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RESEARCH ARTICLE

EFFECT OF REFRIGERATION PROCESS ON ANTINUTRIENTS AND HCL EXTRACTABILITY OF CALCIUM, PHOSPHORUS AND IRON DURING PROCESSING AND STORAGE OF TWO MILLET CULTIVARS

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Abstract

Whole and dehulled flour of millet cultivars Ashana and Dembi was stored for 30 and 60 days before and after refrigeration 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 ($P \leq 0.05$) reduced phytate and polyphenols contents for both cultivars. Refrigeration process alone had no effect on phytate and polyphenols contents but when followed by cooking significantly ($P \leq 0.05$) reduced the level of such antinutrients for the whole and dehulled flour of both cultivars. Dehulling alone significantly ($P \leq 0.05$) decreased Ca and P content but slightly decreased Fe content. Refrigeration 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 refrigeration of whole or dehulled raw flour significantly ($P \leq 0.05$) improved the extractable Ca but had no significant ($P \leq 0.05$) effect on extractable P and Fe for both cultivars.

Key Words: Calcium, iron, phosphorus, refrigeration, HCl extractability, polyphenols, phytate, millet.

Introduction

Pearl millet (*Pennisetum gluucum* 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 inhibit proteolytic and amylolytic enzymes, limit protein and starch digestibility and make poor human bioavailability of minerals. Pearl millet is also a versatile foodstuff used mainly as cooked, whole, dehulled or ground flour dough or as a grain like rice. 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), kisra (unleavened bread) from fermented or unfermented dough. Moreover, meals

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such as Jiria and Damierga are prepared from fermented dehulled pearl millet flour. Among millets, pearl millet contains a higher protein content and better amino acid balance than sorghum. Large variations in protein and mineral contents have been observed (Abdel Rahaman *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 Elyas *et al*., (2002) gave a range of 10.8-14.9% 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 (Abdel Rahaman *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. Phytates and polyphenols have been considered as antinutrients because they interact with food constituents such as minerals and make them unavailable. Abdel Rahaman *et al*. (2007) reported that millet contains some antinutrients (phytate and polyphenols) that affect nutrient absorption by the human body. The food industry has become increasingly interested in novel food processing technologies which promise to preserve and improve the quality of food without the use of heat or chemical additives while still retaining the food quality such as refrigeration and irradiation (Mohamed *et al*., 2010). 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, the refrigeration process emerges as an attractive and healthy alternative when compared to chemical conventional treatments. The literature has many reports demonstrating that thermal processing methods improve the nutritional quality of food due to reduction in anti-nutrients. However, there is a scarcity of information relating to the effects of processing with refrigeration. Therefore, the present work was undertaken to explore the effects of refrigeration on anti-nutrients, total and extractable Ca, P and Fe of raw and processed whole and dehulled millet flour during storage.

Materials and methods

Sample collection and refrigeration

Grains of Ashana and Dembi millet (*Pennisetum gluucum* 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 and ground to pass a 0.4 mm screen and stored in polythene bags for refrigeration. The seeds were refrigerated at 4 $^oC \pm 1$ throughout the storage period (30 or 60 days). All chemicals used for the experiments were of reagent grade.

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 a polythene bags at room temperature (25 ^oC) or at 4 $^oC \pm 1$ 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 at room temperature (25 ^oC) or at 4 $^oC \pm 1$.

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 $Fe(NO_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming a 4:6 iron to phosphorus molar ratio.

Polyphenols determination

Total polyphenols were determined according to the Prussian blue spectrophotometric method (Price and Butler 1977) with a minor modification. A quantity of 60 mg of ground sample was shaken manually for 1 min in 3.0 ml methanol. The mixture was filtered quantitatively through Whatman No. 42 filter paper. The filtrate was mixed with 50 ml distilled water and analysed within an hour. About 3.0 ml of 0.1 M $FeCl_3$ in 0.1 M HCl were added to 1 ml filtrate, followed immediately by timed addition of 3.0 ml freshly prepared $K_3Fe(CN)_6$. The absorbance was monitored on a spectrophotometer (Pye Unicam SP6-550 UV, London, UK) at 720 nm after 10 min from the addition of 3.0 ml of 0.1 M $FeCl_3$ and 3.0 ml of 0.008 M $K_3Fe(CN)_6$. A standard curve was prepared, 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.

