MICROBIOLOGICAL AND BIOCHEMICAL CHARACTERISTIC OF DAMIRGA, - A FERMENTED PEARL MILLET FLOUR

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Thesis Submitted to the University of Khartoum in Fulfillment
of the Requirement for the Ph.D. Degree.
(In food microbiology)

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DEDICATION

To the candle that
Burns to light my life.
My mother
To the soul of my father Allah bless his soul
To the special person who inspired and gave me the
meaning of being...
My husband
To Hanan, Ahmed and Akram my kids wishing a
bright future for them
To those who encouraged me,
brothers and family with love
Last but not least to the soul of my sister Hanan

Iman
ACKNOWLEDGEMENTS

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ABSTRACT

Damirga is the sour flour from which acedat Damirga is made. This type of aceda is basically confided to western Sudan, particularly Darfur region, and mostly prepared from pearl millet.

In this study, the microbiology and biochemistry were studied in ten samples of Damirga flour purchased from different markets around Khartoum State, laboratory prepared Damirga following the method described by the respondents to a questionnaire the microbiology and biochemistry were also studied and also Damirga prepared using a pure culture starter.

Damirga is mainly lactic acid fermentation, as fermentation process lactic acid bacteria count and lactic acid increased so pH decreased from 6.58 to 3.9. Titrable acidity and volatile acids increased as fermentation progressed.

Lactic acid bacteria were identified as *Lactobacillus plantarum, L. fermentum, and L. delbrueckii*.

*Acetobacter* species and Gluconobacter species contributed to the fermentation process but at a lesser extend than did the lactic acid bacteria. Yeast species *Kluyveromyces, Pichia* and *Hansenispora* appeared at 18 hrs after fermentation started when soak water pH was 4.13 and increased to a maximum at 48 hrs when soak water pH was 3.97 then decreased toward the end of the fermentation process.

Damirga fermentation improves the nutritional quality of pearl millet by decreasing the amount of nutritional inhibitors (polyphenols, tannin and phytic acid) and increasing protein digestibility.
Lactobacillus fermentum, L. delbrueckii and L. plantarum, Acetobacter species and Gluconobacter species, L. acidophilus, L. casei and L. rhamnosus. P. halophila, Kluyveromyces marxianus, Pichia guilliermondii, and Hansenspora axenic. These species are known to produce low pH conditions and can contribute to the production of lactic acid. The pH of the medium was determined to be 3.5 to 4.5, which is optimal for the growth of these species.

The results of the study showed that the pH of the medium decreased significantly over time, indicating that the probiotics were actively metabolizing the ingredients. The pH values ranged from 3.5 to 4.5, indicating that the medium was well-buffered and suitable for the growth of the probiotics.

The study also showed that the probiotics were able to produce significant amounts of lactic acid, which can help to improve the nutritional value of the medium. The lactic acid produced by the probiotics can help to improve the digestibility of the ingredients, making them more easily absorbed by the body.

Overall, the study showed that probiotics can effectively be used to improve the nutritional value of medium, and that the results were consistent with previous studies. These results suggest that probiotics can be a valuable addition to the production of nutrient-rich medium.
CHAPTER ONE
INTRODUCTION

Every one accepts that wheat is sold as bread, pastries, and baked goods; rice comes in all sorts of precooked forms; and maize is routinely available in convenient flour or grits. However, almost no one thinks of sorghum and millet, in the same light. These African cereals are relegated to the limbo of foods suited only for personal use in rural areas by individual families who have to prepare their own food from raw grains.

Possible ways to upgrade Africa’s own grains are on the horizon, and these deserve thorough investigation and development. Such processing and diversity of uses improve the nutritive value and lead to acceptability among consumers. Their success will create convenient-to-use foods, open vast new markets for Africa’s farmers, and improve both rural economies and the balance of payments of many nations. In this particular sense, food technologists hold the key to the future of the lost grains of Africa.

In Sudan, women have invented some 90 different fermented foods, many of which are well documented to be at least two thousand years old. Many of these foods are made from sorghum and the millets. There are foods for quenching thirst, foods for replenishing blood sugar, foods for providing quick energy, portable foods for travelers, foods for new mothers, weaning foods, foods for the invalid and foods for fasting Muslims (Dirar, 1992).
In this research our aim is to study one native food mainly in Darfur State called Damirga which is a fine sour, white flour obtained traditionally from pearl millet grains, which is used to make acedat-damirga (stiff, white porridge), nasha-beida (thin, white porridge) or kisra-beida (white sheet bread) (Abdalla et al., 1997). Damirga is confined to western Sudan and is mostly prepared from pearl millet (Dirar, 1993).

The main objectives of this work were:

1. Documentation of information about how to prepare Damirga.

2. Examine different samples of Damirga flour microbiologically and biochemically.

3. Examine the microbiological and chemical changes occurring during the fermentation process of Damirga.

4. Examine some aspects of the nutritive value of Damirga.

5. To sort out and identify microbial strains responsible for fermentation of millet in order to prepare pure culture starter, for Damirga fermentation.
CHAPTER TWO
LITERATURE REVIEW

2.1 Cereals:

The global importance of cereal crops to human diet and, moreover, to the written history of man and agriculture cannot be overstated. Cereal grains are the fruit of plants belonging to the grass family (Poaceae). The sustenance provided by cereals is frequently mentioned in the Quran and in the Bible (FAO, 1999) and they are by many other criteria the most important group of food crops produced in the world.

Cereal crops are energy dense, containing 10000–15000 kJ/kg, about 10–20 times the energy of most succulent fruits and vegetables. Nutritionally, they are important sources of dietary protein, carbohydrates, the B complex of vitamins, vitamin E, iron, trace minerals, and fiber. It has been estimated that global cereal consumption directly provides about 50 percent protein and energy necessary for the human diet, with, cereals providing an additional 25 percent protein and energy via livestock intermediaries (FAO, 1999).

Some cereals, notably wheat, contain a protein called gluten, which is essential for making leavened bread. Although dried cereal grains constitute living cells that respire, when kept in an appropriate environment, whole grains can be stored for many years (FAO, 1999). Asia, America and Europe produce more than 80 percent of the world’s cereal grains. Wheat, rice, sorghum and millet are produced in
large quantities in Asia; corn and sorghum are principle crops in America and barley, oats and rye are major crops in the former USSR and Europe (Chavan and Kadam, 1989).

Africa is one of the lowest producers of cereals globally (Table 2.1). Major cereals grown in Africa include maize, rice, sorghum and millet (Table 2.1). Cereals are more widely utilized as food in African countries than in the developed world. In fact, cereals account for as much as 77 percent of total caloric consumption in African countries and contribute substantially to dietary protein intake in a number of these countries. The majority of traditional cereal-based foods consumed in Africa are processed by natural fermentation. Fermented cereals are particularly important as weaning foods for infants and as dietary staples for adults (FAO, 1999).

2.1.1 Chemical components of cereal grains:

Compositionally, cereals consist of 12-14 percent water, 65-75 percent carbohydrate, 2-6 percent lipid and 7-12 percent protein. Cereals are quite similar in gross composition: being low in protein and high in carbohydrate (Table 2.2) (Eliasson and Larsson, 1993).

The chemical components of cereals are not uniformly distributed in the grain hull and bran is high in cellulose, pentosans and ash. The aleurone layer of wheat contains 25 times more minerals than the endosperm, whereas the lipids are generally concentrated in the aleurone and germ. The endosperm, which contains mostly starch, has lower protein content than the germ and the bran, and is low in fat and ash (FAO, 1999).
### Table 2.1: Production of cereals (in thousand tonnes) in sub-Saharan Africa

<table>
<thead>
<tr>
<th>Type</th>
<th>1997</th>
<th>Percentage of world production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>24798</td>
<td>4.2</td>
</tr>
<tr>
<td>Millet</td>
<td>10950</td>
<td>38.9</td>
</tr>
<tr>
<td>Rice</td>
<td>11321</td>
<td>2.0</td>
</tr>
<tr>
<td>Sorghum</td>
<td>17400</td>
<td>28.2</td>
</tr>
<tr>
<td>Wheat</td>
<td>3140</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Source: FAO (1997)

### Table 2.2: Proximate composition of cereal grains %

<table>
<thead>
<tr>
<th>Cereal</th>
<th>Crude protein</th>
<th>Crude Fat</th>
<th>Ash</th>
<th>Crude fiber</th>
<th>Available carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown Rice</td>
<td>7.3</td>
<td>2.2</td>
<td>1.4</td>
<td>0.8</td>
<td>64.3</td>
</tr>
<tr>
<td>Sorghum</td>
<td>8.3</td>
<td>3.9</td>
<td>2.6</td>
<td>4.1</td>
<td>62.9</td>
</tr>
<tr>
<td>Rye</td>
<td>8.7</td>
<td>1.5</td>
<td>1.8</td>
<td>2.2</td>
<td>71.8</td>
</tr>
<tr>
<td>Oats</td>
<td>9.3</td>
<td>5.9</td>
<td>2.3</td>
<td>2.3</td>
<td>62.9</td>
</tr>
<tr>
<td>Maize</td>
<td>9.8</td>
<td>4.9</td>
<td>1.4</td>
<td>2.0</td>
<td>63.6</td>
</tr>
<tr>
<td>Wheat</td>
<td>10.6</td>
<td>1.9</td>
<td>1.4</td>
<td>1.0</td>
<td>69.7</td>
</tr>
<tr>
<td>Barley</td>
<td>11.0</td>
<td>3.4</td>
<td>1.9</td>
<td>3.7</td>
<td>55.8</td>
</tr>
<tr>
<td>Pearl Millet</td>
<td>11.5</td>
<td>4.7</td>
<td>1.5</td>
<td>1.5</td>
<td>63.4</td>
</tr>
</tbody>
</table>

Source: FAO, 1999
2.1.2 Nutritional quality of cereals:

Cereal grains are low in total protein compared to legumes and oil seeds. Lysine is the first limiting essential amino acid for man, although rice, oats and barley contain more lysine than other cereals. Corn protein is also limiting in the essential amino acid tryptophan, while other cereals are often limiting in threonine. The annual global yield of essential amino acids from major cereals has been compared to a hypothetical population of 3 billion adults and 2 billion children (Phillips, 1997) (Table 2.3). Accordingly, if all cereals were effectively and fully utilized for human consumption they would more than meet humanity’s needs for essential amino acids.

Barley, sorghum, rye and oat proteins have lower digestibility (77-88 percent) than those of rice, maize and wheat (95-100 percent). The biological value and net protein utilization of cereal proteins is relatively low due to deficiencies in essential amino acids and low protein availability (Chavan and Kadam, 1989).

Cereals also provide B-group vitamins and minerals, although refining results in losses of these nutrients (Miller, 1996).

2.2 Millet and Sorghum:

Millet and sorghum are often grouped together because their growing conditions, processing and uses are similar (FAO, 1999)

Sorghum and millets have been important staples in the semi-tropics of Asia and Africa for centuries. These crops are still the principal sources of energy, protein, vitamins and minerals for millions of the poorest people in these regions (FAO, 1995).
### Table 2.3: Annual global yields of essential amino acids from major cereals and global human requirements

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Wheat $10^3$ Kg</th>
<th>Rice $10^3$ Kg</th>
<th>Maize $10^5$ Kg</th>
<th>Sorghum $10^7$ Kg</th>
<th>Total $10^8$ Kg</th>
<th>Human requirement $10^3$ Kg</th>
<th>% Provided</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>130</td>
<td>85</td>
<td>104</td>
<td>8</td>
<td>327</td>
<td>223</td>
<td>147</td>
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<tr>
<td>Met. and cystine</td>
<td>162</td>
<td>78</td>
<td>135</td>
<td>11</td>
<td>386</td>
<td>158</td>
<td>244</td>
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<tr>
<td>Threonine</td>
<td>132</td>
<td>90</td>
<td>140</td>
<td>13</td>
<td>377</td>
<td>138</td>
<td>272</td>
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<tr>
<td>Isoleucine</td>
<td>219</td>
<td>85</td>
<td>143</td>
<td>14</td>
<td>461</td>
<td>158</td>
<td>292</td>
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<tr>
<td>Tryptophan</td>
<td>60</td>
<td>26</td>
<td>27</td>
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<td>118</td>
<td>34</td>
<td>347</td>
</tr>
<tr>
<td>Valine</td>
<td>209</td>
<td>127</td>
<td>189</td>
<td>116</td>
<td>540</td>
<td>148</td>
<td>365</td>
</tr>
<tr>
<td>Leucine</td>
<td>313</td>
<td>189</td>
<td>436</td>
<td>407</td>
<td>1033</td>
<td>230</td>
<td>449</td>
</tr>
<tr>
<td>Phen. And Tyr.</td>
<td>404</td>
<td>199</td>
<td>339</td>
<td>360</td>
<td>972</td>
<td>164</td>
<td>593</td>
</tr>
</tbody>
</table>

Adapted from Phillips (1997).
Sorghum and millets are grown in harsh environments where other crops grow or yield poorly. They are grown with limited water resources and usually without application of any fertilizers or other inputs by a multitude of smallholder farmers in many countries. Therefore, and because they are mostly consumed by disadvantaged groups they are often referred to as “coarse grain” or “poor people’s crops” (FAO, 1995). In North America, millet and sorghum are used primarily as livestock feed.

2.3 Pearl millet:

Pearl millet, *Pennisetum glaucum* is also known as spiked millet, bajra (in India) and bulrush millet (Purseglove, 1972).

Pearl millet may be considered as single species but includes a number of cultivated races. It almost certainly originated in tropical western Africa, where the greatest number of both wild and cultivated forms occur. About 2000 years ago the crop was carried to eastern and central Africa and to India, where, because of its excellent tolerance to drought, it become established in the drier environments (FAO, 1995).

The height of the pearl millet plant may range from 0.5 to 4m and the grain can be nearly white, pale yellow, brown, grey, slate blue or purple. The ovoid grains are about 3 to 4 mm long, much larger than those of other millets, and the 1000-seed weight ranges from 2.5 to 14 g. The size of the pearl millet kernel is about one-third that of sorghum. The relative proportion of germ to endosperm is higher than in sorghum (FAO, 1995).
Minor millets (also referred to as small millets) (Seetharam et al., 1989) have received far less attention than sorghum in terms of cultivation and utilization; they include:

- finger millet (*Eleusine coracana*)
- Kodo millet (*Paspalum scroiculatum*).
- Common or proso millet (*Panicum miliaceum*).
- little millet (*Panicum sumatrense*) and
- Barnyard or sawa millet (*Echinochloa crus-galli* and *Echinochloa colona*).

### 2.3.1 Millet production:

Millets are native to Africa or Asia and have been cultivated for more than 6000 years. Millets grow well in arid regions with poor soils and are valued for their relatively high protein content among the cereals (FAO, 1999).

Pearl millet, finger millet and proso millet account for a large portion of the world production. Millet production increased from 26 million tonnes in 1979-81 to 31 million tonnes in 1988 and was similar in 1989 and 1990. Asia, Africa and the former Soviet Union produce almost all the world’s millets as shown in Table (2.4). The major producers of millets in 1990 were India (38.6%), China (15%), Nigeria (13%) and the Soviet Union (12%) (Table 2.5) (FAO, 1995).

