Comparative evaluation of the microbial safety of boiled locally vended ready-to-drink, HTST pasteurized and UHT bovine milk sold in Nakawa Division of Metropolitan Kampala

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Abstract: This study evaluated the microbial safety of vended boiled, pasteurized and UHT milk sold in Nakawa, Kampala-Uganda. 15 milk samples were analyzed; 2 samples had *Salmonella*, 5 had *S. aureus* with a count of 1.66 ± 0.02 $\log_{10}$CFU/ml. *E. coli* was detected in 8 samples with 1.0 ± 0.02 to $3.0\pm0.01\log_{10}$CFU/ml count. A high load of $3.0\pm0.01\log10$ CFU/ml was obtained in 3 samples with *E. coli*. Four *E. coli* positive samples had a contamination load of $2.0\pm0.015\log_{10}$CFU/ml of which one was pasteurized milk. Only a pasteurized milk showed a low E. *coli* load at $1.0\pm0.02\log_{10}$ CFU/ml. All UHT milk had no microbial contamination. Both boiled and pasteurized milk had *Salmonella, S. aureus* and *E. coli* in levels above the set threshold limits. Milk consumers in Nakawa stand a potential public health risk of food poisoning reflected by presence of *Salmonella, S. aureus* and *E. coli* in some milk sold in the area.

Key-words: Contamination, *Escherichia coli*, fecal coliforms, Nakawa, *Salmonella, Staphylococcus aureus.*

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1.0 Introduction

Uganda, the pearl of Africa is blessed with a copious produce of milk that is consumed locally either directly or further processed by an unexhaustive list of licensed milk processing companies. Street vendors locally prepare ready-to-do (RTD) milk and vend them amongst other street foods to earn a livelihood. However, inadequately prepared comestible street-vended foods, including milk, meat and their products are implicated vectors for the transit of resistant bacteria and pathogenic genes into humans [1-3]. Superbugs, such as *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Listeria monocytogenes* and *Bacillus cereus* in street vended foods have threatened public health and have been an unsurpassable subject of continuous obsessive research [4-8]. Milk is chemically an emulsion of butterfat globules entrained in an aqueous milieu with dissolved biological molecules of life [9]. Albeit its culinary and nutritional completeness, milk and its products ought to be obtained, handled and processed under good and strict sanitary conditions as it is highly ephemeral [10, 11] and its contamination thereof is a cause of grave human health concern [12]. Microbial contamination of milk is exacerbated by its complex biochemical profile and high-water activity, which provides a good milieu wherein microbes thrives [1], thereof triggering milk-borne infections [4, 13, 14]. Interestingly, milk possess an inherent antimicrobial mechanism known to beneficially retard microbial growth in the first few hours of milk holding without treatment and/or preservation [15, 16]. Raw bovine milk can reportedly house up to $3.0\log_{10}$ counts of bacteria per millilitres [16, 17]. Etiologic agents, notorious in raw bovine milk are *Salmonella* (Typhimurium) and other *Salmonella* serovars, *Enterobacter* species (spp), *Campylobacter* spp, *Listeria monocytogenes*, contagious mastitis pathogens: *S. aureus, Streptococcus agalactiae* and *Mycoplasma bovis*, *E. coli* 0157:H7, *Brucella* spp, *Coxiella burnetii, Proteus* spp, *Leptospira* spp, *Corynebacterium ulcerans, Clostridium* spp, yeasts and moulds [3, 18-23]. Coliforms, including *E. coli* 057:H7 are innocuous colon inhabitants whose detection in bovine milk are implicatory of faeces-related contamination [11]. An Australian survey in 2007 on 183 raw milk samples recorded a heightened prevalence of *E. coli,* coagulase-positive *S. aureus* and *Salmonella* species at 8% [10]. *E. coli* O157:H7 peculiarly has been isolated from bovine dairy farms and bulk raw bovine milk globally at 1-33.5% prevalence [7] and reportedly implicated for outbreaks of fatal Hemolytic-uremic syndrome (HUS) and hemorrhagic colitis [18, 21]. Microbial milk contamination is multifactorial originating from animal foods, exposure to faecal and environmental contamination as well as the holding containers and water sources [24] and directly from lactating dairy animals with mastitis [25].

