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SURVIVAL OF *ESHERICHIA COLI* O157:H7, *STAPHYLOCOCCUS AUREUS, SHIGELLA FLEXNERI* AND *SALMONELLA* SPP. IN BORDE AND SHAMITA: TRADITIONAL ETHIOPIAN FERMENTED BEVERAGES

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ABSTRACT: The survival or inhibition of foodborne pathogens in different fermented products are well documented. This prompted the study to evaluate survival of *Esherichia coli* O157:H7, *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella* spp. in two Ethiopian traditional fermented low-alcohol beverages, Shamita and Borde. The pH of ready-to-consume Shamita and Borde was 4.0 and 3.8, respectively. Samples were separately inoculated with 10^3 cfu/ml of the test strains for Borde and 10^6 cfu/ml for Shamita and these were maintained at 32^{o}C. In Shamita, *Staphylococcus aureus, Shigella flexneri,* and *Salmonella* survived until 24 h, but were not detectable at 48 h. *Esherichia coli* O157:H7 could survive until 48 h but was undetectable at 72 h. In Borde, *Staphylococcus aureus*, and *Shigella flexneri* survived only until 16 h, whereas *Salmonella* was eliminated within 12 hours. *Esherichia coli* O157:H7 survived longer in Borde and was eliminated only at 24 h. The presence of the test strains in the fermented products resulted in decrease of pH by an average of 0.35 units within 48 hours in Shamita and 0.16 units within 24 hours in Borde. The fermented products are low-alcohol beverages and are consumed in large amounts as meal replacements. As they are supposed to be consumed within a few hours after production and pathogens can survive in the product for over 10 hours, contamination of the products with pathogens should be avoided.

Key words/phrases: *E. coli* O157:H7; *Salmonella*; *Shigella flexneri*; *Staphylococcus aureus*; Traditional fermented beverages.

INTRODUCTION

Fermentation is one of the oldest and known effective methods 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. It is estimated that 60% of the diet in many developing countries consists of fermented foods (Holzapfel *et al*., 1995).

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A variety of traditionally fermented foods and beverages are produced and consumed in Ethiopia. The beverages are produced on fairly small scale and usually for local consumption. Among Ethiopian indigenous fermented beverages, Tella, Tej, Borde and Shamita 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 and served for consumption the next morning. Borde is consumed while in an active stage of fermentation. It is usually consumed by low-income groups as food replacement and, on the average, a person consumes about three liters of Borde per day. Shamita is a widely consumed low alcohol beverage with a thick consistency and is consumed as meal replacement by most people who cannot afford a reasonable meal. For Shamita preparation, lightly roasted barley is ground to which salt, ground linseed and small amounts of spices are added. These are mixed with water, usually in the evening, and the product is ready for consumption in the morning. Malt is not commonly used in Shamita fermentation, although local Shamita brewers in Addis Ababa use it frequently, and starch is the only principal fermentable carbohydrate. The microorganisms responsible for the fermentation come mostly from back-slopping using a small amount of Shamita from a previous fermentation as well as from ingredients and equipment. The microbial dynamics and the chemical changes occurring during the fermentation of Borde and Shamita have been reported by Mogessie Ashenafi and Tetemke Mehari (1995), Ketema Bacha *et al*. (1998; 1999) and Kebede Abegaz (2002a, b).

Survival and inhibition of *Escherichia coli* O157:H7, *Salmonella*, *Shigella* and *Staphylococcus aureus* during the storage of various fermented products have been reported by various workers (Mensah *et al*., 1988; Estrada *et al*., 1999; Chang *et al*., 2000; Issa and Ryser, 2000; Tetteh and Beuchat, 2001; 2003; Hsin-Yi and Chou, 2001; Yuste and Fung, 2003). Behaviour of these pathogens during the fermentation of various Ethiopian traditional foods has also been documented (Meaza Girma *et al*., 1989; Mogessie Ashenafi, 1993; Ayele Nigatu and Berhanu Abegaz Gashe, 1994; Gulilat Dessie *et al*., 1997; Mekonnen Tsegaye, 2003).

The fate of various food-borne pathogens during the fermentation of Borde and Shamita and the inhibitory potential of the lactic acid bacteria on the food-borne pathogens has also been reported (Girum Tadesse *et al*., 2005 a, b). As Borde and Shamita are produced through an overnight fermentation, acid and alcohol production is limited. This study, therefore, aimed to assess the microbiological safety of these products with respect to survival or elimination of *Escherichia coli* O157:H7, *Salmonella*, *Shigella flexneri* and *Staphylococcus aureus* in the ready-to-consume fermented products.

