DIVERSITY OF NON-STARTER LACTIC ACID BACTERIA IN LATVIAN SEMI-HARD CHEESES

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Abstract

The most non-starter lactic acid bacteria (NSLAB) isolated from semi-hard cheeses are heterofermentative and handled as one of the reasons of cheese off-flavours and yield defect but at the same time majority of researches argued positive effect of NSLAB on cheese flavour formation and diversification due to rendered compounds of chemical reactions. The amount of NSLAB in cheese varies from 10 at the beginning of ripening to 10^5 cfu ml^{-1} within 6-8 weeks.

The aims of this paper were to establish diversity of NSLAB in commercial samples of Krievijas and Holandes cheeses, and to evaluate the effect of ripening temperatures on NSLAB in the trials of Krievijas cheese.

A total of 12 commercial cheese samples from seven different Latvian manufacturers and trials from one cheese manufacturer were examined. The trials were ripened for 60 days at different temperatures – 6 °C and 12 °C. Serial dilutions of each cheese sample (1:1000 and 1:10 000) in saline were made. NSLAB were cultivated using MRS media. Strain identification was performed by the API 50 CHL system (BioMerieux, Marey l'Etoile, France).

In commercial samples of Krievijas cheese dominance of *L. curvatus* was observed, simultaneously *L.plantarum* and *L.paracasei subsp. paracasei* were isolated. Whereas in Holandes cheese samples dominance of *L.paracasei subsp. paracasei* was noted and *L.plantarum, L.curvatus, L.rhamnosus* and *L.acidophilus* were isolated. In the trials ripened at different temperatures prevalence of *L.curvatus* was noted. Concentration of *Lactobacillus spp.* varied from 10^4 cfu ml^{-1} on the first day of ripening and reached the highest concentration (10^6 cfu ml^{-1}) after 6 weeks of ripening.

Key words: API, ripening temperatures, Krievijas cheese, Holandes cheese.

Introduction

The contribution of non-starter lactic acid bacteria (NSLAB) to cheese ripening and quality is a vexed question' remains topical. Their effects are strain-specific, strongly affected by the technological issues and probably by the interactions with other microorganisms in cheese.

NSLAB affect cheese quality and contribute to the intensity of flavour, although sometimes they may cause off-flavours in cheese (Fox et al., 1998). The most NSLAB isolated from semi-hard cheeses are heterofermentative lactobacilli. Heterofermentative lactobacilli are regarded as an adventitious flora in cheese, and they originate in raw milk and factory environment. This flora can reach 10^8 cfu ml^{-1} in most, if not all, ripened cheeses (Beresford et al., 2001; Cogan et al., 2007; Fox, 1999). Many factors influence cfu of *Lactobacillus spp.* and variety of derived products. One of them is ripening temperature, which can intensify or slowdown the ripening process and influence sensory properties of cheese (Kujawski et al., 2003; White, 2000).

Differences in aroma, taste, consistence and holes of Holandes and Krievijas cheeses even from one manufacturer have showed necessity to examine the main factors influencing the quality parameters of above-mentioned cheese brands. Therefore the aims of this paper were to establish diversity of NSLAB in commercial samples of Krievijas and Holandes cheeses, and to evaluate the effect of ripening temperatures on NSLAB in the trials of Krievijas cheese.

Materials and Methods

Twelve cheese samples from seven different Latvian cheese manufacturers were selected for examination and identification of NSLAB (see Table 1). 10 g of analysed cheese samples were diluted in 90 ml of saline solution, and samples were homogenized in 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 colony forming units (cfu) was performed by ACOLYTE colony counter. Grown cultures were examined microscopically and seeded onto MRS agar at the same conditions as previous for multiplication. Grown *Lactobacillus spp.* colonies were identified on the basis of carbohydrate fermentation patterns with API 50 CHL system (BioMerieux, Marey l'Etoile, France) as recommended by the manufacturer. The APILAB Plus version 4.0 program (bioMerieux) was used to analyze the fermentation profiles obtained with the identification strips.