Efforts of thermal treatment sought thus is always geared towards the reduction of this microbial loads to none-lethal or better still, safer levels [6], thereby increasing its shelf life [26] and thus comestibility. The shelf life of pasteurized milk is affected by large numbers of psychotropic bacteria that reportedly predominates the microbial profile of raw milk and which profile positively translate into concentrations of plasmin (a heat-stable protease) and lipoprotein lipase in the milk; activities of these extracellular enzymes augment those of bacterial hydrolases, shortening the storage life of milk [27, 28]. Albeit a vast array of microbial pathogens incessantly encountered in raw and processed bovine milk, there is finite published data on the prevalence and levels of pathogens in bovine milk [29]. In Uganda, studies of Wawa *et al* [7] and Mugampoza *et al* [8] reported the prevalence of bacteriological contaminants in selected processed bovine milk products vended in selected regions of Uganda. Further, Mugampoza *et al* [30] reported the microbial load and prevalence of *E. coli* and *Salmonella* spp. in ready-to-eat street-vended foods in Nakawa and Naguru parishes of Metropolitan Kampala. This study augmented the aforeacknowledged reports by investigating the microbial load and safety of locally vended RTD, high temperature short time (HTST) pasteurized and UHT milk sold in Nakawa division of Kampala.

2.0 Materials and methods

2.1 Sample size and sample collection

The study was conducted in Banda parish of Nakawa division of Metropolitan Kampala, Uganda between July to October 2018 on a total of 15 representative bovine milk samples bought from local supermarkets and local people vending RTD boiled milk. They included 5 different brands of UHT milk samples (500ml each), 5 assorted brands of pasteurized milk (500ml each), and 5 locally boiled RTD milk obtained from 5 different local people vending RTD milk. All the samples were obtained according to ISO/DIS 707 [31]. Thus, the milk samples were aseptically collected and examined for microbial contamination using ISO Analytical methods at the laboratory of Uganda Industrial Research Institute, Department of Microbiology, 42A Mukabya Rd, Nakawa Industrial Area, Nakawa-Kampala.

2.2 Microbial Analysis

Microbial analysis involved isolation, detection and total colony count (TCC) of *Salmonella*, *S. aureus*, total fecal coliforms (TFC) and thus *E. coli*.

2.21 Isolation and detection of *Salmonella* in the milk samples

Isolation of *Salmonella* was carried out using the ISO 6759 [32] standard method for detection and isolation of *Salmonella* species in dairy and food products. The milk samples were pre-enriched by inoculating 25ml of the samples into 225ml of Buffered Peptone Water (BPW) followed by serological incubation at 37^0C for 24 hours. Following pre-enrichment, 1ml of the samples were selectively enriched in 10ml of Selenite Cysteine Broth, SCB (Sigma Aldrich) and incubated for 24hrs at 37^0C. Selective plating on Xylose Lysine Deoxycholate, XLD (Oxoid, UK) agar was done by streaking a loop full of the selectively enriched contents and incubating at 37^0C for 48hours. The XLD plates were observed for colonies that appeared pinkish in color with or without a dark or black center of hydrogen sulphite as a characteristic feature for *Salmonella*. Suspected colonies were then subjected to citrate and urease biochemical tests for identification of *Salmonella* spp. Citrate test was performed to determine the ability of the isolates previously obtained on XLD plates to utilize citrate as a carbon source. The isolated suspect microorganisms were subsequently inoculated on Simmon's citrate agar (SCA) slant and incubated at 37^0C for 48hours. After incubation, the tubes were examined for change in coloration of slant. A change in coloration from green to blue indicate positive test for citrate utilization and thus confirming *Salmonella.* Urease agar was inoculated with the colonies of the test isolates and incubated at 37℃ for 24 hours. The change of color of the slant from light orange to magenta within the hours of incubation was taken to show the presence of *Salmonella*.

