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QUALITY ASSESSMENT OF BUTTER PRODUCED USING TRADITIONAL AND MECHANIZED CHURNING METHODS

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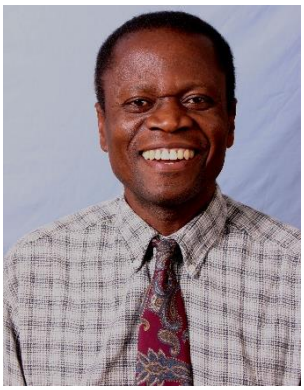

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ABSTRACT

Traditional butter/ghee-making, predominantly done by women, is labor-intensive. To reduce this labor and/or increase incomes among these women, a hand-operated churner was previously developed with the capacity to reduce labor eight-fold. The present study was carried out to compare the quality of butter/ghee made using traditional churning in locally harvested plant containers (gourds and calabashes) and mechanized churning in the new device. As opposed to shaking the whole vessel, churning in the new device is achieved through a hand-operated crank connected to mixing baffles. Butter samples were aseptically collected from four locations (Kiboga1, Kiboga2, Kotido, and Ngoma) along the cattle corridor of Uganda. A "control" butter sample was made under laboratory conditions following standard procedure. The five samples were analyzed with respect to microbial safety, type and concentration of free fatty acids, and sensory attributes. Total viable count (TVC), Total coliforms (TC), *Staphylococcus aureus*, Salmonella, yeasts and molds counts were determined using International Organization for Standardization (ISO) standards. Fatty acid profile was determined by gas chromatography. Sensory evaluation of aroma, smell, taste, mouth feel, and overall acceptability of the products were also conducted. In the sensory evaluation, two commercially marketed ghee products (Sameer, and Lubega brands) were added. Total viable counts in all the samples were in the range of 10^2-10^7 cfu/g. Total coliforms were detected in Kiboga samples in the 10^1-10^3cfu/g range while none were detected from other regions' samples. Yeasts and molds were detected in the 10^2-10^5 cfu/g range. *Staphylococcus aureus* was detected only in butter samples from Kiboga region (10^2 cfu/g) while Salmonella was not detected in any of the samples. The fatty acid profile consisted of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, trans-fatty acids, omega 3 fatty acids, omega 6 fatty acids, and omega 9 fatty acids. Saturated fatty acids were most dominant in the butter and ghee samples ranging from 70-82% whereas trans-fatty acids were present in the least concentration. From the overall acceptability dimension, the butter/ghee made using traditional churning and the new device scored the highest. However, Student's *t*-test analysis showed no significant difference in the organoleptic parameters analyzed in all the samples ($p>0.05$). Therefore, the butter/ghee produced using mechanized churning is as acceptable and as microbiologically safe as butter/ghee produced using traditional churning and two representative marketed products.

Key words: Butter, ghee, microbial safety, churning, traditional processing, human-centered design

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INTRODUCTION

Ghee can be defined as anhydrous milk fat extracted from separation of the milk solid and water [1]. Ghee is a type of clarified butter that originated in India [2] and in Uganda, butter production is mainly at subsistence level and majority of the people are unaware of its health benefits. It is traditionally made by churning fermented milk in naturally grown plant material vessels - calabashes and gourds, to separate butter fat from the rest of the milk [2, 3]. Traditional butter/ghee-making in the cattle corridor of Uganda and other sub-Saharan countries is accomplished mainly by women [3]. These women find the process tiresome, laborious and time consuming. It takes time away from other daily domestic/house work and/or increases the overall labor-burden [3].

The cattle corridor of Uganda diagonally stretches from the Southwest to the Northeast and is home to cattle-herding *Bantu* and *Nilotic* ethnic groups who practice ghee-making. They produce ghee by melting the butter over low heat and allowing it to simmer until most of the water has been evaporated. Ghee is used in many oil therapies, healthy cooking, herb combinations, and combines well with a wide variety of spices providing an excellent aroma and nutty flavor. Previous studies [4, 5] have shown that ghee harbors antioxidants and contains butyric acid, a fatty acid with antiviral activities. Butyric acid also has anti-cancer properties that have been shown to slow the progress of some types of cancer and heart disease and also helps to reduce the body fat while increasing lean muscle mass.

