Original Research Article

Effect of Radiation Process Followed by Fermentation and/or Cooking on Nutritional Quality of High Tannin Sorghum Cultivar

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Author's contribution

This work was carried out (Planning, experiments, analysis and paper writing) in collaboration between all authors

ABSTRACT

Aims: To study the effect of radiation process on nutritional quality of raw and processed flour of a high tannin sorghum cultivar (Karamaka).

Place and Duration of Study: Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Sudan between December 2012 and March 2013

Methodology: The flour of the cultivar was radiated using gamma radiation at doses of 5, 10 and 15 kGy and thereafter the radiated flour was fermented and/or cooked. Antinutrients, protein digestibility, amino acid contents and score, total and extractable minerals were determined.

Results: Radiation of raw flour decreased the level of antinutrients with a concomitant increase in protein digestibility and minerals extractability. Moreover, radiation increased the level of some amino acids and decreased others. Among amino acids the most limiting ones are lysine, methionine plus cysteine and threonine. Fermentation and/or cooking of radiated and non-radiated flour improved the protein digestibility and minerals extractability as well as the level of some amino acids. Most of the amino acids were slightly stable against all treatments with few exceptions.

Conclusion: Radiation of the flour followed by traditional processing greatly improved the nutritional quality of sorghum flour.

Key words: Sorghum, tannin, radiation, traditional treatments, nutrients

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1. INTRODUCTION

Plant foods such as cereals and legumes have consistently been listed as the major potential sources of dietary protein. However, with increasing interest in new food sources and in improved nutritional quality, the seeds of cereals are now receiving more attention. Most of the African people like consuming sorghum as fermented flat bread (Kisra), thick porridge (Aceda), thin fermented gruel (Nasha), boiled grains (Balella) and beverages (Abreh and Hulumur) as a source of protein and minerals [1]. Irradiation could replace or supplement chemical preservatives; in other cases it may have unique advantages whether dry or frozen foods [2].

In agricultural science and food technology, recent research has elucidated new potential applications for radiation. High doses of ionising radiation have been shown to inhibit growth of microbs [3, 4] and to reduce the level of antinutrients [5] which were reported to reduce the availability of proteins and minerals [6, 7, 8, 9, 10]. There are also many reports supporting the use of gamma irradiation as a fungicidal agent [11].

There are insufficient reports about possible effects of gamma radiation on nutritional value of the seeds associated with seed sterilizations. Seeds of different plants that are consumed as food have varying nutrient values which are dependent on the basic constituents of seed proteins [1]. The amino group ($–NH_2$) is the most radiosensitive portion of the amino acids [12]. Extensive research showed that the macronutrients (carbohydrates, proteins and lipids) content is relatively stable against irradiation dose up to 10 kGy [13]. However, Lee *et al*. [14] reported that gamma irradiation affects proteins by causing conformational changes, oxidation of amino acids, rupturing of covalent bonds and formation of protein free radicals. Also, chemical changes in the proteins caused by gamma irradiation include fragmentation, cross-linking, aggregation and oxidation by oxygen radicals that are generated in the radiolysis of water.

Gamma irradiation has a slight effect on the amino acid profile at recommended doses to foods [13]. This effect could be related to the structure of each amino acid. Simple amino acids increased upon irradiation, such as glycine, which undergo reductive deamination and decarboxylation [15]. In addition, aliphatic amino acids with increasing chain length provide additional C–H bonds for interaction with OH radicals which reduces the extent of oxidative deamination [15]. Wang and von Sonntage [16] reported that sulphur-containing as well as aromatic amino acids are, in general, the most sensitive to irradiation, while simple amino acids could be formed by destruction of other amino acids. Mohamed *et al.* [5] reported that millet flour had a severe problem during

storage and it was observed to produce off-flavour and bitter taste which can be overcome by using radiation.

The literature has many reports demonstrating that thermal processing methods improve the nutritional quality of foods. However, there is a scarcity of information relating to the effects of processing with ionizing energy. Therefore, the present work was carried out to investigate the effect of radiation process followed by traditional treatments on nutritional quality of raw and processed high tannin sorghum cultivar.

2. MATERIALS AND METHODS

2.1 Materials

Grains of the sorghum cultivar (Karamaka) were collected from Department of Agronomy, Faculty of Agriculture, University of Khartoum, Sudan. Collected seeds (4 kg) of the cultivar were ground to pass a 0.4 mm screen. All chemicals used for the experiments were of reagent grade.

