

Preservation of Millet Flour by Refrigeration: Changes in Antinutrients, Protein Digestibility and Sensory Quality During Processing and Storage

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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. The effects of refrigeration and/or cooking on antinutrients, protein digestibility and sensory characteristics of the whole and dehulled flour during storage were examined. Regardless to the storage period, the results showed that tannin and phytate contents of the whole flour of both cultivars were unchanged by refrigeration. However, cooking before and after refrigeration significantly ($P \leq 0.05$) decreased tannin and phytate contents. Regardless to the treatments and storage period, dehulling of the seeds significantly ($P \leq 0.05$) reduced tannin and phytate contents for both cultivars. Further reduction was observed when the treated and untreated dehulled flour was cooked. The protein digestibility was significantly ($P \leq 0.05$) reduced after cooking of the treated and untreated whole and dehulled flour. The sensory quality of the flour and the product reduced with the storage period. However, refrigeration greatly improved the acceptability of the flour before and after cooking. The results obtained demonstrate the benefits of refrigeration on the nutritional as well as sensory quality of millet flour.

Key words: Refrigeration, millet, dehulling, antinutrients, protein digestibility, sensory characteristics

INTRODUCTION

Pearl millet is a multipurpose crop, which is grown for food, feed and forage. Besides supplying calories and proteins in the diet, pearl millet is a good source of essential minerals^[1]. Like other cereal grains, the abundance of antinutrients such as phytic acid and tannins 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), kiswa (unleavened bread) from fermented or unfermented dough. Moreover, meals 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^[2]. A protein content of 15.4%, 14.8% and 16.3% was reported by Klopfenstein *et al.*^[9] for gray, yellow and brown pearl millet, respectively. Local Sudanese cultivars investigated by Elyas *et al.*^[7] gave

a range of 10.8-14.9% protein and were also found to be rich in minerals^[3]. Phytic acid content in pearl millet represents more than 70% of the total phosphorus of the grain^[2]. A value of 990 mg/100 g phytic acid was reported by Khetarpaul and Chauhan^[8] while Kumar and Chauhan^[10] gave a value of 825.7 mg/100 g. Elhag *et al.*^[6] 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. AbdelRahaman *et al.*^[2] 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. Millet flour had a severe problem during storage even if stored for one day; it produces 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, protein digestibility and sensory quality of whole and dehulled millet flour during cooking and storage.

MATERIALS AND METHODS

Sample Collection and Refrigeration: Grains of Ashana and Dembi millet (*Pennisetum 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 and ground to pass a 0.4 mm screen and stored in polythene bags for refrigeration. The seeds were refrigerated at 4 ± 1 °C 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 °C) or at 4 ± 1 °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 at room temperature (25 °C) or at 4 ± 1 °C.

Determination of Tannin Content: Quantitative estimation of tannins was carried out using the modified vanillin-HCl method^[12]. A 200 mg sample was extracted using 10 mL 1% (v/v) concentrated HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 mL) was added to the extract (1 mL) and the absorbance of the colour developed after 20 min at 30 °C was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg/mL) which gives a colour intensity equivalent to that given by tannins after correcting for blank. Then tannin content (%) was calculated according to the equation:

$$\text{Catechin equivalent (CE)\%} = \frac{C \times \text{volume extracted (10 mL)}}{\text{sample weight (g)}} \times 100$$

Where C, concentration obtained from the standard curve (mg/mL).

Determination of Phytic Acid Content: Phytic acid content of the malt, treated and untreated sorghum flour was determined by the method described by Wheeler and Ferrel^[18] using 2.0 g of a dried sample. A standard curve was prepared expressing the results as $\text{Fe}(\text{NO}_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming 4:6 iron to phosphorus molar ratio.

