MICROBIAL ECOLOGY OF BODRE AND SHAMITA FERMENTATION

BY

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To my brother Asfaw Bacha.

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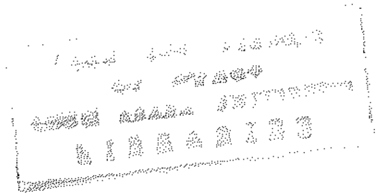

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ABSTRACT

'Borde' and 'Shamita' are two of the traditional Ethiopian fermented beverages commonly consumed in the southern part of the country. They are thick in consistency, consumed while actively fermenting and have served as meal replacement. The ingredients for 'borde' and 'shamita' preparation are usually maize (or wheat) and barely, respectively.

The dominant organisms isolated during 'borde' fermentation belonged to the genus *Bacillus, Micrococcus, Lactobacillus, Streptococcus, Staphylococcus, Saccharmoyces, Rhodotorula* and members of *Enterobacteriaceae.* With drop in pH of 'borde' from 5.2 to 3.6, the count of some of these dominant organisms increased markedly while few were inhibited. Likewise, 'shamita' fermentation was dominated by bacteria of the genus *Bacillus,* both homo- and hetrofermentative *Lactobacillus, Staphylococcus, Micrococcus,* members of *Enterobacteriaceae* and yeasts of the genus *Saccharomyces.* As the pH dropped from 5.82 to 3.98, increase in counts of certain groups and decrease in other groups was noted. Members of *Enterobacteriaceae* were inhibited earlier during fermentation of both beverages.

The ingredients and equipment used for fermentation contributed to the microbial flora. The major groups of organisms isolated from equipment (earthen jar) were *Micrococcus, Saccharomyces, Rhodotorula* and members of *Enterobacteriaceae* with counts as high as 10^4 in most groups. *Bacillus* and bacterial spores dominated spices
and heat treated cereal ingredients with spore counts in the range of $2x10^4$-$9.9x10^4$ and $2x10^2$-$2.5x10^4$, respectively. Barley malt contributed almost all of the dominant microbial isolates including *Bacillus, Staphylococcus, Micrococcus,* heterofermentative *Lactobacillus, Streptococcus,* bacterial spores, *Saccharomyces* and *Rhodotorula.*

Nutritionally, the concentration of reducing sugars decreased with time in both beverages; the availability of soluble protein appeared to increase initially with gradual decrease in the later stages during 'shamita' fermentation (37.2 to 30.6 mg/ml). The difference were not considerable in 'borde'. The percent ash content appeared higher in 'shamita' than 'borde' with moderate lipolytic activities in both beverages.

Locally collected 'shamita' samples were found to have microbial similarity to the laboratory brewed products with slightly higher microbial count in the collected samples.

The low cost of both beverages, the role they play as meal replacement and their popularity among most economically deprived people make these beverages good candidates for production on a large-scale. But identification of microorganisms responsible for their fermentation and assessment of the production procedures are among the preliminary steps that need investigation.

1. INTRODUCTION

Fermentation is one of the effective and the most economical methods of producing and preserving foods and beverages acceptable to man. In fact, fermentation and drying are two of the oldest methods of preparation and preservation of foods and beverages known to mankind. The method is inexpensive, easily acceptable, adaptable and applicable to local house-hold practices in traditional communities (Pederson, 1979; Odunfa, 1987; Samuel and Berhanu, 1991; Nout, 1993; Ayele and Berhanu, 1994; Johansson, 1995). Fermented foods account for 25% of European daily diet and 60% of the daily diet in the developing nations (Holzepfal *et al.*, 1995).

For thousands of years, the people of the world have relied upon foods preserved through fermentation processes. It appears that many of the food preservation practices antedate recorded history (Johansson, 1995). Fermentation yielding alcohol and/or acid generally offer low-cost ways of preserving food in a world where the majority of the people can not afford canned, frozen or dehydrated foods (except those that are sun-dried) (Steinkraus, 1983; Vaughn, 1985). It is certain that fermented products exhibit great stability with the ability to dominate over the wide variety of microbes which will inevitable be present in grains, etc., prepared by traditional methods without the benefits of modern cleaning and sterilizing agents (Wood and Hodge, 1985; Nout *et al.*, 1989). Fermentation involving yeasts and lactic acid bacteria, and which are carried out in closed vessels will rapidly become anaerobic, acidic, saturated with CO_2 and alcohol. This combination of conditions will certainly be inhibitory to many spoilage

microorganisms including filamentous fungi and bacteria associated with various forms of food spoilage (Svanberg *et al.*, 1992).

Fermentation processes improve the nutritional quality of raw ingredients in that they improve the digestibility of nutrients, destroy undesirable flavours, inactivate antinutritional factors such as trypsin inhibitors, tannins, lectins, oligosaccharides (flatulence factors), ODAP and convert perishable products into a product with good keeping quality (Van Veen and Steinkraus, 1970; David and Varma, 1981; Suparmo and Markakis, 1987; Nout, 1993; Biniyam et al., 1995). Natural fermentation is known to reduce the concentration of phytic acid and, thus, enhance the bioavailability of minerals, particularly Fe, Ca, and Mg (Sutardi,- and Buckle, 1985a,b). Fermentation may not greatly alter the amino acids in cereals and legumes, but often may make proteins available (Soni and Sandhu, 1991). The fermentation process usually adds desirable nutrients not present in the original product, and some members of Enterobacteriaceae usually enrich the fermented foods and beverages with vitamins (Plat, 1964; Akinrele, 1970).

Another factor of interest in fermented food product is the production of antibiotics by some organisms involved in the fermentation processes. Certain lactic acid bacteria such as *Lactobacillus acidophilus* produce an antibiotic acidolin (Hamdan and Mikolajcik, 1974) in skim milk. *Rhizopus oligosporus*, fungi important in cassava fermentation, also produce antibacterial compounds. These compounds, thus, have been playing beneficial role in controlling low-grade bacterial infections in people consuming the products (Wang *et al.*, 1969; Jay, 1982). Bacteriocins are produced by

many LAB (Tagg *et al.*, 1976; Blazeka *et al.*, 1991; Motlagh *et al.*, 1991) inhibiting a wide range of organisms.

Practically every civilization has developed some type of fermented food and some type of fermented beverage. The Asians, centuries ago, taught the world how to produce meat like flavours from vegetable proteins. The Indonesians discovered a means of introducing texture into vegetable products, which serves as meat substitute in their diets. In Japan, cultured milks are widely promoted and have been credited with health-giving properties (Pederson, 1979; Wood and Hodge, 1985).

The early men probably used fermented beverages, besides other, as a substitute to safe water. The alcohol content is too low in the early stages of fermentation to produce an intoxicating effect (Rose, 1977). The temporary illness resulting from drinking fermented beverage were less severe than those resulting at times from the consumption of contaminated water (Pederson, 1979). Interestingly, the primitive man learned its preparation through observation and experience without a real understanding of the process. The development of distilled alcoholic beverages was associated with the advent and improvement of distillation techniques. The production of fermented food and alcoholic beverages from a wide variety of fruits and grains, the curing of hay and silage, and the production of lactic and acetic acids were among the early domestic application of fermentation techniques (Harrar, 1964).

The traditionally fermented beverages are low-cost products in all aspects: they are manufactured using only rudimentary equipment such as empty oil vats, earthen vessels, etc., and the handling and consumption often takes place under conditions of poor hygiene (Steinkraus, 1983).

Indigenous fermented foods /or beverages/ are very important in that they are socio-culturally bound, especially in rural house-holds and village community traditions; they could become even more important in feeding additional segments of the increasing world populations in the future (Steinkraus, 1983). Their relative cheapness has a selective effect by providing a cheap alternative for the low income group of consumers.

Ethiopia is one of the countries where varieties of traditionally fermented foods and beverages are produced and consumed. The substrates could be starch (different cereals) or sugar such as honey. The beverages has been produced on a fairly small scale and usually for local consumption. Among Ethiopian indigenous fermented beverages are varieties of *Tella, Tej, Katikala* (Araki), *Korefe, Borde, Shamita, Keribo, Imbushbush, Winetej, Duka*. With various modifications, traditional fermented foods account for 20% - 40% of our food supply (Holzapfal *et al.*, 1995).

In spite of the wide use of the 'borde' and 'shamita' in Ethiopia, very little is known about their microbiology, biochemistry and nutritional properties. There was a study made to evaluate the microbiological and biochemical quality of both 'borde' and 'shamita' produced by village fermentation techniques and as made available to the

consumer in Southern Ethiopia (Mogessie and Tetemke, 1995). In general, there is no report on the systematic studies of changes occurring during the fermentation of 'borde' and 'shamita'.

The objectives of this study were, therefore, to make a detailed examination of the fermentation process with respect to the dynamics of the important groups of microorganisms that initiate and carry out the fermentation; and the major biochemical changes that occur during the process. Findings of this study may serve as base-line data for large-scale production of 'borde' and 'shamita' and also lead to recommendations on the possible role of these products in human nutrition.

2. LITERATURE REVIEW

2.1 Traditional Fermented Beverages

Traditionally Fermented Beverages are those which are indigenous to a particular area and have been developed by the people of that area themselves using age-old techniques from locally available (mostly home-grown) raw materials.

The traditionally fermented foods (beverages) and their preparation; their value for human survival, development and welfare; and importance in counteracting world hunger is considerable. In nearly all areas of the world, some types of alcoholic beverages native to the region are prepared and consumed (Pederson, 1979). As a result, there are a number of traditionally fermented beverages throughout the world.

On the basis of the important role played by the traditional African beverages, the African consumers tend to recognize these beverages as a type of food rather than just beverage(Aucamp *et al.*, 1961; Platt, 1955). Obiolor, Nigerian traditional non-alcoholic beverage, has been consumed daily by the people of Igala land (Achi, 1990). Before the advancement of distillation techniques as introduced into Europe by Arabs, the oldest alcoholic drinks were fermented beverages of relatively low alcoholic content. Among the common traditional fermented beverages in other parts of the world are: Kenyan Urawaya (Harkishor, 1977); Mexican pulque (Sanchez-Marroquin, 1977; Herrera *et al.*, 1977); Bantu beer (Novellie, 1968); Nigerian pito (Ekundayo, 1969); Zambian maize beer (Lovelace, 1977); Korean yakju and takju (Park *et al.*,

1977); Indian jackfruit wine (Dahiya and Prabhu, 1977); Mexican tesgino (Taboda *et al.*, 1977); Kenyan busaa (Harkishor, 1977); American maize drink chicha (Escobar, 1977; Escobar *et al.*, 1977); the Japanese sake (Yoshizawa, 1977) and Sudanese 'Merissa' (Dirar, 1978).

2.2 Some Traditional Ethiopian Fermented Beverages

Tella:- `Tella' is a fermented traditional beverage of variable viscosity and has colour ranging from greyish-white to dark-brown. Its colour is determined by the intensity of some processing stages when preparing it. It is the most commonly consumed alcoholic beverage in Ethiopia (Central Statistical Office, 1988). The common ingredients for its preparation are barley, maize, wheat, millet, sorghum or *tef* (Vogel and Gobezie, 1977; Pederson, 1979; Alemu Fite *et al.*, 1991; Samuel and Berhanu, 1991; Tegegne, 1957). Depending on the type of cereal ingredients used or their locality, tella has different names such as wheat 'tella', barley `tella', millet `tella' (depending on the type of ingredients), and yegojjam `tella' (based on their locality) (Alemu Fite *et al.*, 1991). The process of `tella' preparation and its microbiology has been described elsewhere (Samuel and Berhanu, 1991).

Tej:- Tej is a home-processed honey wine. Quality tej is yellow to olive green in colour, sweet and effervescent. Originally tej was made only in the homes of royality and noblemen and used to be brewed on special occasions. Sometimes, sugar or a mixture of sugar and honey could be used for its preparation. In cases where sugar is

used as a substrate, natural yellow food colouring is added so that the beverage attains a yellow colour similar to that made from honey (Vogel and Gobezie, 1977; Rose, 1977; Desta, 1977; Alemu Fite *et al.*, 1991; Samuel and Berhanu, 1991).

Katikala:- It is colourless, clear, traditional alcoholic beverage distilled from fermented products. Different kinds of grains are used for its preparation (Desta, 1977; Berhanu, 1989; Samuel and Berhanu, 1991). Traditional distillation apparatus, consisting of clay pots, bamboo pots, ropes and a receiver, is used. Usually, katikala is widely served on festive occasions and at social gathering besides its use as a source of income.

Comparison of methanol, fusel oil and ethanol contents of different `tell', `tej', and `katikala' has been reported very recently (Alemu Fite *et al.*, 1991).

Borde:- Borde is a traditional fermented beverage (food) usually made from maize (*Zea mays*) and wheat (*Triticum* spp.). It is a very popular meal replacement in Southern Ethiopia and some parts of the country (Mogessie and Tetemke, 1995). Similar to kaffir beer, traditional South African fermented beverage, `borde' is consumed while actively fermenting. Besides, `borde' has short fermentation period (overnight) though not as short as that of kaffir beer that is ready for consumption in 4-8h (Steinkraus, 1985). On the average, labourers consume about three litres of `borde' per head each day. As compared to `borde', consumption of five pints of kaffir beer is not unusual for a working man each day in South Africa (Steinkraus, 1985).

