OPEN ACCESS

African Journal of Bacteriology Research

August 2018
ISSN 2006-9871
DOI: 10.5897/JBR
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

ABOUT JBR

The African Journal of Bacteriology Research (JBR) (ISSN 2006-9871) is published Monthly (one volume per year) by Academic Journals.

African Journal of Bacteriology Research (JBR), is a peer reviewed journal. The journal is published per article and covers all areas of the subject such as: Bacterial physiology, Bacterial floral for human, Prokaryotes of the environment, Bacterial endotoxin, Cell signalling.

Contact Us

Editorial Office: jbr@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/JBR

Submit manuscript online http://ms.academicjournals.me/

Editorial Board

African Journal of Bacteriology Research

Table of Content: Volume 10 Number 3 August, 2018

ARTICLES

Vol. 10(3), pp. 23-33, August 2018
DOI: 10.5897/JBR2018.0261
Article Number: B16387457880
ISSN: 2006-9871

http://www.academicjournals.org/JBR

African Journal of Bacteriology Research

Full Length Research Paper

Enteric bacterial communities associated with the Omubhira Stream in Kakamega County, Kenya

Menya A. A.*, Shivoga A. W. and Wawire S. A.

Department of Biological Sciences, School of Natural Sciences, Masinde Muliro University of Science and Technology, Kakamega, Kenya.

Received 12 March, 2018; Accepted 9 July, 2018

The present study was undertaken to establish the distribution and diversity of enteric bacterial communities along the Omubhira stream and also determine if physico-chemical parameters influence their distribution in water in terms of total coliforms (TC) and *Escherichia coli*. Stratified random sampling was used and four strata with 15 selected sampling sites identified along the stream. Selection of the sampling sites was in relation to land use activities that are likely to be sources of bacterial contamination to the stream. The results of bacteriological analysis of water quality revealed that water from some of the selected sites of the stream had bacterial loads that exceeded the WHO value/guidelines for water for recreational use. Mean comparison of coliforms counts using a one way ANOVA test revealed that the difference in coliforms among the sampling sites of Omubhira stream was significant (F=18.324, P=0.0005). Pearson product-moment correlation showed that there was a strong positive correlation between *Escherichia coli* and electrical conductivity which was statistically significant (*r*=0.413, *n*=80, *p*<0.0005), total dissolved solids (*r*=0.408, *n*=80, *p*<0.0005), dissolved oxygen (*r* =0.446, *n*=80, *p*<0.0005) and total coliforms (*r*=0.983, *n*=80, *p*<0.0005). However, there was no relationship between faecal coliforms and temperature, total suspended solids and pH which was not statistically significant; temperature (*r* =0.185, *n*=80, *p*>0.101), total suspended solids (*r*=-0.118, *n*=80, *p*>0.298) and pH (*r*=-0.089, *n*=80, *p*>0.433). The bacteria isolated from water samples in this study included *Escherichia coli, Enterobacter* spp*., Citrobacter* spp., *Proteus* spp., *Serratia* spp., *Shigella* spp., *Providencia* spp. *Morganella* spp., *Salmonellae* spp. and *Klebsiella* spp. *Escherichia coli* was the most predominant enterobacterial isolate during both the dry and the wet season. Intervention measures including creating awareness, educating residents on hygiene practices on the use of Omubhira stream water and improvement of sanitation should be implemented.

Key words: Enteric bacteria, Omubhira stream, *Escherichia coli*, total coliforms (TC).

INTRODUCTION

Urban rivers are vulnerable to different urban processes and activities that cause pollution and degradation of the water ecosystem. In the recent past, pollution of water sources in the urban set up with deleterious entero-bacterial communities has been on a steady increase. Major source of these microbes in water is faeces from

*Corresponding author. E-mail: agnetisa@yahoo.com.

human and other mammals (Musyoki et al., 2013)

In Kenya, according to NEMA regulations, all sources of water for domestic uses should comply with stringent standards set out in the first schedule to these regulations. *Escherichia coli, Shigella spp., Pseudomonas aeruginosa* or coliforms should not be detectable in 250 ml of drinking water (Vail et al., 2003). World Health Organization (WHO) developed microbiological quality guidelines based on intended water uses. The guidelines stipulate that faecal coliforms (FC) should not exceed 10^2 per 100 ml of water used in irrigation of crops that are eaten uncooked, sports fields, and public parks in unrestricted regions (WASREB, 2006) . Enteric bacteria in the family Enterobacteriaceae reside normally in the guts of many animals, including humans (Wawire et al., 2013). Some members of Enterobacteriaceae such as *Escherichia coli, Enterobacter spp.,* and *Serratia spp.* are natural inhabitants of the gastro-intestinal tract of human beings and are used as indicators of faecal contamination of the environment. The pathogenic members of Enterobacteriaceae that infect the gastro-intestinal tract of humans include *Salmonella spp., Shigella spp., Proteus spp., Campylobacter spp. and E. coli.* They get access to the human when ingested through contaminated water, food and oral contact with infected surfaces (WASREB, 2006). This raises public health concern. Diarrhoeal cases have majorly been associated with enteric .bacterial groups, this in turn accounts for a substantial degree of morbidity, and mortality in different age groups worldwide (Obi et al., 2003; Eze and Madumere, 2012).

In Kakamega town, the Omubhira stream, like any other stream or rivers is facing pollution problems due to increased human activities along the stream. The stream traverses formal and informal settlements, agricultural farms, learning institution and wastewater treatment plant. Water from the stream is used extensively for watering livestock, washing, bathing and irrigation of crops namely arrow roots, vegetables, plantain and nappier grass grown along it. Although this stream has been providing water for domestic and agricultural purposes over the years, no studies have however demonstrated the distribution and diversity of enteric bacterial along the stream.

The current study therefore establishes the distribution and diversity of enteric bacterial communities along the Omubhira stream. Information gathered provides baseline data that would be applied in controlling and reducing occurrence of disease burdens among stream users, contamination of agricultural produce and reduce pathogen transmission.

MATERIALS AND METHODS

Description of study area

Omubhira stream is a first-order stream that flows in an Easterly direction with its origin situated in Milimani Estate within Kakamega town in Western Kenya. The stream is approximately 0.98 km long and approximately 100 cm wide. Temperature ranges from a minimum of 10.3ºC to a maximum of 30.8ºC with a mean of 20.5ºC. The rainfall ranges between 1250 and 1750 per annum (KARI Kakamega Annual Report, 2011). The community along the stream is majorly engaged in small-scale crop farming, livestock rearing and aquaculture. Omubhira stream joins Lurambi stream at a confluence and both drain into River Isiukhu and eventually into River Nzoia; one of the major rivers draining into Lake Victoria. Important features along the stream include a narrow strip of natural wetland on either side that comprises majorly of the sedges, settlement schemes, fishponds, wastewater treatment plant, farmlands and learning institution (Figure 1).

Study design

Stratified random sampling design was applied in this study. The stream was categorised into four strata in relation to the purported sources of bacterial contamination, with three sampling sites per strata. Strata 4(4S) - downstream, strata 3(3S) - lower midstream, strata 2(2S) - upper midstream and strata 1(1S) - upstream (source) coordinates of sampling sites was taken by “Garmin Etrex” (GPS) and charted using the ArcGIS software.

Sample collection

Collection of samples occurred twice in a month. This was conducted randomly between 9 am and 12 noon during the wet season (April to September-2014) and dry season (December-2014 to February- 2015). Physico-chemical parameters namely: Water temperature, electrical conductivity, ph, dissolved oxygen, total dissolved solids (TDS) and total suspended solids (TSS) were measured *in situ* at the time of sampling using electrical probes (APHA, 2005). Bacteriological analysis involved determination of the levels of total coliforms and *E. coli* using the 3 M *E. coli*/coliforms Petri film count plates. The samples were then transported in ice packed cooler boxes and analyzed within two hours of collection in the Biological Sciences Department - microbiology laboratory within 8 h from the sampling time.

Physico-chemical analysis

Temperature of the water samples was taken at all sampling sites, using a thermometer and recorded in degrees celsius. The thermometer bulb was dipped into the water and allowed to stand for one minute before the reading was taken directly. Electrical conductivity (EC) and total dissolved solids (TDS) were analyzed using Cond/TDS/Salt/Temp meter CTS-406K. The conductivity probe was rinsed with distilled water, immersed into the sample and the reading recorded in a table. PH was analyzed using HI 2211 pH/ORP meter, Hannia instrument. The meter was calibrated by inserting its probe in a standard solution at pH 7.0 then rinsed with distilled water and then at pH 4.0. The probe was then rinsed with distilled water and inserted into the collected sample. The pH was read off above the temperature level displayed on the screen. Dissolved oxygen was analyzed using digital oxygen meter of M.R.C model.

For the determination of total suspended solids (TSS), Whatman GF/C glass microfibre filter papers with 1.2 µm pore size was used. The filter paper was weighed using an electronic digital balance and the initial reading noted. 100 ml of the sample was filtered through and the filter paper oven dried at 50ºC for 1 h. The filter paper was then re- weighed and the final weight of the filter paper gave the value of TSS in grams.

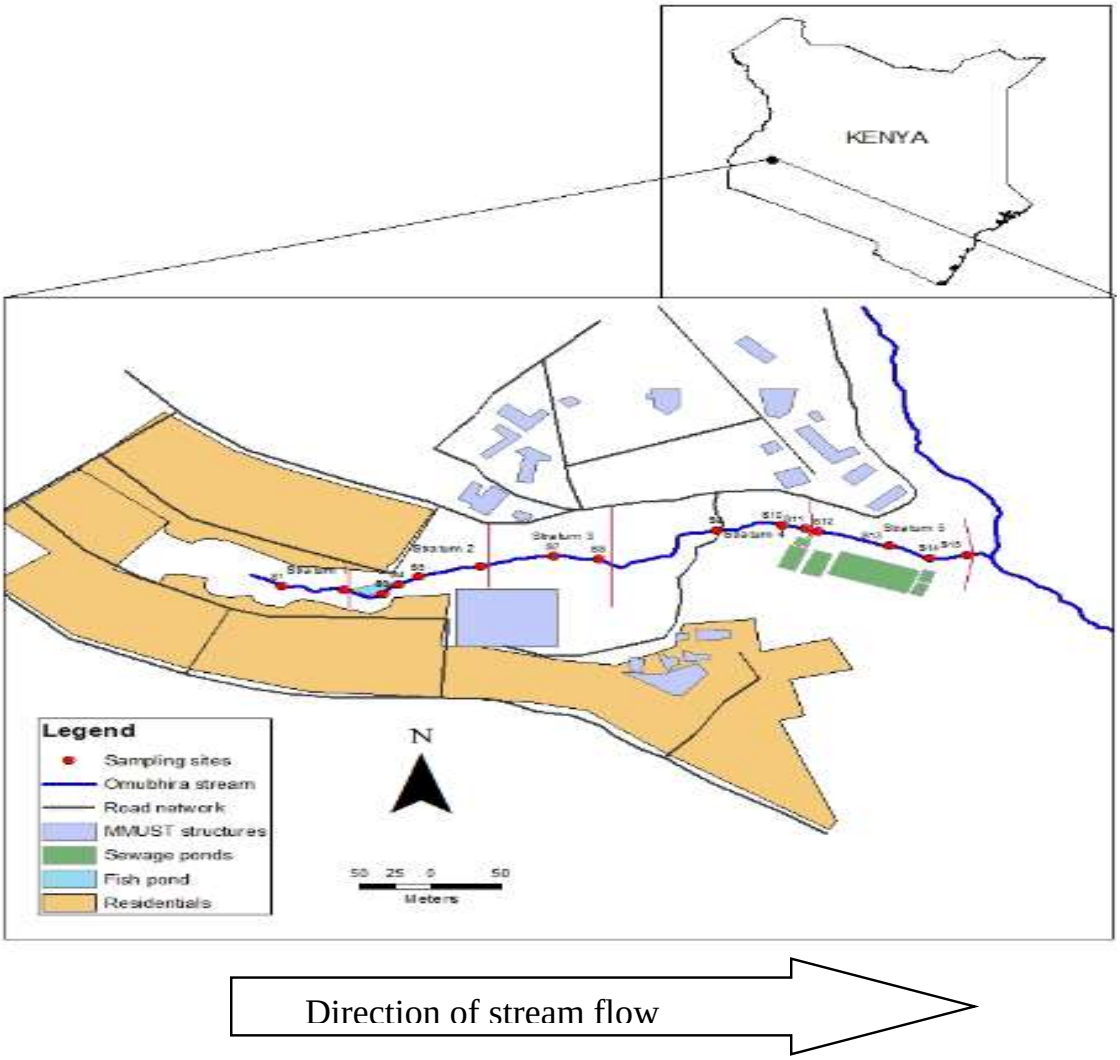

Figure 1. Map of the study area.
Source: ArcGIS generated.

