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Reduction of Microbial Flora to Prolong the Shelf Life of *Cucumis Sativus* Through Gamma Irradiation

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Abstract: Cucumber (*Cucumis sativus* L.) belongs to family of cucurbitaceae. It is an economically important vegetable crop cultivated almost 80 countries with more than 66 million production annually. The current study involves the use of gamma radiation to enhance the shelf life of cucumber. For this purpose, cucumber was subjected to Co-60 gamma irradiation at different doses 1, 1.5 and 2 kGy. Microbial and sensory analysis was carried out for all the treatments. 1kGy dose was ineffective in reducing microbial population while at 2kGy organoleptic attributes were severely affected. Irradiation of 1.5 kGy was found to be the most effective dose at which both microbiological and sensory attributes remained within acceptable limit. Thus, shelf-life of cucumber was extended upto 5 days at 1.5 kGy dose.

Keywords: Cucumber, Dose, Nutrient Agar, Shelf Life, Hedonic Scale

1. INTRODUCTION

Cucumber (*Cucumis sativus*) belongs to the oldest and widely cultivated plant of Cucurbitaceae family also known as gourd family. From the historical perspective, people are familiar about this crop for almost 3000 years due to varied medicinal and nutritional benefits (Vora *et al.*, 2014). Cucumber is considered to be most important summer vegetable of Pakistan. It is grown during spring season and harvest time is from May to June in Punjab province. From July to onward the supply of cucumber is reduced due to unfavorable conditions.

Cucumber is cultivated in more than 80 countries. About 66 million tons produced per annum is used both in fresh and processed form. It mostly consumed in raw or pickled form (Adhikari *et al.*, 2012).World's cucumber production is about 40,000 tonnes and China is one of the largest producer of cucumber (FAO, 2005). From export point of view Pakistan stands at 139 position despite of being the 45th largest producer of cucumber which is due to improper post-harvest handling. Most important postharvest problem for cucumber is shrinkage which occurs due to weight loss. Cucumbers are also prone to various bacterial and fungal diseases which are another major cause of postharvest loss particularly in fruits whose quality is diminished by chilling injury. With the increase in demand and market globalization for fresh fruits and vegetables, food borne illness is increasing because contaminated raw commodities are the main vehicles for transmission of pathogens (Holy *et al.*, 2006). Microbial contamination can occur by variety of sources such as animal, human, environmental during growth, harvest, processing, packaging, transport, handling and retail which can be risk of pathogenic micro-organisms (Lu *et al.*, 2002).

Radiation processing is a method which is involved in preservation of foods by reducing spoilage microorganisms (extending shelf-life) and in eliminating pathogenic bacteria (ensuring safety) in fresh produce without any change in quality (Arvanitoyannis *et al.*, 2009; Mohapatra *et al.*, 2013). Spoilage organisms are usually associated with deterioration of food quality resulting in alteration in the components of food, texture, appearance, flavor, slime formation and color. (Altekruse and Swerdlow, 1996; Raso and Barbosa-Canovas, 2003). Irradiation is advantageous over other treatments because during irradiation, temperature of food is not raised as it is a non-thermal process responsible for killing pathogenic and spoilage causing organisms (Lacroix and Vigneault, 2007).

There are several methods available for irradiation of food commodities but gamma rays are now being widely used and it has surpassed x-rays and e-beam due to many advantages. Food irradiation by gamma rays involves cesium-137 and cobalt-60. Co-60 is most commonly and widely used due to numerous advantages as it has deep penetration power, pose less risk to the environment, maximum energy is available for use and decay product is nickel which is non-radioactive

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possessing 5.3 year half-life. No drawbacks have been reported regarding human and environmental health to the use of Co-60. Electron beam also destroy pathogenic organisms meant for causing food-borne illness. Although e-beam is advantageous but has some drawbacks as it cannot penetrate deeply into food; also it is more expensive and require complex handling as compare to radionuclides-based systems. X-rays work on a same principle as e-beam. Although X-rays have ability to penetrate larger items such as carcasses but this is energy demanding and costly procedure (Arvanitoyannis, 2010).