Determination of in Vitro Protein Digestibility (IVPD): IVPD was carried out according to the method described by Monjula and John^[11] with a minor modification. A known weight of the sample containing 16 mg nitrogen was taken in triplicate and digested with 1 mg pepsin in 15 ml of 0.1 N HCl at 37 °C for 2 h. The reaction was stopped by the addition of 15 ml 10% trichloroacetic acid (TCA). The mixture was then filtered quantitatively, through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-Kjeldahl method^[4]. Digestibility was estimated by using the following equation:

$$\text{IVPD\%} = \frac{\text{N in supernatant} - \text{N in pepsin}}{\text{N in sample}} \times 100$$

Sensory evaluations: The sensory tests were conducted using conventional profiling by a trained panel. Ten judges were selected who had successfully passed standardized tests for olfactory and taste sensitivities as well as verbal abilities and creativity. The panellists were given a hedonic questionnaire to test color, flavor, bitterness and overall acceptability of coded samples of treated and/or processed samples. They were scored on a scale of 1–5 (1 = poor, 2 = fair, 3 = good, 4 = very good and 5 = excellent).

Statistical analysis: Each determination was carried out on three separate samples, on dry weight basis and analysed in triplicate, the figures were then averaged. Data were assessed using anova^[16]. Mean comparisons for treatments were made using Duncan's multiple range tests. Significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of Refrigeration and Storage Period on Antinutritional Factors of Millet Flour: Table 1 summarizes the data for tannin content of treated and untreated whole and dehulled flour of two millet cultivars (Ashana and Dembi). Tannin content of untreated whole flour was found to be 0.38 and 0.34 mg/100g for the cultivars Ashana and Dembi, respectively. Dehulling of the seeds of both cultivars

Table 1: Effect of refrigeration and storage on tannin content (mg/100g) of whole and dehulled flour of pearl millet cultivars.

Samples	Cultivars					
	Ashana			Dembi		
	Storage period (days)					
	0	30	60	0	30	60
<i>Whole seeds flour</i>						
Untreated	0.38 ^a (±0.05)	0.36 ^b (±0.03)	0.41 ^b (±0.01)	0.34 ^{ab} (±0.05)	0.31 ^{ab} (±0.01)	0.35 ^{ab} (±0.02)
Cooked	0.29 ^{bc} (±0.03)	0.28 ^{cd} (±0.01)	0.29 ^{de} (±0.02)	0.30 ^b (±0.08)	0.31 ^{ab} (±0.10)	0.29 ^b (±0.05)
Refrigerated	0.36 ^a (±0.02)	0.30 ^{cd} (±0.02)	0.33 ^{cd} (±0.03)	0.37 ^a (±0.02)	0.29 ^b (±0.01)	0.33 ^b (±0.03)
Refrigerated/cooked	0.25 ^{cd} (±0.02)	0.31 ^c (±0.05)	0.28 ^e (±0.02)	0.36 ^{ab} (±0.06)	0.28 ^b (±0.04)	0.29 ^b (±0.05)
<i>Dehulled seeds flour</i>						
Untreated	0.20 ^e (±0.01)	0.23 ^{ef} (±0.02)	0.20 ^f (±0.01)	0.17 ^e (±0.04)	0.17 ^{cd} (±0.05)	0.18 ^c (±0.06)
Cooked	0.19 ^e (±0.06)	0.19 ^{ef} (±0.09)	0.20 ^f (±0.03)	0.20 ^{de} (±0.11)	0.19 ^{cd} (±0.09)	0.20 ^c (±0.01)
Refrigerated	0.24 ^{de} (±0.05)	0.26 ^{de} (±0.02)	0.27 ^e (±0.05)	0.19 ^{de} (±0.05)	0.19 ^{cd} (±0.06)	0.18 ^c (±0.07)
Refrigerated/cooked	0.23 ^{de} (±0.04)	0.29 ^{cd} (±0.04)	0.28 ^e (±0.03)	0.25 ^{cd} (±0.14)	0.16 ^{cd} (±0.13)	0.23 ^c (±0.06)

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.

