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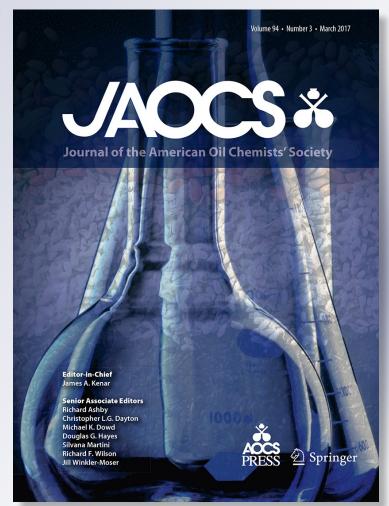
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Lipoxygenase and Tocopherol Profiling of Soybean Genotypes Exposed to Electron Beam Irradiation

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Abstract Electron beam (EB)-irradiation is increasingly being preferred to radioactive-based gamma irradiation in overcoming the constraints that affect the quality of food material. Soybean seeds of 3 soybean genotypes were exposed to 4 doses viz. 4.8, 9.2, 15.3 and 21.2 kGy of EB-irradiation and assessed for the changes in the contents of lipoxygenase isozymes and tocopherol isomers. Densitometry of protein profile revealed decreasing intensity of lipoxygenase with increasing EB dose. All the 3 lipoxygenase isozymes viz. lipoxygenase-1, -2 and -3 registered significant ($P < 0.05$) increasing reduction with increasing dose; though genotypic variation was noted for the magnitude of reduction at the same dose. Concomitantly, all the 3 genotypes exhibited significant ($P < 0.05$) decline in α-, γ- and δ-isomers of tocopherol. δ-Tocopherol was the most sensitive to EB-irradiation. EB dose, which caused minimum and maximum decline in total tocopherol content, was genotype-dependent. Decline in vitamin E activity corresponding to the dose, which induced maximum reduction for total lipoxygenase also varied in 3 genotypes. The study showed the usefulness of EB for significant inactivation of off-flavor generating lipoxygenases in soybean, with a non-significant effect on oil content and varied retention of tocopherol isomers and vitamin E activity depending upon genotype.

Keywords Electron beam · Soybean lipoxygenases · Tocopherols · Vitamin E

Introduction

Soybean is a major oilseed with 260 million tonnes of global production. Besides containing a 20% oil fraction packed with essential fatty acids and tocopherol isomers, soybean is one of the most economical sources of good quality protein for human consumption. However, consumption of soy-food products is constrained by the off-flavor generated during the processing of soy products. The underlying reason of this off-flavor is the release of aldehyde and ketone compounds during the catalytic oxidation of pentadiene-containing polyunsaturated fatty acids viz. linoleic and α-linolenic acid by soybean seed lipoxygenase. This enzyme exists in 3 isozymic forms viz. lipoxygenase-1, lipoxygenase-2 and lipoxygenase-3 and constitutes about 1–2% of total soybean seed protein. Heat inactivation method conventionally employed to denature lipoxygenases affects the solubility and functionality of proteins. Alternatively, gamma rays irradiation employed to disinfest grains, food and feed material of several crops including soybean at low doses [1, 2], has been reported to reduce undesirable components like trypsin inhibitor, lipoxygenases, oligosaccharides etc. in soybean [3, 4]. Lately, EB-irradiation is preferred to radioactive material (^{60}Co and ^{137}Cs) based gamma irradiation. However, compared to gamma rays, EB has a lower penetrating capacity and requires a high dose rate. Studies pertaining to the use of EB-irradiation in reducing soybean seed

