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Original article

# Influence of gamma irradiation on microflora, sensory quaity, nutritional properties and shelf life of Desi chickpea (*Cicer arietinum)*

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**Summary.** Chickpea is one of the highly nutritious pulses. It is a rich source of proteins and carbohydrates, providing dietary as well as health benefits too. Pakistan is the 3rd major producer**,** of chickpea but due to post harvest losses (insect and microbial infestation) huge quantity of Desi chick pea goes to waste. In the present study, the research was directed to increase the shelf life by reducing post-harvest losses of Desi chickpea using gamma radiations. The Desi chick pea were irradiated at different gamma radiation doses (0.5, 1, 2 kGy). Sensory analysis, insect infestation, proximate and microbial analysis were carried out after every 20 days interval for all the treatments. A remarkable reduction in microflora and insect infestation was occurred at 2kGy as compared to control samples stored at ambient temperature without affecting the sensory properties. The variation in proximate composition with gamma radiation treatment was not significant with increase in dose. Sample irradiated at 2kGy showed one month shelf life enhancement as compared to control.

**Key words:** Desi chickpea, micro flora, proximate analysis, gamma radiation

## Introduction

Chickpea (*Cicer arietinum*) is one of the important and highly produced pulses worldwide after dry beans and dry peas. It originated in southeastern Turkey (1). Desi chickpea is highly nutritious grains, brown colored, have a thick, firm seed coat and are small in size (2). They contained protein, fat, carbohydrates. It also supplies important vitamins. It is consumed as condiments, fried, and stewed, snack food, parched, and cooked. Besides human consumption, it also used as animal fodder (3). The Chickpea is the most hypocholesteremic agent and also they maintain good digestion. It also exhibits anti-oxidant activity. In Pakistan it is grown as a main Rabi pulse crop due to which Pakistan ranks 3rd in terms of production and 2nd in terms of area in whole World. They are stored in bags or in baskets, made from twisted rice straw, but during storage they are exposed to insect damage. Production of chickpea in Pakistan has seen a fall over the past few decades due to insect infestation and microbiological diseases. Due to these post-harvest losses, 5-10% damage has been reported in temperate regions, while 50-100% in semi-arid regions (4). More than 50 pathogens of chickpea have been reported worldwide. Fungi are the main pathogen of chickpea among which *Fusarium oxysporum* has been recognized to be the major pathogen (5). Different strategies are present to improve quality of Desi chickpea, but they all have some side effects like use of thermal treatments negatively affects color, taste, and nutritional content of the food products (6). So, Radiation of food is opted as the best and international strategy to improve food quality and its export value. Food Irradiation is a technology in which food products are exposed to a specific dose of ionizing radiation to improve safety and extend shelf life by eliminating or reducing bacteria, molds, insects from foods. Irradiation disrupts DNA in the nuclei of cells (7). Gamma radiation sterilization is mainly used for the sterilization of food because of its high penetration power. Gamma radiation does impart radioactivity to only organisms in food, but not to the processed food (8).

## Materials and Methods

The fresh Desi chickpeas of uniform size, same age group were collected from the wholesale market of Lahore. The samples were divided into two groups; experimental and control. All the samples were weighed, packed in polythene bags and labelled with a particular group and radiation dose in kGy. The packets of experimental group were carried to Pakistan Atomic Radiation Services (PARAS) for irradiation. After that irradiated and control samples were stored at ambient temperature and evaluated periodically after 20 days interval for sensory, infestation, microbial and proximate analysis.

*Sensory analysis*

The samples of both groups were physically analyzed to observe the changes in color, texture and overall acceptability after every 20 days of storage. The physical characteristics of un-irradiated and irradiated samples were also studied by carrying out organoleptic evaluation using a nine point hedonic scale where 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 (9).