significantly ($P \leq 0.05$) reduced tannin content to 0.20 and 0.17 mg/100g for the cultivars, respectively. The storage of untreated whole flour for both cultivars had slight effect on tannin content. Storage of untreated whole flour slightly decreased tannin content after 30 days but slightly increased again after 60 days for both cultivars. However, storage of dehulled flour before treatments was found to have no effect on tannin content even after storage for 60 days. The results indicated that the whole flour may contain tannins chemically unstable. Regardless of the storage periods, cooking of the whole or dehulled flour significantly ($P \leq 0.05$) decreased tannin content of both cultivars. This may be due to the fact that heat treatment may likely inactivate tannins. Regardless of the storage periods, refrigeration of the flour alone insignificantly ($P \leq 0.05$) affected tannin content of both cultivars but cooking of the refrigerated flour significantly ($P \leq 0.05$) reduced tannin content of the whole flour while that of the dehulled seeds was not greatly affected. The results obtained for phytic acid content (Table 2) of treated and untreated whole and dehulled flour of millet cultivars during storage are similar to those reported for tannin. Phytic acid in cereals is one of major concern as it chelates mineral cations and interacts with proteins forming insoluble complexes which lead to reduced bio-availability of minerals and reduced digestibility of protein^[13]. The observations about phytic acid and tannin in the studied samples tend to suggest that refrigeration as a mean of preservation had little or no effects on the level of such antinutrients with the storage period but when combined with cooking the effects increased.

Effect of Refrigeration and Storage Period on in Vitro Protein Digestibility of Millet Flour: Table 3 shows the effect of treatments on *in vitro* protein digestibility (IVPD) of whole and dehulled flour of two millet cultivars (Ashana and Dembi). The IVPD of untreated whole seeds flour was found to be 46.43 and 51.23% of the cultivars Ashana and Dembi, respectively. Dehulling of the seeds of both cultivars significantly ($P \leq 0.05$) increased the IVPD to 50.54 and 55.28% for the cultivars, respectively. The storage of untreated whole flour for both cultivars had slight effect on the IVPD. Storage of untreated whole flour slightly decreased the IVPD throughout the period for Ashana cultivar while that for Dembi cultivars it decreased to 49.56%. However, storage of dehulled flour before treatments was found to have no effect on IVPD even after storage for 60 days. The results indicated that the whole seeds flour contained an appreciable amount of tannin and phytate which reduced the IVPD of both cultivars. Regardless of the storage periods, cooking of the whole or dehulled flour significantly ($P \leq 0.05$) decreased the IVPD of both cultivars flour. Refrigeration of the flour alone was found to have no effect on the whole and dehulled flour IVPD for both cultivars but when combined with cooking it significantly ($P \leq 0.05$) reduced the IVPD of the cultivars. Evidence from *in vitro* studies indicates that digestion of native seed storage protein is limited because of the structure and conformation of the protein^[5]. Also, *in vitro* studies have shown that phytate-protein complexes are insoluble and less subject to attack by proteolytic enzymes than the same protein alone^[13] and subsequently affect the functional

Table 2: Effect of refrigeration and storage on phytic acid content (mg/100g) of whole and dehulled flour of pearl millet cultivars.

