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Original articles

# Influence of gamma radiation on nutritional quality and shelf life of fresh red chilies (*Capsicum annuum*)

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**Summary.** The aim of this work was to optimize the best suitable dose that effectively removes microbes, enhances the shelf life and has least negative effect on nutritional value of fresh red chili. The samples were packed in polyethylene bags and irradiated with selected doses (5, 6 and 7.5 kGy) using gamma radiation. Along with control sample, irradiated samples were stored at their storage temperature 4-7 °C for 15 days and were analyzed weekly. Impact of gamma irradiation on microbial load, sensory attributes like, color, texture, appearance, pungency and overall acceptability as well as on proximate composition was evaluated. 7.5 kGy was proven to be most effective dose as microbial load remained minimal and their nutritional value did not change significantly of fresh red chili, which suggest that gamma irradiation of 7.5 kGy can be used for enhancement of shelf life.

**Key words:** red chili, irradiation, microbial load, proximate analysis, shelf life

## Introduction

Chili is economically very important crop, which is cultivated all over the world. It belongs to the family Solanaceae and named as Capsicum annum / Capsicum frutescens botanically. Chili is primarily used as condiments and spices. There are almost 150 different chili types characterized by their color, shape and pungency (1).

Mexico is considered as a place of origin of red chilies. Chilies was brought from Brazil to Indo-Pak by the Portugues' before 1585 (Pakistan Agriculture Research, 2015). It is mostly distributed by India, Malaysia, Thailand, Pakistan, Philippines, Indonesia, tropical Africa, South America, North Africa as the major chili producing countries. Indians used capsicums as far back as 700 BC. The world's largest red chili producer, consumer and exporter is India, which produces nearly 25 percent of total chili production in the world, but exports only 4 percent of its total production, because of elevated use in their daily life routine. Pakistan is ranked 5th in cultivation of red chili (2), and is ranked the sixth largest exporter of chili peppers in the world (3). This export is mostly done in the form of fresh chilies, stalk less chilies, green chilies and chili powder.

Red chili comprises of numerous chemicals including steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fiber and mineral elements, but it also contains different vitamins (4). Red Chili has the ability to fight against inflammation, helps to clear congestion, boost immunity, cardiovascular health, weight loss and cancer protection (5, 6).

Chili crops can be attacked by numerous of different pests and can be diseased due to different pathogens and some other factor, which may cause heavy losses. Different diseases in the chili crop can reduce the yield of the plant such as bacteria, fungi, viruses, and nematodes. Furthermore, the damage due to fungal diseases are more harmful as compared to pathogenic diseases. Aflatoxins are the most potent natural mycotoxins produced by many species of Aspergillus; *Aspergillus flavus* and *Aspergillus parasticius*. These are the major agents in foodstuff spoilage, leading to huge economic loss and also a prime public health hazard through mycotoxin contamination. Pakistani chilies have been cast off for import into the Japan and the European Union because of elevated concentrations of aflatoxins (7).

Food irradiation is one of the main food processing techniques (8). Extension in shelf life of fruits and vegetables, reduced loss of food through spoilage are the important features of irradiation process. Recently the effect of radiation on pine nuts was investigated, which showed that radiation was effective for long term storage of pine nuts (9). Gamma radiation technique was applied to reduce the microbial load and yield loss of fresh red chili. In this study the effect of gamma radiation on the nutritional value as well as sensory attributes was evaluated to increase the shelf life, which would enable the degree of fresh produce to more distant markets and a substantial amount of foreign exchange would be earned.

## Materials and methods

The samples of red chili were taken from the local market of Lahore, Pakistan. The samples were packed properly in polyethylene bags (100g each for dose). The sample containing bags were subjected to gamma-irradiation by applying 5, 6 and 7.5 kGy dose level, in a cobalt-60 irradiator at Pakistan Radiation Services (PARAS). Fresh red chili samples were stored at 4-7 °C (10).

Stored irradiated samples were evaluated at weekly for sensory, microbial and proximate analysis. Experimental work was done in the research Laboratory of Department of Biotechnology, Lahore College for Women University, Lahore as all facilities and sources were available.

Control and irradiated samples of fresh red chili were put through the sensory evaluation periodically to compare color, texture, appearance, pungency and overall acceptability. Panellists were instructed to score the attributes using 9-point hedonic scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 3 = dislike very much, 1 = dislike extremely) (11).

