DIVERSITY OF *LACTOBACILLUS SPP.* IN KRIEVIJAS CHEESE

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Abstract. Dairy ecosystems have been developing in thousands of years giving us wonderful collection of different types of cheese varieties. On the other hand, modern technology provokes the loss of variability (Adamberg et al., 2007). The only way to keep biological processes under control is to study physiology of microorganisms, and relationships between them systematically, especially non-starter lactic acid bacteria (NSLAB). The number of non-starter lactic acid bacteria increases during cheese ripening and may constitute a dominant population in the mature cheese. The NSLAB diversity, their metabolism and interactions with starter bacteria have an effect on the ripening process of the cheese.
The aim of the present study was to investigate the diversity of *Lactobacillus spp.* during ripening of Krievijas cheese.
The diversity of different profiles varied among the cheese samples matured at different temperatures, and the results indicated a correlation between NSLAB species and aroma development (Miķelsone et al., 2009). A higher metabolism of *Lactobacillus curvatus* at 12 °C leads to faster depletion of nutrients and approaching the last two phases of microorganism growth curve. However, in each of the cheeses, the microflora had a tendency to be dominated by one *Lactobacillus* profile at the end of maturation.
Key words: NSLAB, *Lactobacillus spp.,* ripening temperature, Krievijas cheese.

Introduction

Ripened cheese varieties of Latvia are traditionally made with a mesophilic starter, and non-starter facultative heterofermentative *Lactobacillus spp*. contribute to the development of quality and sensory properties of cheeses during ripening. In spite of not being added deliberately to the milk, heterofermentative lactobacilli grow to high numbers in many hard and semi-hard cheeses. The non-starter microflora has been studied during the last decade in the Norwegian cheeses Jarslberg and Norvegia (Lindenberg et al., 1996), the Swedish Herrgärd (Antonsson et al., 2001), Greve (Lindenberg et al., 1996) and Präst (Østlie et al., 2005), the Danish Danbo cheese (Antonsson et al., 2003; Christiansen et al., 2005), and the Estonian Eesti Kuldne, Eesti, Saare and Arensburg cheeses (Kask et al., 2003).

Non-starter lactic acid bacteria may have different roles in cheese ripening including contribution to flavour development and controlling activities of detrimental bacteria (Ärdo, 2007). Their effects are strain-specific, strongly affected by the technological context and probably by the interactions with other microorganisms in cheese. Some of them also have a potential as probiotics. Diversity of the NSLAB strains in cheese may therefore be of importance for a satisfactory aroma development during ripening (Steele et al., 2006).

The aim of the present study was to investigate the diversity of *Lactobacillus spp.* during ripening of Krievijas cheese.

Materials and Methods

Krievijas cheese (SC "Smiltenes piens") (salt content - 1.5-2.5%, protein content – 17%, fat content – 28.2%, and fat content in dry matter – 50%) was chosen for further analysis.

The cheese was made, cut and wrapped at the factory using traditional Krievijas cheese technology, and afterwards was divided into two groups for further examination at the Laboratory of Microbiology of the Latvia University of Agriculture. The first group was matured at 6 °C, and the second - at 12 °C at the same relative humidity of 85%. Samples of both groups were checked on the first day and then on the 15^{th}, 30^{th}, 45^{th} and 60^{th} day of ripening. In all cases LAB colony forming units (cfu) and identification of *Lactobacillus* species were analyzed.

The following scheme was used for the analysis: 10 g of analysed cheese samples were diluted in 90 ml of saline solution, and samples were homogenized in a bag Mixer 400 MI. Serial dilutions of each cheese sample (1:1 000, 1: 10 000) in saline were made, then plated onto MRS agar and incubated for 48 hours at 37 °C. Calculation of cfu was performed by ACOLYTE colony counter. The grown cultures were examined microscopically and seeded onto MRS agar at the same conditions as previousely for multiplication.

Lactobacillus spp. colonies were identified on the basis of carbohydrate patterns with API 50 CHL system (BioMerieux, Mareyl'Etoile, France) as recommended by the manufacturer. The APILAB plus version 4.0 program (BioMerieux) was used to analyse the fermentation profiles obtained with the API 50 CHL kit. Gram staining was performed as described by Gregersen, and catalase reaction was determined by transferring fresh colonies from a Petri dish to a glass slide and by adding 5% of H_2O_2.

