Application of Ionizing Radiation to Improve the Quality of Vegetable Salad Especially for Immunocompromised Patients

G.M. Khairuzzaman[1], M.Z. Hasan[2], M.A. Akond[1], M.K. Alam[2] and M.K. Pramanik[2*]

[1]*Department of Botany, Faculty of Life Science, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh*
[2]*Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE) Ganakbari, Savar, Dhaka, Bangladesh*

Abstract

Investigation was carried out for the development of "ready-to-eat vegetable salad" by applying gamma radiation for the consumption of immunocompromised patients who are highly susceptible to microbial infections. Different vegetable samples (*viz.* carrot, cucumber, lettuce, tomato and capsicum) were irradiated with a ^{60}Co gamma source at 1.0, 2.0, 2.5 and 3.0 kGy to eliminate microbial contamination. After irradiation, microbiological quality of both irradiated and non-irradiated vegetable samples was determined and compared with the safety criteria as suggested by IAEA for immuno-compromised patients. In non-irradiated samples, highest total aerobic counts, aerobic and anaerobic spore counts were 9.7×10^6, 1.15×10^2, 0.5×10^2 CFU/g in tomato, lettuce and carrot, respectively. In addition, highest *Staphylococcus* and *Listeria* counts were 2.1×10^3, 1.7×10^5 CFU/g, respectively in tomato and carrot whereas the highest count of coliform, faecal coliform and yeast and mold were 3.4×10^4, 3.3×10^3 and 8×10^1 CFU/g, respectively in lettuce. *Salmonella* was detected only in the carrot sample. Irradiation with 1.0 kGy completely eliminated bacteria from cucumber and capsicum whereas 3.0 kGy was required for carrot, lettuce and tomato to eliminate bacterial counts at safe level for immunocompromised patients. Moreover, irradiation treatment renders considerable retardation and proliferation of microorganisms in comparison to the non-irradiated samples, which is of great benefit of irradiation technique to increase the shelf life of these food items.

Keywords: Immunocompromised patients, Irradiation, Pathogens, Ready-to-eat, Vegetable salad

1. Introduction

One of the safe and effective methods for food preservation is food irradiation which is a cold process. Radiation treatment can reduce microbial load which ultimately results in the control of foodborne illness. Fruits and vegetables are the foods which are often eaten raw and may harbor pathogenic microorganism resulted from the usage contaminated water for irrigation and fertilizer [1]. Combination of low dose radiation and modified storage temperature was found effective for the preservation of easily perishable fruits [2, 3]. Consumption of fruits and vegetables can reduce the risk of non communicable diseases [4, 5]. These products can become contaminated at any step of harvesting and processing. Postharvest practices, water, local environment, fertilizer, workers health and hygiene, and consumption patterns and practices-are the factors identified by WHO as they can influence the presence of hazard in the production system [6]. Contaminating microorganisms can persist in diverse environmental condition [7]. According to Center for Disease Control and Prevention (CDC), leafy greens and other vegetables are major source of several pathogens such as *E. coli* O157, norovirus, *Salmonella, Listeria* and *Cyclospora*. Although in healthy individuals, pathogens can produce self limiting diarrhea, they possess serious threat for adults aged over 65 years, children under 5 years, immunocompromised patients and pregnant women [8]. The most vulnerable immunocompromised patients include those treated with chemotherapy or radiation therapy, patients with transplants, leukemia or with diseases of the immune system and even malnourished people [9]. Chemotherapy can cause damage to intestinal mucosa which may make the patient susceptible to bacteremia [10]. These incidents can be prevented by limiting contamination in food items. Along with other different raw foods, raw vegetables containing different vitamins and minerals, are not allowed in the diet of immunocompromised patients [11, 12]. Diet with either of the three safely levels such as sterile, low bacterial count and prepared with hygiene condition- can be given to these patients. Keeping the natural appearance of food is also important. Ionizing radiation can ensure the safety and nutritional and sensorial quality of different food items [13]. Food irradiation can be the tool of interest in such situations. This can be defined as the application of ionizing radiation to food and to make it safer with extended shelf life while microorganisms and insects are eliminated [14, 15]. Application of radiation in combination with other treatment is important for effective elimination of pathogens and quality retention of foods [16]. In a previous Coordinated Research Project (CRP) of the International Atomic Energy Agency (IAEA), effect of irradiation on ready to eat food was analyzed [17]. In most developed nations, 'low microbe diets' or 'clean diets' are used. Definition of low microbe diets varies as <500 colony-forming units (CFU) per gram of food, or <l000 CFU/ml of coagulase-negative staphylococci or *Streptococcus viridans* and <10000 CFU/ml *Bacillus* species, diptheroids or Micrococcus [18].