Enumeration of the total coliforms, faecal coliforms and *E. coli* levels in water

Levels of total coliforms and *E. coli* were enumerated using the 3 M *E. coli*/coliforms petrifilm count plates. Serial dilution of 1:10 dilution of sample collected was prepared using saline solution (0.85%). Briefly, the petrifilm plate was placed on level surface with top film lifted and using a pipette, 1 ml of sample was placed onto the center of bottom film. The top film was then rolled down to avoid entrapping air bubbles making sure the top film does not drop. With flat side down, spreader was placed on top film over innoculum and pressure gently applied on spreader to distribute innoculum over circular area before gel formed. Spreader was lifted and a minimum of one minute given for gel to solidify.

Plates were placed with clear side up in stacks of no more than 20. The incubator was humidified to minimize moisture loss. The plates were incubated for 24 h ± 2 h at 37°C.

Petrifilm plates were counted on a standard colony counter or other illuminated magnifier. Typical coliforms colonies appear pink/red whereas the *E. coli* appear dark blue. The colonies were enumerated, characterized and recorded. The results were expressed as the number of total coliforms and *E. coli* in 100 ml of water.

Isolation, purification and characterization of bacterial isolates

Colonies were purified by sub-culturing using streaking method. Characteristic *E. coli* and other coliforms colonies from the petrifilm plate were picked using a sterile wire loop and streaked onto Mac-Conkey Agar (HIMedia Lab. Pvt. Mumbai, India).

The inoculated Mac-Conkey plates were then incubated at 37°C overnight. Four distinct lactose and non-lactose fermenters colonies based on morphological characteristics were further picked and streaked onto nutrient agar (HIMedia Lab. Pvt. Mumbai, India) and incubated at 37°C. Preliminary characterization was performed using morphological characteristics as described by (Holt et al., 1994). Morphological identification of the isolate was done under the dissecting and compound microscope to observe cell size, shape and arrangement characteristics after classical staining of bacteria (Bartholomew, 1962). Biochemical tests that were also conducted included; citrate utilization using Simmons Citrate Agar

Table 1. The overall mean values of physico- chemical and bacteriological analysis of Omubhira stream system.

Parameter	Dry season	Wet season	NEMA standards	WHO standards
Temperature (°C)	21.1192	12.45	<25	<25
EC (µs/cm)	156.6250	64.8974	0-250	NS
TDS (mg/l)	104.6125	43.2051	1200	500
pH	7.5387	7.1085	6.5-8.5	6.5-8.5
TSS (mg/l)	0.033293	0.017837	30	NS
DO (mg/l)	6.4538	6.7436	6-8	NS
TC (cfu/100 ml)	3691.25	4780	1000	Zero(0)
E. coli(Cfu/100 ml)	1096.25	651.5	Nil(0)	Nil(0)

EC, Electrical conductivity; TDS, Total dissolved solids; TSS, Total suspended solids; DO, Dissolved solids; TC, Total coliforms; NS, Not specific.

test all from HIMedia Lab. Pvt. Mumbai, India, , motility, indole and lysine (MIL) test and Triple Sugar Iron agar (TSI Agar test) for H_2S production and sugar utilization test. The biochemical tests were used to classify the isolates to genera level (Cheesbrough, 2002).

Statistical analysis

Data was subjected to analysis by majorly using Correlation analyses and ANOVA analyses. Statistical Package for the Social Sciences (SPSS) version 20 for Windows was used to calculate means and standard deviations and the data tabulated. Pearson product moment correlation procedure was used to perform correlation analysis and determine whether there were significant relationships between different physicochemical parameters, total coliforms and *E. coli* levels.

To check whether there was any significant difference between the values of physico-chemical parameters at different stations, one way Analysis Of Variance (ANOVA) was performed.

RESULTS

General overview

Assessment of Omubhira stream environment during study revealed that sampling locations were mostly frequented by the nearby residents and livestock as watering points and especially strata four (4S) that represents downstream. Observations also revealed that some people living in the stream basin and downstream use stream to water their crops with a number of fishponds constructed along the stream.

Physico-chemical water quality gradients along the Omubhira stream course in Kakamega town

Data obtained from all samples was in triplicates. Calculation of mean and the average for each stratum was calculated. The results were presented in tables. Table 1 shows the overall averaged mean values of physico- chemical and bacteriological characteristics of the examined water samples. Results obtained for most of the physico-chemical parameters conformed to the NEMA and WHO standards for drinking water quality except for the total coliforms and *E. coli* that were above the recommended standards.

Table 2 shows the descriptive analysis of the physicochemical and bacteriological properties of Omubhira stream during the dry season in regard to the strata along the stream. The physic-chemical conditions in the four strata conformed to the NEMA and WHO standards for water with 3S recording the lowest mean value of electrical conductivity and the total dissolved solids. Bacteriological analysis however indicated that the mean values of total coliforms and *E. coli* were above the recommended standards with 4S recording the highest values while 1S the lowest mean values with a minimum of zero value.

Table 3 indicates the descriptive analysis of physicochemical and bacteriological properties of Omubhira stream during the rainy season in regard to the strata along the stream. The physico-chemical properties conformed to the NEMA and WHO standards for water with fourth stratum (4S) recording the highest mean value for electrical conductivity and total dissolved solids. However, bacteriological analysis indicated that the mean values for total coliforms and faecal coliforms (*E. coli)* were far above the recommended standards for drinking water with 4S recording the highest values while 1S recording the lowest mean value with a minimum of zero value.

Figure 2 shows the mean values of faecal coliforms (*E. coli*) counts during the study periods. The values range from 20- 1593 Cfu/100 ml during the dry season and 93-1229 Cfu/100 ml during the wet season. Fourth stratum (4S) recorded the highest counts in both seasons while first stratum (1S) recorded the least in both season with a minimum of zero (0) coliforms count. Generally, the mean coliforms counts were higher than the WHO standard (≤ zero Cfu/100 ml) for water for recreational use.

Figure 3 shows the mean values of total coliforms counts during the study periods. The values range between 300 and 4476 Cfu/100 ml during the dry season

Table 2. Descriptive analysis of physicochemical and bacteriological properties of Omubhira stream during the dry season.

Parameter		Mean	Std. error	Variance	Minimum	Maximum
Temperature	4S	21.15	0.1515	0.0918	20.85	21.5
	3S	21.2	0.2281	0.2082	20.816	21.4667
	2S	21.09	0.2041	0.1667	20.80	21.7
	1S	20.7	0.4473	0.8	20.1	21.2
Electrical conductivity	4S	178.8332	4.8480	94.013	178.8332	183.667
	3S	126.33314	2.4073	23.180	126.333	129.333
	2S	168.39988	9.4535	357.4698	168.39988	183.333
	1S	173.4	3.7683	56.8	172	180
TDS	4S	119.3	3.419	46.75	119.3	123.667
	3S	84.37	1.693	11.46517	84.36668	86.1667
	2S	111.9	6.346	161.0751	111.9334	121.667
	1S	116.2	2.924	34.2	116.2	121
PH	4S	7.527	0.019	0.001	7.525	7.575
	3S	7.474	0.03	0.003568	7.425	7.563
	2S	7.62	0.01	0.000426	7.596	7.653
	1S	7.654	0.007	0.00018	7.654	7.66
TSS	4S	0.0345	0.000164	0.00000010	0.0343	0.0349
	3S	0.0335	0.00029	0.00000	0.03286	0.03391
	2S	0.0312	0.0000010	0.000000	0.03116	0.0312
	1S	0.0308	0	0	0.0308	0.0308
DO	4S	5.99	0.054	0.012	5.8	6.0667
	3S	6.393	0.005	0.000008	6.383	6.4
	2S	6.706	0.081	0.026297	6.533	6.866
	1S	9.2	0.071	0.02	9.1	9.3
TC	4S	4477	382	0.00000005	3650	5533.333
	3S	3480	332.3	441583.2	3150	4600
	2S	3673	270.9	293543.9	3300	4333.3
	1S	300	61.24	15000	100	400
E. coli	4S	1593	232.2	0.0000005	766.667	1850
	3S	886.7	243	236166.5	366.667	1450
	2S	880	247.9	245888.6	333.333	1533.33
	1S	20	22.36	2000	0	100

1S, first strata; 2S, second strata; 3S, Third strata; 4S, Fourth strata.

and 1160 to 6209 Cfu/100 ml during the wet season. Fourth strata (4S) recorded the highest in both seasons while first strata (S4) recorded the least in both season. The total coliforms counts were above the recommended WHO and NEMA standard for water for recreational use.

During the wet season, Pearson product-moment correlation showed a relationship between *E. coli* and some physico-chemical properties of Omubhira stream. There was a strong, positive correlation between *E. coli* and electrical conductivity. However, there was no relationship between *E. coli* and temperature, dissolved oxygen and pH since there was no statistical significance.

During the dry season, Pearson product-moment correlation showed that there was a strong relationship between *E. coli* and dissolved oxygen, total dissolved solids and electrical conductivity (Table 4).

Table 3. Descriptive analysis of physicochemical and bacteriological properties of Omubhira stream during the wet season.

		Mean	Std. Error	Variance	Minimum	Maximum
Temperature	4S	12.2667	0.129	0.067	11.833	12.667
	3S	12.1666	0.139	0.077441	11.667	12.833
	2S	12.666	0.191	0.146337	12.333	13.333
	1S	14.6	0.245	0.24	13.0	15.0
Electrical conductivity	4S	98.83	19.27	1485	71.333	165.166
	3S	45	10.36	429.6444	26.833	78.5
	2S	41.2	3.536	50.01657	34	51.33
	1S	53.8	6.804	185.2	30	63
TDS	4S	65.73	13.02	678	47	110.667
	3S	29.83	6.47	167.4591	17.166	49.333
	2S	27.4	2.247	20.18991	22.667	32.667
	1S	36.8	4.45	79.2	21	42
pH	4S	7.206	0.068	0.019	7.015	7.33
	3S	7.067	0.103	0.042748	6.773	7.283
	2S	7.103	0.184	0.135839	6.583	7.52
	1S	6.88	0.207	0.1708	6.53	7.42
TSS	4S	0.019	0.005	0.000000005	0.00895	0.0194
	3S	0.015	0.006	0.000132	0.003783	0.0099
	2S	0.014	0.006	0.000127	0.0022	0.0093
	1S	0.01	0.006	0.000152	0.0015	0.0074
DO	4S	6.19	0.317	0.401	5.85	6.916
	3S	6.743	0.333	0.44371	6.383	7.5167
	2S	7.16	0.397	0.631891	6.866	7.8
	1S	8.2	0.489	0.955	7.8	9.3
TC	4S	6210	1481	8773280	4716.667	11500
	3S	4203	567.8	1289501	3500	6200
	2S	4280	268.4	288111.8	3700	5066.667
	1S	1160	653.5	1708000	400	3400
E. coli	4S	1230	164.2	107832.56	933.333	1733.33
	3S	356.7	71.83	20638.99	283.33	550
	2S	294.7	54.59	11919.79	133.333	433.33
	1S	93.33	79.41	25222.27	0	366.667

1S, first strata; 2S, second strata; 3S, Third strata; 4S, Fourth strata.

