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Preservation of Millet Flour by Refrigeration: Changes in Total Protein and Amino Acids Composition during Storage

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Abstract—This work describes refrigeration effects during storage on total protein and amino acids composition of raw and processed flour of two pearl millet cultivars (Ashana and Dembi). The protein content of the whole raw flour was found to be 14.46 and 13.38% for Ashana and Dembi cultivars, respectively. Dehulling of the grains reduced the protein content to 13.38 and 12.67% for the cultivars, respectively. For both cultivars, the protein content of the whole and dehulled raw flour before and after cooking was slightly decreased when the flour was stored for 60 days even after refrigeration. The effect of refrigeration process in combination with the storage period, cooking or dehulling was found to be vary between amino acids and even between cultivars. Regardless of the storage period and processing method, the amino acids content was remained unchanged after refrigeration for both cultivars.

Keywords**—**Amino acids, Dehulling, Irradiation, Millet, Protein content.

I. 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]. 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 such as Jiria and Damierga are prepared from fermented dehulled pearl millet flour. 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. [3] for gray, yellow and brown pearl millet, respectively. Local Sudanese cultivars investigated by Elyas et al. [4] gave a range of 10.8-14.9% protein and were also found to be rich in minerals [5]. The objectives of heat treatments, developed primarily for fruits, are to achieve insect disinfestations, to control diseases, to modify tissue response to other types of stress and to maintain product quality during storage [6]. These aims are the same as those set for the post-harvest of vegetables. To put them into practice, several times–temperature combinations must be tested in advance, taking into account that the botanical origin of edible parts (fruits, stems, seeds, petioles, leaves and buds) is, in vegetables and cereals, considerably broader. Moreover, the optimum time and temperature combination chosen to extend fresh product quality during storage depends on cultivars, maturity stage, size and growing conditions [7]. In addition, selection of treatment type among heating in dry air, steam or water, may depend on product characteristics. Application of heat treatments whether high or low in minimally processed products would comprise additional objectives, namely to reduce physiological alterations in the plant, induced by mechanical and oxidative damage, as well as to lessen the responses linked to cicatrisation or wounding protection [8]. Pearl millet flour had a severe problem during storage and it 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 foods 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 protein and amino acids contents of raw and processed pearl millet flour during storage.

II. Materials and Methods

A. 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 flour was refrigerated at 4°C ± 1. All experiments were repeated three times and 3 replicates of each flour type were refrigerated. All chemicals used for the experiments were of reagent grade.

B. Processing and storage of the samples

Treated and untreated samples of whole and dehulled raw

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flour of each cultivar were divided into two portions. One portion was stored for 60 days in polythene bags at room temperature 25°C or at 4°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 60 days at room temperature 25°C or at 4°C.

C. Determination of protein content

Total crude protein of the samples was determined by following the AOAC method [9].

D. Amino acids composition

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 [10].
About 200 mg of the sample was taken in a hydrolysis tube. Then 5 mL of 6 N HCl was added to and the tube tightly closed and incubated at 110°C for 24 h. After incubation, the solution was filtered and 200 mL of the filtrate was evaporated to dryness at 140°C for 1 h. The hydrolysates after dryness were diluted with 1.0 mL of 0.12 M citrate buffer (pH 2.2). Aliquot of 150 μL of the sample hydrolysates was injected in an action separation column at 130°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°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 g/100g protein.

E. Statistical analysis

Each determination was carried out on three separate samples, on dry weight basis and analyzed in triplicate, the figures were then averaged. Data were assessed using ANOVA [11]. Mean comparisons for treatments were made using Duncan's multiple range tests. Significance was accepted at P ≤ 0.05.