MATERIALS AND METHODS

Sample collection

Samples of Borde and Shamita (250 ml/brewer) were separately collected from ten Borde and Shamita household brewers in Addis Ababa using sterilized flasks and brought to the laboratory for isolation of lactic acid bacteria and for assessment of antimicrobial properties. The samples were kept for 2-4 h in the refrigerator until analysis was conducted.

Test strains

Salmonella sp., *Shigella flexneri*, *Staphylococcus aureus* and *E. coli* O157:H7 were used as test strains to evaluate the antimicrobial effects of ready-to-consume Borde and Shamita. *Salmonella* sp., *Shigella flexneri* and *Staphylococcus aureus* were clinical isolates obtained from the Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa. *E. coli* O157:H7 was isolated from hamburger meat which was obtained from the Food Microbiology Laboratory of Howard University, USA.

Determination of antimicrobial activity of ready-to-consume Borde and Shamita

Ready-to-consume fermented products of Borde and Shamita were separately filtered through a cheese cloth. The filtrate from each product was centrifuged and the resulting supernatant was filter-sterilized using millipore filters. The sterile filtrate was used to evaluate the inhibitory properties of fermented ready-to-consume Borde and Shamita on the test strains. As the substrates were made free of any fermenting microorganism, only the antimicrobial activity of the microbial metabolites was considered in this experiment.

Each test strain was inoculated into 250 ml sterile screw-capped bottles, each containing 100 ml of the filter-sterilized product to give an initial inoculum level of 10^3 cfu/ml for Borde and 10^6 cfu/ml for Shamita. The two initial inoculum levels were used to assess survival of test organisms when inoculated at high and low levels. All samples were incubated at 32°C.

Enumeration of the test strains

A volume of 1ml of sample was separately taken from bottles inoculated with the test strains at four-hour intervals and a volume of 0.1ml of appropriate dilutions was spread-plated in duplicate on pre-dried surfaces of Plate Count agar (OXOID) plates. The plates were incubated at 32°C for two hours to allow recovery of injured cells. The inoculated plates were then over-laid with the following media to make counting easier: Salmonella-Shigella agar (OXOID) for *Salmonella* and *Shigella flexneri*, Mannitol Salt agar (OXOID) for *Staphylococcus aureus*, and MacConkey agar (OXOID) for *E. coli* O157: H7. Counting was done after incubation at 32^{o}C for 24 to 48 hours.

Measurement of pH

The pH of the various product filtrates was measured during the sampling times using a digital pH meter.

Determination of titratable acidity

The titratable acidity of the product filtrates was determined at each sampling time according to the method of Antony and Chandra (1997). Five ml of the substrate was titrated against 0.05M NaOH to the end point of phenolphthalein. Titratable acidity was expressed as % lactic acid.

Statistical analysis

Coefficient of variation (%CV) was calculated to see if significant variation occurred in counts of a test strain during the sampling times.

RESULTS AND DISCUSSION

To evaluate the inhibitory potential of ready-to-consume Borde and Shamita or to confirm the presence of extracellular antimicrobial metabolites produced during the fermentation process in this study, sterilization of the finished products was achieved through filter-sterilization method. This method was preferred to sterilization by heat (autoclaving) to remove only the diverse microorganisms found in the ingredients of the products without affecting heat-labile antimicrobial metabolites and any alcohol produced

during the fermentation process.

In ready-to-consume Shamita, counts of *Staphylococcus aureus* declined by about 2 log units within the first four hours, but decrease in count was gradual thereafter. At 48 h, *Staphylococcus aureus* was not detectable in the fermented product. Survival of *Staphylococcus aureus* in Shamita resulted in a 0.2 unit reduction in pH and the initial titratable acidity of 0.39% increased to 0.53% by the time the test strain was eliminated (Table 1). Counts of *Staphylococcus aureus* in ready-to-consume Borde decreased gradually and it was not detectable after 16 h, where the pH of Borde reached 3.75. Its presence in Borde resulted in 0.1 unit increase in % titratable acidity (Table 1). *Staphylococcus aureus* was reported to survive 8 hours of yogurt fermentation with lactic acid bacteria, but its population decreased from the first day of storage in the product (Estrada *et al*., 1999). Survival of *Staphylococcus aureus* in a fermented product was much shorter in our study than in the study of Estrada *et al*. (1999) where *Staphylococcus aureus* required 9-10 days to be completely inhibited in stored yogurt. Elimination of *Staphylococcus aureus* within 48 hours in Thai-style fermented pork sausage was also reported by Petchsing and Woodburn (1990). As *Staphylococcus aureus* was shown to survive at pH 3.4-3.8 for about 7 days in apple juice at 20^{o}C (Yuste and Fung, 2003) and at titratable acidity of >1.3% for 72 hours in Moroccan cheese (Hamama *et al*., 2002), the fast elimination of the test strains in our study could not be attributed to pH or acidity alone, but also to other metabolites produced by lactic acid bacteria during the overnight fermentation of the fermented beverages. Ketema Bacha *et al*. (1998; 1999) have shown that lactic acid bacteria are responsible for the fermentation of Shamita and Borde, and are also the dominant bacteria in the final products.