Figures 4 and 5 shows the different microorganisms recovered and identified from stream water in both the wet and dry season. These included *Escherichia coli, Enterobacter* spp., *Citrobacter* spp*., Salmonellae* spp*., Providencia* spp*., Proteus* spp*., Klebsiella* spp*., Shigella* spp*., Morganella* spp*.* and *Serratia* spp*.* Microorganisms recovered were present in both wet and dry season accordingly except for *Citrobacter freundii* and *Proteus spp*. *Escherichia coli* was the most predominant enterobacterial isolate in both seasons and across all the study sites. This showed that there was a common source of these microorganisms.

DISCUSSION

The physico-chemical parameters of water in Omubhira stream exhibited significant variations in the dry and wet

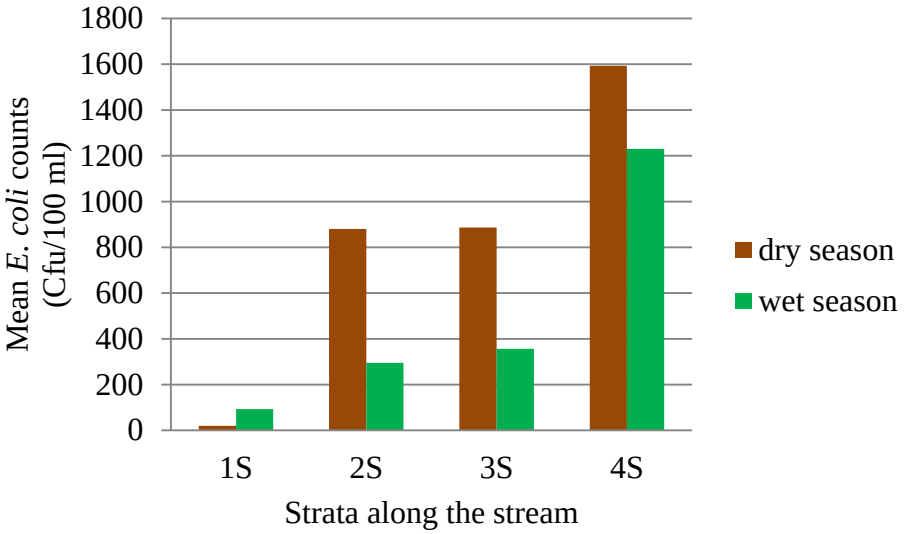

Figure 2. Mean values of *E. coli* counts from Omubhira Stream.

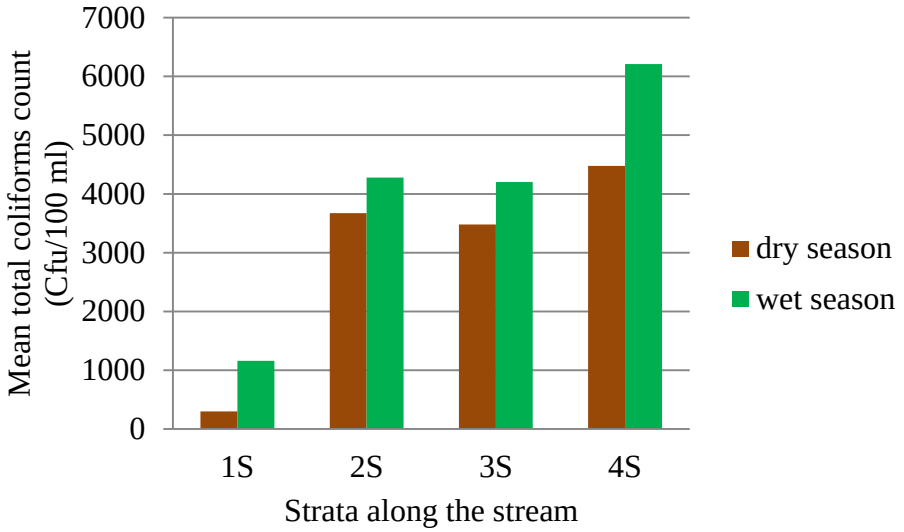

Figure 3. Mean values of total coliforms count from Omubhira Stream.

Table 4. Pearson correlation coefficients between physical chemical parameters and E. coli in the dry and wet season for Omubhira stream (*designate significant correlation at α=0.05).

Parameter	Temperature	pH	EC	TDS	DO	TSS	*T. coliforms*
E. coli (Dry season)	-0.128	-0.135	0.307	0.302*	-0.421*	0.159	0.545*
E. coli (Wet season)	-0.081	-0.002	0.349*	0.351*	-0.198	0.072	0.241*

*, Designate significant correlation at α=0.05.

seasons. Furthermore, there were spatial variations of the physico- chemical parameters from upstream to downstream.

All the physico-chemical parameters studied were within the maximum permissible limit as per the WHO standards (WHO, 2006) and NEMA regulations for water for recreational use. Electrical conductivity, TDS and TSS had higher values in the dry season than in wet season.

However, strata four (4S) that is situated at the lower reaches of the stream had significantly high mean levels

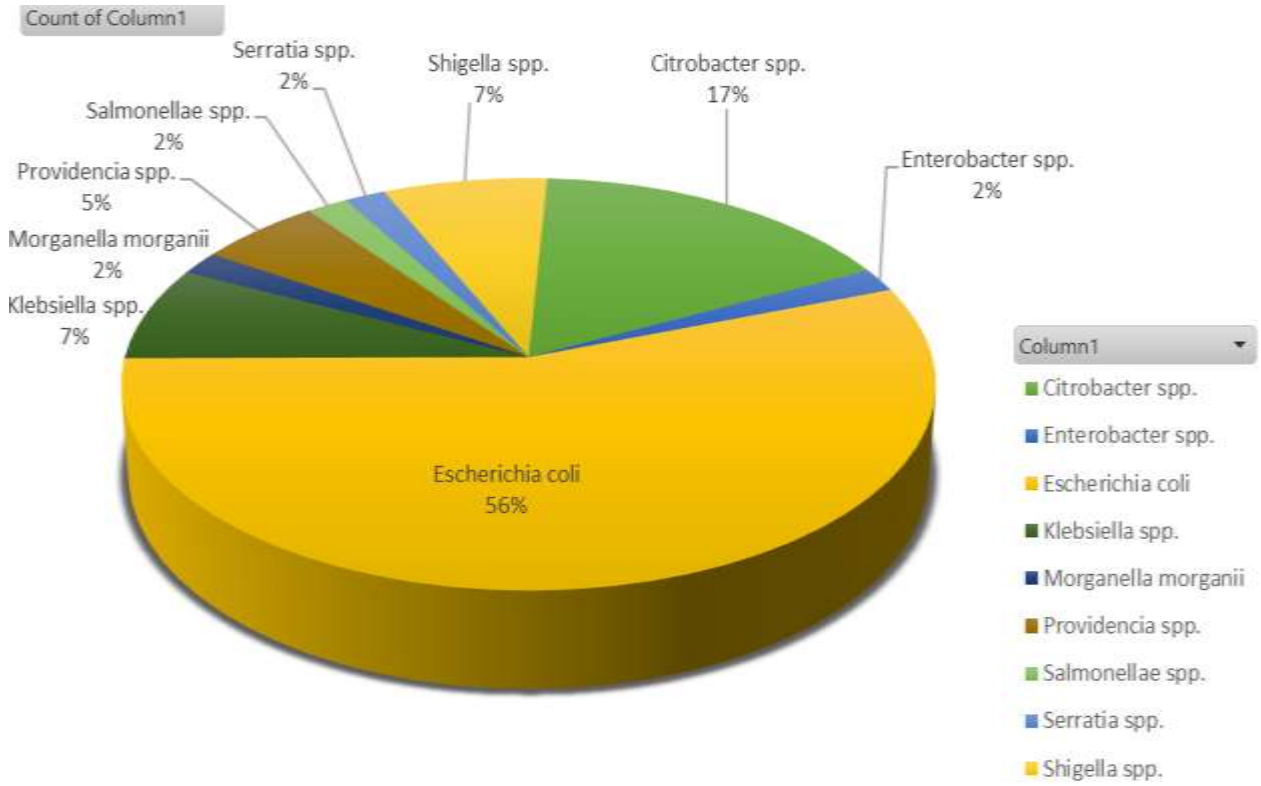

Figure 4. Bacterial isolates recovered in the dry season.

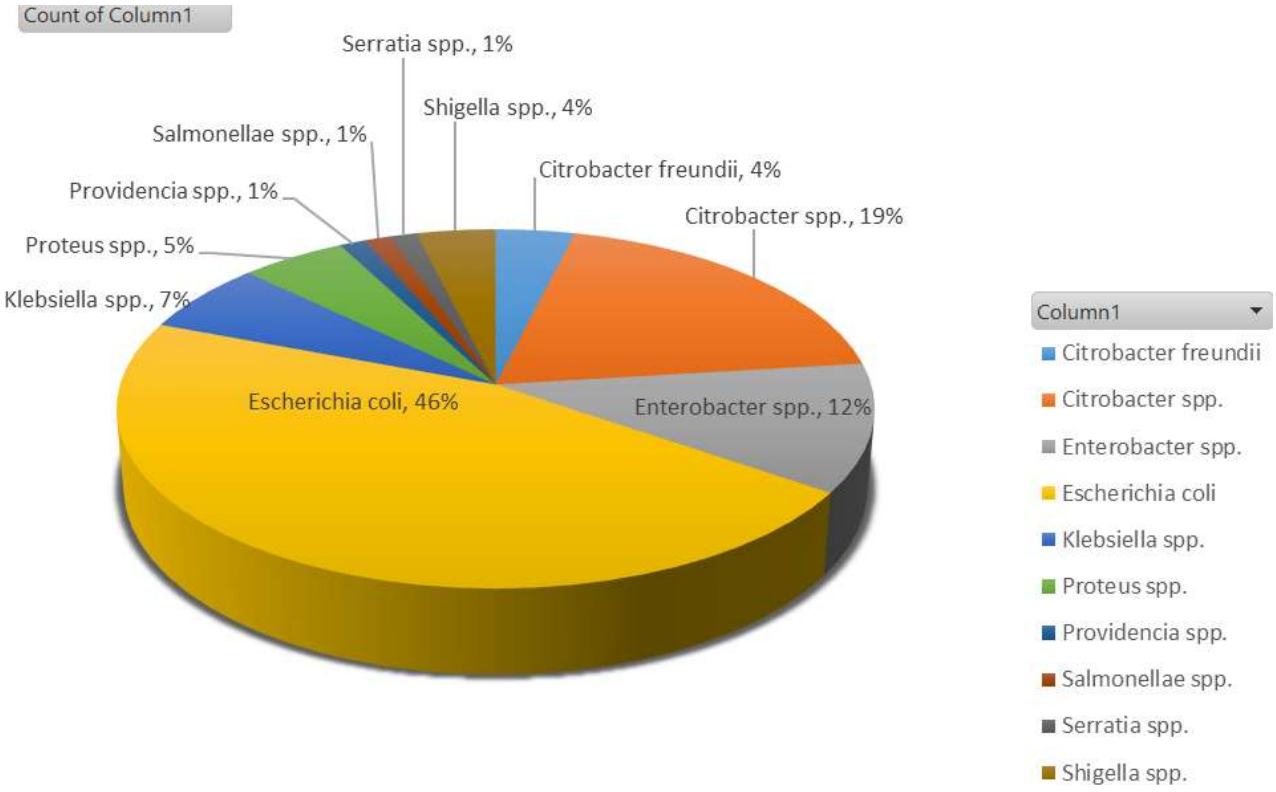

Figure 5. Bacterial isolates recovered in the wet season.

of electrical conductivity and TDS than in the other three sampling strata. High levels of electrical conductivity and total dissolved solids could have been due to the high organic load, which in this study was from the wastewater effluent discharge and livestock watering points. This corresponds to a study on Nyangores stream, Mara river basin whereby the levels of electrical conductivity, total suspended solids and total dissolved solids increased downstream with the low temperature, conductivity, TSS and BOD levels recorded in upstream station increasing downstream due to the increased run-off from agricultural activities and sewage effluents (Gichana et al., 2014)

The temperature values obtained attributed mainly to the atmosphere and weather conditions of the study area. Water temperature variation at the various sampling sites could have been influenced by the differences in the quantity of water present in the site and due to the presence of vegetation shielding water source from direct radiation.