Shamita:- It is produced by fermenting barley. Like `borde`, `shamita' is thick in consistency and is consumed as meal replacement for most people who cannot afford a reasonable meal. It has short shelf-life and the product turns too sour four hours after being ready for consumption (Mogessie and Tetemke, 1995). Usually, neither sugar nor *malt* is used for `Shamita' fermentation. Starch is the only principal fermentable carbohydrate. The source of the microorganisms responsible for its fermentation could be the ingredients used, equipment, and mostly the starter used from previous fermentation as observed in a Mexican fermented product (Watcher *et al.*, 1993)

Many factors could account for the role that traditional fermented beverages, in general, and `borde' and `shamita', in particular, play as meal replacement. High microbial loads (10^6-10^8 CFU/ml) of yeast and lactic acid bacteria are reported from both `borde' and `shamita' samples analysed for its microbiology. These could make the products good sources of microbial protein. The relatively high lysine content of yeasts protein would improve the nutritive value if added to grains such as corn, wheat or rice. Moreover, fermented products of both `borde' and `shamita' are known to have higher protein, fat, and vitamin contents than the ingredients (Mogessie and Tetemke, 1995).

2.3 Substrates for Beverage Fermentation

The fermentable carbohydrate for preparation of beverages could be sugars or starch. In some cases, however, a beverage could be made both from sugar and starch. Fruit wines, for instance, have been made from sweet fruits such as grapes, apples, oranges,

and related fruits. In the preparation of wines from the starchy raw materials such as wheat, barley, rice, or corn, the raw materials must be degraded into sugars in order to ferment them by yeasts. Thus, traditionally fermented beverages throughout the world could be grouped in to two main categories on the basis of the types of substrates used for their preparation and production of ethyl alcohol.

The first group of fermented alcoholic beverages in which sugars are the principal fermentable carbohydrates includes: Ethiopian Tej (Vogel and Gobezie, 1977); Honey wines (Mead; Metheglin) (Morse and Steinkraus, 1977); Indian jack fruit wine (Dahiya and Prabhu, 1977); Mexican pulque (Sanchez-Marroquin, 1977; Herrera *et al.*, 1977; Goncalves de Lima, 1977); Kenyan urawaya (Harkishor, 1977); Palm wines (Toddys) (Okafor, 1977; Odeyemi, 1977; Faparusi, 1977; Theivendirarajah *et al.*, 1977a, b; Samarajeewa, 1977; Nyako, 1977; Merican, 1977; Shuaib and Azmey, 1977; Wong and Jackson, 1977; Ekmon and Nagodawithana, 1977); Philippine basi (Sanchez, 1977; Tanimura *et al.*, 1978) and Kenyan muratina (Harkishor, 1977), both of which are sugar cane wines; and other related alcoholic beverages like Mexican mezcal (Pennington, 1969).

The second principal fermentable carbohydrate, as already mentioned, is starch. For fermentation process to occur, the starch should be hydrolyzed in to simple sugars. Such hydrolysis could be achieved either by using saliva as a source of amylolytic agent, by malting (germination) or by using amylolytic molds and yeasts (Steinkraus, 1983).

Saliva has been used as a source of diastase (amylase) for conversion of starch to sugars in fermented alcoholic beverages such as Japanese Sake (Kodama and Yoshizawa, 1977) and South American fermented maize chicha (Escobar, 1977; Escobar *et al.*, 1977). Malting or germination of cereals is a major means by which starch is converted to simple sugars. Alcoholic beverages in which starch hydrolysis is accomplished by malting (germination) includes African kaffir beer or bantu beer (Novellie, 1966a; 1968); Mexican tesquino (Taboada *et al.*, 1977); Egyptian bouza (Morcos, 1977); Nigerian pito (Ekundayo, 1969); Ethiopian Tella (Vogel and Gobezie, 1977; Samuel and Berhanu, 1991); Kenyan busaa (Harkishor, 1977); Zambian opaque maize beer (Lovelance, 1977); and Zambian munkoyo (Lovelance, 1977; Mbugua, 1977). Malting (germinating) the corn kernels to produce the amylases needed for starch conversion is an alternative procedure, to saliva, that is widely used today in chicha fermentation (Steinkraus, 1983).

In the other category of alcoholic beverage where the fermentable carbohydrate is starch, starch hydrolysis and fermentation are accomplished by amylolytic molds and yeasts. This category ranges from very primitive Thai rice wines to highly sophisticated Japanese sake (Yoshizawa, 1977; Kodama and Yoshizawa, 1977). Also included here are the Korean Yakju and Takju (Park *et al.*, 1977). The conversion of starch to sugars during steeping and malting appears to be due partly to enzymes in the grain and partly to moulds (*Penicillium* spp. *Rhizopus oryzae*, and *Aspergillus* spp.) (Ekundayo, 1969).

2.4 Major Microorganisms Involved in Fermentation of Beverages

Microorganisms of various groups appear to involve in the fermentation of beverages indigenous to different parts of the world.

In fermentation of the Nigerian pito, different species of the genera of *Leuconostoc, Lactobacillus, Saccharomyces, candida* and *Geotrichum* are known to participate (Ekundayo, 1969). *Saccharomyces cerevisiae* dominates the alcoholic fermentation of Kenyan Busa. and the lactic acid fermentation involves various *Lactobacillus spp.* (Nout, 1980).

The microbiology of Japanese sake fermentation appears to involve nitrate-reducing microorganisms succeeded by lactic acid bacteria. Under traditional fermentation conditions, the first organisms to develop in the mash are *Pseudomonas, Achromobacter, Flavobacterium*, or *Micrococcus* spp. (Murakami, 1972) followed or possibly accompanied by *Leuconostoc* and *Lactobacillus spp.* (Kodama and Yoshizawa, 1977).

Although yeasts and bacteria are important in the fermentation of the actual Korean yakju, the production of the starter involves the activities of *Aspergillus* and *Rhizopus* spp., aerobic bacteria and yeast (Kim, 1968). A variety of yeasts, aerobic spore formers and lactic acid bacteria (LAB) are involved in the fermentation. In general, *Saccharomyces cerevisiae* and *Hansenula* spp. are important among these microflora for

production of alcohol and flavour development, respectively (Kim and Lee, 1970; Kim, 1970, Lee and Rhee, 1970; Shin and Cho, 1970).

Kozaki (1976) reported that the dominant organisms in Philippine Basi are *Saccharomyces, Endomycopsis*, and Lactic acid bacteria. Yeasts are the dominant groups in the fermentation of Kenyan Muratina (Harkishor, 1977). Among the honey wines, the fermentation of Dwojniack and Trojniack are carried out by osmophilic yeasts (Tanimura *et al.*, 1978).

Fermentation of palm wine involves lactic acid bacteria, yeasts, acetic acid bacteria as well as *Zymomonas* and *Micrococcus* spp. (Okafor, 1972a, b). Faparusi (1977) reported the involvement of *Acetobacter* spp. and a variety of yeasts and molds in the fermentation of Nigerian wine. Similarly, various groups of yeasts are reported from Ghanian wine among other bacteria such as *Micrococcus* spp.,and LAB (Nyako, 1977). Swings and Delay (1977) isolated *Zymomonas mobilis* from African wines.

For the alcoholic fermentation of kaffir beer, strains of *Saccharomyces cerevisiae* are responsible. Souring is caused by lactic acid bacteria that are introduced from previously soured malt /water slurry/ (Vander-Walt, 1956). Fermentation of Indian jack fruit wine is assumed to involve yeasts that resemble Endomycopsis (Dahiya and Prabhu, 1977).

In the alcoholic fermentation of tesquino, the yeast *S. cerevisiae* is an important microorganism (Herrera and Ulloa, 1973). In Mexican pulque fermentation, *S. cerevisiae* is the primary ethanol producing yeast (Herrera and Ulloa, 1975). In addition, homo-and hetero fermentative lactobacilli closely related to *Lactobacillus plantarum* and *L. brevis* were reported (Sanchez - Marroquin and Hope, 1953). Swings and Delay (1977) consider *Zymomonas mobilis* sub sp. *mobilis* as the causal organism for pulque fermentation.

The important microorganism in `Tella' fermentation are *Arthrobacter, Acetobacter, Lactobacillus, Bacillus, Proteus, Achromobacter* and the yeast *Saccharomyces* (Samuel and Berhanu, 1991). Recent reports on the microbiology of 'borde' and 'shamita' samples collected from local brewers as made available for consumers show that *Bacillus, Micrococcus,* and *Lactobacillus* spp. have been the dominant bacterial genera in 'borde'. In 'shamita', however, *Bacillus* spp. dominated the aerobic microflora. In both beverages, high counts of Enterobacteriaceae and yeasts were isolated. *Staphylococcus, Acinetobacter* and *Streptococcus* spp. were among the isolates that were important next to the dominant groups (Mogessie and Tetemke, 1995).

It appears that systematic studies have not been made of the microbiology of Kenyan urwaga (Harkishor, 1977), Egyptian bousa (Morcos, 1977), and Zambian opaque maize beer and Zambian Munkoyo (Lovelance, 1977; Mbugua, 1977). Lactic acid bacteria and yeast are assumed to be involve in the fermentation of Kenyan urwaga (Harkishor,

1977) and Egyptian bousa (Morcos, 1977) only because of the low pH (or high acidity) and alcohol contents of the products.

Fermentation of guinea corn (*Sorghum bicolor*-Linn) in the preparation of "Sorghum Stout", involves bacterial genera and yeasts of the *Saccharomyces* genus both during mashing and fermentation of the wort (Kolawole *et al.*, 1987). Fermentation of 'burukutu' and 'raphia' palm wine involves *Saccharomyces cerevisiae* and species of lactobacilli (Owuama, 1991). *Bacillus* spp., *Lactobacillus plantarum* and *Streptococcus lactis* are reported to actively involve in `obiolor' fermentation, Nigerian acidic non-alcoholic beverage (Achi, 1990), the later two being the most important.

2.5 Nutritional Value of Fermented Beverages

The nutritional value of any food and/or beverage must be looked at in terms of the nutritional status of the consumers of such a products. While the affluent western world can afford to enrich its basic foods with chemically available vitamins, most of the developing world must rely upon biological enrichment of food through fermentation. It is worth noting that the nutritional status of consumers varies very much with the time of the year and with the success or failure of agricultural efforts. In some parts of Africa, when the harvest fails, porridge and/or fermented beverages are the choice of a society where cereals constitute a staple food staff (Novellie & De Schapdrijver, 1979).

There are two views as to the nutritional value of fermented beverages, such as beer. On the one hand, fermentation has been said to waste grain since starch is respired to carbon dioxide both during malting and alcoholic fermentation (Chavan and Kadam, 1989a cited in Nout, 1993). In addition, the beer strainings often go to feed pigs or other animals rather than directly nourishing humans (May, 1968). On the other hand, fermented beverages extend the available grain supply, since a given amount provides a greater bulk and hence a greater feeling of satiety than could be obtained from an equivalent amount of porridge (Bohannan and Bohannan, 1968). It could also be enriched by the yeast present. Thus, it is important to optimise germination process and to use it only for those ingredients which will be nutritionally improved by it (Nout, 1993).

Fermentation processes increase digestibility and availability of nutrients. For instance, those beverages that use malt are known to contain much more free amino nitrogen than does the original grain. i.e. the partial degradation of reserve proteins in cereals makes the free amino nitrogen available (Chavan and Kadam, 1989a cited in Nout, 1993). Besides, proteolytic activity increases considerably during germination, roughly ten fold in sorghum (Novellie and De Schapdrijver, 1979).

Fermentation does not increase the protein nutritive value but that digestibility and organoleptic characteristics of certain fermented foods (Van Veen and Steinkraus, 1970). The proteins are partially hydrolyzed, made more soluble and available nutritionally. The original physical and chemical characteristics of the foods may be altered during fermentation but their nutritive value are usually retained to a great extent (Pederson, 1979).

Among some population which are near the margin of vitamin deficiency, fermented beverages can also serve as source of vitamins (Okafor, 1977; Escobar, 1977; Herrera *et al.*, 1977; Sanchez-Marroquin, 1977; Steinkraus, 1983; Samuel and Berhanu, 1991). Fermentation of kaffir beer almost doubles the riboflavin and nicotinic acid content of the grain used for fermentation (Platt, 1964). In fact, thiamine and niacin remains fairly constant during chicha fermentation (Escobar *et al.*, 1977).

Evidence show that consumption of 2 litres of kaffir beer per day, which is a rather common level of consumption, would supply 14%, 72% and 36% of the recommended allowance for adult males of thiamine, riboflavin and nicotinic acid, respectively (NAS/NRC, 1980). According to Nout (1980), some traditionally fermented beverages can cover at least 13% of vitamin B_2 (riboflavin) and 25% of niacin recommended for daily intake per adult person.

Nutritionally, alcoholic drinks have an appreciable caloric content. In some parts of the tropics, consumption of local beer has been known to provide 10% of the caloric requirement of the individual (Steinkraus, 1983). One must, of course, acknowledge that excessive consumption of any alcoholic drink can be dangerous.

