BIOCHEMICAL AND MICROBIAL CHANGES DURING FERMENTATION OF "GURRASSA MURRA"

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1995

A Thesis Submitted to the University of Khartoum in Partial Fulfillment of the Requirements for the Degree of Master of Science in Agricultural Biotechnology

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October, 2003

Dedication

To my brother Eissa

With love

Namia

ACKNOWLEDGEMENT

First and foremost I thank Allah who gave me health and strength to conduct the present study.

I would like to express my deepest gratitude to my supervisor Prof. Hamid Ahmed Dirar for his critical supervision, keen advice and guidance.

Great thanks are for Dr. Izzeldeen Mohamed Siddig who offered me great support.

Grateful and thanks for Dr. Hatel Hashim and Nagwa Babiker who were helpful to me.

Special thanks are due to the technicians in the Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum, and for Ekhlass Elamin Bashir who typed this thesis.

Also I wish to express my appreciation to all those who have contributed, in many ways, to make my study possible.

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ABSTRACT

The present study was conducted to investigate the microbiological and biochemical aspects of gurrassa murra (a fermented food made from wheat and dates) that was collected from different households and from travellers and laboratory-made samples.

Isolation, identification and enumeration of microorganisms involved in the fermentation of gurrassa murra were also carried out.

The total viable count of household and traveller specimens ranged from 10^5 to10^6 cfu/g.

The more dominant bacterial genera were *Lactobacillus*, *Bacillus*, *Staphylococcus* and *Micrococcus,* in descending order. The total viable yeast count was 10^2-10^4 cfu/g. The more dominant yeast isolates species were *Debaryomyces pseudopolymorphus* and *Debaryomyces hansenii.* The bacterial isolates of the laboratory-adapted samples were *Bacillus sp*. and *Staphylococcus sp.* and the yeasts *were Debaryomyces hansenii*.

The fermentation was carried out by *Lactobacillus* and the yeasts.

Biochemical changes during fermentation of gurrassa murra were characterized by a decrease in pH from 6.4 to 4.2 and there was a notable increase in acidity from 1.3 to 2.9 % and a decrease to 2.1% after addition of the dates. Alcohol increased from 0.4 to 2.1 % and then decreased to 1.2 after addition of dates.

خلاصة الأطروحة

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10^5 - 10^6

Staphylococcus *Bacillus* *Lactobacillus*

Micrococcus . 10^2-10^4 .

Debaryomyces pseudopolymorphus *Debaryomyces hansenii* .

Bacillus *Staphylococcus* *Debaryomyces hansenii*.

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4.2 6.4 pH

%1.2 % 2.1 0.4 % 2.1 %2.9 1.3

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Chapter One

INTRODUCTION

Food fermentation is an old technique and has been known for thousands of years in some parts of the world. Information on it can be gathered from elderly rural women who traditionally hand down such knowledge from generation to generation (Dirar, 1993).

In a fermented food microorganisms grow on the raw materials and by doing so bring about important changes in nutritional values and in other qualities of the food.

Cereals are major staples in many countries and people ferment cereal grains as a food for thousands of years. These fermented products are used for children as weaning foods as well as for adults.

The intensive use of ground grain in the Nile and Sahara regions between 12,000 and 10,000 BC (Dirar, 1997) suggested that man was already accustomed to acid fermented cereal foods 13,000 years ago.

The reason why people in Africa use fermentation to preserve their food is the high costs and infra-structural requirements of many advanced food preservation methods such as refrigeration, freezing, canning and irradiation which are common in developed countries (Cooke *et al*., 1987).

Sudan has over 80 indigenous fermented foods and beverages that are distinctly different from each other. The variations are brought about by the nature of the raw materials from which the foods are made and from the types of microbes that ferment them (Dirar, 1993).

The sweet-sour bread called gurrassa murra, is confined to the northern Sudan because this area is the date-producing region of the country and dates form an essential ingredient of the product.

Gurrassa murra fermentation is a traditional spontaneous process.

The main targets of this research work are:

1. To collect information about gurrassa murra making methods and its consumption.
2. To isolate and identify the microbes from collected samples.
3. To isolate and identify the microbes involved in the fermentation process.
4. To carry out biochemical analyses of collected samples.
5. To examine the biochemical and microbiological changes in gurrassa murra during fermentation.

CHAPTER TWO
LITERATURE REVIEW

2.1 The fermentation

Fermentation is one of the first methods used by Man to produce and preserve foods.

Microbial fermentations by bacteria, yeast and moulds play an important role by modifying the material physically, nutritionally, and organoleptically. Lopez (1992) mentioned that the traditional fermented foods might be divided into two broad categories:

A. Submerged-culture fermentations (SCFs) like kaffir beer and rice wine.
B. Solid–substrate fermentations (SSFs) like tempeh and natto. One of the major characteristics that distinguish SSFs from SCFs is that SSF processes usually occur at low moisture content.

For many indigenous fermentations the microbial interactions are complex and mixed fungal-bacterial, mould-yeast and yeast-bacterial combinations occur (Rogers, 1989). These interactions play an important role in the nutritional, safety and sensory characteristics (Hall, 1989).

2.1.1 Advantages of fermentation

Fermentation provides a way to preserve food products, to enhance nutritive value, destroy undesirable factors in the raw products to make safer products, to improve the appearance and taste of some foods, to salvage materials otherwise not usable for human consumption, and reduce the energy required for cooking (Dirar, 1993).

2.1.2 Lactic acid fermentation technology as a household technology to improve food safety in Africa

In Africa, diarrhea related to poor hygienic conditions has long been recognized as a major health hazard to infants. Many studies have shown that contamination of imported infant weaning formulas constitute potential source of diarrhea disease in African children (Rowland *et al*., 1978; Barrell and Rowland, 1979). Most of these imported weaning foods are milk-based and their preparation is prone to easy contamination as a result of poor sanitation and low knowledge of personal hygiene by many mothers in the developing countries. A possible solution to this problem is the possibility of promoting lactic-fermented weaning foods in Africa.

In 1957, Gordan *et al.* suggested the use of food preparations of *Lactobacillus acidophilus* to offset the side effects of antibiotic for such infants.

Zychowicz *et al*. (1974) fed acidophilus milk to polish children with *Salmonella* and *Shigella* infections.

