Biochemical Changes during Traditional Fermentation of Saudi Sorghum (*Sorghum bicolor* L.) Cultivars Flour into Khamir (Local Gluten Free Bread)

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Abstract: Effect of traditional fermentation on pH, titratable acidity, proteins, amino acids, and sugars contents of three local sorghum cultivars namely Hamra, Biadah and Shahla used in making khamir local bread were investigated. During 24 fermentations, the pH of fermented dough dropped sharply and this was coincided with increase in total acidity. Fermentation was found to cause no significant change in protein content of the cultivars. Amino acid analysis, revealed slight insignificant improvement in lysine, and leucine content but there was a slight decrease in valine, phenyl alanine and arginine content in fermented dough. Glucose, fructose and maltose content of the three sorghum cultivars increased considerably in the early stages of fermentation, followed by decrease towards the end of fermentation. Low amount of sucrose detected in the three cultivars and it was completely utilized after 8 hrs. of fermentation.

Key words: sorghum, fermentation, amino acids, sugars

1 Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the most important staple food for more than 500 million people in the semi-arid and arid lands in Africa and Asia, primarily the low income people¹. Sorghum produces well with limited moisture and tolerant to salinity and high temperature stress. The sorghum production is around 54 million tons, from crop grown in about 40 million ha, and ranked the fifth most important cereal grown in the world². The nutrient composition of the sorghum revealed that it is an important source of energy, protein and mineral, vitamins and phytochemicals. Recently, sorghum has drawn special attention due to its gluten-free nature³, low glycemic index⁴, and as rich source of health promoting antioxidants because due the presence of phenolic acids and tannins specially, in the pigmented varieties⁵.

Sorghum is traditionally fermented or germinated prior to consumption. Fermented sorghum is used in making various traditional foods, alcoholic and nonalcoholic beverage around the world, such Kisra in Sudan, Injera in Ethio-

pia, fin in India, Mahewu in Zimbabwe, Dolo and pito in west Africa and lahloh and Khamir in Saudi Arabia⁶. Fermentation process of sorghum flour had been reported to improve its nutritional quality and reduce antinutritional factors. Fermentation of sorghum for 36 or 24 hrs was also found to increase protein and starch *in vitro* digestibility and decrease antinutritional factors such as phytic acid, tannins, enzyme inhibitors^{6,7}. Inyang and Zakary⁸ reported an increased in protein during natural fermentation of millet. Similarly, Cui *et al.*⁹ observed an increase in protein content and most of amino acids especially lysine in maize after three days' fermentation.

In Saudi Arabia, there are three sorghum cultivars Hamra, Bidaha and Shahla are grown in South-West region of Jazan, where it is traditionally utilized to prepare fermented traditional bread known as Khamir. The bread is prepared by mixing the sorghum flour with water, onion, lemon juice and fenugreek and fermented for 24 hr. The microbiological analysis of the micro-organisms involved in fermentation process of Khamir bread showed that lactic

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acid bacteria dominated over Enterobacteriaceae groups, yeasts and molds¹⁰. Fermentation process was also found to decrease antinutritional factors and increase protein digestibility^{11, 12}. However, there is no information is available regarding the biochemical changes occurring during traditional fermentation of sorghum in to Khamir bread. Therefore, the objectives of this study were to investigate the changes in pH, titerable acidity, proteins content, soluble sugars and amino acids content, occurring during traditional fermentation of sorghum cultivars mainly Hamra, Baidha and Shahla in to Khamir bread

2 Materials and Methods

2.1 Materials

The three sorghum varieties, Hamra, Baidha and Shahla used for this study were obtained from a local market in Jazan province (South-west Saudi Arabia). The grain was manually cleaned from impurities and milled and the flour was then transferred to laboratory in Riyadh and stored at 25°C until used.

2.2 Fermentation

Natural fermentation was carried out using the traditional process used by housewives as described by Osman¹¹, briefly sorghum dough was prepared by mixing the flour with water and spices (fenugreek, onion and lemon juice) (1:0.8 ratio). The fermentation was carried out by adding 3% inocula from previous fermentation as a starter to the dough and incubated at 37°C for 24 hours. The fermented dough is made into pancake shape with 2-3 cm thickness by hand and baked in clay or mud oven known as tann our for 15 min. Fermentation was performed in duplicate and sampled every 4 hr during fermentation period (24 hr). Samples were dried in a vacuum oven at 50°C (Heraucus LBS Co.), and milled to fine powder using a coffee miller then passed through a 60 mm mesh and kept at 4°C in polyethylene bags for analysis.

