EFFECT OF GAMMA RADIA
TION AND STORAGE ON TOTAL ANTI
OXIDANT CAPACITY AND PARAMETER RESPONSIBLE FOR GENERATION OF OFF-FLAVOUR IN SOYBEAN SEEDS VARYING IN SEED COAT COLOUR

Gaurav Kumar, Anil Dahuja and I.M. Santha*
Division of Biochemistry,
Indian Agriculture Research Institute, New Delhi, 110 012, India

Received: 07-02-2013
Accepted: 21-08-2013

ABSTRACT
The total antioxidant potential and degree of off-flavour generation, measured by TBA number, Carbonyl value and LOX activity, were estimated in seventeen varieties of soybean seeds differing in seed coat colour. The antioxidant potential was high in seeds with black and brown seed coat colour as compared to yellow and green seed coat colour. Six selected varieties were irradiated with gamma radiation and were analysed for above mentioned parameters. The relative enhancement in antioxidant potential was maximum in yellow seed coat coloured genotype and minimum in black and brown seed coat coloured ones at lower dose of 0.5 KGy. At higher dose of 1.0 and 2.0 KGy the total antioxidant and FRSA were decreased in all varieties. The TBA number, Carbonyl value and LOX activity decreased at 0.5 KGy whereas these values increased at 1.0 and 2.0 KGy. The total antioxidant capacity of different soybean cultivars effectively controls the off-flavour generating compounds at lower dose of gamma irradiation.

Key words: Antioxidant capacity, Gamma irradiation, Off-flavour development, Soybean seeds.

INTRODUCTION
The delicious, slightly nutty flavoured soybean, known for its versatile food source is the most widely grown and utilized legume in the world and one of the most well researched, health-promoting foods available today. Like other beans, soybeans grow in pods, featuring edible seeds which can be green, yellow, brown or black in colour.

In spite of its excellent protein content, high levels of essential fatty acids and other important antioxidant molecule like isoflavons the use of soybean as a food product is limiting due to the presence of protease inhibitors, flatulent factors, nutrient-binding phytic acid and off-flavour (Kumar et al., 2010). The off-flavours are due to the presence of medium chain aldehydes and ketones produced by the action of lipoxygenase on polyunsaturated fatty acids linoleic and linolenic acids (Dahuja and Madaan, 2003).

A number of studies have been conducted to develop soybean cultivars without LOX by plant breeders to control off-flavour, but this strategy seems to be inadequate as LOX has also been reported to be involved in generation of various important secondary products and is also involved in maintaining appropriate level of protease inhibitors, which are crucial for the protection of plants against insects and pests (Carvalho et al., 1999). Gamma radiation has been used to prevent fungal and bacterial infection of seeds and food stuffs, for this reason radiation up to a dose of 1KGy have been recommended for quarantine treatment of legumes including soybean (Variyar et al., 2004). Soybean seeds treated with gamma radiation showed higher content of total phenolic compounds (Villavicencio et al., 2000) and increased the antioxidant constituents like isoflavones, anthocyanin and total phenols and hence antioxidant potential of soybean seed (Dixit et al. 2010). However, very few studies have been carried out to know any correlation between inherent total antioxidant capacity and level of off-flavour generation in various soybean cultivars exists or not on gamma radiation treatment. Therefore in the present study Indian soybean...
varying in seed coat colour were treated with gamma irradiation at 0.5, 1 and 2 KGy in order to find out the effect of irradiation on the level of total antioxidants and off-flavour causing compounds.

**MATERIALS AND METHODS**

Seventeen varieties of soybean varying in seed coat colour (Table 1) collected from Division of Genetics, IARI; New Delhi was used in the present study. These varieties were analysed for total antioxidant capacity, LOX activity, TBA No. and Carbonyl value and based on the observations, 6 varieties differing in seed coat colour were selected for further analysis.

**Gamma irradiation treatment**: 10gm of selected soybean seeds were irradiated at doses of 0.5, 1 and 2 KGy gamma rays in a gamma cell GC 5000 with C\(^{60}\) source installed at NRL, IARI, and New Delhi. The non-irradiated seeds served as control and all the seeds were packed in polyethylene bags.