Samples	Cultivars					
	Ashana			Dembi		
	Storage period (days)					
	0	30	60	0	30	60
<i>Whole seeds flour</i>						
Untreated	768.21 ^a (±0.50)	768.92 ^a (±0.70)	769.56 ^a (±0.95)	722.31 ^a (±0.24)	723.53 ^b (±0.35)	724.95 ^a (±0.50)
Cooked	761.68 ^b (±0.75)	761.87 ^d (±0.11)	762.16 ^d (±0.88)	715.42 ^b (±0.21)	715.89 ^e (±0.10)	716.31 ^e (±0.30)
Refrigerated	768.19 ^a (±0.30)	767.90 ^c (±0.28)	767.46 ^c (±0.65)	722.28 ^a (±0.24)	718.79 ^c (±0.15)	718.15 ^c (±0.36)
Refrigerated/cooked	761.64 ^b (±0.76)	759.64 ^e (±0.98)	760.53 ^e (±0.55)	715.40 ^{bc} (±0.21)	715.85 ^e (±0.16)	716.88 ^d (±0.54)
<i>Dehulled seeds flour</i>						
Untreated	302.79 ^c (±0.22)	302.52 ^b (±0.44)	303.13 ^b (±0.33)	284.69 ^e (±0.25)	283.87 ^e (±0.26)	285.89 ^f (±0.10)
Cooked	293.55 ^e (±0.31)	293.83 ^j (±0.22)	293.72 ^j (±0.15)	279.08 ^d (±0.23)	278.87 ⁱ (±0.71)	279.54 ^h (±0.65)
Refrigerated	302.85 ^c (±0.27)	301.80 ⁱ (±0.45)	301.34 ^k (±0.66)	284.65 ^e (±0.25)	282.64 ^h (±0.31)	283.41 ^g (±0.54)
Refrigerated/cooked	293.50 ^e (±0.38)	291.83 ^h (±0.07)	291.74 ^l (±0.76)	279.18 ^d (±0.23)	278.65 ^j (±0.22)	278.79 ⁱ (±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 3: Effect of refrigeration and storage on *in vitro* protein digestibility (%) of whole and dehulled flour of millet cultivars.

Samples	Cultivars					
	Ashana			Dembi		
	Storage period (days)					
	0	30	60	0	30	60
<i>Whole seeds flour</i>						
Untreated	46.43 ^b (±1.07)	45.65 ^e (±0.47)	45.23 ^f (±0.18)	51.23 ^b (±0.23)	50.92 ^e (±0.15)	49.56 ^g (±0.30)
Cooked	36.35 ^c (±0.65)	35.40 ⁱ (±0.45)	35.39 ^j (±0.55)	37.68 ^d (±0.40)	37.21 ^j (±0.45)	36.65 ^j (±0.76)
Refrigerated	46.50 ^b (±1.07)	47.56 ^d (±0.15)	49.84 ^c (±0.36)	51.27 ^b (±0.23)	51.95 ^c (±0.04)	51.84 ^e (±0.15)
Refrigerated/cooked	36.31 ^c (±0.65)	37.88 ^h (±0.80)	40.37 ^h (±0.18)	37.64 ^d (±0.40)	38.70 ^h (±0.07)	38.93 ⁱ (±0.55)
<i>Dehulled seeds flour</i>						
Untreated	50.54 ^a (±0.80)	49.83 ^c (±0.30)	50.24 ^c (±0.43)	55.28 ^a (±0.15)	55.58 ^b (±0.20)	55.63 ^c (±0.49)
Cooked	46.33 ^b (±0.17)	45.37 ^e (±0.18)	43.37 ^g (±0.23)	50.33 ^c (±0.13)	51.36 ^d (±0.13)	49.23 ^h (±0.65)
Refrigerated	50.50 ^a (±0.80)	52.39 ^a (±0.50)	56.49 ^a (±0.39)	55.25 ^a (±0.15)	56.40 ^a (±0.05)	56.88 ^a (±0.09)
Refrigerated/cooked	46.70 ^b (±0.17)	47.72 ^d (±0.37)	48.81 ^d (±0.14)	50.35 ^c (±0.13)	51.50 ^d (±0.13)	53.35 ^d (±0.30)

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.

properties of the protein. Moreover, the partial removal of tannin and phytate probably created a large space within the matrix, which increased the susceptibility to enzymatic attack^[14] and consequently improve the digestibility of protein. Higher protein digestibility after dehulling of the seeds may be due to increased accessibility of the protein to enzymatic attack. However, this effect could also be due to inactivation of proteinaceous antinutritional factors after cooking^[17]. The apparent improvement in the *in vitro* digestibility that being ensured through treatments, may be attributed to appreciable effect of treatments on the antinutritional factors present naturally in untreated

studied whole and dehulled flour which are more sensitive to enzyme action. It could be concluded that the refrigeration process offers a good treatment for millet to reduce or eliminate their antinutritional factors when combined with cooking with consequent increase in the protein digestibility and thereby increase the utilization of the proteins.

