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Adaptation of *Escherichia coli* 0157:H7 to acid in traditional and commercial goat milk amasi

Bhekisisa C. Dlamini, Elna M. Buys*

Department of Food Science, University of Pretoria, Lynnwood Road, Pretoria 0002, South Africa

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ABSTRACT

Acid resistance of *Escherichia coli* O157:H7 strains UT 10 and UT 15 were determined in traditional Amasi fermented for 3 days at ambient temperature (*ca* 30 °C) and commercial Amasi fermented at 30 °C for 24 h and stored at 7 °C for 2 days. *Escherichia coli* O157:H7 counts in commercial Amasi were detected at 2.7 $\log_{10}$ cfu/ml after 3 days while those in traditional Amasi could not be detected after the same period. There was no significant difference ($p \leqslant 0.05$) in the survival of acid adapted (AA) and non-adapted (NA) *E. coli* O157:H7 in traditional Amasi, while in commercial Amasi, the NA strain survived significantly ($p \leqslant 0.05$) better than its AA counterpart. Regardless of prior adaptation to acid, *E. coli* O157:H7 can survive during fermentation and storage of fermented goat milk Amasi. Also, the fermentation time, pH and storage temperature affects the survival of *E. coli* O157:H7 in the fermented milk.

1. Introduction

Amasi is a traditional fermented milk product consumed in all parts of South Africa. Wide variations in taste have been reported to be caused by the use of different fermenting pots such as clay pots, calabashes and gouards (Kebede et al., 2007). Fermentation temperatures and fermenting microflora also contribute to different tastes. Traditionally, Amasi is produced from raw milk that ferments naturally at ambient temperature (Bryant, 1949). However, due to the ever escalating population size, and the advent of commercial starter cultures, commercial Amasi is now produced from pasteurized milk, under controlled processing conditions. Large-scale commercial production of traditional fermented milk in South Africa is in the form of Amasi also known as 'Maas' and 'Inkomasi' (Keller and Jordaan, 1990). Numerous types of lactic acid bacteria (LAB) and other microorganisms such as yeasts have been cited to be responsible for fermentation in Amasi (Beukes et al., 2001; Kebede et al., 2007). Among the fermenting pots used to make fermented milk traditional Amasi in South Africa, Kebede et al. (2007) reported that those made of clay provided a greater diversity of yeasts. This is believed to influence the characteristics of the end product, hence the focus on their use as starter cultures (Fleet, 1990; Jakobsen and Narvhus, 1996; Loretan et al., 1998).

The inhibitory effect of fermented foods on contaminating organisms is attributed to the production of antimicrobial compounds and the reduction of pH (Gulmez and Guven, 2003). Lactic acid bacteria are the major producers of inhibitory metabolites in fermented foods. Fermented milk products were found to be more effective in reducing the growth of acid adapted (AA) *E. coli* O157:H7 than acidic fruit juices (Hsin-Yi and Chou, 2001). Of concern, however, is the implication of fermented foods such as yoghurt in food-borne outbreaks caused by *E. coli* O157:H7. Several studies have also demonstrated that AA *E. coli* O157:H7 may survive during processing and storage of fermented dairy foods such as lactic cheese and Ergo (Vernozy-Rozand et al., 2005; Tsegaye and Ashenafi, 2005). Refrigeration has also been reported to increase the survival of AA *E. coli* O157:H7 in acidic foods (Cheng and Kaspar, 1998; Clavero and Beuchat, 1996; Faith et al., 1998). While the behavior of most food-borne pathogens has been investigated in other fermented foods, no study has been undertaken to study the adaptation of *E. coli* O157:H7 in Amasi. The aim of the study was to assess the survival and growth of AA *E. coli* O157:H7 in traditional and commercial Amasi.

2. Materials and methods

2.1. E. coli *serotypes*

A cocktail of *E. coli* O157:H7 strains, UT 10 and UT 15, obtained from the Agricultural Research Council, Onderstepoort Veterinary Institute, Pretoria, South Africa, were used in this study. The strains were cultured in Tryptone Soy Broth (TSB) from Oxoid for 24 h at 37 °C and then stored at 4 °C. The stock cultures were sub-cultured

* Corresponding author. Tel.: +27 12 4203209; fax: +27 12 4202839.
E-mail address: elna.buys@up.ac.za (E.M. Buys).

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through at least two 24 h incubation cycles in fresh broth medium before use in experiments.

2.2. Preparation of acid adapted E. coli strains

Acid adapted and non-adapted *E. coli* were prepared to the stationary-phase by following the procedure outlined by Buchanan and Edelson (1996). The working stock cultures (1 ml) were inoculated into 100 ml of either TSB supplemented with glucose (Associated Chemical Enterprises, Glenvista, South Africa) to a concentration of 10 g/l (1%) and to pH 4.6 (TSB + G) or TSB without glucose (TSB-G) buffered with 100 mM 3-(N-Morpholino) propanesulfonic acid (MOPS), pH 7.4 (Sigma-Aldrich Chemie, Steinheim, Germany) and incubated for 18 h at 37 °C. The former served as the acid adapted strain whereas the latter served as the non-adapted strain.

