Changes in physico-chemical properties of native and toasted defatted soy flour on submission to electron beam radiation

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ABSTRACT

Ionizing radiations are increasingly being used to disinfest raw material for several food products. Toasted and native soy flour, the major food ingredients in bread and bakery industries, can be disinfested prior to use through electron beam. However, this can induce changes in the nutritional and functional properties, which can ultimately affect the quality and the nutritional value of the final products. In the present study, toasted and native soy flour were submitted to electron beam (EB) irradiation at 4.8, 9.2, 15.3 and 21.2 kGy; and assessed for water absorption capacity (WAC), protein dispersibility index (PDI), protein solubility, trypsin inhibitor (TI) content, isoflavones content, in vitro protein digestibility (IVPD), glycinin (11S) to β-conglycinin (7S) ratio, and lipoxygenase. WAC declined slightly (P < 0.05) in toasted soy flour, but increased significantly (P < 0.05) at low doses in native soy flour. In both toasted and native soy flour, slight decline was noted in protein solubility while TI and lipoxygenase declined significantly (P < 0.05). However, the decline noted in TI content was not proportionate to the increase in IVPD. PDI remained unchanged in toasted soy flour but declined significantly (P < 0.05) in native soy flour. 11S to 7S ratio increased significantly (P < 0.05) in toasted flour at all the doses. In general, significant (P < 0.05) decline in isoflavones was noted in both toasted and native soy flour. In conclusion, the results showed that EB-irradiation could induce desirable changes in the nutritional/functional properties of toasted and native soy flour, though at the expense of some of the physical properties.

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1. Introduction

Globally, soybean is the major oilseed and the de-oiled meal obtained after extraction of the oil from its grains is used as feed for animals. USA, Brazil, Argentina, China and India are the leading contributors to the total global soybean production. In India, according to 3rd estimate of Department of Agriculture, Ministry of Agriculture, Government of India, soybean production stands at 14.03 million tonne in 2016. Oil extracted from soybean produce is entirely used in domestic consumption; however, protein-rich de-oiled meal (48% or more) obtained after extraction is mainly exported and the remaining used as feed for domestic poultry industry. Being packed with 20% oil, 40% protein, several minerals and vitamins, and nutraceutical components such as isoflavones, tocopherols, and bowman-birk, soybean has gained the "functional food" status. As a result, there is growing interest in soy-based food products like soy milk, tofu etc. across the world including India. Lately, toasted and native, which are variants of commercially available, defatted soy flour are increasingly being used as functional ingredients in bakery and food industry. Toasted defatted flour is manufactured after exposing the defatted flour at l00–110 °C, which is lower

than the temperature applied in roasting (120–150 °C). In bread-making, the addition of toasted defatted soy flour has been reported to increase the water absorption and improve the dough elasticity and crust color (Stauffer and Ph, 2008). Defatted soy flour has been reported to improve the batter smoothness and distribution of air cells in cake-making (Kulp, 2000), and impart crispiness in cookies-preparation. Native soy flour is added in wheat flour to bleach carotenoids pigments in *pasta*-making. Besides, it is also an excellent raw material for manufacturing soymilk. Native soy flour is also used in dietetic food, baby food and drinks and fortification of cereal. Physical and chemical properties, namely, water absorption capacity (WAC), protein dispersibility index (PDI), protein solubility, in-vitro protein digestibility (IVPD), contents of bio-molecules like trypsin inhibitor (TI), isoflavones, lipoxygenase and ratio of glycinin (11S) to β-conglycinin (7S) protein fraction, play important role in the quality of the final products prepared from toasted and native soy flour. Protein solubility and PDI determine the quality of soy milk and tofu processed from these raw materials (Poysa et al., 2006). TI is an anti-nutritional factor that affects protein digestibility. Lipoxygenase is responsible for the off-flavor associated with the soy-based products but its bleaching property makes it a desirable trait in bakery application. Isoflavones in toasted and untoasted soy flour impart astringency to the final products (Huang et al., 1982). The ratio of glycinin to β-conglycinin protein fraction is responsible for gelling property (Poysa et al., 2006; Tang et al., 2006). Ionizing radiations like gamma and electron beam irradiation are useful in disinfesting and preserving the food products and the raw material (Rahman, 2007). Both toasted and native soy flour are vulnerable to spoilage during storage. If these radiations are to be used for disinfesting the toasted and native soy flour to enhance shelf life during storage, the possible effects of electron beam on the physical and chemical properties of toasted and native soy flour need to be investigated. Gamma and EB radiations have been reported to affect the quality traits in soybean seeds (Wang et al., 2010; Dixit et al., 2011; Kumar et al., 2016). However, the reports focusing on their effects on the above-mentioned physico-chemical and functional properties of toasted and untoasted soy flour are not available. In the present investigation, industrially-manufactured toasted and native soy flour were submitted to varying doses of high-speed EB radiation and the changes in WAC, PDI, protein solubility, TI, IVPD, lipoxygenase protein, isoflavones content and ratio of glycinin (11S) to β-conglycinin (7S) protein fraction, were assessed.

