COMPARATIVE STUDY ON MICROBIOLOGICAL EVALUATION OF CHEESE COLLECTED FROM TWO DIFFERENT MARKETS

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Abstract

The microbiological quality of milk and dairy products is influenced by the initial flora of raw milk, the processing conditions, and post-heat treatment contamination. Therefore, poor sanitary practices in local cheese processing and distribution results in public or consumers health hazard due to the presence of pathogenic bacteria, mold and yeast. In Hawassa two different markets provide cheese for consumers one from local market and the other is from super markets. This research activity was initiated with the objective to assess microbial quality and hazards of cheese in relation to environmental condition of cheese markets. In this study a microbial load of 12 samples collected from the local market and 12 samples collected from super markets were determined. 1 gram cheese was added to 9 ml peptone water and homogenized in stomacher bag. Then appropriate serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} & 10^{-5}) of all samples were prepared and then 0.1 ml of the odd power dilutions were taken and plated using Standard Medias for each bacteria, mold and yeast. The result indicate that the total aerobic bacterial count(APC) of all samples from local markets(LM) and 5 samples from super markets(SM) shows the highest count($>10^5$cfu/g) from recommended level. Despite three samples from local market and four samples from super markets which show mold growth at the highest level of microbiological risk category the rest samples from both markets didn't show any growth. The highest Lactic acid bacteria (LAB) and Staphylococcus species from local market was found ($3.1x10^5$cfu/g) and ($6.2\ x10^5$cfu/g) respectively and the lowest was found ($<1x10^1$ cfu/g) and ($<1x10^1$ cfu/g) from respectively. Pathogenic bacteria, Salmonella Species was isolated from 9 samples of local markets and 3 samples from super markets while Shigella Species was isolated from 8 samples collected from local markets and 5 samples collected from super markets. The highest microbial load observed from local market except total yeast count could be due to human contact through air particles breathed, coughed or sneezed out during the course of work or from food handlers or from other sources in the air within the vending area. The results indicate the unhygienic conditions prevailing during distribution or sale where most of the products are sold in open containers at local market. Therefore, precautions should be taken to prevent contamination during post harvest handling and processing of cheese.

Key words: *Cheese, risk, microbial load, local market, super market*

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1. Introduction

Cheese is a fresh or matured product obtained by draining after coagulation of the whole, skimmed or partially skimmed milk. Its principle of processing is based on the coagulation of the protein in milk, during which about 90% of the milk fat is encapsulated. The coagulated mass is called curd, the remaining liquid is called whey. Curd

consists mainly of milk proteins (casein) and milk fat; while whey mainly contains water, milk sugar (lactose), protein (serum proteins) and B-vitamins (Pauline, 2006).

The characteristics and the technology which are described suggest in developing countries traditional milk product like butter and cheese are made in general under primitive conditions which results in low yield and poor quality of the product. Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms (Ruegg, 2003 ; Rajagopal, 2005). The microbiological quality of milk and dairy products is influenced by the initial flora of raw milk, the processing conditions, and post-heat treatment contamination (Richter, 1992).Therefore, poor sanitary practices in local cheese processing and distribution results in public or consumers health hazard due to the presence of pathogenic bacteria, mold and yeast. Several microorganisms like spoilage and pathogens such as coli-forms, lactobacilli, heat resistant staphylococcus can grow in cheese or on the surface (Zottola and Smith, 1979).

In Hawassa two different markets provide cheese for consumers one from traditional market(local market) and the other is from super markets. The super markets has special site for selling of cheese where modern facilities like refrigerator, electric, attractive packaging, clean environment and salesman where as the local market have dirty, damp and unhealthy place, poor storage and packaging facilities which encourage microbial contamination from different sources. But collection of cheese in both types of market is from traditional cheese processors. Thus, a comparative study of microbial load in cheese of two types of market environments may give a clear idea about health hazards.

