Assessment of Storage Stability of Brown Top Millet Treated with Gamma Irradiation

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Abstract
Flour stability is largely influenced by microbial contamination, enzymatic activity, and the storage conditions. To address these issues, millet flour samples were gamma-irradiated at a previously optimized dose of 7.5 kGy to assess its shelf-life extension potential. Both irradiated and untreated samples were stored in two distinct conditions: at 25 °C (65% relative humidity) and at 37 °C (75% relative humidity) for three months. Throughout the storage period, samples were evaluated for moisture, free fatty acids (FFA), titratable acidity, color, peroxide value (PV), and microbial count. In this study, flour stored at 37°C (control) had a higher moisture content than the irradiated samples throughout the storage period. Titratable acidity, peroxide value, and free fatty acid levels of the control samples increased significantly ($p \leq 0.05$) at 37°C. Hunter color parameters (L*, a*, and b*) exhibited varying trends for both control and irradiated samples. Gamma irradiation significantly reduced the initial microbial load and effectively inhibited microbial proliferation during storage. In conclusion, irradiated samples stored at lower temperatures exhibited superior microbial stability and shelf-life quality during 3 months of storage. Thus, irradiation represents a cost-effective and practical method for reducing post-harvest losses of millets.
Keywords: Gamma-irradiation, Titratable acidity, Peroxide value, Colour score, Microbial count

1. Introduction

Millet is typically classified as major millets, minor millets, and pseudo millets. Major millets are pearl millet, sorghum, and finger millet, while minor millets include foxtail, little, proso, brown top, barnyard, kodo, teff, and fonio. The most common pseudo-millets are amaranth and buckwheat [1]. Millets, once called coarse grains are now popularly named as "nutri-cereals" because of their significance in combating malnutrition, ensuring food security and enhancing nutritional quality [2]. Millet consumption not only provides the essential nutrients but also helps in sustainable agriculture [3].

Among the various millets, brown top millet is especially appreciated for its high fiber content, its richness in vitamins-particularly B-complex vitamins-its low glycemic index, and its low levels of simple carbohydrates, such as monosaccharides and disaccharides, which are quickly digested and absorbed, resulting in a rapid increase in blood glucose levels. In food systems, they are an immediate source of energy and influence sweetness, browning reactions and microbial growth [1, 4]. It also contains important phenolic and flavonoid compounds such as gallic acid, caffeic acid, ferulic acid, kaempferol and myricetin [5]. Due to these nutritional and functional qualities, brown top millet, along with a few other millets, has been recognised as a SMART food by ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) [6]. In view of these benefits, brown top millet flour may be employed to make a wide variety of food products.

Flour is a widely consumed staple, but its storage life is restricted by microbial spoilage, oxidative rancidity, and enzymatic reactions. Millet flour, in particular, deteriorates faster than many other flours because of its relatively high fat content. Conventional preservation methods, such as refrigeration or the use of chemical preservatives, may be expensive or undesirable for consumers seeking minimally processed foods. Hence, gamma irradiation is a promising non-thermal alternative which helps in retaining nutrients and extending shelf life by reducing spoilage and pathogenic microorganisms by damaging DNA. The success of this technique is dependent on both the dose of radiation and the storage conditions after treatment, particularly temperature. Although doses between 1 and 10 kGy have been applied, the optimal level varies according to crop quality and desired shelf life [7]. Hence, the existing research is designed to evaluate the combined effects of gamma irradiation and storage temperature on the storage stability of brown top millet flour.

2. Materials and methods

Brown top millet variety HBR-2 (Hungry Brown Top Millet-2) was procured from ICAR–Indian Institute of Millets Research, Hyderabad, India. All other ingredients, including refined wheat flour (Fortune brand), soy flour (Pansari brand), and gluten, were obtained from the local market in Greater Noida. Brown top millet flour was prepared by grinding whole grains in an electric grinder (Sujata, India). The ground material was fractionated through a 0.25 mm mesh and

kept in LDPE (low-density polyethylene) pouches until further use. The flour samples were exposed with irradiation at 7.5 kGy using a Gamma Chamber GC-5000 (Shriram Institute for Industrial Research, Delhi, India). The selected dose was based on the results of an earlier optimized irradiation treatment study conducted on brown top millet, in which the authors used different doses of gamma irradiation to evaluate alteration in the physicochemical properties of brown top millet flour (under review).

