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Survival of *E. coli* O157:H7 *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella* spp. in fermenting 'Borde', a traditional Ethiopian beverage

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Abstract

The survival and inhibition of food-borne pathogens during the fermentation of different foods and beverages is documented. This prompted the study to evaluate survival of *Staphylococcus aureus*, *Shigella flexneri*, *Salmonella* spp. and *Escherichia coli* O157:H7 during the fermentation of 'Borde', a traditional Ethiopian fermented low-alcohol beverages. The pH of mixtures of 'Borde' ingredients at the start of fermentation was between 4.28 and 4.31. At ambient temperatures all test strains grew well in the control and reached counts as high as log 6.6 cfu/ml for *E. coli* O157:H7 and >log 7 cfu/ml for *S. aureus*, *S. flexneri*, and *Salmonella* spp. When co-inoculated with lactic acid bacteria (LAB), counts of *E. coli* O157:H7, *S. aureus* and *S. flexneri* were >log 2 cfu/ml and >log 1.5 cfu/ml at 12 h and at 16 h, respectively. At 24 h, these counts were <log 1.3 cfu/ml. The count of *Salmonella* spp., however, was <log 2 at 16 h and they were eliminated at 24 h. LAB-fermented 'Borde' had final pH of 3.85 and titratable acidity of 0.41%. At refrigeration temperature, there was no marked growth of test strains in the control. In the presence of LAB, counts of *S. aureus* were reduced by 2 log units whereas the counts of the other test strains were reduced only by <1 log unit. LAB increased by 1 log unit between 12 and 16 h. The survival of test strains at levels of >2 log cfu/ml between 12 and 16 h indicated health hazard as fermentation of 'Borde' is completed within 12 h and consumed within 4 h thereafter.
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Keywords: *E. coli* O157:H7; *Salmonella*; *Shigella flexneri*; *Staphylococcus aureus*; Borde; Traditional beverage fermentation

1. Introduction

Fermentation is one of the oldest and effective method of preparing and preserving foods and beverages. Fermented products are generally considered more attractive and desirable than the unfermented raw materials from which they are prepared. The method of fermentation is inexpensive and easily adaptable to local household practices in traditional communities (Steinkraus, 1985). It is estimated that 60% of the diet in many developing countries consists of fermented foods (Holzapfel, Geisen, & Schillinger, 1995).

A variety of traditionally fermented foods and beverages are produced and consumed in Ethiopia. The beverages have been produced on fairly small scale and usually for local consumption. Among Ethiopian indigenous fermented beverages '*Tella*' (Sahle & Gashe, 1991), '*Tej*' (Bahiru, Mehari, & Ashenafi, 2001), 'Borde' (Abegaz, Beyene, Langsrud, & Narvhus, 2002a, 2002b; Ashenafi & Mehari, 1995; Bacha, Mehari, & Ashenafi, 1998) and '*Shamita*' (Ashenafi & Mehari, 1995; Bacha, Mehari, & Ashenafi, 1999) are the most common.

'Borde' is a traditional fermented beverage made from maize or wheat. It is a very popular meal replacement in southern Ethiopia and some other parts of the country. 'Borde' is prepared mainly from maize. Maize flour is soaked in excess water and then deeply roasted on a hot metal pan. After cooling, ground malt is thoroughly mixed into it, put into a large clay jar and further blended in boiling water. At this stage, ground barley whipped in hot water is added to it and allowed to ferment overnight. The fermenting mixture is filtered through cheese clothe and served for consumption the next morning (Fig. 1). 'Borde' is consumed while in an active stage of fermentation. It is usually consumed by low-income groups, as meal replacement, and, on the