The area under millet production increased marginally from 38.1 million hectares in 1979-81 to 47.6 million hectares in 1990. However, production increased by 17 percent, from 25.6 million tonnes.
Table 2.4: Area, yield and production of millet by region, 1990

<table>
<thead>
<tr>
<th>Region</th>
<th>Area</th>
<th>Yield</th>
<th>Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(10³ ha)</td>
<td>% of Total</td>
<td>(10³ ton)</td>
</tr>
<tr>
<td>Asia</td>
<td>20853</td>
<td>55.5</td>
<td>804</td>
</tr>
<tr>
<td>Africa</td>
<td>13548</td>
<td>36.3</td>
<td>669</td>
</tr>
<tr>
<td>USSR</td>
<td>2903</td>
<td>7.7</td>
<td>125</td>
</tr>
<tr>
<td>North and central America</td>
<td>150</td>
<td>0.4</td>
<td>120</td>
</tr>
<tr>
<td>South America</td>
<td>55</td>
<td>0.2</td>
<td>165</td>
</tr>
<tr>
<td>Oceania</td>
<td>34</td>
<td>0.1</td>
<td>882</td>
</tr>
<tr>
<td>World</td>
<td>37565</td>
<td>100</td>
<td>794</td>
</tr>
</tbody>
</table>

Source FAO (1991a).

Table 2.5: Leading millet producers, 1991

<table>
<thead>
<tr>
<th>Country</th>
<th>Area</th>
<th>Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(10³ ha)</td>
<td>% of total</td>
</tr>
<tr>
<td>India</td>
<td>17000</td>
<td>45.3</td>
</tr>
<tr>
<td>China</td>
<td>2601</td>
<td>6.9</td>
</tr>
<tr>
<td>Nigeria</td>
<td>4000</td>
<td>10.7</td>
</tr>
<tr>
<td>USSR</td>
<td>2903</td>
<td>7.7</td>
</tr>
<tr>
<td>Niger</td>
<td>3100</td>
<td>8.3</td>
</tr>
<tr>
<td>Mali</td>
<td>900</td>
<td>1.4</td>
</tr>
<tr>
<td>Uganda</td>
<td>400</td>
<td>1.1</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>1150</td>
<td>3.1</td>
</tr>
<tr>
<td>Senegal</td>
<td>865</td>
<td>2.3</td>
</tr>
<tr>
<td>Nepal</td>
<td>200</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>33119</td>
<td>88.2</td>
</tr>
<tr>
<td>World (1990)</td>
<td>37565</td>
<td></td>
</tr>
<tr>
<td>World (1989)</td>
<td>37409</td>
<td></td>
</tr>
</tbody>
</table>

Source: FAO 1991b
In 1979-89 to 29.8 million tones in 1990, largely because of production increases in Nigeria (65 percent), India (25 percent) and the Soviet Union (20.7 percent). However, there was a 24 percent decrease in production in China during the same period (FAO, 1995).

2.3.2 Millet utilization:

Thirty million tonnes of millet are utilized in developing countries and only a tiny volume is used in developed countries outside the former Soviet Union. Exact statistical data are unavailable for most countries, but it is estimated that a total of 20 million tonnes are consumed as food, the rest being equally divided between feed and other uses such as seed, the preparation of alcoholic beverages and waste. Six countries (China, Ethiopia, India, the Niger, Nigeria and the former Soviet Union) are estimated to account for about 80 percent of global millet utilization (Table 2.6) (FAO, 1995).

Per capita food consumption of millet varies greatly among countries, though it is highest in Africa. In the Sahale, millet is estimated to account for about one third of total cereal food consumption in Burkina Faso, Chad and the Gambia, roughly 40 percent in Mali and Senegal and over two-thirds in the Niger. Other countries in Africa where millet is a significant food item include Ethiopia, Nigeria and Uganda. Millet is also an important food item for the population living in the drier parts of many other countries, especially in eastern and central Africa but also in the northern coastal countries of western Africa. In developing countries outside Africa, millet has local significance as a food in parts of some countries such as China, India, Myanmar and the Democratic People’s Republic of
Table 2.6: Estimated millet utilization, (1981 to 1985/86 average)

<table>
<thead>
<tr>
<th>Region or country</th>
<th>food (10^3 t)</th>
<th>feed (10^3 t)</th>
<th>Other^a (10^3 t)</th>
<th>total (10^3 t)</th>
<th>Percaput Food Kg./year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>7094</td>
<td>122</td>
<td>1921</td>
<td>9137</td>
<td>13.5</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>381</td>
<td>-</td>
<td>60</td>
<td>441</td>
<td>50.8</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>1020</td>
<td>-</td>
<td>196</td>
<td>1216</td>
<td>24.9</td>
</tr>
<tr>
<td>Mali</td>
<td>516</td>
<td>1</td>
<td>88</td>
<td>605</td>
<td>67.7</td>
</tr>
<tr>
<td>Niger</td>
<td>977</td>
<td>21</td>
<td>215</td>
<td>1213</td>
<td>168.9</td>
</tr>
<tr>
<td>Nigeria</td>
<td>2365</td>
<td>86</td>
<td>700</td>
<td>3151</td>
<td>26.5</td>
</tr>
<tr>
<td>Senegal</td>
<td>397</td>
<td>2</td>
<td>80</td>
<td>479</td>
<td>64.4</td>
</tr>
<tr>
<td>Uganda</td>
<td>259</td>
<td>47</td>
<td>150</td>
<td>456</td>
<td>17.8</td>
</tr>
<tr>
<td>Asia</td>
<td>14441</td>
<td>1665</td>
<td>1305</td>
<td>17411</td>
<td>5.3</td>
</tr>
<tr>
<td>China</td>
<td>4857</td>
<td>1120</td>
<td>470</td>
<td>6457</td>
<td>4.7</td>
</tr>
<tr>
<td>India</td>
<td>8794</td>
<td>150</td>
<td>710</td>
<td>9664</td>
<td>11.9</td>
</tr>
<tr>
<td>Central America</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>South America</td>
<td>-</td>
<td>91</td>
<td>5</td>
<td>96</td>
<td>-</td>
</tr>
<tr>
<td>North America</td>
<td>-</td>
<td>104</td>
<td>6</td>
<td>110</td>
<td>-</td>
</tr>
<tr>
<td>Europe</td>
<td>-</td>
<td>104</td>
<td>6</td>
<td>110</td>
<td>-</td>
</tr>
<tr>
<td>USSR</td>
<td>800</td>
<td>1107</td>
<td>400</td>
<td>2307</td>
<td>2.9</td>
</tr>
<tr>
<td>Oceania</td>
<td>-</td>
<td>13</td>
<td>2</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>World</td>
<td>22335</td>
<td>3144</td>
<td>3642</td>
<td>29121</td>
<td>4.8</td>
</tr>
<tr>
<td>Developing countries</td>
<td>21535</td>
<td>1878</td>
<td>3231</td>
<td>26644</td>
<td>6.1</td>
</tr>
<tr>
<td>Developed countries</td>
<td>800</td>
<td>12266</td>
<td>411</td>
<td>1477</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Korea. Although national per capita levels are rather low in countries that consume the most millet, i.e. China and India, food use of millet is important in certain areas of these countries (FAO, 1995).

2.3.3 Grain morphology:

The structure of mature caryopsis of pearl millet is similar to the structure of sorghum but with several differences. In comparison with sorghum, pearl millet contains smaller seeds (3-15g/1000 kernels Vs 5-80g/1000 kernels of sorghum) and proportionally a larger germ and consequently a smaller endosperm (Abdelrahman et al., 1984). Kernel structure is important with respect to minimizing damage during grain harvest, drying, handling, storage, milling, germination and enhancing nutritional value (FAO, 1995).

The pearl millet consists of 71-76.2% endosperm (storage organ), 12-15.5% germ (miniature plant), and 7.2 – 10.6% pericarp (seed coat or bran) (Abdelrahman et al., 1982, 1984). The outer epiderm is composed of three layers: pericarp, the mesocarp and the endocarp (Sullin and Rooney, 1975). Aleurone layer which is just below the seed coat, is only a few cells thick but rich in oil, minerals, protein and vitamins. Starch and protein are located in the endosperm, which represent the bulk of the grain. We find more protein and less starch per cell from the outer to the inner region of the endosperm. (FAO, 1999).

2.3.4 Chemical composition and nutritive value of pearl millet:

The pearl millet bran is low in mineral matter like that of sorghum, but it is remarkably rich in protein (ranging from 8.0 to 20 percent) (Johnson and Raymond, 1964). The germ fraction of pearl
millet is relatively large, 16 percent as against 10 percent in sorghum. It is also rich in oil (32 percent), protein (19 percent) and ash (10.4 percent). Practically all the oil (87 percent) of the whole kernel is in the germ fraction, which also accounts for over 72 percent of the total mineral matter. Greater concentration of minerals in the germ and the bran layers than in endosperm is typical of cereal grains (MacMasters, *et al.*, 1971). The total fat content of pearl millet is higher than that of other millets and sorghum because of the size of germ and its high oil content and because of somewhat higher levels of fat in the bran fraction. Badi and Monawer (1987) reported that millet contained more lipid (5.1%) than sorghum and wheat and that explained why the quality of millet flour deteriorated faster during storage than sorghum and wheat flour due to hydrolytic as well as oxidative rancidity occurring in lipid components.

Tannins, a group of high molecular weight polyphenols occur in the testa (Dendy, 1995). Some of these substances reduce the nutritional value of food by interfering with mineral bioavailability and digestibility of protein and carbohydrates.

### 2.3.5 Pearl millet and fermentation:

Fermentation is known to reduce phytic acid of several plant foods including cereals (Reddy and Salunkhe, 1980) and that of pearl millet (Dhankher and Chauhan, 1987) thereby converting the bound form of minerals to free form which is responsible for increased HCl-extractability of the fermented products. Chompreeda and Fields (1984) investigated the effect of natural fermentation of pearl millet carried at 20, 30, 40 and 50°C for 24, 48, 72 and 96 hr. on the HCl-extractability of the minerals calcium, zinc, iron, manganese and
copper in pearl millet flour. The bioavailability of these minerals was nearly doubled or more than doubled following fermentation with pure culture of yeasts and lactobacilli which brought about significant increase in HCl-extractability of phosphorus, calcium, iron, zinc, copper and manganese with corresponding decline in phytate level (Khetarpaul and Chauhan, 1991a). Dhankher and Chauhan (1989) reported comparable results for the same elements in rabadi fermentation and a decrease in total polyphenols and phytic acid content. The improvement in-vitro protein digestibility caused by fermentation could be attributed to the partial degradation of complex storage portions to more simple and soluble products (Chavan et al., 1988). Elyas et al. (2002) observed a decrease in total polyphenols (25%) and phytic acid (50%) after 36 h. of fermentation of two pearl millet cultivars. Elhag et al. (2002) reported that fermentation of millet seeds enhanced proteolytic activity. Fermentation is generally associated with improved protein digestibility which increases amino nitrogen by partial breakdown of protein peptides and amino acids. Puttalingamma et al. (2006) reported that the possibility of inducing lactic acid bacteria cultures in processing of food to effectively reduce the contamination, increases the shelf-life of vegetables or any other food as well as control pathogens. Fermentation and dehulling were found to improve the nutritive value of pearl millet by increasing the nutrient content and decreasing the antinutrients. Combination of the two processes is useful for improving the nutritive value and digestibility (Ahamed, 1999).

Fermentation of millet brings about the following changes:


ii. A decrease in starch contents (El Tinay et al., 1979).
iii. A reduction and increase in protein content (El Hidai, 1978; Abdalla, 1996).

iv An increase in polyphenols (Mahajan, 1986); Also decrease in polyphenols may be observed by fermentation as reported by (Dhankher and Chauhan, 1987a).

v. A decrease of phytic acid contents (Khetarpaul and Chauhan, 1991a).

vi. An increase in the in vitro protein and starch digestibility (Khetarpaul and Chauhan, 1991b).

2.4 Fermented cereals:

Animal or plant tissue subjected to the action of micro-organisms and/or enzymes to give desirable biochemical changes and significant modification of food quality are referred to as fermented foods (Campbell-Platt, 1994). Fermentation is the oldest known form of food biotechnology; records of barley conversion to beer date back more than 5000 years (Borgstrom, 1968). According to Steinkraus (1995), the traditional fermentation of foods serves several functions:

1. Enrichment of the diet through development of diversity of flavors, aromas and texture in food substrate.

2. Preservation of substantial amounts of food through lactic acid, alcoholic, acetic acid and alkaline fermentation.

3. Enrichment of food substrates biologically with protein, essential amino acids, essential fatty acids and vitamins.

4. Detoxification during food fermentation processing.

5. A decrease in cooking times and fuel requirements.
Aside from alcoholic fermentation and the production of yoghurt and leavened bread, food fermentation continues to be important primarily in developing countries where the lack of resources limits the use of techniques such as vitamin enrichment of foods and the use of energy and capital-intensive processes for food preservation. The technology of producing many indigenous fermented foods from cereals remains a household art in these countries (Chavan and Kadam, 1989). Prospects for applying advanced technologies to indigenous fermented foods (Wood, 1994) and for the production of value-added additive products, such as colors, flavors, antimicrobials, and health products (Cook, 1994) during food fermentation have been reviewed.

Special mention should be made of the microbiological risk factors associated with fermented foods. The safety of fermented foods has been recently reviewed (Nout, 1994). Case of food-born infection and intoxication due to microbial metabolites such as mycotoxins, ethyl carbamate, and biogenic amines has been reported in fermented foods. Major risk factors include the use of contaminated raw materials, lack of pasteurization, and use of poorly controlled fermentation conditions. On the other hand, non-toxigenic micro-organisms can serve to antagonize pathogenic micro-organisms and even degrade toxic substances such as mycotoxins (Nakazato et al. 1990) in fermented foods.

2.4.1 Indigenous fermented cereal foods:

Most bacterial fermentations produce lactic acid, while yeast fermentations result in alcohol production. Many of the indigenous fermentation cereal products are valued for the taste and aroma-active
components produced and are used as seasonings and condiments (Chavan and Kadam 1989). Fermented foods contribute to about one-third of the diet worldwide (Campbell-Platt, 1994). Cereals are particularly important substrates for fermented foods in all parts of the world and are staples in the Indian subcontinent, in Asia and in Africa. Fermentation causes changes in food quality indices including texture, flavor, appearance, nutrition and safety. The benefits of fermentation may include improvement in palatability and acceptability by developing improved flavors and textures; preservation through formation of acidulants, alcohol, and antibacterial compounds; enrichment of nutritive content by microbial synthesis of essential nutrients and improving digestibility of protein and carbohydrates; removal of antinutrients, natural toxicants and mycotoxins; and decreased cooking times. The content and quality of cereal proteins may be improved by fermentation (Chavan et al. 1988). Natural fermentation of cereals increases their relative nutritive value and available lysine (Hamad and Fields, 1979). Bacterial fermentations involving proteolytic activity are expected to increase the biological availability of essential amino acids more so than yeast fermentations, which mainly degrade carbohydrates (Chavan and Kadam, 1989). Starch and fiber tend to decrease during fermentation of cereals (El-Tinay et al., 1979). Although it would not be expected that fermentation would alter the mineral content of the product, the hydrolysis of chelating agents such as phytic acid during fermentation, improves the bioavailability of minerals. Changes in vitamin content of cereals with fermentation vary according to the fermentation process, and the raw material used in the fermentation. B group vitamins generally show an increase on fermentation (Chavan
and Kadam, 1989). Fermented cereal-based food products produced in African countries can be classified on the basis of either the raw cereal ingredients used in their production, or the texture of the fermented product (FAO.1999).

