



## Biochemical changes occurring during traditional Sudanese processing of Kisra bread

Magdi A. Osman <sup>1\*</sup>, Ibrahim E. Abdel Rahman <sup>1</sup>, Siddig H. Hamad <sup>1</sup> and Hamid A. Dirar <sup>2</sup>

<sup>1</sup> Department of Food Science & Nutrition, College of Food & Agricultural Sciences, King Saud University, P.O. Box 2460 Riyadh, 11451 Saudi Arabia. <sup>2</sup> Department of Botany & Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum, Khartoum North, 13314, Shambat, Sudan. \*e-mail: magdios@ksu.edu.sa

Received 12 August 2009, accepted 12 April 2010.

### Abstract

Changes in crude protein, soluble sugars and organic acids during traditional Kisra bread preparation from three sorghum varieties were investigated. Fermented dough was prepared in the traditional way used by Sudanese housewives. For the three varieties the protein content was not significantly increased during the fermentation. Traditional fermentation caused decrease in soluble sugars content, whereas the main by-products of the fermentation, lactic acid, acetic acid and ethanol increased. The pattern of change in the level of soluble sugars of the three sorghum varieties were similar. During the first 4 h of fermentation, glucose concentration increased by 259, 102 and 73% for Fetarita, Safra and Ahmer variety, respectively. Thereafter it started to decrease at a steady rate towards the end of the fermentation process. The fructose concentration was decreased and completely utilized after 12, 16 and 20 hours for Safra, Ahmer and Fetarita varieties, respectively. Sucrose in three varieties was completely utilized after 4 hours, while maltose was completely utilized after 8 h. Lactic acid production started from the beginning of fermentation to the end, while production of acetic acid and ethanol started after 4 hours of fermentation and continued to increase at slow rate. Fetarita variety had the least amount of lactic acid, acetic acid and ethanol, while there was no variation between Safra and Ahmer variety.

**Key words:** Sorghum, fermentation, glucose, maltose, sucrose, lactic and acetic acids, ethanol.

### Introduction

Grain sorghum (*Sorghum bicolor* L. Moench) is an important basic food in many parts of Africa and Asia, it is widely grown in the semiarid regions because of its drought tolerance. Sorghum is the fifth most important cereal in world production, being exceeded by wheat, rice, maize and barley in that order <sup>11</sup>. Sorghum and millet are cultivated in many parts of the Sudan mainly in the Gezira region. According to the Ministry of Agriculture <sup>19</sup> the annual production of sorghum is about 3.7 million tons and millet is about million tons while area under cultivation is 6.5 to 15.3 million feddans for sorghum and 1.5 to 7.7 million feddans for millet. The annual consumption of sorghum and millet in the Sudan is about 1.5 to 2 million tons from sorghum and about 0.23 to 0.28 million tons from millet and about 90% of this quantity is used as human food and hence goes mainly to Kisra production <sup>13</sup>. Fermentation of food is one of the oldest and most economical methods of food processing; it often leads to an improvement in the nutritional value of foods through bioenrichment with microbial proteins, amino acids, lipids and vitamins <sup>18</sup>.

The food preparation methods of sorghum are generally simple, the basic diet in most Africa being a porridge or stiff paste prepared by adding pounded flour to hot water, whereas in Ethiopia and the Sudan a flat cake is made. The grain may also be parched, popped or boiled as a whole grain <sup>7</sup>. Fermentation and germination are two classical technologies commonly used to improve on the protein digestibility and the B6 vitamin content of cereals, either

by decreasing the amount of inhibitors or releasing the nutrients for absorption <sup>6</sup>. The objective of this study was to investigate the changes in crude protein, soluble sugars and organic acids during traditional fermentation of sorghum (Kisra).

### Materials and Methods

**Materials:** Sudanese sorghum (*Sorghum bicolor*) of the cultivars locally known as Fetarita, Safra and Ahmer were purchased from local grain markets in Omdurman. All samples were carefully cleaned and freed from dirt, stones, chips and other extraneous grains or grits. Sorghum grains were milled at the local grain market to fine flour using a Diamant Mill, model 500-mm (Denmark). The flour was transferred to the laboratory and stored at 25°C until used.

