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Research Article

Effect of γ Radiation on Seed storage Proteins of Chickpea using SDS-PAGE

Pramod Kumar Singh[1]*; Dharmendra Singh [1]
Department of Biosciences Christian Eminent College, Indore (M.P.) India.

***Corresponding author**
Pramod Kumar Singh
Email: pramod.raju21@gmail.com

Abstract: Food irradiation (controlled application of ionizing radiation such as x-rays, gamma rays, electron beam, etc.) refers to improve hygiene, safety and to reduce microbial load in order to extend the shelf life of perishable food products. The aim of the experiment was to study the effect of γ radiation of (dose levels 1- 8 KGy) on seed storage proteins of chickpea. Effects of γ radiation on proteins were analyzed using (SDS-PAGE). Electrophoregrams shown that in seed storage protein only the higher molecular weight proteins band intensity decreases with increasing doses.
Keywords: SDS-PAGE, γ radiation, Total protein, Electrophoregrams.

INTRODUCTION

Food irradiation has been employed to ensure food safety or food sterility, extend its shelf-life and reduce the losses due to sprouting and ripening or pests however, some disadvantages are frequently associated with them, especially regarding unwanted changes in the organoleptic characteristics and nutrient loss[1].

Chickpea is a cheap source of energy, dietary fiber, protein, mineral and vitamins required for human health. Such qualities have been resulted in increased interests in research and possibly greater use of chickpea in the daily diet and in a variety of food products. In spite of good protein sources chickpea are also known to contain some anti-nutritional factors (ANFs) due to these properties, harmful effects are noticed in humans if consumed in raw state[2]. An anti-nutritional factor is defined as a substance which when present in human or animal feed interferes with assimilation of certain nutrients showing toxic or undesirable physiological effects, such as flatulence [2].

In order to inactivate or reduce these anti-nutritional substances, various conventional, simple processing methods have been used such as dry heating, roasting, boiling, soaking in water [3]. However, none of these methods is able to completely remove all these anti-nutrients that are present in seeds, grains or feed materials. Gamma irradiation treatment of legumes may be one of the possible alternative and additional processing techniques for reducing these anti-nutrients and improves the nutritive quality of legumes [4]. Radiation processing at dose up to 10 kGy is accepted safe and improvement in quality such as reduction in cooking time and improvement in texture without production of off-flavor dose in wheat (*Triticum vilgare*), barley (*Hourdeum vulgare*) and sorghum (*Sorghum biocoler*) [1].

The present work was undertaken to explore the effects of radiation to determine the changes in protein isolates and the electrophoretic protein pattern of chickpea following radiation process.

MATERIAL AND METHOD:

The chickpea seed was procured from R.A.K. College of Agriculture Sehore, Madhya Pradesh. M.P. India.

Irradiation of seeds:

250 gm seeds were sealed in polythene bags for irradiation following the method described by [5].The seeds were exposed to a ^{60}Co gamma source for irradiation at 25°C using exposure doses in the range of 1-8 KGy (0.12 KGy/h). The facility used was the Indian Gamma Chamber 400 A ^{60}Co facility at Choithram Hospital and Research Institute, Indore (M.P.), India. The radiation doses were measured with a Fricke Dosimeter [5]. The processed and raw samples were ground to fine powder and stored in a freezer until used.

CHEMICAL ANALYSIS:

Total proteins:

The total seed storage proteins were isolated following the methods of [6]. Powdered sample (100 mg) was mixed with 500 µl of 0.1 M Tris-HCl buffer (pH 7.5). Total protein was extracted after centrifugation at 17,600g for 20 min at 4°C and supernatants were used for analysis. Protein estimation was done following the method of [7].

Protein profiling using SDS-PAGE:

Proteins profiling of samples was performed using SDS-polyacrylamide gels as described by [8]. Equal quantities of proteins (150 µg) from each sample along with protein molecular weight marker (SM0671, Fermentas) were loaded into 10% gels. Electrophoresis was performed at constant voltage (100 volts). Staining of gels was done in 0.025% Coomassie Brilliant blue 250 containing 40% methanol and 7% acetic acid, while destaining was done in the same solution without dye.