High temperatures during the dry season could have been influenced by factors that majorly include solar radiation and turbidity. Bacteriological and physico-chemical studies of the rural catchments of the Lake Victoria showed high temperature levels during the dry season (Ouma et al., 2016). In general, the entire Omubhira stream system surface water samples had temperature means that were within the permissible limit in both seasons and averagely suitable for survival of most tropical aquatic organisms.

Increased turbidity increases water temperature (Ogendi et al., 2015). This was observed during the dry season whereby defecation by livestock directly into the water during feeding and watering increased the amount of suspended solids which absorbed heat from solar radiation and thus increasing temperature

During this study, it was observed that compared to other types of water sources, this water source was mainly located in high altitude areas where they are on most occasions sheltered by trees. Such an environment is likely to experience cool air, which influences water temperature.

This study showed negative correlation between temperature and faecal coliforms and therefore is in agreement to the assessment of physico-chemical properties and sewage pollution indicator bacteria in surface water of River Gomti (India) whereby temperature showed significant negative correlation with faecal coliforms, total coliforms and biological oxygen demand (Anukool and Shivani, 2011).

Electrical conductivity values were higher in the dry season when compared to the rainy/wet season. This is consistent with the findings in River Moiben, Kenya whereby electrical conductivity values were higher in dry season (Masese et al., 2009). This was due to the high solute concentrations in dry season because of evapo-transpiration losses from the channel. Moreover, run-off is supplied from ground water reservoirs, where water has a long residence time and solute release is prompted. However, during the rainy season, run-off is generally flows much more rapidly to the stream channel, has less opportunity for solute pick up and therefore has lower dissolved solids content.

The values of electrical conductivity observed during sampling periods are however within the range prescribed by WHO (WHO, 2006). The correlation coefficient for the electrical conductivity and dissolved solid values is 0.99 which implies that the presence of the total dissolved solids is a major contributing factor to the electrical conductivity of water. However, relatively high conductivity of water observed at some sampling sites could have been due to the effect of released effluent from sewage treatment plant (Gichana et al., 2014)

This study showed significant correlation between conductivity and coliforms and therefore is in agreement to a study of physico-chemical properties and sewage pollution indicator bacteria in surface water of River Gomti (India) whereby results revealed that EC showed significant positive correlation with faecal coliforms, total coliforms and biological oxygen demand (Anukool and Shivani, 2011).

Oxygen availability in an aquatic ecosystem is an indication of the systems health and general well-being and dissolved oxygen usually reflects the physical and biological processes prevailing in the water (Cohen and Hillel, 1972). Dissolved oxygen values for the fourth stratum (4S) of 5.99 and 6.12 mg/l in dry and wet season respectively indicated that there was slight pollution occurrence at this sampling location. The decreased dissolved oxygen levels downstream could have been attributed to the high organic load and slightly due to increased water temperature that decreases solubility of oxygen in water (Gichana et al., 2014). DO values of 9.2 and 8.2 for the dry and wet season respectively recorded at the first stratum (1S) which is the source of the stream indicated that the waters at this sampling location were high quality water. Dissolved oxygen concentrations above mug/l in all the studied strata implies that the water is not stressful to fish growth since fish kills are usually observed at below 5 mg/l concentrations and thus suitable for fish farming.

TDS values in this study were within the prescribed limit given by WHO and the NEMA. This could not interfere with the osmo-regulation of fresh water organisms in the stream. Excess sediment can harm the water quality since high level of solids in water increases water density and affect osmo-regulation of fresh water organisms thereby reducing the solubility of gases such as oxygen.

The TSS levels were high for the dry season than for the wet season. This could have been due to the stampede of the stream basin by the livestock that are directly watering and feeding just by the stream banks during the dry season. However, low values of TSS

during the wet season could account for the reason why the entire appearance of the water samples was clear, not turbid and having no odour.

The Omubhira stream had a measured pH ranging from 6.88 to 7.654 .The pH of water samples indicated that it was within the range set by WHO. pH influences the survival of aquatic organisms in the water bodies since their metabolic activities are pH dependent and drastic changes in pH can have detrimental effects on stream health (Ouma et al., 2016). The presence of total and faecal coliforms counts in the stream water indicated contamination by raw sewage or defecations in the bush in the catchment or rather defecation by livestock during watering. This was similar to the findings in the study of Nyanchwa- Riana River (South West Kenya) (Ogendi et al., 2015).

The average mean for total coliforms and *E. coli* (faecal coliforms) counts were far above the NEMA and WHO recommended standards for bacteria for portable and recreational water. Moreover, the four strata representation of the stream also recorded TC and E.coli counts that were above the recommended NEMA and WHO standards for drinking water. Generally, higher mean counts for TC were recorded in the wet season than in the dry season while *E. coli* (faecal coliforms) mean counts were higher in the dry season than in the wet season.

High flows in streams tend to increase bacterial counts due to run-offs (Muhibbu et al., 2011) However, high levels of *E. coli* counts were recorded in the dry season compared to the wet season. The high levels of *E. coli* could have been due to increased turbidity from suspended particles during stampede by livestock during watering and feeding at the stream banks, which facilitate the survival and growth of coliforms bacteria as they are protected from ultra violet radiation and attack by bacteriophage (Medema et al., 2003). Moreover, the high counts in the dry season compared to the wet season of *E. coli* could be associated with rainfall occurrences that diluted and weakened the effects of point source pollution. While also increasing the contribution of non-point sources or diffuse pollution through land run-off from agricultural fields and leaches from refuse dumps. (Muhibbu et al., 2011). The high mean E. coli counts during the dry season could be associated with non-human warm-blooded animals' origin since domestic animals especially cows were a common precincts of sampling locations considered as watering and feeding points. This is in agreement with findings in the study of patterns and sources of faecal pollution in the heavily impaired river Njoro watershed (Kenya) (Jenkins, 2008). Moreover, *E. coli* counts were associated with humans as a result of the open defecation evident along the stream and sewage treatment plants discharge point (Jenkins, 2008).

Concentrations of coliforms counts studied shows a strong increase from the upstream (source) (S1) to downstream (4S). This indicates that there was input of raw sewage or animal waste at certain points along the stream transect. Zero *E. coli* counts at the source of the stream (S1) during various sampling occasions may be an indication that stream source is clean, safe and not contaminated. Generally, the findings of the total coliforms (TC) and *E. coli* counts revealed that the human activities on most occasions increased the bacterial load of the water. Although total coliforms organisms may not always be directly related to the presence of faecal contamination or pathogens in the drinking water, this study found that all water samples contained both total coliforms and *E. coli*.

In addition, the Omubhira stream is not well protected from direct access by animals, because most of the animals move along the laggas in search of water, salt licks and pasture. In the process, they deposit a lot of organic wastes directly into the stream or rather on the laggas floor. When it rains, the seasonal floods wash off bacteria and organic water into the Omubhira stream hence contaminating them (Musyoki et al., 2013)

However, presence of a significant difference in TC and *E.coli* counts in Omubhira stream samples from the four strata suggests the level of hygiene in the different sampling sites.

According to Kavka et al. (2006), although some community members enclose their streams to protect them from direct faecal contamination by livestock, the high population of livestock and wildlife that visit the stream beds at different times exposes the stream to some contamination implying that each morning, these streams have to be cleaned before drawing drinking or livestock water. The fourth strata (4S) that represents the downstream had the highest loads of total coliforms and *E. coli,* while the first stratum had the least and at some occasions zero counts. Microbiological studies of river Danube showed similar characteristic with high levels of faecal pollution being particularly downstream with the main sources of pollution being raw discharges, discharges from wastewater treatment plants, impaired tributaries and impact by diffuse sources. It is therefore clear that should the water be qualified as portable, Omubhira stream must be fully protected from pollutants accordingly from its source in the first stratum to downstream at the fourth stratum.

Various bacterial isolates of public health concern were also identified from stream water samples in this study. *E. coli* were the most predominant enterobacterial isolate during both wet and dry seasons and across all the study sites. It is evident that the occurrence of pathogenic organism in stream water indicates the contamination of stream water with human or animal wastes and thus of public health significance (Shitu et al., 2008). Though these bacteria are naturally found in the intestinal tract, in soil and water, they can cause primary and opportunistic infections in humans and animals (Cheesbrough, 2002). Most are faecal-oral route transmitted and cause number

of diseases from diarrhoeal, urinary tract infections, inflammation and ulceration of intestinal tract, enteric fever to chest infections. However, *Serratia spp.*, though found mostly in soil and water, has been reported to cause pulmonary and urinary infection.

Conclusion

Although the physico-chemical water quality of Omubhira stream was within the acceptable limits as per NEMA and WHO standards, the bacteriological water quality was above the recommended standards levels. This implies that the application of physico-chemical water quality analysis of water alone cannot be used to determine the safety of water as portable and suitable for recreational purposes. The widespread practice of cattle watering in the Omubhira stream appears to be one of the major causes of gross faecal pollution in the watershed. Defecation by cattle while drinking water directly from the stream and the transport of the faecal matter during major rainfall- run-off events of accumulated cattle faecal deposits from contributing areas along stream are likely to explain large spikes in faecal contamination during peak run-off .This may pose a health risk to several communities which rely on the receiving water body primarily as their source of domestic water. Therefore, control of human activities to prevent faecal matter from entering water body is the key to avoiding bacterial contamination. Though the bacterial levels render stream water unfit for human consumption before treatment, the water can be used for other purposes depending on the particular use.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The author extends their thanks to the microbiological department for the effort during microbial analysis.

REFERENCES

American Public Health Association (APHA) (2005). Standard methods for the examination of water and wastewater. 20th Edition. Published jointly by the American Public Health Association, American Water Works Association, and Water Environment Federation. Washington, D.C.

Anukool S, Shivani S (2011). Assessment of Physico-Chemical properties and sewage pollution indicator bacteria in surface water of River Gomti in Uttar Pradesh. International Journal of Environmental Sciences 2(1):325.

Cheesbrough M (2002). District Laboratory Practice in Tropical Countries- Part 2. Cambridge University Press.

Cohen J, Hillel IS (1972). Coliforms, Faecal Coliforms, and Faecal Streptococci as indicators of water pollution, Environmental Health laboratory. Hebrew University. Israel.

Eze SO, Madumere IC (2012). Physicochemical and microbiological analysis of water bodies in Uturu, Abia State-Nigeria. Asian Journal of Natural and Applied Sciences 1(4):58-65.

Gichana ZM, Njiru M, Raburu PO, Masese FO (2014). Effects of Human Activities on Microbial Water Quality in Nyangores stream, Mara River basin. International Journal of Scientific and Technology Research 3(2):153-157.

Jenkins MW (2008). Gross fecal pollution of a rural watershed in Kenya: Research identifying cattle as a major source in the River Njoro Watershed. Research brief, 08-01. Available at: http://crsps.net/resource/gross-fecal-pollution-of-a-rural-watershed-in-kenya-research-identifying-cattle-as-a-major-source-in-the-river-njoro-watershed/

Kavka GG, Kasimir GD, Farnleitner AH (2006). Microbiological water quality of the River Danube (km 2581-km 15): Longitudinal variation of pollution as determined by standard parameters. Available at: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.505.1676&rep=rep1&type=pdf

Masese FO, Muchiri M, Raburu PO (2009). Macroinvertebrate assemblages as biological indicators of water quality in the Moiben River, Kenya. African Journal of Aquatic Science 34(1):15-26.

