Scientific African 10 (2020) e00583

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Microbiological safety and physicochemical composition of *Bongo*, a traditional fermented milk product from Lyantonde district, Uganda

Ivan Muzira Mukisa*, George William Ssendagala, Stellah Byakika

Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bioengineering, College of Agricultural and Environmental Sciences, Makerere University, P.O. Box 7062, Kampala, Uganda

ARTICLE INFO

Article history:
Received 26 May 2020
Revised 11 September 2020
Accepted 30 September 2020

Keywords:
Bongo
Milk
Fermented foods
Safety
Public health

ABSTRACT

Traditional fermented dairy products, including *Bongo,* are an important emerging market owing to their nutritional benefits and probiotic potential. Production of *Bongo* is unstandardized, unregulated and may raise public safety concerns. Microbial counts and physicochemical parameters ($n = 30$ *Bongo* samples), production protocol, and hygiene practices of processors ($n = 15$) were evaluated. Over 93.3% of the products were contaminated with potentially pathogenic microbes including Enterobacteriaceae, coliforms, Sulphur reducing clostridia, *Enterococcus* spp. and *Staphylococcus* spp. About 40% of the samples had pH $\geq$ 4.0. Most processors had inadequate hygiene practices which compromised product safety. These results indicate the need for developing product specifications, training processors in good food safety practices and enforcing relevant regulations.

Introduction

Consumers are increasingly recognizing the contribution of diet on their health. They are more conscious of disease prevention than cure and consequently many are embracing fermented foods. Fermented foods contain bioactive compounds and probiotics which confer health benefits such as antioxidant and anti-hypertensive properties, alleviation of lactose intolerance, enriched vitamin content and improved protein digestibility among others [7,37,38,46,52]. They are also popular for their unique taste and shelf stability.

There are numerous types of fermented foods around the world with milk-based products representing a significant portion. Examples include; *Kule naoto* (Kenya), *Nunu* (Ghana), *Kivuguto* (Rwanda), *Ergo* (Ethiopia), Kefir and Koumiss (Eastern Europe and Central Asia), *Amasi* (Zimbabwe and South Africa), *Omashikwa* (Namibia), *Mabisi* (Zambia), *Dadiah* (Indonesia), *Kumis* (Central Asia) and *Bongo* (Uganda) [3,40,41,57].

Bongo is a popular beverage in western and central Uganda. It is produced traditionally by fermenting unpasteurized cows' milk [41]. Although it is originally from the rural cattle keeping regions of Uganda, *Bongo* is gaining popularity across the country. Consequently, there is an unregulated increase in its commercial production which may present a serious public

* Corresponding author.
E-mail address: ivanmukisa@caes.mak.ac.ug (I.M. Mukisa).

https://doi.org/10.1016/j.sciaf.2020.e00583

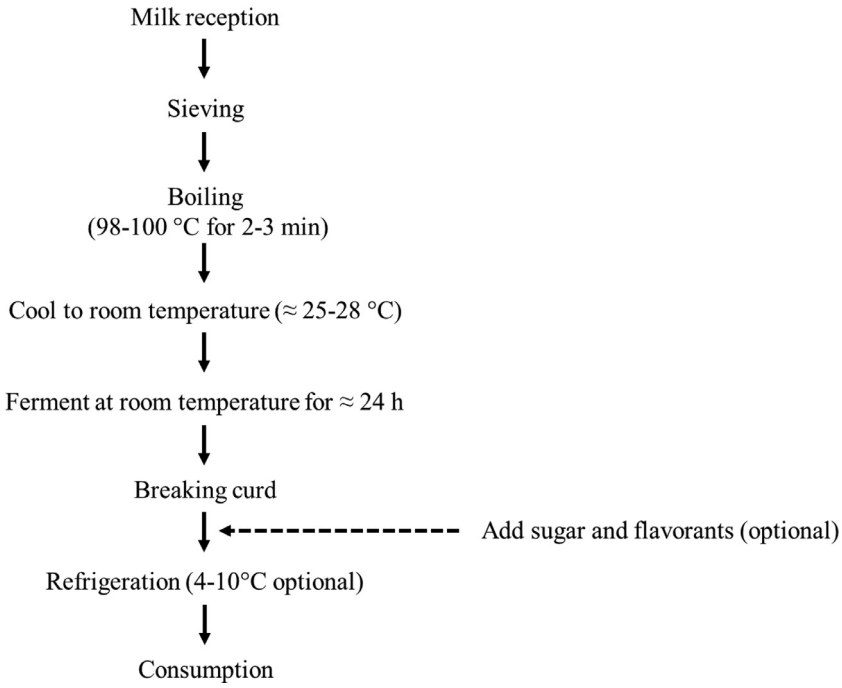

Fig. 1. Protocol for traditional processing of *Bongo*.

health challenge. More so, since it is spontaneously fermented implying its safety is not a guarantee. Indeed, the occurrence of contaminants like *Escherichia* (*E). coli, Pseudomonas, Listeria, Salmonella, Shigella*, and *Staphylococcus* in spontaneously fermented foods is reported [8,33,41,45]. To-date there is no safety standard for traditional fermented milk products in Uganda and there is also very limited literature on the safety of *Bongo*. Therefore, this study evaluated the quality aspects of *Bongo* produced in Lyantonde, a Ugandan town where the beverage is commercially produced. The results of physicochemical characteristics and microbial quality of this product are essential for developing product specifications.

Materials and methods

Study design and sample size

The study was carried out in Lyantonde Town Council, a sub-county of Lyantonde district in southern central Uganda. This sub-county is known for pastoralism and commercial *Bongo* production. A descriptive cross-sectional study was carried out between March and April 2019. The estimated sample size was obtained by use of an automated online calculator [48]. The predetermined margin of error and confidence level were 5% and 95%, respectively. Findings from a preliminary survey in Lyantonde town indicated that there were about 20 commercial *Bongo* processors so the minimum calculated sample size was 20 processors. Considering a 10% non-responsive rate the required sample size was further increased to 22 but only 15 respondents agreed to participate in the study.

Interviewing Bongo *processors*

Data on the production protocol, demographic characteristics and hygiene practices of *Bongo* processors was obtained through face-to-face interviews using a structured questionnaire. The questionnaire was peer-reviewed and pre-tested before use.

Traditional processing of Bongo

A summary of the *Bongo* processing protocol that was followed by the processors is shown in Fig. 1. The processors generally produced 5 to 20 L of *Bongo* per day. Raw milk for *Bongo* processing was initially tested upon reception for foul odors and adulteration (dilution with water). To check for dilution, about 5 mL of the milk was poured on the ground. The extent of dilution is proportional to the ease with which the milk soaks into the ground. After testing for odor and dilution, the milk was sieved to remove physical impurities prior to boiling in a sauce pan for 2 - 3 min. The milk was subsequently allowed to cool to room temperature (about ≈25–28 °C) and then transferred to a fermenting vessel (sauce

pan or gourd). The milk was covered with a cloth or plastic lid and left to ferment for about 24 h. Most processors placed their fermentation vessels on raised platforms (e.g. a wooden table) away from the floor which tends to get cold in the night thus slowing down the fermentation process. After fermentation whey was poured off and the curd stirred into a uniform consistency using a wooden ladle. At this stage some of the processors added sugar and artificial flavorings prior to pouring the finished product into jerrycans. Processors generally stored *Bongo* at room temperature but most of them preferred to refrigerate *Bongo* during storage to enhance its shelf life.

