EFFECT OF FERMENTATION ON THE NUTRITIONAL AND MICROBIOLOGICAL QUALITY OF DOUGH OF DIFFERENT SORGHUM VARIETIES

By

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ABSTRACT
Three varieties of sorghum grain flours namely; Feterita, Tabat and Wad Akar were obtained from Khartoum North local market. The proximate analysis of these varieties showed that the contents of moisture ranged between 7.33-8.00%, ash 1.71-1.79%, protein 9.13-12.71%, fat 4.01-4.67%, crude fiber 2.46-3.26% and carbohydrates 70.00-80.00%. Significant drop in pH was recorded during fermentation of sorghum dough. Microbial analysis of the sorghum varieties before fermentation showed that the total bacterial counts and the counts of yeast and molds increased significantly after fermentation time (24 hours), while, there was a decrease in staphylococcal counts. The microbiological analysis also revealed that E. coli counts exceeded 2.400 cfu/g in the raw sorghum flour but the counts were very low in the fermented dough. Salmonella was detected in the three sorghum varieties disappeared in the fermented dough after 24 h fermentation.

Key words: Sorghum, fermented dough, quality
INTRODUCTION

For human consumption, sorghum (Sorghum bicolor L.) is the fifth most important cereal crop produced in the world after wheat, rice, maize and barley. It is an important source of calories and protein for a large segment of the human population in the semi-arid tropics (Axtel et al., 1981) is grown in all countries of the world, except in the cool north-western part of Europe. The leading producing countries are the United States, India, Nigeria, Argentina, Mexico and Sudan (Dirar, 1991).

Traditionally, Africa has employed sorghum in both the malted and un-malted form in wide varieties of porridge and beverages, often using lactic and alcoholic fermentation to enhance their appeal.

In the Sudan, sorghum is the most important food crop. It is the staple food of the vast majority of the population and is produced mainly in the central clay plains of the Sudan under rain, with limited amount being produced in the irrigated schemes of Gezira, Rahad and New Half. The annual production of sorghum in Sudan is about 1.4 million tons with cultivated areas ranging from 2.73-6.43 million hectares.

The chemical composition of sorghum grain is more variable than that of many other cereal crops (Rooney, 1973). Yousif and Magboul (1972) analyzed fifteen different varieties of sorghum grown in the Sudan and they gave the following ranges; 5.7-10.5% moisture, protein 6.9-12.8%, fat 3.0-4.1%, crude fiber 1.2-2.6%, ash 1.3-1.8% and carbohydrates 72.3-78.8%. Eggum et al. (1983) analyzed Tetron, Dabar and Feterita grain and they found that the fat content varied between 4.0 and 5.0%, crude fiber level ranged from 2.0-2.1%, ash between 1.7 and 2.1% and protein 10.9 and 13.4%. El Sharif (1993) analyzed sorghum flour variety Debar
and gave the following results: moisture content 2.4%, ash 1.3%, protein 10.8, crude fiber 0.9%, fat 3.3%, total sugar 2.6% and carbohydrates 81.35%.

Sudan seems to have the greatest number of fermented sorghum products. There are about 30 such products that are basically different from one another (Dirar, 1991). Most varieties of sorghum have gained universal fame for production of fermented foods, because of the wide adaptability and low cost of production.

The objectives of the present study are to evaluate the chemical composition of three varieties of sorghum grains, grown in the Gezira Scheme namely Feterita, Tabat and Dabar and to study the microbiological changes occurring during fermentation of flour dough prepared from them.

MATERIALS AND METHODS

Materials
Sorghum flour
Three local varieties of sorghum (Tabat, Feterita and Wad Akar) were purchased from the Central Market, Khartoum North (Shambat), milled and kept in previously hot-sterilized containers at 4°C.

Methods
Dough fermentation
Sorghum flour fermentation was achieved by adding sterile water to the sorghum flour 2:1 (v/w) and left to naturally ferment after inoculation with a previous batch of fermented sorghum dough at 37°C.

Proximate analysis
Determination of moisture content, crude fiber, fat, protein and ash content of the different sorghum flour samples were carried out using AOAC (1990). The total carbohydrate content was obtained by subtraction of the sum mentioned components from 100, i.e.