Little is known about the microbial quality and acceptability of the locally made cheese in Hawassa. It is thus, imperative to assess and determine the microbial quality and safety of the cheese in order to determine its level of hazardousness towards the consumer's health. Therefore, this research activity was initiated with the objective to assess microbial quality and hazards of cheese in relation to environmental condition of cheese markets.

2. Materials and Method

2.1 Study Area

Hawassa town is situated in Sidamo Zone, Southern Nations Nationalities and Peoples of Ethiopia region, Ethiopia 273 Km far from Addis Abeba to the south. Four local markets and for super markets were selected for the sample collection.

2.2 Sample Collection

Cheese samples were collected from four local and four super markets three times each using a sterile aseptic glass jar and cool transported using ice and ice box to Hawassa University Food Science laboratory and Biology laboratory.

2.3 Sample Analysis

Microbial load analysis was conducted at Food Science laboratory three times for twelve samples and Biology laboratory three times for twelve samples. Immediately after arrival, 1 gram cheese was added to 9 ml peptone water and homogenized in stomacher bag. Then appropriate serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} & 10^{-5}) of all samples were prepared and then 0.1 ml of the odd power dilutions were taken and plated using spread and pour plate technique in duplicate by using a Standard Medias for each bacteria, mold and yeast for a total of ten parameters (Richardson, 1985; APHA, 1984).

2.4 Microbial Count

Finally, it was incubated and then number of colony was counted and colony forming units was calculated by multiplying number of colony by its serial dilution factors.

2.5 Statistical analysis

Average colony forming units of microbial load was calculated using descriptive statistics of spread sheet Microsoft excel.

3. Result

The individual results of microbiological analysis conducted on samples collected from two different markets were presented on table 1 and table 2.

Among the local market samples, the highest count of APC was found ($8.3x10^7$cfu/g) collected from LM-4 and the lowest count ($2.2x10^4$ cfu/g) from LM-2(Table 1) where as among the super market samples, the highest count of APC was found ($3.4x10^6$ cfu/g) from SM-1 and the lowest count ($2.0x10^4$ cfu/g) from SM-2 (Table 2). Besides, APC was beyond the acceptable limits ($>10^5$ cfu/g) almost all the samples of local markets while only 3 samples were beyond the acceptable limit from super market.

Mold count in samples collected from the local markets, the highest count was found ($2.4x10^3$cfu/g) collected from LM-4 and the lowest count ($<1x10^1$cfu/g) from LM-1,2,3,4 (Table 1) and from super markets the highest count was found ($5.6x10^3$cfu/g) collected from LM-3 and the lowest count ($<1x10^1$cfu/g) from LM-1,2,3,4.

LM-2 shows the highest count ($3.6x10^5$cfu/g) and LM-1shows the lowest count ($<1x10^1$ cfu/g) whereas SM-1 shows the highest count ($7.1x10^5$cfu/g) and LM-1shows the lowest count ($<1x10^1$ cfu/g) for total yeast count.

Highest E-coli count from local markets was found ($6.1x10^3$cfu/g) from LM-1and the lowest was ($<1x10^1$ cfu/g) from LM-4 while for super markets ($3.9x10^3$cfu/g) from SM-1 was the highest and ($<1x10^1$cfu/g) from SM-2,3 was the lowest count. Highest total coliform count from local markets was found ($1.6x10^3$cfu/g) from LM-4and the lowest was ($<1x10^1$ cfu/g) from LM-3 while for super markets ($8.2x10^2$cfu/g) from SM-4 was the highest and ($<1x10^1$ cfu/g) from SM-2,3 was the lowest count.

Fecal coliform count from local markets were ranging between ($8.5\ x10^3$ cfu/g) from LM-1 and ($<1x10^1$ cfu/g) from LM-4 ; from super markets ranging between ($2.5\ x10^2$ cfu/g) from SM-4 and ($<1x10^1$ cfu/g) from SM-1,2.

