Abstract

Milk is a complex biological fluid which is rich in nutrients, by its nature it is a good growth medium for many micro-organisms. Bacterial contamination of milk may arise from various sources due to poor storage and handling. The coliform bacteria count in milk is a major feature in determining its quality. This study sought to investigate microbial contamination of raw and boiled milk sold at Baraton Center. Standard plate count and coliform count was used to analyze the presences of coliform bacteria. The study results showed that all the raw milk samples contained ≥100000CFU/ml of total bacteria count while 60% samples had >50000CFU/ml coliform bacteria. The results of this study is a clear indication that the milk sold at Baraton center could be contaminated with pathogenic microbes and is an eye opener to the consumers on the quality of milk and the potential health hazards.

Key words: Coliform Bacteria, milk quality, Standard plate count (SPC)

Introduction and Literature Review

Milk plays a major role in the human diet because of its rich nutritional content. Microbial quality of milk is therefore a major concern to consumers of milk and milk products. Bacterial contamination of raw milk can generally occur from three main sources; within the udder, outside the udder, and from the surface of equipment used for milk handling and storage. (Arenas et al., 2004). Many milk-borne epidemics of human diseases have been spread by contamination of milk by dirty hands of dairy workers, unsanitary utensils, flies and polluted water supplies. (Parekh & Subhash, 2008). Presence of bacteria in raw milk reduces the keeping quality of milk and certain bacteria with their associated enzymes and toxins may even survive pasteurization creating health hazards (Salman & Hamad, 2011). In Kenya it is estimated that about 85 percent of marketed milk is sold raw (FAO 2011) and like any other developing country there is a great public health risk associated with milk due to inadequate monitoring of the unprocessed milk industry (FAO, 2011). Coliforms E.g. *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter* are considered as normal flora of intestinal tract of human and animals. They have been used as indicator organisms for bacteriological quality of milk and its products (Chatterjee et al., 2006). Generally, counts greater than 100000 CFU/ml would indicate poor milking hygiene or other sources of contaminants (Wallace, 2008).

The purpose of this study was to determine the microbial quality of raw and boiled cow’s milk from different sampling points at Baraton Center in Nandi County of Kenya.

Materials and Methods

Sample Collection and Preparation

Milk samples were collected from 10 different selling points at Baraton center. About 100 ml of milk were collected in sterile glass bottles and transported directly to the laboratory at the University of Eastern Africa Baraton, Department Biological Sciences within one hour for analysis. Each sample was divided into two parts and one part boiled and the other treated as raw sample.

Methylene Blue Reduction (MBR) Test

To determine the ability to reduce methylene blue 10 ml of each milk sample transferred to a new sterile test tubes. To each tube 1 drop of methylene blue added the tubes were tightly capped and inverted several times to mix. the tubes were then incubated in water bath at 37°C and checked for color change from blue to white 30 minutes after incubation and there after at hourly intervals for 8 hours (Oliver et
Reduction was demonstrated by a change of color of the samples from blue to white.

Standard Plate Count and Coliform Count

Milk samples were serially diluted in peptone water. From the tubes with dilutions of $10^{-1} – 10^{-5}$, 1ml was pipetted and inoculated in standard plate count agar using pour plate method. The plated sample was allowed to solidify and then incubated at 37°C for 48 hours. Negative control was done using plate count agar only (Harley, 2013). For coliform count the milk samples were shaken 25 times then diluted in using the same dilution procedure used for standard plate count. The samples were inoculated on MacConkey’s agar using the spread plate method. The plates were then incubated at 37°C for 36 hours (Harley, 2013). The number of colonies were recorded using a colony counter. Only the plates with 30-300 colonies were considered in calculating the colony forming units (CFU) per ml of sample.

Statistical Analysis

Analyses of variance (ANOVA) for the comparison of geometric means were performed using SAS software (www.sas.com) based on the results of microbiological analyses. The significance level adopted was 5%. The data was entered. The CFU/ml of sample was determined using the formula

\[ \text{CFU per ml of sample} = \frac{\text{number of colonies}}{\text{amount plated} \times \text{dilution}} \]

Results

Methylene Blue Reduction (MBR) Test

Methylene blue was reduced in less than 30 minutes of incubation in 80% of the raw milk samples and 60% of the boiled milk samples which is an indication of presence of microbes.

Standard Plate Count and Coliform Count

In all the samples the total bacterial count was significantly higher (P<0.005) than the coliform count (Figure 1).

![TOTAL BACTERIAL COUNT vs COLIFORM COUNT](image)

*Figure 1. Total bacterial count compared to coliform count in raw samples.*

Boiling reduced significantly both total bacterial count and coliform count (P=0.034). The total bacterial count in raw milk was high with 70% of the samples having ≥ 100,000 CFU/ml while after boiling, only 40% of the samples had ≥ 100,000 CFU/ml total bacterial count (Figure 2).
In addition, the coliform count in 60% of the raw milk samples was > 50000 cfu/ml while after boiling the coliform count reduced and only 40% of the boiled samples had > 50000 CFU/ml coliforms (Figure 3). In the boiled milk samples, 40% of the samples had > 100,000 CFU/ml total bacterial count while 40% of the samples had 50000 CFU/ml coliforms.

Figure 2. Total bacterial count in boiled and raw milk samples.

Figure 3. Coliform count in boiled and raw milk samples.
Discussion

Total bacterial count is a rough gauge to measure the quality of milk, herd health, efficacy of farm sanitation, milk handling and storage and transportation temperature while coliform counts are especially associated with level of hygiene since they are mainly of fecal origin (Omore et al., 2002). The results of this study clearly shows that the total bacterial count in the milk sold at Baraton Center is below the Kenya Bureau of Standards (KEBS, 1996) and East African Community Standard (EACS, 2007) set values for good milk which is (<200000 CFU/ml). However, the coliform count is above the standards (<50000 CFU/ml). These high counts are linked with poor sanitation during milking and milk handling along the value chain. It may have also arisen due to contamination from mixing of milk from different farms. Coliform organism can rapidly build up in the moist residues on the milking equipment and become a source of contamination for the milk (Omore et al. 2001). The milk sold at Baraton Center is usually transported in plastic containers which may also serve as a source of contamination as a result of milk residues remaining in the parts of the container that are not easy to reach during cleaning. These residues serves as the growth media for bacteria which contaminates fresh milk added to the containers. Keeping milk in clean containers at refrigerated temperatures immediately after milking process may delay the increase of initial microbial load and prevent the multiplication of micro-organisms in milk between milking at the farm and transportation to the processing plant (Bonfoh et al., 2003). Lack of cold storage facility may also have enhanced the microbial growth. High coliform counts is indicative of faecal contamination of milk with either from animal or human origin and environmental materials (Robinson, 2002) this clearly reveals the risk of presence of pathogenic enterobacteria in the samples (Chye et al., 2004) This studies are in line with studies done in other parts of Kenya (Omore et al., 2002; Wambua et al., 2011).

Conclusion

In conclusion the raw milk sold in the study area has poor microbial quality and therefore poses great health risk to the consumers. There is need for the milk handlers within the value chain implement good hygiene practices and proper storage of milk before it is finally delivered to the consumers. Further studies need to be done to analyze the microbial contamination within the value chain of milk to clearly identify the origin of contaminants.

Acknowledgement

The authors acknowledge and are very grateful to all the vendors who supplied the milk samples used in the study. We also thank University of Eastern Africa, Baraton department of biological sciences for supporting the project.

References

the United Nations.