Total carbohydrate = 100 - (% moisture + % crude protein + % crude fat + % crude fiber + % ash). However, the pH of the suspension was measured using pH-meter model 7020 (AACC, 1983)
Microbiological methods

Preparation of serial dilutions

Ten ml from each sample of raw sorghum flour and fermented dough were transferred to 90 ml sterile peptone water (0.1%) and thoroughly mixed to give 1:10 dilution 'first dilution'; serial dilutions were prepared by transferring one ml from first dilution \((10^{-1})\) to 9 ml peptone water, \((10^{-2})\) and so on \((10^{-3}, 10^{-4}, \ldots)\) as described by Harrigan and McCane (1976).

Total viable count

Total viable count was carried out using the pour plate method described by Harrigan (1998).

Yeast and mold enumeration

From suitable dilution of sample, 0.1 ml was transferred onto solidified malt-extract agar containing 0.1 g chloramphenicol per one liter of medium to inhibit bacterial growth. Samples were spread all over the plates using sterile bent glass rod. Plates were then incubated at 27°C for 48 hours described by as described by Harrigan and McCane (1976). Colony forming units (CFU) were counted using a colony counter and the results were presented as cfu/g.

Staphylococcus aureus

The Staphylococcus count was done by plating from suitable dilutions, 0.1 ml onto plates containing manitol salt agar medium and the inoculum was distributed evenly using sterile glass rod. The plates were then incubated at 37°C for 24 -48 hours and the counts were presented as colony forming units per gram (cfu/g).

Coliform bacteria, E. coli

The coliform test was done according to Harrigan (1998) by plating one ml sample onto MacConky agar media. The plates were incubated at 37°C for 48 hours and the counts were presented as colony forming unites per gram (cfu/g). Plates showing positive coliform were subjected to the confirmed test using Brilliant green bile lactose broth in test tubes with Durham tubes. The test tubes were then incubated at 44°C for 48 hours. Each confirmed positive tube was subcultured into E.C. broth medium and then incubated at 44.5°C for 24 hours.
Tubes showing any amount of gas production were considered to be positive *E. coli* presence.

**Detection of Salmonella**
The stage of the pre-enrichment of *Salmonella* medium was done by mixing 25 g of sample with 225 ml of buffer peptone water in a sterile microbiological bag. The pre-enrichment culture was incubated for 24 hours at 37°C. The stage of the selective enrichment of *Salmonella* was done by peptideing one ml of the pre-enrichment culture to 10 ml of selective selenite broth and incubating at 37°C for 24 hours. The stage of plating on selective agar media was done by transferring a loopful of the selective enrichment media to the surface of each one of the three selective agar media (xylem lysine deoxycholate, bismuth sapphire agar and brilliant green agar) and spreading to obtain isolated colonies.

**Statistical analysis**
The data were subjected to Statistical Analysis System (SAS) Software and Randomized Complete Design (RCD) was used with factorial design and then means separated according to Mead and Grown (1982).

**RESULTS AND DISCUSSION**

**Proximate composition of sorghum flour**
Table (1) shows the proximate composition of sorghum flour. The moisture content of *Feterita*, *Tabat* and *Wad Akar* whole sorghum flour were 8.00%, 7.36% and 7.33%, respectively. These values are within the range of 5.7-10.4 reported by Yousif and Magboul (1972) for Sudanese sorghum. The values were higher than the range of 6.8-7.8% reported by El-Hidai (1978). However, the ash contents of *Feterita*, *Tabat* and *Wad Akar* whole sorghum flour were 1.79, 1.50 and 1.70%, respectively. These values are within the ranges of 1.1-2.7% and 1.3-1.9% reported by Yousif and Magboul (1972).

The crude protein content of *Feterita* and *Wad Akar* sorghum flours were 12.7% and 9.1%, respectively. These values were within the range of 6.9-12.8% reported by Yousif and Magboul (1972) for Sudanese sorghum.

Fat content of *Feterita*, *Tabat* and *Wad Akar* sorghum flours were 4.6, 3.3 and 4.0%, respectively. These values were within the range of 2.5-5.1% and the range
of 2.5-3.5% reported by El-Tinay et al. (1972). Crude fiber content of Feterita, Tabat and Wad Akar sorghum flours were 2.4, 3.2 and 3.8%, respectively. These values were higher than the range of 1.2-1.9% reported by El-Tinay et al. (1972). The carbohydrate contents of the various sorghum flour varieties ranged between 70 and 80%.