Odugbemi *et al*., (1991) confirmed that enteropathogenic *Esherichia coli*, *Salmonella typhi* and *Salmonella paratyphi* were incapable of multiplying in "*ogi*", a lactic fermented maize product used for feeding infants in West Africa.

Mensah *et al*. (1988) and Mensah *et al*. (1991), in their investigation of simulated unhygienic conditions of typical rural community in a developing country by inoculation of fermented maize dough porridge with *Shigella flexneri* and enteropathogenic *Escherichia coli* found that fermented maize dough inhibited the growth of these pathogens. They concluded that fermentation of maize dough is a useful strategy for reducing contamination of weaning food in developing countries.

2.2 The effect of fermentation on nutrient composition

During fermentation the microorganisms secrete hydrolytic enzymes into the substrate and assimilate some of the fatty acids, amino acids and the simple sugars. Fukushima (1985) reported that during fermentation lactic acid bacteria accumulate with concomitant increase in acidity and decrease of dry matter.

Due to the crude methods of analysis usually followed in research, the proximate composition of food does not seem to have changed much during fermentation. However there is almost always a high increase in the soluble fraction of a food during fermentation. The proteoltic activity of bacteria in traditional fermentations degrades complex protein into simpler proteins, peptides and amino acids. The bacteria used in *natto* fermentation cause substantial

increase in the level of free amino acids and soluble carbohydrates. On the other hand, *Rhizopus* spp., used in the fermentation of various types of *tempeh,* are highly hydrolytic and outstanding increase in soluble fat, protein, and carbohydrate are observed (Steinkraus, 1983; Lopez *et al*., 1987).

Traditional fermentations dramatically improve the vitamin content of a wide variety of substrates; only *injera* showed decline in vitamin content (Lopez *et al*., l988; Soni and Sandhu, 1989).

Unwanted food components such as phytic acid, trypsin inhibitor, flatus factor and lectins can be destroyed by fermentation process. These components can be hydrolyzed and their levels reduced by microorganisms, as reported by Mital and Garga (1990).

Khetarpaul and Chauhan (1990) reported that fermentation increased the quantity of soluble protein in foods, might improve the amino acid profile and reduced the levels of certain anti-nutritional factors that interfere with digestion.

During lactic fermentation of cassava, Giraud *et al*. (1992) and Giraud *et al*. (1993) confirmed that certain strains of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* had the abilities to produce the linamarase enzyme responsible of breaking down the toxic linamarin.

Nout (1990) reported that fermentation increased energy density in porridges and reduced anti-nutritional factors.

Lactic acid, acetic acid and ethanol are the main flavouring and preserving products, although other metabolic by-products like ethanoic, butanoic and propanoic acids are supposed to account for much of the organoleptic quality of the fermented dough (Dirar, 1993).

Household lactic fermentations of cereals have been found to effectively reduce the amount of phytic acid, polyphenols and tannins and improve protein availability in sorghum (Chauhan *et al*., 1988) and millet (Khetarpaul and Chauhan, 1989).

2.3 Microorganisms involved in fermentation

The microorganisms implicated in fermentation may either be added as pure or mixed cultures or may be present naturally in sufficient numbers in raw materials (Frazier and Westhoff, 1978).

The microorganisms involved in the lactic acid fermentation of food in Africa belong to four genera namely *Lactobacillus, Lactococcus, Leuconsotoc*, and *Pediococcus*. Oyewole (1995) reported the microbial fermentations of different lactic-fermented products follow patterns in which:

1. A mixed flora of microorganisms usually initiates fermentation process
2. The non-lactics are eliminated with increasing acid production in the medium.
3. There is a microbial succession trend among the lactic acid bacteria that survive in the acidic medium.

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

The predominant microflora in kishk, an Egyptian product, consists of lactobacilli, mainly: *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus brevis* and yeast. Coliforms were not detected (El- Malek, 1978). The predominant microorganisms in the dough body at the optimum time of fermentation (i.e. 24 hours or 48 hours and very rarely 72 hours) were *Leucostoc mesenteroid* followed by species of *Lactobacillus, Saccharomyces* and *Pediococcus* and appeared to be of equal numbers; their presence could be attributed to souring of the dough. *E. coli, Staphylococcus* and *Streptococcus* were found at the initial hours of fermentation but later disappeared and were viewed as contaminants (IFS, 1985).

Mbugua (1981) studied uji, a fermented maize product from Kenya, and showed complete inhibition of all coliforms which formed 40% of resident flora. This occurred after 24 hours when the pH of the maize slurry had decreased.

Sour dough bread is leavened by a mixture of various microorganisms naturally present in flour, instead of bread yeast. These microorganisms are wild yeasts, coliform bacteria, saccharolytic *Colstridium* species, and heterofermentative lactic acid bacteria (Frazier and Westhoff 1978). These authors mentioned that the leavening

microorganisms in San Francisco sour dough bread are *Torulopsis holmii* yeast and heterofermentative *Lactobacillus spp*.

Odunfa and Adeyele (1985) examined the microorganisms associated with the different stages of the fermentation of ogi-baba, a fermented sorghum gruel, and they found that the microbial population was mainly *Streptococcus lactis* and *Candida krusei*.

Frazier (1967) mentioned that a pH of about 4.0 to 4.5 favours most fermentation yeasts. Besides, some of the bacteria like the acid-formers are favoured by moderate acidity. In point of fact, most bacteria are favoured by pH near neutrality.

2.3.1 Spoilage of fermented cereal products

Bacteria, yeast and mould can all cause the spoilage of food by growing in it and altering its chemical composition. Foods, which humans eat are also often a lush pasture for microorganisms which consume the chemicals which alter the colour, flavour and texture of the food. These changes may cause the food to lose nutritional value, or to become unpleasant or even positively harmful to the consumer.

Mainly lactic acid bacteria cause the spoilage of fresh refrigerated dough products including biscuits, dinner and sweet rolls, and pizza dough. Hesseltine *et al*. (1979) reported that 92% of isolates were Lactobacillaceae, with more

than one-half belonging to the genus *Lactobacillus*, 36% to genus *Leuconostoc*, and 3% to *Streptococcus.* Molds were found generally in low numbers in spoiled products.