For determination of microbial load irradiated chilies were analyzed weekly. Three growth media (Nutrient agar for bacterial determination; MacConkey agar for Gram-negative enteric bacilli determination; PDA for fungi determination) were used. Isolation of microflora is carried out by serial dilution method. 100 µl of the diluted sample was poured to sterilized petriplates containing the respective media followed by incubation for 24 hours at 37°C (bacterial isolation) and for 72 hours at 30°C (fungal isolation). After completion of the incubation time, all colonies were counted. Viable bacterial count is calculated by the method in accordance to Yousef and Carlstrom (12).

Fresh red chili sample was studied to determine the nutritional contents like carbohydrates, ash, moisture, protein and fiber. The moisture content of the chili sample was determined by utilizing a hot-air oven (13), ash test was performed by using Muffle furnace. Fat content was determined by Soxhlet reflux apparatus. Protein content was determined by the Kjeldahl method (14). Carbohydrates were determined by the overall difference between the total content and determined lipid and protein content. The AOAC (2006) official methods were used for proximate analysis of red chili sample.

The results were obtained by analysis of variance (ANOVA) was employed by SPSS version 20.0 and the average values were compared with three replicates with the aid of new multiple range Duncan's test and expressed as mean ± standard deviation. The mean square error of triplicates of the average value was also calculated and significance was accepted at $p \leq 0.05$.

## Results

Sensory evaluation of red chili was carried out at different time intervals to evaluate the different parameters such as color, texture, pungency, appearance and overall acceptability. Table 1 shows the results that 7.5kGy sample exhibited slight change in color immediate after radiation and slight change in texture after

**Table 1.** Effect of gamma radiation using 9-point hedonic scale

| Attributes* | Irradiated Red chili | Control | P value** |
|---|---|---|---|
| **Overall acceptability** $^{s}$ | 5.75±1.78 | 2.5±1.8 | 0.01 |
| **Color liking** $^{s}$ | 5.75±1.47 | 2.5±0.5 | 0.007 |
| **Texture liking** $^{s}$ | 6±1.22 | 3±1.22 | 0.01 |
| **Appearance liking** $^{s}$ | 5.25±1.47 | 2.75±1.90 | 0.04 |
| **Pungency liking** $^{s}$ | 5.25±0.82 | 2.5±1.5 | 0.02 |

± indicates mean square error and significance level for panelists acceptance (n=25)
** Student t-test 5%
*Attributes are significant at p≤0.05

7 days but no change in other sensory parameters over the 15 days of storage. Overall acceptability of un-irradiated and irradiated samples of red chili were interpreted by 9-point hedonic scale which uses parametric statistical analysis of data. Overall acceptability of irradiated sample was observed to be more as compared to control sample of red chili. Fig. 1 (a and b) shows the overall acceptability of control and irradiated sample after two weeks of irradiation and storage respectively.

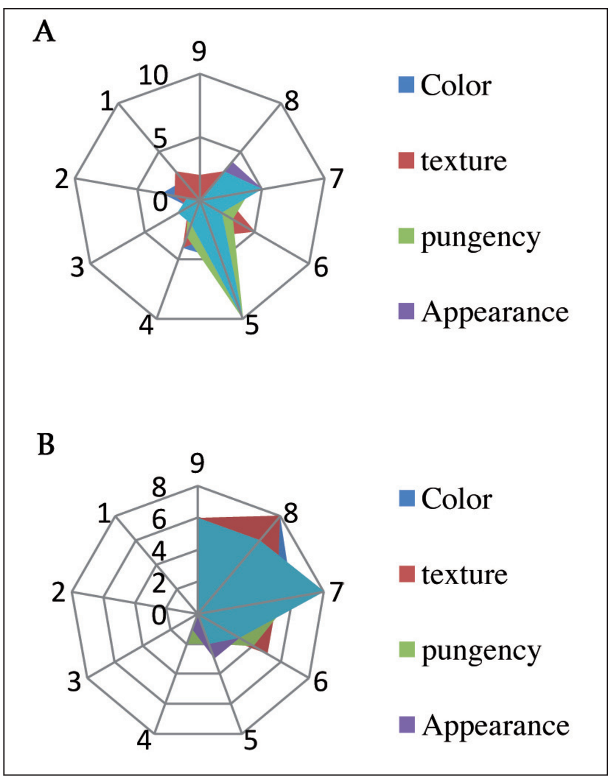


**Figure. 1** Sensory evaluation of red chili using 9-point hedonic scale. (a) Control at 15th Day (b) Irradiated at 15th Day

