GAMMA IRRADIATION AS A QUARANTINE TREATMENT TO PREVENT POST HARVEST SPOILAGE OF *DAUCUS CAROTA L.*

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Abstract

In the present study, the effect of gamma irradiation on the micro flora and shelf life of carrots (*Daucus carota L*) were studied. The samples were subjected to various irradiation doses 1.0, 1.5 and 2.0 kGy and products were kept in a refrigerator for 21 days. For the treated and untreated samples, sensory properties, microbiological and physical analyses were carried out on weekly basis. Our results indicated that bacterial, mould and yeast count was reduced in all irradiated carrot samples as compared with control samples. The maximal microbial flora elimination was noticed at a dose of 1.5 kGy. A complete decay of samples receiving 2.0 kGy was observed within one week.

Introduction

Carrots (Apiacae, Umbelliferae) are an important root vegetable cultivated in most of the temperate regions of the world. Carrot was first cultivated in Central Asia, approximately 1,100 years ago, it is a frost-tolerant and a nutritious vegetable (Ilyas *et al.*, 2013). It is a widely distributed crop cultivated over an area of 13.9 thousand hectares, with an annual production of 242.3 thousand tons (FAO, 2008). Carrot belongs to genus *Daucus* with more than 80 species which can be categorized into six main varieties, including Imperator, Danvers, Nantes, Chantenay, Amsterdam, Paris Market (Simon *et al.*, 2008). There are mainly two types of carrots: Eastern carrots and western carrots. Eastern carrots are confined to Afghanistan, Russia, Iran, Pakistan and India, while western carrots were domesticated firstly in Turkey (Bradeen and Simon, 2007). Its public preference is due to increasing sugar content as well as for its medicinal properties, however, an increased ratio of carotenoids can overwhelm its sweetness (Al-Safadi and Simon, 1990). Different varieties with a high anticancer β-carotene (provitamin A) can enhance its vitamin A availability (Stein and Nothnagel, 1995).

Since antiquity, preservation of food is a major human concern. Food with huge microbial load and pests, leads to post-harvest losses during transportation, storage and marketing. For example, carrots, a major source of vitamin A, suffer 40% post-harvest loss. For preservation of carrots, the present study suggests the use of gamma irradiation which is most effective as compared to traditional preservation methods including pasteurization, freezing, chemical, refrigeration, or canning processes (Nilsson, 2000; Agrios, 2005). Cold storage carrots are susceptible to chilling effect but gamma irradiation can be employed to enhance shelf-life of carrots. Foodstuff irradiation not only preserves food but it also helps in food safety and maintain its quality. γ-Irradiation makes use of Cobalt-60 with no undesirable side effects on food or any significant changes in physicochemical properties including nutritional value of the treated product. The current study reports the effect of γ-irradiation on microflora of carrots.

Materials and Methods

Red colored imperator variety carrot samples were collected from the farms and transported to the laboratory after being wrapped in sterilized aluminum sheets. Carrot samples were divided into four groups (A-D) with 20 carrots in each group. Group A was taken as control (without γ-irradiation) while groups B-D were subjected to 1kGy, 1.5kGy and 2kGy dose of γ-radiation, respectively. The samples were cooled in fridge and microbial, sensory and physicochemical analyses were carried out weekly for all groups of samples.

Sensory evaluation was considered against parameters including color, flavour, taste, texture, odor and overall acceptance of the vegetable. During storage of samples, decay assessment was analyzed and results were recorded based on visual inspection for each sample. For this, sample decay was arrayed into following categories: The value assigned to each sample in the table represents the following 0 = superficial fleck (no decay), 1 = 1- 24 % of the surface decay, 2 = 25-49 % of the surface decay, 3 = 50-74 % of the surface decay, 4 = 75% or more of the surface decay. Using 9-point Hedonic scale, organoleptic qualities of raw samples were evaluated. Sample testing was carried out to determine how much each sample was liked based on 9-point Hedonic scale for a set of attributes: overall acceptance, color, crunchiness, odor, texture and taste where 9

(extremely liked), 8 (very much liked), 7 (moderately liked), 6 (slightly liked), 5 (neither liked nor disliked), 4 (slightly disliked), 3 (moderately disliked), 2 (very much disliked), 1 (extremely disliked) (Stone *et al.*, 2012).