Bread generally lacks sufficient amount of moisture to allow the growth of any microorganism except moulds. One of the most common mould-spoilers is *Rhizopus stolonifer*, often referred to as “the bread mould”. Ropiness type of spoilage is caused by the growth of certain strains of *Bacillus subtilis* (*Bacillus mesentericus*)

Cakes of all types rarely undergo bacterial spoilage due to their unusually high concentrations of sugar which restricts the availability of water. The spoilage of these products is caused by moulds either from ingredients such as sugar, nuts and spices or from handling or air. Continued growth of mould on breads and cakes results in hardening of the products (Jay, 1986).

Ropiness of bread is fairly common in home-baked bread, especially during hot weather, but it is rare in commercially baked bread because of preventive measures now employed.

Ropiness is caused by a mucoid variant of *Bacillus subtilis* or *Bacillus licheniformis* formerly called *Bacillus mesentericus, Bacillus panis* and other species names. The spores of these species can withstand the temperature of the bread during baking, which does not exceed 100°C, and can germinate and grow in the loaf if conditions are favorable. The ropy condition apparently is the result of capsulation of the bacillus together with hydrolysis of the flour proteins

(gluten) by proteinases of the organism and of starch by amylase to give sugars that encourage rope formation (Frazier and Westhoff, 1978).

Dirar (1992) reported that in the traditional production of sour bread called kisra, local women occasionally used some traditional devices to encourage the growth of lactic acid bacteria. Where this fails the dough that develops has a bad odour defect caused by spore-forming proteolytic *Bacillus* species. These spoilage organisms are effectively inhibited when lactic production in kisra is successful.

Sour bread defect is usually due to an over-time fermentation resulting in the production of acids in the dough. Lactic acid bacteria are responsible for this condition and no doubt are introduced either in the flour or starter or by dirty equipment. *Serratia marcescens* or *Torula glutinis* grows on the surface of the bread and gives the product distinct red, patchy areas which are undesirable. These organisms are neither heat-resistant nor spore-formers. Their presence in bread comes after the bread has been baked (Weiser, 1962).

2.4 Fermentation of cereals in different countries

2.4.1 General aspects

The fermentation of cereal grain has a long history in many parts of the world. Fermented products from maize are usually found in Africa, Central and South America and those from sorghum and millet in Africa and south Asia.

Food fermentation based on rice are practiced in India, China, South East Asia and Far East, while those from wheat are particularly, important in Middle East, Turkey and the Far East (Hesseltine, 1979).

2.4.2 Examples of fermented foods in the world

a) Minichin

Fermented *minichin* is made from wheat gluten from which starch has been removed. The moist, raw gluten is placed in a closed jar and allowed to ferment 2-3 weeks after which it is salted.

A typical specimen was found to contain seven species of mould, nine of bacteria, and 3 of yeasts. The final product is cut into strips to be boiled, baked or fried (Frazier and Westhoff, 1978).

b) Indian idli and dosa

Indian *idli* is a small, white, acidic, leavened, steam-cooked cake made by lactic acid fermentation of a thick batter made from polished rice and dehulled black gram dhal, a pulse (*Phaseolus mungo*). *Dosa*, a closely related product, is made from the same ingredients, but the batter is generally thinner, and *dosa* is fried like pancake.

The initial step in the fermentation is to wash both rice and black gram dhal, and then they are soaked for 5-10 hours and drained. The coarsely ground rice and black gram are then combined with water and 1% of salt to make a

thick batter. The batter is then fermented in a warm place (30-32°C). The batter is then placed in small cups and steamed or fried.

Idli and *dosa* are both lactic acid fermentations. *Leuconostoc mesenteroides* and *Sterptococcus faecalis* develop during soaking (Steinkraus, 1992).

c) Nigerian ogi

It is a smooth-textured sour porridge made by lactic acid fermentation of corn, sorghum or millet. Soybeans may be added to improve nutritive value. Clean grains are fermented for 1-3 days. The grains are then ground with water and filtered to remove coarse particles. Optimum pH for ogi is 3.6 to 3.7. The concentration of lactic acid may reach 0.65% and that of acetic acid 0.11% during fermentation.

The essential microorganisms in *ogi* fermentation are *Lactobacillus plantarum* followed by *Saccharomyces cerevisiae* and *Candida mycoderma* (Steinkraus, 1992).

d) Obusera

It is a refreshing beverage for both adult and infant consumption, widely distributed in Uganda and southern African countries.

Maize or sorghum is milled into flour without the soaking step. Then it is cooked into porridge to which an old stock of fermented porridge is added as a starter for fermentation to produce this product (Novellie, 1982).

e) Ethiopian injera

It is usually made from teff (*Eragrostis tef*) but other cereals such as wheat, barley, maize and sorghum may be used.

In the preparation the flour and water are mixed in especial container (*bohaka*). Then the starter is added and allowed to undergo fermentation for 20-72 hours at room temperature. About 10% of the fermented paste is mixed with three parts water and boiled for 2-5 minutes to give a porridge. The porridge is next mixed with the dough in the *bohaka* and the mixture allowed to ferment for 1.5-2 hours as a second stage of fermentation, then the batter is baked (Gashe, 1985)

f) Kesra

It is a home- made bread commonly consumed throughout the Maghreb countries. It is made from flour usually extracted from hard wheat varieties but it can also be prepared from barely or corn flour. Wheat flour is mixed with salted water and a small amount of fermented dough is added. The dough is allowed to rise for one to two hours then

divided into balls of approximately 250 g each. One ball is kept as a leaven for the next day's bread and the rest are baked. (FAO, 1991)

g) Date bread

Date bread (*Khoubez al-tamer*) is one of the traditional foods commonly consumed in the Arabian Gulf countries. Sugar is first dissolved in water and then date flour or date syrup and yeast are added. The ingredients are mixed until a dough of good consistency has formed. The dough is then cut into pieces and flattened before baking at 400° C in an earthenware oven until brown (FAO, 1991)

g) Egyption kishk

Kishk, a popular food in Egypt is prepared from *laban zeer* and wheat. The ratio of wheat to *laban zeer* is 2:1. The wheat is parboiled at some stage in the process and then it is added to *laban zeer*. The product may be eaten with bread or fried alone with eggs or meat or used as refreshing drink. Kishk is a balanced and complete food (El-malek, 1978).

