Effect of radiation process on antinutrients and HCl extractability of calcium, phosphorus and iron during processing and storage

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Whole and dehulled flours of millet cultivars Ashana and Dembi were stored for 30 and 60 days before and after radiation and/or cooking. Phytic acid and polyphenols contents were assayed for all treatments. The results revealed that the storage period was found to have no effect on phytate and polyphenols contents. Moreover, dehulling of the grains reduced more than 50% of phytate and polyphenols of both cultivars. Cooking of the raw whole and dehulled flour significantly (P ≤ 0.05) reduced phytate and polyphenols contents for both cultivars. Radiation process alone had no effect on phytate and polyphenols contents but when followed by cooking significantly (P ≤ 0.05) reduced the level of such antinutrients for the whole and dehulled flour of both cultivars. Dehulling alone significantly (P ≤ 0.05) decreased Ca and P content but slightly decreased Fe content. Radiation alone or in combination with cooking was found to have slight effect on minerals content of the whole and dehulled raw flour for both cultivars. Cooking alone or in combination with radiation of whole or dehulled raw flour significantly (P ≤ 0.05) improved the extractable Ca but had no significant (P ≥ 0.05) effect on extractable P and Fe for both cultivars.

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1. Introduction

Pearl millet (Pennisetum glaucum L.) is a staple food for a large section of the population in Asian and African countries. Besides supplying calories and proteins in the diet, pearl millet is a good source of essential minerals (Abdalla et al., 1998). Like other cereal grains, the abundance of antinutrients such as phytic acid and polyphenols inhibits proteolytic and amylolytic enzymes, limits protein and starch digestibility and makes poor human bioavailability of minerals. In Sudan, millet is a staple diet of the people in the Western region (Darfur) and is consumed as thick porridge (aseeda), a thin porridge (nasha) and kisra (unleavened bread) from fermented or unfermented dough. Moreover, meals such as Jiria and Damierga are prepared from fermented dehulled pearl millet flour. Large variations in protein and mineral contents have been observed (AbdelRahaman et al., 2007). A protein content of 15.4%, 14.8% and 16.3% was reported by Klopfenstein et al. (1991) for gray, yellow and brown pearl millet, respectively. Local Sudanese cultivars investigated by Eljas et al. (2002) gave a range of 10.8–14.5% protein and were also found to be rich in minerals (Ali et al., 2003). Phytic acid content in pearl millet represents more than 70% of the total phosphorus of the grain (AbdelRahaman et al., 2007). A value of 990 mg/100 g phytic acid was reported by Khetarpaul and Chauhan (1990), while Kumar and Chauhan (1993) gave a value of 825.7 mg/100 g. Elhag et al. (2002) reported values of 943 and 1076 mg/100 g phytic acid for two Sudanese cultivars. AbdelRahaman et al. (2007) reported that millet contains some antinutrients (phytate and polyphenols) that affect nutrient absorption, especially minerals, by the human body. It has been reported that irradiation of phytate (IP6), known to bind essential minerals in beef, soy and soy-extended beef, at an absorbed dosage of less than 4 kGy caused no difference (P > 0.05) in the level of IP3-6 compared to non-irradiated samples (Engeljohn et al., 1999).

Research on the basic interaction of radiation with biological systems has contributed to human society through applications in medicine, agriculture, pharmaceutical uses and other technological developments. In agricultural science and food technology, recent research has elucidated new potential applications for radiation. For example, high doses of ionising radiation have been shown to inhibit growth of microbial infestations in seeds. There are also many reports supporting the use of gamma irradiation as a fungicidal agent (Aziz et al., 2007). However, the viability, and sometimes the developmental process, of the seedling or the plant has been seriously hampered by radiation (Casarett, 1996).

There are insufficient reports about possible effects of gamma radiation on nutritional value of the seeds associated with seed radiation. Seeds of different plants that are consumed as food...
have varying nutrient values, which are dependent on the basic constituents of seed proteins. The chemical structure of irradiating food is less modified than heat-treated food and this technique avoids the use of potentially harmful chemicals (Siddhuraju et al., 2002).

Millet flour had a severe problem during storage and was observed to produce off-flavor and bitter taste. In order to minimize losses occurring during storage of millet flour, the radiation process emerges as an attractive and healthy alternative when compared with chemical conventional treatments. Therefore, in this study we would like to investigate the effect of radiation process as a preserving agent on the antinutritional factors and total and extractable minerals during storage of raw and processed whole and dehulled millet flour.

2. Experimental

2.1. Sample collection and preparation

Grains of Ashana and Dembi millet (P. glaucum L.) cultivars were collected from Nyala Agricultural Research Station, Southern Darfur State, Sudan. Collected seeds (4 kg) of each cultivar were either ground to pass a 0.4 mm screen or dehulled using mechanical dehuller and ground to pass a 0.4 mm screen. All chemicals used for the experiments were of reagent grade.

