Impact of γ -irradiation on physicochemical characteristics, lipoxygenase activity and antioxidant properties of finger millet

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Abstract In the present study, the finger millet grains were subjected to γ -irradiation at four different dosage levels: 2, 5, 10, and 15 kGy. Effect of γ -irradiation on the proximate composition, pasting properties, lipoxygenase activity, and antioxidant properties of finger millet flour was evaluated. Moisture, protein, fat, ash, and carbohydrate content of native flour was 12.96, 6.85, 2.73, 1.57, and 73.69 g/100 g, respectively. According to the results of proximate analysis, γ -irradiation significantly ($p < 0.05$) reduced the moisture contents and increased protein contents; however, the fat content of the irradiated flour was equal to or lower than that of the native flour. Pasting properties of the finger millet flour was reduced with the γ -irradiation. Compared with the native flour, lipoxygenase activity and malondialdehyde content decreased and radical scavenging activity, catalase, and superoxide dismutase activities increased in the irradiated flour with increasing γ -irradiation doses. Finally, the present study suggested that the γ -irradiation process improved the antioxidant enzymes and physicochemical characteristics; which could be helpful in formulating tailored made food products.

Keywords Finger millet · γ -irradiation · Antioxidant enzymes · Lipoxygenase · Pasting properties

Introduction

Finger millet (*Eleusine coracana* L.), which is also known as “ragi,” belongs to the family *Poaceae*, and originated in Ethiopia before reaching India. Finger millet is a one of the most important members of millets, is widely cultivated in Asia and Africa (Saleh et al. 2013), and it is a stable food for populations in these developing countries (Chandra et al. 2016). Among millets, finger millet contains vital nutritional components, mainly carbohydrates, calcium, methionine, phytochemicals, seed storage proteins with essential amino acids and dietary fiber (Ramashia et al. 2018). Further, finger millet has been inversely associated with the total cholesterol level, metabolic syndrome, insulin resistance, mortality from cardiovascular diseases, and incidence of type 2 diabetes (Chandra et al. 2016). Finger millet is a resilient crop because it can grow in semi-arid regions and exhibits a substantial level of tolerance to drought stress and severe diseases (Vadez et al. 2012). Despite its significance, finger millet is considered as an orphan crop compared with rice, wheat, and maize because researchers have focused majorly on staple crops during and after the green revolution.

Because of the excellent nutritional value of finger millet, it is increasingly being consumed in various forms. Finger millet is commonly pulverized, and the whole meal is used for traditional food preparations. Besides, finger millet is used to prepare popped and malted varieties as well as noodles for diabetic patients (Shukla and Srivastava 2014); the seed coat matter of finger millet is used to prepare biscuits (Saleh et al. 2013); and fermented products

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of finger millet are prepared using processing technology in the food industry (Saleh et al. 2013; Shukla and Srivastava 2014). Although finger millet is an excellent source of minerals, macro- and micro-nutrients, and provides various health benefits, the tiny millet grain that has a dark-brown seed coat contains some antinutritional components and digestive enzyme inhibitors (Chandra et al. 2016; Kotapati et al. 2016). The consumption of finger millet food products by populations has declined due to rancidity of these processed products leading to the development of an unpleasant aroma or taste (Subba Rao and Muralikrishna 2002). The food products become rancid due to the hydrolysis or autoxidation of polyunsaturated fatty acids (PUFAs) into short-chain fatty acids, which are subsequently converted into flavor-producing aldehydes and ketones that are offensive in taste and aroma (Kotapati et al. 2016). The undesirable aroma and taste results from the action of oxidative enzymes, such as lipoxygenases, on PUFAs.

Lipoxygenases (LOXs) are a group of non-heme iron- or manganese-containing oxidoreductases that catalyze the stereo-specific insertion of molecular oxygen into PUFAs through the β 1-4-pentadiene system (Liavonchanka and Feussner 2006). Volatile flavor compounds produced by PUFAs due to the action of LOXs are important in food products. However, most volatile flavor compounds have a positive effect on the sensory experience; few compounds, such as short-chain C6- or C9-aldehydes and alcohols, may exert a harmful effect, even at low levels; and hexanal has an exceptionally low taste threshold (Hayward et al. 2017). During the preparation of food products, care should be taken to reduce off-flavor production.