III. RESULTS AND DISCUSSION

A. Effect of refrigeration on protein content of raw and processed millet flour during storage

Fig. 1 shows the effect of refrigeration process on protein content of whole and dehulled flour of two millet cultivars, Ashana (Fig. 1A) and Dembi (Fig. 1B) during processing and storage. For Ashana cultivar (Fig. 1A), the protein content of the whole raw flour was 14.46% and 13.90 before and after storage, respectively. Refrigeration process of the whole raw flour slightly decreased the protein content. Cooking of the whole flour reduced the protein content of the cultivar to 13.64% and further decreased to 13.11% during storage for 60 days. However, cooking of the refrigerated flour slightly increased the protein content even after storage for 60 days. Dehulling of the grains reduced the protein content of the cultivar to 13.38% and further reduction was observed after storage for 60 days (12.88%). Refrigeration of the dehulled flour slightly increased the protein content to 13.30% compared to the stored samples. Cooking of the dehulled flour reduced the protein content of the cultivar to 13.15% and further reduction (12.66%) was observed during storage (60 days). However, cooking of refrigerated flour slightly increased the protein content even after storage for 60 days (13.12%). The results obtained for Dembi cultivar (Figure 1B) are similar to those reported for Ashana (Fig. 1A). The results obtained showed that refrigeration process had little or minor effect on the protein content of both cultivars. However, cooking of the flour before or after storage was found to cause a significant (P ≤ 0.05) change in the protein content of the whole and dehulled flour for both cultivars but cooking of refrigerated flour alleviates such effect. Refrigeration of food is essential to maintain the inherent quality of the product, gradual changes in physicochemical, microbiological and sensory quality are certain. The oxidative and lipolytic deterioration lower the shelf life of the product [12]. A high level of oxygen in the packaging atmosphere was found to increase both lipid and protein oxidation during storage as evaluated by the analysis of secondary lipid oxidation products and by 2,4-dinitrophenylhydrazine derivatisation of protein carbonyls [13]. The storage temperature affected microbial development and production of biogenic amines [14]. Increased storage days gradually decrease the sensory quality due to chemical changes occurred to the food product [15].

A

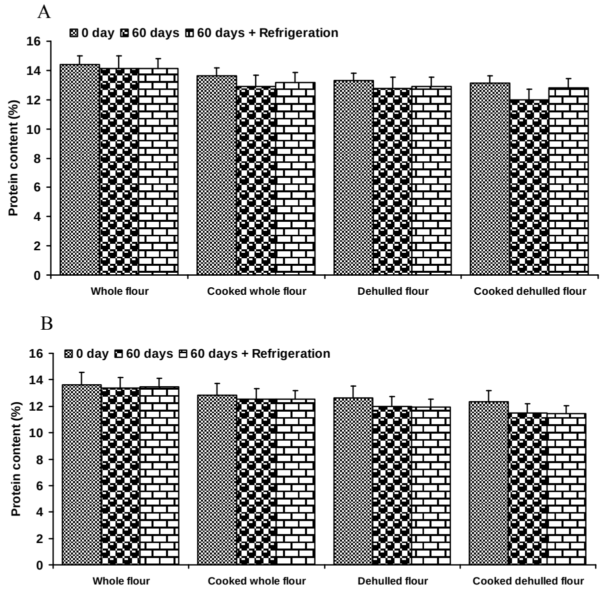

Fig. 1 Effect of refrigeration process on protein content (%) of raw and processed flour of Ashana (A) and Dembi (B) cultivars during

storage. Error bars indicate the standard deviation of triplicate samples.

B. Effect of refrigeration process on amino acid composition of raw and processed millet flour during storage

Fig. 2 shows the effect of refrigeration on amino acids composition of whole and dehulled raw flour of Ashana (Fig. 2A) and Dembi (Fig. 2B) cultivars during storage. As shown in Figure 2, glutamic acid is the dominant amino acid of the whole raw flour of Ashana (Fig. 2A) and Dembi (Fig. 2B) cultivars followed by leucine. For Ashana cultivar (Fig. 2A) storage of raw and refrigerated flour for 60 days was slightly reduced the level of most amino acids. For Dembi cultivar (Fig. 2B) the storage of the whole raw flour for 60 days slightly affected isoleucine, phenylalanine and serine. However, storage of refrigerated flour for 60 days had no effect on amino acids levels except glutamic acid, which was slightly decreased. Generally refrigeration process had no profound negative effect on amino acid level of raw flour of both cultivars during storage.