Microbiological studies: Microorganisms were isolated by using serial dilution method (Molins, 2001). The carrots were washed carefully with 100 ml sterilized water and serial dilutions were made. Each dilution was transferred into petri plates with sterilized MacConkey agar (for Gram negative enteric bacteria isolation), Nutrient agar (for non-fastidious bacteria), Potato Dextrose agar (for fungi) and Salmonella-Shigella agar (for *Shigella* spp and *Salmonella* spp.). After spreading with nutrient agar, MacConkey agar and SS agar, petri plates were allowed to incubate for 24 hours at 37°C for bacterial growth. While petri plates containing PDA were incubated for 72 hours at 30°C for fungal growth. The total viable count of fungal and bacterial growth were calculated using the following equation (Rico *et al.*, 2007).

$$\text{Colony forming unit/gram (cfu/g)} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Amount plated}}$$

Bacteria were identified on the basis of morphological characteristics and by using the analytical profile index (Holmes *et al.*, 1978). Fungi were identified on the basis of micro- and macroscopic characteristics.

Statistical analysis: The data were analyzed by statistics software Costat 6.4 using completely randomized block design and mean values were compared using Duncan's New Multiple Range test at $p \leq 0.05$ with five replicates.

Results

Gamma irradiation at different doses differentially enhanced the shelf life by lowering the decay progression of carrots. The samples treated with a dose 1.0 kGy were completely decayed by the end of third week while those which were treated with higher dose 2.0 kGy were completely destroyed within four days, while maintaining in a refrigerated storage. Irradiation at 1.5 kGy seemed to be better for minimizing the percent decay as carrots exposed to this dose retained their texture properties for 21 days. Fig.1 shows the overall percent decay of samples at different γ-irradiation doses.

Physiological weight loss of carrots after irradiation: Table 1 shows the maximum percent weight loss (1.29%) found in controls while minimum weight loss (0.16%) was recorded in carrots irradiated at 1.5 kGy dose. However, weight loss at irradiation 1.0 kGy dose was 0.9 % which is also smaller than that of weight loss in control samples. The weight loss after 7, 14 or 21 days of storage was 0.46%, 0.71%, 1.18%, respectively.

Sensory analysis of *Daucus carota:* Table 2. shows sensory analyses using 9 point hedonic scale, physical parameters including texture, color, odor, taste, crunchiness and overall suitability of the vegetable.

Significance test for the acceptance of consumer was carried out to analyze the attributes for both nonradiated and irradiated samples. The consumer acceptance for radiated samples was found to be more tolerable compared with non-irradiated samples. A panel of 25 experts analyzed the samples for sensory evaluation. The experts' opinion signposted that the samples irradiated at 1.5 kGy, with minimal variation in the sensory attributes, was more acceptable. Fig 2 (a) and (b) show the evaluation of control and irradiated carrot samples respectively.

Enumeration of micro-organisms

Impact of different doses of gamma radiation on bacterial count on nutrient agar: The control carrot samples showed an initial bacterial count of 8×10^5 cfu/g on nutrient agar medium after their storage for seven days. The bacterial count elevated to 1.2×10^6cfu/g during the following week at a storage temperature c 8°C (Fig. 3a). A significant diminution in viable bacterial count was observed at 1.5 kGy irradiation dose. The initial bacterial count calculated for 1.0 kGy and 1.5 kGy dose irradiation was 3×10^5 and 4×10^4cfu/g, respectively. While at a higher dose 2.0 kGy, the overall acceptance exceeded the consumer acceptable limit as the texture of the vegetable was unacceptable. The samples exposed to 1.5 kGy dose and their storage for three weeks at low temperature, showed a total viable bacterial count 1.8×10^5cfu/g that was even less than the control samples stored for seven days at the same temperature. The effect of radiation dose on overall viable bacterial count radically fluctuated from each other at $p \leq 0.05$. The bacteria were chiefly Gram-negative rods and Gram-negative cocci but a few Gram-positive rods were also observed.