2.3. Processing of commercial Amasi and enumeration of lactic acid bacteria and E. coli test strains

Raw goat milk was sourced from the University of Pretoria (UP) experimental farm (Pretoria, South Africa). Commercial Amasi was prepared using a method that purely simulates the processing of commercial Amasi. Pasteurized goat milk was inoculated with a commercial LAB starter culture of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (MO 0.30) (Sacco, Cadorago, Italy) to give an initial inoculum level of 10^6 cfu/ml. Skim milk (3%) and gelatine (0.5%) (Davis, Gauteng, South Africa) were added before pasteurization to stabilize the product. *Escherichia coli* test strains (AA and NA) were inoculated at a level of 10^6 cfu/ml into separate screw cap bottles when the pH of the Amasi reached 5.6. Inoculation was done at pH 5.6 to prevent the non-adapted strain from adapting. After 24 h of incubation at 30 °C, the samples were kept at 7 °C for 48 h. Enumeration of *E. coli* and LAB was done after inoculation at days 0, 1, 2, and 3. Samples (1 ml) were serially diluted in 9 ml of 0.1% Buffered Peptone Water (BPW) and appropriate dilutions were surface plated onto Sorbitol MacConkey agar (SMAC) (Oxoid) for *E. coli* and de Man, Rogosa and Sharp (MRS) agar (Merck) for LAB. SMAC plates were incubated at 37 °C for 24 h while MRS agar plates were incubated anaerobically at 37 °C for 48 h.

2.4. Preparation of starter culture for traditional Amasi production

The inoculum used for making traditional Amasi was isolated from traditional fermented milk sourced in Botswana. The traditional fermented milk inoculum was chilled and transported to the UP. Upon arrival at the laboratory, the total plate count, yeasts and moulds count, titratable acidity and pH of the fermented milk was determined. The traditional fermented milk inoculum was further tested for the presence of pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* and coliforms, following the methods outlined in 3.2.2.5 below. The fermented milk was then put in 90 mm Petri dishes (Merck), frozen to −20 °C, freeze-dried (Instruvac lyophilizer, model 13KL) and then stored at −20 °C. Before inoculation into being used for the manufacture of the traditional Amasi, the traditional fermented milk inoculum was activated by placing 10 g of freeze dried fermented milk in 90 ml sterile skim milk and incubating at 30 °C for 24 h.

2.5. Detection of pathogens in the original traditional fermented milk inoculum

Coliforms and faecal *E. coli* were detected by using the Most Probable Number (MPN) method (Canadian Health Products and Food Branch, MFHPB-19) by inoculating 10 ml of the sample into 5 tubes of double strength Lauryl Sulfate Tryptose (LST) broth (Oxoid) with Durham tubes and by inoculating 1 ml or 0.1 ml of the sample into 5 tubes of single strength LST broth (10 ml) with Durham tubes. The tubes were incubated for 24 h at 35 °C. On LST positive tubes, a loopful from the LST positive tubes was transferred to Brilliant Green Lactose 2% Bile Broth (BGLB) (Oxoid) tubes (10 ml) which were incubated at 35 °C for 24 h. A loopful from the BGLB positive tubes was transferred into Enterococcosel (EC) broth (Oxoid) tubes (10 ml) which were incubated in a water-bath at 45 °C for 24 h. A loopful from EC positive tubes was streaked onto Eosins methylene Blue agar (EMB) (Oxoid) plates which were subsequently incubated at 35 °C for 18–24 h and typical colonies counted. Gas production from BGLB broth confirmed the presence of coliforms while gas production from EC broth confirmed the presence of faecal *E. coli*. *L. monocytogenes* was detected using the International Standard Organization (ISO) 11290-1 method, by inoculating 1 ml of the sample into 9 ml half strength Frazer *Listeria* selective enrichment (Oxoid) and incubating the suspension at 30 °C for 24 h. After incubation, 0.1 ml of the culture was transferred into a 10 ml tube with full strength Frazer and incubated at 35 °C for 48 h. From the half Frazer or full Frazer culture, a loop of the culture was plated on Oxford agar (Oxoid) or PALCAM agar (Oxoid) and incubated microaerobically at 35 °C for 24 h. Typical colonies were further streaked on Tryptone Soy Agar plates, incubated at 35 °C for 18–24 h and confirmed with Gram strain (Harrigan and McCance, 1966) and catalase test (Harrigan and McCance, 1966).

Salmonella was detected using the South African Bureau of Standards (SABS) ISO 6579:1993 method, by transferring 0.1 ml of pre-enriched (BPW) sample to a tube containing 10 ml *Salmonella* enrichment broth, Rappaport and Vassiliadis (RVS) (Merck) and by transferring 10 ml of the pre-enriched broth to a bottle containing 100 ml Selenite cystine medium (Oxoid). The tubes containing enrichment broth and Selenite cystine medium were incubated at 42 °C for 24 h and 35 °C for 24 h, respectively. A loopful from the *Salmonella* enrichment broth and Selenite cystine medium was streaked onto pre-dried Phenol Red/Brilliant Green Agar (Oxoid) or XLD agar (Oxoid) and incubated at 35 °C for 20–24 h.

