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FULL LENGTH ARTICLE

Changes in protein nutritional quality as affected by processing of millet supplemented with *Moringa* seed flour

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Abstract Pearl millet flour was supplemented with 5%, 10% and 15% defatted *Moringa* seeds flour (DMSF). Raw and supplemented flour were fermented and/or cooked for 0, 8 and 16 h. Changes in protein content and digestibility and amino acid compositions and scores of the samples were investigated. Supplementation of raw flour increased significantly ($p \leq 0.05$) the protein content and digestibility. Further increase in protein content and digestibility was observed in the fermented dough of raw flour and higher values were obtained after cooking of 16 h-fermented dough ($p \leq 0.05$). Amino acids were increased significantly ($p \leq 0.05$) with supplementation level. Cooking of the flour supplemented with 10% DMSF lowered both essential and non-essential amino acids with lysine and glycine reduced to 25.68 and 12.09 mg/100 g, respectively. Fermentation for 16 h increased amino acids except isoleucine, phenylalanine, arginine, serine and proline compared to cooked composite flour. All amino acids were significantly ($p \leq 0.05$) increased after cooking of 16 h-fermented dough. The chemical scores of the essential amino acids of the flour were fluctuated after supplementation. The majority of the amino acids scores were decreased after cooking of 10% DMSF fermented dough except histidine, lysine and threonine.

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1. Introduction

In developing countries, the high price of animal foods and limited income earned by majority people has resulted in their dependency on cereal grains as staple food. Also increase in human population in developing countries and short-falls in cereal production in several developed countries had resulted

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in increase in demand for cereals as foods, feed and industrial raw materials (Sokrab et al., 2012).

Millet is a cereal grain used in the production of various traditional foods and beverages and as major food components such as bread, snack foods and porridges in many African and Asian countries (Chandrasekara et al., 2012). Studies have shown that millet also possesses some health benefits such as reducing blood pressure, heart diseases and cholesterol and supplying gastrointestinal bulk (Gupta et al., 2012). Millet grains and products are now receiving great interest from researchers in food scientist and nutritionist due to their potential health benefit and contribution to national food security (Saleh et al., 2013). However there is inadequacy in the nutritive value of millet, like other cereals, because of its deficiency in essential amino acids like lysine (Ali et al., 2009). Millet flour also has low *in vitro* protein digestibility (45.75%) and this varies among fractions of the grain (Nour et al., 2015b).

Several researches or approaches have been carried out in improving the low protein quality and biological utilization of nutrients in millets which serve as a basic staple food for majority of people in developing countries (Ali et al., 2009; Mohamed et al., 2010a–c, 2011). Fortification and processing are some of the approaches that can also be used to solve such problems. Cheap and readily available plant proteins from legumes are large replacing animal protein as suitable source of high quality protein (Annan and Plahar, 1995). *Moringa oleifera* is a fast growing drought resistant leguminous crop commonly grown in Africa and Middle East. Moringa are rich in protein source and are being recommended by nutritionist to solve the problem of malnutrition worldwide (Thurber and Fahey, 2009). The legume contains high amount of essential amino acids such as lysine which are deficient in most cereal grains such as millet. Supplementation of millet flour with Moringa seeds flour has been reported to increase *in vitro* protein digestibility and antinutritional factors of the millet flour (Nour et al., 2015a).

Improvement in the nutritional value of plant grains has been achieved by various simple processing methods such as cooking, fermentation and soaking (Nour et al., 2010; Yagoub and Abdalla, 2007). Research has shown that fermentation increased the *in vitro* protein digestibility and mineral contents of pearl millet cultivars flour (AbdelRahaman et al., 2005). Cooking on the other hand has been reported to decrease the *in vitro* protein digestibility and values of antinutritional factors of pearl millet flour (Nour et al., 2015a). There is still dearth of information on the combined effect of fermentation and cooking coupled with supplementation on the protein quality and digestibility of millet flour. Therefore this study was conducted to investigate the effect of fermentation and/or cooking of millet flour supplemented with DMSF on protein quality and *in vitro* protein digestibility.

2. Materials and methods

2.1. Sample preparation

Grain sample of millet seeds (*Pennisetum glaucum* L.) was obtained from Department of Agronomy, Faculty of Agriculture, University of Khartoum. The seeds were cleaned, freed from foreign, broken and shrunken seeds, milled into fine flour using house blender and mortar to pass through a 0.4 mm

screen and then stored in polyethylene bags at 4 °C for further analysis. *Moringa* (*M. oleifera*) was obtained from a private agricultural company, Khartoum North, Sudan. The seeds were cleaned, freed from extraneous matter, milled into fine powder, and defatted with cold (4 °C) acetone (flour to solvent ratio 1:5 w/v) with constant magnetic stirring provided for 4 h. The defatted flour was placed inside a fume cupboard for 6 h to dry and to remove any trace of residual acetone. The flakes were then milled into fine flour to pass a 0.4 mm sieve and kept at 4 °C for further analysis. The defatted freeze-dried *Moringa* seeds flour was added individually to millet flour using Pearson square to increase nutritive value of millet flour by 5%, 10% and 15%. All chemicals used in this study were of reagent grade.

2.2. Cooking

Cooking of the samples was carried out by suspending the flour of the sample in distilled water in the ratio of 1:2 (flour:water, w/v) and the mixture was shaken to avoid lumps while boiling in a water bath (Karle Kolb, 777015, Frankfurt, Germany) for 20 min. The viscous mass was spread out thinly in a dish and oven dried at 70 °C. The dried flakes were milled into fine flour using house blender (BLG-450, Binatone, Shenzhen, China) and mortar to pass through 0.4 mm screen and stored at 4 °C for further analysis.

2.3. Fermentation

Natural fermentation of millet flour and composite flours was carried out by mixing the flour with distilled water (1:2 w/v). About 250 g of each sample was mixed with 500 ml distilled water in 750 ml beaker and incubated (Gallenkamp, England) at 37 °C for periods 0, 8 and 16 h. After the incubation periods the samples were mixed using a glass rod and transferred to aluminum dishes (30 cm diameter), and dried in a freeze drier (12525, Virtis Company, Gardiner, New York). Dried samples were ground to pass through 0.4 mm screen and stored at 4 °C for further analysis.

2.4. Cooking of fermented dough

Slurry of the fermented dough of each sample was cooked for 10 min, cooled and dried in a freeze drier. The dried flakes were milled into fine flour and stored at 4 °C for further analysis.

2.5. *In vitro* protein digestibility

In vitro protein digestibility of the samples was measured according to the method described by Manjula and John (1991), using pepsin and pancreatin digestion method. The digested protein was analyzed for nitrogen using micro Kjeldahl procedure (AOAC, 1995) and expressed as a percent of the total N.

2.6. Amino acid compositions and scores determination

Amino acid composition of the samples was measured on hydrolysates using amino acids analyzer (Sykam-S7130/

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