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Effects of gamma and electron radiation on chemical composition and some phyto-chemical properties of whole flaxseed

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Abstract
The effects of gamma (GR; 0, 5, 10, 15 and 20 kGy) and electron irradiation (ER; 0, 5, 10, 15 and 20 kGy) on proximate compositions, fatty acid profiles, cyanid, total phenolic compounds, flavonoids and γ-tocopherols (γ-toc) contents of flaxseed (FS) were investigated. Irradiation had no effect on the proximate composition, cyanid content and fatty acid profiles of FS. Both GR and ER at high applied doses decreased ($P < 0.05$) total phenolic compounds and flavonoid content of FS. All applied doses of GR and ER decreased ($P < 0.05$) γ-toc content of FS compared to the control.

Keywords Flaxseed · Irradiation · Total phenolics · Flavonoid · Cyanid · Tocopherol · Fatty acids

Introduction

Because of high amounts of α-linolenic acid (52% of the total fatty acids) and phenolic compounds, flaxseed (FS) is considered as an important functional food for human [1, 2]. There is also increasing trends to fortify animal products, and especially poultry meat and eggs with omega-3 fatty acid for human consumption by inclusion the FS in their diets [3].

Alpha-linolenic acid from flaxseed oil is also known as an anti-cardiovascular agent. Some studies have indicated that daily dose of ground FS in human caused a reduction in blood triglyceride and low density lipoprotein [4, 5]. In spite of high nutritive value, FS contains anti-nutritive components, like cyanogenic glycosides and anti-vitamin B6. To prevent toxic effects of cyanogenic glycosides, daily FS intake should be limited to 1–2 table spoons [6]. It has been reported that microwave heating [7] and solvent extraction [8] are carried out to eliminate cyanogenic glycosides of flaxseed and flaxseed meal, respectively.

The radiation techniques have been widely used to prevent agricultural products from spoilage microorganism and insect during storages. Applying ionizing irradiation up to 10 kGy is permitted in many countries for food processing [9].

In recent years many studies have shown remediating effect of ionizing radiation on phyto-chemicals and toxic substances of plants and plants production [10, 11]. Compared to electron beam, γ-irradiation is used more widely for irradiation of plants materials due to its higher penetrating capability and negligible heat production [12]. But irradiation of herbal products especially at doses more than 10 kGy may has adverse effect on the content of functional nutrients like fatty acids, vitamins and etc. Yalcin et al. [13] showed a decrease in crude protein and oil content, and irregular changes in palmitic and stearic acid content of gamma irradiated FS up to 7 kGy. El-Shennaway et al. [14] showed a decrease in fiber content and irregular changes in some of fatty acids of gamma irradiated FS up to 10 kGy. To the knowledge of authors there are no data available on the effect of electron beam on chemical composition of FS.

Hence, the aims of the present study was to evaluate and compare the effect of gamma and electron beam irradiation, on the proximate composition, total phenolic compounds, fatty acid composition, cyanide and gamma-tocopherol of FS irradiated at doses of 5, 10, 15 and 20 kGy.

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Materials and methods

Sample preparation and experimental design

The raw Canadian flaxseed (*Linum usitatissimum*) was purchased from Zarbal Company (Amol, Mazandaran, Iran) and was packed into 32 poly ethylene bags (15×20 cm^2). Sixteen of the bags were exposed to various doses (5, 10, 15 and 20 kGy; four bags each per dose step) of electron radiation (ER) with a fixed beam energy of 10 MeV using a Rhodotron accelerator. A dose rate of 180 kGy/min was used as determined by cellulose triacetate films [15]. Remaining of the sixteen bags were irradiated by ^{60}Co-gamma cell at similar doses to those used for ER (Four bags each per dose step) and with a dose rate of 9.06 Gy/min as measured using a fricke dosimetery system [16]. Uncertainties for ER and gamma radiation (GR) were 5% and 3%, respectively. The measured dose uniformity ratios (D_{max}/D_{min}) were 1.10 and 1.20 for ER and GR, respectively. After irradiation, the content of each bag was ground and analyzed for further analysis.

Proximate analysis

Proximate analysis was down for determination of crude protein (CP; #955.04), organic matter (OM; #942.05), crude fiber (CF; #985.29) and ether extract (EE; #920.39) of flaxseed according to AOAC [17] analytical methods.