*Infestation damage analysis*

100 grains from each sample were examined periodically after every 20 days of storage. The percentage grain damage by insect was determined according Jat *et al*. (10) by using the following formula

$$\text{\% Grain damage by insect} = \frac{\text{Number of damaged grains}}{\text{Total number of grains counted}} \times 100$$

*Determination of microflora*

For micro flora determination, serial dilution method was used (11). 1 g of sample was added in 9 ml of 0.9% saline water. 0.1 ml of this stock solution was poured into different petri plates containing Nutrient agar (for bacterial identification), MacConkey agar (for gram negative bacteria), SS agar (for Salmonella and Shigella identification and differentiation) and PDA (for fungal identification). The 0.2 ml of each dilution was poured and spread on the plates with the help of the spreader. Nutrient agar and MaCconkey agar plates were placed in an incubator at 37°C for 24 h. PDA plates were placed in an incubator at 30°C for 3 days for fungal growth. Total viable bacterial and fungal count was counted, according to Ben-David (12). The isolated bacteria were identified using analytical profile index while fungi were identified on the basis of micro and macroscopic features.

*Proximate analysis*

The proximate analysis (moisture, ash, crude fiber and protein contents) of both groups was determined according to the Association of Official Analytical Chemists (2005) official methods (13). Carbohydrate content was estimated by the difference method (14). The energy value was calculated by Khan *et al*. (15)

*Statistical analysis*

All the data was tabulated and results analysis was performed statistically (standard deviation, Duncan multiple range under one way ANOVA) by using SPSS version 18.0. Significance has been calculated at the level of ($P \leq 0.05$).

## Results and Discussion

*Effect of gamma radiations on sensory attributes*

In the current study, the gamma radiations (0.5, 1 and 2kGy) had no effect on sensory attributes (color and texture) of Desi chickpeas. Our findings are in line with Nagar *et al.* (16) who reported gamma radiation has very minor or no effect on sensory quality of chickpea. The dark brown color of un-irradiated and radiated Desi chickpeas became light with the passage of time and texture firmness was remained unchanged in most grains of both control and experimental group (Table 1). The lightening in color might be due to environmental conditions after seed ripening, as they influence the rate of loss of seed coat's color. High ambient temperature and relative humidity accelerated color loss (17). The texture of control, 0.5kGy and 1kGy irradiated samples was defected during storage, whereas 2kGy irradiated sample had normal texture. The breakage or splitting of the seed coat of a few grains of all groups (un-irradiated and irradiated) was also observed during their storage. The reason for this breakage might be weather conditions during seed maturation and harvest time, which were determinant for propensity of seed to break (17). So, overall acceptability of the 2kGy irradiated sample was good as compared to other samples. Therefore the 2kGy irradiated sample had the highest score for their color, texture and overall acceptability and were "liked very much" by the judges, whereas dis-likeness for control sample was increased at the end of the experiment (Table 2; Figure 1a and 1b).

**Table1.** Effect of Gamma radiations on sensory attributes of desi chickpeas stored at ambient temperature

| Radiation Doses (kGy) | Days of storage | | | |
|---|---|---|---|---|
| | **0** | **20** | **40** | **60** |
| | | **Color** | | |
| Control | Dark brown | Dark brown | Medium brown with black spot | Medium brown with black spot |
| 0.5 | Dark brown | Dark brown | Medium brown with black spot | Medium brown with black spot |
| 1 | Dark brown | Dark brown | Medium brown | Medium brown with black spot |
| 2 | Dark brown | Dark brown | Medium brown | Medium brown |
| | | **Texture** | | |
| Control | Hard | Hard | Hard, few have split seed coat, few have mouldy and soft surface | Hard, few lost seed coat, some have mouldy and soft surface |
| 0.5 | Hard | Hard | Hard, few have split seed coat | Hard, few lost seed coat, some have mouldy and soft surface |
| 1 | Hard | Hard | Hard, few have split seed coat | Hard, few lost seed coat, some have mouldy and soft surface |
| 2 | Hard | Hard | Hard, few have split seed coat | Hard, few lost seed coat |
| | | **Overall acceptability** | | |
| Control | Very like | Very like | Dislike | Dislike |
| 0.5 | Very like | Very like | Like | Dislike |
| 1 | Very like | Very like | Like | Dislike |
| 2 | Very like | Very like | Very like | Like |