2.3 Determination of pH and titratable acidity

The pH was monitored every 4 h interval using a digital pH-meter (JenwayPH10 flected UK). Titratable acidity was determined according to AOAC¹³. The titratable acidity was calculated as percent lactic acid.

2.4 Protein content

The crude protein of raw and fermented sorghum flour was determined by micro-Kjeldahl method as described by AOAC¹³.

2.5 Amino acid

Amino acids composition was determined following the AOAC¹⁴ method. Using HPLC (Shimadzu LC-10AD, Shi-

madzu Corporation, Kyoto, Japan) in duplicate samples after hydrolyzed with 6.0 N HCL at 100°C for 24 hrs.

2.6 Determination of sugars

Glucose, sucrose, fructose and maltose content of raw and fermented sorghum flour were analyzed according to AOAC¹³, using HPLC Shimadzu LC from Shimadzu, Kyoto-Japan. Sugar standards were purchased from Sigma (Sigma Chemical Co., St. Louis, Mo).

2.7 Statistical analysis

Data, expressed as mean and standard deviation, were statistically analyzed using one-way ANOVA Steel and Torri¹⁵. Duncan's multiple tests were used to compare means and significance was accepted at $p < 0.05$.

3 Results and Discussion

3.1 Effect of fermentation on the pH and titratable acidity of sorghum cultivars

The effect of fermentation on pH and titratable acidity of Hamra, Baidha and Shahla is presented in Figs. 1 and 2. In general, there was progressive decrease in pH with increase of fermentation time in the three sorghum cultivars. The pH of fermented dough of the three cultivars were was decreased and reached 4.43, 4.65 and 4.47 at the end of 24 hr fermentation for Hamra, Bidah and Shahla respectively. The pH dropped sharply at first from 8 h and then, there was slight insignificantly ($p < 0.05$) decrease up to 24 h in the three cultivars. The decrease in pH was concomitant with increase in titerable acidity. The titerable increased significantly ($p < 0.05$) from initial values 0.26, 0.27 and 2.0 to final values 1.37, 1.70 and 1.65 during 24 h fermentation for Hamra, Biadha and Shahla respectively. Similar observations were reported for several other cereals. Fadlalla *et al.*¹⁶ observed that the pH of sorghum dropped from 6.05 to 3.79 and that of the sorghum supplemented with chickpea from 6.07 to 3.81 at the end of fermentation period (24 h). Similarly, Tou *et al.*¹⁷ reported decrease in pH and increase titratable acidity during preparation ben-saalga, a traditional fermented millet-based gruel. According to Gassem¹⁰ increase in titerable acidity at the end fermentation of sorghum dough in to Khamir bread is due to accumulation of lactic acid produced by increased number of lactic acid bacteria during fermentation. The final pH values of fermented sorghum cultivars dough mix were slightly higher than the safe inhibitory pH (3.6 to 4.1) for pathogenic bacteria. The high pH could be attributed to low lactic acid concentration due to shorter period of fermentation, the temperature or to the presence of other ingredients such as onion, lemon juice and fenugreek in dough mix that may slow the fermentation process. Lactic acid is responsible for the desirable sour taste and the

Fig. 1 Effect of fermentation for different period of time (hours) on pH of sorghum varieties. Values are means ($\pm$ SD) of triplicate samples. Values followed by the same letter are not significantly different ($p < 0.05$) as assessed by LSD.

Fig. 2 Effect of fermentation for different period of time (hours) on total acidity of sorghum varieties. Values are means ($\pm$ SD) of triplicate samples. Values followed by the same letter are not significantly different ($p < 0.05$) as assessed by LSD.

special flavor of the bread that are important for in utilization and acceptability.