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lipoxygenase are limited in the literature [5, 6]; moreover, these studies did not focus on individual isozymes. The present investigation aimed at monitoring the changes in each of the 3 isozymes of lipoxygenase in seed samples exposed to varying dose of EB. Soybean being an oilseed crop, its economic value in international market depends upon oil content, fatty acid composition, tocopherol isomers and vitamin E activity. Tocopherol exists in 4 different forms viz. α, β, γ and δ. These isomers have a special niche in nutraceutical market due to their role in reducing the risk of onset of atherosclerosis, cancer, Alzheimer, and Parkinson [7]. EB-irradiation has been reported to cause a decrease in vitamin E activity of soybean [8]; however, this study did not assess changes in concentration of 4 tocopherol isomers, which is important as not only the contribution of each isomer to total vitamin E activity varies, but the sensitivity of each isomer to irradiation may also vary. To the best of our knowledge, studies pertaining to the changes in tocopherol isomers in soybean seeds submitted to EB-irradiation are not available, though a decrease in tocopherol isomers on exposure to EB irradiation has been reported in other crops viz. pecan kernels [9] and Portuguese chestnut [10]. In the present investigation, soybean seeds of 3 genotypes viz. IC210, NRC107 and JS95-60 were exposed to varying doses (4.8, 9.2, 15.3 and 21.2 kGy) of EB, and the changes in the content of 3 isozymic forms of lipoxygenase and tocopherol isomers and vitamin E activity were studied. As the oil content and fatty acid composition of soybean are equally important from the commercial perspective, these parameters were also monitored in the soybean seeds submitted to EB-irradiation.

Materials and Methods

Material

Seeds of 3 soybean genotypes viz. IC210, NRC107 and JS95-60 were from the crop raised in the fields of the Indian Council of Agricultural Research-Indian Institute of Soybean Research. The packages with a size $17 \times 12 \times 1.8$ cm, containing the soybean seeds were exposed to EB-irradiation. No additional gas was used in the package.

Irradiation Set-up

Electron accelerator (10 MeV) LINAC (Linear Accelerator, Raja Ramanna Centre for Advanced Technology, Indore, India) was used for irradiating the soybean seeds at ambient temperature which was 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 soybean seeds were exposed to EB radiation for 3, 6, 10 and 13 passes, which resulted in absorbed doses of 4.8, 9.2, 15.3 and 21.2 kGy, respectively. The dose delivered per pass was 1.57 kGy. Alanine pellets were used as a dosimeter and absorbed doses were measured using e-scan EPR (Electron paramagnetic resonance, Bruker BioSpin, Silberstreifen, Germany) spectrometer system. The overall uncertainty in dose measurement was less than 3%. Calibration of the dosimetry system is traceable to the National Physical Laboratory, UK.

SDS-PAGE Analysis for Lipoxygenase Concentration

Soybean seeds were ground and passed through a 30 mesh screen. A known amount (50 mg) of finely ground soy flour was suspended in 1 ml of extraction buffer containing 125 mM Tris–Cl buffer (pH 6.8), 0.2 M SDS and 1 M 2-mercaptoethanol and kept in a boiling water-bath for 10 min, followed by centrifugation. Extracted protein was subjected to SDS-PAGE consisting of 5% stacking and 12% resolving gel. Lipoxygenase standard (L7395, Mol. wt. 108 kDa) procured from Sigma-Aldrich; Bangalore, India was run in one lane to identify lipoxygenase protein. The gel was stained overnight with 0.1% Coomassie brilliant blue R-250 followed by destaining using methanol:water:acetic acid (45:45:10). The protein profile pattern was scanned and density of lipoxygenase was quantified through densitometer (Bio-Rad G900, Bio-Rad Laboratories, California, United States) using *Image Lab* software version 5.2.1 (Bio-Rad Laboratories, California, United States).

Quantitative Estimation of Lipoxygenase-1, Lipoxygenase-2 and Lipoxygenase-3 Isozyme Activity

Crude extract was prepared by homogenization of 0.5 g of the defatted soy flour in 50 ml of sodium phosphate buffer (0.2 M, pH 6.8) in a micro-tissue polytron homogenizer (PT2100, Luzern, Switzerland) at 20,000 rpm for 20 min at 0–4 °C. The homogenized solution was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant so obtained was used as the crude extract for the enzymatic assay following Axelrod *et al.* [11]. Lipoxygenase-1 was assayed under alkaline conditions with 2.8 ml sodium borate buffer (0.2 M, pH 9.0), suitable volumes of crude extract and 10 mM sodium linoleate. Reaction mixture for lipoxygenase-2 consisted of 2.8 ml sodium phosphate buffer (0.2 M, pH 6.1), suitable volumes of crude extract and 10 mM sodium arachidonate; while assay conditions for estimating lipoxygenase-3 consisted of 2.8 ml sodium phosphate buffer (0.2 M, pH 6.5), suitable volumes of

