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Full Length Research Paper

Effects of glucose and clove treatment on the microbial load of *Bunyi Youri* during fermentation and solar tent drying of the product

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“Bunyi youri” is a Nigerian fermented sun-dried fish product used as condiment in soups. This study was carried out to determine the effects of glucose and clove treatment on the microbial quality of the fermented and solar tent-dried product. A total of 24 isolates were obtained from the “Bunyi youri” samples analyzed, *Micrococcus* sp. was the predominant constituting 45.83% of the isolates, while *Bacillus* sp., *Staphylococcus* sp., *Leuconostoc* sp. and *Streptococcus* sp. constituted 29.17, 12.5, 8.33 and 4.17% of the isolates, respectively. Treatment of the samples with glucose resulted in an increase of the total aerobic plate count from an initial 2.11×10^3 cfu/g in unfermented raw fish samples to an average of 2.05×10^5 cfu/g during a 24 hour fermentation; the mean total plate count decreased to 9.73×10^2 cfu/g after the solar tent drying of the product. An improvement in the fermentation was observed as a result of the incorporation of glucose which increased the growth rate of the lactic acid bacteria (LAB) and which, in combination with clove resulted in a reduction of the spoilage bacteria in the final processed products.

Key words: “Bunyi youri”, fermentation, microbial, glucose, clove.

INTRODUCTION

Fish is one of the most important animal protein foods available in the tropics (Eyo, 2001). The less developed countries (LDCs) capture about 50% of the world's harvest and a large proportion of that catch are consumed locally (FAO, 1985). In many Asian countries, over 50% of the animal protein intake comes from fish while in Africa the proportion is only 17.5% (William et al.,

1998). Fish and other seafood constitute an important and popular part of the diet of many Nigerians which is made up of 60% of animal protein intake, particularly in regions where livestock is relatively scarce (Adeniyi 1987; Igwegbe, 2015). This means that shortfall in fish availability will affect the animal protein intake of people in Nigeria and other tropical countries. In addition, the

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harvesting, handling, processing and distribution of fish provide livelihood for millions of people across the globe (Eyo, 2001; Aljufaili and Opara, 2006). Furthermore, well processed fish from the tropics have a ready market in developed countries and are thus good foreign exchange earners (Akande and Odogbo, 2005).

The domestic fish production in Nigeria in the year 2007 was 615,000 metric tonnes (FAOSTAT, 2009). Nigeria was reported to spend over ₦100 billion on the importation of frozen fish in 2010. Estimated annual demand was about 2.66 million metric tonnes as against the annual domestic production of about 0.70 million metric tonnes giving a demand-supply gap of about 1.8 million metric tonnes (Tiamiyu, 2012). Despite the fact that Nigeria has a compact landmass of 923,762 km²; 860 km of coastline on a major gulf of the South Atlantic, abundant water resources with major rivers of the Niger and the Benue traversing its territory in addition to numerous smaller rivers and streams crisscrossing its vast terrains (Olaosebikan and Raji, 1998; Igwegbe *et al.*, 2014), the country imports over 780,000 metric tonnes of frozen fish annually from Europe, Latin America and eastern countries. The shortfall in fish supply in the country had led to a low annual per capita fish consumption rate of only 7.5 kg as against 15 kg per annum recommended by the FAO. There are many health benefits that can be derived from fish consumption. One of such merits is that fish is less tough and more digestible compared to beef, mutton, chicken and bush meat. This is possible because of the greater ratio of muscle protein to connective tissue protein in fish as compared with other animals (Alais and Linden, 1999) thus making fish acceptable to infants and adult alike (Eyo, 2001). Because of its greater digestibility, fish is usually recommended to patients with digestive disorders such as ulcers (Eyo, 2001); cardiovascular benefits which reduce the risk of heart attack, and anti-inflammatory properties of fish oil due to the presence of omega-3 fatty acid (Igwegbe, 2015). Apart from this, fish is abundant and to some extent occurs “free” in nature. This may account for its relatively low cost compared with other animal products. In addition, there is hardly any religious taboo affecting the consumption of fish, unlike pork and beef. Immediately the fish dies, a number of physiological and microbial deterioration set in and thereby degrade the fish. Most of the fish harvested in the tropics are either used in direct human consumption or processed into fish meal for use in compounding feeds. A significant quantity is also lost through the absence of adequate technology to prevent post-harvest losses common in most tropical countries. An estimated 20 to 50% of the fish produced in the remote coastal centers and hinterlands of many tropical countries deteriorate before they get to the consumers due to poor handling, preservation and processing practices adopted by the artisanal fishermen, fish farmers and fisheries entrepreneurs in those areas (Negbenebor, 1990; Eyo, 2001;

Igwegbe, 2015). The gap between fish demand and supply is unfortunately widening due to increasing population, drop in meat and fish supply, thus prompting the search for methods of improving fish quantity and quality. Thus, fish spoilage could be reduced if fish are traditionally processed to products that will have extended shelf life such as “Bunyi youri”.

“Bunyi youri” is a putrefied sun dried fish product prepared from Nile perch (*Lates niloticus*) or Clarias species and is used as condiment in the flavouring of soups and is very popular among the Kanuri Tribe of North Eastern Nigeria (Negbenebor *et al.*, 1995). These authors reported the proximate composition of this traditional product as 32.63±1.05% protein, 40.59±0.12% fat, 24.80±2.55% moisture and 1.88±0.32 ash. It is therefore evident that “Bunyi youri” is a highly nutritious fish product that could be processed very easily using the abundant sun light energy in the tropics including Nigeria. The processing and distribution of “Bunyi youri” can also serve as a source of income as well as offer employment opportunities to many Nigerians that may be engaged in the business. The traditional “Bunyi youri” processing involves the removal of the fish scale, gutting, washing and fermentation of the fish for 6 to 8 days at ambient temperatures of between 31 and 49°C.

Since partial putrefaction of the fish is involved during the traditional processing of “Bunyi youri”, uncontrolled chemical and microbial changes in the product can be expected. Also, the production of very strong odour which can attract flies and even rodents, during the processing, could lead to a serious contamination by these agents. Therefore, the control of both chemical and microbial activities during the processing is paramount to obtaining a shelf-stable product. Idakwo *et al.* (2016) reported the proximate composition and acceptability of “Bunyi youri” treated with glucose and clove stored for 24 weeks in which the product was acceptable within these weeks. The aim of the present study is therefore to determine the possible effects of combination of glucose and clove particularly on microbial activities during fermentation and subsequent solar tent-drying of “Bunyi youri”. This is targeted at improving the shelf-stability of the product as influenced by the microbial load. Although glucose and clove have been used either separately or in combination with other spices to enhance the quality of meat and fish products, there is paucity of information on the combined effects of the two ingredients on enhancing the quality of fermented fish and this is thus the major reason for the present study.

MATERIALS AND METHODS

Acquisition of the raw materials

Fresh fish samples of Nile perch (*Lates niloticus*) species were obtained directly from the River Benue fish landing sites in Yola, Adamawa State Nigeria. Samples were collected using a clean



Plate 1. The fresh fish samples.

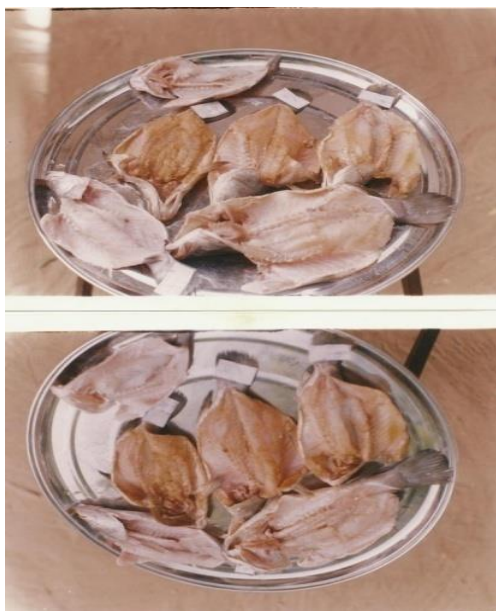


Plate 2. The prepared samples.

plastic container (80 × 20 × 45 cm) with tight lid, and packed with crushed ice to minimize deterioration of the fish during transportation to the laboratory in Maiduguri. For the purpose of comparison, samples were equally divided and a portion of it was taken to a local processor in Gaboru ward, for “Bunyi your”i to be produced using the normal traditional method, this product served as the commercial control sample. Cloves (*Eugenia carryophyllata*) were obtained from Maiduguri Monday Market while glucose of analytical grade (BDH Chemicals Ltd, U.K.) was obtained from chemicals supplier in Maiduguri.