Medema GJ, Shaw S, Waite M, Snozzi M, Morreau A, Grabow W (2003). Catchment characterization and source water quality. In Assessing Microbial Safety of Drinking Water. World Health Organization Geneva, Switzerland. pp. 111-158.

Muhibbu-Din OI, Aduwo AO, Adedeji AA (2011). Study of physiochemical parameter of effluent impacted stream in Obafemi Awolowo University, Ile-Ife, Nigeria. Published Msc Thesis.

Musyoki MA, Suleiman MA, Mbithi JN, Maingi JM (2013). Waterborne Bacterial Pathogens in surface waters of Nairobi River and Health Implications to communities downstream Athi River. https://erepository.mku.ac.ke/handle/123456789/112

Obi CL, Potgieter N, Bessong PO, Matsaung G (2003). Scope of potential bacterial agents of diarrhoea and microbial assessment of quality of river water sources in rural Venda communities in South Africa. Water Science and Technology 47(3):59-64.

Ogendi GM, Getabu AM, Onchieku JM, Babu JM (2015). An Assessment of Some Physical, Chemical and Biological Characteristics of Nyanchwa- Riana River Flowing through Kisii Town in South West Kenya. International Journal of Applied Science and Technology 5(2):68-79.

Ouma SO, Ngeranwa JN, Juma KK, Mburu DN (2016). Seasonal variation of the physicochemical and bacteriological quality of water from five rural catchment areas of lake victoria basin in Kenya. Journal of Environmental Analytical Chemistry 3:170.

Shitu OB, Olaitan JO, Amusa TS (2008). Physico-Chemical and Bacteriological Analyses of Water Used for Drinking and Swimming Purposes in Abeokuta, Nigeria. African Journal of Biomedical Research 11:285-290.

Vail JH, Morgan R, Merino CR, Gonzales F, Miller R, Ram JL (2003). Enumeration of waterborne *Escherichia coli* with Petrifilm plates. Journal of Environmental Quality 32(1):368-373.

Water Service Regulatory Board (WASREB) (2006). Drinking Water Quality and Effluent Monitoring Guidelines, Water Service Regulatory Board, Kenya. Available at: http://www.waterfund.go.ke/toolkit/Downloads/2.%20Drinking%20Water%20Guidelines.pdf

Wawire SA, Miruka DO, Nelson N, Ofulla A (2013). Antimicrobial susceptibility patterns of Enterobacteriaceae isolated from domesticated animals and the environment in Lake Victoria, Kenya. Ecohydrology and Hydrobiology 13(4):246-252.

World Health Organization (WHO) (2006). Guidelines for drinking-water quality. Vol. 1, Recommendations: addendum, 3rd ed. Geneva: World Health Organization. Available at: http://www.who.int/iris/handle/10665/43242

Vol. 10(3), pp. 34-43, August 2018
DOI: 10.5897/JBR2018.0268
Article Number: 7CB80FA57882
ISSN: 2006-9871

http://www.academicjournals.org/JBR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Bacteriology Research

Full Length Research Paper

Physicochemical analysis and microbial quality of cow butter obtained from Menz district of Amhara region, Ethiopia

Lina Gazu[1]*, Tewodros Eshete[1] and Getachew Kassa[2]

[1]Department of Animal Science, Debre Berhan University, College of Agriculture and Natural Resource Sciences, P. O. Box 445 Debre Berhan, Ethiopia.
[2]Department of Biotechnology, Debre Berhan University, College of Natural and Computational Science, Ethiopia.

Received 4 January, 2018; Accepted 4 April, 2018

Menz has long been known for its quality butter production but traditional milk products are generally reported to be of substandard quality. Therefore, this investigation was conducted to access physiochemical and microbial quality of butter from Menz district along the market value chain. The study was conducted by using laboratory analysis of physiochemical and microbial quality. The microbiological count data were transformed to log10 values before statistical analysis. Overall values of 15.05%:82.62%:2.09%, 14.26%:83.44%:2.77%, 14.25%:83.30%:1.03%, 14.58%:83.82%:3.58%, and 12.52%:83.96%:2.82% for moisture, fat and free fatty acid contents were observed in samples from farmers, traders, made by investigators, Tarmaber and Addis Ababa, respectively. In general, an overall mean of 3.94 ×10^9 : 2.66×10^6 : 1.83×10^6, total aerobic mesophilic bacterial count, total coliform and yeast and mold counts were observed in samples from farmers. Total aerobic mesophilic bacterial count, total coliform and yeast and mold counts values were 3.44×10^9:3.03×10^6:1.31×10^6 and 3.26×10^9:1.61×10^6:1.77×10^6 for samples collected from traders and for butter made by invigilators, respectively. For samples collected from Tarmaber and Addis Ababa, these values were 4.19×10^9:2.69×10^6:1.56×10^6 and 4.20×10^9:2.10×10^6:1.45×10^6, respectively. There is unhygienic production and processing of butter in the study area. Both physiochemical and microbial analysis shows the substandard traditional production system of the area which calls for improvement. Improvements are required on introduction of modern butter production technologies and awareness creation on hygienic production, processing and handling of butter.

Key words: Bacterial count, fat content, market value chain, butter from Menz district, moisture content.

INTRODUCTION

Ethiopia has one of the largest livestock inventories in Africa with a national herd estimated at 49.2 million cattle, 46.8 million sheep and goats, and 9 million pack animals. All livestock currently support and sustain livelihoods for 80% of all rural poor. Of the total population, 35 to 40% of all livestock are located in the pastoral areas (MoARD,

*Corresponding author. E-mail: linagazu@yahoo.com.

2007). Female cattle constitute about 55.48% of the national herd and of the total female cattle population dairy and milking cows total 16,941,361 with 14.24% dairy cows and 20.12% milking cows (CSA, 2008).

Eighty-three percent of all milk produced in Ethiopia comes from cattle with the remainder coming from goats and camels (MoARD, 2007). The Central Statistics Agency (CSA, 2008) estimates 2.76 billion liters of cow milk produced by sedentary populations annually, while camel milk is estimated at 16.2 million liters annually. The Ministry of Finance and Economic Development (MOFED) estimated the gross value of ruminant livestock production in 2008/2009 at Birr 32.64 billion. The estimate includes the values of livestock off-take (Birr 9.653 billion), milk and milk products (Birr 19.471 billion) and other products. Given the considerable potential for smallholder income and employment generation from high-value dairy products, development of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country (MoARD, 2007).

Fresh milk is easily perishable if it is not consumed immediately. So when surplus amount of milk is produced, it should be processed into different products like butter, soured milk and cheese. Butter has long shelf life as compared to fresh milk, especially when heated to higher temperature (100-120°C) for 30 min; it can stay for several months without spoilage (Lejko et al., 2009). Butter is one of the primarily fat sources and an important source of dietary energy. It has been produced since ancient times and was an internationally traded commodity as early as the 14th century (Vernam and Sutherland, 1994; Rady and Badr, 2003).

The quality of butter is closely related to its physico-chemical and microbiological characteristics. Besides fats, butter contains small percentages of proteins, milk sugar and water which make it a suitable substrate for microorganisms (Mahendra et al., 2016, Singh et al., 2011). Although butter spoilage is most often due to the development of chemical rancidity, microbiological problems do also occur in the form of cheesy, rotten or fruity odors and the rancid flavor produced by hydrolysis (Rady and Badr, 2003).

The primary spoilage factors in butter are moulds and the majority of the moulds growing in butter are composed of the species of *Thamnidium*, *Cladosporium* and *Aspergillus*. Through the application of a proper heat treatment, moulds cannot survive in cream even if contamination exists. So, the presence of mould contamination in butter indicates contamination by water or air after production (Bereda et al., 2014). Moreover, some pathogenic microorganisms like *Listeria monocytogenes*, verocytotoxin-producing *Escherichia coli* and *Stapayloccocus. aureus* which are known to cause food borne illness in human beings were also detected in butter (Pal, 2014).

In most areas of North Shoa zone, livestock production is the main income source of the farmer among those dairy, sheep and equines are mainly adapted in the area. Livestock and livestock products like milk, butter and meat are the main income generators for farmers living specially in the highland areas of the zone. Among these areas, Angolelana Tera, some parts of Basonaworana and Menz districts are known by their dairy production and the livelihood of the farmer also depend on dairy products (milk and butter). Angolelanatera and Basonaworena district are known in cow milk production and supply their product to the surrounding milk processing plant. But Menz districts are well known by their butter production in addition to milk and this product is also preferred by consumers to cow milk butter collected from other areas. Even if the areas have good potential in butter and milk production, there is no enough scientific information or study that inform about the quality of milk and butter made from cow milk. In this background, the present study is intended to describe the physicochemical property and microbiological composition of cow butter collected from different area of the Menz districts.

MATERIALS AND METHODS

Study area

The study was conducted in three purposively selected district of North Shoa zone (Menz Gera, Menz Mama, and Menz Keya) based on their butter production capacity from November 2016 to March 2017. North Shoa zone is one of the ten administrative zones of Amhara National Regional State. This zone covers 17.7 thousand km^2 land areas. Of the total land area, 38.2% is arable, 42.1% grazing and browsing, 7.5% natural vegetation, 2.1% unproductive, and 11.0% unutilized. Traditionally, the zone is divided into Dega (37.4%), Woina Dega (30.1%) and Kola (32.5%) agro-climatic zones (ANRS -BOFED, 2001).

The study was conducted in three districts of Menz, namely, Menz Gera Midir (also called Mehal Meda) with administrative center of Mehal Meda and a total population of 120,469, of whom 58,827 are men and 61,642 are women; 11,055 or 9.18% are urban inhabitants, Menz Mam Midir (also known as Molale) with administrative center of Molale and a total population of 85,129, of whom 42,102 are men and 43,027 are women; 6,513 or 7.65% are urban inhabitants and Menz Keya Gebreal (also called Zemero) with administrative center of Zemero and a total population of 46,219, of whom 22,965 are men and 23,254 are women; 2,623 or 5.68% are urban inhabitants (Figure 1).

Data collection

Laboratory analysis

Traditional butter was elaborated in the laboratory as described by Idoui et al. (2010). Butter samples were collected from each value chain actors (starting from the producer to the traders of different locations of Menz districts, Addis Ababa). In each of the three districts, three samples from the farmers, one sample from trader and fresh milk sample was collected. In addition, two butter samples were collected from the butter market value chain one from Tarmaber and other from Addis Ababa. The fresh milk was processed to butter by investigators following the traditional way of

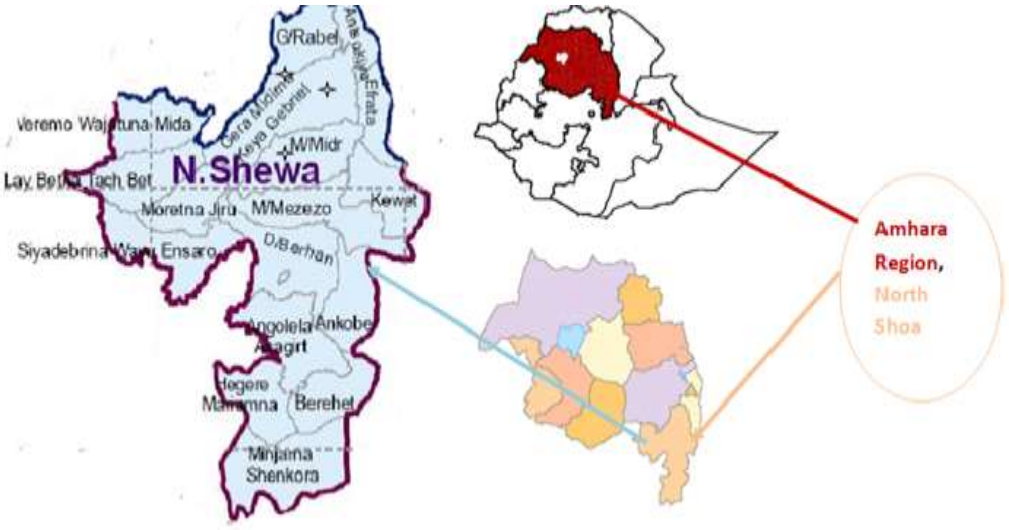

Figure 1. Study area (left: map od Amhara region; right: North Shoa with the study area signaled by *symbol).

butter making process. After the milk is left to turn into yoghurt, it was churned by hand churner till whipped cream became coarser and semi-solid butter granules were formed that rapidly increased in size and separated sharply from the liquid buttermilk. Butter was washed with cold water several times and the excess water was removed. Butter was filled in sterile disposable polyethylene bags and stored at 4°C till analysis. After collection, the butter samples were brought to the Dairy Laboratory in Holeta by placing it under ice box. Three microbial (TAMBC, total coiform, and yeast and molds) and three physiochemical (%FFA, %Moisture content, and %Fat) were conducted at Holeta Dairy Laboratory following standard procedures according to Richardson (1985).