2.2 Radiation process

The flour with a moisture content of 5.45% was spread uniformly and stored in polyethylene bags of mass of 100 gm, gamma radiation process was conducted at Kaila irradiation processing unit, Sudanese Atomic Energy Corporation (SAEC). The flour was exposed to gamma rays generated by a cobalt-60 source (Gammacell 220, MDS Nordion, Ottawa, Canada). The flour was radiated at 0, 5, 10 and 15 kGy following the procedures described by Helinski *et al*. [17] with a dose rate of ca. 3.2 kGy/h at 24±1 ^{o}C and normal relative humidity. Double side irradiation (exposure to both sides) was performed for uniform dose delivery. A dosimetry system was used to measure the dose received by the batch based on the Gafchromic HD-810 film (International Specialty Products, NJ, USA; FAO/IAEA/USDA 2003). Three dosimeters were included with each batch of flour and read after irradiation with a Radiachromics reader (Far West Technology Inc., CA, USA). All experiments were repeated 3 times and 3 replicates of each flour type were irradiated. Radiated and non-radiated flour of the cultivar was naturally fermented till the pH of the dough reached 4.50 and thereafter cooked for 20 min in a water bath and then dried and ground to pass a 0.4 mm screen for further analysis.

2.3 Tannin determination

Quantitative determination of tannins was carried out using the modified vanillin-HCl method according to Price and Butler [18] using 200 mg sample. A standard curve was prepared expressing the results as catechin equivalents, i.e amount of catechin (mg per

ml) which gives a colour intensity equivalent to that given by tannins after correcting for blank.

2.4 Phytate determination

Phytic acid content was determined by the method described by Wheeler and Ferrel [19] using 2.0 gm dried sample. A standard curve was prepared expressing the results as $Fe(NO_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming a 4:6 iron to phosphorus molar ratio.

2.5 *In vitro* protein digestibility (IVPD) determination

The IVPD of the samples was carried out using single enzyme (pepsin) digestion according to the method of Maliwal [20] in the manner described by Monjula and John [21]. Digestibility was calculated using the following equation:

$$\text{Protein digestibility (\%)} = \frac{\text{N in supernatant} - \text{N in pepsin}}{\text{N in sample}} \text{X100}$$

2.6 Total and extractable minerals determination

For total content, minerals were extracted from the samples by the dry ashing method described by Chapman and Pratt [22]. Minerals were determined using atomic absorption spectrophotometer (Perkin-Elmer 2380). Na and K contents were determined using flame photometer (corning 400). For extractability, minerals in the samples were extracted by the method described by Chauhan and Mahjan [23] using 1.0 gm of the sample. HCl extractability (%) was determined as follows:

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in 0:03NHCl (mg/100g)}}{\text{Total minerals (mg/100g)}} \text{X100}$$

2.7 Amino acid content and score determination

The amino acids composition of the samples was measured on hydrolysates using amino acids analyzer (Sykam-S7130, Tokyo, Japan) based on high performance liquid chromatography technique. Sample hydrolysates were prepared following the method of Moore and Stain [24]. About 200 mg of the sample was taken in a hydrolysis tube. Then 5 ml of 6 N HCl was added to the sample and the tube was tightly closed and incubated at 110 ^{o}C for 24 h. After incubation, the solution was filtered and 200 ml of the filtrate was evaporated to dryness at 140 ^{o}C for 1 h. The hydrolysates after dryness were diluted with 1.0 ml of 0.12 N citrate buffer (pH 2.2). Aliquot of 150 □l of the sample hydrolysates was injected in an action separation column at 130 ^{o}C. Ninhydrin solution and an eluent buffer (solvent A, pH 3.45 and solvent B, pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m length) at a flow rate of 0.7

ml/min. The buffer/ninhydrin mixture was heated in the reactor at 130 ^{o}C for 2 min to accelerate chemical reaction of amino acids with ninhydrin. The products of the reaction mixture were detected at wavelengths of 570 and 440 nm on a dual channel photometer. The amino acids composition was calculated from the areas of standards obtained from the integrator and expressed as gm/100 gm protein.

The essential amino acid (EAA) score was determined by applying the formula:

$$\text{AAS score \%} = \frac{\text{gm of EAA in 100 gm test protein}}{\text{gm of EAA in 100 gm FAO/WHO reference pattern}} \text{X100}$$

2.8 Statistical analysis

Each determination was carried out on three separate samples and analysed in duplicate on dry weight basis; the figures were then averaged. Data were assessed by the analysis of variance [25]. Comparisons of means for treatments were made using Duncan's multiple range tests. Significance was accepted at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