2.22 Isolation and enumeration of *S. aureus*

ISO 6888-1 [33] laboratory test method for *S. aureus* enumeration and isolation by spread plate method was used. Briefly, 10ml portion of each sample was diluted with 90ml of sterile Baird Parker Medium to form 10^{-1} sample homogenate. 1ml of serially diluted samples were used as test portions on Mannitol Salt Agar, MSA (Oxoid, U.K). Then 1.0 ml test portion of 10^{-1} sample homogenate was aseptically distributed and spread onto three poured plates of MSA. The plates were incubated aerobically for 48hours at 37^0C. After the incubation, plates carrying sample portions, colonies of *S. aureus* appearing to be circular, smooth, convex, moist, 2-3 mm in diameter (on uncrowned plates), above all with unique features of gray to jet-black frequently with light-colored (off-white) margin which is surrounded by opaque zone were enumerated as *S. aureus*. The total count of *S. aureus* in each milk sample was computed as the number of organisms of colony forming units per ml (CFU/ml) of samples from equation (1)

$$N \ = \ A \ x \ D \hspace{4cm} (1)$$

Confirmation for *S. aureus* (Slide Agglutination and Catalase test)

Rapid Latex Slide Agglutination (Staphurex kit) and catalase tests were used for the confirmatory identification of presumptive *Staphylococcus aureus* colonies as per the standard ISO 6888-1&2 [33] procedure. Isolated colonies of the suspected *S. aureus* were emulsified in a 3.0ml of Brain Heart Infusion broth and incubated at 37^0C for 18hours. One drop of overnight cultures was mixed with Latex coagulase reagent on the Latex Slide and rocked for 60 seconds observing for agglutination. For catalase test, exactly 0.1 ml of the overnight culture portions were transferred onto clean glass slides followed by equal parts of 3% hydrogen peroxide and observed for effervescence.

2.23 Detection of indicator organisms of fecal contamination

Chromocult® Coliform Agar (CCA) was used following Pour Plate Technique for the enumeration of Total coliforms and *E. coli* [34]. In this method, a 10ml portion of milk sample was aseptically diluted with 90ml of sterilized Buffered Peptone Water (BPW). Serial dilutions from 10^{-1} to 10^{-5} were aseptically prepared using a 10-fold serial dilution series. Then, 0.1 ml of the homogenate was spread onto prepared plates of Chromocult® Coliform Agar (Pronadisa). Plates were then incubated for 48 hours at 37°C. Blue colonies on CCA were identified and counted as *E. coli*, whereas purple-pink colonies as other coliforms. A sample of purple-pink colonies from CCA were subjected to indole tube test. Sterilized test tubes containing 4ml of tryptophan broth were aseptically inoculated with a colony and incubated at 37°C for 24 hours. After incubation, 0.5ml of Kovac's reagent was added to the broth culture. Appearance of pink colored ring was taken as confirmatory presence of *E. coli*. Colonies were counted promptly after the incubation period. Plates with 0-300 colonies were selected and colonies counted with the use of a colony counter. For the dilution, whose average count (arithmetic mean) of the duplicate plates was near 30 CFU were computed using equation (1).

3.0 Results and statistical analysis

The experiment was done in triplicate and statistically significant differences between the microbial load of the milk samples was established using analysis of variance (ANOVA) performed using Minitab statistical software (v6, Minitab Inc., USA).

Salmonella

There was total absence of *Salmonella* in 13 (86.7%) of the 15 samples analyzed. One (20.0%) sample of the locally boiled RTD milk and one (20.0%) sample of the HTST pasteurized milk

was positive for *Salmonella*. The overall point occurrence of *Salmonella* in milk samples under this study was 13.3%.