2.1 Storage conditions and analysis

The grounded brown top millet flour samples were stored at two different temperatures (25°C at 65% RH and 37°C, at RH 75%). The samples were evaluated at monthly intervals for three months to determine moisture content, color characteristics, free fatty acid content (as an index of rancidity), peroxide value, total titratable acidity and total plate count. Sigma-Aldrich chemicals were used for the analysis.

2.2 Analysis of flour moisture percentage during storage

The moisture present in flour samples was estimated using a standard protocol [8].

2.3 Total titratable acidity (TTA)

TTA was determined using the methodology of Tamani et al. [9]. An aliquot (10 ml) of millet flour was put to an Erlenmeyer flask, along with two mL of phenolphthalein. The light pink color was obtained by titrating the mixture with 0.1 N NaOH solution. The titration volume was measured and used to calculate the total titratable acidity. Total titratable acidity was measured in mL of 0.1 M NaOH/10 g of suspension.

$$\%\ \text{Total titratable acidity} = \frac{\text{IV} \times \text{factor of oleic} \times 100 \times \text{N}}{\text{Sample weight}}$$

Where IV denotes the indicator amount of NaOH utilized during titration, Factor of oleic is the conversion factor used to express acidity as oleic acid, 100 is used for percentage conversion, N represents normality of NaOH solution.

2.4 Determination of peroxide value

The peroxide value was examined with minor modifications of the method recommended by Jalgaonkar et al. [10]. Around 2.0 g of powder and 12 mL of a solvent mixture (chloroform and acetic acid) were taken in a properly washed dried flask. The mixture was filtered under vacuum to get rid of insoluble particles. The filtrate was thoroughly mixed with 0.5 mL of saturated potassium iodide solution and left in the dark for about one minute. Thereafter 30 mL of purified water was added. 0.01% starch was used as indicator and free iodine was titrated with 0.1 N sodium thiosulphate. The peroxide value was expressed as milliequivalents of peroxide per 100 g of sample (S, mL of titrant used for sample; B, the titer value (ml); N, the normality of $Na_2S_2O_3$ (mEq/cm3); W, the sample weight).

$$\textit{Peroxide value} = \frac{(S - B) \times N \times 1000}{W}$$

2.5 Analysis of FFA

The free fatty acid (FFA) content as a percentage calculated as oleic acid was determined by the method of Kamble et al. [11]. About 2.0 g of flour, 100 mL of ethanol and 2 mL of phenolphthalein indicator solution were placed in a 250 mL conical flask. The contents of the flask were titrated with 0.01 N NaOH solution until a persistent pink color appeared. The FFA content was estimated using the formula:

$$\textit{FFA(\% oleic acid)} = \frac{\text{V} \times \text{M} \times 28.2}{\text{W}}$$

Where V is the volume of NaOH solution consumed (mL), M is the molarity of the NaOH solution, and W is the initial mass of the sample.

2.6 Total plate count

Microbiological analysis of stored flour for total bacterial counts was performed at 30-day intervals using the method of Chaturvedi et al. [12]. Microbial populations were enumerated using a standard serial dilution and bacterial count method. Bacterial colonies were represented as logarithmic colony-forming units (CFU)/g of flour. After the end of incubation time, the numbers of microorganisms were calculated with the help of following formula:

$$N = \frac{\Sigma C}{V \times 1,1 \times d}$$

Where, Σ C = total number of colonies retained on the dishes; n_1 = the total number of dishes used for first dilution, V = Inoculum volume used for each dish, d = dilution factor equivalent to the first dilution.

2.7 Color analysis

The color estimation was observed throughout the storage period and compared with the corresponding control samples. Color measurements were performed using a portable colorimeter (CR-400, Konica Minolta Co., Osaka, Japan). The following color constraints were recorded: brightness (L*), red/green (a*), and yellow/blue (b*), where L* ranges from black (0) to white (100).

2.8 Statistical analysis of data

The experimental data, attained from three observations, were exposed to statistical analysis through (ANOVA). The significance of individual terms was determined using Duncan's test in SPSS software, Chicago, IL, USA.