Heat treatment is a well-recognized traditional method. However, its use is limited because heat treatment might remove a considerable amount of natural nutrients as bioactive compounds are comparatively unstable to heat. Non-thermal processing technologies, such as high-intensity ultrasound, ozone processing, ionizing radiation, and oscillating magnetic fields, do not require high temperatures, are effective at ambient temperatures, and can maintain the nutritive value, texture, flavor, and aroma of processed foods (Pereira and Vicente 2010). Among these non-thermal technologies, irradiation is a physical process with a high potential and is perhaps the utmost versatile technology (Pereira and Vicente 2010). The irradiation process typically involves the exposure of packaged or nonpackaged food to ionizing or non-ionizing radiation. In general, irradiation of food products creates a change in their physicochemical characteristics; however, the level of modification depends on the food ingredients used, irradiation dose, and type of radiation source (Bhat et al. 2009; Mukisa et al. 2012). Among the three ionizing radiation sources, namely X-rays, γ -rays, and high-energy electrons,

we used γ -rays to treat finger millet grains in the present study.

In recent years, irradiation has been used as a technique for the functional modification of polymers and improving safety and shelf stability of food products (Mukisa et al. 2012). In addition, the γ -irradiation process creates a change in their quality characteristics of flours and enhance the overall nutritional attributes and some desired changes in functional characteristics (Wani et al. 2015). The study of irradiated foods by the joint FAO/IAEA/WHO study group concluded that the application of ionizing radiation at 10 kGy or higher doses will be safe and nutritionally acceptable. Various researches have been carried out to determine the impact of γ -irradiation on the physicochemical properties of flour from different botanical sources (Manjula et al. 2015; Mukisa et al. 2012).

To the best of our knowledge, limited studies have been carried out to study the effects of γ -irradiation on the antioxidative activities, physicochemical characteristics, and LOX activity of finger millet. With this limited information, the present study examined the proximate composition, pasting properties, LOX activity, and antioxidative activities of finger millet treated with different γ -irradiation doses to understand the effect of increasing irradiation doses on finger millet.

Materials and methods

Source of millet samples

Finger millet (variety: Sri Chaitanya VR-847) was procured from the Regional Agricultural Research Station, Vizianagaram, India. The grains were dried and cleaned to remove all damaged grains and foreign matter. The dried and cleaned grain samples were packed in Ziploc double-zipper pouches and stored at room temperature in light condition.

Chemicals

Linoleic acid, L-Methionine, Riboflavin, Malondialdehyde (MDA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Phenyl-methylsulfonyl fluoride (PMSF), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Polyvinylpyrrolidone (PVP), Ethylenediaminetetraacetic acid (EDTA), Hydrogen peroxide (H_2O_2), and Nitroblue tetrazolium (NBT) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Gamma-irradiation of whole finger millet grains

The irradiation process was done at the Central Instrumentation Facility (CIF), Pondicherry University, Puducherry, India, using cobalt-60 as the γ -source at room temperature. Finger millet grains (250 g) were packed in polyethylene pouches, and subjected to irradiation doses of 2, 5, 10, and 15 kGy with a dose rate of 2 kGy/h. The sample without irradiation taken as control.

Flour preparation

After irradiation, the grains were milled into flour by using a hammer mill (CMC-20, Thailand) and sieved through a 250- μ m sieve. The obtained flour was packed in Ziploc double-zipper polyethylene bags and stored at 4 °C till use.

Proximate composition

Proximate composition of native and irradiated flours was determined as per the AOAC standards (AOAC 2000). Moisture content was done by the gravimetric method in a hot air oven at 105 °C until constant weight, and the amount of ash was determined using a muffle furnace at 550 °C. Fat content of the flour was determined using solvent extraction technique. Crude protein was determined according to the micro-Kjeldahl method using a nitrogen conversion factor of 6.25. Carbohydrate content was calculated by difference between 100 and total sum of the percentage of moisture, protein, fat, and ash.

