

MICROBIOLOGICAL EVALUATION STUDY OF MILK AND DAIRY PRODUCTS IN THE CITY OF KORÇA, ALBANIA

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ABSTRACT

The study of microbiological evaluation of milk and dairy products (butter, cheese, yoghurt, cottage cheese (curd), and sour cream) in the city of Korça was conducted during three consecutive years, 2011-2013, based on the requirements of European Standards of the Quality of Food Products. The aim of this study was the assessment of microbial quality of milk and its products and to identify risky products for the consumer health. There were evaluated 90 samples of milk, 50 samples of butter, 60 samples of cheese, 70 samples of yoghurt, 50 samples of cottage cheese, and 40 samples of sour cream, taken randomly in Korça. Milk, soft cheese and ice-cream have a maximum two or three week consumption period (shelf life), and, after that, there occurs a fast deterioration and microorganism development, while yoghurt and sour cream have a slightly longer shelf life because of fermentation and acidity. Samples were examined analytically for mesophyll aerobic microorganisms, coliforms, *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus*. There was found that the microbiological quality of milk and dairy products was not at the appropriate level for human consumption because of contamination by different microorganisms. Results showed that 52.1% of samples were contaminated with MAM, 55% of samples were contaminated with coliforms, 40% of samples were contaminated with *E. coli*, and 45% of samples were contaminated with mold.

Key words: *Escherichia coli*, microorganism, milk, milk products, *Salmonella*, *Staphylococcus aureus*.

INTRODUCTION

Milk, destined for human consumption, was determined since 1909 at the International Congress of Alimentary Products as “... an integral product of total and continuous milking of a female milking animal with good health conditions...It must be clean and free of foodborne illnesses...” (OFSP, 1989). Milk is the first food for infant mammalian. The biological function of milk is to supply nutrition and immunological protection to the young mammals. In some species, milk is the only food consumed for weeks or months. The consumption of liquid milk by infants, children and adults is well established in European dietary culture, as it is in North America, Australia, New Zealand, and various other parts of the world (Early, 1998; Varnam and Sutherland, 2001). It is a nutritional complete food that provides all nutritive requirements for maintenance and growth, such as energy (lactose), proteins [casein (alpha and beta caseins) and whey (alpha and beta lactalbumin), amino acids, minerals (calcium, iron, magnesium, phosphorus, potassium, sodium, zinc) and vitamins (A, D, E, C, B₁, B₂, B₆, B₁₂, pantothenic acid, niacin, biotin, and folic acid) (Jensen, 1995; Patton, 2005; Bylund, 2003). Factors affecting milk composition are species, breed, feed, season, and stage of lactation (O’Brien *et al.*, 2009). Physical and chemical properties of milk are: density (1.028-1.036), freezing point (-0,51°C to -0,55°C), color, pH (20°C) 6.5–7), titratable acidity (15-18 D), and flavor.

Microbiological quality of milk and milk-based products depend on the initial flora of raw milk, conditions of technological milk processing and contamination after pasteurization, until the products reach the consumer (Little *et al.*, 2008; Samson, 2000). Milk producers are

