

Effects of Irradiation on Protein Electrophoretic Properties, Water Absorption and Cooking Quality of Dry Bean and Chickpea

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Effects of gamma-irradiation at doses of 1, 5, 10 kGy on electrophoretic patterns of insoluble proteins, water absorption properties and cooking quality of dry bean and chickpea samples were investigated. SDS-PAGE patterns of the samples in each variety did not differ in terms of relative mobilities. The densitometric analysis results indicated that the effects of irradiation on SDS-PAGE patterns of dry bean and chickpea proteins seem to be minor. Generally, lower irradiation doses did not significantly affect the water absorption properties of the food legumes. On the other hand, the dry and wet cooking times of the irradiated samples were found to be significantly reduced in all dry bean and chickpea samples.

Keywords: gamma-irradiation, chickpea, dry bean, SDS-PAGE, cooking quality

Introduction

Cereal grains and food legumes serve as the major food sources in almost all countries. Although cereals, grains and food legumes are susceptible to attack by a variety of insects and microorganisms during storage, they can be stored as dry seeds for considerable periods without deteriorating if necessary protective measures are taken. Commonly used methods for preventing and controlling infestation of stored grain involves physical, chemical and biological methods and sanitation (Bulla *et al.*, 1978).

Gamma-irradiation is a physical technique of food preservation and seems to have a potential to protect foods from insect attack during storage. It has been reported that low doses of radiation are effective in controlling insects, at doses far below the tolerance of the product (Goresline, 1973). Irradiation at 1 kGy dosage was proposed for insect disinfestation of grain (Murray, 1990). Irradiation at 2.5–5.0 kGy dose levels has been recommended for complete disinfestation of dry legumes and thereby to increase their storage life (Rao & Vakil, 1983). A Joint Expert Committee on the Wholesomeness of Irradiated Food convened by FAO, IAEA and WHO stated that irradiation of any food commodity upto 10 kGy presents no toxicological hazard (Anonymous, 1981). Therefore, the effect of ionizing radiation upto this recommended dose level on the quality and constituents of food legumes needs to be investigated.

Besides its protective role against insects, gamma-irradiation may also have important effects on the physico-chemical and functional properties of macromolecules and various quality criteria of cereal grains and food legumes (MacArthur & D'Appolonia, 1983; Rao & Vakil, 1985; Sabularse *et al.*, 1991; Köksel *et al.*, 1996; Köksel *et al.*, 1998a; Rao *et al.*, 2000). It was suggested that irradiation of wheat results in molecular degradation of proteins

(Srinivas *et al.*, 1972; Köksel *et al.*, 1998). Nutritive value of beans was significantly improved by gamma-irradiation due to inactivation of anti-nutritional factors (Reddy *et al.*, 1979). The rates of digestibility of proteins and α -amylolysis of starch were shown to increase by gamma-irradiation in red gram samples (Nene *et al.*, 1975 a,b).

The loss of the external membrane integrity due to irradiation (Dadaylı *et al.*, 1997) and the changes in the properties of macromolecules are expected to cause water to enter through the cell membrane of food legumes easily. Thus, it was decided to investigate the effects of gamma-irradiation on water absorption properties and cooking quality.

Legumes contain major protein classes defined by Osborne (1924). Globulins are the major storage proteins of legumes. They are soluble in low concentration of salt solutions but insoluble in water. Albumins also exist as storage proteins in some legumes. The globulins are investigated in two major classes according to their sedimentation coefficients. These are legumin-like (11S globulins) and vicilin-like (7S globulins) proteins (Deshpande & Damodaran, 1990). The solubility of legume proteins is affected from pH to a large extent. Solubility profiles of legume proteins as a function of pH indicated that solubility was lowest around at pH 4.0 (pI region) and highest at pH 10 (Zayas, 1997).