The effect of gamma irradiation and/or traditional processings on tannin and phytate contents and *in vitro* protein digestibility of sorghum cultivar (Karamaka) are shown in Table 1. Ten cultivars of sorghum were investigated for tannin content; Karamaka was found to contain higher amount of tannin (2.90%) and accordingly designated as a high tannin cultivar. Tannin content of raw flour was significantly ($P \leq 0.05$) reduced after radiation and the level of reduction increased with an increase in radiation dose as reported by Mohamed *et al*. [5] for millet cultivars. The reduction in tannin content after radiation is likely to be due to chemical degradation of tannin by the action of free radicals produced by the radiation. Traditional processing (fermentation and/or cooking) of raw flour significantly ($P \leq 0.05$) decreased the level of tannin which agree with that reported by Idris *et al*. [26], who stated that combination of fermentation and cooking of sorghum improved the nutritional quality and drastically reduced tannin content to safe levels than any other processing methods. Also Osman [27] reported that fermentation markedly reduced tannin content of sorghum cultivars. The reduction in tannin content due to cooking is likely to be due to denaturation of tannin by heat treatment while that due to fermentation is likely to be due to biochemical activity of fermenting organisms [28]. However, when traditional treatments were applied in combination with radiation process, further reduction in tannin content was observed with a minimum value of

0.465% obtained for fermented and cooked seeds that radiated at a dose of 10 KGy. The results are in agreement with those reported by Brigide and Canniatti-Brazaca [29] who observed that tannin content was inversely correlated with the applied irradiation doses.

Radiation of raw flour significantly ($P \leq 0.05$) reduced the level of phytate and the rate of reduction increased with an increase in radiation dose as reported by Mohamed *et al*. [5] for millet cultivars. This reduction in phytic acid content is probably due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by the radiation or cleavage of the phytate ring itself [30]. Traditional processing (fermentation and/or cooking) significantly ($P \leq 0.05$) decreased the level of phytate as reported by Idris *et al.* [26], who found that combination of cooking and fermentation of sorghum significantly ($P \leq 0.05$) reduced the level of phytate. Also Osman [27] reported that fermentation markedly and significantly ($P \leq 0.05$) reduced phytate content of sorghum cultivars. The reduction in phytate content due to fermentation is likely due to biochemical activity of the microbial enzyme phytase which hydrolyzed phytate. However, when traditional treatments were applied in combination with radiation process, further reduction was observed with a minimum value of 105.40 mg/100gm obtained for fermented flour radiated at 15 KGy. Osman *et al*. [31] reported that phytic acid and tannin contents of broad bean were significantly ($P \leq 0.05$) reduced as a result of radiation. El- Niely [32] stated that radiation after processing significantly ($P \leq 0.05$) decreased the level of phytic acid of legumes. The reduction in phytic acid during radiation process is likely to be due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by radiation or might be due to cleavage of the phytate ring itself [12]. Both cereal and microbial phytases can contribute to a reduction in phytate during fermentation process [28]. Duodu *et al*. [33] reported that cooking did not decrease phytic acid in sorghum porridge, but cooking and irradiation caused a significant decrease (40%).

The effect of gamma irradiation on *in vitro* protein digestibility (IVPD) of Karamaka cultivar is shown in Table 1. Radiation alone of raw flour was found to be effective in improving the IVPD at low dose of 5 KGy and as the level of radiation increased the IVPD significantly ($P \leq 0.05$) increased. Traditional processing (fermentation and/or cooking) of raw flour significantly ($P \leq 0.05$) increased the level of IVPD as reported by AbdElhaleem *et al*. [34]. When traditional treatments were applied in combination with radiation process, further improvement in IVPD was observed with a maximum value of

42.71% for fermented flour radiated at 15 KGy. The results obtained agree with results obtained by El-Niely [32] who studied the influence of irradiation on *in vitro* protein digestibility of broad beans irradiated at 2.5, 5, 10 and 20 KGy. He observed that the *in vitro* protein digestibility had been improved by 4.5, 10, 16 and 20%, respectively. The improvement in IVPD of fermented flour before and after radiation is likely to be due to reduction in antinutrients, especially phytate, which degraded by phytase during fermentation as reported by Sokrab *et al.* [10]. On the other hand, the reduction in IVPD after cooking of raw ad radiated flour may be due to disulphide bond formation resulting in disulphide cross-linked protein oligomers and polymers. These polymers form a coat reducing the accessibility of the protein bodies to enzymatic attack [33].

