Gamma radiation and magnetic field mediated delay in effect of accelerated ageing of soybean

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Abstract Soybean seeds were exposed to gamma radiation (0.5, 1, 3 and 5 kGy), static magnetic field (50, 100 and 200 mT) and a combination of gamma radiation and magnetic energy (0.5 kGy+200 mT and 5 kGy+50 mT) and stored at room temperature for six months. These seeds were later subjected to accelerated ageing treatment at 42 °C temperature and 95–100 % relative humidity and were compared for various physical and biochemical characteristics between the untreated and the energized treatments. Energy treatment protected the quality of stored seeds in terms of its protein and oil content. Accelerated aging conditions, however, affected the oil and protein quantity and quality of seed negatively. Antioxidant enzymes exhibited a decline in their activity during aging while the LOX activity, which reflects the rate of lipid peroxidation, in general, increased during the aging. Gamma irradiated (3 and 5 kGy) and magnetic field treated seeds (100 and 200 mT) maintained a higher catalase and ascorbate peroxidase activity which may help in efficient scavenging of deleterious free radical produced during the aging. Aging caused peroxidative changes to lipids, which could be contributed to the loss of oil quality. Among the electromagnetic energy treatments, a dose of 1–5 kGy of gamma and 100 mT, 200 mT magnetic field effectively slowed the rate of biochemical degradation and loss of cellular integrity in seeds stored under conditions of accelerated aging and thus, protected the

deterioration of seed quality. Energy combination treatments did not yield any additional protection advantage.

Keywords Soybean · Gamma irradiation · Magnetic energy · Accelerated ageing

Introduction

Soybean is an excellent source of protein for either direct human consumption or indirect consumption through food processing or livestock production. It is also a rich source of edible oil. Soybean is structurally weak, inherently short lived and easily subjected to damage (Delouche and Baskin 1973). Under storage, soybean seeds lose their quality faster than any other major crop seed except for shelled peanuts (Delouche 1982). Maintenance of the quality of seeds during post-harvest storage is a great challenge in soybean and many other agricultural crops. It depends greatly on the condition of storage. High temperatures, high relative humidity is considered by many researchers as the most important abiotic factors associated with seed deterioration in soybean. When these factors act in concert their effect can be more severe. The performance capability of seeds in general and soybean in particular decline during storage. This ageing or loss of vigor is evidenced by delayed germination and emergence, slower growth, increased susceptibility to environmental stresses, and ultimately, a decline in germinability. Seed ageing is therefore a serious problem in agriculture, and is receiving increased research interest. Ageing process can get enhanced by certain treatment or environmental condition and is called accelerated aging. Likhatchev et al. (1984) suggested that physiological changes in seeds subjected to accelerated ageing were largely similar to those during natural ageing with the main difference being the rate at which they occur. The rate of deterioration depends on

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either seed moisture content or temperature of storage (Kapoor et al. 2010).

A large number of reactive oxygen species are generated in the seed during ageing which cause lipid peroxidation (Ahuja et al. 2014). A number of different events or processes have been suggested as causal mechanisms, including chromosomal damage, loss of various enzymes, degradation of the respiratory system, loss of ATP production/storage capacity, and deterioration of membranes. The rate at which seeds lose vigour during storage is affected by environmental factors such as temperature, moisture, and O_2/CO_2 concentrations (Villiers 1973). Harrington (1972) has suggested that within the normal range of moisture and temperature for stored seed, each 1 % reduction in seed moisture or each 5 °C reduction in temperature doubles the storage life of the seed. High temperature, high humidity storage provides a convenient method for accelerated aging of seed. Those age-correlated physiological changes which appear earliest would seem most likely to have a causative relation to ageing.

Electromagnetic energies have been used for varied applications in agriculture. Radiation processing by gamma radiation at dose of 1 kGy has been recommended for quarantine treatment of legumes including soybeans (Variyar et al. 2004), whereas exposure to higher doses (up to 5 kGy) resulted in reduction in cooking time and improvement in texture without production of off-flavor (Wilkinson and Gould 1996). There are some reports which indicate that low doses of γ -irradiation could induce antioxidant activities in plant material including soybean (Variyar et al. 2004). Electric or magnetic treatments are assumed to enhance vigor of fresh and stored seed by influencing the biochemical processes that involve free radical quenching or by stimulating the activity of related proteins. Magnetic field treatment for improving preservation of fluid food is already a potential technology (Qin et al. 1994). However, whether or not these electromagnetic energies are capable of yielding any positive influence on the quality or protection against accelerated aging induced physical and physiological deterioration of seed is not known.

The aim of our study was to investigate the effect of γ -irradiation (0.5–5 kGy) and static magnetic field either alone or in combination on lipid and protein quantity and quality, free radical scavenging ability lipoxigenase activity under accelerated aging of soybean seeds.

Material and methods

Soybean (*Glycine max* [L.] var. SL 595) seeds (6–8 % moisture) were obtained from the Division of Genetics and Plant Breeding, IARI New Delhi. Seeds were exposed to gamma irradiation (kGy) and magnetic energy individually and in selected combinations viz. 0.5, 1, 3 and 5; 50, 100, 200 mT; 0.5 kGy+200 mT and 5 kGy+50 mT. Untreated seeds were

considered as control. Two separate experiments were conducted to ascertain the effect of gamma radiation and static magnetic field, both alone or in combination, on post-harvest storage quality of soybean seeds (a) after 6 months at room temperature (25–35 °C) and (b) at 10 and 20 days of accelerated ageing (42 °C, 95–100 % RH). Accelerated aging was obtained by modification of the method proposed by Parrish and Leopold (1978) where seeds were placed in a single layer muslin cloth bag and placed in a covered water bath maintained at 42 ± 2 °C temperature and 95–100 % relative humidity. Water was taken up hygroscopically from the humid atmosphere within the water bath, but no free water came in contact with the seeds. After accelerated aging for varying duration (0, 10 and 20 days), the seeds were removed and allowed to return to air dryness (6–8 % moisture) at room temperature before further investigations. All the observations were taken in 3 replications.

