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CHARACTERIZATION OF AUTOCHTHONOUS LACTIC ACID BACTERIA FROM AN ARTISANAL ITALIAN CHEESE

Giuseppe ZEPPA [a)], Maria Grazia FORTINA [b)], Paola DOLCI [a)], Anna ACQUATI [a)], Annibale GANDINI [a)] and Pier Luigi MANACHINI [b)]

[a)] DI.VA.P.R.A., Settore Microbiologia e Industrie agrarie, Università degli Studi di Torino, Via L. da Vinci, 44, 10095, Grugliasco, Italy.

[b)] DI.S.T.A.M., Settore Microbiologia Industriale, Università degli Studi di Milano, Via Celoria 2, 20133, Milano, Italy.

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ABSTRACT

We studied the natural lactic bacteria population of an artisanal Italian cheese, Toma piemontese POD, from Piedmont (Northwest Italy), in order to select new strains to be used as starters in large-scale production. Isolates collected from curd and ripened artisanal cheeses were identified by the combined use of PCR 16S-23S rDNA spacer analysis, species-specific probes and 16S rDNA sequencing. Lactococci constitued 67% of the coccal isolates. Enterococci were also isolated together with strains of *Streptococcus macedonicus* and *S. thermophilus*. Lactobacilli were only detected in three samples of curds. Acidification and proteolytic activity and aroma production were also determined for each isolate. On the basis of the results a few isolates were selected and used as starters in cheesemaking trials with both raw and pasteurised milk. The produced cheeses were sensory evaluated and two of them showed typical Toma piemontese taste and flavour. The results suggest the possibility to use these new starters in both dairy industry and artisanal cheesemaking to improve product quality.

Key words: milk products / autochthonous cheese / cheese Toma piemontese / microbiology / lactic acid bacteria / strains / starters / Italy

KARAKTERIZACIJA MLEČNOKISLINSKIH BAKTERIJ V AVTOHTONEM KMEČKEM ITALIJANSKEM SIRU

IZVLEČEK

Preučevali smo naravne populacije mlečnokislinskih bakterij v avtohtonem kmečkem italijanskem siru “Toma piemontese POD” iz Piemonta v severozahodni Italiji, da bi pridobili nove seve, ki bi jih lahko uporabljali v industrijski proizvodnji. Izolate, ki smo jih osamili iz sirnine in zrelega avtohtonega sira smo identificirali s pomočjo kombinirane uporabe različnih metod in sicer analizo vmesne regije 16S-23S rDNA z verižno reakcijo s polimerazo, uporabo vrstno specifičnih začetnikov in sekvenciranjem 16S rDNA. Lactococci so predstavljali 67 % vseh izoliranih kokov. Enterokoke smo izolirali skupaj s sevi vrst *Streptococcus macedonicus* in *S. thermophilus.* Laktobacile smo odkrili samo v treh vzorcih sirnine. Za vsak izolat smo ugotavljali tudi proteolitično aktivnost, acidifikacijsko sposobnost in proizvodnjo arome. Na osnovi rezultatov smo izbrali nekatere isolate in jih kot starterske culture uporabili v poskusih izdelave sira in sicer iz surovega in pasteriziranega mleka. Sire smo senzorično ocenili in dva med njimi sta imela za “Toma piemontese» značilen okus in vonj. Naši rezultati nakazujejo možnost izboljšanja kakovosti proizvodov z uporabo teh novih starterskih kultur v sirarski industriji in v kmečki avtohtoni proizvodnji sirov.

Ključne besede: mlečni izdelki / avtohtoni sir / sir Toma piemontese / mikrobiologija / mlečnokislinske bakterije / sevi / starterske kulture / Italija

INTRODUCTION

In recent years several studies have been carried out to isolate and identify autochthonous lactic bacteria from both raw milk and artisanal cheeses produced with no addition of any starter cultures (Cogan *et al*., 1997; Coppola *et al*., 2001). Increasing information on the natural microbial population present in dairy products can help to prevent the loss of microbial biodiversity in typical foods and consequently the loss of a wide range of cheeses produced by different methods whose typical features depend on local and regional traditions and on the indigenous microbial population present in raw milk and selected by the cheesemaking environment.

Due to cheesemaker's increasing demand for new strains to improve cheese quality, we isolated and identified strains from Toma piemontese POD (Protected Origin Denomination) an artisanal cheese produced in Piedmont (Northwest Italy) to be selected and used as starters in both large-scale and artisanal cheesemaking.

Toma is a semi-cooked cheese which is produced in Piedmont from raw milk warmed to 37–40 °C. Rennet is added at a concentration of 0.15–0.20 mL L^{-1} and the clotting time is established visually by the cheesemaker. The curd is cut into 5–10 mm particles and collected with muslin, pressed and drained for 24 h. Cheese is ripened at 6–10 °C and 85% relative humidity for 30–40 days. The production and the ripening process depend entirely on the natural microbial population present in the milk.

The first objective of this work was to study the natural bacterial population present in the production of Toma piemontese cheese, while the second objective was to select new starters to be used in both artisanal and larger-scale cheesemaking.

MATERIALS AND METHODS

Sampling and isolation of bacteria

We collected samples from 7 dairy farms in different regions of Toma piemontese POD production area; 5 curd samples on the day of the production during the summer alpine pasture and 6 cheese samples at 30–40 days of ripening were analysed.

M17 agar medium was used to isolate enterococci, after incubation at 37 °C for 24–48 h and mesophilic and thermophilic cocci, after incubation at 30 °C and 37 °C for 48 h. We used MRS agar pH 5.8 to obtain lattobacilli, after incubation at 30 °C and 37 °C for 48 h.

Randomly selected colonies were purified and then submitted to microscopic examination, Gram staining. The catalase test, production of gas from D-glucose, growth at 6.5% NaCl, at 10 °C and 45 °C were evaluated as well.

DNA extraction

Genomic DNA for PCR reactions were extracted from 100 µl of an overnight culture diluted with 300 µl of TE 1X buffer (10 mM Tris-HCl, 1 mM Na_2EDTA, pH 8.0) as described by Mora *et al.* (2000).

Identification of isolates

The isolates were identified by the combined use of PCR 16S-23S rDNA spacer analysis (RSA), species-specific PCR and 16S rDNA sequencing according to the methods suggested by the following authors: Jensen *et al.* (1993) for RSA analysis; Ke *et al.* (1999) for species-specific PCR on enterococci, Cheng *et al.* (1997) on *Enterococcus faecium*, Dutka-Malen *et al.* (1995) on

E. faecalis, Lick *et al.* (1996) on *Streptococcus thermophilus*, Corroler *et al.* (1999) on *Lactococcus lactis*, Zlotkin *et al.* (1998) on *L. garvieae*, Ward and Timmins (1999) on *Lactobacillus casei*, *L. paracasei* and *L. rhamnosus*, Berthier and Ehrlich (1998) on *L. curvatus* and *L. sakei*.

A 500 bp portion of the 16S rRNA gene was sequenced for some isolates. Amplification was performed according to the protocol used by Lane (1991). PCR products were purified and sequenced using the dideoxy chain-termination principle (Sanger *et al.*, 1977). Taxonomic identification was performed using the Ribosomal Database Project (RPD-II) (Maidak *et al.*, 2001).

Aroma analysis

Aroma analysis was performed as follows: 3 g of inoculated milk for each isolate, 10 mL of internal standard (1-heptanol: 10 μg mL^{-1}) and 28% (w/w) of NaCl were mixed and stirred at 42 °C for 40 min.

Extraction was carried out at 42 °C for 20 min in head space by SPME by DVB/Carboxen/PDMS fiber (2 cm) and desorbtion at 270 °C for 4 min in splitless.

The analysis was carried out by DB-WAX capillary column according to the following operation conditions: 35 °C for 5 min; 2 °C/min to 183 °C; 5 °C/min to 210 °C; 3 min at 210 °C. Mass spectra was recorded in TIC mode, ionisation voltage of 70 eV and 33–300 amu mass range.

Acidification activity

In order to evaluate acidification activity of the isolates, we inoculated milk at 2% for each strain and pH measures were taken for 24 h, at incubation temperature of 37 °C.

Protease activity

Protease activity of the isolates was observed for each strain inoculated in milk at 2% and incubated at 37 °C for 24 h, by colorimetric determination with the Hull method.

Cheesemaking trials

A few isolates were selected and used as starter in cheesmaking trials.

Eight cheesemakings were carried out on pasteurized cow milk by using 7 different starter mixtures of lactic bacteria isolates and one commercial starter.

Two cheesemakings were carried out on raw cow milk by using starter n.7 and without the addition of any starter culture.

Sensory evaluation was carried out on Toma cheese obtained at 60 days ripening.

RESULTS

Isolates

Altogether 116 coccal isolates were collected, 53 from curd samples and 63 from cheese samples (Table 1). The viable counts on M17 plates varied from 10^5 to 10^6 cfu g^{-1} for curd samples; higher levels were reached in cheese samples, 10^8 cfu g^{-1}. Lactobacilli were only detected in three samples and their incidence was very low, 1 to 7 cfu g^{-1}.

Table 1. Identification of cocci from Toma samples
Preglednica 1. Identifikacija kokov v siru Toma

Identification	Number of strains
L. lactis subsp. *lactis*	24
L. lactis subsp. *cremoris*	10
L. garvieae	44
S. suis	6
S. agalactiae	2
S. dysgalactiae	2
S. macedonicus	6
S. thermophilus	3
S. uberis	1
E. faecium	9
E. durans	4
E. faecalis	2
Enterococcus spp.	4

Identification of isolates

On the basis of PCR amplification of the 16S-23S rRNA spacer region (RSA) it was possible to cluster the coccal isolates in 5 groups (Fig. 1).

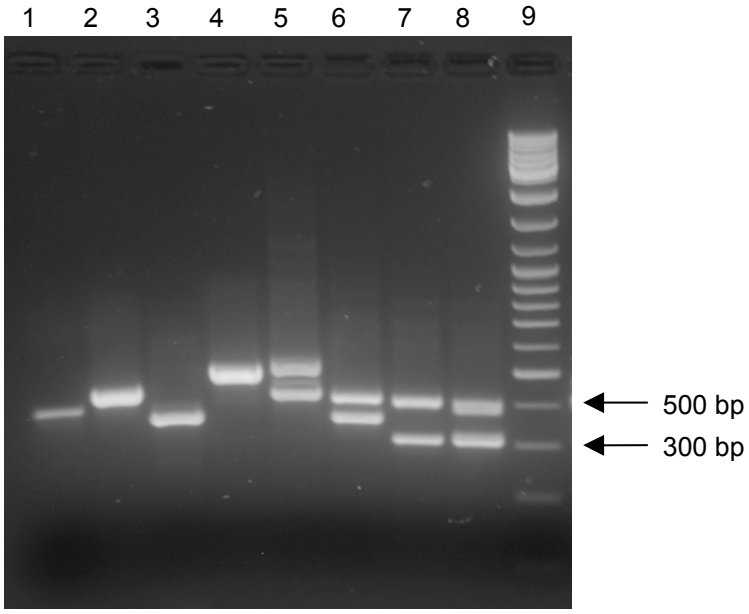

Figure 1. RSA profiles of representative coccal strains of each cluster obtained. Lanes 1–4: cluster I, II, III, IV; lanes 5–8: cluster V, subgroups a, b, c, d; lane 9: DNA Ladder Mix.
Slika 1. Profili regije med rRNA geni pri izbranih sevih, ki predstavljajo posamezne skupine kokov. Steze 1–4: skupine I, II, III in IV; steze 5–8: podskupine a, b, c in d; steza 9: DNA standard.

The isolates grouped in cluster I showed typical spacer region referable to *Lactococcus* lactis, the cocci belonging to cluster II showed a typical spacer region referable to *L. garvieae*, the ones of group III to *S. thermophilus*, the ones of group V to genera *Enterococcus*. In cluster IV six isolates were grouped showing a band at 500 bp.

16S rRNA sequence analysis was necessary to identify isolates included in cluster IV, for which no indication was obtainable by RSA analysis. The analysis of the 16S rRNA sequence together with species-specific PCR was also necessary to identify some dubious isolates belonging to the other groups.

Specific PCR and RSA analysis were also used to identify lactobacilli: three strains belonged to the species *Lactobacillus fermentum*, one to *L. coryniformis*, two to *L. paracasei*, two to *L. curvatus* and two to *L. rhamnosus*.

Aroma analysis

Genetically identified isolates were submitted to aroma analysis; they were chosen among the strains obtained from curd and belonging to the species *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *S. thermophilus*, *L. paracasei*,

GC-MS analysis highlighted the presence of about 100 organic compounds mainly belonging to aldehydes, alcohols, acids, of which over 40 are not present in the milk.

The processing of these data by Principal Component Analysis allowed us to group these isolates in 4 sets by using the first three components (Fig. 2).

A group is formed by B18 strains belonging to the species *S. thermophilus*. *L. lactis* supsp. *lactis* strains A4, A1, A18 and A13, A20, A6 form respectively the second and third group. The fourth group is constituted by the only *L. lactis* supsp. *cremoris* A8 strains.

Milk aroma is quite different therefore it is located in a different space (M) of the score plot.

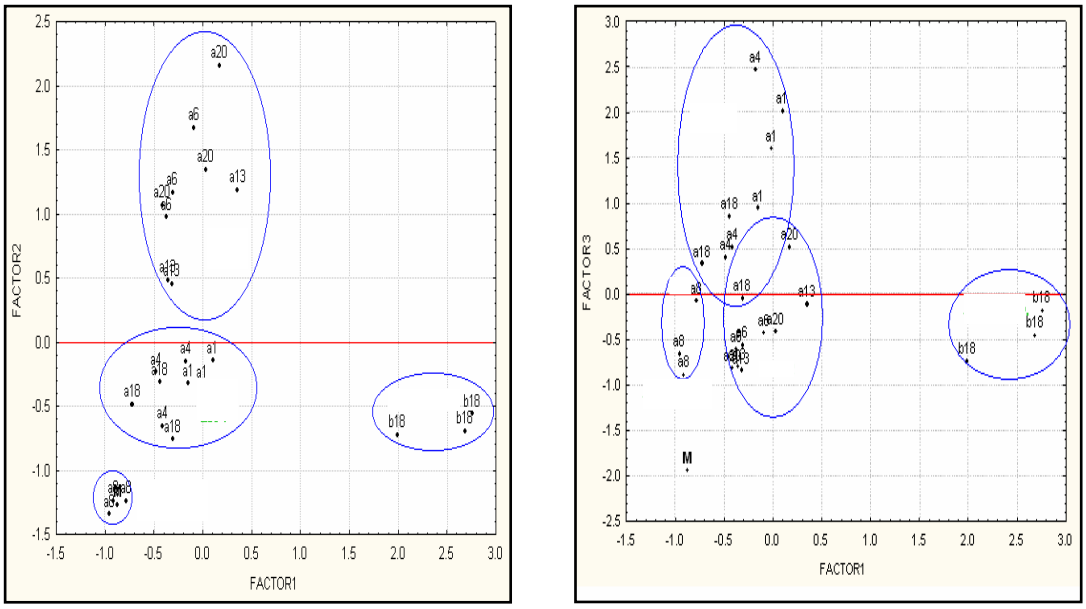

Figure 2. Score plots of the first three PCA (Principal Component Analysis) factors performed on the basis of the concentration of aromatic compounds produced by lactic acid bacteria strains.

Slika 2. Predstavitev vrednosti prvih treh dejavnikov (analiza glavnih komponent) na osnovi koncentracij aromatskih sestavin, ki jih proizvajajo sevi mlečnokislinskih bakterij.

Starters

The selection of strains to be used as starters in cheesemaking trials was carried out by the choice of, at least, one isolate for each PCA group. For each group was considered the strain showing quicker acidification activity in the 24 h and higher protease activity. One *L. paracasei* strain has been added to starters n.2, n.4, n.6 for the high acidification and proteolytic activities showed. The bacterial composition of the seven starter mixes, chosen according to the above criteria and to the results obtained, is shown in Table 2.

Generally each mix contained only two strains with the exception of starter n. 7 composed of five different strains. The mix combination of two strains of *L. lactis* supsp. *lactis* has not been considered while a commercial starter, generally used for Toma piemontese POD cheesemaking, was used.

Cheesemaking trials

We obtained the best sensory results from n.7 in trials with both pasteurized and raw cow milk. In both cases cheese produced from starter n.7 was better in structure, taste and aroma than cheese without the addition of any starter.

Table 2. Starter compositions used in cheesemaking trials
Preglednica 2. Sestava starterskih kultur, ki so bile uporabljene v sirarskem poskusu

Starter	Isolates
n.1	B18 (*S. thermophilus*) – A18 (*L. lactis* subsp. *cremoris*)
n.2	G1 (*Lb. paracasei*) – A18 (*L. lactis* subsp. *cremoris*)
n.3	A6 (*L. lactis* subsp. *lactis*) – B18 (*S. thermophilus)*
n.4	A6 (*L. lactis* subsp. *lactis*) – G1 *(Lb. paracasei)*
n.5	A8 *(L. lactis* subsp. *lactis)* – B18 *(S. thermophilus)*
n.6	A8 *(L. lactis* subsp. *lactis)* –G1 *(Lb. paracasei*)
n.7	A6 *(L. lactis* subsp. *lactis)* – A8 *(L. lactis* subsp. *lactis)* – A18 (*L. lactis* subsp. *cremoris*) – B18 *(S. thermophilus)* – G1 *(Lb. paracasei*)
n.8	Commercial starter

Toma cheese obtained by using starter n.7 in raw milk compared to the one without starter is shown in Fig. 3. The relative sensory evaluations showed that the first one is a good product characterized by typical aroma and taste, proper eyes and structure while the other one showed defective structure and strong and bitter aroma due to lipolysis and proteolysis.

CONCLUSIONS

The results of the present work represent the first approach to understanding the bacterial population involved in traditional Toma piemontese POD cheese.

The technological performance of these strains suggest the possibility of their use in the production of Toma piemontese POD cheese in order to improve and standardize product quality.

Further investigations to prove the technological characteristics of these strains and their stability will be needed.

New cheesemakings are still in progress to test new starter combinations.

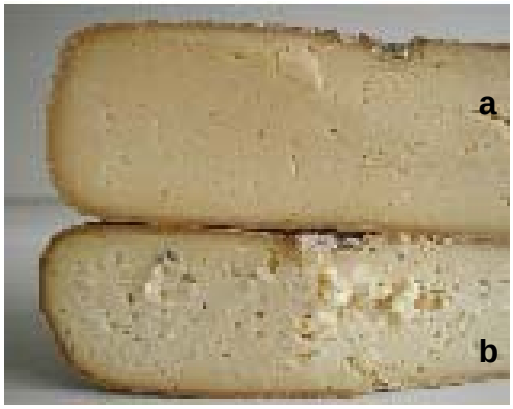

Figure 3. Toma cheese produced from raw cow milk using starter n.7 (a) and with no addition of any starter (b).

Slika 3. Sir Toma, izdelan iz surovega mleka ob uporabi starterske kulture št. 7 (a) in brez starterske culture (b).

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TRADITIONAL MANUFACTURING OF HARD CHESE – KACHKAVAL ON STARA PLANINA MOUNTAIN

Zora MIJAČEVIĆ [a)] and Snežana BULAJIĆ [b)]

[a)] Univ. of Belgrade, Fac. of veterinary medicine, Dept. of hygiene and technology of food products of animal origin, Bulevar JA 18, 11000 Belgrade, Serbia, Prof., Ph.D.

[b)] Univ. of Belgrade, Fac. of veterinary medicine, Dept. of hygiene and technology of food products of animal origin, Bulevar JA 18, 11000 Belgrade, Serbia, M.Sc.

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ABSTRACT

Nomads from Greece implemented the tradition of kachkaval cheese making on Stara Planina, 100 years ago. At the beginning, the kachkaval was made exclusively from sheep's milk, during the grazing period. In the past, kachkaval was made on «bačija« dairies, and besides the kachkaval the significant by-products were ghee and «urda«. The historical data showed that in the year 1903, 160 wagons of kachkaval were exported to Vienna and Budapest. The changes in animal breeding conditions and increasing number of cattle in this region lead to significant reduction in number of sheep, and consequently caused the discontinuance in production of kachkaval. Nowadays, there are few "bačija"-dairies on Stara Planina Mountain which are producing kachkaval in the traditional way. The characteristics of chemical content of traditionally produced kachkaval are: 60.79±3.37% dry matter, 29.50±2.25% milk fat, 55.57±3.55% moisture on a fat-free basis, 48.52±2.41% fat in dry matter, 24.99±1.60% proteins, 3.8±2.57% NaCl and pH value 5.62±0.09. The traditionally made kachaval has a shape of a flatten cylinder, with the weigh of 6–7 kg, bright-yellow crust and firm dough. The traditional cheese making of kachkaval comprises the hand made process of «pasta filatta« cheese and for the end product a spicy and salty taste are typical. Revitalizing the traditional kachkaval production also means the preservation of cultural identity of the region of Stara Planina.

Key words: milk products / cheese / kachkaval / Stara Planina / traditional cheese making / Serbia

TRADICIONALNA IZDELAVA TRDEGA SIRA KAČKAVALJ NA STARI PLANINI

IZVLEČEK

Tradicija proizvodnje sira kačkavalj na Stari Planini izvira od nomadskih pastirjev iz Grčije, ki so jo tja prinesli pred več kot 100 leti. Na začetku so kačkavalj izdelovali izključno iz ovčjega mleka v pašnem obdobju. Kačkavalj so izdelovali v mlekarnah – "bačijah", poleg sira pa sta bila tipična stranska proizvoda še malso in "urda". Po zgodovinskih podatkih so leta 1903 izvozili 160 vagonov kačkavalja s Stare Planine na Dunaj in v Budimpešto. S spremembo pogojev v živinoreji in s povečanjem staleža govedi, se je število ovac na Stari Planini bistveno zmanjšalo, zato je tudi proizvodnja kačkavalja skoraj zamrla. Danes še obstoja nekaj mlekarn –"bačij" na področju Stare Planine, ki še vedno proizvajajo kačkavalj po tradicionalnem postopku. Kačkavalj, izdelan na tradicionalen način, vsebuje 60,79±3,37 % suhe snovi, 29,50±2,25 % maščobe, 48,52±2,41 % maščobe v suhi snovi, 24,99±1,60 % beljakovin, 3,8±2,57 % NaCl, in ima pH vrednost 5,62±0,09. Tradicionalna oblika kačkavalja je sploščen cilinder, ki tehta 6–7 kilogramov, ima svetlo rumeno skorjo, in čvrsto testo. Tradicionalna izdelava kačkavalja temelji na ročni pripravi pasta filata sira (kuhan sir), za končni izdelek pa je značilen pikanten in slan

okus. Oživljanje tradicije izdelave kačkavalja na področju Stare Planine je pomembno tudi zaradi ohranjanja kulturne identitete te regije.

Ključne besede: mlečni izdelki / sir / kačkavalj / Stara Planina / tradicionalna izdelava sira / Srbija

THE HISTORY

Hard cheese, so called kachkaval, is produced in Mediterranean region, including Adriatic and Black Sea regions, in other words territory once under the rule of Ancient Greece, Roman Empire and their eastern and southern colonies. Based on written historical monuments, we can conclude that kachkaval has a very long history. Columella, the roman writer (Pejić, 1956) from 68 AD described the process of cheese making »manum pressum«, which is almost identical to the production of kachkaval.

The region where kachkaval was manufactured in the past and where it is manufactured today is in the fact the region where during the second century BC and all up to the seventh century of the new era great migrations of nomadic tribes have occurred. Their major agricultural activity was cattle and sheep breeding.

On the Balkan Peninsula, especially in Romania, Bulgaria, Greece and Serbia, the animal breeding was mostly in the hands of Tzintzars, who were manufacturing predominantly hard cheese – kachkaval. According to historical data Tzintzars were natives of Balkan Peninsula, who settled there before Romans, and even perhaps before the ancient Greeks.

According to the historical material (Pejić, 1956), it can be most certainly presumed that the manufacturing of cheese was brought to the Balkan Peninsula by nomadic tribes from the East. Later, the manufacturing of this cheese was brought to Italy and from there to Britain during the period of Julius Caesar. In Britain, the technology of making cheese has been adapted to climatic conditions and leading to the development of the technology for Cheddar cheese production.

This ancient technology of cheese making is mostly spread on the Balkan Peninsula and in Italy, as well as in the southern regions of Russia (the Crimea, Be Arabia, South Ukraine), in Turkey, Algeria, Tunisia, Egypt and Morocco, that is in the regions with warm and dry climate, hilly relief and developed sheep breeding (Pejić, 1956).

In each of these countries with developed manufacturing of hard cheese during the time some varieties or types of hard cheese developed distinguishing themselves according to the properties and quality, such as hard cheese from Šarplanina Mountain, Pirot hard cheese in Serbia, Pirdop cheese from Bulgaria, Penlu from Romania and Tesalia and Epiria cheese from Greece.

The origin of the name kachkaval can be found in the language of the people who most probably brought kachkaval to the Balkan Peninsula from where it spread further. In the Tzintzar language the word ″kač″ means cheese.

The story about hard cheese production on Stara Planina Mountain necessarily includes the nomadic sheep breeders, who are known as ″Crnovunci″ (Blackwool people). They were named after the black colour of the wool of their sheep. In the period from the end of the 19th century up to the third decade of the 20th century "Crnovunci" populated the pastures of Stara Planina Mountain with their flocks of 500–1000 sheep. Large quantities of sheep milk, which gave their flocks, "Crnovunci" processed into white cheese and kept it in bellows. In order to prolong the durability of this cheese, it was soaked into hot water, mixed and salted, that is, in other words, the processing of milk into hard cheese – kachkaval had started. The skill of making kachkaval "Crnovunci" have passed on to the people of Dojkinci village, who improved it to the perfection and kept until today (Petrović, 1997).

Production of kachkaval on Stara Planina Mountain is connected with small dairies called «bačija». «Bačija» dairy represents cooperative organization for joint sheep keeping, pasture, production and processing of all products – wool, meat and milk. Peasants realized that small

number of sheep is of no use, therefore they associated in all activities relating to sheep breeding and production at dairies – «bačije» (Stojanović et Katić, 2003).

Manufacturing of hard cheese – kachkaval and other products was the same at all "bačija" dairies on Stara Planina Mountain. It is known that they were made from light material that could be transported, premises were exposed to draft, and their size varied depending on the quantity of milk. Processing of milk was carried out in wooden tubs containing warm water where the «baskia» was fumed, and also dairies – «bačija» had tables for casting and salting as well as a table for cheese ripening (Pejić, 1956).

PRODUCTION STAGES IN MANUFACTURING OF HARD CHEESE – KACHKAVAL IN DAIRY PLANT IN THE VILLAGE OF DOJKINCI ON STARA PLANINA MOUNTAIN

On Stara Planina Mountain, in the village of Dojkinci, dairy plant has a daily capacity for processing of 2000 l of milk into kachkaval. Altogether there are 7 similar dairies on Stara Planina Mountain at present that are processing larger or smaller quantities of milk. Milk is combined sheep's and cow's milk, although according to the original technology of kachkaval production only sheep milk is to be used. Prepared quantity of milk (800 l) from Dojkinci is heated until the temperature of 65–70 °C is achieved. This temperature is achieved in approximately two hours and subsequently warmed milk is mixed with remaining milk coming from other villages. Temperature of mixed milk is 30 °C and acidity 8–8.2 °SH. The curdling temperature does not change during the whole phase of curdling which lasts for 60 minutes. The phase of processing curd starts by curd chopping up by means of the cross and lasts for 5 minutes, the temperature of separated whey is 29.6 °C and acidity 6.6°SH, while the temperature of the curd is 29 °C and acidity 5.2 °SH. After separating a part of whey, the curd is again intensively mixed to separate the grains and again a part of whey is being separated, with acidity of 6.4 °SH, and temperature 29.9 °C, while the curd has acidity of 10.8 °SH and temperature of 29.5 °C. After that the warm water from the boiler is added and the temperature of curd is lifted to 34.9 °C. The duration of the phase of drying the curd is 10 minutes, until the required strength of the grains has been reached. The acidity of whey is 9.6 °SH. The curd is then left in the bath, pressed for 10 min to separate whey and then cut, taken out from the bath and transferred to the drainer on to the cheese-making table, grained, allotted and pressed for 20 minutes, the pressing being achieved by screwing the press according to the experience. After pressing the lump is cut into pieces, taken out from the drainer and left on the draining table until the next day. The temperature of the room is 20.7 °C, and the period of ripening of baskia is 19–20 hours. The room temperature during the night is 15–16 °C. After the acidity has been inspected by spreading (Fig. 1), "baskia" is chopped on the cutter like that for cabbage and steamed in the water whose temperature is 75 °C. Steaming is performed in weaved baskets (Fig. 2), containing about 6 kg of baskia, devaporated and put onto the cheese-making table (Fig. 3), salted (Fig. 4), formed into the ball (Fig. 5) and put into casts (Fig. 6). The cheese remains in the casts until the next day. Than it is taken out, weighed and transferred into the room at 20 °C for drying. After drying for 1–2 days, it is transferred into the room for cheese ripening. The temperature in the ripening room 20 °C, and the cakes of cheese are put one onto the other and salted with dry salt. Finally, the ripened kachkaval is packed into vacuum bags.