Churning is the most time consuming component of the ghee-making process [3]. Such high labor burden faced by women, in comparison to their male counterparts, is one of the reasons behind their financial inequality, an issue termed as “the gender asset gap” that has caught the attention of development scholars and practitioners [6]. Mechanization of the labor-intensive components of their daily work such as churning and hand-tools has been proposed as the first step toward narrowing this gender gap [7]. In response to such calls, a hand-operated butter churner (Fig. 1) was designed for processing fermented milk or cream to obtain butter. The butter can be processed into ghee as described above.

In comparison to traditional churning in gourds or calabashes [5], the churner in Fig. 1 is capable of reducing labor eight-fold [7]. Introduction of a new tool in any traditional food production process comes with anxiety about possible changes in the food product that may impact consumer acceptability. The purpose of this study was to assess the safety of butter/ghee produced by application of mechanized churning in comparison to the traditionally produced product, in terms of microbial safety, free fatty acid content and concentration, and sensory attributes.

Although, there are over 120 different compounds that contribute to butter/ghee’s unique flavor, fatty acids are among the five primary factors responsible for the flavor, and a measure of the amount of these acids is very important [3]. Certain microbial presence in butter facilitates the formation of butter/ghee aroma and flavor. However, excessive levels of microbes may put the consuming public at risk. Therefore, the safety aspect of these bacteria has to be considered and thus the need for their analysis. For butter/ghee produced using mechanized churning to be acceptable, it has to present minimal

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deviation from the traditionally produced product, hence the need for sensory evaluation. In all the results reported in this paper, both butter and its derived ghee were analyzed separately.

MATERIALS AND METHODS

Traditional gourd and mechanical churner butter samples were aseptically collected from the four locations (Kiboga1, Kiboga2, Kotido and Ngoma); previously described in detail by Sempiira *et al*. [3]. The two samplings in Kiboga were conducted in sub-regions of Kiboga. A "control" butter/ghee sample was prepared at the Makerere University Food Technology and Business Incubation Center (FTBIC) and was also included in the analysis. The "control" sample production involved filtering the milk, batch pasteurization with high temperatures short time (HTST) process, followed by cooling to room temperature. The milk at room temperature was inoculated with the starter culture and kept for a period of over 24 hours. The fermented milk was churned in the device presented in Fig. 1 and the resulting butter was kept for a month in a cold room. For all the samples, a portion of the butter was heated as previously described to produce ghee.