Table 1 Relative concentration of lipoxygenase protein in irradiated seeds compared to non-irradiated seeds through SDS-PAGE analysis

Dose (kGy)	Genotype		
	IC210	NRC107	JS95-60
Control (Non-irradiated)	1.00[a]	1.00[a]	1.00[a]
4.8	0.67 ± 0.02[b]	0.85 ± 0.03[b]	0.85 ± 0.03[b]
9.2	0.57 ± 0.03[c]	0.73 ± 0.02[c]	0.70 ± 0.01[c]
15.3	0.36 ± 0.04[d]	0.60 ± 0.01[d]	0.62 ± 0.01[d]
21.2	0.22 ± 0.04[e]	0.30 ± 0.04[e]	0.57 ± 0.02[e]

Values given are means of triplicate samples ± standard deviation; values with different superscript letters in the same column are significantly ($P < 0.05$) different

The SDS-PAGE profile is given in Supplementary Fig. 1

crude extract and 10 mM sodium linoleate. The reaction was initiated by the addition of sodium arachidonate/linoleate. Changes in absorbance were recorded every 15 s at 234, 238 and 280 nm for lipoxygenase-1, -2 and -3, respectively. One unit of enzyme was taken as equivalent to the amount of enzyme that generated an increase in absorbance of 1.0 min^{-1}.

Oil Content and Fatty Acid Composition

Oil from finely ground flour (30 mesh) was extracted with 180 ml hexane in an automated Soxhlet unit (Pelican Equipments, Chennai, India) for 3 h. Percentage oil content was determined by weight differences. For fatty acid analysis, soybean oil was extracted by incubating the vial containing the mixture of soy flour and petroleum ether (boiling point 30–50 °C) at 40 °C. Fatty acid methyl esters prepared using 1 N sodium methoxide were estimated through Gas Chromatography using a Shimadzu GC17A instrument (Kyoto, Japan), fitted with a capillary column (SGEBPX70, 30 m × 0.32 mm × 0.25 µm). For distinct resolution of fatty acid methyl esters, oven temperature was programmed at 140 °C for 3.6 min, then increased to 170 °C at a rate of 13.5 °C min^{-1} and maintained for 3.8 min and finally increased to 182 °C at a rate of 5 °C min^{-1}. The flame ionization detector (FID) and injector were maintained at 240 °C. Nitrogen was used as the carrier gas. Peaks obtained for fatty acid methyl esters were identified by comparing the retention times with those of standard fatty acid methyl esters (Sigma-Aldrich, Bangalore, India).

Extraction and Determination of Tocopherols Using HPLC

Oil from the finely ground soy flour (30 mesh size) was extracted by soaking the flour in HPLC-grade petroleum

ether for 8 h at 40 °C. The mixture was transferred into vials and the solvent was evaporated under vacuum at 30 °C. The weight of the oil was determined gravimetrically in each vial and the samples were re-dissolved in a fixed volume of HPLC grade *n*-hexane. Tocopherol composition was determined using a Shimadzu HPLC system (Kyoto, Japan) equipped with a UV detector and a silica–NH$_2$ column (Inert Sustain 5 µm; 4.6 × 250 mm C/N 5020-16628). Twenty microliters of a syringe-filtered sample were injected into the column and eluted isocratically with HPLC-grade *n*-hexane and ethyl acetate (70:30 v/v) at a flow rate of 0.5 ml/min Tocopherols were detected with UV detector (SPD 10 AT *vp*) at a wavelength of 295 nm. The resolution of the tocopherol isomers and the relative amounts of tocopherols were calculated by comparing their peak areas with a standard curve generated using different amounts of external standards of α-, β-, γ- and δ-tocopherol (Sigma-Aldrich, India). Tocopherols were expressed as µg/g oil basis and total tocopherols content was computed by summing up the values of all the four isomers.

Computation of Vitamin E Activity

The vitamin E activity of soybean oil from different genotypes was taken as the sum of multiplication of α-, β-, γ- and δ-tocopherol content by 1.0, 0.5, 0.1, and 0.03, respectively, as previously reported [12]. Taken into consideration the guidelines of United States Pharmacopeia, according to which 1 mg of α-tocopherol is equivalent to 1.49 International Unit of vitamin E, the value obtained as µg/g of oil was multiplied by 0.149 for conversion into International Units per 100 g of oil.