Table 2 shows the content and HCl extractability of some major minerals (Na, K and Mg) of high tannin sorghum cultivar (Karamaka) as affected by radiation and/or traditional processings. For the raw flour, Na content was found to be 22.60 mg/100 g and out of this amount about 67.4% was found to be extractable; K was 470.10 mg/100 g with extractability of 50.0% and Mg was 50.10 mg/100 g and out of this amount about 52.50% was extractable. Variations in extractability between Na, K and Mg are likely to be due to the binding ability of such minerals with antinutrients especially phytate which are observed to form complexes with minerals and reduced their extractability as reported by Idris *et al*. [26]. Radiation of raw flour had no significant ($P \leq 0.05$) effect on total Na, K and Mg content but significantly ($P \leq 0.05$) increased the extractability of such minerals. The increment in minerals extractability after radiation could be attributed to reduction in the antinutrients. Fermentation of raw flour significantly ($P \leq 0.05$) increased both total and extractable Na, K and Mg. Fermentation leads to lowering of pH as a consequence of bacterial production of lactic and acetic acids, which is favourable for phytase activities, resulting in lowering of phytate. The reduction in phytate resulted in an improvement in minerals extractability.

Cooking of raw flour decreased both total and extractable minerals. Cooking of raw fermented dough alleviates the effect of cooking and significantly ($P \leq 0.05$) increased minerals extractability. Fermentation of radiated flour significantly ($P \leq 0.05$) increased both total and extractable minerals. Radiation of the flour at 5, 10 or 15 KGy followed by cooking decreased minerals content but increased their extractability as compared to raw samples. Cooking of irradiated fermented dough caused a significant ($P \leq 0.05$) improvement in extractable minerals. Moreover, as the level of radiation increased the degree of improvement in minerals extractability was significant ($P \leq 0.05$). The results

obtained showed that processing of radiated flour significantly ($P \leq 0.05$) increased the extractable Na, K and Mg with an increase in radiation dose but had no significant ($P \leq 0.05$) effect on total minerals. Generally, the increase in extractable minerals during traditional processing before and after radiation could be attributed to the reduction in the level of antinutrients as a result of such treatments [6, 9].

Table 3 shows the content and HCl extractability of some trace minerals (Zn, Cu, Mn and Co) of sorghum cultivar (Karamaka) as affected by radiation and/or traditional processings. The raw flour of the cultivar contained 2.54, 0.42, 3.35 and 0.15 mg/100 g of Zn, Cu, Mn and Co, respectively, and out of this amount about 49.29, 34.17, 39.42 and 62.07 were found to be extractable for the minerals, respectively. Variations in extractability between trace minerals are likely to be due to the binding ability of such minerals with antinutrients as reported by Idris *et al*. [26]. Radiation of raw flour had no significant ($P \leq 0.05$) effect on total minerals but significantly ($P \leq 0.05$) increased the HCl extractability of such minerals. Fermentation of raw flour slightly increased total minerals and significantly ($P \leq 0.05$) increased the extractable ones. Cooking of raw flour had no significant ($P \leq 0.05$) effect on total and extractable minerals. Cooking of fermented dough alleviates the effect of cooking and significantly ($P \leq 0.05$) increased the extractability of trace minerals but did not significantly ($P \leq 0.05$) affect total minerals. Radiation at all levels followed by fermentation of raw flour gave varying changes in total and extractable minerals for the cultivar but, generally, there is a significant ($P \leq 0.05$) increase in total as well as in extractable minerals compared to raw flour. However, cooking of radiated flour had no significant effect on content but significantly ($P \leq 0.05$) increased extractability of trace minerals. Cooking of radiated/fermented dough caused a significant ($P \leq 0.05$) improvement in extractable minerals but did not affect total minerals. The results obtained showed that as the level of radiation increased the degree of improvement in minerals extractability was significant ($P \leq 0.05$). The results showed that processing of irradiated flour significantly ($P \leq 0.05$) increased the extractable trace minerals with an increase in radiation dose but had no significant ($P \leq 0.05$) effect on total trace minerals. The increment in extractable minerals during traditional processing before and after radiation could be attributed to the reduction in the level of antinutrients as a result of such treatments.

Table 4 shows the effect of radiation process on amino acids composition of raw and treated flour of Karamaka cultivar. Radiation of raw flour of the cultivar at 5, 10 and 15 kGy decreased the level of all amino acids except phenylalanine plus tyrosine with a

concomitant decrease in amino acid score except that of phenylalanine + tyrosine. Joseph *et al*. [35] reported that with the exception of tyrosine the amino acids in cowpea (acidic, basic, polar and non-polar amino acids) were decreased significantly with increase in gamma radiation compared to their respective controls. Hooshmand and Klopfenstein [36] reported that lysine content decreased by 7 and 13%, respectively, when maize and wheat flours were irradiated at 7.5 kGy. The observed increase in some free amino acid content due to exposure to ionizing radiation is in agreement with the findings reported by Satter *et al*. [37], who documented increases in essential and nonessential amino acids of soybean when irradiated at a dose level of 10 kGy.