In Southern Nigeria, around 4 million people each drink about 500 ml palm wine a day. A litre of good potable oil palm wine provides approximately 300 calories from sugars and alcohol (Bassir, 1968). It also provides 0.5-2 gram of protein and important amounts of water soluble vitamins. These properties make palm wine an important

addition to the diets of pregnant women and teenagers among which vitamin B deficiencies are highly wide spread (Bassir, 1968).

Platt (1964) has attempted to compare diet with and without maize beer in which the grains has been consumed directly. Interestingly, the diet containing beer has been known to double its riboflavin and almost double the nicotinic acid content due to synthesis of vitamins during malting and fermentation. Consuming usual amount of kaffir beer minimizes pellagra, which is relatively common in people subsisting on maize diets (Platt, 1964).

Besides vitamins, energy, and protein, fermented beverages could serve as a source of minerals. Sorghum beer, for instance, is an excellent source of iron, magnesium, manganese and phosphorous. A litre of beer can also contribute to the daily requirements for copper, zinc, calcium and potassium (Novellie and de Schaepdrijver, 1979).

In general, the traditionally fermented foods and beverages are important elements in the diets of millions of people especially in the developing countries. With our present and expanded future needs for food, in general, and protein, in particular, the important role that traditionally fermented foods and beverages play can neither be over-looked nor ignored. Evidence show that the value of indigenous fermented foods as a potential source of food supply for many parts of the world is widely recognized (Hesseltine, 1981 as cited in Hasan *et al.*, 1993; Ogbadu *et al.*, 1990).

3. MATERIAL AND METHODS

3.1 Sampling

Samples were separately taken from ingredients (25g) used for both `borde' and `shamita' fermentation; rinse of the equipment (100ml) used for brewing. Moreover, samples (about 500 ml) were aseptically removed from the fermenting mash at four hours interval for 24 hours both for microbiological and biochemical analyses. Samples of `shamita' were also collected from local brewers in Addis Ababa as made available to consumers. Although `shamita' was prepared in the laboratory, sample of `borde' (about 500 ml in sterile 1 litre flask covered with aluminium foil) was rushed with in few minutes to the laboratory from where it was prepared. Part of `borde' and `shamita' samples removed in the course of fermentation and those collected from local brewers were freeze-dried and stored at 4°c for protein, fat and ash content. analyses.

3.2 Microbiological Analyses

Twenty-five gram of ingredients used for `borde' and `shamita' fermentation and twenty-five ml of `borde' and `shamita' samples drawn in the course of fermentation were separately blended in 225ml of sterile physiological saline solution. Moreover, ten-ml of the 100ml rinse solution from equipment (earthen jar) was diluted in 90ml sterile physiological saline solution. The samples were homogenized using a vortex mixer and surface-plated on the following media for counting.

3.2.1 Aerobic Mesophilic Count

Samples were further diluted in sterile saline solution and 0.1ml of appropriate dilution was spread-plated in duplicate on pre-dried surfaces of Plate Count Agar(PC, Merck) with a bent glass rod. Colonies were counted after incubation at 37°c for 48 hours.

3.2.2 Coliform Count

Volume of 0.1ml of appropriate dilution were spread-plated in duplicate on pre-dried surfaces of Violet Red Bile Agar (Oxoid) plates. The plates were incubated at 37°c for 24 hours. Purplish red colonies on VRB agar surrounded by a reddish zone of precipitated bile were counted as coliforms.

3.2.3 Counts of Lactic Acid Bacteria

Volume of 0.1ml of appropriate dilutions were spread plated in duplicates on pre-dried surfaces of de Man-Rogossa and Sharpe (MRS) agar (Oxoid) plates. Colonies were counted after incubation in anaerobic jar (BBL) at 37°c for 48 hours.

3.2.4 Bacterial Spores Count

Portion of four ml and 0.4ml from 1:10 homogenates were added to two different flasks containing 40ml of molten PCA and heat shocked in a water bath at 80°c for 10 min according to Hobbs and Cross (1983). The contents of each flask were then distributed

between two pertri-dishes and incubated at 37°c for 48 hours. Two countable plates of the same dilution were used to count colonies.

3.2.5 Count of Enterobacteriaceae

For the enumeration of *Enterobacteriaceae,* of 0.1ml of appropriate dilutions were spread plated in duplicate on pre-dried surfaces of Violet Red Bile Glucose (VRBG) Agar (oxoid) plates. The plates were incubated at 37°c for 24 hours. Purple red colonies were counted as members of *Enterobacteriaceae*.

3.2.6 Count of Staphylococci

Appropriate dilutions were spread-plated on duplicate plates of Mannitol Salt Agar (MSA) (Oxoid) and incubated at 37°c for 48 hours. The counts were further confirmed after biochemical tests was done on ten to fifteen colonies picked from countable plates.

3.2.7 Yeast and Mold Count

Volume of 0.1ml of appropriate dilutions were spread-plated in duplicate on pre-dried surfaces of chloramphenicol-bromophenol-blue agar (CBB).

Ingredients:- Yeast extract, 6.0g, glucose, 20.0g, chloramphenicol, 0.1g; bromophenol blue, 0.01g, agar, 15g; distilled water, 1000ml; pH, 6.0-6.4.

Yeast colonies were counted after incubating plates at 25-27°c for 5 days. Molds were counted at the early stage of their appearance on plates.

3.2.8 Flora Assessment

After colony counting, ten to fifteen colonies were randomly picked from countable plates of PC, MRS, VRBG ,MSA and CBB agar. The isolates were further purified by repeated plating on appropriate medium. Pure isolates were maintained on appropriate agar slants, and finally differentiated into various bacterial groups using morphological and biochemical characteristics listed below.

3.2.8.1 Cell Morphology

Morphological characterization of the pure culture was made by observing wet mount and/or Gram stained preparation of young culture under microscope. The morphological criteria considered during observation were:

a) Cell shape: regular: rods, coccobacilli, cocci

irregular: branched, coryneform, pleomorph

b) Cell grouping: singles, pairs, chains, clusters, tetrads

c) Endospores: present, absent

d) Motility: Motile, non-motile

3.2.8.2 KOH-test (test on lipopolysaccharide)

KOH-test was made according to Gregersen (1978) to distinguish between Gram-positive and Gram-negative bacteria. In principle, the application of KOH is meant to dissolve the lipopolysaccharide present in the cell wall of Gram-negative bacteria which is absent in Gram-positive bacteria. The dissolved lipopolysaccharides stretche when pulled with a needle.

One or two drops of 3% KOH solution were placed on a clean glass slide. A colony of pure isolate was picked with an inoculating loop and stirred in the KOH solution for 5-10 seconds. The loop was then raised slowly from the mass. In Gram-negative isolates, the KOH solution became viscous and the thread of slime followed the loop for 0.5 to 2cm or more. In gram-positive, there was no slime that follow the loop.

3.2.8.3 Cytochrome Oxidase Test

Cytochrome oxidase was tested by the method of Kovacs (1956). Reagents were prepared for immediate use.

Reagents: Tetramethyl-phenylenediamine, 1.0g; ascorbic acid, 0.1g; distilled water, 100ml.

Here, filter paper strips were socked with 1-2 drops of the reagents. Then, one loopful of the test organism from culture not older than 24 hours was taken and streaked on the

soaked filter paper. The appearance of a dark blue colour within 20 seconds indicated a positive reaction.

3.2.8.4 Catalase Test

Catalase test was made with 3% (v/v) H_2O_2 solutions (MacFaddin, 1980). Formation of bubbles was considered as positive reaction.

3.2.8.5 Oxidation-fermentation Test

Glucose metabolism was investigated by the O/F test of Hugh and Leifson (1953).

Ingredients: Casein Peptone, 2.0g; yeast extract, 1.0g; NaC1, 5.0g; K_2HPO_4, 0.2g; glucose, 10.0g; bromothymoleblue, 0.08g; agar, 2.5g; distilled water, 1000ml; pH, 7.1.

The above medium was freshly prepared (in 10ml amounts in test tubes), cooled down to about 35°c under tap water, inoculated by stabbing with a straight wire to the bottom and incubated at 32°c. Acid formation, gas production and growth were recorded for 2-5 days.

Glucose metabolism in Staphylococci and Micrococci was investigated using O/F medium prepared following the Baird-Parker modification (Collins and Lyne, 1976).

Ingredients:- Tryptone, 10.0g; glucose, 10g; yeast extract, 1.0g; agar, 2.0g; distilled water, 1000ml; bromocresol purple (0.2%), 20ml; pH, 7.2.

All Gram-positive cocci in singles or pairs, which were catalase positive with oxidative reaction in their O/F test were considered as members of the Genus *Micrococcus*; the fermentatives being species of the Genus *Staphylococcus*.

3.2.9 Grouping of Lactic Acid Bacteria

Gram-positive, non-sporing, catalase-negative, cocci or rod shaped isolates picked from MRS and PC plates were considered as lactic acid bacteria. Further grouping in to different genera was made by testing for gas production from 5% glucose in MRS broth. Gas production was detected in Durham tubes after incubation at 37°c for 5 days.

3.2.10 Grouping of Yeasts

Yeast isolates were physiologically characterized based on their fermentation of and gas production in glucose, galactose, sucrose, maltose, lactose and raffinose using the peptone-yeast extract basal medium of Wickerham (1951) as cited in Lodder (1971).

Ingredient:- Peptone, 7.5g; powdered yeast extract, 4.5g; distilled water, 1000ml; Bromothylmole blue, 0.1g; pH, 7.0.

Two ml aliquotes of the unsterilized basal medium was placed in 150x12mm tubes carrying Durham's tubes, sterilized, cooled, 1ml filter-sterilized sugar solutions were aseptically added, inoculated with yeast isolate and incubated at 25-27°c. The tubes were regularly shaken and observed for percent of gas in the inserts and for change in colour of the indicator.

Based on the information already gathered, standard manuals for bacterial identification such as *Bergey's Manual of Systematic Bacteriology* (Sneath *et al.*, 1986), Collins and Lyne (1976), Buchanan and Gibbons (1974) Pederson (1979); and the manual entitled `The Yeasts' (Lodder, 1971) were consulted for the proper naming of bacteria and yeast isolates, respectively, to the genus level.

3.3 Biochemical Analyses

3.3.1 Physical Parameters

3.3.1.1 pH:-

The change in pH of the fermenting mash in the course of fermentation and that of samples collected from local brewers was measured by aseptically placing the electrode of a pH metre (Beckmann) in the fermenting mash and samples.

3.3.1.2 Moisture Content

Moisture content of each sample was determined by drying known quantities of the samples to constant weight in an oven at 80°c for 24 hours.

3.3.1.3 Temperature

Changes in temperature within the fermenting vat in the course of fermentation and changes in ambient temperature were recorded using Beckmann pH metre (recording both pH and temperature at the same time) and a Philip-Harris model thermometer, respectively.

3.3.2 Chemical Parameters

3.3.2.1 Soluble Protein Content

The soluble protein content of samples were determined according to Lowry *et al.*(1951). One ml of the sample was mixed with 5ml of the working reagent that was made by combining reagents " A"," B" and "C" in the ratio of 98:1:1 respectively. [Reagent A was prepared by dissolving 2g sodium hydroxide (NaOH) and 10g sodium carbonate in 500 ml of distilled water; Reagent "B" was 1g sodium tartarate dissolved in 50ml of distilled water (2%); Reagent "C" was constituted by dissolving 1g hydrated copper sulphate ($CuSO_4SH_2O$) in 100ml of distilled water (1%)].

The mixed samples and working reagent were left to react for 5-10 min. After reaction, 0.5ml of freshly prepared Folin's phenol reagent (reagent made by mixing one part of Folin - ciocalteu's phenol reagent with one part of water) was added and mixed thoroughly. After 30 minutes the mixture was centrifuged (5000 RPM for 10 min) and the colour produced was read on spectronic-2 UVD spectrophotometer (Bausch and Limb) at 750 nm against blank that contained all the ingredients except the sample. Bovine serum albumin was used to construct the standard curve.

3.3.2.2 Fat Content

Lipid was determined by the method described in Golterman *et al.*, (1978) as modified by Getachew (1987). Sample (100mg) was placed in folded filter paper, and stapled.

The lipid was extracted with diethyl ether for 3 hours. The sample was then air dried for 10 minutes and oven dried at 100°c for 30 minutes and cooled in a desiccator. The filter paper with the sample was then weighed and the difference in weight taken as the weight of lipid.

3.3.2.3 Ash Content

Ash was determined by igniting a five gram sample, placed in a crucible, in a muffle furnace (Fisher Isotemp Muffle Furnace 184 A) at 550°c for 4 hours according to A.O.A.C. 923.1 (A.O.A.C., 1990). The weight loss after ignition was considered to be the weight of total organic matter (Ash free dry weight, AFDW) in the sample, the remaining being the weight of ash.

3.3.2.4 Reducing Sugars' Content

The reducing sugar content of the samples drawn at different period in the course of fermentation and that of 'shamita' samples collected from local brewers were estimated by Nelson's method (Nelson, 1944) as modified by Clark and Switzer (1977).