Bongo *samples*

A total of thirty samples were collected from the processors on two separate days (1 sample per processor x 15 processors x 2 days = 30). Samples (about 900 mL per processor per day) were collected in 1 L glass bottles (Pyrex, Karter Scientific, LA, USA) previously sterilized at 121 °C for 15 min. The samples were held in cooler boxes and transported to the laboratory within 24 h. In the laboratory, samples were refrigerated at 4 °C and analyzed within the next 24 h after reception.

Analyses

Microbiological analyses

To prepare the first serial dilution, 10 mL of *Bongo* were added to 90 mL of sterile peptone water and homogenized for 1 min at 230 rpm in a stomacher (Seward, Wagtech International, Thatcham, England). Further ten-fold dilutions, up to 10^{-8}, were prepared by using 1 mL of previous dilution in 9 mL of peptone water. All microbial analyses were performed following International Standards Organization (ISO) methods.

Total Plate Count (TPC) was performed by pour plating 1 mL of each selected dilution in Plate Count Agar (PCA) followed by incubating at 37 °C for 24 h according to ISO 4833–1 [29]. All distinct visible colonies were counted. Psychrophiles were enumerated by pour plating 1 mL of each selected dilution in PCA and incubating at 4 °C for 7 days according to ISO/TS 11,059 [26]. All distinct visible colonies were counted. Lactic acid bacteria (LAB) were enumerated by pour plating 1 mL of each selected dilution in MRS agar followed by incubation at 30 °C for 48 h according to ISO 15,214 [21]. Straw colored colonies were counted. Enterobacteriaceae were enumerated by pour plating 1 mL of each selected dilution in Violet Red Bile Glucose agar followed by overlaying with the same agar and incubation at 37 °C for 24 h according to ISO 21,528–2 [30]. Purple colonies were counted. Sulphur reducing clostridia (SRC) were enumerated by pour plating 1 mL of each selected dilution of a heat treated sample (80 °C for 10 min) in Iron Sulphite agar followed by anaerobic incubation at 37 °C for 24 h as described in ISO 15,213 [23]. Black colonies were counted. Total coliforms and *E. coli* were enumerated by pour plating 1 mL of each selected dilution in *E. coli*-coliforms chromogenic agar and incubating at 37 °C for 24 h as described in ISO 4832 [25] and ISO 16,649–1 [32], respectively. Pink and blue/purple colonies were counted as total coliforms and *E. coli*, respectively. Enterococci were enumerated by pour plating 1 mL of each selected dilution in Slanetz and Bartley agar and incubating at 44.5 °C for 48 h as described in ISO 7899–2 [22]. Deep red colonies were counted. Yeasts were enumerated by surface plating 0.1 mL of each selected dilution on Potato Dextrose Agar acidified with 1% lactic acid followed by incubation at 25 °C for 5 days according to ISO 21,527–2 [27]. Only typical yeast colonies (raised smooth white or creamy waxy colonies) were observed and therefore counted. Staphylococci were enumerated by surface plating 0.1 mL of each selected dilution on Baird Parker Agar containing tellurite egg yolk emulsion and incubating at 30 °C for 48 h as described in ISO 6888–3 [24]. Black/gray colonies with a halo were counted. *Salmonella* was detected following ISO 6579–1 [31]. Briefly, 25 mL of *Bongo* were added to 225 mL of Buffered Peptone Water followed by incubation at 37 °C for 24 h. Thereafter, 0.1 mL of the mixture was added to 10 mL of Rappaport Vassiliadis Soy broth and incubated at 41.5 °C for 24 h. This was followed by streaking on Xylose Lysine Deoxycholate (XLD) agar and Brilliant Green Agar (BGA) and incubation at 37 °C for 24 h. Red colonies with black centers on XLD agar and red/pink colonies on BGA were considered presumptive *Salmonella*. To confirm their identity, the presumptive *Salmonella* colonies were streaked on Triple Sugar Iron (TSI) slants and stubbed in the butts of Simmons Citrate agars followed by incubation at 37 °C for 24 h. Formation of a pink slant and yellow butt for TSI agar and change from green to blue for Simmons Citrate agar were considered positive reactions. All media were supplied by Laboratorios CONDA (Madrid, Spain) and prepared according to manufacturer's instructions.

Physicochemical analyses

Crude fat (Method 920.85; [4]), crude protein (Method 920.87; [4]) and ash (Method 924.05; [4]) content were determined using the Soxhlet, Kjeldahl and dry ashing methods, respectively. A conversion factor of $N = 6.02$ was used for calculation of protein content [54]. Total solids/dry matter were determined following ISO 6731 [28]. Total carbohydrate content was determined by subtracting the sum of% crude proteins,% crude fat and% ash from% total solids [42]. The milk Solids Not Fat (SNF) was determined by subtracting fat from the total solids. The pH of *Bongo* was determined using a digital pH meter (pH 98,107, USA). Titratable acidity (TA) was determined by titrating 10 mL of *Bongo* against a standardized solution of 0.1 M NaOH with phenolphthalein as an indicator. Total soluble solids were determined using a refractometer (RSG-100/ATC, China).

Data analysis

Means of microbiological counts and physicochemical parameters of products from the different processors were analyzed using one-way analysis of variance to test for significance differences at an α level of 5%. The Tukey's HSD test was used to separate the means. Descriptive statistics (means, standard deviations and frequencies) were also used to compile data on the hygiene practices of the processors, microbiological and physicochemical properties of the *Bongo* samples. The Fisher's Exact Test [35,53] was used to determine associations (α=0.05) between carrying out recommended food safety practices and products meeting the microbiological specifications for safety. All data were analyzed using XLSTAT software version 2017 (Addinsoft, New York, USA).

Results

Microbiological safety and physicochemical composition of Bongo

Table 1 shows the microbiological quality of *Bongo* from different processors. Apart from yeast, sulphite reducing clostridia, enterococci and LAB counts, the counts of the rest of the microbes evaluated varied significantly ($p < 0.05$) among processors. The TPC was 6.3–9.0 log cfu/mL with the dominant microbial community comprising of LAB (4.9–7.8 log cfu/mL) and yeasts (2.7–6.1 log cfu/mL). Although none of the samples were positive for *Salmonella* over 93.3% were contaminated with Enterobacteriaceae, coliforms, SRC, *Enterococcus* spp. and *Staphylococcus* spp. Samples also contained psychrophiles (undetected up to 3.9 log cfu/mL).

Table 2 shows the physicochemical parameters of *Bongo.* Only titratable acidity, total soluble solids, dry matter and milk solids non-fat varied significantly ($p < 0.05$) among processors. About 40% of the *Bongo* did not conform to the pH specification (pH $\leq$ 4.0) but all had TA above 0.3%, the minimum requirement. Close to a third (33.3%) of *Bongo* samples did not conform to the milk solids non-fat specification. All samples had crude protein and crude fat levels within the specifications.

Demographic characteristics of processors and traditional processing of Bongo

Four of the processors were males and eleven were females. All *Bongo* processors were *Banyankole,* a pastoralist tribe. They had 4 to 30 years' experience in the *Bongo* production and reported to have learnt the practice from their forefathers.