**Extraction of antioxidants from seeds**: 1g of finely ground soy flour was extracted with 15 ml of 70% acetone at 25°C in the dark overnight (Malencic et al. 2008). The mixture was centrifuged at 3000 g for 10 min and the supernatant was stored at 4°C in the dark for further analysis.

**Sample preparation for determination of Carbonyl value, TBA No., and LOX activity**: 1g of overnight soaked soybean seeds in distilled water were homogenized in 10ml ice-cooled distilled water using a pestle and mortar. The homogenate was allowed to stand for 10 min at 4°C and centrifuged at 15000 g for 30 min at 4°C. The resultant supernatant was kept at 4°C for further use.

**CUPRAC method of antioxidant assay**: CUPRAC assay is a simple and versatile antioxidant capacity assay for dietary polyphenols, vitamin C and vitamin E as described by Apak et al. (2004). The antioxidant capacity was expressed as Trolox equivalent (mmol TE/g) using the formula, mmol TE/g = (A\(_f\)/c) (V\(_f\)/V\(_s\)) r (V\(_{\text{initial}}\)/m) \(\varepsilon\) = 1.67 \times 10\(^4\) Lmol\(^{-1}\)cm\(^{-1}\)

**Free radical scavenging activity (FRSA) using 2, 2-diphenyl-1- picrylhydrazyl (DPPH)**: The free radical scavenging capacity of the extract was estimated using ethanolic solution of DPPH following Mellors and Tappel (1966). To 3 ml of ethanolic DPPH solution, 0.1 ml of sample extract was added and the decrease in absorption at 517 nm was measured from 0 to 10 min. The DPPH radical scavenging activity (%) was calculated using the following formula:

\[ \text{Radical scavenging activity} \% = \frac{(A_0 - A_1)}{A_0} \times 100, \]

where \(A_0\) and \(A_1\) are the absorbances at 0 and 10 min respectively.

**Lipoxygenase (LOX) assay**: LOX activity was assayed by a modified method of Axelrod et al. (1981) where increase in OD was measured for 10 minutes at 234 nm. The LOX activity was expressed as \(\Delta\text{OD/min/mg of protein}\). Protein concentrations were determined by the Lowry's method (Lowry et al. 1951) using bovine serum albumin as a standard.

**Estimation of Thiobarbituric acid (TBA) number**: TBA number was determined using the method developed by Ohkawa et al. (1979) which determines total malonaldehydes generated during lipid peroxidation. Thiobarbituric acid thus formed shows maximum absorbance at 532 nm. The standard curve was prepared using TEP ranging from 10 to 70 nmoles and the level of lipid peroxides was expressed as nmoles MDA/g fresh wt.

**Estimation of carbonyl value**: In carbonyl value determination, 2, 4-dinitrophenyl hydrazine derivative was determined as described by Henicks et al. (1954) with slight modification. To a volumetric flask, 2ml 2,4-nitrophenylhydrazine and 2ml of soybean extract were added and incubated for 30 min at 60°C. After cooling, 6ml of 4% KOH solution was added and exactly after 10 min, the absorbance was measured at 480 nm against a blank prepared in the same manner, using 5 ml of double distilled water in place of the sample. Calculations were made using the formula

\[ A = \varepsilon \times C \times L \]

**RESULTS AND DISCUSSION**

Among the 17 genotypes (Table 1) analysed for total antioxidant activity (Fig.1) using CUPRAC method, the antioxidant capacity was found to range from 2.26 to 15.06 (µmol TE/g) and was found to be maximum in BR2 (15.06 µmol TE/g) having brown seed coat colour and minimum in PK1347 (2.26µmol TE/g) with yellow seed coat colour. Among the different seed coat colour varieties
TABLE 1: List of soybean varieties used in the present study