Beans and chickpeas are the most widely consumed food legumes in traditional dishes of Turkey and surrounding countries. Some losses might occur due to insect infestation and microbial growth during post harvest storage. Gamma-irradiation might be an efficient preservation method. However, not enough is known about what happens to constituents of food legumes when they are irradiated. Therefore, this research was designed to evaluate the effects of irradiation on the constituents and cooking properties of these legumes. The main objective of this study was to examine the effects of gamma-irradiation at

different levels (upto 10 kGy) on proteins of dry bean and chickpea samples by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Water absorption properties and cooking quality of dry bean and chickpea samples were also investigated.

Materials and methods

Materials Two dry bean (*Phaseolus vulgaris*) and two kabuli chickpea (*Cicer arietinum*) samples were used in this study. The dry bean (cvs. Yalova and Yunus) and chickpea (cvs. Akçin and Camitez) samples were grown at Experimental Research Farm of Field Crops Improvement Center (Ankara, Turkey). The samples were cleaned, placed in polyethylene bags and irradiated with the doses of 1, 5, 10 kGy from the ^{60}Co source at Sarayköy Nuclear Research Institute. Unirradiated seeds served as controls. The dose rate was 6.0 kGy/h. The absorbed dose was checked by Fricke's dosimetry (Chadwick *et al.*, 1977). All food legume samples were stored in a refrigerator before laboratory analyses.

Methods

Extraction of proteins Seeds of each cultivar were dehulled and ground to pass through a 212 μm screen. Salt soluble and insoluble proteins of ground dry bean samples were obtained by the following extraction procedure; 40 mg ground sample was mixed with 300 μl 0.5 M NaCl (pH 1.3, adjusted with 1 M HCl) in order to adjust the final pH to 3.6. Extraction was carried out for 1 hour by vortexing each sample every 15 minutes followed by centrifugation at $11,600 \times g$. (Sanyo MSE, Japan) for 5 minutes. The supernatants were decanted and used for the determination of soluble proteins (Lowry *et al.*, 1951). Salt insoluble fraction was dissolved in 500 μl buffer solution (pH 6.8), for another 1 hour extraction. The buffer solution contained 0.063 M Tris-HCL, 2% (w/v) SDS, 7% (v/v) 2-mercaptoethanol, 20% (w/v) glycerol, and 0.01% (w/v) Pyronin Y. The supernatants (12 μl) were applied to the SDS-PAGE. The extraction procedure for dry bean samples was also used for the extraction of salt soluble proteins of chickpea sample with some modifications. Ground chickpea samples (40 mg) were mixed with 250 μl 0.5 M NaCl (pH 1.3) in order to adjust the final pH to 3.1. After centrifugation at $11,600 \times g$ for 5 minutes, the supernatants were decanted and used for the determination of soluble proteins (Lowry *et al.*, 1951). The salt insoluble fraction was used for SDS-PAGE procedure.

SDS-PAGE method The salt insoluble fraction was dissolved in 500 μl Tris-HCL buffer solution (pH 6.8). The supernatants (15 μl) were applied to the gel. SDS-PAGE was performed according to the method of Laemmli (1970) as modified by Ng & Bushuk (1987). The acrylamide concentrations of stacking and separating gels were 4.0% and 12.5%, respectively. SDS-PAGE was performed in a cooled slab gel unit (Hoefer Scientific Instruments, San Francisco, CA, USA). High molecular weight (HMW) marker proteins were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). The gels were scanned by a scanning densitometer (The Imager BL, Biolab) with GeneTools

software (SynGene-Version 3.02). The relative quantities of bands from the scanned patterns were calculated from the respective areas on the densitograms and normalized to facilitate the comparison of the effects of irradiation at different doses.