After storing chilies at 4 °C, the chili control sample showed an initial bacterial count of 12.6 × $10^5$ cfu/ml on nutrient agar. This count increased up to 15.4 × $10^5$cfu/ml after one week (Fig. 2). While the irradiated chilies exhibited a significant reduction in the total viable bacterial count to great extent. The highest reduction was shown in the results of highest dose subjected to chilies i.e. 7.5 kGy. The initial bacterial count observed were 2.4 × $10^5$ cfu/ml, 1.9 × $10^5$ cfu/ml and 1.2 × $10^5$ cfu/ml for the applied doses of 5 kGy, 6 kGy and 7.5 kGy respectively. After 15 days of storage, the number of bacterial colonies increased up to 15.4 ×$10^5$ cfu/ml for control and 5.3 × $10^5$ cfu/ml, 2.1× $10^5$ cfu/ml and 1.7 × $10^5$ cfu/ml 5 kGy, 6 kGy and 7.5 kGy respectively. In case of irradiated chilies, there was a gradual increase in the bacterial count week-wise as well but that was less as compared to their respective control groups. The error bars indicate the standard deviation from the mean value. The values vary significantly at p £ 0.05.

Fig. 2b shows the initial bacterial count observed in control was 6.6 × $10^5$ which gradually increased up to 7.3 × $10^5$cfu/ml. Fresh red chilies irradiated at 5 kGy and 6 kGy had an initial total viable bacterial count of 4.2 × $10^5$cfu/ml and 3.8 × $10^5$cfu/ml respectively. There was no colony appeared on fresh red chili irradiated at 7.5 kGy. The irradiated fresh red chilies showed less viable count than the non-radiated ones even after 15 days of irradiation. For example, the highest dose 7.5 kGy harbored the bacterial count of 1.1 × $10^5$ cfu/ml after 15 days which was less than that of control group of first week.

Fungi require higher doses for their complete elimination. However, Fig. 2 shows that the selected doses also exhibited reduction in fungi count. Selected dose harboured less fungal count 2.5 x $10^5$ after 15 days. Based on the macroscopic and microscopic characteristics, the identified fungi were *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium*, *Geotrichum*, *Alternaria alternata*, *Alternaria solani*, *Fusarium spp*. and a few yeasts species were also observed and microscopically examined.

In the conducted study evaluation of proximate composition of fresh red chili exposed the non-significant variations in moisture, ash, fat, crude fiber, protein and carbohydrate content. It was witnessed that fat,

