NON STARTER LACTIC ACID BACTERIA IN RAW MILK, THERMALLY TREATED MILK AND SWISS TYPE CHEESE

Agita Bluma, Inga Ciprovica
Latvia University of Agriculture
agitabluma@inbox.lv

Abstract
The diversity of non starter lactic acid bacteria in raw, pasteurised milk and maturated Swiss type cheese was tested. The aim of the present study was to analyse the concentrations and representatives of non-starter lactic acid bacteria in raw milk and to evaluate the changes of their concentrations and representatives during pasteurisation of cheesemilk and Swiss-type cheese production. The analysis carried out in the study showed a variation in the microbial composition and quantity of raw milk. The most frequently isolated lactobacilli were found at low level in raw milk (mean 27.5×10^4 CFU mL^{-1}) and the most frequently identified species were *Lactobacillus brevis* and *Lactobacillus paracasei.* The microflora of raw and pasteurised milk is similar analysing lactic acid bacteria representatives in the samples. *Lactobacillus brevis* and *Lactobacillus paracasei* were detected in the same samples in raw milk, pasteurised milk and maturated cheese. Our study showed that lactic acid bacteria concentration was quite low in pasteurised milk (1-12 CFU mL^{-1}), but they grow rapidly in cheese during ripening reaching $1.1\text{-}1.8*10^6$ CFU g^{-1}. The present study has shown that NSLAB in Swiss-type cheese mainly derive from raw milk, and only a few strains survive the processing conditions and grow during ripening.
Key words: lactic acid bacteria, lactobacilli, raw milk, pasteurised milk, cheese.

Introduction

The raw milk is a natural growth medium for microorganisms. An integral part of raw milk microflora is the non-starter lactic acid bacteria (NSLAB) - *L.casei subsp. paracasei, L.plantarum, L.rhamnosus, L.curvatus, L.brevis, L.fermentum; Leu. lactis, Leu.cremoris; E.faecium, E.faecalis, E.durans and Pediococcus spp.: P.pentosaceus, P.acidilactici.* It provides milk with its microflora and enriches the microflora of cheesemaking environment (Montel *et al*., 2014).

Pasteurization regimes selected in cheese manufacturing are able to destroy essential microflora (also lactic acid bacteria), enzymes and pathogens in milk. Inactivation level of microorganisms depends on the count of microorganisms, growth phase and other factors. Some NSLAB strains also withstand heat treatment, mainly resulting in damaged cells that recover and proliferate in the curd during ripening (De Angelis *et al*., 2004). According to R. Coppola and co-authors study (1997), mesophilic lactobacilli are present in relatively low numbers in pasteurised milk and our previous study (Bluma & Ciprovica, 2015) showed that it was from 0 to 76 CFU mL^{-1}, however, they grow rapidly in cheese during ripening.

A lot of reviews approve that the significance of non starter lactic acid bacteria is still controversial and several approaches have been used in attempts to elucidate the role of NSLAB in cheese production (Shakeel-Ur-Rehman *et al*., 2000; Beresford & Williams, 2004; Mikelsone, 2011; Gobbetti *et al*., 2015). Species and, particularly, biotypes of NSLAB may vary between dairy plants, within a dairy plant, depending on season and day of manufacture, and even vary between batches of cheese (Gobbetti *et al*., 2015; Østlie *et al*., 2016). The aim of the present study was to analyse the concentrations and representatives of non-starter lactic acid bacteria in raw milk and to evaluate the changes of their concentrations and representatives during pasteurisation of cheese milk and Swiss-type cheese production.

Materials and Methods

Research was performed from January 2015 to February 2016 at:

- ✓ the Laboratory of Microbiology of the Department of Food Technology of Latvia University of Agriculture;
- ✓ the Laboratories of the dairy processing company 'Latvijas piens' and 'Bauskas piens' Ltd.