Statistical Analyses

All the statistical analyses were carried out using SAS version 9.3 (SAS Institute of India Pvt Ltd, Mumbai, India).

Results and Discussion

Changes in Lipoxygenase Isozymes

Total lipoxygenase protein in the irradiated seeds compared to non-irradiated seeds was quantified through densitometry (Online Resource 1). Concentration of lipoxygenase protein declined significantly ($P < 0.05$) with increasing dose of EB in all the 3 genotypes as evident from the SDS-PAGE analysis (Table 1). These results were corroborated by quantitative determination of each of 3 lipoxygenase isozymes through standard assay conditions. Table 2 details the content of lipoxygenase-1, -2 and -3 in the EB

Table 2 Lipoxygenase isozymes' content (units/g soy flour) in EB-irradiated and non-irradiated seeds of soybean genotypes

Genotype	Irradiation dose kGy	Lipoxygenase-1	Lipoxygenase-2	Lipoxygenase-3	Total Lipoxygenase
IC210	Control (non-irradiated)	4061 ± 125^a	675 ± 29^a	416 ± 13^b	5152 ± 175^a
	4.8	3335 ± 95^b (17.9)	611 ± 22^b (9.5)	258 ± 8.4^d (38.0)	4204 ± 118^b (18.4)
	9.2	3257 ± 120^c (19.8)	604 ± 28^b (10.5)	263 ± 7.2^{ed} (36.8)	4124 ± 105^c (20.0)
	15.3	2993 ± 66^d (26.3)	464 ± 18^e (31.3)	218 ± 5.2^f (47.6)	3675 ± 76^e (28.7)
	21.2	2356 ± 49^e (42.0)	409 ± 15^g (39.4)	213 ± 4.7^f (48.8)	2978 ± 85^f (42.2)
NRC107	Control	1638 ± 50^h	543 ± 18^c	430 ± 16^a	2611 ± 55^h
	4.8	1335 ± 24^i (21.5)	498 ± 12^d (8.3)	443 ± 16^a (-3.0)	2276 ± 63^j (12.8)
	9.2	1341 ± 33^i (21.2)	305 ± 7.6^f (43.8)	213 ± 9.8^f (50.5)	1859 ± 40^k (28.8)
	15.3	1034 ± 35^j (39.9)	287 ± 8.8^k (47.1)	164 ± 5.5^h (61.9)	1485 ± 45^l (43.1)
	21.2	803.5 ± 22^k (54.0)	234 ± 10^l (56.9)	290 ± 12^d (32.6)	1327 ± 40^m (49.2)
JS95-60	Control	3256 ± 77^c	490 ± 15^{de}	328 ± 11^c	4074 ± 114^c
	4.8	3221 ± 79^c (1.1)	474 ± 19^e (3.3)	259 ± 8.4^d (21.0)	3954 ± 127^d (2.9)
	9.2	2070 ± 65^f (36.4)	458 ± 20^f (6.5)	274 ± 9.2^e (16.5)	2802 ± 7^g (31.2)
	15.3	1922 ± 47^g (41.0)	395 ± 12^h (19.4)	270 ± 7.6^{ef} (17.7)	2587 ± 88^h (36.5)
	21.2	1925 ± 55^g (40.9)	356 ± 15^i (27.3)	237 ± 9.4^g (27.7)	2518 ± 90^i (38.2)

One unit is expressed as the change in absorbance $1.0 \, \mathrm{min}^{-1} \, \mathrm{g}^{-1}$ of flour