Hygienic practices of Bongo *processors*

Table 3 summarizes the hygienic practices of *Bongo* processors. It shows the percentage of processors with correct and wrong practices. All processors did not have: a HACCP plan, suitable hand washing and sanitizing facilities and garbage bins with covers. All did not wash their hands in between handling money and *Bongo*. About 20% of them did not wash their utensils before and after processing. Although all processors refrigerate their *Bongo* immediately after production none refrigerated it during transportation to the selling points.

Association of hygienic practices with microbial quality

Table 4 shows results of the Fisher's exact test for association between hygienic practices by processors and their products meeting specifications for microbial quality. None of the practices considered in this study was associated (p>0.05) with *Bongo* products meeting microbial specifications.

Discussion

Microbiological safety and physicochemical composition of Bongo

Total plate counts (TPC) are generally used to evaluate the microbial quality of foods and to predict product stability/shelf life [47]. This may not be applicable in unpasteurized fermented products because their TPC could be largely comprised of the fermenting flora or starter cultures. Values of TPC of these products also vary widely. For instance, our results of TPC (Table 1) were comparable to values (3.8 – 6.9 log cfu/mL) reported for *Bongo* [17], *Nunu* [2], yoghurt and *Lebnah* [49]. However, our results were lower than values (9.0 – 10.9 log cfu/mL) reported for *Bongo* [41], fermented milk from camels, cows and goats [9], *Nono* and *Wara* [58]. Interpreting results of TPC for unpasteurized fermented products may require comparing these with the constituent microbial community groups and/or levels of the fermenting flora/starter cultures. From this study, the dominating microbial communities in *Bongo* were the LAB and yeasts implying that they are most likely the principle fermenters of the product. Similar observations have been made for some traditional fermented dairy products like *Kule naoto* [40] and *Nunu* [3].

The values of LAB observed in this study are similar to those (6.4 $\pm$ 0.6 log cfu/mL) previously reported for *Bongo* [17]. Values in the range 6.4 – 8.9 log cfu/mL have been reported for other traditional fermented dairy products [9,18,39]. Fermented milks should contain a minimum of 6 – 7 log cfu/mL LAB population constituting the starter culture [11]. This was only achieved in less than 50% of the *Bongo* samples (Table 2). LAB are important for producing lactic acid which contributes

Table 1
Microbial counts of *Bongo* from different processors and their conformance with specifications.

Processor	Microbial counts (log cfu/ml)										*Salmonella* spp (In 25 mL)
	TPC	TC	*E. coli*	Yeasts	SRC	Enterococci	Enterobac	LAB	Psychrophiles	Staph	
Processor 1	$8.3^{abc}\pm0.1$	$2.8^{abcd}\pm1.0$	$2.5^{abcd}\pm0.7$	$6.1^{a}\pm0.1$	$1.1^{a}\pm1.3$	$2.1^{ab}\pm1.5$	$2.3^{bcd}\pm0.2$	$6.0^{a}\pm0.8$	$1.4^{ab}\pm1.7$	$3.7^{abcd}\pm0.1$	Negative
Processor 2	$8.7^{ab}\pm0.1$	$3.6^{abc}\pm0.5$	$3.6^{abc}\pm0.5$	$5.5^{a}\pm0.2$	$0.9^{a}\pm0.4$	$2.0^{ab}\pm1.4$	$3.2^{abc}\pm1.3$	$5.8^{a}\pm0.9$	$3.9^{a}\pm0.2$	$3.3^{abcd}\pm0.3$	Negative
Processor 3	$8.6^{ab}\pm0.4$	$3.4^{abc}\pm1.0$	$3.3^{abc}\pm1.2$	$4.1^{a}\pm2.3$	$1.2^{a}\pm1.3$	$2.9^{ab}\pm0.6$	$3.6^{ab}\pm0.5$	$6.7^{a}\pm0.2$	$3.7^{a}\pm0.5$	$3.4^{abcd}\pm0.4$	Negative
Processor 4	$8.4^{abc}\pm0.4$	$5.0^{a}\pm0.1$	$5.0^{a}\pm0.2$	$4.3^{a}\pm2.4$	$1.8^{a}\pm1.6$	$4.3^{a}\pm0.4$	$5.0^{a}\pm0.3$	$7.8^{a}\pm0.8$	$0.0^{b}\pm0.0$	$4.8^{a}\pm0.3$	Negative
Processor 5	$8.2^{abc}\pm1.5$	$0.6^{cd}\pm0.7$	$0.4^{cd}\pm0.4$	$2.7^{a}\pm0.2$	$1.1^{a}\pm1.3$	$2.3^{ab}\pm0.1$	$1.1^{cd}\pm0.6$	$5.5^{a}\pm1.2$	$1.3^{ab}\pm1.5$	$2.6^{bcd}\pm0.4$	Negative
Processor 6	$9.0^{a}\pm0.5$	$0.0^{d}\pm0.0$	$0.0^{cd}\pm0.0$	$3.9^{a}\pm0.9$	$1.2^{a}\pm1.4$	$2.6^{ab}\pm0.3$	$1.0^{cd}\pm0.5$	$5.7^{a}\pm1.3$	$0.0^{b}\pm0.0$	$3.4^{abcd}\pm1.3$	Negative
Processor 7	$8.4^{abc}\pm0.5$	$1.8^{bcd}\pm2.0$	$1.7^{bcd}\pm2.0$	$5.4^{a}\pm1.4$	$1.7^{a}\pm0.7$	$2.7^{ab}\pm0.8$	$2.4^{bcd}\pm1.1$	$5.9^{a}\pm0.6$	$3.2^{ab}\pm0.1$	$2.9^{abcd}\pm0.3$	Negative
Processor 8	$6.6^{bc}\pm0.1$	$1.3^{bcd}\pm0.2$	$1.2^{bcd}\pm0.2$	$5.4^{a}\pm0.1$	$2.8^{a}\pm1.8$	$1.6^{b}\pm1.8$	$0.7^{d}\pm0.8$	$5.2^{a}\pm1.1$	$2.7^{ab}\pm3.1$	$4.1^{abc}\pm0.7$	Negative
Processor 9	$7.6^{abc}\pm1.2$	$1.7^{bcd}\pm1.9$	$1.6^{bcd}\pm1.9$	$4.9^{a}\pm0.3$	$1.2^{a}\pm1.4$	$4.0^{ab}\pm1.8$	$2.2^{bcd}\pm0.9$	$5.0^{a}\pm1.8$	$1.5^{ab}\pm1.8$	$3.4^{abcd}\pm0.3$	Negative
Processor 10	$8.1^{abc}\pm0.9$	$3.8^{ab}\pm0.2$	$3.7^{ab}\pm0.2$	$3.1^{a}\pm3.6$	$0.7^{a}\pm0.6$	$3.2^{ab}\pm1.1$	$3.5^{ab}\pm0.2$	$4.9^{a}\pm1.7$	$3.2^{ab}\pm0.1$	$4.4^{ab}\pm0.3$	Negative
Processor 11	$6.4^{c}\pm1.4$	$1.1^{bcd}\pm0.7$	$1.0^{c}\pm0.8$	$4.3^{a}\pm2.0$	$3.2^{a}\pm1.2$	$2.6^{ab}\pm0.2$	$1.9^{bcd}\pm1.7$	$5.4^{a}\pm1.3$	$0.0^{b}\pm0.0$	$2.8^{bcd}\pm0.2$	Negative
Processor 12	$6.8^{bc}\pm0.1$	$0.8^{bcd}\pm0.4$	$0.2^{cd}\pm0.2$	$4.2^{a}\pm1.1$	$1.7^{a}\pm0.4$	$2.7^{ab}\pm0.1$	$2.1^{bcd}\pm0.1$	$6.1^{a}\pm0.8$	$1.4^{ab}\pm1.6$	$2.3^{cd}\pm1.1$	Negative
Processor 13	$6.8^{bc}\pm1.3$	$1.8^{bcd}\pm2.1$	$1.5^{bcd}\pm1.7$	$3.3^{a}\pm3.8$	$2.1^{a}\pm2.4$	$2.7^{ab}\pm1.0$	$2.3^{bcd}\pm1.3$	$5.5^{a}\pm1.4$	$1.2^{ab}\pm1.4$	$2.6^{bcd}\pm0.5$	Negative
Processor 14	$6.3^{c}\pm1.3$	$2.7^{abcd}\pm1.7$	$2.3^{abcd}\pm1.2$	$4.2^{a}\pm2.1$	$1.1^{a}\pm1.3$	$2.5^{ab}\pm0.4$	$2.4^{bcd}\pm1.0$	$5.3^{a}\pm2.2$	$0.0^{b}\pm0.0$	$2.9^{abcd}\pm0.4$	Negative
Processor 15	$7.0^{abc}\pm0.1$	$2.9^{abcd}\pm1.8$	$2.4^{abcd}\pm1.4$	$6.1^{a}\pm0.8$	$1.1^{a}\pm1.2$	$3.1^{ab}\pm0.7$	$3.9^{ab}\pm0.8$	$6.4^{a}\pm0.7$	$1.2^{ab}\pm1.4$	$1.8^{d}\pm2.0$	Negative
p –value	< 0.0001	< 0.0001	< 0.0001	0.262	0.369	0.049	< 0.0001	0.184	< 0.0001	< 0.0001	NA
Overall mean± SD	7.6 ± 1.2	2.2 ± 1.7	2.0 ± 1.7	4.5 ± 1.9	1.6 ± 1.3	2.8 ± 1.1	2.4 ± 1.4	5.8 ± 1.3	1.6 ± 1.7	3.3 ± 1.0	Negative
Conformance with specification											
Specification	–	Absent	Absent	Min = 4.0	Absent	Absent	Absent	Min =6	–	Absent	Absent in 25 mL
% Non-conformance	NA	93.3	93.3	26.7	100	100	100	33.3	NA	100	0
Reference specification	NA	*	EAC [15]	Codex [11]	*	*	*	Codex [11]	–	EAC (2006)	EAC (2006)