One ml of the samples were mixed with one ml of reagent D (Reagent A-D are described below) using vortex-mixer. The mixed samples were then immersed in boiling water for 20 minutes. At the end of 20 minutes the boiled samples were immersed in running water for immediate cooling. Then, the cooled samples were mixed with 1ml of reagent C (Aresno molybdate reagent) and allowed to stand for 5

minutes at room temperature. Thereafter, the final volume of the samples was adjusted to 10ml with distilled water and left to stand for 30 minutes. It was then centrifuged for 10 minutes (GallenKamp Centrifuge 200). Finally, absorbance of the test solutions were read on a spectronic 2'UVD spectrophotometer (Bausch and Lomb) at 540 nm against a blank solution containing all the reagents used with the same proportion as test solutions but lacking sample. A known concentration of glucose was used to construct the standard curve.

The reagents used to estimate the reducing sugars content of the sample were:

I) Reagent A:- made by dissolving 12.5g sodium carbonate (Na_2CO_3), 12.5g potassium sodium tartarate, 10g sodium bicarbonate (Na_2HOC_3) and 100g sodium sulphate in distilled water whose final volume was adjusted to 500 ml.

ii) Reagent B:- A solution made by dissolving 7g hydrated copper sulphate ($CuSO_45H_2O$) and one drop of concentrated sulphuric acid in 50ml distilled water.

iii) Reagent C:- (Arseno Molybdate Reagent):- A solution made by dissolving 25g ammonium molybdate in 450 ml distilled water to which 20 ml of concentrated sulphuric acid was added. To the above, 3g sodium arsenate dissolved in 25 ml of distilled water was further added.

iv) Reagent D:- Combination of 12.5ml of reagent A and 0.5ml of reagent B.

3.4 Borde Fermentation

For the investigation of `borde' fermentation, the main ingredients used were wheat (*Triticum* spp.) and barley (*Hordeum vulgare*). Wheat (*Triticum* spp.) was used to

prepare an adjunct, and barely (*Hordeum vulgare*) as a raw material for preparation of malt (or `Bikil'). One litre of `borde' from previous fermentation was used as starter. Optionally, ground bird's-eye chili (*Capsicum minimum*) has been used traditionally either to improve the taste or flavour of the fermented product. In our case, the likely microbial contribution of the spice was investigated.

For adjunct preparation, wheat flour was soaked in excess water and then deeply roasted in a hot flat metal pan. Usually, ground barley (*Hordeum vulgare*) whipped in hot water is used along other adjuncts. The later is uncommon among local brewers in Addis Ababa, hence it was omitted from the preparation process in this study.

For malting (or `Bikil' preparation) barley was first cleaned to remove dirt and extraneous materials. This was steeped in clean water for about a day. Then, excess water was strained-off and allowed to germinate for five days after spread on and covered with banana leaves. The extent of germination was monitored using the length of plumule in judging when germination has gone far enough.

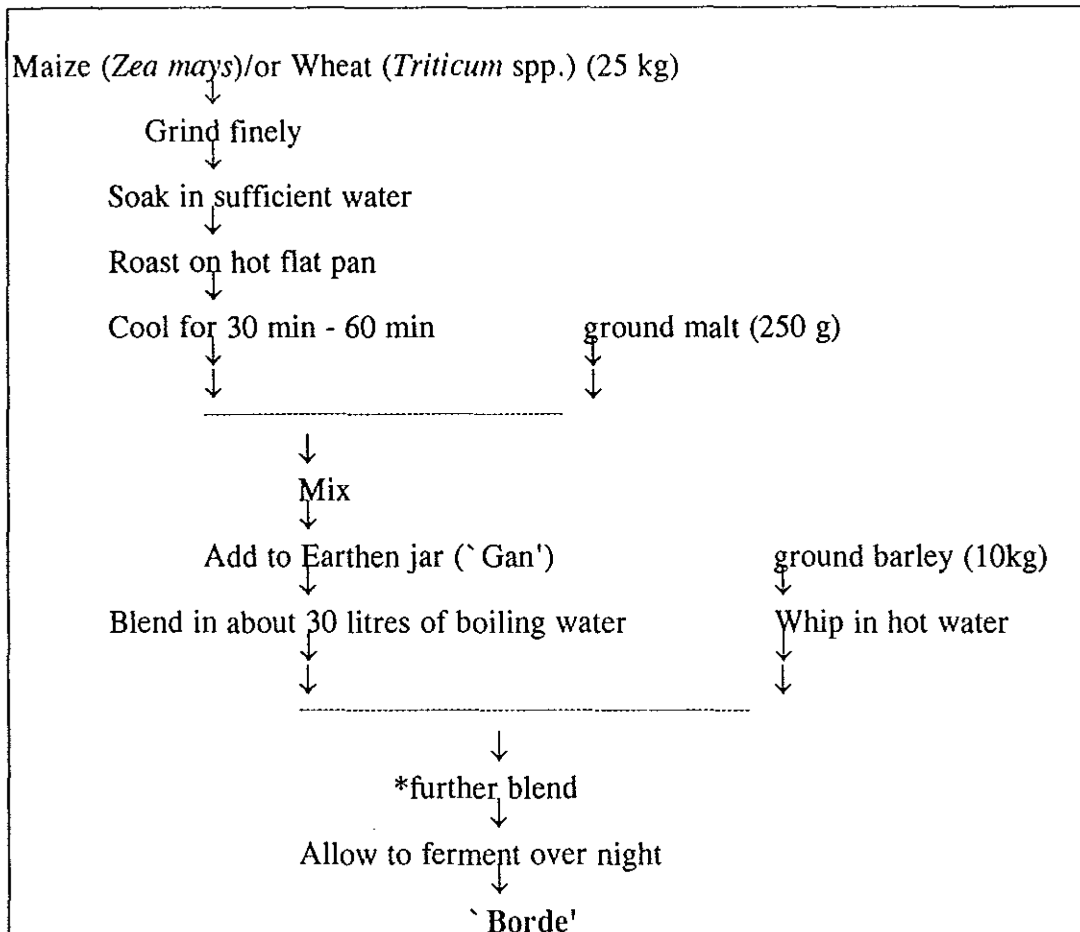

Fig. 1. Flow chart of `borde' fermentation process. * Not used in this study.

After germination, the malt was sun-dried and ground finely. The major equipment used for `borde' fermentation was `Gan' (earthen jar) with a capacity of about 90 litre. It was washed and dried before use.

During the study period, `borde', was prepared on three occasions by an experienced woman brewer following the traditional fermentation technique. Arrangements were made with an experienced `borde' brewer to prepare `borde' for the purpose of this study.

The traditional fermentation technique used in `borde' fermentation employing maize as ingredient was followed (as summarized by Mogessie and Tetemke, 1995). Twenty-five kg of wheat flour was soaked in excess water and then deeply roasted in a hot flat pan. After cooling for an hour, about 250g of malt was thoroughly mixed in to it. The whole mixture was put in to an earthen jar (`Gan') and further blended in about 30 litres of tap water. Usually, 10kg of ground barley whipped in hot water is added at this stage. About one litre of `borde' from previous fermentation was added to it as starter, sealed-well with plastic sheets and clothe, and allowed to ferment for over 24 hours (Fig.1). Traditionally, the fermenting mash is allowed to ferment for not more than 12 hours (i.e. over-night fermentation).

3.5 `Shamita' Fermentation

To study the fermentation process of `shamita', the basic ingredients used were: Barley (*Hordeum vulgare*) - as an adjunct; ground linseed to ensure thick consistency of the product; salt (sodium chloride) - mainly to improve taste of the fermented product; and different kinds of spices to impart characteristic aroma to the produce. Some of the spices might serve to improve the keeping quality of the beverages (Caragay, 1992; Olojede, *et al.*, 1993). The spices used here include, Ethiopian - caraway (*Trachysperum ammi*) and false cardamon (*Aframomum korarima*).

For adjunct preparation, 125kg of barley (*Hordeum vulgare*) was soaked in water for 15 minutes. Then, the excess water was strained-off and dehulled using mortar and pestle. The whole dehulled was allowed to air dry for short period of time and then the hull

was removed. The dehulled barley was roasted on flat metal pan. The extent of heat treatment during roasting was controlled by the brewer. For the purpose of this study, it was roasted to light brown. After further dry-dehulling, the roasted barley was ground finely.

To ensure thick consistency of the product during fermentation, as it has been used traditionally, nine kg of linseed was ground and made ready for use. Moreover, the `base ingredient' was made by mixing certain proportions of each of the ingredients. Accordingly, 100kg of barley flour, three kg of salt (sodium chloride), nine kg of ground linseed and small amount of the spices (20-25g) were mixed. These constituted the stock ingredient out of which the required amount were taken for single preparation of `shamita'.

`Gan' (Earthen jar) with capacity of about 90 litre was the equipment used through out the `shamita' preparation processes. It was washed with tap water and dried before use.

For laboratory preparation of `shamita', 25kg of the `base ingredient' was mixed with about 50 litres of water. Although it has been uncommon to use malt for `shamita' fermentation in Awassa (Mogessie and Tetemke, 1995), it has been frequently used in Addis Ababa. Therefore, about 150gm of malt was added to the above mixture. One litre of `shamita' from an over-night fermented `shamita' was added as starter and the whole system was allowed to ferment over 24 hours after tight sealing. Traditionally, `shamita' is ready for consumption after an over-night fermentation. Optionally, depending on the consumers' interest, ground birds-eye chili (*Capsicum minimum*) has been added to the product before serving (Fig.2).

`Shamita' was prepared on three occasions in the laboratory by an experienced woman brewer following the traditional fermentation technique.

All the raw materials used in this study were purchased from local market in Addis Ababa.

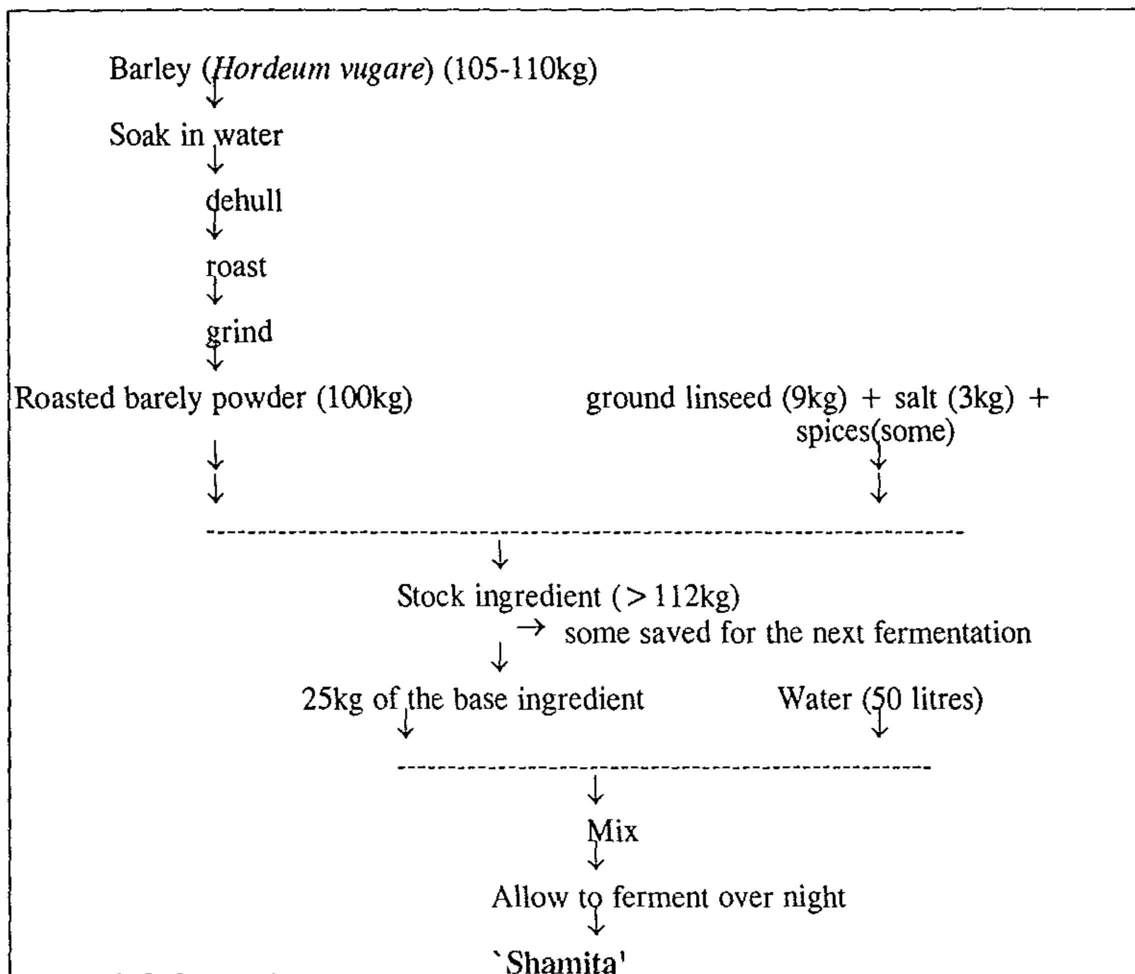

Fig. 2 Flow Chart of `Shamita' fermentation process.