Here different fresh-cut vegetable and fruits were collected from open market and chain super shops. Microbiological quality of these vegetables and fruits was determined before and after applying ionizing radiation by Cobalt-60 gamma irradiator to determine safety status and quality retention of these ready to eat vegetable and fruit salad ingredients for immunocompromised patients.

*Corresponding author: *kpramanik2003@yahoo.com*

2. Materials and Method

2.1 *Samples collection*

Diffrent vegetable samples e.g., Cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.), carrot (*Daucus carota* L.), green leaf lettuce (*Lactuca sativa* L.) and green capsicum (*Capsicum annuum* L.) were collected from kitchen market of the chain store "AGORA" of Dhaka city. It is noted that the samples were fresh, good quality and free of any type of physical injury or deterioration.

2.2 Samples preparation

The vegetable samples were washed into running tap water three times. Then cucumbers and carrots were first peeled with a sterile peeler then uniformly sliced with a sterile knife on a clean sterile chopping board. 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). For five different irradiation doses (0, 1.0, 2.0, 2.5 and 3.0 kGy), there were five packets for every type of vegetable sample each containing 5 g of sample. All the procedures were done inside laminar hood to prevent processing contamination.

2.3 Irradiation of samples

Vegetable samples were irradiated at the doses of 0, 1.0, 2.0, 2.5 and 3.0 kGy at room temperature from the Co-60 gamma irradiator source (Located at Atomic Energy Research Establishment, Institute of Food and Radiation Biology, Dhaka, Bangladesh). Absorbed dose level in irradiated samples was assured by Ceric-cereus dosimetry system.

2.4 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 and after 7 days storage at 4°C. The microbiological procedures used to analyse the samples were decimal dilution technique followed by pour plating [19] and spread plating [20]. All the microbiological procedures were done inside a laminar hood. Five (5) g of sample (25 g for *Listeria* spp.) was homogenized by a autoclaved mortar and pestle and filtered through a sterile filter paper 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. Ten (10) fold serial dilution was performed for all samples. Both raw and diluted samples (0.1 ml of each sample) were spreaded on different types of selective and differential media. Then the Petri dishes were placed in upturned position in incubator at 37°C for bacteria and at 30°C for yeast and mold for 24-48 hr. Enumeration of total aerobic bacteria using Nutrient Agar (Scharlau Chemie S.A.,Spain), total anaerobic bacteria using Thioglycollate media (Scharlau Chemie), total yeast and mold using Potato Dextrose Agar (Scharlau Chemie), total coliform using MacConkey Agar (Scharlau Chemie), *Listeria* spp. using *Listeria* Selective Agar Base (Oxoid, England) and *Staphylococcus aureus* using Mannitol Salt Agar (Scharlau Chemie) was performed. Qualitative detection of *Salmonella* was also carried out in different vegetative samples using Lactose Broth, Selenite Cystine Broth and Bismuth Sulphite Agar (Scharlau Chemie).

For anaerobic bacteria, after spreading, plates were kept into an anaerobic jar. After closing the jar, 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. The jar was put inside the incubator for incubation at 37°C for 24 hours.