2.4.3 Fermented products of the Sudan

a) Kissra

This bread is the staple of the Sudan; it is prepared from sorghum or millet flour. By far, however, the bulk of kissra is made from the various types of sorghum. The flour is mixed with water and a small portion of the previous lot of fermented sorghum dough is added as a starter. The dough is incubated in a warm corner of the house, covered and left to ferment over-night. Fresh flour may be added to previous dough and it is baked on a hot plate to give thin sheets of bread (Dirar, 1993 and 1996). Fermentation of the dough was found to be mixed, with lactic acid and acetic acid bacteria playing the major role but yeast have a minor role, (FAO, 1991).

Average values for the composition of *kissra* are: 50% moisture, 80% carbohydrates 12% crude protein, 2% crude fiber and 1.5% ash (Dirar, 1993).

b) Dakkai and sherbot

Dakkai, which is made from whole date fruits, is regarded as a kind of wine. The dates are mixed with clean boiling water in an impervious earthenware jar which is sealed with mud after the contents have cooled; fermentation takes 7-10 days.

Another wine drink called *sherbot*, is made from date syrup and spices. It is specially consumed during I`id Kebir; fermentation takes 2-3 days (Dirar, 1993).

c) Gergoush

Gergoush is a traditional fermented food. It is prepared from wheat. It is widely consumed in the Sudan especially in the Northern State.

The first stage is to prepare a primary starter culture by mixing a few legume beans with boiled milk in a small closed container and incubated in a warm and dark place about 12 hours. Then the primary starter is used to inoculate a small amount of flour to prepare the secondary starter (adapted starter). Finally, the adapted starter is used to ferment the final dough. Spices are added and the fermented dough is baked and dried (Sherfi, 1997).

d) Gurrassa –murra

This sweet sour bread is confined to Northern Sudan, specifically to the area lying between the towns of Dongla and Karima because this area is 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 starter then the batter is placed out in the sun for 4-6 hours to undergo fermentation. The proportion of wheat to dates is 2:1. The dates are boiled to make a 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 storing.

Chapter Three

MATERIALS AND METHODS

3.1 Information gathering

A questionnaire (appendix) was distributed among various respondents in Atabra, Shendi, Marawi, Kosti, Medani, Dongla, Omudrman, Umbadda, Khartoum, Elhajyousif, Elsahafa, Elimtadad, aljireif, Alsalama, Elsagana bus station and Khartoum North railway station. As the present study was not particularized to any ethnic group, respondents were chosen at random, contacted through the cooperation of ten old women (above 35 year). Some of the local uses and names of gurrassa murra have not been recorded in published Sudanese literature and hence it was thought imperative to record these uses.

3.2 Sampling method

Thirty samples of gurrassa murra were collected in sterile foil from different households in different parts of the central and northern Sudan, Khartoum North railway station and Elsagana bus station.

The samples were collected from bus station and railway station were left-over from travellers. Microbiological and biochemical analyses were carried out on arrival of samples at the laboratory after one or two days from collection. During this time they were refrigerated.

3.3 Sterilization of glassware

According to Harrigan and MacCance (1976) glassware were washed thoroughly and left to dry, then they were sterilized in a hot air oven at 160°C for at least three hours.

Inoculating wires, loops and metal instruments such as spatulas, knives and forceps were sterilized by flaming after dipping in ethanol.

3.4 Culture media used for bacterial counting

3.4.1 Solid media

3.4.1.1 Nutrient agar (Oxoid)

This medium was used to determine total viable count and for sub-culturing of bacteria. It was obtained in a dehydrated form. The components of the medium were peptone, yeast extract, Lab-Lemco powder, sodium chloride and agar. It was prepared according to the manufacturer's directions by using 40g in one liter of distilled water and boiled in a water bath until it was completely dissolved. The pH was adjusted to pH 7.2 and then autoclaved at 121°C for 15 minutes.

3.4.1.2 Malt extract agar (Oxoid)

It was used to cultivate, count and isolate the yeasts and moulds. It was obtained in a dehydrated form. The medium was composed of malt extract, yeast extract, peptone, glucose and agar. It was prepared according to the manufacturer's directions by using 50 g in one liter of distilled water. The medium was allowed to boil in water bath until it was completely dissolved. The pH was adjusted to pH 5.4 and then autoclaved at 115°C for 10 minutes.

3.4.1.3 M R S agar

Mann, Rogosa and Sharpe (M R S) agar was used to enumerate and isolate lactic acid bacteria. The constituents of the medium were peptone, Lab Lemco meat extract, yeast extract, D- glucose, tween 80, dipotassium hydrogen phosphate, sodium acetate, triammonium citrate, magnesium sulphate, ($MgSO_4.7H_2O$) and manganese sulphate ($MnSO_4.4H_2o$). This medium was used according to the manufacturer's instruction. It was prepared by using 55g in one liter of distilled water. The medium was allowed to boil in water bath until it was completely dissolved. The pH was adjusted to pH 6.5 and then autoclaved at 121°C for 15 minutes.

3.4.1.4 Nutrient milk agar

This medium was used to determine spore-forming bacteria. It consisted of nutrient agar, 1% separated milk and 1% soluble starch.

3.4.2 Semi-solid media

3.4.2.1 Hugh and Leifson's medium

This medium was used to differentiate the oxidative and fermentative metabolism of carbohydrates (Harrigan and MacCance, 1976). This medium consisted of peptone, sodium chloride dipotassium hydrogen phosphate, bromthymol blue and agar. The ingredients were added to one liter distilled water and dissolved by steaming. The pH was adjusted to 7.1 and then the medium sterilized by autoclave and sterile glucose (sterilized by tyndallization) of 10% concentration was added aseptically to the previously sterilized basal medium. The medium was steamed for 10 to15 minutes to expel dissolved oxygen.

3.4.3 Liquid media

3.4.3.1 MacConkey Broth (Oxoid)

This selective and differential medium was used for detection of coliform bacteria by multiple tube technique (FAO, 1992). It was obtained in a dehydrated form. The medium was composed of peptone, lactose, bile salt, sodium chloride and bromocresol purple. It was prepared according to the manufacturer's instructions by suspending 40g in one liter distilled water. The medium was distributed in 5ml amounts in 150×16mm test tubes with inverted Durham tubes. The pH was adjusted to 7.0 and then the medium sterilized in an autoclave at 121°C for 20 minutes.