2.   Materials and methods

2.1.   Samples

Toasted and native (untoasted) defatted flour samples were procured in the sealed bags from a local soy flour manufacturing unit. The grains used for manufacturing, contained freshly harvested (cropping season 2015) soybean seeds of mixture of popular soybean varieties grown in Central India. Industrial preparation of toasted and untoasted defatted soy flour samples packed in the sealed polyethylene bags of size 17 cm × 12 cm and 1.8 mm thickness and these sealed samples were exposed to EB-irradiation.

2.2.   Irradiation set-up

Electron accelerator (10 MeV) LINAC was used for irradiating toasted and native (untoasted) defatted soy flour at ambient temperature (28–30 °C). Accelerator parameters viz beam energy, peak current, and pulse repetition rate were set at 8.3 MeV, 400 mA and 52 Hz, respectively. Polyethylene bags containing industrial preparation of toasted and native (untoasted) defatted soy flour samples were passed before electron beam repeatedly to achieve the absorbed doses of 4.8, 9.2, 15.3 and 21.2 kGy. The dose delivered per pass was 1.57 kGy and alanine electron paramagnetic resonance (Bruker, e-scan

EPR spectrometer) dosimetry was used to measure the doses absorbed by soy flour samples.

2.3.   Water absorption capacity (WAC)

WAC of soy flour samples was determined through standard protocol (Sosulski, 1962). One gram of flour was weighed in a centrifuge tube, and 10 ml of water was added, the tubes were capped and vortexed for 5 min to suspend the flour. Tubes were then centrifuged at 10,000 rpm for 10 min and then inverted to drain excess water; inner sides of the tubes were dried by lint-free tissues. The tubes were then weighed and water absorption is calculated by the difference in weight of tubes before and after absorbing water and the values were expressed in g of water absorbed by 1 g of soy flour.

2.4.   Trypsin inhibitor (TI) analysis

TI content of soy flour samples was determined following standard procedure (Hammerstrand et al., 1981).

2.5.   Protein dispersibility index (PDI)

PDI of soy flour samples was determined as described earlier (Palic et al., 2010). Initially, 1 g (dry weight basis) of soy flour was blended in 15 ml of distilled water for exactly 10 min, followed by the centrifugation at 2700 rpm (815 g). Nitrogen in the residue was estimated by the micro-Kjeldahl method. For that 1.5 ml supernatant so obtained was subjected to digestion at 380 °C in digestion tubes with digestion mixture (2 g) and conc. H_2SO_4 (10 ml), after cooling the digested samples were diluted with 30 ml distilled water and distillation was done with 40% NaOH (60 ml), the resulted NH_4OH was trapped in a flask containing 4% boric acid (20 ml) and titrated against 0.01 N HCl, the amount of acid consumed are used to calculate total nitrogen and then multiplied by the factor 6.25 to obtain crude protein. The PDI was calculated as the ratio of protein in the supernatant to total protein in the original toasted and native soy flour.

2.6.   Protein solubility

The protein solubility of soy flour samples was determined after extraction in 0.2% KOH following the standard procedure. Initially, 1.5 g (dry weight basis) of soy flour was weighed and 75 ml of 0.2% (0.36 N, pH 12.5) KOH was added into it. Stirred on magnetic stir plate for 20 min, followed by the centrifugation at 2700 rpm (815 g) for 15 min. Supernatant so obtained (15 ml) was taken into Kjeldahl tubes, for duplicate analysis. Conc. H_2SO_4 (12.5 ml) and H_2O (2 ml) was added to each tube and estimated for total nitrogen content analysis. The protein solubility was expressed as the ratio of protein of the supernatant and total protein in the original toasted and native (untoasted) soy flour.

