(April-June, 2015)



GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES (Published By: Global Institute for Research & Education)

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Survival of Indicator Organisms (Coliforms) and Index Bacteria (*Escherichia Coli*) in Swaziland's Traditionally Fermented Emasi

A.M. Dlamini^{a*}, A.R. Amusan^b, A.B. Magongo^c

^a Department of Animal Science, University of Swaziland, Private Bag Luyengo, Luyengo Swaziland. ^b Department of Food Science and Agricultural Chemistry, Faculty of Agricultural and Environmental Science, McGill University, Quebec, Canada, H9X3X9

^c Ministry of Education, Schools Agriculture, Government offices, Mbabane, Swaziland

*Corresponding Author

Abstract

Traditionally processed emasi is made by fermenting raw milk thus raising public health concerns. The objective of this study was to determine survival of coliforms and *Escherichia coli* (*E. coli*) in traditionally made emasi. Coliforms and *E. coli* from emasi were isolated and enumerated using the spread plate method. Coliforms and *E. coli* survived fermentation during traditional production of emasi. Coliform counts increased by at least 4 log cycles in 72 hours, from $3.8*10^4$ cfu.mL⁻¹ to $5.69*10^8$ cfu.mL⁻¹. *Escherichia coli* also proliferated in traditionally made emasi. It increased by at least two log cycles (R² = 0.82). Coliforms and *E. coli* survived the fermentation despite acid production (R² = 0.92). The survival of coliforms, demonstrated that the traditional technique for emasi production is unhygienic. The survival of *E. coli* indicated that the traditional emasi is a potential public health hazard. Attempts should be done to produce traditional emasi hygienically.

Key words: emasi, coliforms, Esherichia coli, raw-milk

1. Introduction

Milk in the udder of a healthy cow is free from microorganisms. Despite being a highly nutritious food, milk however, may present a favourable physical environment for the multiplication of microorganisms (Robinson, 1990; Murinda, *et al.*, 2004; Oliver, *et al.*, 2005). There are several sources of milk contamination including the cow, the milker, the milking equipment and the environment in general (Robson, 1990; Rodojcic-Prodaova & Necev, 1991; Ali & Abdelgadir, 2011). Reports have shown that food spoilage, intoxicating and pathogenic bacteria can be found in raw milk (Fakudze & Dlamini, 2001; Ogwaro, *et al.*, 2002; Jayarao, *et al.*, 2006; D'Amico & Donnelly, 2010). Coliforms have been defined (Robinson, 1990; Walstra, *et al.*, 2006) as facultative anaerobic gram-negative, oxidase-negative, non-spore forming rods which can grow in the presence of bile salts or other surface active agents with similar growth inhibiting properties. They may include well known pathogens belonging to several genera within the family Enterobacteriaceae such as, *Escherichia, Enterobacter* and *Citrobacter*. It is well established that presence of high coliform counts in raw milk indicates production under unhygienic conditions (Arnold & Kaspar, 1995). Reports have shown that some coliforms may survive acid production in foods (Arnold & Kaspar, 1995; Ogwaro, *et al.*, 2002).

As reported before, Ashbolt, *et al.* (2001), index microorganisms are those whose presence in products implies the presence of a similar, but pathogenic organism. For example, the presence of *Escherichia coli* (*E. coli*) may indicate the possible presence of *Salmonella* and other pathogenic Enterobacteriaceae, the presence of male specific coliphages (F-RNA coliphages) may indicate a possible presence of human enteric viruses and the presence of *Listeria innocua* may indicate the presence of *Listeria monocytogenes* (Ashbolt, *et al.*, 2001). The significance of the isolation of index microorganisms from products that are ready for human consumption is the indication of a product that is a public health hazard. Previous reports (Dlamini and Dlamini, 1992; Nkambule & Dlamini, 2012) have shown that raw milk sold to consumers in Swaziland may contain high loads of coliform bacterial counts, including very high counts of *Escherichia coli*. Although coliforms are readily killed by pasteurisation (Elmer, 1978), there is evidence that some dairy products are produced from unpasteurised milk (Masarirambi, *et al.*, 2009; Pinto, *et al.*, 2009; Pinto, *et al.*, 2011). Emasi is a typical example of such a product. Emasi is fermented milk that is widely consumed in southern Africa and it has its distinct physical, chemical, microbiological and organoleptic attributes (Nsibandze & Dlamini, 2002; Masarirambi, *et al.*, 2009). Traditionally produced emasi is fermented from unpasteurised milk (Masarirambi, *et al.*, 2009). Previously, the hygienic safety of traditionally fermented emasi has not been studied. The purpose of this study was to investigate the survival of indicator organisms, coliforms and index organism, *Escherichia coli*, in traditionally fermented emasi.