**Table 2.** Evaluation of sensory attributes using 9-point hedonic scale

| Attributes* | Un-radiated desi chickpeas | Radiated desi chickpeas | p value** |
|---|---|---|---|
| **Overall acceptability** | $8\pm1.24^{a}$ | $2\pm0.20^{c}$ | 0.004 |
| **Color liking** | $6.08\pm0.71^{b}$ | $1.16\pm0.31^{c}$ | 0.003 |
| **Texture liking** | $7.33\pm0.94^{ab}$ | $1.91\pm0.42^{c}$ | 0.005 |

*± means standard deviation and significance for consumer acceptance (n=25)*
**Attributes are significant at p ≤ 0.05*
*** Student t-test 5*

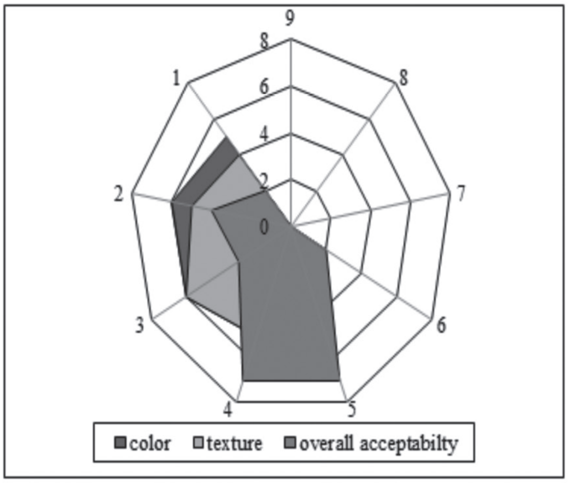

Figure 1a. Sensory evaluation of un-irradiated desi chickpeas by using 9 point hedonic scale

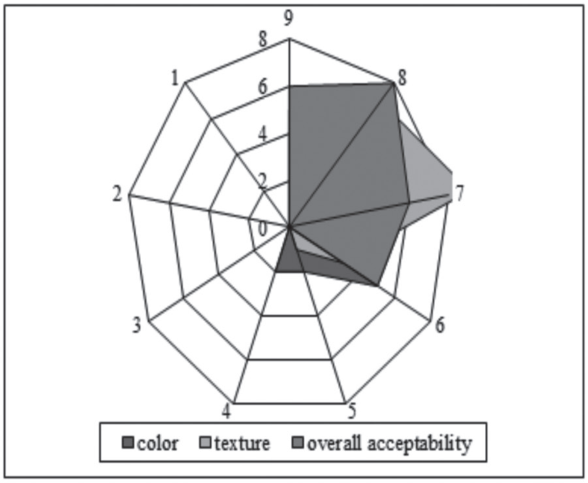

Figure 1b. Sensory evaluation of irradiated desi chickpeas by using 9 point hedonic scale

*Effect of gamma radiations on infestation damage*

The shelf life of Desi chickpeas was increased by gamma radiation as decay percent of radiated chickpeas was low as compared to un-irradiated chickpeas. The control, 0.5 and 1kGy radiated sample had shown decaying process with the passage of time, whereas 2kGy irradiated sample had retained its quality and remain safe from insect attack, till the 120$^{th}$ day of storage (Figure 2). This might be due to the deterioration effect of radiation on the developmental period of the insects, which was increased with an increase in the radiation doses (18). In short, dose of 2kGy was useful for disinfestation by gamma radiation, as compared to other applied doses.