Estimation of total phenolic compounds and flavonoid

Total phenolic compounds of flaxseed (FS) was determined by the Folin–Ciocalteu micro-method [18] on FS acetone extracts. Briefly, 20 µl of the extract solution were mixed with 1.16 ml distilled water and 100 µl of Folin–Ciocalteu reagent, followed by addition of 300 µl of Na_2CO_3 solution (20%) within 1–8 min. Subsequently, the mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The total phenolic compounds was expressed as gallic acid equivalents using the following linear equation based on the calibration curve: $A = 0.98\ C + 9.321 \times 0.001$, $R^2 = 0.9965$. Where A is the absorbance and C is concentration as gallic acid equivalents (mg/g).

The total flavonoid content was determined using the method of Dowd as adapted by Arvouet-Grand et al. [19], with some minor modifications. Briefly, 5 ml of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of a sample solution (20 mg/ml). Absorption readings at 415 nm were measured in UV–visible spectrophotometer after 10 min against a blank sample consisting of a 5 ml sample solution with 5 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with chrysin (0–0.05 mg/ml) as the standard. The mean of three readings was used and expressed as mg of chrysin equivalents (CE)/100 g of sample.

Cyanid

Cyanide content was determined using the picrate method [20, 21] using a commercial cyanide kit. Briefly, 25–100 mg ground FS sample was mixed with phosphate buffer (0.5 ml, 0.1 M at pH 4–10) and beta-glycosidase enzyme. A picrate paper attached to a plastic backing strip [20] was inserted into the sample and after about 16 h (30 C°), the picrate paper was removed and immersed in 5.0 ml water for 30 min. The absorbance of picrate solution was measured at 510 nm and the total cyanide content (ppm) determined (1).

$$\text{Total cyanide content (ppm)} = \frac{360 \times \text{Abs} \times 100}{z} \quad (1)$$

where z = sample weight (mg), Abs = absorbance value.

Gamma-tocopherol assay

Gamma-tocopherol (γ-toc) assay analysis was done at the Vitamin E laboratory (Linus Pauling Institute, Oregon State University, Corvallis, USA). Gamma-tocopherol was extracted from FS samples by the method described by Podda et al. [22]. Briefly, 0.2 g of FS were saponified by addition of 0.3 ml saturated KOH at 70 °C and were incubated for 30 min. Samples were cooled on ice and extracted with *n*-hexane, dried under nitrogen, re suspended in ethanol-methanol (1:1), then injected into an HPLC system. A Shimadzu LC-10AD VP HPLC system was used with a Shimadzu SIL-10AD VP auto injector. A C18, 4.6×100 mm, 3 µM, isocratic 1 ml/min column was used, with 99% methanol as a mobile phase at a 1.0 ml/min flow rate. A Shimadzu Prominence UFLC (Shimadzu USA MFG, INC) with fluorescence detection was used to quantify γ-toc. Excitation and emission wavelengths were 295 and 325 nm, respectively. Resulting values were compared to a standard curve from an α or γ-toc standard and quantified. Values were reported as µg/g.

Fatty acid analysis

All the fatty acids (FA) analysis was done at the lipid laboratory (Oregon State University, Corvallis, USA). About 2 g of FS was taken for total lipid extraction using chloroform: methanol (2:1) following the method of Folch et al.

[23]. Fatty acid methyl esters were prepared from total lipid extract using boron trifluoride-methanol [24]. Fatty acid analysis was performed with an HP 6890 gas chromatograph (Hewlett-Packard Co., Wilmington, DE) equipped with an auto sampler, flame ionization detector, and SP-2360 fused silica capillary column. Samples in hexane (1 µl) were injected with helium as a carrier gas into the column programmed for ramped oven temperatures. Initial oven temperature was set at 150 °C, held for 1.5 min, then ramped at 15 °C/min to 190 °C and held for 20 min, then ramped again at 30 °C/min to 230 °C and held for 3 min. Inlet and detector temperatures were both 250 °C. Fatty acid methyl esters were identified by comparison with retention times of authentic internal or external standards (Nuchek Prep, Elysian, MN). Peak areas and percentages were calculated using Hewlett-Packard ChemStation software (Agilent Technologies Inc., Wilmington, DE). Fatty acid values were reported as percentage of methyl esters.

Statistical analysis

The data were analyzed one-way ANOVA. Significant differences among the treatments means were analyzed by Tukey's test when $P < 0.05$. Computations were done using the General Linear Models procedure of SAS [25].

