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# THE MICROBIAL DYNAMICS OF '*BORDE*' FERMENTATION, A TRADITIONAL ETHIOPIAN FERMENTED BEVERAGE

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**ABSTRACT:** The growth pattern of various microbial groups was analyzed during preparation of '*borde*', a traditional Ethiopian fermented beverage. The ingredients consisted of wheat flour and barley malt and the product was ready for consumption within 12 h of fermentation. Malt contained high counts of aerobic mesophilic bacteria (about $10^6$ cfu $g^{-1}$) along with considerable numbers of lactic acid bacteria (LAB), yeasts and micrococci. The aerobic mesophilic bacterial flora at the start of fermentation was dominated by micrococci, staphylococci, members of Enterobacteriaceae and *Bacillus* spp. Gram positive cocci and rods dominated after 4 h. Coliforms and other members of Enterobacteriaceae were not detected after 4 h. LAB had initial counts of $10^5$ cfu(ml)$^{-1}$ and reached counts as high as $10^9$ cfu(ml)$^{-1}$ at 24 h. Hetero-fermentative lactobacilli dominated the lactic flora throughout the fermentation, although streptococci were considerably high until 12 h. The yeast count was low at the initiation of fermentation and a sharp increase was noted after 12 h. The pH of fermenting '*borde*' declined from 5.2 at the start of fermentation to 3.6 at 24 h.

**Key words/phrases: Beverage, *'borde'*, fermentation, microbial dynamics**

## INTRODUCTION

Traditional fermented beverages are those which are indigenous to a particular area and have been developed by the people of that area themselves using

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age-old techniques from locally available (mostly home-grown) raw materials. In nearly all areas of the world, some types of alcoholic beverages native to the region are prepared and consumed (Pederson, 1979; Steinkraus, 1983). As a result, there are a variety of traditional 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 (Platt, 1955; Aucamp *et al.*, 1961). Among the common traditional fermented beverages in other parts of the world are Kenyan Bussa (Nout, 1980), Mexican pulque (Sanchez-Marroquin, 1977), Bantu beer (Novellie, 1968), Nigerian pito (Ekundayo, 1969), Zambian maize beer (Lovelace, 1977), Japanese sake (Yoshizawa, 1977) and Sudanese merissa (Dirar, 1978).

Ethiopia is one of the countries where a variety of traditional fermented foods and beverages are produced and consumed. The beverages are produced on a fairly small scale and usually for local consumption. Among Ethiopian fermented beverages are varieties of *tella*, *tej*, *katikala (areki)*, *korefe*, *borde*, *shamita*, *keribo*, *imbushbush*, etc.

'*Borde*' is a traditional fermented beverage usually made from maize or wheat. It is a very popular meal replacement in Southern Ethiopia and some other parts of the country (Mogessie Ashenafi and Tetemke Mehari, 1995). '*Borde*' is consumed while actively fermenting and has a short fermentation period, usually overnight. On the average, a labourer consumes about three litres of '*borde*' per day.

Many factors could account for the role that many traditional fermented beverages play as meal replacements. The high microbial load of yeasts and lactic acid bacteria qualify '*borde*' to be a good source of microbial protein (Mogessie Ashenafi and Tetemke Mehari, 1995). The relatively high lysine content of yeast protein would improve the nutritive value if added to grains such as maize, wheat or rice.

This study aims at elucidating the microbial dynamics of '*borde*' fermentation in order to identify the major microorganisms which initiate the fermentation and those that carry out the process and determine the nature of the product.

## MATERIALS AND METHODS

### *Preparation of* 'borde'

The main ingredients used in this study for the preparation of '*borde*' were wheat, the major fermenting component, and barley for the preparation of malt. For malt preparation, barley was cleansed to remove dirt and extraneous materials and steeped in water for about a day. Excess water was strained-off and the soaked barley was allowed to germinate for five days wrapped in banana leaves. After germination the malt was sun-dried and ground finely.

Wheat flour (25 kg) was soaked in excess water and the thick coarse paste was deeply roasted on a hot flat metal pan. After cooling for one hour, about 250 g of ground malt was thoroughly mixed into it. The whole mixture was put into an earthen jar and further blended in about 30 litres of tap water. About one litre of '*borde*' from a previous fermentation was added to it as starter, sealed well with plastic films and clothe and allowed to ferment at ambient temperature for 24 hours (Fig. 1).