Total minerals determination

Minerals were extracted from the samples by the dry ashing method that described by Chapman and Pratt (1982). About 2.0 g of sample was acid-digested with diacid mixture (HNO_3:$HClO_4$, 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.

Calcium content determination was carried out for each extract according to Chapman and Pratt (1982). A volume of 2 ml of the extracted sample was placed in a 50 ml conical flask. Then 10 ml of distilled water was added and also drops of 4 M NaOH were added with small amount of meroxide indicator. The content of the flask were then titrated with 0.01M EDTA until violet color (indicating the end point) was obtained.

The determination of phosphorous content was carried according to the method of Chapman and Pratt (1982). A volume of 2 ml of the extract was transferred to a 50 ml volumetric flask. Then 10 ml of ammonium molybdate- ammonium vanadate reagents were added. The contents of the flask were mixed and diluted to 50 ml. The density of the dye was read after 30 minutes at 470 nm using a spectrophotometer (Corning, 259). A standard curve of different KH_2PO_4 concentration was plotted to calculate the ion phosphorous concentration.

Iron was determined by atomic absorption spectrophotometer (Perkin–Elmer 2380) following the standard official method (AOAC, 1990). The wavelength used for the iron was 248.3 nm.

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 temperature of 37 ^{o}C and then filtered by filter paper (Whatman No. 42). The clear extract obtained was oven-dried at temperature of 100 ^{o}C and then acid-digested. The amount of the extractable minerals was determined by the methods described above. HCl extractability (%) was determined as follows:

Mineral extractability, % = (mineral extractable in 0.03 M HCl (mg/100g)/total minerals (mg/100g)) $\cdot$ 100

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). Means comparisons for treatments were made by using Duncan's multiple range tests. Significance was accepted at $P \leq 0.05$.

Results and discussion

Effect of refrigeration process on anti-nutritional factors of raw and processed millet flour during storage

Table 1 presents the effect of refrigeration 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/100g while that of the dehulled raw flour was 302.79 and 284.69 mg/100 g for Ashana and Dembi cultivars, respectively. The results revealed that dehulling of the grains significantly ($P \leq 0.05$) reduced phytate content of both cultivars which indicated that the seed coat contained an appreciable amount of phytate. As shown in Table 1, storage of treated and untreated whole and dehulled flour had insignificant effect on phytate content of both cultivars. Cooking of the whole raw flour significantly ($P \leq 0.05$)

reduced phytate content to 761.68 and 715.42 mg/100g for the cultivars, respectively. Further reduction in phytate content was observed when the dehulled raw flour was cooked and it was found to be 293.55 and 279.08mg/100g for the cultivars, respectively.

Table 1. *Effect of refrigeration on phytic acid content (mg/100g) of raw and processed whole and dehulled flour of pearl millet cultivars during storage*