$N = 30$ (2 samples per processor). Values are means ± standard deviations. Means in the same column with similar superscript letters are not significantly different ($p > 0.05$). NA; Not applicable, –; Not available for *Bongo* or related fermented dairy products. *No specifications for this or related products therefore the limits were set by the authors basing on known history of pathogenicity of several members of these groups of microorganisms. TPC = Total plate counts; TC = Total coliforms; SRC = Sulphur reducing clostridia; Enterobac = Enterobacteriaceae; LAB = Lactic Acid Bacteria; Staph = *Staphylococcus* spp.

Table 2
Physicochemical properties of *Bongo* from different processors and their conformance with specifications.

Processor	Physicochemical attributes								
	pH	%Acidity	% Crude Fat	% Crude Protein	Total Soluble Solids (°Brix)	%Dry Matter	%Ash	%Carbohydrates	%Milk Solids Non-fat
Processor 1	4.0 ^{a} ± 0.1	1.1^{ab}±0.1	3.3^{a}±0.2	3.2^{a}±0.1	7.3^{bc}±0.2	10.9^{cde}±0.1	0.78^{ab}±0.04	3.9^{a}±0.8	7.5^{c}±0.1
Processor 2	3.9 ^{a} ± 0.2	1.0^{abc}±0.2	3.5^{a}±0.2	3.0^{a}±0.1	7.3^{bc}±0.1	11.8^{b}±0.3	0.78^{ab}±0.04	4.5^{a}±0.3	8.4^{a}±0.1
Processor 3	4.1^{a}±0.2	0.8^{d}±0.1	2.9^{a}±0.1	2.9^{a}±0.1	6.9^{cd}±0.2	11.0^{cde}±0.1	0.70^{ab}±0.07	4.3^{a}±0.1	8.2^{ab}±0.3
Processor 4	4.0^{a}±0.2	1.1^{ab}±0.1	3.8^{a}±0.4	3.0^{a}±0.4	6.7^{cd}±0.3	10.7^{de}±0.2	0.73^{ab}±0.03	3.7^{a}±0.2	8.4^{a}±0.1
Processor 5	4.2^{a}±0.1	1.0^{abc}±0.1	3.2^{a}±0.5	3.2^{a}±0.2	7.2^{bc}±0.1	11.2^{bcd}±0.1	0.69^{ab}±0.07	4.0^{a}±0.7	7.9 ^{abc}±0.1
Processor 6	4.0^{a}±0.1	1.2^{a}±0.1	2.9^{a}±0.1	3.3^{a}±0.1	7.0^{bcd}±0.2	10.2^{e}±0.3	0.82^{a}±0.02	4.2^{a}±0.8	8.1^{abc}±0.2
Processor 7	4.2^{a}±0.1	1.1^{ab}±0.1	3.5^{a}±0.2	3.2^{a}±0.1	7.0^{bcd}±0.3	10. ^{e} ± 0.1	0.73^{ab}±0.02	4.0^{a}±0.8	7.6^{c}±0.2
Processor 8	4.0^{a}±0.3	1.0^{abc}±0.1	3.1^{a}±0.2	3.2^{a}±0.1	7.1^{bcd}±0.3	10.5^{de}±0.2	0.80^{a}±0.04	4.6^{a}±0.1	8.2^{ab}±0.2
Processor 9	3.9^{a}±0.1	0.9^{bc}±0.1	3.3^{a}±0.5	3.3^{a}±0.2	6.9^{cd} ±0.2	11.7^{bc}±0.2	0.76^{ab}±0.01	4.0^{a}±0.2	7.9^{abc}±0.1
Processor 10	3.9^{a}±0.1	1.0^{abc}±0.1	3.5^{a}±0.9	3.1^{a}±0.2	7.5^{bc}±0.1	11.0^{bcde}±0.1	0.73^{ab}±0.02	4.5^{a}±0.2	8.4^{a}±0.1
Processor 11	4.0^{a}±0.2	1.0^{abc}±0.1	3.1^{a}±0.7	3.0^{a}±0.1	7.6^{bc}±0.1	13.6^{a}±0.1	0.70^{ab}±0.03	3.9^{a}±0.5	8.5^{a}±0.2
Processor 12	3.8^{a}±0.2	1.0^{abc}±0.1	2.9^{a}±0.2	3.2^{a}±0.1	7.8^{b}±0.4	13.9^{a}±0.2	0.77^{ab}±0.05	4.0^{a}±0.3	7.8^{abc}±0.3
Processor 13	3.7^{a}±0.1	1.0^{abc}±0.2	3.1^{a}±0.2	2.9^{a}±0.1	9.0^{a}±0.1	13.0^{a}±0.7	0.64^{b}±0.02	4.2^{a}±0.4	8.4^{a}±0.1
Processor 14	4.1^{a}±0.1	0.9^{cd}±0.2	3.7^{a}±0.5	3.4^{a}±0.1	8.9^{a}±0.2	10.2^{e}±0.1	0.65^{b}±0.01	4.^{1a}±0.2	8.2^{ab}±0.4
Processor 15	4.2^{a}±0.2	0.9^{cd}±0.1^{cd}	2.7 ^{a} ± 0.2	3.2^{a}±0.3	6.3^{d}±0.4	10.4^{de}±0.2	0.65^{b}±0.01	4.6 ^{a} ± 0.2	8.3^{a}±0.1
p-value	0.138	< 0.0001	0.356	0.270	< 0.0001	< 0.0001	0.002	0.769	0.000
Overall mean± SD	4.1 ± 0.2	1.0 ± 0.1	3.2 ± 0.3	3.1 ± 0.3	7.0 ± 1.0	11.2 ± 2.3	0.7 ± 0.1	4.2 ± 0.7	8.1 ± 0.6
Conformance with Specification									
Specification	≤4	Min = 0.6	<10	Min = 2.7	–	–	–	–	Min = 8.2
% Non-conformance	40	0	0	0	NA	NA	NA	NA	33.3
Reference specification	Steinkraus [57]	Codex [11]	Codex [11]	Codex [11]	–	–	–	–	EAC (2006)