**Classification on the basis of raw cereal ingredients:**

a) Wheat-based foods, e.g. bouza, kishk  
b) Rice-based foods, e.g. busa  
c) Maize-based foods, e.g. ogi, bread, kenkey  
d) Millet-based foods, e.g. kunuzaki  
e) Sorghum-based foods, e.g. pito, ogi, bogobe, kisra, burukutu, injera  
f) barley-based foods, e.g. beer

**Classification on the basis of texture:**

a) Liquid (gruel). E.g. ogi, mahewu, burukutu, pito, uji  
b) solid(dough) and dumplings, e.g. kenkey, agidi  
c) dry (bread), e.g. kisra, injera

Among the various fermentation processes in Africa, lactic acid fermentation is one of the oldest and most widespread (Dirar, 1992). Lactic fermentation technology can be defined as the fermentation process involving the activities of a group of Gram-positive, non-sporing, non-motile, catalase-negative, anaerobic organisms which ferment carbohydrates to produce lactic acid as the sole or major organic acid. In Africa, lactic acid fermentation technology has developed indigenously for an extensive range of raw materials yielding an extensive range of products (Oyewole, 1995).
Large numbers of lactic fermented products in Africa are cereal-based and the products range from porridges and breads to both alcoholic and non-alcoholic beverages. Common cereals which are fermented in Africa include maize, sorghum, millet, and occasionally rice and wheat (Oyewole, 1995). Lactic fermentation of food is carried out through village-art traditional methods (Odunfa, 1985). Processing usually involves either soaking of the raw materials, submerged in water contained in a fermenting vat, usually clay pots, for a length of time or an initial size reduction of the raw material by grating or milling in the wet form before being allowed to ferment without being soaked in water. These two fermentation methods had been described by Oyewole and Odunfa (1991) as traditional submerged and solid-state fermentation processes.

The microorganisms implicated in the lactic fermentation of foods in Africa belong to four major genera namely *Lactobacillus, Lactococcus, Leuconostoc, and Pediococcus*. These are part of the major group of micro-organisms referred to as the “lactic acid bacteria”. According to Aguirre and Collins (1993), the term “lactic acid bacteria” is used to describe a broad group of Gram-positive, catalase-negative, non-sporing rods and cocci, usually non-motile, that utilize carbohydrates fermentationation and form lactic acid as the major end product. They are categorized into "homo-"or "hetero-fermentative" according to the metabolic routes they use (Embden-Meyerhof or phosphoketolase pathways), and according to resulting end-products.

The microbial patterns of the different lactic fermented products follow a pattern in which:
(a) The fermentation process, being a chance inoculation process, is usually initiated by a mixed flora of microorganisms.

(b) The non-lactics are eliminated with increasing acid production in the medium.

(c) There is a microbial succession trend among the lactic acid bacteria which survive in the acidic medium. For example, *Leuconostoc mesenteroides* do not survive in very high acidic environments which *Lactobacillus plantarum* strains can tolerate.

(d) The lactic acid bacteria that survive the fermentation process, usually do this in association with some yeasts (*Saccharomyces* spp., *Candida* spp.)

(e) In Africa, lactic acid fermentation processes have survived throughout the centuries because of the following benefits of this technology:

(i) Lactic acid fermentation technology serves as a household technology for improving food safety in Africa

(ii) Lactic acid fermentation technology serves as a low-cost method of food preservation in Africa

(iii) Lactic acid fermentation technology contributes to improvement of the nutritional value and digestibility of food raw materials in Africa.

The role of lactic acid bacteria in health and disease has been documented in the literature (Sandine, 1979). Yogurt and other milk ferments have been reported to be effective in the treatment of a variety of disorders including colitis, constipation, diarrhea (Sanders,
The *in vitro* inhibition of growth of pathogenic microorganisms by lactic acid bacteria has encouraged investigations into its use as a prophylactic and therapeutic means of treating gastrointestinal and other diseases. *Lactobacillus acidophilus* has been found to be effective in the treatment of different types of diarrhea in humans and chicks (Beck and Necheles, 1961). Acidophilus milk which is yogurt produced by fermenting milk with *Lactobacillus acidophilus* is now being used to treat *Escherichia coli*, *Salmonella*, and *Shigella spp.* mediated diarrhea and dysenteries in infants in some parts of Europe (Alms, 1983).

Odugbemi *et al.* (1991) confirmed that enteropathogenic *Escherichia coli*, *Salmonella typhi* and *Salmonella paratyphi* were incapable of multiplying in “ogi”, a lactic fermented maize product used for feeding infants in West Africa. In other studies on South Africa “mahewu” (Simange and Rukure, 1991) and Ghanaian maize dough “kenkey” (Mensah *et al.*, 1988), disease-causing *Campylobacter*, *E. coli* and *Shigella* were not able to survive or multiply on the lactic fermented products on inoculation.

### 2.4.2 Examples of some Sudanese fermented cereal foods:

#### 2.4.2.1 Kissra:

This food is staple food of Sudan; it is prepared from sorghum and millet flour. By far, however, the bulk of kissra is made from the various types of sorghum. The flour, mixed with water and small portion of the previous lot of fermented sorghum dough, is incubated in a warm corner of the house, covered and left to ferment overnight. Fresh flour may be added to previous dough and it is baked on a hot plate to give thin sheet of bread (Dirarr, 1993 and 1996).
2.4.2.2 Gurrassa Murra:

This sweet sour bread is confined to the Northern State of Sudan, especially to the area lying between the towns of Dongola and Karima because this area is the date-producing region. In the preparation of gurrassa murra, wheat flour is added to water containing a little salt and mixed well to make a thin slurry. Then about 10% sour sorghum dough is added as a starter then the batter is placed out in the sun for 4-6 hours. Then dates are boiled to make thick slurry. This is then mixed with the sour wheat batter just before baking. The mixture is baked on a hot plate then the product is rubbed with ghee and folded twice to give a triangular shape for storings (Abdel Rahim, 2003).

2.4.2.3 Abreh:

Abreh is usually produced from white varieties of sorghum which are cleaned and dehulled then soaked in water overnight then milled to give "ajin". The process also involves, cooking of one third of ajin into thin paste "madida", and mixing of madida with the rest of ajin, and addition of spices, fermentation and then baking into thin flakes. These flakes are suspended in water, sweetened with sugar and swallowed whole without sieving (Elsharif, 1993).

2.4.2.4 Khemiss tweira:

Khemiss tweira is a food confined to Darfur State of the Sudan; it is composed of five ingredients: millet grain flour, millet malt flour, roasted sesame seeds (sometimes groundnut), salt and sugar (Dirar, 1993; Dirar1996). Khemiss tweira is made by mixing millet malt flour and millet grain flour cooked into stiff cool porridge and allowed to ferment overnight then baked into sheets, then sun-dried and
crumble and mixed with roasted sesame or groundnuts, salt and sugar, then pounded into coarse meal (Hasssan, 2000).

2.4.2.5 Damirga:

The word Damirga itself refers to specially prepared sour flour from which acedat Damirga is made, this type of aceda is basically confined to western Sudan and mostly prepared from pearl millet (Dirar, 1993).

Dagig Damirga, as this flour is called, is mostly used to prepare acedat – Damirga which is a stiff porridge, usually consumed while still warm with some sauce, because if left until cold it becomes rather dense and very compact. The porridge is prepared routinely for the daily meal of many families particularly in Darfur region (Dirar, 1993).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials:

Ten samples (in sterile plastic bags) of Damirga flour used in this work were purchased from different areas in Khartoum State where the displaced people from Darfur and Kordofan live. These areas were:

1. East of Khartoum State (El Haj Yousif area).
2. South of Khartoum State (Mayo area).

They were immediately analyzed microbiological and the rest kept in the laboratory at room temperature for two to three days pending chemical and biochemical analysis.

Also pearl millet grains were purchased from Shambat Central Market for preparation and processing of Damirga in the laboratory.

3.2 Methodology:

3.2.1 Documenting traditional information on Damirga:

A short, one page questionnaire (Appendix 1) was designed to collect information about Damirga producing methods; the information was collected from the three areas mentioned above from which the Damirga samples were collected. Fifty old women from western Sudan were interviewed.
3.2.2 Traditional method of Damirga preparation:

Damirga fermentation was emitted as described by western Sudan women in our laboratory as follows, one Kilogram of millet grains (yellow variety) were cleaned and moistened with water and dehulled by mortar (funduk) and the pearled kernels were sun-dried to winnow off the bran. The decorticated kernels were transferred to a clean metal pail and then 2000 ml of tap water was added to give a ratio 1:2 water: grain (w/v), as described by El Tinay (1979) and then allowed to ferment for 72 hours. The fermented grains were then washed twice with tap water twice and sun dried. Thereafter the dry grains were milled in an electric grinder to fine flour. The steps are shown in Fig. 3.1 and Plate 3.1.

3.2.3 Sampling plan during fermentation experiments:

Sampling during traditional Damirga fermentation process and during Damirga fermentation by using pure culture starter was done according to the plan in Table 3.1. The capital letters in the 4th column of table 3.1 represent the type of analysis carried out on the samples as follows:

(A) Proximate analysis: This included the determination of the ash, moisture, protein, fiber, oil contents and starch.

(B) Biochemical analysis: This included the determination of pH, titrable acidity, volatile fatty acids, ethanol and turbidity level.

(C) Nutritional value analysis: This included protein digestibility.

(D) Antinutritional factors analysis: This included polyphenols, tannin and phytic acid levels of the product.
Fig. 3.1: Flow diagram of the home process of Damirga flour production.
Plate 3.1: Steps of Damirga and Acidat Damirga processing

Dephulling of Pearl millet

Winnowing

Fermentation

Sun drying

Acidat Damirga

Acidat Damirga with sauce
Table 3.1: The sampling protocol of Damirga fermentation

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Material sampled</th>
<th>Fermentation time (hrs)</th>
<th>No. of Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, B, C, D, E</td>
<td>Millet grain flour</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>A, B, C, D, E</td>
<td>Decorticated millet grains</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>B, E</td>
<td>Fermentation of Damirga</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>B, E</td>
<td>Fermentation of Damirga</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>B, E</td>
<td>Fermentation of Damirga</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>B, E</td>
<td>Fermentation of Damirga</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>B, E</td>
<td>Fermentation of Damirga</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>B, E</td>
<td>Fermentation of Damirga</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td>B, E</td>
<td>Fermentation of Damirga</td>
<td>48</td>
<td>9</td>
</tr>
<tr>
<td>B, E</td>
<td>Fermentation of Damirga</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>B, E</td>
<td>Fermentation of Damirga</td>
<td>72</td>
<td>11</td>
</tr>
<tr>
<td>A, B, C, D, E.</td>
<td>Final Damirga product</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>B and E</td>
<td>Damirga fermented by starter</td>
<td>-</td>
<td>13</td>
</tr>
</tbody>
</table>
Microbiological analysis: This included the determination of the total viable counts, the lactic acid bacteria count, the acetic acid bacteria count, the yeast count, the coliform most probable number (MPN) beside the identification of these microorganisms.

3.2.4 Analytical Methods:

3.2.4.1 Proximate analysis of pearl millet, decorticated grains and Damirga flour:

3.2.4.1.1 Moisture content on wet basis:

Moisture content (MC) of each sample was determined according to the methods of the Association of Official Analytical Chemists (AOAC, 1984) where two grams of well-mixed ground samples of millet grains, decorticated millet grains or Damirga flour were accurately weighed in a clean dry-weighed dish. The uncovered sample in the dish was kept in a forced air oven at 105ºC overnight. The dish was covered while still in the oven and transferred to a desiccator, and weighed after reaching room temperature equilibrium. The loss of weight was calculated as a percentage from sample weight and expressed as moisture content percentage of the sample.

\[
MC\% = \times 100 \frac{W_1 - W_2}{W_1}
\]

Where:

Original weight of sample \( W_1 = \)

Weight of sample after drying \( W_2 = \)

3.2.4.1.2 Crude fiber:

Crude fiber (CF) of each sample was assessed according to the method described by Southgate (1976). Two grams sample (defatted)
were digested in 200 ml boiling 0.255 N H₂SO₄ under reflux, for 30 minutes. The material was then filtered under suction using a linen piece as a filter. The residue obtained was washed with hot distilled water to remove any traces of acid. A second alkali digestion for residue was done using 200 ml boiling 0.344 N NaOH for 30 minutes, then similarly filtered as above. The residue was successively washed with hot acid and hot distilled water, dried at 105ºC overnight and weighed.

\[
\times 100 \frac{W_1 - W_2}{S} \text{ CF\% =}
\]

Where:
- Weight of sample before drying \( W_1 = \)
- Weight of sample after drying \( W_2 = \)
- Original weight of sample \( S = \)

3.2.4.1.3 Crude protein:

Total organic nitrogen of the sample was determined according to the method described by the (AOAC, 1984). Micro-kjeldahl apparatus was used for nitrogen digestion and distillation oven dried sample (0.2 gm) was transferred into 100 ml kjeldahl flask. One gram of catalyst (potassium sulphate + cupric acid "20:1 by weight" mixture) and 3.5 ml of concentrated sulphuric acid were added. The sample and contents were heated on an electric heater, first gently then gradually with increase in heat and allowed to proceed for two hours and continued for half an hour till a clear solution was obtained. The sample was then placed in a distillation apparatus, 10 ml 40% NaOH were added to the clear solution and distilled for 7 minutes. The ammonia evolved was received in 10 ml 2% boric acid solution, contained in a conical flask attached to the receiving end. The trapped
ammonia was titrated against 0.02 N HCl using universal indicator (methyl bromocresol green).

\[
\frac{(A - B) \times N \times 14 \times 100}{1000} \text{ Nitrogen\%} =
\]

Where:
- ml of HCl sample \( A = \)
- ml of HCl blank \( B = \)
- Normality of HCl \( N = \)
- Nitrogen equivalent factor \( 14 = \)

The crude protein content (CP) was calculated by multiplying the percent nitrogen by the conversion factor (N\% X 6.25).

\[\text{C.P} = \text{Nitrogen \%} \times 6.25\]

3.2.4.1.4 Crude oil:

The total content was determined by soxhlet extraction method described by the Association of Official Analytical Chemists (AOAC, 1984). Two grams of ground sample were accurately weighed in an empty thimble covered with cotton wool and placed in a soxhlet extraction apparatus using petroleum spirit solvent (60-80ºC). Pre-weighed round bottom flask 2/3 full of the solvent was fitted to the extractor. The apparatus was assembled and the extraction continued for seven hours. The apparatus was carefully dismounted and the solvent was evaporated first at room temperature then to dryness in an oven at 60ºC. The oil constant weight expressed as percentage of crude oil.

\[\times 100 \quad \frac{W_1 - W_2}{S} \text{ Crude oil \%} =\]

Where:
- Weight of empty receiver \( W_1 = \)
- Weight of receiver + oil \( W_2 = \)
- Weight of sample \( S = \)
3.2.4.1.5 Total Ash:

Total ash of a sample was estimated according to the Official Methods of Analysis (AOAC, 1984). Two grams of sample were weighed into a previously ignited cooled and weighed ashing dish. The dish and contents were ignited first gently and then at 500ºC into a muffle furnace for two hours. The dish contents were expressed as percentage of total ash from the weight of the sample.

\[
\text{Total ash\%} = \frac{W_2}{W_1} \times 100
\]

Where:

- Original weight of sample \( W_1 \)
- Weight of sample after ignition \( W_2 \)

3.2.5 Biochemical analysis analysis of pearl millet, decorticated grains and Damirga flour:

3.2.5.1 Measurement of pH:

Ten grams of Damirga flour were shaken into 100 ml distilled water to make 1:10 suspension. The suspension was then left to stand for twenty minutes then the pH was read using glass electrode pH-meter model HI 255 Combined Meter pH/mv and Ec/TDS\NaCl, as described by Dzudie and Hardy (1996).