Major characteristics of chemical composition of hard cheese from Stara Planina Mountain are shown in Table 1.

Chemical composition of the kachkaval from Stara Planina Mountain varied and ratio between the minimum and maximum value of dry matter was 10%. It must also be pointed out

that percentage of salt in certain kachkavals also varied and that there were samples of cheese with over 7% of salt.

Table 1. Chemical composition of kachkaval from Stara Planina Mountain (Mijačević *et al.*, 2003)
Preglednica 1. Kemična sestava sira kačkavalj s Stare Planine (Mijačević in sod., 2003)

Parameters / Parametri	n	X±s	min	max
% dry matter % suhe snovi	7	60.79±3.37	54.55	64.05
% fat % maščobe	7	29.50±2.25	25.00	32.50
% water without fat % vode brez maščobe	7	55.57±3.55	50.07	60.60
% fat in dry matter % maščobe v suhi snovi	7	48.52±2.41	45.83	53.03
% protein % beljakovin	7	24.99±1.60	25.07	27.38
% dissolved protein % raztopljenih beljakovin	7	5.72±0.22	5.42	6.02
% NaCl	7	3.80±2.57	1.66	7.67
pH	7	5.62±0.09	5.50	5.75
oSH	7	37.82±8.41	23.20	51.20

Table 2. Sensory evaluation of cheese in different ripening stages
Preglednica 2. Senzorična ocena sira v različnih fazah zorenja

Ripening period of cheese Čas zorenja sira	Crust Skorja	Cross section Prerez	Consistency Konzistenca	Smell Vonj	Taste Okus
2 days 2 dneva	Characteristic for young cheese	Technological holes, grain of not devaporated baskia, layers well expressed	Soft, elastic	Smells like milk	Bitter
30 days 30 dni	Yellow crust, integrity preserved	Technological holes, layers preserved	Firm	Smell of sheep and cow milk	Salty, bitter, fine melting in the mouth
60 days 60 dni	Yellow crust, integrity preserved	Technological holes	Firm	Smell of sheep and cow milk	Salty, bitter, fine melting in the mouth

THE ANALYSIS OF SENSORY CHARACTERISTICS OF KACHKAVAL FROM STARA PLANINA MOUNTAIN

Sensory evaluation of 2 days old cheese in drying process, cheese that ripened for 30 and 60 days, respectively, was carried out by three evaluators. Sensory evaluation of investigated cheese is presented in Table 2.

Sensory evaluation indicates that 30 days old cheese can be placed on the market labeled as cheese, ripe for consumption. However, true ripeness is achieved after the period of 60 days. In general, cheese is salty, and the cross section shows technological holes. Occurrence of technological holes is characteristic for manual processing of steamed dough, however, number of holes should be low and their size relatively small.

Analysis of cheese, ripe for consumption and ripe cheese has revealed the absence of coliform bacteria and *E. coli.* However, some useful microorganisms were isolated that are responsible for specific taste of the cheese. In the cheese, originating from Stara Planina Mountain, the most often isolated bacteria are *Enterococcus spp*, *Lactococcus spp*., *Leuconostoc spp* and *Lactobacilus spp*., with specific characteristic being able to grow in conditions of high and low temperatures and in the broth with increased quantity of salt.

CONCLUSION

Since there is preserved tradition of manufacturing hard cheese – kachkaval on Stara Planina Mountain, with already famous Senokos, Dojkinci, and Pirot kachkaval, this is also the justification of the necessity for protection of kachkval produced in this region with certain label defining geographical origin of the product. Preservation of the tradition of cheese manufacturing means at the same time also preservation of the cultural identity of the region (Dozet *et al.*, 1996, Mijačević *et al.*, 2003a and 2003b)

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USE OF MONO- AND SESQUITERPENES FOR CHARACTERISATION OF MOUNTAIN CHEESES

Giuseppe ZEPPA [a)], Manuela GIORDANO [a)], Marta BERTOLINO [a)] and Vincenzo GERBI [a)]

[a)] University of Turin, Department of Exploitation and Protection of the Agricultural and Forestry Resources – Laboratory of Food Technology, Via L. da Vinci 44, 10095, Grugliasco (TO), Italy.

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ABSTRACT

Terpenes are volatile compounds originating exclusively from plants that can be found in dairy products. Several studies have therefore been conducted to use the terpenes to trace the geographical origin or the nature of the feed supplied to animals. The vegetation of mountain pastures varies which results in different types of foraging. Our aim was to determine if the terpene profile can be used to distinguish the mountain pasture of origin. The mono- and the sesquiterpenes of ricotta cheese samples produced during the summer in three mountain farms were then determined with SPME-GC-MS. The ricotta cheeses were produced with cow and goat milk and every three days of production a sample was analysed. Obtained results showed that these compounds vary widely between samples due to the stage of plant development but discrimination among the farms was still possible. Hence mono- and sesquiterpenes can be used not only to distinguish between summer pasture cheeses and the ones produced during other periods but also as markers of each mountain pasture.

Key words: milk products / cheese / ricotta / characterisation / terpenes / sesquiterpenes / markers

UPORABA MONO- IN SESKVITERPENOV ZA KARAKTERIZACIJO SIROV S PLANIN

IZVLEČEK

Terpeni so hlapne snovi izključno rastlinskega izvora, ki jih lahko najdemo v mlečnih izdelkih. Opravljene so bile študije, v katerih so terpene uporabljali za sledenje in ugotavljanje geografskega porekla krme, ki so jo zaužile živali. Razlike v vegetaciji na planinskih pašnikih se odražajo tudi v krmi. V tej raziskavi smo želeli proučiti možnost, če na osnovi profila terpenov lahko ugotovimo ali so se krave pasle na planinskem pašniku. Poleti smo s SPME-GC-MS ugotavljali mono- in seskviterpene v vzorcih sira "ricotta" s treh planinskih farm.. Vzorce sira "ricotta", ki so ga izdelovali iz kravjega in ovčjega mleka smo zbirali vsak tretji dan in opravili analize. Čeprav rezultati kažejo, da se vsebnost proučevanih snovi v vzorcih, zaradi razlik v razvojni fazi rastlin, zelo razlikuje, smo lahko ločevali vzorce z različnih farm. Mono- in seskviterpene lahko uporabimo za ločevanja med siri, ki so bili izdelani med pašno sezono in izven nje pa tudi kot markerje za posamezne planinske pašnike.

Ključne besede: mlečni izdelki / sir / ricotta / karakterizacija / terpeni / seskviterpeni / markerji

INTRODUCTION

Animal feeding is a very important factor in cheese characterisation due to its action on bacteria and milk compounds such as fats, proteins, flavours and so on. Several studies have

highlighted the possibility to define a relation between cheese and its production area with the study of animal feed and above all with the study of terpene compounds (Dumont and Adda, 1978; Moio *et al.*, 1996; Mariaca *et al.*, 1997; Schehovic *et al.*, 1998; Viallon *et al.*, 1999; Bugaud *et al.*, 2000; Viallon *et al.*, 2000; Bugaud *et al.*, 2001a; Bugaud *et al.*, 2001b). These molecules are secondary metabolites of plants, well-known for their disinfectant (medicinal) and odorant (spice) properties. In the plant kingdom their qualitative and quantitative distribution is highly variable, but they are specie-specific thus many researchers have suggested that the analysis of such substances might improve the traceability of dairy products or meats originating from animals raised in specific geographical areas. Results have shown that the milk and the cheese from different production sites (lowland vs. highland) and seasons (winter vs. summer) can be distinguished (Jeangros *et al.*, 1997; Bosset *et al.*, 1999; Buchin *et al.*, 1999; Cornu *et al.*, 1999; Buchin *et al.*, 2002). In fact terpene compounds are more abundant in dicotyledones than in monocothyledons which are more abundant in alpine pastures. Terpenes are also more abundant in fresh grass than in hay. The aim of this work was to verify if mono- and sesquiterpens can also be used to distinguish the products from different highlands pastures and to define a traceability system for a typical mountain cheese.

MATERIALS AND METHODS

Three mountain farms (Alpe Bancet, Alpe Gianna, Alpe Pra) were used in this study. The farms are located in Pellice Valley near Torino (Piedmont, North West Italy) at 1000–2500 m. A sample of ricotta cheese has been taken every four days for the period July 12/August 23 for Alpe Pra, for the period July 12/September 16 for Alpe Bancet and for the period July 14/September 16 for the Alpe Gianna. These differences of sampling are due to the different time of mountain grazing.

Each sample was vacuum packed in polyethylene bags and stored to –20 °C. Samples of 2.5 g of ricotta cheese were taken and placed in a 10 mL glass vial (38 mm high and 22 mm in diameter) and sealed with 20 mm PTFE/Silicone septum caps (Supelco, Bellefonte,USA).

For the conditioning, the sample vial was placed in a 53 °C water bath for 10 min. The fiber used for the extraction was a DVB/Carboxen/PDMS 2 cm stable flex fiber (Supelco, Bellefonte, USA).

The volatile components were extracted using the static headspace method. During this step the fiber was exposed for 60 min in the headspace of the cheese with the vial maintained at 53 °C in a thermostatic bath. Every sample was analysed in triplicate.

The adsorbed molecules were desorbed by introducing the SPME fiber into the injector of a gaschromatograph (GC17A, Shimadzu, Tokyo, Japan) at 270 °C for 6 min in splitless mode. The volatile components were separated on a DB-WAX capillary column (30 m × 0.25 mm ID; film thickness 0.25 μm; J&W Scientific Inc., Folsom, CA, USA). The oven temperature program and the operating conditions were as follow: carrier gas helium at 1 mL min^{-1}; the column was maintained at 35 °C for 5 min, ramped at 2 °C min^{-1} to 173 °C, maintained at 173 °C for 1 min, ramped at 15 °C min^{-1} to 210 and finally maintained at 210 °C for 5 min. Mass spectra were recorded in the electron impact mode at an ionisation voltage of 70 eV in the 33–300 amu mass range. The ion source and the interface were maintained at 220 °C. Compounds identification was carried out with the mass spectra and retention times of standard compound, when available, or the NIST 12 and NIST62 mass spectral data base.

Statistical analysis was performed with Statistica ver. 6.0 (Statsoft Inc., Tulsa, OK, USA).

RESULTS

The analysis of volatile compounds highlighted the presence of twenty-two monoterpenes (over all α-pinene, β-pinene, camphene, *p*-cymene, β-myrcene and limonene) and sixteen sesquiterpenes such as α-caryophyllene, α-copaene and 9-epi-caryophyllene. Twenty-one monoterpenes were detected in the Alpe Bancet ricotta, 16 in the Alpe Gianna ricotta and 20 in the Alpe Pra ricotta. In these products also 13, 11 and 16 sesquiterpenes, respectively, were detected (Table 1).

Table 1. Monoterpenes (t) and sesquiterpenes (s) detected in the ricotta cheeses analysed for each mountain farm (n.i. – not identified)

Preglednica 1. monoterpeni (t) in seskviterpeni (s), odkriti v siru "ricotta" s posameznih gorskih farm (n.i. – ni identificirano)

		BANCET	GIANNA	PRA			BANCET	GIANNA	PRA
α-phellandrene	t	*	*	*	9-epi-caryophyllene	s	*	*	*
α-pinene	t	*	*	*	α-caryophyllene (α-humulene)	s	*	*	*
α-terpineol	t	*	*	*	α-copaene	s	*	*	*
β-myrcene	t	*		*	α-isocomene	s			*
bornyl acetate	t	*			β-maaliene	s			*
β-pinene	t	*	*	*	epi-cedrane	s	*	*	*
Camphene	t	*	*	*	isocaryophyllene	s		*	*
δ-3-carene	t	*	*	*	selinene	s	*	*	*
dihydro carveol acetate	t			*	sesquiterpene (n.i.)	s	*	*	*
D-verbenone	t	*		*	sesquiterpene (n.i.)	s	*		*
γ-terpinene	t	*	*	*	sesquiterpene (n.i.)	s	*	*	*
Limonene	t	*	*	*	sesquiterpene (n.i.)	s	*	*	*
Linalool	t	*	*	*	sesquiterpene (n.i.)	s	*	*	*
Myrtenol	t	*	*	*	sesquiterpene (n.i.)	s	*		*
p-cymene	t	*	*	*	sesquiterpene (n.i.)	s			*
Sabinene	t	*	*	*	valencene	s	*	*	*
terpene (n.i.)	t	*	*	*					
terpene (n.i.)	t	*	*	*					
terpene (n.i.)	t	*		*					
terpene (n.i.)	t	*	*	*					
terpene (n.i.)	t	*	*	*					
terpene (n.i.)	t	*		*					

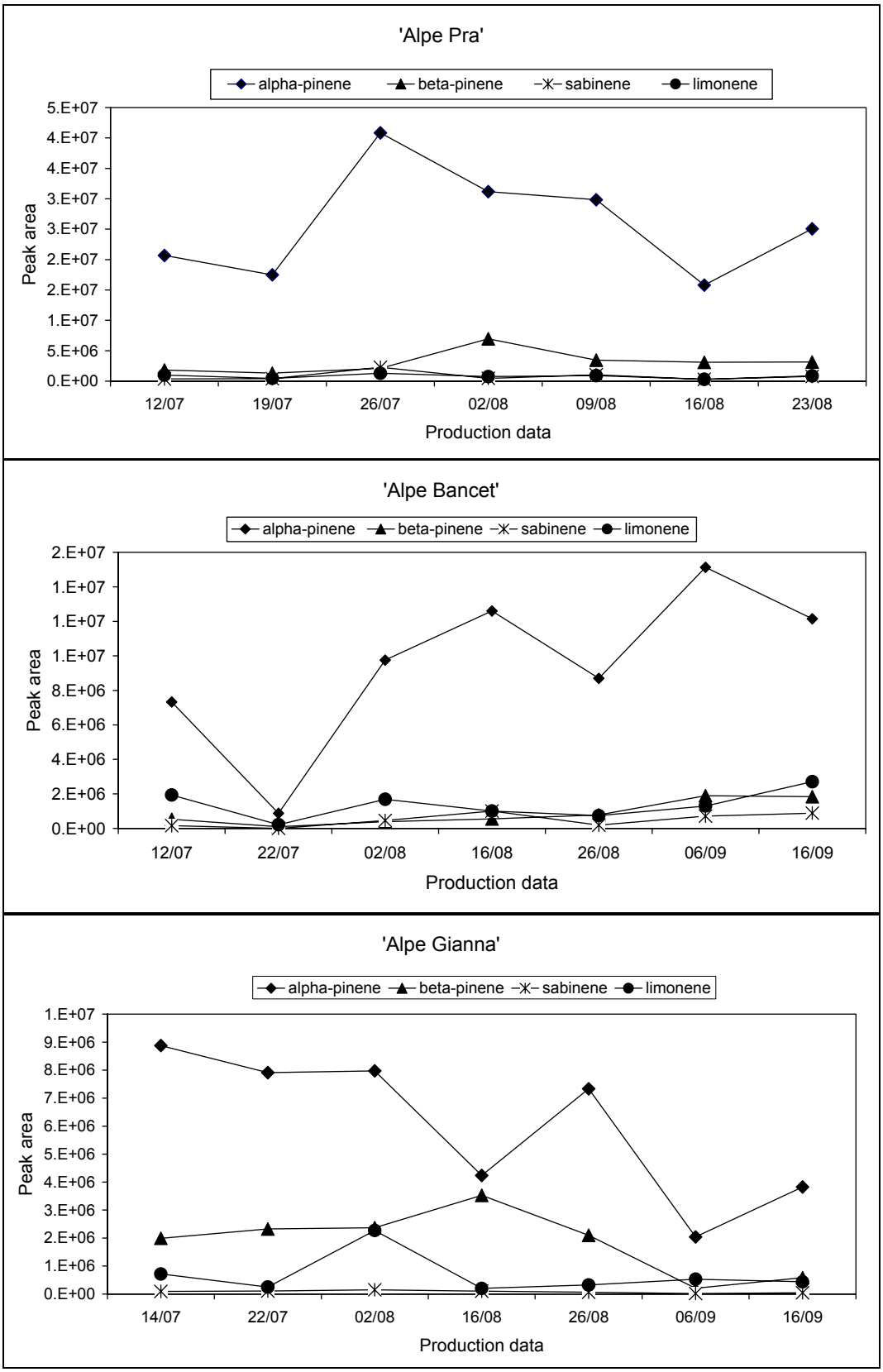

Figure 1. Trend of some terpenes (reported as area of base peak ion) for the three mountain farms ricotta cheeses during the grazing.

Slika 1. Trend gibanja posameznih terpenov v vzorcih sira "ricotta" s treh planinskih farm v pašni sezoni.

All these compounds showed a wide concentration variability due to the plant stage development and the pastured area during the mountain grazing. In Fig. 1 this effect is reported for some mono- and sesquiterpenes.

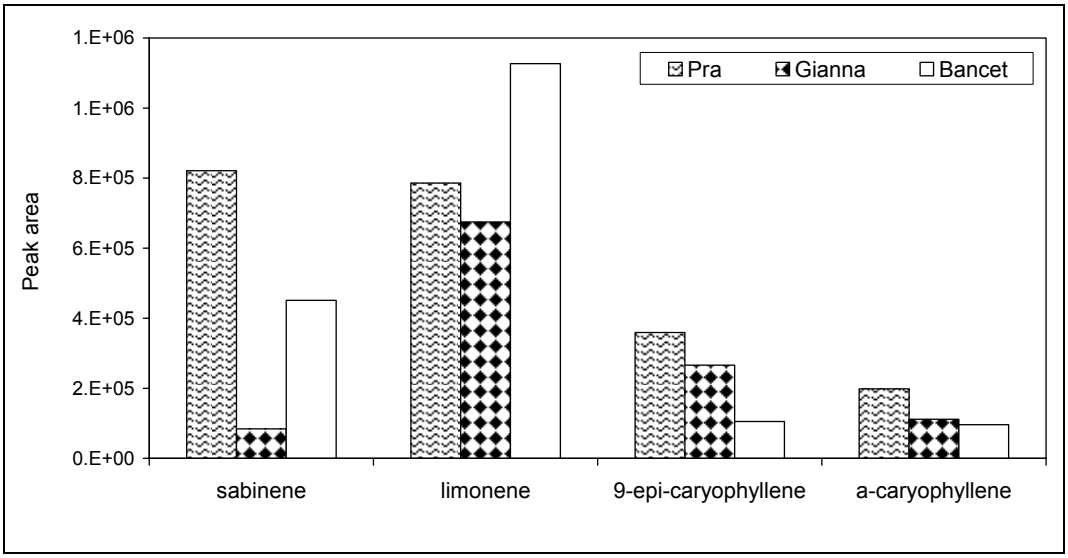

Figure 2. Area of base peak ion of two monoterpenes and two sesquiterpenes for the three mountain farms.
Slika 2. Površina vrhov dveh monoterpenov in dveh seskviterpenov na treh planinskih farmah.

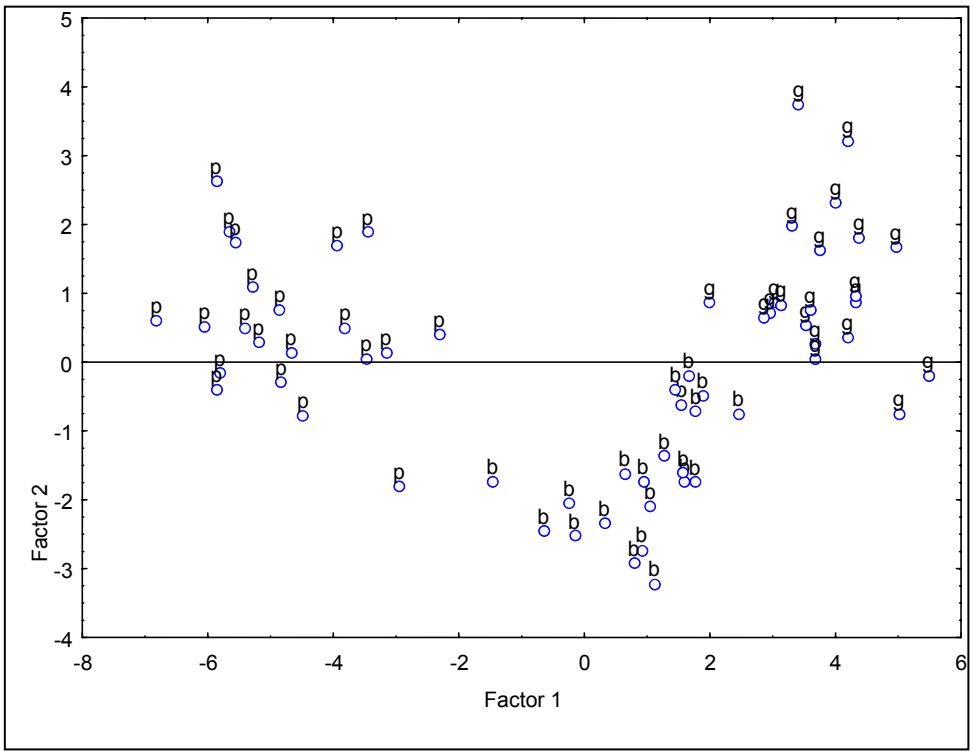

Figure 3. Score-plot with the ricotta cheese samples of the three mountain farms (g - Alpe Gianna; b - Alpe Bancet; p - Alpe Pra) calculated by terpene concentrations.
Slika 3. Porazdelitev vzorcev sira "ricotta" s treh planinskih farm (g - Alpe Gianna; b - Alpe Bancet; p - Alpe Pra) na osnovi koncentracije terpenov.

Although the concentrations of these compounds vary during the pasturing season, there are high differences between the three mountain farm ricotta cheeses (Fig. 2). Mono- and sesquiterpenes are particularly abundant in the Alpe Pra products and this is due to the mountain pastures with a small percentage of graminacee and the presence of goat and ewe flocks. Concentration of mono- and sesquiterpenes in Alpe Bancet and Alpe Gianna is generally lower for the presence of cow herds and a high percentage of graminacee in the pasture.

The differences between the three mountain farms can be highlighted with the Linear Discriminant Analysis calculated by areas of base peak ions for each detected terpenes (Fig. 3). The discrimination of Alpe Pra samples is excellent while for the other two farms some samples are misclassified. The refiling percentage is approximately 99% for Alpe Pra samples and 95% for the other farms. The differences between Alpe Bancet and Alpe Gianna are more evident at the end of the pasturing period (September) when, due to its northern exposure, there is still fresh grass in Alpe Gianna while the pasture is very reduced in Alpe Bancet.

CONCLUSIONS

This study has confirmed the presence of mono- and sesquiterpenes in the mountain ricotta cheese in the mountain ricotta cheese much like those highlighted for other cheeses. These compounds can be used not only to distinguish between summer pasture products and the ones produced during other periods but also as markers of each mountain pasture. The study has also confirmed that SPME-GC-MS is a simple and effective technique for the study of terpene compounds.

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ENTEROCOCCI IN CHEESE – PHENOTYPIZATION AND ANTIBIOTIC RESISTANCE

Snežana BULAJIĆ [a)] and Zora MIJAČEVIĆ [b)]

[a)] Univ. of Belgrade, Fac. of veterinary medicine, Dept. of hygiene and technology of food products of animal origin, Bulevar JA 18, 11000 Belgrade, Serbia, M.Sc.
[b)] Same address as [a)], Prof., Ph.D.

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ABSTRACT

The material of investigation was consisted of samples of fresh and ripened cheeses made from raw and cooked milk subjected to rennet or acid coagulation. The primary isolation of enterococci was carried out on kanamycin-aesculine-azide agar at 37 °C and 42 °C during 24 hours. It was isolated totally 42 strains of enterococci. The examination of antibiotic resistance/sensitivity profiles was performed by applying disk-diffusion procedure on Muller-Hinton agar. The number of enterococci determined in cheese samples depended on applied technological process. In the samples of fresh, by rennet coagulated cheese made from raw and cooked milk the number of enterococci was ranged from 8.0×10^4 to 9.0×10^6 cfu g^{-1}, and from 4.0×10^7 to 4.4×10^7 cfu g^{-1}, respectively. In the case of ripened cheeses made from raw and cooked milk subjected to acid coagulation the number of enterococci was ranged from $<10^2$ to 5.0×10^4 cfu g^{-1} and from 2.5×10^4 to 1.5×10^5 cfu g^{-1}, respectively. In the samples of fresh cheeses made from raw and cooked milk subjected to acid coagulation the number of enterococci was ranged from 2.4×10^5 to 2.12×10^7 cfu-g, and from 5×10^4 to 6×10^4 cfu g^{-1}, respectively.
The phenotypic identification of isolated enterococcal strains was performed according to the following biochemical-physiological characteristics: microscopic examination (cell morphology), catalase activity, growth in MRS broth at 10 °C and 45 °C, growth at pH 9.6, growth in broth containing 6.5% NaCl, growth in 0.1% methylen blue milk, resistance at 60 °C/15 and 30 minute, Voges/Proskauer reaction, fermentation of ribose. The isolated strains of enterococci were resistant to following antibiotics: penicillin (65.82%), tetracycline (62.02%), lincomycin (68.35%), gentamycin (27.84%), neomycin (31.64%), erythromycin (31.64%) and chloramphenicol (65.82%).

Key words: milk products / cheese / microbiology / enterococci / phenotypization / antibiotics / resistance

ENTEROKOKI V SIRIH – FENOTOPIZACIJA IN REZISTENCA PROTI ANTIBIOTIKOM

IZVLEČEK

V raziskavi smo proučevali vzorce svežih in zorenih sirov, izdelanih iz surovega ali toplotno obdelanega mleka s potopkom encimske ali kislinske koagulacije. Začetno izolacijo enterokokov smo opravili na kanamicin-eskulin-azidnem agarju pri temperaturi 37 °C in 42 °C in 24-urni inkubaciji. Izolirali smo 42 sevov enterokokov. Za ugotavljanje rezistence oziroma občutljivosti izoliranih sevov proti antibiotikom smo uporabili difuzni test z diski, na Muller-Huntonovem agarju. Število enterokokov, ki so jih ugotovili v vzorcih sirov, je bilo odvisno od tehnološkega postopka izdelave sira. V svežih sirih, narejenih z encimsko koagulacijo surovega in toplotno obdelanega mleka, se je število enterokokov gibalo med $8{,}0\times10^4$ in $9{,}0\times10^6$ cfu g^{-1} ter $4{,}0\times10^7$ in $4{,}4\times10^7$ cfu g^{-1}. Pri zorenih sirih, narejenih s kislinsko koagulacijo, se je število enterokovov

gibalo od < 10^2–$5{,}0\times10^4$ cfu g^{-1} v sirih iz surovega mleka in od $2{,}5\times10^4$ do $1{,}5\times10^5$ cfu g^{-1} v sirih iz toplotno obdelanega mleka. Sveži siri, narejeni s kislinsko koagulacijo surovega in toplotno obdelanega mleka so vsebovali med $2{,}4\times10^5$ in $2{,}12\times10^7$ cfu g^{-1}, ter med 5×10^4 in 6×10^4 cfu g^{-1}. Fenotipsko smo izolirane seve opisali na osnovi morfologije celic, katalaznega testa, rasti pri 10 °C in 45 °C, rasti v mleku z 0,1 % metilenskega modrila in pri pH 9,6, rasti v bujonu s 6,5 % NaCl, rezistence ob izpostavitvi temperaturi 60 °C za 15 in 30 minut, Voges-Proskauerjeve reakcije in fermentacije riboze. Izolirani sevi enterokokov so bili v 65,82 % primerov odporni proti penicilinu, 62,02 % sevov je bilo odpornih proti tetraciklinu, 68,35 % proti linkomicinu in 27,84 % proti gentamicinu. Odpornost proti neomicinu je bila prisotna pri 31,64 % proučevanih sevov, proti eritromicinu pri 31,64 % in proti kloramfenikolu pri 65,82 % sevov.