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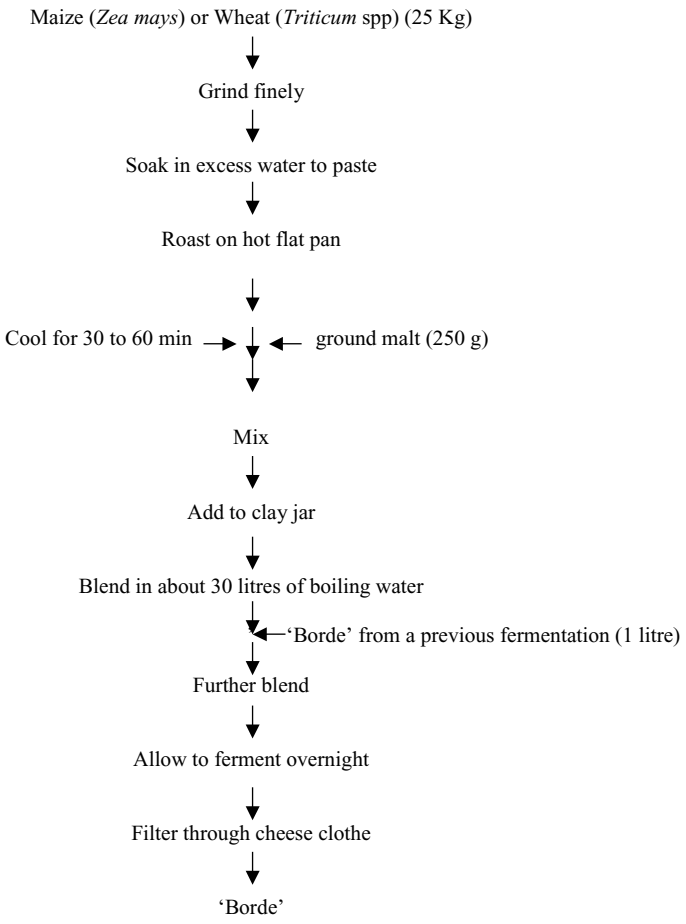

Fig. 1. Flow chart of 'Borde' fermentation process.

average, a person consumes about three liters of 'Borde' per day (Ashenafi & Mehari, 1995).

The microbiology, biochemistry, nutritional properties and processing of 'Borde' and the effect of fermentation on the products with regards to aroma, flavor and improvement of nutritional value were studied and documented (Abegaz et al., 2002a, 2002b; Ashenafi & Mehari, 1995; Bacha et al., 1998). Studies on traditional fermented foods of Ethiopia have shown that LAB have inhibitory effect on various food-borne pathogens (Ashenafi, 1993; Dessie, Abegaz, & Ashenafi, 1997; Girma, Gashe, & Lakew, 1989; Idris, Mehari, & Ashenafi, 2001; Nigatu & Gashe, 1994; Tsegaye, 2003). But the inhibitory properties of 'Borde' against specific food-borne pathogens were not studied. As 'Borde' is produced through an overnight fermentation, production of acid and alcohol during fermentation is limited.

In the cooler regions of the northern highlands of Ethiopia, the gruel of fermentable mixtures of 'Tella', is kept in a clay pot and stored underground. The process enhances the keeping quality of the gruel for months. A portion is mixed with a appropriate volume of water and maintained at ambient temperatures for several hours for instant fermentation and subsequent consumption. It may, thus, be useful to evaluate survival of pathogens in fermentable 'Borde' ingredients at low temperatures. The aim of this study was, therefore, to assess the action of LAB in fermenting 'Borde' on the survival of some food-borne pathogens at ambient and refrigeration temperatures.

2. Materials and methods

2.1. Sample collection

Samples of 'Borde' (250 ml/brewer) were collected from 10 'Borde' traditional household brewers in Addis Ababa using sterilized flasks and brought to the laboratory for isolation of lactic acid bacteria. The samples were kept for 2–4 h in the refrigerator until analysis was conducted.

2.2. Isolation and grouping of lactic acid bacteria

For the isolation of lactic acid bacteria, a volume of 0.1 ml of appropriate dilutions of 'Borde' was plated on MRS (OXOID) agar plates. Inoculated plates were

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