Figure 1 XLD plates positive for *Salmonella*

Staphylococcus aureus

Five samples (33.3%) of the 15 milk samples analyzed had *Staphylococcus* with a mean count of $1.66\pm0.02\log_{10}$CFU/ml. Of these, were 3 samples (60.0%) of the locally boiled RTD with a mean count of $1.89\pm0.01\log_{10}$CFU/ml and 2 (40.0%) pasteurized milk samples with a mean count of $1.46\pm0.03\log_{10}$ CFU/ml. *S. aureus* was not detected in all the five (100%) UHT milk samples analyzed. The overall point occurrence of *Staphylococcus* in the assorted milk samples in this study was 33.3%.

Table 1 Occurrence and mean count of *S. aureus* in the milk samples

Sample	*Staphylococcus* occurrence (%)	[a]Mean count ($\log_{10}$CFU/ml)
Locally Boiled RTD milk	60	1.89 ± 0.01
HTST Pasteurized milk	40	1.46 ± 0.03
UHT milk	0.00	0.00 ± 0.00

[a] Mean count are presented as Mean$\pm$S.E (Standard error) of experiments done in triplicate.
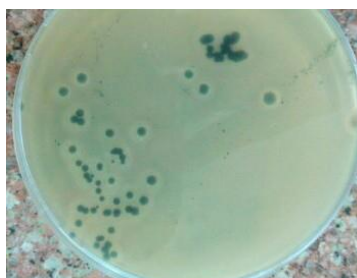

Figure 2 Baird Parker Agar plate positive for *S. aureus*

Escherichia coli

E. coli was detected in 8 (53.3%) of the 15 samples analyzed with counts in the range of 1.0 ± 0.02 to $3.0\pm0.01 \log_{10}$ CFU/ml. A high load of $3.0\pm0.01 \log10$ CFU/ml was obtained in 3 (37.5%) of the samples that showed positive for *E. coli*; all were samples of the boiled locally vended RTD milk. Four (50.0%) of the *E. coli* positive samples had a contamination load of $2.0\pm0.015 \log_{10}$ CFU/ml of which one sample was a pasteurized milk sample. Only one (12.5%) sample showed a low load of E. *coli* at $1.0\pm0.02 \log_{10}$ CFU/ml and this was a pasteurized milk sample. All the 5 (100%) UHT milk samples showed negative for *E. coli*. A significant difference ($p<0.05$) in occurrence of *E. coli* was observed between locally boiled RTD and HTST pasteurized milk.

Table **2** Occurrence and mean count of *E. coli* in the milk samples

Sample	Prevalence of *E. coli* (%)	Mean *E. coli* count ($\log_{10}$CFU/ml)
Locally boiled RTD milk	100	3.52 ± 0.015
HTST Pasteurized milk	60	2.002 ± 0.02
UHT milk	0.00	0.00 ± 0.00

4.0 Discussion

Thirteen (86.7%) milk samples did not have *Salmonella*. However, one (20.0%) sample of the locally boiled RTD milk and one (20.0%) sample of the HTST pasteurized milk samples analyzed were positive for *Salmonella* implying that they are not safe for human consumption since some strains of *Salmonella* are known to cause food poisoning and salmonellosis in humans [35].

Five samples (33.3%) of the total samples tested in the study had *S. aureus*. Three samples (60.0%) of the locally boiled RTD milk had *S. aureus* with a mean count of $1.81.46\pm0.01$ $\log_{10}$CFU/ml while two (40.0%) pasteurized milk samples had a mean count of $1.46\pm0.03\log_{10}$CFU/ml. A significant difference ($p<0.05$) in occurrence of *S. aureus* was observed between locally boiled RTD and pasteurized milk. The study findings on *S. aureus* in the milk samples indicated a possible health risk because *S. aureus* produces a heat stable toxin in milk [13] that may lead to food poisoning. More so, its prevalence is of great concern to dairy milk processors as it is seemingly an innocuous microflora of the mammalian mucus, and skin and thus suggests there is deficient hygienic practices during milk processing [35]. The study showed that *S. aureus* is common in milk in Nakawa and may impose a public health hazard.