2.4.3.2 Brilliant green bile lactose broth (Oxoid)

This selective medium was used for detection *Escrichia coli* by the multiple tube technique. It was obtained in dehydrated form. The medium was composed of peptone, lactose, purified bile and brilliant green. It was prepared according to the manufacturer's instructions by suspending 40g in one liter distilled water. The pH was adjusted to 7.4 distributed in 5ml amounts in 150×16ml test tubes with inverted Durham tubes and then sterilized in the autoclave at 121°C for 15 minutes.

3.5 Microbiological analysis

3.5.1 Preparation of serial dilutions

Ten grams of the gurrassa murra were weighed aseptically in a sterile bottle and then mixed with 90 ml sterile distilled water by using a sterile electric blender. The emulsion was shaken for 5 minutes to obtain 1/10 dilution. Further serial dilutions were made by mixing one ml with 9 ml of 0.9% NaCl saline.

3.5.2 Total viable count (TVC)

The total viable count per gram of sample was obtained by the pour plate method as described by Harrigan and MacCance (1976). One ml from a suitable dilution was transferred into sterile Petri dishes, two replicates for each dilution and melted and cooled (42°C) nutrient agar was added. Then inocula were mixed with the medium and allowed to solidify. The plates were then inverted and incubated in an incubator at 37°C for 48 hours.

3.5.3 Enumeration of total spores

The colony count method to determine the total spores was followed as described by Harrigan and MacCance (1976). A test tube of suitable dilution is heated in water bath at 80°C for 10 minutes. Then the tube is cooled and 1 ml from this dilution was aseptically transferred into sterile Petri dishes. Then to each plate melted nutrient milk agar was added. The inocula were mixed with the medium and allowed to solidify. The plates were then incubated at 37°C for 2 days.

3.5.4 Lactic acid bacteria

From suitable dilution of sample 0.1 ml aseptically transferred on solidified MRS agar containing nystatin of 10g in liter concentration (Elsharif, 1993). The sample was spread all over the plate using sterile bent glass rod. The plates were incubated by using anaerobic jars and gas generating kits for anaerobic growth.

3.5.5 Enumeration of yeasts and moulds

From suitable dilutions of sample 0.1ml was aseptically transferred onto solidified malt extract agar containing 0.1g chloramphenicol per one liter to inhibit bacterial growth. The sample was spread all over the plates using sterile bent glass rod and then the plates were incubated at 28°C for 48 hours (Harrigan and MacCance 1976).

3.5.6 Coliform group

The presumptive coliform test was carried out by inoculating tubes containing MacConkey broth. Tubes were then incubated at 37°C for 48 hours. Tubes producing acid and gas were used for further tests. A loopfull from each positive tube was subcultured into two tubes containing brilliant green bile lactose broth. One set of the tubes was incubated at 37°C for 48 hours, while the other group was incubated at 44.5°C for 24 hours. The most probable number (MPN) tables were used to determine the numbers of colifroms and faecal coliforms (FAO, 1992). For further confirmation of faecal coliforms tubes giving positive reaction at 44.5°C for 24 hours were streaked onto eosin methylene blue (EMB) agar. Colonies with metallic green sheen showed a positive test.

3.6 Identification of bacteria

Identification of pure culture of each organism was carried out according to Cowan and Steel (1974). The purified colonies were subjected to the following tests:

3.6.1 Oxidation fermentation (O/F) test

A fresh culture (18 –24 h) of the isolate was tested for O/F test by stab inoculation onto pairs of freshly steamed Hugh and Leifson medium contained in test tubes. 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.

3.6.2 Motility

Hanging drop technique was used to determine motility of bacteria as described by Harrigan and MacCance (1976). A drop of the culture (18–24 hours) was suspended from a cover slip over the depression in a cavity slide. The slide was sealed to the cover slip using vaseline and then it was microscopically examined for motility.

3.6.3 Gram staining method

The bacterial smear of culture (18–24 hours) was prepared. The colony was emulsified. A drop of bacterial emulsion was placed on clean 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. Then the smear was stained as described by Harrigan and MacCance (1976).

3.6.4 Staining of bacterial spores

A fixed thin smear was stained with a saturated aqueous solution of malachite green for 10 minutes and placed in a water bath. 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 microscope (Harrigan and MacCacne 1976).

3.6.5 Catalase test

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

3.6.6 Oxidase test

A few drops of oxidase test solution were added to a piece of filter paper. Then a loopful from a 24 hours culture was streaked onto the filter paper. Positive result was recorded as a purple colour production after 5 to 15 seconds.

3.7 Methods used in the identification of yeast isolates

Yeast isolates were identified according to the methods described by Lodder (1970), Barnett *et al*. (1983) and Kreger – Van Rij (1984). The purified cultures were subjected to the following tests:

3.7.1 Vegetative cells budding

A sterile culture medium composed of 20g glucose, 5g yeast extract and 10g peptone was used. Active yeast isolates were inoculated into 10 ml of the above medium and incubated at 28°C. The cell shapes, form of the budding and type of growth in liquid medium were recorded.

3.7.2 Formation of extra-cellular starch like compounds

The above medium was used to detect the formation of starch-like compounds. Fifty ml of the medium were dispensed into cotton-plugged flasks and inoculated. They were incubated at 28°C for 72 hours and a few drops of dilute Lugol's iodine solution were added. A blue or green color indicated the presence of starch (Van der Walt and Yarrow, 1984).

3.7.3 Pseudomycelium formation

A sterile petri dish containing a U-shaped glass rod that supported two clean microscope slides with clean cover slips was used. Apiece of wetted filter paper was put in the dish to avoid drying. The slides were dipped aseptically into malted sterile potato glucose agar (Barnett *et al*., 1983), and then placed on the glass rod support. Yeasts from actively growing cultures were inoculated with a straight wire along the length of the slides and the slides were covered with cover slips, incubated at 28°C for 72 hours and then microscopically examined for filamentous growth.

3.7.4 Spore formation

According to Barnett *et al*. (1979) the following media were used:

1. Yeast extract-Malt Extract-Glucose Peptone Agar.
2. Acetate Agar which was prepared by dissolving 9.8g potassium acetate, 10g glucose, 1.2g sodium chloride, 0.7g magnesium sulphate, 2.5g yeast extract and 20g agar in a liter of distilled water.
3. Potato Dextrose Agar (PDA)

Each of the above media was formed into slopes after autoclaving at 121°C and 1.06 kg/cm^2 for 15 minutes. Each isolate was then inoculated in each medium and incubated at 28°C for one to four weeks, then they were examined microscopically for ascospore formation.