Table (2) shows significant drop (P< 0.05) in pH during the fermentation of the three varieties of sorghum flours. Fermentation of Feterita sorghum dough resulted in pH drop from 6.24 at zero time to 4.00 and 3.86 at 19 and 24 hours, respectively, while the pH dropped during fermentation of Tabat sorghum dough from 6.34 at zero time to 4.05 and 3.88 at 19 and 24 hours, respectively, and in Wad Akar sorghum dough fermentation pH dropped from 5.77 at zero time to 3.81 and 3.71 at 19 and 24 hours, respectively. The pH value 6.24 in sorghum flour dough is quite suitable for the growth of lactic acid bacteria, but not for yeast growth as indicated by Dirar (1978) who stated that during fermentation, the pH dropped to 3.71 possibly due to the acid production by bacteria creating acidic conditions necessary for yeast growth, which in turn provide vitamins for the bacterial growth.

Table (1): Proximate composition of sorghum flour

<table>
<thead>
<tr>
<th>Type of flour</th>
<th>Moisture content (%)</th>
<th>Ash content (%)</th>
<th>Protein content (%)</th>
<th>Fat content (%)</th>
<th>Crude fiber (%)</th>
<th>CHO (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feterita</td>
<td>8.00</td>
<td>1.799</td>
<td>12.71</td>
<td>4.677</td>
<td>2.467</td>
<td>70.00</td>
</tr>
<tr>
<td>Tabat</td>
<td>7.367</td>
<td>1.501</td>
<td>6.644</td>
<td>3.374</td>
<td>2.33</td>
<td>80.00</td>
</tr>
<tr>
<td>Wad Akar</td>
<td>7.333</td>
<td>1.708</td>
<td>9.133</td>
<td>4.045</td>
<td>3.267</td>
<td>75.00</td>
</tr>
</tbody>
</table>

Table (2): The pH-values of fermented dough

<table>
<thead>
<tr>
<th>Type of flour</th>
<th>Zero time</th>
<th>19 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feterita</td>
<td>6.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.86&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tabat</td>
<td>6.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.88&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wad Akar</td>
<td>5.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means having different superscript letter in columns and rows differ significantly (P<0.05).
Table (3): Changes in microbiological characteristics during different fermentation periods of sorghum flour

<table>
<thead>
<tr>
<th>Fermentation period</th>
<th>Feterita</th>
<th>Tabat</th>
<th>Wad Akar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hour 0</td>
<td>19 hour</td>
<td>hour24</td>
</tr>
<tr>
<td>Total bacterial count (cfu/g)</td>
<td>5.60 x 10⁶</td>
<td>6.83 x 10⁷</td>
<td>5.03 x 10⁷</td>
</tr>
<tr>
<td>Staph. spp count (cfu/g)</td>
<td>2.04 x 10⁴</td>
<td>9.05 x 10⁵</td>
<td>5.03 x 10⁷</td>
</tr>
<tr>
<td>Coliform (E. coli)</td>
<td>-2,400</td>
<td>15</td>
<td>-2,400</td>
</tr>
<tr>
<td>Yeast and mould count (cfu/g)</td>
<td>2.50 x 10⁴</td>
<td>9.11 x 10¹</td>
<td>6.15 x 10⁷</td>
</tr>
</tbody>
</table>

Means having different superscript letter in each row differ significantly ($p < 0.05$).

Table (4): Detection of Salmonella in fermented sorghum dough

<table>
<thead>
<tr>
<th>Type of flour</th>
<th>Zero time</th>
<th>19 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feterita</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Tabat</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Wad Akar</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

Table (3) shows that, there was a significant increase ($P<0.05$) in total bacterial count of Feterita sorghum flour (5.60 x 10⁶ cfu/g) when compared with that of Tabat sorghum flour, which contained 6.25 x 10⁷ cfu/g at zero time. Also it shows that, there was a significant increase ($P<0.05$) in total bacterial count of Feterita sorghum flour (2.04 x 10⁴ cfu/g) when compared with that of Tabat sorghum flour which contained (6.40 x 10⁵ cfu/g) after 19 hours of fermentation. On the other hand, after 24 hours, the total bacterial count of Feterita, Tabat and Wad Akar sorghum flour was 6.25 x 10⁵ cfu/g, 5.53 x 10⁷ cfu/g and 6.32 x 10⁵ cfu/g, respectively. These results were in agreement with Ahmed (1994) who found that the total bacterial count in dough fermentation was (5.20 x 10⁶ cfu/g) at zero time fermentation.

