

RESEARCH ARTICLE

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Contamination with *Escherichia coli* of fresh butter produced by cow's milk in KosovoENVER BAJRAMI^{1*}, KAPLLAN SULAJ²^{1*}Veterinary Doctor, Gjilan, Kosovo²Faculty of Biotechnology and Food, Agricultural University of Tirana, Kamez, Tirana, Albania

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Abstract

Processing of milk may produce undesirable effects and some microorganisms produce food infections carrying the pathogens that will increase the likelihood of infection of the consumer's food. Contamination of milk and milk products is largely due to human factor and unhygienic conditions. Fresh butter is usually contaminated with different kinds and levels of pathogens. In Kosovo fresh butter is produced in home conditions and sometimes in not appropriate hygienic conditions. Sixty two butter samples were randomly collected from different localities of Kosovo for the isolation of *E. coli* a notorious contaminant. All the samples were inoculated on different bacteriological media and a number of biochemical tests were performed for the confirmation of the isolates. The results revealed that out of 62 fresh samples 22, 5% (16/62) showed growth of *E. coli*. The highest number of butter samples contaminated with *E. coli* is recorded from butter samples obtained from vending shops and houses. Among the 16 butter samples showed growth of *E. coli* the highest rate of contamination was found in butter samples collected in Ferizaj region. 5 butter samples collected in Ferizaj or 8% showed the values of *E. coli* from 10cfu/g to 100cfu/g.

Keywords: butter, contamination, *E. coli*, cow, milk, Kosovo.

1. Introduction

The quality of fresh butter is determined by aspects of milk composition, hygiene, storage and way of production. Because of complex biochemical composition and water activity, butter can serve as a good culture medium for the growth and multiplication of different kinds of microorganisms [4, 11]. The production of butter by the milk processing may produce undesirable effects and contamination with microorganisms that cause food infections. The production of butter is based on traditional method without any regard to the quality of raw material (milk) and the hygienic conditions applied during production and storage. Under such conditions many microorganisms can find access to this milk product [5, 9, 11]. On this context, *Escherichia coli* is frequently contaminating organism, and is a reliable indicator of fecal pollution generally in insanitary conditions of water, food, milk, equipments, people etc. Cases of hemolytic uremic syndrome caused by *E. coli* O157:H7 in butter are reported by some authors [4, 8, 7]. Detection of *E. coli* from butter is an indicative of bacterial contamination [4, 7]. Enteropathogenic or toxigenic strains could constitute a public health hazard. Contamination of fresh butter produced in home conditions is more

frequent than butter produced by processing of milk [6, 9]. There are many studies reporting cases of contamination of fresh butter with *E. coli*. Quality of fresh milk is considered as main cause of contamination. However, contaminated water, unclean equipments, unhealthy people can also be a possible source of contamination. The World Health Organization (WHO) confirmed the strain of enterohaemorrhagic *Escherichia coli* (EHEC) O104:H4 isolated from cases in the infection outbreak in Germany [9]. The outbreak sickened more than 1,600 and killed 30 people in 11 European countries in 2011. The Robert Koch Institute identified 470 positive cases and for nine deaths was caused from Hemolytic Uremic Syndrome (HUS), a form of acute kidney failure [9]. Even though the high incidence of *E. coli* O157:H7 in food including also and milk products, a possible risk caused by other pathogenic strains as enterohaemorrhagic *Escherichia coli* (EHEC) O104:H4 is evident [12, 13]. According to study report the South Carolina department of Health and Environmental Control reported 73,000 cases of *E. coli* infection and 61 deaths occur in USA each year [7, 11]. In 2012, *E. coli* bacteria were found in butter at four kindergartens in the city of Samara in Russia. In Kosovo there are very few studies reporting sporadic cases of *E. coli* in food.

2. Materials and Methods

A total of 62 random samples of refrigerated fresh butter were aseptically collected from different farmer's houses in different villages, in regions of Kosovo during 2013. All samples were taken in sterilized plastic bags and transported under refrigerated condition to the laboratory. Analyses were started without any delay. The samples were collected in the sterile sampling bottles that were kept in a cold box and were immediately shifted to the laboratory of Microbiology, Institute of Veterinary in Kosovo. After that, the serial decimal dilution was prepared from fresh butter samples using 10g butter and 90 ml physiological solution at temperature 30°C [1, 2]. The samples were inoculated on MacConkey Agar (Difco laboratories, USA) and incubated aerobically conditions at 37°C for 24 hours. The plates were controlled for the growth of *E. coli*. A single, isolated colony was picked and subcultured again on MacConkey agar for purification of the isolate [3]. All the samples positive for *E. coli* contamination were confirmed firstly by using Gram's staining, cultural and differential biochemical characteristics. For culture characterization was used MacConkey Agar. The culture of *E. coli* was appeared with smooth, circular pink colonies with spreading growth. In Blood Agar culture was non hemolytic, grey white moist, glistening, opaque, circular, convex colonies with entire edge. Culture characteristics of *E.*

coli were confirmed using different media as: Violet Red Bile Agar, Nutrient Agar and peptone broth 1% [4]. Another single colony with similar characters was picked for the examination of staining and morphological characters of the isolate using bright field microscope for Gram's stain. The biochemical characterization of *E. coli* was achieved by using catalase test and commercial kit API 20E.