However, the absence of *S. aureus* in all the five UHT milk samples analyzed is an indication that the elevated temperature used in UHT treatment is effective in killing *S. aureus* and such milk is very safe for human consumption. Matter-of-factly, UHT milk should be devoid of microorganisms capable of proliferation at conditions encountered before consumption in their packagings at 25-37℃ for a week or more.

The presence of *E. coli* and other faecal coliforms in eight (86.7%) of the total samples analyzed in this study is a clear implication of the possible faecal contamination of the milk [36] probably due to inapt cleanliness while milking, preparing, storing and handling of milk as well as inapt or no reheating and inapt hawked milk temperatures during sale [37, 38]. This could be because the hawkers must move around in search of potential customers. All the five (100.0%) UHT milk samples had no detectable *E. coli.* A significant difference ($p<0.05$) in occurrence of *E. coli* was observed between locally boiled vended RTD and HTST pasteurized milk. These findings imply that consumers of locally boiled RTD milk and some of the pasteurized milk might be at risk of Enterohaemorrhagic infections if the process of boiling and pasteurization are not well effected. *E. coli* can be destroyed by thorough cooking of foods until 70°C or higher. *E. coli* O157: H7 is the most important STEC serotype of daily increasing clinical importance in relation to public health. Therefore, boiling should be made effective by ensuring ample holding temperature and the milk should be allowed to cool in a sufficiently clean container to avoid post-processing contamination. Despite the existence of pathogenic strains, the observed presence of *E. coli* is an alarm indicator of contamination. Strategies should be laid to reduce microbiological contamination during milking and storage to minimize microbial growth and maintain quality milk products. It should be overemphasized that the bacterial contamination observed in some of the pasteurized milk samples in this study is a deleteriously serious observation as the raw milk had undergone heat treatment and is mostly preferred by consumers as UHT treatment causes milk to lose its natural flavour and the economically prohibitive purchasing costs make them not a first choice of some consumers [39].

The results observed with UHT milk in this study are comparable with the report of Nascentes and Araújo [5] who found that the UHT milk in Patos de Minas MG-Brazil had no detectable microbial contaminants. The elevated microbial load of RTD milk could supposedly be due to the entry of the pathogens into the milk through inadequate sanitation during milking, handling, and local preparation. Furthermore, the poor hygienic conditions noted at the sale points for the locally boiled vended RTD milk during sampling might have contributed to such elevated occurrence of the microorganisms in the RTD milk.

5.0 Conclusions and recommendations

Both boiled RTD and pasteurized milk in this study had *S. aureus, E. coli* and *Salmonella* in levels above the threshold limits by WHO and FDA and therefore poses a potential public health risk. The bacteriological profile of UHT milk samples in the study conformed to the safety requirements as set by FDA and WHO. *E. coli* is more prevalent than *S. aureus* and *Salmonella* in the milk samples investigated in this study. Local milk consumers in Nakawa Division stand a potential public health risk of food poisoning as shown by the presence of *S. aureus* and *Salmonella* encountered in the tested samples. Due to the observed presence of pathogens in the milk samples, proper handling and preservation of milk should be observed with respect to proper hygiene as recommended by WHO. Public health sensitization programs in milk handling should be launched. From a food safety point of view, food handlers at different points in the milk value chain should be educated on how to reduce contamination of milk and milk products from *Staphylococcus species* and other pathogens through personal protective equipment hygiene as well provision of cold-chain equipment in the milk supply chain. A comparative evaluation of the microbial safety of other milk brands on the market should be assessed using a larger sample size.