The highest Lactic acid bacteria and Staphylococcus species from local market was found ($3.1x10^5$cfu/g) from LM-3 and ($6.2\ x10^5$cfu/g) from LM-4 respectively and the lowest was found ($<1x10^1$ cfu/g) from LM-3,4 and ($<1x10^1$ cfu/g) from LM-1 respectively.

Salmonella Species was isolated from nine samples of local markets and three samples from super markets while Shigella Species was isolated from eight samples collected from local markets and five samples collected from super markets.

Local markets mean log value of all microbial analysis parameters except yeast species were greater than the super market (Fig. 1).

The present study shows that the total aerobic bacterial count of all samples collected from local markets and five samples collected from super markets left under highest microbiological risk category as it shows highest count($>10^5$cfu/g) from recommended level for human consumption. Despite three samples from local market and four samples from super markets which show mold growth at the highest level of microbiological risk category the rest samples from both markets didn't show any growth. For yeast count six samples from local markets and seven samples from super markets show highest microbiological risk category through the growth beyond the acceptable limit ($<10^4$cfu/g) which could potentially injurious to health and/ or unfit for human consumption while one samples from local market and one sample from super market left under moderate risk. As regard E-coli nine samples, total coliforms ten samples and fecal coliforms nine samples collected from local markets and three, five and five samples respectively collected from super markets shows growth that exceeds the acceptable limit where as the rest samples left under the acceptable microbial load. Unlike six samples from local markets and seven samples from super markets show the highest growth for lactic acid bacteria the rest samples left under the acceptable limit which fits for human consumption while two samples from each markets left under moderate microbiological risk

category. Seven samples from local market and five samples from super markets were show the highest risk through the growth of Staphylococcus Species beyond the acceptable limits ($<10^4$cfu/g).

Table 1 Average CFU/gram of twelve cheese samples collected from local markets

No	APC	Mold	Yeast	E.coli	Coliform	Fecal Coliform	LAB	Staphylococcus Spp*	Salmonella Spp*	Shigella Spp*
$LM1_1$	$9.0x10^5$	$<1x10^1$	$1.1x10^4$	$6.1x10^3$	$4.1x10^2$	$8.5\ x10^3$	$6.1x10^4$	$1.8\ x10^4$	**Isolated**	**Isolated**
$LM1_2$	$6.7x10^5$	$<1x10^1$	$<1x10^1$	$1.2x10^2$	$3.1x10^2$	$1.4\ x10^2$	$2.4x10^2$	$<1x10^1$	**Isolated**	**Not isolated**
$LM1_3$	$3.4x10^6$	$<1x10^1$	$<1x10^1$	$4.1x10^2$	$1.2x10^2$	$<1x10^1$	$5.7x10^2$	$5.4\ x10^4$	**Isolated**	**Isolated**
$LM2_1$	$1.9x10^6$	$1.9x10^3$	$<1x10^1$	$2.5x10^3$	$3.6x10^2$	$1.0\ x10^2$	$3.1x10^4$	$2.1\ x10^5$	**Isolated**	**Isolated**
$LM2_2$	$2.2x10^4$	$<1x10^1$	$3.6x10^5$	$<1x10^1$	$1.3x10^3$	$<1x10^1$	$5.4x10^3$	$<1x10^1$	**Not isolated**	**Not isolated**
$LM2_3$	$5.3x10^6$	$<1x10^1$	$6.2x10^4$	$3.6x10^2$	$1.5x10^2$	$4.7\ x10^3$	$1.6x10^3$	$<1x10^1$	**Isolated**	**Isolated**
$LM3_1$	$4.1x10^6$	$<1x10^1$	$7.0x10^2$	$1.2x10^2$	$<1x10^1$	$4.1x10^3$	$<1x10^1$	$8.6\ x10^4$	**Isolated**	**Isolated**
$LM3_2$	$3.9x10^6$	$<1x10^1$	$<1x10^1$	$4.5x10^3$	$3.6x10^2$	$1.0\ x10^3$	$<1x10^1$	$4.2\ x10^5$	**Not isolated**	**Not isolated**
$LM3_3$	$4.8x10^6$	$<1x10^1$	$2.7x10^4$	$5.3x10^2$	$<1x10^1$	$1.0\ x10^2$	$3.1x10^5$	$<1x10^1$	**Isolated**	**Not isolated**
$LM4_1$	$2.7x10^5$	$2.4x10^3$	$<1x10^1$	$2.0x10^2$	$4.7x10^2$	$<1x10^1$	$<1x10^1$	$6.2\ x10^5$	**Isolated**	**Isolated**
$LM4_2$	$7.3x10^6$	$<1x10^1$	$1.3x10^4$	$<1x10^1$	$1.6x10^3$	$1.5\ x10^2$	$1.1x10^4$	$<1x10^1$	**Isolated**	**Isolated**
$LM4_3$	$8.3x10^7$	$6.1x10^2$	$4.1x10^4$	$<1x10^1$	$7.4x10^2$	$<1x10^1$	$<1x10^1$	$4.4\ x10^4$	**Not isolated**	**Isolated**