### Methods:

**The proximate analysis:** The chemical composition of sorghum grains, moisture, total ash and crude fiber were determined according to the cereal laboratory methods recommended by the American Association of Cereal Chemists <sup>1</sup>. Fat and crude protein (N× 6.25) were determined by the Soxhlet and Kjeldahl methods, respectively; total carbohydrates were calculated by difference <sup>4</sup>.

**Preparation of fermented dough:** Fermented dough was prepared in the traditional way used by Sudanese housewives. In the

laboratory, sorghum flour was mixed with sterile distilled water in a 1:2 (wt/vol) ratio. A small amount of the previously fermented dough was then added to the mixture of flour and water to act as a starter (about 5%). This mixture was incubated at 30°C for 24 h in a sterile covered flask (2 kg flour + 4 litres water). Fermentation was performed in duplicate and sampled every 4 h during the fermentation period (24 h). For determination of crude protein, samples were dried in a vacuum oven at 50°C for 24 h [Heraeus LBS-Co]. The dried samples were milled to a fine powder using a coffee miller, passed through a 60 mm mesh and kept at 4°C.

**Determination of sugars:** For glucose, sucrose, fructose and maltose estimation, the dough was centrifuged at 10,000 r.p.m. for 20 min and passed through a membrane filter 0.45 µm. The clear supernatant was analyzed using HPLC Shimadzu LC from Shimadzu, Kuoto-Japan. The mobile phase (20% water and 80% acetonitrile, HPLC grade) was introduced by a delivery pump model LC-10AD (Shimadzu) at a flow rate of 2.5 ml/min. The system was attached to an injector (Model SIL - 10A, Shimadzu) through which a 5µl sample was injected. The running time was 15 min. The peak areas for the calibration curves and for the calculations of sugar amounts in the samples were measured by an integrator model C-R7A (Shimadzu Chromatopac data processor). Sugar standards were purchased from Sigma (Sigma Chemical Co., St. Louis, Mo). Sample preparation and chromatographic procedure were conducted as described in AOAC<sup>4</sup>. Results were reported as a percentage (w/w).

**Determination of organic acids and ethanol:** For determination of organic acids and ethanol, the dough was centrifuged at 10,000 r.p.m. for 20 min. The supernatant was filtered through membrane filter 0.45 µm dia 25 mm (Schleicher & Schüll, Germany) and analyzed by HPLC used for the determination of sugars using the column PL Hi-plex H (from Polymer Laboratories Amherst, M.A. 01002, U.S.A) fast acid column, mobile phase 0.001 M H<sub>2</sub>SO<sub>4</sub> at 57°C flow rate 0.7 ml/minutes. Results were reported as percentage (w/w).

## Results and Discussion

**Chemical composition of the sorghum grains:** The chemical composition of the whole grain of the three varieties studied is shown in Table 1. The moisture content ranged from 7.0% (Fetarita) to 8.2% (Safra) and that of crude fat from 2.7% for (Fetarita) to 3.0% for Safra and Ahmer. The ash content varied from 1.4% (Safra) to 1.8% for (Fetarita) and the protein content was 13.6, 10.1 and 11.1% for Fetarita, Safra and Ahmer, respectively, while the crude fiber ranged from 1.4 (Fetarita) to 2.0% (Ahmer). The carbohydrate content was 73.5, 75.7 and 74.2% for Fetarita, Safra and Ahmer, respectively. These results agree with those reported by Yousif and Magboul<sup>25</sup> and Rooney *et al.*<sup>20</sup>. The protein content of the

**Table 1.** Proximate analysis of three varieties of sorghum.

Cultivar	Moisture %	Carbohydrate %	Crude Fat %	Crude Fiber %	Protein %	Ash %
Fetarita	7.0	73.5	2.7	1.4	13.6	1.8
Safra	8.2	75.7	3.0	1.6	10.1	1.4
Ahmer	8.0	74.2	3.0	2.0	11.1	1.7

Results are means of triplicate determinations.  
Carbohydrates content calculated by difference.  
Protein % (N x 6.25).