Effect of Refrigeration and Storage Period on Sensory Quality of Millet Flour: Table 4 and 5 show the consumer panel data of treated and untreated whole and dehulled flour of Ashana and Dembi cultivars, respectively (Storage of the flour for 30 and 60 days

Table 4: Effect of refrigeration and storage on sensory characteristics of whole and dehulled flour of millet cultivar (Ashana).

Samples	Overall acceptance		Bitterness		Flavour		Color	
	Storage period (days)							
	0	30	0	30	0	30	0	30
<i>Whole seeds flour</i>								
Untreated	3.8 ^{ab} (±0.71)	2.6 ^d (±1.06)	3.3 ^{cd} (±1.16)	2.5 ^c (±1.41)	3.9 ^a (± 0.83)	2.9 ^b (± 0.83)	4.0 ^a (± 0.76)	3.1 ^b (± 1.13)
Cooked	3.6 ^{bc} (±1.06)	4.3 ^{ab} (±1.04)	4.3 ^a (±0.89)	4.0 ^a (±0.76)	3.1 ^b (± 1.25)	3.4 ^{ab} (± 1.41)	3.1 ^b (±1.13)	3.1 ^b (± 0.83)
Refrigerated	3.3 ^c (±0.71)	3.9 ^{ab} (±1.25)	3.8 ^{ab} (±1.16)	4.1 ^a (±1.13)	4.0 ^a (± 0.53)	2.6 ^c (±1.30)	3.8 ^{ab} (±1.04)	4.1 ^a (±1.36)
Refrigerated/cooked	3.2 ^c (±1.06)	3.8 ^{bc} (±0.71)	4.0 ^{ab} (±0.89)	3.6 ^{ab} (±1.06)	3.6 ^a (± 1.25)	2.6 ^c (± 0.92)	3.6 ^{bc} (±1.13)	2.6 ^c (±1.06)
<i>Dehulled seeds flour</i>								
Untreated	3.4 ^{bc} (±1.06)	3.6 ^{bc} (±1.19)	2.8 ^{de} (±1.04)	2.8 ^{bc} (±1.28)	2.8 ^b (± 0.59)	2.8 ^{bc} (± 0.89)	2.4 ^c (±1.06)	1.9 ^{cd} (± 0.83)
Cooked	3.5 ^{bc} (±0.93)	4.4 ^a (±0.74)	3.3 ^{cd} (±0.46)	4.1 ^a (±0.64)	2.8 ^b (± 0.89)	3.6 ^a (±1.41)	3.6 ^{bc} (±1.06)	3.7 ^b (±1.25)
Refrigerated	3.6 ^{bc} (±1.26)	4.0 ^{ab} (±0.53)	2.6 ^e (±0.92)	3.1 ^{bc} (±0.99)	2.5 ^b (± 0.89)	3.5 ^{ab} (± 0.76)	2.8 ^c (±1.06)	2.4 ^c (±1.51)
Refrigerated/cooked	3.8 ^{ab} (±0.93)	3.5 ^c (±0.76)	3.6 ^{bc} (±0.46)	3.3 ^b (±1.04)	2.8 ^b (± 1.16)	2.8 ^{bc} (± 0.71)	3.8 ^{ab} (±1.04)	4.3 ^a (±1.16)

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 5: Effect of refrigeration and storage on sensory characteristics of whole and dehulled flour of millet cultivar (Dembi).