Physical parameters

Water activity (HYGROLAB Bench Top Humidity Temperature Indicator, V 2.0) and Seed coat hardness (texture analyser, Model: TA+Di, Stable Micro Systems, UK) were determined in the soybean seeds at 0, 10 and 20 days of accelerated ageing.

Biochemical parameter

Biochemical parameters like protein quantity (Lowry et al. 1951), total nitrogen (modified Kjeldahl method), total free amino acid (Misra et al. 1975), Methionine content (Thimmaiah 1999), Oil extraction (Soxlet method) Acid value (Thimmaiah 1999), Free fatty acids percent (Sarvet and Ercosun 2011), Saponification value (Horowitz 1975) Iodine value (Horowitz 1975) were estimated. In addition to this fatty acid composition, Antioxidant enzyme activities like Superoxide Dismutase (SOD) (Dhindsa et al. 1981), Catalase (Dhindsa et al. 1981), Ascorbate peroxidase (Nakano and Asada 1981), Free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Mellors and Tappel 1966), Lipoxigenase (LOX) assay (Axelrod et al. 1981) were measured in aged seed and compared with the control and 0 day samples for ascertaining the deterioration in the storage quality of soybean seeds.

Statistical analysis

Statistical analysis was done using one way ANOVA with two factors viz. duration of aging and radiation doses with 3 replication in experiment ($n=3$). Using SPSS 16.0 significant effects were noted. Further the means were compared using DMRT (Duncan's multiple range test) to assess the significance.

Results and discussion

Physical parameter

Water activity and seed coat hardness

Water activity of seeds stored for six months in general, was higher than that of the initial water activity at the start of the experiment. Gamma irradiated seeds maintained significantly lower water activity (Table 1) even after six months of storage as compared to the aged control. However, the radiation induced decline in the water activity was independent of the dose of exposure. Magnetic field yielded a lower seed water activity. However, the difference was significant only at higher dose of 100 and 200 mT. Energy combination treatment did not influence the seed water activity significantly when compared with untreated control. Similar pattern of variation was observed for seed coat hardness (Table 1) which in general declined significantly during the storage period. Seed coat hardness, measured and presented in terms of amount of force required to break the seed was highest for the radiated treatments particularly at dose above 0.5 kGy. Magnetic field alone was unable to prevent the softening of seed coat during storage. This was also apparent from the result of energy combination treatment where 0.5 kGy+200 mT revealed significantly lower seed coat hardness than 5 kGy+50 mT. Sustenance of seed coat hardness during storage was determined by the dose of gamma irradiation and was unrelated to static magnetic field. Under accelerated aging condition water activity of soybean seeds varied under different treatment. Seeds treated with higher doses of gamma and magnetic energy showed a slow rate of increase in the water activity. During the same period of observation, the combination of gamma and magnetic energy showed a better

performance than individual treatment in the early period of study. In general 5 kGy alone and in combination with 50 mT caused statistically significant lesser water activity over other treatments avoiding the day of treatment (Table 2). Gamma irradiation dose of 1 kGy and above and all the magnetic treatment alone having stronger seed coat than the lower gamma irradiated seed. Combination of higher gamma dose (5 kGy) and low magnetic field (50 mT) treatment showed strongest seed coat (Table 2). Results also indicated that with aging the seed coat become brittle. Seeds having a harder seed coat are more suited to withstand accelerated aging. In the present investigation a high dose of gamma radiation (5 kGy) alone or in combination with magnetic field helped seeds maintain a stronger seed coat than the control.

Biochemical parameters

Oil content and quality

In general, Oil content of the untreated seeds declined (Table 1) during storage. However, the variations were insignificant when compared to within and between the gamma radiation and static magnetic field treatments. Acid value of untreated seeds increased significantly (Table 1) during storage when compared to respective value of zero time control. High dose of gamma particularly 3 & 5 kGy resulted in lower acid value compared to other treatments. Free fatty acid (%) also behaved in a similar manner and was highest for the untreated aged seeds than the zero time control. Both magnetic and gamma irradiation treatment although measured lower contents of free fatty acid than the aged control. The differences were insignificant. These results clearly indicate that gamma radiation and magnetic field were unable to have any positive influence on the acid value or free fatty acid content

Table 1 Individual and combined effect of gamma radiation and static magnetic field on water activity (dS), seed coat hardness (kgF), oil (%), acid value (mg/g), free fatty acid (%), saponification value (mg KOH) and iodine value (g I₂/100 g oil) after six months of storage of soybean seeds at room temperature

Treatments	Water activity	Seed coat hardness	Oil (%)	Acid value	Free fatty acid	Saponification value	Iodine value
*Control	0.532	121.3	20.8	2.5	1.2	210.0	128.0
#Control	0.593	110.5	20.0	3.6	1.8	165.0	120.0
0.5 kGy	0.576	111.7	20.3	3.2	1.6	170.7	122.0
1 kGy	0.576	115.1	20.4	3.1	1.6	180.0	124.0
3 kGy	0.576	117.7	20.7	2.9	1.5	199.0	123.8
5 kGy	0.568	117.9	20.7	3.0	1.5	201.0	125.5
50 mT	0.581	117.0	20.1	3.3	1.7	179.5	123.0
100 mT	0.577	112.3	20.3	3.2	1.6	191.3	123.5
200 mT	0.576	112.9	20.4	3.1	1.6	197.0	124.6
0.5 kGy+200 mT	0.583	111.9	20.6	3.3	1.7	195.0	124.6
5 kGy+50 mT	0.583	119.3	20.7	3.3	1.6	203.0	125.0
CD (5 %)	0.016	0.38	1.11	0.48	0.56	23.04	4.85
SE (M)	±0.005	±0.12	±0.421	±0.169	±0.197	±8.14	±1.71

*Control: untreated sample at zero time of storage #Control: untreated sample at six months of storage

[§] kGy: kilogray; mT: milli tesla

Table 2 Individual and combined effect of gamma irradiation and static magnetic field on water activity (dS), Seed coat hardness (kgF), Oil content (%) of soybean seeds under accelerated aging