Values given are means of triplicate samples $\pm$ standard deviations. Values given in parenthesis indicate the reduction compared to the control (non-irradiated seeds). Values with different superscript letters in the same column are significantly ($P < 0.05$) different

irradiated and non-irradiated seeds of 3 soybean genotypes. Among 3 isozymic forms of lipoxygenase, lipoxygenase-1 was at the highest concentration and constituted about 80% of total lipoxygenase content. In general, all 3 genotypes exhibited an inverse relationship between the EB dose and the content of the 3 lipoxygenase isozymes. Significant ($P < 0.05$) genotypic variation was noted for the magnitude of reduction at different doses. For lipoxygenase-1, at the lowest dose of 4.8 kGy, IC210 and NRC107 showed 17.9 and 21.5% reduction, respectively. Maximum reduction in this isozyme was induced by 21.2 kGy dose and was found to be 42.0, 54.0 and 40.9% in IC210, NRC107 and JS95-60, respectively. Though, the reduction induced by 15.3 and 21.2 kGy dose in JS95-60 was not significantly ($P < 0.05$) different. With regard to lipoxygenase-2, which is the principal contributor to off-flavor, significant ($P < 0.05$) reduction in this isozyme also was noted at 4.8 kGy (the lowest dose) in 2 genotypes viz. IC210 and NRC107; but the reduction for this isozyme in IC210 and NRC107 was 9.5 and 8.3% which was far lower compared to the 17.9 and 21.5% observed for lipoxygenase-1, respectively. JS95-60 did not register any significant ($P < 0.05$) change for both lipoxygenase-1 and lipoxygenase-2 at this low dose. Similar to lipoxygenase-1, maximum reduction for lipoxygenase-2 was observed at the highest dose of 21.2 kGy in all the 3 genotypes, though the significant ($P < 0.05$) genotypic variation was observed for magnitude of reduction. For lipoxygenase-3, the lowest dose of 4.8 kGy caused 38 and 21% reduction in IC210 and JS95-60, respectively, and non-significant ($P < 0.05$) change

in NRC107. Unlike lipoxygenase-1 and lipoxygenase-2, varying EB doses caused a maximum reduction for lipoxygenase-3 in the 3 genotypes. IC210 registered a maximum reduction for this isozyme at 21.2 kGy (48.8%) while NRC107 (61.9%) and JS95-60 (27.7%) at 15.3 and 21.2 kGy, respectively. We compared these results with the available studies wherein EB-irradiation had been reported to induce changes in lipoxygenase activity in soybean seeds. Decline in lipoxygenase activity of 38.2–49.2% in 3 genotypes due to 21.2 kGy of EB dose in the present study is commensurate with the results in Wang *et al.* [6] who irradiated soybean seeds using EB doses of 0,1,5,7 and 10 kGy and reported a decrease in lipoxygenase activity with an increase in dose. However, the authors did not investigate the effects of EB-irradiation on the individual isozymes of soybean seed lipoxygenase. Our results are also in agreement with the study of Barros *et al.* [13], who reported gamma irradiation-induced increasing reduction in lipoxygenase activity with increasing dose from 2 to 10 kGy. The authors reported 39.47 and 45.17% reduction in 2 Brazilian cultivars, namely BRS-258 and EMB48, respectively, at minimum dose of 2.5 kGy, which is higher than the magnitude of reduction observed in IC210 (18.4%), NRC107 (12.8%), and JS95-60 (2.9%) at EB dose of 4.8 kGy in the present study. Decline in the level of lipoxygenase activity due to EB-irradiation may be due to the oxidative disruption of secondary/tertiary structure of lipoxygenase isozymes by the free radicals generated by EB-irradiation as suggested in earlier study with gamma irradiation on protein [14].

Table 3 Tocopherol isomer content in EB-irradiated and non-irradiated seeds of soybean genotypes