Samples	Overall acceptance		Bitterness		Flavour		Color	
	Storage period (days)							
	0	30	0	30	0	30	0	30
<i>Whole seeds flour</i>								
Untreated	3.1 ^{bc} (±0.83)	3.4 ^c (±1.06)	2.9 ^{bc} (±0.59)	3.0 ^c (±1.20)	2.5 ^d (±0.53)	2.6 ^{bc} (±0.52)	4.5 ^a (±0.70)	3.6 ^{ab} (±0.74)
Cooked	3.5 ^{ab} (±0.43)	2.9 ^d (±0.99)	3.0 ^{ab} (±0.53)	2.9 ^{cd} (±1.13)	2.6 ^{cd} (±1.06)	2.8 ^{ab} (±0.89)	2.1 ^e (±0.83)	1.9 ^{cd} (±0.83)
Refrigerated	3.3 ^{ab} (±0.71)	3.4 ^c (±1.30)	2.8 ^c (±0.89)	3.5 ^b (±1.07)	2.7 ^{cd} (±0.33)	3.0 ^{ab} (±0.76)	3.5 ^{bc} (±1.27)	3.9 ^{ab} (±1.25)
Refrigerated/cooked	3.0 ^a (±0.76)	3.4 ^c (±1.19)	3.1 ^{ab} (±0.33)	4.0 ^a (±0.76)	2.8 ^{cd} (±1.04)	2.6 ^b (±0.92)	2.7 ^{de} (±0.23)	2.3 ^c (±1.39)
<i>Dehulled seeds flour</i>								
Untreated	3.5 ^{ab} (±1.07)	4.3 ^a (±0.71)	3.5 ^{ab} (±1.07)	3.9 ^{ab} (±0.99)	2.5 ^d (±0.93)	3.0 ^{ab} (±0.76)	2.6 ^{de} (±1.30)	4.0 ^{ab} (±0.93)
Cooked	3.5 ^{ab} (±0.93)	4.3 ^a (±0.71)	3.6 ^a (±1.19)	3.9 ^{ab} (±0.64)	3.0 ^{bc} (±1.20)	3.4 ^a (±0.52)	4.0 ^{ab} (±0.51)	1.4 ^{de} (±0.74)
Refrigerated	3.6 ^{ab} (±0.57)	4.0 ^{ab} (±0.53)	3.6 ^a (±0.77)	3.6 ^{ab} (±0.74)	2.9 ^{bc} (±0.43)	3.0 ^{ab} (±0.76)	3.6 ^{bc} (±0.30)	4.3 ^a (±0.71)
Refrigerated/cooked	3.5 ^{ab} (±0.93)	3.9 ^{ab} (±0.83)	3.5 ^{ab} (±0.19)	3.4 ^{ab} (±0.92)	3.2 ^{ab} (±0.20)	3.1 ^{ab} (±0.99)	3.0 ^{cd} (±1.51)	1.8 ^{cd} (±1.16)

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.

gave similar panel data, therefore in Tables 4 and 5 we presented the data for 30 days only). The consumer panel data for the treated and untreated whole and dehulled flour of Ashana cultivar (Table 4) indicated that significant ($P \leq 0.05$) differences in color, flavor, bitterness or overall acceptability were observed between fresh flour and that stored for 30 days for the whole flour. However, for the dehulled flour no significant ($P \leq 0.05$) differences in color, flavor, bitterness or overall acceptability were observed between fresh dehulled flour and that stored for 30 days. Moreover, the whole flour accepted by the panellists better than the dehulled one. Cooking of the whole flour significantly ($P \leq 0.05$) reduced the score obtained for the color, flavor and overall acceptability of the whole flour but for the dehulled one significantly ($P \leq 0.05$) increased. Refrigeration alone had low score for color and overall acceptability but had high one for flavor and bitterness for the whole flour while that of the dehulled had high score for color and overall acceptability and low score for color and flavor. When refrigeration combined with cooking, the score for bitterness increased for both whole and dehulled flour. The results obtained for sensory characteristics of Dembi cultivar (Table 5) are similar to those reported for Ashana cultivar. The effect of

refrigeration on the sensory characteristics of millet flour has not been reported previously. We found that refrigeration caused noticeable changes in these characteristics especially the bitterness attribute which affect the quality of the flour during storage.

Conclusion: The observations about phytic acid and tannin in the studied samples tend to suggest that refrigeration had little effects on their value and had no effects on the protein digestibility but improved the sensory quality of the flour whether whole or dehulled. Therefore, refrigeration can be applied to alleviate the severe problem of off-flavor and bitter taste during storage.