3. Results and discussions

The results of the present study are summarized in the Table 1. According to these results the 16 samples out of 62 showed the contamination of *E. coli* (22.5%). The highest contamination was recorded from the samples of the Ferizaj which showed 5 out of 16 samples positive (53.33%), The studies provided the evidence that *Escherichia coli* is frequently occurring organism in sweet milk products such as different Balkan countries: Turkey, Greece and Macedonia[5, 7]. The method of butter production and hygienic conditions, as well as, storage and transportation of this product are mainly the factors indicating to bacterial contamination [6, 9]. The microflora of butter reflects the quality of cream, the sanitary conditions of equipment used to manufacture the butter and the environmental and sanitary conditions during packaging and handling of such product. Psychrotrophic bacteria such as *Pseudomonas spp* and *Flavobacterium spp* may cause odour formation and rancidity [6, 9, 10].

Table 1: The contamination with *Escherichia coli* of fresh butter samples

<i>Fresh butter samples</i>	<i>No. of positive samples</i>	<i>Percentage of positive samples</i>	<i>Percentage of positive samples E. coli (10cfu/g to 100cfu/g)</i>
62	16	(16/62) 22.5%	5/62
Total	16	22.5%	8%

Growth of *Flavobacterium malodoris* cause surface taints very quickly affecting the mass of the product and can develop a putrid, decomposed or cheesy flavour making product unmarketable and leading to economic losses [7, 11]. Spoilage of butter may result from the presence of heat resistant proteases and lipases produced by psychrotrophic bacteria during storage of raw milk or cream even after death of spoilage organisms [4, 7, 8]. In this study, *E. coli* is an indicator of faecal contamination and the possibility of the presence of enteric pathogens. 22.5% of fresh butter samples contained *E. coli*. In previous studies, higher incidence rates were

reported by some authors in Egypt. They found until 37.7 % of the samples contaminated with *E. coli* [6]. The presence of *E. coli* in cooking butter may be from an endogenous source, using raw cream for manufacture of butter as result of handling and inadequate personnel hygiene of farmer's wife [5, 8]. Generally, the microbial contamination of butter is caused from raw cream, in addition to the primitive way of processing, handling, storage and marketing. Butter usually contains 1.5–2% NaCl. This concentration strongly inhibits most microorganisms [6, 9]. On the other hand, lactic acid level produced as a result of natural souring during storage.

Conclusion

The results concluded that fresh butter in Kosovo is produced under unhygienic conditions. The *E. coli* was found as contaminant in 22.5% of fresh butter samples collected from different localities in Kosovo. 8% of fresh butter samples were contaminated with *E. coli* more than 10cfu/g. The counts of *E. coli* above the recommended criteria and the presence of pathogenic bacteria may pose a risk for public health. Therefore, there is a necessity for improving the hygienic status of locally produced butter through education of rural women and consumers on good processing hygiene and on handling of their foods including correct storage protecting them from contamination.

References

1. IDF, author: **Milk and milk products. Preparation of samples and dilutions microbiological examination:** Belgium; 1992.
2. Frank JF, Christen GL, Bullerman LB: **Tests for groups of microorganisms.** In: **Marshall RT, editor: Standard methods for the examination of dairy products:** American Public Health Association, Washington DC; 1992: 271–286.
3. Flowers RS, Andrews W, Donnelly CW, Koenig E: **Pathogens in milk and milk products.** In: **Marshall RT, editor. Standard methods for the examination of dairy products:** American Public Health Association, Washington DC; 1992: 103–212.
4. Kornacki JL, Flowers RS, Bradley RL: **Microbiology of butter and related products.** In: **Marth EH, Steele JL, editors. Applied Dairy Microbiology. 2nd edition.** USA: Marcel Dekker, Inc.; 2001: 127–150.
5. Karagozlu N, Ergonul B: **Microbiological attributes of Turkish butters sold under markets conditions.** *J Verbr Lebensm* 2008, **(3)**:376–379.
6. Kumar R, Prasad A: **Detection of *E. coli* and *Staphylococcus* in Milk and Milk Products in and around Pantnagar.** *Veterinary World* 2010, **3 (11)**:495-496.
7. Kumar V, Sinha RN: **Incidence of coliforms in indigenous milk products.** *Indian Journal of Dairy Science* 1989, **(42)**: 579-580.
8. Kulshrestha SB: **Prevalence of enteropathogenic sero groups of *E. coli* in milk products samples from Bareilly and their multiple drug resistance.** *Indian. Journal of Dairy Science* 1990, **(43)**: 337-378.
9. Ryu SH, Lee JH, Park SH, Jung HW, Park GY, Choi SM, Kim MS, Chae YZ, Park SG, Lee YK: **Antimicrobial resistance profiles among *Escherichia coli* strains isolated from commercial and cooked foods.** *International Journal of Food Microbiology* 2012, **159(3)**:263-266.
10. Varnam AH, Sutherland JP: **Milk and milk products: 1st edition. Butter margarine and spread;** Chapman and Hall publisher, London; 1994: 224–274.
11. Wilbey RA: **Microbiology of cream and butter: in Robinson RK editor: Dairy Microbiology Handbook, the microbiology of milk and milk products, 3rd edition.** New York, USA; 2002: 123–174.
12. WHO: **EHEC outbreak: increased cases in Germany.** Geneva, Switzerland; 2011.
13. Robert Koch Institute: **Technical report, EHEC/HUS 0104 outbreak,** Berlin Germany; 2011.