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Short communication

Alkaline phosphatase activity and microbiological quality of heat-treated goat milk and cheeses

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ABSTRACT

The importance of goat milk and products of goat milk origin in human nutrition has increased in recent years. To provide a high quality and safety of this kind of food and to avoid a risk for public health, the criteria referring to microbiological quality of raw milk and final products, as well as to heat treatment have to be met. In the present study the alkaline phosphatase activity (ALP) in pasteurized goat milk and cheeses was determined. A hygiene quality and a presence of selected bacteria in goat milk products were also tested. The samples of milk ($n = 100$) and cheese ($n = 105$) originated from Polish dairies were analyzed. Ninety seven percent of milk and 94.3% of cheese samples showed ALP activity below 350 mU/L and 10 mU/g, respectively. The absence of *Salmonella* spp., *Listeria monocytogenes* and coagulase-positive staphylocci was observed in all samples tested, whereas in some of them *Escherichia coli* and Enterobacteriaceae were identified. The present study showed that pasteurized and UHT goat milk as well as goat cheeses from pasteurized milk were safe for consumers in terms of the absence of bacterial pathogens if proper pasteurization of raw milk is applied.

1. Introduction

Goat milk and products of goat milk are important in human nutrition and have become a part of the current trend of healthy eating around the world (Hassan et al., 2014; Yangilar, 2013). Goat milk consumption is increasing and consequently, the goat population has considerably increased during recent years (Slacanac et al., 2010).

Besides of direct consumption, a large volume of goat milk is used for cheese production as well as for thermally treated milk and milk powder (Slacanac et al., 2010). Cheeses, depending on traditions of the country, are produced from raw or heat treated milk, ripened or non-ripened (Slacanac et al., 2010). Casla et al. (1996) described the antimicrobial effect of lactid acid bacteria isolated from some traditional cheeses what confirms the healthy properties of goat milk products. A considerable number of goat milk products have a strong regional and artisanal character (Slacanac et al., 2010). Many traditional production technologies do not include a heat treatment step. The consumption of raw milk or cheeses and other products made from unpasteurized goat milk has been identified as the reason of some epidemics caused by *Listeria monocytogenes*, enterotoxin-producing *Staphylococcus* spp., Shiga toxins-producing *Escherichia coli* and *Salmonella enterica* (Silanikove et al., 2010; Yangilar, 2013). To avoid the risk of consumer's poisoning by microbiologically contaminated cheeses, it is recommended to produce them only from pasteurized milk (Rosenthal et al., 1996).

To confirm the completeness of the pasteurization process of raw milk the activity of naturally occurring in raw milk enzymes, such as alkaline phosphatase (ALP), lactoperoxidase or γ-glutamylotransferase is investigated (Lorenzen et al., 2010; Raynal-Ljutovac et al., 2007). Fluorimetric determination of ALP activity has been specified as the reference method (European Commission, 2006). The European Union legislation defines a level of 350 mU/L of ALP activity as safe for the consumption of cow milk (European Commission, 2006). However, for milk from other animals than cows and for cheeses, the limits have not been established yet. The tentative limit adopted for pasteurized goat milk by the European Union Reference Laboratory for Milk and Milk Products is equal to the cow milk (350 mU/L) and for cheeses from pasteurized milk is 10 mU/g. Because most studies on ALP determination have been conducted with cow milk and cow milk products, the verification of pasteurization in nonbovine products is needed (Rankin et al., 2010).

To provide a high quality of milk products and avoid the risk of consumer's poisoning the European Commission established a limit of total bacteria count as 1.5×10^6 CFU/mL for raw goat milk and 5.0×10^5 CFU/mL if milk is intended for the manufacturing of

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products made with raw milk by a process that does not involve any heat treatment (European Commission, 2004). Food safety criteria for microbial quality of milk products are established in relation to the presence of *Salmonella* spp. and *L. monocytogenes* and the absence of these microorganisms in cheeses and in ready-to-eat foods is specified. Process hygiene criteria are related to the number of coagulese-positive staphylococci, Enterobacteriaceae and *E. coli*. The Commission Regulation No. 2073/2005 defines the limit for the number of coagulase-positive staphylococci as 1.0×10^2 CFU/g in cheeses made from heat-treated milk and 1.0×10^1 CFU/g in unripened soft cheeses made from pasteurized milk. The limit of Enterobacteriaceae equalled to 1.0×10^0 CFU/mL in pasteurized milk and *E.coli* to 1.0×10^2 CFU/g in cheeses made from heat treated milk (European Commission, 2005).

The objective of this study was to determine the ALP activity as well as microbiological quality in heat treated goat milk and cheeses obtained from dairies located in the western and central parts of Poland.

2. Materials and methods

2.1. Collection of samples

One hundred heat-treated goat milk samples, including 61 pasteurized and 39 ultra high temperature (UHT) treated as well as 105 samples of cheese, including 54 unripened cheeses and curd, 29 ripened soft cheeses Camembert, 8 melted cheeses and 14 ripened hard cheeses were tested. The pasteurized milk was declared by producers to be heated at 72 °C for 15 s, whereas UHT milk was homogenized and treated at 135 °C for 5 s. The technology of unripened cheeses and curds included pasteurization at 75–76 °C for 20 s. The hard cheeses were also manufactured from milk after heating at the same conditions and ripened for 6 weeks. Soft cheeses Camembert were made from milk treated at 72 °C for 20 s and then ripened for 2 weeks. For melted cheeses production curd and hard cheese were used and thermized at 80 °C for 180 s. All products were made of goat milk obtained in Poland from three goat breeds: the Polish white improved, Saanen and Alpine.