Ključne besede: mlečni izdelki / sir / mikrobiologija / enterokoki / fenotipizacija / antibiotiki / rezistenca

INTRODUCTION

Enterococci are ubiquitous bacteria which colonize different niches. The primary habitat is considered to be the gastrointestinal tract of animals and humans, thus via fecal contamination reach the raw milk and meat. The wide distribution of the enterococci, their resistance to high temperatures, tolerance to high salt concentration argumented their survival through the production process and implication that this group of organisms, may also be isolated from heat-treated milk and dairy products, especially cheese made from raw milk. Enterococci, present in raw milk, may develop during cheese making process and ripening period and may represent the predominant microflora found in cheese made from raw milk (Neviani *et al.*,1982a, Neviani *et al.*,1982b). Depending on the stage of ripening, enterococci can reach numbers of up to 10^6–10^8 cfu g^{-1} (Fontecha *et al.*, 1990; Basso *et al.*, 1994). In cheeses like Manchego (Ordonez *et al.*, 1978), Mozzarella (Coppola *et al.*, 1988), Kefalotyri (Litopoulou-Tzanetaki, 1990), Feta and Teleme (Tzanetakis and Litopoulou-Tzanetaki, 1992), Serra (Macedo *et al.*, 1995), Cebreiro (Centeno *et al.*, 1996) and Comte (Bouton *et al.*, 1998) enterococci represent a major part of the fresh cheese curd microflora and particularly they are the predominant microorganisms in the fully ripened cheese. In fresh Feta cheese, the normal microflora of lactic acid bacteria consists of starter cultures that are gradually replaced by salt resistant lactobacilli and enterococci, mainly *Enterococcus faecalis* and *Enterococcus faecium* (Tzanetakis *et al.*, 1995).The predominant microorganism in Cebreiro cheese

is *Enterococcus faecalis*, which is also the most frequently isolated *Enterococcus* in acidified raw milk (Wessels *et al.*, 1988; Jiwoua and Milliere, 1990) and fresh Italian cheeses (Soncini and Piantoni, 1992). There are contradictory reports on the influence of enterococci on sensory characteristics of cheese. High contamination levels of enterococci are considered to cause the deterioration of organoleptic properties in some cheese (Thompson and Marth, 1986; Lopez-Diaz *et al.*, 1995). On contrary, many authors claim that enterococci may have a positive role in cheese making process (Jensen *et al.*, 1975b and 1975c; Ordonez *et al.*, 1978; Trovatelli and Schliesser, 1987; Centeno *et al.*, 1999). The high proteolytic activity presented by some strains of *Enterococcus faecalis* could contribute to the sensorial and textural properties of cheese (Centeno *et al.*, 1999). In addition, enterococci produce esterases, which can play an important role in flavour formation (Tsakalidou *et al.*, 1993). According to many authors, *Enterococcus faecalis* has been successfully used to accelerate maturation and to improve organoleptic characteristics of cheeses (Jensen *et al.*, 1975a; Neviani *et al.* 1982b; Hegazi, 1990; Ledda *et al.*, 1994;Villani and Coppola, 1994; Tzanetakis *et al.*, 1995). On the basis of well-documented desirable biochemical properties, which argumented technological acceptability of enterococci, they have been proposed as part of defined starter cultures for different European cheeses, such as Water-Buffalo Mozzarella (Villani and Coppola, 1994), Feta (Litopoulou-Tzanetaki *et al.*, 1993), Venaco (Casalta and Zennaro, 1997) and Cebreiro (Centeno *et al.*, 1996) cheese.

Moreover, many enterococci produce one or more bacteriocins, and may be considered as protective towards spoilage and pathogenic bacteria (De Vuyst, 1994; Cintas *et al.*, 1997; Aymerich *et al.*, 2000; Giraffa, 1995). Furthermore, a strain of *E. faecium* SF68 has been confirmed as a probiotic according to its positive effects against diarrhea in man and pigs (Underdahl, 1983). But, in spite of all this, there is no consensus whether these bacteria pose the risk in food fermentation process, because of their ability to develop resistance against most antibiotics currently used, in combination with known virulence factors. The strains of enterococci are naturally tolerant to β-lactams, cephalosporins, lincosamides and polymyxins. A specific cause for concern and a factor contributing to the pathogenesis of enterococci is the resistance they acquire to aminoglycosides, tetracyclines, macrolides, chloramphenicol, penicillin, and ampicillin (Gray *et al.*, 1991), and their capacity to exchange genetic information by conjugation. This paper reports on enterococci, isolated from cheese originated from Serbia and its patterns of susceptibility to selected antibiotics.

MATERIAL AND METHODS

Cheese samples

The material of investigation was consisted of samples of fresh and ripened cheeses made from raw and cooked milk subjected to rennet or acid coagulation.

Microbiological analysis

Ten grams of each sample were homogenized with sterile solution of sodium citrate (20 g l^{-1}), adequately diluted in sterile Ringer solution and spread on kanamycin aesculine azide (KAA; Oxoid,**)** plates.

After 24 h incubation at 42 °C in aerobic conditions, colonies that displayed the typical enterococcal growth and cell morphology were picked up and purified twice on KAA plates. The phenotypization of isolated strains was performed according to following tests: catalase activity, growth in MRS broth at 10 °C and 45 °C, growth at pH 9.6, growth in broth containing 6.5% NaCl, growth in 0.1% methylen blue milk, resistance at 60 °C/15 and 30 minutes, Voges/Proskauer reaction, and fermentation of ribose.

Antibiotic resistance patterns

Antibiotic resistance was tested by the agar diffusion method on plates of Muller-Hinton agar supplemented with antibiotic disks (BD BBL Sensi-Disc Antimicrobial Susceptibility Test Discs), according to the directions of the manufacturer of disks.

RESULTS AND DISSCUSION

The number of enterococci determined in cheese samples depended on applied technological process. In the samples of fresh, by rennet coagulated cheese made from raw and cooked milk, the number of enterococci was ranged from 8.0×10^4 to 9.0×10^6 cfu g^{-1}, and from 4.0×10^7 to 4.4×10^7 cfu g^{-1}, respectively. In the case of ripened cheese made from raw and cooked milk subjected to acid coagulation, the number of enterococci was ranged from $<10^2$ to 5.0×10^4 cfu g^{-1} and from 2.5×10^4 to 1.5×10^5 cfu g^{-1}, respectively. In the samples of fresh cheese made from raw and cooked milk subjected to acid coagulation the number of enterococci was ranged from 2.4×10^5 to 2.12×10^7 cfu g^{-1}, and from 5×10^4 to 6×10^4 cfu g^{-1}, respectively.

Similar enterococcal number determined in cheese samples was reported by Fontecha *et al.* (1990) and Basso *et al.* (1994). Teuber *et al.* (1999) highlighted the finding that the contaminating enterococci may multiply to high number, e.g. more than 1.10^7 cfu per gram in soft cheeses. A preliminary study of 67 European yielded 40% samples containing enterococci in the range from 10^3 to 10^7 cfu g^{-1} (Sievers *et al.* 1993).

Table 1. Number of enterococci determined in cheese samples
Preglednica 1. Število enterokokov v vzorcih sira

	N (cfu / g)		
	Fresh cheeses		Ripened cheeses
	Rennet coagulation	Acid coagulation	Acid coagulation
Raw milk	8.0×10^4 to 9.0×10^6	2.4×10^5 to 2.12×10^7	$<10^2$ to 5.0×10^4
Cooked milk	4.0×10^7 to 4.4×10^7	5×10^4 to 6×10^4	2.5×10^4 to 1.5×10^5

Fourty two strains of enterococci were isolated, which were subjected to antibiotic susceptibility testing. The isolated strains of enterococci were resistant to following antibiotics: penicillin (65.82%), tetracycline (62.02%), lincomycin (68.35%), gentamycin (27.84%), neomycin (31.64%), erithromycin (31.64%), and chloramphenicol (65.82%). Sievers *et al.* (1993) reported that in 55% of analysed cheeses, *E. faecalis, E. faecium*, and *E. durans* strains were isolated showing resistance to one or more following antibiotics: penicillin, cefalotin, furadoin, fucidin, erytromycin, tetracycline and chloramphenicol. Antibiotic resistant enterococci were present in different food items, including raw milk cheese (Emmenthal, Appenzell, Gruyere, Tilsit and soft cheeses) (Baumargartner *et al.*, 2001). In the same study, resistance to chloramphenicol, erytromycin and tetracycline was prominent in *E. faecalis*, while *E. faecium* showed resistance to ampicillin, nitrofurantoin, penicillin and tetracycline. In order to evaluate the potential risk which food contaminated with resistant strains of enterococci represent to human health, it is important to distinguish between intrinsic and acquired antibiotic resistance (Clewell, 1990; Murray, 1990). Further investigations are needed to address this question.

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ENUMERATION, ISOLATION, AND IDENTIFICATION OF BIFIDOBACTERIA FROM DAIRY PRODUCTS

Eva VLKOVÁ [a)], Vojtěch RADA [b)] and Iva TROJANOVÁ [a)]

[a)] Department of Microbiology and Biotechnology, Czech University of Agriculture Prague, Kamýcká 129, 165 21 Prague 6, Czech Republic.
[b)] Same address, Ass. Prof.

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ABSTRACT

Six dairy products were tested. Bifidobacteria were enumerated and isolated using TPY agar modified by the addition of mupirocin (100 mg l^{-1}). Isolates were identified to the genus level by the detection of fructose-6-phosphate phosphoketolase (F6PPK) and by the FISH method. Bifidobacteria were characterised using API 50 CHL and API ID 32 A Rapid tests. In addition, grow at 46 °C was tested. Subsequently, all strains were identified to the species level using computer program Bacter. The identification was also carried out by PCR method using genus- and species-specific primers.
The bifidobacterial counts in products tested varied from 2.37 to 7.17 log CFU/ml. High selectivity was seen for modified TPY agar from which all isolates were identified as bifidobacteria. While most strains were identified as B. animalis using Bacter program, the same isolates had positive reaction with B. lactis-specific primers. Strain isolated from Rajo yogurt was identified as B. longum by Bacter as well as using PCR, but its bacterial counts were too low. Our results showed, that most of the bifidobacterial strains currently used in food products could be of animal origin.

Key words: milk products / dairy products / microbiology / bifidobacteria / enumeration / identification / isolation

ŠTETJE, IZOLACIJA IN IDENTIFIKACIJA BIFIDOBAKTERIJ V MLEČNIH IZDELKIH

IZVLEČEK

Testirali smo šest mlečnih izdelkov. Štetje bifidobakterij in izolacijo smo izvedli na TPY agarju, ki smo mu dodali muriprocin (100 mg l^{-1}). Izolate smo identificirali na ravni rodu z detekcijo fruktoza-6-fosfat fosfoketolaze (F6PPK) in z metodo FISH. Bifidobakterije smo opisali s hitrima testoma API 50 CHL in API ID 32. Preverili smo tudi rast pri 46 °C. Nato smo vse isolate identificirali na ravni vrst z računalniškim programom Bacter. Dodatno smo izvedli identifikacijo z metodo PCR ob uporabi za posamezen rod, oziroma vrsto specifičnih začetnih oligonukleotidov.
Število bifidobakterij v izdelkih je variralo med 2,37 in 7,17 log ke/ml. Opazili smo visoko selektivnost modificiranega TPY agarja, na katerem smo izolirali izključno bifidobakterije. Kljub temu, da smo večino izolatov z računalniškim programom Bacter identificirali kot *B. animalis*, smo DNA nekaterih izolatov lahko pomnožili z začetnimi oligonukleotidi, specifičnimi za *B. lactis*. Izolat iz jogurta Rajo smo s programom Bacter in vrstno specifično PCR identificirali kot *B. longum*, vendar je bilo število celic zelo nizko. Naši rezultati kažejo, da bi bila lahko večina sevov bifidobakterij, ki so v uporabi prehranski industriji, živalskega izvora.

Ključne besede: mlečni izdelki / mikrobiologija / bifidobakterije / štetje / identifikacija / izolacija

INTRODUCTION

Bifidobacteria are Gram-positive, non-sporeforming, non-motile, anaerobic, irregular rods. The typical habitat of bifidobacteria is human, warm-blooded animal and honeybee intestinal tract (Scardovi, 1986). Members of genus *Bifidobacterium (B.)* are among the most common microorganisms in the human gut, comprising up to 3% of the total faecal microflora of adults (Sghir *et al.*, 2000). They are more numerous in the infant gut, where they form up to 91% of the total microflora in breast-fed babies being supported by bifidogenic factors presented in human milk and up to 75% in formula-fed infants (Harmsen *et al.*, 2000). Using classical culturing methods it has been found that *B. adolescentis* and *B. longum* are major bifidobacterial species in the adult intestine (Gavini *et al.*, 2001; Biavati *et al.*, 1986; Mutai and Tanaka, 1987) and that *B. infantis* and *B. breve* are predominant species in the intestinal tract of human infants (Benno *et al.*, 1984; Biavati *et al.*, 1984; Mutai and Tanaka, 1987). In addition, *B. bifidum*, *B. catenulatum*, *B. pseudocatenulatum*, *B. angulatum, B. gallicum*, and *B. dentium* have also been reported to be human intestinal bifidobacteria (Scardovi, 1986). Matsuki *et al.* (1999) who used for the detection of bifidobacteria in human gut species-specific polymerase chain reaction (PCR) reported, that the most common species in the breast-fed infants are *B. breve*, *B. infantis*, *B. longum*, and *B. bifidum*. In adult intestinal tracts, the *B. catenulatum* group was the most common taxon, followed by *B. longum* and *B. adolescentis*.

The genus *Bifidobacterium* constitutes a significant proportion of the probiotic cultures used in the food industry (Langhendries *et al.*, 1995; Saavedra *et al.*, 1994). The employment of strains belonging to *B. animalis*, *B. longum*, *B. bifidum*, and *B. infantis* as probiotic starter cultures is due to their important role played in the gut (Gibson and Roberfroid, 1995; Modler *et al.*, 1990). They suppress harmful bacteria by controlling pH of the large intestine through the production of lactic and acetic acids (Gibson *et al.*, 1997). Bifidobacteria have antitumoral activity (Reddy and Rivenson, 1993; Rastall and Gibson, 2002), anticholesterolemic (Pereira and Gibson, 2002), and immune system activation effects (Mitsuoka, 1992). Other effects that have been described to this genus are the alleviation of lactose intolerance and vitamin production (Hughes and Hoover, 1995; Fooks *et al.*, 1999).

The presence of high number of bifidobacteria in the large intestine is desirable and can be influenced by dietary supplementation with probiotics and/or prebiotics. Probiotics have been defined as living microorganisms, which beneficially affect the host by improving its intestinal microbial balance (Fuller, 1989). Prebiotics are nondigestible dietary supplements that modify the balance of the intestinal microflora, stimulating the growth and/or activity of beneficial organisms and suppressing potentially deleterious bacteria (Gibson and Roberfroid, 1995). In order to exert a beneficial effect, probiotic bacteria should be viable and present at high numbers in the product at time of consumption (McBrearty *et al.*, 2001).

Industrial interest in the use of bifidobacterial strains as food additives in dairy products is rapidly growing. This development leads to the requirement for accurate quantity and quality control of the probiotic products and hence methods for specific identification of probiotic strains. Consequently, the aim of our work was to enumerate and isolate bifidobacteria from dairy products, and to compare biochemical and molecular biology methods for the identification of these isolates.

MATERIAL AND METHODS

Six dairy products were tested, three yogurts and three fermented milk products. Five products were made in the Czech Republic and one (Rajo) in the Slovak republic. The list of products tested is in Table 1.

Table 1. Tested products

Product	Product type	Made in
Activia	yogurt	Czech Republic
Hollandia	yogurt	Czech Republic
Olma – Dr. Bio	fermented milk product	Czech Republic
Yoplait	fermented milk product	Czech Republic
Kefir-like milk	fermented milk product	Czech Republic
Rajo	yogurt	Slovak Republic

Bifidobacteria in dairy products were enumerated and isolated using TPY agar (Sharlou, Barcelona, Spain) modified by the addition of mupirocin at a concentration of 100 mg/L (Rada and Koc, 2000). Pure isolates were enriched in TPY broth, and were identified as members of the genus *Bifidobacterium* by the demonstration of fructose-6-phosphate phosphoketolase (EC 4.1.2.22) activity, as described by Orban and Patterson (2000).

The genus identification was performed also using fluorescence *in situ* hybridisation (FISH) kit for *Bifidobacterium* sp. (RiboTechnologies, Groningen, the Netherlands). The component of the kit is a genus-specific oligonucleotide DNA probe labelled by fluorescein isothiocyanate (FITC), which binds to bifidobacterial rRNA. After the hybridization, the samples were analysed with a Nikon E-800 epifluorescence microscope. Another method used for the genus identification was PCR with genus specific primers which was performed as described previously (Kok *et al.*, 1996).

All isolates were tested for the ability to grow at 46 °C. Testing of grow at this temperature is a method recommended for distinguishing of human and animal strains. Human isolates are not able to grow at 46 °C and most of animal isolates are able to grow at this temperature (Gavini *et al.*, 1991). Subsequently, the isolates were characterised using API 50 CHL and API ID 32 A Rapid kits (BioMérieux, France). On the basis of the results from these tests, all strains were identified to the species level using computer program Bacter (http://kounou.lille.inra.fr, INRA, Lille, France).

The identification to the species level was also carried out by PCR method using species-specific primers. The genomic DNA of the strains was extracted by heating at 100 °C for 10 minutes in 1% Triton X-100 (Sigma, USA) by the method described by Wang *et al.* (1996). Amplifications were performed with a thermal cycler (Techne, Techgene, UK) with solutions, species-specific primers and temperature profiles described by Matsuki *et al.* (1999). Amplified PCR products were analyzed by 1% agarose gel electrophoresis at a constant voltage of 7 $V.cm^{-1}$ and visualized with ethidium bromide (0.5 μg/mL) under UV light (wavelength, 260 nm).

RESULTS AND DISCUSSION

Bifidobacterial counts determined in tested products are shown in Table 2. The counts varied from 2.37 to 7.17 log CFU/mL. Recommended lower limit of International Dairy Federation (IDF) for bifidobacterial counts in dairy product is 10^6 CFU per one mL. In Japan this recommendation is even at least 10^7 viable probiotic cells per gram or millilitre (Ishibashi and Shimamura, 1993). Generally, bifidobacteria show poor viability in fermented dairy products and various studies have indicated that not all probiotic products contain the recommended levels of viable microorganisms (Kailasapathy and Rybka, 1997; Dave and Shah, 1997). Only four of our product tested fulfil the recommendation of IDF, while two products did not meet these criteria. In Rajo yogurt the counts of bifidobacteria were only 2.37 log CFU/mL.

High selectivity was observed for modified TPY agar from which all isolates were F6PPK-positive and were identified as bifidobacteria. These results were confirmed by FISH and PCR methods.

Table 2. Bifidobacterial counts and species isolated from dairy products

Product	Bifidobacterial counts	Species isolated
Activia	7.17 ± 0.09^{a}	*B. animalis*/*B. lactis*
Hollandia	6.31 ± 0.38^{a}	*B. animalis*/*B. lactis*
Olma – Dr. Bio	6.22 ± 0.20^{a}	*B. animalis*/*B. lactis*
Yoplait	6.03 ± 0.12^{a}	*B. animalis*/*B. lactis*
Kefir-like milk	5.44 ± 0.41^{b}	*B. animalis*/*B. lactis*
Rajo	2.37 ± 0.47^{c}	*B. longum*

Results are means (n=3) ± SD of log CFU/mL
[a,b,c]Data in column with no common superscripts differ ($P < 0.05$)

While five strains were identified as *B. animalis* using Bacter program, the same isolates had positive reaction with *B. lactis*-specific primers. Hence, it is not clear when *B. lactis* and *B. animalis* are the same species, because Bacter database does not contain species *B. lactis* and on contrary, there are no available *B. animalis*-specific primers. Cai *et al.* (2000) reported that the relative taxonomic position of *B. lactis* is still under discussion and that *B. lactis* could be considered a junior subjective synonym for *B. animalis*. In contrary, Ventura *et al.* (2001) demonstrated clear differences in rDNA sequences between *B. lactis* (DSM 10141) and the type strain of *B. animalis* (ATCC 25227). A decision of this issue by the International Committee on Systematic Bacteriology is still outstanding (Anonym, 2001).

Strain isolated from Rajo yogurt (1?) was identified as *B. longum*, which is the human origin species, by both Bacter as well as using PCR. But its bifidobacterial counts were too low. Five from six dairy-related isolates were identified as species of animal origin, although it is recommended that the bifidobacterial strains used in fermented milk products should be of human origin. Especially *B. animalis* is often found in dairy products (Bonaparte, 1997).

A modified TPY agar was found to be highly selective and suitable for isolation and enumeration of bifidobacteria from dairy products, as all isolates in our study were identified as bifidobacteria. Our results also showed that most of the bifidobacterial strains currently used in food products are probably of animal origin. Only one strain was identified as *B. longum*, which is of human origin, but its survival in fermented milk product was poor. Further investigations should be focused on the selection of human bifidobacterial isolates which are able to survive in milk for longer period of time. Also, the identity and origin of currently used strains should be clearified.

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DIAGNOSTIC USE OF PROFICIENCY TESTING IN DAIRY LABORATORY

Oto HANUŠ [a)], Václava GENČUROVÁ [b)], Pavel HERING [c)], Miloš KLIMEŠ [c)] and Radoslava JEDELSKÁ [b)]

[a)] Research Institute for Cattle Breeding, Ltd., Rapotin, Vyzkumniku 267, 788 13 Vikyrovice, Czech Republic, Ph.D.
[b)] Same address as [a)].
[c)] Czech-Moravia Breeders Association, a.s., Prague, 252 09 Hradistko pod Mednikem, Czech Republic.

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ABSTRACT

The composition of raw milk (RM) is important for milk recording, herd improvement, payment of milk and for quality evaluation. The reliability of routine analytical results is therefore significant. A mistake occurrence could jeopardize the efficiency of results. Milk laboratories (ML) perform the systems of analytical quality assurance. Proficiency tests are carried out usually by 10 RM samples with modified variability of components. At evaluation the system of Euclidian distance ($Re=(\bar{d}^2+sd^2)^{0,5}$) for participants order according to their analytical result reliability is used. Two specificaly modified types (designs) of systematic diagnostical graphs were constructed on the Re basis. The first type of flow diagnostical diagram for RM analysators is based on Shewhart´s diagram principles for Re parameter (alternating system of result rendering before and after calibrations). The second type is based on analysator set comparisons before (proficiency test results) and after calibration (success of calibration) by Re. The opposite comparison is possible and valuable as well. It makes possible the diagnosis and incidental co-ordinating of the corrections in the case of the unconformity occurrence. The positive contributions of the developed diagnostic graphics system are expected to: – improvement of estimation of unconformity sources of RM routine analytical results, their origin and character; – determination and improvement of incidental result corrections and instrument repairs; – improvement of reliability of routine results of RM analyses in general.

Key words: milk / dairy laboratories / proficiency testing / quality

DIAGNOSTIČNA UPORABA PREVERJANJA USPOSOBLJENOSTI MLEKARSKIH LABORATORIJEV

IZVLEČEK

Sestava surovega mleka (SM) je pomembna za kontrolo proizvodnje, selekcijo, plačevanje in vrednotenje kakovosti mleka. Zanesljivost rutinskih metod je ključnega pomena, ker bi napake ogrozile verodostojnost rezultatov. Mlekarski laboratoriji vpeljujejo sisteme za zagotavljanje kakovosti analitskih postopkov. Preverjanje usposobljenosti se izvaja navadno na 10 vzorcih SM z različno vsebnostjo osnovnih sestavin. Za preverjanje zanesljivosti analitskih rezultatov se pogosto uporablja sistem Evklidskih distanc ($Re=(\bar{d}^2+sd^2)^{0,5}$). Na osnovi Re sta bili zasnovani dve modifikaciji modela diagnostičnih grafov. Prvi tip diagramov poteka za analizo SM temelji na Shewhart ovem diagramu za parameter Re (sistem za določitev rezultata pred in po kalibraciji). Drugi tip temelji na primerjavi serij rezultatov pred in po kalibraciji z uporabao Re. Primerjava omogoča diagnozo in korekcije v primeru neskladja rezultatov. Od razvoja diagnostičnega grafičnega sistema si obetamo izboljšano določitev virov in narave neskladnosti analitskih rezultatov rutinske kontrole sestave SM, izboljšanje in korekcij rezultatov in instrumentov ter izboljšanje zanesljivosti rutinski rezultatov analize SM na splošno.

Ključne besede: mlekarstvo / laboratoriji / usposobljenost / preverjanje / mleko / kakovost

INTRODUCTION

The main composition and properties of raw milk and their testing systems are very important: – for performance of the milk recording; – for monitoring of dairy cow health state and controlling (prevention and treatments of the dairy cow production disorders); – for milk quality evaluation; – for animal genetic appreciation and improvement (cattle breeding); – for milk quality payment; – for dairy processing; – for foodstuff chain safety in general. The milk foodstuff chain is one of the most monitored and controlled foodstuff chains in terms of: – the number of routinely checked milk parameters (microbiological, compositional, technological); – regularity and relatively high frequency of the mentioned investigations; – mostly biological character of the mentioned investigations. In accordance with such information, there are assumptions, that the milk foodstuff chain could be one of the most safe of all known foodstuff chains. Therefore, the reliability of the referential and routine milk analytical results is very important as well. Incidental mistake occurrence could jeopardize the efficiency of the dairy production.

In the framework of supporting of the mentioned facts the milk laboratories carry out the accreditation according to the international standard (EN ISO/IEC 17025) in the Czech Republic. The referential and routine milk laboratories perform**s** the systems of the analytical quality assurance of the calibrations and measure functions of the master and routine milk analysators as well. It means, laboratories improve the analytical result**s** reliability runningly. A similar situation is at using of all referential and routine milk analytical methods in general. The last development works about above mentioned topics are introduced in many scientific and professional papers (Grappin, 1985, 1993; Hanuš and Ficnar, 1990; Hanuš and Kaššovicová, 1992; Leray, 1993; Wood, 1994; Golc-Teger, 1996, 1997; Hanuš *et al.*, 1996, 1998, 2000, 2001, 2002; Klopčič *et al.*, 1999). Mentioned systems are currently developed and improved also in the framework of the projects MZe-ČR, NAZV, QF 3019 and MŠMT-ČR, INGO, LA103.

MATERIAL AND METHODS

The Czech milk referential and routine laboratories (laboratories of the milk recording = individual milk samples; central laboratories = bulk milk samples) take part regularly in the interlaboratory proficiency testing on the national and international levels as well. RICB Rapotin organizes proficiency testing of the routine milk recording laboratories for some raw milk components: fat (F); protein (P); lactose (L); urea (U). Tests are performed on the basis of ten raw milk samples with necessary modified variability of the relevant introduced components.

The calculation system of the Euclidian distance to the origin (Re; according to Leray, 1993; Fig. 1) is used. Euclidian distance represents the distance of each laboratory to the origin (0,0) corresponding to the reference. Re is used for evaluation of the proficiency testing results and for order of the participants (laboratories) according to accuracy and reliability of their analytical results for the mentioned purposes. The Euclidian distance (Leray, 1993) from the origin calculation system is prefered for the mentioned purposes in comparison for instance to the often used Z-score system (Wood, 1994) because of its advantage to separate an incidental analytical mistake into two parts: – systematic error part and random error part. Such a differentiation ability could be important for suggested necessary diagnostical purposes at analytical mistake investigation, identification and specification.

The design of diagnostical diagram system for a better identification of mistakes in milk analysis and specification was proposed, created and developed according to: – results of twenty two proficiency tests performed during the year (2002 and 2003) by RICB referential milk laboratory; – knowledge of result variability of reliability parameter (Re) in regularly (monthly) performed proficiency tests; – knowledge of result dynamics of reliability parameter in regularly

performed proficiency tests; – knowledege and consideration about interpretation efficiency of different evalution systems (such as Re, Z-score, Youden plot, Shewhart´s diagram) for interlaboratory proficiency testing results in terms of their real graphical ability to analytical mistake investigation, identification and specification.

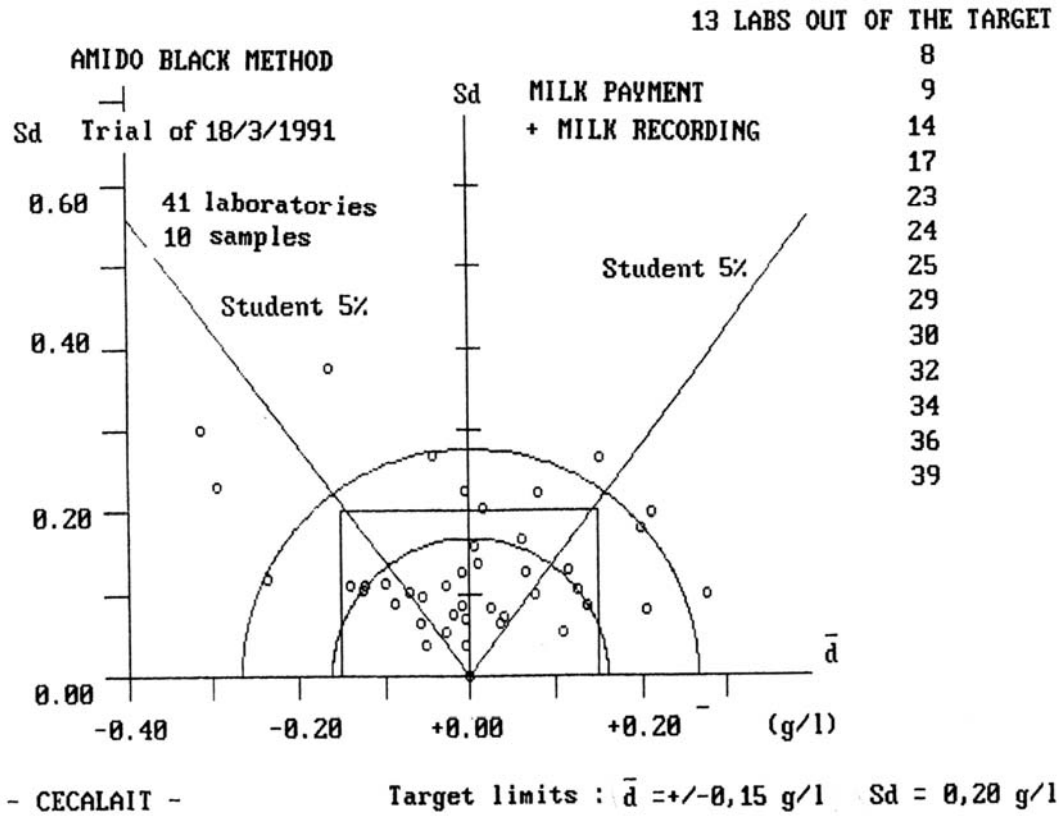

Figure 1. The expression system of Euclidian distance to the origin (Re) as accuracy parameter of milk analytical results in the proficiency testing (according to Leray, 1993).

RESULTS AND DISCUSSION

The diagnostical diagram systems for raw milk analysators were constructed as specific purpose modifications on the basis of the Shewhart´s diagram principles (Kupka, 1997) for the mentioned Re system (Fig. 2) and Re time diagram reciprocal comparisons (Fig. 3). The Re discrimination limits (HDLRe) were calculated for F, P, L and U according to long-term analytical result rows (2 years = 22 tests at month intervals) for Fig. 2 and according to results of instrument sets of proficiency tests and calibration process (Fig. 3; the half circle lines). The graphs of the individual instruments (Fig. 2), and calibration process and proficiency test comparisons (Fig. 3) are accessible for workers of the routine milk laboratories (workers of laboratory network) in the electronical way:

– the Re situations (Fig. 2) before and after calibration are shown by the alternating system of the symbols (o×o×…). It makes possible the diagnose and incidental co-ordinating of the necessary corrections in the case of the result unconformity occurrence. It is possible to show separately two components of the instrument result reliability (Re): the mean difference ($\bar{d}$); the variability of the differences (sd); by the same type of the graph as well. Such projection improves the diagnosis of incidental occurrence of the result error in terms of the effect estimation of the systematical and random error parts on the total

value of the unconformity. There are the protein graphs chosen for four types of the instruments (Fig. 2) for instance:

- master instrument of the referential-routine laboratory system (network) with good stability in the referential laboratory;
- routine instrument with good stability in the routine laboratory;
- routine instrument with usual stability in the routine laboratory;
- routine instrument with unconformity occurrence;

– further, the mentioned modificated system shows the situations of the whole set of the analysators according to Re (Re=$(\bar{d}^2+sd^2)^{0,5}$; by the original Re rendering (according to Leray, 1993) Fig. 1) by the mutual comparison of two graphs after the last calibration and before the next calibration (×o). The opposite comparison (o×) is possible and very valuable for relevant analytical mistake diagnoses as well (Fig. 3). Such rendering improves the further diagnostical possibilities for investigation, identification and specification of incidental unconformities.

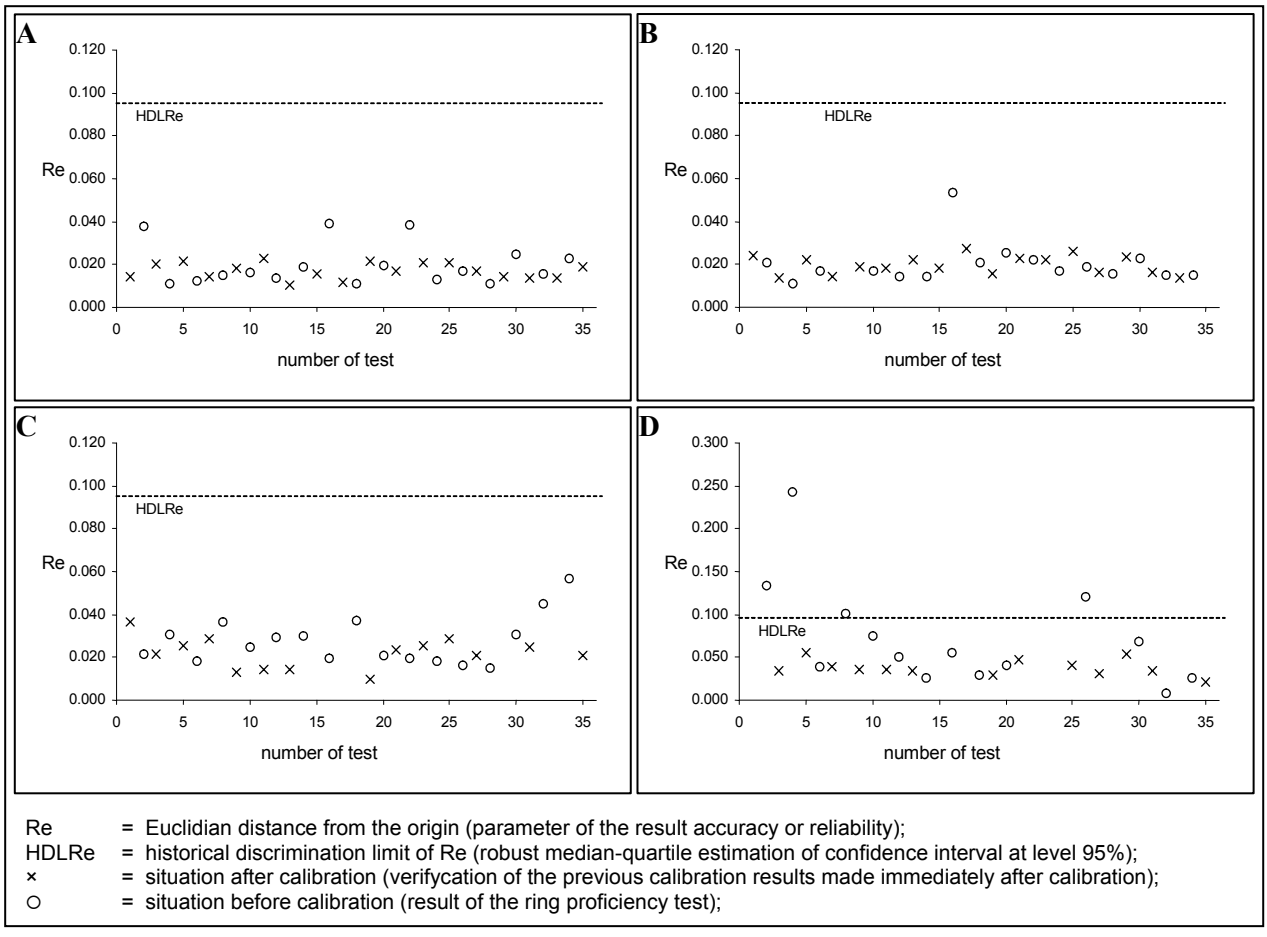

Figure 2. Individual diagnostical flow graphs for protein content at instruments (A,B,C,D) with different measurement stability.

The results of the proficiency testing are anonymous, of course. Nevertheless, if the laboratory managers know the key numbers of their own instruments and further they can compare the result reliability (accuracy) situations by different types of mentioned developped diagnostical graph combinations, they could be able to guess the type, character and source of

incidental analytical mistakes according to their own knowledge about analytical method principles and simultaneous laboratory facts.

CONCLUSIONS

It is expected, that the mentioned developped diagnostical graphical system for the analytical mistake identification and speification will contribute positively in the milk referential-routine laboratory networks to:

- improve of the estimation of unconformity sources of the raw milk routine analytical results in terms of their origin and character;
- determine and improve the incidental corrections of the results and necessary reparations of the instruments;
- improve the quality (reliability) of the routine results of the raw milk analyses in general.

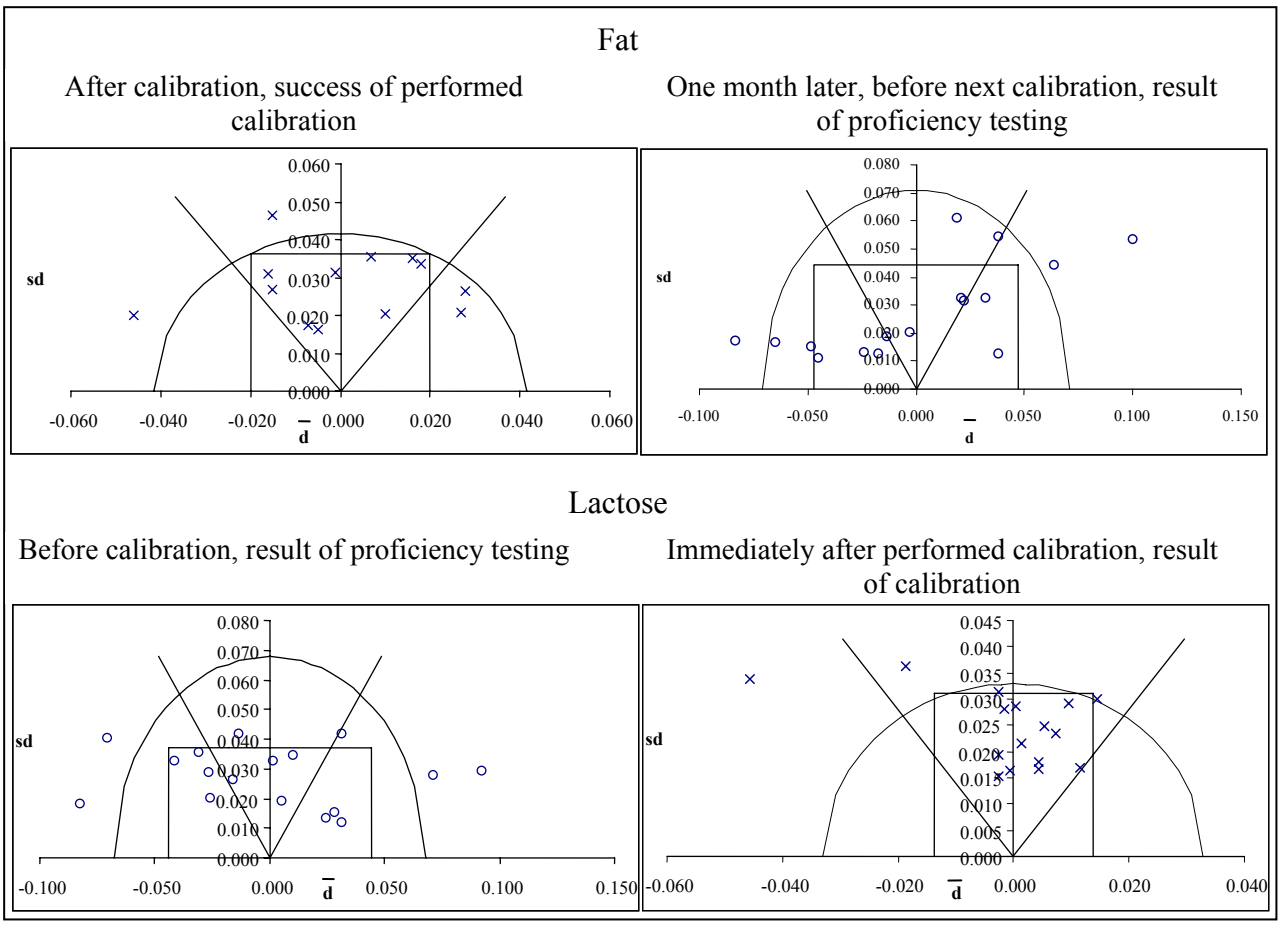

Figure 3. Pairs of group diagnostics comparison graphs (fat: comparison of analysator group after calibration and before next calibration by two different sets of milk referential standard samples; lactose: comparison of analysator group immediately before calibration and after calibration by the same set of milk referential standard samples).

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THE MICROBIOLOGICAL QUALITY OF SOME CRITICAL CONTROL POINTS IN THE CHEESE PRODUCTION OF INDIVIDUAL SLOVENIAN CHEESE-MAKERS

Karmen GODIČ TORKAR [a)] and Slavica GOLC TEGER [b)]

[a)] Univ. of Ljubljana, Biotechnical Fac., Zootechnical Dept., Groblje 3, SI-1230 Domžale, Slovenia, Ph.D., M.Sc., Consultant, e-mail: karmen.torkar@bfro.uni-lj.si.

[b)] Same address as [a)], Ass.Prof., Ph.D., M.Sc.

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ABSTRACT

The microbiological quality of 98 samples taken at some critical control points during the milking and processing of 14 semi-hard cheese made from raw cow milk by individual Slovenian producers was studied. The sampling points were: swabs from cows' udders, milking machines inner surfaces before and after milking, fresh raw and mixed milk from vats, whey immediately after curdling, brine, cheese after one month of ripening and after the following month of being kept vacuum packed at 6 °C. The high number of micro-organisms on the inner surfaces of washed milking machines before milking revealed ineffective cleaning (washing) by about 60% of cheese producers. There were no seasonal differences in the number of micro-organisms, except that the number of coliforms was higher in spring. The average of total number of micro-organisms was $4.9 \cdot 10^5$ cfu/ml in raw milk and $5.5 \cdot 10^6$ cfu/ml in mixed milk from a vat (raw fresh milk mixed with milk kept for about 18–24 hours at room temperature), which did not grow significantly during cheese-processing. The number of coliforms in raw and mixed milk was in the range of $3.4 \cdot 10^5$ cfu/ml and fell to $5.4 \cdot 10^4$ cfu/ml in whey. The average number of enterococci, aerobic spore-forming micro-organisms, yeasts and moulds, lactobacilli, lactococci, proteolytic and lipolytic micro-organisms in milk and in whey were in the same logarithmic range of about $2.2 \cdot 10^4$, 310, 3.5, $31.2 \cdot 10^4$, $2.1 \cdot 10^6$, $6.2 \cdot 10^3$ and $1.7 \cdot 10^4$ cfu/ml of the sample, respectively. *Listeria* spp. was isolated from 5.3% (cows' udders, milking machine, milk and whey), while none of the examined samples were positive to the presence of *Salmonella* spp. and *Campylobacter* spp. *Proteus* was present in 7 (7%) cases of milk and whey. Clostridia were detected in 10 (10%) samples (swabs, raw milk, whey). *E. coli* was isolated from 12 (12%) samples of swabs, raw and mixed milk, whey and brine. After one month of ripening the average total bacterial count was $9.2 \cdot 10^7$ cfu g^{-1} of cheese, of these $6.8 \cdot 10^7$ represented lactic-acid producers and $2.2 \cdot 10^7$ represented non-lactic acid producers. The average number of coliforms, enterococci, aerobic spore-forming micro-organisms, yeasts and moulds, lactococci, lactobacilli, proteolytic and lipolytic micro-organisms were $2.0 \cdot 10^5$, $6.3 \cdot 10^6$, 280, 960, $2.5 \cdot 10^7$, $9.8 \cdot 10^7$, 450 and $9.8 \cdot 10^4$ cfu g^{-1} of cheese, respectively. *Salmonella* spp., *Listeria* spp., *Proteus*, sulphite-reducing clostridia and *Campylobacter* spp. were not detected in cheese samples. *E. coli* was found in 4 (30%) of samples while coagulase positive staphylococci were present in 9 (64%) of cheese samples. A high number of enterococci (from a min. 3.10^3 to a max. 15.10^7 cfu g^{-1}) and coliforms (from a min. 10 to a max. 19.10^5 cfu g^{-1}) were detected as well. After one month of keeping vacuum-packed ripened cheeses at 6 °C, the number of micro-organisms did not rise significantly, except for the number of yeasts and moulds which grew to $3.6 \cdot 10^4$ cfu g^{-1} of cheese. Because of improper milking and processing hygiene conditions, three (21%) of the tested cheese samples did not correspond to the microbiological criteria according to the applicable regulations.

Key words: cheese-making / critical control points / microbiological quality / Slovenia

MIKROBIOLOŠKA KAKOVOST NA NEKATERIH KRITIČNIH KONTROLNIH TOČKAH PROIZVODNJE SIRA PRI POSAMEZNIH SLOVENSKIH SIRARJIH

IZVLEČEK

Proučevali smo mikrobiološko kakovost 98 vzorcev, odvzetih na nekaterih kritičnih kontrolnih točkah molže in proizvodnje 14-poltrdih sirov iz surovega mleka pri posameznih slovenskih sirarjih. Jemali smo vzorce brisov površine vimena krav molznic, notranjih površin molznih strojev pred in po molži, surovega mleka takoj po molži, mešanega mleka iz sirarskega kotla pred sirjenjem, sirotke takoj po koagulaciji, slanice, sirov po enomesečnem zorenju in vakumsko pakiranih sirov po nadaljnjem enomesečnem skladiščenju pri 6 °C. Visoko število mikroorganizmov na površini opranih molznih strojev pred molžo kaže na neučinkovito čiščenje (pranje) pri okrog 60 % sirarjev. Sezonskih razlik v številu mikroorganizmov nismo zasledili, razen nekoliko povišanega števila koliformnih mikroorganizmov v spomladanskem obdobju. Skupno število mikroorganizmov je bilo $4{,}9 \cdot 10^5$ kolonijskih enot ke/ml v surovem mleku in $5{,}5 \cdot 10^6$ ke/ml v mešanem mleku iz sirarskega kotla (sveže pomolženo surovo mleko primešano mleku, hranjenem 18–24 ur pri sobni temperaturi) in ni statistično značilno naraščalo med sirjenjem. Število koliformnih mikroorganizmov v surovem in mešanem mleku se je gibalo v območju okrog $3{,}4 \cdot 10^5$ ke/ml in se znižalo do vrednosti $5{,}4 \cdot 10^4$ ke/ml v sirotki. Povprečno število enterokokov, aerobnih sporotvornih mikroorganizmov, kvasovk in plesni, lactobacilov, laktokokov, proteolitičnih in lipolitičnih mikroorganizmov je bilo v mleku in sirotki v enakem logaritemskem območju $2{,}2 \cdot 10^4$, 310, 3,5, $31{,}2 \cdot 10^4$, $2{,}1 \cdot 10^6$, $6{,}2 \cdot 10^3$ in $1{,}7 \cdot 10^4$ ke/ml vzorca za vsako skupino mikroorganizmov. *Listeria* sp. je bila izolirana v 5,3 % vzorcev (vimena, molzni stroji, mleko, sirotka), medtem ko v nobenem od preiskanih vzorcev nismo zasledili bakterij vrst *Salmonella* spp. in *Campylobacter* spp. *Proteus* je bil prisoten v 7 (7 %) vzorcih mleka in sirotke. Sulfid-reducirajoči klostridiji so bili ugotovljeni v 10 (10 %) vzorcih (brisi, surovo mleko, sirotka). *E. coli* je bila izolirana iz 12 (12 %) vzorcev brisov, surovega in mešanega mleka, sirotke in slanice. Po enomesečnem zorenju je bilo povprečno število aerobnih mezofilnih mikroorganizmov okrog $9{,}2 \cdot 10^7$ ke g^{-1} sira, od teh je bilo $6{,}8 \cdot 10^7$ kislinotvornih in $2{,}2 \cdot 10^7$ nekislinotvornih mikroorganizmov. Povprečno število koliformnih mikroorganizmov, enterokokov, aerobnih sporoformnih mikroorganizmov, kvasovk in plesni, laktokokov, laktobacilov, proteolitičnih in lipolitičnih mikroorganizmov je bilo $2{,}0 \cdot 10^5$, $6{,}3 \cdot 10^6$, 280, 960, $2{,}5 \cdot 10^7$, $9{,}8 \cdot 10^7$, 450 in $9{,}8 \cdot 10^4$ ke g^{-1} sira. Bakterij vrst *Salmonella* spp., *Listeria* spp., *Proteus* in *Campylobacter* spp. nismo zasledili v nobenem od vzorcev sirov. *E. coli* smo našli v 4 (30 %) vzorcih, medtem ko so bili koagulaza pozitivni stafilokoki prisotni v 9 (64 %) vzorcih sirov. Ugotovili smo tudi visoko število enterokokov (od najmanj 3.10^3 do največ 15.10^7 ke g^{-1}) in koliformnih mikroorganizmov (od najmanj 10 do največ 19.10^5 ke g^{-1}). Po enomesečnem skladiščenju vakumsko pakiranih vzorcev sirov pri 6 °C se število mikroorganizmov ni statistično značilno zvišalo, le število kvasovk in plesni je poraslo do $3{,}6 \cdot 10^4$ ke g^{-1} sira. Zaradi neustrezne higiene molže in postopka sirjenja trije (21 %) vzorci sirov niso ustrezali kriterijem mikrobiološke kakovosti po veljavnih predpisih.

Ključne besede: sirarstvo / kritične kontrolne točke / mikrobiološka kakovost / Slovenija

INTRODUCTION

About 40% of cow milk produced in Slovenia each year is processed into different sorts of cheese (Valjavec, 2000; Valjavec, 2003). Some of these kinds of cheese are made from raw milk at small, artisanal cottage cheese-makers. Using raw instead of pasteurised milk keeps a larger proportion and diversity of strains belonging to endogenous lactic acid flora and secondary flora that may play an important role in the development of many desirable characteristics in cheese, particularly its specific sensory properties. The native, particularly lactic-acid flora, can also be used as protective cultures to inhibit harmful micro-organisms in milk (Salmeron *et al*., 2002).

The number and types of micro-organisms present in milk and dairy products at any particular period depended on the microbial quality of the raw materials, the conditions in which the

products were produced and the temperatures and duration of storage, feeding of the animals, season, area, using different starter cultures etc. (Anonim., 1994). Rinsing water for milking machine and cheese-making equipment washing also involve some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk and raw milk products (Bramley, 1990).

Testing for the presence and number of specific micro-organisms is therefore an integral part of any quality control or quality assurance plan and it may be applied to a number of areas: raw materials, intermediate samples, finished products, or environmental/equipment sites. The most common spoilage micro-organisms in milk and dairy products are *Pseudomonas* spp, coliforms, *Bacillus* spp, *Clostridium* spp, lactic-acid producing bacteria, yeasts and moulds, enterococci, etc. On the other hand, milk-borne and milk-product borne outbreaks, caused mostly by cheeses, represent 2–6% of the bacterial food-borne outbreaks reported by surveillance systems from several countries (De Buysier *et al*., 2001). Cheese represents a large risk of bacterial food-borne outbreaks because of pathogen micro-flora, divided into pathogens of current concern (*Salmonella* spp., *Campylobacter* spp., coagulase-positive staphylococci, *Listeria monocytogenes* etc.), and those which cause disease only occasionally (*Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium botulinum*, *Streptoccoccus zoepidemicus* etc.) (Anonim., 1994).

For this reason the production of milk products should be in accordance with legal regulations for good sanitary practice. According to the standards to be met when collecting raw milk from production, holding or for the acceptance at a treatment or processing establishment, raw milk intended for direct human consumption and raw cow milk for the manufacture of products made with raw milk whose manufacturing process does not involve any heat treatment must only meet a few microbiological standards: the plate count at 30 °C should be ≤ 100 000 micro-organisms per ml (geometric average over a period of two months, with at least two samples a month), *Staphylococcus aureus* per ml n=5, m=500, M=2000 and c=2, somatic cell count ≤ 400 000 and absence of antibiotics. The microbiological criteria for cheese made from raw milk are the absence of *Listeria monocytogenes* and *Salmonella* spp. in 25 g of sample (n=5, c=0), *Staphylococcus aureus* (m=1 000, M=10 000, n=5, c=2) and *Escherichia coli* (m=10 000, M=100 000, n=5, c=2) (Off. J. of the European Communities, 1992; Pravilnik.., Ur. l. RS, 2004). For milk that does not comply with the standards, pasteurisation is the primary mean of ensuring that related cheese does not represent a health risk. Still, even industrial pasteurisation cannot guarantee the absence of pathogenic micro-organisms because they are present in large numbers in raw milk or due to post-pasteurisation contamination. Pasteurisation also reduces a large proportion of lactic acid bacteria and secondary flora that may play an important role in the development of many desirable characteristics in cheese (Salmeron *et al*., 2002).

The aim of the present study was to determine variations in the different microbial groups affecting the manufacture or sanitary quality of cheese at different critical control points of milking and cheese production.

For this purpose we wanted to find out the presence of pathogens and indicator micro-organisms in 14 semi-hard cheeses after one month of ripening made from raw cow milk by individual Slovenian producers.

The same micro-organisms were established in a total of 98 samples taken during milking and processing of cheese mentioned before: swabs from udders and milking machines, fresh raw and mixed milk from vats, whey and brine. Drinking water used for cleaning milking machines and cheese production was examined as well.

MATERIAL AND METHODS

Sampling

The milk and milk product samples were taken in accordance with the instructions given in ISO/DIS 707 (1995). The samples of water were taken in accordance with the instructions given in ISO 5667-2 (1991).

98 samples were taken from sampling points of milk producing and cheese manufacturing: swabs from cows' udders surfaces before milking, swabs from the surfaces of cleaned milking machines and milking machines after milking (liners and claws), raw fresh milk after milking, mixed milk from vats before starting cheese manufacturing (fresh milk mixed with 12–24-hour-old milk kept at room temperature), whey after curdling, brine, cheese after one month of ripening, ripened cheese after one month of being kept vacuum packed at 6 °C, drinking water used for cleaning the milking machine and cheese vat.

Detection and enumeration of micro-organisms

Preparation of test samples, initial suspensions and decimal dilutions were carried out according to ISO/FDIS 8261 (E) (2001).

Swabs, milk, whey, brine samples

For the detection of ***Listeria monocytogenes*** in swabs, milk and milk products according to EN ISO 11290-1 (1996), we used *Listeria* enrichment broth as pre-enrichment (inc. 30 °C/24–48 h) and 1 Fraser broth as enrichment broth (inc. 37 °C/24–48 h). Palcam (Biokar Diagnostics, France), Oxford (Biokar Diagnostics, France) and ChromAgar Listeria (Mast Diagnostica, Germany) were used for isolation. The immunological method Tecra Unique Listeria (Tecra, Australia) and API Listeria strips (Biomerieux, France) were used for confirmation and identification.

For the detection of *Salmonella* in swabs, milk and milk products we used Buffered peptone water as a non-selective pre-enrichment medium and Selenite cystein buffer as an enrichment medium (ISO 6579, 2002). XLD agar (Biokar Diagnostics, France), BSA agar (Biokar Diagnostics, France) and Rambach agar (Merck, Germany) were used for isolation. The immunological method Tecra Unique Salmonella (Tecra, Australia) and API 10 S strips (Biomerieux, France) were used for confirmation and identification.

Detection of *Proteus* spp. in swabs, milk and milk products was carried out with inoculation of the sample into Nutrient broth (inc. 37 °C/24 h), spreading the colonies on the Brilliant Green Agar according to Edel and Kampelmacher (Biokar Diagnostics, France), typical colonies were confirmed and identified on a Kligler iron slant agar (Merck, Germany) and with API 10 S strips (Biomerieux, France).

For the detection of thermotolerant *Campylobacter* species in swabs, milk and milk products the Preston broth, Karmali agar and Columbia blood agar (Oxoid) were used according to ISO 10272 (E) (1995). The identification was carried out by using API Campy strips (Biomerieux, France).

For the enumeration of bacteria *Escherichia coli* in swabs, milk and milk products the chromogenic medium COLI ID (Biomerieux, France) (inc. 37 °C/24 h) was used.

For the enumeration of coagulase positive staphylococci (*Staphylococcus aureus* and other species) in swabs, milk and milk products the Baird Parker with RPF supplement agar (Biokar Diagnostics, France) was used (SIST EN ISO 6888-2, 1999). The Petrifilm^{TM} Staph Express Count System (3 M^{TM}, USA) was used for confirmation.

The enumeration of coliform micro-organisms in swabs, milk and milk products was carried out on VRBL agar (Merck, Germany) according to the standard IDF 73B, (1998).

For the enumeration of faecal enterococci in swabs, milk and milk products the KF Streptococcus agar with a TTC supplement (Biokar Diagnostics, France) was used according to the standard FIL- IDF 149A (1997).

The presence of sulphite-reducing clostridia spores in swabs, milk and milk products was detected after heating the samples at the temperature of 80 °C/10 minutes, inoculating the SPS agar according to Angelotti (1962) (Merck, Germany) and incubation in anaerobic conditions (inc. 35 °C/24–48 h).

The presence of total bacterial count at 30 °C and aerobic bacteria spores in swabs, milk, whey, cheese and brine was enumerated on PCA agar (Merck, Germany) with the addition of 0.1% w/v (1 g per 1 l of medium) of skimmed milk powder, according to the standards EN ISO 4833 (2003) and Anonim. (2002).

For the enumeration and differentiation of lactic-acid- and non-lactic-acid-producing micro-organisms at 30 °C in swabs, milk, whey, cheese and brine the CLA agar with Chinablue and lactose was composed and used according to the Methodenbuch, M 7.16.2 (1985).

For the enumeration of lactococci in swabs, milk, whey, cheese and brine the M17 agar (Merck, Germany) was used according to Terzaghi and Sandine (1975).

For the enumeration of lactobacilli in swabs, milk, whey, cheese and brine the MRS agar (Merck, Germany) was used according to De Man *et al*. (1960).

For the enumeration of yeasts and moulds in milk, whey, cheese and brine the YGC agar (Merck, Germany) was used according to the standard ISO 6611(E) (1992).

The enumeration of lipolytic micro-organisms in milk, whey, cheese and brine the Tributyrin agar supplemented with Glycerintributyrat (Merck, Germany) was determined according to the Methodenbuch, M 7.6 (1985).

For the enumeration of proteolytic micro-organisms in milk, whey, cheese and brine the Milk Agar was composed and used according to the Methodenbuch, M 7.3.3 (1985).

The enumeration of lactolitic clostridia spores in milk, whey, cheese and brine after heating the samples at the temperature of 80 °C/10 minutes pH-modified RCM agar was proceeded according to the Methodenbuch, M 7.18.3.1 (1995).

For the detection of inhibitory substances in raw and mixed milk the Delvotest SP (DSM, the Netherlands) was used.

Somatic cell count in raw and mixed milk was done using the Fossomatic 5000 (Foss Electric, Denmark).

Water samples

The enumeration of viable micro-organisms was carried out with a colony count on PCA agar culture medium (Merck, Germany) after aerobic incubation at 22 °C/72 h (first set of plates) and at 37 °C/24 h (second set of plates) (SIST EN ISO 6222, 1999).

For the detection and enumeration of intestinal enterococci in water samples the membrane filtration method on a Slanetz-Bartley medium (Biokar Diagnostics, France) for isolation and Bile Esculin Azide agar (Biokar Diagnostics, France) for confirmation (SIST EN ISO 7899-2, 2000) were used.

Coliform micro-organisms and presumptive *Escherichia coli* in water were detected by the MPN method (ISO 9308-2, 1990).

The presence of sulphite-reducing anaerobes (clostridia) spores was detected after heating the samples at a temperature of 75 °C/10–15 minutes according to ISO 6461/2 (1986).

Statistical analyses

The results were analysed with SAS/STAT (1990) statistical procedure. The basic statistical parameters (mean, median, standard deviation, coefficient of variation, maximum and minimum values) and correlation procedure of log values of different groups of micro-organisms were calculated. F-test according to Scheffer for estimation of differences for log values of groups of micro-organisms depending on the area, season, type of feeding, type of cheese-making procedure, individual cheese-maker was used.

RESULTS AND DISCUSSION

The microbiological quality of milking machine surfaces, raw and mixed milk, whey, brine and cheese samples

Milking machines

Cousins *et al*. (1981) reported that inadequately disinfected milk-contact surfaces of milking equipment, including milk cans and bulk tanks, were the major sources of bacteria in milk after it left the udder until collection. The proportion of number of bacteria recovered by rinsing a milking machine during milking is known to be at least 10% or more of the number available to the milk because of the rough inside surfaces with bacterial biofilms.

In our study swabs were taken at two different critical points: liners and claw surfaces. The represented results are the averages of both measurements. It also has to be admitted that washing and in some cases disinfection immediately followed after an earlier milking.

The total number of micro-organisms on 1 cm^2 of milking machine surfaces before and after milking was $14.7 \cdot 10^3$ and $15.0 \cdot 10^3$ cfu, respectively. The number of non-lactic-acid producers was 3 times higher than lactic-acid producers, while the number of coliforms and enterococci was 230 cfu/cm^2 and 120 cfu/cm^2, respectively. It is well known that coliforms can rapidly build-up in moist, milk residues in milking equipment, which then becomes the major source of contamination of produced milk. However, relatively low coliform counts in milk do not necessarily indicate effectively cleaned and disinfected equipment (Bramley, 1990). The original source of enterococci in a milking machine is not clear because cow faeces are not considered the source of enterococci in cheese. Their natural habitats are human and animal intestinal tracts, yet they are also found in soil, on plants, and in the intestines of insects and birds. At the production of farmhouse raw-milk cheese, the enterococcus strains are found in cheese, in milk, on milking machines even after chlorination, on milking equipment surfaces, in water used on the farm and on cows' teats (Gelsomino *et al*., 2002).

The results showed very small differences between the number of micro-organisms on milking machine surfaces before and after milking. According to the applicable regulations (Pravilnik., Ur. l. SFRJ, 1989) the high number of micro-organisms on the surfaces of milking machines even after being cleaned shows improper washing (cleaning) by about 60% of cheese producers. Bramley (1990) also reported that a higher ratio of non-lactic-acid producers reveals the presence not only of coliforms and enterococci but probably also other harmful micro-organisms like micrococci, N group streptococci and mastitis streptococci, asporogenous Gram positive rods like *Microbacterium, Corynebacterium*, sporeforms, Gram negative rods like *Pseudomonas*, enterobacteria etc., which are mostly also present in raw milk (Bramley, 1990).

There were no seasonal differences in number of micro-organisms, except that the number of coliforms was higher in spring (Fig. 1).

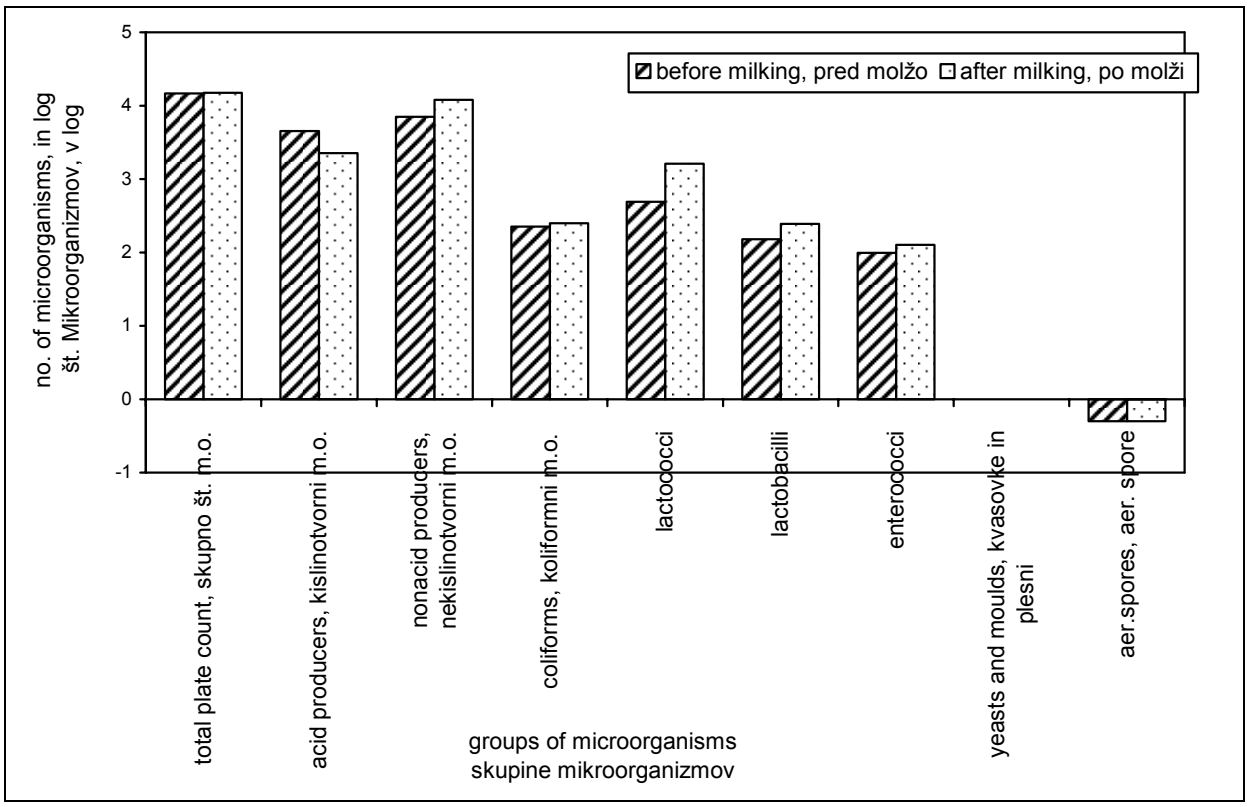

Figure 1. The average number (in log) of micro-organisms belonging to different groups expressed as the number of colony-forming units cfu per 1 cm^2 before and after milking of the inner surface of milking machines.

Slika 1. Povprečno število (v log) mikroorganizmov različnih skupin mikroorganizmov pred in po molži, izraženo kot število kolonijskih enot ke na 1 cm^2 notranje površine molznih strojev.

Raw and mixed milk and whey samples

The total number of micro-organisms was $4.9 \cdot 10^5$ cfu/ml in raw milk and $5.5 \cdot 10^6$ cfu/ml in mixed milk from a vat and did not rise significantly during cheese-processing. Arenas *et al*. (2003) reported on the same values ($5.5 \cdot 10^6$ cfu/ml) of the total number of micro-organisms in raw milk for Genestoso cheese production. The lactic-acid and non-lactic-acid producers in both types of milk samples were at a ratio of 1 to 1. The number of non-lactic-acid producers is unexpectedly high, which showed a possible contamination during cheese-processing. Particularly the number of coliforms in raw and mixed milk was high (in the range of $3.4 \cdot 10^5$) and fell to $5.4 \cdot 10^4$ in whey. The incidence of coliforms in raw milk has received considerable attention, partly due to their association with contamination of faecal origin and the consequent risk of more pathogenic faecal organisms being present, partly because of the spoilage their growth in milk at ambient temperatures can produce, and not least due to the availability of sensitive and rapid tests for detecting and enumerating coliforms. Coliform counts regularly in excess of 100 cfu/ml are considered by some authorities as evidence of unsatisfactory production hygiene. Sporadic high coliform counts may also be a consequence of unrecognised coliform mastitis. Some species of the genera making up the coliform group of bacteria are psychrotrophic and constitute 10–30% of the whole group of micro-organisms, the majority of these coliforms are *Aerobacter* spp. (Bramley, 1990).

The average number of enterococci, aerobic spore-forming micro-organisms, yeasts and moulds, lactobacilli, lactococci, proteolytic and lipolytic micro-organisms in milk and in whey

was in the same logarithmic range of about $2.2 \cdot 10^4$, 310, 3.5, $31.2 \cdot 10^4$, $2.1 \cdot 10^6$, $6.2 \cdot 10^3$ and $1.7 \cdot 10^4$ cfu/ml of the sample, respectively. The number of lactobacilli and lactococci did not increase much during the whole cheese-processing (Fig. 2).

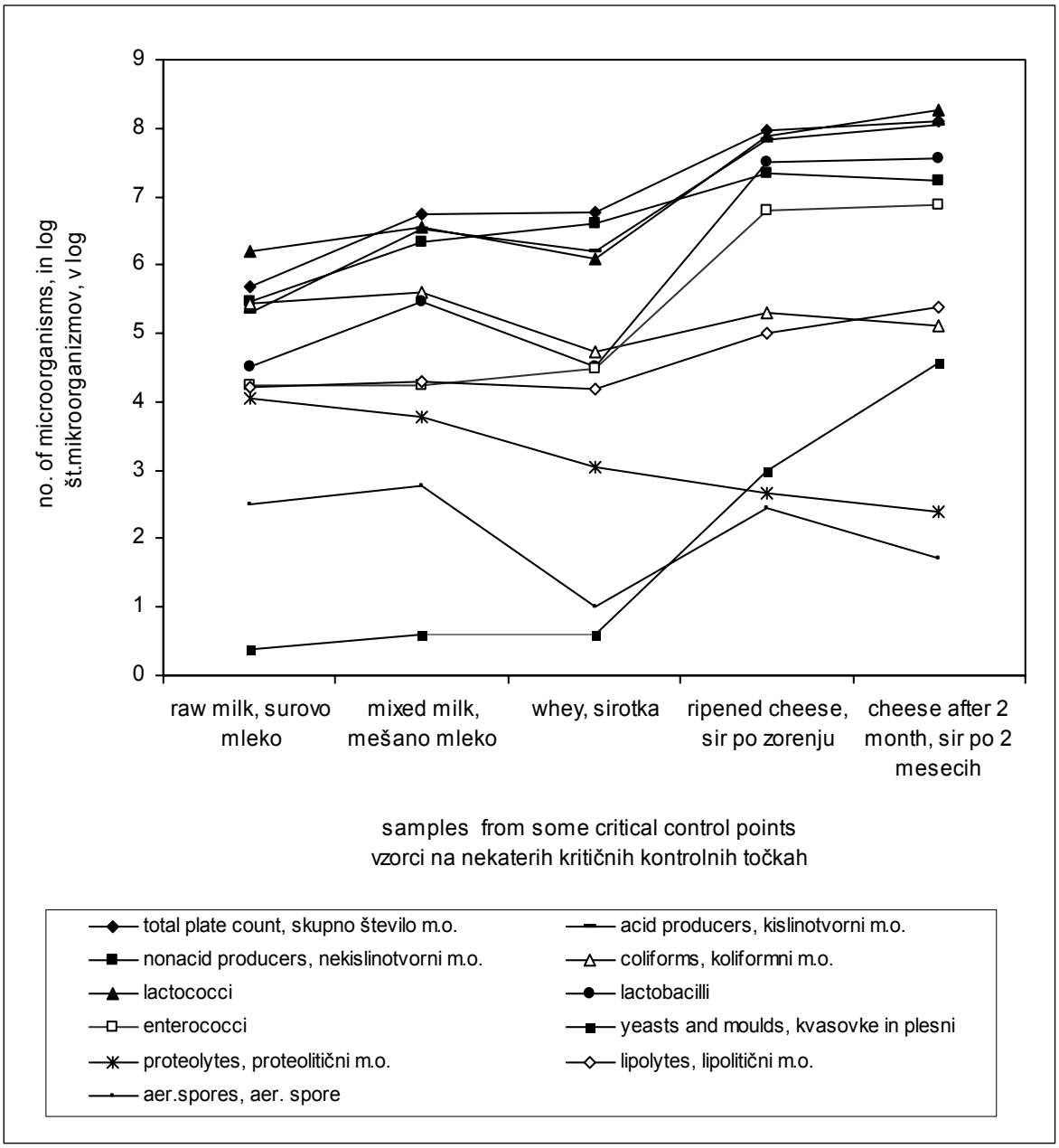

Figure 2. The average number (in log) of different groups of micro-organisms expressed as the number of colony-forming units cfu per 1 ml (g) of samples.

Slika 2. Povprečno število (v log) mikroorganizmov različnih skupin, izraženo kot število kolonijskih enot ke v 1 ml (g) vzorcev.

Arenas *et al*. (2003) found a lower number of lactococci on M17 medium ($2.6 \cdot 10^4$) and enterococci ($2.3 \cdot 10^3$) than we did in our experiment. Estepar *et al*. (1999) studied the quality of Penamellera cheese where the average of total counts on a PCA medium in starting milk was close to 10^6 cfu/ml, the number of coliforms, lactococci, lactobacilli, enterococci and yeasts and moulds was at about 10^4, 2.10^5, 5.10^3, 8.10^3, 8.10^4, respectively. Comparing the results we saw

that the number of the total count of micro-organisms, enterococci and coliforms was relatively close to our results, the numbers of lactococci and lactobacilli were lower and the number of yeasts and moulds was much higher than in our experiment.

In about 60% of tested raw milk samples the number of total plate count of micro-organisms at 30 °C was higher than 50 000 cfu/ml in (without a geometric average calculation). These samples were according to the norms (Council Directive 92/46/EEC, 1992; Pravilnik…, Ur. l. RS, 2004) not appropriate for cheese production. The number of somatic cells per ml were higher than 400 000 in 15.3% of samples. No inhibitory substances were detected in milk samples.

Cheese samples

After one month of ripening the average of total bacterial count was $9.2 \cdot 10^7$ cfu g^{-1} of cheese, of these $6.8 \cdot 10^7$ represented lactic-acid producers and $2.2 \cdot 10^7$ represented non-lactic-acid producers. The average number of coliforms, enterococci, aerobic spore-forming micro-organisms, yeasts and moulds, lactococci, lactobacilli, proteolytic and lipolytic micro-organsims were $2.0 \cdot 10^5$, $6.3 \cdot 10^6$, 280, 960, $2.5 \cdot 10^7$, $9.8 \cdot 10^7$, 450 and $9.8 \cdot 10^4$ cfu g^{-1} of cheese, respectively. After one month of keeping vacuum-packed ripened cheese samples at 6 °C, the number of micro-organisms did not rise significantly, but the number of yeasts and moulds grew to $3.6 \cdot 10^4$ cfu g^{-1} of cheese (Fig. 2).

A high number of enterococci (from a min. $3 \cdot 10^3$ to a max.$15 \cdot 10^7$ cfu g^{-1}) and coliforms (from a min. 10 to a max. $19 \cdot 10^5$ cfu g^{-1}) were detected (Fig. 3, 4). Gelsomino *et al*. (2002) reported that enterococci were widely distributed in raw milk cheese and were generally thought to positively affect the development of flavour.

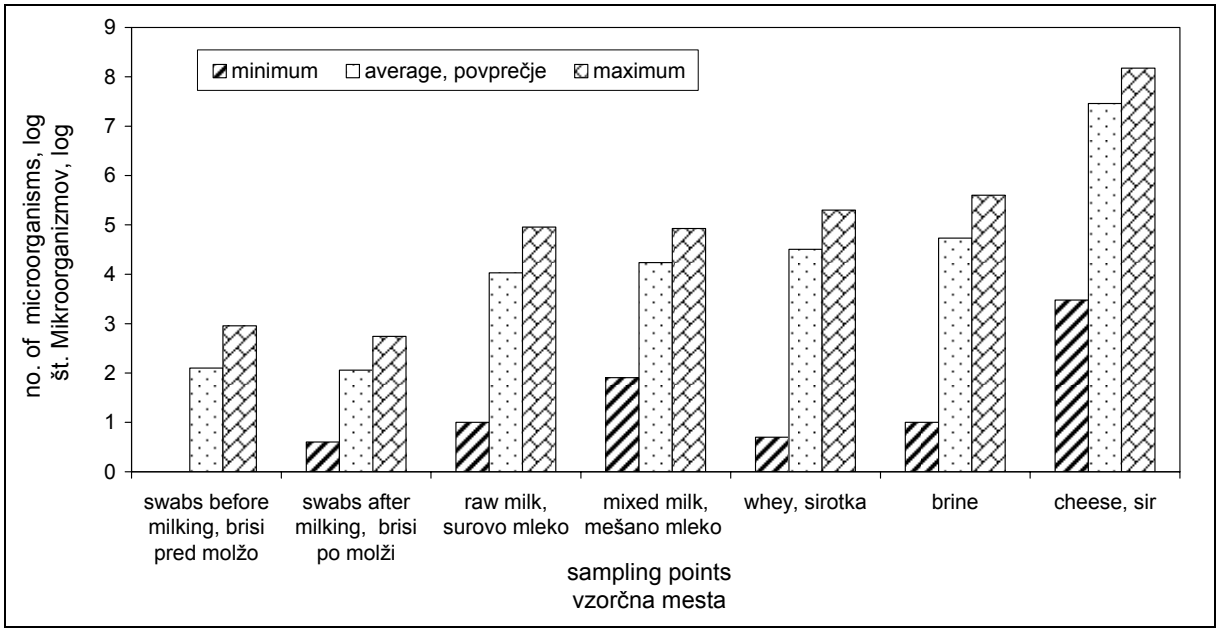

Figure 3. The average number (in log) of enterococci in 98 samples taken at different sampling points of milking and cheese manufacturing (the results of swabs before and after milking are expressed in number of colony-forming units cfu per 1 cm^2 of milking machine surfaces and in other samples as the number of cfu per 1 ml or 1 g).

Slika 3. Povprečno število (v log) enterokokov v 98 vzorcih, odvzetih na različnih kontrolnih točkah molže in sirjenja (brisi površine molznih strojev pred in po molži so izraženi kot število kolonijskih enot ke na 1 cm^2, a pri ostalih vzorcih kot število ke na 1 ml ali g).

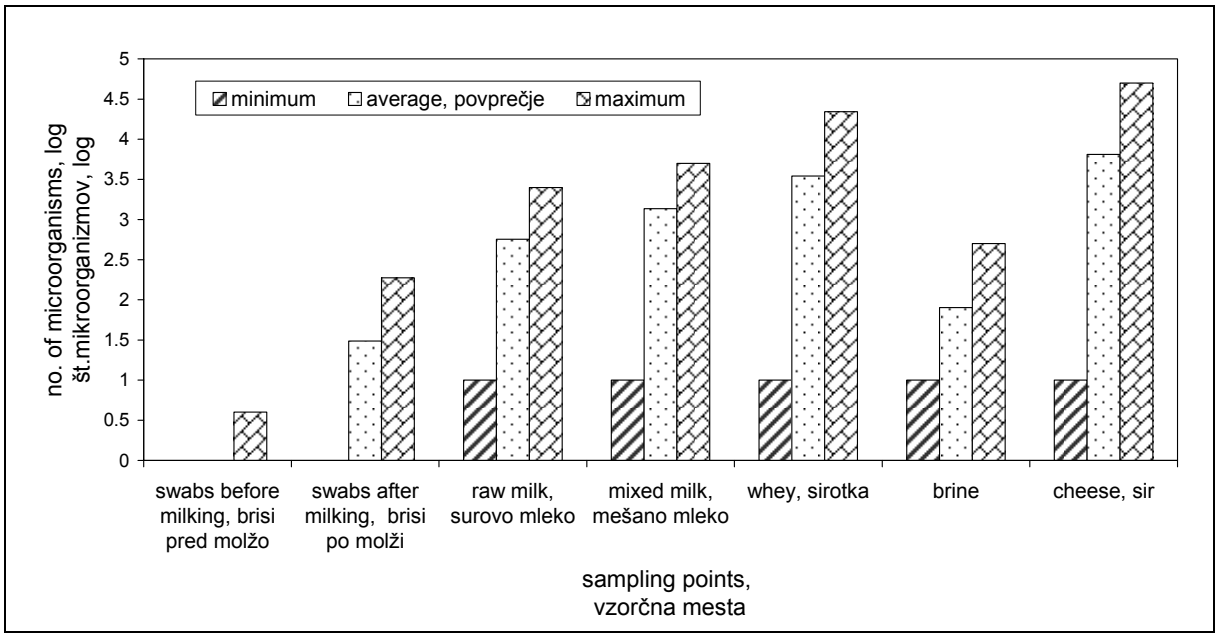

Figure 4. The average number (in log) of coliforms in 98 samples taken at different sampling points of milking and cheese manufacturing (the results of swabs before and after milking are expressed in number of colony forming units cfu per 1 cm^2 of milking machine surfaces and in other samples as the number of cfu per 1 ml or 1 g).

Slika 4. Povprečno število (v log) koliformnih mikroorganizmov v 98 vzorcih, odvzetih na različnih kontrolnih točkah molže in sirjenja (brisi površine molznih strojev pred in po molži so izraženi kot število kolonijskih enot ke na 1 cm^2, a pri ostalih vzorcih kot število ke na 1 ml ali 1 g).

The results of Estepar *et al*. (1999) showed a somewhat higher average number of total bacterial count, coliforms, enterococci and particularly yeasts and moulds, while the number of lactobacilli and lactococci was the same or a little lower than our results. Estepar also reported that the lactic acid bacteria soon became dominant after manufacturing, both on the surface and the interior of cheese. The growth of lactococci was parallel to total aerobic counts. The same data were found in our study (Fig. 2).

Arenas *et al*. (2003) reported on somewhat higher values of total count of micro-organisms ($2.6 \cdot 10^8$ cfu g^{-1} of cheese), while the number of lactococci ($3 \cdot 10^4$) and enterococci ($4.6 \cdot 10^5$) was lower than in our cheese samples.

Menendez *et al*. (2001) studied the characteristics of 24 Tetilla raw cow milk cheese, where the number of total count of micro-organisms, lactococci on M17 agar and enterococci was $3 \cdot 10^9$ cfu/ml, $2 \cdot 10^9$ cfu/ml and $2 \cdot 10^7$ cfu/ml, respectively. High mean counts of coliforms ($1.2 \cdot 10^6$ cfu/ml), and yeasts ($2.7 \cdot 10^4$ cfu/ml) were also measured.

Brine

The total number of micro-organisms in brine was about $4.0 \cdot 10^6$ cfu/ml, while a high number of lactococci (10^8 cfu/ml), lactobacilli ($2.0 \cdot 10^7$ cfu/ml), enterococci ($5.0 \cdot 10^4$ cfu/ml), and yeasts and moulds ($6.0 \cdot 10^4$ cfu/ml) was also established (Fig. 5).

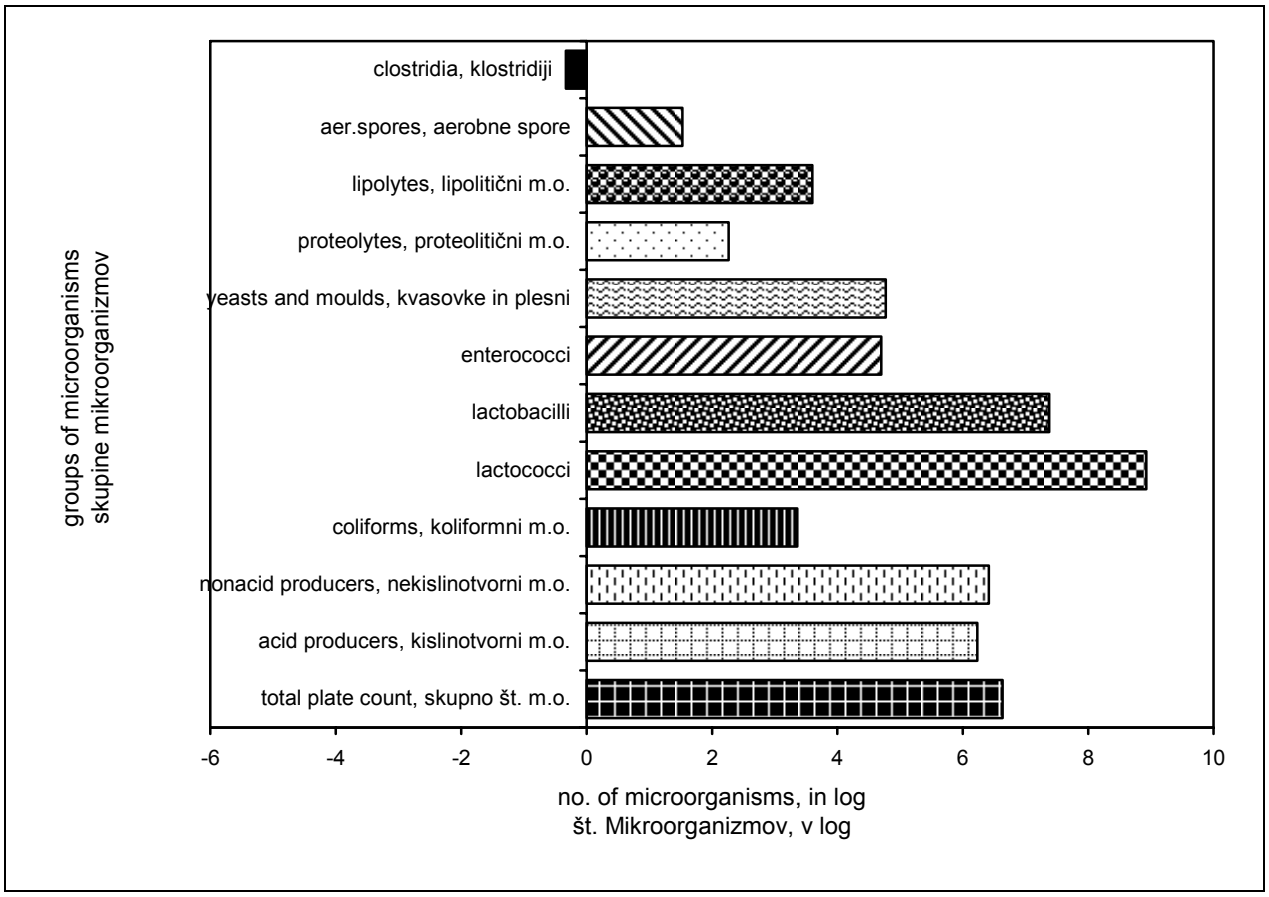

Figure 5. The average number (in log) of different groups of micro-organisms expressed as the number of colony-forming units cfu per 1 ml of brine samples.

Slika 5. Povprečno število (v log) mikroorganizmov različnih skupin, izraženo kot število kolonijskih enot ke v 1 ml vzorca slanice.

Pathogen micro-organisms in samples taken at sampling points in milking and cheese manufacturing

In this experiment the presence of pathogen micro-organisms *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, *Campylobacter* spp., sulphite-reducing clostridia and coagulase-positive staphylococci (*Staphylococcus aureus* and related species) was established in samples of udder surfaces, milking-machine surfaces, raw and mixed milk, whey after curdling, as well as cheese and brine.

Listeria spp. was isolated from 7% of samples of four cows' udder surfaces, one swab from milking machine inner surfaces, two milk and one whey samples, while none of the samples examined were positive to the presence of *Salmonella* spp. and *Campylobacter* spp. *L. monocytogenes* is a food-borne pathogen that can contaminate dairy products (Menendez *et al*., 2001). *Listeria monocytogenes* is in contrast to *Salmonella* a psychrotrophic microorganism and can survive at low temperatures. The growth of this organism on contaminated cheese can occur. The most commonly occurring species in food are *L. innocua* and *L. monocytogenes*. Outbreaks of listeriosis resulting from the consumption of dairy foods contaminated with *L. monocytogenes* have prompted concern about the behaviour of this organism during processing and the subsequent storage of various dairy products and about control of the hazard the bacterium poses to the dairy industry. Although *Listeria* is inactivated under normal conditions of pasteurisation (Schaack and Marth, 1988), problems can arise from post-pasteurisation contamination. Bacteria

can enter cheese at many stages during its processing. Meyer Broseta *et al*. (2003) reported that the presence of *L. monocytogenes* in farm raw milk was low (only 2.4% of samples taken monthly from milk tankers). A seasonal effect (with peaks in winter) was observed. The farm milk contamination is, most often, a sporadic event. The number of bacterial cells of *Listeria* was also very low (below 3 bacteria per millilitre with a most probable concentration of 0.1 cfu/ml). Such low levels are very likely to be due to environmental contamination.

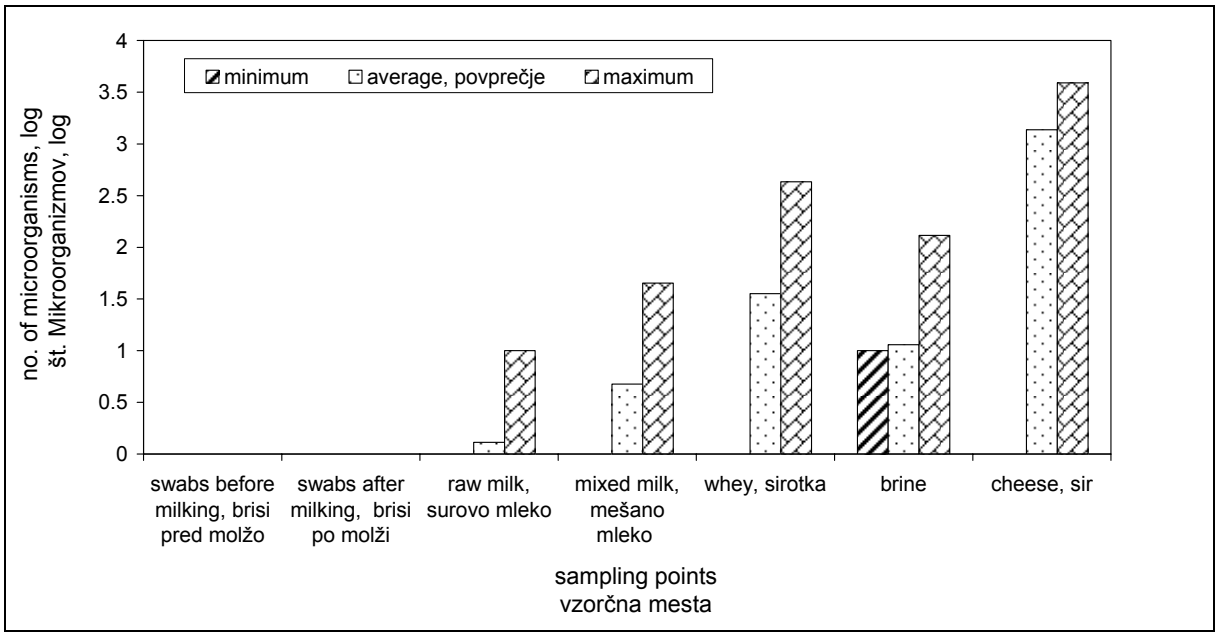

Figure 6. The average number (in log) of coagulase-positive staphylococci (*S. aureus*) in 98 samples taken at different sampling points of milking and cheese manufacturing (the results of swabs taken before and after milking are expressed in number of colony forming units cfu per 1 cm^2 of milking machine surfaces and in other samples as number of cfu per 1 ml or 1 g).

Slika 6. Povprečno število (v log) koagulaza-pozitivnih stafilokokov v 98 vzorcih, odvzetih na različnih kontrolnih točkah molže in sirjenja (brisi površine molznih strojev pred in po molži so izraženi kot število kolonijskih enot ke na 1 cm^2, a pri ostalih vzorcih kot število ke na 1 ml ali 1 g).

The contamination of raw milk with *Salmonella* usually occurs as a result of the transfer of faeces from an animal to milk via unclean teats and udders. Such contamination can pass into milk during milking and, once present, on milking parlour equipment that can then readily proliferate and spread if such equipment is not adequately cleaned and sanitised. Its growth in milk should be limited by effective refrigeration (<8 °C). Effective milking parlour hygiene (cleaning and disinfection of udders and teats), cleaning and sanitisation of milking equipment and subsequent milk storage systems are essential elements in preventing the spread of this organism (McManus and Lanier, 1987).

Campylobacter jejunii and *Campylobacter coli*, which cause *Campylobacter* enteritis, may be commonly isolated from cow faeces and this is considered to be the main source of infection of raw milk. *Campylobacter* species do not generally grow at temperatures below 30 °C and are sensitive to the conditions necessary for growth. Therefore, growth is unlikely to occur in milk and dairy products. The infectious dose for these micro-organisms is, however, low and consequently growth may not be a prerequisite of infection. In raw milk, the *Campylobacter*

number will normally be reduced during cold storage. Both *Campylobacter* species are sensitive to milk pasteurisation (Anonim., 1994).

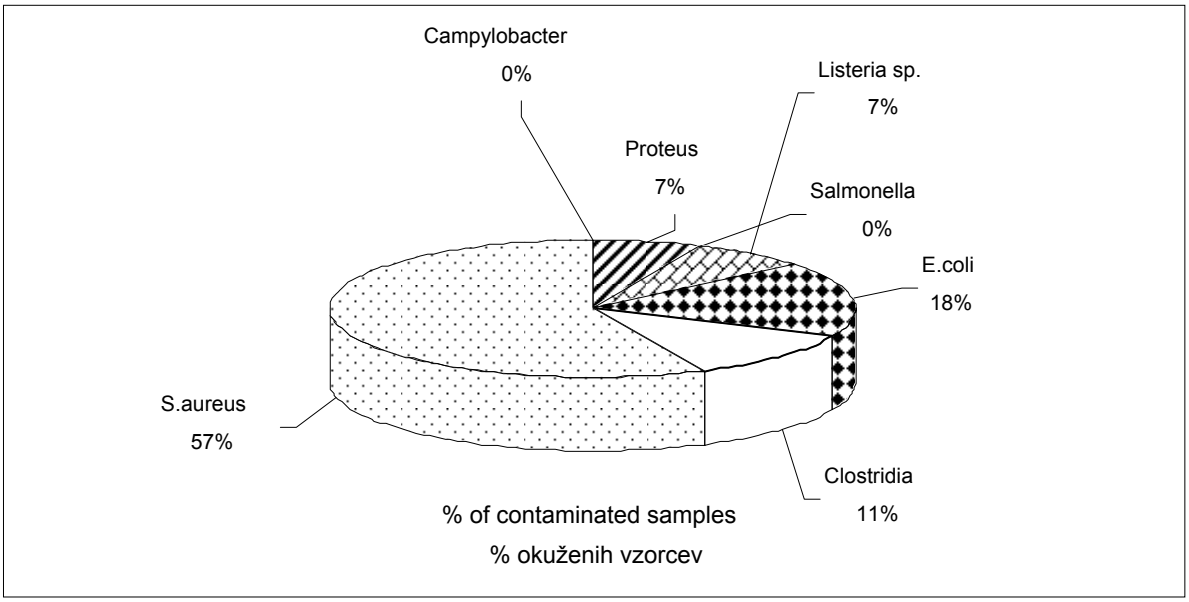

Figure 7. The percentage (%) of samples contaminated by pathogen micro-organisms of all 98 samples tested.
Slika 7. Odstotek (%) s patogenimi mikroorganizmi kontaminiranih od skupno 98 preizkušenih vzorcev.

Proteus was present in 7 (7%) cases of milk and whey. Habeš (2002) reported similar results. In his study *Proteus* spp. was present in 7.28% of 840 raw and pasteurised milk samples taken in Bosnia in a 4-year period.

Sulfite-reducing clostridia (mostly *Cl. perfringens*) were detected in 10 (10%) samples (swabs, raw milk, whey). These spore-forms are present in sediment of various types, and in the intestinal tracts of men and animals. They gain entry to milk via faeces, soil and feedstuff, especially silage. Strains may be psychrotrophic, mesophilic or thermophilic. Since most strains are strictly anaerobic, they have the greatest potential importance as spoilage organisms of cheese and canned milk products. They produce a number of soluble toxic substances (Gilmour and Rowe, 1990).

Escherichia coli was isolated in our experiment from 12 (12%) samples of swabs, raw and mixed milk, whey and brine. Testing for *E. coli* as an indicator of faecal contamination and/or poor hygienic practices has traditionally been done in dairy industry. It is well known that some strains may be enteropathogenic or enterotoxigenic. Both of these groups have been responsible for outbreaks involving cheese and milk (Anonim., 1994).

The number of coagulase-positive staphylococci (*Staphylococcus aureus* and related species) exceeded the norms of the European Communities (M=2 000 bacteria per ml of sample) in 14.4% of raw milk samples. The average number of these organisms in raw milk was $5.7 \cdot 10^2$ and increased to $3.5 \cdot 10^3$ in whey samples (Fig. 6). The presence of coagulase-positive staphylococci was examined in 57% of samples (raw milk, mixed milk, whey, brine, cheese samples) (Fig. 7).

De Buysier *et al*. (2001) reported that *Staphylococcus aureus* was by far the most frequent pathogen associated with food pathogen outbreaks (85.5% of the outbreaks) in France, followed by *Salmonella* (10.1%), *E. coli* (3%), *L. monocytogenes* (3%) and *C. jejuni* (1.5%) outbreaks.

Coagulase-positive staphylococci (*Staphylococcus aureus* and related species) may cause human disease through the production of toxins. The formation of effective levels of toxin requires a high number of micro-organisms (approximately 10^5–10^6 micro-organisms per gram of food) at a pH value greater than 5 and so the presence of coagulase-positive staphylococci at a low level does not necessarily constitute a hazard. Dairy-related outbreaks of staphylococcal intoxication have been attributed to raw milk, dried milk, cheese and ice cream. Coagulase-positive staphylococci may be present in raw milk from the udder and teat canals of a cow, particularly if lesions are present. Also, the nasal area and hands of humans are recognised sites of contamination: poor personal hygiene can result in the contamination of milk and dairy products. Essential to the production of toxin is the growth of micro-organisms. In general, *Staphylococcus aureus* and the related species *Staphylococcus intermedius* and *Staphylococcus hyius* do not multiply at temperatures below 8 °C, and 10 °C is the minimum for toxin production. These micro-organisms are, however, resistant to salt. Pasteurisation will be effective against them but, if toxins are present, the toxins will not be inactivated. Therefore, toxins may be present in the absence of viable micro-organisms. The higher counts of *Staphylococcus* recorded in spring, when milk yields are at their peak, are a cause for concern and mammary infections (Anonim., 1994) (Fig. 7).

Pathogen micro-organisms in cheese samples

Salmonella, *Listeria* spp., *Proteus*, sulphite-reducing clostridia, *Campylobacter* were not detected in cheese samples. *E. coli* was found in 4 (30%) of samples on levels from 10–3400 cfu g^{-1} of cheese, while the coagulase positive staphylococci (*S. aureus* and related species) were present in 9 (64%) of samples and ranged from 100 to 50 000 cfu g^{-1} of cheese. Their average number in cheese samples ($6.5 \cdot 10^3$) was in the same log range as in the whey samples (Fig. 6). These results showed a high contamination with these two types of micro-organisms in comparison with the results of Menendez *et al*. (2001) who established low average numbers of *Staphylococcus aureus* (<61 per g/cheese) and *Escherichia coli* (<52 per g/cheese) in 24 Tetilla cheese samples. *Listeria monocytogenes* was detected in two of 24 samples. None of the samples yielded *Salmonella* spp.

A significant correlation (correlation coefficient $r>0.80$, $P<0.0001$) between the total bacterial count, the number of lactic-acid, non-lactic-acid-producers, enterococci, lactococci and lactobacilli in swabs, milk and cheese samples was established (data not shown).

There were no significant differences in the number of micro-organisms between spring and autumn seasons, except for enterococci (P=0.0004**). Significant differences between the microbiological quality of samples from individual cheese-makers were also established (P=<0.0001***). There is no statistically significant influence on microbiological quality between different Slovenian areas where cheese production takes place, using starter cultures in cheese production or not, and between types of feeding (pasture, feeding in a cowshed, etc).

Highly statistically significant differences in number of enterococci between cheese producers (P=<0.0001***) and between seasons (P=0.0041**) were found. There were also statistically significant differences between the number of bacteria *E. coli* between seasons (P=0.041), while the differences in *E. coli* numbers between producers were not statistically significant (P=0.36).

Water samples

Water used in the process of milk production should be of bacteriologically potable quality. The purity of properly treated supplies taken direct from the mains is assured, but bacterial contamination can be introduced from storage tanks not properly protected against rodents, birds, insects and dust. Bacteria may also come from dirty wash troughs, or the carrying of buckets and hoses. Many farms rely on untreated water supplies from boreholes, wells, lakes, springs and rivers; some of these may be contaminated at source with micro-organisms of faecal origin, e.g.

coliforms, faecal streptococci and clostridia. In addition, a wide variety of saprophytic micro-organisms derived from soil or from vegetation may be present, including *Pseudomonas* spp., coliforms and other Gram-negative rods, *Bacillus* spores, coryneform bacteria and lactic acid bacteria. The numbers of these contaminants vary widely. If untreated water gains access to milk or is used for rinsing equipment and containers, any micro-organisms present in the water will contaminate the milk although the numbers of micro-organisms added may not be significant in terms of the cfu/ml of milk. However, multiplication of some of the water-borne bacteria in any residual water in the equipment will result in a more serious contamination and may lead to the establishment and development of some undesirable types of micro-organisms, e.g. psychrotrophic Gram-negative rods, in the milking equipment.

For these reasons, in areas where farm water supplies are bacteriologically unsatisfactory the chemical disinfection or sanitation of milking equipment is always delayed until just before the next milking, and the disinfectant solution is merely drained from the equipment before it is used for milking. This practice prevents recontamination resulting from rinsing with untreated water. Chlorination, by dosing with hypoclorite, is frequently recommended for water of unsatisfactory bacteriological quality used for the final rinsing of equipment, because it helps to reduce the risk of bacterial multiplication in residual water left in milking machines that are cleaned and sanitised in the one operation (Cousins *et al*., 1981).

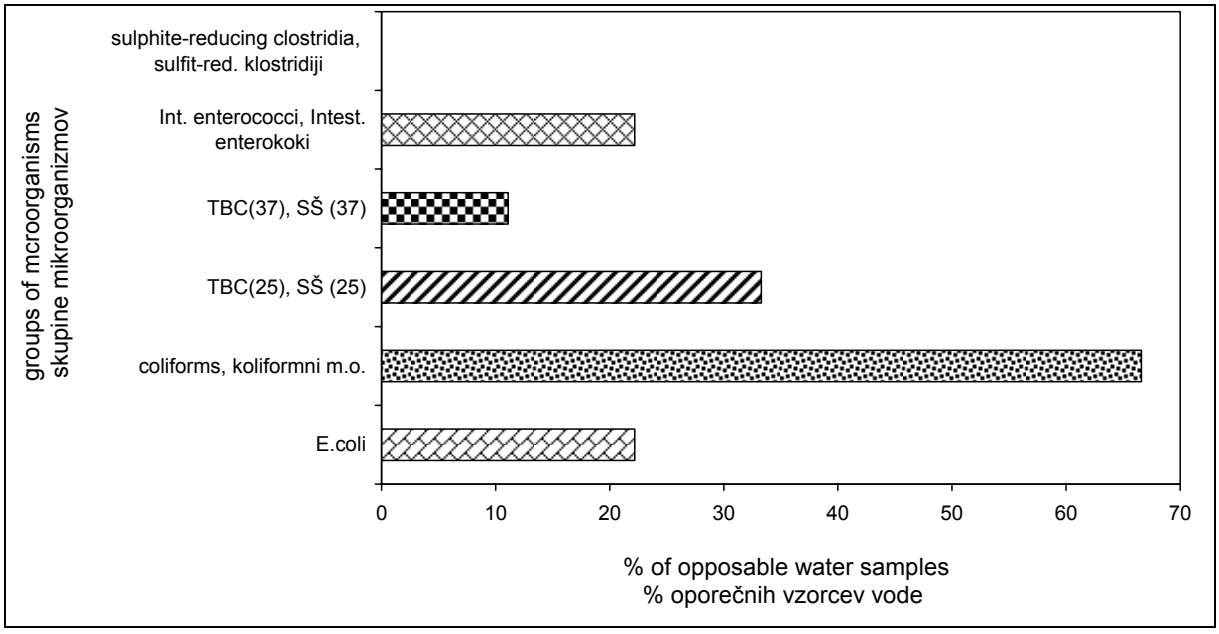

Figure 8. The percentage (%) of water samples not corresponding to the applicable regulations due to the presence or higher number of indicator micro-organisms: TBC (37): total bacterial count inc. at 37 °C; TBC (25): total bacterial count inc. at 25 °C, sulfite-reducing clostridia, coliforms, intestinal enterococci and *E. coli.*

Slika 8. Odstotek (%) vzorcev vode, ki niso ustrezali veljavnim predpisom zaradi povišanega števila indikatorskih mikroorganizmov: SŠ (37): skupno število m.o. ob inkubaciji pri 37 °C; SŠ (25): skupno število m.o. ob inkubaciji pri 25 °C, sulfit-reducirajočih klostridijev, koliformnih m.o., intestinalnih enterokokov in *E. coli*.

The number of total bacterial count and the presence of *E. coli*, coliforms and faecal streptococci in water samples taken at taps or water pipes in cheese-makers and in milking parlours were established in our study. The results showed that 78% of water samples did not correspond to the microbiological criteria according to the applicable regulations (Pravilnik, Ur.

l. RS, 2004). *E. coli* was present in 22% of samples, coliforms in 67% and faecal enterococci in 22% of the samples. The number of viable micro-organisms at 37 °C was exceeded in 11% of samples, while the number of micro-organisms at 22 °C was exceeded in 33% of samples (Fig. 8).

System of critical control points

In recent years, the hazard analysis critical control points (HACCP) concept has been proposed as the best approach to ensure food safety. The results of this study also underline the need to achieve food safety and reduce risk, to implement the hazard analysis critical control points (HACCP) concept and quality assurance from the farm to the dairy plant, and to set up and apply EU directive 92/46 on milk hygiene (Silva, 2003; De Buyser, 2001). It is very important for cheese-makers to set up the system of critical control points and to investigate the direct and cross-contamination sources in their cheese production. The milking machines, production pipelines, equipment such as vats, plastic wraps, pressing cloths, starter cultures and the hands of workers were direct contamination sources. In addition, the hands of workers and the water used in a facility played a role in direct cross-contamination. The air in the facility was a critical control point for yeast and mould contamination.

Routine microbiological monitoring of the hygienic quality of raw milk should be employed using not only the total plate count, but also indicator bacteria such as *E. coli* or coliforms and incentive payment schemes should be considered where milk is intended to be used without a bacterial destruction stage in the process, i.e. for raw milk cheese, to encourage the adoption of high hygienic standards (McManus and Lanier, 1987). The inclusion of other pathogen micro-organisms like *L. monocytogenes* on the list of organisms subject to the HACCP has recently also been called for (Silva *et al*., 2003).

CONCLUSIONS

- In our study 21% of tested cheese samples did not correspond to the microbiological criteria according to the applicable EU and Slovenian regulations.
- The high number of micro-organisms on the surfaces of washed milking machines before milking showed ineffective cleaning (washing) by about 60% of cheese producers.
- Greater contamination usually appeared during cheese-processing and not during milking.
- In 78% of drinking water samples the results exceeded the microbiological criteria according to the applicable regulations so greater attention should also be paid to water quality.
- It is suggested that more importance should be given to milking and cheese production hygiene, as well as to the determination and control of critical points in firms for improving cheese quality and preventing food-borne pathogenic outbreaks.
- The authors believe that milk and cheese producers should employ the HACCP and quality assurance practices in the production stages of milk from the farm up to and including the dairy plant, while also setting up and implementing EU Directive 92/46.

POVZETEK

V Sloveniji je posebno v odročnejših predelih kar nekaj individualnih majhnih sirarn, kjer proizvajalci mleko sami predelajo v sire, včasih tudi v skuto. Pogosto uporabljajo za sirjenje surovo, nepasterizirano mleko, kar omogoči boljši izkoristek mleka, ohrani pa se tudi naravna, za tisto področje značilna mlečnokislinska mikroflora, ki igra pomembno vlogo pri senzoričnih

značilnostih proizvedenih sirov. Higienska kakovost in zdravstvena ustreznost proizvedenih sirov zavisi od ustrezne mikrobiološke kakovosti mleka kot surovine, razmer, v katerih se mleko predeluje v sire, kakovosti molže, temperature hranjenja mleka in sirov, krme, sezone, uporabe različnih starterskih kultur, vode, uporabljene za napajanje, pranje molznega in mlekarskega pribora itd.

Preverjanje prisotnosti in števila specifičnih mikroorganizmov v različnih fazah prireje mleka in njegove predelave je pomemben dejavnik pri kontroli in sistemu zagotavljanja kakovosti proizvodnje.

Namen našega dela je bil ugotoviti mikrobiološko kakovost, oziroma prisotnost posameznih skupin mikroorganizmov na nekaterih kritičnih kontrolnih točkah prireje mleka in sirjenja. V ta namen smo ugotavljali prisotnost patogenih in indikatorskih mikroorganizmov v 14 vzorcih poltrdega sira, proizvedenih pri posameznih sirarjih na različnih področjih Slovenije ter v 98 vzorcih, odvzetih v postopku molže in predelave mleka v sir. Odvzeli smo brise površine vimena krav molznic ter notranjih površin molznih strojev, vzorce surovega mleka takoj po molži, mešanega mleka iz sirarskega kotla, sirotke po usirjanju, slanice, sirov po enomesečnem zorenju in vakuumsko pakiranih sirov po naknadnem enomesečnem skladiščenju pri temperaturi 6 °C. Odvzeli smo vzorce vode, namenjene pranju molznega in mlekarskega pribora.

Ugotovili smo, da 3 (21 %) vzorci sirov glede mikrobiološke kakovosti niso ustrezali kriterijem slovenske zakonodaje. Visoko število mikroorganizmov na notranjih površinah molznih strojev (kolektorji, sesne gume) pred molžo kažejo na neučinkovitost pranja (čiščenja, dezinfekcije) pri kar 60 % proizvajalcev. V postopku predelave mleka v sir je prišlo pogosto do večje okužbe kot v postopku molže. Kar 78 % vzorcev vode glede mikrobiološke kakovosti ni ustrezalo predpisanim kriterijem, zato je potrebno veliko pozornost usmeriti na problem zagotavljanja kakovosti vode v odročnejših predelih. Prav tako predlagamo, da posamezni sirarji posvetijo večjo pozornost izboljšanju higiene pri molži in sirjenju ter se tako izognejo slabi mikrobiološki in senzorični kakovosti ter zdravstveni oporečnosti svojih proizvodov. Vzpostavitev sistema kontrole kritičnih točk v posameznih stopnjah prireje in predelave mleka v smislu sistema HACCP in zagotavljanja kakovosti je zaželena in tudi zakonsko predpisana z direktivami EU.

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THE CONSEQUENCES OF CHANGING CONDITIONS OF THE EUROPEAN DAIRY SECTOR FOR THE STRATEGIES OF DAIRY COMPANIES

Hannes WEINDLMAIER [a)]

[a)] Technical Univ. of Munich, Professor for Dairy and Food Industry Management, Weihenstephaner Berg 1, D-85350 Freising, Prof., Ph.D., E-mail: h.weindlmaier@wzw.tum.de.

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ABSTRACT

This contribution deals with the consequences of changing conditions of the European dairy sector. Important influences discussed are the decisions of the Mid-Term-Review of the Common Agricultural Policy, the enlargement of the European Union to include the countries of Central and Eastern Europe and the concentration and globalisation of the food trade. Based on these changes, the dairy industry has to adjust their strategies. Of great importance for future competitiveness is the size of dairy companies and plants, as large enterprises and plants have several economic advantages, if an optimal size corresponds with a good job performed by the management of the company. Important alternative strategies like the cost leadership strategy, the differentiation strategy, the focus strategy and internationalisation are discussed and assessed with respect to their possible contribution to retain respectively to improve competitiveness of dairy enterprises. It is concluded that within a period of ten years the face of the European dairy sector will change quite extensively. Only those enterprises will survive which in time actively implement strategies, which are a suitable answer to the changing conditions.

Key words: milk production / dairy sector / conditions / EU enlargement / globalisation / strategies / competitivity

IZVLEČEK

Prispevek analizira posledice spremenjenih pogojev, v katerih deluje evropska mlekarstvo. Obravnava pomemben vpliv odločitev srednjeročnega poročila o Skupni kmetijski politiki, širitev Evropske Skupnosti z vključitvijo držav Srednje in Vzhodne Evrope ter koncentracijo in globalizacijo trgovine. Upoštevaje te spremembe mora mlekarska industrija prilagoditi svoje strategije. Velikega pomena za konkurenčnost v prihodnosti je velikost mlekarskih firm in obratov, ker imajo ob dobrem upravljanju veliki obrati in podjetja številne prednosti. Podan je pregled pomembnih alternativnih strategij, kot so strategija cene vodenja, strategija diferenciacije, strategija fokusiranja in strategija internacionalizacije ter diskusija možnega doprinosa teh strategij k večji konkurenčnosti mlekarskih podjetij. Podana je napoved, da se bo stanje v evropski mlekarski industriji v naslednjih desetih letih močno spremenilo. Preživela bodo samo tista podjetja, ki bodo aktivno in pravočasno uveljavila strategije, ki pomenijo ustrezen odgovor na spremenjene pogoje.

Ključne besede: mlekarstvo / pogoji / širitev EU / globalizacija / strategije / konkurenčnost

INTRODUCTION

In order to analyse the future competitiveness of the dairy sector Porters diamond is most helpful to be used as a framework (Porter, 1998, Pitts and Lagnevik, 1998, Weindlmaier, 2000). This approach identifies six sources of comparative advantage, i.e. the factor conditions, firm

strategy, structure and rivalry, demand conditions, related and supporting industries, government and chance.

In this contribution, the analysis is limited to changes in the economic environment of the European dairy sector which are of specific current relevance. First of all, the changes in the Common Agricultural Policy according to the decisions of the Mid Term Review (MTR) of June 26th, 2003 are discussed. Then the enlargement of the European Union to include the countries of Central and Eastern Europe and developments in food retail trade are presented and analysed. The second part discusses the main strategic options which are at the disposal of the dairy industry as a reaction to these changes. In this connection, the main emphasis is given to structural changes in the dairy processing sector.

THE CHANGING CONDITIONS FOR THE EU DAIRY INDUSTRY

Forthcoming changes in the common market order for the dairy sector and its implications

With respect to the changing policy environment affecting the dairy industry the decisions of the MTR of the Common Agricultural Policy of June 26th, 2003 have to be taken into account. Referring to the dairy sector, the following decisions are of specific importance:

First of all, the dairy quota system was prolonged until 2014/15. The start of the general quota increase of 1.5% decided within the Agenda 2000 was postponed until 2006 (0.5% per year). Secondly, starting in 2004, the intervention prices of butter will be cut by 25% (3×7% and 1×4%) and the intervention price of skimmed milk powder by 15% (over three years, 5% each). The third important decision refers to limitations of the intervention of butter: Intervention purchases of butter will be suspended above a limit of 70,000 tons per year in 2005/06. This amount will be reduced by further 10,000 tons per year until 2008/09 (30,000 tons thereafter). Furthermore, butter intervention will be restricted to the period March 1st to September 1st.

A new element for the EU dairy policy is the introduction of direct payments to at least partly compensate the price reduction of intervention prices. Direct payments to dairy farmers will be introduced, starting in 2004/05 with 1.18 Cent/kg and increasing to 3.55 Cent/kg in 2006/07 and after. In connection with the introduction of direct payments it has been decided that these so called Single Farm Payments will be decoupled from the volume and kind of production. This decoupling is expected to reduce output by EU dairy farmers. However, member states may choose to maintain a limited link between subsidy and production under well defined conditions and within clear limits. Furthermore, the principle of Cross Compliance has been introduced, which means that direct payments are conditional on compliance of production with environmental, food safety, animal welfare and occupational safety standards.

The implications of the MTR for milk producers (for milk production)

For milk producers because of falling intervention prices and the extension of quotas a significant drop in producer milk prices is expected. Fig. 1 shows that over time the producer milk prices followed quite closely the support prices by intervention. Therefore, if these support prices decrease from about 28 Cent/kg in 2003 to 22 Cent/kg in 2007, a significant drop in producer milk prices is likely. This drop in producer milk prices will only be partly compensated by direct payments to the farmers.

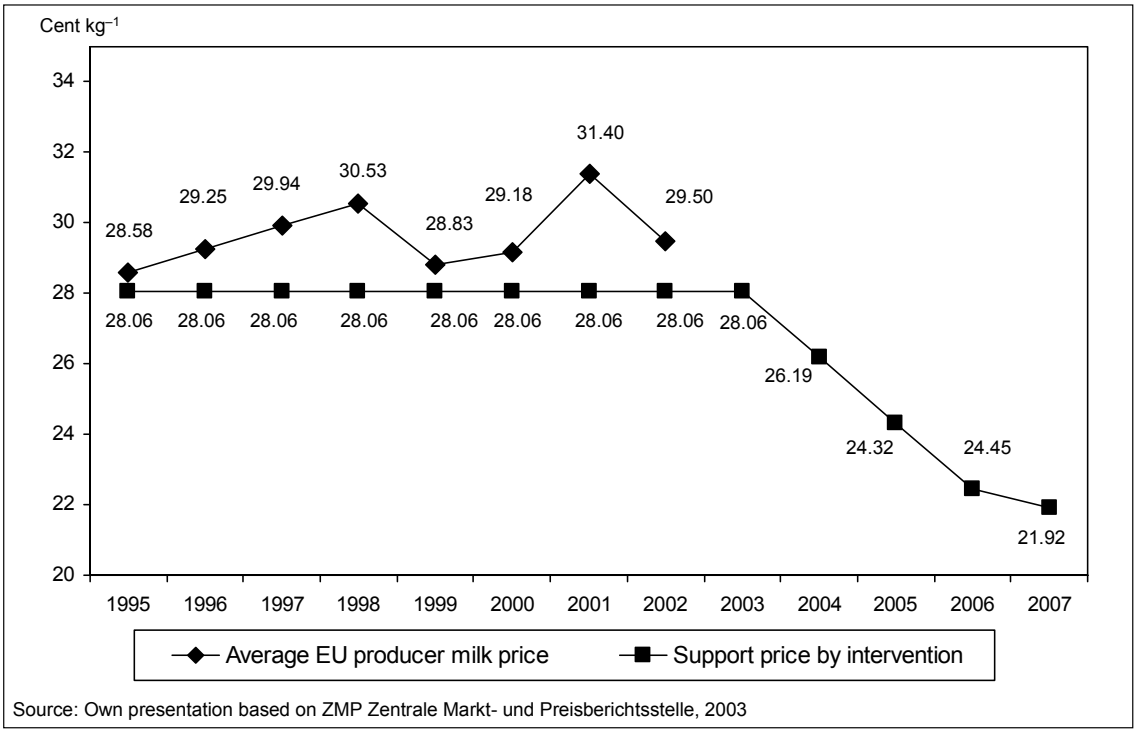

Figure 1. Development of the average producer milk prices in the EU and of the EU support price by intervention.
Slika 1. Razvoj povprečnih stroškov proizvajalcev mleka v ES in podpornih cen z intervencijami ES.

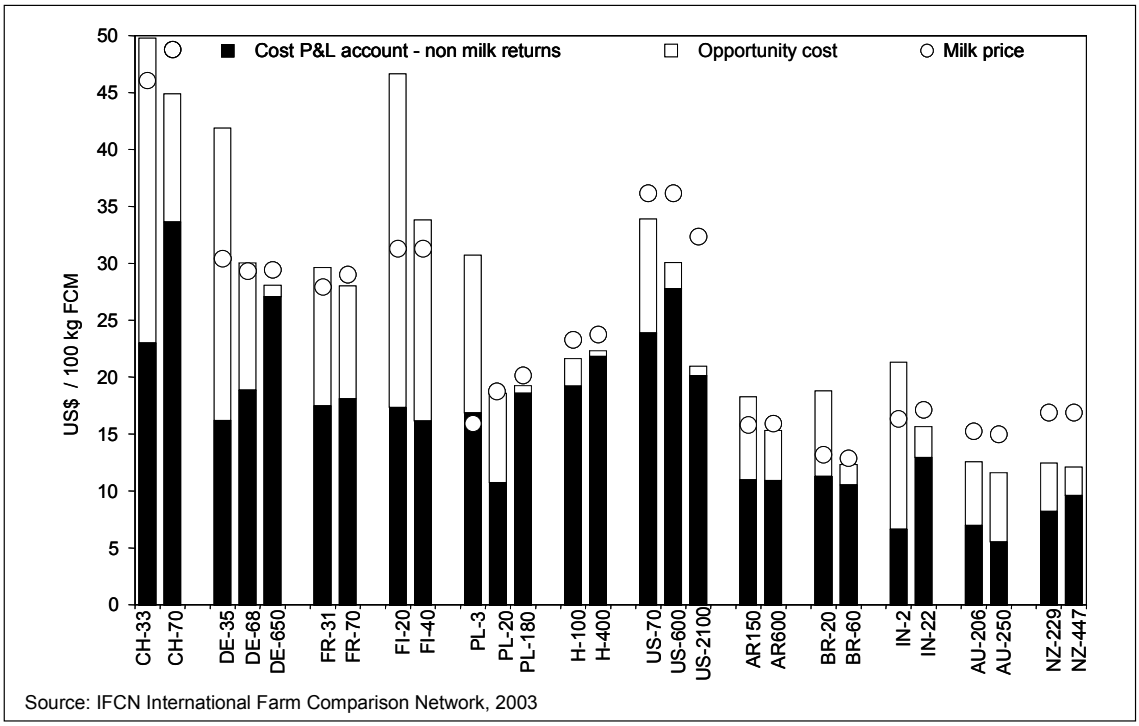

Figure 2. Costs of Milk Production and Producer Milk Price.
Slika 2. Cena proizvodnje mleka in cena mleka.

A second outcome for dairy farmers will be the fact that a comprehensive coverage of the costs of milk production by the returns received for milk will further decline**.** Subsequently, the consequences are most probably significant decreases in farmer incomes. Fig. 2 indicates that already in 2002 in some European countries the total costs of milk production are not covered entirely by the returns received for milk. Therefore, if prices will decline as shown, this coverage of full economic costs will further decrease. In this figure, the opportunity costs refer to costs for own production factors inside the enterprise (own land, family labour, own capital), the abbreviations below the bars refer to farm codes, i.e. DE-35 means a German 35-cow farm.

From this, the third consequence is quite likely: In regions with high production costs and low competitiveness for milk production, like for instance in mountainous regions of Southern Germany, France and Austria, milk production will be significantly reduced in the long run. This furthermore means that a universal coverage of grassland management, which has been an objective for agricultural policy for a long time, cannot be continued.

The implications of the MTR for the dairies

If the MTR is realised, dairies potentially have an advantage because of the fact that producer milk prices will decrease. Consequently, the production costs of the dairies will decrease, too. A growing international competitiveness could be the outcome. This indeed is one of the most important aims of the EU commission for changing Common Agricultural Policy for the dairy sector.

However, in regions with a low profitability of milk production, e.g. if small farms are prevailing, the density of milk production will probably be cut dramatically as a consequence, leading to a corresponding loss of raw milk for the dairies. A relocation of dairy plants connected with the need for regional disinvestment programs will probably be the logical consequence. As an effect of the decisions of the MTR, the need for structural change in the dairy sector will further be intensified.

The accession of the Central and Eastern European Countries to the European Union

The European Union realised its biggest enlargement ever in terms of scope and diversity. 13 countries have applied to become new members. Ten of these countries including Slovenia joined the EU on May 1st, 2004.

In order to join the EU, the new countries need to fulfill the economic and political conditions known as the "Copenhagen criteria". The dairy sector of the new member states will have full and immediate access to CAP market measures, such as export refunds and skimmed milk powder and butter intervention, which will contribute to stabilising the markets.

Of great importance are the decisions concerning the milk quotas for the new member states in relation to the consumption expectations and the decision concerning the quality of products and the hygienic standards (table 1). The table shows that the CEEC accession countries got a milk quota of 18.3 million tons. This quota can be extended by a reserve to a maximum of 19.0 million tons in 2006. If this amount is compared with the estimates concerning the demand of dairy products, this decision means that there is a potential need for imports of 1.2 million tons in 2004.1

Of similar importance are the decisions concerning quality and hygienic standards, as with the admission into the EU all EU-quality and hygienic standards are relevant and have to be fulfilled. Exports to EU- and other countries are only allowed for processors with an export license.

In most of the CEEC-countries intensive activities are performed to increase quality standards and to get export licenses. However, from the experience Germany made with the integration of the German Democratic Republic we know that this is a rather long and expensive process. This

is especially true for Poland, the largest milk producing country of the accession countries. In 2003, out of the 330 dairies in Poland only 49 had an export licence to EU-countries (Pieniadz, 2004).

Table 1. Relevant decisions concerning EU-extension to the CEEC-countries
Preglednica 1. Pomembne odločitve povezane s širitvijo ES

(1) Decisions concerning milk quotas

Milk quota after 2004	18.3 million tons
Milk quota inclusive reserve after 2006	19.0 million. tons
Actual dairy production in access countries	21.5 million tons
Estimated demand of the CEEC-countries	19.5 million tons
Potential need for imports 2004	1.2 million tons

(2) Decisions concerning milk quality

- With the admission into the EU all rules concerning quality and hygienic standard are relevant –however only a small part of the dairies has licenses for exports.
- Special quality and hygienic arrangements until 2006, but for national markets only.

Source: Weindlmaier, 2003 based on Richarts, 2003.

From this situation several consequences for the dairies are expected. First of all, the degree of competition in the markets for dairy products in the CEEC countries will increase quite extensively. Dairy companies in the accession countries which are not able to adjust to the hygienic standards set by the EU and to increase the quality of their products very fast, will not be competitive in the common European market. It is expected, that many dairy companies in the accession countries will get out of business during the next few years.

The EU enlargement will also have consequences for the trade flows within Europe. At the one hand it is likely that the CEEC countries will increase the export of basic dairy products like cheese and milk powder to the countries of Western Europe. In the adjacent regions to Western European countries even raw milk might be transported for processing in processing plants of the West. At the other hand it is quite likely that Western European dairy companies will increase the export of branded dairy products and specialities to the CEEC countries. In addition, the number of joint ventures and direct investments will further rise.

Concentration and globalisation in the food trade

During the last few decades significant changes took place within the global food trade. Table 2 illustrates first of all the enormous, border-spreading concentration processes in the food trade. The largest food trade company world-wide, *Wal-Mart*, has a yearly turnover of 243 billion Euros. In addition to this huge, US based enterprise with several affiliates in Europe, we note very large European companies like *Carrefour*, *ITM Enterprises*, and *Auchan* in France, *Ahold* in the Netherlands, *Metro, Rewe, Aldi, Edeka,* and *Tengelmann* in Germany, *Tesco* and *Sainsbury* in Great-Britain.

Based on strong bargaining power, the food retail trade is more or less fixing purchase prices for the products delivered by the dairy industry. In addition, the food trade forces suppliers to

accept increasing price deductions to support the advertising expenses of the food trade and to perform specific payments for including articles of the company into the assortment of outlets.

Table 2. The Top-20 food trade companies world-wide in 2002
Preglednica 2. Vodilnih 20 prehranskih trgovskih podjetij na svetu v letu 2002

	Company	Country	Turnover 2001, million EUR	Share of food, %	Foreign sales, %
1	Wal-Mart	USA	243,281	40.0	18.0
2	Carrefour	France	69,486	70.5	50.6
3	Ahold	Netherlands	66,593	92.0	86.5
4	Kroger	USA	55,959	92.0	86.5
5	Metro	Germany/CG	49,522	49.7	44.0
6	Albertson's	USA	42,781	90.0	0.0
7	Kmart	USA	38,655	37.0	0.0
8	Safeway	USA	48,314	92.0	10.1
9	Costco	USA	38,131	41.0	18.0
10	Tesco	Great-Britain	38,059	90.0	15.0
11	Rewe	Germany	37,540	70.3	20.5
12	Aldi	Germany	32,400*	84.0	39.4
13	ITM Enterprises	France	31,900*	82.4	25.5
14	J. Sainsbury	Great-Britain	29,743	90.0	15.0
15	Ito-Yokado	Japan	29,624	47.0	36.0
16	Edeka/AVA	Germany	28,035*	84.0	8.6
17	Aeon (Jusco)	Japan	26,680	44.0	11.8
18	Tengelmann	Germany	25,670*	74.6	57.6
19	Auchan	France	25,500*	70.0	35.0
20	Supervalu	USA	23,243	76.0	0.0

* = Estimate; Source: M+M Planet retail, www.planetretail.net

Table 2 shows that a high percentage of the turnover of these companies is not realised by outlets in the home country of the respective company but by their foreign subsidiaries. For example, *Metro*, the largest German food trade enterprise, has affiliates in 18 European countries. As a consequence of the national and international concentration processes only large suppliers are adequate partners for these food trade companies. Purchasing activities of these food trade giants are continually centralised which means that large quantities are needed for the great number of outlets. In the future, international sourcing will gain importance.

A second important development refers to changes in retailing food products. The typical supermarket lost market shares while large self-service department stores and low-price discount stores quickly gained importance. In addition, the establishment and penetration of private labels has become a preferred sales strategy. The fast growing introduction of private labels by the food trade consequently forced even leading suppliers of brands to produce private labels in spite of the risks associated with such activities. Fig. 3 illustrates the fast growth of discount stores in different European countries.

By far the highest percentage of discount stores we find in Germany, as shown in Fig. 4. Meanwhile more than 50% of dairy products are sold in stores of *Aldi, Lidl, Penny*, etc. This development is particularly important insofar as low prices represent important sales arguments of these discount stores. Intense price struggles are performed between some of the low-price

chains. Important for the dairy industry is the fact that these competitors attempt to realise their low price strategy primarily by low purchasing prices of the products supplied by the dairies.

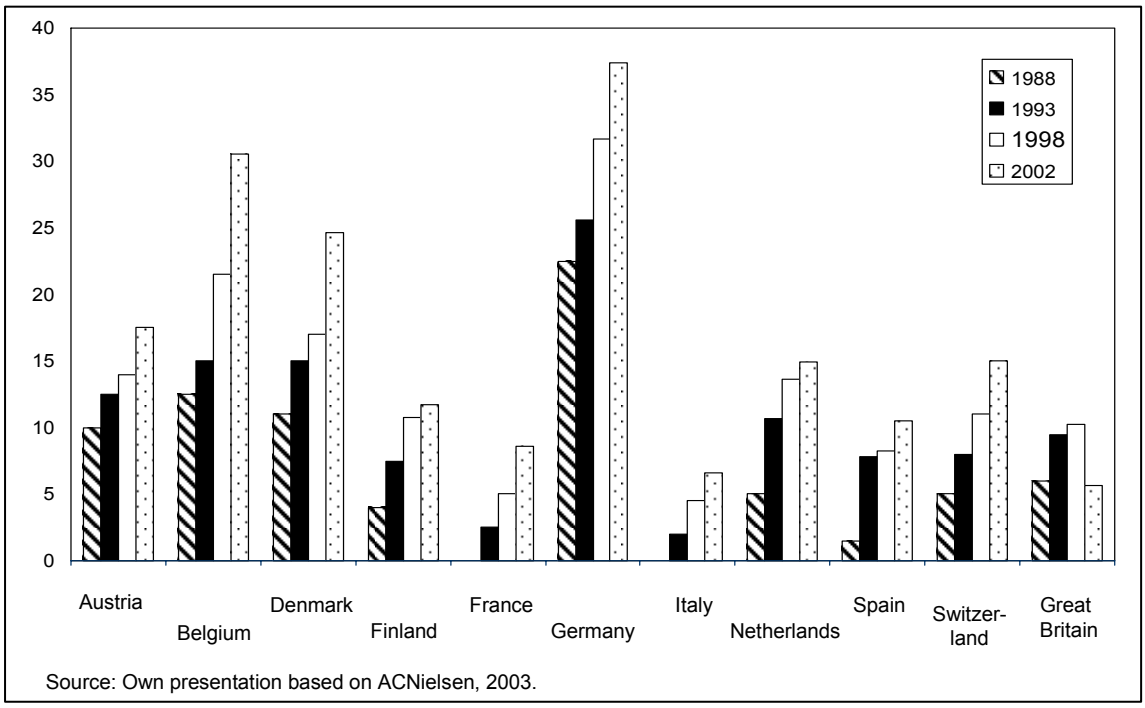

Figure 3. Development of market share of discount stores in Europe.
Slika 3. Razvoj tržnega deleža veletrgovin v Evropi.

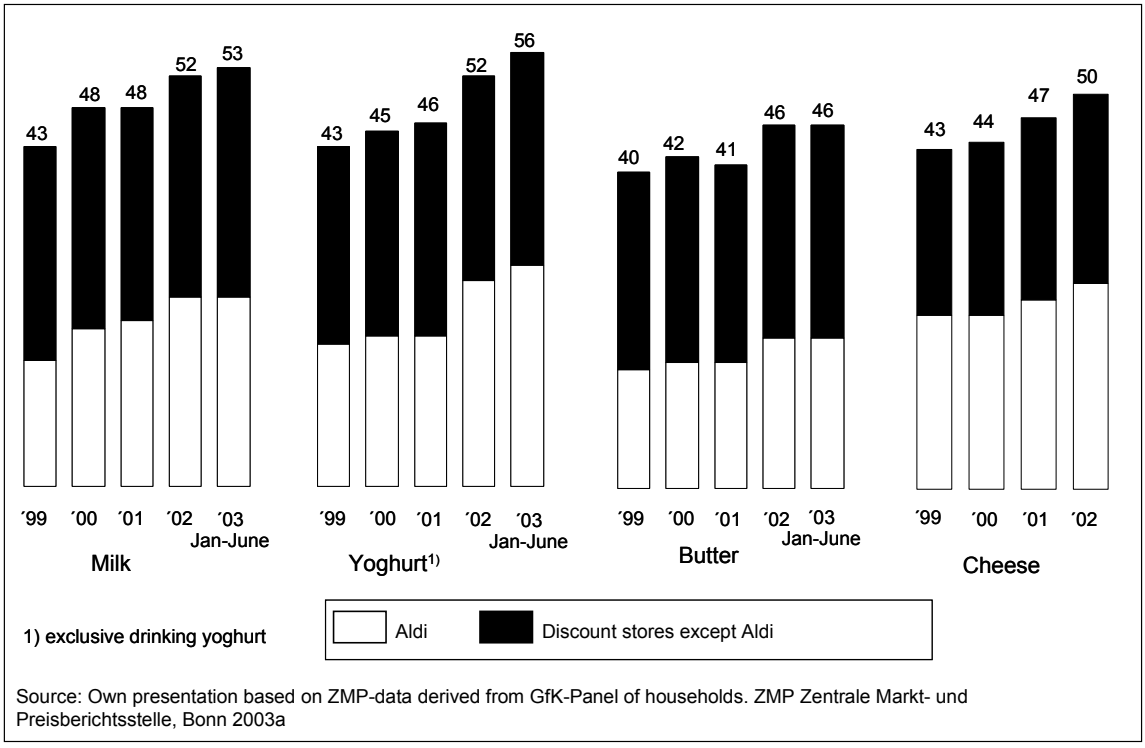

Figure 4. Percentage of dairy products sold in low price discount stores in Germany
Slika 4. Odstotek mlečnih izdelkov prodanih v veletrgovinah znizkimi cenami v Nemčiji

A third development is the ongoing reorganisation of the relationship between the food trade and the dairy industry. One aspect of this process is the establishment of ECR (Efficient Consumer Response) to optimise the supply chain. Dairy companies are asked to introduce such systems together with the food trade companies. Behind this development stands the demand for closer co-operation in the ranges product development, assortment optimisation, logistics and storage.

Another aspect is a reaction of the diverse food scandals in the 90ies: To improve the safety of the food products sold, the food trade companies nowadays demand from the suppliers the implementation of quality management standards. Food safety systems of the manufacturers, e.g. IFS or BRC, are more or less presupposed and aspects of food security are becoming increasingly important (Schiefer, 2003; Weindlmaier, 2003).

STRATEGIES OF THE DAIRY INDUSTRY TO IMPROVE COMPETITIVENESS UNDER THE GIVEN CONDITIONS

Structural change and concentration in the dairy industry

Past structural changes and concentration in the dairy industry on the one hand are an important issue for the present conditions of the dairy sector today. However, the growth of companies is on the other hand an important strategy to retain and improve competitiveness.

Past developments of the dairy structure

In general a strong decline in the number of companies and plants in the dairy industry in almost all countries of the Western World can be observed . Table 3 illustrates the structural change in the dairy industry of selected countries between 1983 and 1997 in terms of milk collection by dairy firms. During this period the number of companies decreased in all countries quite extensively, i.e. between 25% in Ireland and 52% in Germany. The right side of the table shows that at the same time the volume of milk collected increased by up to 156% in the United States. These data also illustrate that there are big differences concerning the size of dairies. While average milk collection in French companies has been 30.2 million litres only, in the Netherlands nearly 500 million litres have been collected per average company.

Table 3. Structural change in the dairy industry of selected countries
Preglednica 3. Strukturne spremembe v mlekarski industriji izbranih držav

	No. of fiems in milk collection			Average volume collected, mio. lit.		
	1983	1997	% Change	1983	1997	% Change
Germany	528	256	–52	57.3	106.0	85
France	1570	734	–50	15.5	30.2	95
Netherlands	42	21	–50	317.5	498.0	57
Ireland	51	39	–25	100.3	131.0	30
United States [1]	435	226	–48	100.0	256.0	156
Canada	250	171	–32	28.9	43.4	50

[1] = United States data refer to 1980 rather than 1983 and relate only co-operatives. Source: Pitts and Krijger, 2001.

Table 4 shows Europe's major milk processors in 2001/2002 in terms of milk purchases per year. The largest companies like *Arla Foods* and *Group Lactalis* process more than seven billion litres per year, that means more than 15 times the milk deliveries of 461 million litres to all dairy

companies in Slovenia in 2001 (ZMP, 2003). Out of the Top-15 companies about half are Co-op's and half are private dairy enterprises.

From Fig. 5 we can derive that the largest dairy producers worldwide are at the one hand multinational companies like *Nestlé*, *Danone*, *Kraft Foods* and *Unilever*. Several very large companies are operating in the United States, but also in some of the European countries, in which the concentration processes progressed very far. Examples are the Scandinavian countries like Denmark and Sweden with the largest European dairy company *Arla Foods* or the Netherlands with *Friesland Coberco* and *Campina*.

Table 4. Europe's major milk processors in 2001/2002*
Preglednica 4. Največji predelovalci mleka v Evropi v letih 2001/2002

Rank	Company	Co-op (C) or Private (P)	Country of origin	Milk purchases million lit./year
1	Arla Foods	C	Denmark/Sweden	7,200
2	Group Lactalis	P	France	7,000
3	Campina	C	Netherlands	5,750
4	Friesland Coberco	C	Netherlands	5,600
5	Nordmilch	C	Germany	4,200
6	Bongrain/CLE	P	France	4,100
7	Nestlé	P	Switzerland	2,800
8	Dairy Crest	P	UK	2,700
9	Humana Milchunion	C	Germany	2,460
10	Glanbia	C/P	Ireland	2,450
11	Danone	P	France	2,430
12	Sodiaal	C	France	2,300
13	Entremont	P	France	1,950
14	Müller	P	Germany	1,850
15	Laita Group	C	France	1,730

* = Source: Barry Wilson's Dairy Industry Newsletter Online, 2003.

Typical for the recent concentration processes is the fact that those are not only characterised by takeovers of small or medium sized companies by the large ones. In addition, mergers and takeovers of very big enterprises are at the agenda. Examples are the merger between *MD foods* and *Arla* in 2000 and the takeover of the English *Express Dairies* by *Arla Foods* and of the German dairy part of *Unilever* by the French giant *Bongrain* in 2003.

Another characteristic of recent concentration processes is the fact of transnational mergers, acquisitions, joint ventures, takeovers and strategic alliances. Recent examples of transnational alliances are the transnational strategic alliance between the French *Lactalis* and the Danish *Tholstrup Cheese* in 2001 and the alliance between *Nestlé* and the *New Zealand Dairy Group (Fonterra)* for the American market in 2002.

If these developments in concentration are analysed, the question about the future development of concentration arises. There are indeed no signs that the speed of these concentration processes have diminished in recent years. For 2000 to 2003 the Danish Dairy Board (2004) has listed 37 mergers, acquisitions, takeovers, joint ventures and alliances in the global dairy industry.

