Compliance by SMEs to Existing Tanzania Standards with Respect *Escherichia coli* and *Staphylococcus aureus* in Cultured Milk: a Case of Dar Es Salaam, Tanzania

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Abstract

Small and medium enterprises are important for economic growth not only in Tanzania but all over the world. Most of them are informal, without official registration. However, they provide tremendous contribution to food security, nutrition, income generation and poverty reduction in the country. Therefore, upgrading and modernization of Small and Medium Enterprises (SMEs) to ensure food security, safety and quality of food has becomes one of the priorities of Tanzania development visions of 2025, which is the blueprint of structural economic transformation of the country. To help achieve this, the present study was conducted to assess the safety of cultured milk. A total of 33 cultured milk samples were collected from 11 SMEs which were analyzed in triplicate to make 99. Two microorganisms, Escherichia coli and Staphylococcus aureus were analysed in cultured milk. Results were compared against the existing Tanzania standards. The isolates had an overall mean of 2.02 × 102 ± 1.73 × 102CFU/g, 1.91×102 ± 1.85 ×102CFU/g respectively for E. coli and S. aureusc count. The results obtained were higher than the limits set by the Tanzanian Standard (TZS1625:2013). It may thus be concluded that sampled cultured milk was of poor microbial quality and hence not safe for consumption. Poor hygienic practices during milk handling pose a high risk to cultured milk and render it unsafe for human consumption.

Keywords: *Escherichia coli* and *Staphylococcus aureus*, SMEs, cultured milk, food safety.

Introduction

Milk and other dairy products are high-quality foodstuffs that contain proteins, fats, carbohydrates, vitamins and mineral salts (Rahman *et al.,* 2015). For this rich nutritional composition, bacteria can easily multiply in milk, and therefore an agent in the spread of human diseases (Hadžić *et al.,* 2017). Microbiological quality of milk products is influenced by the initial flora of raw milk, transport equipment, processing conditions and factory personnel and their occurrence in milk and milk products is of great significance they serve as an index of hygienic standards maintained during production, processing, packaging and transportation (Osman *et al.,* 2020).

The value chain of milk and milk products is dominated by small and medium-sized producers who mainly produce milk for domestic consumption. 90% of the milk produced is consumed at the farm level, while 10% goes through collection points. There are 221 milk collection points across the country, 173 of which have the cooling capacity and 64 are not in operation. Only 2.7% of the milk produced is processed. There are 99 milk processing units in the country that process 862,100 liters per day which are SMEs. The most important production and processing regions are Mara, Tanga, Arusha, Dar es Salaam and Iringa (Nell *et al.,* 2014). The main products from these processing industries (SMEs) are cultured milk (mtindi), pasteurized milk, ghee and butter, yoghurt and UHT milk (Paraffin *et al.,* 2019).

Cultured milk like other fermented dairy products is one of the most readily available and widely consumed foodstuffs in several

developing countries (Chimuti *et al.,* 2016), particularly in urban areas such as Dar es Salaam Nell *et al.,* (2014). Such products are not only available in supermarkets, mini-stores and cafeterias/restaurants, but are also sold by street vendors. This is probably due to increased awareness of the benefits of consumption of milk and milk products. In addition, it does not need high levels of expertise or processing equipment as compared to the other dairy by-products (Paraffin *et al.,* 2019).However, recent and reliable data about the safety of cultured milk in Tanzania is lacking. In view of the above, a microbiological study was conducted in order to investigate compliance by SMEs to existing Tanzania standards with respect to *Escherichia coli* and *Staphylococcus aureus* in cultured milk processed in Dar es Salaam.