**Local market(LM), Aerobic bacterial count(APC), Lactic acid bacteria(LAB), Species(Spp*)

Table 2 Average CFU/gram of twelve cheese samples collected from super markets

No	APC	Mold	Yeast	E.coli	Coliform	Fecal Coliform	LAB	Staphylococcus Spp*	Salmonella Spp*	Shigella Spp*
$SM1_1$	$5.7x10^4$	$2.1x10^2$	$7.1x10^5$	$<1x10^1$	$<1x10^1$	$<1x10^1$	$6.1x10^3$	$<1x10^1$	**Isolated**	**Not isolated**
$SM1_2$	$3.4x10^6$	$<1x10^1$	$<1x10^1$	$3.9x10^2$	$7.4x10^2$	$<1x10^1$	$3.9x10^4$	$<1x10^1$	**Not isolated**	**Isolated**
$SM1_3$	$7.3x10^6$	$<1x10^1$	$1.3x10^4$	$<1x10^1$	$<1x10^1$	$1.0x10^2$	$1.1x10^4$	$7.0\ x10^4$	**Not isolated**	**Not isolated**
$SM2_1$	$2.9x10^4$	$1.6x10^3$	$<1x10^1$	$<1x10^1$	$1.5x10^2$	$1.0x10^2$	$4.7x10^3$	$2.4\ x10^5$	**Not isolated**	**Isolated**
$SM2_2$	$4.4x10^4$	$<1x10^1$	$2.0x10^5$	$<1x10^1$	$<1x10^1$	$<1x10^1$	$1.6x10^4$	$5.3\ x10^5$	**Not isolated**	**Isolated**
$SM2_3$	$2.0x10^4$	$<1x10^1$	$5.4x10^3$	$3.0x10^2$	$<1x10^1$	$<1x10^1$	$3.3x10^4$	$<1x10^1$	**Isolated**	**Not isolated**
$SM3_1$	$6.1x10^5$	$<1x10^1$	$1.8x10^4$	$<1x10^1$	$<1x10^1$	$1.0x10^2$	$<1x10^1$	$<1x10^1$	**Isolated**	**Isolated**
$SM3_2$	$2.9x10^4$	$5.6x10^3$	$<1x10^1$	$<1x10^1$	$<1x10^1$	$<1x10^1$	$4.7x10^3$	$<1x10^1$	**Not isolated**	**Not isolated**
$SM3_3$	$2.4x10^5$	$2.4x10^2$	$<1x10^1$	$<1x10^1$	$4.3x10^2$	$1.1x10^2$	$7.8x10^2$	$3.1\ x10^5$	**Not isolated**	**Not isolated**
$SM4_1$	$5.4x10^4$	$<1x10^1$	$3.1x10^4$	$<1x10^1$	$6.4x10^2$	$2.5\ x10^2$	$<1x10^1$	$<1x10^1$	**Not isolated**	**Not isolated**
$SM4_2$	$6.2x10^4$	$<1x10^1$	$7.1x10^5$	$4.1x10^2$	$8.2x10^2$	$<1x10^1$	$<1x10^1$	$2.3\ x10^4$	**Not isolated**	**Not isolated**
$SM4_3$	$6.7x10^5$	$<1x10^1$	$<1x10^1$	$<1x10^1$	$<1x10^1$	$<1x10^1$	$6.1x10^2$	$<1x10^1$	**Not isolated**	**Isolated**