3. Results and Discussion

3.1 Effect of irradiation on total aerobic plate count

In non-irradiated vegetable samples, total aerobic bacterial counts were 4.01×10^6, 4.05×10^6, 1.4×10^5, 9.7×10^6 and 4.45×10^4 CFU/g in carrot, cucumber, lettuce, tomato and capsicum samples respectively. Effect of irradiation at different doses on total aerobic plate count was observed immediately (Fig. 1a) and after 7 days of storage (Fig. 1b). Total aerobic plate counts in the tomato and green leaf samples tested immediately after irradiation with 2.0 and 2.5 kGy were found within the safe level for immunocompromised target group. For other vegetables, 1.0 kGy was enough to achieve the same result. After storage for 7 days at 4°C, aerobic plate count in the cucumber sample irradiated with 1.0 kGy was within the desired range. In case of lettuce, carrot and tomato, 2.0, 2.5 and 3.0 kGy radiation dose level respectively resulted into bacterial count at safe level for immunocompromised patients.

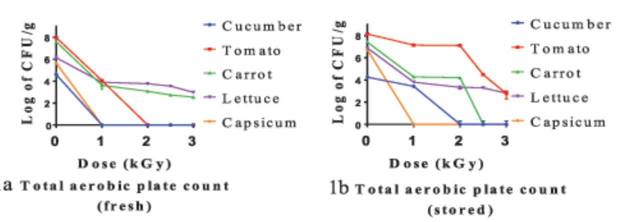

Fig. 1: (a) Aerobic plate counts of the five vegetables samples irradiated with different doses (0, 1.0, 2.0, 2.5 and 3.0 kGy) and analysed on the same day of irradiation (b) after 7 days of storage at 4°C

3.2 Effect of irradiation on total aerobic and anaerobic spore count

In non-irradiated vegetable samples, aerobic spore counts were 1.0×10^1, 1.5×10^1, 1.15×10^2, 0 and 0 CFU/g; anaerobic spore counts were 5.0×10^1, 0, 0, 0 and 0 CFU/g in carrot, cucumber, lettuce, tomato and capsicum samples, respectively. In carrot and lettuce, 2.0 kGy was enough to meet the criteria for immunocompromised patients (Fig. 2a). In other vegetables like cucumber 1.0 kGy was enough to obtain the same effect. In case of 7-days stored samples, applied radiation doses produced almost same effect with respect to total aerobic count (Fig. 2b). In fresh samples, anaerobic spore was absent in all the vegetables except carrot (Fig. 2c). At 1.0 kGy dose of radiation, no anaerobic spore was detected. In case of stored sample, 2.5 kGy radiation dose was required to eliminate spores from lettuce sample. In the case of other vegetables, 1.0 kGy was enough to produce the same effect (Fig. 2d).

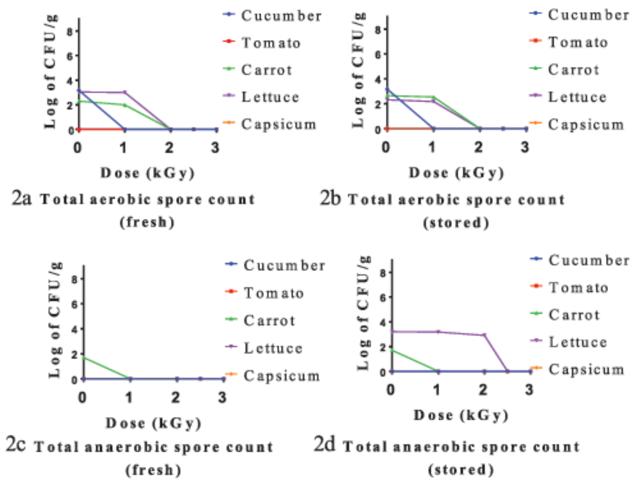

Fig. 2: Effect of different irradiation doses on total spore count of five vegetables

Total aerobic and anaerobic spore counts of vegetable samples irradiated with different doses (0, 1.0, 2.0, 2.5 and 3.0 kGy) analysed on the same day of irradiation (a, c) and after 7 days of storage at 4°C (b, d).