Fetarita variety was similar to that found by Eggum *et al.*<sup>8</sup> and Ahmed and Ramanatham<sup>3</sup> who reported 13.4 and 13%, respectively, but it was higher than that reported by Yousif and Magboul<sup>25</sup> and Khattab *et al.*<sup>16</sup> who found 12.4 and 9.4%, respectively. Yousif and Magboul<sup>25</sup> reported the following chemical composition for the Ahmer variety: moisture 9.1%, protein 11.2%, fat 3.8%, fiber 2.0%, ash 1.6% and carbohydrates 72.3%. These values are quite similar to ours. Our results of Safra variety in Table 1 are comparable to the results found by Khattab *et al.*<sup>16</sup>, except for the protein content (12.1%) which is slightly higher than that in the present study (10.1%).

**Effect of fermentation on crude proteins:** Table 2 shows changes in protein content during fermentation of the three sorghum varieties. Protein content of unfermented samples were 13.6, 10.2 and 11.2% for Fetarita, Safra and Ahmer, respectively. Fermentation for 24 h did not alter the protein content for the three varieties. These results indicate that fermentation does not seem to be viable process for increasing protein content in Kisra bread. The results obtained in this work agree with those reported by Hamad *et al.*<sup>14</sup>, who found no significant effect on the protein content as a result of Kisra fermentation. Similarly Chavan and Kadam<sup>5</sup> reported that the total protein content of cereals were not affected by fermentation. Trend has been observed in pearl millet<sup>26,27</sup>. In contrast, Abasiokong<sup>2</sup> found an increase in the protein content of sorghum from 15 to 30% after 4 days of fermentation. In a similar study, El-Tinay *et al.*<sup>10</sup> found a slight increase in the protein content as a result of Kisra fermentation.

**Table 2.** Effect of fermentation on crude protein % of sorghum varieties.

Cultivar	Time (h)		
	0	12	24
Fetarita	13.6	13.7	13.8
Safra	10.2	10.4	10.5
Ahmer	11.2	11.3	11.3

Results are means of triplicate determinations.  
Protein % (N x 6.25).

**Changes in soluble sugar content:** Results for soluble sugars change of the three sorghum varieties as function of traditional fermentation are illustrated in Fig. 1a-d. The HPLC analysis of monosaccharides and disaccharides during fermentation revealed the presence of glucose, fructose, sucrose and maltose in the three sorghum varieties (Fetarita, Safra and Ahmer). Previous studies on sorghum grain showed the presence of many types of sugars, which included those found in our study. El-Hidai<sup>9</sup> reported the presence of glucose, fructose, maltose and raffinose in unfermented sorghum grain while Hamad *et al.*<sup>14</sup> found glucose and maltose. In a similar study, five soluble sugars were identified from sorghum grain (maltose, raffinose, sucrose, glucose and fructose)<sup>15</sup>. In the three varieties glucose content ranged from 0.54 to 1.51 g/100 g, sucrose from 0.09 to 0.36 g/100 g, whereas fructose and maltose ranged from 0.08 to 0.14 g/100 g and from 0.44 to 0.66 g/100 g, respectively. Sugar concentration of the three varieties fall within the range reported<sup>15</sup>. Fetarita variety had the highest amount of sucrose (0.36 g/100 g) and maltose (0.66 g/100 g) while Safra had the highest amount of fructose (0.14 g/100 g) and Ahmer was higher in the amount of glucose (1.51 g/100 g). The change in the soluble sugar content in the three sorghum