2. Materials and Methods

2.1 Milk Samples

Raw milk samples were obtained from the University of Swaziland Dairy Farm in the Faculty of Agriculture, Luyengo Campus. The campus is located on latitude 26° 32' south and longitude 31° 14' east. The annual rainfall of the area ranges from 850 to 1000 mm and average maximum and minimum temperature is 23° C and 11° C respectively (Monadjem & David, 2005; Dlamini, *et al.*, 2008). Triplicate samples of milk were collected five times from the farm and a total of 15 samples were fermented to emasi and analysed for the presence and behaviour of coliforms and *E. coli*.

2.2 Fermentation studies

Each of the three samples collected at a time was split into three treatments, in 2L plastic buckets. The samples of

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the different treatments were accordingly fermented at 30° C for 120 hrs. Inoculum size of 5% (v/v) was appropriately added. The three treatments were:

- a) Treatment A: 3x2 litre unpasteurised milk allowed to ferment naturally.
 b) Treatment B: 3x2 litre pasturised milk inoculated with commercial emasi lactic acid bacteria (*Streptococcus lactis*) and *E. coli* culture.
- c) Treatment C: 3x2 litre pasteurised milk inoculated with commercial emasi lactic acid bacteria (*Streptococcus lactis*) and no *E. coli*.

2.3 Enumeration of microorganisms

Coliform counts were determined using the spread plate technique on MacConkey agar (Oxoid, UK). Samples were diluted on ¹/₄ strength ringer solution and 0.1 mL samples were spread on the agar plates. Inoculated plates were incubated at 37° C for 48hours. Enumeration of *E. coli* was done using the spread plate technique on Eosin –Methylene Blue (EMB) agar (Oxoid, UK). Plates were incubated at 35° C for 48 hours after which metallic sheen green colonies were counted. Colony counts were done on plates with samples from dilutions that had growth of 25-250 colonies.

2.4 Source of Escherichia coli for fermentation studies

Escherichia coli for fermentation studies was isolated from raw milk samples obtained from the University farm contaminated with cow dung. The isolation procedure involved three steps, enrichment, isolation and confirmation. Enrichment and differentiation tests were carried out using MacConkey broth (Oxoid, UK) to stimulate the growth of coliforms and to select for it by discouraging the growth of other organisms. Isolation of the organism was done by growing the presumptive *E. coli* in Eosin Methylene Blue (EMB) agar (Oxoid, UK). Confirmation tests for *E. coli* were carried out by conducting Biochemical and Physiological tests. These included Gram reaction, Catalase, Indole, Methyl Red (MR), Voges Proskuer (VP), Citrate Utilization tests and incubation in MacConkey Agar at 44^{0} C and in Eosin Methylene Blue agar at 35^{0} C. The procedures for these tests were followed as outlined by Johnson & Case (1995). The growth and biochemical results of the *E. coli* isolate were positive for catalase, indole, MR, growth on EMB at 35° C, growth on MaConkey agar (Oxoid, UK) at 44° C and they were negative for VP test and the gram reaction was negative.

2.5 Determination of pH

The pH was determined on 10mL milk samples using the pH meter model Hanna 211.

2.6 Titratable acidity determination

Titratable acidity was determined using 0.1N NaOH, 10mL samples and 4 drops of phenolphthalein indicator following the procedure described by ILRI (1994).