3.7.5 Fermentation of carbohydrates

A test tube containing sterile carbohydrate fermentation broth was used. This medium was composed of 0.5% yeast extract and 2% of test sugars. The tubes were inoculated with active yeast culture. The tubes were covered with vaseline-paraffin layer. Then the broth culture was incubated at 28°C for 5-7 days. Fermentation was indicated by elevation of the vaseline –paraffin layer.

3.7.6 Utilization of carbohydrate aerobically

Selected yeast cultures were inoculated into nitrogen-base agar medium, composed of 5g ammonium sulphate, 1g potassium hydrogen sulphate, 0.5g magnesium sulphate, 20g agar and 1000 ml distilled water (Kiss, 1984).

The medium was poured into sterile Petri dishes and then left to solidify. Five ml of each sugar (2%) were placed on the surface of the medium, the growth being observed every two days for about one week.

3.7.7 Utilization of nitrogen compounds for aerobic growth

The method used for assessing growth is similar to those described for carbon sources. The base agar medium used was free from nitrogen sources and consisted of 20g glucose, 1g potassium dihydrogen phosphate, 5g magnesium sulphate, 20g agar in 1000 ml distilled water (Kiss, 1984)

3.7.8 Starch hydrolysis

Selected yeast isolates were grown on starch-agar plates. The medium was composed of 10g peptone, 10g yeast extract, 5g soluble starch, 5g dipotassium hydrogen phosphate and 15g agar in one liter of distilled water. The plates were inoculated at the center and were then incubated at 28°C. After 3-7 days of incubation the plates were flooded with Lugol’s iodine solution. A clear zone around the colony of the microorganism indicated hydrolysis of starch.

3.8 Chemical analysis

3.8.1 pH determination

Ten grams of the sample of gurassa murra were shaken into 100 ml of distilled water, left to stand for twenty minutes and the pH of the suspension was read using glass electrode model 79 pH meter (PYE and Co. Ltd., Cambridge).

3.8.2 Dry matter

Two grams of gurrassa murra sample were placed into a dried crucible of known weight. Then they were heated in an oven at 105C°overnight then the crucible was taken out, cooled in a desiccator and weighed. The dry matter was calculated as follows:

$$\text{Dry Matter \%} = \frac{\text{crucible wt+ sample wt-crucible wt+ sample wt after drying}}{\text{sample wt before drying}} \times 100$$

3.8.3 Crude protein

This was determined using Kjeldahl method as described in A.O.A.C. (1980). The crude protein was calculated by multiplying the total nitrogen by the factor 6.25.

3.8.4 Ash content

The ash content was determined according to the method of AOAC (1980). Two grams of gurrassa murra were placed into crucible of known weight in an oven at 600C° for 3 hours then the crucible was taken out and weighed. The ash percentage was calculated as follows:

$$\text{Ash \%} = \frac{\text{crucible wt+ incinerated gurrassa murra- wt of empty crucible}}{\text{Gurrassa murra sample wt before incineration}}$$

3.8.5 Crude fat

Crude fat percentage was determined by ether extract. Two grams of the sample were extracted for 5 hours. Fat percentage was then calculated as:

$$\text{Fat \%} = \frac{\text{Extracted weight}}{\text{Sample wt}} \times 100$$

3.8.6 Crude fiber

Crude fiber was determined according to AOAC (1980)

3.8.7 Carbohydrates

The percentage of carbohydrates was determined by subtraction from 100 % after the determination of the contents of the other components.

3.8.8 Titratable acidity

Titratabale acidity was estimated according to AOAC (1980). Ten grams of the sample were mixed in 100ml distilled water and the suspension was titrated with 0.1 N sodium hydroxide. The results were expressed as percent lactic acid.

3.9 Preparation of gurrassa murra

3.9.1 Preparation of sorghum flour, wheat flour and starter

Sorghum and wheat grains were milled to fine flour in a local grain mill.

Fermented dough was prepared in the traditional way used by Sudanese housewives. Sorghum flour was mixed with water in the ratio of 1:2 (W/V), then starter from previous dough was added to sorghum flour at the rate of 10% of the dough. The fermentation was carried out for 24 hours. This dough was used as starter to prepare gurrassa murra.

3.9.2 Preparation of wheat dough

Wheat flour was added to water containing a little salt in plastic container and mixed well to make thin slurry, then the starter (10% from sour sorghum dough) was added. The plastic container was covered and put under the sun for 4 hours to undergo fermentation.

3.9.3 Preparation of date paste

Dates, which were used to sweeten the sour wheat, were cleaned, washed and boiled to obtain paste (madida). Proportion of wheat to date was 2:1.

3.9.4 Preparation of gurrassa murra

The date paste was added to fermented wheat dough. The mixture was baked on a hot plate.

3.9.5 Microbiological analysis

Microbiological analysis was carried out after addition of the starter, date paste and after cooking. Total viable count, moulds and yeast, lactic acid bacteria, total coliforms, *E. coli* and endospores were enumerated and checked as described previously and were obtained per gram of sample.

3.9.6 Chemical analysis

Moisture content, protein, fat, ash content, carbohydrates, dry matter, titratable acidity, pH and fiber were determined according to AOAC (1980) and alcohol by Table Ebulliometre.

3.9.7 Preparation of serial dilution

The standard pour plate method as described by Harrigan and MacCance (1976) was used for total viable bacterial count. Ten grams of the Gurrassa murra were weighed aseptically in a sterile bottle and then mixed with 90 ml sterile distilled water by using an electric blender. The emulsion was shaken for 5 minutes to obtain 1/10 dilutions. Further serial dilutions were made by mixing one ml of dilution with 9 ml of 0.9% NaCl saline.

Table (1): Microbial counts of home-made gurrassa murra fermented in aluminium containers.