varieties showed similar pattern. There were sharp increase in glucose content during the first 4 h of fermentation, corresponding to decrease in sucrose and maltose content. The glucose content increased by 259, 102 and 73% for Fetarita, Safra and Ahmer variety, respectively. After 4 hours the amount of glucose started to decrease at a steady rate towards the end of fermentation process (Fig. 1a). Similar to glucose, fructose concentration increased slightly after 4 hours (0.08 to 0.11 g/100 g) for Fetarita variety and started to decrease rapidly to reach 0.01 g/100 g after 16 hours. For Ahmer variety the fructose content decreased from 0.12 to 0.01 g/100 g after 12 hours while it was completely utilized after 12 hours in Safra variety (Fig. 1d). Hamad and Fields<sup>12</sup> reported similar finding that during the natural lactic acid fermentation of cereal the level of reducing sugars increased and then decreased as the fermentation period increase. Similarly, Khetarpaul and Chauhan<sup>17</sup> observed significant decrease in reducing sugars during 72 h fermentation. The increase and decrease in glucose and fructose content during Kiswa fermentation could be attributed to the action microorganisms. Maltose concentration decreased in the three varieties during fermentation process, after 4 hours the amount of maltose dropped nearly to half its initial amount for Ahmer variety (from 0.44 to 0.22 g/100 g), while in Fetarita and Safra it decreased about 70% of its initial concentration, and was completely utilized after 8 h in the three varieties (Fig. 1c). Sucrose content was very low in the three sorghum varieties. Fetarita had the highest sucrose content (0.36 gm/100 g) followed by Ahmer (0.10 g/100 g) and Safra (0.09 g/100 g). After 4 h fermentation sucrose was completely utilized in the three varieties indicating that sucrose is main substrate for lactic acid fermentation (Fig. 1b). A decrease in maltose and sucrose has also been reported for pearl millet traditional process into ben-saalga, a fermented gruel<sup>22,23</sup>.

**Organic acids and ethanol:** The HPLC analysis of organic acids and alcohol during traditional fermentation of Kiswa bread showed that lactic acid was the major product formed followed by acetic acid and ethanol in the three sorghum varieties. Both organic acids and alcohol showed significant increase as fermentation process and the highest values were attained after 24 h. The production of lactic acid started from the beginning of fermentation to the end, while production of acetic acid and ethanol started after 4 h of fermentation and continued to increase slowly. The concentration of lactic acid in the fermented dough after 24 h

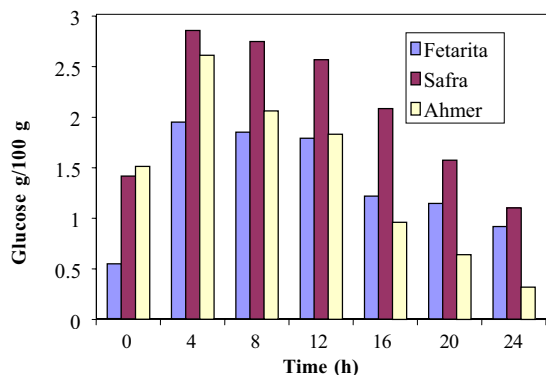


Figure 1a. Change in glucose concentration during fermentation period of sorghum varieties.

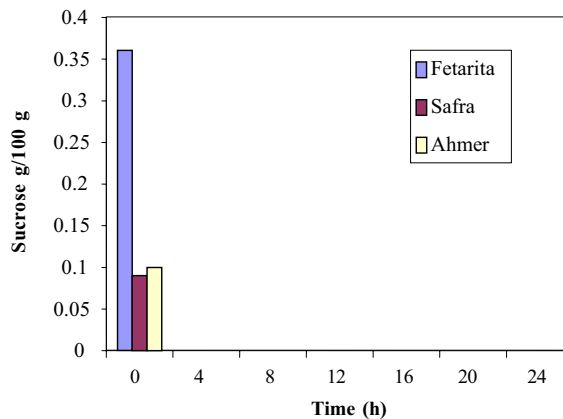


Figure 1b. Change in sucrose concentration during fermentation period of sorghum varieties.

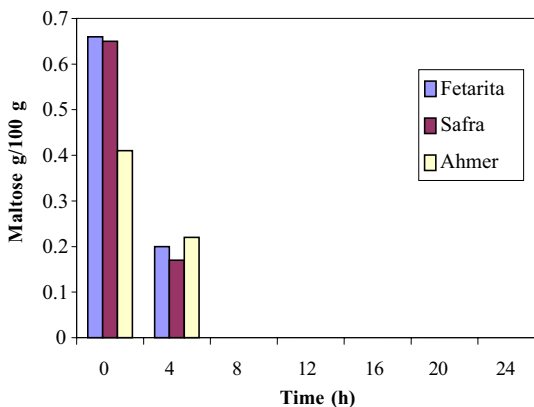


Figure 1c. Change in maltose concentration during fermentation period of sorghum varieties.