The precise effect of ionizing radiation on free amino acid content depends on various factors, such as sensitivity of the exposed system, the type of particular functional tissue and even other conditions, such as aqueous soaking after irradiation, as has been indicated by Siddhuraju *et al*. [12]. Wang and von Sonntage [16] reported that sulphur-containing as well as aromatic amino acids are, in general, the most sensitive to irradiation, while simple amino acids could be formed by destruction of other amino acids. Bhat *et al*. [38] reported that radiation is an efficient process in maintaining the nutritional potential of *Mucuna pruriens* seeds. Fermentation of raw flour of the cultivar increased the level of lysine and phenylalanine plus tyrosine (Table 4). It has been reported that fermentation of Sicklepod leaves significantly ($P \leq 0.05$) increased alanine, valine, cystine, isoleucine, leucine and ammonia contents while aspartic acid, threonine, serine, glutamic acid, tyrosine, histidine, lysine and arginine contents were significantly ($P \leq 0.05$) decreased [7]. Radiation at 5, 10 and 15 kGy followed by fermentation significantly ($P \leq 0.05$) increased the level of phenylalanine plus tyrosine of the cultivar compared to the respective control.

Cooking of raw flour of the cultivar slightly increased the level of leucin and significantly ($P \leq 0.05$) that of phenylalanine plus tyrosine. Fageer *et al*. [39] observed that cooking of corn in water increased lysine, valine, leucine and phenylalanine while threonine, methionine and isoleucine were decreased. Radiation at 5, 10 and 15 kGy followed by cooking significantly ($P \leq 0.05$) increased phenylalanine plus tyrosine of the cultivar. Cooking of fermented dough of radiated and non-radiated flour increased phenylalanine plus tyrosine. Osman *et al*. [7] reported that cooking of the fermented Sicklepod leaves significantly ($P \leq 0.05$) decreased aspartic acid, threonine, serine, glutamic acid, glycine, tyrosine, phenylalanine and arginine contents. The results obtained showed that the effect of radiation alone on amino acids composition was minor as both fermentation and

cooking had a negative effect on some amino acids. Moreover, radiation process is an ideal method in preserving material as reported for millet flour [5].

4. Conclusion

The nutritional quality of the studied samples suggested that radiation processing up to 15 kGy had reduced the level of antinutrients with a concomitant increase in protein digestibility and minerals extractability. Therefore, radiation can be used to alleviate the severe problem of antinutrients. Moreover, when accompanied by fermentation or cooking of fermented dough an improvement in protein and minerals availability was observed. However, cooking alone of radiated or non-radiated flour had an adverse effect on protein and minerals availability as well as amino acid content.

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Table 1. Tannin (%), Phytate (mg/100g) and percent *in vitro* protein digestibility (IVPD) of sorghum cultivar Karamaka as influenced by radiation followed by traditional treatments

Radiation dose (KGy)	Treatment	Parameter		
		Tannin	Phytate	IVPD
0	Raw	2.90 ±0.23^{a}	272.00 ±0.16^{a}	35.20 ±0.17^{h}
	Fermented	1.752 ±0.07^{e}	129.20 ±1.98^{m}	40.69 ±0.10^{d}
	Cooked	1.137 ±0.00^{i}	217.60 ±0.23^{d}	14.12 ±0.09^{p}
	Fermented/cooked	0.586 ±0.32^{m}	160.480 ±0.1^{h}	20.49 ±0.16^{l}
5	Raw	2.572 ±0.22^{b}	236.37 ±2.13^{b}	37.75 ±1.86^{g}
	Fermented	1.657 ±0.01^{f}	112.53 ±0.11^{n}	41.51 ±1.85^{c}
	Cooked	1.050 ±0.02^{j}	191.92 ±0.21^{f}	15.01 ±1.06^{o}
	Fermented/cooked	0.528 ±0.03^{n}	138.98 ±0.13^{i}	21.56 ±0.01^{k}
10	Raw	2.242 ±0.01^{c}	226.58 ±0.05^{c}	38.47 ±0.09^{f}
	Fermented	1.352 ±2.91^{h}	132.30 ±1.95^{k}	42.18 ±1.82^{b}
	Cooked	0.913 ±0.05^{k}	109.69 ±0.11^{o}	15.56 ±2.91^{n}
	Fermented/cooked	0.465 ±0.15^{o}	136.41 ±0.84^{j}	21.70 ±0.01^{j}
15	Raw	2.227 ±0.020^{d}	217.33 ±0.04^{e}	38.86 ±0.05^{e}
	Fermented	1.358 ±0.02^{g}	104.40 ±0.12^{p}	42.71 ±1.99^{a}
	Cooked	0.840 ±0.05^{l}	176.91 ±1.56^{g}	15.83 ±0.01^{m}
	Fermented cooked	0.444 ±0.01^{p}	131.11 ±0.92^{l}	22.08 ±0.08^{i}