*Effect of gamma radiations on microbial analysis*

Post-harvest losses in Desi chickpea cause high damage due to insect infestation and microbial diseases. Color intensity (light brown) change, increased micro flora, insect infestation is the problems which occurred during storage (19). In the current study, a sample of Desi chickpea was irradiated at different radiation doses (0.5, 1, 2 kGy) to extend the shelf life by controlling the growth of spoilage microorganisms. Total viable bacterial and fungal count was calculated both in irradiated and un irradiated Desi chickpea sample (Figure 3 and 4). Samples irradiated at 0.5, 1 and 2 kGy showed prominent reduction in bacterial growth on nutrient agar as compared to control sample. At ambient temperature, total viable count was found maximum on control group and it decreases linearly with increasing radiation doses as 0.5 kGy chickpea sample showed a little decrease in bacterial load while at 1 kGy it reduced significantly. But 1 kGy dose rejected due to bad sensory properties and insect infestation. 2 kGy was found to be optimum as the minimum bacterial population and good sensory properties were observed up to 2months of storage. No growth was shown on MacConkey and *Salmonella Shigella* agar for both control and irradiated sample. All the isolated bacteria were identified by using the analytic profile index strip. The bacteria identified include *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Staphylococcus epidermidis*. Among them, *Bacillus subtilis* and *Staphylococcus aureus* were present in high proportion in control and reduced significantly in gamma irradiated sample. Our studies were in accordance to Viswanathan *et al.* (20) who found *Staphylococcus aureus* to be the dominant bacteria present in Desi chickpea and responsible of its low quality and associated diseases such as various infections, food poisoning etc. The reason behind inhibition of micro flora in gamma irradiated sample might be that the DNA of microorganisms was susceptible to irradiation as irra-

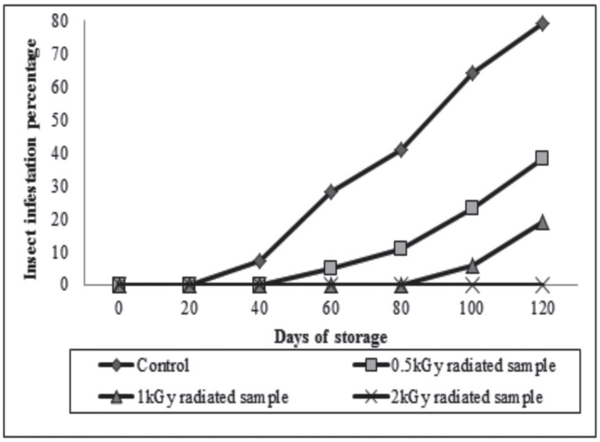


Figure 2. Effect of gamma radiations on insect infestation of desi chickpeas stored at ambient temperature

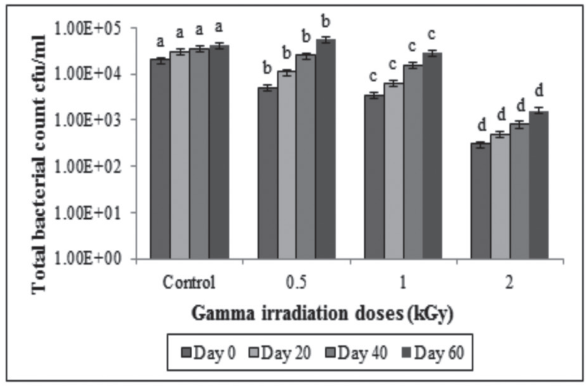


Figure 3: Effect of different gamma radiation doses on total viable bacterial count of desi chickpea sample kept at ambient temperature at different time intervals using nutrient agar

Each value is mean of three replicates. The error bars indicate standard error mean (± SEM) among the replicates. Superscripts show difference at the level of $p \leq 0.05$ by Duncan multiple range tests.