Table 1. Effect of different Gamma radiation doses on the percent weight loss of carrot.

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Storage Duration	Gamma irradiation doses				
	Control	**1.0 kGy**	**1.5kGy**	**2.0kGy**	**Mean***
Week$_1$	0.52	0.74	0.13	Decayed	0.46[a]
Week$_2$	1.07	0.9	0.16	Decayed	0.71[b]
Week$_3$	2.28	1.08	0.19	Decayed	1.18[c]
Mean*	1.29[a]	0.90[b]	0.16[c]		

Table 2. Sensory Evaluation of carrots using 9-point Hedonic scale.

Attributes	Control	Radiated	p value**
Color liking[s]	2.8 ± 0.5[a]	8.2 ± 1.47[a]	0.007
Taste liking[s]	4.8 ±1.22[b]	7.8 ± 1.80[b]	0.032
Odour liking[s]	5.5 ±1.5[c]	8.0 ± 1.47[c]	0.023
Crunchiness liking[s]	3 ± 0.70[d]	6 ± 1.4[d]	0.001
Texture liking[s]	4.2 ± 3.37[e]	5.7 ± 1.6[e]	0.035
Over all acceptance[s]	5.75 ± 1.92[f]	7.75 ± 2.2[f]	0.032

Sample means ± standard deviation and significance for consumer acceptance (n = 25)
****** Student t-test 5% [s] Attributes are significant at $p \leq 0.05$

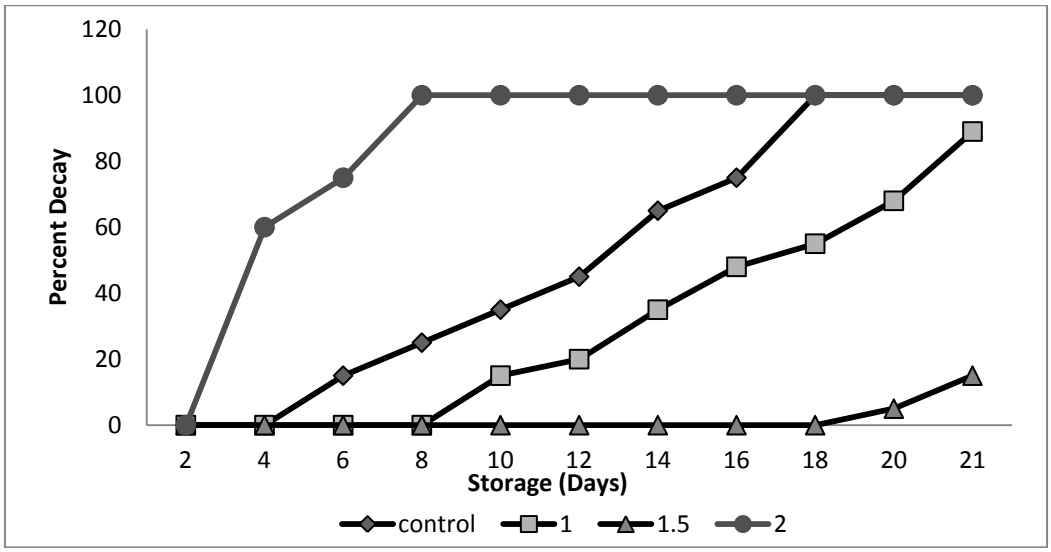

Fig. 1. Impact of gamma radiations on percent decay of carrot kept at refrigerated temperature.

