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Effect of Gamma, Electron Beam and Infrared Radiation Treatment on the Nutritional Value and Anti-Nutritional Factors of Sorghum Grain

Research Article

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ABSTRACT

The present study describes the radiation-induced effects on nutritional quality and on anti-nutritional factors of sorghum grain: effects of electron beam (ER) and gamma irradiation (GR) at doses of 10, 20 and 30 kGy and infrared irradiation (IR) at 60, 90 and 120 s on the chemical composition, mineral content and bioavailability, *in vitro* protein and starch digestibility, total phenolic compound, phytate and tannins contents of sorghum grain, were investigated. The results showed that chemical composition were unchanged (P>0.05) by the irradiations. GR, ER and IR resulted in an increase in the digestibility of starch by 3.8, 5.4 and 6.8%, respectively. *In vitro* protein digestibility was reduced by 23% in IR, where as GR and ER increased crude protein digestibility of sorghum grain by 6.6 and 5%, respectively (P>0.05). The levels of phytic acid and tannins were significantly reduced by irradiations while the total phenols were increased relative to unprocessed control samples. The results indicated no substantial change in mineral content and bioavailability amongst the sample, with exception of significant (P>0.05) increase in extractable calcium, phosphorus, zinc and iron.

KEY WORDS anti-nutritional factors, chemical composition, irradiation, sorghum.

INTRODUCTION

Sorghum (*Sorghum bicolor*) ranks fifth in worldwide production among cereal crops following wheat, rice, corn and barley. Popularity of sorghum is due in part to its ability to produce reasonable yields in warmer, drier regions. Sorghum is a possible source of energy and protein for millions of the world's poorest people, but it has some limitations for application due to significant amounts of anti-nutritional compounds such as phytate, tannins and phenolics that may affect the utilization of nutrients. Numerous factors contribute to the digestibility problem (Duodu *et al.* 2003).

Exogenous aspects include the interaction of protein with other constituents, such as starch, non-starch polysaccharides, polyphenols, phytate, tannins, dietary fiber and lipids. These compounds are known to interfere with the digestibility of mineral bioavailability, protein and starch (Sade, 2009). Endogenous factors arise from the nature of the proteins themselves and their organization within the grain (Duodu *et al.* 2003). As in other cereals, the low amount of protein relative to starch, i.e., approximately 10% protein, *vs*. 70-80% starch, based on grain dry weight, affects the functional properties of starch, such as gelatinization and digestion rate, to a greater extentin sorghum than in other

cereals (Ezeogu *et al.* 2008). Nuclear and related biotechnological techniques have played, a significant role in improving livestock productivity. In the past four decades, a vast knowledge has been accumulated on the chemical and biological effects of ionizing irradiation, which has contributed to promote its utilization. Application of ionizing radiation treatment of foods on an industrial scale started at the beginning of the 1980s after the joint FAO / IAEA / WHO expert committee accepted the application of a 10 kGy overall average dose for foods (WHO, 1981). Irradiation has been identified as a reliable and safe method to improve the nutritional value of food and feed. Gamma, electron beam and infrared irradiation have also been shown to reduce or inactivate some of the anti-nutritional factors in seeds or meals, thereby enhancing their edibility (Siddhuraju *et al.* 2002; Shawrang *et al.* 2011; Keya and Sherman, 1997). Many attempts have been done to improve the nutritional quality and inactivate orreduce anti-nutritional substances of sorghum and plant-origin feeds such as dry heating, roasting (Khattab *et al.* 2009), graindehulling (Mwasaru *et al.* 1988), mailing (Hemanalini *et al.* 1980), germination (Al-Kaisey *et al.* 1997), cooking (Urbano *et al.* 1995) and fermentation (Zamora and Veum, 1979). However, most of these various conventional, simple processing methods adversely affect the sensory characteristics of the final product and there is always some loss of nutrient quality in these processing and also, none of these methods is able to completely remove all the detected anti-nutrients that are present in seeds, grains or feed materials. Compared to other degradation methods, ionizing radiation is free of initiators and side products which results in chemically pure degradation product (Choi *et al.* 2009). Therefore, this technology is simpler and more environmentally friendly than the current conventional ones. However, there is a rareness of information relating to the effects of processing with ionizing energy. This study was designed to assess the effects of irradiations on some of the nutritional and anti-nutritional qualities of sorghum grain.

MATERIALS AND METHODS

Sample preparation
Sorghum grains were obtained from Seed and Plant Improvement Institute, Karaj, Iran and store at 4 ˚C until the experiments were conducted.