Color characteristics

Surface color of native and irradiated flours was measured using a Color Flex Spectrocolorimeter (D-25, Hunter Lab Associates Inc., USA) as per the method of Reddy et al. (2016). Color parameters namely, L^* represents the lightness from 0 (black) to 100 (white); a^* and b^* represents redness (+ a) to greenness (− a) and yellowness (+ b) to blueness (− b), respectively.

Pasting properties

Pasting properties of native and irradiated flours was measured using a Rapid Visco Analyzer (Newport Scientific Pvt. Ltd., Australia) as per the method of Wang et al. (2018). Briefly, finger millet flour slurry was prepared by mixing the 3.5 gm of flour with 25 mL distilled water. Then, it was heated at 50 °C with continuous stirring for 10 s and held for 1 min. The temperature was elevated to 95 °C (heating rate 6 °C/min) for 7.3 min and held for 5 min and finally cooled at 50 °C (Cooling rate 6 °C/min).

Lipoxygenase activity

For extraction of lipoxygenase (LOX), finger millet grains (100 mg) were homogenized with phosphate buffer (pH 6.4, 50 mM, 100 mL) containing 1 mM PMSF. The resultant dispersion was centrifuged at 12,000 $\times g$ for 10 min at 4 °C. The supernatant (crude extract) was used in the analysis of LOX activity.

The specific activity of LOX was determined as per the method of Kotapati et al. (2016). Briefly, a stock solution of linoleic acid was prepared by solubilizing linoleic acid (100 mg) with Tween-20 (100 mg) in deionized water, and its final volume was adjusted to 5 mL. Care was taken to avoid bubble formation, and turbidity was cleared by adding a few drops of 2 N NaOH. The substrate stock solution was stored at − 20 °C in a dark tube.

To determine LOX activity, crude extract (10 μ L) and phosphate buffer (3 mL, pH 6.4, 50 mM) was taken in a cuvette; after 10 s, 20 μ L of the substrate was added to the reaction mixture. Absorbance was measured using a UV–visible spectrophotometer (Shimadzu-1800, Japan) by monitoring the increase in absorbance at 234 nm at 20 s intervals for 1 min.

Lipid peroxidation

The lipid peroxidation (LPO) levels of finger millet grains were analyzed as per the method of Heath and Packer (1968). Briefly, finger millet grains (100 mg) were homogenized with 5 mL of TCA, and the solution was centrifuged at 10,000 $\times g$ for 10 min at 4 °C. An equal amount of TBA was added to the supernatant. The mixture was boiled for 30 min and cooled, and the absorbance was measured at 532 nm by using a UV–visible spectrophotometer (Shimadzu-1800, Japan). LPO levels were expressed as μ mol of MDA/g of sample.

DPPH radical scavenging capacity

DPPH radical scavenging activity of native and irradiated flours was measured according to the method of Larrauri et al. (1998). Stock solutions of millet flour were prepared in methanol to achieve a concentration of 1 mg/mL (w/v). Briefly, 200 μ L of the extract was mixed with 1.4 mL of 0.2 mM DPPH in methanol. The mixture was vigorously shaken and allowed to stand for 30 min in a dark room at room temperature. The absorbance at 517 nm was measured against blank of 200 μ L methanol in 1.4 mL DPPH radical solutions.

$$\text{Radical scavenging activity (\%)} = ((A_0 - A_s)/A_0) \times 100 \quad (1)$$

where A_S = absorbance of the extract, and A_0 = absorbance of blank.

Preparation of extract for antioxidant bioassay

For enzyme extraction, the finger millet grains (100 mg) were homogenized with phosphate buffer (50 mM, pH 6.4) containing EDTA (1.0 mM) and PVPP (2% w/v) at 4 °C for 5 min. The resulting mixture was centrifuged at 15,000×g for 30 min at 4 °C, and the supernatant was used for determining enzyme activity.

Catalase activity

Catalase (CAT) activity of the finger millet grains was determined as described by Cakmak and Marschner (1992) with a minor modification. Briefly, 0.7 mL of the extract was mixed with phosphate buffer (pH 7.0, 50 mM, 0.1 mL), H_2O_2 (100 mM, 0.1 mL), and EDTA (1 mM, 0.1 mL). The decline in H_2O_2 was calculated as a reduction in absorbance at 240 nm, and activity was expressed as μmol of H_2O_2 consumed per minute.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity of the finger millet grains was assayed using a UV–visible spectrophotometer (Shimadzu-1800, Japan) according to the method described by Botelho et al. (2015). One unit of SOD was defined as the amount of enzyme that inhibits 50% NBT photo reduction at a wavelength of 560 nm.