Table (3) indicates that the coliform bacterial (E. coli) in fermented dough of Feterita, Tabat and Wad Akar sorghum flour decreased from >2,400 cfu/g to 34 cfu/g, 34 cfu/g and 28 cfu/g, respectively, at 19 hours, while after 24 hours, these microbial groups decreased to 15 cfu/g, 11 cfu/g in Feterita, Tabat and Wad Akar fermented sorghum dough, respectively. These results were in agreement with Hamad et al., (1992) who found that, the coliform bacteria (E. coli) in fermented dough decreased to 11 cfu/g at 19 hours.
Significant difference ($P<0.05$) existed between fermented sorghum dough of different varieties in Staphylococci count (Table 3). The Staphylococci count was found to be $2.04 \times 10^3$ cfu/g, $6.87 \times 10^2$ cfu/g and $2.81 \times 10^2$ cfu/g in *Feterita* sorghum dough at zero time, 19 hours and 24 hours, respectively, compared with that of *Tabat* sorghum dough which contained $9.05 \times 10^3$ cfu/g, $6.7 \times 10^2$ cfu/g and $4.51 \times 10^2$ cfu/g at zero time, 19 hours and 24 hours, respectively. These results were in agreement with Hamad *et al.* (1992) who found that the staphylococci in fermented dough decreased from $2.7 \times 10^4$ at the beginning of fermentation to $2.11 \times 10^2$ at 21 hours.

The counts of tested pathogenic bacteria (*E. coli* and staphylococci) were dropped sharply with increased time of fermentation and that was mainly due to the increase of acidity. Dirar (1992) reported that, the coliform bacteria (*E. coli*) in fermented *Tabat* sorghum dough, dropped from (27 cfu/g) at 18 hours to (11 cfu/g) at 24 hours fermentation as a result of reduction in pH. Moreover, Hamad *et al.*, (1992) reported that the staphylococci in *Dabar* flour at initial time of fermentation was $6.05 \times 10^4$ cfu/g, decreased to $5.11 \times 10^3$ cfu/g at 24 hours of fermentation.

There was significant decrease ($P<0.05$) in yeast and moulds count of *Feterita* sorghum flour, which contained $2.56 \times 10^4$ cfu/g, $7.30 \times 10^7$ cfu/g and $9.11 \times 10^7$ cfu/g, compared to that of *Tabat* sorghum flour, which contained $6.15 \times 10^5$ cfu/g, $6.86 \times 10^6$ cfu/g and $7.93 \times 10^7$ cfu/g at zero time, 19 hours and 24 hours fermentation, respectively. However *Wad Akar* sorghum flour contained $4.95 \times 10^4$ cfu/g, $6.70 \times 10^4$ cfu/g and $6.27 \times 10^5$ cfu/g at zero time, 19 hours and 24 hours fermentation, respectively. These results are in close agreement with that of Hamad *et al.*, (1992) who found that, the yeast and moulds count in *Tabat* fermented dough was $5.0 \times 10^4$ cfu/g at 18 hours fermentation.

Table (4) shows that the Salmonella was +ve at zero time and 19 hours fermentation of the three types of sorghum dough. While at 24 hours fermentation absence of Salmonella was observed in all sorghum dough samples, indicating that, the fermentation might destroy these pathogenic bacteria via reduction of pH.
CONCLUSION
The chemical analysis of the three sorghum varieties (Feterita, Tabat and Wad Akar), showed that there were some differences in their chemical components. Also there were significant changes occurred in pH of sorghum flour dough due to fermentation process.

Bacteria, yeasts and moulds were increased with progressing time of fermentation, while the number of harmful microorganisms (staphylococci, coliform bacteria "E.coli" and Salmonella) were decreased significantly decreased with the increase of fermentation period. This might be due to the reduction of pH and accumulation of organic acids in the fermented sorghum flour, to production of certain microbial by products which eliminated these pathogens.

In view of the substantial contribution of fermented foods in human diets in Africa it is recommended that different types of fermentation should be investigated so that the optimal condition could be determined taking into consideration the method used and time of fermentation in local households. And as bacterial contamination of sorghum flour is likely to happen during fermentation, careful supervision overall the process is indispensable. More research efforts should be directed to study the effect of fermentation on quality of dough from different sorghum varieties.

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REFERENCES