Data analysis

The microbiological count data were transformed to log10 values. Mean values and frequencies were used to compare data.

RESULTS AND DISCUSSION

Physiochemical property

Traditionally and legally, however, butter must contain >81% of only milk fat (Gebremedhin et al., 2014). As shown in Table 1, the mean value of moisture content in the three districts was 14.17, 15.25, and 15.05 in Mehal Meda, Molale, and Zemero respectively with an overall mean value of 15.05. Highest values of moisture content were observed in Zemero (16.90% at kebele 01) and Molale (16.75% at kebele 06). All of these results are below the maximum legal compositional standards for butter moisture (16%). Table 2 shows almost equal mean value of moisture content both in butter samples from traders (14.36%) and butter made by investigators (14.25%). This is lower than the moisture content registered in samples from farmers.

Legal compositional standards for butter are a minimum of 80% butter fat and a maximum of 16% moisture. Moisture and impurities values of butter samples ranged from 16 to 35.73% and 9.25 to 12.25%, respectively. The moisture level in all butter samples is higher than the international standard (0.05 to 2%). The high level of moisture in traditional butter may have an influence on its microbiological and physicochemical quality since the presence of water in butter can activate lipases, stimulate the growth of micro organisms and cause the hydrolysis of triglycerides spoilage when stored at room temperature (Ronholt et al., 2013). The moisture content of traditional butter ranges from 20 to 43%. Ashenafi has reported that traditional butter has 17.2% moisture, 1.3% protein, 81.2% fat, 0.1% carbohydrate, 0.2% ash, 0.024% calcium, and 0.0015% iron (Ashenafi, 2006). The average moisture content of butter collected from open markets of Delbo and Kucha was 18.86±1.02%/g of butter samples. Generally, there is scanty information on chemical composition of butter in the country (Mekdes, 2008).

The mean fat content of butter collected from Mehal Meda, Molale and Zemero farmers were 83.5, 82.52 and 81.74%, respectively with an overall mean of 82.68%. Highest values of 85.94% (at Molale, kebele 01) and 84.47% (at Mehal Meda, kebele 07) fat% were observed. The value of fat percentage in samples from traders (83.44%) and sample from butter made by investigators (83.30%) were higher than the overall mean seen in samples from farmers. The food value of butter depends on its butterfat content. The fat content of butter is reduced by the incorporation of excess water and most countries protect the consumer by prescribing a legal limit

Table 1. Moisture and fat content of butter samples from farmers.

District	%Moisture content	%Fat
Mehal Meda		
Kebele 02	14.22	83.889
Kebele 03	14.83	82.431
Kebele 07	13.451	84.47
Mean	14.17	83.59
Molale		
Kebele 01	12.561	85.94
Kebele 02	16.45	80.937
Kebele 06	16.75	80.688
Mean	15.25	82.52
Zemero		
Kebele 01	16.90	79.33
Kebele 02	14.604	83.769
Kebele 08	15.674	82.127
Mean	15.72	81.74
Overall mean	15.05	82.62

Table 2. Moisture and fat content of butter samples from traders and other markets.

Type of butter	District	%Moisture content	%Fat
	Mehal Meda	15.42	83.44
Trader	Molale	12.706	84.92
	Zemero	14.672	81.9550
Mean	-	14.26	83.44
	Mehal Meda	14.387	83.749
Churned	Molale	16.00	81.127
	Zemero	12.38	85.05
Mean		14.25	83.30
	Tarmaber	14.58	83.82

for water content. The higher fat content in buttermilk within the traditional methods might be attributed to the long churning time and/or the mechanism of churning that allows the incorporation of large volumes of air. A short churning time corresponds with low churning efficiency (Zelalem et al., 2007)

As shown in Figure 2, the moisture content of all samples was below the maximum standard (16%) expected in butter. Lower moisture content were seen at Tarmaber and Addis Ababa which may be due to long time of storage and evaporation moisture of butter at the final markets away from the production area. The fat content of the samples is also above the minimum required value (81%). But the free fatty acid content indicates unhygienic processing and handling of butter in all samples with the lowest value attained from butter sample made by investigators.

Free fatty acid composition

Hydrolysis is the liberation of free fatty acids from the glycerol backbone in the presence of a lipase enzyme. A large amount lipoprotein lipase is present naturally in milk, but fortunately fat globules with an intact milk fat globule membrane are not susceptible to hydrolysis by the enzyme. Spoilage bacteria provide a heat stable lipase, but the spoilage bacteria must exceed normal levels. Fat that has been lipolysed tastes rancid and smells rancid. The fat globules can be damaged by

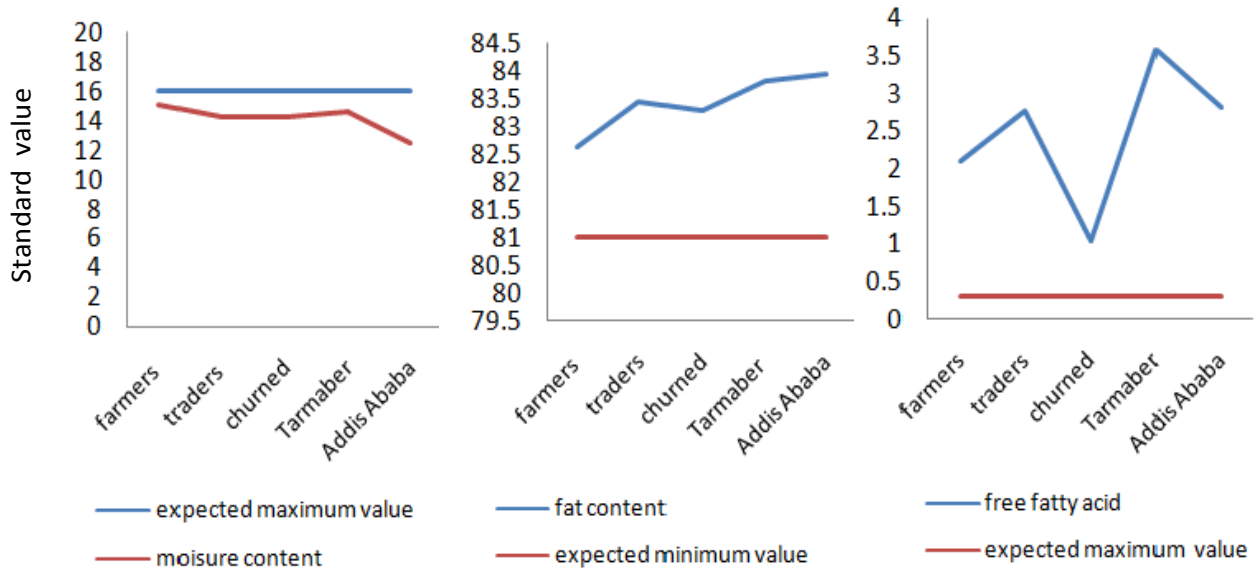

Figure 2. Comparison of moisture, fat and free fatty acid contents of butter with standard values.

Table 3. Free fatty acid composition of butter samples collected from producers (farmers).

Parameter	Districts								
	Mehal Meda			Molale			Zemero		
	Kebele 02	Kebele 03	Kebele 07	Kebele 01	Kebele 02	Kebele 06	Kebele 01	Kebele 02	Kebele 08
% Free fatty acid	2.87	1.49	1.81	2.00	1.10	1.56	3.01	3.77	1.24
Mean		2.06			1.55			2.67	

pumping, stirring, or splashing the milk. Therefore, unnecessary agitation of unpasteurized milk should be avoided to prevent the damage of the fat globules. The free fatty acids of milk fat are believed to be involved in imparting flavor properties to milk and other dairy products. The reduction in quality was caused by rancidity and bitterness that are related to high levels of free fatty acids and break down of proteins (Dieffenbacher et al., 2000). The standard specifies butter to have 0.3% maximum free fatty acids expressed as oleic acid, and a peroxide value less than 1.0 (Sserunjogi et al., 1998).

The mean value of free fatty acid in Mehal Meda, Molale and Zemero are 2.06, 1.55 and 2.67, respectively with an overall mean of 2.09%. In Table 3, the mean value of free fatty acid in butter from traders and butter made by investigators were 2.77 and 1.03%, respectively. But the highest values were seen in samples from Tarmaber (3.58%) and Addis Ababa (2.82%) (Table 4). The content of free fatty acids in the butter sold in rural markets varied from 0.23 to 1.20%. Older butter sold in the Addis Ababa market had free fatty acids content of as high as 23%. In Debre Zehit content, free fatty acids was between 0.07 and 3.32% (O'Mahony and Ephraim, 1985).

A study conducted in Algeria by Idoui et al. (2010) reviled that traditional cows' butter contained a high percentage of saturated fatty acids (SFA) and palmitic acid was the major SFA (24.33 to 36.95%), followed by myristic acid (18.49 to 27.35%) and stearic acid (7.68 to 14.05%). In other study, palmitic acid was reported to be the major SFA (22.81%) followed by stearic acid (10.21%) (Rady and Badr, 2003).

Microbial properties of butter from Menz district

Microbial criteria require that specific microorganisms or toxins produced by a microorganism must not be present at all, are allowed in a limited number per gram of samples, or be present at less than a specified number or amount in a given quantity of a food ingredient (Michael and Joseph, 2004). Average value of total aerobic mesophilic bacterial count (TAMBC) from farmers sample was 3.94×10^9 with the maximum value of 4.11×10^9 at Zemero district Kebele 01. A mean value of 3.89×10^9, 3.99×10^9 and 3.94×10^9 TAMBC was recorded from Mehal

Table 4. Free fatty acid composition of butter samples from traders and other markets.

Parameter	District	Free fatty acid
Trader	Mehal Meda	1.89
	Molale	3.02
	Zemero	3.4
	Mean	2.77
Churned	Mehal Meda	1.2
	Molale	0.92
	Zemero	0.96
	Mean	1.03
-	Tarmaber	3.58
	Addis Ababa	2.82

Table 5. Microbial quality of butter samples collected from farmers.