Staphylococcus aureus was detected using the ISO 6888-1 method, by transferring 0.1 ml of the sample to Baird-Parker medium (Merck) plates supplemented with Egg-yolk Tellurite Emulsion (Oxoid) solution. The plates were allowed to dry for 15 min and incubated for 24 h at 35 °C. Typical colonies which are black or grey, were enumerated and confirmed with coagulase test (Harrigan and McCance, 1966).

Faecal enterococci was detected using the South African National Standard (SANS) 7899-2:2004 method, by plating 0.1 ml of the sample on Slanetz and Bartley medium (Oxoid) supplement with triphenyltetrazolium chloride solution. The plates were then incubated at 35 °C for 44 h and typical colonies (red or maroon) after incubation were further streaked on Bile Esculin Azide (Oxoid) plates and incubated at 44 °C for 2 h. All colonies showing a tan to black color after the incubation for 2 h were regarded positive and counted.

2.6. Processing of traditional Amasi and enumeration of LAB and E. coli test strains

Amasi was prepared in hand-made pots that are traditionally used to make Amasi. Two clay pots were purchased from an informal market at Bosman station, Pretoria, South Africa. The clay pots were conditioned by fermenting 3 successive batches of raw goat milk sourced from the University of Pretoria experimental farm (Pretoria, South Africa) in the pots. This was done by allowing raw goat milk to ferment using the freeze dried fermented milk described in Section 2.4, as an inoculum, at ambient temperatures for three days, after which, the fermented milk was discarded and fresh milk added for the next fermentation.

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Traditional Amasi was prepared following modification of the method outlined by Bryant (1949). It was prepared in two handmade clay pots at the dairy factory of the University of Pretoria, South Africa. The clay pots were filled with raw goat milk sourced from the University of Pretoria experimental farm (Pretoria, South Africa) and inoculated with a fermented milk inoculum with an initial level of 10^6 cfu/ml LAB. The milk was allowed to ferment naturally at ambient temperature (*ca* 30 °C) for 3 days. Inoculation of the product with *E. coli* test strains was done after 6 h, when the pH reached 5.6.

Enumeration of *E. coli* and LAB was done at days 0, 1, 2, and 3 using the same method detailed in 3.2.2.3. SMAC was used for *E. coli* while MRS agar and M17 (Oxoid) agar were used for enumeration of LAB. MRS plates were incubated anaerobically using the anaerocult system (Merck, Darmstadt, Germany) at 37 °C for 48 h for the enumeration of mesophilic lactobacilli and leuconostocs and at 42 °C for the enumeration of thermophilic lactobacilli and streptococci while SMAC plates were incubated aerobically for 24 h at 37 °C. The M17 agar plates were incubated aerobically for 48 h at 30 °C for the enumeration of lactococci. Total viable counts were performed using Plate Count Agar (Merck) incubated at 25 °C for 24 h. Yeast and moulds were enumerated using Yeasts and Moulds Petrifilm (Microbiology products, St. Lous, USA). The petrifilms were inoculated with 1 ml of serially diluted sample and incubated at 25 °C for 24 h. At each sampling time, the pH (Sentron, Gig Harbor, USA) and titratable acidity was also determined by the titration method using NaOH (0.1 mol/l) in the presence of phenolphthalein.

2.7. Statistical analysis

Analysis of variance (ANOVA) was used to determine whether factors such as acid adaptation and time, affected the survival and growth of the bacteria significantly (95% confidence levels). All samples were analyzed in duplicate and each experiment was repeated three times ($n = 6$). ANOVA was performed using Statistica software for windows (Tulsa, Oklohama, USA, 2003).

3. Results

3.1. Enumeration of the microbial population and detection of potential pathogens in the traditional fermented milk

The fermented milk had total aerobic counts of 5.8 $\log_{10}$ cfu/ml, LAB counts of 6.4 $\log_{10}$ cfu/ml as well as a yeast and mould count of 4.1 $\log_{10}$ cfu/ml (data not shown). Presumptive *L. monocytogenes* was detected in the traditionally fermented milk at 3.2 $\log_{10}$ cfu/ml. *Salmonella*, coliforms, *S. aureus* and faecal enterococci were not detected. The original fermented milk had a pH of 4.0 while its titratable acidity (T.A) was 1.4%.