4. RESULTS AND DISCUSSION

Various genera of microorganisms including *Bacillus* spp., *Staphylococcus* spp. *Micrococcus* spp., *Streptococcus* spp., members of *Enterobacteriaceae* and yeasts, mainly represented by *Saccharomyces* spp. and *Rhodotorula* spp., were isolated from malt used for fermentation of `borde' and `shamita' (Tables 1 and 2). Related genera were identified also from wheat flour and roasted ground barley. Basically, cereal grains and meals are prone to contamination by molds, yeasts and bacteria (Nout, 1980; Ogundiwin *et al.*, 1991). Although the traditional technique of fermentation was followed in this study, attempts were made to reduce the load of natural microflora associated with ingredients: the barely used for malting was steeped in water repeatedly; barely for preparation of roasted ground barley was washed and dehulled before further processing. Despite all these efforts, however, considerable numbers of bacteria, *Bacillus* spores and molds were isolated from ingredients. According to Frazier and Westhoff (1978), bacteria in wheat flour include spores of *Bacillus*, coliforms, and a few representatives of the genera *Achromobacter, Flavobacterium, Sarcina, Micrococcus, Alkaligenes*, and *Serratia*.

Thermoduric microorganisms including *Bacillus* spores were frequently isolated at the early stage of `borde' fermentation. This could be due to elimination of most of the other bacteria as a result of heat-treatment of the adjuncts (or ingredients) (Fig 3, Table 4). Achi (1990) isolated two species of *Bacillus*, particularly *Bacillus*

stearothermophilus and *B. subtilis* from boiled mash during `obiolor' fermentation study. In our case, addition of starter, malt and/or spices during fermentation appeared to replenish some of the genera removed during heat treatment and also introduced other bacteria and yeasts. Although *Bacillus* have been associated with spoilage (Crielly *et al.*, 1994) in heat processed foods, they may not play any significant similar role here as the products are consumed within a few hours after completion of fermentation.

A high load of aerobic mesophilic bacteria as high as $9x10^5$ and *Bacillus* spores were isolated from spices used for the study (Table 3). The presence of high load of aerobic bacteria and spores has been reported for spices used for seasoning (Tjaberg *et a.*, 1972). According to the report, over 10^7 cfu/g each of aerobic mesophilic bacteria, yeasts, molds and spore formers were isolated from ginger. Giaccone *et al.*, (1996), cited in Lindberg (1997) also found upto 10^7 spores per gram spices. In agreement with observation of Banwart (1979) that the total aerobic count of flour sampled at the mills was generally in the range of 10^2 to 10^3/g, the spore count of wheat flour and roasted ground barely used in this study was in the range of $2\text{-}4x10^3$ and $1\text{-}2x10^4$, respectively. In general the counts of the various groups of microorganisms in our study were, however, much lower than those reported elsewhere.

Among yeasts, species of the genus *Saccharomyces* were the very dominant isolates. Less frequently, however, *Rhodotorula* spp. were encountered at different stages of `borde' fermentation (Table 4). These two yeast genera have been recently reported

from `Tella' fermentation (Samuel and Berhanu, 1991). The *Saccharomyces* spp. may be responsible for the small but insignificant amount of alcohol produced during the fermentation. The possible source of *Rhodotorula* spp. were ingredients and equipment used for fermentation (Table 1 and 2).

Table 1

Dominant microorganisms isolated from ingredients and equipment used for `borde' fermentation (CFU/ml)

Ingredients and Equipment		*Bacillus* spp.	*Entero-bacter laceae*	*Micrococcus* spp.	*Lactobacillus* spp.		*Streptoco-ccus*spp	Bacterial spore	*Saccharom-yces* spp.	*Rhodotorula* spp.	Molds
					Homo-ferment.	.Hetroferment ative					
Ingre-dients	Weat (*Triticum* spp.) powder	$2.8x10^3$ $1x10^3$-$4.1x10^3$)	$3.2x10^3$ ($2x10^3$-$5.6x10^3$)	$4.4x10^4$ ($3x10^4$-$6x10^4$)	-	-	-	$3.3x10^2$ ($2x10^2$-$4x10^2$)	-	-	$1.2x10^3$ ($1x10^3$-$3x10^3$)
	Barley (*Hordeum vulgare)* malt (1Bikil').	$1.8x10^4$ ($1x10^4$-$3.2x10^4$)	$1.8x10^5$ ($3x10^4$-$5x10^5$)	$2.1x10^4$ ($1x10^4$-$4x10^4$)	-	$1.6x10^4$ ($1.2x10^3$-$3x10^4$)	$2.1x10^5$ ($3x10^4$-$5x10^5$)	$5.2x10^3$ ($4.7x10^3$-$6x10^3$)	$1.5x10^4$ ($3x10^3$-$4.2x10^4$)	$2.2x10^3$ (0-$4x10^3$)	$1.4x10^4$ ($3x10^3$-$3x10^4$)
	Starter*	$4.2x10^5$ ($3x10^5$-$6x10^5$)	-	$2.5x10^6$ ($9x10^5$-$7x10^6$)	$1.2x10^4$ ($1x10^4$-$3.2x10^4$)	$1.1x10^8$ ($1.1x10^7$-$3x10^8$)	$2.2x10^6$ ($4x10^5$-$5.1x10^6$)	$3.5x10^5$ ($2.8x10^5$-$5x10^5$)	$1.2x10^7$ ($5x10^6$-$2.6x10^7$)	$1.3x10^4$ ($8.8x10^3$-$2.1x10^4$)	-
Equip ment	Earthen Jar 'Gan'	-	$2.2x10^2$ ($1x10^2$-$3x10^2$)	$7.2x10^3$ ($6x10^3$-$1x10^4$)	-	-	--	-	$1.2x10^4$ ($2.3x10^3$-$3.2x10^4$)	--	-

*About a liter of over-night fermented `borde' sample.

Table 2

Dominant microorganisms isolated from ingredients and equipment used for `Shamita' fermentation

Ingredients and Equipment		*Bacillus spp.*	*Staphylococcus spp.*	*Micrococcus spp.*	*Streptococcus spp.*	*Lactobacillus spp.*		Enterobacteriaccae	*Saccharomyces* spp.	*Rhodotorula* spp.
						Homofermentative	Heteroferm			
Ingredients	Roasted barley powder	$1.8x10^4$ ($9.8x10^3$-$3x10^4$)	5.8x103 ($9.5x10^2$-$8.3x10^3$)	$5.1x10^4$ ($3.4x10^4$-$6x10^4$)	-	-	-	$2.1x10^3$ $1x10^3$-$3x10^3$	-	-
	Ground linseed	$1x10^4$ ($9.6x10^3$-$1.3x10^4$)	$3.2x10^2$ ($2.4x10^2$-$5x10^2$)	$1.6x10^4$ ($1x10^4$-$2.4x10^4$)	-	-	-	$6x10^3$ ($5x10^3$-$8x10^3$	-	-
	Ground chili (Capsicum minimum)	$4.6x10^4$ ($4x10^4$-$5x10^4$)	-	$3.3x10^3$ ($1.9x10^1$-$4.3x10^3$)	$9x10^3$ ($8.2x10^1$-$9.6x10^1$)	-	-	$3.3x10^4$ ($3x10^4$-$4.3x10^4$)	-	$4x10^2$ ($2.9x10^2$-$5x10^2$)
	thiopian caraway (Trachyseprum ammi)	$1.2x10^4$ ($9.4x10^3$-$2x10^4$)	-	$2.6x10^4$ ($2x10^4$-$3.6x10^4$)	$2.2x10^3$ ($1.9x10^3$-$3x10^3$)	-	-	-	-	$3x10^3$ ($2.4x10^3$-$5x10^3$)
	False cardamom (Aframomum korarima)	$3.4x10^3$ ($9.7x10^2$-$5x10^3$)	-	$2.6x10^4$ ($1x10^4$-$3x10^4$)	-	-	-	-	-	$3.3x10^3$ ($2.4x10^3$-$5x10^3$)
	Malt ('Bikil')	$2x10^4$ ($5.5x10^3$-$2.2x10^4$)	$5.5x10^4$ ($3x10^4$-$9.5x10^4$)	$3x10^4$ ($1.9x10^4$-$5x10^4$)	$1.4x10^5$ ($9.1x10^4$-$2.4x10^5$)	-	-	$9.5x10^4$ ($8x10^4$-$1.2x10^5$)	$5.9x10^5$ ($3.6x10^5$-$8x10^5$)	-
	Starter	$1.6x10^4$ ($5.5x10^3$-$2.2x10^4$)	$3.2x10^3$ ($2x10^3$-$5x10^3$)	$5.1x10^3$ ($4x10^3$-$7x10^3$)	-	$3.5x10^6$ ($2x10^6$-$5x10^6$)	$1.5x10^5$ ($2x10^4$-$4.5x10^5$)	-	$4x10^6$ ($2.8x10^6$-$6x10^6$)	-
Equipment	`Gan' (Earthen Jar)	-	-	1.6x104 ($8.1x10^3$-$2x10^4$)	-	-	-	$3.5x10^2$ ($2..8x10^2$-$4x10^2$)	$6.5x10^4$ ($6.1x10^4$-$7.2x10^4$)	$1x10^4$ ($1.1x10^3$-$1.7x10^4$)

Despite its presence along ingredients, none of the *Rhodotorula* spp. were isolated from `shamita'. Basically, *Rhodotorula* spp. has no fermentative ability and they are only contaminants (Collins and Lyne, 1976). In African beers, isolates of *Saccharomyces cerevisiae* predominates varieties of yeast flora (Novellie and De Schaepdrijver, 1979).

An increase in the count of lactic acid bacteria (as high as 10^9 CFU/ml) was accompanied by fall in pH of `borde' and `shamita' fermentation. In both cases, the initial phase of fermentation appeared to be carried out solely by members of *Enterobacteriaceae* (Tables 4 and 5). Their presence, along with other bacteria, lowered the pH to below 4.47 and 4.4 in `shamita' and `borde', respectively at the end of fermentation. Similarly, the initial fermentation of `tef' dough is known to be run by gram-negative aerogenic rods including *Enterobacter, Hafnia, Citrobacter, Klebsiella, Escherichia* and *Proteus* reducing the pH to 5-5.5 (Berhanu *et al.*, 1982). Although *Bacillus* spp. may not have major fermentative role at the presence of high counts of lactic acid bacteria, both *Micrococcus* species and *Bacillus* spp. are also important to certain degree at the initial stages of fermentation. Micrococci may acidify the flour-and-water paste while *Bacillus* spp. may metabolise heterofermentatively, producing lactic acid, gas, alcohol, acetoin and small amounts of esters and aromatic compounds (Anon, 1980 as cited in Mogessie and Tetemke, 1995). Spores of *Bacilus subtilis, B. firmus, B. Coagulans* and *B. Licheniformis* have been reported, to be consistently isolated from kocho and bulla samples of high acidity (pH 4-5) (Meaza and Berhanu, 1985).

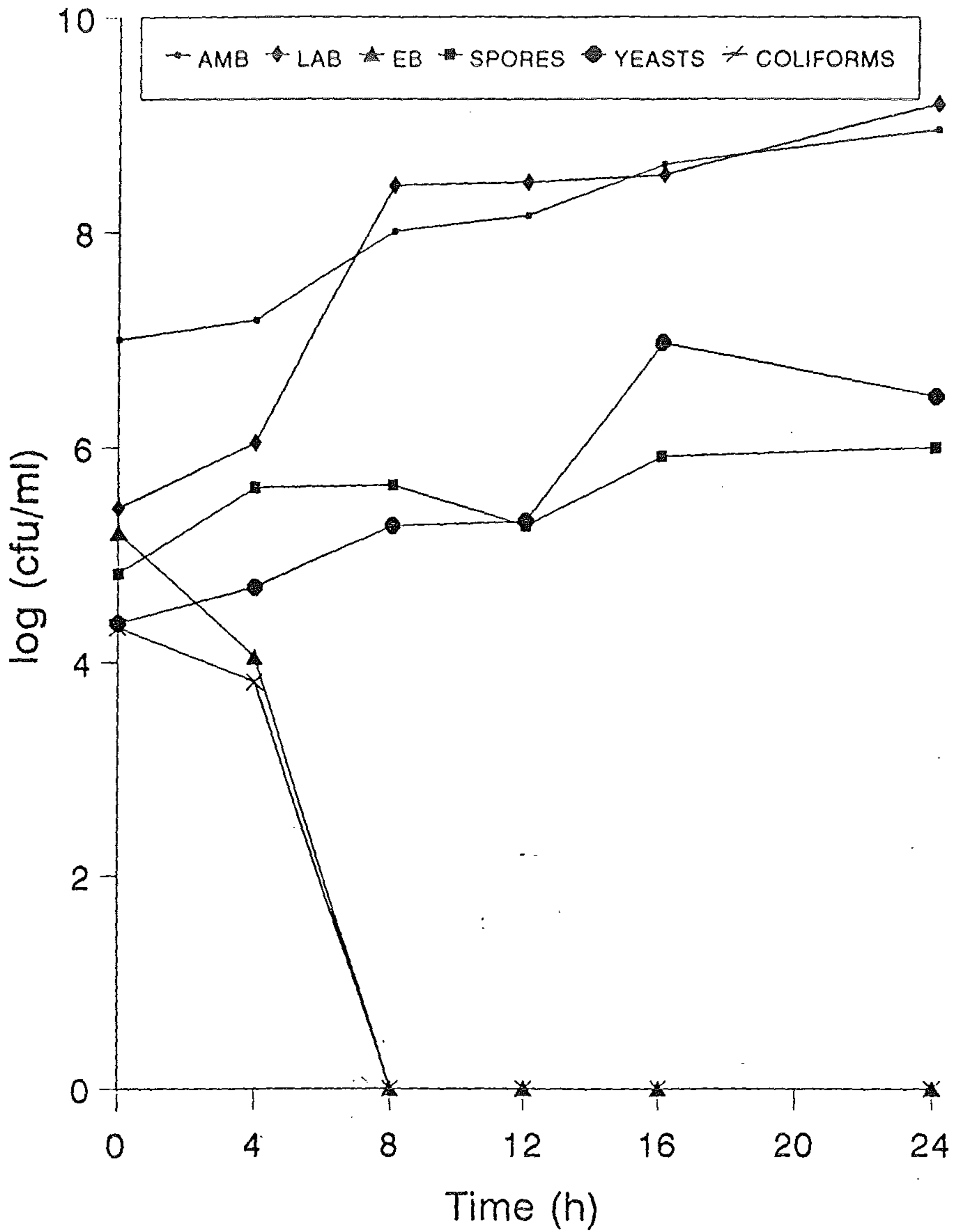

Fig. 3. Growth dynamics of various groups of microorganisms during 'Borde' fermentation. (AMB, Aerobic Mesophilic Bacteria; LAB, Lactic Acid Bacteria; EB, Enterobacteriaceae