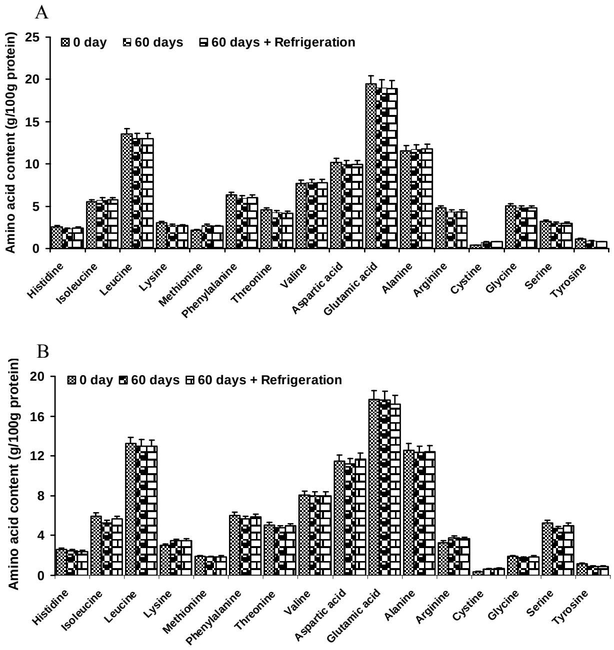

Fig. 2. Effect of refrigeration process on amino acid composition (g/100g protein) of whole raw flour of Ashana (A) and Dembi (B) cultivars during storage. Error bars indicate the standard deviation of triplicate samples.

Fig. 3 shows the effect of refrigeration process on amino acids composition of dehulled raw flour of Ashana (Fig. 3A) and Dembi (Fig. 3B) cultivars during storage. Dehulling of millet grains significantly ($P \leq 0.05$) decreased the protein as well as the amino acids contents of both cultivars. The amino acid content of dehulled raw flour of Ashana cultivar (Fig. 3A) was not greatly affected by storage except glutamic acid, which was slightly decreased. However, storage of the refrigerated flour insignificantly ($P \leq 0.05$) increased the level of glutamic acid. For Dembi cultivar (Fig. 3B) the amino acids level of dehulled raw flour during storage was not greatly affected except leucine but storage of refrigerated dehulled raw flour alleviates this effect. Dehulling had been reported to decrease the nutrients content of most plants seeds and grains; therefore the level of amino acids when compared to the raw flour was greatly decreased.

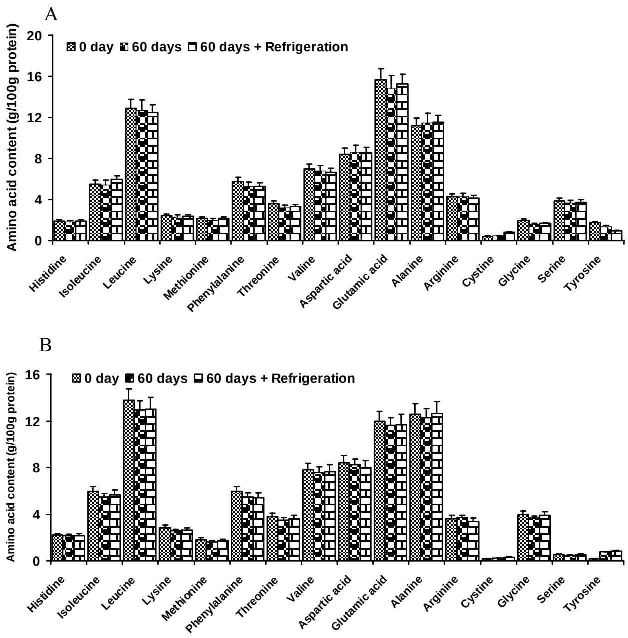

Fig. 3 Effect of refrigeration on amino acid composition (g/100g protein) of dehulled raw flour of Ashana (A) and Dembi (B) cultivars during storage. Error bars indicate the standard deviation of triplicate samples.