Statistical analysis

All measurements were carried out in triplicate. All data are presented as means $\pm$ standard deviations. Statistical analysis was performed using SPSS statistical software version 22.0 (SPSS Inc., IL, USA), using one-way ANOVA. Significant differences between samples were evaluated using Duncan's test at a probability level of 0.05.

Results and discussion

Effect of γ -irradiation on proximate composition

Table 1 shows the proximate values of native and irradiated finger millet flours. In general, the moisture content of flour plays a vital role in upholding its quality and extending its shelf life. The data showed that moisture content of flour significantly ($p < 0.05$) decreased on γ -irradiation up to a dose of 15 kGy (12.96 g/100 g in

control and 8.92 g/100 g at 15 kGy). The changes in moisture content of flour might be associated to deterioration in the hydration capability of the protein fractions induced by γ -rays (Bhat et al. 2009; Reddy et al. 2015). In addition, reduction in the moisture on irradiation can be ascribed to the time and dose of radiation delivered. Besides, the water content is excluded as drip and leftovers on the surface of foods, declining the moisture at higher irradiation doses (Chung and Liu 2010; Wani et al. 2015). As shown in Table 1, the amount of crude protein significantly ($p < 0.05$) elevated in finger millet flour on irradiation up to a dose of 15 kGy (6.85 g/100 g in control and 8.51 g/100 g at 15 kGy). The increase in the crude protein on irradiation might be credited to high extractability resulting from the dissociation of complex protein molecules into simple forms (Bhat et al. 2009; Chung and Liu 2010). However, the percent moisture content in individual samples might have also contributed to the observed increase in crude protein level.

The fat content of finger millet flour was not dependent on the irradiation dose applied to finger millet grains (Table 1). The results revealed that the fat content of irradiated flour (range = 2.63–2.78 g/100 g) was equal to or lower than that of native finger millet flour. A study reported that γ -irradiation did not induce any change in the fat content of cowpea and soybeans (Dar et al. 2018). However, the decrease of fat content might be credited to the fact that γ -rays are an ionizing source of radiation and are capable of breaking the ordered structure of large lipid molecules, resulting in the degradation of lipids as well as conversion to other forms, thus contributing to an overall decrease in the documented levels (Bhat et al. 2009). The ash content of finger millet flour samples varied significantly ($p < 0.05$), with values ranging from 1.57 to 1.92 g/100 g. As shown in Table 1, the carbohydrate content significantly differed between native and irradiated finger millet flours at all doses (73.69 g/100 g in control and 77.86 g/100 g at 15 kGy). In flour, carbohydrates exist in a complex form with protein and lipid molecules, and γ -irradiation may facilitate the breakdown of these complex molecules into simple forms, which might have amplified the carbohydrate content (Bhat et al. 2009).

Effect of γ -irradiation on flour color

Table 1 shows the color values of the native and irradiated finger millet flours. The highest L^* value was observed in the native flour (80.87), and this value reduced significantly ($p < 0.05$) when the irradiation dose was increased from 2 to 15 kGy, with values ranging from 72.06 to 76.81. The a^* and b^* values of native flour were 1.89 and 4.69, respectively. However, a^* and b^* values increased significantly ($p < 0.05$) when the irradiation dose was increased

Table 1 Proximate composition and color values of native and γ -irradiated finger millet flours