Table 1

The characteristic of analysed cheese samples

Manufacturer	Cheese brand name	Protein content, %	Fat content, %	Fat content in dry matter, %	Salt content, %
JSC 'Smiltenes piens'	Krievijas	17	28.2	50	1.5-2.5
	Holandes	17	26.0	45	1.5-3.0
JSC 'Rīgas piena kombināts'	Edamjuusto (Holandes)	37	19	40	1.5-3.0
	Old Farmer (Krievijas)	23	29	50	1.3-1.8
	Limbažu (Krievijas)	23	29	50	1.3-1.8
JSC 'Trikātas piens'	Krievijas	23	29	50	1.3-1.8
	Holandes	26	26.8	45	1.5-3.0
LTD 'Mālpils piensaimnieks'	Holandes	25	25.2	45	1.5-3.0
JSC 'Cesvaines piens'	Holandes	26	26.8	45	1.5-3.0
JSC 'Valmieras piens'	Holandes	26	26.8	45	1.5-3.0
JSC 'Rankas piens'	Holandes	25	25.2	45	1.5-3.0
	Krievijas	23	28.5	50	1.3-1.8

The trials of Krievijas cheese were selected from JSC 'Rīgas piena kombināts' with fat content of 50% in dry matter. All trials were divided into two groups. Cheese samples of the first group were ripened at 6 °C but of the second group - at 12 °C for 60 days. The ripening temperature of cheese samples at 6 °C was selected according to ordinary practice of Latvian cheese producers. The ripening temperature of cheese samples at 12 °C was selected according to Dutch type cheese maturation parameters to achieve classical sensory properties. Analyses of NSLAB in trials were made at first day and after 15, 30, 45 and 60 days of ripening. The scheme of NSLAB identification was the same as for analyses of the commercial samples described above.

Results and Discussion

In commercial samples of Latvian cheeses dominance of *L.curvatus* (28.6%) and *L.paracasei subsp. paracasei* (38%) was detected, moreover *L. plantarum* (14.3%), *L. rhamnosus* (14.3%), *L. acidophilus* (4.8 %) were isolated (Tables 2 and 3).

Table 2

***Lactobacillus spp.* in Krievijas cheese samples**

Manufacturer	Cheese brand	*Lactobacillus spp.*
JSC 'Smiltenes piens'	Krievijas	*L.curvatus, L.plantarum 2*
JSC 'Rīgas piena kombināts'	Old Farmer (Krievijas)	*L.curvatus*
	Limbažu Krievijas	*L.curvatus, L.plantarum 2*
JSC 'Trikātas piens'	Krievijas	*L.curvatus*
JSC 'Rankas piens'	Krievijas	*L.curvatus, L.acidophilus 3*

Table 3

***Lactobacillus spp.* in Holandes cheese samples**

Manufacturer	Cheese brand	*Lactobacillus spp.*
JSC 'Smiltenes piens'	Holandes	*L. paracasei subsp.paracasei 1, L. paracasei subsp.paracasei 2*
JSC 'Rīgas piena kombināts'	Edamjuusto (Holandes)	*L.paracasei subsp.paracasei 1, L.rhamnosus*
JSC 'Trikātas piens'	Holandes	*L.paracasei subsp.paracasei 1, L.rhamnosus*

Table 3 continued

Manufacturer	Cheese brand	*Lactobacillus spp.*
LTD 'Mālpils piensaimnieks'	Holandes	*L. paracasei subsp.paracasei 1, L. paracasei subsp.paracasei 2, L.rhamnosus*
JSC 'Rankas piens'	Holandes	*L. paracasei subsp.paracasei 1, L. paracasei subsp.paracasei 2*
JSC 'Valmieras Piens'	Holandes	*L. curvatus, L. plantarum 2*

It is generally recognised that *L. paracasei subsp. paracasei, L. rhamnosus, L. plantarum* and *L. curvatus* are the main species of facultative heterofermentative lactobacilli in cheese (Cogan et al., 2007).

Colony forming units of *Lactobacillus spp.* in analysed commercial cheeses are presented in Figures 1 and 2.