2.7.   In vitro protein digestibility (IVPD)

IVPD of un-irradiated and irradiated toasted and untoasted soy flour samples was determined by pepsin digestion method (Kayembe and Jansen van Rensburg, 2013). Soy flour (300 mg) were weighed into a series of test tubes. A solution of 5 ml of 0.075 N HCl and 0.5 ml of pepsin solution (2.0 mg/ml) in 0.075 N HCl was added to each tube. The tubes were incubated at 37 °C and enzyme action was stopped after 24 h by adding 5 ml

Table 1 – Physico-chemical properties of toasted and native (untoasted) soy flour submitted to varying EB doses.

Soy flour	EB dose (kGy)	WAC g/g	Protein solubility (%)	Urease index	PDI (%)	IVPD (%)	TI (mg/g)	Lipoxygenase (% of the total protein bands)
Native (untoasted)	0	2.56 ± 0.08^c	94.43 ± 4.1^a	1.22 ± 0.02^a	54.94 ± 2.2^a	50.96 ± 2.1^b	65.1 ± 2.1^a	0.8 ± 0.04^a
	4.8	3.17 ± 0.06^b	93.45 ± 3.9^{ab}	1.22 ± 0.02^a	47.6 ± 1.2^b	51.40 ± 1.9^b	55.57 ± 1.8^b	0.70 ± 0.03^b
	9.2	3.74 ± 0.1^a	92.83 ± 2.9^b	1.18 ± 0.05^b	47.83 ± 1.4^b	51.84 ± 1.7^b	56.42 ± 1.5^b	0.33 ± 0.02^d
	15.3	2.71 ± 0.05^c	92.68 ± 2.6^b	1.19 ± 0.04^b	48.32 ± 1.8^b	52.50 ± 1.3^b	54.42 ± 1.2^b	0.4 ± 0.02^c
	21.2	2.63 ± 0.09^c	93.51 ± 3.4^{ab}	1.18 ± 0.03^b	43.16 ± 1.6^c	52.71 ± 1.5^b	52.09 ± 1.7^c	0.4 ± 0.03^c
Toasted	0	2.84 ± 0.04^{bc}	90.1 ± 3.1^c	0.06 ± 0.002^c	24.2 ± 0.9^d	54.03 ± 2.2^{ab}	10.2 ± 0.3^d	0.3 ± 0.01^d
	4.8	2.76 ± 0.06^e	87.2 ± 2.6^d	0.06 ± 0.002^c	24.6 ± 0.7^d	56.21 ± 2.1^a	07.1 ± 0.4^e	0.1 ± 0.00^f
	9.2	2.78 ± 0.07^{bc}	87.03 ± 4.5^d	0.04 ± 0.001^e	24.6 ± 0.6^d	54.62 ± 1.9^{ab}	07.0 ± 0.2^e	0.1 ± 0.00^f
	15.3	2.59 ± 0.09^c	88.7 ± 2.8^{cd}	0.05 ± 0.001^d	24.6 ± 0.8^d	54.90 ± 2.4^{ab}	06.5 ± 0.1^e	0.2 ± 0.01^e
	21.2	1.82 ± 0.08^d	86.0 ± 1.9^e	0.05 ± 0.002^d	24.9 ± 1.1^d	54.46 ± 2.3^{ab}	06.7 ± 0.3^e	0.2 ± 0.01^e

Values given are mean ± standard deviation of replicated samples. Values within the same column with different superscripts are significantly different from each other at $P < 0.05$.

Table 2 – Proportion (%) of storage protein fractions of toasted and native (untoasted) soy flour submitted to varying EB doses.