$N = 30$ (2 samples per processor). Values are means ± standard deviations. Means in the same column with similar superscript letters are not significantly different ($p > 0.05$). NA; Not applicable, –; Not available for *Bongo* or related fermented dairy products.

Table 3
Summary of hygienic practices of traditional *Bongo* processors.

Practice	Response% (n)	
	Done/Available	Not done/Not available
Follow a Hazard Analytical Critical Control point (HACCP) plan	0.0 (0)	100.0 (15)
Availability of suitable hand washing and sanitizing facilities	0.0 (0)	100.0 (15)
Washing of utensils before *Bongo* processing	100.0 (15)	0.0 (0)
Washing of utensils after *Bongo* processing	80.0 (12)	20.0 (3)
Sanitization of utensils after *Bongo* processing	0.0 (0)	100.0 (15)
Availability of racks for drying processing utensils	13.3 (2)	86.7 (13)
Wearing clean clothes during processors	53.3 (8)	46.7 (7)
Clean and clipped hand nails of processor	40.0 (6)	60.0 (9)
Refrigeration of *Bongo* immediately after production	100.0 (15)	0.0 (0)
Refrigeration of *Bongo* during transport to selling point	0.0 (0)	100.0 (15)
Use of clean bulk *Bongo* holding vessels	66.7 (10)	33.3 (5)
Processor blows into polythene bag before putting *Bongo* for retail buyers	20.0 (3)	80.0 (12)
Processor touches inside polythene bag while packing *Bongo* for retail buyers	66.7 (10)	33.3 (5)
Washing of hands in between handling money and *Bongo* during sale	0.0 (0)	100.0 (15)
Availability of garbage containers	6.7 (1)	93.3 (14)
Garbage containers with covers	0.0 (0)	100.0 (15)

$N = 15$ processors.

to coagulum formation (Dewan and Tamang, 2007), sour taste [50] and pathogen inactivation [57]. LAB also produce aroma compounds [16,50] that contribute to the characteristic flavor of *Bongo.* Thus variations in LAB counts of *Bongo* may result in inconsistences in product characteristics thus necessitating the development and use of commercial starter cultures.

Yeasts are contaminants in yoghurt because they produce unwanted flavors and limit product shelf life [5]. However, in some traditional fermented dairy products, yeasts are not regarded as contaminants since their metabolism contributes to the unique characteristic flavor profile [50]. For such products, a minimum of 4 log cfu/mL of yeasts in the starter is recommended [11]. The yeast counts in this study exceeded 4 log cfu/mL and were close to those previously reported for *Bongo* [17] and traditional fermented milks from Burkina Faso [9]. The high counts of yeasts in *Bongo* suggests that they may contribute to the characteristic flavor profile of *Bongo*.

Psychrophile counts in this study were close to those reported for yoghurts produced in Kano, Nigeria [56] but much lower than those for buffalo and dairy yoghurts (up to 4.9 log cfu/mL) from Egypt [13]. Psychrophiles are cold-tolerant microorganisms with a minimum growth temperature of $\leq$ 0 °C and an optimum growth temperature of $\leq$ 15 °C [6]. Psychrophile counts in milk products could indirectly indicate contamination by *Listeria* spp. Indeed, Mugampoza et al. [41] reported the presence of *Listeria* spp.in *Bongo* suggesting a potential health threat to the consumer. Psychrophiles also indicate possible contamination by *Pseudomonas* spp. which causes proteolytic and lipolytic spoilage in refrigerated milks [1,14].

Enterobacteriaceae counts of *Bongo* were much lower than values (6.2–7.5 log cfu/mL) reported for *Nunu* [2]. Total coliform and *E. coli* counts of *Bongo* were similar or close to those reported for other traditional fermented milk products [9,18,49]. Enterobacteriaceae is a family of more than 30 genera of gram-negative, facultatively anaerobic rods including *Salmonella, Escherichia, Klebsiella, Shigella, Enterobacter, Cronobacter, Yersinia* and *Citrobacter,* which are associated with animals and plants [20,43]. Coliforms are a sub-group of Enterobacteriaceae which typically include *Citrobacter, Enterobacter, Klebsiella* and *Escherichia* [20]. These are associated with water, soil, plants and gastrointestinal tracts of animals. Their presence in food suggests possible contamination by pathogenic organisms of fecal origin. *E. coli* on the other hand is exclusively of fecal origin and thus indicates fecal contamination. Most samples of *Bongo* (77 – 80%) evaluated in this study contained *E. coli,* coliforms and Enterobacteriaceae indicating poor process hygiene and the likelihood of contamination with pathogens of fecal origin.

The agar-based detection of sulphur reducing clostridia (SRC) is used for indicating the possible presence of pathogenic or spoilage *Clostridium* spp. (Doyle, O'Toole and Cotter, 2018). Using 16S rRNA gene sequencing, most (76%) of the sulphur reducing bacteria isolated from the dairy chain were identified as SRCs and others as the non-clostridia species *Bacillus licheniformis, B. cereus, Paenibacillus lactis, Proteus mirabilis* and *S. enterica* which possess sulphur reducing genes (Doyle, O'Toole and Cotter, 2018). Thus results of this study suggest that *Bongo* could be contaminated by pathogenic and/or spoilage clostridia.

All *Bongo* samples contained enterococci. Enterococci are LAB comprising both pathogenic and commensal microorganisms found in the environment and gastrointestinal tracts of animals [19]. Although enterococci are currently not recognized as safe (GRAS), some are used as probiotics, starter cultures, or as sources of bacteriocins [19]. The identity and properties of enterococci in *Bongo* need to be evaluated further to establish if they are safe or potentially pathogenic.

The *Staphylococcus* spp. counts in this study were similar to values (3.3 – 4.3 log cfu/mL) reported by Cissé et al. [9] for different fermented milks but higher than 1.3 – 1.6 log cfu/mL reported for yoghurt and *Lebnah* produced in Jordan [49]. The presence of *Staphylococcus* spp. in *Bongo* could suggest the possibility of contamination with toxigenic *S. aureus* which is associated with using milk from mastitic cows or infected human processors [49].