Values are means of three replicates ± SD. Means not sharing a common superscript(s) in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple Range Test**.**

Table 2. Total (mg/100gm) and extractable (%) sodium (Na), potassium (K) and magnesium (Mg) of Karamaka cultivar as influenced by gamma radiation followed by traditional treatments

Radiation dose (kGy)	Treatment	Minerals					
		Na		K		Mg	
		Total	extractable	Total	extractable	Total	extractable
0	Raw	22.60 ±0.01^{d}	67.40 ±0.02^{j}	470.10 ±0.05^{fg}	50.00 ±0.01^{m}	50.00 ±1.98^{ef}	52.50 ±0.03^{j}
	Fermented	23.90 ±0.11^{c}	73.40 ±0. 02^{h}	495.32 ±1.35^{c}	73.30 ±0.01^{d}	62.20 ±1.97^{ab}	75.00 ±0.19^{e}
	Cooked	19.87 ±2.04^{n}	69.90 ±0.11^{i}	458.00 ±0.08^{k}	46.20 ±0.78^{n}	47.40 ±0.01^{i}	50.60 ±0.25^{k}
	Fermented/cooked	20.30 ±0.03^{n}	72.30 ±2.01^{h}	469.80 ±1.06^{g}	70.00 ±0.03^{e}	56.80 ±2.03^{b}	72.30 ±0.14^{g}
5	Raw	22.10 ±1.09^{def}	76.60 ±2.97^{g}	470.00 ±1.76^{fg}	57.40 ±0.43^{i}	49.80 ±0.05^{fg}	75.00 ±2.42^{e}
	Fermented	24.50 ±0.43^{b}	88.60 ±0.09^{b}	479.40 ±0.53^{d}	75.10 ±0.52^{c}	64.40 ±0.98^{a}	76.10 ±0.07^{d}
	Cooked	21.33 ±0.75^{g}	70.50 ±0.52^{i}	450.00 ±0.87^{l}	53.30 ±0.01^{l}	43.80 ±0.08^{j}	50.00 ±0.09^{k}
	Fermented/cooked	22.31 ±0.98^{def}	79.90 ±0.52^{f}	466.00 ±0.09^{h}	67.10 ±0.06^{f}	53.80 ±1.23^{c}	73.30 ±0.01^{f}
10	Raw	22.52 ±1.18^{de}	81.40 ±3.03^{e}	473.00 ±0.38^{e}	60.00 ±0.42^{h}	50.40 ±1.23^{e}	67.30 ±0.78^{h}
	Fermented	25.3 ±0.87^{a}	97.70 ±0.52^{a}	496.40 ±0.42^{b}	81.20 ±0.64^{b}	65.50 ±0.42^{a}	100.0 ±0.07^{a}
	Cooked	21.91 ±0.76^{f}	86.60 ±0.64^{c}	460.60 ±0.98^{j}	56.20 ±0.92^{j}	47.20 ±0.76^{i}	55.90 ±0.09^{i}
	Fermented/cooked	21.93 ±0.23^{f}	86.60 ±0.85^{c}	465.00 ±0.65^{i}	80.40 ±0.21^{b}	51.20 ±0.01^{d}	92.50 ±0.05^{b}
15	Raw	22.5 ±1.97^{de}	84.00 ±2.43^{d}	470.50 ±0.12^{f}	63.40 ±0.91^{g}	49.60 ±1.13^{g}	75.10 ±0.23^{de}
	Fermented	25.00±0.76^{ab}	88.00 ±0.53^{b}	500.00 ±0.75^{a}	86.00 ±0.23^{a}	66.40 ±0.18^{a}	83.50 ±0.55^{c}
	Cooked	21.90 ±1.87^{f}	81.40 ±0.09^{e}	450.00 ±0.12^{l}	54.80 ±0.43^{k}	48.20 ±0.98^{h}	73.90 ±0.91^{f}
	Fermented /cooked	22.00 ±0.23^{ef}	86.63 ±0.65^{c}	473.20 ±0.76^{e}	85.80 ±0.98^{a}	53.80 ±0.65^{c}	91.00 ±0.01^{b}

Values are means of three replicates (± SD). Means not sharing a common superscript(s) in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple Range Test**.**

Table 3. Total (mg/100gm) and extractable (%) trace minerals of Karamaka cultivar as affected by gamma irradiation followed by traditional treatments