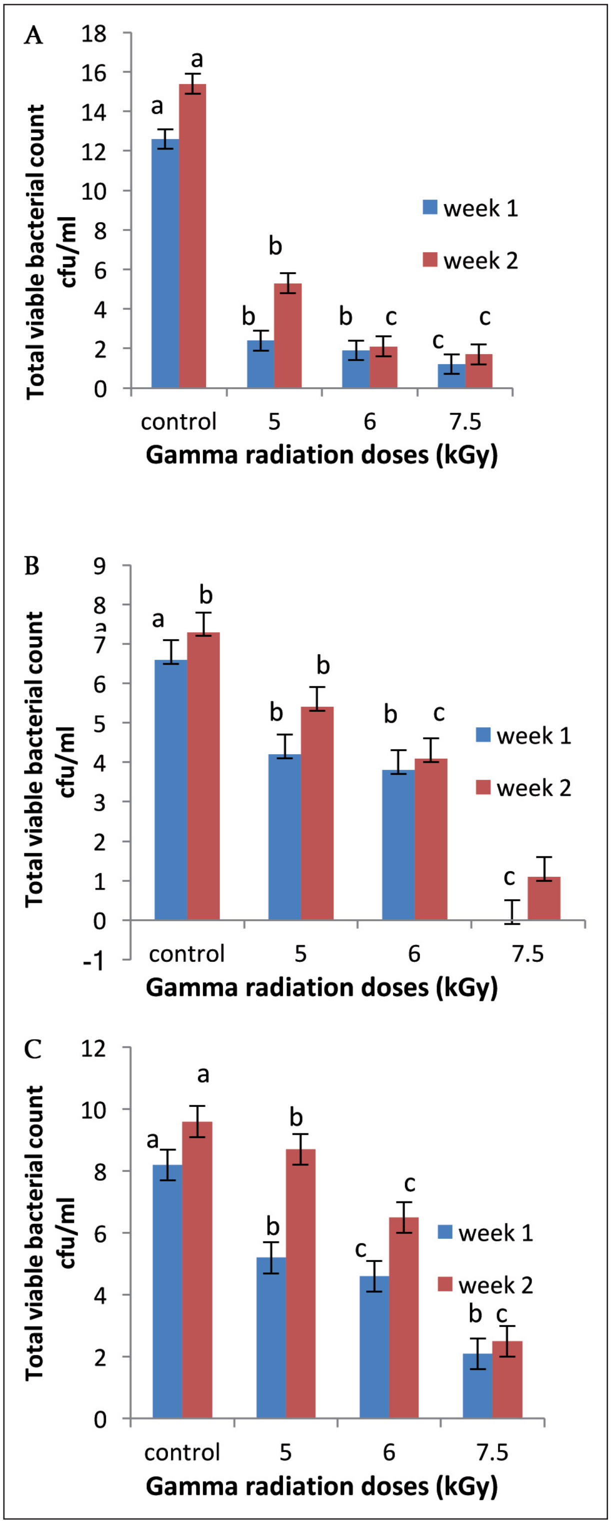


**Figure. 2** Effect of gamma radiation on (a) total viable count of bacteria on nutrient Agar, (b) Total viable count of bacteria on fresh red chilies using MacConkey agar as testing medium (c) fungal count of fresh red chilies using potato dextrose agar.

protein and carbohydrates are main nutritional components of any food and in case of fresh red chili at 7.5 kGy were not affected much (Table 2).

## Discussion

This study was aimed to optimize a minimum dose of gamma radiation to enhance the shelf life of fresh red chili. Fresh red chilli samples were subjected to various doses of gamma radiation in order to minimize microflora and analyse nutritional composition by proximate analysis.

Color is an important attribute of the quality of red chili. Decrease in color value is due to loss of yellow carotenoids by irradiation, which was higher than for the red carotenoid (15). Based on the current study sample irradiated at 7.5 kGy exhibited slight change in color immediate after radiation, while irradiated sample with 5 and 6 kGy showed no change in color after radiation treatment as compared to the control. This suggest that radiation of red chili had no immediate effect. The change in the color of the red chili with time were the same in radiated and non-radiated, which is in agreement with Munasiri *et al.* (16), who reported that, the irradiated spices were found to retain their quality after six-month storage. Pungency of red chili is caused by the chemical capsaicin (17). It was observed that the pungency of red chili was not affected by irradiation as well as storage. Change in texture was observed during storage, though the irradiation did not affect the texture of red chili either as the change during storage occurred both in control as well as irradiated chili peppers. There was a significant difference observed in the firmness of the stored chili pepper. This may be caused by the breakdown of polymeric carbohydrates especially pectin substances (18). Overall acceptability of irradiated sample was observed to be more as compared to the control sample of red chili.

The morphological characteristics of fungi were analyzed for their identification. Microscopic examination showed that *Aspergillus flavus* was abundantly present in control as well as irradiated samples. Other identified fungi were Fusarium species (particularly *Fusarium oxysporum*), *Aspergillus niger*, *Cladosporium*

**Table 2.** Influence of gamma radiation on proximate composition of fresh Red chili.
Each value is the mean of three replicates with ± indicating standard deviation (Mean ± SD). Mean value in table having different letters in superscript demonstrating mean difference is significant at the level of ($p \leq 0.05$) found by Duncan's new multiple range tests. Decay *

| Parameters (g/100g) | Time interval after irradiation | Different Radiation Doses (kGy) | | | |
|---|---|---|---|---|---|
| | | Control | 5 kGy | 6 kGy | 7.5 kGy |
| Moisture | 1st day | $13.20 \pm 0.015^{a}$ | $13.16 \pm 0.015^{b}$ | $13.15 \pm 0.015^{b}$ | $13.12 \pm 0.015^{c}$ |
| | 15th day | $13.41 \pm 0.02^{a*}$ | $13.38 \pm 0.015^{a*}$ | $13.35 \pm 0.01^{b}$ | $13.29 \pm 0.01^{c}$ |
| Ash | 1st day | $5.44 \pm 0.015^{c}$ | $5.46 \pm 0.015^{bc}$ | $5.48 \pm 0.01^{ab}$ | $5.50 \pm 0.015^{a}$ |
| | 15th day | $5.25 \pm 0.015^{c*}$ | $5.28 \pm 0.01^{a*}$ | $5.34 \pm 0.01^{b}$ | $5.37 \pm 0.01^{a}$ |
| Fat | 1st day | $5.62 \pm 0.015^{a}$ | $5.58 \pm 0.01^{b}$ | $5.55 \pm 0.01^{c}$ | $5.52 \pm 0.015^{d}$ |
| | 15th day | $5.40 \pm 0.02^{a*}$ | $5.31 \pm 0.01^{b*}$ | $5.23 \pm 0.015^{c}$ | $5.16 \pm 0.01^{d}$ |
| Crude Fiber | 1st day | $30.19 \pm 0.015^{d}$ | $30.23 \pm 0.015^{c}$ | $30.26 \pm 0.02^{b}$ | $30.29 \pm 0.015^{a}$ |
| | 15th day | $29.88 \pm 0.01^{d*}$ | $29.92 \pm 0.02^{c*}$ | $29.96 \pm 0.01^{b}$ | $29.99 \pm 0.015^{a}$ |
| Protein | 1st day | $12.45 \pm 0.015^{a}$ | $12.42 \pm 0.015^{b}$ | $12.40 \pm 0.01^{bc}$ | $12.38 \pm 0.015^{c}$ |
| | 15th day | $12.28 \pm 0.01^{a*}$ | $12.25 \pm 0.01^{b*}$ | $12.20 \pm 0.01^{c}$ | $12.15 \pm 0.02^{d}$ |
| Carbohydrates | 1st day | $33.10 \pm 0.02^{b}$ | $33.15 \pm 0.015^{a}$ | $33.16 \pm 0.025^{a}$ | $33.18 \pm 0.01^{a}$ |
| | 15th day | $33.78 \pm 0.015^{d*}$ | $33.86 \pm 0.01^{c*}$ | $33.92 \pm 0.015^{b}$ | $34.04 \pm 0.025^{a}$ |