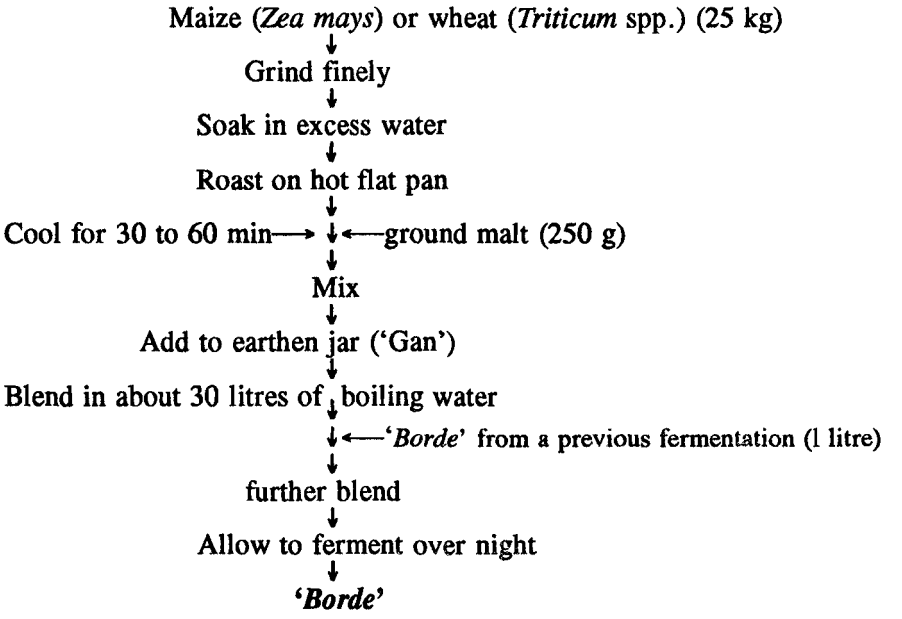


**Fig. 1. Flow chart of '*borde*' fermentation process.**

All the materials used for the fermentation in this study were purchased from a local market in Addis Ababa. All the preparation was made following the traditional fermentation procedures by an experienced female '*borde*' brewer. A total of three different fermentations were carried out in this study and values were averages of the processes.

### *Sampling*

Samples were separately taken from ingredients (25 g) used for '*borde*'. In addition, 500 ml of sample was aseptically removed from the fermenting mash at four-hour intervals for 24 h for microbiological and other analyses.

### *Microbiological analyses*

Twenty-five grams of ingredients and 25 ml of samples drawn at intervals in the course of fermentation were separately blended in 225 ml of sterile physiological saline solution. The samples were homogenized and appropriate dilutions were surface plated on the respective media (all from OXOID) for counting.

Aerobic mesophilic bacteria were counted on Plate Count (PC) agar after incubation at 32° C for 24–48 h. Violet Red Bile (VRB) agar was used to count coliforms. After 24 h incubation at 32° C, purplish red colonies surrounded by red zone of precipitated bile were counted as coliforms. Enterobacteriaceae were counted on Violet Red Bile Glucose (VRBG) agar and incubated at 32° C for 24 h. Purple red colonies were counted as members of Enterobacteriaceae. Staphylococci were counted on Mannitol Salt agar after incubation at 32° C for 36 h. Lactic acid bacteria were counted on de-Mann, Rogossa and Sharp (MRS) agar plates after incubation in an anaerobic jar at 32° C for 48 h. Yeasts and molds were counted on Chloramphenicol-Bromophenol Blue (CBB) agar plates (yeast extract, 5.0 g; glucose, 20.0 g; chloramphenicol, 0.1 g; bromophenol blue, 0.01 g; agar, 15 g; distilled water, 1000 ml; pH, 6.0–6.4). The chemicals were obtained from Sigma. Colonies were counted after incubation at 28–30° C for 4 to 5 days. For spore counting, appropriate dilutions were heat-treated at 80° C for 10 min in a water bath, and plated on PC agar. Colonies were counted after incubation at 32° C for 48 h.