3.2 Effect of fermentation on crude proteins of sorghum cultivars

The change in protein contents of sorghum cultivars as function of fermentation time is shown in Table 1. The protein content of unfermented Hamra, Biadha and Shahla were 14.7, 15.0 and 14.95 respectively. Results obtained were within the range reported by Gassem and Osman¹²⁾ and Basahy¹⁸⁾. For the three cultivars, fermentation up to 24 h caused insignificant ($p > 0.05$) change in the content of protein. The protein content ranged from 14.71 to 14.90% at the end of fermentation period (24 h). This finding agree

with that of Abdel Rahman and Osman¹⁹⁾, who reported the total protein content of was not significantly changed when three sorghum varieties fermented for 24 h for making Kissra. Hassan *et al.*²⁰⁾ also reported that the protein content of millet was not affected by fermentation. In same way, Yousif and El Tinay²¹⁾ and Ibrahim *et al.*²²⁾ reported that the protein content of protein content of Dabar sorghum cultivar was fluctuated during the 24 h fermentation. However, prolonged fermentation time to 32 h slightly increase the protein content of sorghum dough. The slight insignificant reduction in protein content observed in this study could be attributed to the utilization of protein by microorganism for growth.

Table 1 Effect of fermentation for different period of time (hours) on crude protein (%) of sorghum varieties.

Fermentation time (h)	Sorghum cultivars		
	Hamra	Baidah	Shahla
0	14.17 ± 0.10 ^a	15.00 ± 0.11 ^a	14.95 ± 0.21 ^a
8	14.00 ± 0.07 ^a	15.09 ± 0.17 ^a	15.79 ± 0.17 ^a
16	15.13 ± 0.34 ^a	14.79 ± 0.17 ^a	15.90 ± 0.11 ^a
24	14.09 ± 0.04 ^a	14.86 ± 0.00 ^a	14.71 ± 0.36 ^a

Values are means ± SD of three replicates. Values not sharing a common superscript letter are significantly different at $p < 0.05$.

Table 2 Effect of fermentation for different period of time (hours) on amino acid profile (g/100 g protein) of sorghum varieties.