Soy flour	EB dose (kGy)	β-Conglycinin (7S)			Total (7S)	Glycinin (11S)		Total (11S)	Ratio of 11S:7S
		α'	α	β		Acidic	Basic		
Native (untoasted)	0	5.4 ± 0.16^e	7.5 ± 0.16^d	6.5 ± 0.27^b	19.4 ± 0.6^e	15.9 ± 0.1^{de}	18.5 ± 0.4^f	34.4 ± 2.1^d	1.77 ± 0.04^e
	4.8	5.6 ± 0.13^e	8.2 ± 0.16^c	6.7 ± 0.23^b	20.5 ± 0.9^d	15.9 ± 0.3^{de}	16.3 ± 0.6^h	32.2 ± 1.8^e	1.57 ± 0.03^g
	9.2	4.8 ± 0.12^f	7.1 ± 0.2^d	6.8 ± 0.21^b	18.7 ± 0.8^e	15.4 ± 0.2^e	16.3 ± 0.2^h	31.7 ± 1.5^e	1.69 ± 0.01^f
	15.3	4.4 ± 0.15^g	6.4 ± 0.19^d	6.6 ± 0.25^b	17.4 ± 0.3^e	16.5 ± 0.9^d	20.9 ± 0.7^e	37.4 ± 1.9^d	2.14 ± 0.05^c
	21.2	4.7 ± 0.16^f	7.1 ± 0.31^d	6.0 ± 0.22^c	17.8 ± 0.2^e	14.5 ± 0.7^f	17.4 ± 0.5^g	31.9 ± 0.9^e	1.79 ± 0.03^e
Toasted	0	9.0 ± 0.31^a	11.0 ± 0.4^a	7.8 ± 0.23^a	27.8 ± 0.9^a	24.8 ± 0.4^a	21.1 ± 0.4^e	45.9 ± 1.8^{bc}	1.65 ± 0.01^f
	4.8	7.2 ± 0.23^b	9.9 ± 0.13^b	5.9 ± 0.18^c	23.0 ± 0.8^b	22.5 ± 0.6^b	25.0 ± 0.5^b	47.5 ± 1.6^b	2.06 ± 0.04^d
	9.2	5.9 ± 0.12^{de}	8.1 ± 0.32^c	5.7 ± 0.16^c	19.7 ± 0.7^d	21.7 ± 0.7^c	23.6 ± 0.8^c	45.3 ± 0.9^c	2.29 ± 0.05^a
	15.3	6.7 ± 0.21^c	9.8 ± 0.34^b	6.6 ± 0.25^b	23.1 ± 1.1^b	24.2 ± 0.5^a	26.8 ± 0.9^a	51.0 ± 1.2^a	2.20 ± 0.02^b
	21.2	6.2 ± 0.14^d	9.2 ± 0.29^b	6.0 ± 0.28^c	21.4 ± 0.4^{cd}	22.3 ± 0.4^{bc}	22.7 ± 0.9^d	45.0 ± 1.5^c	2.16 ± 0.03^{bc}

Values given are mean ± standard deviation of replicated samples. Values within the same column with different superscripts are significantly different from each other at $P < 0.05$.

10% (w/v) trichloroacetic acid (TCA). Digestion was performed in triplicate. The mixture then filtered through Whatmann no. 1 filter paper and the residues were washed twice with warm water and the residue is oven dried for complete drying. Nitrogen in the residue was estimated by the micro-Kjeldahl method. For that 100 mg of the above dried residue or 100 mg of dried soybean flour was subjected to digestion at 380 °C in digestion tubes with digestion mixture (2 g) and conc. H_2SO_4 (10 ml), after cooling the digested samples are diluted with 30 ml distilled water and distillation is done with 40% NaOH (60 ml), the resulted NH_4OH was trapped in a flask containing 4% boric acid (20 ml) and titrated against 0.01 HCl, the amount of acid consumed was used to calculate total nitrogen and then multiplied by the factor 6.25 to obtain crude protein. IVPD was obtained by calculating the difference between the amount of total nitrogen in the sample before and after in vitro digestion with pepsin.

2.8. Densitometry of storage proteins for determining 11S:7S ratio and lipoxygenase protein

Soy flour samples (50 mg on dry wt basis) were extracted with buffer containing 125 mM Tris–Cl buffer (pH—6.8), 0.2 M SDS and 1 M 2-mercaptoethanol and kept in boiling water bath for 10 min, followed by centrifugation. A fixed amount of soluble protein was resolved on stacking (5%) and running gel (12.5%) by employing 30 and 70 mA current during the movement of the protein in the former and latter portion, respectively. After completing the run, the gel was stained with 0.1% coomassie brilliant blue R250 followed by destaining using methanol: water: acetic acid in the ratio of 45:45:10. The protein profile pattern was scanned and quantified using densitometer Bio-Rad G900 using *Image Lab*.

2.9. Estimation of isoflavones through HPLC

Contents of isoflavones isomers, namely, daidzein, glycitein and genistein were determined through acid hydrolysis of soy flour samples as described elsewhere (Kumar et al., 2011). The method converts 12 endogenous isoflavones isomers to their respective aglycones forms i.e. daidzein, glycitein and genistein (Vyn et al., 2002).