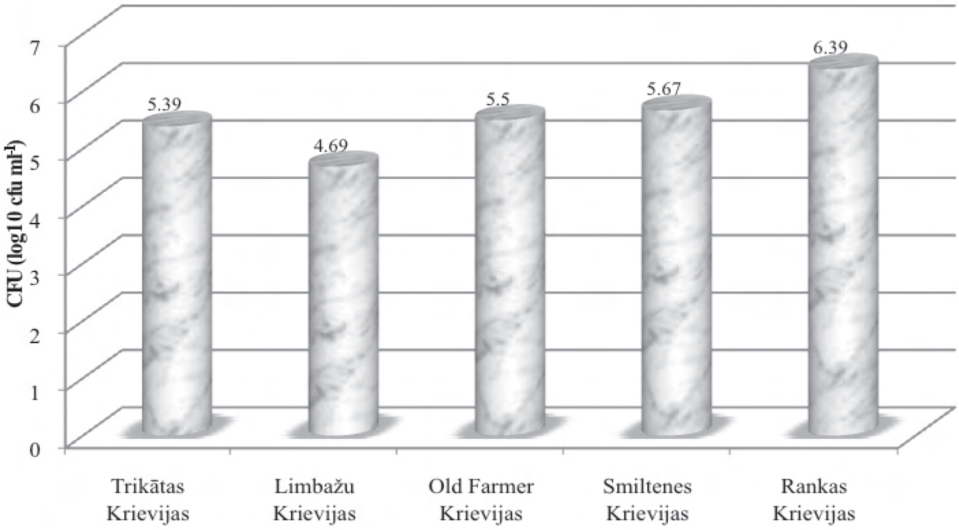

Figure 1. Colony forming units of *Lactobacillus spp.* in Krievijas cheese samples.

The obtained results showed that the lowest number of colony forming units was detected in the sample of Krievijas cheese with brand name 'Limbažu' in comparison with other sample from this manufacturer 'Old Farmer'. The highest value of colony forming units was noted in the sample from JSC 'Rankas Piens'. Among samples of Holandes cheese the lowest number of cfu was detected in the sample from JSC 'Valmieras Piens' while almost the same was the sample from JSC 'Cesvaines Piens' where count of colony forming units was only by 7% higher than in the first sample. The highest value of cfu was observed in sample from JSC 'Rankas Piens', difference between two extreme values was 17.6%. The same result for this manufacturer was etablished in the sample of Krievijas cheese, where difference between lowest and highest value was 26.6%.

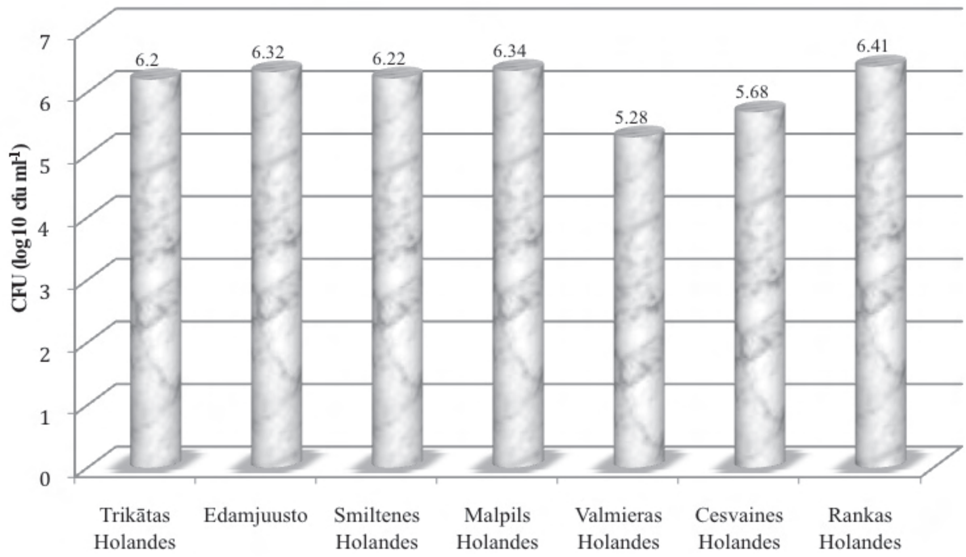

Figure 2. Colony forming units of *Lactobacillus spp.* in Holandes cheese samples.