The samples were transported to the laboratory at 1–5 °C and microbial analyses were performed within two hours or the cheeses were frozen below −20 °C up to 3 months for ALP investigation.

2.2. Determination of alkaline phosphatase activity

ALP activity in milk and cheese samples was determined using a fluorimetric method according to the EN-ISO 11816-1 and EN-ISO 11816-2 standards, respectively. The results of the analysis were expressed in mU/L or mU/g of ALP activity, where 1 U is an amount of the enzyme that catalyses the transformation of 1 μmol of non-fluorescent aromatic monophosphoric ester substrate per minute (ISO, 2003; ISO, 2013b). The analyses were performed using Fluorophos FLM 200 (Advanced Instruments, USA). Each sample was examined twice and the final result of ALP activity is expressed as the mean of two determinations.

2.3. Microbiological analyses

The microbial quality of the samples was assessed by determination of the number of coagulase-positive staphylococci, beta-glucuronidase-positive *E. coli,* Enterobacteriaceae and total bacteria count. The analyses were conducted according to EN-ISO 6888-2, ISO 16649-2, EN-ISO 21528-2, and EN-ISO 4833-2, respectively (ISO, 1999; ISO, 2001; ISO, 2004; ISO, 2013a). The presence of *Salmonella* spp. was tested according to EN ISO 6579 (ISO, 2002) with Brilliant Green Agar (BGA) (bioMerieux, France) as the second selective medium. Identification of *L. monocytogenes* was done with Vidas LMO2 (bioMerieux) procedure based on enzyme linked fluorescent assay.

2.4. Statistical study

The statistical significance of the differences between ALP activity in pasteurized and UHT milk was evaluated by the student's *t*-test analysis.

3. Results

3.1. Alkaline phoshatase activity

The vast majority (97 of 100, 97.0%) of the milk samples showed ALP activity below 350 mU/L, mainly between 100 and 200 mU/L (Fig. 1). A higher enzyme activity was demonstrated in UHT than pasteurized milk, but these differences were not statistically significant ($p > 0.05$). In case of cheeses, most samples (99 of 105, 94.3%) had ALP activity under 10 mU/g, mainly below 1.0 mU/g (67; 63.8%) (Fig. 2). The highest ALP activity was observed in ripened soft cheeses, whereas the lowest one in melted cheeses (Table 1).

3.2. Microbiological contamination

Microbiological studies showed the absence of *Salmonella* spp., *L. monocytogenes* and coagulase-positive staphylocci in all examined samples. Enterobacteriaceae were present in 15 out of 29 (51.7%) samples of ripened soft cheese Camembert and in 3 of 54 (5.6%) unripened cheese and curd at the level up to 8.5×10^6 CFU/g. These bacteria were also detected in 3 of 61 (4.9%) pasteurized milk samples. *E. coli* were found in two samples of Camembert cheese with the number below 2.0×10^3 CFU/g. Determination of total bacterial count showed the presence of microorganisms in each type of products, even sterilized. The highest level of bacterial contamination was identified in ripened soft cheese Camembert (2.1×10^{10} CFU/g), followed by unripened cheese and curd (2.7×10^9 CFU/g) and ripened hard cheese (4.4×10^8 CFU/g). On the other hand, the lower bacterial number were found in pasteurized milk (5.6×10^5 CFU/mL), melted cheese (2.6×10^3 CFU/g) and UHT milk (2.4×10^1 CFU/mL).

4. Discussion

Determination of ALP activity in goat milk as an assessment of pasteurization completeness was performed by Lorenzen et al. (2010) and Wilinska et al. (2007). In their investigations the samples after pasteurization process showed the enzyme activity up to 62 mU/L. The ALP activity depends on the pasteurization conditions and milk that does not exceed 350 mU/L is regarded as properly pasteurized (European Commission, 2006; Lorenzen et al., 2010). A higher mean ALP activity in UHT (140.2 mU/L) compared to pasteurized milk (120.1 mU/L) demonstrated in the present study was also obtained by Lorenzen et al., 2010, 2011. Such results may be explained by reactivation of ALP in the presence of magnesium and zinc ions which occurs in UHT milk stored at above 25 °C (Fox and Kelly, 2006; Lorenzen et al., 2010).

It is difficult to compare the results of ALP activity in cheeses obtained in the present and other studies because of limited literature data (Rankin et al., 2010). Furthermore, the authors often expressed their results in different units, e.g. rate of fluorescence produced per minute or quantity of μg of phenol/g of cheese. Moreover, most studies were related to cheeses from cow milk (Yoshitomi, 2004; Rankin et al., 2010). Therefore, the present study is unique and important for further development of ALP analyses in milk and milk products other than made of cow milk.