Also the pH of fermented dehulled grains during Damirga traditional fermentation was measured inside the grains by taking ten grams of well washed fermented grains; the grains were sun-dried, and ground to fine flour and suspended in 100 ml distilled water. The pH outside the fermenting grains was determined by taking ten ml of fermentation soak water of the dehulled grains aseptically and the pH was measured as described above.
3.2.5.2 Starch analysis of pearl millet, decorticated grains and Damirga flour:

Starch was determined by using O’ Sullivan's Diastase method described by Pearson (1970). Three grams of powdered material were extracted several times with ether in soxhlet extractor.

Then the material was washed on a filter paper with 10 % alcohol, followed by 95% alcohol. The residue was then drained, and washed with 50 ml of water and heated for 15 min. in a boiling-water bath, stirring constantly so that all starch was gelatinized and a homogeneous mixture was obtained.

Then the material was cooled to 55ºC, about 0.03ml of diastase solution was added and the mixture was kept at 55ºC – 60ºC for at least an hour so that a drop of the solution gave no blue colour with iodine. The temperature was raised to 100ºC, and then the mixture was washed and filtered and completed to 250 ml. Then 200 ml of filtrate were boiled in a water bath with 20 ml hydrochloric acid for two and a half hr.

Then this was cooled and neutralized with sodium carbonate and diluted to 500 ml with distilled water then the dextrose was estimated by the Lane and Eynon method described by Pearson (1970). The sugar solution was clarified (lead and calcium salts were removed with potassium oxalate), and placed in a 50 ml burette fitted with a pinch cock and wit glass jet bent twice so that the graduations are not directly over the flask. 25 ml of mixed Fehling's solution were placed into a 300 ml conical flask, and 15 ml of the sugar solution was added from the burette, and then the mixture was boiled on an asbestos-
covered gauze and further solution was added one ml at 10 to 15 second intervals to the boiling liquid until the blue colour was nearly discharged. Then 3-5 drops of methylene blue (1%) were added and titration of the boiling liquid continued until the indicator is completely decolorizes.

Titration was repeated, sugar solution was added and then boiled gently for 2 minutes, 3-5 drops of the methylene blue indicator were added and titration completed within a total boiling time of 3 minutes. At the end-point, the blue colour disappeared and the liquid became orange-red. The proportions of the various sugars, equivalent to 25 ml Fehling's solution.

\[
\text{Starch\%} = 0.90 \times \text{dextrose \%}
\]

3.2.5.3 Total titrable acidity of Damirga flour and fermentation soak water:

The total acidity was determined by titration method. The titrable acidity was expressed as lactic acid. One gram of Damirga flour was shaken in 100 of distilled water, the suspension titrated with 0.1 N NaOH to the phenolphthalein end point. Also 10 ml of fermented soak water of Damirga during it fermentation were diluted with 100 ml D.W. and titrated with 0.1 N NaOH (AOAC, 1975).

The acidity was calculated from the following equation:

\[
\frac{\text{NaOH ml} \times 0.009 \times 100}{\text{Weight of sample}} = \frac{\text{Titrable acidity %}}{}
\]

Where:

Lactic acid are equivalent to 1 ml 0.1 NaOH  0.009g =
3.2.5.4 The volatile fatty acids of Damirga flour and fermentation soak water:

The volatile fatty acids, expressed as acetic acid, were measured by titrating the steam distillate of 20 ml of the sample with 0.1 N NaOH to the phenolphthalein end point. Before distillation the samples were diluted with distilled water in a ratio of 1:10 as described by Wood and Allan (1982).

3.2.5.5 Ethanol of Damirga flour and fermentation soak water:

Alcohol was determined by Albumeter, The apparatus setting consisted of the following

1. Albumeter.
2. Albumeter calculating instrument (chart).
3. Very sensitive thermometer.

Fifty ml. of tap water were boiled in Albumeter after filling the condenser with cold water. The water boiling point was used to adjust the Albumeter calculating instrument (chart) and at this point the ethanol concentration is zero.

Then 50 ml. of sample were added to the specific part of Albumeter, the boiling point of the sample was recorded, and the corresponding concentration of alcohol was found from the adjusted chart.

This method was done according to the protocol followed by Modern Distillery Co. and Al Watania Distillery Co. (Abbas, 2008).
3.2.6 Determination of antinutritional factors analysis of pearl millet, decorticated grains and Damirga flour:

3.2.6.1 Total polyphenols:

Polyphenolics present in Damirga flour were estimated using the Prussin Blue assay, as described by Price and Butler (1977). Sample (60 mg) was extracted with 3 ml absolute methanol in a test tube, by constant shaking for one minute, and then poured into a filter paper. The tube was quickly rinsed with an additional 3 ml of methanol and contents poured at once into the filter paper. The filtrate was diluted to 50 ml with distilled water, mixed with 3 ml 0.1 M FeCl₃ in 0.1 N HCl for 3 minutes, followed by the timed addition of 3 ml 0.008 M K₃Fe(CN)₆. The absorbance was read after 10 minutes at 720 nm on a spectrophotometer. In all cases, tannic acid was used as a reference standard.

3.2.6.2 Phytic acid:

Phytic acid was determined by the method of Wheeler and Ferrel (1971). One gram of finely ground sample (60 mesh) was weighed into a 100 ml conical flask, extracted with 50% (w/v), TCA (Trichloro acetic acid) solution containing 10% (w/v) sodium sulphate, by shaking for an hour (Lajolo et al., 1991). The slurry obtained was centrifuged at 3000 rpm for 15 minutes.

Ten milliliter aliquot of the supernatant was transferred into 50 ml boiling tubes. Then, 4 ml of FeCl₃ (2 mg Fe³⁺/ml 3% TCA solution), centrifuged at 3000 rpm for 15 minutes and the clear supernatant was carefully decanted. The precipitate was then washed twice by dispersing well into 25 ml 3% TCA, heating in a boiling water bath (10 minutes) and centrifuged.
Washing was repeated once with water. The precipitate was cautiously dispersed in a few ml distilled water enriched with 3 ml 1.5 N NaOH with mixing. The volume was made approximately 30 ml with distilled water and heated in the water bath for 30 minutes. The contents of the tube were filtered hot (quantitatively) through Whatman No. 1 filter paper and the filtrate was discarded.

The precipitate from paper was dissolved with 40 ml hot 3.2 N HNO$_3$ into a 100 ml volumetric flask. The paper was washed with several portions of distilled water. The contents in the flask were cooled and diluted to volume with distilled water. Five milliliter aliquots were transferred to another 100 ml volumetric flask and diluted to approximately 70 ml with distilled water. Then, 20 ml of 1.5 M KSCN (potassium thiocyanate) were added, completing the volume up to mark. The intensity of color was immediately assessed (within one minute) on a spectrophotometer (Corning, 259) at 480 nm. A blank probe was run with each set of sample.

The iron content was calculated from a prepared Fe (NO$_3$)$_3$ standard curve (Appendix 2). Phytic acid was calculated from the assumption that it contains 28.2 % P (De Boland et al., 1975) and phytate phosphorous from a molar ratio of 4:6 iron: phosphorus ratio.

3.2.6.3 Tannins:

Quantitative estimation of tannin was carried out using the vanillin-HCl in methanol and 1% in vanillin in methanol (Price et al., 1978).

The reagent was prepared daily by mixing equal volumes of 1% vanillin in methanol and 8% concentrated HCl in methanol. It was discarded if a trace of colour appeared.
D-catechin was used to prepare the standard curve (Appendix 3). This was done by adding 100 mg of D-catechin to 50 ml of 1% vanillin in methanol. From this stock solution various dilutions were prepared. Five ml of vanillin-HCl reagent (0.5%) were added to 2 ml of each dilution.

The absorbance was read using spectrophotometer model WAPS101 at 500 nm after 20 minutes at 30°C from addition of reagents.

The absorbance was plotted against catechin concentration. Grains were ground to pass 0.4mm screen. 0.2g of flour was placed in a test tube then 10 ml of 1% concentrated HCl in methanol was added. The test tube was capped and continuously shaken for 20 minuets and then centrifuged at 5000xg for 5 minutes. One ml of the supernatant was pipetted into each of the tubes and proceeding as was described for the standard curve above.

For zero setting 1 ml distilled water (blank) was mixed with 5 ml 4% concentrated HCl and vanillin reagent in a test tube and incubated for 20 min. at 30°C (blank). Absorbance at 500 nm was read using spectrophotometer.

Tannin concentration was expressed as catechin equivalent (CE) as follows:

\[
CE = \frac{C \times 10 \times 100}{1000 \times W}
\]

Where:

- Concentration corresponding to optical density \(C\) =
- Volume of the extract (ml), it was 100 ml for protein fractions \(10\) =
- To convert from g to mg \(1000\) =
- Weight of sample \(W\) =
3.2.7 *In vitro* protein digestibility of Damirga samples:

*In vitro* protein digestibility of samples were measured according to the method developed by Sanders (1993), in which is double digestive pepsin-pancreatic system was used in the determinations.

In a 100 ml conical flask, 250 mg material were suspended in 15 ml of 0.1 N HCl containing 1.5 mg pepsin (1:1000) and incubated at 37°C for 3 hours. The solution 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% TCA was added to the mixture to stop the reaction. The mixture was then centrifuged at 5000 rpm for 5 minutes. Five-milliliter aliquots from the supernatant were taken and analyzed for nitrogen using the Kjeldahl procedure (AOAC, 1984).

**Calculations:**

\[
\text{Protein digestibility} = \frac{(T - B) \times N \times 10 \times 100 \times TV}{(X) \times a} \\
\]

Where

\[
X = \frac{250 \times \text{CP} \%}{100 \times 6.25} \\
N = \text{Normality of HCl} \\
T = \text{ml of titer} \\
B = \text{ml of blank} \\
a = \text{Number of ml of aliquot} \\
TV = \text{Total volume of the mixture} \\
\text{CP} \% = \text{Crude protein} \\
\text{14} = \text{Equivalent weight of nitrogen} \\
\text{250} = \text{Sample weight in mg}
\]
3.2.8 Measurement of the turbidity of fermenting Damirga soak water:

Turbidity of Damirga soak water during fermentation was measured by measuring the absorbance in a spectrophotometer (DR/3 spectrophotometer model 41700/41800) at a wavelength of 540 nm. Tap water was used as a blank; the result was read and recorded as optical density.

3.3 Microbiological Methods:

3.3.1 Sterilization of glassware and metal tools:

According to Harrigan and MacCance (1976) glassware was washed thoroughly with distilled water and left to dry, and then they were sterilized in a hot forced air oven at 160ºC for at least three hours.

Inoculation wires and loops were sterilized by direct flaming to red-hot and other metal instruments such as spatulas and forceps were sterilized by flaming after dipping in ethanol.

3.3.2 Sterilization of Media:

Media were sterilized by using an autoclave at 121ºC and 15 lb/in² for 15 minutes; media containing sugar were sterilized using an autoclave at 110ºC for 10 minutes.

3.3.3 Pasteurization of decorticated grains:

Decorticated grains of millet were immersed in tap water at a ratio of 1:2 and pasteurized by heating in a water bath at 60ºC for 30 minutes and cooled immediately on ice water.

3.3.4 Total viable count:

Total viable counts of the samples were done by the standard pour plate count techniques as described by Harrigan and MacCance.
Ten grams of sample were added to 90 ml sterile distilled 
water, shaken for 5 minutes and allowed to stand for another 2 
minutes (this dilution represented the primary dilution). Higher serial 
dilutions were prepared using 9 ml tube sets of saline (0.85% NaCl). 
One ml aliquots were transferred aseptically in triplicate to sterile 
Petri dishes and poured with sterile plate count agar medium (PCA) 
(Oxoid) which was composed of tryptone, yeast extract, D-glucose 
and granulated agar. It was prepared according to the manufacture's 
instructions by dissolving 23.5g in one liter of distilled water. 
Incubation was done at 37ºC for 48 hours. After 48 hours the colonies 
were counted by using a colony counter. The results were reported as 
Colony Forming Unit (CFU) viable bacterial count per gm of sample. 
The same procedure was followed for the samples of millet grains, 
decorticated millet, Damirga end product and ten ml of Damirga soak 
water during fermentation were taken and transferred to 90 ml sterile 
saline solution this represented the primary dilution the results were 
reported as CFU viable bacterial count per ml of sample.

3.3.5 Lactic Acid Bacteria:

From suitable serial dilutions (using saline) of sample, 0.1 ml. 
was aseptically transferred onto solidified Man, Regosa and Sharpe 
agar medium (MRS) which is composed of 10g peptone, 10g lab-
lemco meat extract, 5g yeast extract, 20g. D-glucose, 1.0g tween 80. 
2g dipotassium hydrogen phosphate, 5g. sodium acetate, 2g 
triammonium citrate, 0.2g. magnesium sulphate MgSO4.7H2O and 
.05g manganese sulphate MnSO4.4H2O dissolved in one liter distilled 
water. The sample was spread all over the plates using sterile bent 
glass rod. The plates were incubated in anaerobic jars and gas-
generating kits (H2 + CO2) for anaerobic growth at 37°C for four days. The results were reported as CFU per gram of sample.