2.7 Statistical analysis

Colony counts were converted to Log_{10} using Microsoft Excel 5.0 (Microsoft Corporation Inc. 2003) statistical package. Trend analysis was done using regression relationship (R^2).

3. Results and Discussion

3.1 Behaviour of coliforms in traditionally fermented emasi

Previous reports have shown that consumer raw milk produced in Swaziland may have very high coliform counts of $>10^5$ cfu.mL⁻¹ (Dlamini, *et al.*, 1997; Fakudze & Dlamini, 2001) and high *E. coli* counts (Nkambule & Dlamini, 2012). The results presented on Figure 1 shows that during the traditional production of emasi, coliforms survived fermentation. The results have shown that Log₁₀ coliform counts increased with fermentation time. As can be seen from these results, Figure 1, the regression relationship between log₁₀ counts and fermentation time is R² = 0.920, indicating a strong positive relationship between increases in fermentation time and coliform counts. Coliform counts increased from $3.8*10^4$ cfu.mL⁻¹ at time 0 hours to $5.69*10^8$ cfu.mL⁻¹ after 72 hours.



Figure 1. Relationship between fermentation time and survival of coliforms during traditional fermentation of raw milk to emasi.

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It has been reported before that coliforms are unlikely to grow when the pH of milk has been brought down by lactic acid bacteria to < 4.5 (Walstra, *et al.*, 2006). Results from this study however (Figures 1-3), have shown that despite that during the fermentation of emasi, titratable acidity increased to 1.2% (v/v) and pH dropped to pH 3.8, coliforms proliferated and survived regardless of acid production. This is evidenced by the observation from these results that the lactic acid during the fermentation increased with time (R^2 = 0.92). Figure 3 also shows the relationship between the coliform counts and pH of the fermented milk. The coliform counts increased as the pH decreased (R^2 = 0.914). These results have shown that coliforms may survive the traditional fermentation process used for emasi production. The presence of coliforms in the fermented dairy product that is ready for human consumption indicates that the traditional method for emasi production is unhygienic.



Figure 2. Titratable acid production during the traditional fermentation of raw milk to emasi.



Figure 3. Relationship between pH and coliform counts during the traditional fermentation of raw milk to emasi.

3.2 Behavior of Escherichia coli in traditionally fermented emasi

Figure 4 indicates that *E. coli* counts increased from $5.34*10^5$ cfu.mL⁻¹ to $1.92*10^6$ cfu.mL⁻¹ after 48 hours. Even when raw milk was inoculated with the commercial emasi production culture, *Streptococcus lactis*, *E. coli* still grew and survived during the fermentation (Figure 5).



Figure 4. Survival of Escherichia coli in traditionally fermented emasi

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Figure 5. Survival of Escherichia coli in raw milk fermented using Streptococcus lactis as inoculum.

The results have shown that *E. coli* had increased by at least two log cycles after 60 hours of fermentation. *Escherichia coli* survived and increased, $R^2 = 0.82$, during the fermentation of raw milk in the presence of lactic acid bacteria. Figure 6 shows the relationship between titratable acidity and fermentation time of raw milk inoculated with *Streptococcus lactis*. It can be seen from the results that despite the increase in lactic acid production ($R^2=0.87$) during the fermentation, *Escherichia coli* survived and increased.



Figure 6. Relationship between fermentation time and titratable acid production during fermentation of raw milk inoculated with *Escherichia coli* and *Streptococcus lactis*.