Object of the research

In order to study the non-starter lactic acid bacteria representatives in raw milk (n = 15), pasteurised milk (n = 15) and cheese samples after maturation (n = 15) all samples were tested. The samples were taken from raw milk tanks in the dairy company. Bulk milk was kept at 2-4 °C prior treatment. Bulk milk samples were analysed in the dairy company 'Latvijas piens'.

For better understanding the proliferation of lactic acid bacteria representatives during thermal treatment, raw milk was pasteurized at +74 °C 30 s in the dairy company 'Latvijas piens'. Treated milk samples (n = 15) were taken from cheese vats before renneting. Ripened Swiss type cheese (ripening regimes: 8-12 °C, 20-25 °C, 8-12 °C temperature, 85-90% relative humidity and ripening period 6-8 weeks) was analysed at the end of maturation determining NSLAB representatives and their concentrations.

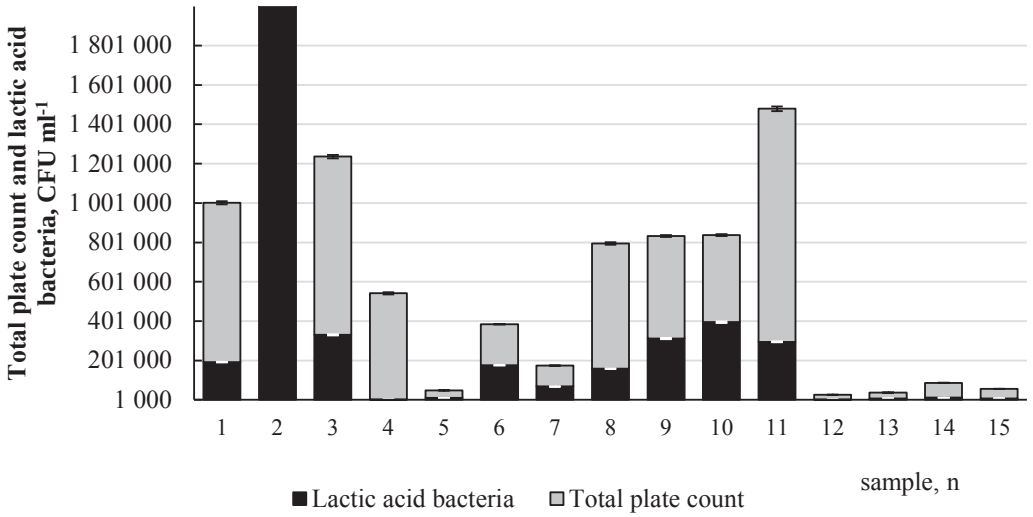

Figure 1. Total plate and lactic acid bacteria count (thous CFU mL^{-1}) in analysed bulk milk samples.

According to information given in literature (Demarigny *et al*., 1996), all studied cheese samples belong to young cheese group.

Methods of analyses

Determination of total plate count (TPC) was performed in all analyzed bulk milk samples according to LVS EN ISO 4833-1:2014 using PCA (plate count agar) (OXOID, UK). Sample dilutions were performed according to ISO 6887-5:2010 using salt-peptone solution. The chosen parameters for cultivation of bacteria in PCA agar were 72 hours at 30 °C.

Determination of *Lactobacillus* spp. was performed in all analyzed samples (raw milk, pasteurised milk, cheese) according to LVS ISO 15214:1998 using MRS agar (de Man Rogosa and Sharpe with Tween) media (OXOID, UK). Media were prepared according to LVS CEN ISO/TS 11133-1:2009. Sample dilutions were performed according to ISO 6887-5:2010 using salt-peptone solution.

The cultivation media were prepared according to LVS CEN ISO/TS 11133-1:2009.

The chosen parameters for cultivation of lactic acid bacteria in MRS agar were 72 hours at 37 °C, taking as a basis regimes recommended in Coeuret *et al*. (2003) work.

Identification of *Lactobacillus* spp. colonies was performed taking as a basis the fermentation of carbohydrates using API 50 CHL (BioMerieux, France). The program APILAB Plus version 4.0 (BioMerieux) was used for identification of the isolated colonies up to species.