The aim of this research is to minimize post-harvest loss by use of gamma radiation which will help to extend the shelf life of cucumber and contribute to the national economy.

2. MATERIALS AND METHODS

Sample collection and gamma irradiation

Fresh cucumbers sample of uniform size and age were collected from the local market of Lahore. The dealer was requested to make sure that the selected samples for all experiments were taken from the same plantation. It was make sure that the fruits did not encounter any kind of damage, bruises or physical injury. These samples were packed in polythene bags and accurately labeled with the desired doses. The samples were then sent to the radiation unit of PARAS (Pakistan Atomic Radiation Services Lahore) for irradiation. One group was referred as control and the other group as experimental. The doses at which experimental cucumbers subjected for irradiation were 1, 1.5 and 2 kGy. Both control and irradiated cucumbers were stored in refrigerator at 10°C. Sensory evaluation was carried out after every 2 days interval and microbial flora at 5 days interval.

Decay assessment

The decay percentage of cucumber fruit during the storage period was calculated according to Li *et al.* (2014). % of fruit rotting = Number of affected cucumbers per treatment × 100
Total number of cucumbers in that treatment

Isolation of microorganisms

Serial dilution method was used for the isolation of microorganisms. Cucumber was washed thoroughly in a beaker containing 300 ml sterile saline water and then serial dilutions were made from it. 100 µl of each dilution was transferred to the petri plates containing various media (Cappuccino *et al*., 1983). Nutrient agar was used for growth of non-fastidious microorganisms, MacConkey agar for isolation of gram negative bacteria and Potato Dextrose agar for fungal isolation. The plates of Nutrient agar and MacConkey agar were placed in an incubator at 37°C for 24 h after specific time bacterial growth was observed. However, PDA plates were incubated at 30°C for 72 hours after wards fungal colonies were noted. Total viable bacterial and fungal count (CFU/g) was calculated according to Viswanathan and Kaur (2001).

Bacteria were identified on the basis of morphological and biochemical characteristics. Gram negative bacteria were identified using API 20E. Fungi were identified on the basis of micro and macroscopic characteristics.

Statistical analysis

The results were statically analyzed by using Costat 6.4 program. One way analysis of variance (ANOVA) was applied using completely randomized design. The standard deviation of replicates from mean value was also calculated. Mean values were compared using Duncan's New Multiple Range test at $p \leq 0.05$ (Al-Juhaimi *et al*., 2012).

2. RESULT AND DISCUSSION

***Decay assessment*:** Effect of Gamma radiation on decay percent and shelf life improvement of fresh green cucumbers kept at refrigerated temperature was determined (Table1) which showed no decay upto 5 days of storage for both the irradiated and non irradiated cucumbers. On 6th day, control sample started to decay and by the end of 10th day, it was completely decayed. 2kGy and 1kGy cucumber samples were also decayed with the passage of time. The reason of decay might be due to the changes in various quality attributes most probably due to senescence or physiological breakdown. The dose of 1.5 kGy sample exhibited good sensorial characteristics and remained fresh by the end of 10th day. So, the dose of 1.5 kGy was found to be better as it resulted in enhancement of shelf life and controlled microbial spoilage to an appreciable degree. **Fig. 1 and 2** shows the effect of gamma radiation on the shelf life of cucumbers at various time intervals.