Sample No	Source	Total viable count (cfu/g)	Endospore Count/g	Yeast and mould Count (cfu/g)
3*	Shendi	8.0×10^{5}	3.0×10^{3}	_
4	Alrekabia (Northern state)	3.6×10^{5}	5.1×10^{3}	6.4×10^{3}
11	Al Imtidad (Khartoum)	1.8×10^{6}	1.2×10^{3}	2.33×10^{4}
17*	Medani	2.2×10^{6}	1.11×10^{4}	4.2×10^{3}
18	Medani	3.6×10^{5}	6.7×10^{3}	1.10×10^{4}
21	Omdurman	2.90×10^{6}	4.1×10^{4}	7.3×10^{3}
26	Haj Yousif (Khartoum North)	2.20×10^{6}	5.8×10^{3}	6.1×10^{3}

* stored sample

Table (2): Microbial counts of home -made gurrassa murra fermented in plastic containers

Sample no	Source	Total viable count (cfu/g)	Endospore Count (cfu/g)	Yeast and mold count (cfu/g)
1	Al Sahafa (Khartoum)	2.2×10^5	3.9×10^3	4.7×10^2
5*	Al Salama (Khartoum)	6.0×105^5	1.2×10^3	2.8×10^3
7	Haj Yousif (Khartoum North)	8.0×10^3	9.0×10^2	6.1×10^3
9	Al Jireif	5.0×10^5	2.5×10^3	1.28×10^3
12	Gelas (NorthState)	4.3×10^5	5.4×10^2	1.9×10^4
13	Gelas (North State)	1.7×10^6	1.12×10^4	6.1×10^4
15*	Dongola (North State)	1.5×10^6	9×10^3	8.2×10^4
19	Kosti	5.9×10^5	2.3×10^4	6.6×10^3
20*	El Damer	1.44×10^6	6.2×10^4	1.2×10^3
22	El Kalakla	1.17×10^6	1.93×10^4	6.7×10^3
23	El Dabba	2.4×10^6	2.3×10^4	1.23×10^4
24	Omdurman	1.12×10^6	3.9×10^3	8.2×10^2
27*	Atbara	1.23×10^6	1.10×10^4	6.4×10^3
29	Um badda	4.4×10^5	7.3×10^3	2.6×10^4
30	Helffa	9.3×10^5	9.4×10^3	4.2×10^3

* stored sample

Table (3): Microbial counts of home-made samples of spiced gurrassa murra fermented in plastic containers.

Sample No	Source	Treatment	Total viable count(cfu/g)	Endospore Count /g	Yeast and mould Count (cfu/g)
2	Um Badda	Hilba was added	6×10^{5}	5.4×10^{2}	6.4×10^{3}
6	Haj Yousif	Cumin And Hilba were added	6.8×10^{2}	1.2×10^{2}	2.8×10^{3}
8	Al Sahafa	Cumin and Hilba were added	4.5×10^{3}	1.1×10	3.0×10^{2}
10	Al Emtedad	Cumin and Hilba were added	8.0×10^{5}	7×10	6.0×10^{4}

Table (4): Microbial count of spiced gurrassa murra samples collected from travellers.

Sample No	**Source**	**Treatment**	**Total viable count (cfu/g)**	**Endospore count/g**
14	bus station	Cumin and Ghee	2.4×10^{6}	7.3×10^{3}
16	bus station	Hilba, Ghee and Cumin	2.2×10^{6}	9.4×10^{3}
25	railway station	Hilba, Ghee and Cumin	4.5×10^{5}	5.8×10^{3}
28	railway station	Cumin and Ghee	7.5×10^{6}	5.8×10^{4}

Table (5): Identification of bacteria isolated from collected samples.

Isolate no.	Gram staining	Catalase Test	Oxidase Test	Endospore Test	Motility	O/FTtst	Aerobic growth	Genus
1	+Rod	+	-	+	+	F	+	*Bacillus*
2	+Rod	+	-	+	+	F	+	*Bacillus*
3	+cocci	+	-	-	-	F	+	*Staphylococus*
4 (b)	+cocci	+	-	-	-	O	+	*Micrococcus*
4(A)	+cocci	+	-	-	-	F	+	*Staphylococus*
9	+Rod	+	-	+	+	F	+	*Bacillus*
10	+Rod	+	-	+	+	F	+	*Bacillus*
13	+Rod	+	-	+	+	F	+	*Bacillus*
14	+Rod	+	-	+	+	F	+	*Bacillus*
15	+Rod	+	-	+	-	F	+	*Bacillus*
18(A)	+Rod	+	-	+	+	F	+	*Bacillus*
18(B)	+cocci	+	-	-	-	F	+	*Staphylococus*
19	+cocci	+	-	-	-	F	+	*Staphylococus*
20	+Rod	+	-	+	+	F	+	*Bacillus*
22	+Rod	+	-	+	+	F	+	*Bacillus*
23	+cocci	+	-	-	-	F	+	*Staphylococus*
24	+Rod	+	-	+	+	F	+	*Bacillus*
25	+Rod	+	-	+	-	F	+	*Bacillus*
26	+Rod	+	-	+	+	F	+	*Bacillus*
27	+cocci	+	-	-	-	F	+	*Staphylococus*
28	+cocci	+	-	-	-	F	+	*Staphylococus*
29	+Rod	+	-	+	+	F	+	*Bacillus*
30	+Rod	+	-	+	+	F	+	*Bacillus*

Table (6): Biochemical and physiological tests for tentative identification of yeasts isolated from collected samples

Tests	Isolate							
	S1	**S5**	**S7**	**S9**	**S10**	**S12**	**S13**	**S15**
Assimilation of carbohydrates anaerobically:								
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	-	+	+	+	-	+
Galactose	+	+	-	+	+	+	-	+
Lactose	-	-	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-	-
Assimilation of carbohydrate acrobically:								
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Maltose	+	+	v.w	+	+	+	v.w	+
Starch-like compounds	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-
Growth on:								
KNO_3	-	-	-	-	-	-	-	-
KNO_2	+	+	+	+	+	+	+	+
Growth in liquid medium	sediment	sediment	sediment	sediment	sediment	sediment	sediment	sediment
Pseudomycelium	+	+	+	+	+	+	+	+
Vegetative cell	Budding	Budding	Budding	Budding	Budding	Budding	Budding	Budding
Sexual reproduction ascospore	+	+	+	+	+	+	+	+

formation								

v.w: very weak

Table (7): Chemical analysis of gurrassa murra collected from different households