diation causes molecular destruction within the organism resulting in breakdown of DNA strands and cross linking ultimately causing death of the organism or making them devoid of their any important function without which they can't survive or reproduce (21). Effect of radiation on fungal count of Desi chickpea was also observed. Positive results were shown with much decreased growth in irradiated Desi chickpea as compared to the control sample at ambient temperature. Sample irradiated at 2kGy showed the best results with no fungal growth up to 1 month and with minimum growth in next intervals. The main pathogens of chickpea are fungal species. Control sample and samples irradiated at 0.5 kGy and 1 kGy showed fungal growth from the 1st interval to the last interval. The fungal species identified were *Aspergillus niger, Mucor sp., Rhizopus sp., Fusarium sp., Penicillum sp,* and *Saccharomyces sp.*(yeast). Our studies are in accordance with Diaz *et al.* (22) who found that *Fusarium oxysporum* is the main pathogen of chickpea deterioration producing black spores on chickpea surface. 2 kGy was found to be the best dose for fungi as there was no incidence of fungal growth on 2 kGy sample up to 1 month. Furthermore, only slight growth was seen after 1 month on 2 kGy chickpea sample as compared to control and other irradiated samples. This is because fungal population decreases proportionally with the increasing dose due to the sensitivity of fungi to radiation (23).

*Effect of gamma radiations on proximate composition*

Proximate analysis of the present study showed that overall there was no significant effect of applied gamma radiation doses (0.5, 1 and 2kGy) on Desi chickpeas. The change in proximate composition of control sample was also observed during storage at different times of analysis. This is showing that storage also had effects on proximate composition of Desi chickpeas. Our findings were similar to Ubairah et al. (24) who study the effect of storage time on proximate composition of chickpea cultivars. During this study, slight reduction in moisture, fat, fiber and protein had occurred, whereas ash and carbohydrate contents were slightly increased with increase in doses.

The fluctuation in the moisture content of unirradiated Desi chickpeas along with slight reduction due to gamma radiation in radiated Desi chickpeas was observed during storage. The moisture content of control sample was increased from 8.29 g/100 g up to 8.41 g/100 g at 40th day and then decreased to 8.36 g/100 g at 60th day. Probably the reason for this fluctuation is that during storage the moisture content depends on relative humidity and temperature. The grains of Desi chickpeas are hygroscopic, and they either absorb moisture from, or lose moisture to the atmosphere until the vapour pressure of moisture in seed and surrounding air are in equilibrium (17). The radiated samples had lower moisture content than control one. Like, on the first day of analysis, 0.5kGy, 1kGy and 2kGy radiated sample showed slight reduction in moisture from 8.29 g/100 g to 8.24g/100 g, 8.20 g/100 g and 8.17 g/100 g, respectively. On the last day of analysis, control had 8.36 g/100 g moisture content which was reduced in radiated samples to 8.33 g/100 g, 8.28 g/100 g and 8.24 g/100 g with the increase in radiation doses (0.5, 1, 2kGy) (Figure 5). This might be happening due to radiolysis of water, which is good for storage of chickpeas as high moisture content is responsible for fungal and insect attack. Furthermore, storage at lower moisture contents is suggested to minimize darkening and color loss from the seed coat (25).

In the current study, the gamma radiation increased the ash content with an increase in dose. The ash of control was also increased with the passage of time (Figure 6). The minimum ash 2.88 g/100 g was evaluated in control on the first day of analysis and maximum ash 3.39 g/100 g was evaluated in a 2kGy irradiated sample on the 60th day. Actually ash is a measure of the total amount of minerals present within a food. Legume seeds contain a broad mix of minerals, but these are bound to other compounds like tannin, protein and fiber. Phosphorus is mostly bound with phytic acid. With the passage of time, reduction in protein and fiber occurred. So, increase in ash content of control, during storage was observed (26). Gamma irradiation seemed to further enhance the availability of minerals in the radiated samples as phytic acid and tannin were also reduced by them (27). In short, both storage period and gamma radiation reduce the mineral chelators and availability of the minerals was increased.