Table 1: Effect of gamma radiation on decay percent of fresh green cucumbers kept at refrigerated temperature

Days Of Storage	Irradiation Doses (Kgy)			
	0	1.0	1.5	2.0
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	1	0	0	0
7	2	0	0	1
8	3	1	0	2
9	4	2	0	3
10	Decayed	3	0	4

The value assigned to each sample in the table represents the following

0 = superficial fleck (no decay)
1 = 1- 24 % of the surface decayed
2 = 25-49 % of the surface decayed
3 = 50-74 % of the surface decayed
4 = 75% or more of the surface decayed

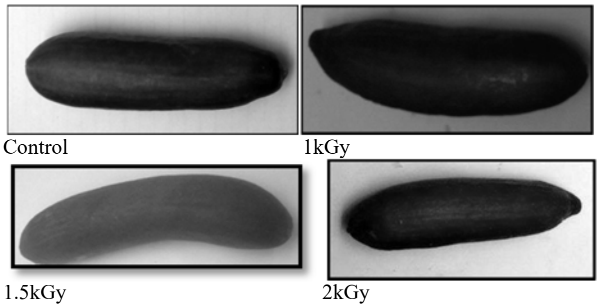

Fig. 1: Effect of different gamma radiation doses on the shelf life of cucumber (0 day)

Fig. 2: Effect of different gamma radiation doses on the shelf life of cucumber (10th day)

Sensory evaluation using 9-point hedonic scale

Sensory analysis of cucumber was carried out at different time intervals to assess the various parameters such as texture and color because these are the two essential attributes which determine quality, consumer preference and purchasing of cucumber (Itohan *et al.*, 2011; Yoshioka *et al.*, 2010). Results indicated that 1.5kGy sample showed no change in sensory parameters over the 10 days of storage period while remarkable change in texture and color was observed in control, 1 and 2kGy radiated sample with the passage of time. Texture (firmness) of the cucumbers depends upon the nature of its constituent tissues which may be grouped into exocarp , endocarp and mesocarp. The change in texture in control, 1 and 2 kGy samples might be due to enzymatic degradation of components that were linked with structural rigidity of the fruit such as insoluble pectin and proto-pectin. This texture change might also be attributable to the radiation degradation of carbohydrates. Free radical species, when exposed to gamma radiation was probably responsible for depolymerization of the pectic substances. Our findings are in accordance to the study of Al-Juhaimi *et al.* (2012) who reported that firmness of control was usually decreased with storage time. 2kGy sample also showed decreasing trend as higher radiation doses usually reduce firmness. Softening of cucumbers was also observed in control, 1 and 2kGy samples due to textural changes. This softness might be associated with the breakdown of seed area tissue by the activity of natural endopolygalatcuronase after the fruit development (Glegg and Kertesz, 1956) or softening of fruit might also be due to degradation of structure of cell, composition of cell wall and intracellular materials by a biochemical process which involves hydrolysis of pectin and starch by wall hydrolases (Seymour *et al.*, 1993). Greater degree of yellowing was observed at 2kGy dose which might be due to the radiation-induced ethylene generation and increase in ripening leading to degreeness (Gould, 1996). The reason behind might also be the degradation of chlorophyll during senescence resulting in exposure of lighter yellowish pigments as yellow carotenoids also exist with chlorophyll. Chlorophyll breakdown was mediated by chlorophyllase enzyme (Matile *et al.*, 1999; Moalemiyan and Ramaswamy, 2012). 2kGy dose also showed higher wilting probably due to the increase in electrolyte leakage resulting in damage of cell membrane (Al-Juhaimi *et al.*, 2012).

Overall visual quality of both radiated and un-irradiated cucumbers was also interpreted by 9-point hedonic scale which uses parametric statistics for analysis of data and for discussion of cross tabulation percentage results. 9-point hedonic scale categorized as follows 9=Like extremely, 8=Like very much, 7=Like moderately, 6= Like slightly, 5= neither like nor dislike, 4=Dislike slightly, 3=Dislike moderately,2=Dislike very much,1= Dislike extremely (Peryam and Pilgrim, 1957).