Table 3

Count of the various microbial groups isolated from ingredients and equipment used for `Shamita' fermentation (CFU/ml)

Ingredients and Equipment		Aerobic mesophilic count	Lactic acid bacteria	*Enterobacteriaceae*	Coliforms	Bacterial Spore	Yeas	Mold
Ingredients	Roasted barley powder	8.8×10^{4} (8×10^{4}-9.6×10^{4})	-	2.1×10^{3} (1×10^{3}-3×10^{3})	-	1.5×10^{4} (9.7×10^{3}-2.5×10^{4})	-	
	Ground Linseed	3.5×10^{4} (2×10^{4}-5×10^{4})	-	6×10^{3} (5×10^{3}-8×10^{3})	4×10^{3} (2×10^{3}-6×10^{3})	2.0×10^{4} (1×10^{4}-2.7×10^{4})	-	
	Ground chili (*Capsicum minimum*)	9.3×10^{4} (9×10^{4}-9.6×10^{4})	9×10^{3} (8.2×10^{3}-9.6×10^{3})	3.3×10^{4} (3×10^{4}-4.3×10^{4})	2×10^{3} (8×10^{2}-5×10^{3})	2.2×10^{4} (2×10^{4}-3.1×10^{4})	4×10^{2} (2.9×10^{2}-5×10^{2})	$1X10^{3}$ ($2.8X10^{2}$-3.10^{3})
	Ethiopian Caraway (*Trachysperum ammi*)	4.3×10^{5} (5×10^{4}-9×10^{5})	2.2×10^{3} (1.9×10^{3}-3.0×10^{3})	-	-	8.5×10^{4} (7.8×10^{4}-9.1×10^{4})	3.3×10^{3} (2.4×10^{3}-5×10^{3})	$1X10^{3}$ ($2.5X10^{2}$-$1.5X10^{3}$)
	False cardamom (*Aframomum korarima*)	2.6×10^{5} (1×10^{5}-4×10^{5})	-	-	-	9.8×10^{4} (9.5×10^{4}-9.9×10^{4})	-	$2.6X10^{4}$ ($2X10^{3}$-$4X10^{4}$)
	Malt (or `Bikil')	3.1×10^{6} (2×10^{6}-4×10^{6})	1.4×10^{5} (9.4×10^{4}-2×10^{5})	9.5×10^{4} (8×10^{4}-1.2×10^{5})	5.2×10^{4} (3×10^{4}-7×10^{4})	3.6×10^{3} (2.5×10^{3}-4.2×10^{3})	5.6×10^{5} (3.6×10^{5}-8×10^{5})	$4X10^{3}$ ($2.7X10^{3}$-$4.8X10^{3}$)
Equipment	'Gan' (Earthen jar)	1.2×10^{5} (9×10^{4}-1.6×10^{5})	-	3.5×10^{2} (2.8×10^{2}-4×10^{2})	1.1×10^{3} (8×10^{2}-2×10^{3})	-	7.5×10^{4} (6×10^{4}-8.6×10^{4})	$4X10^{3}$ ($2X10^{3}$-$5X10^{3}$)

Table 4

Variation in the population of major organisms in the course of `borde' fermentation (CFU/ml)

Duration of fermentation (hrs)	*Bacillus* spp.	*Lactobacillus* spp.		*Straptococcus* spp.	*Staphylococcus* spp.	*Micrococ-cus* spp.	Entero-Baecteriaceae	*Sacchromyces* spp.	*Rhadotorula* spp.
		Homo-fermentative	Hetero-fermentative						
0	$2.6x10^5$ $(5x10^4-7x10^5)$	-	$2.5x10^5$ $(1.2x10^5-3.4x10^5)$	$3.4x10^4$ $(1x10^4-4.8x10^4)$	$3.6x10^4$ $(2x10^4-5x10^4)$	$9.3x10^4$ $(2x10^4-1.6x10^5)$	$1.6x10^5$ $(1.5x10^4-2.7x10^5)$	$3.1x10^4$ $(1.8x10^4-4x10^4)$	-
4	$3x10^5$ $(7x10^4-7.7x10^5)$	-	$1.1x10^6$ $(5.8x10^5-1.6x10^6)$	$3.3x10^5$ $(0-1x10^6)$	$1.2x10^5$ $(7.2x10^4-1.9x10^5)$	$8.6x10^5$ $(5x10^5-1.1x10^6)$	$1.1x10^4$ $(9x10^1-1.5x10^4)$	$4.6x10^4$ $(2.8x10^4-6x10^4)$	-
8	$5.1x10^6$ $(7x10^4-7.6x10^5)$	$1.4x10^6$ $(0-4.2x10^6)$	$1.7x10^8$ $(7.6x10^7-2.5x10^8)$	$9.5x10^7$ $(6x10^7-1.2x10^8)$	$1.4x10^5$ $(2x10^5-2.2x10^5)$	$1x10^6$ $(6x10^5-1.6x10^6)$	-	$1.8x10^5$ $(9x10^4-2.5x10^5)$	-
12	$5.1x10^6$ $(4.5x10^5-6x10^6)$	-	$1.7x10^8$ $(9.1x10^7-2.2x10^8)$	$1.2x10^8$ $(5x10^6-2.2x10^8)$	$5.7x10^5$ $(4x10^4-8.7x10^5)$	$1x10^7$ $(5x10^6-1.7x10^7)$	-	$1.9x10^5$ $(1x10^5-2.5x10^5)$	$2.2x10^4$ $(0-6x10^4)$
16	$7.2x10^6$ $(6x10^6-9.2x10^6)$	-	$2.1x10^8$ $(1x10^8-2.8x10^8)$	$1.4x10^8$ $(7x10^6-2.3x10^8)$	$7x10^5$ $(1x10^5-1x10^6)$	$6.3x10^7$ $(2x10^7-1.6x10^8)$	-	$9.2x10^5$ $(8x10^5-1x10^6)$	-
24	$1x10^6$ $(8x10^5-1.8x10^6)$	-	$9.8x10^8$ $(8.6x10^8-1.1x10^9)$	$7x10^5$ $(1x10^5-1x10^6)$	$7x10^5$ $(1x10^5-1x10^6)$	$3.1x10^8$ $(5.5x10^7-8x10^8)$	-	$3.6x10^6$ $(9.5x10^5-5.5x10^6)$	$3.3x10^4$ $(0-1x10^5)$

Heterofermentative *Lactobacillus* spp. and *Streptococcus* spp. were the most abundant group of lactic acid bacteria in `borde' fermentation (Table 4). Their number increased throughout the 24 hours of fermentation. They appeared to increase further until the pH of the fermenting mash limited their multiplication. In lactobacilli, growth usually ceases when pH 4.0-3.6 is reached (Kandlar and Weiss, 1986). Likewise, *Lactobacillus plantarum* and *Streptococcus lactis* are reported to be the most abundant and predominant lactic acid bacteria that increase progressively to the end of `obiolor' fermentation (Achi, 1990).

In `shamita' fermentation too, the hetrofermentative *Lactobacillus* spp. were found important. Thus, the fermentation is largely carried out by hetrofermentative lactic acid bacteria (Table 5). Furthermore, the absence of *Streptocociss* spp. and *Pediococcus* spp. among isolates of lactic acid bacteria suggests that hetero and/or homofermentative *Lactobacillus* spp. are the major groups in `shamita fermentation. The simultaneous fermentative importance of both homofermentative lactobacilli (eg. *Lactobacillus plantarum* and *L. casei*), and heterofermentative lactobacilli (eg. *Lactobacillus brevis* and *L. buchneri*) in Kenyan busaa has been reported (Nout, 1980). *Lactobacillus plantarum* is the commonest lactic acid bacteria in the fermentation of Mexican `pulque' (Sanchez-Marroquin and Hope, 1953), Ghanian wine (Nyako, 1977), Korean yakju and takju (Kim, 1968), Nigerian burukutu (Owuama, 1991) and `obiolor' (Achi, 1990). Lactobacilli are responsible for the first phase of `pito' and kaffir beer fermentation, namely the souring phase. In kaffir beer, thermophilic lactobacilli are particularly important (Van der Walt, 1956).

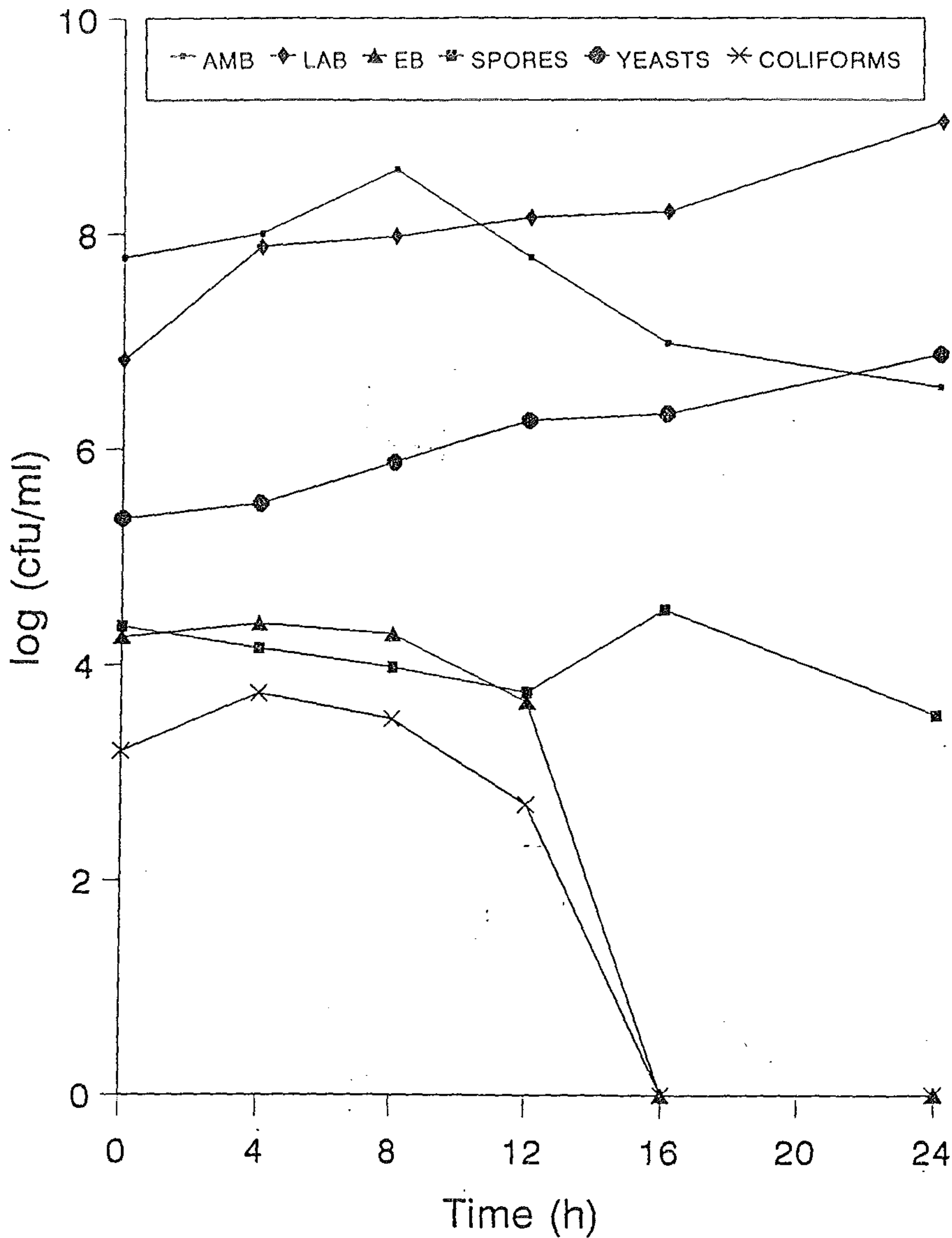

Fig. 4. Growth dynamics of various groups of microorganisms during 'Shamita' fermentation. (AMB, Aerobic Mesophilic Bacteria; LAB, Lactic Acid Bacteria; EB, Enterobacteriaceae