Material and methods

This study was conducted in Dar es Salaam, which is a major commercial city, an administrative and industrial Centre of Tanzania (Chijoriga, 2017). The study involved a cross section design; where by data was collected at specific points in time. Purposive sampling technique was used to collect cultured milk from the Dairy Processing Units. There are 99 milk processing units in the country. However, only 11 SMEs were currently processing cultured milk in Dar es Salaam and hence were purposively drawn from the list of 99 Units and were included in this study. Sample for microbial analysis were randomly collected from cultured milk SMEs in Dar es Salaam from January 2021 to March 2021. Production per day was normally an average of 20 to 500 liters depending on the market outlets to their customers and availability of materials particularly raw milk. From each enterprise, three samples (500 mL each) were drawn making a total of 33 cultured milk samples from the 11 enterprises that were analyzed in triplicate to make a total of 99 data for each parameter (*Escherichia coli* and *Staphylococcus aureus*). Samples were collected from production lots not exceeding one week from production date as described by Chijoriga (2017). The samples were coded and transported in a cool box to the Department of Food Technology, Nutrition and Consumer Science (DFTNCS) SUA, Morogoro. They were immediately analyzed for physio-chemical and microbial qualities. The pH of each sample was determined by using a pH meter. The enumeration was carried out according to procedures described by ISO 16649-1 (2001). and ISO 6888:1983 for *E. coli* and *S. aureus* respectively. *E. coli* and *S. aureus* were identified by colony counting using colony counter device whereby the number of colonies obtained was expressed as colony forming units per gram of sample (CFU/g). Data was analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM-SPSS Inc., Chicago, USA). All values for microbial parameters and pH values were presented as Mean ± SD. Statistical differences between SMEs samples were determined by one-way ANOVA and Duncan multiple comparison post hoc test; $P < 0.05$ was considered statistically significant. One sample T-test was carried out to determine compliance of microbiological parameters with TZS 1625:2013 at $P<0.05$.

Results and discussion

pH of cultured milk samples

The pH values of cultured milk sample are presented in Table 1. Statistical analysis showed significant difference ($P<0.05$) in pH between cultured milk samples. Though the mean pH was found to be 5.5, sample collected from E3 had the lowest pH and was statistically different from all other samples. Sample collected from E11 were the second lowest and was also statistically different from other samples. These samples from (E3 and E11) were statistically different from all other samples at $P<0.05$. Studies by Olugbuyiro *et al.* (2011) and Akeem *et al.* (2018) indicated that under normal circumstances fermented milk should have a pH between 4.2 and 5.1. The pH recorded in this study majority were within the range (4.23 to 5.63) reported by De *et al.* (2014) but higher than the range (2.38 ± 0.81 to 3.2 ± 0.08) reported by Dirisu *et al.* (2015). This high pH could be attributed to low acidity of cultured milk which favors the growth of bacteria hence their presence in the product. Akeem *et al.* (2018) reported that variation of pH between fermented milk products is an indication of age

of the products and manner in which they have been handled. Other study Haj *et al.* (2007) found that types of culture (artificial culture or wild culture), specific time for incubation and temperature affect the desirable pH and acidity of the final products. The latter author explained that the higher and lower pH could be due to improper culture dosage, which largely affects the acidity and microbial quality of the final products (Akeem *et al.,* 2018). In case of cultured milk samples in the study area, proper fermentation conditions were not fully controlled and the pH does not fulfil the acceptable level of fermented standards.

Table 1: pH of the cultured milk samples from 11 SMEs in Dar es salaam, Tanzania

SMEs	N	n	Mean pH ± SD
E1	3	9	5.63 ± 0.16^{cde}
E2	3	9	5.85 ± 0.9^{f}
E3	3	9	4.86 ± 0.38^{a}
E4	3	9	5.63 ± 0.14^{cde}
E5	3	9	5.68 ± 0.04^{def}
E6	3	9	5.64 ± 0.59^{cde}
E7	3	9	5.72 ± 0.02^{ef}
E8	3	9	5.51 ± 0.14^{cd}
E9	3	9	5.5 ± 0.11^{cd}
E10	3	9	5.45 ± 0.63^{c}
E11	3	9	5.07 ± 0.35^{b}
Total	**33**	**99**	**5.5 ± 0.33**

Key: *N: Total number of samples analyzed for each SME*

n: Total number of data analyzed per SME

ND: Not detected

Each value reflects mean count ± standard deviation of the mean.

Mean values bearing different superscripts in each column are significantly different (P<0.05) according to Duncan Multiple Range Test

Microbial analysis of cultured milk sample

Escherichia coli

The current study revealed that 27 of all cultured milk samples were contaminated with *Escherichia coli* and only six samples were free. *Escherichia coli* is commonly used as an indicator, whereby its presence in food generally indicates fecal contamination and may also indicate the possible presence of disease-causing pathogens such as bacteria, viruses, yeast/moulds and parasites (Chijoriga, 2017).