Treatments	Water activity			Mean	Seed coat hardness			Mean	Oil content			Mean
	Days in to Accelerated ageing				Days in to Accelerated ageing				Days in to Accelerated ageing			
	0	10	20		0	10	20		0	10	20	
Control	0.593	0.822	0.996	0.803 ^a	110.5	86.0	76.2	90.9 ^g	20.0	19.2	18.2	19.1 ^b
0.5 kGy	0.576	0.810	0.939	0.775 ^{ab}	111.7	91.0	81.1	94.6 ^f	20.3	19.6	18.7	19.5 ^{ab}
1 kGy	0.576	0.783	0.917	0.759 ^{bc}	115.1	94.7	84.9	98.2 ^d	20.4	19.8	19.1	19.8 ^{ab}
3 kGy	0.576	0.785	0.941	0.767 ^{bc}	117.7	97.0	87.0	100.6 ^b	20.7	20.1	19.4	20.1 ^a
5 kGy	0.568	0.774	0.912	0.751 ^{cd}	117.9	97.7	88.1	101.2 ^b	20.7	20.1	19.6	20.1 ^a
50 mT	0.581	0.796	0.923	0.767 ^{bc}	117.0	95.98	86.0	99.7 ^c	20.1	19.3	18.8	19.4 ^{ab}
100 mT	0.577	0.801	0.929	0.769 ^b	112.3	92.33	83.2	95.9 ^c	20.3	19.5	19.0	19.6 ^{ab}
200 mT	0.576	0.774	0.901	0.750 ^{cd}	112.9	96.67	87.9	99.2 ^c	20.4	19.8	19.2	19.8 ^{ab}
0.5 kGy+200 mT	0.583	0.796	0.886	0.755 ^{bc}	111.9	96.12	87.1	98.4 ^d	20.6	20.0	19.1	19.9 ^{ab}
5 kGy+50 mT	0.583	0.793	0.865	0.747 ^d	119.3	111.30	101.0	110.5 ^a	20.7	20.1	19.7	20.2 ^a
Mean	0.579 ^c	0.793 ^b	0.921 ^a		114.6 ^a	95.9 ^b	86.3 ^c		20.4 ^a	19.8 ^b	19.1 ^c	
CD (5 %)	0.016				0.38				1.11			
SE(M)	±0.005				±0.13				±0.42			

*Means with same letter are not significantly different at $p < .05$

^g kGy: kilogray; mT: milli tesla

of the seeds. Both magnetic field and gamma radiation, either alone or in combination, helped the seeds to maintain a relatively higher saponification value at levels much closer to that of zero time control and significantly higher when compared to that of untreated stored sample. Iodine value also declined significantly during storage, however, the differences were significant only for the gamma radiated and aged control treatments. Oil quantity during the accelerated aging (Table 2) showed a little decline in the first 10 days in the treated seed then the control. Among the magnetic field and irradiation treatment, irrespective of the stage of sample, oil content was unaffected by dose of gamma dose (Table 4). Similar to the seed coat hardness, 5 kGy+50 mT combination treatment showed significantly higher mean oil content compared to unirradiated control. Magnetic field exposed seeds were not showing significant difference in oil content compared to control (Table 2). This decline may be due to an increase in respiration of cells during accelerated aging. The seed coat is one of the principal conditioning factors for germination, vigour and longevity of seeds, with its characteristics being associated with susceptibility to mechanical damage, longevity and potential for seed deterioration, which may be influenced by degree of seed coat permeability and changes under different environmental condition (Parrish and Leopad 1978).

In all the treatments the acid value of the oil was observed to increase significantly during the process of ageing, however, it was in general low for all the magnetic and irradiation treatments when compared to control. Least mean acid value was measured at 3 kGy (4.3) and highest was observed for

0.5 kGy (5.5) treatment which was still lower than the unirradiated control seeds (7.0) of soybean (Table 3). Acid value reached a maximum of 11.04 mg/g at 20 days after treatment in aged control and was the highest among all the treatments. Acid value is a useful parameter for characterizing the quality of oil as measures the liberation the fatty acids from their ester linkage following hydrolysis to a triglyceride molecule. Acidity is extensively referred in terms of the percentage of free fatty acids in the oil or fat. Free fatty acids are important constituents of oilseeds (Bourland et al. 1988). Free fatty acid content exhibited a pattern similar to that observed for acid value of the oil (Table 3). Free fatty acid content of the seeds exposed to gamma and magnetic field alone or in combination was significantly lower than that of control irrespective of the days of ageing. Gamma irradiated seeds particularly at 1, 3 and 5 kGy measures a significantly lower free fatty acid level than all other treatments at all the stages of sampling during the ageing process. In general, saponification value decreased with aging duration irrespective of treatment (Table 3). Irrespective of aging duration, magnetic field or gamma radiation exposed seeds had a significantly higher saponification value than the untreated control. Rate of decline in saponification value was less rapid for energy treated than untreated seed during the accelerated aging. Combination of gamma and magnetic energy yielded best performance in terms of quality. These treatment had significantly higher saponification value at all the stages and when compared to all other treatments. A similar age and treatment effect was measured for seed iodine value (Table 3). The present data also indicates a gradual

Table 3 Individual and combined effect of gamma irradiation and static magnetic field on Acid value (mg/g), Free fatty acid (%), Saponification value (mg KOH), Iodine value (g I₂/100 g oil) of soybean seeds under accelerated aging