The materials for the construction of the solar tent drier were

obtained from Maiduguri Timber and Monday markets. Finally, plastic buckets of five litre capacity were purchased from the Gaboru plastic container depot in Maiduguri and used for the fermentation. The constructed solar tent-drier used in this study is presented in Plate 3.

Preparation of fish samples

The fresh fish samples (Plate 1) were gutted and thoroughly washed with potable water. Five treatments were used in each experimental processing. For each of the five treatments, three replicates were made. The prepared fish (Plate 2) was divided into five different groups and four groups were treated separately with concentrations 2.0% glucose and 0.1, 0.3, 0.5 and 0.6% of clove, dipping the fish samples into the glucose-clove solution for 20 min (Adams et al., 1987); while the fifth group, which served as the control, was treated by dipping it only in distilled water. The prepared samples were allowed to ferment for 24 h at mean ambient temperature and relative humidity (RH) of 38.5 ± 1.7 and $25.9 \pm 2.1\%$ respectively (Adams et al., 1987). The fermentation was carried out in covered plastic buckets of 5 L capacity in the laboratory. The fermented fish were dried in the solar tent-drier (Plate 4) at the mean temperature and RH of 65.0 ± 5.2 and $21.9 \pm 0.35\%$, respectively, for two to three days until the samples were crisp dried (Plate 4). The humidity was measured using an electronic temperature/humidity meter (ELE, EA 506-124). The dried samples were stored in plastic containers until analyzed.

Sequence of operation in “Bunyi your”i processing

The traditional method of “Bunyi your”i processing was utilized with modifications by including the dipping of the fish samples in mixtures of glucose and clove for 20 minutes prior to fermentation for 12 to 24 h (Figure 1), and drying in a solar tent drier instead of the usual traditional practice of spreading on mats and direct exposure to the sun. The drying was continued for two to five days until the “Bunyi your”i was crispy-dried. The clove samples were ground using a hammer mill, wrapped in aluminum foil and then autoclaved at a temperature of 121°C and pressure of 15 psi for 15 min, to ensure its freedom from mould and bacterial spores before its application to the fish samples.

Construction of solar tent drier

The solar tent-drier was constructed as described by Clucas and Ward (1996) but with some modifications to include raising the drier on a platform table to prevent dust contamination and attacks by domestic animals like cats and dogs. The dimensions of the drier were as described by FAO (1983) and this is shown in Plate 3 as improved solar tent-drier. This consisted of a rectangular table measuring 210 × 240 cm as the base and a tent roof with a height of 40 cm. The top of the table was covered with a black polythene sheet for absorption of heat while the roof was vented to allow the exit of saturated air from the tent. These vents were covered with wire net in order to prevent flies and insect infestation of the drying products. The table was supported on 100 cm woods at the four angles. A window was also fitted on one side of the tent-drier to facilitate loading and offloading of the samples (Plate 3).

Microbiological media

The bacteriological and mycological media which included de-man Rogosa Sharpe agar (Oxoid Ltd, Basingstoke, UK), Plate count agar (Oxoid) nutrient agar (Biotec Laboratories, Suffolk, UK), Brain-

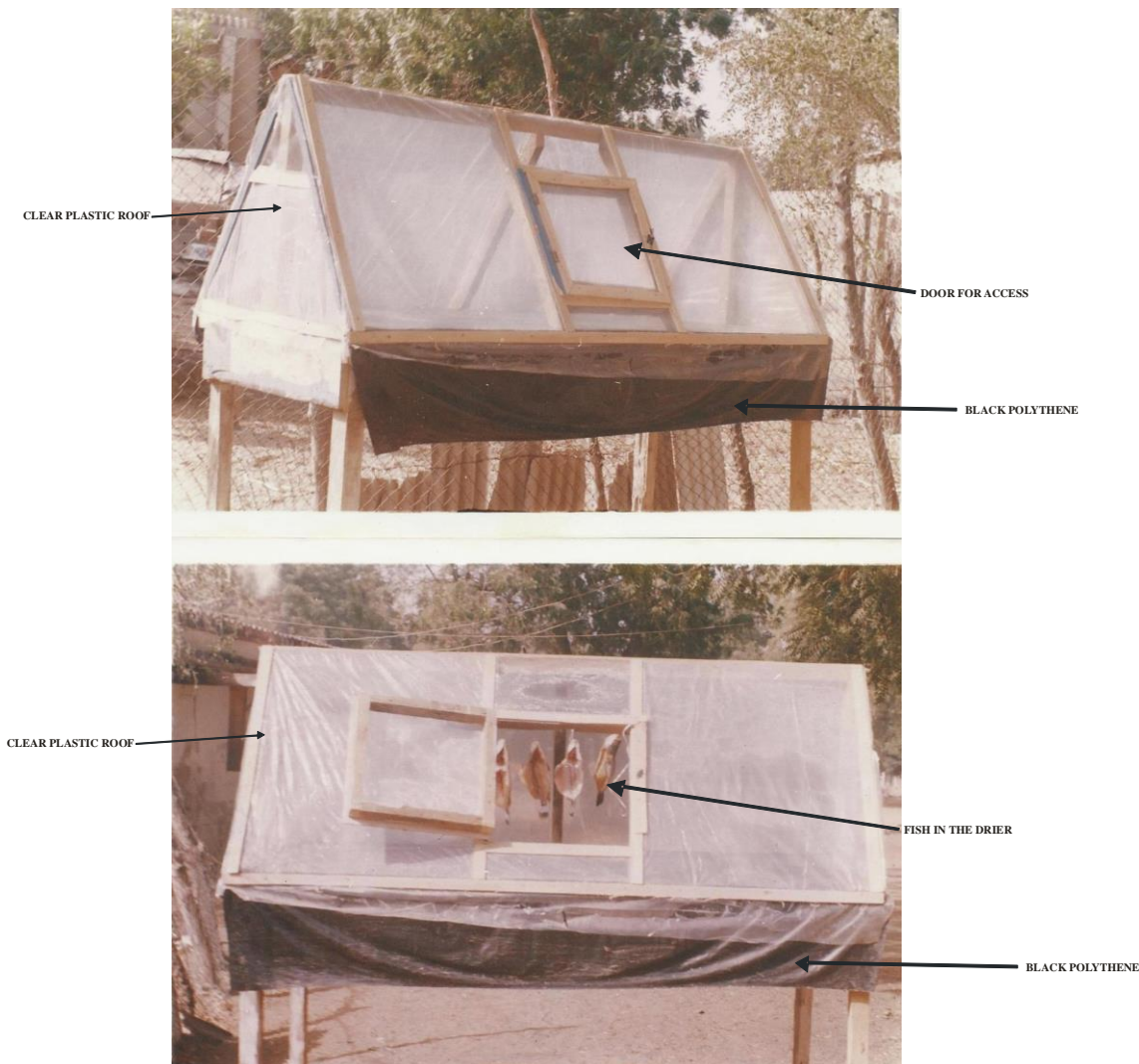


Plate 3. The solar tent-drier during drying.

heart infusion agar (Oxoid) Sabourand agar (Topley House, England), Lauryl sulphate tryptose broth, Brilliant green lactose broth and Eosin methylene blue agar (Biotec) were obtained and used for the isolation, enumeration and identification of the microorganisms associated with “Bunyi youri” processing. All glass wares including petri-dishes, test tubes, pipettes, flasks and bottles, were sterilized in a hot air oven at $170 \pm 5^\circ\text{C}$ for at least two hours, while the media and distilled water were sterilized by autoclaving at 121°C for 15 min and at 15 psi (Marshall, 1992; Quinn et al., 2002), and each medium was prepared and used according to the manufacturer’s instructions. The media were allowed to cool to about 40°C before pouring or plating.

Microbiological analysis

“Bunyi youri” sample (25 g) was aseptically transferred to a sterile plastic bag and pummeled for 1min in a stomacher (IUL Instrument, Spain), with 225 ml of 0.1% sterile peptone water. Appropriate decimal dilutions of the sample were prepared using the same diluents and 0.1 ml of each dilution was plated in triplicate on

different growth media.

