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RESEARCH ARTICLE

Contamination with *Escherichia coli* of homemade fresh butter in rural areas of Ferizaj and Gjilan in Kosovo

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Kosova'nın Ferizaj ve Gjilan kırsal bölgelerinde üretilen ev yapımı taze tereyağlarının *Escherichia coli* ile kontaminasyonu

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Öz

Amaç: Kosova'da bazı kırsal bölgelerde üretilen taze ve ev yapımı tereyağının *E.coli* ile kontaminasyon düzeyinin belirlenmesi amaçlandı.

Gereç ve Yöntem: 187 tereyağı örneği, 5-bromo-4-chloro-3-indolyl- ß-D-glucuronic acid (BCIG) agar besi yerlerinde sulandırılarak analiz edildi. Daha ileri identifikasyon, farklı besi yerlerine ekimler ve biyokimyasal testler ile yapıldı ve *E.coli* izolatlarının identifikasyonunda API 20E stripleri kullanıldı.

Bulgular: Bu araştırma 2014 yılında Kosova'da Gjilan ve Ferizaj kırsal bölgelerinde yaşayan ailelerden toplanan taze tereyağı örneklerinde gerçekleştirildi ve β-glucuronidase-positif E. coli sayıları belirlendi. Çalışma ile tereyağı örneklerinin % 34 (64/187)'ünde *E.coli* kontaminasyonu belirlendi. Ferizaj bölgesinde E.coli izolasyonu % 22 (20/189) oranı ile yüksekti. Toplamda 187 tereyağı örneğinin 36 (%19)'ının 5 log cfu/g'den yüksek E.coli içerdiği tespit edildi.

Öneriler: Taze tereyağı Kosova'nın kırsal alanlarında çoğu aile tarafından ev yapımı metot üretilen bir süt ürünüdür. Geleneksel yöntem ile üretilen taze tereyağı çoğunlukla patojen *E.coli* suşlarını da içeren çeşitli mikroorganizmalar ile kontamine olur. Kosova'nın bu kırsal alanlarında zayıf hijyenik koşullar altında ev üretiminin tereyağının kalitesine etkisi vardır ve zayıf hijyen tereyağında artan sayıdaki E.coli ile ilişkilidir.

Anahtar kelimeler: Tereyağı, *E. coli*, inek, ev yapımı, Kosova.

Abstract

Aim: It was aimed to evaluate level of contamination of *Escherichia coli* in homemade fresh butter in some rural areas in Kosovo.

Materials and methods: A hundred eighty seven butter samples were analyzed inoculating diluted volumes in selective agar plates containing 5-bromo-4-chloro-3-indolyl- ß-D-glucuronic acid (BCIG). The further identification of isolates was performed using other media and some additional biochemical tests as well as API 20E strips are used for identification of *E. coli* isolates.

Results: This research was carried out in 2014 to evaluate the number of β-glucuronidase-positive Escherichia coli in homemade fresh butter samples collected by families in rural areas of regions; Gjilan and Ferizaj in Kosovo. This analytical check concluded that 34% (64/187) of fresh butter samples were contaminated with *E. coli*. The high number of *E. coli* was detected in 22% (20/189) butter samples collected in rural areas of Ferizaj. In total, 36 out 187 butter samples or 19% showed values of *E. coli* higher than 5log cfu/g.

Conclusion: Homemade fresh butter produced from fresh cow's milk in rural areas of Kosovo is commonly contaminated with different kinds of microorganisms as well as by pathogenic strains of E. coli. The poor hygienic home conditions in some rural areas in Kosovo have impact in the butter quality and are associated with the increasing number of *E. coli*.

Keywords: Butter, Escherichia, coli, cow, homemade, Kosovo.

Introduction

The strict hygienic measures during milk handling prevent contamination and improve fresh milk quality. However, the number of microorganisms in fresh milk produced by dairy farms remains higher (Hahn 1996, Ahmed and Sallam 1991). For that reason the proper heat treatment is applied to reduce the number of microorganisms in milk ensuring protection of consumers against food borne infections. The contamination level of fresh milk products is depending on load of microorganism present in raw milk (Adesiyun 1994). Due to its complex, the processing milk is changing the number of microorganisms and its biochemical composition (Hahn, 1996; Robinson, 2002). On this context, high water activity of milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms (Farrokh et al 2013). Therefore during the processing of milk are produced undesirable effects. The fresh milk can either carry the pathogens that will increase the likelihood of food infections (N'Guessan et al 2015).