required to exclude bacteria from milk, but some may still gain entry. In the US, the bacterial count in Grade A raw milk may not exceed 300,000/ml. When the cow has mastitis, microorganisms associated with infections are found, including *Staphylococcus aureus*, *Streptococcus uberis*, and *Streptococcus agalactiae* (Delbes *et al.*, 2006; Heidinger *et al.*, 2009). Milk from cows with mastitis cannot be used for human consumption. Pasteurization destroys most of the microorganisms in milk and all of the pathogens. In the US, the upper limit of bacteria in pasteurized milk is 20,000/ml (D'Amico and Donnelly, 2010; D'Amico *et al.*, 2008; AAS, 1984; AAS, 1985). Milk has a pH of 6.5–7, which is ideal for the growth of many organisms. Milk is sterile at secretion in the udder, but it becomes contaminated by bacteria before it leaves the udder. At this point, the bacteria are few and harmless, unless the cow has mastitis. Further infection of the milk by microorganisms happens during milking, storage, handling, and other activities. *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Salmonella* spp., *Bacillus cereus*, *Yersinia enterocolitica*, *Escherichia coli* O157:H7, *Campylobacter jejuni* are a concern pathogenic bacteria in raw milk (Lejeune and Rajala-Schultz, 2009; Oliver *et al.*, 2009). These pathogenic bacteria can cause foodborne illnesses by ingesting raw milk that contains these organisms or milk that was not pasteurized properly or was contaminated after processing (Brooks *et al.*, 2012; Lindqvist *et al.*, 2002). Some pathogens such as *Listeria monocytogenes* can survive and thrive in post-pasteurization processing environments, thus leading to recontamination of dairy products (Oliver *et al.*, 2005). Milk-based products with high water content, such as milk, soft cheese, ice cream, etc., have a 2-3 weeks maximum shelf life, because of contamination by pathogenic bacteria, even at good processing and storage conditions. Some other milk-based products, such as yoghurt and sour cream have a slightly longer shelf life because of fermentation and acidity, while condensed milk, powder milk, yellow cheese, and butter have a longer shelf life because of UHT treatment during technological processing. Spoilage microorganisms cause degradation of proteins, fats, and carbohydrates and the milk's texture, color, taste, and smell is deteriorated (Dijksterhuis and Samson, 2007). Psychrotrophs (bacteria that grow at refrigeration temperatures) are usually involved in spoilage reactions. Most are destroyed by pasteurization, but can still produce heat stable lipolytic and proteolytic enzymes that can cause spoilage. Some strains and species of *Clostridium*, *Bacillus*, *Arthrobacter*, *Cornebacterium*, *Microbacterium*, *Micrococcus*, *Streptococcus*, and *Lactobacillus* can survive pasteurization and grow at refrigeration temperatures and cause spoilage (Brither, 1994; Samson *et al.*, 2001). Coliforms are closely associated with the presence of pathogens, but they are not always pathogenic themselves, thus they are called “indicator organisms”. They are killed by proper pasteurization treatment. Their presence indicates improper or inadequate pasteurization or it indicates contamination after pasteurization. *Escherichia coli* is an example of a coliform. They are facultative anaerobes and grow best at 37°C. Coliforms are able to degrade milk proteins and ferment lactose, causing milk to be spoiled quickly (Samson *et al.*, 2004; De Boer, 1995). Lactic acid bacteria can produce chemical changes that are desirable in the production of fermented dairy products because they are able to ferment lactose to lactic acid. They are naturally present in the milk and are used as starter cultures in the production of cultured dairy products, such as yoghurt and cottage cheese. Lactic acid bacteria include lactococci, lactobacilli, and *Leuconostoc*.

Unpasteurized milk is considered as one of the most hazardous risk for human health by EU and Albanian health authorities, as well (EEC, 1994; MoH, 1985; FAO & OMS, 1992). After privatization in recent years, the Albanian dairy processing factories are supplied by individuals or farmers groups, according to individual contracts. The whole system of collection, processing and distribution of milk and milk-based products to the consumer has been changed. The aim of the study was to determine the microbiological characteristics of milk and milk-based products in Korça.

MATERIALS AND METHODS

The study of microbiological evaluation of milk and dairy products was conducted during three consecutive years, 2011-2013, in Korça.

The main objectives of the study were: the microbiological evaluation of milk and milk-based products, detection and identification of the risky milky products for human health, and giving recommendations for improving the quality of milk and milk-based products.

There were evaluated 90 samples of raw milk, 50 samples of butter, 60 samples of cheese, 70 samples of yoghurt, 50 samples of cottage cheese, and 40 samples of sour cream, taken randomly in Korça's open markets.