Treatments	Acid Value			Mean	Free Fatty Acids			Mean	Saponification value			Mean	Iodine value			Mean
	Days in to Accelerated ageing				Days in to Accelerated ageing				Days in to Accelerated ageing				Days in to Accelerated ageing			
	0	10	20		0	10	20		0	10	20		0	10	20	
Control	3.6	6.5	11.0	7.0 ^a	1.8	3.3	5.6	3.6 ^a	165.0	143.5	119.9	142.8 ^c	120.0	106.0	80.8	102.3 ^d
0.5 kGy	3.2	5.4	7.8	5.5 ^{bc}	1.6	2.7	3.9	2.7 ^{bc}	170.7	149.3	127.7	149.2 ^{bc}	122.0	108.3	86.5	105.6 ^c
1 kGy	3.1	4.5	6.5	4.7 ^{de}	1.6	2.3	3.3	2.4 ^d	180.0	160.0	135.0	158.3 ^b	124.0	111.0	86.0	107.0 ^c
3 kGy	3.0	4.0	5.8	4.3 ^f	1.5	2.0	2.9	2.1 ^d	199.0	178.8	156.6	178.1 ^a	123.8	110.9	96.9	110.5 ^{ab}
5 kGy	3.1	4.5	6.9	4.8 ^e	1.5	2.3	3.1	2.3 ^d	201.0	181.0	159.0	180.3 ^a	125.5	111.0	97.0	111.2 ^{ab}
50 mT	3.3	5.4	8.5	5.7 ^b	1.7	2.7	4.3	2.9 ^b	179.5	158.4	133.0	157.0 ^{bc}	123.0	108.5	87.0	106.2 ^c
100 mT	3.2	5.0	8.2	5.5 ^{bc}	1.6	2.5	4.1	2.7 ^{bc}	191.3	170.1	155.0	172.1 ^a	123.5	110.9	90.1	108.2 ^{bc}
200 mT	3.1	4.7	7.0	4.9 ^d	1.6	2.4	3.5	2.5 ^{cd}	197.0	176.0	161.0	178.0 ^a	124.6	117.8	106.9	116.4 ^a
0.5 kGy+200 mT	3.3	5.1	7.8	5.4 ^c	1.7	2.6	3.9	2.7 ^{bc}	195.0	179.0	161.0	178.3 ^a	124.6	110.9	89.7	108.4 ^{abc}
5 kGy+50 mT	3.3	5.4	7.5	5.4 ^c	1.7	2.7	3.8	2.7 ^{bc}	203.0	184.2	165.5	184.2 ^a	125.0	111.9	97.1	111.3 ^a
Mean	3.2 ^c	5.1 ^b	7.7 ^a		1.6 ^c	2.6 ^b	3.8 ^a		188.2 ^a	168.0 ^b	147.4 ^c		123.6 ^a	110.7 ^b	91.8 ^b	
CD (5 %)	0.48				0.56				23.04				4.85			
SE(M)	±0.17				±0.19				±8.14				±1.71			

*Means with same letter are not significantly different at $p < .05$

^s kGy: kilogray; mT: milli tesla

increase in FFA with accelerated aging of soybean seeds which was highest in control then the treated seeds due to the slower rate of conversion of triglycerides to free fatty acids, as reported by earlier workers (Bangash et al. 2004). Many biochemical investigations have proven that lipid peroxidation and fat acidity (free fatty acid percentage) are the major causes of seed deterioration, including cellular membrane disruption (Lehner et al. 2008; Priestley 1986). Seed quality is known to decline with an increase in the levels of free fatty acids (Gholami and Golpayegani 2011). Several researchers have noticed an increase in lipase activity which cause an increase in FFA content during accelerated aging (Leshem 1987). These FFA, especially linoleic and linolenic acids, may be subsequently oxidized by lipoxygenases, producing hydroperoxides and active oxygen forms. Iodine value indicates the stability of the oil towards oxidation. Higher the iodine value, the greater will be the unsaturation or the number of double bonds present in the fatty acids. Accelerated ageing caused a reduction in the iodine value (Table 3) indicating a lower number of double bonds in magnetic field or gamma radiated seeds and apparently related to protection against seed aging. Saponification value of soybean oil showed a gradual decrease. The decrease in saponification value under accelerated ageing might be due to breakage of bonds in oil which results in the reduction of average molecular weight of fatty acids in the oil and lowers the oil quality. Elevated levels of free fatty acids, which are toxic to most cells, are not present in healthy seed tissues (Priestley 1986) so, it can be concluded

that both lipid peroxidation and free fat acidity contribute to seed deterioration. Lipid peroxidation produces highly reactive free radical intermediates and lipid peroxides.

Protein content and protein quality

Protein degradation was noticed during storage (Table 4) and was independent of the dose of application. Zero time control had the highest protein level when compared with the aged control and all other energy treatments. The magnetic field or gamma radiation treatment did not show significant difference between themselves during storage for seed protein content. Total free amino acid content was expectedly higher for the aged control than when compared with the respective zero time control. More than the gamma radiation it was magnetic field which has positive effect on the free amino acid content of the seeds. Gamma radiation treatment alone showed relatively higher free amino acid content than the magnetic treatment or their combination. A much lower amino acid accumulation in seeds of the energy combination treatments could be linked to the magnetic field advantage rather than the gamma radiation. Seed methionine content of the gamma radiated or magnetic treatment alone was significantly higher than the aged control and could be grouped more closely to the zero time control. Gamma radiation effect on seed methionine content was significant at exposures above 1 kGy. More or less a similar pattern of decline was observed in nitrogen content between fresh and aged seed with or without exposure

Table 4 Individual and combined effect of gamma radiation and static magnetic field on protein (mg g⁻¹fw), total free amino acids (mg g⁻¹), methionine (mg g⁻¹) nitrogen (%), activity of LOX (Δ OD/g fw/min $\times$ 10⁻³), catalase (μ mol g⁻¹ fw min⁻¹), peroxidase (μ mol g⁻¹ fw min⁻¹) andSOD (a.u.g⁻¹fw min⁻¹) after six months of storage of soybean seeds at room temperature after six months of storage of soybean seeds at room temperature

Treatments	Protein	Total free amino acid	Methionine	Nitrogen	LOX	Catalase	Peroxidase	SOD
*Control	106.2	3.4	6.9	6.1	18.0	34.5	12.0	32.2
#Control	95.6	4.3	6.2	5.1	24.0	26.3	8.4	24.8
0.5 kGy	98.4	4.1	6.3	5.2	21.3	28.4	9.1	25.1
1 kGy	99.1	3.9	6.3	5.4	18.7	29.4	9.8	28.2
3 kGy	99.5	3.6	6.6	5.6	20.9	30.4	7.7	29.4
5 kGy	99.7	4.0	6.6	6.0	21.3	32.0	9.1	29.7
50 mT	100.4	3.6	6.7	5.6	19.2	31.0	10.6	26.7
100 mT	98.7	3.6	6.8	5.4	18.9	31.0	10.7	24.8
200 mT	100.1	3.6	6.5	5.4	18.6	31.2	10.9	29.4
0.5 kGy+200 mT	97.6	3.6	6.2	5.4	19.2	28.4	10.5	22.4
5 kGy+50 mT	96.0	3.6	6.4	5.2	19.2	28.4	10.1	21.1
CD (5 %)	2.90	1.08	0.52	0.36	1.89	0.92	0.86	1.04
SE (M)	$\pm$ 1.02	$\pm$ 0.38	$\pm$ 0.20	$\pm$ 0.12	$\pm$ 0.57	$\pm$ 0.38	$\pm$ 0.30	$\pm$ 0.41