3. Results and discussion

3.1 Moisture content

Moisture content is a substantial factor in regulating the keeping quality of foods. Moisture levels may rise or fall throughout storage, reliant on the place and the packing material. Table 1 illustrates how storage affects the moisture content of brown top millet flour. The

moisture content of the control flour significantly increased at both 25 °C and 37 °C, from 0.24% and 0.91% to 6.76% and 6.95%, respectively. This may be due to atmospheric packing conditions such as changes in temperature and relative humidity [13]. Whereas the irradiated sample showed reduced moisture content from 0.18 to 5.16% at 25 °C and 0.17 to 5.34% at 37 °C. The Codex Alimentarius, which recommends a maximum limit of 15.5% for safe storage, has not been exceeded by these values [14]. Bhatt et al. [15] similarly observed a significant rise in the moisture content of millet flour after 16 days of storage, especially in samples kept at room temperature in cotton bags. Veena et al. [16] reported that moisture gain in papad increased throughout a 90-day storage period. Likewise, Mali and Satyavir [17] discovered that the moisture percentage of millet grain raised from 5.15 to 13.7% after one year of storage at 25°C.

Table 1: Moisture percentage of control and irradiated samples throughout the storage

Moisture content (%) at 25°C		
Storage period	**Control sample**	**Irradiated sample**
0 Day	0.91 ± 1.15^{a}	0.18 ± 0.40^{a}
30 Days	2.77 ± 0.30^{b}	1.87 ± 0.47^{b}
60 Days	4.06 ± 0.55^{c}	3.29 ± 0.25^{c}
90 Days	6.76 ± 0.36^{d}	5.16 ± 0.41^{d}
Moisture content (%) at 37°C		
Storage period	**Control sample**	**Irradiated sample**
0 Day	0.24 ± 0.02^{a}	0.17 ± 0.03^{a}
30 Days	2.88 ± 0.03^{b}	1.72 ± 0.30b
60 Days	4.9 ± 0.30^{c}	3.56 ± 0.04^{c}
90 Days	6.95 ± 0.40^{d}	5.34 ± 0.03^{d}

Readings presented indicate the mean ± SD of triple measurements. Average in columns with distinct superscripts are significantly different ($p \leq 0.05$).

3.2 Total titrable acidity of samples during storage

Titrable acidity (%) 25°C		
Storage period	**Control sample**	**Irradiated sample**
0 Day	0.41 ± 0.03^{a}	0.14 ± 0.03^{a}
30 Days	0.65 ± 0.05^{b}	0.22 ± 0.04^{b}
60 Days	0.78 ± 0.04^{c}	0.44 ± 0.04^{c}
90 Days	0.93 ± 0.03^{d}	0.67 ± 0.04^{d}
Titrable acidity (%) 37°C		
Storage period	**Control sample**	**Irradiated sample**
0 Day	$0.44 \pm 0.04_{a}$	$0.16 \pm 0.05_{a}$
30 Days	$0.73 \pm 0.03_{b}$	$0.58 \pm 0.06_{b}$
60 Days	$1.06 \pm 0.05_{c}$	$0.51 \pm 0.04_{c}$
90 Days	$1.37 \pm 0.04_{d}$	$1.16 \pm 0.04_{d}$

The titrable acidity of the flour increased with increased storage time due to an increase in free fatty acids by enzymatic lipolysis (Table 2). The millet flour sample at 0 days showed 0.41% for the control and 0.14 % for the irradiated flour. Increased storage time and temperature led to a substantial rise in the titrable acidity of flour from 0.41 to 0.93% for the control sample and 0.14 to 0.67% for the irradiated sample at 25°C. A similar trend was followed at 37 °C, where the control sample showed a rapid increase from 0.44 to 1.37% and 0.16 to 1.16 % for the irradiated sample. The titrable acidity was less at 25°C. Similar findings were reported by Akoja et al. [18]

Table 2: Titrable acidity of samples during storage
Average in columns with distinct superscripts are significantly different ($p \leq 0.05$).

3.3 Peroxide value

The peroxide content is used to assess the number of free peroxides produced (hydroxylated radicals and hydroperoxides). Peroxide value found in the control sample of millet flour is 0.72 (control), and in the irradiated sample is 0.71 meq O_2/kg at 0 day. Both control and irradiated samples exhibit a notable rise in peroxide value following a 90-day storage period (Table 3). The control sample exhibited an increase from 0.28 at 25°C to 6.3 at 37°C. The irradiated samples exhibited an increase from 0.67 to 1.97 at 25°C and from 0.67 to 2.31 at 37°C. This may be ascribed to the decomposition of primary oxidation initiation products, such as peroxides [19]. According to Shobha et al. [20] peroxide values should remain within 20-40 meq/kg fat to avoid noticeable rancidity.