Table 1 Counts (log cfu/ml) of *Staphylococcus aureus* in ready-to-consume Shamita and Borde.

Time	Shamita			Borde		
(h)	pH	%T.A.	Count	pH	%T.A.	Count
0	4.0	0.39	6.16	3.85	0.48	3.16
4	4.0	0.41	4.30	3.80	0.52	3.13
8	3.98	0.44	4.13	3.80	0.55	2.55
12	3.95	0.45	3.72	3.78	0.56	1.72
16	3.90	0.48	3.39	3.75	0.58	1.16
20	3.90	0.51	3.22	3.70	0.59	<1
24	3.84	0.51	2.63	3.70	0.59	<1
48	3.80	0.53	<1			

T.A. – titratable acidity

Shigella flexneri showed no decrease in count during the first 4 hours in ready-to-consume Shamita (Table 2). Reduction in counts was gradual thereafter and it was not detectable after 24 h. Its survival in Shamita was accompanied by a slight reduction in pH and a slight increase in titratable acidity. In ready-to-consume Borde, reduction in counts of *Shigella flexneri* was gradual after 4 h and the test strain was undetectable after 16 h (Table 2). The pH dropped from an initial value of 3.8 to 3.64. A slight increase in titratable acidity was also noted. Reports on the inhibition of *Shigella* spp. in acid environments vary markedly. Similar to our observation, Mensah *et al*. (1988) reported that strains of *Shigella flexneri* could survive for 24 h in Ghanian fermented maize dough (pH 3.2), although most strains were eliminated within 6 hours. Tetteh and Beuchat (2003) reported that *Shigella flexneri* could survive for more than 6 hours in Tryptone Soya Broth when the broth was acidified to pH 4.0, but were eliminated in less than 2 hours when acidified to pH 3.5. Survival of *Shigella flexneri* for 8 hours in fermented cassava dough (pH 3.8-3.9) was also observed by Mante *et al*. (2003). Several other studies have shown that *Shigella* could survive in high acid or high temperature conditions for many hours (Smith, 1987; Small *et al*., 1994). The survival of *Shigella* for about 24 hours in acidic environments could increase its tolerance to acidic conditions which may enhance survival in acidic foods and in the human gastric environment (Tetteh and Beuchat, 2001; Gorden and Small, 1993).

Table 2 Counts (log cfu/ml) of *Shigella flexneri* in ready-to-consume Shamita and Borde.

Time	Shamita			Borde		
(h)	pH	%T.A.	Count	pH	%T.A.	Count
0	4.00	0.38	6.32	3.80	0.50	3.13
4	3.98	0.41	6.31	3.80	0.53	2.16
8	3.95	0.42	5.81	3.70	0.54	2.13
12	3.90	0.44	5.28	3.70	0.55	2.13
16	3.85	0.45	4.21	3.65	0.57	1.43
20	3.80	0.48	3.44	3.64	0.60	<1
24	3.75	0.48	2.13	3.64	0.60	<1
48	3.70	0.52	<1			

T.A.-titratable acidity

Salmonella decreased only by 0.35 log units during the first 4 hours in ready-to-consume Shamita, but reduction in number was rapid thereafter, followed by elimination after 24 h (Table 3). A slight gradual decrease in pH and increase in titratable acidity was noted during its survival in Shamita. In ready-to-consume Borde, presence of *Salmonella* resulted in decrease in pH by 1.2 units within 12 hours. Increase in titratable acidity during this period was marked. Our *Salmonella* was eliminated within 12 hours in Borde (Table 3). Different workers have come up with different

findings regarding survival of *Salmonella* spp. in acid environments. *Salmonella* could survive for 4 days in labneh, a fermented milk (Issa and Ryser, 2000). In ogi, a fermented weaning food (pH 3.5-3.7), *Salmonella* spp. were inhibited within 24 hours (Bakare *et al*., 1998). In another study, *Salmonella* test strains inoculated in a 48-hour fermented cassava dough (pH 3.8-3.9) could not be detected after 4 h (Mante *et al*., 2003). However, *Salmonella typhimurium* could survive until day 7 in pasteurized pineapple juice (pH 3.64), stored at 20^{o}C (Yuste and Fung, 2003). The varying inhibition or survival can not, thus, be explained only in terms of pH or acidity. Other metabolic products of fermentation may rather play a more important role in the inhibition of *Salmonella* spp. in fermented products.