The minor effect of gamma radiation was observed on the fat content of Desi chickpeas. The fat content was decreased with the time interval in both radiated and control samples. Fat content was also decreased with the increase in the gamma radiation dose. On the first day of analysis, the fat of Desi chickpeas was reduced from 3.78 g/100 g to 3.73 g/100 g, 3.69 g/100 g and 3.63 g/100 g when irradiated at 0.5, 1 and 2kGy, respectively. The reduction in fat of control was also observed with the passage of time. The control had 3.78 g/100 g fat, which was reduced up to 3.56 g/100 g on the last day of analysis (Figure 7). On the last day of analysis, control and 2kGy irradiated sample had 3.56 g/100 g and 3.42 g/100 g fat, respectively. So, radiation dose up to 2kGy had a slight effect on the fat content of chickpea and this small decrease might be occurred due to radiolysis of water by ionizing radiation which generates free radicals all of which react with the food constituents. Irradiation has been shown to accelerate lipid oxidation. In lipid molecule, the most susceptible site for free radical attack is adjacent to the double bonds. Therefore, most affected lipids during irradiation are polyunsaturated fatty acids that bear two or more double bonds (28) and it was known that chickpea is rich in unsaturated fatty acids such as linoleic and oleic acids so more susceptible to radical attack and reduction was occurred.

Similarly, radiation had little effect on fiber content. The first day analysis showed 10.65 g/100 g fiber of Desi chickpeas was reduced to 10.60 g/100 g, 10.58 g/100 g and 10.53 g/100 g after irradiation at 0.5, 1 and 2kGy, respectively. Likewise slight reduction was observed in irradiated Desi chickpeas throughout the experiment period. The last day analysis showed fiber of control was reduced from 10.65 g/100 g to 10.46 g/100 g. A Similar trend of reduction was observed on the last day analysis of irradiated chickpeas samples. At 60th day, 0.5, 1 and 2kGy irradiated sample had 10.42 g/100 g, 10.38 g/100 g and 10.35 g/100 g fiber content respectively (Figure 8). This dose-dependent decrease in fiber on irradiation had been attributed to depolymerization and delignification of the plant matrix (29).

The protein concentration of Desi chickpeas was decreased after radiation treatment. The slight reduction in protein content was observed in irradiated chickpeas with the increase in doses (Figure 9). On the first day of analysis, the protein of Desi chickpeas was reduced from 22.83 g/100 g to 22.79, 22.72 and 22.68 g/100 g after irradiation at 0.5, 1 and 2kGy, respectively. The protein was also decreased in control with the passage of time. On the 20th day of storage, protein of control was reduced to 22.75 g/100 g which was further decreased to 22.71, 22.68 and 22.63 g/100 g in 0.5, 1 and 2kGy irradiated samples, respectively.

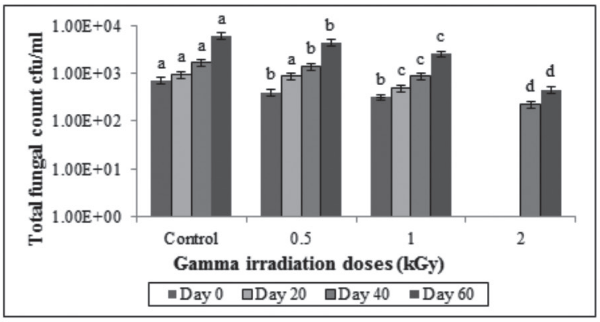


Figure 4: Effect of different gamma radiation doses on total fungal count of desi chickpea at different time intervals using potato dextrose agar