Parameters*	Irradiation dose (kGy)				
	0	2	5	10	15
Moisture (g/100 g)	12.96 $\pm$ 1.0 ^a	12.01 $\pm$ 0.5 ^b	11.23 $\pm$ 0.4 ^c	9.52 $\pm$ 0.3 ^d	8.92 $\pm$ 0.3 ^e
Protein (g/100 g)	6.85 $\pm$ 0.05 ^c	6.96 $\pm$ 0.04 ^c	7.04 $\pm$ 0.1 ^{bc}	7.55 $\pm$ 0.05 ^b	8.51 $\pm$ 0.1 ^a
Fat (g/100 g)	2.73 $\pm$ 0.05 ^{ab}	2.65 $\pm$ 0.02 ^c	2.75 $\pm$ 0.03 ^a	2.63 $\pm$ 0.03 ^c	2.78 $\pm$ 0.03 ^a
Ash (g/100 g)	1.57 $\pm$ 0.09 ^c	1.65 $\pm$ 0.03 ^d	1.72 $\pm$ 0.03 ^c	1.85 $\pm$ 0.06 ^a	1.92 $\pm$ 0.02 ^a
Carbohydrates (g/100 g)	73.69 $\pm$ 0.9 ^e	75.28 $\pm$ 0.7 ^d	76.93 $\pm$ 0.3 ^c	77.27 $\pm$ 0.5 ^{ab}	77.86 $\pm$ 0.6 ^a
<i>Color parameters</i>					
<i>L*</i>	80.87 $\pm$ 1.0 ^a	76.81 $\pm$ 0.5 ^b	76.38 $\pm$ 0.4 ^b	75.93 $\pm$ 0.3 ^{bc}	72.06 $\pm$ 0.3 ^d
<i>a*</i>	1.89 $\pm$ 0.2 ^e	2.59 $\pm$ 0.4 ^c	2.24 $\pm$ 0.2 ^d	2.65 $\pm$ 0.3 ^b	3.55 $\pm$ 0.3 ^a
<i>b*</i>	4.69 $\pm$ 0.9 ^e	5.68 $\pm$ 0.7 ^d	5.93 $\pm$ 0.3 ^c	7.27 $\pm$ 0.5 ^b	7.66 $\pm$ 0.6 ^a

Values expressed are mean $\pm$ standard deviation. Data of different alphabets in the same row were different with statistical significant ($p < 0.05$)

L, *a** and *b** represent the lightness, redness and yellowness, respectively

from 2 to 15 kGy, with the *a** value ranging from 2.59 to 3.55 and *b** value ranging from 5.68 to 7.66. Furthermore, a decrease in the *L** value with an increase in *a** and *b** values indicated that flour samples were darker in color with a higher level of redness and yellowness. The changes in color values of flour may be due to the caramelization of monosaccharides or maillard browning between sugars and amino acids cleaved from polymers upon γ -irradiation.

Effect of γ -irradiation on pasting properties

Table 2 illustrates the details on the pasting properties of the native and irradiated finger millet flours. In general, pasting parameters and pasting temperature of flour mostly affected by the cultivar type and γ -irradiation dose. As shown in Table 2, the native finger millet flour had the highest values for pasting parameters, namely peak, hold, breakdown, setback, and final viscosities. Except for breakdown viscosity and pasting temperature, the pasting properties of irradiated flours significantly ($p < 0.05$) decreased and steadily with an increase in the irradiation dose from 2 to 15 kGy. From the findings, the peak

viscosity of the native flour was the highest (1933 cP), and peak viscosity steadily decreased from 936 to 34 cP with an increase in the irradiation dose from 2 to 15 kGy (Table 2). The reduction of peak viscosity with irradiation might be attributed to the degradation of amylose and amylopectin in starch by irradiation caused a significant decrease in water binding capacity, and thus a reduction of the swelling ability of starch granules (Ezekiel et al. 2007; Singh et al. 2011). The native flour had the highest hold viscosity (1901 cP), which decreased significantly from 806 to 19 cP with an increase in the irradiation dose from 2 to 15 kGy. Decrease in hold viscosity was due to the disruption of starch granules by the action of shearing and heating.

As shown in Table 2, the breakdown viscosity of the native flour was 32 cP, which increased to up to 152 cP upon irradiation (5 kGy). The increase in breakdown viscosity did not follow any specific pattern and was found to increase initially with an increase in the irradiation dose from 2 to 5 kGy; however, it declined significantly at irradiation doses of 10 and 15 kGy. Previous studies reported that the breakdown viscosity provides an

Table 2 Pasting properties of native and γ -irradiated finger millet flours

Parameters	Irradiation dose (kGy)				
	0	2	5	10	15
Pasting temp (°C)	80.1 $\pm$ 1.0 ^b	80.9 $\pm$ 0.5 ^a	81.1 $\pm$ 0.4 ^a	nd	nd
Peak viscosity (cP)	1933 $\pm$ 10 ^a	936 $\pm$ 5 ^b	433 $\pm$ 4 ^c	69 $\pm$ 4 ^d	34 $\pm$ 2 ^e
Hold viscosity (cP)	1901 $\pm$ 15 ^a	806 $\pm$ 4 ^b	281 $\pm$ 3 ^c	48 $\pm$ 2 ^d	19 $\pm$ 1 ^e
Final viscosity (cP)	2718 $\pm$ 12 ^a	1298 $\pm$ 6 ^b	500 $\pm$ 5 ^c	92 $\pm$ 3 ^d	44 $\pm$ 3 ^e
Breakdown viscosity (cP)	32 $\pm$ 6 ^c	130 $\pm$ 4 ^b	152 $\pm$ 3 ^a	21 $\pm$ 3 ^d	15 $\pm$ 4 ^e
Setback viscosity (cP)	817 $\pm$ 8 ^a	492 $\pm$ 2 ^b	219 $\pm$ 2 ^c	44 $\pm$ 1 ^d	25 $\pm$ 1 ^e

Values expressed are mean $\pm$ standard deviation. Data of different alphabets in the same row were different with statistical significant ($p < 0.05$). nd, not detected

information about the stability of the starch pastes and the tendency of starch granules to resist shear force during heating process (Chung and Liu 2010; Wani et al. 2015). Further, during irradiation, higher breakdown viscosity might be possible due to disintegration of the hydrogen bonds in the starch granules. Besides, the lower breakdown viscosity specifies that the starch granules have good stability (Wani et al. 2015).

In general, the final viscosity represents the capacity of starch granules to produce viscous paste or gel after heating and cooling. The final viscosity of the finger millet flours significantly ($p < 0.05$) decreased from 2718 to 44 cP with increasing irradiation doses, and the reduction was dose dependent (Table 2). Setback viscosity gives the tendency of the starch-based foods to retrograde which affects the texture and other physicochemical properties of foods. It measures the gelling capacity or retrogradation tendency of the paste and is often used as an indicator of the firmness of cooked products (Ezekiel et al. 2007; Wani et al. 2015). As shown in Table 2, the setback viscosity of the finger millet flours significantly ($p < 0.05$) decreased from 817 to 25 cP by γ -irradiation. This result shows that the food products made from irradiated finger millet flour will be soft in texture. In addition, the pasting temperature ranged from 80.1 to 81.1 °C and varied significantly among the flours with increasing irradiation doses. The decrease in pasting temperature might be due to degradation of starch especially amylopectin resulting in its reduced resistance towards swelling (Bhat et al. 2009; Wani et al. 2015).

Effect of γ -irradiation on LOX activity

The effect of γ -irradiation on LOX activity in native and irradiated finger millet grains are shown in Fig. 1. The data shows that the decrease in LOX activity levels of finger millet was observed with increasing doses of γ -irradiation.

Fig. 1 Effect of γ -irradiation on LOX activity of native and irradiated finger millet. Data with different letters were different with statistical significance ($p < 0.05$)

Compared with the native flour, LOX activity levels in irradiated flour decreased significantly by 35% ($p < 0.05$) at 2 kGy but by 30%, 30%, and 26% at 5, 10, and 15 kGy, respectively. The decrease in LOX activity levels was almost the same in samples treated at 5, 10, and 15 kGy.