Amino acid	Sorghum cultivars								
	Hamra			Baidah			Shahla		
	0 h	12 h	24 h	0 h	12 h	24 h	0 h	12 h	24 h
Aspartic acid	6.5 ± 0.44 ^a	6.35 ± 0.53 ^a	6.6 ± 0.10 ^a	6.1 ± 0.05 ^a	6.3 ± 0.05 ^a	6.1 ± 0.10 ^a	6.37 ± 0.06 ^a	5.89 ± 0.10 ^a	6.22 ± 0.12 ^a
Threonine	2.9 ± 0.20 ^a	2.85 ± 0.15 ^a	2.9 ± 0.05 ^a	2.8 ± 0.05 ^a	2.8 ± 0.10 ^a	2.8 ± 0.05 ^a	2.94 ± 0.05 ^a	2.73 ± 0.02 ^a	2.88 ± 0.12 ^a
Serine	3.7 ± 0.30 ^a	3.7 ± 0.20 ^a	3.8 ± 0.10 ^a	3.5 ± 0.05 ^a	3.5 ± 0.15 ^a	3.6 ± 0.30 ^a	3.76 ± 0.04 ^a	3.47 ± 0.06 ^a	3.58 ± 0.14 ^a
Glutamic acid	20.1 ± 1.3 ^a	20.15 ± 0.9 ^a	21.2 ± 0.4 ^a	20.0 ± 0.3 ^a	20.1 ± 0.6 ^a	20.2 ± 0.6 ^a	20.4 ± 0.21 ^a	19.23 ± 0.2 ^a	20.5 ± 0.07 ^a
Glycine	2.7 ± 0.15 ^a	2.65 ± 0.15 ^a	2.8 ± 0.21 ^a	2.6 ± 0.05 ^a	2.8 ± 0.05 ^a	2.3 ± 0.20 ^a	2.75 ± 0.14 ^a	2.42 ± 0.02 ^a	2.55 ± 0.11 ^a
Alanine	8.6 ± 0.65 ^a	8.45 ± 0.51 ^a	8.8 ± 0.21 ^a	8.3 ± 0.10 ^a	8.4 ± 0.13 ^a	8.3 ± 0.10 ^a	8.73 ± 0.09 ^a	8.14 ± 0.05 ^a	8.50 ± 0.25 ^a
Valine	5.0 ± 0.21 ^a	4.9 ± 0.30 ^a	4.9 ± 0.15 ^a	4.8 ± 0.10 ^a	4.6 ± 0.32 ^a	4.5 ± 0.31 ^a	5.02 ± 0.07 ^a	4.60 ± 0.08 ^a	5.00 ± 0.30 ^a
Methionine	1.5 ± 0.31 ^a	1.35 ± 0.5 ^a	1.4 ± 0.1 ^a	1.8 ± 0.05 ^a	1.7 ± 0.02 ^a	1.5 ± 0.31 ^a	1.75 ± 0.03 ^a	1.57 ± 0.01 ^a	1.65 ± 0.05 ^a
Isoleucine	3.7 ± 0.15 ^a	3.55 ± 0.25 ^a	3.5 ± 0.10 ^a	3.8 ± 0.05 ^a	3.7 ± 0.02 ^a	3.6 ± 0.05 ^a	3.70 ± 0.06 ^a	3.46 ± 0.05 ^a	3.73 ± 0.10 ^a
Leucine	12.6 ± 0.9 ^a	12.5 ± 0.8 ^a	12.9 ± 0.1 ^a	12.5 ± 0.2 ^a	12.7 ± 0.2 ^a	12.7 ± 0.5 ^a	13.18 ± 0.1 ^a	12.17 ± 0.1 ^a	13.65 ± 0.9 ^a
Tyrosine	3.1 ± 0.25 ^a	3.0 ± 0.20 ^a	3.3 ± 1.21 ^a	3.2 ± 0.05 ^a	3.2 ± 0.15 ^a	3.2 ± 0.05 ^a	3.36 ± 0.06 ^a	2.70 ± 0.20 ^a	3.27 ± 0.07 ^a
Phenylalanine	5.0 ± 0.40 ^a	4.5 ± 0.81 ^a	5.2 ± 0.11 ^a	5.0 ± 0.10 ^a	5.3 ± 0.12 ^a	4.9 ± 0.05 ^a	5.06 ± 0.06 ^a	4.70 ± 0.10 ^a	4.98 ± 0.02 ^a
Histidine	2.1 ± 0.10 ^a	2.1 ± 0.10 ^a	2.1 ± 0.00 ^a	2.1 ± 0.10 ^a	2.1 ± 0.04 ^a	1.9 ± 0.11 ^a	2.00 ± 0.02 ^a	1.84 ± 0.04 ^a	2.00 ± 0.03 ^a
Lysine	2.1 ± 0.15 ^a	2.2 ± 0.10 ^a	2.2 ± 0.05 ^a	1.9 ± 0.05 ^a	1.9 ± 0.10 ^a	2.0 ± 0.08 ^a	1.85 ± 0.00 ^a	1.85 ± 0.02 ^a	2.00 ± 0.03 ^a
Arginine	5.2 ± 0.25 ^a	5.1 ± 0.20 ^a	4.8 ± 0.05 ^a	5.1 ± 0.05 ^a	4.9 ± 0.31 ^a	3.2 ± 0.20 ^a	4.87 ± 0.03 ^a	4.47 ± 0.10 ^a	4.80 ± 0.14 ^a
Proline	6.7 ± 0.15 ^a	6.6 ± 0.25 ^a	6.8 ± 0.40 ^a	5.3 ± 0.15 ^a	5.8 ± 0.10 ^a	5.6 ± 0.05 ^a	6.18 ± 0.06 ^a	5.65 ± 0.05 ^a	5.91 ± 0.12 ^a

Values are means ± SD of three replicates. Values sharing a common superscript letter are insignificantly different at $p < 0.05$.

3.3 Effect of fermentation on Amino acids profile of sorghum cultivars

The effect of fermentation on amino acids content of Hamra, Biadha and Shahla sorghum cultivars is presented in **Table 2**. The amino acids composition of the unfermented sorghum flours showed that glutamic acids, leucine, alanine and aspartic acids were the major amino acids. The amino acids profile of the sorghum the three cultivars are in agreement with those reported by Abdel Rahman and Osman²³⁾. Fermentation process resulted in slight insignificant increase, leucine and lysine. Similarly, Fadlallah *et al.*¹⁶⁾ could not find any definite trend in amino acids during 24 h fermentation of sorghum and sorghum-chickpea blends. Cui *et al.*⁹⁾, however, reported significant increase in most of the amino acids with largest increase in lysine, during 3 days' fermentation of maize. Similarly, Hamad and Field²⁴⁾ reported that natural fermentation of wheat,

oats, sorghum and rice for 6 days significantly increased the essential amino acids, and available lysine content. On the contrary, Osman²⁵⁾, observed significant decrease in lysine, arginine and glycine after 24 h fermentation in millet dough. The slight change in amino acids content observed is probably due to short fermentation period used in this study.