Previous reports have suggested that the suppression of *E. coli* and *Aerobacter aerogenes* may be achieved by rapid lactic acid production from active starter cultures (Gadaga, *et al.*, 2004), provided that the lactic acid bacteria responsible for producing the lactic acid dominate the fermentation process (Walstra, *et al.*, 2006). In this study, it was observed that *E. coli* may survive fermentation in traditionally produced emasi. The surviving *E. coli* reached high population of $1.92*10^{6}$ cfu.mL⁻¹ after 60 hours of fermentation (Figure 5). These results have shown that the production of traditionally fermented emasi could be a public health hazard. The *E. coli* counts from traditionally fermented emasi with no lactic acid bacteria inoculated was slightly higher than that from raw milk fermented with *Streptococcus lactis* inoculated. This may be due to the slight inhibition of *E. coli* growth by the lactic acid bacteria (Gadaga, *et al.*, 2004). This, however, shows that the use of starter cultures in fermenting raw milk does not completely inhibit the growth of the index microorganism. Hence, the pasteurization of milk before inoculation with a starter culture is very important.

Ashenafi, (1993) reported that the use of starter cultures during fermentation of fish sausage resulted in a significant reduction in *E. coli, Salmonella typhimurium, Salmonella sofia* and *Staphylococcus aureus* cell numbers where the pH was quickly reduced to < 4 within 48 hours. The high population of lactic acid bacteria and the rapid increase in acidity during inoculated fermentations were strong deterrents to the survival and growth of the various microbial pathogens. However, this was not observed in the present study, probably the acidity developed during traditionally fermented emasi was insufficient to inhibit the growth of the index bacteria. It was observed that during the fermentation of the emasi, acid production may not be quick enough to reduce the pH to < 4 within 48 hours of the fermentation time. It was also observed from the study that *E. coli* can survive in 1.17% (v/v) lactic acid. The findings from this study have implied that emasi produced using the traditional fermentation technology may contain high *E. coli* counts and most probable, other similar but pathogenic organism thus making the product a health hazard to consumers.

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4. Conclusion

Coliforms survived during the traditional fermentation of emasi. It can thus be concluded that the traditionally fermented emasi is unhygienic. *Escherichia coli* also survived during traditional fermentation of emasi. The survival of this index organism shows that consumption of this ready to eat product is a public health hazard. Acid production during the fermentation of emasi is not sufficient to inhibit growth coliforms and *E. coli*. Since both coliforms and *E. coli* are destroyed by pasteurization, there is need to enforce legislation requiring that heat treated milk should be used to produce emasi in the traditional sector.

Acknowledgement

The authors wish to thank the University of Swaziland Research Board for funding this research work.

Reference

Ali, A.A., Abdelgadir, W.S. 2011. Incidence of *Escherichia coli* in raw cow's milk in Khartoum State. *British Journal of Dairy Sciences*, 2, 23-26.

Arnold, K.W., Kaspar, C.W.1995. Starvation and stationary phase induce acid tolerance in *Escherichia coli 0157: H 7. Journal of Applied and Environmental Microbiology*, 61, 2037 - 2039.

Ashbolt, N.J., Grabow, W.O.K., Snozzi M. 2001. indicators of microbial water quality. in 1 fewtrell, j. iwa. batram, (Eds), *Water quality guidelines standards and health*. World Health Organization (WHO) Publishing, London UK.

Ashenafi, M. 1993. Fate of *Salmonella enteritidis* and *Salmonella typhimurium* during the fermentation of ergo, a traditional Ethiopian sour milk. *Ethiopian Medical Journal*, 31, 91 - 98.

D'amico, D.J., Donelly, S.W. 2010. Microbiological quality of raw milk used for small-scale artisan cheese production in Vermont: effect of farm characteristics and practices. *Journal of Dairy Science*, 93, 134-147.

Dlamini, A.M., Dlamini, C. 1992. Detection of coliform bacteria in consumer milk inSwaziland. UNISWA Journal of Agriculture, 1, 83-85

Dlamini, A.M., Shandomo, M.N., Hadebe, M. 1997. Bacteriological quality of consumer's raw milk in the Malkerns Valley dairy farms of Swaziland. UNISWA Research Journal of Agriculture, 6, 36 – 42.

Dlamini, A.M., Dube M.A., Nkambule N. 2008. Stakeholder's perceptions and application of biotechnology in agricultural based industries in Swaziland. *Scientific Research and Essays*, 3, 477 – 487.