The homemade fresh butter is used as common food in villages and rural areas and it is produced from cow's milk in traditional way, so the quality of fresh butter is determined by aspects of hygienic conditions. The homemade butter is usually contaminated with high number of microorganisms because milk is indicated to human factor and poor hygienic conditions (Henin and Kaldes 1992). Usually milk is contaminated with different kinds of microorganisms during milking, collecting places, processing, storage and packaging. As milk product, the homemade fresh butter usually has increased number of microorganism including also and *Escherichia coli* (Kornacki et al 2001). *E. coli* is present ever with increasing number of strains in milk. So, it is clear that *E. coli* as part of harmless intestinal flora is identified as the serious causal agent of various illnesses (Henin and Kaldes 1992).

The production of butter is based on traditional method without any regard to the milk quality or standard hygienic conditions. Under such conditions many microorganisms can find access to be present and to be multiply (Anjum et al 1989). Among all micro-organisms, *E. coli* is frequently contaminating organism, and it is considered indicator of fecal pollution generally in insanitary conditions of water, food, milk and fresh dairy products (Marrier 1973, Kulshrestha 1990, Hahn 1996, Kornacki et al 2001). *E. coli* O157: H7 is reported by Martin et al (1986) and Singh (2016) as main cause of hemolytic uremic syndrome providing information that raw milk and fresh butter may be a vehicle of transmission in persons consuming raw milk and fresh butter. *E. coli* was isolated from milk products such as cheese, butter, cream and other dairy products (Robinson 2002). Some studies reported the incidence of *E. coli* as fecal pollution indicator in fresh butter until 35% of analyzed samples (N'Guessan et al 2015).

Considering the above arguments this study aimed to evaluate contamination level through isolation of *E. coli* from fresh butter produced in home conditions in rural areas of Ferizaj and Gjilan in Kosovo.

Material and Methods

Samples

In 2014, a total of 187 random samples of fresh butter were aseptically collected from different farmer's houses in different villages, in Ferizaj and Gjilan regions in Kosovo. All samples were packaged in sterilized plastic bags and transported under refrigerated conditions to the laboratory. Analyses were started without any delay. Fresh butter samples were immediately shifted to the laboratory of Microbiology, Institute of Veterinary in Kosovo and to the Food Safety and Veterinary Institute in Albania.

Bacterial isolation

From fresh butter samples was prepared the serial decimal dilution using 10g butter and 90 ml physiological solution at temperature 30oC (Diliello 1982). The enumeration of *E. coli* in butter samples was based on this colony count technique involving the inoculation of pour plates and mixing of specified volumes of the sample or dilutions of the sample with a cooled molten selective culture medium containing 5-bromo-4-chloro-3-indolyl- ß-D-glucuronic acid (BCIG). Incubation was done at 37°C for 4 h followed by 44 °C for 21 h to allow for the selective growth of *E. coli*. Detection of ß-glucuronidase activity through the generation of E. coli blue colonies was achieved by the use of the chromogenic substrate BCIG. The number of colony forming units (CFU) of β-glucuronidase-positive *E. coli* per gram (g) or per milliliter (mL) of sample was calculated accorded to serial decimal solutions. ß-glucuronidase activity in Gram negative bacteria is restricted to *E. coli* (90 %), Shigella species, Yersinia species and Salmonella species. A single, isolated colony was then picked and subcultured again on MacConkey agar for purification of isolates (Difco 1999). Firstly, all positive samples were tested by using Gram's staining, cultural and differential biochemical characteristics. For culture characterization was used MacConkey Agar. The culture of *E. coli* appeared smooth, circular pink colonies with spreading growth. In Blood Agar culture colonies were non hemolytic, grey white, moist, glistening, opaque, circular, and convex with entire edge. Culture characteristics of *E. coli* were confirmed using different media as: Violet Red Bile Agar, Nutrient Agar and peptone broth 1% (Difco 1999). Another single colony with similar characteristics was picked for the examination of staining and morphological characteristics using bright field microscope for Gram's stain. The biochemical characterization of *E. coli* was achieved by using catalase test and API 20E commercial kit.

Results

The percentage of fresh butter samples contaminated with *E. coli* is higher for Ferizaj dhe Gjilan. However, both fresh butter samples produced in home conditions were highly contaminated with *E. coli*.