Lactic acid bacteria (LAB) were enumerated on de-man Rogosa Sharpe agar (Oxoid) using the pour plate technique (Harrigan and McCance, 1976), catalase negative, gram positive, opaque, white colonies were counted as lactic acid bacteria (Adams et al., 1987). Spoilage organisms were enumerated on plate count agar (Oxoid) using the pour plate technique as described by Harrigan and McCance (1976). Catalase positive, large and medium sized colonies were counted as spoilage organisms (Adams et al., 1987), on the same plate count agar, lactic acid bacteria were enumerated as very translucent or white, catalase negative colonies. These small colonies were enumerated so as to determine the proportion of lactic acid bacteria involved in the fermentation process. All the plates were incubated at 37°C for 24 h. *Staphylococcus aureus* were enumerated as described by FAO (2013) utilizing 0.25 ml of the fish homogenate and the subsequent decimal dilutions on the surface of Baird Parker agar. A confirmatory test or coagulase test was used to establish the identity of *Staphylococcus aureus*. Also, the enumeration of coliforms was as described in FAO (2013) utilising the Lauryl sulphate tryptose broth, brilliant green lactose broth and Eosin methylene blue agar (EMBA) which consisted of



Plate 4. Solar tent-dried samples.

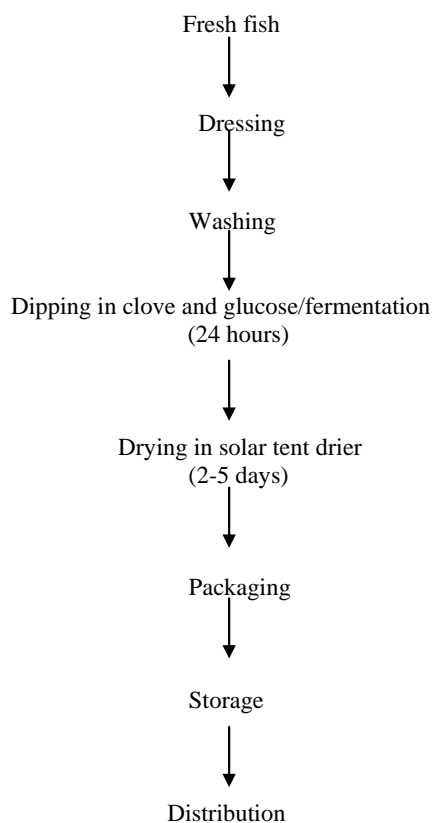


Figure 1. Schematic diagram of improved “bunyi youri” processing (improved from traditional method).

presumptive, confirmatory and completed tests. Sabouroud agar was incubated at 25°C for 3 to 4 days for fungal isolation

The other inoculated plates were inverted and then incubated at 37°C for 24 h, after which the cultural and morphological characteristics of colonies were observed. Colonies were selected at random and sub-cultured to obtain pure isolates on fresh plates

containing nutrient agar and then incubated again at 37°C for 24 h. The stock cultures were obtained, labeled carefully and were used for conventional identification of the organisms using Gram’s staining, motility, indole production, urease, carbohydrate utilization, catalase, citrate utilization, oxidase, coagulase and methyl red tests.

Biochemical identification of Isolates

The colonies of bacteria were isolated, purified and subjected to different biochemical tests using methods described by Harrigan and McCance (1976). However, mould colonies appearing on the plates were sub-cultured to give pure colonies employing the method of Onions et al. (1980). The moulds were identified following the guidelines prescribed by Gupta et al., (2013) and Onions et al. (1980).

Statistical analysis of the data

The statistical package for Social Science (SPSS version 10) was used. Data analysis involved one-way analysis of variance (ANOVA). The mean differences were determined using Duncan’s multiple range test. A significant difference was established.

RESULTS AND DISCUSSION

Microbial analysis

The microbial contents described as the total aerobic plate count (TAPC), lactic acid bacteria (LAB) and spoilage bacteria (SB) counts in *Bunyi youri* are presented in Table 1. The mean total bacterial counts recorded in the control samples ranged between 1.76×10^3 and 1.803×10^3 cfu/g whereas that of the treated samples ranged between 1.79×10^5 and 2.08×10^5 cfu/g. Increase in the viable count of micro flora from 10^6 to 10^9 during the 72 h of fermentation using starter culture was reported in African fermented fish (Aseidu and Sanni, 2002). Suchitra and Sarojinalini (2012) also reported a viable count of up to 10^6 cfu/g in samples of “Ngari” Aryanta et al. (1991) also observed a population of 10^7 to 10^8 cfu/ g after 48 h in fermented fish sausage using *Pediococcus acidilactici* as starter culture. ICMSF (1986) mentioned the limit for standard plate count (SPC) for microorganisms per gram weight of different fish samples to be 10^5 cfu/g while Cho et al. (1988) reported a range of viable bacterial count between 10^3 and 10^7 cfu/g in dried fish. In this study, the significant increase in total bacterial counts recorded is an indication of the bacterial role in fermentation, which ultimately results in the production of flavours.

Generally, there was an increase in the microbial population in all the treated samples because the microorganisms responsible for fermentation were multiplying as a result of abundant substrate particularly glucose and suitable conditions of low pH for their growth and this observation is in agreement with that made by Pelczar et al. (1986). Thapa et al. (2004) also fermented

Table 1. Microbiological counts (cfu/g) of “Bunyi youri” after fermentation.

Treatment ²	Microbial Group (cfu/g) / Percentage ¹				
	TAPC	LAB	LAB %	SB	SB%
Control 1	1.76×10 ³ ±4.9×10 ^{2b}	4.43×10 ² ±6.8×10 ^{1b}	25.71±2.9 ^c	1.32 × 10 ³ ±4.2×10 ^b	74.29±2.9 ^a
Control 2	1.80×10 ³ ±2.9×10 ^{2b}	5.09×10 ² ±1.8×10 ^{1b}	28.72±4.6 ^c	1.29×10 ³ ±2.8×10 ^{2b}	71.29± 4.6 ^a
A	1.79×10 ⁵ ±4.0×10 ^{4a}	1.22×10 ⁵ ±1.5×10 ^{4a}	68.16±15.8 ^a	5.70×10 ⁴ ±3.5×10 ^{4a}	31.84±15.8 ^b
B	2.05×10 ⁵ ±4.0×10 ^{4a}	1.55×10 ⁵ ±4.6×10 ^{4a}	74.39±8.2 ^a	5.03×10 ⁴ ±6.1×10 ^{3a}	25.54±8.1 ^b
C	1.92×10 ⁵ ±7.5×10 ^{4a}	1.69×10 ⁵ ±9.0×10 ^{4a}	74.80±10.0 ^a	5.30×10 ⁴ ±3.7×10 ^{4a}	24.53± 9.7 ^b
D	2.08×10 ⁵ ±5.0×10 ^{4a}	1.48×10 ⁵ ±4.0×10 ^{4a}	71.27±11.1 ^a	5.97×10 ⁴ ±2.9×10 ^{4a}	28.73±11.2 ^b

¹In any column, means bearing similar alphabetical superscripts are not significantly different ($P \geq 0.05$); TAPC = Total aerobic plate count; LAB = lactic acid bacteria; SB = spoilage bacteria. ²Control 1 = commercial “Bunyi youri”; Control 2 = laboratory prepared “Bunyi youri”; A = 2% glucose + 0.1% clove; B = 2% glucose + 0.3% clove; C = 2% glucose + 0.5% clove; D = 2% glucose + 0.6% clove.

fish into ‘Ngari’, ‘Hentak’ and ‘Tungtap’, which are traditional fermented fish products, and recorded increased microbial load in the fish samples. The predominant microorganisms were in the order of *Micrococci* followed by *Bacilli* spp. Anihouvi et al. (2006) similarly reported high microbial population during the fermentation of “Lanhoun” a traditionally processed fermented fish product popularly consumed in Benin Republic. The presence of this similar micro flora ‘Momone’ has also been reported by many researchers including Yankah (1988), and Abbey et al. (1994) and in other fermented fish products (Essuman, 1992).