The mean *Escherichia coli* value for each SME is presented in Table 2. Significant difference in *Escherichia coli* contamination was observed among SMEs at (P<0.05). *Escherichia coli* were not detected in neither sample from E1 nor sample E3. E8 was statistically different from all other samples except E10, both of which had values less than 100 CFU/g. The rest of the samples had higher values.

Non detection of *Escherichia coli* in samples from (E1 and E3) confirms that the products were well handled and prepared under GHP. The prevalence of *Escherichia coli* in some of the tested samples, suggest that it could be due to contaminated environment and unhygienic handling or preparation and implies a risk that other enteric pathogens may be present in the product. Similar observation was reported by Pyz-Łukasik *et al.* (2015). Detection of *Escherichia coli* in cultured milk samples in this study consonance with a study done by Kumar and Prasad (2010). However, they concurred with the previous study in Zimbabwe that showed lowest *Escherichia coli* count in milk and milk products Osman *et al.* (2020). Tadesse *et al.* (2020) reported that, absence of suitable area for storage and documented hygienic practice to be followed by handlers might contribute to high levels of contaminants in the samples. Other researchers reported that, poor hygiene in the environment resulted in the presence of *Escherichia coli* in their samples Yuen *et al.* (2012). Presence of *Escherichia coli* in foods and milk products violates regulations which prohibit the presence of pathogenic and spoilage microorganisms in any food item intended for human consumption (Pal *et al.,* 2016). Thus, effective hygienic handling of food intended for human consumption is of paramount as it helps to produce safe and quality products for the consumers, thereby reduce microbial contamination and loss of product economies (Tadesse *et al.,* 2020).

Table 2: *Escherichia coli* and *Staphylococcus aureus* (CFU/g) of the tested cultured milk from 11 SMEs in Dar es salaam, Tanzania

SMEs	N	n	Parameters	
			Escherichia coli **(Mean CFU/g)**	***Staphylococcus aureus*** **(Mean CFU/g)**
E1	3	9	ND	ND
E2	3	9	$3.35 \times 102 \pm 6.67 \times 101^{de}$	$2.81 \times 102 \pm 9.59 \times 101^{d}$
E3	3	9	ND	ND
E4	3	9	$3.48 \times 102 \pm 9.85 \times 101^{e}$	$5.63 \times 102 \pm 1.81 \times 102^{e}$
E5	3	9	$2.6 \times 102 \pm 7.47 \times 101^{c}$	$1.97 \times 102 \pm 8.16 \times 101^{c}$
E6	3	9	$3.33 \times 102 \pm 5.65 \times 101^{de}$	$2.63 \times 102 \pm 5.09 \times 101^{cd}$
E7	3	9	$2.8 \times 102 \pm 1.0 \times 102^{cd}$	$3.31 \times 102 \pm 6.2 \times 101^{d}$
E8	3	9	$1.43 \times 101 \pm 4.7^{a}$	$6.3 \times 101 \pm 6.3 \times 101^{ab}$
E9	3	9	$1.11 \times 102 \pm 7.9 \times 101^{b}$	$1.12 \times 102 \pm 1.4 \times 101^{b}$
E10	3	9	$6.33 \times 101 \pm 2.4 \times 101^{ab}$	ND
E11	3	9	$4.8 \times 102 \pm 7.1 \times 101^{f}$	$2.85 \times 102 \pm 9.11 \times 101^{d}$
Total	**33**	**99**	**$2.02 \times 102 \pm 1.73 \times 102$**	**$1.91 \times 102 \pm 1.85 \times 102$**

Key: *N: Total number of samples analyzed for each SME*
n: Total number of data analyzed per SME
ND: Not detected
Each value reflects mean count ± standard deviation of the mean.
Mean values bearing different superscripts letters in each column are significantly different ($P<0.05$) according to Duncan Multiple Range Test

Staphylococcus aureus

In this study, the presence of *Staphylococcus aureus* was also tested as an important microbiocidal parameter. Over 24 of cultured milk samples handled were contaminated with *Staphylococcus aureus* while the remaining nine were free from *Staphylococcus aureus*. All of the isolates were coagulase positive. The mean *Staphylococcus aureus* value for each SME is presented in Table 2. Significant difference in *Staphylococcus aureus* contamination was observed between SMEs at ($P<0.05$). Sample from E4 had the highest *Staphylococcus aureus* count and were statistically different from all other samples. Likewise, E8 had the lowest *Staphylococcus aureus* count and was also statistically different from all other samples except sample from E9. All other samples were statistically different from the three samples previously mentioned. *Staphylococcus aureus* was not detected in the sample collected from E1, E3 nor E10. These might be attributed to good sanitary practices during manufacturing, storage or packaging. Similar results were reported by Befekadu (2019). A previously study by Malavi *et al.* (2018) suggested that adherence to personal hygiene practices by food handlers is important in preventing cross contamination in food processing.

