiated cucumber and 2-3 kGy irradiated tomato was below acceptability threshold.

Patterson and Stewart (2003) were experimented the effect of ionizing radiation on the microbiological and nutritional quality of chilled ready meals normally produced for consumption in a number of hospitals in the Belfast area or for use as 'mealson- wheels' for elderly people. Results showed that an irradiation dose of 2 kGy can be used to extend the shelf-life of ready meals for up to 14 days and that the irradiated meals must be stored under good refrigeration conditions (<3°C) in order to obtain maximum benefit from the irradiation treatment. Higher irradiation doses used for any fresh produce were found to be better for controlling microbial counts than lower doses. But a CRP of IAEA was demonstrated that in general, fruits can be exposed to doses between 1.0-2.0 kGy without affecting the sensory attributes. It was also demonstrated that most of the studied minimally processed vegetables could be irradiated with doses up to 2 kGy. These doses were effective in reducing the initial microflora in 4–5 logs and at the same time extending the shelf-life of the products without adverse effect on their sensory characteristics (IAEA, 2006).

From our observation we may come into decision that freshly-cut cucumber and tomato irradiated over 1 kGy should not be recommended after one day of storage at refrigeration temperature. Freshly-cut carrot, green leaf lettuce and green capsicum irradiated up to 3 kGy could be supplied to the hospitals between two days of storage. But 3 kGy dose could not make green leaf lettuce microbiologically safe.

5. CONCLUSIONS

Bangladesh has the seventh largest population in the world (PRB, 2009). The number of immunocompromised patients is steadily increasing due to increased incidence of diseases like cancer, HIV/AIDS, diabetes, and increase in age expectancy. There is a need to develop microbiologically safe and nutritionally wholesome food products for this segment of the population (IAEA, 2010).

Like many 3rd world countries hygienic quality of the fruits and vegetables of Bangladesh are maintained so poorly. From this study, it was also observed, initial microflora of the samples even after washing with water were so high that, minimum doses required to meet the microbiological criteria (Table 1) were 2, 2.5, 2.5 and 2 kGy in cucumber, tomato, carrot and green capsicum respectively. In green leaf lettuce the criteria were not met at our given radiation doses. But our study showed biochemical, nutritional and sensory quality altered for some vegetables in those doses. EFSA Panel on Biological Hazards (2011) recommend that upper dose limits for pathogen reduction should not be specified, since other constraints, such as undesirable chemical changes, will limit the doses applied. Currently maximum allowable dose for uncut vegetables in USA is 1 kGy (EPA, 2013; FAO/IAEA, 2012; FDA, 2007), in Russia 0.03 kGy, in UK 1 kGy (FAO/IAEA, 2012), in EU 1 kGy (FSA, 2012). And advisory technological dose limit given by IAEA (IAEA, 1998) for self-life extension of uncut fresh fruits and vegetables was 2.5 kGy but not for microbial food safety.

So, in Bangladesh supplying ready-to-eat low microbial vegetable salads to immunocompromised patients will not be possible if only irradiation technology is used. Further research should be done with various disinfecting treatment to reduce initial microbial load along with modified atmosphere packaging before irradiation to meet the microbiological criteria at low dose irradiation treatment.

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