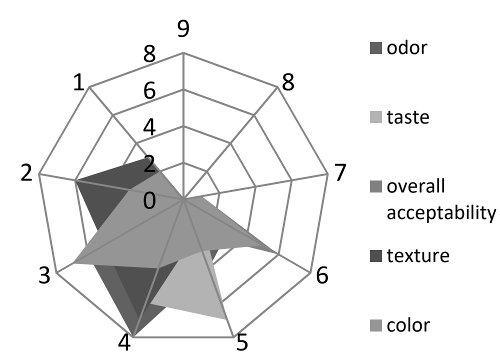

Fig. 3 (a and b) shows that the overall acceptance by consumers for radiated sample was found to be more as compared to un-irradiated cucumber samples.

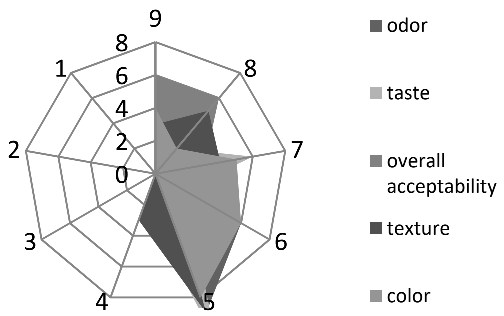

Fig. 3: Sensory evaluation of cucumber (a) non-irradiated (b) irradiated

Total viable count of bacteria

(Fig. 4) shows significant difference of total viable bacterial count between control and irradiated samples stored at refrigerated temperature. The control group showed initial bacterial count of 3.1×10^5 cfu/g which increased progressively as the storage period was prolonged. On 5th day control cucumber sample showed high bacterial count of 1.20×10^6 cfu/g. Irradiated cucumbers manifested considerable reduction in total viable bacterial count. With increase in radiation dose microbial count became lower and maximum reduction was observed in 2kGy sample. Although as the days passed, irradiated samples depicted increase in bacterial count but the value was still lower as compared to control samples. CFU values of cucumbers irradiated at 1kGy was 11.2×10^5 cfu/g which increased to 56.2×10^5 cfu/g on 5th day. The bacterial colony count of sample irradiated at 1.5 kGy initially was 6.2×10^4 cfu/g, 13.6×10^4 cfu/g on 5th day and 13.6×10^5cfu/g on 10th day. CFU values for 2 kGy irradiated samples were 1×10^4 and 7×10^4; less than that of control sample. Similar trend of decrease in bacterial count with increase in radiation dose was observed in MacConkey agar **(Fig 5).** Microbial load of control on the first day of spreading on macConkey agar was 2.2×10^4cfu/g which increased to 9×10^4 cfu/g by the end of 5th day of storage. Cucumber sample irradiated at 1, 1.5, 2 kGy doses showed no bacterial colony.

The bacteria isolated from cucumber samples were *E. coli*, *Staphylococcus aureus, Bacillus sp.* and *Streptococcus sp.* Among all the isolated bacteria *Staphylococcus aureus* was present in high proportion in control samples and greatly reduced by radiation. Our findings were in accordance to Bari *et al*. (2015) who found *S. aureus* to be predominant and the most hydrophobic bacterium (96%) in cucumber responsible for various diseases ranging from minor skin infections to life threatening diseases such as pneumonia, endocarditis, etc. *E.coli* which is responsible for diahorrea, gastroenteritis and urinary tract infections was also present in greater proportion in control samples but inhibited at all radiation doses.1.5kGy was found to be the optimized dose at which microbial and textural attributes remained within acceptable limit upto 10 days of storage. Although at 2kGy microorganisms were inhibited but quality of cucumber was severely affected resulting in rejection of this dose. The reason behind inhibition of microbial load might be that DNA of microorganisms was very susceptible to irradiation as irradiation causes base damage resulting in breakdown of DNA strands and cross linking due to which organisms became unable to reproduce and hence microbial population was reduced (da Silva Aquino, 2012)

