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Effect of Processing Treatments Followed by Fermentation on Protein Content and Digestibility of Pearl Millet (*Pennisetum typhoideum*) Cultivars

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Abstract: Two pearl millet cultivars namely Gadarif and Gazeera were used in this study. The effect of soaking, debranning, dry heating and germination of the grains before and after fermentation on protein content and digestibility was investigated. The effect of processing treatments on the protein content was fluctuated and varied between the cultivars. For both cultivars germination of the grains increased the protein content and digestibility (except course ground grains). For both cultivars fermentation of the germinated and course ground grains increased the protein content while fermentation of other treated grains fluctuated between the cultivars. The protein digestibility of the treated grains after fermentation was greatly improved. For both cultivars fermentation of the germinated grains gave higher protein digestibility (> 90%) compared to all other treatments.

Key words: Cultivars, fermentation, protein, digestibility, pearl millet

Introduction

Pearl millet (*Pennisetum typhoideum*) is a staple food in large segment population in Asian and African countries where it contributes a major part of directory nutrients (Burton *et al*., 1972). Pearl millet is grown annually on about 26 million ha in the arid and semi-arid tropical areas of Africa and India principally for grain and forage. Among millets, pearl millet is known to have a higher protein content and better amino acid balance than sorghum. The higher ratio of germ to endosperm is responsible for the higher protein content (Dendy, 1995). Fermented cereal products are widely consumed in India and many countries of Central and Southern Africa. Fermentation usually involves malting and souring by mixed cultures of yeast and lactobacilli. Fermentation causes degradation of grain components, especially starch and soluble sugars, by both grain and fermented media enzymes (Chavan and Kadam, 1989a,b). Pearl millet is no doubt superior to cereal with respect to some of nutrients especially average protein, mineral and fat (Usha *et al*., 1996). However, the presence of various antinutrients, poor digestibility of the protein and carbohydrates and low palatability greatly affected its utilization as a food. Various processing treatments are known to affect the chemical composition of food, improve its digestibility and nutritive value (Alka-Sharma and Kapoor, 1996). Fermentation (Khetarpaul and Chauhan, 1990 and 1991; Chavan *et al*., 1988 and Usha *et al*., 1996) and sprouting (Chavan and Kadam, 1989b) have been reported to increase the protein digestibility of millet. Considering the nutritional value, ease to culture, low investment and proportionally high returns, the present study was carried out to develop techniques for improving the protein digestibility of pearl millet grains by applying various processing treatments followed by natural fermentation for different period of time.

Materials and Methods

Seeds of two millet cultivars (Gadarif and Gazeera) were obtained from Khartoum North local market, Sudan. Seeds were cleaned, freed from foreign materials as well as broken seeds. The two cultivars seeds were divided into five parts and kept for processing treatments. Unless otherwise stated all reagents used in this study were reagent grade.

Processing treatments

Grinding: The cultivars seeds were ground to fine and coarse particles to pass through 0.2 and 1mm screen, respectively.

Soaking: The seeds were soaked in water for 18 h. Then the soaked grains were dried at 60°C and ground to pass a 0.2 mm screen.

Debranning: The seeds were soaked in water for 18 h and then hand pounded to separate the bran. The debranned grains were then dried at 60°C and ground to pass a 0.2 mm screen.

Dry heating: The cultivars grains were ground to pass a 0.2 mm screen and autoclaved at 110°C for 10 min.

