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Effect of physical mutagen on the *salmonella* inactivation, sensory evaluation and proximate analysis of chickpea (*Cicer arietinum* L.)

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world. It is a good as well as the cheapest source of protein, soluble, insoluble fibers, vitamins, potassium and phosphorus. The present research was done to check the efficacy of gamma irradiation on chickpea's proximate components and how the physical mutagen helped in *Salmonella* inactivation without any significant change in the proximate components of chickpea. The samples were treated with three different doses of gamma radiation (0.5, 1.0 and 2.0 kGy). Screening and evaluation of native micro flora on chickpea was performed and the viable counts of the microbes detected on samples of chickpea were *Bacillus subtillis, Escherichia coli, Streptococcus pyogenes, Staphylococcus aureus and Salmonella sp.* which were confirmed by biochemical test (API strips) before and after gamma irradiation. Results showed that 2 kGy is the optimum dose for chickpea at which complete elimination of *Salmonella* was recorded with no significant effect on sensory property as well as the proximate content of *Cicer arietinum* L.

Key woreds: Biochemical test, Chickpea, Gamma irradiation, Proximate analysis.

INTRODUCTION

Chickpea also known as Chana (*Cicer areitinum* L.) is an important grain legume and pulse crop that is rich in protein belonging to the leguminous family grown over 10.2 million hectare. The demand for chickpea has increased over the last few years due to its notable nutritional value as a source of vegetable protein, carbohydrates, dietary fibre, vitamins and minerals, (Jukanti *et al*., 2012).

Chickpea is grown as a winter crop in the Indian subcontinent, which accounts for nearly 85% of the chickpea area sown worldwide. It is also an important crop in West Asia and Mediterranean region (Rizvi *et al*., 2014). In Pakistan 90% of chickpea grown is of desi type and only 10% of kabuli type. Major producing provinces are Punjab and NWFP, constituting 87% and 7% of the area for chickpea cultivation, respectively (Shah *et al*., 2007). At present the world's chickpea production is about 1.43 million tonnes on an area of about 1.48 million hectares yielding about 9620 hg/ha in the year 2014. Pakistan's chickpea production is about 750 thousand tonnes on an area of about 990 thousand hectares yielding about 7576 hg/ha in the year 2014 (FAO, 2014).

Proximate analysis is a partitioning of compounds in a feed into six categories based on the chemical properties of the compounds which include moisture content, ash content, crude protein, crude lipid, crude fiber and digestible carbohydrates (Jeremiah *et al*., 2015).

Normally there are about 50 pathogens that attack the chickpea crop including 35 fungi, 9 viruses, 2 bacteria and 4 nematodes (Iqbal *et al*., 2002). The major pathogen that attacks the chickpea is *Fusarium oxysporum* L. Being rich in protein; chickpea plant is susceptible to a number of insect pests, which attack on roots, foliage and pods. However, bacteria are also involved in producing diseases in chickpea like bacterial blight cause by *Xanthomonas compestris* and bacterial leaf spot produce by *Burkholderia andropogonis* (Malik, 1984). Gamma rays with the seeds of and *kabuli* chickpea genotypes were treated with 10 doses of gamma rays ranging from 100 to 1000Gy with an interval of 100Gy by a 60Co source the self disintegration (Shah *et al*., 2008). *Xanthomonas* species is one of the mostly microbial species associated with low moisture foods like chickpeas. It can cause illness even if present in low numbers (Chen *et al*., 2009).

Food irradiation is the use of ionizing radiation to enhance food storage life, lessen post-harvest food losses and abolish food poisoning microorganisms. Food sterilization by gamma can destroy microorganisms, bacteria or insects that might be present in the food. Irradiated food does not become radioactive, but in some cases there may be subtle chemical changes. Over 50 countries currently permit food irradiation, and the volume of food treated is estimated to exceed 500,000 metric tons annually worldwide (Aquino, 2012).

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Hence the present study was focused to optimize such a dose for chickpea which is safe for the consumption without harming the nutrient content and minimizing the microbial load particularly *Salmonella sp.*

MATERIALS AND METHODS

Sample collection and gamma irradiation: Chickpeas (Kabuli type) were collected from the local market of Lahore and were apparently of good quality, without any physical injury. Chickpeas were then packed in polythene bags and were carried to the radiation unit of PARAS (Lahore) for irradiation. The doses administered were 0.5, 1and 2 kGy. During the present work, Harwell Amber 3042 dosimeter was used for dose measurement. The measurement uncertainty was 3% at 95% confidence level. Control was kept under identical conditions for comparison. Both the control as well as mutated chickpeas was stored at ambient temperature (30-37°C).