Quality evaluation The quality criteria; dry and wet seed weights, dry and wet seed volumes, hydration capacity, hydration index, swelling capacity and swelling index, and the dry and wet cooking times were determined according to the methods of Williams *et al.* (1983, 1986). The seed weights and seed volumes were determined in mg and μl , respectively. For the determination of hydration capacity, 50 seeds were weighed and transferred to a 250 ml Erlenmeyer flask and 100 ml water was added and left overnight at room temperature. Next day the seeds were drained, surplus water removed with filter paper and the swollen seeds reweighed. For the determination of swelling capacity, dry and wet volumes of the 50 seeds were determined. Hydration capacity, hydration index, swelling capacity and swelling index were calculated according to the following equations (Williams *et al.*, 1983).

$$\text{Hydration capacity} = (\text{weight after soaking} - \text{weight before soaking})/50 \quad (1)$$

$$\text{Hydration index} = \text{hydration capacity} / \text{original seed weight} \quad (2)$$

$$\text{Swelling capacity} = (\text{volume after soaking} - \text{volume before soaking})/50 \quad (3)$$

$$\text{Swelling index} = \text{swelling capacity} / \text{original seed volume} \quad (4)$$

Labconco crude fiber testing equipment was used for the determination of the cooking times. Berzelius beakers (600 ml) were used for boiling the samples under continuous reflux. Cooking time means the time taken between starting to boil the seeds until they are ready to eat, which means that at least 90% of the seeds are soft enough to masticate without having to chew. The cooking of the seed involves gelatinization of the starch, and the simultaneous reduction of the cell wall tissues to the extent that they are soft and friable enough to disintegrate easily in the mouth. In the present study, the cooking time was determined by watching the progress of the gelatinization. During cooking the white area becomes smaller due to gelatinization as the samples are boiled for progressively longer time (Williams *et al.* 1983).

Statistical evaluation Data related to protein solubility, hydration capacity, hydration index, swelling capacity, swelling index, dry and wet cooking time values were analyzed for variance using the MSTAT statistical package (Anonymous, 1988). When significant differences were found, the Least Significant Difference (LSD) test was used to determine the differences among means.

Results and Discussion

Effects of gamma-irradiation on dry bean and chickpea proteins During the preliminary studies, effects

of different conditions (pH values and NaCl concentrations) on electrophoretic properties of soluble legume proteins were investigated. However, resolution of the protein bands especially at alkaline conditions was not good in electrophoregrams and masked the minor differences in electrophoretic patterns due to irradiation treatment. Therefore, soluble proteins of the dry bean and chickpea samples were extracted at pH 3.6 and pH 3.1, respectively and insoluble proteins are used in further SDS-PAGE experiments. In the present study, the proteins observed in the SDS-PAGE patterns probably represent the globulins that were salt-insoluble because the extraction was carried out near isoelectric points. Albumins may also be present.

Effects of gamma-irradiation at doses of 1, 5, 10 kGy on solubilities of the proteins of dry beans and chickpea samples (extracted at pH 3.6 and pH 3.1, respectively) were determined according to the method of Lowry *et al.* (1951) and presented in Table 1. The results indicated that there were no significant differences in the protein solubility values of unirradiated and all irradiated samples of both dry bean cultivars. Effect of irradiation on the protein solubility of the two chickpea cultivars was also statistically insignificant.

Table 1. Soluble protein content (mg/100 mg sample) of irradiated samples* determined according to the method of Lowry *et al.* (1951).

Radiation level (kGy)	Soluble protein content (mg/100 mg sample)			
	Chickpea cultivars		Dry bean cultivars	
	Canitez	Akçin	Yalova	Yunus
Control	2.82	2.47	9.67	9.62
1.0	2.36	2.58	9.75	8.50
5.0	2.34	2.57	10.25	8.69
10.0	2.65	2.52	9.48	8.90

*Mean of duplicate determinations.

No significant differences were observed among the samples within each cultivar ($p > 0.05$).