Samples	Cultivars: Ashana			Dembi		
	Storage period (days)					
	0	30	60	0	30	60
Whole seeds flour						
Untreated	768.21 ^{a} (±0.50)	768.92 ^{a} (±0.70)	769.56 ^{a} (±0.95)	722.31 ^{a} (±0.24)	723.53 ^{a} (±0.35)	724.95 ^{a} (±0.50)
Cooked	761.68 ^{b}(±0.75)	761.87 ^{c} (±0.11)	762.16 ^{c} (±0.88)	715.42 ^{b} (±0.21)	715.89 ^{c} (±0.10)	716.31 ^{c} (±0.30)
Refrigerated	768.19 ^{a} (±0.30)	767.90 ^{b} (±0.28)	767.46 ^{b} (±0.65)	722.28 ^{a} (±0.24)	718.79 ^{b} (±0.15)	718.15 ^{b} (±0.36)
Refrigerated/cooked	761.64 ^{b} (±0.76)	759.64 ^{d} (±0.98)	760.53 ^{d} (±0.55)	715.40 ^{b} (±0.21)	715.85 ^{c} (±0.16)	716.88 ^{c} (±0.54)
Dehulled seeds flour						
Untreated	302.79 ^{c} (±0.22)	302.52 ^{e} (±0.44)	303.13 ^{e} (±0.33)	284.69 ^{c} (±0.25)	283.87 ^{d} (±0.26)	285.89 ^{d} (±0.10)
Cooked	293.55 ^{d} (±0.31)	293.83 ^{f} (±0.22)	293.72 ^{g} (±0.15)	279.08 ^{d} (±0.23)	278.87 ^{e} (±0.71)	279.54 ^{f} (±0.65)
Refrigerated	302.85 ^{c} (±0.27)	301.80 ^{e} (±0.45)	301.34 ^{f} (±0.66)	284.65 ^{c} (±0.25)	282.64 ^{d} (±0.31)	283.41 ^{e} (±0.54)
Refrigerated/cooked	293.50 ^{d} (±0.38)	291.83 ^{g}(±0.07)	291.74 ^{h} (±0.76)	279.18 ^{d} (±0.23)	278.65 ^{e} (±0.22)	278.79 ^{f} (±0.17)

Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at ($p \leq 0.05$) as assessed by Duncan's multiple range tests.

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

Samples	Cultivars: Ashana			Dembi		
	Storage period (days)					
	0	30	60	0	30	60
Whole seeds flour						
Untreated	455.53 ^{a} (±0.20)	455.84 ^{a} (±0.90)	456.12 ^{a} (±0.49)	443.38 ^{a} (±0.20)	443.85 ^{a} (±0.45)	444.57 ^{a} (±0.38)
Cooked	410.44 ^{b} (±0.12)	410.90 ^{c} (±0.50)	411.15 ^{c} (±0.70)	385.58 ^{b} (±0.24)	385.77 ^{c} (±0.10)	386.52 ^{c} (±0.17)
Refrigerated	455.40 ^{a} (±0.26)	453.56 ^{b} (±0.13)	454.25 ^{b} (±0.25)	443.16 ^{a} (±0.29)	440.78 ^{b} (±0.90)	442.65 ^{b} (±0.78)
Refrigerated/cooked	410.10 ^{b} (±0.17)	408.90 ^{d} (±0.05)	409.84 ^{d} (±0.09)	385.38 ^{b} (±0.27)	384.45 ^{c} (±0.18)	384.74 ^{d} (±0.50)
Dehulled seeds flour						
Untreated	233.35 ^{c} (±0.11)	233.77 ^{e} (±0.19)	234.78 ^{e} (±0.21)	218.55 ^{c} (±0.23)	219.52 ^{d} (±0.50)	219.87 ^{e} (±0.80)
Cooked	208.29 ^{d} (±0.23)	209.80 ^{f} (±0.50)	209.60 ^{g} (±0.67)	201.20 ^{d} (±0.15)	202.54 ^{e} (±0.21)	203.11 ^{f} (±0.56)
Refrigerated	233.30 ^{c} (±0.04)	233.18 ^{e} (±0.15)	232.71 ^{f} (±0.17)	218.15 ^{c} (±0.20)	218.72 ^{d} (±0.11)	218.86 ^{e} (±0.21)
Refrigerated/cooked	208.26 ^{d} (±0.20)	208.67 ^{f} (±0.16)	209.23 ^{g} (±0.80)	201.55 ^{d} (±0.45)	202.47 ^{e} (±0.33)	201.92 ^{f} (±0.70)

Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at ($p \leq 0.05$) as assessed by Duncan's multiple range tests.