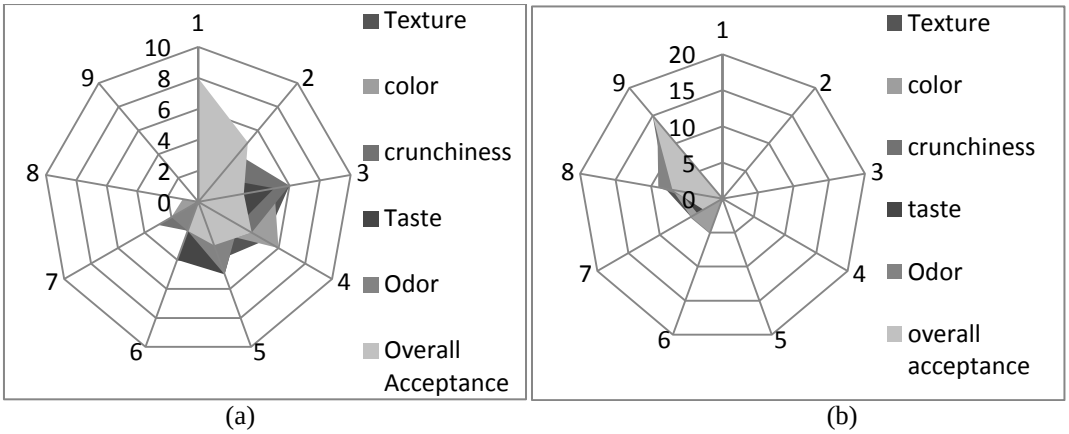

Fig. 2. Sensory evaluation of carrot samples (a) control (b) Irradiated.