3.2. Enumeration and characterization of the microbial population and detection of the AA and NA E. coli in traditional Amasi

The presence of adapted bacteria had a significant effect ($p \leqslant 0.00$) on the total aerobic counts (TAC) in traditional Amasi over 3 days (Table 1). TAC in the clay pot inoculated with AA *E. coli* O157:H7 decreased with 2.3 $\log_{10}$ cfu/ml while counts in the clay pot inoculated with NA *E. coli* O157:H7 decreased with 1.4 $\log_{10}$ cfu/ml after 3 days (Fig. 1). TAC increased from 7.5 $\log_{10}$ cfu/ml to 8 $\log_{10}$ cfu/ml after 1 day followed by a decrease to 5.2 $\log_{10}$ cfu/ml in the clay pot inoculated with AA *E. coli* O157:H7, while a count of 7.9 $\log_{10}$ cfu/ml was maintained after 1 day followed by a decline to 6.4 $\log_{10}$ cfu/ml in the clay pot inoculated with NA *E. coli* O157:H7 (Fig. 1).

Yeast and mould counts in the traditional Amasi clay pot inoculated with AA *E. coli* O157:H7 were not significantly different ($p \leqslant 0.73$) from those in the NA *E. coli* O157:H7 inoculated clay pot (Table 1). Yeast and mould counts in the AA *E. coli* O157:H7 inoculated clay pot increased from 4.5 $\log_{10}$ cfu/ml to 6.2 $\log_{10}$ cfu/ml after 3 days, while those in the NA *E. coli* O157:H7 inoculated clay pot increased from 4.5 $\log_{10}$ cfu/ml at day 0 to 6.4 $\log_{10}$ cfu/ml after 1 day followed by a decrease to 5.4 $\log_{10}$ cfu/ml after 3 days (Fig. 1).

Similar to the yeasts and moulds, survival of mesophilic or thermophilic LAB in the clay pot inoculated with AA *E. coli* O157:H7 did not differ significantly ($p \leqslant 0.15$) from that in the clay pot inoculated with NA *E. coli* O157:H7 (Table 1). Mesophilic counts in the clay pot inoculated with AA *E. coli* O157:H7 increased from 8.4 $\log_{10}$ cfu/ml to 8.8 $\log_{10}$ cfu/ml after 1 day followed by a decrease to 6.8 $\log_{10}$ cfu/ml after 3 days, while those from the NA *E. coli* O157:H7 inoculated clay pot increased from 8.8 $\log_{10}$ cfu/ml at day 0 to 8.9 $\log_{10}$ cfu/ml after 1 day followed by a decrease to 6.8 $\log_{10}$ cfu/ml after 3 days (Fig. 1). Thermophilic LAB counts in the clay pot inoculated with AA *E. coli* O157:H7 increased from 7.5 at day 0 to 7.9 $\log_{10}$ cfu/ml after 1 day followed by a decrease to 5.3 $\log_{10}$ cfu/ml after 3 days. Those in the NA *E. coli* O157:H7 clay pot increased from 7.6 at day 0 to 7.8 $\log_{10}$ cfu/ml after 1 day followed by a decrease to 5.3 $\log_{10}$ cfu/ml after 3 days (Fig. 1).

Lactococcus counts decreased from 7.6 $\log_{10}$ cfu/ml to 6.5 $\log_{10}$ cfu/ml and from 7.6 to 6.1 $\log_{10}$ cfu/ml after 3 days in the clay pots inoculated with AA or NA *E. coli* O157:H7, respectively (Fig. 1). The survival of lactococci in the AA *E. coli* inoculated clay pot did not differ significantly ($p \leqslant 0.05$) from that in the NA *E. coli* O157:H7 inoculated clay pot (Table 1).

In general, the survival of yeasts and moulds, lactococci, mesophilic and thermophilic LAB in the clay pot inoculated with AA *E. coli* O157:H7 was not significantly ($p \leqslant 0.05$) different from that in the clay pot inoculated with NA *E. coli* O157:H7 (Table 1). However, the 3-days time of challenge had a significant effect ($p \leqslant 0.00$) on the survival of the counts of all microorganisms.

Reduction in pH was similar in both traditional Amasi clay pots inoculated with AA or NA *E. coli* O157:H7 (Table 2). The pH dropped from 5.6 to 4.1 and from 5.6 to 4.0 in the clay pots inoculated with AA and NA *E. coli* O157:H7, respectively over the 3-day period (Table 2).

3.3. Enumeration of LAB in commercial Amasi

There was no significant difference ($p \leqslant 0.52$) between LAB counts in both the AA and NA *E. coli* O157:H7 inoculated in commercial Amasi. LAB counts in commercial Amasi inoculated with AA and NA *E. coli* O157:H7 increased from 7.0 $\log_{10}$ cfu/ml to

Table 1
Statistical analysis of total aerobic counts, yeasts and moulds, lactococci, mesophilic and thermophilic lactic acid bacteria (LAB) from traditional Amasi after 3 days at ambient temperature ($n = 3$)

Treatment	Degrees of freedom	*P* value				
		Total aerobic count	Yeasts and moulds	Lactococci	Mesophilic LAB	Thermophilic LAB
Acid-adaptation (AA, NA)	1	0.001	0.731	0.723	0.149	0.441
Time (3 d)	3	0.000	0.000	0.000	0.000	0.000
Time × acid-adaptation	3	0.029	0.074	0.509	0.696	0.621