Fig. 4 shows the effect of refrigeration process on amino acids composition of cooked whole flour of Ashana (Fig. 4A) and Dembi (Fig. 4B) cultivars during storage. Storage of the raw cooked whole flour of Ashana (Fig. 4A) for 60 days had no significant effect on the amino acid composition but slightly decreased the level of leucine, lysine, phenylalanine, threonine, glutamic acid and alanine. However, storage of refrigerated and cooked whole flour alleviates the effect of storage by causing slight increase in some amino acids such as phenylalanine. Storage of raw cooked whole flour of Dembi cultivar (Fig. 4B) had no great effect on the amino acid composition even when refrigerated flour was stored for 60 days. Fig. 5 shows the effect of refrigeration process on amino acids composition of cooked dehulled flour of Ashana (Fig. 5A) and Dembi (Fig. 5B) cultivars during storage. Storage of the raw cooked dehulled flour of Ashana cultivar (Fig. 5A) for 60 days had no effect on the amino acid composition except that leucine was slightly decreased even after refrigeration. Storage of raw cooked dehulled flour of Dembi cultivar (Fig.5B) had no great effect on amino acid composition except leucine and phenylalanine, which were slightly decreased. It has been reported that cooking of corn flour significantly (P $\leq$ 05) reduced threonine and histidine contents and significantly increased phenylalanine content while other amino acids were only slightly affected by cooking [16].

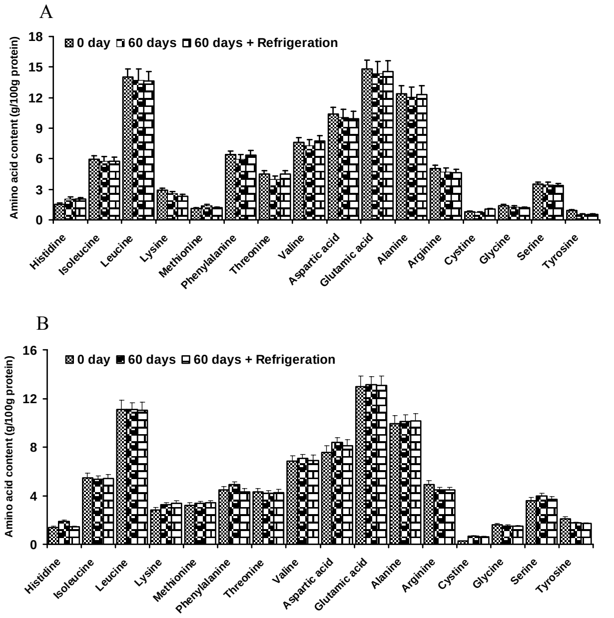

Fig. 4 Effect of refrigeration on amino acid composition (g/100g protein) of cooked whole flour of Ashana (A) and Dembi (B) cultivars during storage. Error bars indicate the standard deviation of triplicate samples.

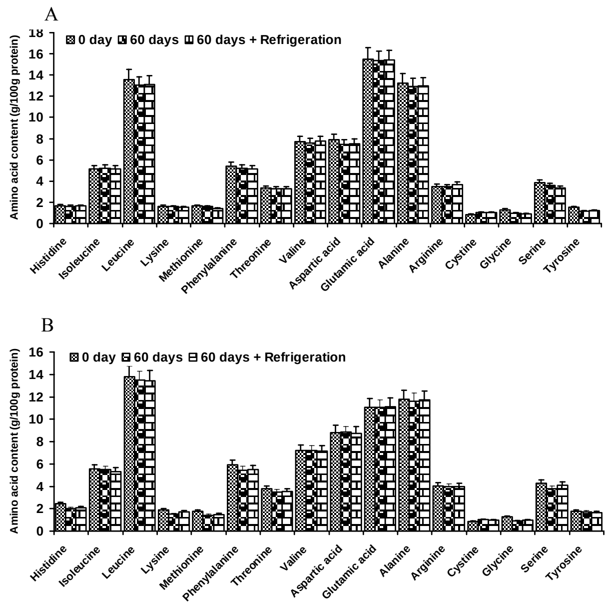

Fig. 5 Effect of refrigeration process on amino acid composition (g/100g protein) of cooked dehulled flour of Ashana (A) and Dembi (B) cultivars during storage. Error bars indicate the standard deviation of triplicate samples.

IV. Conclusion

The observations about protein and amino acid compositions in the studied samples tend to suggest that refrigeration process had little effects on their value and had no effects on the protein 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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