***Flora assessment***

After colony counting, ten to fifteen colonies were picked from countable plates of PC, MRS and CBB agar plates and further purified by repeated plating. Cell shape, cell grouping pattern, motility and presence or absence of endospores were determined microscopically on wet mounts. KOH test was made according to Gregersen (1978) to distinguish between Gram-positive and Gram negative bacteria. Presence of cytochrome oxidase was tested by the method of Kovacs (1956), and catalase test was performed by flooding young cultures with 10% $H_2O_2$ solution (MacFaddin, 1980). Oxidation/fermentation test for Gram negative rods was done on Hugh and Leifson O/F medium as described by Collins and Lyne (1976). For Gram positive cocci, Baird-Parker's modification of Hugh and Leifson medium was used (Collins and Lyne, 1976).

Gram positive, non-spore forming, catalase-negative cocci, coccobacilli or rods isolated from MRS agar plates were considered as lactic acid bacteria and further placed into different groups by testing for gas production in 5% glucose in MRS broth. Incubation was at 32° C for 5 days. Based on the obtained information, identification of the various microbial groups was made according to Sneath *et al.*, (1986), and Lodder (1971). Identification was used to make corrections of counts, particularly of LAB on MRS agar. Counts on MRS agar during 0 h and 4 h of fermentation and counts of LAB from malt were reduced by 1 log unit after identification.

Change in pH in fermenting mash was measured using a pH meter (Beckmann).

## RESULTS AND DISCUSSION

The aerobic mesophilic bacterial count of ground malt used for this study was considerably high (about $10^6$ cfu $g^{-1}$). The flora was dominated by members of Enterobacteriaceae and lactic acid bacteria (LAB) mainly consisting of streptococci. Malt also contained a considerable number of yeasts and micrococci (about $10^4$ cfu $ml^{-1}$) (Fig. 2). As grains are usually contaminated with various types of microorganisms (Nout, 1980; Ogundiwin, 1991), they may also be sources of those found in malt. A variety of microorganisms were also isolated from wheat used for '*borde*' fermentation. *Bacillus* spores,

coliforms, micrococci and a variety of Gram negative rods were reported from flours (Frazier and Westhoff, 1978). '*Borde*' from a previous fermentation used as starter contained high counts of LAB, yeasts and micrococci (Table 1).