Proximate analysis: Chickpeas were being analyzed to find out moisture content, ash, fat, protein and carbohydrates. Official methods of analysis (AOAC, 2005) were used for proximate analysis of sample.

Microbial analysis: Irradiated chickpeas were analyzed for a week to determine the microbial load. Four growth media were used for the enumeration and identification of bacteria and fungi associated with chickpeas. Nutrient agar (for bacterial isolation), MacConkey agar (for Gram-negative enteric bacilli isolation), Potato dextrose agar (for fungi isolation) and *Salmonella-Shigella* Agar (for *Salmonella* spp. and *Shigella* spp. isolation) were used. Both the control and radiated samples were tested for the microbial load. The chickpea was weighed 1gm and was suspended in 9ml of sterilized distilled water for the isolation of micro flora. 100 µl of aliquots were transferred in petri plates containing sterilized nutrient agar media. Plates were incubated at 37°C for 24 hours. The crowing or excessive stacking of plates was avoided to permit rapid equilibration of plates with incubator temperature. The colonies were counted promptly after incubation period. The average colony count (arithmetic mean) of all replicates was calculated. Viable bacterial count is determined by standard formula of Colony Forming Unit per ml (CFU/ml) (Gent and Schwartz, 2005).

$$CFU/ml = \frac{\text{Colony count on plate}}{\text{total dilution of tube(used to make plate)} \times \text{amount plated}}$$

Statistical Analysis:

All the experiments were arranged in a completely randomized design and data was analyzed using the Costat software data (mean + SD) and was collected from experiments with five replicates based on Duncan's new multiple range test.

RESULTS AND DISCUSSION

Proximate analysis: The proximate analysis (moisture content, ash content, fiber content, fat content, protein content and carbohydrates) of control and mutated chickpea was determined and mentioned in Table 1.

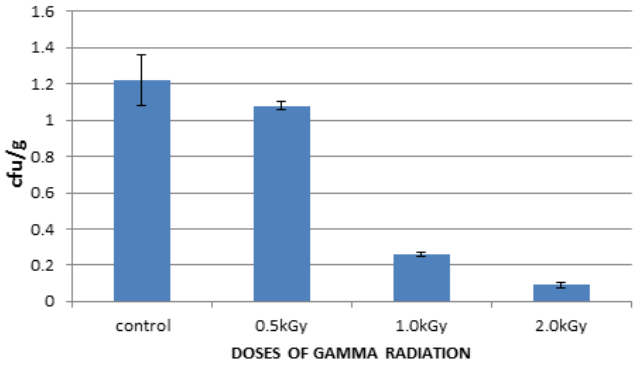
The radiation dose varied from 0.5 kGy to 2kGy to check the effect on the proximate components of chickpea and the difference was calculated in following three weeks. At dose 2 kGy the moisture content of control and mutated chickpea in 3rd week ranged from 7.33-7.00 g $100g^{-1}$, respectively. A variation in the ash content of non-irradiated and irradiated chickpea at dose 2 kGy in 3rd week ranging from 2.74-2.54 g $100g^{-1}$ was recorded in the samples but this change was statistically non-significant. The fiber content of control and mutated chickpea in 3rd week at dose 2kGy ranges from 10.00-9.76 g $100g^{-1}$ respectively. The obtained mean of fat content of non-irradiated and irradiated chickpea in 3rd week at dose 2 kGy ranges from 4.89-4.75 g $100g^{-1}$ was also indicating no statistical differences. The protein content of non-irradiated and irradiated chickpea in 3rd week ranges at dose 2kGy from 22.9-22.32 g $100g^{-1}$ respectively. The carbohydrate content of non-irradiated and irradiated chickpea ranged from 3rd week at dose 2kGy 51.61-51.63 g $100g^{-1}$ respectively. Arab *et al.* (2010) also reported decrease in protein content at higher dose. Wood and Grusak (2007) in their research on the proximate analysis of chickpea concluded that the dietary fiber content in chickpea was found up to 6g 100g-1, or decrease with the increase in gamma dose.

Microbial analysis: The total microbial content (cfu/g) of chickpea samples (control and mutated) was spread on nutrient agar initially (Figure 1).