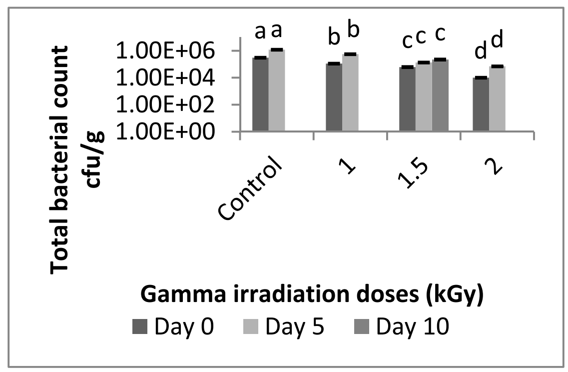

Fig. 4: Effect of different gamma radiation doses on the epiphytic bacteria of cucumber using nutrient agar as testing medium

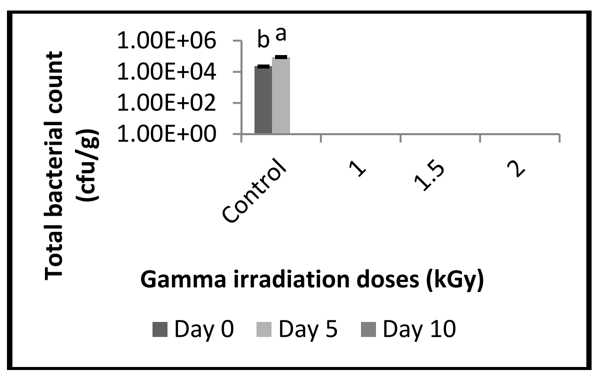

Fig. 5: Effect of different gamma radiation doses on the epiphytic bacteria of cucumber using MacConkey agar as testing medium testing medium

Reduction in fungus follows the similar pattern as in case of bacteria **(Fig.6).** Lower fungal count was observed in all irradiated sample as compared to control. On the first day, total viable fungal count of control group was 1.6×10^5 cfu/g which increased gradually on further storage and found to be 6.94×10^5 cfu/g on 5th day. Sample irradiated at dose 1kGy showed total viable fungal count of 1.00×10^5 cfu/g and 1.36×10^5cfu/g less than that of control cucumber sample.

Those irradiated at 1.5 kGy exhibited significant reduction in fungal count as 3×10^4cfu/g on first day, 5×10^4 cfu/g on 5th day and 6×10^3 cfu/g on 10th day of storage. Almost similar reduction pattern was observed for 2kGy irradiated sample; 0.6×10^3 on first day which increased upto 1.6×10^4 with the storage duration. The effects of all the treatments on total viable bacterial and fungal count vary significantly from each other at $p \leq 0.05$.

Microscopic and macroscopic analysis of fungal growth was carried out and following fungi were identified which included *Aspergillus sp., Rhizopus sp., Penicillum sp., Fusarium sp. Botrytis sp*, *Candida sp.* and *Saccharomyces sp.*

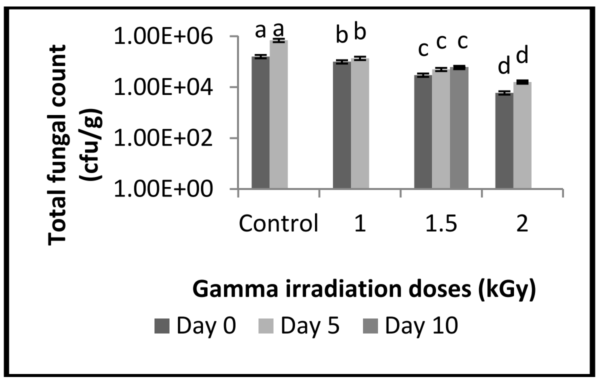

Fig. 6: Effect of different gamma radiation doses on total viable fungal count of cucumber kept at refrigerated temperature using potato dextrose agar as a testing medium

4. CONCLUSION

1.5 kGy proved to be the most effective dose as both microbiological and sensorial characteristics remained within acceptable limits due to which shelf life was extended up to 5 days reducing the decay and suppressing the epiphytic microbial load of cucumber.

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