Recently, the use of finger millet grains has increased in the food industry. To date, studies have not reported accurate results for LOX activity in finger millet, except for one study that reported the characterization of a LOX (*EcLOX*) during seed germination (Kotapati et al. 2016). The amount of PUFAs (74%) is higher than that of saturated fatty acids (26%) in finger millet, and these PUFAs act as substrates for LOX. The inhibition of LOX activity is a significant factor for the food industry because undesirable flavors are produced under the effect of LOX activity (Yang et al. 2016). Hydroperoxides produced by the LOX reaction act as a substrate for downstream enzymes for producing volatile compounds, mainly hexanal being the major volatile compound associated with undesirable flavors. In the present study, we observed that LOX activity levels in irradiated finger millet samples were inversely proportional to the irradiation dose. Kumar et al. (2017) analyzed the LOX-1, -2, and -3 activity levels in soybean and found that the LOX protein content and activity levels decreased considerably when the γ -irradiation dose was increased from 4.8 to 21.2 kGy. Tewari et al. (2015) suggested that the LOX activity level significantly decreased in dark-colored varieties of soybean with different seed coat colors; however, in yellow soybean varieties, the LOX activity level first amplified at lower doses and then reduced slightly at a dose of 1.0 kGy. The decrease in LOX activity levels under an increasing irradiation dose may be due to degradation, damage, or conformational changes in the protein (Cho and Song 2000). The DNA sequence of LOX-free soybean mutant lines (*LOX-1*, -2 and -3) induced by γ -irradiation was compared with that of normal soybean lines; mutations in these genes affected the active site domain of the LOX protein, particularly a highly conserved group of six histidine residues (His) essential for enzymatic activity. These studies have shown the usefulness of irradiation for the substantial inactivation of undesirable flavors generated by LOXs. According to our results, the application of different γ -irradiation doses to finger millet samples reduced LOX activity levels, which may further reduce the production of off-flavor components.

Effect of γ -irradiation on LPO levels

The effect of γ -irradiation on LPO levels in native and irradiated finger millet samples was compared, and results are presented in Fig. 2. The MDA content significantly decreased ($p < 0.05$) to 18%, 26%, 26% and 25% at 2, 5, 10 and 15 kGy, respectively, in irradiated samples

Fig. 2 Effect of γ -irradiation on lipid peroxidation (LPO) levels of native and irradiated finger millet. Data with different letters were different with statistical significance ($p < 0.05$)

compared with the native sample. Similar results have been observed in soybeans having different seed coat colors and in two chickpea varieties (Hameed et al. 2008; Tewari et al. 2015). Tewari et al. (2015) reported that the MDA content significantly decreased in soybeans with brown and black seed coats but significantly increased in soybeans with yellow seed coat. Hameed et al. (2008) reported that the irradiation reduced the MDA content in kabuli chickpea but increased the MDA content slightly in desi chickpea. During cold storage, an irradiation dose of 2.5 kGy mostly postponed membrane lipid peroxidation, therefore confirming the integrity of cell membranes. In wild mushrooms, LPO was inhibited by the antioxidant system under γ -irradiation of 2 kGy, which controlled senescence, browning, water loss, and microbial attack (Fernandes et al. 2013). MDA has been recognized as the final product of LPO, and MDA can destruct the integrity and reduce the fluidity of cell membranes. However, the decrease in the MDA content with an increase in the irradiation dose in finger millet samples may be due to the activation of the cellular defense system. This may also be due to the formation of carbonyl groups by γ -irradiation.

DPPH radical scavenging capacity

The DPPH radical scavenging capacity of native and irradiated finger millet samples is shown in Fig. 3. The results showed a significant increase in the inhibition of free radicals in irradiated finger millet samples compared with native sample when exposed to irradiation at doses of 2, 5, 10 and 15 kGy. We found that free RSA (radical scavenging activity) significantly increased ($p < 0.05$), particularly to 32%, 61%, and 72% at 2, 5, and 10 kGy, respectively, but slightly decreased (54.16%) at 15 kGy when compared with the remaining doses.

Fig. 3 Effect of γ -irradiation on DPPH radical scavenging activity of native and irradiated finger millet. Data with different letters were different with statistical significance ($p < 0.05$)