3.3 Effect of irradiation on total coliform and fecal coliform count

In non-irradiated carrot, cucumber, lettuce, tomato and capsicum samples, total coliform counts were 3.7×10^1, 4.2×10^2, 3.4×10^4, 8.6×10^2 and 1.01×10^3 CFU/g; fecal coliform counts were 3.1×10^1, 4.0×10^1, 3.3×10^3, 0 and 1.7×10^2 CFU/g, respectively. In lettuce and carrot, 2.0 and 3.0 kGy radiation doses were enough to eliminate coliform, respectively (Fig. 3a). In other vegetables tested, 1.0 kGy dose was enough to obtain the same effect. In case of 7-days stored samples, 2.0 kGy radiation dose was required for tomato, carrot and lettuce to make them coliform free (Fig. 3b) and 1.0 kGy removed coliform from other vegetables. To eliminate fecal coliform from fresh carrot and lettuce sample, 2.5 and 2.0 kGy radiation doses were required, respectively (Fig. 3c). In stored samples, 2.0 kGy was required to eliminate fecal coliform from all stored vegetable samples except tomato which required 2.0 kGy for the same effect (Fig. 3d).

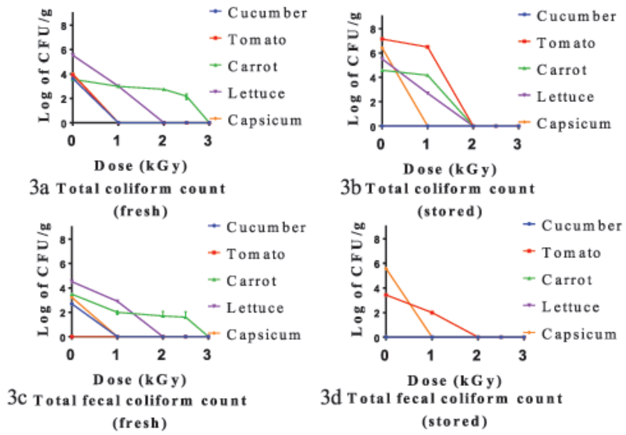

Fig. 3: (a, c) Total coliform and fecal coliform counts of five vegetables samples irradiated with different doses (0, 1.0, 2.0, 2.5 and 3.0 kGy) analysed on the same day of irradiation (b, d) after 7 days of storage at 4°C

3.4 Effect of irradiation on total Staphylococcus aureus count

Staphylococcus counts in non-irradiated vegetable samples were 6.5×10^2, 1.0×10^2, 9.7×10^2, 2.1×10^3 and 1.75×10^2 CFU/g in carrot, cucumber, lettuce, tomato and capsicum samples, respectively. Irradiation dose levels at 1.0 kGy for cucumber and capsicum, 2.5 kGy for tomato and lettuce, 3.0 kGy for carrot were required to make them edible for immunocompromised patients (Fig. 4a). After storage, irradiation at 3.0, 2.5 and 2.0 kGy effectively eliminated *Staphylococcus aureus* from lettuce, carrot and cucumber, respectively (Fig. 4b). For other two vegetables, 1.0 kGy radiation dose produced the same effect.

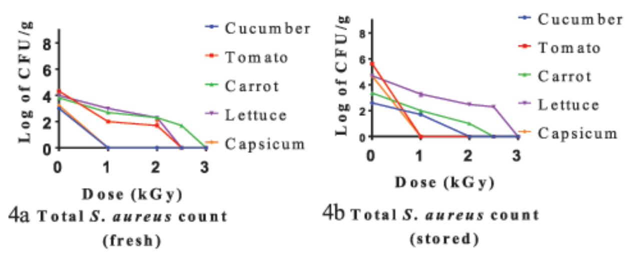

Fig. 4: (a) Staphylococcal counts of the five vegetables sample irradiated with different doses (0, 1.0, 2.0, 2.5 and 3.0 kGy) analysed on the same day of irradiation (b) after 7 days of storage at 4°C

3.5 Effect of irradiation on total Listeria spp. count

Listeria counts in non-irradiated carrot, cucumber, lettuce, tomato and capsicum samples were 1.7×10^5, 4.8×10^2, 8.8×10^2, 2.5×10^1 and 1.3×10^2 CFU/g, respectively. In case of fresh lettuce, a radiation dose of 3.0 kGy was required to eliminate *Listeria* spp. (Fig. 5a). For other vegetables, 1.0 kGy showed the same results. In case of stored samples, 2.0 kGy radiation dose completely eliminated *Listeria* spp. from lettuce and 1.0 kGy showed the same effect for other vegetables (Fig. 5b).