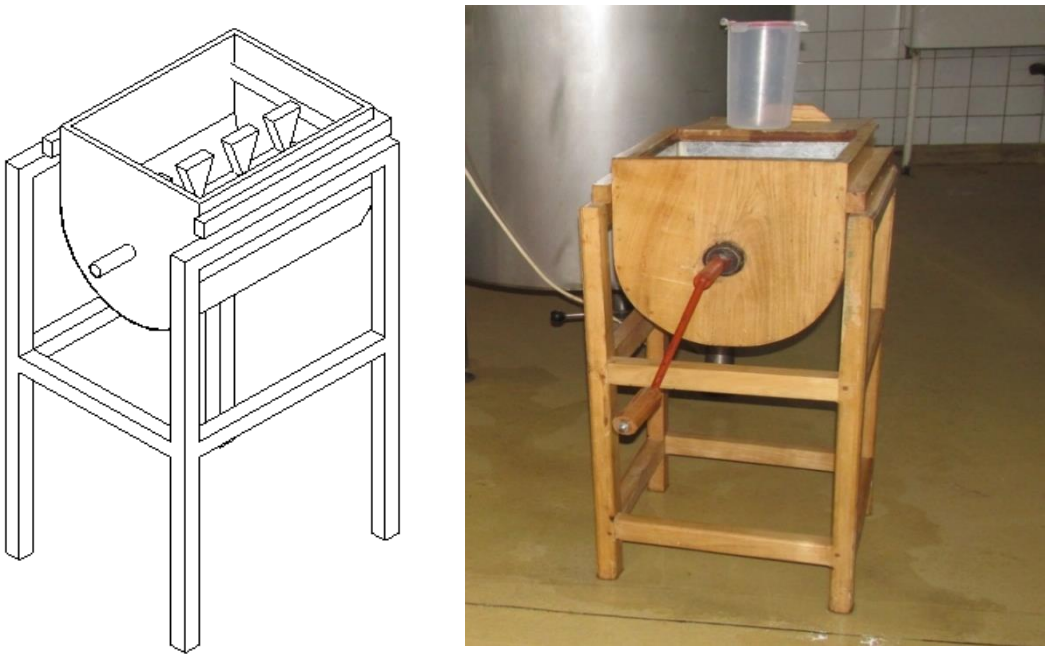

Figure 1: Hand-operated churner made from wood and lined with aluminum sheet. It consists of a shaft fitted with baffles. A woman performed the churning when seated and rotated the shaft through the crank to allow the baffles to cause a vigorous mixing that results in coagulation of the butter and separation from the rest of the fermented milk. The churner shown has a capacity of 15 liters, but higher or lower capacity units can be made

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Microbial Analysis

Selected butter/ghee samples (25 g) were placed in 225 ml of buffered peptone water (Conda S.A) and stomached (Seward stomacher). The samples were then used to make serial dilutions. Enumeration of total viable count (TVC) in the samples was performed following the International Standard ISO-4833 colony counting protocol. Using a sterile pipette, 1 ml of the test sample of the chosen dilution was aseptically transferred to each Petri dish. Molten nutrient agar (20 ml) was poured into each Petri dish. The inoculums were carefully mixed with the agar by rotating and then the mixture was allowed to solidify at 25°C. After solidification, the dishes were inverted and incubated at 37°C for 24 hours. Counts ranging from 30-300 were taken using a colony counter (Stuart, Bibby Sterlin Ltd, UK) and the results expressed as colony-forming units per gram (cfu/g).

Yeasts and molds enumeration in the samples was done using International Standard Method ISO-21527-2 protocol. Briefly, with a sterile pipette, 0.1 ml of the sample of the appropriate dilution was transferred to the center of solidified Potato Dextrose Agar (PDA) (Conda). Using a sterile spreader, the inoculums were evenly spread on the surface of the solidified PDA. The upright plates were incubated at 30°C for 3 days. A control plate with about 20 ml of the medium for checking its sterility was also prepared. Colony counts were performed as described above. Spreading colonies and clusters were considered as single colonies.

The total coliforms enumeration in the samples was performed using International Standard ISO-4832 protocol. Briefly, using a sterile pipette, 1 ml of the sample of the appropriate dilution was transferred to the center of each dish. About 10 ml of the medium were poured into each Petri dish, carefully mixed with the inoculums, and allowed to solidify at 25°C. After complete solidification, about 5 ml of the Violet Red Bile Agar (VRBL) (Conda) were poured onto the surface of the inoculated medium and allowed to solidify as before. A control plate was included as described for yeast and molds. After complete solidification, the dishes were inverted and incubated at 30°C for 24 hours for coliforms. The purplish red colonies were considered as typical colonies of coliforms and did not require further confirmation. Colony counts were performed as described above and spreading colonies were treated the same way.

The procedure for enumeration of *Staphylococcus aureus sp* was based on International Standard ISO 6888-1 protocol. Briefly, dilutions (0.1 ml) were aseptically transferred onto the center of the solidified Baird Parker Agar (BPA) (Himedia) plate. Using a sterile spreader, the inoculums were evenly spread as quickly as possible on the agar surface. The plates were inverted and incubated for 24 hours at 37°C and re- incubated for a further 24 hours at 37°C. Counts ranging from 30-300 with typical characteristic of black /grey colonies surrounded by an opaque clear zone were taken using a colony counting equipment and the results expressed as cfu/g.