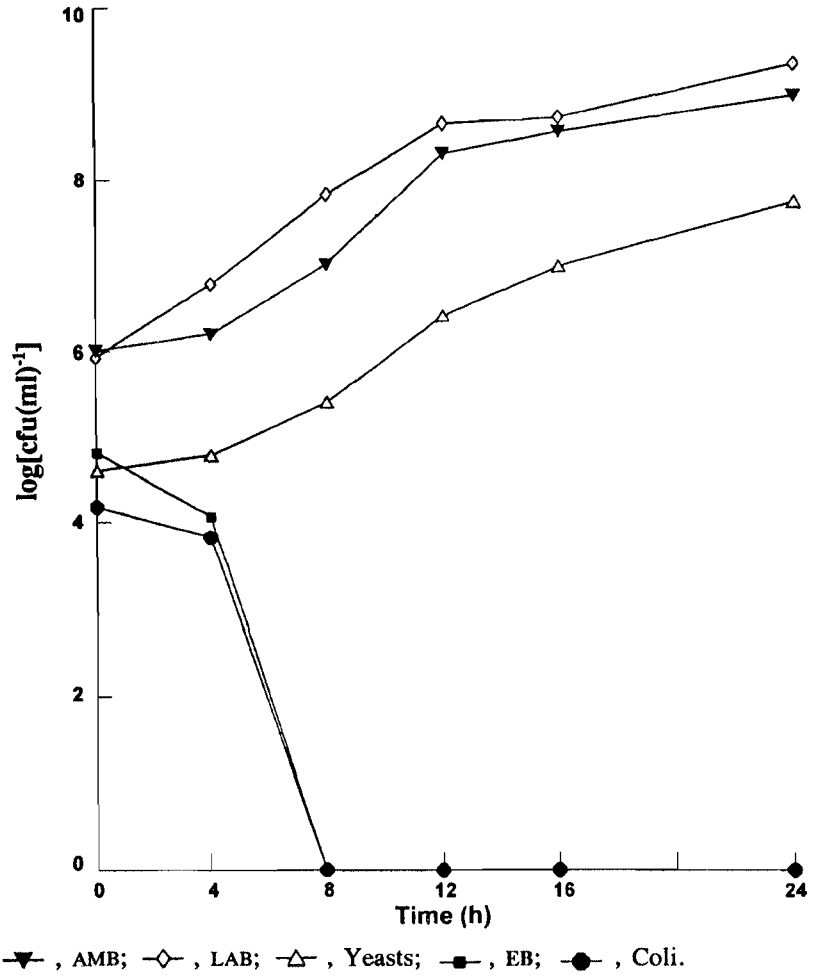


**Fig. 2. Increase in counts of aerobic mesophilic bacteria (AMB), lactic acid bacteria (LAB) and yeasts and elimination of members of Enterobacteriaceae (EB) and coliforms (Coli) during '*borde*' fermentation.**

**Table 1. Dominant microorganisms from ingredients and equipment used for '*borde*' fermentation.**

| Ingredient/ equipment | AMB | *Bacillus* spp | Entero-bacteriaceae | micrococci | LAB | Yeasts | Molds |
|---|---|---|---|---|---|---|---|
| Wheat flour | $6.5x10^4$ | $2.8x10^3$ | $3.2x10^3$ | $4.4x10^4$ | - | - | $1.2x10^3$ |
| Barely malt | $3.2x10^6$ | $1.8x10^4$ | $1.8x10^5$ | $2.1x10^4$ | $1.9x10^5$ | $1.5x10^4$ | $1.4x10^4$ |
| Starter | $5.2x10^8$ | $4.2x10^5$ | $< 10^2$ | $2.5x10^6$ | $1.1x10^7$ | $1.2x10^6$ | $< 10^2$ |
| Clay jar [cfu(ml)$^{-1}$] | $8.1x10^3$ | < 10 | $2.2x10^2$ | $7.2x10^3$ | < 10 | $1.2x10^4$ | < 10 |

AMB, aerobic mesophilic bacteria; LAB, lactic acid bacteria.

The aerobic mesophilic bacteria at the start of fermentation were dominated by staphylococci, micrococci, members of Enterobacteriaceae, and *Bacillus* spp and this pattern persisted for the first four hours (Table 2). This indicated that the roasting temperature of the wheat flour paste was not high enough to eliminate even Gram negative rods. Gram positive cocci and rods dominated thereafter. The Gram positive bacteria, particularly *Micrococcus* and *Bacillus* spp., may contribute to breaking of starch and production of acids at the initial stages of fermentation (Anon, 1980). Since the aerobic mesophilic bacterial flora had high counts all through the fermentation process, they may be important in producing certain metabolites which impart characteristic flavour to the product. However, the aerobic spore formers may not be active at the later stages as they could not proliferate in acidic conditions.

Members of Enterobacteriaceae and coliforms were not detected after 4 hours of fermentation although they were initially present at levels around $10^4$ cfu ml$^{-1}$ (Fig. 2). They could contribute to the initiation of fermentation by utilizing simple sugars produced after the action of amylase from malt and other amylolytic microorganisms. Their complete inhibition after 4 h may be indicative of the possible safety of '*borde*' from enteric pathogens. In other indigenous fermentation processes members of Enterobacteriaceae including the enteric pathogens are known to be inhibited by the action of LAB (Mogessie Ashenafi and Busse, 1989).