*Control: untreated sample at zero time of storage . # Control: untreated sample at six months of storage

§ kGy: kilogray; mT: milli tesla; LOX: Lipoxigenase; SOD: Superoxide dismutase; OD: optical density

to the electromagnetic energy. Although nitrogen content of seeds declined during storage this decline was much higher for untreated control and at low doses of gamma radiation and magnetic fields. Nitrogen content of seeds were improved for the magnetic field treatments. This advantage was negated at high radiation dose as evident from low nitrogen in seeds exposed to 5 kGy+50 mT energy combination which was insignificantly different from aged control. A decrease in the seed protein content (Table 5) was observed during the aging across the treatment. Control showed the lowest mean protein content (85.5 mg/g fw) which was significantly lower than all other irradiated treatments. Combined energy treatment had a negative effect on seed protein content.

In general, total free amino acid increased and methionine content decreased (Table 5) with aging in all the treatment irrespective of dose. Control seeds possessed significantly higher free amino acid content (8.3 mg/g dw) than all other treatments. 100 mT (5.9 mg/g), 200 mT (5.5 mg/g) and 3 kGy (5.9 mg/g) treatments measured least free amino acid content then the control. At 20 days in to aging 1 and 3 kGy irradiated seeds maintained lower free amino acid content. This advantage is however, lost in combination treatment (5 kGy+50 mT). Different doses of gamma and magnetic field affect methionine content of the seeds when compared over aging duration. . Higher dose of gamma (3 and 5 kGy), magnetic energy (100 and 200 mT) and its combination (5 kGy+50 mT) was not significantly different in terms of methionine content but, lower doses of gamma (0.5 and 1 kGy) and magnetic energy (50 mT) had significantly higher amount of

mean methionine content then untreated one (5.22 mg/g). Lipid oxidation products have pronounced effects on other important cellular systems and can damage DNA (Wilson and McDonald 1986). The protein content in the aged seed decreased significantly, irrespective of the treatment. This may possibly be due to the ageing induced deterioration in soluble proteins (Sun and Leopold 1995). The decrease in the total protein content (Kapoor et al. 2011) of aged seed reasons for the lesser enzymatic activity during the advanced period of study. Findings of the present experiment find support from Nautiyal et al. (1985), who showed that the protein loss depends upon the severity of aging condition. Total free amino acid content was higher in accelerated aging condition which was also supported by Ravikumar et al. (2002). A decrease in the methionine content of the aged seeds is in tune with the decrease in the protein content. High lipoxigenase activity of the aged seed shows an increase the deoxygenation of lipid polyunsaturated fatty acids and leads to deterioration of seed quality. In this regard Priestley and Leopold (1979) reported that the changes associated with aging are probably many and variable. Significance of the aging and energy related changes observed in the present study need to be determined. Certainly any oxidation of lipids and loss of membrane integrity must be a contributing factor.

Over the period of accelerated aging nitrogen content showed declining trend (Table 5) and it seems dose dependent in gamma radiated seeds with highest value in 5 kGy (5.7 %) and lowest in 0.5 kGy (4.8) which is statistically similar to control. 50 and 100 mT exposed seed had highest mean

Table 5 Individual and combined effect of gamma irradiation and static magnetic field on Protein content (mg /g fw), Total free amino acid (mg/g), Methionine (mg/g), Nitrogen (%) of soybean seeds under accelerated aging

Treatments	Protein content			Mean	Total free amino acid			Mean	Methionine			Mean	Nitrogen			Mean
	Days in to Accelerated ageing				Days in to Accelerated ageing				Days in to Accelerated ageing				Days in to Accelerated ageing			
	0	10	20		0	10	20		0	10	20		0	10	20	
Control	95.6	86.5	74.5	85.5 ^d	4.3	6.0	14.6	8.3 ^a	6.2	5.4	4.1	5.2 ^c	5.1	4.6	4.2	4.6 ^e
0.5 kGy	98.4	92.0	84.3	91.6 ^a	4.1	5.1	11.0	6.7 ^b	6.3	5.7	5.1	5.7 ^b	5.2	4.8	4.4	4.8 ^{de}
1 kGy	99.1	92.9	85.0	92.3 ^a	3.9	4.8	9.9	6.2 ^{bcd}	6.3	5.9	5.2	5.8 ^b	5.4	5.0	4.6	5.0 ^{cd}
3 kGy	99.5	92.8	85.4	92.6 ^a	3.6	4.4	9.8	5.9 ^{cd}	6.7	6.3	5.8	6.3 ^a	5.6	5.2	4.9	5.2 ^{bc}
5 kGy	99.7	93.0	86.2	93.0 ^a	4.0	4.8	11.1	6.6 ^b	6.6	6.3	5.9	6.3 ^a	6.0	5.6	5.4	5.7 ^a
50 mT	100.4	91.9	83.9	92.1 ^a	3.6	4.5	10.1	6.1 ^{bcd}	6.7	6.0	5.1	5.9 ^{ab}	5.6	5.3	4.8	5.2 ^{bc}
100 mT	98.8	91.4	83.6	91.3 ^{ab}	3.7	4.4	9.7	5.9 ^{cd}	6.8	6.1	5.7	6.2 ^a	5.4	5.2	4.9	5.2 ^{bc}
200 mT	100.1	93.1	84.2	92.5 ^a	3.6	4.0	8.8	5.5 ^d	6.5	6.3	5.9	6.2 ^a	5.4	5.2	5.0	5.2 ^{bc}
0.5 kGy+200 mT	97.6	89.3	81.8	89.6 ^b	3.6	4.9	10.9	6.5 ^{bc}	6.2	5.8	5.3	5.8 ^b	5.4	5.0	4.7	5.0 ^{cd}
5 kGy+50 mT	96.0	87.1	80.3	87.8 ^c	3.6	4.7	10.8	6.4 ^{bc}	6.4	5.9	5.4	5.9 ^{ab}	5.2	4.8	4.7	4.9 ^{cd}
Mean	98.5 ^a	91.0 ^b	82.9 ^c		3.8 ^c	4.8 ^b	10.7 ^a		6.5 ^a	6.0 ^b	5.4 ^c		5.4 ^a	5.1 ^b	4.8 ^c	
CD (5 %)	2.90				1.08				0.52				0.36			
SE(M)	±1.02				±0.38				±0.20				±0.12			

*Means with same letter are not significantly different at $p < .05$

^s kGy: kilogray; mT: milli tesla

nitrogen content (5.2 %) among the magnetic field treated seeds.