Table 5
Variations in the populations of dominant organisms in the course of `Shamita' fermentation

Duration of Fermentation (hrs)	*Bacillius spp.*	*Lactobacillus* spp.		*Streptococcus* spp.	*Staphylo-Coccus* spp.	*Micrococ-cus* Spp.	*Entero bacteriaceae*	*Saccharomyces* spp	*Rhodotorula* spp. + other yeasts
		Homo-fermentative	Hetero-fermentative						
0	2.4×10^4 (1×10^4-3.2×10^4)	6.4×10^6 (1×10^6-9.2×10^6)	3×10^4 (0-6×10^4)	-	2.1×10^4 (1.5×10^4-2.5×10^4)	1.5×10^4 (1.2×10^4-2×10^4)	1.8×10^4 (3×10^3-5×10^4)	2.2×10^5 (1.5×10^4-3.5×10^5)	-
4	7.5×10^3 (1.5×10^3-2×10^4)	6.9×10^6 (5×10^6-8×10^6)	3×10^6 (2×10^6-8×10^6)	-	5.7×10^3 (1×10^3-1.5×10^4)	7.2×10^4 (3.3×10^4-1.5×10^5)	2.1×10^5 (4.5×10^3-6.5×10^4)	3.0×10^5 (8×10^4-4.3×10^5)	-
8	1.3×10^4 (4.2×10^3-3×10^4)	7.6×10^6 (2×10^6-1.1×10^7)	1.1×10^8 (9.7×10^7-1.5×10^8)	-	5.4×10^3 (3×10^3-1×10^4)	1.1×10^6 (2×10^4-2×10^6)	1.7×10^4 (3×10^3-5×10^4)	7.4×10^5 (2.5×10^4-1.2×10^6)	-
12	3.1×10^4 (6×10^3-2×10^4)	3.2×10^7 (8.8×10^7-1.5×10^8)	1.3×10^8 (7×10^7-2.0×10^8)	-	1.8×10^4 (5×10^3-4.5×10^4)	1.6×10^4 (5×10^3-4×10^4)	4.7×10^3 (2.5×10^3-8.6×10^3)	1.8×10^6 (3×10^4-2.8×10^6)	-
16	2.6×10^4 (8.2×10^3-1×10^4)	4.4×10^7 (1×10^7-6.3×10^7)	1.2×10^8 (9.5×10^7-1.59×10^8)	-	1.9×10^4 (8×10^3-4×10^4)	2.9×10^6 (4×10^5-4.3×10^6)	-	2.1×10^6 (1.5×10^5-3.3×10^6)	-
24	4.6×10^3 (0-1×10^4)	1.0×10^9 (8.7×10^8-1.2×10^9)	2.2×10^8 (1.8×10^8-2.8×10^8)	-	1.4×10^4 (0-4×10^4)	5×10^3 (0-1×10^4)	-	7.6×10^6 (2.5×10^6-1.0×10^7)	-

In as much as the *Lactobacillus* spp. appear desirable for fermentation, they may also cause spoilage in most other foods and beverages. It has been reported (Sharpe, 1981 as cited in Sneath *et al*, 1986; Steinkraus, 1983; Kandler, 1984 as cited in Sneath *et al*, 1986) that lactobacilli are responsible for the production and spoilage of fermented vegetable feed and foods (eg. Silage, Sauerkraut, mixed pickles) and beverages (eg. beer, wine and juices). The chiefly isolated species of *Lactobacillus* from these fermented beverages and vegetables have been *Lactobacillus plantarum, L. brevis, L. coryniformis, L. casei, L. sake* and *L. fermentum*. Both fermented products in our study are consumed within a few hours time due to their short shelf life and poor keeping quality. The lactobacilli may result in undesirable souring at longer keeping.

Morphologically distinct molds of low count were encountered both in ingredients and at the early stage of fermentation. As the activities of bacteria in the mash resulted in drop in pH in the course of fermentation, and as the yeasts created anaerobiosis by quick absorption of oxygen, the molds were inhibited and finally eliminated from the mash. Usually, molds are associated with grains and malts where conditions are conducive for their proliferation. It appears that different molds involve in the conversion of starch to sugar during steeping and malting. This could be the case at least in various alcoholic beverages including Japanese sake (Yoshizawa, 1977; Kodama and Yoshizawa, 1977), Korean yakju and takju (Park *et al.*, 1977) and `pito' (Ekundayo, 1969) where the fermentable carbohydrate is starch. *Pencillium* spp; *Rhizopus oryzae*, and *Aspergillus* spp. are isolated from the surface of cereal grains during steeping and malting of grains for `pito' fermentation (Ekundayo, 1969).

During malting, the saccharifying agent could be the molds and cereal amylases (Ekundayo, 1969) or only the cereal amylases, fungal activities having no importance (Novellie, 1965; 1966a,b, 1968; Van der Walt, 1956). The result of observation made by Novellie and De Schaepdrijver (1979) has made the importance of fungi in African brewing doubtful. According to the report, a heavy growth of mold on a malt bed has yielded a product of greatly reduced diastic activity.

The overall change in pH during `borde' fermentation within the 24 hours duration were in the range of 5.2-3.6 (Table 6). In `obiolor' fermentation, beverage related to `borde', the activities of lactic acid bacteria has reduced the pH from 6.81 to 4.9 within the same duration of fermentation (Achi, 1990).

In `shamita' fermentation, however, it were between 5.82-3.98 having `low' acidity as compared to `borde' (Table 7). In principle, `shamita' that was dominated by the hetrofermentative *Lactobacillus* spp. in the presence of homofermentatives should have more acidic product than `borde' where the heterofermentative *Lactobacillus* spp. play the primary fermentative role. In fact, the count of lactic acid bacteria could have an influence on the rate of drop in pH. With this respect, the higher count of *Lactobacillus* spp. in the starter used for `borde' fermentation could be responsible for the rapid drop in pH of `borde' in the course of fermentation. Similarly Nout *et al.* (1989, 1992), suggested the recycling of inoculum (`back sloping') for *accelerated* fermentation as a simple technique particularly suitable for house-hold small scale commercial fermentation operations with only rudimentary facilities.

Table 6

Some Physical and chemical parameters determined in the cours of `borde' fermentation

Duration of fermentation (hrs)	pH	Temperature (^{0}c)		Moisture content (%)	Total solid (%)	Reducing sugars (mg/ml)	Protein (mg/ml)	Fat (%)	Ash (%)
		Room	Mash						
0	5.00 (4.82-5.20)	19 (18.0-19.6)	21.9 (21-22.5)	80.3 (79.5-81.2)	16.69 (18.7-20.4)	0.82 (0.6-0.92)	80.5 (77.5-85.5)	10.92 (10.35-11.42)	1.83 (1.70-1.94)
4	4.28 (4.17-4.43)	20 (19.8-20.5)	22.5 (22.1-23.0)	82.29 (80.8-83.1)	17.8 (16.8-19.2)	0.97 (0.86-10.6)	88.1 (85.5-91.0)	13.0 (12.7-13.47)	1.66 (1.60-1.72)
8	3.96 (3.76-4.20)	23.4 (22.5-24)	22.6 (22-23)	83.38 (82.2-84.3)	16.61 (14.7-17.8)	1.10 (1.05-1.2)	84.3 (83.7-85.5)	13.76 (13.1-14.2)	1.58 (1.50-1.64)
12	3.80 (3.75-3.91)	23.1 (22.5-24.2)	22.4 (21.9-23)	81.38 (78.1-83.8)	18.61 (16.2-21.9)	1.06 (1.00-1.26)	84.1 (82.7-86.0)	9.41 (7.89-10.36)	1.47 (1.16-1.75)
16	3.76 (3.72-3.82)	23 (22.5-24)	22.3 (21.6-22.8)	82.93 (79.8-84)	17.06 (16-20.2)	0.88 (0.78-0.96)	82.8 (82.0-84.0)	14.09 (13.89-14.4)	1.92 (1.80-2.10)

Microbiologically, the locally collected samples had an overall similarity to the laboratory prepared ones (Table 8). It has been common experience to observe local brewers adding new adjunct (usually roasted ground barley) to the over fermented product to "re-fresh" it. By so doing, they tend to reduce sourness at least temporarily for immediate serving. Samples taken under such a circumstance could have mixed microbial population. On the whole, the microbial content of the locally fermented product was slightly higher than that prepared in the laboratory as the case is with `obiolor' (Achi, 1990). In traditionally fermented beverages, the handling and consumption often takes place under conditions of poor hygiene.

It appears that the ingredient used for `borde' fermentation varies from place to place and/or communities accustomed to `borde' preparation. According to earlier study (Mogessie and Tetemke, 1995), maize (*Zea mays*) has been the common ingredient of `borde' preparation among people in Awassa (a town 275 km South of Addis Ababa) and its surrounding. But in Addis Ababa, were this study was conducted, wheat (*Triticum* spp.) is the ingredient most preferred. In principle, the procedures involved in its preparation are similar except that addition of ground barley (*Hordeum vulgare*) whipped in hot water was uncommon (Fig. 1). The purpose of addition of whipped ground barely is meant to enhance gas production (Mogessie and Tetemke, 1995) even though its absence in preparation process for this study did not markedly affect gas production. Vigorous gas production was observed in `shamita' fermentation too.

Table 7

Some physical and chemical parameters measured in the course of `Shamita' fermentation

Duration of Fermentation (hrs)	pH	Temperature (°c) Room	Temperature (°c) Mash	Moisture content (%)	Total solid (%)	Reducing sugar (mg/ml)	Protein (mg/ml)	Fat (%)	Ash (%)
0	5.80 (5.79-5.82)	21.7 (21.5-22.0)	18.1 (17.0-18.7)	83.3 (81.5-84.4)	16.6 (15.5-18.5)	1.57 (0.73-2.0)	37.2 (35-38.6)	10.3 (9.2-11.9)	2.49 (2.27-3.02)
4	5.52 (5.50-5.56)	24.3 (24.0-25.0)	18.7 (18.0-19.2)	81.7 (80.9-82.4)	18.1 (17.5-19.1)	0.88 (0.1-1.33)	48.0 (45.5-49.5)	20.2 (19.2-21.6)	1.64 (1.51-1.93)
8	4.77 (4.76-4.80)	23.7 (23.0-25.0)	20.9 (20.0-21.4)	81.3 (79.8-82.2)	18.6 (17.7-20.2)	0.58 (0.16-0.8)	42.1 (40.3-43.2)	15.0 (13.6-16.9)	2.85 (2.83-2.92)
12	4.43 (4.41-4.47)	22.9 (22.8-23.2)	20.6 (20.0-21.0)	81.2 (79.5-82.3)	18.7 (17.7-20.4)	0.24 (0.03-04)	39.2 (37.2-40.5)	14.3 (13.4-15.0)	3.38 (3.03-4.12)
16	4.26 (4.23-4.31)	22.9 (22.8-23.0)	21.2 (20.5-21.7)	81.9 (81.0-83.0)	18.0 (17.0-19.8)	0.20 (0.02-0.50)	(32.4 (31.1-33.2)	13.4 (12.2-14.8)	3.54 (3.23-4.2)
24	4.03 (3.98-4.12)	22.4 (22.1-23.0)	22.0 (21.0-22.6)	87.0 (86.8-88.3)	12.3 (11.7-13.2)	0.20 (0.01-0.46)	30.6 (29.5-31.5)	13.1 (11.3-14.9)	3.82 (3.44-4.62)

Table 8

Microflora of Locally Collected `Shamita' Samples

Sample No.	Aerobic mesophilic count	Lactic acid bacteria	*Enterobacteriaceae*	Yeasts	Molds	*Bacillus* spp.	*Lactobacillus* spp.	*Streptococcus* spp	*Staphylococcus* spp.	*Mirococcus* spp.	*Saccharomyces* spp.
1.	$2.6x10^7$	$1.4x10^7$	$1.2x10^4$	$8.2x10^6$	$2.8x10^3$	$1.6x10^6$	$1.4x10^7$	-	$1x10^5$	$1.4x10^6$	$8.2x10^6$
2.	$4.2x10^6$	$5x10^7$	$5.1x10^3$	$2.7x10^6$	-	$2.6x10^5$	$4.9x10^7$	$1x10^6$	$2.1x10^4$	$2.5x10^4$	$2.7x10^6$
3.	$4.8x10^8$	$2.3x10^6$	-	$3.8x10^7$	-	$2.8x10^7$	$2.1x10^6$	$2x10^6$	$2.6x10^3$	$2.3x10^4$	$3.8x10^7$
4.	$4.3x10^9$	$3.5x10^6$	-	$3.6x10^7$	$1x10^3$	$2.4x10^7$	$3.3x10^6$	$2x10^6$	$2.3x10^4$	$4.5x10^5$	$3.6x10^7$
5.	$3.9x10^7$	$5.1x10^5$	$6.2x10^3$	$1.8x10^6$	$1x10^4$	$2.3x10^6$	$5.1x10^6$	-	$1.7x10^5$	$3.5x10^6$	$1.8x10^6$
6.	$4.2x10^8$	$6.7x10^7$	-	$4.1x10^7$	-	$2.2x10^7$	$6.7x10^7$	-	$1.8x10^4$	$4x10^5$	$4.1x10^7$
7.	$2.5x10^7$	$6.8x10^5$	$1.5x10^4$	$2.9x10^6$	$2.1x10^3$	$1.3x10^6$	$6x10^5$	$8x10^4$	$1x10^4$	$2x10^5$	$2.9x10^6$
8.	$3.8x10^6$	$5.8x10^6$	-	$3.2x10^7$	-	$2x10^5$	$5.8x10^6$	-	$1.8x10^3$	$5x10^3$	$3.2x10^7$
9.	$1x10^8$	$5.6x10^5$	-	$4.7x10^6$	$1.4x10^2$	$5.4x10^6$	$5.6x10^5$	-	$5x10^4$	$9x10^4$	$4.7x10^6$
10.	$2.3x10^7$	$3.2x10^6$	-	$2.9x10^7$	$1.3x10^3$	$1x10^6$	$3.2x10^6$	-	$1x10^5$	$3x10^4$	$2.9x10^7$
Lab.* brewed	$4.4x10^7$	$1.4x10^8$	$4.5x10^3$	$1.8x10^6$	$6.5x10^4$	$3.1x10^4$	$1.6X10^8$	-	$1.8X10^4$	$1.6X10^4$	$1.8X10^6$

*Represented by the sample taken at the 12th hour of fermentation.

Table 9

Some physical and chemical parameters of locally collected `Shamita' samples

Sample No.	pH	Moisture content (%)	Total solid (%)	Reducing sugar (mg/ml	Protein (mg/ml	Fat (%)	Ash (%)
1	4.43	79.17	20.8	0.34	35.0	25.3	3.00
2	4.38	87.98	12.0	0.10	35.0	25.1	3.82
3	3.98	74.55	25.4	0.14	30.0	23.9	2.76
4	4.15	77.08	22.9	0.21	41.0	24.9	2.78
5	4.41	82.96	17.0	0.22	34.5	25.8	2.76
6	3.99	76.12	23.8	0.19	30.5	23.7	2.48
7	4.36	82.12	17.8	0.23	35.5	24.8	2.86
8	4.06	78.00	22.0	0.18	31.0	23.0	3.28
9	4.22	79.92	20.0	0.19	42.2	24.2	3.08
10	4.18	79.69	20.3	0.21	41.8	23.8	3.1
*Lab. Brewed	4.43 (4.41-4.47)	81.2 (79.5-82.3)	18.7 (17.7-20.4)	0.24 (0.03-0.4)	39.2 (37.2-40.5)	14.3 (13.4-15.0)	3.38 (3.03-4.12)

Represented by the sample taken at the 12th hour of fermentation.

The concentration of reducing sugars increased for the first few fermentation hours of 'borde' followed by substantial drop (Table 6 and 7). An increase in reducing sugar concentration in the early part of fermentation could be attributed to an activity of amylase that originate from the microorganisms and malt used. Kanazas and Field (1981) have showed that fermentation of sorghum improves the amount of available amino acid and total reducing sugars. A six-fold increase in reducing sugar concentration in the first part of *tef* dough fermentation has been reported (Berhanu *et al.*, 1982).

The fall in reducing sugar concentration at the later part of both `borde' and `shamita' fermentation could be attributed to the continuous utilization of these sugars from the mash during fermentation and/or inhibition of microbial amylase by the drop in pH. Amylase producing *Bacillus* spp. are usually inhibited at lower pH. Through spontaneous lactic acid fermentation of carbohydrates by lactic acid bacteria under almost oxygen-free conditions, the pH usually drops to 3.5-4.0. But already at pH value of 4.5 the carbohydrate source is preserved from further spoilage (Johansson, 1995; Lindberg, 1997). Although most of the microorganisms involved in `borde' and `shamita' fermentation could produce amylases that hydrolyze starch, some of the microorganisms only ferment the sugars. *Saccharomyces* spp. mainly *Saccharomyces cerevisiae* is known to ferment sucrose usually in preference to other sugars.

Glucose and maltose have been used up through out `burukutu' fermentation unlike the unfermentable malto-oligosaccharides (Faparusi, 1970). Thus, it could be the continuous fermentation of some of the reducing sugars (eg. glucose and maltose) from

the fermenting mash that accounts for the over all fall in reducing sugar concentration in the course of fermentation of `borde', `shamita', `burkutu' (Faparusi, 1970), `pito' (Ekundayo, 1969) and `obiolor' (Achi, 1990). On the other hand, in most of African traditionally fermented beverages, amylolytic enzymes of malt make sugar in ample amount before an extreme fall in pH of the fermenting mash occurs (Novellie and De Schaepdrijver, 1979). In this case, it appears that the drop in reducing sugar concentration in the later parts of `borde' and `shamita' fermentation after tremendous rise at the very start could not be due to total depletion of carbohydrate source from the mash, but inhibition of carbohydrate hydrolysis by the extreme fall in pH of the mash. In support of this, starch amylosis failed to continue during later stage of `burukutu' fermentation despite the presence of carbohydrate source (Faparusi, 1970).

Some of the preparatory techniques being implemented during traditional fermentation of foods or beverages appear to enhance microbial growth and enzymatic activities, hence nutrient availability. The roasting of wheat flour moistened in sufficient water during `borde' preparation could serve to gelatinize the substrate so that the breakdown of starch is rapid in producing sugar. Likewise, the importance of gelatinization of starch during `gari' preparation has been stressed (Novellie and Schutte, 1969; Okafor, 1977). Evidence show that pre-heat treatment, and longer fermentation time increases the crude protein contents of maize and sorghum irrespective of the type of microorganisms involved in the fermentation process (Abasiekong, 1991). This has been attributed to rise in microbial count, hence unicellular protein, as heat treatment makes grain a better substrate for microbial growth. Heat disruption of starch granules is believed to speed up its break down by amylolytic enzymes.

Both in `borde' and `shamita' the concentration of soluble protein increased, at least for the first 12 hours of fermentation when the products were ready for consumption, as compared to its concentration at the very beginning of the fermentation. It appeared to decrease in the later periods of fermentation (Table 6 and 7). In `borde', although there was tendency for soluble proteins concentration to fall from fourth hours onward, the concentration was still high at the 16th hour of fermentation (Table 6). Unlike amylolytic enzymes, the optimum pH for proteolysis is on the acid side. Thus, the falling pH of the souring mash appears to have no limiting effect until the very last stage of acidification. With this in mind, the slight drop in the concentration of soluble proteins in the later parts of both `borde' and `shamita' fermentation (at the time of spoilage) could be attributed to its utilization by microorganisms at the rate that exceed proteolytic activity in the mash. Microorganisms use protein not only as a source of carbon and energy, but also as the sole source of nitrogen. Protein make 40-50% of dry weight of microorganisms. In yeast, it varies from 30-65% depending on the yeast strain and substrate used (Laskin, 1977).

Loss of nitrogen during fermentation is reported in various traditional fermented products. Abraham, Haile and Taye (1979) reported loss of about 16% of nitrogen in kocho fermentation. They attributed the loss to biological degradation and leaching. Furthermore, 4-13% loss in nitrogen from *tef* dough fermented for about 96 hours has been reported (Berhanu *et al.*, 1982). In this study loss in soluble nitrogen within the 24 hours of fermentation of `shamita' amounted to 15.7-18.3% and there was hardly any loss of nitrogen from `borde' (Tables 6 and 7). Wang and Hesseltine (1966) have found out that the concentration of protein remains constant during wheat flour fermentation for bread making.

With reference to the 12 hour duration of fermentation, the soluble protein content of each beverage increased (these beverages are ready for consumption after an over-night fermentation). In agreement with this result, fermentation of spent sorghum mash and brewer's dry grain have been reported to end up with increment of protein content (Oyegbile, 1988 as cited in Abasiekong, 1991).

From this study, it could be suggested that further loss in the protein content of both `shamita' and `borde' could be avoided by terminating the fermentation process before the pH of the fermenting mash drop beyond 4.41 and 3.72-3.83, respectively (Table 6 and 7).

Both `borde' and `shamita' appeared to have short shelf-life as the product turned too sour to consume within less than a day. `Kaffir ' beer and `pito' also deteriorate rapidly on storage, the products become undrinkable within about 24 hours of preparation. In some alcoholic beverages, hops has been used to make beverages biologically stable (Prescott and Dunn, 1959). Addition of hops to honey wines is a very old technique. Hops are quite popular for the flavour and aroma that it impart to beverages, and its tannin composition. In Ethiopia, leaves and stems of "Gesho" plant (*Rhamunus prinoides*) chopped, sun-dried, and pounded into finer powder appears to serve the purpose of hops. Unlike some of traditional Ethiopian fermented alcoholic beverages (`Tella', `Tej', `Korofe', `Arak', etc.) `Gesho' is not used during `borde' and `shamita' preparation. There is no report, as far as this literature review covered, on the antimicrobial role of `Gesho'.

5. CONCLUSION AND RECOMMENDATION

Clear understanding of the procedures involved in the fermentation, and the identification of microorganisms responsible for `borde' and `shamita' fermentation could help to design mechanism for production of an industrially based finished product. It is worth noting, however, that the scaling-up of indigenous fermented products of `borde' and `shamita' types should be undertaken with great care in order not to lose the nutritive value as well as public acceptance of the beverages. Kaffir beer produced at large-scale breweries with less emphasis to the traditional techniques has shown dramatic decrease in vitamin values (Novellie, 1966c); Kenyan busaa produced by modern technique has lost acceptance by those accustomed to traditional busaa. Thus, if a successful modern process is to be developed, it should utilized the essential steps of the traditional process, make them more hygienic and must yield an authentic product (Novellie and De Schaepdrijver, 1979). Some traditional fermented beverages are already scaled-up. Urbanization and industrialization have been the driving force behind the large-scale development of some traditional fermented beverages such as `bantu' beer. In Nigeria, bottled and preserved palm wine was successfully introduced recently by the Federal Institute of Industrial Research (Nout, 1980). `Pulque' fermentation has been modernized (Sanchez-Marroquin, 1977) with better quality, natural vitamin content and shorter fermentation period. (The product is ready within 48-72 hours unlike the 8-30 days required, otherwise). After an investigation on the microbiology and biochemical changes in the course of `borde' and `shamita' fermentation; and analysis of samples collected from local brewers, the following could be recommended:

1. The spices used in this study were found to harbour significant number of spores and spore-forming bacteria which should not be overlooked. Besides, *Bacillus* spp. were consistently isolated in the course of both `borde' and `shamita' fermentation. But some *Bacillus* spp. such as *Bacillus cereus* are known to be pathogenic to human being. To that effect, the microbiology of spices and the prevalence of human pathogens among Ethiopian spices needs further investigation.

2. One of the major problems associated with `borde' and `shamita' is the poor keeping quality of the products. Lactic acid bacteria are known to eliminate or prevent the growth of pathogenic Gram negative aerogens. On the other hand, however, they render the products - undrinkable (or unacceptable by consumers) by making it too sour. Pasteurization has been reported to greatly lengthen shelf-life of sorghum beer (Novellie and De Schaepdrijver, 1979). Using pasteurization as a preservative method, the "South African Sorghum beer unit" has succeeded in developing two new, non-conventional sorghum product with good keeping quality (the products extend to three or more months which otherwise spoil within not more than three days) (Novellie and De Schaepdrijver, 1979). Thus, there is a need for an evaluation of preservative method(s) applicable to `borde' and `shamita'.

3. Microorganisms responsible for the fermentation of `borde' and `shamita' were described in this study. Still, however, information pertaining to the association between these organisms is rather limited.

Wood and Hodge (1985) and Gobbetti *et al.*,1994 have attempted to make survey of the interaction between yeast and lactic acid bacteria in some fermented foods and beverages. For a fuller and more controlled utilisation of the desired fermentation of both `borde' and `shamita', there is a need for investigation of the physiology and biochemistry of the interaction among microorganisms responsible for the fermentation.

4. The frequency of isolation of mold from malt and early stage of fermentation is high. The moist, warm, and aerobic conditions prevailing during malting encourage malt growth. Although the mere presence of moulds is no indication of mycotoxin formation, mold infection can produce mycotoxins in dangerous quantities without noticeably affecting the fermentations or the taste of fermentation product. Mycotoxin contamination may be possible during growth, harvesting. storage and malting. Mycotoxins, thus constitute a hidden danger to the consumer. To this effect, the prevalence and extent of mycotoxin contamination of ingredients that has been used by local `borde' and `shamita' brewers need analyses. For economic reasons, low-grade cereals could be the choice of brewers.

5 In this study, the general pattern of changes in soluble proteins, reducing sugars and minerals was investigated. The types of amino acids, sugars, and the nature of their dynamics during preparation of borde and shamita need further study. Changes in vitamin content and possible synthesis of vitamins by microorganisms during fermentation need to be evaluated.

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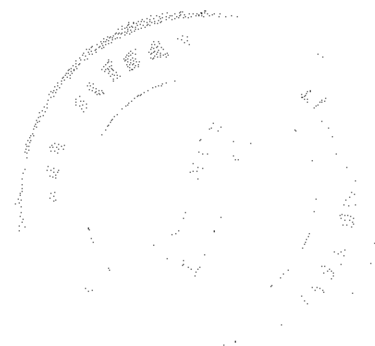

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