Discussion

Indeed, the above results confirmed incidence of *E. coli* for 34% of total analyzed samples and higher value of incidence in 37.7% of fresh butter samples collected in Gjilan. Production and handling of this product is entirely depending upon traditional system in rural areas of Kosovo. Such system could pose favorable environment for bacterial contamination because of the unclean hands of worker, poor quality of milk, unhygienic conditions of home dishes. On the other hand inferior quality of water supplied for washing the utensils could be the source of accelerating the bacterial contamination of fresh butter during the production and after it (Kornacki et al 2001, Oporto et al 2008).

According to Center of Disease Control (CDC) in USA between1999-2006 more than 1500 people became sick from drinking raw milk or eating milk products made from raw milk. Unpasteurized milk is 150 times more likely to cause food borne illness (Oporto et al 2008). Butter is accessory food used for direct consumption or for cooking. Butter consists of butter fat, milk proteins and water. People eat the butter on bread or in dishes. According the prevention of food adulteration in 1 gram *E. coli* must be absent. The butter produced in rural areas in developing countries content higher number of *E. coli* than the value recommended by CDC (Robinson 2002).

Table 1. Results for determination and MPN of E. coli in fresh butter samples collected in Gjilan and Ferizaj in Kosovo.

Regions	No. of fresh butter samples	Positive for *E. coli* (%)	Negative for *E. coli* (%)	MPN of E. coli (>10^5 cfu/g)
Gjilan	98	37.7% (37/98)	62. 3% (61/98)	(16/98) 16%
Ferizaj	89	30.3% (27/89)	69.7% (62/89)	(20/89) 22%
Total	187	34% (64/187)	66% (123/187)	(36/187) 19%

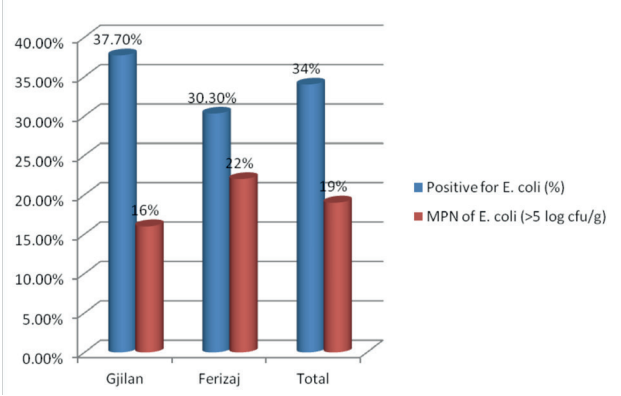

Figure 1. Percentage (%) of butter samples confirmed positive with E. coli compared with samples with MPN of E. coli (5 log cfu/g).

A survey carried out in Belgium in 2015 reported results on the microbiological safety standards and on the hygienic indicator microorganisms like *E. coli*. The results exceeded the defined limits of E. U in 35% of butter samples (N'Guessan et al 2015). This survey showed that most farm dairy products investigated were microbiologically safe. However, high levels of hygiene indicators (e.g., *E. coli*) in some products, like butter, remind us of applying good hygienic practices at every stage of the dairy production process to ensure consumer safety (Hahn 1996).Comparing our study results with the incidence of *E. coli* found in other countries, it is likely to the values reported in above mentioned countries (Kulshrestha 1990, Kornacki et al 2001, Hussein and Sakuma 2005). In 2008, in Turkey is reported the incidence of *E. coli* in 66.7% of butter samples collected in rural areas (Karagozlu and Ergonul 2008).

In the table 1, is showed number of positive cases confirmed with values of MPN of *E. coli* (>105 cfu/g). 18% of fresh butter samples are confirmed with high number of *E. coli* exceeded the European Regulation EC 2073/2005 limit for *E. coli* (ranging from < 1to 105 CFUg- signalizing the possible risk for consumers. In some other countries are reported different values of incidence of *E. coli* in fresh butter produced from cow's milk. Milk quality and hygienic conditions in processing units are remaining two main factors affecting butter quality (Sharma and Joshi 1992). Thus, the results of the present study warn the need for applying preventive measures regarding to regular clean and sanitation of dairy equipments, washing of utensils, milkier's hands, udders, eradication of diseases in animals, improving of milk delivery conditions and applying the safety milk processing practices.