2.10. Statistical analysis

All steps and biochemical assays were performed in triplicate samples and data presented in Tables 1–3 are mean ± standard deviation of three independent replicates. All the statistical analyses were carried out through SAS 14.0 with significance at $P < 0.05$.

3. Results and discussion

3.1. Water absorption capacity (WAC)

High WAC is one of the important characteristic of soy flour for its use in bread-making industry. Table 1 shows that WAC of

Soy flour	EB dose (kGy)	Isoflavones (µg/g flour)			
		Daidzein	Glycitein	Genistein	Total isoflavones
Native	0	544.14 ± 12[b]	476.93 ± 16[b]	587.20 ± 22[f]	1608.2 ± 63[b]
(untoasted)	4.8	562.21 ± 18[a]	505.79 ± 17[a]	627.56 ± 25[e]	1695.5 ± 59[a]
	9.2	534.95 ± 19[b]	454.23 ± 18[b]	600.81 ± 24[ef]	1589.9 ± 51[b]
	15.3	505.41 ± 15[c]	439.30 ± 19[c]	545.90 ± 18[g]	1490.6 ± 45[cd]
	21.2	516.22 ± 17[c]	441.98 ± 14[c]	552.53 ± 19[g]	1510.7 ± 42[c]
Toasted	0	433.52 ± 11[d]	271.06 ± 08[d]	795.50 ± 27[a]	1500.09 ± 59[c]
	4.8	425.31 ± 16[de]	255.1 ± 10[d]	747.14 ± 30[b]	1427.57 ± 42[d]
	9.2	435.96 ± 13[d]	233.94 ± 12[e]	706.40 ± 29[c]	1376.31 ± 47[d]
	15.3	404.05 ± 14[e]	197.86 ± 06[f]	662.40 ± 22[d]	1264.33 ± 37[e]
	21.2	388.44 ± 12[e]	202.95 ± 07[f]	650.28 ± 35[e]	1241.69 ± 51[e]

Table 3 – Isoflavone content of toasted and native (untoasted) soy flour submitted to varying EB doses.

Values given are mean ± standard deviation of replicated samples. Values within the same column with different superscripts are significantly different from each other at P < 0.05.

toasted flour was significantly (P < 0.05) higher than untoasted soy flour. In toasted soy flour, this property remained unaffected at the low doses but showed significant (P < 0.05) decline at 15.3 and 21.2 kGy. On the contrary, untoasted soy flour exhibited significant (P < 0.05) increase at low doses of 4.8 and 9.2 kGy, though at high doses the increase was non-significant. WAC of protein products depends on the size of the protein, stearic interactions, ionic strength, which may vary in different variants of soy flour at different doses of EB; thereby, leading to differential changes in this property in toasted and untoasted flour.

3.2. Trypsin inhibitor (TI) content and urease index

TI is a heat labile anti-nutritional factor (ANF) present in soybean seeds. This ANF may be left active in soy products due to faulty/insufficient processing treatments. Changes in TI content in toasted and untoasted soy flour due to EB irradiation are presented in Table 1. TI content in toasted soy flour was 10.2 mg/g while in untoasted soy flour the level of this ANF was 65.1 mg/g. In toasted soy flour, EB-irradiation at all the doses resulted significant (P < 0.05) reduction in the TI content; however, the increase in the EB dose did not result in a higher reduction in TI content. In untoasted soy flour also, all the doses induced significant (P < 0.05) reduction in TI content; however, higher (P < 0.05) reduction was noted at 21.2 kGy compared to other 3 EB doses. In a recent study from our laboratory (Kumar et al., 2016), significant reduction in TI content in soybean seeds on exposure to EB-irradiation was found and the magnitude of reduction was genotype-dependent. In the present study, maximum reduction up to 20% for TI content in untoasted soy flour (at 21.2 kGy) and 36.3% (at 15.3 kGy) in toasted soy flour was noted. Hamza et al. (2012) reported 12.9 and 34.3% decline in TI content in raw soy flour at 5 and 10 kGy irradiation, respectively, which is comparable to the reduction in TI content in both toasted and untoasted soy flour at similar doses. Reduction in TI content may be due to the breaking of the disulfide bonds present in the kunitz trypsin inhibitor and bowman-birk polypeptides of the trypsin inhibitor. Further, urease index of toasted and untoasted soy flour was 0.06 and 1.22, respectively. In both toasted and untoasted soy flour, urease index improved at all the EB doses except 4.8 kGy (Table 1). Slight but significant decline (P < 0.05) in urease index on exposure to EB irradiation corresponded to the reduction in TI content.