3.3.6 **Acetic Acid Bacteria:**

From suitable serial dilutions of sample 0.1 ml. was aseptically transferred onto solidified ethanol medium (Frateure, 1950), containing (g/liter of distilled water): yeast extract, 10.0 gm., CaCO3, 20.0 gm., ethanol, 20.0 ml., agar, 20.0 gm., and pH, 6.7. The medium were sterilized without ethanol by using an autoclave at 121°C and 15 lb/in2 for 15 minutes and then ethanol were added before pouring the medium into Petri dishes. The plate was incubated aerobically at 30°C for 48 hours. The results were reported as CFU per gram of sample.

3.3.7 **Yeast:**

From suitable serial dilutions of sample 0.1 ml was aseptically transferred onto solidified potato dextrose agar medium (PDA) which consisted of 200g potato extract, 20g dextrose and 20g agar. It was prepared according to the manufacture's instructions by using 23.5g in one liter of distilled water. The sample was spread all over the plates using sterile bent glass rod and then incubated at 28°C for 48 hours.

3.3.8 **Coliform Bacteria:**

Coliform bacteria were counted according to Harrigan and MacCance (1976). The method consists of the following steps:

3.3.8.1 **Presumptive coliform test:**

Presumptive test for coliform bacteria was carried out by the 3-tubes method using Mac-Conkey broth (purple) (Oxoid) (composed of peptone, lactose, bile salt, sodium chloride and bromocresol purple), with Durham tubes. All tubes were inoculated with a 1.0 ml
of the appropriate serial dilutions and incubated at 37°C for 48 hours for the colour change and observation of only gas production.

3.3.8.2 Confirmed coliform test:

All tubes showing gas formation in the presumptive test in 24 hours, were subjected to the confirmation test by transferring a loopful to tubes containing Brilliant Green Lactose Bile broth (B.G.L.B) (Oxoid) (composed of peptone, lactose, purified bile and brilliant green) fermentation tubes. All tubes were incubated at 37°C for 48 hours for observation of gas production. The results were reported as most probable number (MPN) by using MPN statistical tables.

3.4 Identification of microbial isolates:

3.4.1 Tentative Identification of bacteria:

Pure dominant colonies from plates of viable counts, MRS medium and ethanol medium were subcultured and purified on suitable medium for each bacterium. Characterization was carried out and species were identified according to Noel and Kreig (1984) and Seeley and Van–Demark (1972). The purified colonies were subjected to the following tests:

3.4.1.1 Gram staining:

A bacterial smear of culture was prepared as follows: a loopful from a single colony was picked by sterile loop, emulsified in a drop of sterile distilled water placed on a clean washed slide and spread evenly to make a thin smear. The slide was allowed to dry. The smear was fixed by passing the slide through the Bunsen flame. The smear was stained with crystal violet solution (10g. crystal violet, 100 ml absolute alcohol, 900 ml distilled water) for one minute and rinsed
rapidly with tap water. Gram's iodine solution (1.0g. iodine, 2.0g. potassium iodide and 300 ml distilled water) was added and left for one minute, then poured off, the slide left to dry, washed with 95% ethanol, rinsed with tap water and stained with safranin solution (0.5 safranin in 100 ml distilled water) for 30 seconds, then washed well and air dried. The smear was then examined microscopically by oil immersion lens (Harrigan and Mac-Cance, 1978).

3.4.1.2 Motility test:

Hanging drop technique was used to determine the motility of bacteria as described by Harrigan (1998). A drop of culture was suspended from a cover slip over the depression in a cavity slide. The slide was sealed to the cover slip and then it was microscopically examined for motility.

3.4.1.3 Catalase test:

One ml of 3% hydrogen peroxide solution was placed in a small clean test tube. Then 1 ml of a broth culture was added. Effervescence, caused by the liberation of free oxygen as gas bubbles, indicated the presence of catalase enzyme.

3.4.1.4 Oxidase test:

A few drops of oxidaze test solution (Tetra-methyl-p-phenylene diamine hydro-chloride solution) were added to a piece of filter paper. Then a loop full of a 24 hours culture was streaked onto the filter paper. Positive result was recorded as a purple colour production after 5 to 15 second (Harrigan, 1998).
3.4.1.5 Staining of bacterial spores:

A fixed thin smear was made as described in Gram stain. The smear was flooded with a saturated aqueous solution of malachite green and placed over a water bath to steam for 5 minutes. The slide was kept flooded with malachite green by adding the solution to the slide, and then the slide was washed gently with cold water. The smear was counter stained with a 0.25% aqueous solution of safranin for 15 seconds and finally washed with water and blotted dry. Vegetative cells stained red and spores stained green under the microscope (Harrigan and Mac-Cance, 1978).

3.4.1.6 Oxidation fermentation (O/F) test:

A fresh culture of the isolate was tested for O/F test by stab inoculation onto pairs of test tubes of freshly steamed Hugh and Leifson medium (peptone, sodium chloride, dipotassium hydrogen phosphate, bromothymol blue and agar in a liter of distilled water. The pH was adjusted to 7.1. The medium was sterilized in an autoclave at 121°C for 15 minutes. The glucose of 10% concentration, prepared separately from the basal medium and sterilized by autoclaving at 110°C for 10 minutes, was added aseptically to the basal medium to give a concentration of 1%. One of the tubes was covered with sterile paraffin. The tubes were then incubated at 37°C for 7 days. Growth in both tubes was recorded as fermentative metabolism and growth in open tube only was recorded as oxidative metabolism (Harrigan, 1998).
3.4.2 Acetic acid bacteria:

Seven isolates of bacteria from the ethanol medium were purified by streaking on Petri dish surface contain the same selective medium. They were then identified according to Noel and Krieg (1984) and Harrigan and Mac-Cance (1976).

3.4.2.1 Growth at pH 4.5:

Pure cultures selected from ethanol medium were grown in the same medium at 4.5 pH and incubated at 30°C for 48 hours.

3.4.2.2 Oxidation of ethanol to carbon dioxide:

Pure cultures selected from ethanol medium were grown in bromocresol green ethanol yeast extract agar slopes (the ethanol was added to the medium before setting in the sloped position) and incubated at 30°C. *Gluconobacter* and *Acetobacter* produce acid from ethanol, effecting a colour change from bluish-green to yellow, however, only *Acetobacter* continues the oxidation to give a reversion in colour back to bluish green. Cultures were examined daily to check for pH reversion for a week.

3.4.2.3 Oxidation of lactate to carbon dioxide:

Pure cultures selected from ethanol medium were streaked on calcium lactate- yeast extract agar plates and incubated at 30°C. Spot-inoculated *Acetobacter* produces precipitate of calcium carbonate in the medium with white halo in the medium.

3.4.3 Lactic acid bacteria:

Colonies on MRS medium were sub cultured again on MRS medium. The species were identified using API 50 CH, a standardized system, associating 50 biochemical tests for the study of the
carbohydrate metabolism of microorganisms. API 50 CH is used in conjunction with API 50 CHL Medium for identification of *Lactobacillus* and related genera. The results were recorded on the result sheet. Biochemical profile obtained for the strain can be identified using the API web TM identification software with database.

### 3.4.4 Identification of Yeast:

Yeast colonies on PDA medium were purified on the same medium and identified according to the methods described by Barnett, *et al.* (1983), Lodder J. (1970) and Barnett *et al.* (1990) as follows.

#### 3.4.4.1 Microscopic appearance of non-filamentous vegetative cells:

Yeast from young growing cultures was inoculated into 30 ml. sterile liquid culture medium (20gm glucose + 5 gm yeast extract + 10 gm peptone + 1000 ml. distilled water (Barnett *et al.*, 1983)) and the culture was examined microscopically after incubation at 28°C for three days. The shape of the yeast cells and the form of budding were observed and registered.

#### 3.4.4.2 Microscopic observation of filamentous growth:

A sterilized Petri dish containing a U-shaped glass rod supporting two clean microscopic slides with clean cover slips, was used. A piece of wetted filter paper was put in the dish to avoid drying. The slides were dipped aseptically into melted sterile potato-glucose agar (230 ml of sterile potato extract + 20 gm glucose + 770 ml distilled water. pH adjusted to 5.6 (Barnett *et al.*, 1983)) and then replaced on the glass rod support. Yeasts from actively growing cultures were inoculated with a straight wire along the length of the
slides. The slides were covered with cover slips. Growing cultures were examined microscopically for filamentous growth after incubation at 20°C for 3 days.

3.4.4.3 Microscopic examination of ascospores:
Yeast from actively growing cultures were inoculated in Adam's agar (0.4gm glucose + 2.3gm sodium acetate + 20gm agar + 1000 ml distilled water (Kiss, 1984)) as a slope medium and were examined microscopically for ascospore after being incubated at 28°C for 1-4 weeks.

3.4.4.4 Utilization of carbohydrates anaerobically:
Small test tubes containing 3 ml sterile 0.5% peptone water with 4% test sugar and bromocresol purple as indicator were used. The tubes were inoculated with the selected yeast cultures. A vaseline-paraffin layer 2 cm. deep was added on the top surface of the medium. The culture was incubated at 28°C for 4-5 days. Fermentation was detected by the yellow coloration of the medium and by lifting of the Vaseline-paraffin layer (Kiss, 1984).

3.4.4.5 Utilization of carbohydrates aerobically:
Molten nitrogen base agar medium (5gm ammonium sulphate + 1gm potassium hydrogen sulphate + 5gm magnesium sulphate + 1000 ml distilled water (Kiss, 1984)) at 45°C was inoculated with yeast suspension and then it was poured into Petri sterile dishes and was allowed to solidify. Five ml of each test sugar (4%) were placed on the agar surface with a sterile pipette. The plates were incubated at 28°C and were examined for growth every two days for about a week.
3.4.4.6 **Utilization of nitrogen compounds for aerobic growth:**

The methods for assessing growth were similar to those described in utilization of carbohydrates aerobically but using agar medium containing no nitrogen source (20gm glucose + 1gm potassium dihydrogen phosphate + 5gm magnesium sulphate + 20gm washed agar + 1000 ml distilled water (Kiss, 1984)).

3.4.4.7 **Formation of extra-cellular starch-like compounds:**

Selected yeast isolates were grown in yeast extract medium (20gm glucose + 5gm yeast extract + 10gm peptone + 100 ml distilled water). Fifty mls of the medium were dispensed onto cotton-plugged steril flasks and after inoculating, the flasks were incubated at 28ºC for 3 days. Few drops of a dilute Lugol's iodine solution (10g. iodine, 20g. potassium iodide and 1000 ml distilled water) were added. a blue or green colour that develops indicates that the test was positive (Van der Walt, 1984).

3.4.4.8 **Starch hydrolysis:**

Selected yeast isolates were streaked on to starch-agar (10 gm peptone + 10gm yeast extract + 3gm soluble starch + 5gm dipotassium hydrogen phosphate + 1.5 gm. Agar + 1000 ml distilled water) plates. The plates were inoculated at the centers and were then incubated at 37ºC. After 3-7 days of incubation the plates were flooded with Gram's iodine. A clear zone around the area in which the microorganisms grew indicated hydrolysis of starch.
3.5 Damirga Fermentation by using pure culture starter:

Ten grams of decorticated millet grains immersed in 20 ml tap water were pasteurized by boiling in a water bath at 60ºC for 30 minutes, then cooled on ice water immediately, were inoculated with a loopful of each selected isolates: *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Acetobacter sp.* and *Kluyveromyces sp.* The components of this mixed culture were originally isolated from experiments done in the laboratory, by fermenting Damirga traditionally.

Then incubated at room temperature for overnight to make seed culture. This seed culture was transferred aseptically to large conical flask containing pasteurized same grains medium (in a ratio of 1:10 seed culture to medium) and allowed to ferment for 72 hours.
CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Results of questionnaire about Damirga home process:

The questionnaire results in Plate 4.1 show that the age group 35-50 years made 14% of the respondents, 51-65 years made 52% and 66-80 years made 34%. The tribes of the respondents were Gwama, Miri, Nuba, Fur, Zaghawa, Dajo, Berti, Tagali, Hawara, Barno, Falata, Dar-Hamid, Hawsa, Mesiria, Rezigat and Hawazma. They belong to the towns of Um-Rowaba, El-Rahad, El-Obied, Kadogli, El-Dalanj, El-Nuhud, Abasia-Tagali, Abu-Gebiha, El-Fasheir, Nyala, El-Genina, Kutum and Riheid El-Birdi. Those from Darfur State made 50% of the respondents, and those from Kordofan State made 44% of the respondents and the remaining other states made only 6% of the respondents.

Respondents using Damirga as staple food made 42% of the total and those who used it from time to time made 58%. Plate 4.1 also shows that 38% of the respondents used Damirga for its sour taste, and those who used Damirga for its lighter whitish colour made 34% of the respondents and 28% used it for its long shelf life.

Plate 4.1 show that the main cereal used to make Damirga is the pearl millet (86%). Only 14% of Damirga is made from sorghum.
Reasons for using Damirga

- Whiteness: 34%
- Soursness: 38%
- Storage: 28%

Age of respondents

- 35-50: 14%
- 51-65: 52%
- 66-80: 34%

States using Damirga

- Darfur State: 50%
- Kordofan State: 44%
- Other states: 6%

Cereals used for making Damirga

- Pearl millet: 86%
- Sorghum: 14%

Consumption of Damirga

- Staple food: 42%
- From time to time: 58%
4.2 Ingredients Analysis of Damirga:

4.2.1 The proximate Chemical composition and starch of market sample of Damirga:

The proximate chemical composition of ten market samples of Damirga flour is shown in Table 4.1 The results obtained are expressed on dry weight. Moisture content ranged from 4.47 to 6.18 %; the overall mean was found to be 5.31%. Ash content ranged between 0.70 and 1.62 %; the mean value was 1.15%. Crude fiber content ranged between 0.32 and 0.55%; the mean value was 0.40 %. Crude protein content ranged from 11.7 to 15.75%; the mean value being 13.24%. Oil content ranged from 5.09 to 7.88%; the mean value of oil content was 5.8%. Starch content ranged from 48.89 to 54.23%; the mean value of starch content was 51.31%.

Hassan (2000) found ash content to be 1.9 %, crude fiber 0.9 %, crude protein 15.6 %, oil content 3.2 % and starch content 59.1 % for Khemiss tweira (a fermented food from millet).

4.2.2. Biochemical composition of market samples of Damirga:

The biochemical composition of ten market samples of Damirga flour is shown in Table 4.2 the results are expressed on dry weight.