Refrigeration alone was found to have a minor effect on phytate content of the whole and dehulled flour for both cultivars. However, when refrigeration combined with cooking significantly ($P \leq 0.05$) reduced phytate content of the whole flour to 761.64 and 715.40 mg/100g while that of the dehulled flour reduced to 293.50 and 279.18 mg/100g for both cultivars, respectively. Toledo *et* *al.*, (2007) observed a decrease in antinutritional factors after cooking of soybean grains. Similar observations were obtained by Mardia *et al.*, (2002) when they studied the effect of dehulling on antinutritional factors of pearl millet cultivars. Phytate loss rate of pearl millet cultivars remain low at the beginning of decortications and then the rate increased to become proportional to dry matter loss rates in millet grains. Thus it appears that phytates are mainly distributed in starchy endosperm and germ (Lestinne *et al.*, 2007). The results obtained for polyphenols content during treatments, cooking and storage of the whole and dehulled flour (Table 2) are similar to that obtained for phytate.

Effect of refrigeration process on total and extractable minerals of raw and processed millet flour during storage

Table 3 shows the content and HCl extractability of Ca of whole and dehulled millet flour of Ashana and Dembi cultivars as affected by refrigeration process and/or cooking.

Table 3. *Effect of refrigeration on total (mg/100g) and extractable (%) calcium of raw and processed whole and dehulled flour of pearl millet cultivars during storage*

Samples	Cultivars											
	Ashana						Dembi					
	Storage period (days)											
	0		30		60		0		30		60	
	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable
Whole seeds flour												
Untreated	10.23^{a} (±0.18)	54.46^{b} (±0.21)	10.62^{b} (±0.11)	56.96^{c} (±0.28)	9.76^{b} (±0.08)	53.27^{c} (±0.26)	8.73^{a} (±0.60)	54.89^{b} (±0.42)	9.26^{c} (±0.22)	56.40^{a} (±0.39)	9.39^{a} (±0.55)	55.94^{a} (±0.76)
Cooked	10.71^{a} (±0.38)	60.67^{a} (±0.16)	11.41^{a} (±0.07)	58.31^{a} (±0.24)	10.63^{a} (±0.17)	57.63^{a} (±0.20)	8.80^{a} (±0.05)	58.03^{a} (±0.23)	10.53^{a} (±0.45)	53.22^{c} (±0.34)	9.65^{a} (±0.10)	51.73^{b} (±0.15)
Refrigerated	10.18^{a} (± 0.07)	54.35^{b} (±0.21)	9.83^{c} (±0.07)	55.17^{d} (±0.19)	9.85^{b} (±0.05)	54.37^{b} (±0.33)	8.70^{a} (±0.60)	54.84^{b} (±0.42)	8.63^{d} (±0.50)	55.67^{b} (±0.26)	8.84^{b} (±0.60)	55.55^{a} (±0.23)
Refrigerated/cooked	10.56^{a} (±0.32)	60.60^{a} (±0.16)	9.36^{c} (±0.19)	57.40^{b} (±0.13)	9.45^{b} (±0.08)	57.74^{a} (±0.09)	8.85^{a} (±0.05)	58.00^{a} (±0.23)	9.92^{b} (±0.02)	57.07^{a} (±0.10)	9.85^{a} (±0.05)	55.75^{a} (±0.05)
Dehulled seeds flour												
Untreated	5.13^{b} (±0.01)	48.29^{d} (±0.04)	6.25^{d} (±0.06)	49.24^{f} (±0.18)	6.52^{c} (±0.05)	48.76^{e} (±0.23)	5.12^{b} (±0.02)	40.31^{d} (±0.22)	6.14^{e} (±0.14)	41.44^{f} (±0.24)	6.19^{c} (±0.22)	41.27^{d} (±0.11)
Cooked	5.22^{b} (±0.19)	49.39^{c} (±0.15)	6.19^{d} (±0.10)	48.33^{g} (±0.29)	6.37^{c} (±0.08)	48.86^{d} (±0.08)	5.60^{b} (±0.08)	42.43^{c} (±0.10)	6.44^{e} (±0.01)	43.74^{e} (±0.04)	6.23^{c} (±0.02)	41.68^{d} (±0.26)
Refrigerated	5.08^{b} (±0.05)	48.24^{d} (±0.04)	5.75^{d} (±0.01)	48.70^{g} (±0.13)	6.32^{c} (±0.03)	49.35^{d} (±0.34)	5.15^{b} (±0.02)	40.35^{d} (±0.22)	5.56^{f} (±0.05)	43.35^{e} (±0.15)	6.18^{c} (±0.05)	45.76^{c} (±0.08)
Refrigerated/cooked	5.45^{b} (±0.16)	49.34^{c} (±0.15)	5.83^{d} (±0.03)	50.24^{e} (±0.18)	6.15^{c} (±0.06)	50.37^{d} (±0.25)	5.63^{b} (±0.08)	42.42^{c} (±0.10)	5.81^{f} (±0.02)	45.29^{d} (±0.04)	6.63^{c} (±0.09)	45.90^{c} (±0.20)

Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at ($p \leq 0.05$) as assessed by Duncan's multiple range tests.

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

Samples	Cultivars											
	Ashana						Dembi					
	Storage period (days)											
	0		30		60		0		30		60	
	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable
Whole seeds flour												
Untreated	789.34 ^{a} (±0.60)	83.22 ^{a} (±0.06)	789.04 ^{a} (±068)	76.48 ^{b} (±0.33)	791.09 ^{a} (±0.23)	81.39 ^{a} (±0.18)	757.31 ^{a} (±0.76)	72.60 ^{a} (±0.05)	761.28 ^{a} (±0.60)	74.19 ^{a} (±0.14)	762.97 ^{b} (±0.09)	80.09 ^{a} (±0.04)
Cooked	754.73 ^{b} (±0.67)	82.23 ^{b} (±0.12)	769.92 ^{d} (±0.87)	75.53 ^{c} (±0.11)	792.41 ^{a} (±0.90)	78.70 ^{b} (±0.30)	750.37 ^{b} (±0.15)	69.87 ^{b} (±0.07)	760.16 ^{b} (±0.64)	73.19 ^{b} (±0.19)	768.59 ^{a} (±0.64)	75.76 ^{b} (±0.22)
Refrigerated	789.30 ^{a} (±0.60)	83.28 ^{a} (±0.06)	785.33 ^{b} (±0.34)	78.35 ^{a} (±0.13)	785.86 ^{c} (±0.12)	72.35^{c} (±0.11)	757.38 ^{a} (±0.76)	72.65 ^{a} (±0.05)	755.37 ^{d} (±0.22)	70.42 ^{c} (±0.17)	756.80 ^{d} (±0.36)	70.45 ^{c} (±0.11)
Refrigerated /cooked	754.77 ^{b} (±0.67)	82.27 ^{b} (±0.11)	780.94 ^{c} (±0.75)	78.36 ^{a} (±0.17)	786.70 ^{b} (±0.86)	79.68 ^{a} (±0.08)	750.33 ^{b} (±0.15)	69.84 ^{b} (±0.07)	758.38 ^{c} (±0.35)	70.58 ^{c} (±0.33)	760.52 ^{c} (±0.37)	71.26 ^{c} (±0.16)
Dehulled seeds flour												
Untreated	338.85 ^{c} (±0.57)	63.32 ^{c} (±0.11)	339.87^{e} (±0.78)	64.63 ^{d} (±0.11)	343.77^{d} (±0.34)	64.54 ^{d} (±0.35)	318.68 ^{d} (±0.16)	57.61 ^{c} (±0.10)	318.92 ^{f} (±0.26)	55.75 ^{e} (±0.09)	319.27 ^{f} (±0.16)	56.52 ^{e} (±0.15)
Cooked	335.48 ^{d} (±0.60)	49.39 ^{d} (±0.07)	336.58^{f} (±0.43)	45.71^{g} (±0.05)	338.02 ^{e} (±0.58)	49.41 ^{g} (±0.06)	328.83 ^{c} (±0.62)	57.58 ^{c} (±0.15)	327.87 ^{e} (±0.10)	53.31 ^{f} (±0.06)	329.30 ^{e} (±0.18)	53.93 ^{f} (±0.04)
Refrigerated	338.88 ^{c} (±0.57)	63.35 ^{c} (±0.11)	337.56 ^{f} (±0.36)	62.44 ^{e} (±0.10)	338.22 ^{e} (±0.20)	60.42 ^{e} (±0.16)	318.65 ^{d} (±0.16)	57.60 ^{c} (±0.10)	318.65 ^{f} (±0.22)	60.28 ^{d} (±0.08)	318.00 ^{f} (±0.40)	60.48 ^{d} (±0.37)
Refrigerated /cooked	335.49 ^{d} (±0.60)	49.36 ^{d} (±0.07)	336.17 ^{f} (±0.83)	57.33 ^{f} (±0.15)	337.83 ^{f} (±0.75)	58.40 ^{f} (±0.13)	328.80 ^{c} (±0.62)	57.55 ^{c} (±0.15)	316.53^{g} (±0.17)	55.57 ^{e} (±0.23)	317.38 ^{f} (±0.20)	57.43 ^{e} (±0.13)

Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at ($p \leq 0.05$) as assessed by Duncan's multiple range tests.

For the whole raw flour, Ca content was found to be 10.23 and 8.73 mg/100 and respectively out of this amount about 54.46 and 54.89% was found to be extractable for Ashana and Dembi cultivars, respectively. Calcium content of the dehulled raw flour was 5.13 and 5.12 mg/100g and respectively out of this amount about 49.29 and 40.31% was found to be extractable for the cultivars, respectively. 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. Refrigeration alone was found to have a minor effect on Ca content of the whole and dehulled flour for both cultivars and when refrigeration 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 to 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.

Table 5. *Effect of refrigeration on total (mg/100g) and extractable (%) iron of raw and processed whole and dehulled flour of pearl millet cultivars during storage*

Samples	Cultivars											
	Ashana						Dembi					
	Storage period (days)											
	0		30		60		0		30		60	
	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable
Whole seeds flour												
Untreated	7.47 ^{b} (±0.04)	30.31 ^{d} (±0.13)	8.14 ^{b} (±0.07)	31.37 ^{i} (±0.34)	8.17 ^{b} (0.05)	35.19 ^{g} (±0.06)	7.39 ^{c} (±0.80)	29.20 ^{d} (±0.48)	8.08 ^{c} (±0.12)	27.68 ^{f} (±0.62)	8.75 ^{b} (±0.45)	32.72 ^{d} (±0.32)
Cooked	8.15 ^{a} (±0.07)	31.71 ^{c} (±0.27)	8.36 ^{a} (±0.05)	36.73 ^{b} (±0.19)	8.39 ^{b} (0.05)	39.10 ^{c} (±0.06)	8.47 ^{a} (±0.50)	30.55 ^{c} (±0.37)	8.71 ^{a} (±0.90)	37.78 ^{a} (±0.24)	9.15 ^{a} (±0.50)	37.64 ^{b} (±0.19)
Refrigerated	7.45 ^{b} (±0.07)	30.30 ^{d} (±0.18)	8.31 ^{a} (±0.05)	31.21 ^{j} (±0.07)	8.71 ^{a} (0.05)	37.14 ^{e} (±0.05)	7.35 ^{c} (±0.80)	29.24 ^{d} (±0.48)	7.20 ^{d} (±0.34)	30.17 ^{e} (±0.05)	7.96 ^{c} (±0.24)	31.20 ^{e} (±0.06)
Refrigerated /cooked	8.13 ^{a} (±0.04)	31.76 ^{c} (±0.27)	8.20 ^{b} (±0.04)	36.23 ^{c} (±0.11)	8.40 ^{b} (0.03)	37.51 ^{d} (±0.77)	8.42 ^{a} (±0.50)	30.51 ^{c} (±0.37)	8.43 ^{b} (±0.20)	35.93 ^{c} (±0.13)	8.75 ^{b} (±0.40)	37.27 ^{c} (±0.08)
Dehulled seeds flour												
Untreated	6.62 ^{c} (±0.05)	32.67 ^{b} (±0.13)	6.76 ^{c} (±0.08)	35.08 ^{e} (±0.03)	6.96 ^{c} (0.04)	35.22 ^{h} (±0.08)	6.23 ^{b} (±0.20)	35.46 ^{b} (±0.19)	6.24 ^{e} (±0.37)	37.82 ^{a} (±0.04)	6.58 ^{d} (±0.56)	37.58 ^{b} (±0.07)
Cooked	6.52 ^{cd} (±0.04)	33.21 ^{a} (±0.21)	5.97 ^{d} (±0.55)	34.43 ^{g} (±0.05)	5.77 ^{d} (0.15)	34.95 ^{j} (±0.08)	6.16 ^{b} (±0.10)	36.16 ^{a} (±0.04)	6.44 ^{e} (±0.25)	36.14 ^{b} (±0.07)	6.26 ^{d} (±0.65)	36.76 ^{bc} (±0.21)
Refrigerated	6.60 ^{c} (±0.05)	32.60 ^{b} (±0.10)	6.67 ^{c} (±0.03)	38.19 ^{a} (±0.06)	6.87 ^{c} (0.06)	40.36 ^{b} (±0.07)	6.20 ^{b} (±0.20)	35.43 ^{b} (±0.19)	6.18 ^{e} (±0.54)	37.75 ^{a} (±0.05)	6.00 ^{d} (±0.52)	37.77 ^{b} (±0.08)