Enzymes and fatty acid profile

At biochemical level, the activities of LOX increased (Table 4) while that of catalase, peroxidase and SOD were decreased during seed storage. Magnetic field and gamma radiation alone or in combination helped the seeds to maintain significantly lower LOX activity when compared to the aged control. Between the electromagnetic energies, magnetic field was more efficient in protecting the seed metabolism than gamma radiation. Gamma radiation at 5 kGy and magnetic field treatment irrespective of the dose measured catalase activities that were closer to the zero time control and were significantly higher than the aged control. Peroxidase activity was higher for the magnetic field treatment and related energy combinations. Gamma radiation alone did not improve the seed peroxidase activity significantly over the untreated aged control. Higher peroxidase activity of energy combination treatment appears to be induced by the magnetic field rather than the gamma radiation. On the other hand SOD activity which is related to the removal of deleterious superoxide radicals was maintained at a significantly higher level in gamma irradiated than the magnetic field treated seeds with a few exceptions (Table 4). Energy combination treatment measured SOD activity even much lower than that of the aged control suggesting that the gamma radiation advantage of a high SOD activity

during seed storage withers in combination with the magnetic field treatment.

LOX activity in general increase with the duration of aging across the treatment and its activity decrease in a dose dependent manner (Table 6). Control showed highest amount of LOX activity ($32.0 \Delta \text{OD/g fw/min} \times 10^{-3}$) which was significantly reduced up on gamma and magnetic treatments. Higher doses of gamma irradiation (1, 3, 5 kGy) and magnetic field energy (100 and 200 mT) and their combination (0.5 kGy+200 mT and 5 kGy+50 mT) show significantly lower LOX activity (range from 24.0 to $24.7 \Delta \text{OD g}^{-1} \text{fw min}^{-1} \times 10^{-3}$).

Accelerated aging reduces the activity of antioxidant enzymes quite significantly. Catalase activity in general declined (Table 6) with the duration of aging irrespective of radiation doses and magnetic field treatment. Mean catalase activity averaged over duration of aging improved in a dose dependent manner for both magnetic as well as gamma radiation. In the combine treatment positive effect of gamma and magnetic field were down regulated. Further it was observed that decline in the catalase activity during aging occurred at much slower rate in treated than the control seeds.

Peroxidase activity of soybean seeds, averaged over the time during accelerated aging from $9.7 \mu\text{mol g}^{-1} \text{fw min}^{-1}$ at zero days to 8.0 and $6.4 \mu\text{mol g}^{-1} \text{fw min}^{-1}$ at 10 and 20 day to accelerated aging. However, when averaged over the aging duration magnetic exposed or gamma irradiated seed and their

Table 6 Individual and combined effect of gamma irradiation and static magnetic field on LOX (Δ OD/g fw/min $\times 10^{-3}$), Catalase ($\mu\text{mol g}^{-1}$ fw min^{-1}), Ascorbate Peroxidase ($\mu\text{mol g}^{-1}$ fw min^{-1}), SOD (a.u. g^{-1} fw min^{-1}) of soybean seeds under accelerated aging

Treatments	LOX			Mean	Catalase			Mean	Ascorbate Peroxidase			Mean	SOD			Mean
	Days in to Accelerated ageing				Days in to Accelerated ageing				Days in to Accelerated ageing				Days in to Accelerated ageing			
	0	10	20		0	10	20		0	10	20		0	10	20	
Control	24.0	31.2	40.7	32.0 ^a	26.3	22.4	18.7	22.5 ^f	8.4	6.5	3.5	6.1 ^d	24.8	16.3	5.6	15.6 ^g
0.5 kGy	21.3	30.1	37.3	29.5 ^b	28.4	24.3	20.9	24.5 ^e	9.1	7.8	7.8	8.2 ^b	25.1	19.5	11.9	18.8 ^c
1 kGy	18.7	25.4	28.9	24.4 ^d	29.4	25.3	21.2	25.3 ^d	9.8	9.4	5.4	8.2 ^b	28.2	23.6	15.0	22.3 ^b
3 kGy	20.9	23.9	27.8	24.2 ^d	30.4	26.4	22.7	26.5 ^c	7.7	9.6	6.6	8.0 ^b	29.4	25.1	15.9	23.5 ^a
5 kGy	21.3	23.7	28.9	24.6 ^d	32.0	27.7	24.0	27.9 ^a	9.1	8.4	7.4	8.3 ^b	29.7	25.2	17.0	23.9 ^a
50 mT	19.2	28.3	35.2	27.6 ^c	31.0	27.3	23.3	27.2 ^b	10.6	6.6	6.6	7.9 ^b	26.7	20.7	11.9	19.8 ^d
100 mT	18.9	23.5	29.5	24.0 ^d	31.0	27.4	25.1	27.8 ^a	10.7	9.5	7.5	9.2 ^a	24.8	19.8	13.9	19.5 ^{de}
200 mT	18.6	25.0	31.0	24.9 ^d	31.2	27.9	25.9	28.3 ^a	10.9	9.6	8.3	9.6 ^a	29.4	22.0	11.6	21.0 ^c
0.5 kGy+200 mT	19.2	24.7	29.6	24.5 ^d	28.4	24.6	21.2	24.7 ^c	10.5	6.9	6.1	7.8 ^b	22.4	19.0	11.2	17.5 ^f
5 kGy+50 mT	19.2	24.6	30.4	24.7 ^d	28.4	26.3	22.7	25.8 ^d	10.1	5.8	4.8	6.9 ^c	21.1	18.4	9.2	16.2 ^g
Mean	20.1 ^c	26.0 ^b	31.9 ^a		29.7 ^a	26.0 ^b	22.6 ^c		9.7 ^a	8.0 ^b	6.4 ^b		26.2 ^a	21.0 ^b	12.3 ^c	
CD (5 %)	1.89				0.92				0.86				1.04			
SE(M)	±0.57				±0.32				±0.30				±0.40			