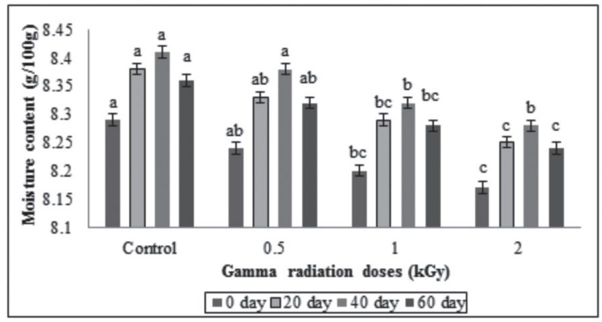


Figure 5. Effect of gamma radiations on moisture content of desi chickpeas stored at ambient temperature

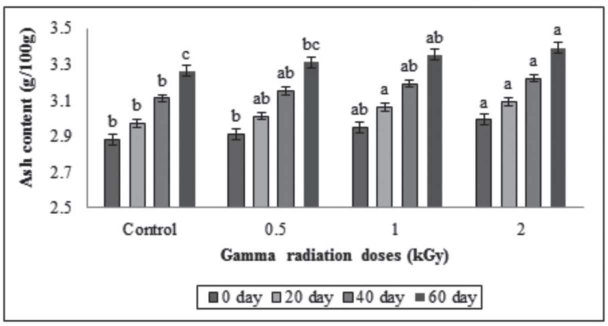


Figure 6. Effect of gamma radiations on ash content of desi chickpeas stored at ambient temperature

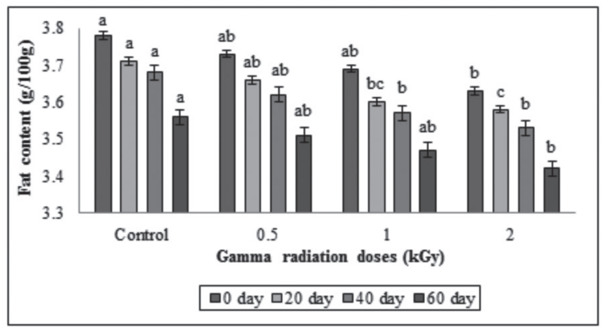


Figure 7. Effect of gamma radiations on fat content of desi chickpeas stored at ambient temperature

Each value is mean of three replicates. The error bars indicate standard error mean (± SEM) among the replicates. Superscripts show difference at the level of $p \leq 0.05$ by Duncan multiple range tests.

Similar trend in reduction in protein content by gamma radiation was also obtained by Singh and Singh (30). The minor changes in the protein fraction might be related to some cross-linking or aggregation of protein as a result of gamma irradiation (31).

In the present study the carbohydrate content of chickpeas was slightly increased by radiation treatment. The 2kGy irradiated sample had highest carbohydrate contents as compared to other samples (Figure 10). At the first day of analysis, the control had 51.57 g/100 g carbohydrate, which was increased to 51.73, 51.86 and 51.98 g/100 g when irradiated at 0.5, 1 and 2kGy respectively. The enhancement in carbohydrate content in radiated samples was significant at 60th day. The carbohydrate content of Desi chickpeas was increased from 51.77 g/100 g up to 51.93, 52.03 and 52.18 g/100 g in 0.5, 1and 2kGy irradiated samples, respectively. This increase in carbohydrate after gamma radiation processing might be due to cleavage of the glycosidic linkage of polysaccharides like starch, fiber, cellulose, pectin and glycogen to produce lower molecular weight fractions such as glucose, maltose and dextrin (32). Furthermore, after radiation the flatulence-producing raffinose family oligosaccharides (RFO), stachyose and verbascose in legumes were degraded and result in accumulation of sucrose and reducing sugars (33). The carbohydrate of control also increased and this might be attributed to the degradation of fiber with the passage of time.