Elmer, H.M. 1978. Standard Methods for Examining Dairy Products. American public Health Association, Washington.

Fakudze, F.M., Dlamini, A.M. 2001. Composition and hygienic quality of consumer Milk from shops in Swaziland. UNISWA Journal of Agriculture, 5, 169.

Gadaga, T.H., Nyange, L.K., Mutukumira, A.N. 2004. Food control activities in fermented foods. *African Journal of Food, Agriculture, Nutrition and Development*, 2, 271 – 288.

ILRI, 1995. Rural Dairy Technology. International Livestock Research Institute, International Livestock Centre, Addis Ababa, Ethiopia.

Jayarao, B.A., Jayarao, B.M., Donaldson, S.G., Straley, A.A., Hegde, N.V., Brown, J.L. 2006. A survey of foodborne pathogens in bulk tank milk and raw milk consumption among families in Pennsylvania. *Journal of Dairy Science*, 89, 2451-2458.

Johnson, T.R., Case, C.L. 1995. Laboratory Experiments in Microbiology, Forth Edition. The Benjamin / Cunmings Publishing Company. California, USA.

Masarirambi, M.T., Mhazo, N., Dlamini, A.M., Mutukumira, A.N. 2009. Common Indigenous fermented foods and beverages produced in Swaziland. *Journal of Food Science and*-*Technology*. 46, 505-508.

Monadjem, A., David, K.G. 2005. Nesting and distribution of vultures in relation to land use in Swaziland. *Biodiversity and Conservation Journal*, 14, 2079 – 2093.

Murinda, S.E., Nguyen, L.T., Man, H.M., Almedia, R.A. 2004. Detection of sorbitol negative and sorbitol-positive shiga toxinproducing *Escherichia coli*, *Listeria monocytogenes, Campylobacter jejuni* and *Salmonella* species in dairy farm environments. *Foodborne Pathogens and Disease*, 1, 97-104.

Nsibandze, R.T., Dlamini, A.M. 2000. The compositional and fermentation quality of Swaziland emasi. UNISWA Research Journal of Agriculture, Science and Technology, 3, 48 – 53.

Nkambule, B.L., Dlamini, A.M. 2012. Determination of milk-borne public health hazards in raw milk from retail outlets around Manzini. UNISWA Journal of Agriculture, 16, 24-40

Ogwaro, B.A., Gibson, H., Whitehead, M., Hill, d.j. 2002. Survival of *Escherichia coli* 0157: H7 in traditional African yoghurt fermentation. *International Journal of Microbiology*, 79, 105 - 112.

Oliver, S.P., Jayarao, B.M., Almedia, R.A. 2005. Food borne pathogens in milk and the dairy environment food safety and public health implications. *Food- borne Pathogens and Disease*, 2, 1115-1129.

O' Mahony, F. 1988. Rural dairy development: Experiences in Ethiopia. ILCA. Addis Ababa.

Pinto, M.S., Calvalho, A.F., Pires A.C.S., Paula, J.C.J., Sobral, D., Magalhaes, F.A.R. 2009. Survival of *Listeria innocua* in Minas traditional Serro cheese during ripening. *Food Control*, 20, 1167-1170.

Pinto, M.S., Calvalho, A.F., Pires A.C.S., Souza A.A.C., Silva, P.H.F., Sobral, D., Paula, J.C.J., Santos, A.L. 2011. The effects of nisin on *Staphylococcus aureus* count and physicochemical properties of traditional Minas cheese. *International Dairy Journal*, 21, 90-96

Robinson, K.R. 1990. *The Microbiology of milk*. Department of Food Science and Technology. University of reading, U.K Elsevier Science Publishers Ltd.

Rodojcic-Prodaova, D., Necev T., 1991. Most common agents of subclinical mastitis in cows on private and communal farms in the republic of Macedonia. *Vet. Glasnik*, 45, 745-747.

Walstra, P., Wouters, J.T.M., Geurts, T.J. 2006 . Dairy Science and Technology (second edition). Taylor and Francis Group, New York, USA.