Table 3 Counts (log cfu/ml) of *Salmonella* spp. in ready-to-consume Shamita and Borde.

Time	Shamita			Borde		
(h)	pH	%T.A.	Count	pH	%T.A.	Count
0	3.99	0.38	6.48	3.80	0.49	3.95
4	3.91	0.40	6.13	3.74	0.52	3.20
8	3.90	0.42	4.26	3.70	0.57	2.39
12	3.88	0.45	3.87	3.68	0.60	1.25
16	3.86	0.47	3.36	3.68	0.60	<1
20	3.86	0.48	2..20	3.68	0.60	<1
24	3.85	0.50	2.16	3.68	0.60	<1
48	3.70	0.51	<1			

T.A.-titratable acidity

E. coli O157:H7 showed a marked reduction in the first 4 hours in ready-to-consume Shamita (Table 4). But its reduction thereafter was very gradual and could be detected only until 48 hours. At this time, the pH value of Shamita dropped to 3.65 with a titratable acidity of 0.52%. *E. coli* O157:H7 survived longer than the other test strains in ready-to-consume Borde (Table 4). Its survival in Borde reduced the pH of the product by 0.2 units in 20 hours and the titratable acidity increased to 0.64%. The survival of *E. coli* O157:H7 in an acidic environment for a more extended period was also observed by other workers. It could survive in mango juice (pH 3.2) for over 5 days and in asparagus juice (pH 3.6) for over 14 days at 25^{o}C (Hsin-Yi and Chou, 2001). Chang *et al*. (2000) reported that some strains of *E. coli* O157:H7 could survive for 2-3 days in diluted cultured milk drink adjusted to pH 3.5. As was observed in our study, a pathogenic *E. coli* strain could survive longer than *Salmonella* spp. at pH 3.8-3.9 in fermented cassava (Mante *et al*., 2003).

Table 4 Counts (log cfu/ml) of *E. coli* O157:H7 in ready-to-consume Shamita and Borde.

Time	Shamita			Borde		
(h)	pH	%T.A.	Count	pH	%T.A.	Count
0	3.98	0.38	6.17	3.85	0.50	3.28
4	3.99	0.42	4.13	3.85	0.53	3.19
8	3.90	0.45	3.87	3.80	0.55	3.13
12	3.85	0.46	3.82	3.75	0.60	2.30
16	3.80	0.49	3.60	3.70	0.60	2.20
20	3.75	0.49	2.95	3.65	0.62	1.39
24	3.72	0.51	2.71	3.65	0.64	<1
48	3.65	0.52	<1			
72	3.60	0.55	<1			

T.A.-titratable acidity

As both fermented beverages are products of an overnight fermentation, they would contain ample fermentable sugars to be utilized by the surviving test organisms. In both fermented beverages, *E. coli* O157:H7 was found to be the most resistant, and the *Salmonella* test strain was the most sensitive, at least in Borde. The lower pH and higher titratable acidity of ready-to-consume Borde might also contribute to faster inhibition of the test strains. The variation of the filtrates of Borde and Shamita in pH is in agreement with the report of Ketema Bacha *et al*. (1998; 1999) who showed that the final product of Borde fermentation had lower pH than that of Shamita fermentation.

Borde and Shamita are low-alcohol products and are consumed in large amounts as meal replacements. They are served immediately after an overnight fermentation and while in an active stage of fermentation. As the products would be too sour to consume after 4 hours, they are supposed to be consumed within hours. Food-borne intoxication from *Staphylococcus aureus* would be improbable in Shamita and Borde due to the high count required ($\geq 10^5$ cfu/ml) to elucidate enough enterotoxins to cause gastroenteritis. But, Borde and Shamita can be vehicles of transmission for *Salmonella*, *Shigella* and *E. coli* O157:H7, as these pathogens have been shown to survive during the short period the products are preferably consumed.

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