Data mathematical treatment was performed by using Microsoft Excel program. The mean and the standard deviation of experimental data were determined.

Results and Discussion

In order to determine the origin of the facultatively heterofermentative lactobacilli we examined the bulk milk and pasteurised milk samples used for the manufacture of cheese for the presence of these bacteria.

The total plate and lactic acid bacteria count of bulk milk samples is summarized in Figure 1.

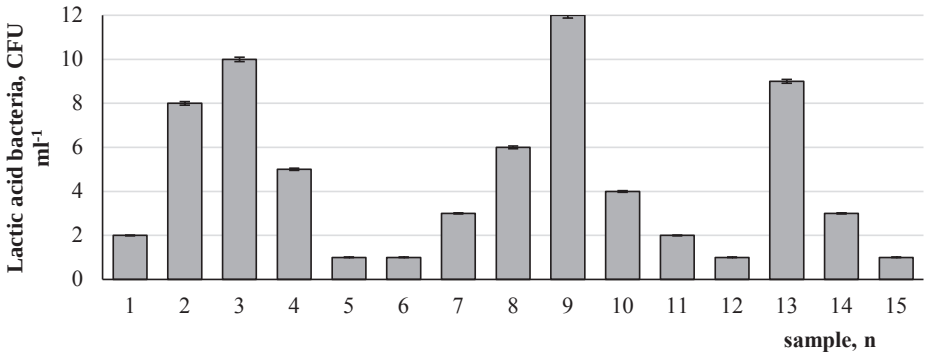

Figure 2. Lactic acid bacteria CFU in pasteurised milk samples (cheese milk).

The microbial quality of evaluated bulk milk samples is appropriate (except samples 3, 11), besides milk is usually pasteurised prior cheesemaking in Latvia, typically bulk milk used for cheese production contains 374 957 CFU mL^{-1} average.

Lactic acid bacteria in pasteurised milk samples are shown in Figure 2. Although pasteurisation reduces the flora of the raw milk to a large extent, small numbers of mesophilic lactobacilli may survive and subsequently grow in cheese made from pasteurised milk.

In cheese produced from pasteurised milk NSLAB typically grow from a few hundred per gram prior ripening to 10^7-10^8 g within 2-3 months of ripening. In analysed Swiss type cheese samples NSLAB vary from $1.10*10^6$ to $1.86*10^6$ CFU g^{-1}. The detected concentration is lower than previously mentioned statement that NSLAB slowly increase and reache a plateau at 10^7-10^9 CFU g^{-1} after a few to several months of cheese ripening (Fitzsimons *et al*., 2001). Our results we could explain with the findings of Gatti and co-authors (2014) when cheese aging is prolonged (Parmigiano Reggiano) the maximum of cell density decreases.

In the present study, microorganisms are identified based on phenotypical criteria and the obtained results also highlighted the absence of isolates in some analysed raw milk samples. The results of the identification are given in Figure 3.

The microflora of pasteurised milk is primarily of bacterial nature, and bacteria commonly isolated from pasteurised milk are of the same type that is found in raw milk (Fig. 3), as well as in cheese.

The bacteria were found at concentrations between 10^6 and 10^7 CFU g^{-1} and the large majority of isolates were *Lactobacillus brevis* and *Lactobacillus paracasei.* Three different species of NSLAB were found in raw milk, whereas 4 species were found in pasteurised milk. All species present in cheese (*Lactobacillus paracasei, Lactobacillus brevis, Lactobacillus curvatus* and *Lactobacillus plantarum*) as well as species in pasteurised milk (*Lactobacillus paracasei, Lactobacillus brevis, Lactobacillus curvatus* and *Lactobacillus plantarum*) were also found in the corresponding raw milk samples. Previously, Casey *et al*. (2006) described the diversity of mesophilic lactobacilli in Gruyère cheese (Swiss type cheese variety). The three genotypes found in the cheese were also found in raw milk, thus indicating that it is the raw milk flora and not the factory environment that principally determines the composition of the NSLAB in Gruyère cheese. It is interesting to note that in the cheese authors could not find the dominant genotype in raw milk, but genotypes were found at much lower concentrations. It can be assumed that some genotypes did not survive the temperatures applied during the cheesemaking process (Casey *et al*., 2006). The microflora of raw and pasteurised milk is similar to analysed lactic acid bacteria representatives in our study, too. Interestingly, we found *Lactobacillus brevis* and *Lactobacillus paracasei* in the same samples of raw milk, pasteurised milk and cheese.

The number of non-starter lactobacilli is higher in Swiss-type cheese made from raw milk than in cheese made from pasteurised milk, but the diversity of non-starter lactobacilli declines during ripening (Beuvier *et al*., 1997). The population of young cheese was comprised of *L.paracesei*, *L.plantarum* and *L.brevis* but as the cheese maturated *L.paracesei* dominated (Demarigny *et al*., 1996; Østlie *et al*., 2016). Species and, particularly, biotypes of NSLAB may vary between dairy plants, within a dairy plant, depending on season and day of manufacture, and even vary between batches of cheese, milk treatment and cheese ripening time (Gobbetti *et al*., 2015, Østlie *et al*., 2016). Although the different species have different growth characteristics (specific growth rate, acidification ability, and final cell number), they are

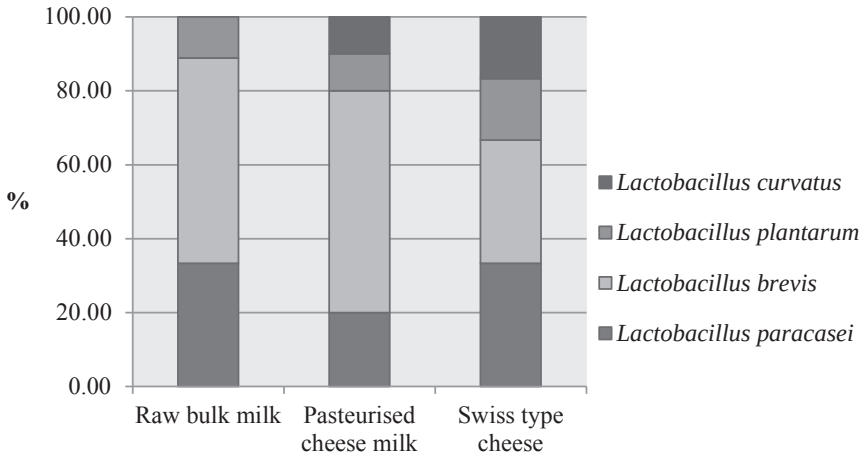

Figure 3. Lactic acid bacteria species isolated from raw, pasteurised milk and cheese samples.

well adapted in changing environmental parameters of ripening cheese (carbohydrate limitation, low temperatures and water activities). The present study has shown that NSLAB in Swiss-type cheese mainly derive from raw milk and generally only a few strains survive the processing conditions and grow during ripening.

Conclusions

In conclusion, the present study has shown that NSLAB in Swiss-type cheese mainly derive from raw milk and generally only a few strains survive the processing conditions and grow during ripening.

Lactic acid bacteria were found at low level in raw milk and the most frequently identified species were *Lactobacillus brevis* and *Lactobacillus paracasei.* Our study showed that lactic acid bacteria concentration was quite low in pasteurised milk (1-12 CFU mL^{-1}), but they grow rapidly in cheese during ripening reaching $1.1\text{-}1.8*10^6$ CFU g^{-1} at Swiss-type cheese samples.