Average colony forming units in all samples of Krievijas and Holandes cheese was 5.53 and 6.06 log10 cfu ml^{-1} respectively, which implies varying manufacturing conditions of these two cheeses. The lowest number of colony forming units in Krievijas cheese infers lower pH and higher lactic acid concentration than in Holandes cheese; however Table 1 shows that salt content in Holandes cheese varies from 1.5 to 3 and upper threshold is twice higher than in Krievijas cheese, which could be one of explanations for differences in the total bacterial count of examined samples (Upreti et al., 2006).

The growth of NSLAB in the trials during different temperatures of cheese maturation is presented in Figure 3.

The prevalence of *L. curvatus* in analysed samples was noted. Concentration of cultures varied from 4.14 log10 cfu ml^{-1} on the first day of ripening to 6.53 log10 cfu ml^{-1} after 4 weeks of ripening.

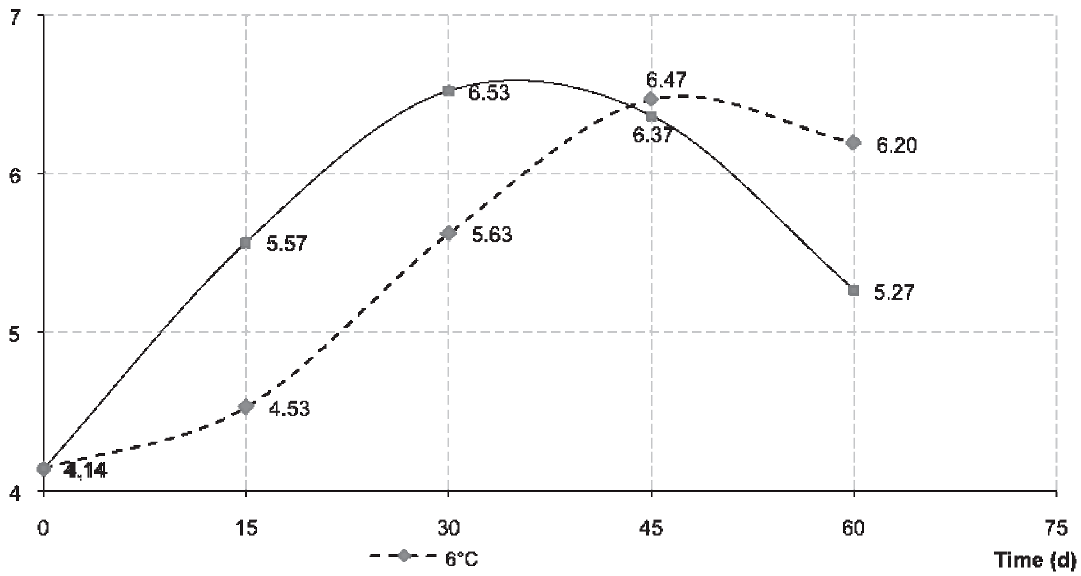

Figure 3. The dynamics of NSLAB in cheese during ripening.

The differences in cfu were observed from the 15th day of ripening. The count of colony forming units increased up to 9.42% and 34.54% for cheeses ripened at 6 °C and 12 °C respectively. Further changes were more obvious: on the 30th day of cheese aging the growth of colony forming units was 35.87% and 57.61% (6.53 log10 cfu ml^{-1}) in comparison with the initial numbers, reaching the peak value for cheese ripened at 12 °C. The decrease in cfu for cheese ripened at 12 °C was noted after 30 days, whereas for cheese matured at 6 °C the cfu increased steadily, and reached its peak value (6.5 log10 cfu ml^{-1}) after 45 days of ripening.