2.2. Irradiation procedure

The flour with a moisture content of 5.45% was spread uniformly and stored in polythene bags of mass of 100 gm, Gamma radiation process was conducted at Kaila irradiation processing unit, Sudanese Atomic Energy Corporation (SAEC). The Gamma radiation process was conducted at Kaila irradiation processing unit, Sudanese Atomic Energy Corporation (SAEC). All experiments were repeated 3 times and 3 replicates of each flour type were irradiated.

2.3. Processing and storage of the samples

Treated and untreated samples of whole and dehulled flour of each cultivar were divided into two portions. One portion was stored for 30 or 60 days in polythene bags at room temperature (25 °C) and the other portion was cooked for 20 min in a water bath and then dried and ground to pass a 0.4 mm screen and then stored for 30 and 60 days.

2.4. Phytic acid determination

Phytic acid content was determined by the method described by Wheeler and Ferrel (1971) using 2.0 g dried sample. A standard curve was prepared expressing the results as Fe(NO₃)₃ equivalent. Phytate phosphorus was calculated from the standard curve assuming a 4:6 iron to phosphorus molar ratio.

2.5. Polyphenols determination

Total polyphenols were determined according to the Prussian blue spectrophotometric method (Price and Butler, 1977) with a minor modification. Sixty milligrams of ground sample were shaken manually for 1 min in 3.0 ml methanol. The mixture was filtered. The filtrate was mixed with 50 ml distilled water and analysed within an hour. About 3.0 ml of 0.1 M FeCl₃ in 0.1 M HCl was added to 1 ml filtrate, followed immediately by timed addition of 3.0 ml freshly prepared K₃Fe(CN)₆. The absorbance was monitored on a spectrophotometer (Pye Uniamic SP6–550 UV, London, UK) at 720 nm after 10 min from the addition of 3.0 ml of 0.1 M FeCl₃ and 3.0 ml of 0.008 M K₃Fe(CN)₆. A standard curve was obtained, expressing the result as tannic acid equivalents; that is, the amount of tannic acid (mg/100 g) that gives a color intensity equivalent to that given by polyphenols after correction for blank.

2.6. Total minerals determination

Minerals were extracted from the samples by the dry ashing method described by Chapman and Pratt (1982). About 2.0 g of sample was acid-digested with diacid mixture (HNO₃:HClO₄, 5:1, v/v) in a digestion chamber. The digested samples were dissolved in double-distilled water and filtered (Whatman No. 42). The filtrate was made to 50 mL with double-distilled water and was used for determination of total calcium, phosphorus and iron. Calcium was determined by a titration method. Iron was determined by atomic absorption spectrophotometer (Perkin-Elmer 2380). Phosphorus was determined spectrophotometrically using molybdoovanadate method.

2.7. HCl extractability of minerals (in vitro bioavailability)

Minerals in the samples were extracted by the method described by Chauhan and Mahjan (1988). About 1.0 g of the sample was shaken with 10 mL of 0.03 m HCl for 3 h at 37 °C and then filtered. The clear extract obtained was oven-dried at 100 °C and then acid-digested. The amount of extractable minerals was determined by the methods described above. HCl extractability (%) was determined as follows:

\[
\text{mineral extractability(%) } = \frac{\text{mineral extractable in 0.03 NHCl (mg/100g)}}{\text{total minerals (mg/100g)}} \times 100
\]

2.8. Statistical analysis

Each determination was carried out on three separate samples and analysed in triplicate on dry weight basis; the figures were then averaged. Data were assessed by the analysis of variance (Snedecor and Cochran, 1987). 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

3.1. Effect of radiation process on antinutritional factors of raw and processed millet flour during storage

Table 1 shows the effect of radiation process of whole and dehulled raw and processed flour during storage periods (30 and 60 days) on phytate content of Ashana and Dembi cultivars. Phytate content of the whole raw flour of the cultivars was 768.21 and 722.31 mg/100 g while that of the dehulled raw flour was...
Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at (P ≤ 0.05) as assessed by Duncan’s multiple range tests.

### Table 2
Effect of radiation process on total polyphenols content (mg/100 g) of raw and processed whole and dehulled flour of pearl millet cultivars during storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cultivars</th>
<th>Ashana</th>
<th>Dembi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Whole seeds flour</td>
<td>Untreated</td>
<td>455.53* (± 0.20)</td>
<td>455.84* (± 0.90)</td>
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<td>Cooked</td>
<td>410.44* (± 0.12)</td>
<td>410.96* (± 0.50)</td>
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<td>Irradiated</td>
<td>455.56* (± 0.25)</td>
<td>454.69* (± 0.26)</td>
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<td>Irradiated/cooked</td>
<td>410.57* (± 0.15)</td>
<td>409.67* (± 0.19)</td>
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<tr>
<td>Dehulled seeds flour</td>
<td>Untreated</td>
<td>233.35* (± 0.11)</td>
<td>233.77* (± 0.19)</td>
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<td></td>
<td>Cooked</td>
<td>208.29* (± 0.23)</td>
<td>209.80* (± 0.50)</td>
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<td>Irradiated</td>
<td>233.72* (± 0.13)</td>
<td>235.39* (± 0.13)</td>
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<td>Irradiated/cooked</td>
<td>208.17* (± 0.23)</td>
<td>207.58* (± 0.13)</td>
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</table>

Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at (P ≤ 0.05) as assessed by Duncan’s multiple range tests.
### Table 3
Effect of radiation process on total (mg/100 g) and extractable (%) calcium of raw and processed whole and dehulled flour of pearl millet cultivars during storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cultivars</th>
<th>Storage period (days)</th>
<th>Total</th>
<th>Extractable</th>
<th>Total</th>
<th>Extractable</th>
<th>Total</th>
<th>Extractable</th>
<th>Total</th>
<th>Extractable</th>
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<td>10.40</td>
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Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at P<0.05 as assessed by Duncan’s multiple range tests.