Considerable difference of total viable bacterial count was observed between controls and irradiated samples on nutrient agar and compared in Figure 2. At first, the

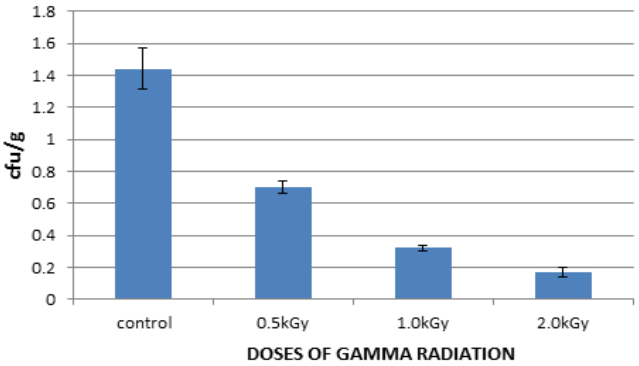
Table 1: Proximate analysis of control and mutated chickpea.

Parameters	Dose (kGy)			
	0.0	0.5	1	2
Moisture	7.67^{a}	7.33^{ab}	7.04^{b}	6.72^{b}
	7.5^{b}	7.16^{b}	6.83^{a}	6.42^{ab}
	7.33^{a}	7.0^{ab}	6.65^{b}	6.39^{b}
Ash	2.96^{b}	2.72^{a}	2.65^{c}	2.46^{a}
	2.85^{c}	2.63^{a}	2.53^{ab}	2.39^{b}
	2.74^{a}	2.54^{b}	2.41^{a}	2.32^{ab}
Fat	5.91^{a}	5.7^{a}	5.67^{b}	5.21^{a}
	5.10^{b}	5.53^{b}	5.51^{b}	5.03^{a}
	4.89^{b}	4.75^{a}	4.35^{a}	4.84^{b}
Fiber	10.50^{a}	10.21^{a}	9.97^{a}	9.73^{a}
	10.19^{a}	9.89^{a}	9.55^{ab}	9.46^{a}
	10.00^{ab}	9.76^{a}	9.32^{a}	8.5^{a}
Protein	23.45^{a}	22.73^{a}	22.37^{a}	21.93^{a}
	23.03^{a}	22.51^{ab}	22.22^{a}	21.54^{a}
	22.9^{a}	22.32^{a}	22.0^{ab}	21.30^{a}
Carbohydrates	50.11^{b}	51.10^{d}	52.30^{a}	54.55^{c}
	51.33^{d}	51.98^{c}	53.63^{a}	55.16^{d}
	51.61^{c}	53.63^{a}	55.26^{a}	56.65^{b}



Each value is the mean of five replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

Fig 1: Total microbial content (cfu/g) of chickpea sample spread on nutrient agar.

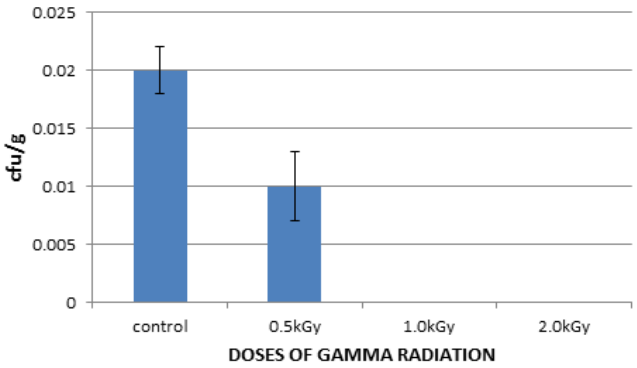


Each value is the mean of five replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

Fig 2: Total bacterial contents (cfu/g) of chickpea sample spread on nutrient agar.

chickpea sample from "local" as control was taken and checked for the presence of microbial load using nutrient agar. The average number of colonies observed was 1.22×10^4cfu/g while at the second week it was 1.44×10^4cfu/g and at the third week it was 2.01×10^4cfu/g. Then the samples were irradiated at 0.5, 1 and 2.0KGy were also observed for the enumeration of microbes on the nutrient agar plates. In that, the average number of colonies counted for dose 2kGy was 9×10^2cfu/g while at the second week average number of colony were 1.7×10^3cfu/g and at third week 18×10^{-4}cfu/g. This shows that radiation dose of 2kGy greatly reduced bacterial count as compared to bacterial count on control samples. Colonies obtained were round, slightly convex, entire, undulant, creamy, and opaque and white in color.

The inhibitory effect of gamma irradiation on the bacterial growth on chickpea surface was also observed on MacConkey agar showed in Figure 3. No colonies were found on radiated sample of chickpea at 2.0 kGy.