Detection of Salmonella spp in the butter/ghee samples was performed using International Standard ISO 6579-1 protocol. Samples of 25 g were placed in 225 ml of buffered peptone water and stomached. The mixture was incubated at 37°C for 24 hours. After incubation, 0.1 ml of the culture was aseptically transferred to 10 ml of Rappaport Vassiliadis Soy (RVS) broth (Hemedia) and incubated at 41.5°C for 24 hours. The purpose of the pre-enrichment was to resuscitate the Salmonella spp cells present in the

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sample that could have undergone sub-lethal injury during processing. After incubation, the culture was then streaked on pre-poured plates of Xylose Lysine Deoxycholate (XLD) (Conda S.A) agar and incubated at 37°C for 24 hours. Upon incubation, black colonies formed on the agar were considered to be presumptive of Salmonella spp. Where black colonies were observed, Triple Sugar Iron (TSI) (Himedia) agar slants were used for confirmation. A loopful of a presumptive colony was stubbed into the butt of the agar slant and the same wire loop was used to streak the slant, then incubated at 37°C for 24 hours. Presence of Salmonella spp was confirmed if the incubated butt turned from orange to yellow and the slant turned from orange to pink. Otherwise the black colony was not Salmonella spp. Simmon's citrate agar (Conda S.A) and urea agars (Conda S.A) were used as additional confirmation media.

Determination of Free Fatty Acid Profile

The fatty acid profile in each of the butter/ghee samples was analyzed using a gas chromatograph (GC) fitted with a Flame Ionization Detector (FID). A representative sample of the homogenized portion of the butter/ghee was weighed into a volumetric flask, a 10 ml volume of chloroform was added and the sample mixed for approximately 2 minutes, using Ultraturax (high-performance single-stage dispersion device). The mixture was centrifuged, filtered and dried. The dried sample was dissolved in di-ethyl ether, saponified and fatty acids extracted with hexane. The excess alkaline was removed from the hexane using demineralized water. The cleanup was repeated three to four more times and the hexane layer transferred into a GC (Column used: sol gel wax column or sp 2560 depending on the type of fatty acid profile of interest; GC conditions: 50°C, held for 5 minutes, ramped to 180°C at 20 degrees per minute, held for 0 minutes, ramped to 200°C at 2 degrees per minute and held for 11 minutes, and finally raised to 250°C at 2 degrees per minute and held for 2.5 minutes) with FID detection.

Sensory Evaluation

Four food samples were prepared for sensory evaluation. Ghee was added to a local dish of cassava and beans. Two food samples were prepared with ghee from traditional (505) and machine churner (606) prepared butter. The other two food samples were prepared with commercially available ghee products: Sameer brand (404) and Lubega brand (707). The two commercial samples were chosen to represent ghee produced in industrial settings (Sameer) and the less sophisticated cottage industrial settings (Lubega). Sensory evaluation was carried out using the nine-point hedonic scale [8], which has nine scales with word descriptions ranging from 'dislike extremely' to 'like extremely'. The instructions were designed to direct the test subject's attention to his/her feeling about the food rather than the food itself. The panelists (n=30) were college students and evaluated the organoleptic parameters of aroma, smell, taste, mouth feel and overall acceptability.

RESULTS

Microbial Analysis

The results from the standard enumeration are shown in Figs. 2A& 2B. Total viable counts in both traditionally made and machine churner made samples were in the 2-7

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Log cfu/g range with the exception of Ngoma sample at 10 Log cfu/g. The coliforms in the 1-3 Log cfu/g range were only detected in Kiboga traditionally made and machine churner made butter samples and none was detected in the other samples. Yeasts and molds counts were detected in all the samples with counts ranging from 2 to 5 Log cfu/g. The butter had more yeast and molds counts than ghee samples. The yeasts and molds were more in traditionally made samples than in machine churner made samples (Figs. 2C & 2D). *Staphylococcus aureus* was detected in all butter samples from Kiboga region (2 Log cfu/g) while butter obtained from other regions did not contain *Staphylococcus aureus*.