Statistical analysis :

All work was done in triplicates and the data presented are means ± S.D. of three independent determinations. Significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

Immense application of gamma radiation in agriculture have been found for reducing post-harvest losses by suppressing sprouting and contamination, eradication or control of insect pest and in extension of shelf life and for breeding of high-performance well adopted and disease resistant agricultural crop varieties [9]. The objective of this investigation was to evaluate the effect of different doses of gamma radiation (1-8) KGy on chickpea protein patterns. The total protein content was found to be depleted by gamma irradiation in dose dependent manner as shown in table 1. The pattern of change in protein content, as a function of ionizing radiation, is different for different doses when compared to their respective controls. Maximum soluble protein loss of JG74 is 33.33 % was observed when seed was irradiated at 8 kGy. Irradiation at higher doses (5 kGy) on black seed showed the slightly greater reduction as 4 kGy radiation showed 10.07 % but as in case of irradiation at 5 kGy showed 13.43% reduction.

The result in Fig. 1 shows that the electrophoretic separation of protein profiles was different according to the concentration of protein obtained in extraction. The protein profiles of total soluble protein were affected by gamma irradiation depending on the dose of irradiation and protein fractions. Electrophoretic analysis of chickpea protein after gamma radiation compared with control sample proved that major protein bands after 4 KGy change its intensity (faint) by gamma radiation. Since radiation is known to cause disintegration and aggregation of protein molecule this may be occur in the present investigation [10] which will effect on the protein solubility. Therefore the irradiation dose used considered very important, since this dose could unfold protein to increase its solubility through the deamination of the protein [11] or aggregate protein during disintegration through the decrease of the sulphahydryl group and increase the disulphide bond [12] and rearrangement of the small molecular weight protein to a high molecular weight and causes decrease in protein solubility [10].