Genotype	Irradiation dose (kGy)	Tocopherols (µg/g of oil)					Vitamin E IU/100 g oil
		α	β	γ	δ	Total	
IC210	Control (non-irradiated)	232.3 ± 20.2^d	33.0 ± 2.8^e	895.2 ± 82.1^a	213.8 ± 18.7^b	1374.3 ± 112.2^a	51.36 ± 4.51^d
	4.8	196.4 ± 15.2^e (15.4)	28.2 ± 2.2^f (14.5)	584.2 ± 51.2^c (34.7)	122.8 ± 10.5^f (42.6)	996.3 ± 83.8^e (27.5)	40.62 ± 5.3^e (20.9)
	9.2	178.6 ± 16.6^e (23.1)	30.6 ± 2.6^e (7.3)	650.4 ± 63.1^b (27.3)	147.9 ± 12.2^e (30.8)	1065.3 ± 98.6^d (22.5)	39.24 ± 3.4^f (23.5)
	15.3	186.1 ± 16.2^e (19.9)	29.5 ± 2.5^{ef} (10.6)	593.3 ± 50.3^c (33.7)	144.8 ± 14.1^e (32.3)	1017.9 ± 96.3^d (25.9)	39.49 ± 4.1^f (23.2)
	21.2	109.9 ± 8.6^g (52.7)	25.7 ± 2.1^f (22.0)	360.9 ± 33.3^g (59.7)	79.9 ± 6.7^h (62.6)	710.8 ± 61.4^g (48.3)	24.02 ± 2.8^h (53.2)
NRC107	Control	424.1 ± 40.2^a	65.9 ± 4.9^a	596.2 ± 54.4^c	248.2 ± 16.3^a	1334.4 ± 120.6^{ab}	78.09 ± 6.9^a
	4.8	422.1 ± 40.3^a (0.5)	60.1 ± 5.3^b (8.8)	540.5 ± 51.2^d (9.3)	225.7 ± 18.7^b (9.1)	1267.4 ± 112.5^b (5.1)	76.43 ± 5.6^a (2.1)
	9.2	350.3 ± 31.2^b (17.4)	$60.0. \pm 5.8^b$ (9.0)	516.4 ± 47.3^d (13.4)	219.4 ± 20.1^b (11.6)	1185.8 ± 109.4^c (11.1)	65.33 ± 5.7^b (16.3)
	15.3	368.0 ± 35.2^b (13.2)	58.8 ± 5.2^b (10.8)	437.0 ± 40.1^f (26.7)	174.4 ± 15.3^d (29.7)	1088.9 ± 98.7^d (18.4)	66.50 ± 5.9^b (14.8)
	21.2	326.6 ± 30.1^c (23.0)	52.0 ± 4.9^c (21.0)	475.1 ± 43.3^e (20.3)	200.3 ± 19.2^c (19.3)	1118.4 ± 93.2^{cd} (16.2)	60.51 ± 4.6^c (22.5)
JS95-60	Control	217.9 ± 20.3^d	54.6 ± 5.1^{bc}	591.3 ± 55.3^c	166.4 ± 14.3^d	1030.1 ± 87.6^d	46.08 ± 3.8^e
	4.8	215.6 ± 20.5^d (1.07)	52.7 ± 5.0^c (3.4)	551.9 ± 51.2^{cd} (6.7)	128.8 ± 12.1^{fg} (22.6)	960.12 ± 82.1^e (6.79)	44.85 ± 3.1^e (2.6)
	9.2	147.3 ± 12.6^f (32.4)	51.9 ± 4.7^c (4.9)	463.1 ± 43.2^e (21.7)	140.3 ± 12.5^e (15.6)	861.5 ± 76.4^f (16.4)	33.34 ± 2.7^g (27.6)
	15.3	208.2 ± 18.3^e (4.5)	53.6 ± 4.8^c (1.7)	565.5 ± 51.1^{cd} (4.4)	136.7 ± 10.9^{ef} (17.9)	974.5 ± 79.4^e (5.4)	44.64 ± 4.6^e (4.4)
	21.2	178.3 ± 15.4^e (18.2)	43.1 ± 3.9^d (20.9)	482.7 ± 43.1^e (18.4)	116.6 ± 10.1^g (29.9)	878.2 ± 69.5^f (14.7)	40.70 ± 3.7^f (11.6)

Values given are means of triplicate samples $\pm$ standard deviation. Values given in parenthesis indicate the decline compared to the control (non-irradiated seeds). Values with different superscript letters in the same column are significantly ($P < 0.05$) different

Oil and Fatty Acid Composition

Oil content for all the 3 genotypes remained unaffected by exposure to EB irradiation at different dose levels (Online Resource 2). These results are in contrast to the study of Barros *et al.* [13] who reported gamma-irradiated increase in lipid content of two Brazilian cultivars (namely 213,48) at 2.5, 5 and 10 kGy and decrease at 2.5 kGy in one cultivar. Changes in fatty acid composition because of exposure to EB-irradiation were also monitored (data not given). Palmitic acid declined significantly ($P < 0.05$) at all the 4 doses in IC210, NRC107 and JS95-60. Oleic and linoleic acid content either increased or remained unchanged in NRC107 and JS95-60. α-Linolenic acid also remained either unchanged or increased slightly in 3 genotypes on exposure to EB. Our observation for fatty acid composition is in agreement with the study of Minami *et al.* [15] who reported a non-significant effect of gamma irradiation up-to 10 kGy on the fatty acid composition of soybean oil.