*Means with same letter are not significantly different at $p < .05$

^s kGy: kilogray; mT: milli tesla; LOX: Lipoxigenase; SOD: Superoxide dismutase

combination had significantly higher peroxidase activity over control treatment. Both gamma and magnetic field affected peroxidase activity to a similar extent which was independent of the dose of energy. Seed peroxidase activity declined at much faster rate between 10 to 20 days of aging than the first day period (Table 6). A trend similar to peroxidase and catalase activity was observed for SOD of soybean seeds during the accelerated aging. Here too the decline in SOD activity was more pronounced during 10–20 day then the first ten days. Mean SOD activity, when averaged over the duration of ageing, in general increased significantly in the energized seed then control (Table 6).

Over the period of accelerated aging, the total antioxidant activity in terms of inhibition of free radicle DPPH (%) varied significantly across the treatments. Lower doses had more scavenging capacity compared to control and it increased in a dose dependent manner (Fig 1). Lower doses of magnetic energy did not showed better performance but as the doses improves the total antioxidant activity start increasing. Combined gamma and magnetic treatment also shows better performance than the control in terms of inhibition of free radicle DPPH (%).

A look into the fatty acid profile (Table 7) of the oil from the freshly harvested and stored seed revealed an increase in levels of palmitic acid, stearic acid and oleic acid while a decline was observed for linoleic and linolenic acid. Aged control seeds showed a higher level of oleic acid which appears to have been guided by a decline in the level of

linolenic acid. When compared to controls the highest level of palmitic acid, stearic acid and oleic acid were measured at 200 mT alone, 3 kGy alone and 5 kGy+50 mT combination treatment respectively. Relative content of different fatty acids in soybean seeds during accelerated aging was affected by magnetic field, gamma radiation and their combination treatments are presented in Table 8. Mean fatty acid level when averaged over treatments increased with aging for palmitic and oleic acid and decreased in case of stearic, linoleic and linolenic acid. When compared between the treatments over control, mean palmitic acid content (of total) increased while stearic acid decreases in response to gamma and magnetic energy. However, with a few exceptions, oleic, linoleic and linolenic acid levels remained unchanged in the energy treatments when compared to control. Unsaturated fatty acids content declined in all the treatment with mean value of 7.4 to 2.9 % in linolenic acid (18:3), compared to the total fatty acids present in the seed. The mean decline in linolenic acid content during the aging in gamma irradiation and magnetic energy treated seed was much less rapid compared to the control. Overall no clear pattern of variation in unsaturated as well as saturated fatty acid was observed with respect to the dose of energy treatment.

Peroxidation of unsaturated fatty acids occurs during aging at high RH. Linolenic acid and linoleic acid declining appreciably on the 10th and 20th day, hence, both these unsaturated fatty acids show significant degradation as the seeds deteriorate. These studies agree with the work on peas

Fig. 1 Individual and combined effect of gamma irradiation and magnetic field, either alone or in combination, on DPPH (%) activity during accelerated ageing (42 ± 2 °C, 95–100 % RH) of soybean. Treatments with different letters are significantly different at the 0.05 level of probability. mean $\pm$ SD, $n=3$

by Harman and Mattick (1976) and on soybean by Stewart and Bewely (1980). In the presence of oxygen, ageing of seed can lead to peroxidative change in polyunsaturated fatty acid (PUFA) (Stewart and Bewely 1980; Wilson and McDonald 1986). This is because the cotyledons are a source of stored lipids, and was deemed undesirable to study polar lipid changes and lipid peroxidation in a tissue that contained considerable quantities of nonmembrane lipids. It has long been suggested that lipid peroxidation (or autoxidation) is increased during storage (Sharma et al. 2011) and is a primary cause of seed deterioration. Major peroxidation damage occurs during

storage (Stewart and Bewely 1980), but only when high RH initiates imbibition process. In this study, it is apparent that some changes occur during aging, but that they occur most obviously in deteriorating aged seeds. Protective mechanism that could scavenge the peroxidase actively produced free radicals and peroxides prevail within the cellular system to minimize seed deterioration reactions. Superoxide dismutase, peroxidase and catalase enzyme systems provide one such protective mechanism and inhibition of activities of these enzymes is reported to cause faster deterioration of seed (Jung and Chiu 1995). During the process of aging a decline the

Table 7 Individual and combined effect of gamma radiation and static magnetic field on palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid (relative percent of total) after six months of storage of soybean seeds at room temperature

Treatments	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
*Control	11.9	5.0	20.7	52.7	9.7
#Control	15.1	6.4	25.3	47.5	5.8
0.5 kGy	16.9	5.4	21.9	47.1	8.6
1 kGy	16.2	5.3	21.7	49.3	7.6
3 kGy	16.6	8.6	24.9	43.3	6.6
5 kGy	17.3	8.2	27.6	39.1	7.8
50 mT	15.8	7.1	24.8	44.6	7.8
100 mT	18.3	6.6	29.0	38.4	7.7
200 mT	16.9	7.1	21.8	44.5	9.7
0.5 kGy+200 mT	12.8	6.4	24.3	50.8	5.7
5 kGy+50 mT	14.0	6.6	31.0	41.5	7.2
CD (5 %)	1.50	1.48	1.43	1.43	1.39
SE (M)	± 0.53	± 0.46	± 0.50	± 0.49	± 0.48

*Control: untreated sample at zero time of storage #Control: untreated sample at six months of storage

^s kGy: kilogray; mT: milli tesla

Table 8 Individual and combined effect of gamma irradiation and static magnetic field on fatty acid profile (relative percent of total) of soybean seeds under accelerated aging