Sample No	Moisture %	Dry matter %	Ash %	Crude protein %	Carbohydrates %	Ether extract %
1	43	57	1.5	7.5	43	1.5
3	49	51	1	9.7	35.4	1.2
4	43	57	1.5	8.2	41.9	1.4
5	44.5	55.5	1.2	9	39.8	1.6
7	49.5	50.5	0.5	9.5	35.9	1
9	50.5	49.5	0.5	8.2	35.9	1.5
11	41	59	1.5	9.6	43	1.4
12	40	60	1	8.4	45.0	1.3
13	45.5	54.5	0.5	7.2	41.3	1.7
15	45	55	1.3	7.9	40.8	1.6
17	42.45	57.55	0.75	8.5	42.9	1.4
18	57.95	42.05	1.75	8.2	26.8	1.5
19	55	45	1.4	8.9	28.8	1.8
20	53.5	46.5	1.4	9.3	30.7	1.9
21	55.5	44.5	1.3	8	30.1	1.5
22	50.5	49.5	0.7	8.5	35.8	1
23	48.7	51.3	1.6	7.3	37.1	1.3
24	51.5	48.5	0.5	7.1	35.5	1.5
26	53.5	46.5	1.7	7.1	35.2	1.8
28	49.5	50.5	1.2	8.7	35.9	1.1
29	41.2	58.8	1.4	9	43.1	1.5
30	48.4	51.9	1.6	8.9	36	1
Averge	48.12	51.89	1.17	8.39	37.27	1.43

Tabble (8): Chemical analysis of spiced gurrassa murra samples collected from different households

ample No.	Moisture %	Dry matter %	Ash %	Crude protein %	Ether extract %	Crude fiber %	*Carbohydrates %*	pH	Acidityas % lactic acid	A
2	58	42	1.0	8.2	1.0	3.3	28.5	5.7	2.2	
5	47.4	52.6	1.5	8.5	1.9	4.1	36.6	5.1	1.9	
3	49.1	50.9	0.5	8.9	1.2	3.4	36.9	5.4	2.6	
0	45.5	54.5	0.5	7.6	1.8	3.2	41.4	5.3	2.1	
verage	50	50	.88	8.3	1.48	3.5	35.85	5.38	2.2	

Table (9): Chemical analysis of spiced gurrassa murra collected from reavellers

Sample No	**Moisture %**	**Dry matter %**	**Ash %**	**Crude protein %**	**Ether extract %**	**Crude fiber %**	*Carbohydrates %*	**pH**	**Acidity as% lactic acid**	**A**
S_{14}	39	61	0..9	8.9	3.8	2.6	44.8	5.8	2.0	
$\mathbf{S_{16}}$	48	52	1.0	9.0	3.5	2.9	35.6	5.7	2.2	
S_{25}	50.5	49.5	1.5	7.1	3.5	2.9	34.5	5.5	1.9	
S_{28}	42	58	0.5	9.6	3.4	2.5	42.0	5.1	2.6	
Average	44.88	55.13	1	8.65	3.55	2.73	39.23	5.53	2.18	

Table (10): Tentative identification of bcteria isolated during fermentation process

Isolate number	Gram staining	Catalase test	Oxidase test	EndoSpore staining	O/F test	Aerobic growth	Motility	Genus
$A_{o(1)}$	+Rod	+	-	+	F	+	+	*Bacillu*
$A_{o(2)}$	+cocci	+	-	-	F	+	-	*Staphyloco*
$A_{1(1)}$	+cocci	+	-	-	F	+	-	*Staphyloco*
$A_{1(2)}$	+Rod	+	-	+	F	+	+	*Bacillu*
A_2	+Rod	+	-	+	F	+	+	*Bacillu*
A_3	+Rod	+	-	+	F	+	+	*Bacillu*
A_4	+Rod	+	-	+	F	+	+	*Bacillu*
A_5	+Rod	+	-	+	F	+	+	*Bacillu*
A_6	+Rod	+	-	+	F	+	+	*Bacillu*
1	+Rod	-	-	-	F	+	-	*Lactobaci*
2	+cocci	-	-	-	F	+	-	*Lactococ*

A_0: After adding the starter

A_1: After one hour

A_2: After 2 hours

A_3: After3 hours

A_4: After 4 hours

A_5: After 4 hours and 15 mintues (after date addition)

A_6: After4hours and half (after frying)

1,2: isolated from MRS madia

Table (11): Biochemical and physiological tests for tentative identification of yeasts isolated during fermentation process

Tests	I S O L A T E

	1	2	3	4
(1) Assimilation of carbohydrates anaerobically				
Glucose	+	+	+	+
Sucrose	-	-	-	-
Galactose	-	-	-	-
Lactose	-	-	-	-
Maltose	-	-	-	-
(2) Assimilation of carbohydrates anrobically				
Glucose	+	+	+	+
Sucrose	+	+	+	+
Galactose	+	+	+	+
Lactose	+	+	+	+
Maltose	vw	vw	vw	vw
Starch-like compounds	+	+	+	+
Starch Hydrolysis	-	-	-	-
Growth on:				
KNO_3	-	-	-	-
KNO_2	+	+	+	+
Growth in liquid medium	sediment	sediment	sediment	sediment
Pseudomycelium	+	+	+	+
Vegetative Cell	Budding	Budding	Budding	Budding
Sexual reproduction ascospore formation	+	+	+	+

Table (12): Chemical analysis of gurrassa murra during fermentation

Code	Moisture %	Dry matter %	Ash %	Crude Protein%	Ether Extract %	Crude fiber %	***Total Crude Carbohydrate %***	***PH***	***Acidity %***	***Alc l***
A_0	40.7	60.3	1.4	11.0	1.1	3	43.8	6.4	1.3	0

A1	38.3	61.7	1.5	11.3	1.3	3.7	43.9	5.9	1.9	0
A_2	39.9	60.1	1.4	10.3	1.3	3.9	43.2	5.0	2.0	1
A_3	42.1	57.9	1.4	9.5	1.2	4.0	42.8	4.9	2.9	2
A_4	42.0	58.0	1.6	9.7	1.0	4.1	41.9	4.3	2.9	2
A_5	48.0	52.0	1.6	9.2	1.0	4.5	45.4	4.2	2.1	1
A_6	45.0	55.0	1.5	9.2	1.9	4.4	38.0	4.2	1.3	0

A_0: After adding the starter

A_1: After one hour

A_2: After 2 hours

A_3: After3 hours

A_4: After 4 hours

A_5: After 4 hours and 15 mintues (after date adition)

A_6: After4hours and half (after frying)