3.4 Effect of fermentation on soluble sugars content of sorghum cultivars

The effect of fermentation on glucose, fructose, sucrose and maltose content of Hamra, Biadah and Shahla is presented in **Fig. 3(A, B and C)**. The analysis of soluble sugars revealed that glucose is major soluble sugar in the three sorghum cultivars. The glucose content of unfermented Hamra, Biadha and Shahla dough was found to be 1.20, 1.58 and 1.12 gm/100 gm sample respectively. In the three

Fig. 3 Effect of fermentation for different period of time(hours) on soluble sugars of sorghum varieties (A) Hamra, (B) Baidah and (C) Shahela. Values are means ($\pm$ SD) of triplicate samples. Values followed by the same letter are not significantly different ($p < 0.05$) as assessed by LSD.

cultivars, the concentration of glucose increased sharply during early stages of fermentation and reached a maximum after 16 h and thereafter decreased. The increase of glucose was greater in Shahla (150.9%) compared to Hamra (116.7%) and Bidah (110.2%). Similar to glucose, there was a sharp increase in fructose content in first 8 hrs of fermentation. It increased from 0.202 to 0.31, from 0.24 to 0.4 and from 0.2 to 0.36 for Hamra, Biadah and Shahla respectively. Increasing fermentation period up to 24 h markedly caused a decrease in fructose content to 0.172, 1.38 and 1.61 for the three cultivars. The result obtained here is in agreement with those of Osman *et al.*¹⁹⁾ who reported that the level of reducing sugars showed an increased and then a decreased as the fermentation time increases, during Kissra preparation. Similarly, Hamad and Field²⁴⁾ observed increase and the decrease in reducing sugars during fermentation of wheat, rice, oats and sorghum for 6 days. The increase glucose content could be attributed to the action of microbial α and β amylase, sucrase and maltase, whereas the decrease at the end fermentation probably due to utilization of glucose as source of energy by microorganism.

Sucrose content was decreased rapidly and completely utilized after 8 h in the three cultivars. In contrast, the content of maltose increased sharply during the first 8 hr; it increased from 0.23 to 0.77, from 0.68 to 0.86 and from 0.51 to 0.80 for Hamra, Baidah and Shahla respectively. However, when the fermentation was continued to 24 h, the amount of maltose started to decrease at a steady rate and completely utilized at the end of fermentation period in both Biadh and Shahla. Similar trend observed during fermentation of pearl millet to ben-salga, a fermented gruel¹⁷⁾. The changes either as an increase or a decrease in maltose could be attributed to action of microorganism amylase that hydrolyzed starch to maltose and to the action of maltase associated with fermentation.

Therefore, in order to increase the protein and essential amino acid content, such as lysine the first limiting amino acids of fermented sorghum, the fermentation process should be carried out between 3-6 days or by using a fermenter with pure bacteria culture and controlled parameters for industrial application. The fermented sorghum flour could be utilized in formulation of weaning food, blend with wheat to produce cheap and nutritious bread, blended with local legumes for combating protein malnutrition in poor countries or help in solving major problem for celiac patient, due to the fact that the local bread is gluten free that has the poetical for commercial application.

4 Conclusion

Fermentation process of the three sorghum cultivars during preparation of the khamir gluten free bread resulted

in decrease in pH, which was accompanied by increase in titrable acidity contents. The traditional fermentation process of sorghum cultivars was found to cause no significant change in protein. Amino acid analysis of fermented sorghum revealed slight insignificant increase in the content of some of the amino acids and decrease in others in three dough samples. Sugars study showed remarkable changes in soluble sugars content in the three sorghum varieties. These results indicated the need for longer fermentation time or addition of other process such as soaking or germination before fermentation to increase nutritional quality. The results indicate that fermentation for longer period would contribute in increasing protein and essential amino acids such as lysine the first limiting amino acid in cereal of the three local sorghum cultivars, that could be used in developing new cheap bakery products by mixing with wheat, in poor countries or weaning food for children in Asia and Africa by blending fermented sorghum flour with local legumes in order to combat protein malnutrition (Kawakshior).

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Conflict of Interest

The authors declare that there is no conflict of interest.

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