Table 4
Association of processors practices with products meeting specific microbial specifications of food safety concern.

Practice	p-values for Fisher's exact test of association of practice with meeting different microbial specifications							
	Coliforms	*E. coli*	yeast	sulphur reducing clostridia*	Enterococci*	Enterobacteriaceae*	*Staphylococcus* spp.*	*Salmonella* spp.*
Having HACCP*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Hand washing and sanitation facilities*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Washing utensils before processing*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Washing utensils after processing	0.200	0.200	0.516	1.000	1.000	1.000	1.000	1.000
Sanitizing utensils*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Having racks for drying	1.000	1.000	0.371	1.000	1.000	1.000	1.000	1.000
Wearing clean clothes	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Clean clipped nails	1.000	1.000	0.229	1.000	1.000	1.000	1.000	1.000
Refrigerating after processing*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Refrigerating during transportation*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Clean bulking vessels	0.313	0.313	0.516	1.000	1.000	1.000	1.000	1.000
Blowing into packaging	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Touching packaging during filling	0.267	0.267	1.000	1.000	1.000	1.000	1.000	1.000
Washing hands after holding money*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Having garbage bins	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Having covered garbage bins*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Note: A p value < 0.05 indicates an association between practice and meeting particular microbial criteria. *For some associations having $p = 1.000$ at least one active row or one active column of the contingency table has a null marginal sum (i.e. there are zero counts/processors or products in some categories either of pass vs fail of specification or practice vs no practice).

Salmonella spp. was not detected in any of the *Bongo* samples tested in this study. Cissé et al. [9] also did not detect *Salmonella* spp. in fermented milk from camels, cows and goats. However, other authors reported presence of *Salmonella* spp. in *Nunu* [2] and other milk products [34]. *Salmonella* spp. contamination is associated with several sources including air, feed, soil, faeces, grass, animals, utensils and water [34].

The pH and acidity of *Bongo* were in the range of 2.9 – 4.8 and 0.48 – 2.8%, respectively that have been reported for related traditional fermented dairy products [9,18,39,41,58]. An optimum pH of 4.1 – 4.4 is desirable for fermented dairy products since it faciliates flavor development, coagulum formation [36] and prevents growth of pathogenic and spoilage microbes [59]. All samples met the minimum recommended acidity of 0.6% [11] which is also the point at which coagulum formation is reported to start [36]. Many pathogenic species in lactic acid fermented beverages are inhibited at a minimum lactic acid content of 0.7% and pH $\leq$4.0 (Steinkraus [57]. The presence of psychrophiles, Enterobacteriaceae, sulphur reducing clostridia and *Staphylococcus* spp., in *Bongo* despite the pH and acidity values of the product can be attributed to survival of acid tolerant species. This necessitates ensuring adequate heat treatment of the milk and improving processing hygiene.

The fat content of *Bongo* was within the range 1.1 – 4.65% as reported for related traditional fermented dairy products [9,18,39]. Fat content in dairy product is influenced by variations in raw milk quality, treatments to reduce fat and adulteration by addition of water to milk [42]. The fat content of *Bongo* met the minimum requiremnt (3%) for whole milk yoghurts [15] suggesting that the processors generally do not dilute their milk.

The protein content of *Bongo* was within the range 2.1 – 3.8% reported for related traditional fermented dairy products [9,18,39]. All *Bongo* samples evaluated met the minimum protein content (2.7%) specified by Codex [11] further indicating that processors do not dilute their milk.

The total soluble solids content of *Bongo* was less than values of 9.6 – 14.3% reported for yoghurts locally produced in Uganda [42]. Total soluble solids is a measure of soluble solutes which in products like *Bongo* include sugars and lactic acid. The difference in values reported for *Bongo* and yoghurt could be due to the fact that the latter could have contained added sugar while the former is produced without added sugars.

The dry matter content of *Bongo* was close to or in the range of 6.3 – 18.6% that has been reported for related fermented dairy products [9,18,39]. The ash content of *Bongo* was within the range of values (0.2 – 1.54%) reported for related products [9,39,58]. Total carbohydrate content was similar to values (3.1 – 5.5%) reported by Mathara [39] and Cissé et al. [9]. The milk solids non-fat content of *Bongo* was close to but lower than values (9.6 – 14.3%) reported for yoghurt on the market in Kampala, Uganda [42]. About 67% of the *Bongo* evaluated met the minimum solids non-fat content set by the East African Community [15] for yoghurt. According to Staff [55], a milk solids non-fat content of 12–15% is required to achieve a desirable texture in yoghurt. The milk solids-non-fat content is mainly influenced by fat and total solids content [36] and can be increased by prolonged evaporation or addition of dry matter in form of carbohydrates.

Traditional processing of Bongo

Although a previous study reported that *Bongo* is traditionally made using unpasteurized milk [41] all the commercial processors in this study used boiled milk. The shift to using boiled milk could be attributed to the increased awareness about the risk of contracting brucellosis from consuming un-boiled milk. None-the-less, according to the processors, the use of un-boiled milk to make *Bongo* is still common in most rural settings.

Traditional processing of *Bongo* is fairly simple and is similar to that followed for other traditional fermented milk products [3,40,57]. For these products, the fermentation vessels (saucepans, jerrycans and gourds) serve as an important source of inoculum. Moreover, the gourds which are preferred for the smoky flavor they impart on the product are seldom washed. It is important to note that in *Bongo* processing the boiled milk is transferred to the vessels after it has cooled to room temperature ($\approx$25–28 °C) and the fermentation proceeds spontaneously. This gives chance to the fermenting flora and potential pathogens available on the vessels to grow. Other stages in the processing of *Bongo* could also contribute to contamination with microorganisms. Breaking the curd and addition of flavorant could be another entry point for microbes into the product. These processes involve mixing by use of ladles/spoons which are not sterilized. The holding containers, usually jerrycans, are also potential sources of contamination. These are used for several batches of *Bongo* and are not adequately sanitized. Jerrycans also have hard-to-clean areas which likely harbor biofilms that could be containing pathogenic microflora. Therefore, despite refrigeration after fermentation, it may be insufficient to subdue the microbial contamination.

Hygienic practices of Bongo *processors*

Out of ignorance, poor attitude or lack of resources some processors engaged in poor hygiene practices which could explain the presence undesirable microbes in *Bongo* (Table 1). All processors were ignorant of HACCP and the need to sanitize their hands and utensils before, during and after production. Some processors associated hypochlorite with hospital use only which agrees with observations by Byakika et al. [8]. Some processors do not wash their utensils, especially the gourds, to preserve the starter culture and/or because of water access challenges. Additionally 86.7% had no racks to drain and dry their processing utensils. Up to 60% had visibility unclean jerrycans in which they placed the *Bongo* after fermentation. About 47% of processors made *Bongo* in dirty clothing and also had long dirty finger nails. Generally, analysis of their productions sites, which were largely makeshift houses, revealed that they lacked basic facilities to support good hygiene

practices. These observations have been reported elsewhere and are known potential contributors to product contamination [8,12,44].

It is also noteworthy that processors lacked means to transport *Bongo* under refrigeration moreover some deliver as far as Kampala which is about 200 km away. Processors reported that for such long distances, the product is transported very early in the morning or late evening when the temperatures are cooler.