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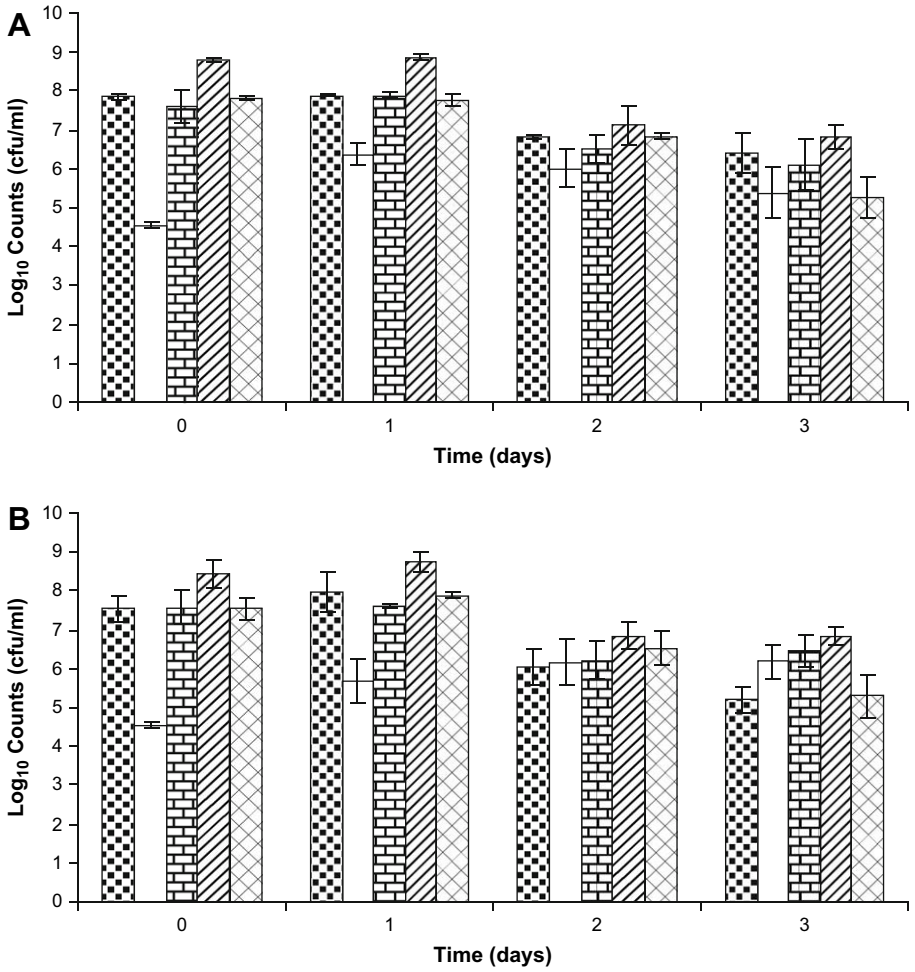

Fig. 1. Total aerobic count, ⊡ yeasts and moulds counts □, mesophilic LAB counts on MRS agar ▨, lactococci counts ⊟ and thermophilic LAB ▩ in traditional Amasi clay pots inoculated with acid adapted *E. coli* O157:H7 (A) and non-adapted *E. coli* O157:H7 (B) ($n = 3$).

8.1 $\log_{10}$ cfu/ml and from 7.7 $\log_{10}$ cfu/ml to 8.4 $\log_{10}$ cfu/ml after 2 days and decreased to 7.9 $\log_{10}$ cfu/ml and 7.3 $\log_{10}$ cfu/ml, respectively, after 3 days (data not shown). Reduction in pH was similar in both AA and NA *E. coli* O157:H7 (Table 2). The initial pH of 5.6 dropped to pH 4.7 after 24 h and was further reduced to pH 4.4 after 3 days (Table 2).

3.4. Acid resistance of acid adapted and non-adapted E. coli O157:H7 in traditional and commercial Amasi products

Acid adaptation had a significant effect ($p \leqslant 0.04$) on *E. coli* O157:H7 counts in traditional and commercial Amasi (Table 3). However, the observed overall significant difference ($p \leqslant 0.04$) in traditional Amasi was only 0.4 $\log_{10}$ cfu/ml. AA and NA *E. coli* O157:H7 counts in traditional Amasi decreased from 6.8 $\log_{10}$ cfu/ml to 3.5 $\log_{10}$ cfu/ml and from 6.4 $\log_{10}$ cfu/ml to 3.2 $\log_{10}$ cfu/ml, respectively, after 2 days, and were not detected after the third day (Fig. 2). A 1 $\log_{10}$ cfu/ml and 0.3 $\log_{10}$ cfu/ml difference was observed in traditional Amasi after 1 and 2 days, between AA and NA *E. coli* O157:H7, respectively, with the AA strain showing better survival. Neither strains could, however, be detected after 3 days, indicating that there was no significant difference ($p \leqslant 0.09$) in their survival over the full period of 3 days.