Districts	TAMBC (CFU/g)	Total coiform (CFU/g)	Yeast and molds (CFU/g)
Mehal Meda			
Kebele 02	4.07×10^9	3.16×10^6	1.90×10^6
Kebele 03	3.43×10^9	2.89×10^6	1.65×10^6
Kebele 07	4.17×10^9	2.45×10^6	1.82×10^6
Mean	3.89×10^9	2.83×10^6	1.79×10^6
Molale			
Kebele 01	4.08×10^9	2.58×10^6	2.32×10^6
Kebele 02	3.85×10^9	2.31×10^6	1.68×10^6
Kebele 06	4.06×10^9	2.48×10^6	1.75×10^6
Mean	3.99×10^9	2.46×10^6	1.92×10^6
Zemero			
Kebele 01	4.11×10^9	2.63×10^6	1.37×10^6
Kebele 02	3.57×10^9	2.12×10^6	1.75×10^6
Kebele 08	4.15×10^9	3.32×10^6	2.22×10^6
Mean	3.94×10^9	2.69×10^6	1.78×10^6
Overall mean	3.94×10^9	2.66×10^6	1.83×10^6

Meda, Molale and Zemero respectively (Table 5). Lower results of 3.64×109, 3.23×10^9 and 3.45×10^9 were obtained in samples collected from traders at Mehal Meda, Molale and Zemero, respectively (Table 6). In samples taken from butter made by the investigators (*churned*), lowest values of 3.26×109, 3.21×10^9 and 3.32×10^9 were recorded as shown in Table 6. But much higher results were seen at Tarmaber (4.19×10^9) and at Addis Ababa (4.20×10^9) (Figure 3).

In general, an overall mean of 3.94×10^9 TAMBC from farmers sample, 3.44×10^9 from traders, 3.26×10^9 from churned samples, 4.19×10^9 from Tarmaber and 4.20×10^9 from Addis Ababa were recorded. These values are higher than the acceptable limit of 5×10^4 cfu/g (Mostert and Jooste, 2002). Here, highly contaminated butter was found at Addis Ababa and Tarmaber followed by butter collected from farmers. At the time of collection, necessary measures were taken to collect butter which is not aged; this may rules out the source of variation to be age of butter. And there will not be seasonal variation since all samples were collected and tested on month of august which is supposed to be rainy season. Accordingly, the source of contamination seems to be at farmers and secondary/final market places (Table 7). Mamo (2007) reported a total microbial load of 3.15×10^7 where he suggests there is a high variability among samples depending on the sources with samples collected from open markets and rural producers for

Table 6. Microbial quality of butter samples collected from traders and churned by investigators.

District	TAMBC (CFU/g)	Total coliform (CFU/g)	Yeast and molds (CFU/g)
Mehal Meda			
Traders	3.64×10^{9}	3.00×10^{6}	1.92×10^{6}
Churned	3.26×10^{9}	1.38×10^{6}	1.99×10^{6}
Molale			
Traders	3.23×10^{9}	3.16×10^{6}	1.37×10^{6}
Churned	3.21×10^{9}	2.10×10^{6}	1.79×10^{6}
Zemero			
Traders	3.45×10^{9}	2.92×10^{6}	6.27×10^{5}
Churned	3.32×10^{9}	1.36×10^{6}	1.52×10^{6}

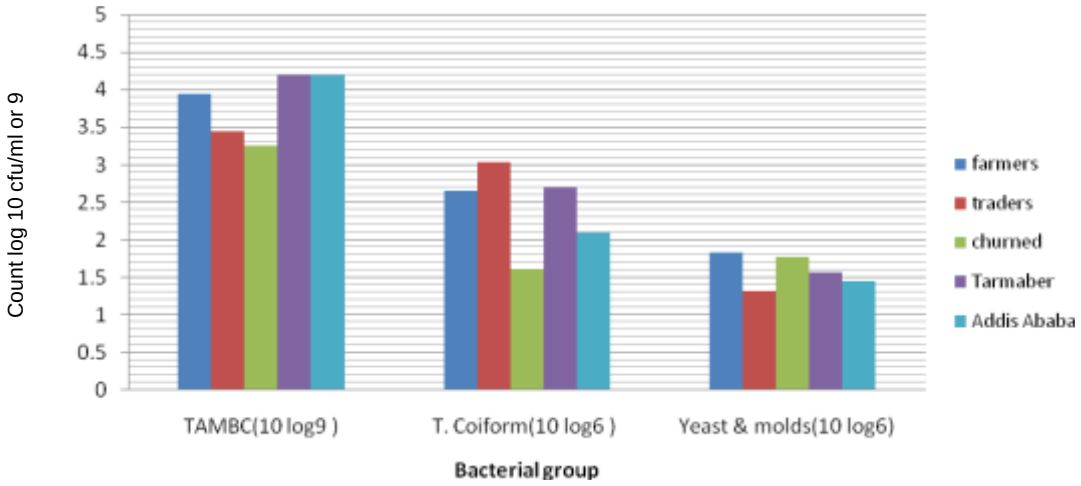

Figure 3. Microbial counts of butter samples from Menz district.

Table 7. Microbial quality of butter samples collected from Tarmaber and Addis Ababa.

Place	TAMBC (CFU/g)	Total coliform (CFU/g)	Yeast and molds (CFU/g)
Tarmaber	4.19×10^{9}	2.69×10^{6}	1.56×10^{6}
Addis Ababa	4.20×10^{9}	2.10×10^{6}	1.45×10^{6}

instance had higher counts as compared to that obtained from dairy farms and urban producers. According to Zelalem (2010), butter samples collected from Selale area average total bacterial counts of 7.25 cfu/g of butter. A study conducted by Mekedes (2008) in Southern Ethiopia on samples collected from open markets in Delbo and Kucha areas in Southern Ethiopia revealed mean total bacterial counts of 8.19±0.12 log cfu/g. The mean AMBC in fresh butter samples collected from Ambo and Dire Inchini districts of west shewa revealed log8.71 cfu/g (Alganesh, 2017).

A study conducted in other parts of the world recorded higher values of TAMBC than the present study. In Sudan, a value of log107.24 cfu/g, log10 7.68 cfu/g and log10 7.89 cfu/g, respectively for butter traditionally made by farmers, butter manufactured in dairy plant and butter made by investigators (Ahmed et al., 2016). Other researchers (Samet-Bali et al., 2009) also reported total microbial count of log 4.70±0.05 in traditional Tunisian butter and total bacteria count of log 5.18 to 6.83 in traditional Algerian cow's and goat's milk, respectively (Idoui et al., 2010). Additionally, a mean total bacteria count of log 5.18 to 6.08 cfu/g in Karın traditional butter from Turkey was reported (Gökce et al., 2010). The high

count of bacteria in these studies may be attributed to the absence of heat treatment (pasteurization) and salt. High total bacteria count in butter may be attributed to high microbial load initially present in the milk, absence of pasteurization and salt, and the effect of both separation and churning processes on the breaking up of bacterial clumps which increases their number (Idoui et al., 2010). Butter was classified according to its total aerobic mesophilic bacteria as very good quality ($<1.0\times10^6$ cfu/g), good quality (1.0×10^6 - 2.0×10^6 cfu/g) and low quality ($>2.0\times10^6$ cfu/g) (Gökce et al., 2010). In this respect, quality of butter collected from the present study belongs to low quality because the lowest value observed in the study area is 3.21×10^9 from churned sample at Molale district. The microflora of butter reflects the quality of cream, the sanitary conditions of equipment used in the manufacture of butter and the environmental and sanitary conditions during packaging and handling. It is advisable to adopt strict hygienic measures during milk handling to prevent contamination and improve its quality, in addition to proper heat treatment of milk (Meshref, 2010).

Total coliforms as hygiene indicator can be used as important criteria for determination of microbiological quality of butter (Zelalem, 2010). Overall average mean value of 2.66×10^6 total coliform counts was observed from farmer's sample. Looking into the three districts separately, mean value of 2.83×10^6, 2.46×10^6 and 2.69×10^6 were gathered from Mehal Meda, Molale and Zemero, respectively. Both the highest (3.32×10^6) and the lowest (2.12×10^6) value were seen in Zemero district (Table 6). Unlike in the case of TAMBC, the values for total coliform counts were higher in samples collected from traders in Mehal Meda (3.00×10^6), Molale (3.16×10^6) and Zemero (2.92×10^6) (overall average being 3.03×10^6). The lowest values were obtained from butter samples manufactured by investigators (1.38×10^6, 2.10×10^6 and 1.36×10^6 in Mehal Meda, Molale and Zemero districts, respectively with overall value of 1.61×10^6). The result in Tramaber (2.69×10^6) is higher than the value of butter manufactured by investigators, butter from farmers, and also butter sample from Addis Ababa. The total coliform count of butter sample from Addis Ababa (2.10×10^6) indicates a lower value than farmers, traders and Tramaber. Therefore, higher total coliform count records were from traders (3.03×10^6) and from Tarmaber (2.69×10^6).

These high deviations from the acceptable value of 10 cfu/g (Mostert and Jooste, 2002) indicate substandard handling conditions at all stages in the milk chain. Higher values were reported from similar studies conducted in Sudan (Ahmed et al., 2016) where values of log10 2.51 cfu/g, log10 2.38 cfu/g and log10 2.41 cfu/g for butter from farmers, butter from dairy plant and butter made by investigators were seen, respectively. Coliforms are indicators of cleanliness of handling of milk and cream, premises and equipment Idoui et al. (2010). Coliform was found in all samples of fresh butter from rural and public butter markets in Addis Ababa which indicates poor hygienic practices ILCA (1992). Zelalem (2010) reported coliform counts ranging from 1.92 to 4.5 log cfu/gram of butter. Similarly high values were reported by Gökce et al. (2010), Meshref (2010), Idoui et al. (2010), Asresie et al. (2013). Kacem and Karam (2006) reported coliform bacteria count of 0.90-1.66 log cfu/g at refrigerator temperature. Karagozlu and Ergonul (2008) reported that coliform and total fecal coliform count of the samples were found between <3->1400 cfu/g. Elkhidir (2003), observed that 41.71% of butter samples examined in Khartoum State had total coliforms in the range of ≥10 to ≤1400 MPN/g. The existence of coliform bacteria in food material is of greatest importance because it indicates that the food product is exposed to an insufficient heat treatment or is re-contaminated afterwards (Gökce et al., 2010).

Average yeast and mold counts of 1.83×10^6 was recorded from farmers butter with the highest record of 2.32×10^6 (Kebele 01, Molale) and lowest value of 1.37×10^6 (Kebele 01, Zemero). The mean yeast and mold count in the three districts was 1.79×10^6, 1.92×10^6 and 1.78×10^6 in Mehal Meda, Molale and Zemero, respectively. Butter collected from traders shows 1.92×10^6, 1.37×10^6 and 6.27×10^5 in Mehal meda, Molale and Zemero districts, respectively. Lower values were observed from traders sample than farmers except in Mehal Meda. Values from butter samples made by the investigators were 1.99×10^6, 1.79×10^6 and 1.52×10^6 with an overall mean value of 1.77×10^6. Lower values of yeast and mold count in butter prepared by investigators were with lower values than farmers except the value at Mehal Meda district. Even lowest values were observed from samples taken from Tarmaber (1.56×10^6) and Addis Ababa (1.45×10^6).

The presence of mould contamination in butter indicates contamination by water or air after production. The mean yeast and mould count observed in the Ethiopian highlands was 8 cfu/g of butter (Zelalem, 2010). According to Mekdes (2008), yeast and mould counts ranged between 4.3 and 6.86 log cfu/g of butter sampled from Wollayta area. In Sudan, higher values of log10 3.39 cfu/g, log10 3.03 cfu/g and log10 3.08 cfu/g were reported for butter samples from traders, butter samples from dairy plants and samples from butter made by investigators (Ahmed et al., 2016). Other studies also report same result like Samet-Bali et al. (2009) who reported yeasts and moulds count of log10 4.80±0.00 in Turkish butter, Karagözlü and Ergonul (2008) who reported yeast and mould counts of butter <log10 1.00-6.62 cfu/g, Idoui et al. (2010) and Gökce et al. (2010). Moulds and yeasts grow faster than bacteria and cause spoilage in food with low water activity. In Egypt, a lower content of molds and yeasts with a mean count of $6.3\times10^3 \pm 1.07\times10^3$ cfu/g was reported (Meshref, 2010). Beside spoilage, mycotoxin risk also exists and the high amount of moulds and yeasts is as an indicator of

incorrect processing and packaging (Gökce et al., 2010). *Geotrichum candidum* is responsible for yeast smell in butter and after a time it causes a disgusting taste and aroma, while *Penicillium, Aspergillus, Mucor, Candida, Cladosporium, Fusarium, Rizopus, Torula* and *Geotrichum* from spots on the surface and mouldy taste in butter, *Mucor stolonifer* causes lipolytic and proteolytic decomposition in butter and *Candida lipolitica* causes a caustic and cheese-like taste by exerting lipolytic activity in butter (Gökce et al., 2010).