3.3. Protein dispersibility index and solubility

High protein dispersibility and solubility of soy flour are desirable traits for processing good quality soy food products such as soy milk and *tofu*. The changes observed in these properties of toasted and untoasted soy flour on exposure to varying doses of EB are given in Table 1. In toasted and untoasted soy flour, PDI was 24.2 and 54.94%, respectively. In an earlier study (Palic et al., 2010), PDI and protein solubility were investigated as indicators of the degree of heat treatment to full-fat soybean flour in 8 laboratories. These authors reported PDI of soybean seeds extruded at 110, 127, 136, 145, 164 °C in the range of 35.86–47.62, 11.84–31.0, 6.15–12.54, 5.01–10.08, 4.66–8.38%, respectively. The values for PDI for the toasted flour in this study was in the range of the values reported by Palic et al. (2010) in the soybean seeds extruded at 127 °C. In toasted soy flour, no significant effect was noted for PDI at all the 4 doses; significant reduction (P < 0.05) was observed at all the doses in untoasted soy flour, with a maximum reduction at the highest dose i.e. 21.2 kGy.

With regard to protein solubility, the untoasted soy flour exhibited higher (P < 0.05) value than toasted soy flour. Protein solubility of soybeans seeds extruded at 110, 127, 136, 145, 164 °C has been reported in the range of 83.54–95.88, 82.31–88.63, 71.45–79.47, 57.22–70.63, 50.18–68.61%, respectively, in a previous investigation (Palic et al., 2010). The values of protein solubility in the toasted and untoasted soy flour were in the range of protein solubility noted for soybean extruded at 110 °C. Slight but significant (P < 0.05) reduction in protein solubility was noted in toasted and untoasted soy flour on exposure to EB-irradiation at all the 4 doses. This observation is supported by the decline reported by Afify et al. (2011) in the protein solubility of soybean seeds on exposure to gamma irradiation at 0.5, 1.0, 2.0, 3.0, 5.0 and 7.5 kGy. The loss in solubility of the protein products may be because of the aggregation of soy proteins due to irradiation.

3.4. In vitro protein digestibility (IVPD)

IVPD of toasted soy flour (54.03%) was slightly higher (P < 0.05) than the untoasted soy flour (50.96%). In toasted soy flour, IVPD improved slightly (P < 0.05) at 4.8 kGy dose; while higher doses (9.2, 15.3 and 21.2 kGy) did not induce significant (P < 0.05) changes in protein digestibility. In untoasted soy flour, exposure to EB irradiation at all the doses improved IVPD slightly but the changes were non-significant.

However, Kumar et al. (2017) reported significant improvement in the IVPD of seeds of 3 soybean genotypes on exposure to EB-irradiation, with maximum improvement observed to the extent of 24% at 21.2 kGy. The reduction in anti-nutritional factors may create a space in the matrix of flour, thereby making it susceptible to enzymatic digestion.

3.5. Ratio of glycinin (11S) to β-conglycinin (7S) fraction

Table 2 presents the changes in the density of each subunit of glycinin (11S) and the β-conglycinin (7S) fraction of toasted and untoasted soy flour on exposure to EB-irradiation. In toasted soy flour, significant ($P < 0.05$) reduction was noted in α', α, and β subunits of β-conglycinin (7S) fraction at all the 4 doses, with a maximum reduction for 3 subunits at 9.2 kGy. This led to significant ($P < 0.05$) decline in β-conglycinin (7S) fraction, with a maximum reduction at this dose. Conversely, glycinin (11S) fraction registered significant ($P < 0.05$) increase at all the doses because of the significant ($P < 0.05$) increase in its basic subunit with slight decline/no effect in the acidic subunit. In another study from our laboratory (Kumar et al., 2017), least degradation of basic subunit was noted in soybean seeds of 3 genotypes on exposure to EB-irradiation. In toasted soy flour, consequently, EB irradiation at all the 4 doses induced significant ($P < 0.05$) increase in the ratio of 11S to 7S fraction. In untoasted soy flour, only 15.3 kGy dose induced significant ($P < 0.05$) increase in 11S/7S ratio due to increase in the 11S fraction but decline in the β-conglycinin (7S) fraction. Increase in the intensity of acidic and basic subunit of 11S fraction has also been reported in soybean seeds exposed to gamma irradiation up to 7 kGy (Afify et al., 2011). Though, the authors did not observe any changes in the intensity of other protein fractions. The reduction and increase in the intensity of protein fractions on exposure to EB radiation may be because of the degradation or cross-linking of proteins due to the free radicals generated as suggested earlier (Afify and Shousha, 1988).