Germination: The whole grains of each cultivar were immersed in water overnight and then the grains were spread on trays lined with cloth. It was kept wet by

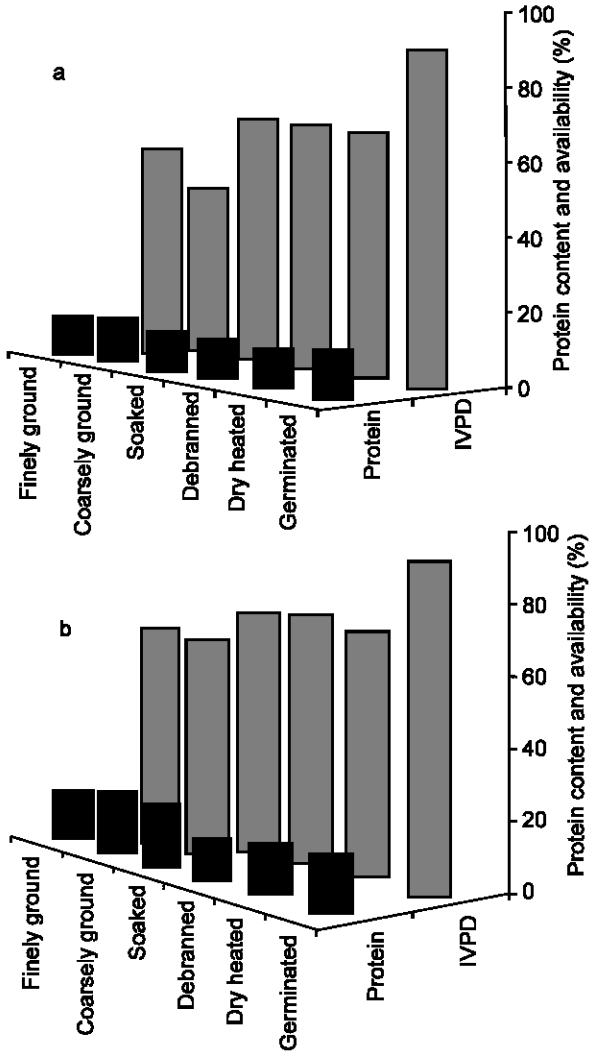

Fig. 1: Effect of processing treatments on percent protein content and *in vitro* digestibility (IVPD) of millet cultivars a. Gadarif and b. Gazeera.

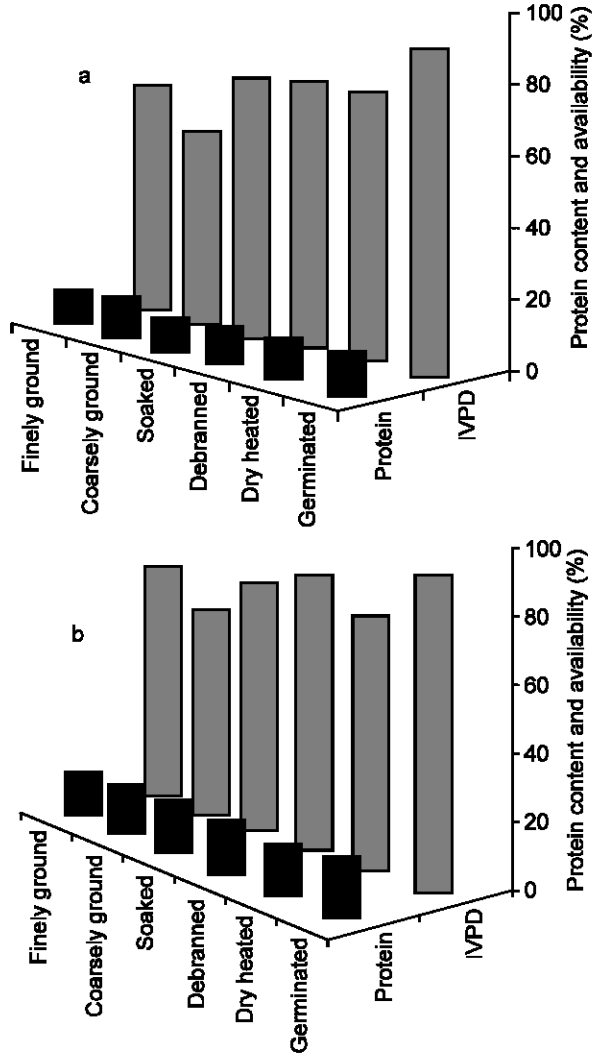

Fig. 2: Effect of processing treatments followed by fermentation for 12 h on percent protein content and *in vitro* digestibility (IVPD) of millet cultivars a. Gadarif and b. Gazeera.

frequent spraying of water. After 36 h the germinated grains were removed from the trays, sun-dried and ground to pass a 0.2 mm screen.