Furthermore, no significant difference ($P \geq 0.05$) was observed in the total aerobic plate count, Lactic acid bacteria and spoilage bacteria counts of the treated samples. Although a similar trend was observed for the control samples, there was however, a significant difference ($P \leq 0.05$) in the TAPC, LAB and SB counts of the controls when compared with the treated samples. This could be due to the favourable environment created for the proliferation of Lactic acid bacteria in the treated samples, which was reflected in the increase in the load of LAB in the treated samples and a reduction in the spoilage bacteria as well. There was no significant difference ($P \geq 0.05$) in the level of spoilage bacteria among the treated samples, however, the two control samples (1 and 2) recorded higher loads of spoilage bacteria, 74.29 and 71.29%, respectively (Table 1), when compared with the samples treated with various levels of glucose and clove. This finding is similar to that recorded on ‘Sharmud’ which is a fermented meat product common in Nigeria, which usually has a pH level of 6.4 (Idakwo, 2004).

The pH of the treated samples may have played a significant role in controlling the type of bacteria found in the treated and untreated control samples. Visessanguan et al. (2006) also reported that as source of lipolytic and proteolytic enzymes, these organisms may contribute to flavour formation of ‘Nham’ a Thai fermented pork sausage. Similarly Beddows et al. (1980) reported that

the complex interaction of enzymatic activity and oxidation during the fermentation along with bacterial production of volatile fatty acids may be responsible for the characteristic flavour and aroma of fermented fish products.

As regards to the microbial characteristics of the raw fish materials, the total aerobic plate count recorded was 2.11×10^3 cfu/g in the fresh fish samples, which decreased during the fermentation and drying of the products to a range between 1.02×10^3 to 1.24×10^3 cfu/g, and 0.87 to 0.92×10^3 cfu/g in the control and all the treated samples, respectively (Table 2). This observation is within the microbiological limit of 10^2 to 10^4 cfu/g stipulated by FAO (1979). Also, the *Staphylococcus* count ranged between 3.98×10^2 and 4.10×10^2 cfu/g for the controls and 0.45×10^2 and 0.54×10^2 cfu/g for the treated samples (Table 2). High count of *Staphylococci* ($10^5 - 10^7$) was recorded in fermented fish sausage by Aryanta et al. (1991). Staphylococcal count exceeding 10^6 cfu/g is considered to be hazardous and a count of 100 million (10^8) cfu/g in any food item is considered unfit for human consumption (Almas, 1981). Iyer (1998), however, noted that the occurrence of a smaller number of *Staphylococcus* in the fishery products is not a serious problem but that food poisoning may occur if the products are handled carelessly during processing. On the other hand, the low counts of coliforms and faecal coliform in all the treated samples showed that “Bunyi youri” was prepared in hygienic environment. The presence of coliforms and *Staphylococci*, even though in few numbers in some of the samples is still significant and shows that there is need for improved handling and processing procedures in the preparation of “Bunyi youri”. Although the absence of *Salmonella* sp. is good sign of the safety the products for consumers, yet the public health importance of the above findings cannot be ignored. It is therefore important to stress here the need to strictly adopt the good manufacturing practices right from the preparation of raw materials, use of utensils, handling practices and processing of “Bunyi youri” to the storage

Table 2. The Microbial Characteristics of the Fresh Fish and “Bunyi youri” prepared with different Levels of Glucose and Clove.

Treatment ²	Type of microorganism ¹				
	TAPC (cfu/g×10 ³)	Staphylococcus (cfu/g×10 ³)	Total coliforms (mpn/g)	Fecal coliform (mpn/g)	Mould count (cfu/g×10 ²)
Fresh Fish	2.110	4.60	23	11	ND
Control 1	1.020	4.10	15	11	1.25
Control 2	1.240	3.98	11	10	1.18
A	0.920	0.54	< 3	< 3	0.39
B	0.884	0.48	< 3	< 3	0.40
C	0.872	0.45	< 3	< 3	0.30
D	0.900	0.52	< 3	< 3	0.45

¹TAPC = Total aerobic plate count; ND = not detected. ²Control 1= commercial “Bunyi youri;” Control 2 = laboratory prepared “Bunyi youri;” A = 2% glucose + 0.1% clove; B = 2% glucose + 0.3% clove; C = 2% glucose + 0.5% clove; D = 2% glucose + 0.6% clove.

Table 3. Types and frequency of occurrence of microbial isolates from “Bunyi youri”.

Type of organism ¹	Frequency of occurrence (n) ²	Number of positive isolates (%)
<i>Micrococcus</i>	24	11 (45.83)
<i>Bacillus</i>	24	7 (29.17)
<i>Staphylococcus</i>	24	3 (12.50)
<i>Leuconostoc</i>	24	2 (8.33)
<i>Streptococcus</i>	24	1 (4.17)

¹Total number of Isolates from nutrient agar = 120; ²n = pooled number of isolates from six sampling.

of the product in order to safeguard the health of the consumers. The use of glucose and clove in this study had greatly reduced the proportion of fecal coliform, thus this practice should be encouraged to ensure the safety of the consumers.

The microbial isolates from “Bunyi youri”

The microorganisms isolated from “Bunyi youri” during fermentation and after solar tent-drying are presented in Tables 3 and 4. Among the bacteria identified were Gram positive rod, namely *Bacillus* and Gram positive cocci, namely *Micrococcus* which were the predominant bacterial flora. Sarojnani and Suchitra (2009) also isolated similar microbial flora from ‘Ngari’ of Manipur. The presence of *Bacillus* suggested that spore forming bacilli might play an active role during the fermentation of “Bunyi youri”. The occurrence of *Micrococcus* species also indicates the possible involvement of non-spore forming microorganisms (Rose, 1982). These bacteria may contribute to the development of flavour and odour in fermented fish products due to their proteolytic

activities and they may also assist in the breakdown of the fish tissue and the development of flavour and aroma (Beaumont, 2002) which are essential for the desirable quality of the final product.

A few numbers of *Staphylococci* were also identified and further subjected to coagulase test which confirmed all the isolates to be coagulase negative, thus indicating that they were *Staphylococcus epidermidis*. *Micrococcus* and *Staphylococcus* are catalase positive and Gottschalk (2012) noted that catalase production inhibits peroxide formation and, in a similar development, Liepe (1983) showed this reaction to also inhibit rancidity. Similarly Steinkraus (1997) reported that *Staphylococcus epidermidis* as well as *Micrococcus* play some roles in meat fermentation by inhibiting rancidity. Thus *Micrococcus* and *Staphylococcus* might actually improve the quality of the final products as a result of the production of flavour compounds (Visessanguan et al., 2006). Among the fungi identified were *Aspergillus*, *Penicillium* and *Cladosporium* species. All the fish samples, irrespective of treatment, contained *Aspergillus* and *Penicillium* spp. In addition *Cladosporium* spp was recorded in the two control samples (1 and 2) but not in

Table 4. Effect of glucose and clove treatments on the mycological flora of “Bunyi youri”.

Treatment ²	Type of organism ¹		
	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Cladosporum</i> spp.
Control 1	+	+	+
Control 2	+	+	+
A	+	+	ND
B	+	+	ND
C	+	+	ND
D	+	+	ND

¹ + = positive; ND = Not detected; ² Control 1= commercial “Bounyi youri”; Control 2 = laboratory prepared “Bounyi youri”; A = 2% glucose + 0.1% clove; B = 2% glucose + 0.3% clove; C = 2% glucose + 0.5% clove; D = 2% glucose + 0.6% clove.

any of the treated samples (Table 4).

Aspergillus and *Penicillium* spp. constituted the dominant genera as shown in Table 4 and these two genera have been shown to be very common in various fermented fish products including fish sauces and fish pastes (Suchitra and Sarojnalini, 2012). The treatment appeared to have inhibited *Cladosporum* growth and activity in the processed samples. Thus, the use of a combination of glucose and clove has helped in the improvement of the microbial quality of the final product.