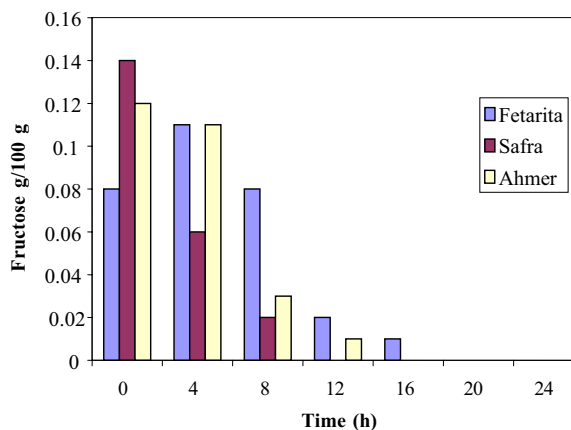


Figure 1d. Change in fructose concentration during fermentation period of sorghum varieties.

fermentation were 1.40, 1.81, and 2.04 g/100 g for Fetarita, Safra and Ahmer, respectively (Fig. 2a).

Acetic acid production, in Safra and Ahmer started during the first 4 h, while there was a delay in Fetarita variety 8 h (Fig. 2b). In both Safra and Ahmer, the final acetic acid concentration was higher (0.37 g/100 g) than that of Fetarita (0.27g/100 g). The production of organic acids such as lactic and acetic acids in

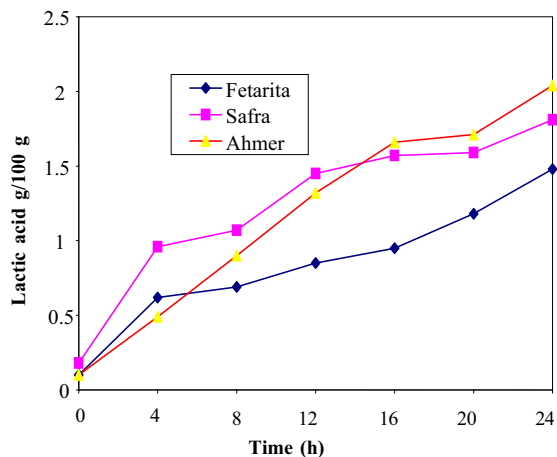


Figure 2a. Changes in lactic acid concentration during fermentation period of sorghum varieties.

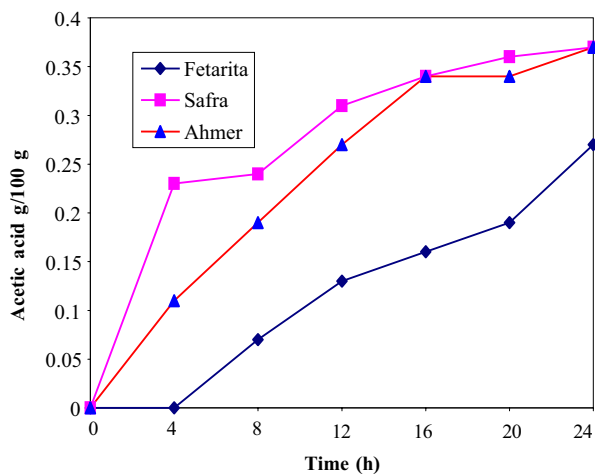


Figure 2b. Changes in acetic acid concentration during fermentation period of sorghum varieties.

traditional fermentation in Kisra bread is desirable because of their role in taste and flavour of the final product. The ethanol concentration in the three sorghum varieties steady increased as fermentation progress (Fig. 2c). Safra showed highest level, followed by Ahmer and Fetarita. The ethanol is undesirable in Kisra bread because of its off-flavour. However, those traces are usually eliminated during the heat treatment in final stage of Kisra bread making. Generally, high lactic and acetic acid was obtained from Ahmer, while high ethanol was obtained from Safra. Similar results were reported by El-Hidai<sup>9</sup> and Hamad *et al.*<sup>14</sup>. They found that lactic and acetic acids and ethanol were the major by-products of fermented sorghum and lactic acid was the main product. However, the amount of lactic acid, acetic acid and ethanol were much higher than that reported in our study. This variation in the results may be due to the amount of starter added to the dough, to the type of microorganisms or sorghum genotype. Zhan *et al.*<sup>24</sup> reported that both sorghum genotype and location had significant effect on ethanol and lactic acid yield. Ethanol and lactic and acetic acids were also found to be the major fermentation products during ben-saalga preparation from pearl millet<sup>21-23</sup>.