*sp.*, *Penicillium* and *Geotrichum*. Different yeast species were also identified and confirmed by morphological and microscopic characteristics. It was observed that γ-irradiation could be an important way to prevent fungal contamination. Results of this study are in agreement with Sung (19) as it was evident that with the increase in radiation dose fungal load was decreased. Different bacterial species like *E. coli*, *S. typhimurium* and *Salmonella spp*. were found in fresh red chili. Every dose of irradiation (5, 6 and 7.5 kGy) showed a reduction in the number of bacterial colonies, but significant ($p<0.05$) reduction was observed at the highest dose 7.5 kGy as both fungus and bacteria were reduced at this radiation level.

The moisture content of red chili increased with passage of time, but decreased with increase in level of radiation dose. This showed close agreement with results of El-Niely (20). A non-significant change in ash content was determined. Results depicted that ash content of red chili sample was reduced with the passage of time and slight increase in the level of ash content was observed with increase of dose. Overall ash content was low. Dose 7.5 kGy was optimized because increase in level of ash content causes the increase in shelf life of sample. Increase in ash content can be explained by loss in moisture content (21). Our results are in agreement with Mohamed *et al.* (22).

The fat content was decreased in the sample of fresh red chili. Decrease in fat content was determined at different irradiation doses. At 6 and 7.5 kGy the fat content was decreased as compared to 5 kGy. This decrease in fat content of red chili could be due the action of high energy radiation on lipid molecules causing lipid peroxidation (23).

Crude fiber content of fresh red chili was increased slightly with increased level of gamma radiation dose whiles crude fiber content decreased during storage period. In contrast according to the study of Sandev and Karaivanov (24) decrease in the crude fiber content has inverse relationship with increase in gamma radiation dose. This might be due to the decomposition of linkage between lignin and other crusted substances by gamma radiation.

The results of current study showed a slight change in protein content of fresh red chili after irradiation during storage period. As the level of gamma radiation dose increased protein content also decreased. Overall, protein content decreased during storage period. Study of Maity *et al.* (25) demonstrated similar results as protein content was decreased with increased level of dose as compared to the control sample. Decrease in protein content with gradually higher irradiation dose is because of high rate of metabolic activities. On the contrary according to Eggum (26) protein content increased due to the application of the radiation. This might be due to the inactivation of the enzymes responsible for autolysis. Slight and non-significant change was observed in the carbohydrate content of the fresh red chili. In the current study it was observed that carbohydrate content was increased with progressively higher dose of gamma radiation as well as with the passage of time. According to the study of Sidhuraju *et al.* (27) increase in carbohydrate content was due to breakdown of oligosaccharides when samples were irradiated.

Gamma irradiation on fresh red chili at different doses such as 5, 6 and 7.5 kGy enhanced the shelf life for days. Although it was determined that 7.5 kGy was optimized dose for fresh red chili because microbial load remained within acceptable limits. There were no detrimental changes in proximate composition and sensory properties of chili irradiated upto 7.5 kGy and stored in polyethylene bags up to 15 days. As irradiation was employed after packing fresh red chili in polyethylene bags probability of recontamination was also reduced. Hence, fresh red chilies can be stored in polyethylene bags at 4-7 °C after irradiation for their better quality preservation at optimum conditions.

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