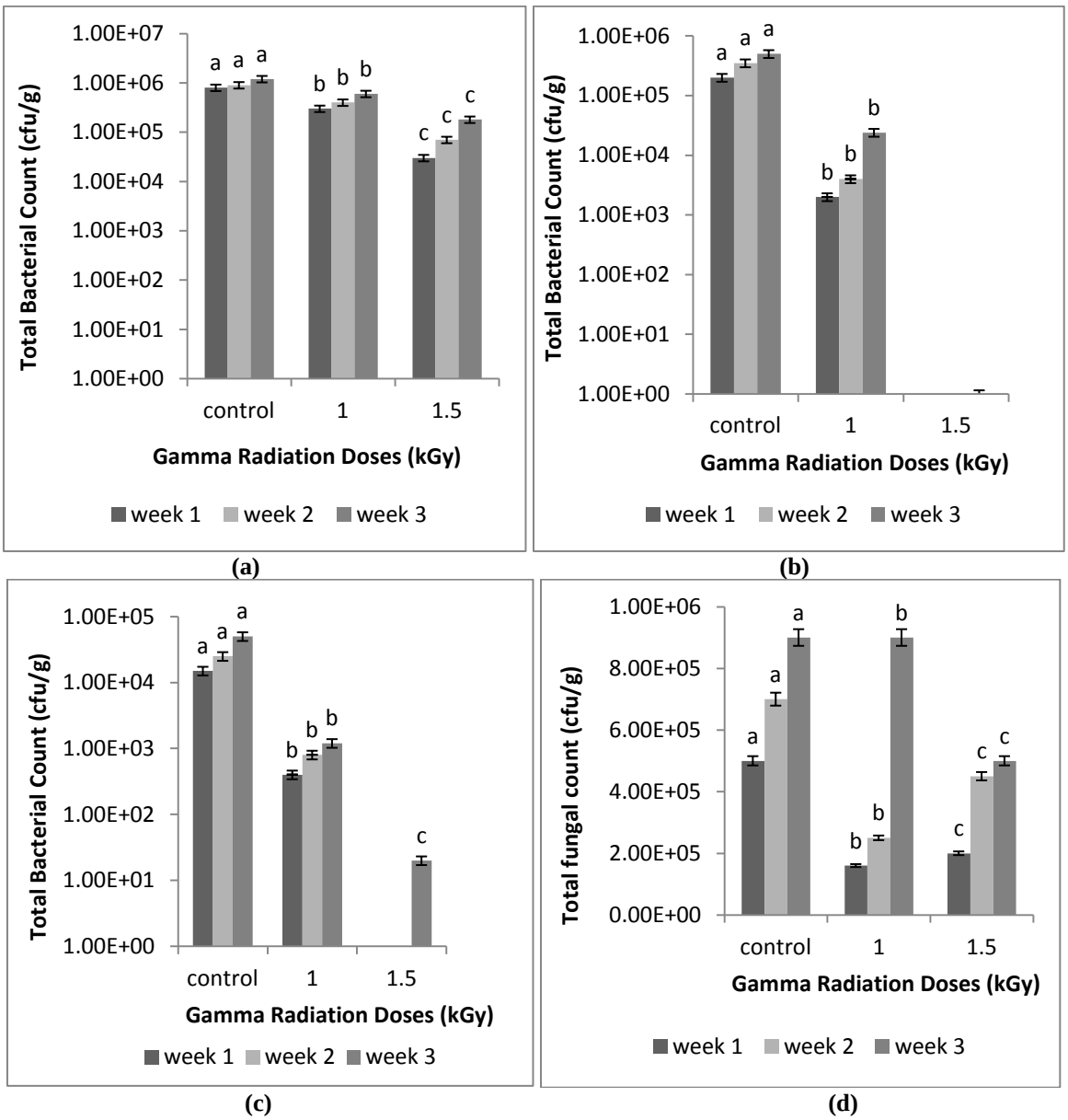

Fig. 3. Effect of gamma radiation on (a) total viable bacterial count of carrot kept at refrigerated storage using nutrient agar, (b & c) epiphytic bacteria present on carrots kept at refrigerated temperature using MacConkey and salmonella shigella agar respectively (d) epiphytic fungi present on carrots kept at refrigerated temperature using Potato dextrose agar as testing medium.

Impact of different doses of γ-radiation on bacterial count on MacConkey agar: The control samples showed a total viable bacterial count 2×10^4 cfu/g whereas carrot samples irradiated at 1.0 kGy showed less total viable bacterial count 1×10^4cfu/g during the first week of refrigerated storage. A complete growth inhibition of Gram-negative bacteria was observed at 1.5 kGy for the first two weeks after irradiation. The total viable bacterial count remained inhibited at 1.5 kGy dose after three weeks of refrigerated storage (Fig. 3b). The effect of all the treatment doses on the overall viable bacterial count remarkably varied from each other at $p \leq 0.05$.

Impact of different doses of γ-radiation on the bacterial count on salmonella shigella agar: A remarkable variation was observed among the viable bacterial count of control and irradiated samples. The untreated samples had the initial total viable count 5×10^4cfu/g, which increased to 2.5×10^5 cfu/g by the end of the second week at refrigerated storage. The irradiated carrot samples showed much less total viable bacterial count than the control group and this was more obvious when the higher doses of γ-radiation were applied. A maximal reduction in bacterial count was observed at 1.5 kGy irradiation because the carrots irradiated at this dose showed no bacterial growth for two weeks in refrigerated storage. However, these samples showed a total viable bacterial count 3.6×10^4 cfu/g after refrigerated storage for three weeks (Fig. 3c). *Salmonella* and *Escherichia coli* colonies were determined from the analyses of both the control and the irradiated samples displaying their presence on the sample surface. However, no *Shigella* colonies were observed on the SS plates of control and irradiated samples indicating their absence on the sample surface.

Total fungal count: *Effect of γ-radiation on fungal count of carrots using potato dextrose agar*
The ability of retarding the fungal growth by γ-radiation was dose depended. Irradiated samples showed fungal count 4.74×10^5 on the 21st day (Fig. 3d). The fungi, identified on the basis of micro and macroscopic characteristics, were *Aspergillus niger*, *Mucor* sp, *Alternaria alternate*, *Sclerotinia sclerotiorum* and various yeast species.