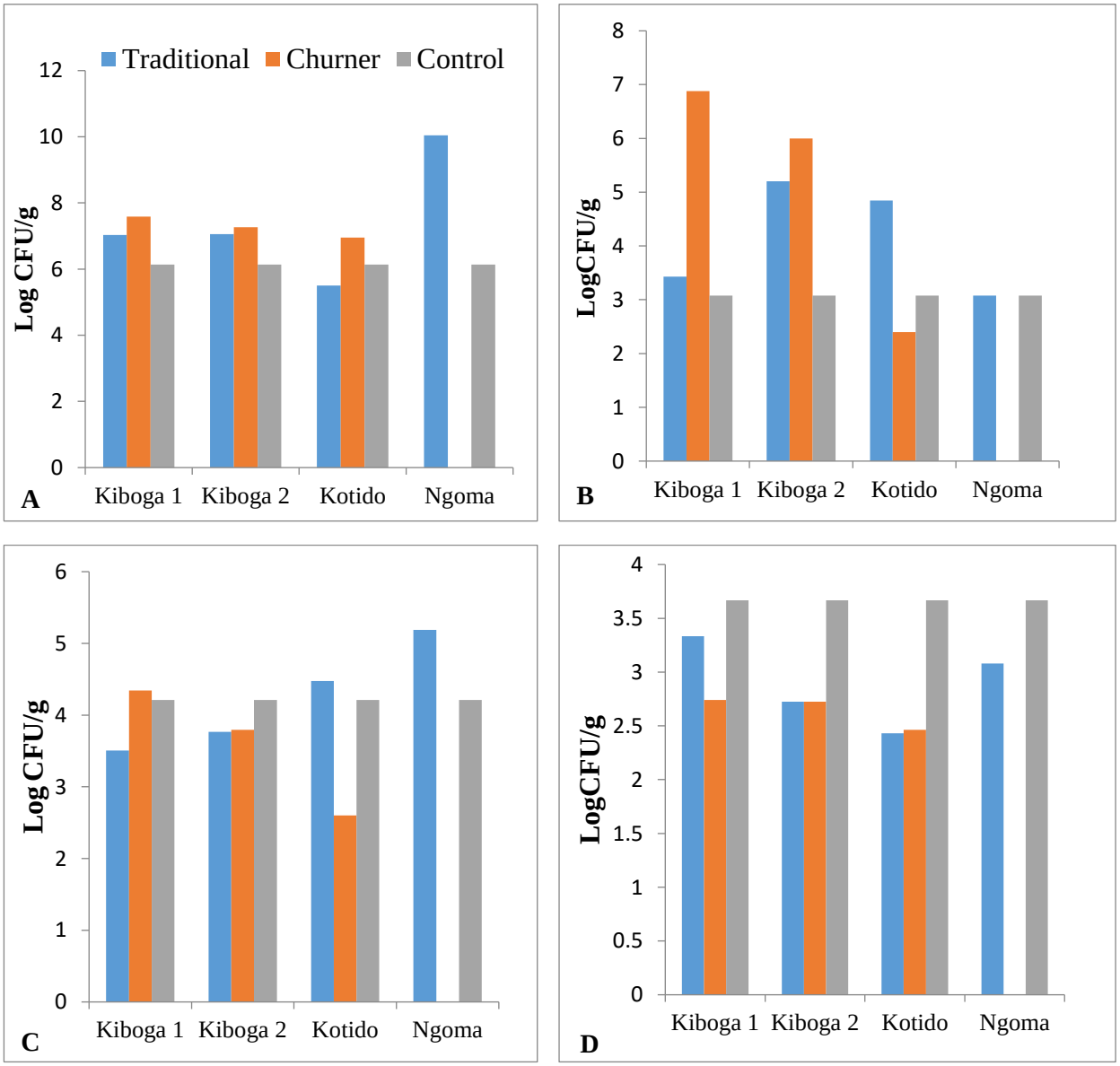

Figure 2: Microbial counts in butter and ghee produced using traditional and mechanized churning methods. A and B represent Total viable count in butter and ghee, respectively; C and D represent yeast and mold counts in butter and ghee, respectively

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The microbial counts were generally lowest in control samples with the exception of butter samples from Kiboga region 2. However, the control sample had the highest yeast/mold counts as observed in ghee samples. There was no machine churner sample collected from Ngoma.