Results and discussion

Proximate composition

The effect of irradiation on proximate composition of FS is shown in Table 1. Gamma and electron irradiation had no significantly effects ($P > 0.05$) on organic matter (OM), ether extract (EE), crude fiber (CF) and crude protein (CP) content of FS. This finding is consistent with some previous studies on radiation processing of agricultural by-products including whole cottonseed and soybean meal [10, 26], but is in contrast to the results of Yalcin et al. [13] who reported a decrease in OM, CP and oil content of gamma irradiated FS (at 2.5–7.0 kGy). El-Shennaway et al. [14] showed that GR up to 10 kGy did not affect CP and EE content of FS, but it decreased CF and ash content of it.

Also, in contrast to the results of the present study, Nayefi et al. [27] and El-Neily and El-Shennawy [28] reported that GR and EB (30–40 kGy) decreased CF content of cottonseed meal. It is generally accepted that irradiation could affect cell wall content of agricultural products via cleavage of the glycosidic bonds [29], but the degree of cleavage could be determine by the level of lignifications of cell wall. Among the compounds of the cell wall, lignin is the most resistant component to the irradiation. Moradi et al. [30] reported that electron beam (10–40 kGy) decreased lignin content of pistachio by-product to the same extent compared to unirradiated sample.

Hydrogen cyanide

The effect of GR and ER on cyanide content of FS is shown in Fig. 1. No significant effect ($P > 0.05$) of GR and EE was observed on the cyanide content of FS. In contrast to the result of the current study Tresina and Mohan [11] showed a significant dose dependent decline in cyanide content of gamma irradiated (2–25 kGy) black cowpea seed. Low dose

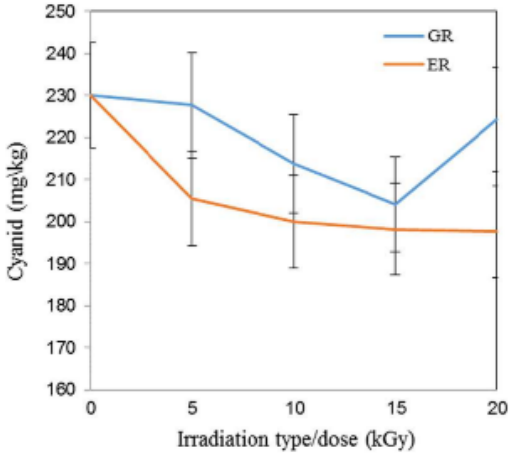

Fig. 1 The effect of gamma and electron radiation on cyanide content of FS (mg/kg of DM)

Table 1 Effects of gamma and electron irradiation on proximate composition of flaxseed (% of DM)

	Treatments									SEM	*P* value
	Control	GR5	GR10	GR15	GR20	ER5	ER10	ER15	ER20		
OM	96.67	96.45	96.49	96.57	96.58	96.61	96.55	96.42	96.55	0.14	0.11
CP	19.11	19.13	18.95	19.12	19.04	18.63	18.04	18.99	18.93	0.07	0.13
EE	36.03	34.32	34.56	33.54	33.91	35.3	34.26	35.74	34.43	0.06	0.66
CF	21.05	20.18	17.75	17.33	19.33	16.14	19.95	16.6	19.42	0.07	0.49

OM organic matters, *CP* crude protein, *EE* ether extract, *CF* crude fiber, *GR* gamma irradiation, *ER* electron irradiation

application of gamma ray (50–150 Gy) also showed a reduction in cyanide content of cassava varieties ranging from 21 to 91% [31]. However, in the present study ER at dose of 20 kGy reduced cyanide content of FS by 14.11% compared to unirradiated FS. The effect of GR on hydrogen cyanide molecule is proven in aqueous system [32, 33]. The effect of GR on hydrogen cyanide is highly depending on the presence of water, oxygen and substance with scavenging activity [33]. In the present study the lack of effect of GR on the amount of cyanide in comparison to the study of Tresina and Mohan [11] can be due to the presence of high levels of phenolic compounds in FS (10.67% of DM) in comparison with the black cowpea (1.24% of DM). Phenolic compounds and flavonoid of FS can act as scavenger against radiolytic effect of GR.

Free radicals due to excitation and ionization of water by gamma radiation can damage or modify important components of plant cells [34]. Hence the effect of lower gamma ray doses (50–150 Gy) in the study of Moradia et al. [30] on hydrogen cyanide can be due to high water content in cassava tubers (about 63–64%) compared with low levels of water in FS (about 9–10%) in the present study.