### Table 4
Effect of radiation process on total (mg/100 g) and extractable (%) phosphorus of raw and processed whole and dehulled flour of pearl millet cultivars during storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cultivars</th>
<th>Storage period (days)</th>
<th>Total</th>
<th>Extractable</th>
<th>Total</th>
<th>Extractable</th>
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<th>Extractable</th>
<th>Total</th>
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<td>789.34</td>
<td>83.22</td>
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<td>84.29</td>
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<td>795.31</td>
<td>82.95</td>
<td>762.58</td>
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Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at P<0.05 as assessed by Duncan’s multiple range tests.
Table 5 shows the effect of radiation process on total and extractable Fe of raw and processed whole and dehulled flour of pearl millet cultivars during storage. For the whole raw flour Fe content was found to be 7.47 and 7.39 mg/100 g, and, respectively; out of this amount about 30.31% and 29.20% was found to be extractable while that of the dehulled raw flour was 338.85 and 318.68 mg/100 g, respectively; out of this amount about 63.32 and 57.61 mg/100 g was found to be extractable. The seed coat P and phytate P are sources of minerals of cowpea. Dehulling has been shown to remove up to 98% of the tannin content and to improve the quality of the protein and minerals of cowpea. The seed coat P and phytate P are sources of total P. Phytate loss rate of pearl millet cultivars remain low at the beginning of decoction and then the rate increased to become proportional to dry matter loss rates in millet grains (Lestin et al., 2007).

Table 4 shows the effect of radiation process on total and extractable P of raw and processed whole and dehulled millet flour of Ashana and Dembi cultivars during storage. Phosphorus content of the whole raw flour was found to be 789.34 and 757.31 mg/100, respectively; out of this amount about 83.22% and 72.60% was found to be extractable while that of the dehulled raw flour was 338.85 and 318.68 mg/100 g, respectively; out of this amount about 63.32 and 57.61 mg/100 g was found to be extractable for Ashana and Dembi cultivars, respectively. Results showed that dehulling significantly ($P \leq 0.05$) reduced both total and extractable P for both cultivars. The results obtained showed that storage of treated and untreated whole and dehulled flour had insignificant effect on total and extractable P of both cultivars. Cooking of the raw whole flour significantly ($P \leq 0.05$) reduced both total and extractable P and further reduction was observed when the dehulled raw flour of both cultivars was cooked. Radiation alone was found to have a minor effect on total and extractable P of the whole and dehulled flour for both cultivars and when combined with cooking only the effect of cooking is applicable. Phlarah et al. (1997) reported that legume seeds are known to contain antinutritional constituents (mostly tannins) that limit their full utilisation. These constituents are concentrated in the seed coat, and dehulling becomes a very critical operation in the processing of most legumes, including cowpea. Dehulling has been shown to remove up to 98% of the tannin content and to improve the quality of the protein and minerals of cowpea. The seed coat P and phytate P are sources of total P. Phytate loss rate of pearl millet cultivars remain low at the beginning of decoction and then the rate increased to become proportional to dry matter loss rates in millet grains (Lestin et al., 2007).

The results obtained showed that dehulling significantly ($P \leq 0.05$) reduced both total and extractable Ca. As shown in Table 3, storage of treated and untreated whole and dehulled flour had insignificant effect on total and extractable Ca of both cultivars. Radiation alone was found to have a minor effect on Ca content of the whole and dehulled flour for both cultivars. Radiation combined with cooking insignificantly ($P \leq 0.05$) affected Ca content of the whole and dehulled flour but significantly ($P \leq 0.05$) improved the extractable Ca level of both cultivars. The improvement of Ca extractability after cooking may likely be due to the fact that cooking had been reported to reduce the antinutritional factors of cereals (Idris et al., 2007). Mardia et al. (2002) reported that dehulling of the seeds significantly reduced the dry matter and especially ash content of the seeds of millet cultivars.
4. Conclusion

The observations about antinutritional factors and minerals content and extractability in the studied samples tend to suggest that radiation processing up to 2 kGy had little effects on their value and had no effects on minerals quality of the flour whether whole or dehulled. Therefore, radiation can be applied to alleviate the severe problem of off-flavor and bitter taste production during storage. Moreover, radiation process when compared with chemicals or heat treatment emerges as an attractive and healthy alternative.

References