Natural fermentation: The cultivars grains were ground to pass a 0.2 mm screen and then mixed with distilled water (1: 3 w/v). The mixture was incubated at 37°C. After 12 or 24 h the fermented mixture was sun-dried and ground to pass a 0.2 mm screen.

Crude protein determination: Total nitrogen content of raw and processed samples was estimated using the semi-microkjeldahl digestion and distillation method as described by AOAC (1984).

***In vitro* protein digestibility (IVPD) determination:** *In vitro* protein digestibility of raw and processed samples was measured according to the method of Saunder *et al.* (1973). About 250 mg sample was suspended in 15 ml of 0.1 N HCl containing 1.5 mg pepsin (1:10,000) in a 100 ml conical flask. The mixture was incubated at 37°C for 3 hours. The mixture was then neutralized with 0.5 N NaOH and treated with 4 mg pancreatin in 7.5 ml of 0.2 M phosphate buffer (pH 8.0), containing 0.005 M sodium azide. The mixture solution was incubated at 37°C for 24 hours. Ten milliliters of 10% trichloroacetic acid (TCA) were added to the mixture to stop the reaction. The mixture was then centrifuged at 5000 rpm for 5 minutes. About 5.0 ml aliquots from the supernatant was pippetted and analyzed for nitrogen content.

$$\text{Protein digestibility \%} = \frac{\text{N in supernatant - enzyme N}}{\text{N in Sample}} \text{ X } 100$$

Results and Discussion

Fig. 1 summarizes the protein content and *in vitro* protein digestibility (IVPD) of treated grains of (a) Gadarif and (b) Gazeera cultivars. The protein content of Gadarif cultivar was found to be 11.4% (Fig. 1a) while that of Gazeera was 14.4% (Fig. 1b). Germination of the grains was found to increase the protein content for both cultivars to 13.2 and 16.3%, respectively. The increment in protein content of the germinated grains may be due to quantitative reduction in antinutritional factors (tannin, polyphenols and phytic acid) as well as other constituents of the grains such as starch. Soaking and dry heating were observed to increase the protein content of Gazeera cultivar while that of Gadarif was decreased. The variations between the cultivars in response to different processing treatments may be attributed to the nature and type of proteins of each cultivar. The *in vitro* protein digestibility of untreated grains (finely ground) of Gadarif cultivar was found to be 63.2% (Fig. 1a) while that of Gazeera cultivar was 66% (Fig. 1b). Germination of the grains was found to increase the IVPD of Gadarif and Gazeera cultivars to 90.1 and 91.7%, respectively (Fig. 1). Coarse grinding of the grains caused great reduction in IVPD of Gadarif (50.2%) and Gazeera (62.9%) cultivars. Dry heating, debranning and soaking of the grains slightly improved the IVPD of both cultivars. The improvement in protein digestibility after germination, soaking, debranning, and dry heating could be attributable to the reduction of antinutrients such as phytic acid, tannins and polyphenols, which are known to interact with proteins to form complexes. Heat processing has been reported to increase the digestibility of proteins by destroying protease inhibitors (Abbey and Berezi, 1988). The reduction in IVPD of coarse ground grains may be attributed to the seed coat antinutritional factors such as tannin and polyphenols (Alonos *et al.*, 2000). The effect of fermentation for different periods of time on the protein content and IVPD of the processed grains is shown in Fig. 2 and 3. Fermentation of the processed grains for 12 h (Fig. 2a and b) was slightly affected the protein content of the cultivars. The effect of fermentation on protein content varied between the cultivars and the processing treatments applied. However, the protein digestibility was significantly improved when the processed grains were fermented for 12 h for both cultivars. Fermentation of the germinated grains improved the IVPD of Gadarif cultivar to 91.1% (Fig. 2a) while that of Gazeera improved to 92.0% (Fig. 2b). Fermentation of dry heated, debranned, soaked and course ground grains for 12 h also improved the IVPD but the rate of improvement varied between the processing treatments and cultivars. Further increase