Similar to what was observed in traditional Amasi, acid adaptation had a significant effect ($p \leqslant 0.04$) on *E. coli* O157:H7 counts in commercial Amasi (Table 3). AA *E. coli* O157:H7 counts in commercial Amasi decreased from 6.6 $\log_{10}$ cfu/ml to 2.2 $\log_{10}$ cfu/ml after over 2 days, followed by an increase to 2.4 $\log_{10}$ cfu/ml after 3 days (Fig. 2). A continuous decline, from 6.7 $\log_{10}$ cfu/ml to 3.0 $\log_{10}$ cfu/ml, was observed for NA *E. coli* O157:H7 counts in commercial Amasi over 3 days (Fig. 2). There was also a significant ($p \leqslant 0.04$) interaction between Amasi, time and acid adaptation (Table 3).

A significant difference ($p \leqslant 0.00$) existed between *E. coli* O157:H7 counts in commercial Amasi and traditional Amasi after 3 days (Table 3). *Escherichia coli* O157:H7 counts in traditional Amasi decreased significantly by 6.6 $\log_{10}$ cycles after 3 days, while only a 2.5 $\log_{10}$ reduction in *E. coli* O157:H7 counts was observed in commercial Amasi after the same period (Fig. 2). A significant difference ($p \leqslant 0.00$) was also observed between Amasi and acid adaptation as well as with time (Table 3).

4. Discussion

4.1. Enumeration and characterization of microorganisms in the traditional fermented milk

LAB in the traditional fermented milk had the highest counts (6.4 $\log_{10}$ cfu/ml) when compared to total aerobic counts (5.8 $\log_{10}$ cfu/ml) and yeast and mould counts (4.1 $\log_{10}$ cfu/ml). Other traditional fermented milks have, however, been reported to have even higher LAB counts (8 $\log_{10}$ cfu/ml) and total aerobic counts that range from 8 $\log_{10}$ cfu/ml to 9 $\log_{10}$ cfu/ml (Beukes et al., 2001; Gran et al., 2003). In the study conducted by Beukes

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Table 2
Changes in pH of traditional Amasi at ambient temperature and commercial Amasi at ambient temperature for 24 h and refrigeration at 7 °C until 3 days, both inoculated with acid adapted or non-adapted *E. coli* O157:H7

Time	Traditional Amasi		Commercial Amasi	
	AA (±SD)	NA (±SD)	AA (±SD)	NA (±SD)
0	5.6 (0.0)	5.6 (0.0)	5.6 (0.0)	5.6 (0.0)
1	4.4 (0.0)	4.4 (0.1)	4.7 (0.1)	4.7 (0.1)
2	4.2 (0.0)	4.2 (0.0)	4.6 (0.1)	4.4 (0.0)
3	4.1 (0.1)	4.0 (0.0)	4.4 (0.0)	4.4 (0.0)

Values are means and standard deviations ($n = 3$).

et al. (2001), fermented milk samples were collected in the cooler months (May–August) which presumably favored the growth of LAB while in the current study, the traditional fermented milk inoculum was collected in November when the temperature was warmer. The difference in LAB counts in the fermented products could also be due to the differences in growth of LAB strains. The presence of presumptive *Listeria* species in the traditional fermented milk inoculum indicates that *Listeria* may survive in traditional fermented milk. *Listeria monocytogenes*, in particular, has been reported to develop acid resistance and survives at pH 3.5, when pre-exposed to mild pH values of pH 5–6 (Foster, 2000). Gulmez and Guven (2003) also reported that *L. monocytogenes* may survive in different yoghurt and kefir combinations when contamination occurs after pasteurization. In South Africa, the sale of dairy products containing pathogenic organisms is not allowed (South Africa, 1997). However, the traditional fermented milk is made for home consumption. Since *L. monocytogenes* is a pathogen that causes disease in pregnant woman, the very young or elderly and in people with immunocompromised systems, the presence of *Listeria* in the traditional fermented milk inoculum warrants further investigation (Adams and Moss, 2000).

4.2. Enumeration and characterization of microorganisms in traditional Amasi

Yeasts and moulds, which were initially regarded as a sign of poor hygiene, have received more attention in the dairy industry, more so, because of their potential use as starter cultures (Jakobsen and Narvhus, 1996; Loretan et al., 1998). Yeast and mould counts in traditional Amasi increased from 4.5 $\log_{10}$ cfu/ml to 5.8 $\log_{10}$ cfu/ml over 3 days. The high initial yeast and mould count was attributed to the traditional fermented milk inoculum that had yeast and mould count of 4.1 $\log_{10}$ cfu/ml. Initial yeast counts of 3.8 $\log_{10}$ cfu/ml were reported in all traditional containers used to ferment milk in South Africa, with clay pots having a wide diversity of yeasts (Kebede et al., 2007). Similar to these results, Gadaga et al. (2000) reported that traditional fermented milk from Zimbabwe had yeast and mould counts ranging from <3 $\log_{10}$ cfu/g to 5.7 $\log_{10}$ cfu/g with average yeast counts of 5 $\log_{10}$ cfu/ml. Yeast counts of 6.0 $\log_{10}$ cfu/ml were also reported by Isono et al. (1994) in Tanzanian fermented milk.