References

1. Parekh TS and Subhash R. Molecular and bacteriological examination of milk from different milch animals with special reference to Coliforms. Curr. Res. Bact.,2008; 1:56-63.

2. Claeys W, Cardoen G.,*et al*. Raw or heated cow milk consumption: Review of risks and benefits. Food Control,2013;31:251-262.

3. Ryser ET. Public Health concerns, in Applied Diary Microbiology. 1998, Marcel Dekker, Inc.: New York. 1998;263-403.

4. Murinda SE, Man HM and Almedia RA. Detection of sorbitol negative and sorbitol-positive shiga toxin-producing Escherichia coli, Listeria monocytogenes, Campylobacter jejuni and Salmonella species in dairy farm environments. Food-borne Path and Dis,2004;1:97-104.

5. Nascentes RM & Arujo BC. Comparação da qualidade microbiológica de leite cru, pasteurizado e UHT comercializados na cidade de Patos de Minas, MG. Perquirere.2016; 9(1):212-223.

6. dos Santos, M.L., Baross, L.S.S, Isabella de Matos, M., Marcos VAVL. Prevalence of pathogens and microbiological quality of milk marketed in the region of the Recôncavo from Bahia, Brazil. Int. J En Agri Res,2016; 2(10), 78-81.

7. Wawa SA, Serunjogi ML, Ogwok P, Mugampoza D. Risk assessment for the occurrence of Escherichia coli 0157:H7 in indigenous fermented milk (Lee naga a agbora) produced in Uganda. Intern. J. Trop. Agric. Food Sys, 2009;68-74.

8. Mugampoza D, Byarugaba GWB, Nyonyintono A & Nakitto P. Occurrence of Escherichia coli and Salmonella spp. In street-vended foods and general hygienic and trading practices in Nakawa Division, Uganda. Am. J. Food. Nutr, 2013; 3(3):167-175.

9. Vargas M, Ca`fer M, Albors A, Chiralt A, Gonza´lez-Martine´z C. Physicochemical and sensory characteristics of yogurt produced from mixtures of cow's and goat's milk. Int Dairy J,2008;18:1142–1152.

10. Kim HJ, Hardy G, Novak, JP, Ramet & Weber F.Off-tastes in raw and reconstituted milk. FAO Animal, 1983:11.

11. Steele ML, Mcnab W, Poppe C, Graffiths MW, Chen S, Degrandis SA, Fruhner LC, Larkin CA, Lynch JA & Odumeru JA. Survey of Ontario bulk tank milk for food borne pathogens. J. Food Prot, 1997;60:1341-1346.

12. Oliveira LP, Ludmilla SSB, Silva VC & Marina GC. Study of Staphylococcus aureus in raw and pasteurized milk consumed in the Reconcavo area of the State of Bahia, Brazil. J Food Pr Technol, 2011; 2(6): 228-239.

13. Normanno TG, La Salandra G, Dambrosio A, Quaglia NC, Corrente M, Parisi A, Santagada G, Firinu A, Crisetti E, Celano GV. Occurrence, characterization and antimicrobial resistance of enterotoxigenic Staphylococcus aureus isolated from meat and dairy products. Int J Food Microbiol, 2007;115:290–296.

14. Oliver SP, B.K., Murphy SC and Murinda SE. Food Safety Hazards Associated with Consumption of Raw Milk. Food borne pathogens and disease, 2009; 9(6): 6534-6558.

15. Schoonman L & Swai ES. Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. Asian Pac. J. Trop. Biomed, 2011;1:217-222.

16. Pandey GS & Voskuil GCS. Manual on Milk safety, quality and hygiene. Zambia.2011.

17. Omore A, Loore T, Staal S, Kutwa J, Ouma R, Arimi S & Kang'ethe E.Addressing the public health and quality concerns towards marketed milk in Kenya, 2005.