In conclusion the Kisra traditional process significantly changed

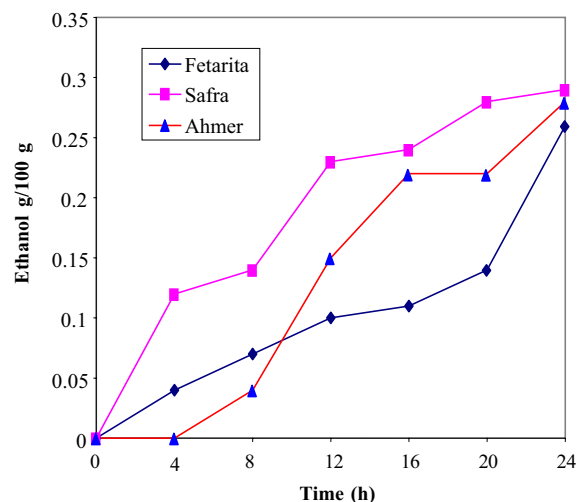


Figure 2c. Changes in ethanol concentration during fermentation period of sorghum varieties.

the profile of mono- and disaccharides. Lactic and acetic acids and ethanol are the main products of the traditional fermentation. In Sudan, ethanol production from sorghum may be good option to solve energy problems and save the environment and forest. Therefore, screening of sorghum genotypes for ethanol and lactic acid production should be considered.

#### Acknowledgements

The authors are grateful College of Food and Agriculture Sciences Research Center, King Saud University, for the financial support.

#### References

- <sup>1</sup>AACC 1983. Approved Methods of the American Association of Cereal Chemists. 8<sup>th</sup> edn. American Association of Cereal Chemists Inc., U.S.A.
- <sup>2</sup>Abasiekong, S.F. 1991. Protein and fat contents of crushed grains of maize and sorghum. *Journal of Appl. Bacteriol.* **70**:391-393.
- <sup>3</sup>Ahmed, A.R. and Ramanatham, G. 1988. Effect of natural fermentation on the functional properties of protein-enriched composite flour. *Journal of Food Science* **53**:218-221.
- <sup>4</sup>AOAC 1995. Official Methods of Analysis. 16<sup>th</sup> edn. Association of Official Analytical Chemists, Washington, DC.
- <sup>5</sup>Chavan, J.K. and Kadam, S.S. 1989. Nutritional improvement of cereals by fermentation. *Crit. Rev. Food Sci. Nutr.* **28**:349-400.
- <sup>6</sup>Dhankher, N. and Chauhan, B.M. 1987. Technical note. Preparation, acceptability and B vitamin content of Rabadi - fermented pearl millet food. *Int. J. Food .Sci. Technol* **22**:173-176.
- <sup>7</sup>Doggett, H. 1970. Sorghum. 2<sup>nd</sup> edn. Longmans Green and Co. Ltd, London and Harlow.
- <sup>8</sup>Eggum, B.O., Monawar, L., Knudsen, K.E., Munk, L. and Axtell, J. 1983. Nutrition quality of sorghum and sorghum foods from Sudan. *Journal of Cereal Science* **1**:127-137.
- <sup>9</sup>El-Hidai, M.M. 1978. Biochemical and Microbiological Investigation on Kisra Fermentation. M.Sc. thesis, University of Khartoum, Sudan.
- <sup>10</sup>El Tinay, A.H., Abdel Gadir, A.M. and El-Hidai, M. 1979. Sorghum fermented Kisra bread. 1. Nutritive value of Kisra. *Journal of Science, Food and Agriculture* **30**:859-863.
- <sup>11</sup>FAO 1977. Year Book of Statistics. Food and Agriculture Organization of the United Nations, Rome, Italy.
- <sup>12</sup>Hamad, A.M. and Fields, M.L. 1979. Evaluation of the protein quality and available lysine of germinated and fermented cereals. *Journal of Food Science* **44**:456-459.