Conclusion

In conclusion, this study has shown that a shelf-stable and acceptable “Bunyi youri” could be produced from Nile perch treated with a combination of different levels of glucose and clove one hand and fermentation and drying under strict hygienic conditions on the other hand. The use of glucose in the processing of “Bunyi youri” reduced the fermentation time, and the use of solar tent drier operated at a higher temperature, when compared to the ambient temperature, resulted to an accelerated process as well as a safer product with regards to the microbial load. Such accelerated processing will lead to an increase in the turnover of the product and more financial benefits to the local fish processors. It will also help in increasing the protein supply which hitherto remains a serious problem in most developing countries, including Nigeria.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Quality control of raw milk in the smallholder collection and bulking enterprises in Nakuru and Nyandarua Counties, Kenya

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Kenya has one of the largest dairy industries in sub-Saharan Africa. Most of the milk marketed by small-scale farmers in Kenya has been reported to be of poor quality and does not meet national and international standards due to high bacterial load, high somatic cell count, adulteration and antibiotic residues. This study was designed to assess status of microbiological and physico-chemical quality of raw milk from two smallholder dairy farmer' groups at four sampling levels. Three hundred and eight raw milk samples were collected and analyzed along the value chain. Microbiological analysis for total bacterial count and coliform count was carried out using 3MTM Petrifilms plates. The average total bacterial and coliform counts Log₁₀ per ml at the processing factory was 8.462 and 6.770 for Ngorika and Olenguruone, respectively. The antibiotic residues especially β -lactam was prevalent with 44.5% of all the analyzed samples being positive. Likewise, 60% of the samples had a range of 150,000 to 500,000 somatic cells/ml. Average water adulteration level for the two collecting and bulking enterprises was 30.3%. TVBC and CC should be used instead of resazurin while freezing point determination should be used for adulteration.

Key words: Raw milk safety, Adulteration, Antibiotic residues Resazurin test.

INTRODUCTION

Kenya produces an estimated volume of 5 billion liters of milk annually and is therefore the leading milk producer in the East Africa region (Muia et al., 2011). Kenya's dairy industry, the single largest livestock production sub-sector contributes 14% of the agricultural gross domestic

product (GDP) and 3.5% of the total GDP (Muriuki et al., 2003).

Kenya's dairy industry is a dynamic and plays an important economic and nutrition role in the lives of many people ranging from farmers to milk hawkers, processors,

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and consumers. Kenya is generally self-sufficient in milk and dairy products. However, the demand for milk and dairy products in developing countries is estimated to increase by 25% by 2025 (Delgado et al., 1999), mainly due to human population growth, further urbanization, increased disposable income, greater diversity of food products to meet nutritional needs, and increased opportunities for domestic and external trade. Indeed, dairy imports in developing countries may reach 38.9 billion litres of milk equivalent by 2030 (Food and Agriculture Organization (FAO) and International Dairy Federation (IDF), 2004). Fortunately, Kenya has the potential to increase milk production from the current 4.2 billion litres in 2009 to over 5.0 billion litres in 2014 (Cherono, 2005).

Kenyan milk production systems can be divided into two general categories: large-scale and small-scale. The small-scale or smallholder dairy production system dominates. The differences between the two dairy systems are in their sizes of operation, level of management and use of inputs. Dairy cattle in smallholdings feed mainly from forage and very small quantities of concentrate, but some small-holder dairy farmers are highly commercial and well versed in dairy production, with high-quality management.

Dairy production is dominated by smallholders who own about 98% of the total dairy herd (Peeler and Omore 1997). Smallholder dairying households estimated to number over 1.5 million households, account for more than 85% of the annual total milk production and 80% of the 1.8 billion litres of milk marketed annually (MoL & FD, 2003; Staal et al., 2001). Over the years, significant changes in the traditional dairying have occurred resulting in a major shift towards market-oriented smallholder production. Farmers' groups handle only about 40 percent of marketed milk production and about 20 percent of total milk (Muriuki, 2003).

Over 800,000 smallholder farmers in Kenya depend on dairy farming for their livelihoods. Small-scale farmers account for 80% of the total milk production and 70% of the total marketed milk in the country. This has positive implications on food security and nutrition and has the potential to reduce poverty, particularly in the rural areas (Chesterman and Neely, 2015).

Milk is synthesized by cells within the mammary gland and is virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, bacterial contamination can generally occur from within the udder, outside the udder, and from the surface of equipment used for milk handling and storage. Cow health, environment, milking procedures and equipment sanitation can influence the level of microbial contamination of raw milk. Equally important is the holding temperature of milk and the length of time milk is stored before testing and processing that allow bacterial growth. All these factors will influence the total bacteria count and the types of bacteria present in raw milk bulk

tank (Murphy and Boor, 2000). Raw milk safety in Kenya has been disputed over a decade but no measurable data exists despite the fact that it requires monitoring from production to consumption. The regulatory institutions are constrained by lack of resources in terms of personnel and equipment (Muriuki et al., 2003) even though, the Kenya Bureau of Standards developed a Hygienic Code of Practice for milk production to assist farmers in producing hygienic milk.

Rejection at market is a result of poor handling and the time taken to reach markets (long distances and bad roads). Rejections are higher during the wet season, when production is high and roads are impassable. Losses at the farm level can be more than 6 percent of total production, which means that at current production levels, national annual losses may reach 60 million kg (Muriuki, 2011). Consequently, most of the milk marketed by small-scale farmers in Kenya has been reported to be of low quality and does not always meet national and international standards due to high bacterial load (Mwangi et al., 2000), antibiotic residues (Omore et al., 2005; Shitandi and Sternesjo, 2004) and water adulteration. The greatest limitations in the whole raw milk collection chain are proper ways to maintain cold collection due to the high investment costs demanded. This particularly affects the informal sector but also relatively in the formal sector (Orregård, 2013).

The formal milk trade is the market segment licensed by KDB. The informal markets controls an estimated 70 percent of the total milk marketed in Kenya (Kenya Dairy Board (KDB), 2009; Government of Kenya, 2006). This sector is important and is driven by among other factors the traditional preferences for fresh raw milk and its relatively lower cost. Raw milk markets offers both higher prices to producers and lower prices to consumers but with several challenges relating to quality control and standards, and the associated health and safety concerns. Other players in milk marketing include informal traders, distributors and retailers. The existence of informal trade results from a combination of the formal system's failure or inefficiency, consumer habits/preferences, and price differences between raw and processed milk (Muriuki, 2011).

Additional factors like unhygienic milking and handling practices, results in poor raw milk quality. According to Orregård, (2013), plastic jerry cans are impossible to clean and are often used for transporting milk by most motor bike transporters. This result in a less hygienic handling compared with the use of aluminum cans whose only limitation is the acquisition cost. Plastic jerry cans which could contribute to milk quality deterioration. This is in line with Gemechu, (2015), who found out that milk producers use plastic containers which are difficult to clean and disinfect and thus it might contribute to poor quality of the milk. The collection and bulking enterprises (CBE's) critical quality control challenges in line with milk bulking are; adulteration (both water and preservatives),

Table 1. The number of samples collected and analyzed from the four sampling levels in each CBE per replicate.

CBE	NGORIKA	Olenguruone
Can	44	24
Collection routes	6	6
Cooling tank	1	1
Tanker	1	1

high bacterial load due to warm collection, potential for contamination with coliforms due to handling, presence of anti-microbial residues and zoonotic diseases like Tuberculosis and Brucellosis (Muriuki, 2011). Owing to the large amount of milk that is marketed unprocessed, and to weak monitoring of the market, public health risks are a concern. The main public health concern is the potential risk of diseases such as brucellosis and tuberculosis (TB). Drug residues are also of concern, even in the processed milk channel.

Nyandarua County produces the highest amount of milk due to its higher population of dairy cows as compared to the other regions in Central Kenya (Muia et al., 2011). However, reports for Central Kenya indicates that dairy production potential for Nyandarua County is the least exploited (Romney, 2004; Staal et al., 2001; Schreiber, 2000; Baltenweck et al., 1998). Nakuru County had many districts; it has an area of 166 square km and human population of 25,800 people (GOK, 2009). The area has now settled down as a productive area with a high potential for dairy farming. The division has a total of 8925 cattle producing 7.5 million litres annually (District livestock production annual report, 2012).

This study was designed to monitor microbiological and physico-chemical quality of raw milk from two smallholder dairy farmer' groups at four sampling levels according to the requirements of the Kenya Bureau of Standards (KEBS).

MATERIALS AND METHODS

Study site

The study was carried out in New Ngorika Milk Producers Limited in Nyandarua County and Olenguruone dairy farmer's cooperative society in Nakuru County. For both CBE's, milk from individual farmers was collected and bulked into milk-cans while warm and transported in the same condition to the CBE cooler. The mode of transport per CBE varied from truck, tractor with trailer, tricycles, donkeys, individual farmer's delivery and motor bikes. Milk collection was only done once in the morning with some few farmers offering their evening milk separately along the routes. Laboratory tests were carried out at the Happy Cow Ltd laboratory and the food chemistry laboratory at the Egerton University department of dairy and food science. The Table 1 shows the number of samples carried out in every CBE during the analysis.