**Super market(SM), Aerobic bacterial count(APC), Lactic acid bacteria(LAB), Species(Spp*)

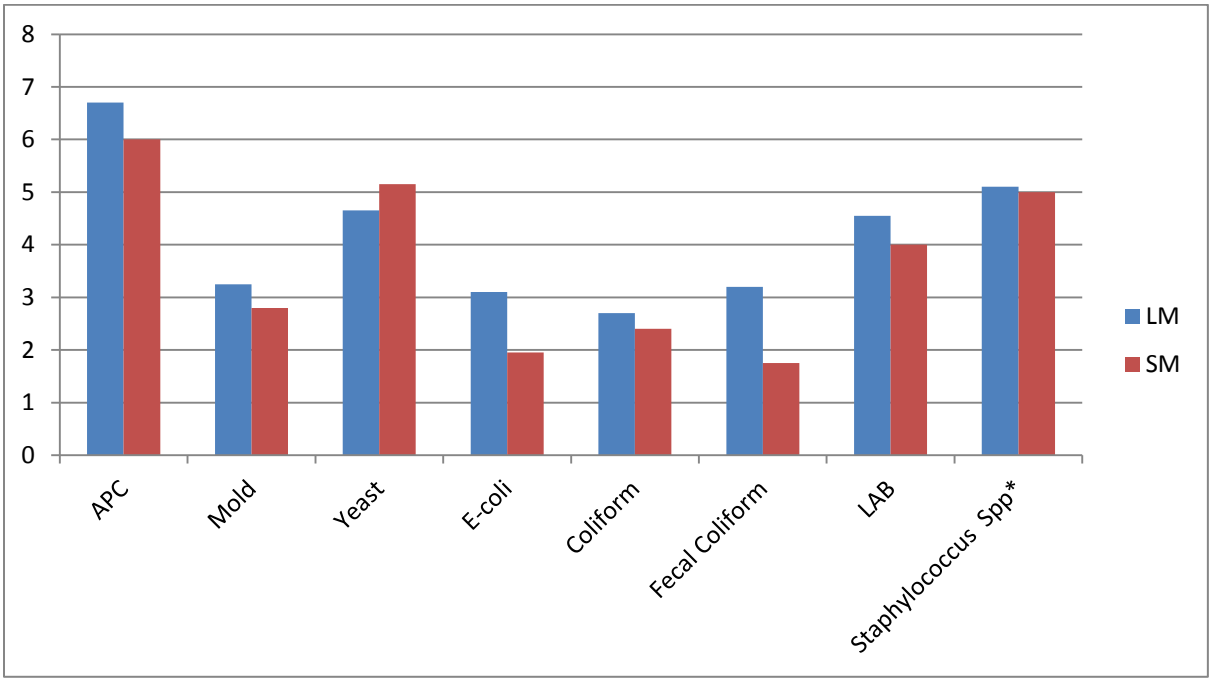

Fig. 1 Mean log value of eight microbial analysis parameters of the two markets.

4. Discussion

This study explored the quality of cheese provided for the consumers in Hawassa two different markets. The microorganisms tested were of ready to eat foods safety concern that included *Staphylococcus aureus (S. aureus), Escherichia coli* (*E. coli*), lactic acid bacteria(LAB),Aerobic plate count (APC), *Salmonella, shigella, mould, yeast, fecal coliform, total coliform.* The highest aerobic plate count (> 10^5 cfu/g) recorded from many of the samples are due to the existence of predominant microorganism (Health Protection Agency, 2009).