Conclusion

The fat content of butter samples collected from different actors in the market value chain are greater than 80% except in one sample collected from farmers in Zemero district (79.33%) ranging from 80.68 to 85.94% which is adequate amount since butter must contain ≥81% of only milk fat. The moisture content of butter in most of the samples is also less than the maximum amount of moisture expected in butter (16%). Only four samples (three from farmers and one sample from butter made by investigators) show higher moisture content than the maximum standard moister content of butter. Moisture content of butter samples vary from 12.38 to 16.9%. Values of free fatty acids which are indicators of quality deterioration are also variable in the samples ranging from 1.10 to 3.77%.

Different values of Total Aerobic Mesophilic Bacterial Count (TAMBC) were observed in samples collected from different butter market actors. 3.94×10^9 cfu/g (mean value of farmers), 3.44×10^9 cfu/g (mean value of traders), 3.26×10^9 cfu/g (mean value of butter made by investigators), 4.19×10^9 cfu/g (Tarmaber) and 4.20×10^9 cfu/g (Addis Ababa) were recorded. Accordingly, total microbial quality is lowest in samples taken at the secondary (Tarmaber) and central markets (Addis Ababa) unlike butter made by investigators which shows best total microbial quality. Even if these records are generally lower than records in previous studies, the results reflect low quality butter as compared to the acceptable limit of 5×10^4 cfu/g. Coliforms were found in all samples that indicate poor hygienic practices. The total coliform counts (cfu/g) were 2.66×10^6, 3.03×10^6, 1.61×10^6, 2.69×10^6 and 2.10×10^6 cfu/g in samples from farmers, traders, butter made by investigators, Tarmaber and Addis Ababa, respectively. Higher value of yeast and mold counts were seen in samples collected from farmers (1.83×10^6 cfu/g) and in butter made by investigators (1.77×10^6 cfu/g).

RECOMMENDATIONS

According to the current study, cooking butter is produced under unhygienic condition. Therefore, there is a necessity for developing the hygienic status of locally produced butter through provision of information to rural women on good process hygiene and to consumers on how to handle their foods including correct storage to protect them from infection and to save a lot of products from being deteriorated. The education on the principles of food hygiene should be imparted to all who form a part of food chain program (Pal, 2014). Many studies indicate that the microbial properties of Ethiopian traditional fermented milk products made from different dairy producer were substandard in quality (Bereda et al., 2014). Maintenance of the proper hygienic conditions during the processing of milk can reduce the prevalence of bacteria, which spoil the milk product (Singh et al., 2011). Good hygiene practices (GHP) during milking and subsequent handling of milk are essential to reduce the risk of contamination on the farm and in the milk processing plant (Sarkar, 2015). Furthermore, the application of hazard analysis and critical control point system (HACCP) seems imperative in all food processing industries from safety point of view (Pal and Jadhav, 2013). Alganesh (2017) stated that the materials used for milking, storage, processing and marketing are local materials which are porous, not easy to clean and disinfect and harbor microorganisms. Consequently, the dairy products are substandard and do not fulfill the safety and quality standards. Moreover, standard operating procedures are not followed on clean milk production and handling. Besides, safety and quality standards were not enforced. The information on safety and quality of dairy products in Ethiopia is not comprehensive. There is a need for further research and authentication policy guidelines of dairy products focusing on spoilage and pathogenic microorganisms, drug and pesticide residues, aflatoxins and adulteration practices. Generally, there is a need to devise means of promotion of modern dairy industry that is responsive to market demand and public health concerns. This would be possible by enforcing quality assurance programs and minimum standard requirements for delivery of authentic dairy products. Therefore, as Teshome and Tesfaye (2016) suggested the need for enriched hygienic practices and educating the public on safety issues and personal hygiene in milk handling. It would be a great interest if further investigations are to be carried out to identify and isolate different species of pathogenic microorganisms that might cause public health importance. There is also a need for assessment of seasonal variation of physiochemical and microbial quality of butter. The study also indicates that high fat content in the fluid milk is a preferred trait by farmers. In this context, animal breeds with higher fat content should be considered for the rural, butter-oriented production systems.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahmed SS, Abdalla MO, Rahamtalla SA (2016). Microbiological Quality of Cows' Milk Butter Processed in Khartoum State, Sudan. British Microbiology Research Journal 11(1):1-10.

Amhara National Regional State -Bureau off Finance and Economic Development (2001). Statistical bulletin. www.amharabofed.gov.et/PDF_Files/Final 2004 development.

Alganesh TG (2017). Status and Challenges in the Safety and Quality of Dairy Products in Ethiopia: A Review. Food Science and Quality Management, ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online) Vol.59

Ashenafi M (2006). A review on the Microbiology of indigenous fermented foods and beverages of Ethiopia. Ethiopian Journal of Science 25(1):97-140.

Asresie A, Seifu E, Kurtu YM (2013). Churning efficiency and microbial quality of butter made from camel milk alone and blending it with goat milk. Net Journal of Agricultural Science 1(3):75-80.

Bereda A, Eshetu M, Yilma Z (2009). Microbial properties of Ethiopian dairy products: A review. African Journal of Microbiology 8(23):2264-2271.

Central Statistical Agency (CSA) (2008). Central statics agency Agricultural sample survey 2008/2009 volume II report on livestock and livestock characteristics (private peasant holding) Addis Ababa, Ethiopia. Available at: www.instepp.umn.edu/.../Ethiopia_2007-08_Vol2.pdf

Dieffenbacher A, Buxtorf P, Derungs R, Friedli R, Zürcher K (2000). Graisses comestibles, huiles comestibles et graisses émulsionnées. In: Neukom Zimmermann (ed) Manuel suisse des denrées alimentaires. Société des chimistes annalistes suisses, Berne (Suisse)

Elkhidir DA (2003). Microbiology Evaluation of Marketed Butter in Khartoum Governorate. M.Sc. thesis, University of Khartoum, Sudan.

Gebremedhin B, Tegegne A, Hoekstra D, Jemaneh S, Shiferaw K, Bogale A, Getahun Y (2014). Developing the butter value chain in Ethiopia. LIVES Working Paper 1. Nairobi, Kenya: International Livestock Research Institute.

Gökce R, Aslanalp Y, Herken EN (2010). Microbiological quality of karın butter, a traditionally manufactured butter from Turkey. Grasas y Aceites 61(2):121-125.

Idoui T, Benhamada N, Leghouchi E (2010). Microbial quality, physicochemical characteristics and fatty acid composition of a traditional butter produced from cows' milk in East Algeria. Grasas y Aceites 61(3):232-236.

Lejko N, Grega T, Sady M, Domagala J (2009). The Quality and Storage Stability of Butter Made from Sour Cream With Addition of Dried Sage And Rosemary. Biotechnology in Animal Husbandry 25(5-6):753-761.

International Livestock Centre for Africa (ILCA) (1992). Alternative milk processing and preservation techniques and the quality of market butter and cheese. Annual program report 1991. International Livestock Centre for Africa. Addis Ababa, Ethiopia. pp. 39-40.

Kacem M, Karam N (2006). Physicochemical and microbiological study of "shmen", a traditional butter made from camel milk in the sahara (Algeria): isolation and identification of lactic acid bacteria and yeasts. Grasas y Aceites 57:198-204.

Karagozlu N, Ergonul B (2008). Microbiological attributes of Turkish butters sold under markets conditions. Journal für Verbraucherschutz und Lebensmittelsicherheit 3(4):376-379.

Mahendra P, Selamawit M, Muluken T, Suneeta V, Pintoo J, Prajapati P (2016). Bacterial contamination of dairy products. Beverage Food World 9(43):40-43.

Mamo W (2007). Composition, microbial quality and acceptability of butter produced from the milk of dairy cows in Hawassa, Southern Ethiopia. Hawassa University. M.Sc. thesis. P 89.

Mekdes A (2008). Assessment of processing techniques and quality attributes of butter produced in Delbo Watershed of Wolayita zone, Southern Ethiopia. MSC thesis, Awassa college of Agriculture, School of Graduate studies, Hawassa University, Ethiopia.

Meshref SM (2010). Microbiological quality and safety of cooking butter in Beni-Suef governorate, Egypt. African Health Sciences 10(2):193-198.

Michael HW, Joseph FF (2004). Standard Methods for the Analysis of Dairy Products. 17th edition, American Public Health Association, Washington, D.C., USA.

MoARD (2007). Livestock Master Plan Study. Phase I Report Volume T – Sociological Aspects.

Mostert JF, Jooste PJ (2002). Quality Control in the Dairy Industry. Dairy Microbiology Handbook. *Third Edition*. Edited by Richard K. Robinson. John Wiley and Sons, Inc., New York. pp. 655-736.

O'Mahony F, Ephraim B (1985). ILCA dairy technology course. International Livestock Centre for Africa (ILCA). Addis Ababa, Ethiopia. pp. 46-60.

Pal M (2014). Spoilage of dairy products due to fungi. Beverage and Food World 41:37:38,40.

Pal M, Jadhav VJ (2013). Microbial contamination of various Indian milk products. Journal of Beverage Food World 40:43-44.

Rady AH, Badr HM (2003). Keeping the quality of cows' butter by x–irradiation. Grasas y Aceites 54:410-418.

Richardson HG (1985). Standard Methods for the Examination of Dairy Products. 6th edn., American Public Health Association, Washington DC. pp. 133-150.

Ronholt S, Mortensen K, Kaudsen JC (2013). The effective factors on the structure of Butter and other milk fat- based products. Comprehensive Reviews in Food Science and Food Safety 12:468-482.

Saad NM, Sabreen MS, Amin WF, Gendi MK (2012). Prevalence of *Escherichia albertii* and Other *Escherichia species* in Raw Milk and Some Dairy Products in Assiut City, Egypt. Journal of American Science 8(11):333-341.

Samet-Bali O, Ayadi MA, Attia H (2009). Traditional Tunisian butter, physicochemical and microbial characteristics and storage stability of the oil fraction. Journal of Food Science and Technology 42:899-905.

Sarkar S (2015). Microbiological Considerations: Pasteurized Milk. International Journal of Dairy Science 10(5):206-218.

Singh V, Kaushal S, Tyagi A, Sharma P (2011). Screening of bacteria responsible for the spoilage of milk. Journal of Chemical and Pharmaceutical Research 3:348-350.

Sserunjogi ML, Abrahamsen RK, Narvhus J (1998). A Review Paper: Current Knowledge of Ghee and Related Products. International Dairy Journal 677-688.

Teshome G, Tesfaye A (2016). Physicochemical Properties and Microbial Quality of Raw Cow Milk Produced by Smallholders in Bench Maji-Zone, Southwestern. Food Science and Quality Management 54:47-54.

Vernam AH, Sutherland JP (1994). Milk and milk products: technology, chemistry and microbiology. Chapman & Hall, London.

Zelalem Y (2010). Quality factors that affect Ethiopian milk business: Experiences from selected dairy potential areas. Netherlands Development Organization, Addis Ababa, Ethiopia.

Zelalem Y, Loiseau G and Faye B (2007). Manufacturing efficiencies and microbial properties of butter and Ayib - Ethiopian cottage cheese. Livestock Research for Rural Development 19:7.

Related Journals: