

THE INFLUENCE OF NON-STARTER LACTIC ACID BACTERIA ON SWISS-TYPE CHEESE QUALITY

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Abstract

The aim of the present study was to analyse the influence of non-starter lactic acid bacteria (NSLAB) on Swiss-type cheese quality.

Lactobacillus brevis, *Lactobacillus plantarum* and *Lactobacillus paracasei* were detected in the same raw and pasteurised milk and cheese samples. Our study showed that non-starter lactic acid bacteria concentration in pasteurised milk varied from 1 to 12 CFU mL⁻¹, but during ripening reaching 3.16*10⁸ CFU g⁻¹. The population of young cheese was comprised of *Lactobacillus paracasei*, *Lactobacillus plantarum* and *Lactobacillus brevis* but in matured cheese *Lactobacillus paracasei* dominated.

NSLAB impact flavour development in Swiss-type cheese. Differences in the detected aroma compounds are observed among the samples of analysed cheeses. Individually detected aroma compounds in cheeses are able to impart different flavour notes, however, in certain combinations uncharacteristic aroma can occur. Among determined volatiles, butanoic acid presents in different quantities, indicating the influence of sporeforming bacteria on Swiss-type cheese production, too. Detected aroma compounds and their concentrations in analysed cheeses do not allow assuring the undesirable impact of NSLAB representative - *L.paracasei* on the Swiss-type cheese flavour, but cheese moisture and salt content, as well as interactions among cheese microflora representatives influence quality of produced cheese.

Key words: mesophilic heterofermentative lactobacilli, raw milk, pasteurised milk, cheese

1. INTRODUCTION

Swiss-type cheese characterises with mild, pleasing, slightly sweet flavour, elastic and firming structure, round or slightly oval-shaped eyes, etc. Taking into account the low salt level in Swiss-type cheeses and small acidity of cheese curd prior ripening, the role of milk indigenous microflora is still actual in cheese production. An important part of raw milk microflora is the non-starter lactic acid bacteria (NSLAB) – *Lactobacillus* spp.: *L. paracasei*, *L.plantarum*, *L.rhamnosus*, *L.curvatus*, *L.brevis*, *L.fermentum*; *Leuconostoc* spp.: *Leu.lactis*, *Leu.cremoris*; *Enterococcus* spp.: *E.faecium*, *E.faecalis*, *E.durans* and *Pediococcus* spp.: *P.pentosaceus*, *P.acidilactici* (Franciosi et al., 2009). Non-starter lactic acid bacteria produce diacetyl and acetoin, as well as high amounts of carbon dioxide. Carbon dioxide forms a lot of small holes in cheese, and sometimes a significant gas pressure results in a sponge-like cheese texture, as well as late blowing. Such defects we may observe under the influence of undesirable bacteria and starter microflora, too. Fröhlic-Wyder and co-authors (2002) investigated propionic acid bacteria interactions between starter and non-starter lactic acid bacteria in Swiss-type cheeses, found that secondary fermentation by production of gas after desired propionic fermentation is affected by propionic acid bacteria and NSLAB. Jimeno et al. (1995) showed that NSLAB inhibit the growth of propionic acid bacteria and reduce the prevention of late blowing, too.

Pasteurisation regimes applied in cheese manufacture are able to partly destroy lactic acid bacteria in milk. Some NSLAB strains withstand heat treatment and proliferate in the cheese during ripening (De Angelis et al., 2004). According to Coppola and co-authors study (1997), mesophilic lactobacilli are presented in relatively low numbers in pasteurised milk and our previous study (Bluma, Ciprovica, 2015) confirmed variations from 0 to 76 CFU mL⁻¹.

A lot of reviews (Shakeel-Ur-Rehman et al., 2000; Beresford & Williams, 2004; Mikelsone, 2011; Gobbetti et al., 2015, Østlie et al., 2016) confirm that the impact of non-starter lactic acid bacteria on

ripened cheese production is still controversial. Species and strains of NSLAB can vary between dairy plants, depending on season and vary between cheese batches (Gobbetti et al., 2015, Østlie et al., 2016).

Methods to reduce the prevalence of the late blowing defects and secondary fermentation in Swiss-type cheeses include different approaches as well as the application of NSLAB to control propionic acid bacteria activity to prevent the excessive gas production. Since the introduction of adjunct cultures of facultatively heterofermentative non-starter lactobacilli in Swiss-type cheese production, the defect of late blowing has decreased considerably (Fröhlich-Wyder & Bachmann, 2004; Daly et al., 2010) as well as some studies have been focused on the effect of bacteriocins producing NSLAB on the cheese microflora particularly sporeforming representatives during ripening (Nascimento et al., 2008; Izquierdo et al., 2009). Findings are of great interest because solutions how to diminish the spores activity and propionic acid bacteria providing secondary fermentation during ripening are still actual.

The aim of the present study was to analyse the influence of NSLAB on Swiss-type cheese quality.

2. MATERIALS AND METHODS

2.1. Materials

Raw milk, corresponding pasteurised milk and Swiss-type cheese samples (n=15) were analysed. The samples were taken from raw milk reservoirs in the one of Latvian milk processing company. Bulk milk was kept at $\leq 4^{\circ}\text{C} \leq 36$ hours prior treatment. Raw milk was pasteurised at 74°C 30 s in the dairy company. Treated milk samples were taken from cheese vats before renneting. Ripened Swiss-type semi-hard cheese (ripening regimes: $8-12^{\circ}\text{C}$, $20-25^{\circ}\text{C}$, $8-12^{\circ}\text{C}$ temperature, 85-90% relative humidity and ripening period 6 weeks for young cheeses and 3 months for matured experimental cheeses) samples were analysed at the end of maturation determining NSLAB representatives and their concentrations. Longer ripening period for experimental Swiss-type cheese samples had chosen for superior comparison of same age commercial cheeses.

For understanding the influence of NSLAB on Swiss-type cheese quality and aroma profile of samples, additionally three different Latvian cheese producers produced Swiss-type cheeses (two with local names and one - Maasdammer) were analysed. Analysed commercial cheeses were ripened at least 8 weeks. The characteristic of cheese samples used for study was summarized in Table 1.

Table 1. Characteristic of analysed semi-hard cheese samples

Cheese	Code	Fat in dry matter, %	Non-fat dry matter, %	Salt content, %
Swiss-type (experimental, 3 months ripening)	E	45	57.0	1.5
Swiss-type	CP	45	58.0	1.2
Swiss-type	RP	45	60.8	1.5
Maasdammer	SP	45	60.0	≤ 3.0

2.2. Methods of analyses

Determination of *Lactobacillus* spp. was made 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 chosen parameters for cultivation of lactic acid bacteria in MRS agar were 72 hours at 37 °C according to Coeuret and co-authors (2003) study recommendations.

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, France) was used for identification of the isolated colonies up to species level.

Aroma compounds were detected with Clarus 500 GC/MS (PerkinElmer®, USA) gas chromatograph with a tandem mass spectrometer.

For extraction of aroma compounds four grams of sample was weighted in glass bottle with volume 20 ml and screwed with aluminium cap. Aroma compounds of the sample were isolated in volatile phase at 70 °C for 30 minutes, then extraction of aroma compounds was provided using the CAR/PDMS coated fiber (75 µm) (Supelco, Bellafonte, PA) during 50 minutes.

Isolation of aroma compounds in headspace was performed by gas chromatograph equipped with capillary column J&W Scientific DB-Wax column (30m x 0.25mm x 0.25 µm). Oven temperature from 40 °C and sample holding time 2 min were gradually increased up to 160 °C with ascent 6 °C min⁻¹ and from 160 °C up to 210 °C with ascent 10 °C min⁻¹. Detector temperature was 250 °C. Flow rate of the carrier gas (helium) 1ml min⁻¹ and mode “*splitless*” was used. Desorption of isolated aroma compounds was two minutes at 250 °C. Identification of isolated aroma compounds was made with selective mass detector. Ionization performed with 70 eV energy and 250 °C input temperature. Identification of aroma compounds was made according to mass spectrum analogy with mass spectra libraries. Aroma compounds were only identified on quality basis.

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

3. RESULTS AND DISCUSSION

NSLAB of analysed samples are summarized in Table 2.

Table 2. NSLAB in raw milk, corresponding pasteurised milk and Swiss-type cheese samples

Indices	Results	NSLAB species
NSLAB in raw milk, CFU mL ⁻¹	$27.5 \cdot 10^4 \pm 5.25 \cdot 10^4$	<i>Lactobacillus plantarum</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus paracasei</i> , <i>Lactobacillus curvatus</i>
NSLAB in pasteurized milk, CFU mL ⁻¹	$4,50 \pm 3.66$	<i>Lactobacillus plantarum</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus paracasei</i>
NSLAB in Swiss-type cheese (6 weeks of ripening), young, CFU g ⁻¹	$1.45 \cdot 10^6 \pm 0.41 \cdot 10^6$	<i>Lactobacillus plantarum</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus paracasei</i>
NSLAB in Swiss-type cheese (3 months of ripening), matured (E), CFU g ⁻¹	$3.16 \cdot 10^8 \pm 0.69 \cdot 10^8$	<i>Lactobacillus paracasei</i>

The pasteurisation process reduces microorganisms in milk to a large extent, only small numbers of mesophilic lactobacilli may survive and subsequently grow in cheese made from pasteurised milk. All species present in pasteurised milk (*Lactobacillus paracasei*, *Lactobacillus brevis* and *Lactobacillus plantarum*) were also found in the corresponding raw milk samples with the exception of *Lactobacillus curvatus*. Bacteria commonly isolated from analysed young cheese samples are of the same type that is found in raw and pasteurised milk (Table 2).

Pasteurisation regimes selected in cheese manufacture are able to destroy lactic acid bacteria and their concentration decreased from $27.5 \cdot 10^4$ CFU mL⁻¹ in raw milk till 4,5 CFU mL⁻¹ in pasteurised milk. Inactivation level of lactic acid bacteria depends on the amount of microorganisms, species, their growth phase and other factors. 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 (Fox et al., 2004; Settanni & Moschetti, 2010; Mikelsone, 2011). In analysed Swiss-type cheese samples NSLAB varies from $1.45 \cdot 10^6$ to $3.16 \cdot 10^8$ CFU g⁻¹ (Table 2). The detected concentration confirms to previous mentioned statement that NSLAB concentration slowly increases and reaches a plateau at 10^7 - 10^9 CFU g⁻¹ after several months of cheese ripening (Fitzsimons et al., 2001). The population of young cheese was comprised of *Lactobacillus paracasei*, *Lactobacillus plantarum* and *Lactobacillus brevis* (Table 2) but in matured cheese only *Lactobacillus paracasei* dominated.

In the present study, microorganisms are identified based on phenotypical analysis and results of all analysed cheese samples are summarized in Table 3.

Table 3. NSLAB concentration and representatives in analysed Swiss-type cheeses

Cheeses	NSLAB, CFU g ⁻¹	Identified species
RP	$3.82 \cdot 10^8 \pm 2.24 \cdot 10^8_a$	<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i> <i>Streptococcus salivarius</i> subsp. <i>thermophilus</i>
CP	$3.48 \cdot 10^8 \pm 0.79 \cdot 10^8_a$	<i>Lactobacillus paracasei</i>
E	$3.16 \cdot 10^8 \pm 0.69 \cdot 10^8_a$	<i>Lactobacillus paracasei</i>
SP	$1.68 \cdot 10^8 \pm 0.35 \cdot 10^8_b$	<i>Lactobacillus paracasei</i>

Different letters in the column indicate significant differences ($p < 0.05$) between samples.

No significant differences ($p > 0.05$) were observed in quantity of NSLAB among cheese samples RP, CP and E. The different species are well adapted to changing environmental parameters of ripening. The present study has shown that NSLAB in Swiss-type cheese mainly derive from raw milk and generally only a few strains survive (see Table 2, Table 3) the processing conditions and grow during ripening. The comparison of the analysed cheeses in this study with the popular in the world produced Swiss-type cheeses confirms that the diversity of non-starter lactic acid bacteria species in semi-hard Swiss-type cheeses produced in Latvia is similar to those species isolated from Emmentaler, Maasdammer, Leerdammer. According to the findings by Fitzsimons et al. (2001) and Williams et al. (2002), the dominating non-starter lactic acid bacteria species usually change during the cheese ripening and represent by one *Lactobacillus* species at the end of ripening. In the opinion of these authors, *L.paracasei*, *L.plantarum* and *L.brevis* dominate in unripened cheeses, but *L.paracasei* – in ripened cheeses. Analysing Cheddar cheese, during the first weeks of ripening *L.paracasei*, *L.plantarum*, *L.rhamnosus* and presence of some unidentified representatives were detected, and by the end – only *L.paracasei* (Fitzsimons et al., 2001). Similar conclusions we made analysing data in Tables 2 and 3.

In ripening several hundreds of different compounds originate. The volatile compounds derive from glycolysis, proteolysis and lipolysis in cheese include volatile short chain fatty acids, ketones, alcohols, etc. form sensory properties of analysed Swiss-type cheeses (Table 4). Essentially important is not as much the amount of one or another compound in cheese, as their interactions. The actual role of aroma compounds in formation of a certain flavour could not be always determined, because each one has sensory properties different from cheese (Ozola & Ciprovica, 2002).

Summarization of the identified aroma compounds in analysed Swiss-type cheeses is given in Table 4.

The characteristic flavour of Swiss-type cheeses is caused primarily by acetic, propionic, butanoic, isovaleric and hexanoic (caproic) acids (Fox et al., 2004). The microbial flora of milk strongly influences the flavour development in cheeses and cheese quality indices. NSLAB slow down propionic acid fermentation. Jimeno et al. (1995) found growth inhibition of propionic acid bacteria in cheese, as consequence, less propionic acid is produced.

Table 4. Volatiles compounds composition (%) in cheese samples at the end of ripening

Compounds	E	SP	RP	CP
3-hydroxy-2-butanone (acetoin)	0.94	1.58	1.52	0.75
acetic acid	18.54	14.61	15.3	12.16
propionic acid	45.65	36.71	33.36	28.25
butanoic acid	4.66	5.73	13.12	39.81
hexanoic acid	8.78	11.91	11.11	4.55
benzylalcohol	0.70	0.29	0.34	n.i.
octanoic acid	8.34	12.15	13.49	5.95
nonanoic acid	1.12	0.54	0.54	1.29
decanoic acid	8.61	13.14	9.97	7.09
2-methyl-butanoic acid	1.03	0.65	n.i.	n.i.
dodecanoic acid	1.64	2.41	1.25	n.i.

n.i. – non identified

Propionic and acetic acid are two volatile compounds that are important for Swiss-type cheeses flavour. Salt and other minerals directly influence the saltiness and indirectly the total aroma intensity. Knowing the influence of salt on inhibition of propionic acid bacteria growing and the diminishing of Swiss-type cheeses aroma profile, the results of our study showed tendency in the intensification of cheese aroma under increased salt concentration, evaluating propionic and acetic acid concentration data. We observed that with the increasing of salt concentration the amount of NSLAB had decreased in cheeses. Beside the higher butanoic acid concentration in Swiss-type cheese (CP) we could explain by the activity of sporeforming representatives' in cheese sample with the lower salt content (CP sample). Some authors indicated that propionic acid bacteria strains which are more tolerant to salt would be more stable to NSLAB and sporeforming representatives' activity than strains that are sensitive to salt (Richoux et al., 1998). Based on previous mentioned statement we concluded that salt content and NSLAB concentration influence sporeforming growth ability and off flavour development in cheese during ripening.

In total, in the samples were detected: acetic acid, propionic acid, butyric acid, hexanoic acid, octanoic acid, nonanoic acid and decanoic acid. Their formation takes place in fat metabolism, imparting the product with different aroma notes. Excessive lipolysis is considered undesirable in many cheese varieties (Emmental, Swiss-type) and cheeses containing even a moderate concentration of free fatty acids may be considered as rancid by some authors (Fox et al., 2004). In Emmental cheese, moderate levels of free fatty acids (in the range 2-7 g kg⁻¹) are liberated during ripening and make an important contribution to the characteristic flavour and aroma of Emmental cheese (Frohlich-Wyder, Bachmann, 2004). Analysing volatile compounds results we make conclusions on excessive lipolysis under the influence of microflora especially in CP and RP samples.

Ketones add flavour of flowers and fruits to the cheese aroma. In the analysed samples was identified 3-hydroxy-butan-2-one. NSLAB seem to be capable of producing flavour compounds from amino acids, but this capacity is highly strain dependent. *Lactobacillus paracasei* subsp. *paracasei* strains generate aldehydes, alcohols and acids from branched-chain amino acids, for example, 2-methyl-butanoic acid from isoleucine (Fox et al., 2004).

Wilkinson and Kilcawley (2007) emphasize formation of butan-2-one derivatives as a result of transformations of citrates in Gouda type and related cheeses under the influence of starter and non-starter lactic acid bacteria.

Differences in the detected aroma compounds are observed among the analysed cheese samples. Individually detected aroma compounds in cheeses are able to impart different flavour notes, however, in certain combinations uncharacteristic aroma can occur. Among detected volatiles, butanoic acid presents in different quantities. In Emmental cheeses butanoic acid varies from 112 to 392 mg kg⁻¹, concentrations over 392 mg kg⁻¹ exhibit distinct flavour defects in commercial samples (Frohlich-Wyder & Bachmann, 2004). Apart from that, sensory properties are influenced negatively by increase of alcohols and ketones concentration in cheeses. The concentration of aroma compounds differs in cheeses of one variety, similarly to the identified non-starter lactic acid bacteria species. Detected aroma compounds and their concentrations in analysed cheeses do not allow assuring the undesirable impact of *L.paracasei* on the Swiss-type cheese flavour, but cheese moisture and salt content, indigenous milk microflora representatives, as well as interactions among cheese microflora representatives influence quality of produced cheese. NSLAB are citrate positive bacteria, it means that the energetic benefit of this metabolism allows bacteria to better withstand and to dominate during cheese ripening. Moreover, the citrate metabolism contributes to the production of toxic compounds, organic acids and diacetyl, active against many undesirable bacteria (Laëtitia et al., 2014). Although the uncontrolled growth of NSLAB may contribute to variable results in cheese maturation, further research is required to understand why Swiss-type cheeses from the same day of production may be free from the defects, while others may not.

4. CONCLUSIONS

NSLAB in Swiss-type cheese mainly derive from raw milk and generally only a few strains survive the processing conditions and grow during ripening.

The comparison of the analysed cheeses confirms that the diversity of non-starter lactic acid bacteria species in semi-hard Swiss-type cheeses produced in Latvia is similar to those species isolated from Emmental, Maasdammer, Leerdammer.

Analysing volatile compounds of cheese samples, the results show excessive lipolysis under the influence of cheese microflora especially in CP and RP samples.

Detected aroma compounds and their concentrations in analysed cheeses do not allow assuring the undesirable impact of NSLAB representative - *L.paracasei* on the Swiss-type cheese flavour, but cheese moisture and salt content, as well as interactions among cheese microflora representatives influence quality of produced cheese.

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