In IC210, which is a specialty soybean variety with high oleic acid content compared to the regular soybean, the lowest dose of 4.8 kGy caused a slight increase of oleic acid content with a small decline in linoleic acid content, though; higher doses caused a significant increase in linoleic acid content at the expense of oleic acid for this genotype.

Changes in Tocopherol Isomers

Tocopherol isomers i.e., α, β, γ and δ present in soybean seeds are known to scavenge free radicals in biological systems in the order α > β > γ > δ due to the variation in the number and position of the methyl groups on the chromanol ring [7]. The highest biological activity for α tocopherol is attributed to 3 methyl groups at all the positions in the chromanol ring, while the lowest vitamin E activity of the δ-isomer is due to the presence of only one methyl group. Both β- and γ-isomers contain 2 methyl

groups but the former possessing methyl groups in *ortho* and *para* position has higher biological activity than the latter. Table 3 shows the contents of all the tocopherol isomers in the non-irradiated and EB-irradiated seeds of 3 soybean genotypes. In the non-irradiated seeds of 3 genotypes, γ-isomer was the most dominant form of tocopherols which is in agreement with earlier studies in soybean [16, 17].

Among the 4 isomers, the content of α-tocopherol remained unaffected ($P < 0.05$) in 2 genotypes, namely, NRC107 and JS95-60 at 4.8 kGy, and declined significantly ($P < 0.05$) in IC210. The maximum decline for this isomer in IC210 (52.7%) and NRC107 (23%) was noted at 21.2 kGy; while in JS95-60 (32.4%) at 9.2 KGy. β-Tocopherol, which was in the lowest concentration, was least affected by EB-irradiation in all the 3 genotypes. The highest dose (21.2 kGy) induced 22.0, 21.0 and 20.9% decline for the content of this isomer in IC210, NRC107 and JS95-60, respectively. With regard to the γ- and δ-isomers, in general, significant ($P < 0.05$) decline was observed for all the 3 genotypes at all the 4 doses. IC210 was the most sensitive for these 2 isomers, as evident from higher decline observed for these isomers in this genotype than NRC107 and JS95-60 at all the 4 doses. Genotypic variation observed for the sensitivity of tocopherols in response to EB-irradiation may be because of the differential level of carotenoids especially lutein, the major carotenoid component quenching the free radicals in non-green soybean seeds; however, the genotypes were not investigated for this carotenoid in the study. In 3 genotypes, minimum and maximum decline for both γ- and δ-isomers was observed at different doses. For the δ-isomer, maximum reduction was observed in IC210, which was about 2 fold higher than for NRC107 and JS95-60. Moreover, among the 4 tocopherol isomers, the δ-isomer was the most sensitive to EB-irradiation. This is in agreement with Lalas *et al.* [18] who also reported the δ-isomer to be the most sensitive when extracted soybean oil was exposed to gamma irradiation.

Total tocopherol content computed by summing up the content for all the isomers in IC210, NRC107 and JS95-60 was 1374.3, 1334.4 and 1030.1 μg/g of oil, respectively. These values were in the range of total tocopherol content reported for Indian soybean genotypes in our earlier study [16]. The results also showed that IC210, in general, was the most sensitive with regard to decline in tocopherols content as exhibited by higher reduction in this genotype than the other 2 genotypes. The results were compared with the studies wherein EB has been used a source of irradiation. Villareal-Lozoya *et al.* [9] reported a decrease in γ-tocopherol content in pecan kernels treated with EB-irradiation and stored under accelerated conditions (40 °C, 55–60% relative humidity) for 134 days. The