18. Thaker HC, Brahbhatt N & Nayak JB.Veterinary world Study On the occurrence and Antibiogram Pattern of Escherichia coli from raw milk sample in Anand, Gujarat, India. Vet World, 2012; 5:556-559.

19. Quigely L, O`Sullivan O, Stanason C, Beresford TF, Ross RF, *et al*.Complex microbiota of raw milk. FEMS microbiol Rev, 2013;37:664-698.

20. Shirima GM, Fitzpatrick J, Cleaveland S, Kambarage DM, Kazwala RR, Kunda J & French NP.Participatory survey on zoonotic diseases affecting livestock keeping communities in Tanzania. J. Ani. Vet. Adv, 2003;2:253 -258.

21. Sivapalasingams S, Friedman CR, Cohen L & Tauxe RV. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States. J. Food Prot, 2004; 67:2342-2353.

22. Al-Tahiri R. A comparison on microbial conditions between traditional dairy products sold in Karak and same products produced by modern dairies. Pak. J. Nutri, 2005;4:345-348.

23. Zadoks R. Streptococcus uberis; environmental or contagious pathogen, in processing. in 42nd Annual meeting of National Mastitis Xouncil incorporated. USA: Natl. Mast. Coun. Madison, 2003.

24. Eberhart RJ. Coliform Mastitis. J Amer Vet Assoc,1977; 70:1160-1163.

25. Rodojcic-Prodaova D and Necev T. Most common agents of subclinical mastitis in cows on private and communal farms in the republic of Macedonia. Vet. Glasnik, 1999;45:745-747.

26. Goff HD & Griffiths MW. Major advances in fresh milk amd milk products. J Dairy Sci, 2006;89:1163-1173.

27. Barbano DM, Ma Y & Santos M. Influence of raw milk quality on fluid milk shelf life. J Dairy Sci, 2005;88:77.

28. Hantsis-Zacharov E & Halpern M. Culturable psychotrophic bacterial communities in raw milk and their proteolytic and lipolytic traits. Applied Environ. Microbiol.,2007;73: 7162-7168.

29. FAO/WHO. Risk assessment of Listeria monocytogenes in ready-to-eat foods. 2004.

30. Mugampoza D, Muyanza CMBK, Ogwok P, Serunjogi ML, Nasinyama GW. Occurrence of Listeria monocytogenes in bulked raw milk and traditionally fermented dairy products in Uganda. African J. Food Agric. Nut. Dev, 2011;11:4610-4622.

31. ISO. ISO/DIS 707. Milk and milk products-Guidence on sampling, Geneva, Switzerland, 1995.

32. ISO. ISO 6759:2002+A1:2007. Horizontal method for the detection of Salmonella species, Geneva, Switzerland,2007.

33. ISO. ISO 6888-1:1999. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) Part 1: Technique using Baird-Parker agar medium, 1999.

34. ISO. ISO 7251:2005. Horizontalmethod for the detection and enumeration of presumptive Escherichia coli, Geneva, Switzerland, 2005.

35. ISO. ISO saftey food brochure. Making food safer according to ISO methods. Geneva: Thermo Scientific Microbiology products, 2003.

36. Gelsomino R, Vancanneyt M, Cogan TM, Condon S, Swings J. Source of enterococci in a farmhouse raw-milk cheese. Appl. Environ. Microbiol, 2002; 68:3560-3565.

37. Umoh VJ & Odoba MD. Safety and quality evaluation of street foods sold in Zaria, Nigeria. Food Cont., 1999;10:9-14.

38. Tambekar DH, Kulkarni RV, Shirsat SD, Bhadange DG.Bacteriological quality of street vended food panipuri: a case study of Amravati city (ms) India. Biosc. Disc, 2011, 2, 350-354.

39. Chapman KW & Boor KJ. Acceptance of 2% Ultrapasteurized milk by consumers. J Dairy Sci, 2001; 84:951-954.