The occurrence of *Staphylococcus aureus* isolated in some of cultured milk samples was in consonance with a study done by Kamana (2016) and also by Tankoano *et al.* (2016) who reported on higher microbial counts in raw milk, sour milk (cultured milk) and artisanal yogurt from Burkina Faso. The reason could be attributed to contamination from the environment and prevalence of the genus on parts of the handler body such as hands, nose, skin and clothing. Other results by Hyera (2015) have indicated that, poor hygiene and lack of refrigeration during storage enables outgrowth of spoilage and pathogenic microbes and thus a potential source of cross contamination during processing. A study by Tarekgne *et al.* (2015) also reported higher counts for ***Staphylococcus***

aureus in their tested samples was influenced by poor sanitary and hygienic practices among handlers.

Higher populations of *Staphylococcus aureus* have been linked to gastroenteritis, boils, skin infections, pneumonia, deep abscesses and meningitis in debilitated persons produced by enterotoxin (Tankoano *et al.,* 2016). Therefore, in order to minimize the health risks associated with *Staphylococcus aureus* contamination, processor and handlers need to take necessary actions to maintain food safety (Kumar and Prasad, 2010). Maintenance of the proper hygienic conditions during the processing of milk can reduce the prevalence of *Staphylococcus aureus* in the milk product (Tarekgne *et al.,* 2015).

Compliance of SMEs to microbial safety with respect to yeast *E. coli* and *S. aureus*

The present study investigated on compliance of cultured milk samples with respect to *Escherichia coli* and *Staphylococcus aureus* against TZS 1625:2013. The overall mean isolates for *Escherichia coli* and *Staphylococcus aureus* respectively are shown on Table 3. The overall average microbial parameters observed in the present study is beyond the acceptable standard set of TZS 1625:2013 for cultured milk, which could potentially be hazardous to human health (Befekadu, 2019). Therefore, samples in the study area were categorized as unsafe for human consumption as the average mean value obtained was significantly higher than the legal limits as stipulated in TZS 1625:2013 at $P<0.05$ (Table 3.) The average mean count of *Escherichia coli* and *Staphylococcus aureus* isolated from cultured milk produced in the study area was in agreement with a study done by Kumar and Prasad (2010); Pyz-Łukasik *et al.* (2015); Chimuti *et al.* (2016) Contamination of cultured milk with *Escherichia coli* and *Staphylococcus aureus* could have originated from poor environmental condition and personal hygiene of the handlers. Modupe *et al.* (2019) reported that contamination of fermented dairy products could also result from inappropriate processing, incomplete heating, or secondary contamination via contact with contaminated equipment and utensils. Thus, effective hygienic handling of food intended for human consumption is paramount as it is a key to preventing the risk of food borne diseases, spoilage and injury to human health and economies (Dirisu *et al.,* 2015).

Conclusion

Based on the findings from this study, cultured milk samples obtained from different SMEs are not fit for human consumption. This is because they were found to be contaminated with the diseases causing organisms above the maximum acceptable level according to TZS 1625:2013. During the surveillance study and also during the period of sample collection, hygiene at workplace, safety and quality control measures and hygienic practices by handlers was observed to be a problem. This might be a greatest link to cultured milk contamination. Therefore, handlers should be educated on the health effects of poor personal and environmental hygiene and good handling practice.

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Table 3: Compliance of microbial count- *E.coli* and *S. aureus* (CFU/g) to TZS 1625:2013

Parameters	Mean ± SD (CFU/g)	Test value (TZS 1625:2013)	P-value
E. coli (CFU/g)	2.02 × 102 ± 1.73 × 102	Shall be absent (0)	0.000*
S. aureus (CFU/g)	1.91×102 ± 1.85 ×102	< 100	0.000*

**Mean significant at P= 0.05 level*

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