Mesophilic LAB in the present study had the highest counts in traditional Amasi reaching 8.9 $\log_{10}$ cfu/ml after 1 day when compared to thermophilic and lactococci LAB which had counts of 7.9 $\log_{10}$ cfu/ml and 7.8 $\log_{10}$ cfu/ml, respectively, after the same period. Similar to the results of this study, Beukes et al. (2001) reported mean LAB counts of 8 $\log_{10}$ cfu/ml in South African traditional fermented milks. In their findings, mesophilic LAB as well as lactococci bacteria had the highest counts, while in the present study, only mesophilic LAB dominated. Differences in experimental conditions such as different fermentation temperatures could have resulted in this difference. A similar decline, with regard to microbial population, was observed after 2 days in all the microorganisms except for yeasts and moulds. This could be due to the interaction of microorganism with each other and depletion of nutrients. Gadaga et al. (2000) also noted a decrease in the final population of yeasts in Zimbabwean fermented milk. The reduction in counts after 2 days also corresponded with reduction in pH which reached pH 4.2 in both clay pots inoculated with AA and NA *E. coli* O157:H7. Low pH increases the proportion of undissociated acid which traverses through plasma membrane, dissociates and acidifies the cytoplasm (Russel, 1992). This decreases cellular energy available to support growth functions hence disrupting intracellular processes.

Table 3
Statistical analyses of the survival and growth of acid adapted and non-adapted *E. coli* O157:H7 inoculated at pH 5.6 after 3 days in traditional and commercial Amasi products ($n = 3$)

Treatment	Degrees freedom	*P* value
Amasi (traditional, commercial)	1	0.003
Acid-adaptation (AA, NA)	1	0.044
Time (3 d)	3	0.000
Amasi × acid-adaptation	1	0.000
Amasi × time	3	0.000
Acid-adaptation × time	3	0.092
Amasi × acid-adaptation × time	3	0.022

4.3. Acid resistance of acid adapted and non-adapted E. coli O157:H7 in traditional and commercial Amasi products

E. coli O157:H7 in commercial Amasi survived to a greater extent than in traditional Amasi, as *E. coli* O157:H7 counts in the latter could not be detected after 3-days of challenge. This could firstly be attributed to the lower pH of traditional Amasi (pH 4.0) after 3 days compared to that of commercial Amasi (pH 4.4). Low external pH in bacteria leads to reduction in internal pH, which subsequently reduces the activity of acid sensitive enzymes, damages proteins and DNA in cells (Adams and Moss, 2000). Secondly, the higher initial level (8.4 $\log_{10}$ cfu/ml) of LAB in traditional Amasi compared to that in commercial Amasi (7.4 $\log_{10}$ cfu/ml) could have had more detrimental effects on *E. coli* O157:H7 in traditional Amasi. LAB are widely known for producing secondary metabolites such as bacteriocins or other antimicrobials that act against the growth of several spoilage and pathogenic bacteria (Bankole and Okagbue, 1992; Adams and Nicolaides, 1997; Borregaard and Arneborg, 1998). The traditional fermented milk inoculum could have also contained antimicrobial properties that aided reduction of *E. coli* O157:H7 in the product. The presence of yeasts (4.1 $\log_{10}$ cfu/ml) in the traditional fermented milk inoculum used for making traditional Amasi could have also contributed to the production of more secondary metabolites. Some yeasts have been reported to produce killer toxins that can kill sensitive bacteria (Polonelli and Morace, 1986). The encouragement of growth of starter cultures by yeasts producing essential growth metabolites such as amino acids and vitamins, has also been observed (Kaminarides and Laskos, 1992; Seiler, 1991; Jakobsen and Narvhus, 1996).

Furthermore, several studies have indicated that low temperature enhances the survival of *E. coli* O157:H7 (Cheng and Kaspar, 1998; Clavero and Beuchat, 1996; Faith et al., 1998). Bachrouri et al. (2002) observed that *E. coli* O157:H7 counts decreased by only 0.8 $\log_{10}$ cfu/g in yoghurt stored at 4 °C for 72 h, while in yoghurt stored at 22 °C, they could not detect any bacteria after 16 h of challenge. Since commercial Amasi was refrigerated at 7 °C for 24 h, survival of *E. coli* O157:H7 could have been enhanced by low temperature, compared to that in traditional Amasi, which was kept at ambient temperature throughout the 3-days of challenge. Low temperature triggers alteration in the