Free radicals generated by γ -irradiation cause oxidative damage to biomolecules present in plants and these radicals can be protected by the antioxidant system. The increase or decrease in the antioxidant content of foods depends on the dose delivered, exposure time, and the raw material used. The DPPH assay is one of the most prevalent methods used to examine the capability of compounds to act as free radical scavengers and to estimate the antioxidant capacity of foods. The purple DPPH reacts with an antioxidant compound through the donation of electrons from the antioxidant compound, changing the color to yellow and forming stable compounds such as DPP hydrazine (Larauri et al. 1998). Due to the DPPH radical's capacity to bind electrons, it is considered to have a radical scavenging property. Similar results were observed for DPPH activity in various foods during γ -irradiation. An increased DPPH radical scavenging activity was detected in soybean under γ -irradiation between 0.5 and 5 kGy (Variyar et al. 2004). By contrast, in Tunisian millet, a slight decrease in DPPH scavenging activity was detected at doses of 1, 2, and 3 kGy (Mustapha et al. 2014). Finger millet is reported as a potent source of antioxidant compounds; particularly, the grain is richer in polyphenols when compared with barley, rice, maize, and wheat. The increased level of DPPH RSA may be due to the presence of polyphenols and tannins.

Estimation of CAT and SOD activity levels

In the present study, we noticed increased activities of CAT and SOD when exposed to γ -irradiation. The activity levels of CAT were found to be increased in irradiated finger millet samples with an increase in the irradiation dose compared with native samples (Fig. 4a). CAT activities were significantly ($p < 0.05$) increased to 17%, 60.5%, 44% and 63.5% at doses of 2, 5, 10, and 15 kGy. A higher activity level was noticed at a dose of 15 kGy compared with the remaining doses.

Fig. 4 Effect of γ -irradiation on catalase (a) and superoxide dismutase (b) activity of native and irradiated finger millet. Data with different letters were different with statistical significance ($p < 0.05$)

SOD activity levels first significantly increased ($p < 0.05$) and then decreased in finger millet samples when exposed to an increased dose of γ -irradiation. The activity levels increased at 2 kGy (23%) and 5 kGy (8.5%) but slightly increased at 10 kGy (2%) and decreased at 15 kGy (6.5%) when compared with native finger millet samples (Fig. 4b). These results indicate that the SOD activity levels increased at a low dose and then decreased at a high dose in finger millet samples.

In general, irradiation causes oxidative stress when an imbalance between the production of free radicals and the alleviated ability of the cellular system. It leads to oxidative stress and causes the harmful effects. Oxidative stress caused by irradiation decreased due to the catalytic activity of various antioxidant enzymes. Among these enzymes, CAT and SOD play a crucial role. Increased activity levels of CAT and SOD in irradiated finger millet samples may be due to the production of large amounts of hydrogen peroxide (H_2O_2) and superoxide radical (O_2^-) in millet grains under γ -irradiation, stimulating the self-defense systems of irradiated finger millet samples, thus increasing CAT and SOD activities. CATs are the most studied enzymes that reduce oxidative damage by catalyzing the breakdown of H_2O_2 produced during irradiation. SOD consecutively catalyzes the partitioning of O_2^- into ordinary molecular

oxygen (O_2) or H_2O_2 . CAT and SOD enzyme activities were compared between the seedlings of two cultivars of finger millet; the results showed that the basal level of enzyme activities were higher in cv. Bhairabi than in cv. Suvra under γ -irradiation (Venugopal et al. 2016). Finger millet grains have the abundance of antioxidant molecules such as phytic acid, phenolic acid and its derivatives flavonoids and tannins. All these compounds have been reported to have effective radical scavenging activity and can therefore serve as better antioxidants than the grain extracts from other millets (Sripriya et al. 1996).

Conclusion

The results of the present study reveal that exposure to appropriate doses of γ -irradiation from 2 to 15 kGy produced significant changes in the physicochemical and antioxidant properties of finger millet flours. The results show that the γ -irradiation reduced moisture contents and increased protein contents; however, the fat content of the irradiated flour was equal to or lower than that of the native flour. An increase in the irradiation dose reduced LOX activity levels, particularly by 35% at 2 kGy; reduced the MDA content by 26% at 5 and 10 kGy; and increased the activities of DPPH, CAT, and SOD by 72% at 10 kGy, by 63.5% at 15 kGy, and by 23% at 2 kGy, respectively. An increased level of the antioxidant system and a decreased level of LOX and MDA may protect finger millet flour from hazardous effect and oxidative stress. Improvement of physicochemical and antioxidant properties of finger millet on γ -irradiation will be helpful in formulating tailored made food products.

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Compliance with ethical standards

Conflict of interest Authors claim that there is no conflict of interest.

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