Effects of gamma-irradiation at doses of 1, 5, 10 kGy on electrophoretic patterns of salt insoluble proteins of dry beans are presented in Fig. 1. Relative band intensities and mobilities of the unirradiated samples of Yalova and Yunus cultivars were quite similar. A distinct difference between the controls of two cultivars was observed at the bands indicated by arrow (Fig. 1). At all irradiation levels, the SDS-PAGE patterns of salt insoluble proteins did not change to a large extent in terms of relative mobilities and intensities. The results indicated that the band intensities of some of the protein bands slightly decreased in both cultivars above 55 kDa as the irradiation level increased. At 5 and 10 kGy irradiation levels, resolution of the two bands just above 55 kDa was slightly deteriorated in cv. Yalova. Thus, the irradiation of dry bean samples might have brought about protein scission in some of the salt insoluble proteins with a molecular weight above 55 kDa (especially around 97, 84 and 55 kDa). By the effect of irradiation, a minor increase in band intensities of the most intense protein bands having molecular weights around 45–55 kDa was observed at 5 and 10 kGy irradiation doses

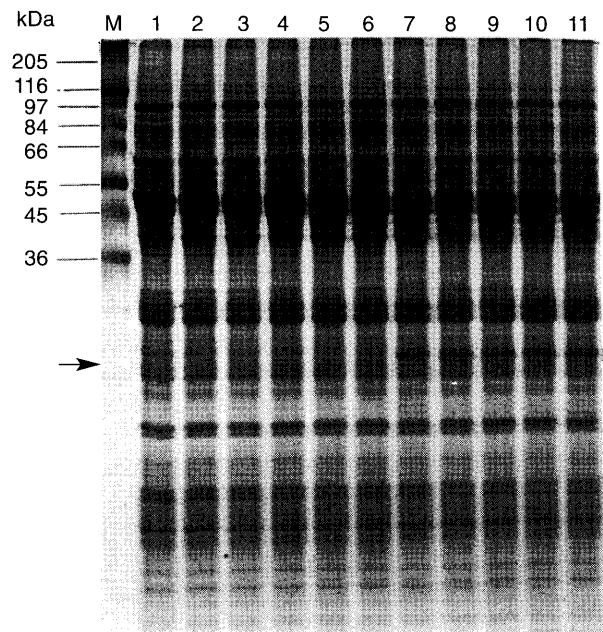


Fig. 1. Effects of gamma irradiation at different levels on salt insoluble proteins of dry bean samples. Lanes: M: Molecular weight markers; 1–6: cv. Yalova; 7–11: cv. Yunus; 1, 2, 6, 7, 11: Control; 3, 8: Irradiated at 1 kGy level; 4, 9: Irradiated at 5 kGy level; 5, 10: Irradiated at 10 kGy level.

in both cultivars. However, this was not significant in densitometric analysis (data not presented). The slight increase in band intensities was more pronounced in cv. Yalova than in cv. Yunus. The faint decrease in band intensities around 36 kDa occurred in both cultivars, especially at 5 and 10 kGy irradiation levels. However, densitometric analysis indicated that most of the protein bands were not affected from irradiation and the effects of irradiation on the overall electrophoretic patterns seem to be minor. This is also supported by the lack of significant differences in the solubility results due to irradiation (Table 1).

Figure 2 shows the effects of gamma-irradiation on SDS-PAGE patterns of salt insoluble proteins of chickpea samples irradiated at 1, 5 and 10 kGy doses. SDS-PAGE patterns of unirradiated samples of Akçin and Canitez did not differ to a large extent in terms of relative band intensities and mobilities. Gamma-irradiation caused faint differences in the band intensities of the irradiated samples at different doses as compared to respective control. In Akçin samples, the band intensities of salt insoluble protein bands just above 66 kDa and at 55 kDa were less intense as compared to the control. However, the changes in the band intensities were not significant in densitometric analysis (data not presented).