- <sup>13</sup>Hamad, S.H. 1995. The commercialization of Kisra production. International Symposium on Development of Small and Medium Enterprises for Biotechnology Commercialization in Developing Countries, Manila, Philippines.
- <sup>14</sup>Hamad, S.H., Boecker, G., Vogel, R.F. and Hammes, W.P. 1992. Microbiological and chemical analysis of fermented sorghum dough for Kisra production. *Appl. Microbiol.* **37**:728-731.
- <sup>15</sup>Subramanian, V., Jambunathan, S.R. and Suryaprakash, S. 1980. Note on the soluble sugars of sorghum. *Cereal Chemistry* **57**(6):440-441.
- <sup>16</sup>Khattab, A.H., Karam-Alla, K.A. and Nour, A.A.M. 1972. Amino acid composition of some sorghum grain varieties. *Sudan Journal of Food Science and Technology* **4**:27-29.
- <sup>17</sup>Khetarpaul, N. and Chauhan, B. M. 1990. Effect of germination and fermentation on available carbohydrate content of pearl millet. *Food Chemistry* **38**:21-26.
- <sup>18</sup>Lorri, W. 1993. Nutritional and Microbiological Evaluation of Fermented Cereal Weaning Foods. PhD. thesis, Chalmers University of Technology, Göteborg, Sweden.
- <sup>19</sup>Ministry of Agriculture 1995. Department of Planning and Agricultural Policy, Khartoum, Sudan.
- <sup>20</sup>Rooney, L.W., Earp, C.F. and Khan, M.N. 1982. Sorghum and millet. In Wold, I.A. (ed.). *CRC Handbook of Processing and Utilization in Agriculture*. Vol. 11. CRC Press, Boca Raton, FL, p. 123.
- <sup>21</sup>Tou, E.H., Mauquet-Rivier, C., Picq, C., Traore, A.S., Treche, S. and Guyot, J.P. 2007. Improving the nutritional quality of ben-saalga, a traditional fermented millet-based gruel, by co-fermenting with groundnut and modifying the process method. *LWT* **40**:1561-1569.
- <sup>22</sup>Tou, E.H., Mauquet-Rivier, C., Rochette, A.S., Traore, A.S., Treche, S. and Guyot, J.P. 2007. Effect of different process combination on the fermentation kinetics, microflora and energy density of ben-saalga, a fermented gruel from Burkina Faso. *Food Chemistry* **100**:935-943.
- <sup>23</sup>Tou, E.H., Guyot, J.P., Mauquet-Rivier, C., Rochette, I., Counil, E., C., Traore, A.S. and Treche, S. 2007. Study through surveys and fermentation kinetics of traditional processing of pearl millet (*Pennisetum glaucum*) into ben-saalga, a fermented gruel from Burkina Faso. *International Journal of Microbiology* **106**:52-60.
- <sup>24</sup>Zhan, X., Wang, D., Tuinstra, M. R., Bean, S., Seb, P.A. and Sun, X. S. 2003. Ethanol and lactic acid production as affected by sorghum genotype and location. *Industrial Crops and Products* **18**:245-255.
- <sup>25</sup>Yousif, B.Y. and Magboul, B.I. 1972. Nutritive value of Sudan food stuffs. Part 1. *Sorghum vulgare* (dura). *Sudan Journal of Food Science and Technology* **4**:39-45.
- <sup>26</sup>Abdalla, A.A., El Tinay, A.H., Mohamed, B.E. and Abdalla, A.H. 1998. Effect of traditional processes on phytate and mineral content of pearl millet. *Food Chem.* **63**:79-84.
- <sup>27</sup>Khetarpaul, N. and Chauhan, B.M. 1991. Effect of natural fermentation on phytate and polyphenolic content on *in-vitro* digestibility of starch and protein of millet (*Pennisetum typhoideum*). *J. Sci. Food Agri.* **55**: 189-195.