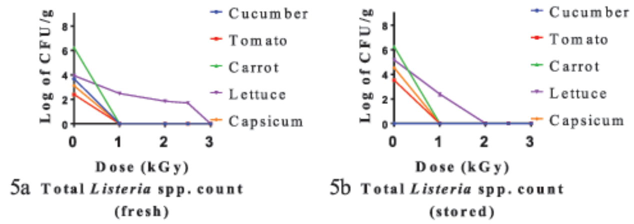

Fig. 5: (a) Total *Listeria* counts of the samples irradiated with different doses (0, 1.0, 2.0, 2.5 and 3.0 kGy) analysed on the same day of irradiation (b) after 7 days of storage at 4°C

3.6 Effect of irradiation on total yeast and mold count

In non-irradiated samples, yeast and mold counts were 1.5×10^1, 0, 8.0×10^1, 0 and 0 CFU/g in carrot, cucumber, lettuce, tomato and capsicum samples, respectively. A radiation dose of 1.0 kGy resulted in the elimination of yeast and mould from all of the fresh and stored samples (Fig. 6a and 6b).

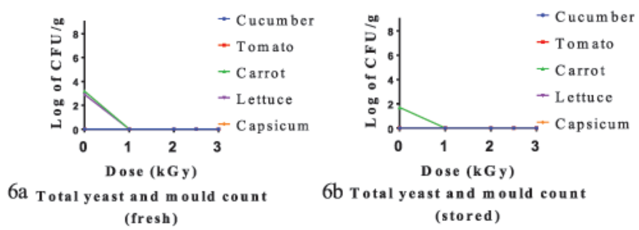

Fig. 6: (a) Yeast and mould counts of the five vegetables samples irradiated with different doses (0, 1.0, 2.0, 2.5 and 3.0 kGy) analysed on the same day of irradiation and (b) after 7 days of storage at 4°C

3.7 Effect of irradiation on Salmonella detection

Effect of irradiation at different doses on *Salmonella* count was also observed in both fresh and 7day stored vegetable samples but this organism was not detected in any of the vegetables.

Fresh vegetables are good sources of phytochemicals and other bioactive compounds those may help to prevent degenerative diseases. Irradiation does not affect antioxidants in plant based items to much extent [21]. Food irradiation has long been used according to history and now many health organizations have started using this technology [22]. Irradiation at non-sterilizing doses can result in low microbe meals which would be more beneficial for the target group of people both nutritionally and psychologically due to less drastic effect [23]. Different vegetables those are usually eaten raw, were subjected to low doses of radiation and its effect on different microbial community was determined. There are many immunocompromised patients who need low microbial diets and fresh fruits and vegetables [24]. Radiation doses with 1.2 -2.0 kGy were reported by previous researchers for mixed salads and alfalfa sprouts [25]. Radiation dose with 3.0 kGy was previously shown to produce a 3 log cycle reduction of total aerobic bacterial count and complete elimination of coliform and *Staphylococcus* from ice-cream [25]. A series of radiation doses up to 3.0 kGy were applied on 5 different vegetables in our study. Total aerobic bacterial counts were reduced to the level of low bacterial diet in all of the vegetable samples by applying 2.5 kGy. Moreover, coliform and fecal coliform were eliminated completely by 2.0 kGy from most of the vegetable samples except carrots though a slight higher dose was required to eliminate *Staphylococcus* from some of the vegetable samples. *Liseteria monocytogenes* incidence has increased in the past decades due to increased immunocompromised people [26]. Effect of irradiation on the presence of this organism in vegetables was determined in this experiment to make vegetables safe for these target people. Lettuce sample was found to be the most contaminated with *L. monocytogenes* and required 3.0 kGy irradiation dose for its complete elimination. In the case of other vegetables tested, 1.0 kGy was enough to make them safe for the immunocompromised people.

4. Conclusion

Safety of irradiated foods is well established and irradiation at 1.0 - 3.0 kGy could effectively eliminate different groups of microorganisms including pathogens from different vegetables while stored under controlled environment. Therefore, irradiation at low dose level can be considered as a suitable option to improve the microbiological quality of these vegetables especially for immuno-compromised patients.

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