The other public health concerns noted in this study were that processors blow and touch inside the polythene bags used to pack *Bongo* for retail buyers. They do not wash and sanitize their hands in between handling money and *Bongo* and lack proper garbage disposal facilities. All these are poor hygiene practices that can contribute to contamination of products.

Association of hygienic practices with microbial quality

The observation that none of the hygienic practices in this study was statistically associated with *Bongo* meeting microbial specification was contrary to expectations. However, similar observations were made in a study of knowledge, attitudes and practices of *Obushera* processors in Uganda [8]. These observations can be attributed to processors falsely reporting that they carry out certain food safety practices [10,51]. Processors are likely to do this so as to impress the interviewer or to appear that they follow regulations. The lack of associations between practice and microbial quality in this study could also be attributed to situations where none of the processors carried out some practices (for example having a HACCP plan or sanitizing utensils among others). In such cases it impossible to compare those that do or do not carry out a certain practice.

Conclusion

Bongo is predominantly a lactic acid bacteria and yeast fermented milk beverage that contains about 11% dry matter, 3% crude protein and 3% crude fat. Its production is still unstandardized and unhygienic thus predisposing the product to contamination by potential foodborne pathogens. Therefore, *Bongo* and similar fermented foods may present a serious public health concern if their production is not regulated. Processors of *Bongo* and related products need to be trained in good manufacturing practices and must be monitored to ensure compliance. A comprehensive food safety standard for *Bongo* and similar fermented milk products is also required to guide processors and regulatory agencies.

Declaration of Competing Interest

The is no conflict of interest to declare

Acknowledgements

The authors thank all *Bongo* processors who were involved in this study.

References

[1] A.H. Abdulghani, Influence of CO_2 addition to raw milk on inhibition of psychrophilic bacteria and some sensory characteristics of pasteurized milk, Anbar J. Agric. Sci. 16 (2) (2018) 43–51.
[2] P.T. Aernan, E.E. Ebah, P. Ukange, Microbial contaminants associated with fermented milk “Nunu” sold in Makurdi metropolis, Benue State of Nigeria, J. Sci. Multidiscip. Res. 3 (2011) 27–34.
[3] F. Akabanda, J. Owusu-Kwarteng, K. Glover R L, K. Tano-Debrah, Microbiological characteristics of Ghanaian traditional fermented milk product, Nunu, Nat. Sci. 8 (2010) 178–187.
[4] AOACOfficial Methods of Analysis of the Association of Analytical Chemists, 1990 Washington, DC.
[5] K.J. Aryana, D.W. Olson, A 100-Year Review: Yogurt and other cultured dairy products, J. Dairy Sci. 100 (12) (2017) 9987–10013.
[6] R. Banerjee, A. Halder, A. Natta, Psychrophilic microorganisms: habitats and exploitation potentials, Eur. J. Biotechnol. Biosci. 4 (2016) 16–24.
[7] L.M. Beltrán-Barrientos, A.F. González-Córdova, A. Hernández-Mendoza, E.H. Torres-Inguanzo, H. Astiazarán-García, J. Esparza-Romero, B. Vallejo-Cordoba, Randomized double-blind controlled clinical trial of the blood pressure–lowering effect of fermented milk with Lactococcus lactis: a pilot study, J. Dairy Sci. 101 (2018) 2819–2825.
[8] S. Byakika, I.M. Mukisa, Y.B. Byaruhanga, D. Male, C. Muyanja, Influence of food safety knowledge, attitudes and practices of processors on microbiological quality of commercially produced traditional fermented cereal beverages, a case of *Obushera* in Kampala, Food Control 100 (2019) 212–219.
[9] H. Cissé, U. Muandze-Nzambe J, S. Somda N, A. Sawadogo, M. Drabo S, F. Tapsoba, C. Zongo, Y. Traoré, A. Savadogo, Assessment of safety and quality of fermented milk of camels, cows, and goats sold and consumed in five localities of Burkina Faso, Vet. World 12 (2019) 295–304.
[10] D.A. Clayton, C.J. Griffith, P. Price, A.C. Peters, Food handlers’ beliefs and self-reported practices, Int. J. Environ. Health Res. 12 (1) (2002) 25–39.
[11] Codex. (2003). Codex standard for fermented milks 242-2003. Available at: www.codexalimentarius.net/download/standards/400/CXS_243e.pdf (accessed 3 August 2020).
[12] T.N. Djéni, A.K. Kouamé, Y. Traoré, R.K. Nevry, K.D. Marcellin, Assessment of knowledge, attitudes and practices of food handlers in Attieke production units in relation to food hygiene and safety in Côte d’Ivoire in 2012, Food Nutr. Sci. 5 (2014) 896.
[13] A.A. El-Shazly, M.A. El-Tahra, M.M. Ismail, M.Z. Eid, Effect of cold storage and mixing various lactations of buffaloe’s and cow’s milk on some properties of yoghurt, J. Agric. Sci. Mansoura Univ. 33 (7) (2008) 5141–5151.
[14] D. Ercolini, F. Russo, I. Ferrocino, F. Villani, Molecular identification of mesophilic and psychrotrophic bacteria from raw cow’s milk, Food Microbiol. 26 (2) (2009) 228–231.
[15] East African Community (EAC). (2007). Yoghurt — Specification (EAS 33:2007). Retrieved from https://www.kdb.go.ke/wp-content/uploads/2019/06/EAC-standard-for-yoghurt.pdf (accessed 9 August 2020).
[16] T.H. Gadaga, B.C. Viljoen, J.A. Narvhus, Volatile organic compounds in naturally fermented milk and milk fermented using yeasts, lactic acid bacteria and their combinations as starter cultures, Food Technol. Biotechnol. 45 (2) (2007) 195.
[17] N. Gulaita, Production Methods, Physico-Chemical Properties and Microbial Quality of Bongo, a Fermented Milk Product Produced in Pallisa District MSc Thesis, Makerere Univeristy, Kampala, 2015.