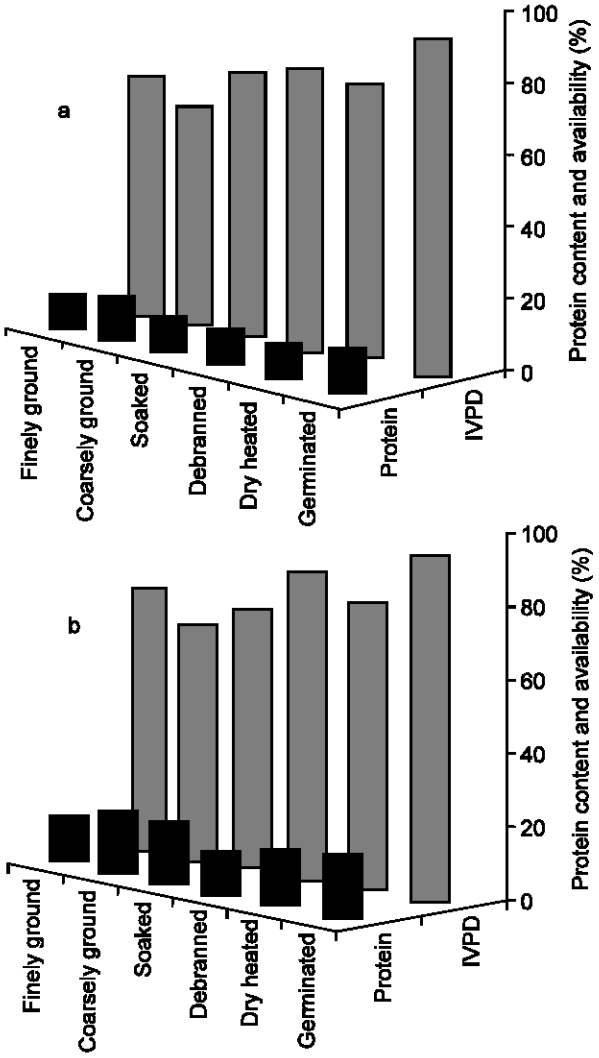

Fig. 3: Effect of processing treatments followed by fermentation for 24 h on percent protein content and *in vitro* digestibility (IVPD) of millet cultivars a. Gadarif and b. Gazeera.

in the fermentation time (24 h) was observed to caused a further improvement in IVPD of the cultivars (Fig. 3a and b). Fermentation of the germinated grains for 24 h improved the IVPD of both Gadarif and Gazeera cultivars to 93.6% (Fig. 3a and b). Fermentation of other processed grains for 24 h also improved the IVPD of the cultivars even the IVPD of the course ground grains. The improvement in IVPD caused by fermentation could be attributed to the partial degradation of complex storage proteins to more simple and soluble products (Chavan *et al.*, 1988); it could also be attributed to the degradation of tannins, polyphenols and phytic acid by microbial enzymes. The results obtained in this study agree with those reported by ElHag *et al.* (2002) who found that fermentation of millet seeds improved IVPD.

Enhanced proteolytic activity during fermentation is generally associated with improved protein digestibility, which increases amino nitrogen by partial breakdown of proteins peptides and amino acid (ElHag *et al.,* 2002).

Conclusion: Fermentation of processed pearl millet grains caused significant reduction in antinutritional factors of the grains, which was accompanied by significant improvement in the protein digestibility. It may be inferred that among various processing treatments germination followed by natural fermentation proved to be more effective in increasing the protein digestibility of the cultivars.

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