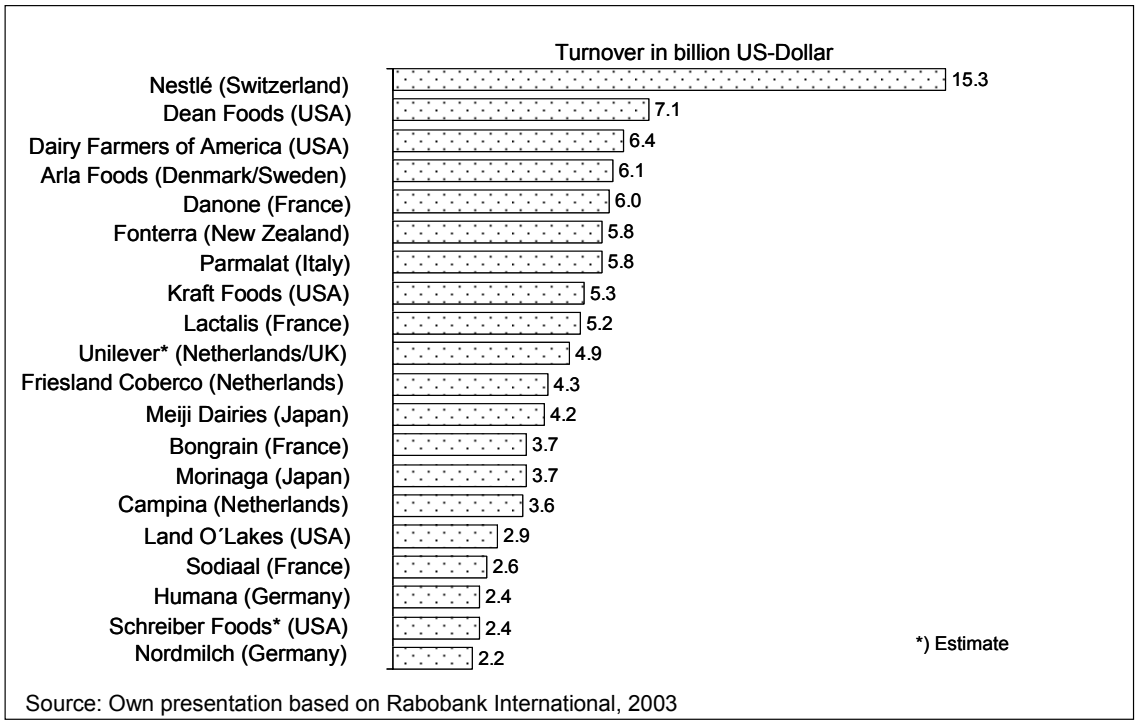

Figure 5. The Top-20 dairy processing companies in 2002/2003.
Slika 5. Vodilnih 20 mlečno predelovalnih podjetij v letih 2002/2003.

The driving forces of the concentration process/potential advantages of large enterprises and/or plants

In the management literature many driving forces, but also some limitations of the concentration process are mentioned and discussed (Schmidt, 1995, Weindlmaier 1999 and 2001).

A first important advantage of large enterprises is the chance to realise economies of scale, respectively lower average costs. These cost reductions can have several origins. A first reason is technical economies as for example large dairy companies can use expensive modern technology and equipment. Secondly, managerial economies can occur in the administration of a large firm by splitting up management jobs and employing specialist accountants, salesmen, IT specialists, etc. Thirdly, financial economies frequently are realised by borrowing money at lower rates of interest than smaller firms. Fourthly, marketing economies are very important nowadays. These are achieved by spreading the high costs of advertising on television and in national newspapers across a large level of output. In Western Europe, today for a national TV-campaign at least 10 million Euros are required if a significant effect and growing sales can be expected. Commercial economies can be made when buying supplies in bulk and therefore gaining a larger discount. As an example, price deductions when buying large quantities of packing material, processed fruits or bacteria cultures can be named. Last but not least, research and development economies are important when developing new and better products. Investigations show that only large enterprises are able to invest in technical equipment and to employ the necessary specialists for an efficient R&D. Furthermore, the high cost of introducing product innovations to the market (e.g. listing fees) presupposes a good financial basis of the company.

Fig. 6 shows as an example the long run cost curve for the production of UHT-milk (excluding raw-material costs). If only 10 million packages of UHT-milk are produced per year, costs of more than 20 Cent per package accrue. In case of a yearly production of more than 180 million packages, the relevant costs are only 14.5 Cent per package, equal to a cost reduction of

nearly 30 per cent. Of course, such a cost difference will be decisive for the competitiveness of a producer of UHT-milk.

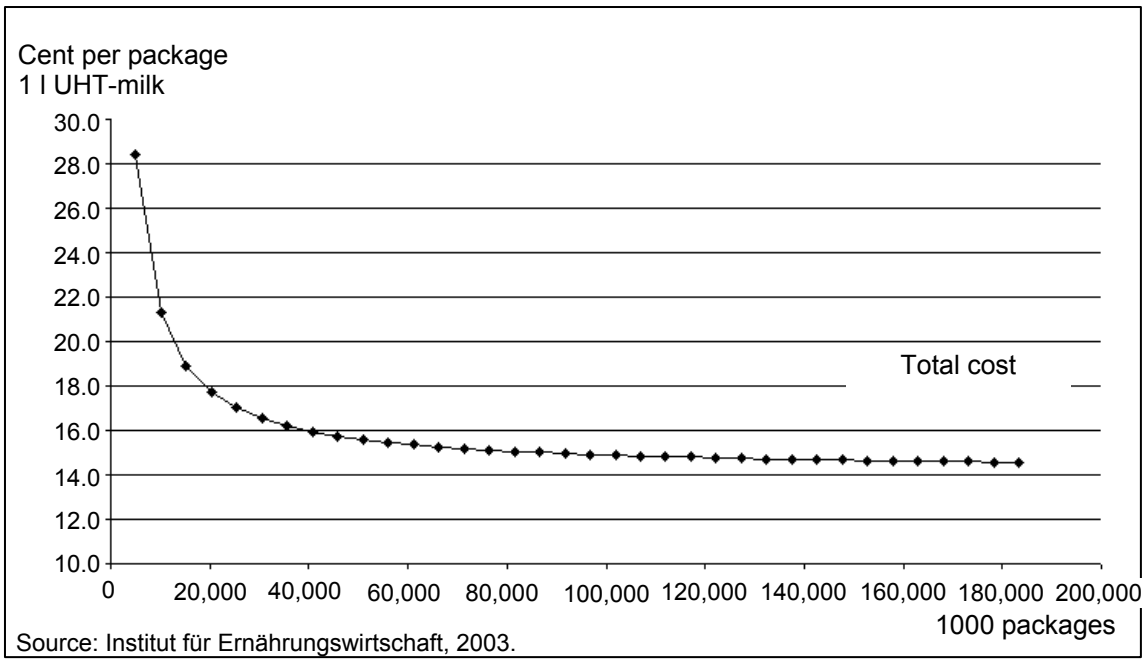

Figure 6. Average long run costs of the production of UHT-milk (except raw milk).
Slika 6. Povprečni stroški proizvodnje UHT mleka (razen surovega mleka).

In the 1960's, management consultants at The Boston Consulting Group observed a consistent relationship between the costs of production and the cumulative production quantity, which is called the effect of Experience Curves (Wikipeda, 2004). Data revealed that the value-added costs decline by 15 to 25 percent each time cumulative volume doubles. The Experience Curve suggests a possibility that systematic cost differences might arise between competitors because increased activity leads to increased learning, which leads to lower costs. The experience curve has important strategic implications**.** If an enterprise is able to gain market share over its competitors, it can develop a cost advantage. Penetration pricing strategies and a significant investment in advertising, sales personnel, production capacity, etc. can be justified to increase market share and gain a competitive advantage.

A further important driver of concentration is the advantage of large companies by realising long run strategies (Maucher, 1993). The point made is that only large enterprises have the opportunity to compensate the risks of different operations. An example is internationalisation of dairy companies, which frequently leads for several years to losses in the newly integrated markets. Only large companies are able to compensate these losses by the profits made in other countries.

Large, diversified dairy companies can more easily adjust their production program to the actual market situation and the development of actual profit margins. That this flexibility can be advantageous could be observed in 1998/99: The sharp tumble of the prices for butter and standard cheese in the EU mainly affected those specialised dairies which had no production alternatives.

A further very important driver for concentration of dairy companies is the high concentration and internationalisation of the food retail trade, which has been discussed before. Only large

dairy enterprises with a high production potential are able to deliver the large quantities of products required by these trade giants.

Limitations of structural changes/potential disadvantages of large enterprises and/or plants

Internal Diseconomies of Scale might be a consequence when the firm has become too large and inefficient. This might be due to the fact that management becomes out of touch with the shop floor and some machinery becomes over-staffed. Also, sometimes decisions are not taken quickly and there is too much form filling. Further more, lack of communication in a large firm is quite frequent, which means that management tasks sometimes get done twice. Finally, poor labour relations may develop in large companies.

External Diseconomies of Scale might occur because of the increasing collection area for milk with the consequence of rising transport costs. In model calculations we performed recently we investigated to what extent collection cost of milk would increase if very large dairy processing plants (with up to 7 billion litres per plant) would be established. An important outcome is that in case of cost efficient milk collection (e.g. collection around the clock, collection per farm only every second day, collection based on route planning) this increase in collection costs is to a high percentage offset by the decrease in processing costs in larger processing plants (Weindlmaier and Huber, 2003).

An important further limitation is psychological and emotional resistance of the full-time and honorary management (e.g. in dairy co-operatives) against the concentration process. It has its origin in the fact that one frequent and necessary consequence of a merger is the reduction of management positions. Therefore, the persons taking the decisions for merger quite often eliminate their own position in the enterprise.

Closely connected with this argument is the fact that the potential advantages of concentration can be realised only if consequently major internal adjustments are performed. These include the shutdown of plants and possibly investments in new, modern processing facilities, the reduction of personnel leading to high expenses for compensation payments, etc. Generally, nowadays the financing of such processes becomes an increasing obstacle.

Summarizing and evaluating these different driving forces and limitations, it seems that the advantages outperform the limitations. Therefore, further growth of dairy plants and enterprises is very likely. In the future, dairy plants of several billion litres per plant are a realistic vision.

Porter's Generic Competitive Strategies

In his famous book "Competitive Strategy" MICHAEL PORTER of the Harvard Business School has argued that a firm's strengths ultimately fall into one of two headings: cost advantage and differentiation (Porter, 1998a; QuickMBA, 2003). By applying these strengths in either a broad or narrow scope, three generic strategies result: cost leadership, differentiation and the focus strategy. These strategies present an ideal vehicle to discuss the options dairy companies have to stabilise respectively increase competitiveness.

Cost leadership strategy

The cost leadership strategy focuses for being the low cost producer for a given level of quality. There are several prerequisites to carry out such a strategy. A cost leader must focus on having a high market share in the respective market and improving process efficiencies by increasing the size of operations and by optimal outsourcing of non core activities. Furthermore, the access to materials and production factors at low costs is important: In the case of the dairy industry cost components of specific importance are low raw milk costs and low costs for packaging materials. A strict cost management and the avoidance of small, marginal customers

are also decisive. In addition, cost leaders tend to minimise the costs in the areas R&D, service, promotion, etc.

If we take into account the changed conditions of the European dairy industry, the importance of the strategy of cost leadership increased remarkably in recent years. It is expected that in the future the production of standard dairy products, of private labels and products for the discount stores will be a domain of large companies able to perform cost leadership for the respective product group. In a recent strategy plan for the Irish Dairy Processing Sector for instance one of the main proposals has been to reduce the number of plants for the production of butter, powder and casein from eleven to four plants to improve cost efficiency (Prospectus and promar International, 2003).

Because of increasing competition even producers of value added products, of brands and specialities nowadays must focus on cost management. The developments on the markets emphasize that in the future the expectable price premium for brands and specialities will allow rather small extra costs only.

Differentiation strategy

A differentiation strategy calls for the development of products that offer unique attributes. Customers perceive that the products offer features that are better than or different from the products of the competitors. The value added by the uniqueness of the products may allow the company to charge a premium price for it. An economic prerequisite is that the price differences between conventional and premium products exceed the relevant cost differences.

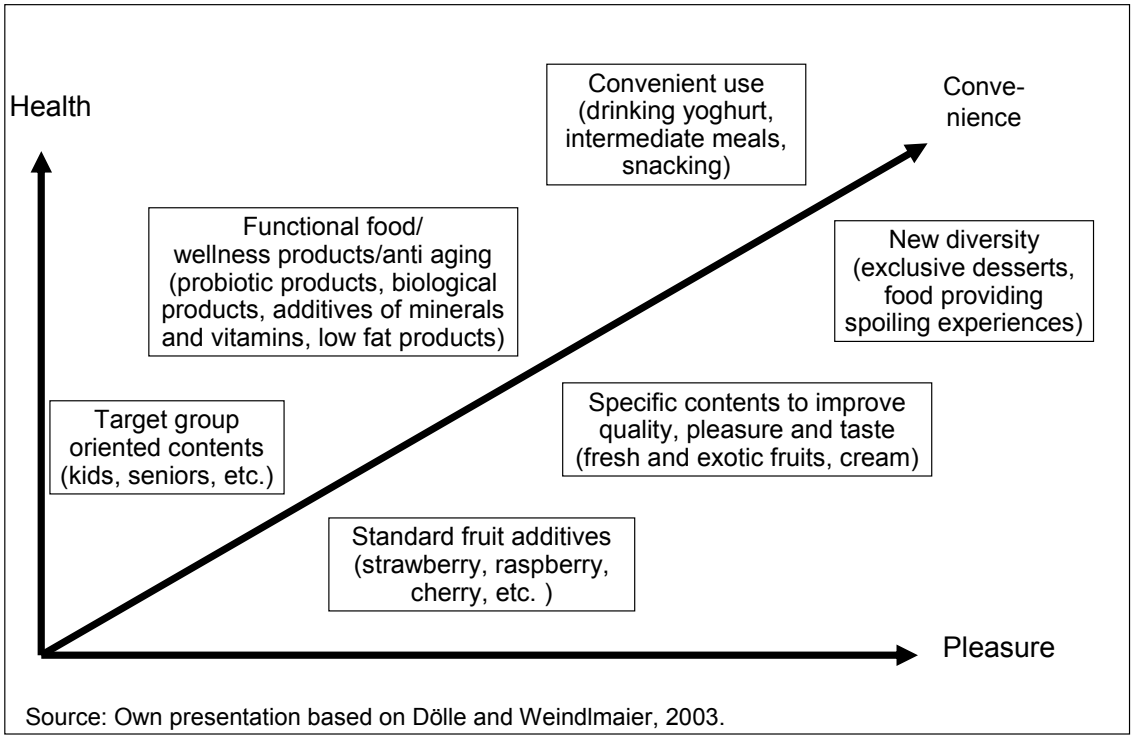

Figure 7. Actual areas for product innovations in the dairy sector.
Slika 7. Aktualna področja inovacij proizvodov v mlekarstvu.

Looking at dairy products, there are several possibilities to offer additional benefits. Important examples are the differentiation of the basic characteristics of the products itself, for instance by use of specific recipes or ripening cultures, by utilising specific raw materials and ingredients or

by employing special processing techniques, allowing a longer shelf life. Fig. 7 illustrates some areas for product innovation in the dairy sector. Actually, there are three main trends in consumer behaviour to be considered if new products are developed: Product innovations have to be healthy and wellness oriented, they are of high quality and incorporate the promise of pleasure and enjoyment by eating them, and last but not least, one of their features is convenience.

However, table 5 demonstrates that consumer attitudes differ extremely between countries. According to a recent survey, convenience for instance is very important in UK, while in France and Italy this characteristic of innovations is less significant. The health aspect is a very important characteristic of new products in Italy, Spain and UK, but it is less essential in France and Germany.

Table 5. Variations in consumer behaviour in different EU-countries
Preglednica 5. Razlike v obnašanju potrošnikov v različnih državah ES

6 FOOD LOGICS people %	% 12501	FRANCE 2452 20	GERMANY 3295 26	ITALY 2560 20	SPAIN 1696 14	U.K. 2497 20
HEALTH	44	39	40	48	47	46
QUALITY	17	20	11	22	18	14
CULTURE	26	36	24	28	26	18
CONVENIENCE	24	35	43	39	44	61
INDULGENCE	13	11	13	14	9	16
CONTINUITY	45	47	48	48	43	37
Index calculated upon European average		High over-representation				Index > 120
		Significant over-representation				105 > Index < 120
		Close to European average				90 > Index < 105
		Significant under-representation				Index < 90

* = Own presentation based on Hasson and Dofour, 2002

A further fundamental approach to reach uniqueness is branding. Brands deliver key signals to the consumers and simplify the buying process. They allow to equip the product with a "unique selling proposition" and to differentiate it from the many varieties of dairy products being more or less exchangeable. However, to develop such a USP nowadays, functional properties of the product have to be supplemented by emotional properties and extensive advertisement.

A third important prerequisite of differentiation are widespread promotional activities. Besides classical advertisement by TV, radio, print and posters, "below-the-Line" promotion by sponsoring, event marketing and marketing via internet is of growing importance, also for dairy brands.

The basic problem associated with a differentiation policy is the fact that product innovation, branding and promotion are very costly. The big players in the dairy sector like *Nestlé* and *Danone* have huge yearly budgets for R&D and promotion. For example, the average yearly

budget for R&D of *Nestlé* during the years 2000–2002 has been more than 770 million Euros. Furthermore, because of the described developments in the food retail trade and its introduction of own labels, the conditions for a branding policy by the dairy industry have deteriorated.

Consequently, a differentiation strategy is for sure a very important option for the large, concentrated enterprises in the dairy sector. The risks associated with it include the growing speed of imitation by competitors and the fast changes in customer tastes leading to short life cycles of the products. For the many small and medium sized companies a differentiation strategy offers only limited or no perspectives. For this group of enterprises, the next strategy discussed, the focus strategy, might achieve better results.

Focus strategy

The focus strategy concentrates on market niches and within those it attempts to achieve either a cost advantage or differentiation. A firm using a focus strategy often enjoys a high degree of customer loyalty, and this entrenched loyalty discourages other firms from competing directly.

A focus strategy might be successful, if it succeeds to offer suitable products focusing on the special needs of the group served. Examples are organic dairy products and special regional cheese varieties. Such cheese varieties are particularly frequently offered in the Southern member countries of the EU, like in France, in Spain and in Italy.

In addition, regional brands of dairy products, based on traditional production processes with a high portion of manual work or environmental advantages in the production region might form the basis for a focus strategy. Such regional brands benefit from the recently intensified preference for products from the home region, having the advantage of special freshness and short distribution distances. An example is the market for dairy products in Austria after the accession to the EU in 1995. The Austrian dairy sector has managed it to a large extent to convince the Austrian consumers that Austrian dairy products are better because of the sound environmental conditions under which Austrian milk production is carried out. Fig. 8 shows that dairy products made in Austria still have a high market share in their home country in spite of the fact that they are frequently sold at higher prices than imported products.

This leads to an important requirement of a focus strategy: Because of their narrow market focus, firms pursuing a focus strategy normally have lower volumes and therefore higher costs. Hence, they must be able to charge higher prices for their products. Taking into account the present market conditions and the increasing competition on the dairy markets, this becomes increasingly difficult. An example is the market for biological dairy products: Recently, we can observe a growing discrepancy between the high costs of production and distribution and the prices accepted by consumers.

In spite of these drawbacks, for a limited number of SME's a focus strategy might even in the long run guarantee the survival in competition to large enterprises. Still, this only will happen if the specific advantages forming the focus strategy can be preserved and if the price differences to conventional dairy products cover the additional cost (Burchardi and Thiele, 2003).

Internationalisation

There is a widespread view that an intensified internationalisation of the dairy industry is among the most important strategies to improve competitiveness. A first important argument in favour of this strategy is the growing internationalisation and globalisation of the markets for dairy products: Even companies, which in the past mainly sold their products regionally or nationally, are confronted with a growing number of competitors in their market area.

Further important drivers are the preferences of European consumers, favouring a broad assortment of products from different countries and the already mentioned concentration and

internationalisation of the European food retail trade. Last but not least the excess supply of dairy products in most of the European countries has to be considered. In spite of the European quota system it is a fact that the degree of self sufficiency for dairy products in the EU is about 108 per cent. Fig. 9 shows that in some of the European countries like Ireland, the Netherlands and Denmark high percentages of excess supply exist which is seeking for markets in other countries.

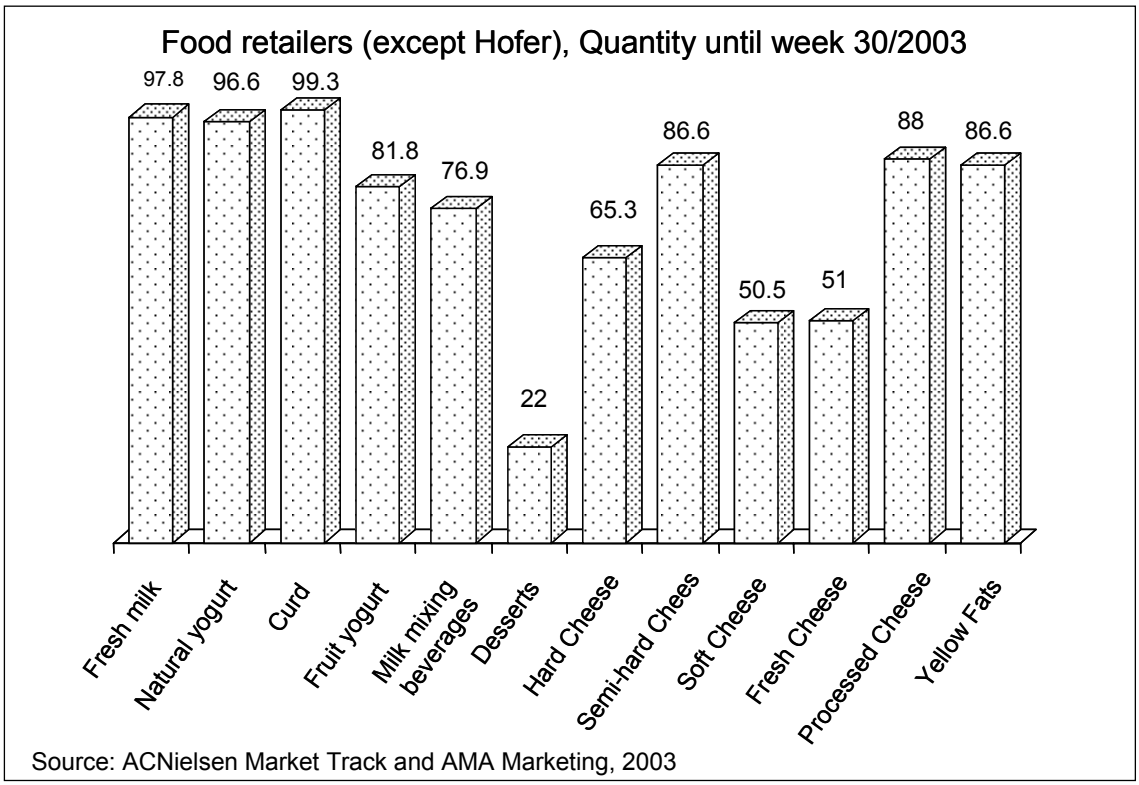

Figure 8. Market shares of Austrian dairy products sold at the Austrian Market.
Slika 8. Tržni delež avstrijskih mlečnih izdelkov, prodanih na avstrijskem trgu.

The necessity of export exists even in countries in witch domestic supply and demand is more or less equal. In Germany, the degree of self sufficiency for dairy products amounts to only 102%. However, there is a remarkable excess supply as high imports add to the production of German enterprises. In 2002 Germany imported 444,600 tonnes of cheese and 137,700 tonnes of butter. As a consequence, the German dairy enterprises are forced to export growing quantities to countries within and outside the EU.

Furthermore, internationalisation is quite often necessary to allow further growth and specialisation of dairy enterprises. For dairy companies specialising their production aiming at the realisation of economies of scale, the homeland market is frequently not large enough. In addition, foreign markets, for example the markets of the CEEC-countries or of Russia, often are characterised by much higher growth rates.

Last but not least: The Western European countries suffer high costs of raw milk, of labour, of energy, etc. Dairy products which are calculated based on these costs offer opportunities for exporting only in case of specialities and premium products. If a dairy company would like to be present with basic products also in low price countries, foreign direct investments are indispensable. Foreign direct investments will be a big challenge for many European dairy

companies in the future. This is especially true for dairy co-operatives which have severe deficits in this connection.

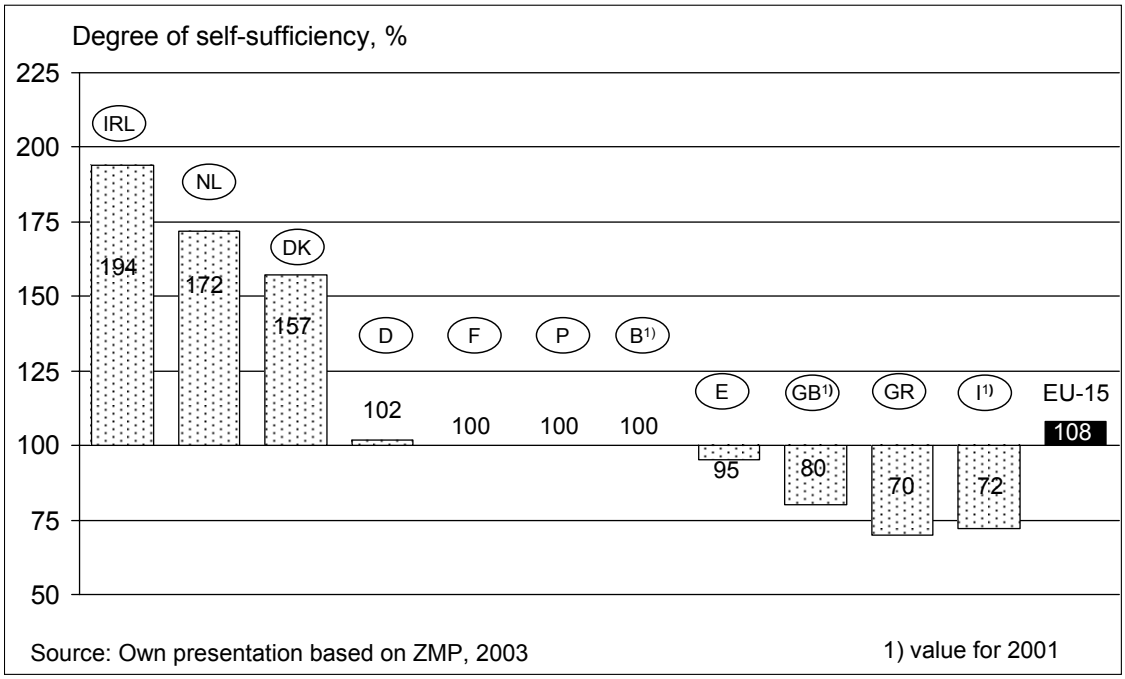

Figure 9. Degree of self-sufficiency for dairy products in European countries in 2002.
Slika 9. Stopnja samooskrbe z mlečnimi izdelki v evropskih državah v letu 2002.

To optimise the internationalisation strategy, many details like the selection of the optimal markets, the market entry strategy, the optimal time for the market entrance and the selection of suitable marketing measures have to be clarified.

CONCLUDING REMARKS

The economic environment of the European dairy sector is changing with high speed and intensity. The decisions of the Mid-Term-Review of the Common Agricultural Policy, the enlargement of the European Union to include the countries of Central and Eastern Europe and the concentration and globalisation of the food trade are the most important current influences. The whole sector, milk producers and dairy enterprises, is confronted with great challenges to retain respectively improve competitiveness. Structural changes towards larger units will continue or even accelerate in dairy farming as well as in dairy processing.

In order to strengthen competitiveness dairy processing enterprises have to seriously reconsider their business strategies. Of specific importance is the further growth of enterprises and processing plants. The other strategies discussed in this contribution are options which can be realised, but they are no guarantee for success. Success can be expected only if on the basis of appropriate external and internal conditions the management of the enterprise selects the correct strategy and implements it with high knowledge, continuity and conviction.

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Tomaž BARTOL [a)]

[a)] Univ. of Ljubljana, Biotechnical Fac., Agronomy Dept., Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia, Ass.Prof., Ph.D., M.Sc., e-mail: tomaz.bartol@bf.uni-lj.si.

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Nataša SIARD [a)]

[a)] Univ. of Ljubljana, Biotechnical Fac., Zootechnical Dept., Groblje 3, SI-1230 Domžale, Slovenia, Ph.D., M.Sc., e-mail: natasa.siard@bfro.uni-lj.si.

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Hvelplund, T. Protein evaluation of treated straws. V: Evaluation of straws in ruminant feeding (ur.: Chenost, M./ Reiniger, A.). London, Elsevier Applied Science, 1989, 66–74.
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Fraser, A.F./ Broom, D.M. Farm animal behaviour and welfare. London, Bailliere Tindall, 1990, 437 p.

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