Treatments	Palmitic acid (16:0)				Stearic acid (18:0)				Oleic acid (18:1)				Linoleic acid (18:2)				Linolenic acid (18:3)				Mean
	Mean				Mean				Mean				Mean				Mean				
	Days in to Accelerated ageing				Days in to Accelerated ageing				Days in to Accelerated ageing				Days in to Accelerated ageing				Days in to Accelerated ageing				
	0	10	20		0	10	20		0	10	20		0	10	20		0	10	20		
Control	15.1	20.6	23.4	19.7 ^f	6.4	7.4	8.7	7.8 ^a	25.3	31.6	33.3	30.0 ^d	47.5	35.3	31.4	38.1 ^b	5.8	5.2	3.3	4.7 ^{bc}	
0.5 kGy	16.9	23.5	29.8	23.4 ^d	5.4	7.5	7.4	6.8 ^{ab}	21.9	36.8	37.7	32.1 ^c	47.1	28.7	21.6	32.5 ^f	8.6	3.5	3.5	5.2 ^a	
1 kGy	16.2	32.6	34.9	27.9 ^a	5.3	7.4	5.8	6.1 ^b	21.7	32.3	22.0	25.3 ^g	49.3	23.1	34.1	35.5 ^c	7.6	4.7	3.2	5.1 ^a	
3 kGy	16.6	26.9	27.2	23.6 ^{cd}	8.6	7.2	5.6	7.1 ^a	24.9	34.3	16.2	25.1 ^g	43.3	26.6	48.6	39.5 ^a	6.6	4.9	2.4	4.7 ^{bc}	
5 kGy	17.3	34.7	32.9	28.3 ^a	8.2	6.0	8.5	7.5 ^a	27.6	28.9	40.6	32.4 ^{bc}	39.1	24.7	15.6	26.5 ^g	7.8	5.8	2.5	5.3 ^a	
50 mT	15.8	23.9	33.4	24.4 ^c	7.1	6.2	4.8	6.1 ^b	24.8	38.2	20.1	27.7 ^e	44.6	27.3	37.5	36.4 ^c	7.8	4.4	4.1	5.4 ^a	
100 mT	18.3	27.8	27.1	24.4 ^c	6.6	6.1	1.8	4.8 ^c	29.0	23.3	47.1	33.1 ^b	38.4	40.1	22.4	33.6 ^e	7.7	2.7	1.6	4.0 ^{bc}	
200 mT	16.9	29.8	32.7	26.5 ^b	7.1	2.6	3.4	4.4 ^c	21.8	23.7	40.4	28.6 ^e	44.5	41.4	21.2	35.7 ^c	9.7	2.6	2.4	4.9 ^{ab}	
0.5 kGy+200 mT	12.8	23.4	30.2	22.1 ^e	6.4	3.9	3.8	4.7 ^c	24.3	20.9	45.3	30.2 ^d	50.8	48.7	17.8	39.1 ^a	5.7	3.2	2.8	3.9 ^c	
5 kGy+50 mT	14.0	23.1	21.8	19.6 ^f	6.3	3.8	3.8	4.6 ^c	31.0	28.5	49.0	36.1 ^a	41.5	40.2	22.1	34.6 ^d	7.2	4.5	3.4	5.0 ^a	
Mean	16.0 ^c	26.6 ^b	29.3 ^a		6.7 ^a	5.8 ^b	5.4 ^c		25.3 ^c	29.8 ^b	35.2 ^a		44.6 ^a	33.6 ^b	27.2 ^c		7.4 ^a	4.1 ^b	2.9 ^c		
CD (5 %)	1.50				1.48				1.43				1.43				1.39				
SE(M)	±0.531				±0.461				±0.501				±0.499				±0.478				

*Means with same letter are not significantly different at $p < 0.05$ ^g kGy: kilogray; mT: milli tesla

activity of antioxidant enzymes (Table 6) shows that efficiency of the free radical scavenging system decrease during the extended period of aging and cause lipid peroxidation, membrane damage and deterioration of the soybean at a much faster pace. However, the levels of catalase, SOD and peroxidase enzymes were greater in energy treated than untreated seeds during accelerated aging. It is possible that the gamma radiation and magnetic field might be playing a role in controlling oxidative processes to enhance the rate of seed deterioration during the aging process. Increased level of total antioxidant during accelerated aging provides evidence for the ability of cells to survive stressful condition as antioxidant system may help to contain or prevent the damage by ROS. Medium dose of gamma irradiation and higher doses of magnetic energy showed a greater activity of DPPH (%) which clearly indicates that in addition to enzymatic a non-enzymatic free radical scavenging system is also present in seeds which inhibit lipid peroxidation and the deterioration of membranes. Inability to contain aging related free radical formation may lead to membrane damage and is likely to cause seed deterioration (Jung and Chiu 1995).

Conclusions

The results indicate that gamma radiation and magnetic field exposed seed had a better storage shelf life both under the ambient and the adverse storage conditions. Electromagnetic energy treatment in form of gamma radiation and static magnetic field were effective in reducing the rate of deterioration of quality of the stored seeds, more so in dose dependent manner. Stored seeds irradiated at 1 kGy and above, and exposed to magnetic field at 100 mT and above maintained a lower water activity and highest seed coat hardness indicating towards a lower metabolic activity in seeds during storage. Under accelerated aging, a higher dose of gamma irradiation (3 and 5 kGy) protects the oil quality quantified in terms of acid value, FFA, saponification value, iodine value and the protein quality of the soybean seeds measured in terms of methionine and free amino acid content, whereas a higher magnetic field protects the quality in terms of the saponification value, iodine value and methionine content. It is clear from the result obtained in this experiment that higher dose of gamma and magnetic field i.e., 3, 5 kGy and 100 and 200 mT respectively of both energy forms is beneficial in maintaining the physical and nutritional quality of seed through a decrease in the activity of LOX which leads to a lower rate of lipid peroxidation as compared to the untreated control and through a better activity of seed antioxidant enzymes, which are useful for scavenging the harmful free radicals. Both the above factors in turn protect the soybean seed deterioration during storage under ambient as well as under conditions of

accelerated aging. Energy combination treatments were not much effective in protecting the seed quality over and above the protection measured at the individual energy treatment of gamma radiation and the magnetic field but were still better than the untreated control.

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