Sampling plan impact on the microbiological assessment of raw milk cheese

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Introduction

The traditional Georgian cheese Imeruli Kveli is produced from cow milk to which 20 % of buffalo or goat milk can be added. It is produced in the Imereti region (western Georgia) and most of the manufacture is based on the artisanal process of rennet curd made from raw milk [1]. Microbial variability in the production environment is particularly important for fermented foods, which rely on the action of microorganisms for their production. Many modern fermentation practices employ starter cultures as a means of standardizing the fermentation process. In the production, Imeruli Kveli producers don't use starter cultures. The cheese made from milk that has not undergone heat treatment may represent a food safety concern, especially pathogenic bacteria, such as *E. coli* and *Enterococcus faecalis* which enter the food by way of the raw ingredients or the food processing environment. They can also participate during fermentation and are sometimes attributed to quality changes in the finished product [2,3].
A primary goal of modern cheese manufacturing is consistent product quality. One aspect of product quality that remains poorly understood is the variability of pathogenic microbial subpopulations due to temporal or facility changes within cheese production environments. Therefore, our aim was to quantify this variability by measuring by days and the storage condition in the cheese microbiome changes.

Methods and Materials

Six cheese samples were prepared under laboratory conditions following the production steps described in the PGI document [1]. The samples were stored in different conditions: in the refrigerator, at room temperature, and in brine. The microbiological indicators of each sample were monitored at each relevant manufacturing step: in raw milk, fresh cheese, and fermented cheese. Detection and enumeration of targeted microorganisms in the samples Total Viable Counts, *Enterobacteriaceae*, and *Escherichia coli* were monitored by standard culture method. Thus, one ml from dilution was inoculated on commercially available selective and differential growth media [4,5,6].

Figure 1. Production steps of cheese Imeruli Kveli

Figure 2. Pathogenic microorganisms in prepared cheese.

Results and Discussion

The traditional cheese Imeruli Kveli are favourite artisanal product of Georgia that have obtained PGI recognition. In their original versions, they are currently produced only by small producers and are distributed only locally or regionally.
This work provides the data distributions and variabilities of microbiological indicators of raw milk, cheese curds according to an artisanal procedure during laboratory preparation. They include the total viable count (TVC), *E. coli* (EC), and *Enterobacteriaceae* (EN) at each relevant manufacturing step: in raw milk, fresh cheese after coagulation, 48 h of fermentation, and 72 h of fermentation. Titric acidity(TA), pH and protein were examined in milk before cheese preparation. Results showed that pH was 5.43, TA 5.4 g/l and protein was 3.57 %.
The microbiological quality of the raw milk examination resulted positive for *Enterobacteriaceae*, and among these, *E. coli* was evidenced.

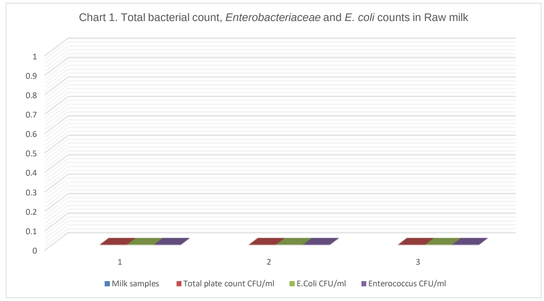

Chart 1. Total bacterial count, *Enterobacteriaceae* and *E. coli* counts in Raw milk

Results of Total Viable Counts, *Enterobacteriaceae*, and *E. coli* enumeration showed that temperature has a consistent effect on the inactivation rate of all microorganisms. Brined cheeses have lower pH and much higher undissociated lactic acid levels and lower pathogen rates were observed. Results of *Enterobacteriaceae* and *E. coli* enumeration showed a bad hygienic level in all samples tested.

Sample number	Total viable count	*E.Coli*	*Enterobacteriaceae*	Total plate count	*E.Coli*	*Enterobacteriaceae*	Total plate count	*E.Coli*	*Enterobacteriaceae*
	CFU/g after 24 hours 10^5			CFU/g after 48 hours 10^5			CFU/g after 72 hours 10^5		
1	15	8	4	911	102	121	655	411	272
2	66	28	21	755	458	263	752	890	257
3	33	14	3	572	123	96	428	72	77
4	67	26	60	1040	581	105	1080	520	227
5	57	6	1	418	17	26	155	5	0
6	79	30	73	191	109	126	400	545	27

Chart 2 . The average counts of microorganisms determined in the cheeses produced from raw milk
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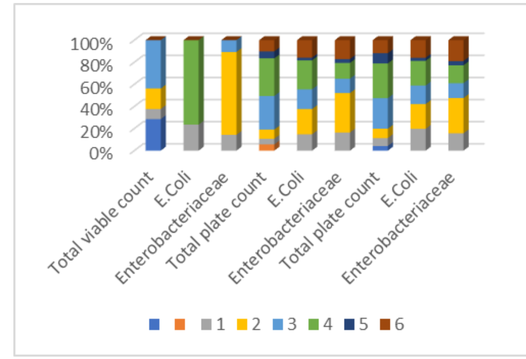

Chart 3. Total bacterial count, *Enterobacteriaceae*, and *E. coli* counts in cheese samples .

Conclusions

In Georgia, traditional cheese-making processes are carried out in small and artisanal enterprises closely linked to the area of origin. Since the traditional method of cheese production technology involves the use of raw milk, it continues to be a concern because small enterprises cannot be controlled properly. This study provides an overview of the behavior of microorganisms during the artisanal production of Georgian fermented cheese Imeruli Kveli. The obtained data point to a potential hazard of microbial growth in an early stage of milk and curd fermentation, which is incorporated into this manufacturing method. Therefore, it is necessary to know the relations between microbiological quality and safety data and artisanal manufacturing conditions, including the efficacy of critical process steps. More data is needed to assess sampling plan impact on microbiological assessment or validate the potential exposure to *E. coli* or *E. Faecalis* from the consumption of artisanal cheese. Estimation of uncertainty of quantitative determinations derived by the cultivation of microorganisms is required to get more accurate results

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