Free Fatty Acid Composition

The different fatty acid percentage compositions for all the samples from the four regions (traditionally made and machine made) for both butter and ghee are shown in Tables 1 and 2, respectively. The saturated fatty acids were the most dominant in the butter and ghee samples and ranged from 70% to 82%. Their distributions were as follows: Palmitic acid (C16:0) varied between 33.26% and 35.67% in butter and between 30.16% and 34.06% in ghee. Myristic acid (C14:0) ranged from 17.29% to 19.18% in butter and 17.87% to 21.42% in ghee. Stearic acid (C18:0) was in the range of 5.62% to 8.30% in butter and 5.02% to 9.61% in ghee. Pentadecanoic acid (C15:0) ranged from 1% to 2.59% in butter and 1.88% to 3.67% in ghee. Heptadecanoic acid (C17:0) varied between 0.85% and 2.52% in butter and 0.68% and 2.39% in ghee whereas arachidic acid (C20:0) was in the 0.32% to 0.98% range in butter and 0.21% to 0.90% range in ghee.

The monounsaturated fatty acids with oleic acid was the most dominant and varied between 14.21%-18.8% in butter and 13.40%-19.29% in ghee. Other monounsaturated fatty acids and their percentage variations included: cis-10-pentadecenoic acid (C15:1), from 0.43% to 0.57% in butter and from 0.41% to 0.55% in ghee; cis- 11- eicosenoic acid (C20:1), from 0.00% to 0.95% in butter and from 0.00% to 0.73% in ghee; cis-10-heptadecanoic acid (C17:1), from 0.28% to 0.35% in butter and from 0.23% to 0.35% in ghee; palmitoleic acid (C16:1), from 0.8% to 2.83% in butter and from 0.94% to 2.65% in ghee; and myristoleic acid (C14:1), at 0.00% to 3.30% in butter and at 0.00% to 2.6% in ghee.

Polyunsaturated acids identified in butter and ghee included linoleic acid (C18:2Cis) at 0.06% to 1.62% in butter and 0.04% to 1.24% in ghee and alfa linolenic acid (ALA) at 0.00% to 0.45% in ghee and 0.00% to 0.34% in butter.

The omega-3-fatty acids included the ALA. The omega-6-fatty acids included the gamma-linolenic acid (0.03%- 0.12%) in butter and 0.00% to 0.11% in ghee while omega-9-fatty acids included the oleic acid in both butter and ghee. Trans-fatty acids were the least dominant for both butter and ghee.

Sensory Evaluation

The Student's *t*-test analysis of sensory evaluation results presented in Table 3 showed no significant difference among the mean scores for aroma, smell, taste, mouth feel and overall acceptability ($p > 0.05$) for all ghee samples. On a scale of 1 – 9, samples were ranked in the order of machine churner, Sameer brand, traditional and Lubega brand. The mean score results of the evaluated component are presented on the chart (Fig. 3). The traditional ghee generally scored higher in all aspects followed by machine churner ghee, Sameer ghee and lastly Lubega ghee brands.