Milk sampling

Milk was initially stirred using a plunger to obtain a homogenous sample. The samples were obtained aseptically per the farmers group cans after acceptance at the reception platform, composite sample per route, CBE cooler and processor tanker. Additional samples were collected from the milk delivered late in the afternoon in Olenguruone, bulk samples from the rejected milk at the CBE platform, composite samples at the dump tank for the farmers that delivered individually to the cooling plant and extra samples for the freezing point determination. Antibiotic residues analysis involved only the bulk samples at the routes and cooler/ tanker levels due to the cost of the analytical method applied.

Microbial analysis

Total viable bacterial count and coliform count were done according to AOAC (2005) methods 991.14 and 990.12, respectively using 3MTM petrifilms plates. Serial dilution was done up to 10⁻⁵ and 10⁻⁶ for coliform count and total viable bacterial count respectively. Incubation was done at 37°C for 24 hr and at 32°C 48 hr for coliform count and total bacterial count, respectively. After the growth, the colony counting was done using the 3M plate reader (6499, 3M health care, Germany) and the total colony forming units (CFU) /ml recorded in an excel sheet.

Adulteration

The freezing point determination was carried out to assess adulteration according to Draaiyer et al. (2009). When it is adulterated with water or other materials are added the density and freezing point of milk change from its normal value causing a detectable elevation of the freezing point of milk from its normal values of -0.54°C. This was done using a cryoscope according to the manufacturers operating instructions. After calibrations, 2.5 ml of the sample were put in the sample vial and placed at the measuring point of the cryoscope. The start measure of the machine was selected and the results presented as a percentage on the display of the machine and as well as printed.

Antibiotic residues

The presence of the antibiotic residues was detected using the delvo test (SP NT and BLF) according to Delvotest technical bulletin (2011). Delvo test is easy to use and covers the broadest spectrum of antibiotic residues in the industry. Moreover, it is reliable and accurate with detection levels closest to maximum residue levels and safe tolerance levels (Hillerton et al., 1999). The milk sample (0.15 ml) was added to the ampule and incubated at 64°C in a delvo incubator for 3 h to observe colour changes (SP NT). The other delvo test (BLF) that involved use of ampules together with strips, was carried out. The incubator was set and the ampules with 0.15 ml milk sample inserted in it for 2 min. The milk sample was swirled again before inserting the strips in the ampules for 3 min and the results recorded.

Somatic cell count

The somatic cell count was done on all the samples using California Mastitis Test (CMT) according to Mellenberger and Roth (2000). An equal amount of commercial CMT reagent was added to each cup and a gentle circular motion applied to the mixture in a horizontal plane. A positive gelling reaction and colour change occurred in 10 s with the positive samples. The gel formation and colour changes

Table 2. Means for Log₁₀ TVBC and CC (CFU /ml), Lactic acid (LA, %) and Resazurin test (RT) for Ngorika samples.

Sampling levels test	Can	Route	Cooler	Tanker	Rejected
TVBC	8.396 ^b	8.818 ^a	8.708 ^a	8.828 ^a	8.889 ^a
CC	6.785 ^b	7.240 ^a	6.953 ^a	7.150 ^a	7.590 ^a
LA	0.151 ^c	0.156 ^b	0.150 ^c	0.150 ^c	0.178 ^a
RT	3.971 ^a	3.647 ^a	3.846 ^a	4.111 ^a	1.778 ^b

Means within a row marked with different letters are significantly different at ($p < 0.05$).

Table 3. Means for Log₁₀ TVBC and CC (CFU/ml), Lactic acid (LA, %) and Resazurin test (RT) for Olenguruone samples.

Sampling levels test	Can	Route	Cooler	Tanker	Rejected
TVBC	6.455 ^c	6.276 ^c	6.369 ^c	7.138 ^b	8.222 ^a
CC	4.137 ^c	4.683 ^{bc}	5.322 ^{ab}	5.390 ^{ab}	6.422 ^a
LA	0.148 ^b	0.152 ^b	0.151 ^b	0.153 ^b	0.167 ^a
RT	4.817 ^a	5.000 ^a	5.000 ^a	4.889 ^a	3.222 ^b

Means within a row marked with different letters are significantly different at ($p < 0.05$).

were observed and compared using the colour and viscosity comparison table.

Ten minute Resazurin test

The Resazurin test was done as per Draaiyer et al. (2009) where a resazurin tablet was completely dissolved in 50 ml of sterile distilled water according to the manufacturer's instructions. One milliliter of the resulting solution was added into 10 ml of the milk sample in a test tube, mixed and then incubated at 37°C in a water bath for 10min in a water bath. The samples were then read using a Lovibond comparator (14 20 00, Tintometer Ltd, England) from a good source of light for colour change and numerical score value ranging from 1 to 6, assigned. A milk sample without the resazurin dye was similarly treated and used as the blank in the comparator. Samples with comparator readings ranging from 4 to 6 were acceptable based on the Kenya Bureau of Standards (KEBS) on milk quality.

Titrateable acidity

The titrateable acidity test was done as per Draaiyer et al. (2009). This measured by titration whereby 3 to 4 drops of 0.5% phenolphthalein were added in 9 ml of milk sample in a beaker on a white tile and titrated against 0.1 equivalents/litre NaOH with constant shaking of the milk until a permanent colour change (pink) was observed. By recording the volume of base used and the volume of the milk sample, the amount of developed lactic acid was calculated and expressed as a percentage lactic acid.

Statistical analysis

The laboratory experimentation employed a randomized complete block design with a 2x5 factorial arrangement having three replications. The blocks of the experiment were the two CBE while the treatments were the four different sampling levels. Analysis of variance (ANOVA) was used to analyze the results obtained. This

was done using PROC general linear model (GLM) procedure of the statistical analysis system (SAS) version 9.0 (SAS, 1999). Means were separated using least significant difference (LSD).

RESULTS AND DISCUSSION

Total viable bacterial count (TVBC) and coliform count (CC)

According to KS 05-1552, bacteriological grades for raw milk grade III for total bacterial count and coliform count are 2,000,000 and 1,000 (CFU/ml), respectively. This study realized results that were generally higher compared to the standards. Mean log₁₀ for total bacterial count and coliform count per ml (CFU/ml) in Ngorika (Table 2) was not significantly different ($p \leq 0.05$) among the milk samples collected from the route composite, cooler and tanker but it was significantly different ($p \leq 0.05$) for the composite cans. This could have been contributed by the warm collection and the much time spent during transportation favouring the bacterial multiplication. Warm collection in the dairy value supply chain creates an optimum environment for microbial growth consequently causing milk quality deterioration (Mwangi et al., 2000; Orregard, 2013). The rejected milk analysis results represent the plates that had countable colonies and it had no significant difference ($p \leq 0.05$) from the route, cooler and tanker samples. Moreover, plates with Too Many To Count (TMT) microbial colony growth were 44.4 and 33.3% for TVBC and CC, respectively for both locations. These uncountable results were not included in the statistical analysis of the data.

In Olenguruone (Table 3), the means log₁₀ for TVBC were significantly different ($p \leq 0.05$) at the tanker level

Table 4. Means for Log₁₀TVBC and CC CFU/ml for all sampling points in the two locations.

Sampling point	TVBC	CC
Rejected	8.889 ^a	7.590 ^a
Tanker	8.462 ^c	6.770 ^{ab}
Cooler	8.305 ^{bc}	6.694 ^{ab}
Route	8.187 ^{bc}	6.474 ^b
Can	8.078 ^b	6.226 ^b

Means within a column marked with different letters are significantly different at ($p < 0.05$).

compared to the can route and cooler levels. Additionally, significant difference ($p \leq 0.05$) was observed between the tanker and the rejected milk. The means log₁₀ CC count CFU/ml for the can and route sample were not significantly different ($p \leq 0.05$) similarly to the means for the route, cooler and tanker.

Nonetheless, significant difference ($p \leq 0.05$) for the rejected milk was observed for both TVBC compared to all the sampling levels (Table 3). These samples were collected from the longest routes with poor infrastructure and took longer time for milk collection in Olenguruone. The microbial quality of the rejected milk samples in Olenguruone was similar to the samples at the tanker level in Ngorika.