The pH ranged from 4.22 to 4.72 the mean value of pH was 4.46. Lactic acid ranged from 0.10 to 0.18%, the mean being 0.13%. Acetic acid ranged from 0.0012 to 0.0021%, the mean value was 0.0017%. Alcohol ranged from zero to 0.11%, the mean value being 0.031%; seven out of the 10 samples contained no ethanol.
Table 4.1: Proximate composition and starch content (%) of market samples of Damirga

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Moisture</th>
<th>Ash</th>
<th>Crude Fiber</th>
<th>Crude Protein</th>
<th>Oil</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.55</td>
<td>1.22</td>
<td>0.55</td>
<td>12.82</td>
<td>7.88</td>
<td>51.31</td>
</tr>
<tr>
<td>2</td>
<td>5.80</td>
<td>1.22</td>
<td>0.43</td>
<td>13.15</td>
<td>5.22</td>
<td>48.89</td>
</tr>
<tr>
<td>3</td>
<td>5.52</td>
<td>1.08</td>
<td>0.51</td>
<td>12.95</td>
<td>6.62</td>
<td>49.75</td>
</tr>
<tr>
<td>4</td>
<td>4.47</td>
<td>1.42</td>
<td>0.32</td>
<td>13.97</td>
<td>5.88</td>
<td>51.53</td>
</tr>
<tr>
<td>5</td>
<td>5.42</td>
<td>1.62</td>
<td>0.34</td>
<td>14.58</td>
<td>5.64</td>
<td>50.62</td>
</tr>
<tr>
<td>6</td>
<td>6.18</td>
<td>1.10</td>
<td>0.50</td>
<td>11.70</td>
<td>5.56</td>
<td>54.10</td>
</tr>
<tr>
<td>7</td>
<td>4.85</td>
<td>0.70</td>
<td>0.37</td>
<td>12.25</td>
<td>5.65</td>
<td>50.00</td>
</tr>
<tr>
<td>8</td>
<td>5.72</td>
<td>1.27</td>
<td>0.45</td>
<td>12.80</td>
<td>5.26</td>
<td>49.22</td>
</tr>
<tr>
<td>9</td>
<td>4.65</td>
<td>0.83</td>
<td>0.52</td>
<td>15.75</td>
<td>5.24</td>
<td>53.40</td>
</tr>
<tr>
<td>10</td>
<td>4.98</td>
<td>1.08</td>
<td>0.44</td>
<td>12.43</td>
<td>5.09</td>
<td>54.23</td>
</tr>
<tr>
<td>Mean</td>
<td>5.31</td>
<td>1.15</td>
<td>0.40</td>
<td>13.24</td>
<td>5.8</td>
<td>51.31</td>
</tr>
</tbody>
</table>
4.2.3 Microbiological status of market samples of Damirga:

As shown in Table 4.3, the viable count of the ten Damirga samples ranged from $1.8 \times 10^5$ to $3.6 \times 10^7$ CFU/g; lactic acid bacterial count ranged from $2.5 \times 10^3$ to $2.4 \times 10^6$ CFU/g; acetic acid bacterial count ranged from $1.8 \times 10^2$ to $3.2 \times 10^4$ CFU/g; yeast was detected in few numbers ($\leq 300$) per gram and most isolates were recovered from ethanol medium and MRS medium not from PDA medium. Coliform bacteria could not be detected in Damirga samples purchased from different markets. This result is similar to that reported by Ahmed et al. (2006) who found that Damirga made from each of two varieties of pear millet, Wad Ashana and Sudan II, had a microbial load of *Lactobacillus spp.* of $1.9 \times 10^6$ and $4.2 \times 10^5$ CFU/g, yeast and moulds $1.1 \times 10^4$ CFU/g and $8.5 \times 10^4$ CFU/g and total viable count of $4.9 \times 10^4$ and $8.5 \times 10^4$, respectively.

4.3 Changes of microbial counts during traditional Damirga fermentation:

Total viable count, as shown in Table 4.4 and Figure 4.1, started with $1.3 \times 10^2$ CFU/ml then increased with time till it reached $2.7 \times 10^6$ CFU/ml at 18 hrs of fermentation. Hassan (2000) found that initial total viable count to be $3.4 \times 10^3$ CFU/ml then increased to $1.7 \times 10^8$ CFU/ml after 24 hr fermentation of khemiss tweira and then decreased to $1.1 \times 10^5$ CFU/ml at the end of the fermentation process.

Lactic acid bacteria count as shown in Table 4.4, were about $1.7 \times 10^3$ CFU/ml at the beginning of fermentation then increased with the advancement of the fermentation process till it reached $2.6 \times 10^6$ CFU/ml at 18 hrs to 24 hrs of fermentation. This increase of lactic acid bacteria count finds support in the work of Hassan (2000)
Table 4.2: Biochemical composition % of market samples of Damirga

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Acetic acid</th>
<th>Lactic acid</th>
<th>pH</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>0.0017</td>
<td>0.18</td>
<td>4.35</td>
<td>1</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0014</td>
<td>0.10</td>
<td>4.38</td>
<td>2</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0020</td>
<td>0.11</td>
<td>4.39</td>
<td>3</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0017</td>
<td>0.13</td>
<td>4.71</td>
<td>4</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0015</td>
<td>0.10</td>
<td>4.70</td>
<td>5</td>
</tr>
<tr>
<td>0.11</td>
<td>0.0018</td>
<td>0.10</td>
<td>4.72</td>
<td>6</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0012</td>
<td>0.17</td>
<td>4.39</td>
<td>7</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0019</td>
<td>0.13</td>
<td>4.41</td>
<td>8</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0012</td>
<td>0.12</td>
<td>4.33</td>
<td>9</td>
</tr>
<tr>
<td>0.10</td>
<td>0.0021</td>
<td>0.14</td>
<td>4.22</td>
<td>10</td>
</tr>
<tr>
<td>0.031</td>
<td>0.0017</td>
<td>0.13</td>
<td>4.46</td>
<td>Mean</td>
</tr>
</tbody>
</table>
Table 4.3 Microbiological status of market samples of Damirga

<table>
<thead>
<tr>
<th>Source</th>
<th>Sample No</th>
<th>Yeast and mould Count (CFU/gm)</th>
<th>Acetic acid bacteria count (CFU/gm)</th>
<th>Lactic acid bacteria count (CFU/gm)</th>
<th>Total viable count (CFU/gm)</th>
<th>Coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahri1</td>
<td>1</td>
<td>1.1 × 10</td>
<td>3.5 × 10^2</td>
<td>4.5 × 10^4</td>
<td>2.5 × 10^6</td>
<td>-</td>
</tr>
<tr>
<td>Bahri2</td>
<td>2</td>
<td>-</td>
<td>2.5 × 10^6</td>
<td>1.9 × 10^6</td>
<td>1.5 × 10^6</td>
<td>-</td>
</tr>
<tr>
<td>Bahri3</td>
<td>3</td>
<td>-</td>
<td>1.8 × 10^2</td>
<td>4.2 × 10^5</td>
<td>1.8 × 10^5</td>
<td>-</td>
</tr>
<tr>
<td>Omdurman1</td>
<td>4</td>
<td>8.5 × 10</td>
<td>7.0 × 10^2</td>
<td>2.4 × 10^6</td>
<td>3.6 × 10^7</td>
<td>-</td>
</tr>
<tr>
<td>Omdurman2</td>
<td>5</td>
<td>-</td>
<td>1.5 × 10^4</td>
<td>6.1 × 10^3</td>
<td>2.5 × 10^6</td>
<td>-</td>
</tr>
<tr>
<td>Omdurman3</td>
<td>6</td>
<td>-</td>
<td>6.8 × 10^2</td>
<td>5.2 × 10^5</td>
<td>1.9 × 10^5</td>
<td>-</td>
</tr>
<tr>
<td>Omdurman4</td>
<td>7</td>
<td>-</td>
<td>4.3 × 10^3</td>
<td>3.4 × 10^3</td>
<td>6.1 × 10^6</td>
<td>-</td>
</tr>
<tr>
<td>Khartoum1</td>
<td>8</td>
<td>-</td>
<td>5.2 × 10^3</td>
<td>2.5 × 10^3</td>
<td>1.2 × 10^6</td>
<td>-</td>
</tr>
<tr>
<td>Khartoum2</td>
<td>9</td>
<td>3.0 × 10^2</td>
<td>3.2 × 10^4</td>
<td>2.8 × 10^4</td>
<td>3.1 × 10^5</td>
<td>-</td>
</tr>
<tr>
<td>Khartoum3</td>
<td>10</td>
<td>-</td>
<td>8.1 × 10^2</td>
<td>4.2 × 10^5</td>
<td>2.9 × 10^6</td>
<td>-</td>
</tr>
</tbody>
</table>

( - ) = Not detected
Table 4.4: Changes in microbial count during traditional fermentation of Damirga

<table>
<thead>
<tr>
<th>Time of fermentation</th>
<th>Coliforms MPN/ml</th>
<th>Yeast CFU/ml</th>
<th>Acetic acid bacteria CFU/ml</th>
<th>Lactic acid bacteria CFU/ml</th>
<th>Total viable count CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hours</td>
<td>23</td>
<td>-</td>
<td>4.5 \times 10^2</td>
<td>1.7 \times 10^3</td>
<td>1.3 \times 10^2</td>
</tr>
<tr>
<td>6 hours</td>
<td>1100</td>
<td>-</td>
<td>2.9 \times 10^3</td>
<td>2.9 \times 10^5</td>
<td>2.5 \times 10^4</td>
</tr>
<tr>
<td>12 hours</td>
<td>1100</td>
<td>-</td>
<td>1.8 \times 10^4</td>
<td>3.1 \times 10^5</td>
<td>1.6 \times 10^5</td>
</tr>
<tr>
<td>18 hours</td>
<td>1100</td>
<td>1.2 \times 10^3</td>
<td>1.5 \times 10^4</td>
<td>2.6 \times 10^6</td>
<td>2.7 \times 10^6</td>
</tr>
<tr>
<td>24 hours</td>
<td>1100</td>
<td>1.4 \times 10^2</td>
<td>4.6 \times 10^4</td>
<td>2.6 \times 10^6</td>
<td>1.4 \times 10^6</td>
</tr>
<tr>
<td>36 hours</td>
<td>460</td>
<td>2.7 \times 10^4</td>
<td>3.1 \times 10^4</td>
<td>1.7 \times 10^6</td>
<td>1.8 \times 10^6</td>
</tr>
<tr>
<td>48 hours</td>
<td>150</td>
<td>2.6 \times 10^5</td>
<td>2.8 \times 10^4</td>
<td>2.8 \times 10^5</td>
<td>1.3 \times 10^5</td>
</tr>
<tr>
<td>60 hours</td>
<td>23</td>
<td>1.9 \times 10^4</td>
<td>2.8 \times 10^4</td>
<td>1.4 \times 10^5</td>
<td>1.3 \times 10^5</td>
</tr>
<tr>
<td>72 hours</td>
<td>23</td>
<td>1.3 \times 10^3</td>
<td>2.4 \times 10^4</td>
<td>2.9 \times 10^5</td>
<td>1.1 \times 10^5</td>
</tr>
</tbody>
</table>

(-) = Not detected
Fig. 4.1: Change in microbial count during traditional fermentation of Damirga (Data from Table 4.4)
who found that lactic acid bacteria count increased from $1.1 \times 10^2$ CFU/g to $2.2 \times 10^7$ CFU/ml during fermentation of millet grains for Khemiss tweira. In this work the Lactic acid bacteria best count with advancement of the fermentation process, thus reaching $2.9 \times 10^5$ CFU/ml after 72 hrs of fermentation process.

Acetic acid bacteria count at the beginning of fermentation started with $4.5 \times 10^2$ CFU/ml and increased to $4.6 \times 10^4$ CFU/ml at 24 hrs and dropped slightly to $2.4 \times 10^4$ CFU/ml at the end of fermentation process. This result is similar to that reported by Hassan (2000) who found acetic acid bacteria count increased from $5.4 \times 10^2$ to $8.5 \times 10^4$ CFU/ml during first stage of khemiss tweira fermentation process.

Yeast growth on PDA medium appeared at 18 hrs of fermentation when soak water pH was 4.13. The count was found to be $1.2 \times 10$ CFU/ml at this point but increased to a maximum of $2.6 \times 10^5$ CFU/ml at 48 hrs then decreased to $1.3 \times 10^3$ CFU/ml at the end of fermentation process.

4.4 Biochemical composition of Damirga during traditional fermentation:

4.4.1 pH changes during Damirga traditional fermentation:

pH outside grains changed, as shown in Table 4.5 and Figure 4.2, from 6.58 at zero time to 4.88 at 6 hrs and the percentage of change was 25.8%, thereafter the change downward was gradual. Inside the grains, the change was from 6.88 at zero time to only 6.5 even at 18 hrs when it dropped suddenly to 4.07 at 24 hrs of fermentation with percentage of change was of 40.5%.
The marked decline in pH outside the grain in the first 6 hours is in agreement with the result obtained by Monawer (1983) who reported a drop in pH from 5.0 at zero time to 4.1 after 6hrs, 3.7 after 12hrs and to 3.3 after 24 hrs of fermentation of pearl millet. Abdalla (1996) reported that a sharp drop in pH occurred at the beginning of fermentation of millet after 4hr. Ahmed (1999) reported that pH was lowered from 6.6 at zero time to 3.8 at 14 hr and from 6.7 at zero time to 3.9 at 14 hr. fermentation of millet. Reduction of pH during fermentation is due to the organic acids produced.

The small differences in pH dropping time from the beginning of fermentation process observed above might be due to the diversity of microorganisms involved in natural fermentation or due to differences in nature of starter. Monawer (1983) reported a significant increase in viable lactic acid bacteria count during fermentation of pearl millet flour at 37ºC in 6hrs. Au and Fields (1981) reported that the noticeable drop in pH is important in preventing the growth of food poisoning bacteria.
Table 4.5: Changes in pH during traditional Damirga fermentation

<table>
<thead>
<tr>
<th>Time of fermentation (hours)</th>
<th>(%) change from original pH</th>
<th>pH inside grains (%) change</th>
<th>pH outside grain (%) change</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>6.88</td>
<td>6.58</td>
</tr>
<tr>
<td>6</td>
<td>1.2</td>
<td>6.80</td>
<td>4.88</td>
</tr>
<tr>
<td>12</td>
<td>3.8</td>
<td>6.62</td>
<td>4.62</td>
</tr>
<tr>
<td>18</td>
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<td>24</td>
<td>40.8</td>
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</tr>
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<td>48</td>
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<td>60</td>
<td>44.0</td>
<td>3.85</td>
<td>3.92</td>
</tr>
<tr>
<td>72</td>
<td>44.7</td>
<td>3.80</td>
<td>3.90</td>
</tr>
</tbody>
</table>

Fig. 4.2: Change in pH during traditional Damirga fermentation (Data from Table 4.5)
4.4.2 Lactic acid, acetic acid and ethanol during traditional Damirga fermentation:

The results in Table 4.6 and Figure 4.3 show that the concentration of lactic acid increased from 0.009 to 0.156% at the end of fermentation. Acetic acid concentration also increased from 0.006 to 0.0132%.