The present study discovered the presence of the non-lactic and lactic acid bacteria, the non-lactic isolates present in the cheese samples may develop during the milking process, milk collection and cheese making process while the lactic isolates are indigenous to raw milk which is the predominant micro flora found in milk , cheese and milk products (Neviani et al, 1982). Therefore, the highest lactic acid bacteria found on thirteen cheese samples could be its indigenousness to the raw milk.

In a recent study on Monte Veronese cheese, an Italian PDO semi-hard cheese made with raw milk, *Staphylococcus aureus* numbers in cheese were higher than the10^4CFU g^{-1}limit in 78% of samples (Poli et al.,2007) while the present study show 58.3% for LM and 41.6% for SM.

Enterobacteriaceae are ubiquitous inhabitants of the gut of human beings and other warm-blooded animals. Members of this group include the generally harmless and commensal E. coli which owing to its occurrence in feces, ready culturability, and typically non-pathogenic character, has been adopted as a universal indicator of fecal contamination. The high viable counts of the *Enterobacteriaceae*, (*Salmonella, Shigella, E. coli*) exhibited by many of the cheeses I sampled from local market may be ascribed to the use of raw milk and linked to poor husbandry of producing animals, poor hygiene practices during milk collection or bad preservation, possibly connected with lack of milk cooling. Otherwise, post-thermal treatment contamination must be hypothesized with organisms originally derived from raw milk or from manufacturing environments as the result shows higher count in local market than super market (Giovanni M. et al.,2011).

Staphylococcus aureus was the most frequent pathogen associated with cheeses from raw or unspecified milk in food-borne disease outbreaks reported in France in 1992-1997 (De Buyser et al., 2001). It is difficult to demonstrate the origin of contamination which could derive from raw milk since *Staphylococcus aureus* is the commonest cause of mastitis in dairy animals but also from post-processing contamination through unhygienic handling of products (Little and De Louvois, 1999).

The presence of human enteric organisms on many cheese products is clear evidence of contamination from a terrigenous source (ICMSF, 1986). The highest feacal coliform observed from eight samples collected from local markets and five samples collected from super markets could be due to feacal contamination of the processing area and water used for processing (this contamination has normally been associated with pollution of natural waters or water environments) or through direct contamination of products during processing.

The appearance of mould and yeast on cheese samples can explained as yeast and moulds are widely distributed in the environment and can enter food through inadequately sanitized equipment or as air borne contaminants. The reason for highest yeast and mould counts in present study is due to long period of storage and / or storage at high temperature of the product (IOM, 1985).

The highest growth for Salmonella species and Shigella species on samples could be attributed to lack of temperature during processing of the product as recent study shows that no growth for soft cheese product treated with thermization (László V., 2007).

5. Conclusion and Recommendation

Cheese can be considered as a good medium for bacterial growth due to their nutrient content and long storage duration. Several steps in their production can cause bacteriological hazards. Though pasteurization of milk can destroy most of the pathogens posing risk to public health, yet, the potential bacteriological hazards can still be found in the final products after pasteurization through the improper handling. The results indicate the unhygienic conditions prevailing during distribution or sale where most of the products are sold in open containers at local market.

Cheese of good quality should have counts of total bacteria of less than 10 per gram faecal coliforms and total coliforms should not exceed 100/gm. Total coliform count of ten samples from LM and five samples from SM, the faecal coliform count of eight samples from LM and five samples from SM and *E. coli* count of nine samples from LM and three samples from SM, in this study exceeded the acceptable limit recommended. This indicates human health risk due to consumption of those cheese products. Therefore, precautions should be taken to prevent contamination during post harvest handling and processing of cheese.

It is recommended to use and implement immediate regulatory measures like good processing practices as well as distribution and retail storage practices for ensuring microbiological safety of cheese provided for Hawassa. Finally, further research work covering wider area and large sample size should be done to identify problems and determine appropriate sale and set standards for production and distribution of cheese. The need of training and capacity building program for cheese processors and cheese vending communities have been suggested.

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