In Table 4, the overall mean log₁₀ for total bacterial count in both locations was 8.078, 8.187, 8.305 and 8.462 TVBC log₁₀ CFU/ml for milk samples collected directly from the can composite, route composite, cooler (bulk) and upon arrival at the processing plant (tanker), respectively. An increasing trend of TVBC was observed at the different points considered from the can to the tanker and consequently the rejected milk samples. However, the means log₁₀TVBC for the two locations indicated that the can level, route level and cooler level were not significantly different ($p \leq 0.05$) unlike the tanker level, but significant difference ($p \leq 0.05$) for TVBC between all the levels and the rejected milk samples was observed. Coliform count indicated no significant difference ($p \leq 0.05$) between can, route composite, cooler and tanker levels. Furthermore, no significant difference ($p \leq 0.05$) was observed between the rejected milk, cooler and the tanker levels. Accordingly, the TVBC increased by 4.75% (0.384 log₁₀ CFU/ml) from the can to the tanker milk sampling points. Increase in TVBC observed along the value chain may be due to several factors like contamination at the farm, storage and transport using improperly cleaned milk cans, and lack of controlled temperature during transportation (Doyle et al., 2015).

The difference between the two CBES might be due to the fact that the Olenguruone was sampled from the smallest radius to the cooling plant and the milk was transported using motor bikes that are very fast in terms

of delivery time. An increase in microbial growth was observed between the cooler and the tanker which could have been majorly contributed by the cooler efficiency that is taking over three hours to cool the milk from 18 to 4°C (which was the required temperature for the processor), the use of the plastic containers in the collection of milk, the milking practices and the handling hygiene through the chain. Additionally, the sample was taken immediately after the cooler filled up. In Ngorika, the sampling was done in all the routes, and collection The raw milk microbial quality was very poor at all levels for this CBE. For both CBE, this may be due the contribution of insufficient pre-milking udder preparation, insufficient cleaning of milk handling equipment, use of poor quality water for cleaning, the storage time and lack of cold chain facility starting from the production site (Doyle et al., 2015). As reported by Van Kessel et al. (2004), the use of insufficient and poor quality water for cleaning of milk handling equipment can result in milk residues on equipment surfaces that provide nutrients for the growth and multiplication of bacteria that can then contaminate the milk. Murphy and Boor (2000) noted that ineffective cleaning, use of water without heat treatment and the absence of sanitizers tend to fasten growth of less heat resistant organisms. Similarly, mastitis infected cows can also contribute to high TVBC.

Generally, the presence of coliforms in milk confirms that the milk has been contaminated with fecal materials and it is an indicator of the sanitary conditions in the production and handling of the milk starting from production (Orregard, 2013) Accordingly, poor herd/farm hygiene, use of contaminated water, unsanitary milking practices, and use of improperly washed equipment for storage and distribution can all lead to elevated coliform count (CC) in raw milk (Gemetchu et al., 2015). The fact that high proportion (90%) of the milk samples taken from all levels had coliform counts more than the upper limit of KEBS standards accepted for CC in raw milk, provides irrefutable evidence that the udder of the cows have been soiled with fecal materials and/or the udder is improperly washed; that is, milk contamination in the study area happened starting from milking of the cows. In addition, the presence of coliform in an aseptically collected sample of raw milk shows the use of bacteriological low quality water, either for washing utensils or mixing in raw milk (Farhan and Salik, 2007). Apart from safety and public health concerns, high contaminations by coliforms results in off-flavours in milk and reduced shelf life of dairy products (Reta and Addis, 2015; Kaindi et al., 2011). Generally, the bacterial generation (doubling) time is between 10 to 15 min depending on the conditions. According to Orregard (2013), aluminum cans allows better hygienic handling unlike plastic jerry cans. In this study, milk was transported while warm and in plastic jerry cans which could contribute to milk quality deterioration, unlike the recommended containers that do not have adhesive properties and are easy to clean when

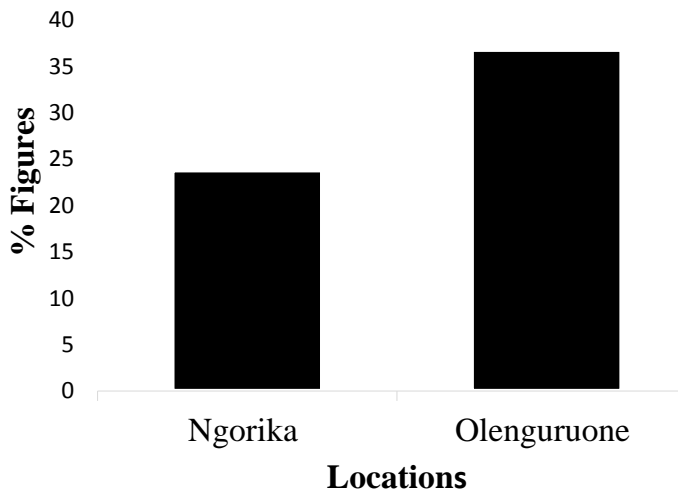


Figure 1. The average percent water adulteration in both CBE's.

compared with plastic containers. Moreover, more than 3 h, where natural lactoperoxidase enzyme could sustain the milk quality, were surpassed in some routes before refrigeration could take place. Cooling of milk is advocated to help in significantly reducing the multiplication of bacteria and in turn reduce spoilage (Kurwijila, 2006).

Adulteration

Freezing point of milk is its most constant property. According to the Kenya Standard (KS 05-1552), approximately 0.545°C; but not less than 0.525°C is the freezing point of milk. Results indicated that adulteration incidences in Ngorika and Olenguruone were 23.8% and 36.8%, respectively (Figure 1). Adulteration in Ngorika was lower than in Olenguruone. This could have been contributed by the effect of the penalty attached to a farmer when caught in this act. Added water can occur in milk due to both unintentional (e.g., poor system drainage) and intentional addition (Kurwijila, 2006). Agents in some areas add water to increase the volume to make a larger profit, especially during the dry period when milk supplies are low and prices are high (Orregård, 2013). This can be detected using a cryoscope by measuring its freezing point. Normally, the freezing point of milk is slightly less than that of pure water and is relatively constant. Typical milk generally has a freezing point below minus 0.542 degrees Hortvett but when water is added to milk, the freezing point increases approximately 0.005°H for every 1% water addition. The lactometer test could not detect water adulteration especially when done at minimal levels. The freezing point determination as a standard gauge for water adulteration (Draaiyer et al., 2009) was applied to

compliment the lactometer test. This study corresponds to a study carried out by Orregård, (2013), where the results showed that about 33% of the samples from farmers were not within the acceptable density limit. According to (KS 05-1552), density of milk of 20°C shall be within the following range: 1.026-1.032 g/ml.

Antibiotics

Antibiotics in milk are a major concern due to the risk of allergic reactions and the development of antibiotic resistant pathogen and inhibition of dairy starter cultures used to develop acid (e.g., lactic acid bacteria), which can result in the loss of significant amounts of product and milk (Popelka et al., 2004). The delvo test was carried out for the composite samples at the route level and the cooler. In total, 74 samples were analyzed in both CBE and out of these, 54 and 35% from Olenguruone and Ngorika, respectively were positive (Figure 2). This study corresponds to another study carried out by Aboge et al. (2000) on antimicrobial residues detected on marketed milk in Kenya. According to Shitandi, (2004), eighteen percent (18%) of the samples from small-scale producers in his study area were β -lactam positive significant ($p < 0.001$) while other studies carried out within Kenya showed that many animal products in Kenyan market have high level of drug residues which is unacceptable (Muriuki, 2001). According to Gallagher (2015), consumption of food with antibiotics residues can lead to bacteria becoming completely resistant to treatment in human beings, a situation referred to as antibiotic apocalypse.

Somatic cell count

Somatic cell count should not exceed 300 000 per ml when tested in accordance with ISO 13366. The California mastitis test (CMT) has been used for more than 50-years and continues to be the most accurate farm screening test for subclinical mastitis (Ruegg and Reinemann, 2002). The heavier the gel the higher the somatic cells in the milk and vice versa, indicative of the leukocyte count (Quinn et al., 1994). Most of the samples were in the range of 150,000 to 500,000 cells/ml which is an indicator of presence of clinical mastitis in the farms. In Ngorika, no sample was within the 0 to 200,000 cells/ml which reflects absence of somatic cells in milk suggesting that it has higher incidences of mastitis compared to Olenguruone which had 5% samples being mastitis negative.