Total phenolic compounds and flavonoid

The effect of irradiation on total phenolic compounds of FS is shown in Fig. 2a. Gamma irradiation at dose of 5 kGy increased ($P<0.05$) phenolic compounds of FS. At higher doses of irradiation (15 and 20 kGy) GR decreased ($P<0.05$) phenolic compounds of FS compared to control. Electron irradiation at doses of 10–20 kGy decreased ($P<0.05$) phenolic compounds of FS. The level of phenolic compounds was increased by the exposure of FS to the GR at dose of 5 kGy. This effect might be due to solubilization of insoluble or cell wall bounded-phenolics [30]. In the present study doses greater than 15 kGy of GR and 10 kGy of ER decreased phenolic compounds of FS. In agreement with these results, an increase in phenolic compounds of GR almond hull (0.5–3 kGy) was noted by Mosavi et al. [35]. These results are in contrast to the other studies in which an increase in total phenol content of gamma irradiated almond skin and electron irradiated *Citrus unshiu* pomaces was reported [36, 37]. This discrepancy can be explained by the differences in phenolic compounds and the level of solubility of phenolic compounds of FS compared to other resources. The effect of GR on total phenols of FS was greater than ER. Reduction of total phenolic compounds in the present study was 37.45% and 26.69% for gamma and electron-irradiated FS at dose of 20 kGy, respectively.

The effect of irradiation on flavonoid content of FS is shown in Fig. 2b. Both GR (15 and 20 kGy) and ER (10–20 kGy) decreased ($P<0.05$) flavonoid content of FS. The decrease in flavonoid content in plant materials treated with ionizing radiation has been reported by Carocho [38] and Mosavi et al. [35]. In the present study gamma and electron irradiation decreased flavonoid content of FS by 33.85% and 22.60% at dose of 20 kGy, respectively. In agreement with this result Carocho [38] showed that GR was more effective in reduction of flavonoid content of chestnut compared to ER.

Fatty acid content

Fatty acid compositions of control and irradiated FS are shown in Table 2. No significant effects ($P>0.05$) of radiation type/dose was observed on fatty acid composition of FS. Few studies have been conducted on the effects of ionizing irradiation on the fatty acids composition of FS. Flaxseed had high concentrations (88%) of unsaturated fatty acids. In agreement with Yalcin et al. [13] the main fatty acid of the FS was alpha-linolenic acid (54.81%). The second and third most abundant fatty acids were oleic (20.97%) and linoleic acids (12.24%), respectively.

As shown in Table 2, no significant effect of GR and ER was seen on fatty acids profile of FS. In contrast to these results El-Shennaway et al. [14] and Yalcine et al. [13]

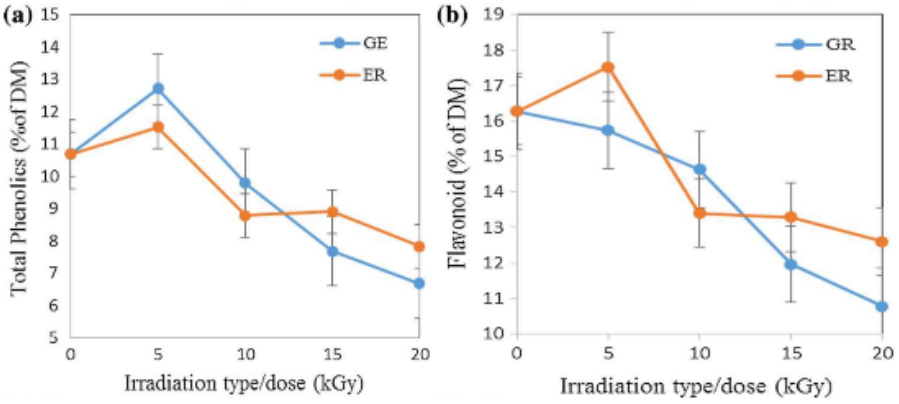

Fig. 2 The effect of gamma and electron radiation on phenolic compounds (**a**) and flavonoid (**b**) content of FS (% of DM)

Table 2 Fatty acid compositions of control and irradiated whole FS

Fatty acid	Treatments									SEM	*P* value
	Control	GR5	GR10	GR15	GR20	ER5	ER10	ER15	ER20		
C16:0	6.33	6.51	6.78	6.64	6.46	6.45	6.63	6.89	6.70	0.02	0.87
C18:0	5.66	5.96	5.64	5.82	6.11	6.10	6.22	5.63	6.16	0.03	0.77
C18:1n-9	20.97	21.00	21.59	22.70	21.07	19.38	20.57	21.53	20.99	0.05	0.39
C18:2n-6	12.24	12.21	12.29	11.99	12.31	12.44	12.24	12.43	12.36	0.02	0.47
C18:3n-3	54.81	54.33	53.75	52.87	54.06	55.63	54.35	53.52	53.78	0.04	0.26
TSFA	12.00	12.47	12.38	12.45	12.57	12.55	12.84	12.52	12.86	0.03	0.97
TMUFA	20.97	21.00	21.59	22.70	21.06	19.38	20.58	21.58	20.99	0.05	0.39
n6	12.24	12.21	12.29	11.99	12.31	12.44	12.24	12.43	12.36	0.02	0.47
n3	54.81	54.33	53.75	52.87	54.06	55.63	54.36	53.52	53.78	0.04	0.26
n6: n3	0.22	0.23	0.23	0.23	0.23	0.22	0.22	0.23	0.23	< 0.01	0.45