**Table 2. Changes in counts [cfu(ml)$^{-1}$] of major microorganisms during 'borde' fermentation.**

| Fermentation time (h) | pH | Lactobacilli | | Streptococci | Micrococci | Staphylococci | Molds |
|---|---|---|---|---|---|---|---|
| | | homo-fermentative | hetro-fermentative | | | | |
| 0 | 5.0 | $< 10^4$ | $2.5x10^5$ | $3.4x10^4$ | $1.0x10^5$ | $3.6x10^4$ | $2.5x10^4$ |
| 4 | 4.28 | $< 10^4$ | $1.1x10^6$ | $3.3x10^6$ | $4.7x10^5$ | $1.2x10^5$ | $1.5x10^4$ |
| 8 | 3.96 | $< 10^4$ | $7.7x10^7$ | $9.5x10^6$ | $2.0x10^6$ | $1.4x10^5$ | $7.1x10^3$ |
| 12 | 3.80 | $< 10^4$ | $3.7x10^8$ | $1.2x10^7$ | $4.7x10^6$ | $5.7x10^5$ | $1.4x10^3$ |
| 16 | 3.76 | $< 10^4$ | $5.1x10^8$ | $1.4x10^6$ | $5.6x10^6$ | $7.0x10^5$ | $< 10$ |
| 24 | 3.60 | $< 10^4$ | $9.8x10^8$ | $7.0x10^5$ | $2.1x10^6$ | $7.0x10^5$ | $< 10$ |

At 0 h, LAB had counts of about $10^6$ cfu(ml)$^{-1}$ and the micro-flora consisted mainly of *Lactobacillus* spp. A sharp increase in LAB count was noted at 8 h and then reached a final count of $10^9$ cfu(ml)$^{-1}$ at 24 h (Fig. 2). Although lactobacilli dominated the lactic flora throughout the fermentation process, streptococci were considerably high at around 12 h. All lactobacilli were hetero-fermentative (Table 2). The high amount of gas in '*borde*' may be due to the action of hetero-fermentative lactobacilli in addition to that produced by yeasts. Lactobacilli and streptococci were found to be the most important in 'obiolor' fermentation (Achi, 1990).

The yeast flora was dominated by *Saccharomyces* spp although *Rhodotorula* spp. were encountered at lower levels. The yeast count was markedly low at the initiation of fermentation and a sharp increase was noticeable after 12 h of fermentation (Fig. 2). As '*borde*' is consumed after an overnight fermentation, the yeast population at this stage may not produce enough alcohol to render the product too alcoholic for consumption.

Morphologically distinct molds were encountered both in ingredients and at the early stages of fermentation. As the activity of bacteria in the fermenting mash resulted in drop in pH and as the yeasts created anaero-biosis 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 appeared that different molds were involved

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) Nigerian pito (Ekundayo, 1969) and Ethiopian 'tella' (Samuel Sahle and Berhanu Abegaz Gashe, 1991).

During '*borde*' fermentation the pH was reduced from 5.2 to 3.6 within the 24 hour duration. In 'obiolor' fermentation, a beverage similar to '*borde*', the activity of LAB reduced the pH from 6.81 to 4.9 within the same duration of fermentation (Achi, 1990). The high count of lactobacilli in the starter used for '*borde*' fermentation could be responsible for the rapid drop in pH. Nout (1980; 1992) recommend the recycling of inoculum ('back slopping') for accelerated fermentation as a simple technique particularly suitable for household small scale fermentations.

It appears that the ingredients for '*borde*' fermentation vary among '*borde*' producing communities. Maize was reported to be the major ingredient of '*borde*' fermentation in Southern Ethiopia (Mogessie Ashenafi and Tetemke Mehari, 1995). In Addis Ababa, where this study was undertaken, wheat was the preferred ingredient. The processing steps were, however, not markedly different.

'*Borde*' has a short keeping quality as the product turns too sour to consume after about 16 to 18 hours of preparation. 'Kaffir' beer (Novellie, 1968) and 'pito' (Ekundayo, 1969) also deteriorate rapidly on storage and the products become unfit for drinking within about 24 hours of preparation. In some alcoholic beverages, hop has been used to make beverages biologically stable (Prescott and Dunn, 1959). In Ethiopia, leaves and stems of 'Gesho' plant (*Rhamunus prinoides*) chopped, sun-dried and pounded into finer powder appear to serve the purpose of hops in some traditional Ethiopian fermented beverages such as 'tella', 'tej', 'araki', etc. 'Gesho' is, however, not used in '*borde*' fermentation. It may be interesting to assess the use of this plant in improving the keeping quality of '*borde*'.

'*Borde*' is one of the various nutritious and low-alcoholic traditional fermented beverages in Ethiopia. The scaling-up of such products, although important, may have to be undertaken with great care so as not to lose the nutritive value

as well as the public acceptance of the beverages. Identification of the strains important for fermentation and optimization of the process parameters should be done in detail to design mechanisms for production of industrial-based products.

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