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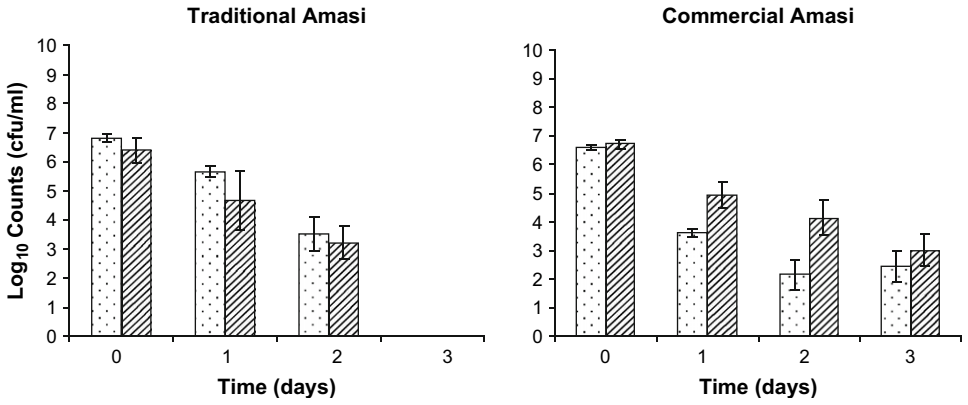

Fig. 2. Survival of acid adapted ▨ and non-adapted ▨ *E. coli* O157:H7 in traditional Amasi at ambient temperature for 3 days and commercial Amasi incubated at 30 °C for 24 h and then refrigerated at 7 °C until 3 days ($n = 3$).

cell membrane fatty acid composition and also results in the production of cold shock proteins (Jones et al., 1987; Berry and Foegeding, 1997). The proportion of unsaturated fatty acids increases, preventing fluid components from forming a gel. Gel-like fluids hinder proper functioning of proteins and cause bacterial membrane leakage (Russel et al., 1995). Contrary to this finding, Gran et al. (2003) reported that fermented milk made from starter cultures was more effective in reducing *E. coli* than fermented milk from back-slopping. In their study, *E. coli* counts were 5.7 $\log_{10}$ cfu/ml after 48 h fermentation at 25 °C in Zimbabwean Amasi fermented by back-slopping while the counts were 0.8–2.8 $\log_{10}$ cfu/ml in Amasi fermented with starter cultures after the same period. The study was, however, done with non-adapted cells and inoculation of *E. coli* was done immediately after milk collection, while in the present study, it was done at pH 5.6 when the level of LAB had increased. Since yeasts were not determined in their study, it remains unknown whether their presence or absence had an impact on the growth of bacteria. Slow pH decrease and lactic acid production, together with the possible existence of AA *E. coli* in the fermented milk product used for back-slopping, were given as reasons for the survival of *E. coli* in the product made with back-slopping.

Acid adaptation enhanced the survival of *E. coli* O157:H7 in traditional Amasi while NA *E. coli* O157:H7 survived significantly ($p \leqslant 0.04$) better than its AA counterpart in commercial Amasi. Concurring with the latter, Hsin-Yi and Chou (2001) reported that acid adaptation reduced survival of *E. coli* O157:H7 (ATCC 43889 and 43895) in Yakult and low-fat yoghurt stored at 7 °C, but enhanced survival of both strains in commercial fruit juice. Although *E. coli* O157:H7 possesses a higher level of acid tolerance compared to other *E. coli* serotypes, strain variations in acid tolerance have been reported (Arnold and Kasper, 1995; Miller and Kaspar, 1994; Benjamin and Datta, 1995). The strains used in the present study could, therefore, be less acid tolerant, hence explaining the failure of acid adaptation to boost survival at low pH.

Furthermore, the better survival of the NA *E. coli* O157:H7 strain compared to the AA *E. coli* O157:H7 strain in commercial Amasi could be due to the possibility that the NA strains simply adapts with changing pH while the AA strain, which had previously adapted to lower pH, had to adapt again to the new environment. The sudden shift of the AA strain to normal optimum growth conditions followed by the subsequent demand to re-adapt could have resulted in its failure to acquire maximum adaptation. It is also worth mentioning that the pH of the AA strain inoculum was 4.6 at the time of inoculation. The loss of adaptation when growing at optimum conditions was reported by Jordan et al. (1999) and Schweder et al. (1996).

In traditional Amasi acid adaptation of *E. coli* had a significant effect ($p \leqslant 0.00$) on the total aerobic counts. This was due to the difference in survival of AA and NA *E. coli* O157:H7 in traditional Amasi. The survival of yeasts and moulds, lactococci, mesophilic and thermophilic LAB in the clay pot inoculated with AA *E. coli* O157:H7 did not differ from that in the NA *E. coli* O157:H7 inoculated clay pot. This indicates that the survival of neither AA nor NA *E. coli* O157:H7 in the clay pots negatively affected the growth of other bacteria.

5. Conclusion

Escherichia coli O157:H7 was detectable in commercial Amasi after 3 days at 7 °C but not in traditional Amasi processed at ambient temperature over the same period. Both AA and NA *E. coli* O157:H7 showed a similar survival over 2 days in traditional Amasi, while in commercial Amasi, the NA strain survived significantly better than its AA counterpart. Regardless of prior adaptation to acid, *E. coli* O157:H7 can survive during fermentation and storage of fermented goat milk Amasi. The fermentation time, pH and storage temperature affects the survival of *E. coli* O157:H7 in the fermented milk.

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