3.6. Isoflavones

Results for the changes in contents of an individual isomer of isoflavones are given in Table 3. In toasted soy flour, daidzein declined significantly ($P < 0.05$) at 15.3 and 21.2 kGy. Glycitein and genistein declined significantly ($P < 0.05$) at 9.2 and 15.3 kGy, while genistein at all the 4 doses. This led to the significant ($P < 0.05$) decline in total isoflavones content of toasted soy flour at high doses of 15.3 and 21.2 kGy. In untoasted soy flour, EB-irradiation at 4.8 kGy increased total isoflavones content slightly (5.1%) due to the small increase registered in the contents of daidzein, glycitein and genistein. Though, higher doses of 9.2, 15.3 and 21.2 kGy induced significant ($P < 0.05$) reduction in these isomers, and hence decline in total isoflavones content, which is in agreement with the slight decline reported earlier in gamma irradiated defatted soy flour (Aguiar et al., 2009). Our results are also in consonance with the study of Popovic et al. (2013) who reported significant ($P < 0.05$) decline in daidzein and genistein content of soybean seeds irradiated at low doses of 4 and 10 kGy. The reduction observed in isoflavones in irradiated soy flour is desirable; especially when soy flour is used as an ingredient in bakery products it may impart undesirable astringency to food products.
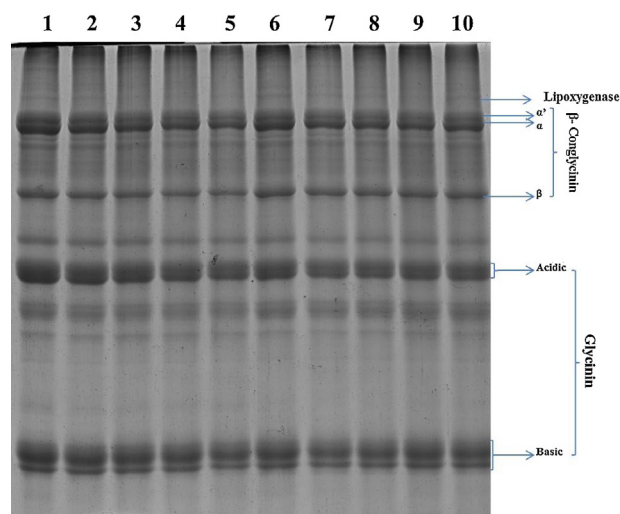

Fig. 1 – Changes in the density of storage protein fractions of native (untoasted) and toasted soy flour at varying doses of EB. Lanes 1–5 and Lanes 6–10 correspond to native (untoasted) and toasted soy flour irradiated with 0, 4.8, 9.2, 15.3 and 21.2 kGy, respectively.

3.7. Lipoxygenase protein

Fig. 1 also depicts the changes in the density of lipoxygenase protein. Numerical values for lipoxygenase protein as determined by relative densitometry are given in Table 1. In both toasted and untoasted soy flour, EB-irradiation at all the doses reduced significantly ($P < 0.05$) the density of lipoxygenase protein. Decline in the level of lipoxygenase protein due to EB-irradiation may be attributed to the oxidative disruption of the secondary/tertiary structure of lipoxygenase by the free radicals generated by EB-irradiation.

4. Conclusions

According to Food and Agriculture Organization/International Atomic Energy Agency/World Health Organization, the low doses of radiation may be used for disinfesting and storage of food and raw material with no toxicological hazards. In the present study, toasted and untoasted soy flour, commonly used ingredients in bakery and bread industry, was EB-irradiated to assess the changes in their physico-chemical properties. The results showed that EB radiation at specific doses enhanced the nutritional value of toasted and untoasted soy flour by reducing ANF and improving IVPD. Decline in isoflavones and increase in glycinin to β-conglycinin ratio suggest that EB irradiation may improve the quality of soy products processed from both the variants of soy flour. Though, physical properties like water absorption capacity, protein solubility, and protein dispersibility may be affected negatively. These results need to be validated at commercial scale to evaluate the viability of use of irradiation at large volume of products.

Acknowledgment

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