Microbiological analysis Microbiological analysis of milk and milk-based products were carried out at the Microbiology Lab of the Agriculture Faculty at the University "Fan S. Noli" Korçë. Samples were analyzed for the mesophyll aerobic microflora, coliforms, *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, and mold, using specific methods (ICMSF, 1986; AAS, 1984; AAS, 1985; Brither, 1994; RIHEIP, 1985), as they are described in Table 1.

Table 1. Methods used for identification and isolation of different microorganisms in milk and milk-based products

Microorganisms	Enrichment	Isolation	Identification
Mesophyll aerobic microflora		PCA 30°C, 72h	
Coliforms		BG 37°C, 24h	
<i>Escherichia coli</i>	BG37°C, 48h	BG44°C, 24h	Indol
<i>Staphylococcus aureus</i>		Ag blood 37°C, 48h	Coagulase
<i>Salmonella</i>	BPW37°C, 18h Selenit 37°C, 24h Rrap vassil 43°C, 24h	DC BG37°C, 24h	Kliger method
Molds		PYA: capek 30°C for 5 days	Microscope

RESULTS AND DISCUSSION

Microbiological evaluation of raw milk and milk-based product samples was based on Albanian Standards and EU recommendations (Table 2).

Table 2. Biological rates of different microorganisms in milk and milk-based products

Microorganisms	Raw milk	Cheese	Butter	Yoghurt	Cottage cheese	Sour cream
Mesofyll Aerobic Microflora	>200000/g					
Coliforms	>50 / g	>50/g	>100/g	>50/g	>50/g	>50/g
<i>E. coli</i>	> 10 / g	>10/g	>20/g	>50/g	>50/g	>50/g
<i>St. aureus</i>	mm/g	mm/g	mm/g	mm/g	mm/g	mm/g
<i>Salmonella</i>	mm/25g	mm/25g	mm/25g	mm/g	mm/g	mm/g
Molds	>1000/g	>1000/g	>100/g	>100/g	>1000/g	>1000/g

Raw milk samples analysis

There was found that from 90 analysed of pasteurized milk samples, 48 samples or 53% contained mesophyll aerobic microflora (MAM) over the accepted limits, 50 samples or 55%

contained coliforms over the accepted limit, 42 samples or 55% contained *Escherichia coli* over the accepted limit, 2 samples or 2.22% contained *Staphylococcus aureus* over the accepted limit, and 41 samples or 45% contained mold over the accepted limit (Table 3). High presence of coliforms and *Escherichia coli* in analysed milk samples indicates a very poor hygiene throughout milk collection process and marketing.

Table 3. Microorganisms found in raw milk samples

Microorganisms	№ of samples	Contaminated samples over limit	Contamination Rate over limit (%)
Mesophyll aerobic microflora	90	48	53
Coliforms	90	50	55
<i>Escherichia coli</i>	90	42	47
<i>Staphylococcus aureus</i>	90	2	2.2
<i>Salmonella</i>	90	0	0
Molds	90	41	45

Microorganism contamination of the analyzed milk and milk-based products samples

There was observed that from 360 analysed samples, 197 samples or 54.7% were contaminated with different microorganisms over the accepted limit, from which 48 samples of raw milk, 44 samples of butter, 31 samples of cheese, 30 samples of yoghurt and 12 samples of sour cream. The highest contamination rate was observed for butter (87%), while the lowest contamination rate was observed for yoghurt (30%) because of higher content of lactic bacteria, which inhibit the proliferation of other microorganisms (Table 4).

High contamination of milk and milk-based products occurs because of high protein content of these products, a specific environment of bacteria, mold and yeast growth and development.