Radiation dose (KGy)	Treatment	Minerals							
		Zn		Cu		Mn		Co	
		Total	extractable	Total	extractable	Total	extractable	Total	extractable
0	Raw	2.54 ±0.01^{a}	49.29 ±0.05^{o}	0.42 ±0.17^{a}	34.17 ±0.09^{l}	3.35 ±1.24^{a}	39.42 ±0.01^{m}	0.15 ±0.88^{a}	62.07 ±1.07^{l}
	Fermented	2.57 ±0.11^{a}	71.02 ±0.23^{g}	0.48 ±0.02^{a}	42.01 ±0.45^{i}	3.42 ±0.17^{a}	64.08 ±0.26^{g}	0.17 ±0.04^{a}	81.89 ±0.71^{e}
	Cooked	2.52 ±0.12^{a}	49.29 ±0.01^{o}	0.42 ±0.01^{a}	33.43 ±0.56^{m}	3.29 ±0.13^{a}	33.47 ±0.11^{n}	0.14 ±0.11^{a}	62.44 ±0.52^{l}
	Fermented/cooked	2.55 ±0.46^{a}	67.43 ±0.98^{i}	0.44 ±0.32^{a}	41.60 ±0.09^{i}	3.37 ±0.98^{a}	63.70 ±0.07^{g}	0.18 ±0.13^{a}	78.80 ±0.08^{f}
5	Raw	2.51 ±0.01^{a}	52.58 ±0.32^{m}	0.41 ±0.02^{a}	40.90 ±0.01^{j}	3.30 ±0.12^{a}	42.90 ±0.16^{i}	0.19 ±0.08^{a}	65.70 ±0.13^{j}
	Fermented	2.57 ±0.75^{a}	84.51 ±0.63^{e}	0.42 ±0.07^{a}	54.90 ±0.31^{e}	3.37 ±0.13^{a}	65.90 ±0.75^{f}	0.21 ±0.54^{a}	86.00 ±0.01^{d}
	Cooked	2.55 ±0.09^{a}	51.12 ±0.51^{n}	0.49 ±0.23^{a}	36.30 ±0.97^{k}	3.31 ±0.98^{a}	48.10 ±0.35^{k}	0.14 ±0.09^{a}	64.30 ±0.06^{k}
	Fermented/cooked	2.55 ±0.16^{a}	82.86 ±0.09^{f}	0.40 ±0.95^{a}	49.10 ±0.94^{f}	3.35 ±0.09^{a}	68.10 ±0.24^{e}	0.15 ±0.87^{a}	82.60 ±0.15^{e}
10	Raw	2.51 ±0.14^{a}	64.14 ±0.81^{k}	0.43 ±0.23^{a}	44.00 ±0.34^{h}	3.32 ±0.04^{a}	50.00 ±1.05^{j}	0.17 ±1.92^{a}	70.00 ±0.05^{h}
	Fermented	2.58 ±0.32^{a}	95.20 ±0.91^{b}	0.46 ±0.81^{a}	63.90 ±0.12^{b}	3.34 ±0.65^{a}	82.30 ±0.86^{b}	0.26 ±0.54^{a}	94.60 ±0.75^{a}
	Cooked	2.51 ±0.43^{a}	60.54 ±0.37^{l}	0.47 ±0.76^{a}	46.90 ±0.98^{g}	3.32 ±0.01^{a}	51.10 ±0.43^{i}	0.15 ±0.98^{a}	69.70 ±0.43^{h}
	Fermented /cooked	2.55 ±0.75^{a}	89.65 ±0.83^{d}	0.41 ±0.01^{a}	61.90 ±0.42^{c}	3.36 ±0.06^{a}	71.90 ±0.09^{d}	0.17 ±0.81^{a}	87.40 ±0.01^{c}
15	Raw	2.54 ±0.23^{a}	69.67 ±0.65^{h}	0.45 ±0.05^{a}	44.20 ±0.16^{h}	3.31 ±0.43^{a}	53.40 ±0.07^{h}	0.15 ±0.06^{a}	73.80 ±0.05^{g}
	Fermented	2.54 ±0.65^{a}	97.62 ±0.71^{a}	0.42 ±0.54^{a}	68.60 ±0.43^{a}	3.32 ±0.01^{a}	88.40 ±0.75^{a}	0.18 ±0.98^{a}	93.10 ±0.43^{b}
	Cooked	2.51 ±0.94^{a}	66.43 ±0.93^{j}	0.42 ±0.91^{a}	44.00±0.53^{h}	3.30 ±0.01^{a}	51.40 ±0.45^{i}	0.19 ±0.61^{a}	68.40 ±0.22^{i}
	Fermented /cooked	2.55 ±0.23^{a}	91.48 ±1.09^{c}	0.44 ±0.38^{a}	60.20 ±0.05^{d}	3.32 ±0.02^{a}	77.70 ±0.11^{c}	0.20 ±0.01^{a}	85.40 ±0.16^{d}