The energy value was decreased with the passage of time in both un-irradiated and radiated Desi chickpeas. The reduction in energy value was observed with the increase in radiation doses (Figure 11). On the first day of analysis, the energy value 378.52kcal/100 g of control was reduced to 378.49, 378.25 and 377.97kcal/100 g in 0.5, 1 and 2kGy samples, respectively. The reduction in energy value of control up to 375.87 kcal/100 g was observed at 60th day. The energy value was further decreased to 375.72, 375.59 and 375.35 g/100 g in 0.5, 1 and 2kGy irradiated samples

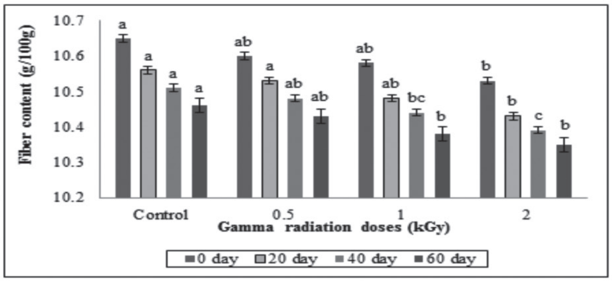


Figure 8. Effect of gamma radiations on fiber content of desi chickpeas stored at ambient temperature

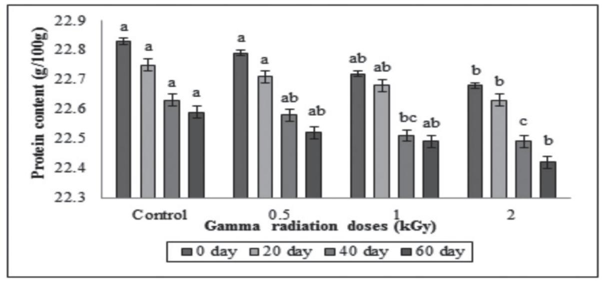


Figure 9. Effect of gamma radiations on protein content of desi chickpeas stored at ambient temperature

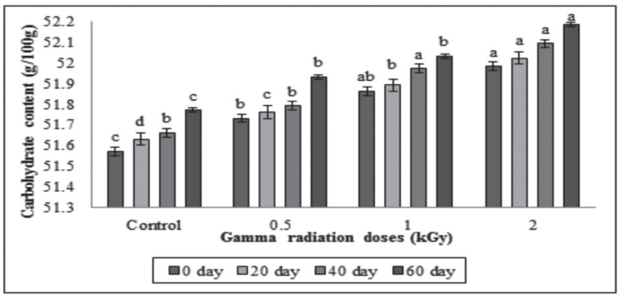


Figure 10. Effect of gamma radiations on carbohydrate content of desi chickpeas stored at ambient temperature

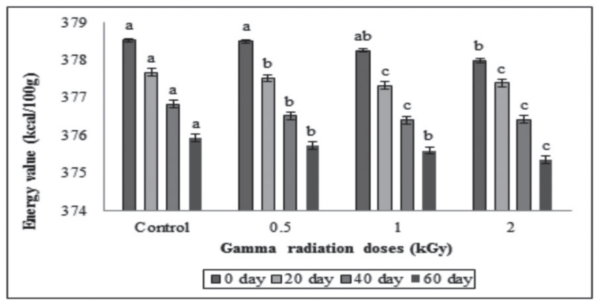


Figure 11. Effect of gamma radiations on energy value of desi chickpeas stored at ambient temperature

Each value is mean of three replicates. The error bars indicate standard error mean (± SEM) among the replicates. Superscripts show difference at the level of $p \leq 0.05$ by Duncan multiple range tests.

respectively. As we observed slight reduction in protein and fat, therefore the energy value of radiated sample was slightly lower than un-irradiated sample. The reason for depletion of the energy value of control was also might be attributed to reduction in fat and protein.

## Conclusion

It was concluded that 2 kGy is the optimum dose for the microbial reduction and insect infestation of Desi chickpea without negatively affecting its sensory properties even up to storage of 2 months. Sample irradiated at 2kGy showed one month shelf life enhancement as compared to control.

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