Table 4. Contaminated samples with different microorganisms

Product samples	№ of samples	Contaminated samples over limit	Contamination Rate over limit (%)
Raw milk	90	48	53
Butter	50	44	87
Cheese	60	31	52
Yoghurt	70	21	30
Cottage cheese	50	30	60
Sour cream	40	24	59
Total	360	197	54.7

Results showed that from 360 analyzed samples in total, 192 samples or 53.3% were contaminated with coliforms (45 samples of raw milk, 43 samples of butter, 30 samples of cheese, 20 samples of yoghurt, 31 samples of cottage cheese, and 23 samples of sour cream), 170 samples or 47.25% were contaminated with *E. coli* (42 samples of raw milk, 41 samples of butter, 26 samples of cheese, 12 samples of yoghurt, 26 samples of cottage cheese, and 24 samples of sour cream), and 8 samples or 2.22% were contaminated with *Staphylococcus aureus* (2 samples of raw milk, 2 samples of butter and 4 samples of cottage cheese). Fecal contamination is what prevails in the products under study (Table 5). There was observed that two samples of butter and four samples of cottage cheese were contaminated with *S. aureus*,

which, according to the Albanian standards were over the accepted limits, while they are accepted according to EU limits (10/g).

Table 5. Contaminated samples with coliforms, *Escherichia coli* and *Staphylococcus aureus*, over accepted limits

Product samples	№ of samples	Contaminated samples over limit					
		Coliforms		<i>E. coli</i>		<i>S. aureus</i>	
		№	%	№	%	№	%
Raw milk	90	45	50	42	47	2	4
Butter	50	43	85	41	81	2	4
Cheese	60	30	50.5	26	43	0	0
Yoghurt	70	20	29	12	17	0	0
Cottage cheese	50	31	61	26	52	4	8
Sour cream	40	23	58	24	59	0	0
Total	360	192	53.3	170	47.25	8	2.22

Almost all samples showed high contamination levels of mold which come from low hygienic conditions during milk collection, preservation and marketing. Observed results showed that 156 samples or 43.4% were contaminated with molds (31 samples of raw milk, 27 samples of butter, 27 samples of cheese, 34 samples of yoghurt, 21 samples of cottage cheese, and 17 samples of sour cream). The highest contamination level was observed for butter (53%) because of its chemical composition (80% fatty acids, 15% water, 0.4% carbohydrates, 0.6% proteins) very appropriate for mold development (Table 6).

Table 6. Contaminated samples with molds over accepted limits

Product samples	Nr. of samples	Contaminated samples with molds over accepted limit	
		№	%
Raw milk	90	31	34
Butter	50	27	53
Cheese	60	27	45
Yoghurt	70	34	48
Cottage cheese	50	21	42.9
Sour cream	40	17	43
Total	360	156	43.4

CONCLUSIONS AND RECOMMENDATIONS

Microbiological evaluation of raw milk and milk-based products sold in the period 2011-2013 in the city of Korça was far away from European Standards.

All the analysed raw milk and milk-based products were contaminated with different contaminants in different rates, which entail the loss of their nutritional values and affect significantly consumer's health. From 360 analysed samples, 197 samples or 54.7% were contaminated with different microorganisms over the accepted limits (48 samples of raw milk, 44 samples of butter, 31 samples of cheese, 30 samples of yoghurt and 12 samples of sour cream). From 90 samples of raw milk, 48 samples or 53% were contaminated with mesophyll aerobic microflora (MAM), 45 samples or 50% with coliforms, 42 samples or 47% with *E.*

coli, 2 samples or 2.22% with *Staphylococcus aureus*, and 41 samples or 45% with molds, over the accepted limits. The same data were shown for milk-based products under study. High presence of coliforms and *Escherichia coli* in analysed raw milk samples indicates a very poor hygiene throughout milk collection, processing and marketing.

In order to provide a high microbial quality of these food products (milk and milk-based products), pasteurization is the most effective method of enhancing the microbiological safety of milk. It is an imperative duty the application of HACCP system in all steps of milk processing, ranging from collection, transport, processing, storage, and marketing.

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