Discussion

In the present study, it was observed that the lower doses of γ-radiation were more effect for the maintenance of sensory properties as well as shelf life of carrots. At 1.5 kGy dose there was no change in color, texture and crunchiness of the carrots for the first two weeks, but during the third week, the samples were soft in texture and discolored and less crispy. The change in color was probably due to damage in various carotenoid pigments. Gamma radiation is known to affect photosynthetic pigments in various vegetables (Marcu *et al.,* 2013a; Vanhoudt *et al.,* 2014). As carrots contain large water content, therefore, during storage, due to dehydration, morphological changes were observed with a loss in crunchiness (Bourke *et al.,* 1967; Ben Mustapha *et al*., 2014) . A radiation dose dependent change in growth and development, which changes photosynthetic pigments, has been reported for lettuce (Marcu *et al.* 2013a). A higher dose of 2.0 kGy resulted in complete tissue decay due to free radical formation (Saakov, 2002; El-Habit *et al.,* 2000). At a dose of 1.5 kGy the reduction in decay ratio of vegetable may be related to the maximal decrease in microbial load due to gradual bacterial growth with deleterious effects on the chromosomal damage, beta-carrotene along with protein coagulation in living cells (Seifter *et al.,* 1982; Abraham *et al.,* 1993; Ray *et al.,* 2011). In the present study the carrots weight loss might be due to transpiration (Bibi *et al.,* 2006). The softness in the texture and loss in crunchiness of carrots may be due cellular damage, loss of essential vitamins such as ascorbic acid, thiamine and amino acids and changes in proteome expression by gamma radiation (Day, *et al.,* 1957; Vincent, 1961; Song *et al.,* 2006; Marcu *et al.,* 2013b; Hayashi *et al.,* 2015).

Total fungal and bacterial count abated at 1.0 kGy and 1.5 kGy dose. This might be due the radiolysis of water resulting in the formation of lethal hydroxyl radical, damaging the nucleic acids (Vavilov, 1992). Irradiation resulting in an increase in the intracellular pH leading to cellular apoptosis has been reported (Dai *et al.,* 1998; Chandna *et al.,* 2013). In the current study it was observed that limited decay of irradiated carrots, may be due to a decrease in microflora and subsequent dehydration caused by irradiation. The cellular decay at higher dose 2.0 kGy could be due to drastic changes in cellular adhesion, cellular and metabolic damage in the vegetable (Ndoti-Nembe *et al.,* 2013). We observed that the difference in percentage decay in carrots varied with the storage time and transpiration. The minimal decay of carrot at a dose of 1.5kGy dose could be justified by the maintenance of water content and consequently turgor pressure. The irradiation dose of 1.5kGy helped to eliminate all enterobacteriaceae after 14 days of storage at refrigerated temperature.

In the control samples, bacterial growth on macConkey agar mostly comprised of *Escherichia coli*. While only *salmonella* spp. were present on SS agar. It is known that *Escherichia coli and salmonella* are generally transmitted by contaminated water in raw vegetables (Pritchard *et al.,* 1992). The growth of *E. coli* and *Salmonella spp* was inhibited through irradiation since total viable *E. coli* and *Salmonella* count were totally eliminated at 1.5 kGy dose after 2 days of refrigerated storage.

The dominating fungi found on the control sample were *Aspergillus niger, Alternaria, Sclerotinia sclerotiorum* and *Mucor spp* along with various yeast species. These results are in line with those of Farkas *et al.* (1997) who determined the gamma irradiation effect on the microflora of carrots deducing that the gamma irradiation significantly reduced the load of bacteria, mold and yeast. Irradiation has been used to extend the shelf-life of carrots by controlling diseases caused by fungal pathogens (da Silva Aquino, 2012). The present study indicates that dose dependent irradiation of food may be a useful low cost method for preserving food.

Conclusion

For preserving carrots, the most effective dose was 1.5 kGy, which enhanced the shelf life to 7 days without causing any kind of detriment to vegetable. This irradiation dose also eliminated most bacterial growth that could damage carrots. The study was helpful in optimization of radiation for improving the shelf-life to overcome trade quarantine barriers in the international market. Therefore, vegetables can be transported and consumed for longer periods of time after harvesting while maintaining desirable sensory qualities longer than non-irradiated products.

Acknowledgements: Authors are thankful to Pakistan radiation services (PARAS) for providing the radiation services during the period of this research work.

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