Peroxide value 25°C (mEq.O_2.kg^{-1}oil)		
Sample ID	**Control sample**	**Irradiated sample**
0 Day	0.68 ± 0.04^{a}	0.67 ± 0.04^{a}
30 Days	1.05 ± 0.02^{b}	0.85 ± 0.03^{b}
60 Days	2.01 ± 0.02^{c}	1.29 ± 0.03^{c}
90 Days	2.3 ± 0.04^{d}	1.97 ± 0.07^{d}
Peroxide value 37°C (mEq.O_2.kg^{-1}oil)		
0 Day	0.68 ± 0.04a	0.67 ± 0.02a
30 Days	1.76 ± 0.03b	0.95 ± 0.02b
60 Days	2.18 ± 0.04c	1.39 ± 0.01c
90 Days	2.85 ± 0.04d	2.31 ± 0.03d

Table 3: Peroxide value of control irradiated sample during storage
Readings presented indicate the mean ± SD of triple measurements. Average in columns with distinct superscripts are significantly different ($p \leq 0.05$).

3.4 Free fatty acids (FFA)

FFA is commonly used to indicate lipid oxidation. FFAs are generated by the breakdown of fats and oils, and owing to their diminished stability relative to neutral lipids, they exhibit a greater susceptibility to oxidation, resulting in rancidity. As shown in Table 4, FFA levels elevated ($p \leq 0.05$) with increasing storage time, with consistently higher values for samples stored at accelerated temperature (37°C) compared to samples kept at room temperature (25°C). From 0 to 90 days,

FFA content rose markedly, and the increase was more noticeable in the control samples, which may be attributed to their higher moisture content. Elevated moisture promotes faster hydrolytic activity of lipase enzymes, resulting in a greater formation of FFAs (Table 4). Similar trends have been reported by Monika and Mridula [21], who observed significant increases in FFA during storage of sunflower-kernel-based and maize-based nutritious bars. However, values remained within acceptable limits for up to three months. According to Shobha et al. [20], FFA levels should not exceed 1.5%.

Table 4: Variations in the free fatty acid content of samples over a three-month storage period

Free fatty acid (%) at 25°C		
Storage Period	**Control Sample**	**Irradiated Sample**
0 Day	0.66 ± 0.92^{a}	0.42 ± 0.18^{a}
30 Days	3.82 ± 0.91^{b}	2.1 ± 0.1^{1b}
60 Days	4.1 ± 0.90^{c}	3.01 ± 0.75^{c}
90 Days	5.1 ± 0.11^{d}	3.3 ± 0.6^{d}
Free fatty acid (%) at 37°C		
Storage period	**Control sample**	**Irradiated sample**
0 Day	0.53 ± 0.32^{a}	0.24 ± 0.35^{a}
30 Days	2.63 ± 0.35^{b}	2.93 ± 0.70^{b}
60 Days	3.07 ± 0.50^{c}	4.85 ± 0.30^{c}
90 Days	3.76 ± 0.35^{d}	6.07 ± 0.70^{d}

Data are presented as mean ± standard deviation (n=3). Means in columns with distinct superscripts are significantly different ($p \leq 0.05$).

3.5 Microbial load of samples during storage

The microbial load in cereal flours affects product quality over time. This study assesses the viability of bacteria in both control and irradiated millet flour samples at a dosage of 7.5 kGy. Irradiation is a viable technique for this purpose and has been authorized by international organizations, specifically FAO, IAEA, and WHO [22]. This study demonstrated that irradiation diminished populations of bacteria during storage. The irradiated millet flour sample had the least total bacterial count. At 0 day, control samples stored at both 25 °C and 37 °C showed higher microbial counts (4.12–4.19 CFU/g), whereas irradiated samples exhibited a substantially lower initial microbial load (1.01–1.11 CFU/g), indicating the immediate effectiveness of irradiation in microbial reduction. At 3 months, the control samples at both 25 °C and 37 °C were spoiled, suggesting unacceptable microbial levels and reduced shelf life. Conversely, irradiated samples remained unspoiled, though an increase in CFU/g was noted, with higher values at 37 °C (2.44 CFU/g) compared to 25 °C (1.52 CFU/g).

The standard microbial count of flour is 5×10^{4}. Results of the present investigation of irradiated millet flour samples are below the Codex standard and are comparable with those found by Veena et al. [16]. Increased bacterial counts in accelerated settings can be attributed to the higher temperature and humidity maintained in storage period. These results suggest that the brown top millet flour can be stored for 45 days under packed conditions without any appreciable decrease in quality. The current outcomes aligned with earlier studies on millet flours [23, 24]. Elimination of spoilage and pathogenic microorganisms upto 7 kGy.