Electron beam irradiation
Seed samples were packed in 30 × 40 × 5 cm nylon bags (0.5 mm thickness) and exposed to electron beam irradiation at the Yazd radiation processing Centre (AEOI, Yazd Centre, Iran) to various doses (10, 20 and 30 kGy) at room temperature by a Rhodotron accelerator model TT 200 (IBA Co., Belgium).

Gamma irradiation
Sorghum grain was packed in polyethylene bags and sealed by heat. They were treated to gamma irradiation at room temperature from a 60 cobalt source (NORDION, IR-136, Canada) at Gamma Irradiation Center, Iranian Nuclear Organization, Tehran, Iran. The delivered doses were supervised by radio chromic film (McLaughlin *et al.* 1985).

Infrared radiation
Infrared radiation was performed using a micronizer equipped with1000 watt infrared lamp at Agricultural Research Institute, Karaj, Iran. The dry Sorghum seeds were exposed to infrared radiations for various time intervals (60, 90 and 120 s).Non irradiated seeds serve as control.

Proximate composition and mineral contents
Determination of moisture, ash, crude protein, total crude fat, crude fiber and starch content of raw and processed sample were determined according to the association of official analytical chemists (AOAC, 2000) procedures. Total mineral content of the samples were extracted by dry ashing method described by Chapman and Pratt (1968). About 2.0 g of sample was acid digested with diacid mixture ($HNO_3:HClO_4$, 5:1, v/v) in a digestion chamber. The digested samples were dissolved in double-distilled water and filtered (What man No. 42). The filtrate was made to 50 mL with double-distilled water and was used for determination of total minerals.

The amounts of Fe, Mn, Cu, Co and Zn were determined according to the atomic absorption spectrophotometer method. Calcium and magnesium was determined by a titration method as described by Chapman and Pratt (1968). Phosphorus was determined spectrophotometerically by using molybdovanadate method. Sodium and Potassium were determined by flame photometer according to AOAC (2000).

HCl-extractability of minerals (*in vitro* availability)
The method described by Chauhan and Mahjan (1988) was used for extraction of mineral sample. About 10 mL of HCl (0.03 *M*) with 1.0 g sample was shaken for 3 hour at 37 ˚C and then filtered. Afterward, the clear extract was oven-dried at 100 ˚C and then acid-digested. The amount of the extractable minerals was determined by the methods described above. HCl extractability of minerals (%) was determined as follows:

Mineral extractability %= (mineral extractable in 0.03 HCl (mg/100 g)) / (total mineral (mg/100 g)) × 100

Tannin content determination
Determination of tannins content was measured by using the modified vanillin-HCl method (Price *et al.* 1978). 200

mg of sample was extracted using 10 mL 1% (v/v) concentrated HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 mL) was added to the extract (1 mL) and the color absorbance developed after 20 min at 30 °C was read at 500 nm using the CE 2021 spectrophotometer (Cecil instruments, Cambridge and England). A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg/mL) which gives a colorintensity equivalent to that given by tannins after correcting for blank. Then tannin content (%) was calculated according to the equation:

Catechin equivalent (CE) %= (C × volume extracted (10 mL)) / (weight of sample (g))

Where:
C: concentration obtained from the standard curve (mg/mL).

Phytic acid content determination
Phytic acid content was determined according to the method described by Wheeler and Ferrel (1971) using 2.0 grams of dried sample. A standard curve of different Fe $(NO_3)_2$ concentrations was plotted to calculate the ferric ion concentration. Phytate phosphorus was calculated from the standard curve assuming 4:6 iron to phosphorus molar ratio.

Extraction of polyphenols
Extraction was done following the method of Santas *et al.* (2008) with minor modifications. Thirty milliliters of methanol: water (70:30 v/v) extraction solvent was added to freeze-dried sorghum powder (3 g). After 20 min of extraction with magnetic stirring at 800 rpm at ambient temperature (23 °C), the extract was centrifuged at 1000 g (accu Spin™ 400, Fisher Scientific, Pittsburgh, PA, USA) for 30 min. Supernatant was pooled and stored at below 4 °C in a dark environment before analysis. Extractions were carried out in triple and kept away from light exposure during the extraction process.

Total phenolic content determination
The amounts of total phenolic concentration in extracts were measured by using the modified Folin-Ciocalteu procedures of Sun *et al.* (2007). In short, the 70% methanolic-sorghum extracts (0.1 mL) were mixed with 0.75 mL of the 10-fold diluted Folin-Ciocalteu reagent with deionized water and incubated for 15 min at room temperature (ca. 21 °C). Then, 0.75 mL of 2% sodium carbonate (w/v) solution was added to the mixture and kept away from light exposure for 45 min before measuring the absorbance at λmax= 765 nm using a spectrophotometer against a blank, containing deionized water instead of sample extract. Total phenolic content values were determined from a calibration line made ready with a series of catechin standards. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue color upon reaction. This blue color is measured spectrophotometrically. Based on the measured absorbance, the concentration was read (mg/mL) from the calibration line; then the content of phenolics in extracts was expressed in terms of catechinequivalent.

Determination of *in vitro* protein digestibility
In vitro protein digestibility was determined according to the method of Akeson and Stahmanna (1964). 15 mL HCl (0.1 *M*) containing 1.5 mg pepsin was added to one gram of groundsamples and then incubated at 37 °C for 2 h. The obtained suspension from incubation was made neutral with 7.5 mL NaOH (0.2 *M*), then treated with 4 mg of pancreatin in 7.5 mL 0.2 *M* phosphate buffer (pH 8.0). In order to prevent microbial growth toluene (1 mL) was added and the mixture was kindly shaken and incubated for 24 hour at 37 °C. After incubation, the sample was treated with trichloro acetic acid (10%, 10 mL) to remove not digested large peptides and protein and centrifuged at 50000 g for 25 min at room temperature. The Kjeldahl method (AOAC, 2000) was used for estimation of protein in the supernatant. The percentage of protein digestibility was calculated by the ratio of protein in supernatant to protein in sample according to the following equation:

IVPD %= (N in supernatant-N in pepsin) / (N in sample) × 100

Determination of *in vitro* starch digestibility
Starch digestibility was determined according to the method of Hagenimana *et al.* (2006) with slight modification. The different starch fractions, resistant starch (RS), slowly digestible starch (SDS) and rapidly digestible starch (RDS), of Sorghum grain were measured in triplicate. Starch (100 mg, dry basis, db) or a dry-ground Sorghum sample equivalent to 100 mg of starch (db), were mixed vigorously in deionized water (5.0 mL) and then equilibrated in a water bath at 37 °C with agitation for 15 min and following cooling at room temperature. Then, the mixture was added to 15 mL of phosphate buffer (0.2 *M*, pH 7.5). After, 1 mL of bacterial α-amylase (A-3403, Type XII-A, Sigma Chemical Co., St. Louis, MO) was added and incubated at 30 °C in a shaking incubator. Following incubation, samples were centrifuged for 15 min at 3000 rpm and supernatant removed. The amount of glucose released after 20 min is defined as RDS. A second measurement of glucose released after further 90 min of incubation is defined as SDS. The starch that remained unhydrolysed after a total of 120 min

of incubation was measured as RS. 3,5-dinitrosalicylic acid method was used to analyze the reducing sugar produced at the end of treatment. Measure of the relative rate of starch digestion (the starch digestion index) was calculated as follows:

SDI= (RDS/TS) × 100.

Energy

Gross energy values of grain and excreta samples were determined by adiabatic bomb calorimeter (Model IKA Calorimeter C400, Adiabatic 2800, Bremen, Germany). The gross heat produced fromignition was calculated with water and compared with benzoic acid as known energy capacity.

Statistical analysis

Statistical analyses were performed using computer program Statistical Packages for Social Science (Murray *et al.* 2003). Each determination was carried out on three separate samples and analyzed in triplicate and results were expressed as mean ± standard deviation (SD). Comparison of the difference in result between irradiated and non- irradiated sample was analyzed using one-way analysis of variance (ANOVA) through the F test and if significant, Duncan's multiple rang test was run to separate means. Significance was accepted at $P \leq 0.05$. Pearson correlation coefficient was used to evaluate the magnitude of relationship between variables.

RESULTS AND DISCUSSION

Effects on nutritional value
Effects on Proximate composition

The results for proximate composition of untreated and irradiated sorghum are shown in Table 1. These results indicated that there were no significant differences in moisture, crude protein, crude fat, crude fiber, starch and ash contents among the gamma and EB irradiated and non-irradiated grains. However, infrared irradiation of the grains caused extensive loss of moisture content (3% to 6%). The increases in protein, fat, fiber and ash, of the infrared irradiated samples were attributed to the reduction in moisture content. The results of this trial are in accordance with previous works concerning the effect of irradiations on proximate composition of sorghum grain and other similar product: on the effect of gamma irradiation on chemical composition of rapeseed and soybean seeds (Ebrahimi *et al.* 2009; Taghinejad *et al.* 2009); on the effects of a brief, intense infrared radiation treatment on proximate composition of maize, rice, sorghum, and beans (Keya and Sherman, 1997); on the effect of electron beam irradiation on sorghum grainschemical composition (Shawrang *et al.* 2011).

Effects on minerals and minerals *in vitro* availability

Table 2 and 3 shows minerals content of sorghum grains. No substantial change in minerals content amongst the samples of the sorghum was recorded. Irradiation did not significantly alter (P>0.05) the concentration of total Ca, P, Fe and Zn in sorghum grain, but it increased HCl extractability of these minerals (P≤0.05). Also, a significant increase in availability of other major and trace minerals was observed.

That may be an indication of their bioavailability to the human and animal digestive system. Higher time and dose level resulted in higher Ca and P extractability. P and Ca cleaved from phytate or decreased content of phytic acid and destruction the phytate ring structure by irradiation, might account for higher extractability after irradiations. The presence of tannin and phytic acid in seed coat as inhibitors was demonstrated to reduce iron absorption (Rao and Prabhavathi, 1982) by chelating the iron ion. A significant negative correlation between level of phytic acid and HCl extractability of minerals in all treatment was observed.

The results are as follows: in IR treatment: P (r=-0.95), Ca (r=-0.99), Fe (r=-0.96), Zn (r=-0.96); in GR treatment: P (r=-0.98), Ca (r=-0.98), Fe (r=-0.97), Zn (r=-0.92) and in ER treatment: P (r=-0.85), Ca (r=-0.89), Fe (r=-0.94), Zn (r=-0.99).

Divalent cations may be present as mineral-phytate chelates in untreated grain, which may explain the lower extractability of those minerals in HCl. Phytic acid contents of several cereals have adversely influence protein and starch digestibility and availability of essential divalent minerals.

Phytate chelates with particular metal ions such as calcium, magnesium, manganese, zinc, molybdenum copper and iron, are insoluble complexes and cannot be dissolved in a liquid, thus they are not readily decompose and may pass through the digestive tract without change (Al-Kaisey *et al.* 2003).

Effects on gross energy and *invitro* digestibility

Table 4 shows the effects of irradiation on gross energy, *in vitro* protein and starch digestibility of sorghum grain. Infrared irradiation at 60, 90 and 120 second reduced protein digestibility by about 6.8, 14.5 and 23.2%, respectively. These differences may be partly attributed to protein denaturation, which could affect nitrogen solubility which may be related to some conformational changes, cross-linking, aggregation, fragmentation, rupturing of covalent bonds, oxidation (by oxygen radicals that are generated in the radiolysis of water), of proteins that were less susceptible to enzyme hydrolysis. The results show that infrared irradiation increased significantly starch digestibility of the grain (P≤0.05).

Table 1 Proximate composition of control and irradiated grains (mean percentage±SD)

Treatment	Dose of irradiation	Component					
		Moisture	Crude protein	Crude fat	Crude fiber	Ash	Starch
Control		8.00±0.3a	11.01±0.4d	3.65±0.3bc	2.55±0.2de	2.20±0.1d	76.92±2.5a
GR	10 kGy	8.12±0.2a	10.95±0.5d	3.54±0.5cd	2.43±0.3e	2.20±0.2d	77.12±2.3a
	20 kGy	8.11±0.5a	11.15±0.5d	3.71±0.2b	2.51±0.2de	2.33±0.2cd	75.83±2b
	30 kGy	7.92±0.5a	11.00±0.6d	3.51±0.2cd	2.40±0.1e	2.21±0.2d	77.22±2.1a
EB	10 kGy	8.13±0.7a	11.03±0.5d	3.41±0.5d	2.40±0.1e	2.34±0.1cd	77.31±1.9a
	20 kGy	8.01±0.1a	10.90±0.3d	3.50±0.5cd	2.63±0.2cd	2.20±0.1d	77.14±2a
	30 kGy	8.04±0.4a	10.95±0.2d	3.43±0.2d	2.64±0.1cd	2.31±0.1cd	76.90±1.5a
IR	60 Sec	5.14±0.3b	11.40±0.3c	3.71±0.4b	2.71±0.2c	2.41±0.1bc	77.81±1a
	90 Sec	4.11±0.2c	11.61±0.5b	3.92±0.3a	2.91±0.2b	2.52±0.2b	77.94±2.2a
	120 Sec	2.12±0.1d	11.84±0.2a	3.90±0.3a	3.11±0.2a	2.74±0.2a	77.90±2.5a

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
GR: gamma irradiation; EB: electron beam irradiation and IR: infrared irradiation.

Table 2 Total and available major minerals contents of control and irradiated grains (as mg/100 g dry matter±SD)

Treatment	Dose of irradiation	Calcium		Phosphate		Magnesium		Sodium		Potassium	
		Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Control		33.0±1.0bc	37.7±0.5h	210.0±7.0a	31.5±0.0g	136.0±1.9a	49.6±0.7c	11.1±0.5ab	39.6±1.3f	314.0±12.0a	61.6±1.0g
GR	10 kGy	32.0±0.9c	45.0±1.5f	209.0±12.0a	33.3±0.4f	137.0±1.2a	47.6±1.0d	11.2±0.7ab	38.6±1.0g	314.0±10.0a	70.1±2.2d
	20 kGy	33.0±1.1bc	49.7±1.0d	212.0±11.0a	40.0±0.0d	135.0±2.0a	47.1±0.8d	11.4±0.8ab	41.6±1.4e	315.0±5.0a	72.6±1.9a
	30 kGy	33.0±1.0bc	57.7±2.1c	213.0±9.0a	44.5±0.7c	137.0±4.0a	50.9±1.1c	11.4±0.8ab	44.6±1.9a	315.0±9.0a	69.9±1.3d
EB	10 kGy	32.0±1.0c	42.7±0.6g	209.0±3.0a	30.0±0.7g	135.0±2.1a	50.0±0.9c	11.0±0.3ab	39.0±1.0f	313.0±4.0a	66.1±1.9f
	20 kGy	34.0±1.0ab	47.0±0.5e	213.0±6.0a	35.5±0.2e	135.0±1.3a	52.5±0.3b	10.7±0.6b	42.6±1.9c	315.0±3.0a	68.9±1.1e
	30 kGy	33.0±0.8bc	45.7±0.9f	214.0±9.0a	39.5±0.4d	137.0±1.4a	52.9±0.5b	10.7±0.1b	43.6±1.5b	313.0±4.0a	71.3±2.0b
IR	60 Sec	34.0±2.0ab	57.6±0.9c	212.0±3.0a	34.5±0.3f	138.0±1.7a	53.9±0.9ab	12.0±0.6a	38.1±1.0g	314.0±6.0a	71.3±1.1b
	90 Sec	35.0±3.0a	69.1±1.5b	214.0±2.0a	49.5±0.7b	137.0±1.0a	54.0±1.7a	12.1±0.5a	45.0±1.6a	316.0±11.0a	70.0±1.7d
	120 Sec	35.0±0.9a	77.0±2.5a	214.0±2.0a	51.5±0.1a	137.0±1.9a	53.6±1.4ab	12.0±0.7a	44.1±1.3a	315.0±8.0a	71.10±2.0c

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
GR: gamma irradiation; EB: electron beam irradiation and IR: infrared irradiation.

Table 3 Total and available trace minerals contents of control and irradiated grains (as mg/100 g dry matter±SD)

Treatment	Dose of irradiation	Iron		Zinc		Manganese		Copper		Cobalt	
		Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Control		4.1±0.4a	24.5±1.2g	2.5±0.9a	51.0±2.3h	1.1±0.4ab	37.5±0.2i	0.8±0.1b	81.1±3.0f	0.05±0.00b	84.3±1.0g
GR	10 kGy	4.2±0.6a	25.5±0.2f	2.6±0.2a	57.7±2.0ef	1.3±0.6a	40.2±0.6h	0.9±0.3ab	82.2±3.6e	0.05±0.00ab	85.5±0.3f
	20 kGy	4.0±0.3a	34.8±0.6c	2.6±0.4a	58.9±1.4cd	1.4±0.2a	47.1±0.9e	0.9±0.3ab	82.7±1.9e	0.05±0.00ab	88.1±0.6d
	30 kGy	4.2±0.1a	39.5±0.9a	2.7±0.5a	63.3±2.0a	1.4±0.7a	51.5±0.7c	0.7±0.4c	84.0±2.2d	0.05±0.00a	90.3±0.2c
EB	10 kGy	4.1±0.6a	25.5±1.0f	2.5±0.1a	54.0±1.4g	1.1±0.2ab	39.8±0.3h	0.8±0.3b	81.8±2.2ef	0.05±0.00ab	85.9±0.2f
	20 kGy	4.2±0.3a	27.7±1.7d	2.7±0.4a	57.0±1.3f	1.2±0.3ab	44.4±0.4f	1.0±0.4a	83.8±3.1d	0.05±0.01ab	87.7±0.5e
	30 kGy	4.2±0.5a	34.9±1.1c	2.7±0.0a	60.0±1.06b	1.4±0.4a	52.5±1.2b	0.7±0.4c	85.8±3.9c	0.05±0.00a	88.7±1.1d
IR	60 Sec	4±0.1a	26.7±1.0e	2.6±0.5a	58.0±2.0d e	1.2±0.5ab	41.3±0.3g	0.7±0.3c	85.9±3.1c	0.05±0.00ab	87.9±1.3e
	90 Sec	4.2±0.1a	34.5±1.9c	2.5±0.1a	59.0±2.1c	1.0±0.5ab	48.7±2.1d	0.8±0.3b	87.1±2.2b	0.05±0.00ab	94.4±0.4b
	120 Sec	3.9±0.3a	38.5±1.5b	2.6±0.4a	64.0±2.5a	0.9±0.1b	58.9±2.2a	0.7±0.2c	88.8±3.9a	0.05±0.00ab	98.3±0.5a

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
GR: gamma irradiation; EB: electron beam irradiation and IR: infrared irradiation.

Starch gelatinization (irreversible loss of the crystalline regions in starch granules) and reduction in anti-nutrients such as phenolic compound, phytic acid and tannin content is the main reason of this increment.

The gelatinization dramatically increases the availability of starch for digestion by amylolytic enzymes. The phytate molecule is highly charged with six phosphate groups and so is an excellent chelator, forming insoluble complexes

with mineral cations, starch and proteins. This leads to reduced protein and starch (energy source) digestibility.

Results of Elkhalil *et al.* (2001) in sorghum showed that malt pre-treatment could reduce phytic acid content and enhance *in vitro* protein digestibility.

Table 4 Grossenergy and invitro digestibility of control and irradiated grains

Treatment	Dose of irradiation	Energy (kcal/g)	Digestibility (%)	
			Protein	Starch
Control		4.3± 0.0[c]	68.9± 3.1[d]	72.5± 1.0[gh]
GR	10 kGy	4.3± 0.0[abc]	68.3± 2.1[d]	72.1± 2.2[h]
	20 kGy	4.4± 0.0[abc]	74.1± 1.7[b]	74.0± 1.5[f]
	30 kGy	4.4± 0.0[ab]	75.5± 1.3[a]	76.3± 2.0[d]
EB	10 kGy	4.4± 0.0[abc]	74.4± 2.2[b]	72.9± 1.8[g]
	20 kGy	4.4± 0.0[abc]	73.1± 1.7[c]	76.5± 2.5[cd]
	30 kGy	4.5± 0.0[a]	73.9± 1.3[bc]	77.9± 2.2[b]
IR	60 Sec	4.4± 0.1[abc]	62.1± 2.2[e]	75.1± 1.4[e]
	90 Sec	4.4± 0.0[ab]	54.4± 1.1[f]	77.2± 1.2[c]
	120 Sec	4.5± 0.0[a]	45.7± 1.4[g]	79.3± 1.6[a]

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
GR: gamma irradiation; EB: electron beam irradiation and IR: infrared irradiation.

Similar significantly increased trends were observed at doses higher than 20 kGy in, *in vitro* protein and starch digestibility of gamma and EB-irradiated sorghum grain compared to control (P≤0.05). The increase in starch digestibility of sorghum grain following gamma and EB irradiation might be due to: 1) changes of starch crystallinity as well as microstructure that can be attributed to the formation of free radicals during irradiation. These substances attack the starch, and disrupt the interaction between amylopectin and amylase causing the cleavage of the glucosyl bonds. Thus, the crystallinity of starch molecules declines and it induces effects such as chain scission (which makes the chain shorter) and cross linking (Shawrang *et al.* 2011); 2) changes in the morphology and molecular weight of polysaccharides following irradiation, have as consequence a change in their solubility that increases / or makes easy the release from other attached components such as proteins or lipid (Choi *et al.* 2009). Shortening of the polysaccharide macromolecular chains can be achieved by various methods such as ultrasound, microwave heating and ionizing irradiation. Among these, an ionizing radiation was found to be effective in degradation by the energy generated from different sources such as cobalt-60 and electron accelerator (Kim *et al.* 2008; Byun *et al.* 2008). This result was well

supported by the reports on depolymerization of polysaccharides by radiolysis on starch (Wu *et al.* 2002). Morphological studies by scanning electron microscope showed that GR-treated tamarind seed polysaccharides (TSP) were characterized by a reduction in interconnection between bunches of fibers and an increase of flat sheet-like structure. In case of EB-treated TSP, the fibril structure was broken down and small particles were produced (Choi *et al.* 2009); 3) the increase in extraction yields and solubility of starch with radiation treatment has also been reported by Huang and Mau (2007). Similarly, Kim *et al.* (2000) found an increase in the extraction yields aftertr eating medicinal herbs with gamma irradiation. They observed that the total extraction yield in Korean medicinal herbs, increased by 5–30% with a 10 kGy dose of gamma irradiation. This drastic increment in digestibility was caused by cleavage of the glycosidic bond through free radical formation to smaller carbohydrate units of dextrins of varying lengths, leading to a reduction in the molecular weight. Therefore, scissions of the chains probably produce short amylose chains, short linear chains from the branches of amylopectin or small branches of amylopectin.

Increase in the protein digestibility can be explained by 1) reduction of anti-nutritional factors in treated sorghum, such as tannins and phytate, is the main reason of enhanced digestibility. Phytic acid induces a decrease of solubility and protein functionality. Duodu *et al.* (2003) reported that tannins have a detrimental effect on digestibility of protein and amino acids. Due to their hydroxyl groups, tannins may interact and form complexes with proteins, which may lead to precipitation because of the large size of the tannins. When this anti-nutritional factor is found at the proportion of 5:1 tannin / protein, all protein is precipitated as consequence of the tannin action (Pino and Lajolo, 2003). In addition to possibly causing a change in protein conformation, study of Siddhuraju *et al.* (2002) showed that tannins may also exert steric effects (due to their large size) and prevent enzymes access to the proteins. Another possible reason for increase in protein digestibility, is the modification in the three dimensional structure of sorghum proteins due to irradiation. Studies of Shawrang *et al.* (2007) and Shawrang *et al.* (2008) illustrated that protein denaturation occur by irradiation that leads to improvement in protein digestibility. Phytic acid chelates mineral cations and proteins, forming insoluble complexes, which lead to a reduced bioavailability of trace minerals and reduced digestibility of proteins (Siddhuraju *et al.* 2002); 2) alteration or removal of natural properties of protein cause decrease in protein hydrophobicity. As mentioned previously, irradiation results in protein denaturation, which exposes hydrophobic amino acids, especially aromatics, which are the position groups for the active site of pepsin. Furthermore, irradiation causes more

peptide bonds to be exposed to proteolytic enzymes by a change in secondary and tertiary structure of protein (Fombang *et al.* 2005). Phytate-protein complexes are insoluble and less prone to be the target of proteolytic enzymes than the protein alone; as a consequence this affects the functional properties of the protein. Moreover, the partial removal of tannin and phytate probably creates a large space within the matrix, which increases the susceptibility to enzymatic attack and consequently improve the digestibility of protein after radiation treatment. Molecular rearrangement and changes in peptide linkages between the amino groups of amino acids could affect the nutritive availability and the biological utilization of the irradiated proteins. Such changes could interfere with the protein digestibility and/or its biological value. The higher digestibility of starch treated by EB irradiation might be due to a higher concentration of radicals, when compared to a GR irradiation system, when the absorbed doses in these two systems are the same. In general, higher steady-state concentration of radicals would lead to higher cross linking and recombination. The average molecular weight of TSP was decreased by the irradiation, and EB treatment degraded more severely than GR, which indicates that EB produce smaller molecular weight TSP than GR (Choi *e tal.* 2009). The higher digestibility of protein treated by GR might be due to a higher penetration of radicals in a GR irradiation system than in an EB irradiation system, when the absorbed doses in these two systems are the same. These two energies have been known to show a similar effect on materials, but there are differences regarding penetration and the method of their use (Choi *et al.* 2009).

Effects on anti-nutritional factors
Effects on phytic acid

Table 5 shows the different mean value of phytic acid, tannins and total phenolic contents in the raw and treated sorghum grain. The results show that irradiation reduce significantly phytic acid content ($P \leq 0.05$). In this case, the maximum losses of phytic acid between treatments were relevant to 120 second IR, 91%. A higher reduction in phytic acid was observed in ER when the absorbed doses in GR and ER are the same. This findings are in agreement with Hassan *et al.* (2009) who reported that gamma irradiation caused significant reduction in phytic acid level of sorghum. Shawrang *et al.* (2011) also reported that phytate content of electron irradiated sorghum, decreased significantly at the doses of 10, 15, 20, 25 and 30 kGy compared to control by 39, 49, 66, 79 and 90%, respectively. This reduction in phytic acid content is probably due to chemical degradation of phytate to lower inositol phosphates and inositol by the action of free radicals, produced by the radiation or cleavage of the phytate ring itself (Siddhuraju *et*

al. 2002). It could also bedue to the formation of insoluble complexes between phytate and other components such as phytate-protein and phytate-mineral complexes according to the amount of free phytate reduced.

Mode of phytate loss by gamma -irradiation has been explained above, whereas mechanism of phytate reduction by electron beam irradiation has not been demonstrated (Shawrang *et al.* 2011).

Table 5 Phytic acid, tannins and total phenolic contents of control and irradiated grains

Treatment	Dose of irradiation	* Phytate	** Tannin	** Phenols
Control		774.0±4.6[a]	921.0±4.6[a]	1155.0±3.5[b]
GR	10 kGy	659.0±5.4[b]	488.0±8.1[c]	594.0±2.5[e]
	20 kGy	458.0±4.1[d]	409.0±3.4[e]	451.0±4.1[f]
	30 kGy	147.0±3.6[g]	191.0±6.7[i]	210.0±2.4[h]
EB	10 kGy	549.0±3.1[c]	774.0±2.2[b]	941.0±1.8[c]
	20 kGy	361.0±2.1[e]	488.0±4.7[c]	609.0±2.5[d]
	30 kGy	91.0±3.5[h]	242.0±7.3[h]	253.0±2.2[g]
IR	60 Sec	456.0±5.1[d]	393.0±2.2[g]	1159.0±2.1[b]
	90 Sec	201.0±4.6[f]	399.0±1.1[f]	1171.0±2.2[b]
	120 Sec	67.0±3.3[i]	419.0±1.4[d]	1228.0±2.4[a]

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
GR: gamma irradiation; EB: electron beam irradiation and IR: infrared irradiation.
* Expressed as mg/100 g dry matter
** Expressed as mg catechin equivalent/100 g dry matter.

Effects on tannins

Table 5 shows that irradiation causes significant reduction in tannin contents. Decrease in tannins was significant at doses of 10, 20 and 30 kGy in GR and ER, compared to control by 47, 55 and 79% and by 16, 47 and 73%, respectively.

Siddhuraju *et al.* (2002) reported an increase in tannins following gamma irradiation while Villavicencio *et al.* (2000) reported a decrease in tannins in foods or feeds. Mechi *et al.* (2005) found that gamma radiation also promoted reduction in the tannin contents as the radiation dose increased until a limited dose. The mechanism of gamma irradiation action on tannin has been related to generation of the hydroxyl and superoxide anion radicals and also related to changes in solubility and chemical reactivity of tannin, but mode of electron beam action on tannins has not been demonstrated (Shawrang *et al.* 2011). Further study is needed to clarify its mode of reduction in tannin contents. Such reduction in tannin content is very favourable, considering that this anti-nutritional factor has the capacity to decrease protein digestibility.

Effects on total phenolic content

Table 5 highlights the percentage of phenolic compounds in the different treatments for raw and treated grains. Their radiation process decreased the content of phenolic com-

pounds in all treated sample with exception of significant increase in 120 second infrared irradiation. Villavicencio *et al.* (2000) reported higher content of phenolic compounds when compared to raw samples. The authors explain such result to the higher extractability of these compounds in cooked samples, due to alterations in cellular components at high temperatures or due to the decomposition of some insoluble phenolic compounds.

The reduction in poly phenols after irradiation might be due to the fact that phenols react with protein during irradiation forming poorly extractable protein-phenolic complexes.

CONCLUSION

The present study reveals that irradiation has no effect on the chemical composition of sorghum grain. The irradiation caused increment of Sorghum grain digestibility directly or indirectly by elimination or reducing of anti-nutritional factors. This occurs through decrease in tannins and phytate contents that could interact with proteins and energy components. Utilizing of irradiation is evaluated as a safe and trustable treatment procedure in poultry and livestock industry. We can use a wide range of irradiated raw materials in poultry and livestock feeding, by development of industrial methods which are based on irradiation and by considering beneficial effects on nutrient digestibility and reducing of anti-nutritional factors. Therefore application and commercialization of irradiation technology is more worthwhile. Though the further study is needed to meet the financials benefits and also technical feasibility to run this process in an industrial scale.

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