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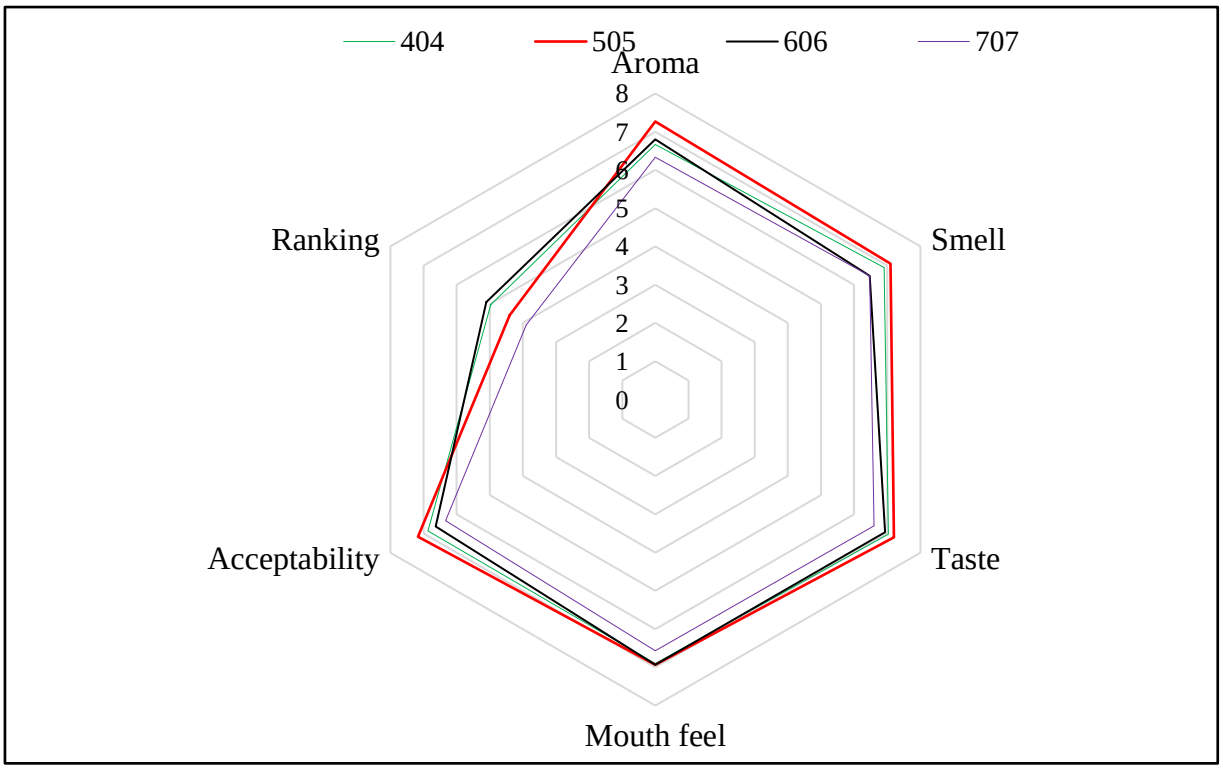

Figure 3: Mean scores for the sensory attributes of the ghee samples. Sameer dairy industry ghee (404), ghee from traditionally made butter (505), ghee from machine churner made butter (606) and Lubega company ghee (707) on market were used

DISCUSSION

Total viable counts observed from the analysis are similar to results reported by Rady and Badr [9] with their highest being 10^6cfu/g in butter. The high total plate count (7 Log cfu/g) observed in both the butter and ghee samples may be attributed to the absence of pasteurization. Adams and Moss [10] attributed the increase of total bacteria to the effect of both separation and churning process on the breaking up of bacterial clumps which increase their number. The total coliforms are in agreement with those of Idoui *et al*. [11] who detected coliforms at the highest with 2 Log cfu/g while Rady and Badr [9] reported 4 Log cfu/g. These results are also similar to those reported by Hassan [12] and Collins [13]. The general decrease in counts from butter to ghee indicated that aseptic boiling of butter to ghee destroyed some microbes. The decreased counts in ghee samples imply improvement of its safety for human consumption in food preparations. Yeasts and molds were high as compared to the 3 Log cfu/g in cow milk butter reported by Rady and Badr [9]. Mold and yeast growths are influenced by factors such as: sanitation during manufacture and ripening, length and degree of ripening, storage conditions (temperature, relative humidity, type and extent of packaging), water activity and composition of the product. The high counts of yeast and molds observed in the butter and ghee samples may be attributed to favorable growth conditions presented both in the ghee and butter samples during processing and under cold storage. Salmonella spp was

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not detected in any of the samples which is in agreement with Idoui *et al.* [11], signifying the safety of the product made with the machine churner.

The saturated fatty acids have detrimental effects on human health since they lead to increased blood cholesterol [14]. The predominance of palmitic acid observed in butter and ghee was similar in trend to that reported for other major fatty acids in milk fat [15, 16] and butter [17,18]. Ghee has higher fatty acid content than butter, which is attributable to the increased amount of medium and short chain fatty acids in ghee.