Non-starter lactobacilli have a generation time of approximatley 8.5 days in cheese ripened at 6 °C (Jordan et al., 1993). Ripening at low temperatures reduces their growth rates. Shakel-Ur-Rehman et al. (2000) found that in cheese ripened at 1 °C, the non-starter lactobacilli population was 3 log lower than in a cheese ripened at 8 °C. NSLAB concentration in cheese is lower than maximum numbers of starter (10^9-10^{10} cfu ml^{-1}). Different concentrations of *Lactobacillus spp.* in cheese ripened at 6 °C and 12 °C of temperature at the end of maturation could be explained by a higher metabolism of *Lactobacillus spp.* at a higher temperature. High metabolic rate results in an early approach of the stationary phase and autolysis during ripening (White, 2000).

The role of heterofermentative lactobacilli in flavour formation in cheese is still unclear compared to the homofermentative starter lactobacilli. Hence the next stage of investigation should be the establishement of the sensory properties of cheese and the evaluation of the level of proteolyses and fat degradation during ripening of cheese at different temperatures.

Conclusions

The main representative of NSLAB in Krievijas cheese is *Lactobacillus curvatus* and in Holandes cheese – *Lactobacillus paracasei subsp. paracasei*.

Ripening of cheese at the temperature of 6 °C reduces NSLAB growth rates due to lower metabolism of *Lactobacillus spp.*

A higher metabolism of *Lactobacillus spp.* at 12 °C results in an early approach of the stationary phase and death phase of microorganisms during cheese ripening.

References

1. Beresford T., Cogan T.M. (2001) Role of Lactobacilli in Flavour Development of Cheddar Cheese. The Dairy Products Research Centre Moorepark, Fermoy, Co. Cork. 14 p.
2. Cogan T.M., Beresford T.P., Steele J., Broadbent J., Shah N.P. and Ustunol Z. (2007) Invited Review: Advances in Starter Cultures and Cultured Foods. *Journal of Dairy Science*, 90, pp. 4005-4021.
3. Fox P.F. (1999) Cheese: Chemistry, Physics and Microbiology (general aspects), 2nd ed., Springer, New York, pp. 415-417.
4. Fox P.F. (2004) Cheese: Chemistry, Physics and Microbiology, 3rd ed., Academic Press, Amsterdam, pp. 326-327.
5. Fox P.F., McSweeney P.L.H. and Lynch C.M. (1998) Significance of non-starter lactic acid bacteria in Cheddar cheese. *Australian Journal of Dairy Technology*, 53, pp. 83-89.
6. Jordan K.N. and Cogan T.M. (1993) Identification and growth of non-starter lactic acid bacteria in Irish Cheddar cheese. *Irish Journal of Agriculture and Food Research*, 32, pp. 47-55.
7. Khalid N.M., Marth E.H. (1990) Lactobacilli – their enzymes and role in ripening and spoilage of cheese: A review. *Journal of Dairy Science*, 73, pp. 2669-2683.
8. Kujawski M., Cichosz G., Podhajna E., Sańko B. (2003) Effect of ripening temperature on proteolysis and organoleptic properties of Edam-type cheese. *Electronic Journal of Polish Agricultural Universities*, 6 (1). Available at: http://www.ejpau.media.pl, 07.02.2009.
9. Shakel-Ur-Rehman, Banksm J.M., McSweeney P.L.H. and Fox P.F. (2000) Effect of ripening temperature on the growth and significance of non-starter lactic acid bacteria in Cheddar cheese made from raw or pasteurised milk. *International Dairy Journal*, 10, pp. 45-53.
10. Upreti P., McKay L.L. and Metzger L.E. (2006) Influence of Calcium and Phosphorus, Lactose, and Salt-to-Moisture Ratio on Cheddar Cheese Quality: Changes in Residual Sugars and Water-Soluble Organic Acids During Ripening. *Journal of Dairy Science*, 89, pp. 429-443.
11. White D. (2000) The Physiology and Biochemistry of Prokariotes, 2nd ed., Oxford Univ. Press, New York, 600 p.
12. Williams A.G., Withers S.E. and Banks J.M. (2000) Energy sources of non-starter lactic acid bacteria isolated from Cheddar cheese. *International Dairy Journal*, 10, pp. 17-23.