<table>
<thead>
<tr>
<th>Variety</th>
<th>Seed coat colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pusa 16</td>
<td>Yellow</td>
</tr>
<tr>
<td>Pusa 22</td>
<td>Yellow</td>
</tr>
<tr>
<td>DS 9814</td>
<td>Yellow</td>
</tr>
<tr>
<td>Pusa 24</td>
<td>Yellow</td>
</tr>
<tr>
<td>Pusa 20</td>
<td>Yellow</td>
</tr>
<tr>
<td>PK 1347</td>
<td>Yellow</td>
</tr>
<tr>
<td>Bragg</td>
<td>Yellow</td>
</tr>
<tr>
<td>Pusa 40</td>
<td>Yellow</td>
</tr>
<tr>
<td>PK 1042</td>
<td>Yellow</td>
</tr>
<tr>
<td>PK 416</td>
<td>Yellow</td>
</tr>
<tr>
<td>Pusa 37</td>
<td>Yellow</td>
</tr>
<tr>
<td>SL 525</td>
<td>Yellow</td>
</tr>
<tr>
<td>EC 439601</td>
<td>Green</td>
</tr>
<tr>
<td>BS 1</td>
<td>Brown</td>
</tr>
<tr>
<td>BR 2</td>
<td>Brown</td>
</tr>
<tr>
<td>EC 457312</td>
<td>Black</td>
</tr>
<tr>
<td>AMSS 34</td>
<td>Black</td>
</tr>
</tbody>
</table>

studied, black and brown seed coat coloured ones showed higher CUPRAC value as compared to varieties having green and yellow seed coat colour. The free radical scavenging activity (FRSA) as measured by reduction of DPPH (Fig. 1) ranged from 2.01 to 12.45 % scavenging/mg of soya flour, and it was maximum in AMSS34 (12.45 % scavenging/mg) having black seed coat colour and minimum in SL525 (2.01% scavenging/mg) having yellow seed coat colour. Similar to CUPRAC values, DPPH values were also found to be higher in brown and black seed coat coloured seeds as compared to yellow and green seed coat coloured seeds. Malonaldehyde content of soybean seeds as measured by TBA number (Fig.2) was found to be maximum in DS9814 (99 nmol/g of seed) genotype having yellow seed coat colour and was minimum in EC439601 (34.57nmol/g of seed) genotype having green seed coat colour. A good index of oxidative change in lipid was measured by Carbonyl value which estimates the secondary oxidation products such as aldehydes and ketones. The Carbonyl value was found to range from 1.18 to 1.55 nmol/g of seed. Among the seventeen varieties analysed Pusa 24 with yellow seed coat colour was found to have maximum carbonyl value and AMSS 34 with black seed coat colour showed the lowest carbonyl value. The LOX activity ranged from 13 to 21.06 ΔOD/min/mg of protein and was found to be maximum in SL525 with yellow seed coat colour and minimum in Pusa 22 with yellow seed coat colour followed by EC 439601 with green seed coat colour.

Effect of different doses of gamma radiation: Out of these 17 varieties 3 varieties having different seed coat colour (black, brown and green) were selected along with 3 yellow seed coat colour varieties for gamma radiation treatment. Effect of radiation doses and storage period on the total antioxidant activity in seeds before and after gamma radiation treatment at 0.5, 1.0 and 2.0 KGy respectively showed that gamma radiation increased the total antioxidant capacity as measured by CUPRAC method in all the seeds and also at all doses studied except in seeds with black and brown seed coat colour where the values decreased at higher dose of 1.0 and 2.0 KGy (Fig.3). The enhancement was more in seeds exposed to 0.5 KGy as compared to higher doses of 1.0 and 2.0 KGy. The relative enhancement was maximum in PK416 (107 %) with yellow seed coat colour and minimum in BR2 (8.7 %) with brown.
Among the selected 6 genotypes of soybean all showed significant increase in FRSA (Fig. 4) except AMSS 34 and BR 2 with black and brown seed coat colour respectively. At radiation dose of 0.5 KGy the maximum relative enhancement of 72% was observed in Pusa 37 with yellow seed coat colour and minimum in BR 2 (0.2%) with brown seed coat colour. Among the different seed coat colour varieties the maximum relative enhancement was found in varieties having yellow seed coat colour at 0.5 KGy, whereas the antioxidant potential decreased at higher doses (1.0 and 2.0 KGy). Gamma radiation was found to decrease the TBA number in all the varieties studied at lower dose of 0.5 KGy (Fig 5). AMSS-34 with black seed coat colour and BR-2 with brown seed coat colour showed significant decrease (56%) in TBA value followed by PK-416 (46%) with yellow seed coat colour, and was lowest (34%) in EC 439601 with green seed coat colour. All the genotypes showed increase in TBA value at higher doses of radiation. Seeds treated with gamma radiation of 0.5 KGy showed significant decrease in Carbonyl value (Fig. 6) and the relative reduction in Carbonyl value was highest in PK 416 (40%) with yellow seed coat colour and minimum in EC 439601 (1.4%) with green seed coat colour. Carbonyl value increased at 1.0 KGy and almost remained same at 2.0 KGy in all the varieties studied. A decrease in LOX activity (Fig. 7) was observed at a lower radiation dose of 0.5 KGy, where maximum relative reduction was found in BR 2 (33%) followed by AMSS 34 (31%) with brown and black seed coat colour respectively and minimum in EC 439601 (10.9%) with green seed coat colour. An increase in
LOX activity was observed at higher radiation dose of 1.0 and 2.0 KGy in all the varieties.