Additionally, 65% of the samples collected Ngorika were lying under the range 150,000 to 500,000 cells/ml which is higher than Olenguruone (55%) (Figure 3). The farmers in Olenguruone have a low average production per cow compared to Ngorika which could be a contributing factor to the high incidences of mastitis observed. *S. agalactiae* is known to be an occasional

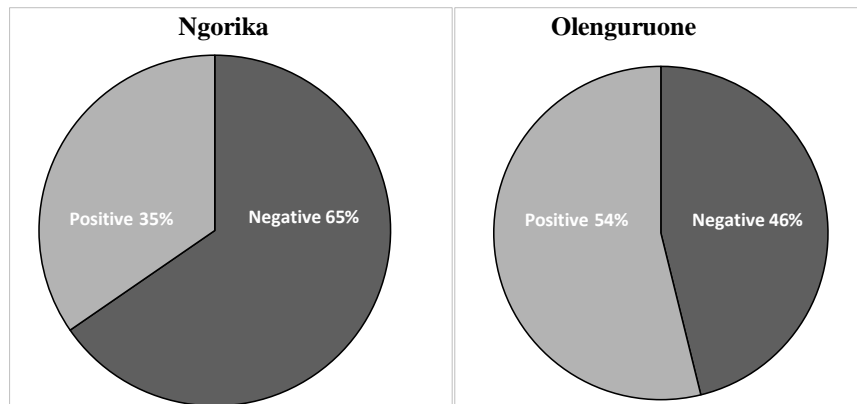


Figure 2. Detection percent of the antibiotic residues in both CBE's.

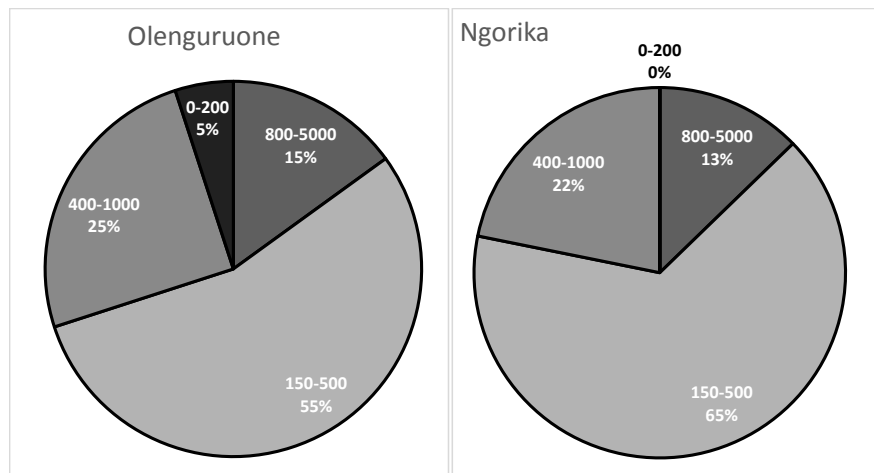


Figure 3. California mastitis test somatic cell count per 1000 mls per CBE.

cause of high bacterial counts and subclinical mastitis problems, hence it should be considered when both the SCC and TVBC are high (Ruegg and Reinemann, 2002). For that reason, the higher somatic cell counts detected could have contributed to the high levels of the total bacterial counts observed in both locations.

Increased somatic cell numbers are positively correlated with concentrations of plasmin, a heat-stable protease, and of lipoprotein lipase in freshly produced milk (Barbano et al., 2005). Activities of these enzymes can supplement those of bacterial hydrolases, hence shortening the time to spoilage. The major determinants of quantities of these enzymes in the milk supply are the initial cell numbers of psychrotrophic bacteria, their generation times, their abilities to produce specific enzymes, and the time and temperature at which the milk is stored before processing. Several conditions must exist for lipolyzed flavor to develop from residual lipases in processed dairy foods, that is, large numbers ($>10^6$ CFU/ml) of lipase producers (Stead, 1986), stability of the

enzyme to the thermal process, long-term storage and favorable conditions of temperature, pH, and water activity.

Resazurin test

It uses the indicator resazurin to measure the bacteriological quality of milk. The majority of the organisms in milk are capable of reducing and decolorizing the resazurin dye. When bacteria grow in the milk they utilize oxygen, the rate of removal or reduction is proportional to the keeping quality (Draaiyer et al., 2009). The Resazurin dye is more sensitive than the methylene blue and for this reason, this test provides a rapid measure of the keeping quality of milk. There was no significant difference ($p \leq 0.05$) for 10 min Resazurin test at the four sampling levels except for the rejected milk for both CBE's (Table 2 and 3). The correlation of the 10 min resazurin test and TVBC was not

Table 5. Correlation of general quality results evaluated at four sampling levels in smallholder dairy farmers supply systems in Kenya.

	TIME	Route	TVBC	CC	LA	RT
TIME	1.00000	-0.74248***	0.42845**	0.53512***	0.22400	-0.47639**
ROUTE		1.00000	-0.46522***	-0.47591***	-0.17855	0.64902***
TVBC			1.00000	0.67682***	-0.06786	-0.23962
CFU				1.00000	0.02256	-0.21445
LA					1.00000	-0.34558*
RT						1.00000

*, **, ***; Indicates significance at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

significantly different ($p \leq 0.05$) at the route level (Table 5). According to KEBS, (2007) raw milk specification, only 20.6% of samples were unacceptable based on this test contrary to the TVBC where all the samples were way above grade III raw milk requirements (2,000,000 CFU/ml) which is unacceptable. The study results agrees with the study carried out by Muliro et al. (2013) on quality assessment of raw camel milk using dye reduction tests that the resazurin test is not reliable as a measure of total viable bacterial count. According to Murphy and Boor (2000), a significant correlation ($p \leq 0.01$) between the total bacterial count and time used to deliver milk for cooling was observed. The Plate count test has been reported to be generally accepted as the most accurate and informative method of testing the bacteriological quality of milk (Kurwijila, 2006; Muliro et al., 2013).

Titratable acidity test

The means separation indicated no significance difference ($p \leq 0.05$) at the can, cooler and tanker level unlike the route level and the rejected milk in Ngorika (Table 2). This could have been contributed to the dilution factor as milk is being bulked together in the cooler. A different scenario was observed in Olenguruone where no significance difference ($p \leq 0.05$) at the can, route, cooler and tanker level but significant difference ($p \leq 0.05$) was observed with the rejected milk (Table 3). All the samples at the can, route, cooler and tanker levels were found to have acidity levels within the range of 0.16 ± 0.02 and therefore judged to be of good quality for the titratable acidity test. This was contrary to all the milk samples rejected at the reception platform that were above 0.18% lactic acid, hence rejected as per the KEBS standards.

CONCLUSION AND RECOMENDATIONS

The study showed that total bacterial count and coliform count were way above the KEBS standards although along the dairy value chain. Factors like famers training

on the clean milk production, milk handling hygiene, use of appropriate containers and reduced delays in milk collection could assist in reducing the microbial load in raw milk. Milk quality tracking and tracing was lacking hence the concept of quality based milk payment system (penalties and premium) would be difficult to introduce. Can labeling and subsequent ownership which was lacking should be enhanced for tracking and tracing. The cooling plants were taking more than 3.5 h to cool the milk to below 5°C which justifies use of plate heat exchangers as an alternative for speedy cooling. The study also revealed higher figures for antibiotic residues which suggests that withdrawal period was not observed. To overcome this, inclusion of the test in quality based payment system and vigorous training to farmers will ensure that the antibiotic residues are within the maximum allowable limit. Adulteration of milk with water was evident which could be due to the fact that the farmers are paid according to supplied quantities. Milk policies should be established and punitive penalties introduced to farmers found to have adulterated the milk unlike the current situation where no action is taken. For raw milk quality based payment system, Resazurin test and lactometer test are not sufficient. TVBC and CC should be used instead of resazurin while freezing point determination should be used for adulteration.

Conflict of Interests

The authors have not declared any conflict of interests.

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The background of the entire page is a photograph of a glass jar filled with a red liquid, possibly a fruit juice or jam, with its lid off and placed to the left. Below the jar is a wooden bowl filled with fresh red berries, likely raspberries or strawberries. The entire scene is set on a dark, textured wooden surface. The text is overlaid on this background.

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