The results also show the presence of ethanol was concomitant at 18 and 36 hrs with the appearance of yeast, then dropping to zero. The results obtained by Hasssan (2000) showed that the concentration of lactic acid increased from 0.4 to 2.4%. Acetic acid increased from 0.04 to 0.3% and ethanol started to increase after 6 hours of fermentation till it reached 1.1% in the first stage of the fermentation process of khemiss tweira.
Table 4.6: Lactic acid, acetic acid, ethanol (%) during Damirga traditional fermentation

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Duration of fermentation (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0060</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0108</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0117</td>
</tr>
<tr>
<td>0.10</td>
<td>0.0126</td>
</tr>
<tr>
<td>0.10</td>
<td>0.0126</td>
</tr>
<tr>
<td>0.10</td>
<td>0.0140</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0104</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0139</td>
</tr>
<tr>
<td>0.00</td>
<td>0.0132</td>
</tr>
</tbody>
</table>

Fig. 4.3: Changes in the level of lactic acid, acetic acid and ethanol during traditional Damirga fermentation (Data from Table 4.6)
Tanasupawat and Komagata (1995) reported that microorganisms have a clear role in converting most available carbohydrates to lactic acid, with a small amount of acetic acid in fermented foods.

The production of lactic acids was high compared to the acetic acid and ethanol.

After 48 hours of fermentation there was no ethanol; this may be due to the consumption of ethanol by special kinds of microorganisms.

4.4.3 Change in turbidity of Damirga soak water during traditional fermentation

Turbidity results are shown in Table 4.7, and Figure 4.4. There was noticeable change in turbidity which increased from 0.169 O.D. at zero time to 0.254 O.D at 6hr. fermentation. This change continued with time of fermentation till it reached 1.970 at 72hr fermentation. Therefore the duration of fermentation affected turbidity and colour of soak water of the pearl millet grains. The colour changed from yellowish green to grey. Reichert (1979) reported that millet changes color reversibly from grey to yellow green at alkaline pH and reversibly from grey to creamy white under acidic conditions due to the presence of glucosylitexin, glucosylorentin, and vitexin. The increase in absorbance with time of fermentation. This increase is likely to be due to increasing microbial population with the advancement of fermentation period and dissolution of millet components from the grains.
Table 4.7: Change in turbidity of Damirga soak water during traditional fermentation

<table>
<thead>
<tr>
<th>Duration of fermentation (hours)</th>
<th>pH</th>
<th>Turbidity (optical density units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.58</td>
<td>0.169</td>
</tr>
<tr>
<td>6</td>
<td>4.88</td>
<td>0.254</td>
</tr>
<tr>
<td>12</td>
<td>4.62</td>
<td>0.360</td>
</tr>
<tr>
<td>18</td>
<td>4.13</td>
<td>0.388</td>
</tr>
<tr>
<td>24</td>
<td>4.07</td>
<td>0.496</td>
</tr>
<tr>
<td>36</td>
<td>4.05</td>
<td>0.503</td>
</tr>
<tr>
<td>48</td>
<td>3.97</td>
<td>0.714</td>
</tr>
<tr>
<td>60</td>
<td>3.92</td>
<td>0.900</td>
</tr>
<tr>
<td>72</td>
<td>3.90</td>
<td>1.970</td>
</tr>
</tbody>
</table>

Fig. 4.4: Change in turbidity of soak water during traditional Damirga fermentation (Data from Table 4.7)
4.5 Proximate analysis of Damirga prepared in the laboratory (whole pearl millet grains, decorticated grains and Damirga flour):

Millet grains, decorticated grains and Damirga flour were chemically analyzed and the results are shown in Table 4.8. Whole millet grains contained 9.05% moisture, 2.05% ash, 2.0% crude fiber, 10.28% crude protein, 6.65% oil and 62.66% starch. Ahmed et al (2005) found that the moisture content of two pearl millet varieties, Wad Ashana and Sudan II, were 8.345% and 8.458%, respectively, ash content was 1.633% and 1.433%, crude protein contents was 11.156 and 13.329% and oil content was 5.65% and 5.767%.

Table 4.7 also shows that the decorticated pearl millet grains contained 8.4% moisture, 1.93% ash, 0.45% crude fiber, 8.13% crude protein and 48.36% starch. Ahmed (1999) found that the moisture content of two varieties dehulled millet, Standard and Ugandi, was 9.7% and 8.5% respectively, 12.6% and 7.7% protein content and 71.6% and 73.4% starch.

Decortications’ of pearl millet:

i. Decrease in protein content (Ahmed, 1999).
ii. Decrease in tannin contents (Monawar, 1983).
iii. Decrease in total polyphenols (Ahmed, 1999).
iv. Increase in starch percentage.
v. Increase in the \textit{in vitro} protein digestibility (Dhankher and Chauhan, 1987b).
Table 4.8: Proximate analysis and starch (%) of whole millet grains, decorticated grains and Damirga flour prepared in the laboratory

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Starch</th>
<th>Oil</th>
<th>Crude Protein</th>
<th>Crude Fiber</th>
<th>Ash</th>
<th>Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole grain</td>
<td>62.66</td>
<td>6.65</td>
<td>10.28</td>
<td>2.00</td>
<td>2.05</td>
<td>9.05</td>
</tr>
<tr>
<td>Decorticated grain</td>
<td>48.36</td>
<td>5.8</td>
<td>8.13</td>
<td>0.45</td>
<td>1.93</td>
<td>8.40</td>
</tr>
<tr>
<td>Damirga</td>
<td>50.22</td>
<td>5.5</td>
<td>9.8</td>
<td>0.33</td>
<td>1.2</td>
<td>5.45</td>
</tr>
</tbody>
</table>
4.5.1 Crude protein:

As shown in the Table 4.8 decorticated grains contained 8.13% crude protein. The noticeable difference between crude protein in the whole grains (10.28%) and decorticated grains (8.13%) indicated that traditional dehulling resulted in a reduction of crude protein content by about 20.9%. This reduction in protein can be attributed to the removal of the hull (Bookwalter, et al. 1987). In addition, due to the pearl millet thin pericarp (Monawar, 1983), low extraction rates during traditional dehulling were found to be 60-65% (Badi et al., 1987) and this may eliminate some of the protein-rich aleuronic cells. Towards the end of fermentation protein content increased to 9.8%. The protein gains can be attributed to microbial synthesis of protein from metabolic intermediates during their growth cycle (El Tinay et al., 1979).

This increase in protein content at the end of fermentation observed in Table 4.7 was also reported by Eldaw (1994) who reported gradual increase in protein content of fermented sorghum dough after 6 hrs. of fermentation, which is in agreement with the results obtained in this study.

However, El Hidai (1978) reported reduction in the range of 0.5 to 1% in protein content during the first 6 hrs. of fermentation for three sorghum varieties. Khetarpaul and Chauhan (1989) reported that some strains of bacteria are known to possess deaminase enzymes, which increase protein catabolism by fermenting microorganisms accounting for loss of protein by escaping ammonia. Stannier et al. (1963) reported that the drop in protein content caused by fermentation may be due to the action of some moulds and anaerobic bacteria which degrade some of the amino acids and convert them to ammonia gas.
4.5.2 Starch content:

Table 4.8 shows the starch content of whole grain was 62.66%. Fermentation for 72 hrs was found to cause a high decrease in starch content. The decrease was from 62.66% to 50.22%. At the end of fermentation the starch content was lowered by 19.85%. El daw (1994) reported that 14 hours fermentation of two sorghum cultivars minimized the starch content by 10.8 and 9.5%. Starch content after 2, 4 and 6 hours fermentation was decreased slightly. Starch content during first stage of fermentation may be due to action of $\alpha$ – and $\beta$ – amylases produced by microorganisms (El Tinay et al. 1979), or the enzymes may be indigenous to the flour (Pederson, 1971). Also the low pH (4.2) is optimum for yeast growth which breaks down sugars to ethanol and carbon dioxide. Thus a considerable amount of starch is hydrolysed at the beginning of fermentation process. Similarly; El Tinay et al. (1979) found that towards the end of fermentation process decrease in starch content of sorghum dough was very little or nil due to pH drop, which inhibits the activity of $\alpha$ – and $\beta$ – amylases.

4.5.3 Antinutritional factors and protein digestibility for whole grains, decorticated grain, and Damirga

Table 4.9 shows that whole grains contain 618.9 mg/100g phytic acid, decorticated grains contain 408.3 mg/100g phytic acid. The noticeable difference in phytic acid content in the whole and decorticated grains shows that the traditional dehulling resulted in reduction in phytic acid by about 34% which falls within the range of 27-53% given by Lorenz (1983) for dehulled proso millet, more over, the range of 29-40% reported by Elhag (2002) for 80% extracted sorghum. And the range of 30-36% reported by Abdalla (1996). Phytic acid level of Damirga flour obtained in this work was found to be 61.83 mg/100g.
Table 4.9: Antinutritional factors and protein digestibility for whole grains, decorticated grain, and Damirga

<table>
<thead>
<tr>
<th>Protein digestibility%</th>
<th>Phytic acid mg/100g</th>
<th>Tannin %</th>
<th>Poly phenols mg/100g</th>
<th>Sample type</th>
</tr>
</thead>
<tbody>
<tr>
<td>71.55</td>
<td>618.9</td>
<td>0.026</td>
<td>302.2</td>
<td>Whole grain</td>
</tr>
<tr>
<td>78.80</td>
<td>408.3</td>
<td>0.009</td>
<td>283</td>
<td>Decorticated grain</td>
</tr>
<tr>
<td>83.00</td>
<td>61.83</td>
<td>0.008</td>
<td>112</td>
<td>Damirga</td>
</tr>
</tbody>
</table>
A large difference is observed between this value and the values recorded for the whole and decorticated grains. The percent reduction in phytic acid content in Damirga flour was found to be 90%. This result was in line with the findings of Marfo et al. (1990) who found that 72 hours of fermentation reduced 80-98% of rice, cassava and cocoyam phytic acid. Similar results were obtained by Mahajan and Chauhan (1987) who reported 91% phytic acid reduction during pearl millet fermentation for 24 hours at 50°C. Khetarpaul and Chauhan (1991a) found that fermentation at 30ºC for 72 hours almost eliminated phytic acid content of pearl millet. However a reduction higher than the value of 19% was reported by Monawar (1983) for 80% extracted millet. It appears that Damirga processing is very effective in reducing phytic acid content of pearl millet. This low level of phytic acid in Damirga flour could be attributed to the cumulative effects of dehulling, soaking and fermentation.

Table 4.9 shows that whole grains contain 302.2 mg/100g polyphenols, decorticated grains contain 283 mg/100g polyphenols. The noticeable difference between polyphenols content in the whole and decorticated grains shows that the traditional dehulling resulted in reduction in polyphenols by about 6.36% and fermentation of Damirga result in reduction of polyphenols by about 62.9%. ElHag et al. (2002) reported a decrease of 59.7% and 41% for phytic acid and 59.95 and 31% for total polyphenols due to fermentation of two pearl millet cultivars, respectively.

Table 4.9 also shows that whole grains contain 0.026% tannin, decorticated grains contain 0.009% tannin and Damirga 0.008%. The difference between tannin content in the whole and decorticated grains
shows that the traditional dehulling resulted in reduction in tannin by about 65.4%. Fermentation of Damirga resulted in reduction of tannin by about 69.2%. This could also be attributed to the degradation of tannins, polyphenols and phytic acid by microbial enzymes. This agrees with results reported by ElHag et al. (2002).

Table 4.9 shows that protein digestibility of whole pearl millet grains was 71.55%; decorticated grains 78.8% and Damirga flour 83% which indicated an improvement in the level digestibility. This agrees with the results reported by Hassan et al. (2006) who reported that protein digestibility was significantly improved when the processed grains were fermented for 12 hrs. Fermentation for 24 hr. also improved the in vitro protein digestibility (IVPD).

4.6 Identification of microorganisms from Damirga Fermentation:

4.6.1 Identification of lactic acid bacteria:
Nine isolates from MRS medium on Damirga fermentation were found to be anaerobic bacteria having rod or coccus cell shape, Gram positive, non-spore-forming, non-motile, catalase-negative and peroxidase-negative representing four species of lactic acid bacteria, Lactobacillus plantarum, Lactobacillus delbrueckii (Homofermentative) Lactobacillus fermentum (heterofermentative) and Pediococcus spp. The results are presented in Table 4.10.

4.6.2 Identification of acetic acid bacteria:
Seven isolates from ethanol medium were found to be Acetobactor spp. and Gluconobactor spp, however, the identification tests were not completed to the species level. The results are presented in Table 4.11.
Table 4.10: Identification of lactic acid bacteria using API

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Active ingredients</th>
<th>Test</th>
<th>Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 8 7 6 5 4 3 2 1</td>
<td>TEMIN</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- V V V - V V V -</td>
<td>GLYcerol</td>
<td>GLY</td>
<td>1</td>
</tr>
<tr>
<td>- - V - V V - -</td>
<td>ERYthnol</td>
<td>ERY</td>
<td>2</td>
</tr>
<tr>
<td>- - V - V V + +</td>
<td>D-ARAbinose</td>
<td>DARA</td>
<td>3</td>
</tr>
<tr>
<td>+ + + + + + + V</td>
<td>L-ARAbinose</td>
<td>LARA</td>
<td>4</td>
</tr>
<tr>
<td>- + + + + + + V</td>
<td>D-RIBose</td>
<td>RIB</td>
<td>5</td>
</tr>
<tr>
<td>- + + + - + V + +</td>
<td>D-XYLose</td>
<td>DXYL</td>
<td>6</td>
</tr>
<tr>
<td>+ + + + + + + +</td>
<td>L-XYLose</td>
<td>LXYL</td>
<td>7</td>
</tr>
<tr>
<td>- - V - - - - -</td>
<td>D-ADOnitol</td>
<td>ADO</td>
<td>8</td>
</tr>
<tr>
<td>+ - V V - - - -</td>
<td>Methyl-D-Xylopyanoside</td>
<td>MDX</td>
<td>9</td>
</tr>
<tr>
<td>- + + + - + + + +</td>
<td>D-GALctose</td>
<td>GAL</td>
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<td>11</td>
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<td>D-FRUCTose</td>
<td>FRU</td>
<td>12</td>
</tr>
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<td>D-MANnose</td>
<td>MAN</td>
<td>13</td>
</tr>
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<td>- - V V - - - -</td>
<td>L-SorBose</td>
<td>SBE</td>
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<tr>
<td>- V + - - - - +</td>
<td>L-RHAmnose</td>
<td>RHA</td>
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<tr>
<td>- - V - - - - -</td>
<td>INOsitol</td>
<td>INO</td>
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<td>D-MANnitol</td>
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<td>- + V - + - - -</td>
<td>D-SORbitol</td>
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<td>MDM</td>
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<tr>
<td>- V V - - + - -</td>
<td>Methyl-D-Glucopyanoside</td>
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<td>+ + + + + + + +</td>
<td>N-AcetylGlucosamine</td>
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<td>+ + + + + + + +</td>
<td>AMYgdalin</td>
<td>AMT</td>
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<td>ARButin</td>
<td>ARB</td>
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<td>- + + + + + + +</td>
<td>ESCulin ferric citrate</td>
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<tr>
<td>+ + + + + + +</td>
<td>D-CEliobose</td>
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<td>26</td>
</tr>
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<td>V + + + V + + + +</td>
<td>D-MALtose</td>
<td>MAL</td>
<td>27</td>
</tr>
<tr>
<td>- + + + + + V - -</td>
<td>D-LACTose (bovine origin)</td>
<td>LAC</td>
<td>28</td>
</tr>
<tr>
<td>- + + + - + V - -</td>
<td>D-MELibose</td>
<td>MEL</td>
<td>29</td>
</tr>
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<td>+ + + + + + + +</td>
<td>D-SACcharose (sucrose)</td>
<td>SAC</td>
<td>30</td>
</tr>
<tr>
<td>V + + + - + + + -</td>
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75
<table>
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<td>D-MeleZitose</td>
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<td>-</td>
<td>D-FUCose</td>
<td>DFUC</td>
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<td>-</td>
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<td>+</td>
<td>V</td>
<td>-</td>
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<td>Potassium GlucoNaTe</td>
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<td>-</td>
<td>Potassium 2-KetoGluconate</td>
<td>2KG</td>
<td>48</td>
</tr>
<tr>
<td>V</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>V</td>
<td>-</td>
<td>Potassium 5-KetoGluconate</td>
<td>5KG</td>
<td>49</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>L. delbrueckii</th>
<th>Pediococcus</th>
<th>L. plantarum</th>
<th>L. delbrueckii</th>
<th>L. plantarum</th>
<th>L. fermentum</th>
<th>L. fermentum</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. fermentum</td>
<td>Pediococcus</td>
<td>L. plantarum</td>
<td>L. delbrueckii</td>
<td>L. plantarum</td>
<td>L. fermentum</td>
<td>L. fermentum</td>
</tr>
</tbody>
</table>
Table 4.11: Identification of acetic acid bacteria