authors reported about 20% decline in γ tocopherol and slight decline in α- and β-isomers at 3 kGy dose of EB; while the minimum dose (4.8 kGy) in our study caused 34.7, 9.3 and 6.7% decline in γ-tocopherol in genotypes IC210, NRC107 and JS95-60, respectively. In agreement with the results of Villareal-Lozoya *et al.* [9], EB dose of 4.8 kGy induced only minor decline for the α-isomer in 2 genotypes viz. NRC107 and JS95-60 and for the β-isomer in JS95-60. However, the decline observed for the α-isomer (15.45%) in IC210 at 4.8 kGy was much higher than reported by Villareal-Lozoya *et al.* [9]. Carocho *et al.* [10] reported non-significant changes in content of the α-, γ-, and δ-isomers in Chestnut due to low EB-irradiation doses of 0.5, 1.0, 3.0 and 6 kGy, which is similar to the observation for the α-isomer in 2 genotypes NRC107 and JS95-60 and for the β-isomer in JS95-60 at the lowest dose (4.8 kGy) in the present study. Significant ($P < 0.05$) decline in total tocopherol content on exposure to EB-irradiation is consistent with gamma irradiation induced decline in other oilseed crops viz, peanut [19], sunflower and maize [20].

Vitamin E activity in IC210, NRC107 and JS95-60 was 51.36, 78.09, 46.08 IU/100 g of oil, respectively (Table 3). With regard to changes in vitamin E activity, 4.8 kGy dose did not cause significant ($P < 0.05$) decline in NRC107 and JS9560, which can be attributed to the negligible changes in α-, β-, and γ-tocopherol content. Taipina *et al.* [21] also reported non-significant change in the vitamin E content of Brazilian pecan nut exposed to low gamma irradiation doses of 1 and 3 kGy. For IC210, the lowest dose (4.8 kGy) caused about 21% loss in vitamin E activity, which is due to 15.4, 14.5, 34.7 and 42.6% decline in the content of α-, β-, γ- and δ-isomers, respectively. In JS95-60, even a high dose of 15 kGy did not cause any significant decline in vitamin E activity, due to non-significant ($P < 0.05$) losses in content for tocopherol isomers except for the δ isomer. The maximum decline for vitamin E in IC210 and NRC107 was 53.2 and 22.5%, respectively, at 21.2 kGy, and 27.6% in JS95-60 at 9.2 kGy. The decline in vitamin E activity in soybean seeds due to EB-irradiation in the present study is in agreement with Wilson *et al.* [8] who reported an approximate 30% decline in vitamin E activity in soybean seeds exposed to EB doses of 5 and 10 kGy.

Conclusions

Our results showed that EB-irradiation induced a significant ($P < 0.05$) reduction in the content of all the 3 isozymic forms of lipoxygenases. In general, maximum reduction in content for all lipoxygenase isozymes was noted at the highest dose of 21.8 kGy. Further, the results demonstrated that EB-irradiation induced reductions in total lipoxygenases, concomitantly, resulted in the loss of

vitamin E activity due to decline in tocopherol isomers. The decline in tocopherol content may be due to their utilization in quenching the free radicals generated in seeds submitted to EB-irradiation. Moreover, of the 4 tocopherol isomers, in general, the δ-isomer, which possesses minimum biological activity, was the most sensitive to EB-irradiation in all the 3 genotypes. Further, genotypic differences were noted for the loss in vitamin E activity at the dose (21.2 kGy), which caused a maximum reduction in total lipoxygenase. In genotype IC210, the maximum reduction of lipoxygenase (42.2%) at 21.2 kGy corresponded to a 53.2% loss of vitamin E activity. In NRC107, 49.2% reduction in lipoxygenase was noted at the expense of a 22.5% loss in vitamin E activity, while JS95-60 incurred only a 11.6% loss of vitamin E for a maximum reduction of 38.2% for total lipoxygenase.

In brief, a drastic reduction in off-flavor generating lipoxygenases in soybean seeds on exposure to EB-irradiation observed in the present investigation would have important implications in soy food processing industries, wherein pre-treatment with EB at specific doses can deliver soy products with reduced off-flavor. The study demonstrated that an expected loss of vitamin E activity due to a decline in tocopherol isomers in soybean seeds submitted to EB-irradiation can be minimized by the right selection of genotypes.

Compliance with Ethical Standards

Conflict of interest Authors declare that they have no conflict of interest.

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