The sensory evaluation results showed that ghee made using machine churning method was not statistically different from that made using traditional churning or the commercial product in terms of taste, smell, aroma, and overall acceptability. This finding confirms that mechanized churning does not alter butter/ghee attributes that are important to consumer acceptability. The authors highly recommend vitamin (especially vitamin A) fortification for enhanced nutritional value, since most vitamins are destroyed by boiling during production of ghee from butter [5]. For most consumers, ghee is just added to the prepared food and not boiled in the food, thus maintaining the availability of the added heat-sensitive nutritional components.

CONCLUSION

Results from this study have shown that that butter made using mechanized churning method or process does not differ from that made following traditional method in terms of the microbial safety, free fatty acid profiles and organoleptic attributes. The results also provide evidence that ghee from the mechanically churned butter is indistinguishable from the traditionally made ghee in terms of taste, smell, aroma and overall acceptability. The microbiological content and fatty acid profiles are within acceptable ranges for consumption purposes.

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Table 1: Mean percentage composition of the different fatty acids in machine made butter and ghee*

	Kiboga 1		Kiboga 2		Kotido		Control	
Fatty acid composition (%)	**Butter**	**Ghee**	**Butter**	**Ghee**	**Butter**	**Ghee**	**Butter**	**Ghee**
Saturated fatty acids	74.4	77.02	74.72	75.84	77.02	77.63	77.73	81.9
Mono-unsaturated fatty acids	24.34	21.52	23.47	22.62	21.52	20.95	18.19	16.75
Polyunsaturated fatty acids	1.25	1.88	1.02	1.65	1.88	1.53	1.59	1.07
Trans-fatty acids	0.00	0.00	0.27	0.78	0.00	0.00	1.42	0.88
Omega-3 fatty acids	0.34	0.36	0.32	0.33	0.36	0.45	0.00	0.00
Omega-6 fatty acids	0.09	0.11	0.10	0.12	0.11	0.00	0.1	0.10
Omega-9 fatty acids	17.83	16.58	17.74	16.31	16.58	18	15.62	13.61

* The top part of the table provides the distribution of the three types of fatty acids. The bottom part of the table breaks down further the saturated fatty acids, the most dominant of which were the Omega-9 fatty acid and the least dominant of which were the trans-fatty acids, for both butter and ghee

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Table 2: Mean Percentage composition of the different fatty acids in traditionally made butter and ghee*

	Kiboga 1		Kiboga 2		Kotido		Ngoma	
Fatty acid composition (%)	**Butter**	**Ghee**	**Butter**	**Ghee**	**Butter**	**Ghee**	**Butter**	**Ghee**
Saturated fatty acids	75.47	76.04	75.84	76.25	75.36	77.52	75.83	80.49
Mono-unsaturated fatty acid	20.5	22.62	22.62	21.19	21.57	21.12	23.14	17.82
Polyunsaturated fatty acids	0.84	1.65	1.65	0.61	0.82	0.65	1.1	1.79
Trans-fatty acid	0.67	0.78	0.78	0.45	0.66	0.59	0.00	1.57
Omega-3 fatty acid	0.00	0.33	0.33	0.00	0.00	0.00	0.30	0.00
Omega-6 fatty acid	0.11	0.12	0.12	0.09	0.10	0.02	0.03	0.12
Omega-9 fatty acid	18.81	16.31	16.31	19.29	18.74	18.58	18.15	13.43

* The top part of the table provides the distribution of the three types of fatty acids. The bottom part of the table breaks down further the saturated fatty acids, the most dominant of which were the Omega-9 fatty acids and the least dominant of which were the Omega-3 fatty acids, for both butter and ghee

Table 3: Student t-test analysis results for the sensory attributes and ranking of ghee from churner butter and other ghee samples used in sensory evaluation

Attribute	P(606 vs. 404)	P(606 vs. 505)	P(606 vs. 707)
Aroma	0.660	0.180	0.236
Smell	0.211	0.104	1.000
Taste	0.603	0.357	0.432
Mouth Feel	0.936	1.000	0.404
Overall acceptability	0.593	0.279	0.514
Ranking	0.870	0.390	0.148

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