[18] N. Guizani, S. Kasapis, M. Al-Ruzeiki, Microbial, chemical and rheological properties of Laban (cultured milk), Int. J. Food Sci. Technol. 36 (2) (2001) 199–205.
[19] H. Hanchi, W. Mottawea, K. Sebei, R. Hammami, The genus enterococcus: between probiotic potential and safety concerns—an update, Front. Microbiol. 9 (2018) 1791.
[20] C.J. Hervert, N.H. Martin, K.J. Boor, M. Wiedmann, Survival and detection of coliforms, Enterobacteriaceae, and gram-negative bacteria in Greek yogurt, J. Dairy Sci. 100 (2) (2017) 950–960.
[21] International Organization for Standardization. (1998). *Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Enumeration of Mesophilic Lactic Acid Bacteria — Colony-Count Technique at 30 Degrees C* (ISO 15214:1998).
[22] International Organization for Standardization. (2000). *Water Quality — Detection and Enumeration of Intestinal Enterococci — Part 2: Membrane Filtration Method* (ISO 7899-2:2000).
[23] International Organization for Standardization. (2003a). *Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Enumeration of Sulfite-Reducing Bacteria Growing Under Anaerobic Conditions* (ISO 15213:2003).
[24] International Organization for Standardization. (2003b). *Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (Staphylococcus Aureus and Other Species) — Part 3: Detection and MPN Technique for Low Numbers* (ISO 6888-3:2003).
[25] International Organization for Standardization. (2006). *Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Enumeration of Coliforms — Colony-Count Technique* (ISO 4832:2006).
[26] International Organization for Standardization. (2008). *Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Enumeration of Yeasts and Moulds — Part 2: Colony Count Technique in Products with Water Activity Less Than or Equal to 0,95* (ISO 21527-2:2008).
[27] International Organization for Standardization. (2009). *Milk and Milk Products — Method for the Enumeration of Pseudomonas spp.* (ISO/TS 11059:2009).
[28] International Organization for Standardization. (2010). *Milk, Cream and Evaporated Milk — Determination of Total Solids Content (Reference Method)* (ISO 6731:2010 [IDF 21:2010]).
[29] International Organization for Standardization. (2013). *Microbiology of the Food Chain — Horizontal Method for the Enumeration of Microorganisms — Part 1: Colony Count at 30°C by the Pour Plate Technique* (ISO 4833-1:2013).
[30] International Organization for Standardization. (2017a). *Microbiology of the Food Chain — Horizontal Method for the Detection and Enumeration of Enterobacteriaceae — Part 2: Colony-Count Technique* (ISO 21528-2:2017).
[31] International Organization for Standardization. (2017b). *Microbiology of the Food Chain — Horizontal Method for the Detection, Enumeration and Serotyping of Salmonella — Part 1: Detection of Salmonella spp.* (ISO 6579-1:2017).
[32] International Organization for Standardization. (2018). *Microbiology of the Food Chain — Horizontal Method for the Enumeration of Beta-glucuronidase-Positive Escherichia Coli — Part 1: Colony-Count Technique at 44 Degrees C Using Membranes and 5-bromo-4-chloro-3-Indolyl Beta-D-glucuronide* (ISO 16649-1:2018).
[33] S.C. Izah, L.T. Kigigha, I.P. Okowa, Microbial quality assessment of fermented maize Ogi (a cereal product) and options for overcoming constraints in production, Biotechnol. Res. 2 (2016) 81–93.
[34] N.S. Karshima, V.A. Pam, S.I. Bata, P.A. Dung, N.D. Paman, Isolation of Salmonella species from milk and locally processed milk products traded for human consumption and associated risk factors in Kanam, Plateau State, Nigeria, J. Anim. Prod. Adv. 3 (3) (2013) 69–74.
[35] H.Y. Kim, Statistical notes for clinical researchers: Chi-squared test and Fisher's exact test, Restor. Dent. Endod. 42 (2) (2017) 152–155.
[36] M. Kroger, Quality of yogurt, J. Dairy Sci. 59 (2) (1976) 344–350.
[37] S.-.M. Lim, Microbiological, physicochemical, and antioxidant properties of plain yogurt and soy yogurt, Korean J. Microbiol. 49 (2013) 403–414.
[38] A. Lorusso, R. Coda, M. Montemurro, G. Rizzello C, Use of selected lactic acid bacteria and quinoa flour for manufacturing novel yogurt-like beverages, Foods 7 (2018) 51.
[39] MatharaStudies on Lactic Acid Producing Microflora in *Murski* and Kule Naoto, Traditional Fermented Milks from Nandi and Masai Communities in Kenya MSc Thesis, University of Nairobi, Kenya, 1999.
[40] J.M. Mathara, U. Schillinger, P.M. Kutima, S.K. Mbugua, W.H. Holzapfel, Isolation, identification and characterisation of the dominant microorganisms of kule naoto: the Maasai traditional fermented milk in Kenya, Int. J. Food Microbiol. 94 (2004) 269–278.
[41] D. Mugampoza, M. Muyanja C, P. Ogwok, M. Serunjogi, G. Nasinyama, Occurrence of *Listeria monocytogenes* in bulked raw milk and traditionally fermented dairy products in Uganda, Afr. J. Food Agric. Nutr. Dev. (2011) 11.
[42] I. Mukisa, R. Kyoshabire, Microbiological, physico-chemical and sensorial quality of small-scale produced stirred yoghurt on the market in Kampala city, Uganda, Nutr. Food Sci. 40 (2010) 409–418.
[43] M.T. Musgrove, D.R. Jones, J.K. Northcutt, N.A. Cox, M.A. Harrison, Identification of Enterobacteriaceae from washed and unwashed commercial shell eggs, J. Food Prot. 67 (11) (2004) 2613–2616.
[44] F.A. Oguntoyinbo, Development of hazard analysis critical control points (HACCP) and enhancement of microbial safety quality during production of fermented legume based condiments in Nigeria, Niger. Food J. 30 (2012) 59–66.
[45] O. Oyelana, A. Coker, Microbial contamination at different stages of production of Ogi in Mowe: a rural community, Southwest, Nigeria, Bacteriol. J. 2 (2012) 1–11.
[46] A. Perna, I. Intaglietta, A. Simonetti, E. Gambacorta, Donkey milk for manufacture of novel functional fermented beverages, J. Food Sci. 80 (2015) S1352–S1359.
[47] A. Parseelan, S. Muthu, P. Kannan, E. Ayyasamy, R. Narayanan, Aerobic plate count of milk and dairy products marketed in different zones of Chennai, Int. J. Livest. Res. 9 (3) (2019) 97–102.
[48] Raosoft Inc (2004) *Sample size calculator* [Internet document] URL http://www.raosoft.com/samplesize.html . Accessed 16/06/2018.
[49] A.T. Riadh, A comparison on microbial conditions between traditional dairy products sold in Karak and same products produced by modern dairies, Pak. J. Nutr. 4 (5) (2005) 345–348.
[50] W. Routray, H.N. Mishra, Scientific and technical aspects of yogurt aroma and taste: a review, Compr. Rev. Food Sci. Food Saf. 10 (4) (2011) 208–220.
[51] S. Samapundo, R. Climat, R. Xhaferi, F. Devlieghere, Food safety knowledge, attitudes and practices of street food vendors and consumers in Port-au-Prince, Haiti, Food Control 50 (2015) 457–466.
[52] F. Saubade, M. Hemery Y, I. Rochette, J.-.P. Guyot, C. Humblot, Influence of fermentation and other processing steps on the folate content of a traditional African cereal-based fermented food, Int. J. Food Microbiol. 266 (2018) 79–86.
[53] M. Scott, D. Flaherty, J. Currall, Statistics: Dealing with categorical data, J. Small Anim. Pract. 54 (1) (2013) 3–8.
[54] F.W. Sosulski, G.I. Imafidon, Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods, J. Agric. Food Chem. 38 (6) (1990) 1351–1356.
[55] M.C. Staff, Cultured milk and fresh cheeses, in: R. Early (Ed.), The Technology of Diary Products, Blackie Academic and Professional, London, UK, 1998 P.446.
[56] A.A. Shehu, Microbiological analyses of some processed yoghurts sold in Kano metropolis, Kano-Nigeria, Techo Sci. Afr. J. 7 (1) (2012) 21–32.
[57] K.H. Steinkraus, Handbook of indigenous fermented foods, Food Sci. Technol. (USA). No. 73 (1996).
[58] R.E. Uzeh, R.E. Ohenhen, A.K. Rojugbokan, Microbiological and nutritional qualities of dairy products: Nono and Wara, Nat. Sci. 4 (3) (2006) 37–40.
[59] F.P. Downes, K. Ito, Compendium of methods for the microbiological, Exam. Foods 4 (2001).