**Sustainability of effect of gamma radiation treatment in treated soybean seeds:** The seeds after radiation were stored at room temperature for a period of 30 days a decrease in the total antioxidant activity (Fig 8 and 9) d in all the 6 varieties. Varieties having yellow seed coat colour showed constant but less decrease (11% in SL525) in antioxidant activity, whereas sharp decrease (37% in BR 2) was found in soybean having brown and black seed coat colour. Seeds treated with 0.5 KGy showed negligible decrease during 1st 15 days of storage but values decreased constantly during next 15 days. However, seeds treated with 1.0 and 2.0 KGy showed sharp decrease from the very beginning of storage period. All the varieties of soybean showed increase in TBA value (Fig. 10) on storage after treatment. It was found that the seeds treated with radiation dose of 1.0 and 2.0 KGy showed substantial increase in TBA number during 1st 15 days but increase was less during latter half of storage. Seeds treated with 0.5 KGy showed constant increase in TBA number during the whole period of storage. The carbonyl value (Fig 11) was found to increase constantly during the storage period of 30 days. The yellow seed coat colour variety PK416 showed maximum (39%) enhancement whereas brown seed coat colour variety BR2 showed minimum (11%) increase in Carbonyl values of seeds treated with 0.5 KGy. A similar trend was observed in seeds treated with higher doses (1 and 2 KGy) of gamma radiation. The increase in LOX activity (Fig. 12) was found to be less during the storage period in the seeds treated with 0.5 KGy whereas the varieties treated with 1 and 2 KGy showed substantial increase during 1st 15 days as compared to latter half. Almost similar relative enhancement of LOX activity was observed in all the varieties at all doses of radiation treatment. Radiation processing of foods by ionizing radiation such as γ and X rays and electron beams has in recent years assumed considerable importance as a technology to reduce postharvest food losses by increasing shelf life and to eliminate food-poisoning microorganisms. The overall goal of this study was to see the combined effect of radiation doses and storage period on total antioxidant activity and off-flavour generating compounds in soybean varieties.
FIG 10: Effect of storage after radiation (0.5 KGy) on total dialdehyde generation as measured by TBA No. in selected six varieties of soybean.

FIG 11: Effect of storage after radiation (0.5 KGy) on total carbonyl compound generation as measured by carbonyl value in selected six varieties of soybean.

FIG 12: Effect of storage after radiation (0.5 KGy) on lipid peroxidation as measured by LOX activity in six selected varieties of soybean.