Conclusion

The number of *Escherichia coli* is above the recommended criteria and may pose a risk for public health. Therefore, imp-rovement of the hygienic conditions and further consumer education on milk processing and handling of locally home-made fresh butter through education of women is necessary. This product should not be manufactured from raw cream. It should be used only for cooking after receiving adequate heat treatment.

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References

Adesiyun AA, 1994. Bacteriological quality and associated public health risk of pre-processed bovine milk in Trinidad. Intr J Food Microbiol, 21, 253-261.

Ahmed AM, Sallam SS, 1991. Prevalence of E. coli serotypes in raw milk and some dairy products. Assiut Vet Med J, 25, 93-97.

Anjum MS, Lodhi K, Raza AA, 1989. Pakistan's Dairy Industry: Issues and Policy Alternatives, Special Report Series 14, Econom. Analysis Network Project Islamabad Anonymous, E. coli Enteritis, Lancet, Islamabad, Pakistan, pp:1131-1132.

Difco 1999. Difco Manual, Difco Laboratories Incorporated Detroit Michigan, USA.

Diliello, LR, 1982. Methods in Food and Dairy Microbiology. AVI Publishing Co. Inc. Westport Connt., USA, pp: 38-39

Farrokh C, Jordan K, Auvray F , Glass K , Oppegaard H, Raynaud S, Thevenot D, Condron R, De Reu K, Govaris A, Heggum K , Heyndrickxi M, Hummerjohann J, Lindsay D, Miszczycha S, Moussiegt S, Verstraete K, Cerf O, 2013. Review of Shiga-toxin-producing Escherichia coli (STEC) and their significance in dairy production. Int J Food Microbiol, 162, 190-212.

Hahn G, 1996. Pathogenic bacteria in raw milk- situation and significance. In: Bacteriological quality of raw milk. Int. Dairy Federation, Brussels, Belgium, pp: 67-83.

Henin AY, Kaldes YT, 1992. Microbiological evaluation of cooking butter manufactured in Minia Governorate. Beni-Suef Vet Med J, 2, 291-297.

Hussein HS, Sakuma T, 2005. Invited review: prevalence of Shiga toxin-producing Escherichia coli in dairy cattle and their products. J Dairy Sci, 88, 450-465.

Karagozlu N, Ergonul B, 2008. Microbiological attributes of Turkish butters sold under markets conditions. J Verbr Lebensm, 3, 376-379.

Kornacki JL, Flowers RS, Bradley JRL, 2001. Microbiology of butter and related products. In: Applied Dairy Microbiology, 2nd edition, Eds; Marth EH, Steele JL, Marcel Dekker Inc, USA, pp: 127-150.

Kulshrestha SB, 1990. Prevalence of enteropathogenic serogroups of E. coli in milk products samples from Bareilly and their multiple drug resistance. Ind J Dairy Sci, 43, 337-378.

Marrier R, 1973. An outbreak of enteropathogenic E. coli food borne disease traced to imported French cheese Lancet, France, 1376-1378.

Martin ML, Shipman LD, Potter ME, Wachsmuth LK, Wells JG, Hedberg K, Tauxe, RV, Davis JP, Arnoldi J, Tilleli J, 1986. Isolation of Escherichia coli O157:H7 from dairy cattle associated with two cases of hemolytic uraemic syndrome. Lancet, 2, 8514-1043.

N'Guessan E, Godrie T, Laubier DJ, Tanna S, Ringuet M, Sindic M, 2015. A survey of bacteria found in Belgian dairy farm products. Biotechnol Agron Soc Environ, 19, 346-354.

Oporto B, Esteban JI, Aduriz, G, Juste RA, Hurtado A, 2008. Escherichia coli O157:H7 and non-O157 Shiga toxin-producing E. coli in healthy cattle, sheep and swine herds in Northern Spain. Zoonoses Public Hlth, 55, 73-81.

Robinson RK, 2002. Dairy Microbiology Handbook, The microbiology of Milk and Milk products. 3 rd edition, John Wiley and Sons, Inc., New York, USA, pp: 722-723.

Sharma DK, Joshi DV, 1992. Bacteriological quality of milk and milk products with special reference to Salmonella and its public health significance. J Food Sci Tech Mysore, 29, 105-107.

Singh NA, Saxena J, 2016. Evaluation of E. coli bacteria in different food samples. Int J Life Sci Bioeng, 3, 13-19.