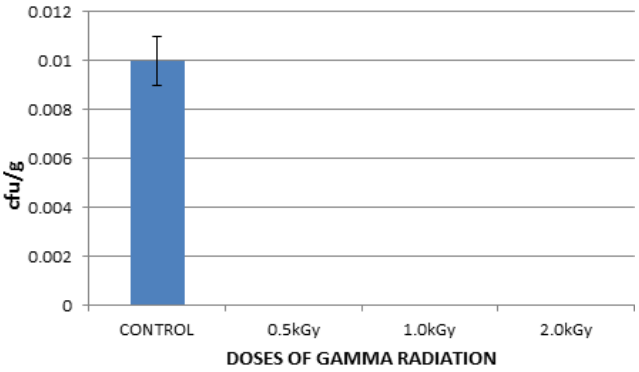


Each value is the mean of five replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

Fig 3: Coliform contents (cfu/g) of chickpea sample spread on Mac-Conkey agar.

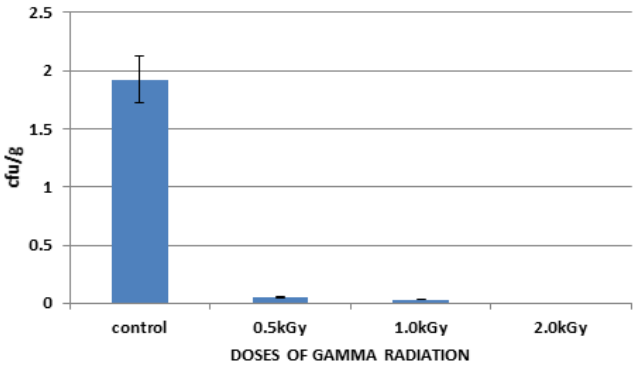
The fungal colonies gradually decrease with increasing gamma radiation doses as shown clearly in Figure 4. The average number of colonies observed was 1×10^4cfu/g. The colonies obtained were round, elevated, and entire, hyphal, opaque and their color was white. The most effective results were noted for the highest dose of 2 kGy. No fungal growth or 0cfu/g was found on chickpea at the radiation dose of 2.0kGy. This indicated that no fungi found on the radiated sample of chickpea. This phenomenon was repeated with dose 0.5 and 1kGy and no fungal hyphae were obtained.

The inhibitory effect of gamma irradiation on the bacterial growth on chickpea surface was also observed on blood agar medium showed in Figure 5. The average number of colonies observed was 1.92×10^4cfu/g. The zones were formed on control sample of chickpea showing the activity of hemolytic bacteria. There were 5 colonies showing the zones at dose 0.5kGy, 3 colonies showing the zones at the radiation dose of 1KGy and no colonies were observed after 30^{th} day of storage.



Each value is the mean of five replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

Fig 4: Fungal count (cfu/g) of chickpea sample spread on PDA.



Each value is the mean of five replicates. The error bars indicate the standard deviation from the mean value. The values vary significantly at $p \leq 0.05$.

Fig 5: Count of hemolytic bacteria (cfu/g) of chickpea sample spread on Blood agar

The highest doses of 2 kGy completely inhibited the growth of bacteria and no colonies were found on growth medium. The bacterial colony count increased after every week, however, substantial difference was observed between control and mutated samples.

Biochemical test (API strips) were inoculated with bacterial suspension and were placed in the incubator for 48 hours. Results were recorded by comparing results of API with characteristics of *Staphylococcus aureus*m *Bacillus subtilis Streptococcus pyogenes* and *Escherichia coli*. They were present only on cotrol samples.

Similar results were reported by Thomas *et al*., (2008), who studied colony formation in black tea irradiated up to 10 kGy absorbed dose. Similarly, Alighourchi *et al*., (2008) reported a progressive reduce in the microbial load of pomegranate juice irradiated to 0.5-10 kGy.

CONCLUSION

The results were indicative of the fact that gamma radiation at low doses does not change the nutritional value. The results of this study showed that gamma irradiation up to an absorbed dose of 0.5kGy did not significantly alter the nutritional components of the chickpea whereas the microbial load was nullified completely at this treatment level. Hence it can be concluded that gamma radiation works well for enhancing shelf life by reducing the microbial load. So in order to preserve the chickpea from disinfection as well as from some other quality oriented deteriorative effects, an appropriate gamma irradiation treatment should be given to the samples before introducing them in the market.

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