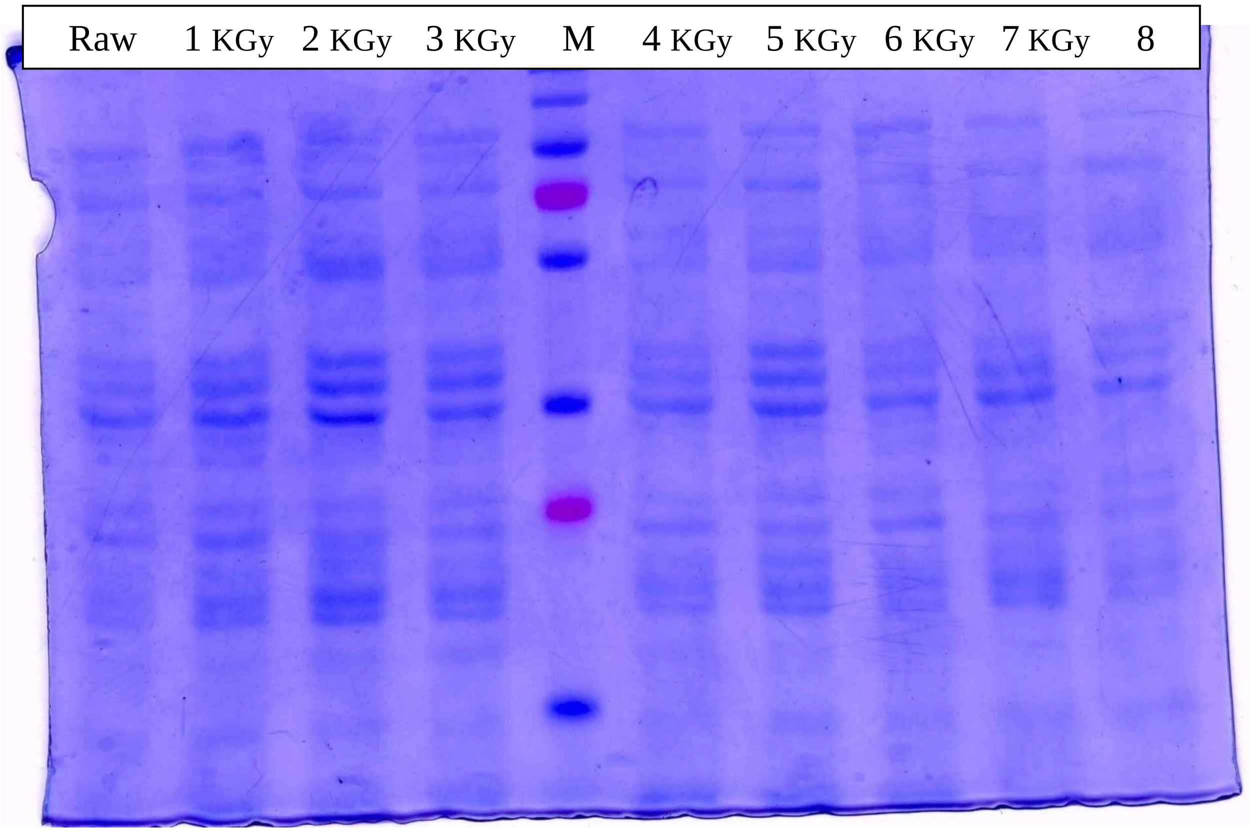

Fig 1: Schematic diagram of SDS-PAGE profile of seed storage proteins of chickpea using γ radiation

Table: 1 Seed storage protein (mg/gm) profiles of chickpea seed and effect of γ radiation.

Sample	Raw	1 KGy	2 KGy	3KGy	4KGy	5 KGy	6 KGy	7 KGy	8 KGy
JG74	3.87±0.03	3.81±0.02	3.67±0.04	3.66±0.03	3.48±0.03	3.35±0.04	2.83±0.04	2.66±0.04	2.58±0.04

Singh *et al.*[3] studied the radiation induced effects on some changes in protein content, as a function of ionizing radiation, is different for different seed types when compared to their respective controls. Maximum soluble protein loss was reported in lentil (19.14%) followed by (10.33 %) in brown chickpea, (6.53%) in mung bean and slightly lesser reduction was showed in kabuli chickpea (3.73 %). They studied the effects of radiation on nutritional as well as anti-nutritional factors of some common legumes which is widely used in India.

In 2009 [13] studied the radiation-induced effects on seed. Their study reflects the effects of radiation induction on edible seed protein profile, carbohydrates, amino acids and genomic DNA during gamma sterilization. They observed that the total protein and carbohydrate was decreased with increasing dose compared to control samples at 6 kGy. Our results are agreement with result of [13] that gamma irradiation had a greater effect on *C. arietinum* in terms of its loss in total soluble protein (30%) and carbohydrate when irradiated at 6 kGy. In our studies also the total protein content decreases with progressively higher doses of gamma radiation due to higher metabolic activities.

In 2010 [14] studied the effect of low dose gamma irradiation on plant and grain nutrition of wheat. They observed that the low dose gamma irradiation induced improvement in plant growth, yield, flag leaf area and photosynthetic attributes is caused by an improvement in plant nutritional characteristics. Gamma irradiated improve plant nutrition in general had higher concentration of both micro and macronutrients in the plant tissues but not in the grains. They further noticed that not only the protein content increased in the irradiated grains, but also a qualitative change in the protein profile was evident from their experimentation. Significantly they noticed that the grain carotene, which is a precursor for vitamin A, was also higher in irradiated grains.

CONCLUSION

In this paper we find an interesting fact that Irradiation dose at 8.0 KGy might be causes fragmentation of protein during radiation. The direct breakdown of the molecule by ionization capacity of the passing photon may be one possibility which will effect on the protein solubility In addition, radiation normally cause the formation of disulphide bridge between polypeptide chain which may be effect on the aggregation of the low molecular weight protein and effect on its conformation.

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