Values are expressed as mean weight percentage of total fatty acid methyl esters

C16:0 palmitic acid, *C18:0* stearic acid, *C18:1n-9* oleic acid, *C18:2n-6* linoleic acid, *C18:3n-3* α-linolenic acid, *TSFA* total saturated fatty acids, *TMUFA* total monounsaturated fatty acids, *n6* omega-6 fatty acids, *n3* omega-3 fatty acids, *GR* gamma irradiation, *ER* electron beam

showed inconsistent changes in fatty acid composition of FS by gamm-irradiation. Yalcin et al. [13] showed a decrease in alpha-linolenic and an increase in the linoleic acid content of gamma irradiated (2.5–7.5 kGy) FS. But, El-Shennaway et al. [14] showed a decrease in stearic and oleic acids and an increase in palmetic and alpha-linolenic acids when FS was irradiated at doses of 2.5–10 kGy of gamma irradiation. In contrast to these results GR of pine nut up to 5 kGy and soybean oil up to 10 kGy did not showed any significant changes in fatty acid composition [39, 40].

Mexis and Kontominas [41] reported that peroxide value and hexanal, as an indicator of partial decomposition of triglycerides, from cashew nut oil were increased with increasing gamma irradiation doses (1–7 kGy). In this study at high irradiation doses stearic acid increased and oleic acid was decreased, while other fatty acids remained unchanged after gamma irradiation. The authors attributed this to the low water content of cashew nut and applied low doses of GR.

It can be suggested that many factors like applied irradiation doses, storage condition, presence of antioxidant compounds and tocopherols may be imparting protection of lipids against ionizing irradiation.

Gamma-tocopherol

The effect of GR and ER on γ-toc content of whole FS is shown Fig. 3. γ-tocopherol content in control FS found 20.65 ± 0.79 ppm in whole grain. Daun and Przybylski [42] reported similar γ-toc content of FS (19.95 ± 5.07 ppm). All doses of GR and ER decreased ($P < 0.05$) γ-toc content of FS compared to unirradiated group. In agreement with these results Lalas et al. [43] showed that GR decreased γ–toc content of some vegetable oils. In the present study, the highest decrease in γ-toc was observed for GR20 and ER 15 by 35%.

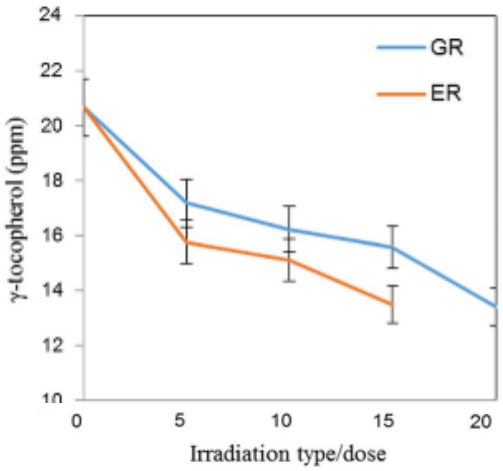

Fig. 3 The effect of GR and ER (ER20 not determined) on γ –toc content of whole FS (ppm)

The reductions of γ-toc for GR and ER at dose of 5 kGy were 17% and 24%, respectively. These results are in line with those reported by Lakritz et al. [44] who reported a 15% reduction in γ-toc after GR (at 3 kGy) of fresh chicken breasts.

Conclusion

The present study showed that GR and EB reduced phenolic compounds, flavonoid and gamma-tocopherol; but had no effects on proximate composition, fatty acids profiles and cyanide content of FS. Minimum effective dose in elimination of total phenolic compounds and flavonoid for GR and

EB were 15 and 10 kGy, respectively. But all studied doses of GR and ER (5–20 kGy) adversely affect γ-toc content of FS and decreased its level compared to unirradiated group. It can be concluded that the most important shortage for the irradiation of FS even at low dose (5 kGy), is that elimination of vitamin E (γ-toc).

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