Refrigerated /cooked	6.60 ^{c} (±0.04)	33.24 ^{a} (±0.21)	6.74 ^{c} (±0.04)	35.44 ^{d} (±0.20)	6.54 ^{c} (0.14)	41.28 ^{a} (±0.32)	6.13 ^{b} (±0.10)	36.15 ^{a} (±0.04)	6.26 ^{e} (±0.47)	34.38 ^{d} (±0.08)	6.35 ^{d} (±0.75)	38.44 ^{a} (±0.38)

Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at ($p \leq 0.05$) as assessed by Duncan's multiple range tests.

Table 4 shows the effect of refrigeration 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 and 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/100g and respectively out of this amount about 63.32 and 57.61 was found to be extractable for Ashana and Dembi cultivars, respectively. Results showed that dehulling significantly ($P \leq 0.05$) reduced the 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. Refrigeration 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. Plahar 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 decortications and then the rate increased to become proportional to dry matter loss rates in millet grains (Lestinne *et al*., 2007). Table 5 shows the effect of refrigeration process on total and extractable Fe of raw and processed whole and dehulled flour of two millet cultivars (Ashana and Dembi) during storage. For the whole raw flour Fe content was found to be 7.47 and 7.39 mg/100g, 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 6.62 and 6.23 mg/100g and respectively out of this amount about 32.67 and 35.46% was found to be extractable for Ashana and Dembi cultivars, respectively. The results revealed that dehulling had slight effect on total Fe but increased the extractable Fe. Storage of the whole flour for different periods of time before and after refrigeration for both cultivars was found to have a minor effect on Fe content and extractability even after cooking. However, cooking of the treated and untreated whole flour was found to cause slight increase in total and extractable Fe. It has been reported that dehulling of millet seeds significantly reduced the dry matter content and the loss of the dry matter was found to be correlated with Fe loss (Lestinne *et al*., 2007). The results obtained showed that refrigeration and/or cooking have a minor effect on total and extractable Fe.

Conclusions

The observations about antinutrional factors and minerals content and extractability in the studied samples tend to suggest that refrigeration processing had little effects on their value and had no effects on minerals quality of the flour whether whole or dehulled. Therefore, refrigeration can be applied to alleviate the severe problem of off-flavor and bitter taste production during storage.

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