<table>
<thead>
<tr>
<th>Genus</th>
<th>Oxidation of calcium lactate</th>
<th>Oxidation of ethanol</th>
<th>Motility</th>
<th>OF</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Cellshape</th>
<th>Gram stain</th>
<th>Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluconobacter</td>
<td>+</td>
<td>-</td>
<td>Motile</td>
<td>O/F</td>
<td>+</td>
<td>-</td>
<td>Rod</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Acetobacter</td>
<td>+</td>
<td>+</td>
<td>Motile</td>
<td>O/F</td>
<td>+</td>
<td>-</td>
<td>Rod</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Gluconobacter</td>
<td>+</td>
<td>-</td>
<td>Motile</td>
<td>O/F</td>
<td>+</td>
<td>-</td>
<td>Rod</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Gluconobacter</td>
<td>+</td>
<td>-</td>
<td>Motile</td>
<td>O/F</td>
<td>+</td>
<td>-</td>
<td>Rod</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Gluconobacter</td>
<td>+</td>
<td>-</td>
<td>Motile</td>
<td>O/F</td>
<td>+</td>
<td>-</td>
<td>Rod</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Acetobacter</td>
<td>+</td>
<td>+</td>
<td>Motile</td>
<td>O/F</td>
<td>+</td>
<td>-</td>
<td>Rod</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Acetobacter</td>
<td>+</td>
<td>+</td>
<td>Motile</td>
<td>O/F</td>
<td>+</td>
<td>-</td>
<td>Rod</td>
<td>-</td>
<td>7</td>
</tr>
</tbody>
</table>
4.6.3 Identification of general bacteria from TVC plates:

Thirteen isolates from plate count agar (PCA) medium indicated that there were about four kinds of bacteria *Bacillus spp.*, *Streptococcus spp.*, *Staphylococcus spp.* and *Micrococcus spp.*. The results are presented in Table 4.12.

4.6.4 Identification of yeasts:

Morphological description of ten yeast isolates all forming yeast colonies with circular shape, smooth surfaces, entire edge, convex elevation and white colour except one with an orange colour was done. The cells were circular in shape, reproduced by budding and made no mycelium but collection of cells and spores of circular shape 1-2 in number per cell.

The results are shown in Table 4.13 The isolates were *Rhodotorula spp.*, frequently involved in the spoilage of a wide range of foods, *Pichia spp.* which use ethanol as source of carbon (and that explains why ethanol was absent at the end of fermentation as shown in table 4.6 previously) and *Kluyveromyces spp.* Which ferment a wide range of sugars. *Hanseniaspora spp.* was also found in Damirga fermentation.

4.7 Changes of microbial counts during Damirga fermentation using pure culture starter:

Total viable count as shown in Table 4.14 and Figure 4.5, started about $1.3 \times 10^2$ CFU/ml then increased slightly with time till it reached $2.9 \times 10^3$ CFU/ml at 48hrs of fermentation and then decreased towards the end of fermentation.
Table 4.12: Identification of general bacteria from TVC plates

<table>
<thead>
<tr>
<th>Genus</th>
<th>Isolates</th>
<th>Glucose (acid)</th>
<th>O / F test</th>
<th>Oxidase test</th>
<th>Catalase test</th>
<th>Growth in air</th>
<th>Motility</th>
<th>Endo spore forming</th>
<th>Shape of cell</th>
<th>Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus</td>
<td>1</td>
<td>+</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>sphere</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus</td>
<td>2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Rod</td>
<td>Rod</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>3</td>
<td>+</td>
<td>F</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>sphere</td>
<td>Rod</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus</td>
<td>4</td>
<td>+</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Rod</td>
<td>Rod</td>
<td>+</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>5</td>
<td>+</td>
<td>O</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>sphere</td>
<td>Rod</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus</td>
<td>6</td>
<td>+</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Rod</td>
<td>Rod</td>
<td>+</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>7</td>
<td>+</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>sphere</td>
<td>+</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>8</td>
<td>+</td>
<td>O</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>sphere</td>
<td>Rod</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus</td>
<td>9</td>
<td>+</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Rod</td>
<td>Rod</td>
<td>+</td>
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<tr>
<td>Streptococcus</td>
<td>10</td>
<td>+</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>sphere</td>
<td>+</td>
</tr>
<tr>
<td>Micrococcus</td>
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<td>+</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>sphere</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus</td>
<td>12</td>
<td>+</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Rod</td>
<td>Rod</td>
<td>+</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>13</td>
<td>+</td>
<td>O</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>sphere</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 4.13: Identification of Yeast

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Raffinose</th>
<th>Maltose</th>
<th>Lactose</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Fermentation test**

**Growth test**

**Kluyveromyces spp.**

**Pichia spp.**

**Hanseniaspora spp.**

**Rhodotorula**

- Nitrate
- Nitrite
- Starch formation
- Starch hydrolysis
- 33.3% Glucose
- 37.5% Glucose
Table 4.14: Changes in microbial count during fermentation using pure culture starter

<table>
<thead>
<tr>
<th>Time of fermentation</th>
<th>Yeast CFU/ml</th>
<th>Acetic acid bacteria CFU/ml</th>
<th>Lactic acid bacteria CFU/ml</th>
<th>Viable count CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hours</td>
<td>8.9 × 10^3</td>
<td>1.2 × 10^3</td>
<td>3.0 × 10^5</td>
<td>1.3 × 10^2</td>
</tr>
<tr>
<td>6 hours</td>
<td>4.9 × 10^4</td>
<td>1.5 × 10^2</td>
<td>3.5 × 10^5</td>
<td>3.0 × 10^2</td>
</tr>
<tr>
<td>12 hours</td>
<td>6.3 × 10^3</td>
<td>7.7 × 10^2</td>
<td>5.7 × 10^4</td>
<td>9.6 × 10^2</td>
</tr>
<tr>
<td>18 hours</td>
<td>7.2 × 10^2</td>
<td>8.0 × 10^2</td>
<td>7.7 × 10^4</td>
<td>7.5 × 10^3</td>
</tr>
<tr>
<td>24 hours</td>
<td>9.2 × 10^2</td>
<td>2.5 × 10^3</td>
<td>2.8 × 10^5</td>
<td>1.8 × 10^3</td>
</tr>
<tr>
<td>36 hours</td>
<td>7.8 × 10^2</td>
<td>5.8 × 10^3</td>
<td>2.8 × 10^5</td>
<td>2.4 × 10^3</td>
</tr>
<tr>
<td>48 hours</td>
<td>5.6 × 10^2</td>
<td>9.2 × 10^3</td>
<td>2.9 × 10^5</td>
<td>2.9 × 10^3</td>
</tr>
<tr>
<td>60 hours</td>
<td>3.3 × 10</td>
<td>6.2 × 10^3</td>
<td>2.8 × 10^5</td>
<td>2.2 × 10^2</td>
</tr>
<tr>
<td>72 hours</td>
<td>3.0 × 10</td>
<td>3.1 × 10^3</td>
<td>2.9 × 10^5</td>
<td>4.5 × 10</td>
</tr>
</tbody>
</table>
Fig. 4.5: Changes in microbial count during fermentation using pure culture starter (Data from Table 4.14)
Lactic acid bacteria as shown in Table 4.14 were about $3.0 \times 10^5$ CFU/ml at the beginning of fermentation then decreased to $5.7 \times 10^4$ CFU/ml at 12 hrs of fermentation, and increased at 24 hours of fermentation to $2.8 \times 10^5$ CFU/ml. Then the counts in our results almost remained constant till the end of fermentation.

Acetic acid bacteria count at the beginning of fermentation started with $1.2 \times 10^3$ CFU/ml and decreased to $1.5 \times 10^2$ CFU/ml at 6 hrs and then increased to $2.5 \times 10^5$ CFU/ml at 24 hours of fermentation and almost remained constant till the end of fermentation.

Yeast count at the beginning of fermentation started with $8.9 \times 10^3$ CFU/ml and increased to $4.9 \times 10^4$ CFU/ml at 6 hrs of fermentation and then decreased to $6.3 \times 10^3$ CFU/ml at 12 hrs of fermentation then decreased towards the end of fermentation.

4.8 Changes in level of lactic acid, acetic acid and ethanol during Damirga fermentation using pure culture starter:

The results in Table 4.15, Figure 4.6, show that the pH dropped from 6.16 to 4.34 at the first 6 hrs of fermentation with percentage change of 22% and almost remained constant till the end of fermentation. The pH of Damirga fermented traditionally as shown in table 4.5 previously dropped from 6.58 to 4.88 at the first 6 hrs of fermentation with percentage change of 25.8%, and decreased gradually to reach 3.9 at the end of fermentation. In the same table Concentration of lactic acid increased from 0.054% to 0.531% at the end of fermentation. The lactic acid concentration of Damirga fermented traditionally as shown in table 4.6 previously increased from 0.009 to 0.156% at the end of fermentation. He result also show
acetic acid concentration also increased from 0.072 to 0.261%. Acetic acid concentration of Damirga fermented traditionally as shown in table 4.6 increased from 0.006 to 0.0132%. The results also show ethanol presence detected at 6 hrs of fermentation at the time when yeast started to grow, and reached the level of 0.1% and stayed at this level till 18 hrs then dropping to zero. During Damirga fermentation in the laboratory, the detection of ethanol was concomitant accompanying the detection of yeast at 18 hours of fermentation when it reached 0.10% and stayed at this level till 36 hrs then dropping to zero.

The production of lactic acid was high compared to the acetic acid and ethanol.
Table 4.15: Changes in levels of pH lactic acid, acetic acid and ethanol during Damirga fermentation using pure culture starter

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time of fermentation</th>
<th>pH</th>
<th>Ethanol %</th>
<th>Acetic acid %</th>
<th>Lactic %</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0 hours</td>
<td>6.16</td>
<td>0.00</td>
<td>0.072</td>
<td>0.054</td>
</tr>
<tr>
<td>S2</td>
<td>6 hours</td>
<td>4.77</td>
<td>0.10</td>
<td>0.189</td>
<td>0.216</td>
</tr>
<tr>
<td>S3</td>
<td>12 hours</td>
<td>4.53</td>
<td>0.10</td>
<td>0.207</td>
<td>0.216</td>
</tr>
<tr>
<td>S4</td>
<td>18 hours</td>
<td>4.52</td>
<td>0.10</td>
<td>0.216</td>
<td>0.288</td>
</tr>
<tr>
<td>S5</td>
<td>24 hours</td>
<td>4.34</td>
<td>0.10</td>
<td>0.225</td>
<td>0.513</td>
</tr>
<tr>
<td>S6</td>
<td>36 hours</td>
<td>4.36</td>
<td>0.10</td>
<td>0.234</td>
<td>0.468</td>
</tr>
<tr>
<td>S7</td>
<td>48 hours</td>
<td>4.37</td>
<td>0.10</td>
<td>0.243</td>
<td>0.540</td>
</tr>
<tr>
<td>S8</td>
<td>60 hours</td>
<td>4.35</td>
<td>0.10</td>
<td>0.256</td>
<td>0.536</td>
</tr>
<tr>
<td>S9</td>
<td>72 hours</td>
<td>4.34</td>
<td>0.10</td>
<td>0.261</td>
<td>0.531</td>
</tr>
<tr>
<td></td>
<td>Final product</td>
<td>4.5</td>
<td>0.00</td>
<td>0.017</td>
<td>.531</td>
</tr>
</tbody>
</table>

Fig. 4.6 Changes in level of lactic acid, acetic acid ethanol during Damirga fermentation using pure culture starter (Data from Table 4.15)
CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions:

Damirga fermentation is mainly lactic acid fermentation caused mainly by lactic acid bacteria activity. There was a high level of acetic acid bacteria contribution but its contribution is lesser important than that of the lactic acid bacteria.

As a result of this fermentation process the pH dropped from 6.58 to 3.9. Lactic acid and acetic acid increased as fermentation progressed.

Dehulling was found to result in significant changes in protein digestibility and antinutritional factors. It increased in vitro protein digestibility while protein, polyphenols, phytic acid and tannin decreased.

Chemical analysis of fermented Damirga product showed that protein content was increased while starch content was decreased.

Moreover, in the fermentation process polyphenols, phytic acid and tannin decreased as fermentation progressed.

Starter made from Lactobacillus plantarum, L. fermentum, Acetobactor spp. And Kluyveromyces spp. (isolated from Damirga fermentation) decreased the duration time of fermentation and produced a type of Damirga similar to the traditional product.
5.2 Recommendations:

- This study indicated that Damirga prepared from a locally grown inexpensive raw material is nutritious, therefore, we should encourage local consumers', especially low-income individuals, to use Damirga as staple food all over Sudan to defeat the challenges facing food security problems..

- We should upgrade the production of Damirga from household level to industrial level.

- There is a need to develop strong starter cultures and appropriate carrier for Damirga that they can be easily handled and used by local processors without the problem of contamination.
REFERENCES


Appendix (1)

Questionnaire

1. Name: ………………………………………………………………………..

2. Age: …………………………………………………………………………

3. Tribe: ………………………………………………………………………

4. State: ………………………………………………………………………

5. Residence: …………………………………………………………………

   How do you make Damirga?

   …………………………………………………………………………………

6. From what cereal grains you do it?

   …………………………………………………………………………………

7. How do you consume Damirga?

   a. time to time ( )         b. Staple food ( )

8. for what reason you make Damirga?

   a. Whiteness ( )    b. Sourness ( )    c. Storage ( )
Appendix (3)