References

1. Bereford, T.P., & Williams, A.G. (2004). The microbiology of cheese ripening. In: P.F.Fox, P.H.L.McSweeney, T.M.Cogan, T.P.Guinee (Eds.) *Cheese: Chemistry, physics and microbiology*, London, UK: Elsevier.
2. Beuvier, E., Berthaud, K., Cegarra, S., Dasen, A., Pochet, S., Buchin, S., & Duboz, G. (1997). Ripening and quality of Swiss-type cheese made from raw, pasteurised or microfiltered milk. *International Dairy Journal*, 7, pp. 311-323.
3. Bluma, A., & Ciprovica, I. (2015). Diversity of lactic acid bacteria in raw milk. Research for rural development 2015: Annual 21st International scientific conference proceedings, Jelgava, Latvia, 13-15 May, 2015 / Latvia University of Agriculture. - Jelgava, 2015. - Vol.1, pp. 157-161.
4. Casey, M.C., Hani, J.P., Gruskovnjak, J., Schaeren, W., & Wechsler, D. (2006). Characterisation of the non-starter lactic acid bacteria (NSLAB) of Gruyère PDO cheese. Lait 86, pp. 407-414.
5. Coeuret, V., Dubernet, S., Bernardieau, M., Gueguen, M., & Vernoux, J.P. (2003). Isolation, characterization and identification of lactobacilli focusing mainly on cheeses and other dairy products. *Lait*, 83, pp. 269-306.
6. Coppola, R., Nanni, M., Iorizzo, M., Sorrentino, A., Sorrentino, E., & Grazia, L. (1997). Survey of lactic acid bacteria isolated during the advanced stages of the ripening of Parmigiano Reggiano cheese. *Journal of Dairy Research*. 64(02), 305-310. DOI: 10.1017/S0022029996002038.
7. De Angelis, M., di Cagno, R., Huet, C., Crecchio, C., Fox, P.F., & Gobbetti, M. (2004). Heat shock response in *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, 70, 1336-1346.
8. Demarigny, Y., Beivier, E., Dasen, A., & Duboz, G. (1996). Influence of raw milk microflora on the characteristics of Swiss-type cheese. 1. Evaluation of microflora during ripening and characterisation of facultatively heterofermentative lactobacilli. *Lait*, 76, pp. 371-387.
9. Fitzsimons, N.A., Cogan, T.M., Condon, S., & Beresford, T. (2001). Spatial and temporal distribution of non starter lactic acid bacteria in Cheddar cheese. *Journal of Applied Microbiology*.90(4), 600-608. DOI: 10.1046/j.1365-2672.2001.01285.x.
10. Gatti, M., Bottari, B., Lazzi, C., Neviani, E., & Mucchetti, G. (2014). Invited review: microbial evoluation in raw-milk, long-ripened cheeses produced using undefined natural whey starters. *Journal of Dairy Science*, 97(2), 573-591. DOI: 10.3168/jds.2013-7187.
11. Gobbetti, M., De Angelis, M., Di Cagno, R., Mancini, L., & Fox, P.F. (2015). Pros and cons for using non-starter lactic acid bacteria (NSLAB) as secondary/adjunct starters for cheese ripening. *Trends in Food Science & Technology*, 45, pp. 167-178.
12. Mikelsone, A. (2011). *Influence on non starter lactic acid bacteria on cheese ripening*: Summary of doctoral thesis for acquiring the doctor degree of engineering sciences in food science. LLU, Jelgava, 61p.
13. Montel, M.C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D., & Desmasures, N. (2014). Traditional cheeses: rich and diverse microbiota with associated benefits. *International Journal of Food Microbiology*.177 (54). DOI: 136-154. DOI: 10.1016/j.ijfoodmicro.2014.02.019.
14. Østlie, H.M., Kraggerud, H., Longra, A.B., & Abrahamsen, R.K. (2016). Characterisation of the microflora during ripening of a Norwegian semi-hard cheese with adjunct culture of propionic acid bacteria. *International Dairy Journal*.54, 43-49. DOI:10.1016/j.idairyj.2015.10.005.
15. Shakel-Ur-Rehman, Fox, P.F., & McSweeney, P.L.H. (2000). Methods used to study non-starter microorganisms in cheese: a review. *International Journal of Dairy Technology*. 53 (3), 113-118. DOI: 10.1111/j.1471-0307.2000.tb02672.x.