Table 5: Aerobic plate count of brown top millet flour samples during storage

Storage period	Control sample (25°C)	Irradiated sample (25°C)	Control sample (37°C)	Irradiated sample (37°C)
0 Day	4.12± 0.02	1.01± 0.02	4.19± 0.01	1.11± 0.01
1 Month	4.22± 0.02	1.8± 0.01	4.45± 0.03	1.21± 0.02
2 Month	4.78± 0.02	1.28± 0.02	4.99.28± 0.02	1.99 ± 0.02
3 Month	Spoiled	1.52± 0.02	Spoiled	2.44± 0.02

Values expressed are average ± SD (n=3)

3.6 Color profile of flour samples during storage

Overall color stability of millet flour was affected by storage time, temperature, and gamma irradiation. During the first month, color parameters showed minimal changes, indicating negligible immediate effects of irradiation and a dominant influence of temperature, with the natural yellow color largely retained. In the second month, significant changes were observed, with a decrease in L* values after irradiation, reflecting reduced lightness and yellowness due to pigment degradation. These effects were more evident at 25 °C, whereas at 37 °C irradiation helped maintain higher lightness, suggesting partial protection against thermal deterioration. By the third month, pronounced color degradation occurred in all samples, marked by high L* values, negative a* values, and a sharp decline or reversal of b* values, indicating extensive pigment loss [25, 26, 27].

Table 6: Color score of samples during storage

The reported values are expressed as mean ± SD (n=3). Averages in columns with distinct superscripts are significantly different ($p \leq 0.05$).

Colour score at 25 °C for control sample			
Storage Period	L*	a*	b*
0 Day	78 ± 0.88^{b}	0.4 ± 0.20^{c}	19.82 ± 0.15^{b}
30 Days	82 ± 1.00^{c}	2.17 ± 0.26^{d}	23.34 ± 0.49^{d}
60 Days	74 ± 1.00^{a}	0.28 ± 0.02^{b}	7.12 ± 0.71^{b}
90 Days	97 ± 0.33^{d}	2.3 ± 0.77^{a}	1.84 ± 1.54^{a}
Colour score at 37 °C for control sample			
0 Days	78 ± 0.88^{a}	0.46 ± 0.20^{b}	19.82 ± 0.15^{c}
30 Days	77.47 ± 1.27^{a}	0.87 ± 0.95^{c}	24.59 ± 1.9^{d}
60 Days	81.43 ± 1.48^{c}	1.99 ± 0.42^{d}	4.49 ± 0.36^{b}
90	97.59 ± 0.33^{d}	2.3 ± 0.77^{a}	1.84 ± 1.54^{a}
Colour score at 25 °C for irradiated sample			
0 Day	76.38 ± 0.9^{b}	0.15 ± 0.25^{ab}	21.06 ± 0.12^{c}
30 Days	82.56 ± 1.55^{c}	2.51 ± 0.18^{d}	23.11 ± 0.30^{d}
60 Days	69.94 ± 0.48^{a}	0.74 ± 0.87^{bc}	8.23 ± 0.38^{b}
90 Days	96.19 ± 0.84^{d}	2.08 ± 2.39^{a}	0.91 ± 0.62^{a}
Colour score at 37 °C for irradiated sample			
0 Day	76.38 ± 0.09^{a}	0.15 ± 0.02^{b}	21.06 ± 0.12^{c}
30 Days	86.85 ± 2.15^{b}	2.22 ± 0.25^{c}	8.55 ± 0.38^{b}
60 Days	96.12 ± 0.77^{c}	2.76 ± 0.34^{a}	0.12 ± 1.85^{a}
90 Days	96.12 ± 0.77^{c}	2.76 ± 0.34^{a}	0.12 ± 1.85^{a}

4. Conclusion

Gamma irradiation is effective in extending the shelf life of millet flour. A dose of 7.5 kGy is recommended as optimal for microbial safety. Higher storage temperatures accelerate deterioration, although irradiation still offers protective effects. The irradiation studies indicated decline of the microbial counts. Thus, irradiation has the potential to reduce post-harvest losses in poor nations with inadequate storage infrastructure. The next step would be to better understand the functionality of food systems and develop millet-based products to maximize the use of millets.

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