Results from the present study suggested that gamma-irradiation of food legumes caused slight differences on the SDS-PAGE patterns of salt insoluble proteins. Reduced band intensities of some of the salt insoluble proteins might be an indication of a very low level of radiolytic breakdown. These observations might be due to the radiosensitivity of some of the proteins. The studies on meat proteins indicated that the most radiosensitive proteins were myofibrillar and radiosensitivity of sarcoplasmic

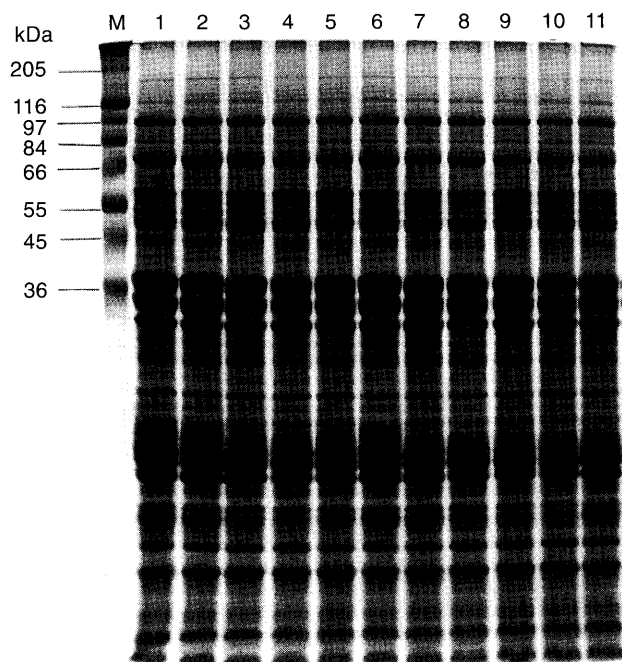


Fig. 2. Effects of gamma-irradiation at different levels on salt insoluble proteins of chickpea samples. Lanes: M: Molecular weight markers; 1–6: cv. Akçin; 7–11: cv. Canitez; 1, 2, 6, 7, 11: Control; 3, 8: Irradiated at 1.0 kGy level; 4, 9: Irradiated at 5 kGy level; 5, 10: Irradiated at 10 kGy level.

proteins was lower (Zabielski *et al.*, 1984). Furthermore, it was observed in one of our previous studies that gamma-irradiation had no effect on wheat gliadins. However, increasing levels of irradiation had greater effect on the largest polymers of glutenin (Köksel *et al.*, 1998b).

Solubility is an important parameter in food protein functionality. It helps to estimate other physicochemical properties of proteins. In the present study, although some variation in the solubility of dry bean and chickpea proteins was observed due to irradiation, the changes were not statistically significant. Hafez *et al.* (1985) reported that irradiation treatment upto 10 kGy caused slight fluctuations in solubility of soybean proteins. Besides this, irradiation treatment (1–10 kGy doses) did not affect the solubility in different solvents (deionized water and salt solutions). Irradiation treatment might affect the relative amounts of

–SH and –SS groups and so might influence the solubility of proteins. There are actually some reports indicating that –SH, –SS conversion during irradiation causes some changes in the solubility of blood plasma proteins (Hayashi *et al.*, 1991). However, more work is needed to understand the effects of gamma-irradiation on protein functionality and physicochemical properties.

Water absorption properties and cooking quality

Average dry seed weights of dry bean cultivars, Yalova and Yunus, were 530 and 510 mg, respectively. Although slight increases were observed in hydration capacity and index, swelling capacity and index values of Yalova cultivar, there was no significant difference among irradiation doses. For cultivar Yunus, the hydration capacity and index of the samples irradiated at 1 kGy were not significantly different from the unirradiated control, while the samples irradiated at 5 and 10 kGy levels had significantly higher hydration capacity and index values. When the swelling capacity was taken into account, only the Yunus sample irradiated at 10.0 kGy was significantly different. In both cultivars, no significant difference was observed between samples irradiated at different levels in terms of swelling index (Table 2).

Average dry seed weights of chickpea cultivars, Canitez and Akçin, were 560 and 430 mg, respectively. Slight increases were observed in hydration capacity and index, swelling capacity and index values of cultivar, Canitez. However, the increases were not significant. In Akçin samples, irradiation treatment did not significantly affect hydration capacity and hydration index values. On the other hand, Akçin samples irradiated at 10 kGy was significantly different in terms of swelling capacity and swelling index (Table 3).