Values are means of three replicates (± SD). Means not sharing a common superscript(s) in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple Range Test**.**

Table 4. Amino acids composition (mg/100gm protein) and score (AAS) of Karamaka cultivar as affected by gamma radiation followed by traditional treatments

Radiation dose (KGy)	Treatment	Amino acid													
		Isoleucine		Leucine		Lysine		Methionine +Cysteine		Phenyl alanine + Tyrosine		Threonine		Valine	
		Total	AAS	Total	AAS	Total	AAS	Total	AAS	Total	AAS	Total	AAS	Total	AAS
0	Raw	3.50^{a}	125.00	12.98^{c}	196.67	1.91^{b}	32.93	1.79^{a}	71.60	6.94^{p}	110.10	1.970^{a}	57.00	4.62^{a}	132.00
	Fermented	3.17^{d}	113.20	12.52^{h}	189.62	1.98^{a}	34.13	1.77^{b}	70.84	8.55^{e}	135.70	1.42^{c}	41.88	4.24^{e}	121.10
	Cooked	3.05^{i}	108.90	13.00^{b}	196.91	0.98^{k}	16.90	1.66^{i}	66.52	8.34^{g}	132.40	1.38^{g}	40.60	4.21^{g}	120.20
	Fermented/cooked	3.09^{g}	110.30	12.87^{d}	195.05	1.22^{g}	21.03	1.76^{c}	70.50	8.06^{j}	127.90	1.31^{i}	38.56	4.15^{i}	118.50
5	Raw	2.72^{j}	97.00	12.16^{j}	184.18	1.33^{f}	22.90	1.71^{h}	68.40	7.33^{n}	116.30	1.11^{m}	32.50	3.77^{n}	107.60
	Fermented	3.30^{b}	117.90	8.91^{k}	134.90	1.88^{c}	32.46	1.72^{f}	68.70	8.68^{b}	137.80	1.41^{d}	41.50	4.47^{b}	127.80
	Cooked	3.08^{h}	110.10	8.130^{m}	123.20	0.79^{n}	13.62	1.64^{k}	65.70	8.42^{f}	133.70	1.40^{f}	41.10	4.24^{d}	121.20
	Fermented/cooked	3.01^{j}	107.30	12.34^{i}	187. 00	1.13^{h}	19.55	1.76^{d}	70.30	8.15^{i}	129.40	1.10^{n}	32.30	4.10^{j}	117.00
10	Raw	2.50^{o}	89.40	12.74^{f}	193.00	0.93^{l}	16.03	1.65^{j}	66.04	7.07^{o}	112.20	0.83^{o}	24.40	3.81^{l}	99.50
	Fermented	2.84^{k}	101.40	6.62^{o}	100.30	1.81^{d}	31.27	1.54^{n}	61.60	7.74^{k}	122.80	1.28^{j}	37.60	3.82^{k}	109.20
	Cooked	3.12^{f}	111.30	8.37^{l}	126.80	0.62^{o}	10.71	1.73^{e}	69.00	8.61^{d}	136.70	1.57^{b}	46.30	4.24^{f}	121.10
	Fermented/cooked	3.19^{c}	113.80	12.59^{g}	190.80	1.09^{i}	18.72	1.62^{l}	65.00	8.25^{h}	130.90	1.40^{e}	41.20	4.38^{c}	125.20
15	Raw	2.50^{p}	89.36	13.00^{a}	196.95	0.92^{m}	15.86	1.58^{m}	62.70	7.36^{m}	116.80	0.83^{o}	24.50	3.38^{p}	96.49
	Fermented	2.70^{m}	96.40	6.38^{p}	96.60	1.81^{e}	31.12	1.51^{p}	60.30	7.73^{l}	122.70	1.19^{k}	34.90	3.78^{m}	107.90
	Cooked	2.62^{n}	93.70	7.16^{n}	108.40	0.54^{p}	9.33	1.51^{o}	60.40	8.67^{c}	137.60	1.34^{h}	39.90	3.51^{o}	100.30
	Fermented/cooked	3.12^{e}	111.50	12.76^{e}	193.30	1.00^{j}	17.28	1.71^{g}	68.60	8.69^{a}	137.90	1.18^{l}	34.70	4.19^{h}	119.60

Values are means of duplicate samples. Means not sharing a common superscript(s) in column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple Range Test.