Varying in seed coat colour. In general the dark colour seeds (brown and black) showed higher antioxidant capacity as compared to yellow and green colour. The same trend was observed in free radical scavenging activity (FRSA) as measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH). This could be due to the presence of more amount of anthocyanin in black and brown seeds of soybean as compared to yellow and green. The anthocyanin content, which is considered as a strong antioxidant as compared to isoflavones is more in dark colour varieties as compared to light coloured ones (Dixit et al. 2010). Dialdehyde content as measured by TBA No. was maximum in DS9814 with yellow seed coat colour and was minimum in EC439601 with green seed coat colour. Kumar et al. (2010) has reported the FRSA and FRAP of the seeds differing in seed coat colour and they have found very less difference in total antioxidant capacity of seeds as measured by FRAP, having black and green seed coat colour. Even though in range the highest value of total antioxidant was found to be more in green seed coat colour variety. The secondary product of lipid peroxidation, malonaldehyde (MDA) as measured by TBA No. and the level of medium chain aldehydes and ketones as measured by Carbonyl value showed that the yellow seed coat colour variety is having highest carbonyl value whereas AMSS34, black colour variety, the lowest. This could be well correlated with the result as antioxidant capacity of black soybean genotype is very high as compared to yellow soybean genotype and it is known that the lipid peroxidation is inversely proportional to the amount of antioxidant present. The increased antioxidant activity of soybean genotypes at a lower dose of gamma radiation may be due to the formation of free flavonoids, which have been shown to have greater antioxidant effect than glycosides. Variyar et al. (2004) suggested a radiation induced break down of glycosides which leads to the formation of free isoflavones. Isoflavones are the phenolic compounds and increase in their concentration at lower dose of gamma radiation sustain by the corresponding increase in total phenolic content. The increased total phenolic content might be responsible for enhancement in the antioxidant activity after gamma irradiation (0.5 KGy). Several studies have also shown increase in phenolic compounds due to
sudden burst in the activities of key enzymes involved in the synthesis of different types of phenolic compounds in other crop plants (Pendharkar and Nair, 1995; Oudefijikh et al. 2000). Heat generated during gamma irradiation could also liberate low molecular weight antioxidant polyphenol compounds (Niwa et al. 1998). In the present study the energy of ionizing radiation could bring the same effect of liberating phenolic compounds. Thus, in the gamma irradiated soybean genotypes, the increased antioxidant effect may be due to the release of free phenolic compounds including flavonoids. However at the higher doses of gamma irradiation, a flux of free radical is generated causing more deleterious effects and a decrease in antioxidant properties (Dixit et al. 2010). The relative increase of total antioxidant activity was found to be more in soybean seeds having yellow seed coat colour, this may be due to the presence of low initial level of antioxidants in yellow seed coat colour genotype as compared to black seed coat colour genotype before treatment. The decrease in off-flavour generating compounds as indicated by the TBA and Carbonyl value and LOX activity at lower dose of radiation could be attributed to the enhancement in total antioxidant capacity at radiation dose of 0.5 KGy. Dahuja and Madaan (2004) have studied the correlation between level of antioxidant and TBA No. where they have found an inverse relationship between the two parameters. It has been reported that low dose of gamma radiation can modulate antioxidant enzyme activities whereas lipid peroxidation intensity and HO· quantities increased in response to increased radiation intensity (Stajner et al. 2009). Higher doses of radiation induced oxidative stress manifested by high hydroxyl radical (HO·) and MDA quantities affect biomolecules by causing conformational changes, oxidation, rupture of covalent bonds and formation of free radicals (Cheftel et al. 1985). The hydroxyl and superoxide anion radicals that are generated by radiation could modify the molecular properties of the proteins and lipid peroxidation (Helliwell and Gutteridge, 1989). Maximum increase in lipid peroxidation was observed with 2KGy dose of gamma radiation which showed its relation with increased LOX activity at 2KGy and ultimately led to the synthesis of large amount of MDA and other carbonyl compounds giving higher values of TBA No. and carbonyl value at higher dose of gamma radiation Stajner et al. (2007). Gamma radiation of lower dose resulted in a decrease in LOX activity whereas higher dose consequences increased LOX activity (Henderson et al. 1990). Byun et al., (1997) reported an increase in TBA value with the increment of irradiation doses in Korean red ginseng powder which signifies indirectly the induced level of LOX activity.

During storage, irradiated soybean seeds showed decrease in antioxidant activity which indirectly signifies the increased LOX activity and probably this could be the reason of getting higher values of TBA No. and Carbonyl value during storage of soybean seeds. However decrease in antioxidant capacity was comparatively less in yellow seed coat colour variety as compared to black and brown seed coat colour variety, hence enhancement in LOX activity was more.

The total antioxidant capacity of different soybean cultivars effectively controls the off-flavour generating compounds. A lower dose of gamma radiation leads to enhancement in the antioxidant potential of soybean seeds, the enhancement being more in yellow seed coat coloured genotypes followed by seeds with green, brown and black seed coat. The effect of gamma irradiation on the off-flavour generating potential of soybean seeds was retained only up to 15 days of storage and it subsided sharply after that.

ACKNOWLEDGEMENTS

We are grateful to Dr S.K. Lal, Senior Scientist, Division of Genetics, IARI, for providing seeds and to Dr. Bhupinder Singh, Principal Scientist, Nuclear Research Laboratory, IARI for providing gamma irradiation facilities.

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