Dry and wet cooking times of the dry bean and chickpea samples irradiated at 1, 5, 10 kGy levels were determined and the results are presented in Tables 2 and 3, respectively. In all dry bean and chickpea samples, the dry and wet cooking times for the irradiated samples were found to be significantly reduced when compared to their respective control. The soaking process reduced the wet cooking time of the samples as expected. The studies by Williams *et al.* (1986) have shown that soaking of food legumes

Table 2. Effects of irradiation on water absorption properties and cooking times of dry beans*.

Variety	Radiation level (kGy)	Hydration		Swelling		Cooking time	
		Capacity	Index	Capacity	Index	Dry	Wet
YALOVA	Control	540	1.09	540	1.42	106 a	37.5 a
	1.0	552	1.09	555	1.42	99 b	28.0 b
	5.0	563	1.15	555	1.52	91 c	13.5 c
	10.0	575	1.19	570	1.54	87 d	12.0 d
LSD	(<i>p</i> < 0.05)					1.1	1.30
YUNUS	Control	503 a	1.02 a	530 a	1.47	84 a	31.5 a
	1.0	506 a	1.02 a	530 a	1.47	78 b	21.0 b
	5.0	550 b	1.07 b	530 a	1.43	69 c	12.5 c
	10.0	568 b	1.07 b	560 b	1.47	60 d	9.0 d
LSD	(<i>p</i> < 0.05)	30.2	0.045	22.5		1.5	1.84

*Mean of duplicate determinations.

For each cultivar, means with the same letter within a column are not significantly different (*p* < 0.05) by least significant difference analysis.

Table 3. Effects of irradiation on water absorption properties and cooking times of chickpeas*.

Variety	Radiation level (kGy)	Hydration		Swelling		Cooking time	
		Capacity	Index	Capacity	Index	Dry	Wet
CANITEZ	Control	542	0.99	544	1.36	178 a	57.5 a
	1.0	539	0.99	550	1.42	164 b	46.5 b
	5.0	566	1.02	575	1.44	143 c	18.0 c
	10.0	568	1.04	575	1.44	134 d	16.0 d
LSD	($p < 0.05$)					7.2	1.84
AKÇIN	Control	416	0.98	410 a	1.37 a	186 a	43.5 a
	1.0	417	0.96	420 a	1.40 a	176 b	38.0 b
	5.0	422	0.98	425 a	1.42 a	139 c	19.0 c
	10.0	431	0.99	460 b	1.53 b	135 c	13.0 d
LSD	($p < 0.05$)			29.1	0.10	4.5	3.38

*Mean of duplicate determinations.

For each cultivar, means with the same letter within a column are not significantly different ($p < 0.05$) by least significant difference analysis.

before cooking, which is a very common practice, reduces cooking time significantly. Rao & Vakil (1985) have also observed appreciable reduction in cooking time of the irradiated legumes. Distinct off-flavor and odor were observed during dry and wet cooking of all legume samples irradiated at 10 kGy level. The formation of off-flavor and odor was also noted in doughs, breads and food legumes especially at high irradiation doses (Lai *et al.*, 1959; Miller *et al.*, 1965; Fifield *et al.*, 1967; Nene *et al.*, 1975a,b).

Cooking time is considered to be a function of permeability of seed coat, followed by the rate at which the hot water causes the gelatinization of starch (Williams & Nakkoul, 1985). Rao & Vakil (1985) reported that the hydration rate of irradiated food legumes was increased. Furthermore, in our recent study the loss of the external membrane integrity due to irradiation was reported (Dadaylı *et al.*, 1997). Therefore, the loss of membrane integrity due to irradiation might have caused water to enter through the cell walls of cotyledons easily, resulting in increases in water absorption properties and gelatinization, hence significant decreases in the cooking times.

Research on the changes in food legumes due to gamma-irradiation as related to properties of proteins, water absorption and cooking quality must be well documented before the irradiation technique can be recommended for industrial applications and as a means of disinfection of the legumes for storage.

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