REFERENCES

1. Abdalla, A.A., A.H. El Tinay, B.E. Mohamed and A.H. Abdalla, 1998. Proximate composition, starch, phytate and mineral contents of 10 pearl millet genotypes. Food Chemistry, 63: 243-246.
2. AbdelRahaman S.M., H.B. ElMaki, W.H. Idris, A.H. Hassan, E.E. Babiker and A.H. El Tinay, 2007. Antinutritional factors content and hydrochloric acid extractability of minerals in pearl millet cultivars as affected by germination. International Journal of Food Sciences and Nutrition, 58: 6-17.

3. Ali, A.M., A.H. ElTinay and A.H. Abdalla, 2003. Effect of fermentation on the in vitro protein digestibility of pearl millet. *Food Chemistry*, 80, 51-54.
4. AOAC, 1990. *Official Methods of Analysis*. Association of Official Agricultural Chemists. 15th edn. Washington, DC.
5. Carbonaro, M., G. Grant, M. Cappelloni and A. Pusztai, 2000. Perspectives into factors limiting in vivo digestion of legume proteins: antinutritional compounds or storage protein? *Journal of Agricultural and Food Chemistry*, 48: 742-749.
6. Elhag, M.E., A.H. El Tinay and N.E. Yousif, 2002. Effect of fermentation and dehuling on starch, total polyphenols, phytic acid content and in vitro protein digestibility of pearl millet. *Food Chemistry*, 77: 193-196.
7. Elyas, S.H., A.H. El Tinay, N.E. Yosif and E.A. Elsheikh, 2002. Effect of fermentation on nutritive value and in vitro protein digestibility of pearl millet. *Food Chemistry*, 78: 75-79.
8. Khetarpaul, N. and B.M. Chauhan, 1990. Effects of germination and pure culture fermentation by yeasts and lactobacilli on phytic acid and polyphenol content of pearl millet. *Journal of Food Science*, 55: 1180-1182.
9. Klopfenstein, C.F., H.W. Leipold and J.E. Cecil, 1991. Semiwet milling of pearl millet flour reduced goitrogenicity. *Cereal Chemistry*, 68: 177-179.
10. Kumar, A. and B.M. Chauhan, 1993. Effects of phytic acid on protein digestibility (*in vitro*) and HCl extractability of minerals in pearl millet sprouts. *Cereal Chemistry*, 70: 504-506.
11. Monjula, S. and E. John, 1991. Biochemical changes and in vitro protein digestibility of the endodermis of germinating *Dolchos lablab*. *Journal of the Science of Food Agriculture*, 55: 229-238.
12. Price, M.L., S. Van Scoyoc and L.G. Butler, 1978. A critical evaluation of the vanillin reactions as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, 26: 1214-1218.
13. Ravindran, V., W.L. Bryden and E.T. Kornegay, 1995. Phytates: occurrence, bioavailability and implications in poultry nutrition. *Poultry Avian Biological Reviews*, 6: 125-143.
14. Rehman, Z.U. and W.H. Shah, 2001. Tannin contents and protein digestibility of black grams (*Vigna mungo*) after soaking and cooking. *Plant Food Human Nutrition*, 56: 265-273.
15. Reyden, P. and R.R. Selvendran, 1993. Phytic acid: properties and determination. In: Macrae, R., Robinson, R.K., Sadler, M.J. (Eds.), *Encyclopedia of Food Science, Food Technology and Nutrition*. Academic Press, London, pp: 3582-3587.
16. Snedecor, G.W. and W.G. Cochran, 1987. *Statistical Methods*, 17th edn. pp: 221-222. Ames, IA: The Iowa State University Press.
17. Van der Poel, A.F.B., 1990. Effect of processing on antinutritional factors and protein nutritional value of dry beans. *Journal of Animal Feed Science and Technology*, 2: 179-208.
18. Wheeler, E.L. and R.E. Ferrel, 1971. A method for phytic acid determination in wheat and wheat fractions. *Cereal chemistry*, 48: 312-320.