Results and Discussion

Starter bacteria until two weeks of age dominated in all cheese samples (Table 1).

Table 1

Species of lactic acid bacteria in Krievijas cheese samples during ripening

Ripening time, days	Species and ripening temperature	
	6 °C	12 °C
Unripened	*Lactococcus lactis ssp.lactis 2, Lactococcus lactis ssp.lactis 1, Lactobacillus curvatus*	
15	*Lactococcus lactis ssp.lactis 2, Lactococcus lactis ssp.lactis 1, Lactobacillus curvatus*	*Lactobacillus plantarum 1*
30	*Lactobacillus curvatus*	*Lactobacillus plantarum 1*
45	*Lactobacillus helveticus*	*Lactobacillus plantarum 1*
60	*Lactobacillus curvatus*	*Lactobacillus plantarum 1*

Three *Lactobacillus* species were found in the Krievijas cheese. The NSLAB species were identified as *Lactobacillus curvatus, Lactobacillus plantarum,* and *Lactobacillus helveticus*. *Lactobacillus helveticus* was detected only after 45 days of ripening. Only two of studied species were found in the mature cheese. Recent studies have shown that the non-starter microflora of Cheddar cheese is dominated by mesophilic lactobacilli, especially *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus plantarum,* and *Lactobacillus curvatus* (Fox et al., 2004). As Krievijas cheese has some similarities with Cheddar cheese production, such as intensification of acidification process during the treatment of cheese grains, the diversity of NSLAB should be analogous.

Although Krievijas cheese samples were matured at different temperatures, the NSLAB varied in diversity, number, and dominance. The microbiological composition in cheese changed during ripening. Although the different species have different growth characteristics (specific growth rate, acidification ability, and final cell count), they are well adapted to changing environmental parameters of cheese ripening (carbohydrate limitation, low temperatures, and water activity).

The changes in cheese pH during ripening are shown in Figure 1.

The study showed that during the ripening of Krievijas cheese there was a relatively low increase in pH - 5.42 - 5.49 at the end of cheese maturation. pH of classical Krievijas cheese is 5.25-5.35. Differences in pH between the analysed and classical Krievijas cheeses depends on the activity of starter bacteria, the concentration and distribution of NaCl in cheese, maturation temperature and availability of nutrients for *Lactobacillus* species in cheese (Ozola et al., 2002). pH is very closely connected with the amount of colony forming units in cheese.

The total amount of lactic acid bacteria reached its maximum number of 10^7-10^8 cfu g^{-1} of cheese. Smith (2003) found that NSLAB from the beginning of ripening to population of 10^7 cfu g^{-1} grow over the first 10-20 weeks of ripening, and then their population remains relatively constant. Beresford (2001) has

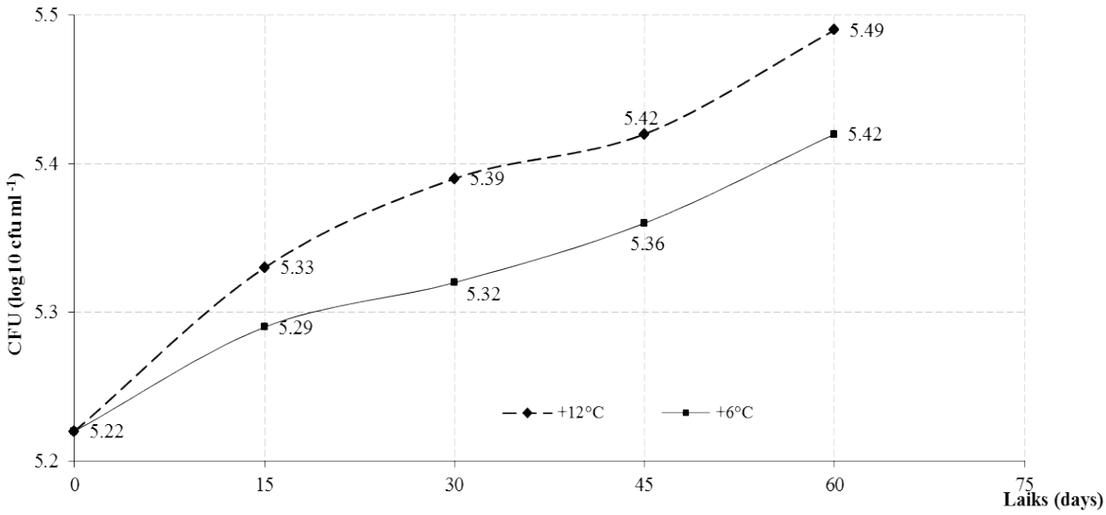


Figure 1. The changes in pH during Krievijas cheese ripening.

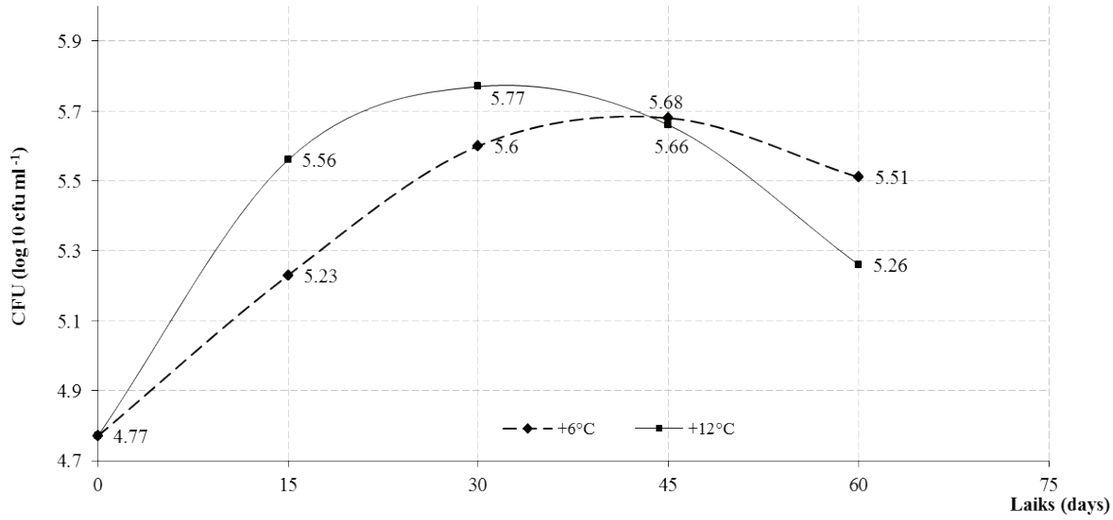


Figure 2. The dynamics of NSLAB in Krievijas cheese during ripening.

indicated that NSLAB reached their maximum value after 6-8 weeks; other authors mention that up to 3 months are needed for NSLAB to reach 10^7-10^8 cfu g^{-1} (Fox et al., 2004). However, all the authors have a common opinion that growth of NSLAB is tightly dependent on the ripening temperature (Crow, 2001; Shakel-Ur-Rehman et al., 2000; Fenelon et al., 1999). The amount of non-starter lactic acid bacteria was relatively low through all ripening period (Figure 2) for Krievijas cheese.

The samples ripened at 12 °C, more rapidly approached the stationary phase of lactic acid bacteria than those ripened at 6 °C. Both samples reached the peak after 30 and 45 days respectively. More sharp elevation of the curve for the sample ripened at 12 °C could be explained by intensification of exponential phase due to the higher ripening temperature; besides, depletion of nutrients comes faster than for the samples ripened at 6 °C. After 30 days of growth the curve reached the stationary phase, and the concentration of lactic acid bacteria fell down intensively. The total amount of LAB in the cheese ranged from $1.60 \cdot 10^5$ to $3.55 \cdot 10^5$ cfu g^{-1}. It indicates that cheese samples also had a weak aroma development, which is in accordance with earlier studies of the numbers of NSLAB versus aroma development during ripening (Antonsson et al., 2001).

Cheese samples also had a lower number of different profiles, which further suggested a relationship between low strain diversity and aroma development of Krievijas cheese during ripening (Miķelsone et al., 2009). However, in each of the cheeses, the microflora had a tendency to be dominated by one *Lactobacillus* profile.

Conclusions

1. The diversity of *Lactobacillus curvatus*, *Lactobacillus plantarum,* and *Lactobacillus helveticus* varied among the cheese samples matured at different temperatures.
2. A higher metabolism of *Lactobacillus curvatus* at 12 °C leads to faster depletion of nutrients and approaching of the last two phases of the growth curve of lactic acid bacteria.
3. However, in each of the cheeses, the microflora had a tendency to be dominated by one *Lactobacillus* profile at the end of ripening.

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