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# Effect of prolonged storage on microbiological quality of raw milk

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**Summary**

The changing of milk microbiota composition in collection tank due to two-day collection was investigated. Each out of 10 independent cycles of every two-day collection was sampled 4 times and microbiologically examined. PCA medium supplemented with skim milk powder was used to determine the total counts of mesophilic aerobic bacteria, proteolytic bacteria, psychrotrophic and proteolytic psychrotrophic bacteria, respectively. MRS and M17 media were used to determine the counts of lactobacilli and cocci (lactococci and enterococci), respectively. As expected the counts of microbial groups increased but particularly the ratio between the groups of microorganisms was changed. The most evident shift in composition of milk microbiota was detected for psychrotrophic, particularly proteolytic psychrotrophic bacteria that are of particular concern to the dairy industry. Proteolytic psychrotrophs are notorious for their proteolytic activities which adversely influence milk proteins. Moreover, secreted proteolytic enzymes cannot be destroyed by heat treatment processes that are normally used in milk processing. The counts of microbial groups sharply increased on the second day of two-day collection. From selected bacterial consortia, the presence of the individual group of microbes was also PCR examined. Results indicate that the microbiological quality of milk in a two-day collection system is remarkably lower in comparison with daily collection system.

*Key words:* mesophilic aerobic bacteria, psychrotrophic bacteria, proteolytic bacteria, PCR identification, two-day collection system

## Introduction

Milk in its natural state is a highly perishable material because it is susceptible to rapid spoilage by the action of naturally occurring enzymes and contaminating microbes. Good hygiene practice from the farm to dairy, effective refrigeration regimes, reduction of storage times and technologies for reduction of spoilage and pathogenic microorganisms are basic measures needed to be accomplished for preserving good quality of raw milk (Sorhaug and Stepaniak, 1997).

Raw milk microbiota is composed of technologically important microbes, such as lactic acid bacteria (LAB) that are of great importance if the milk is processed into cheese, but spoilage and even pathogenic bacteria can also be present. Introduction of cold storage of milk on farms resulted in microbial shift from mesophilic aerobic bacteria towards psychrotrophic microorganisms. Latter are one of the most unwanted milk spoilage bacteria, which adverse affect on milk and milk products is attributed to their ability of producing heat resistant proteolytic and lipolytic enzymes at refrigerating temperatures (Cousin, 1982). Moreover facing the economic crisis, where milk market is of no exception, new solutions for costs lowering are searched for. One of the possible measures is passing from daily collection of milk to two-day collection of milk. The combination of prolonged storage of milk

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at low temperatures even more favours the drastic increase of psychrotrophic bacteria, predominantly *Pseudomonas* spp.

The rate of microbial contamination with mesophilic and psychrotrophic bacteria of cow's raw milk is influenced by health status and hygiene of dairy cows, hygiene of the environment in which dairy cows are housed and milked, methods for udder preparation and milking techniques, methods used for the cleaning and disinfection of milking machines and milk tanks, hygiene of attendant staff (Cempirkova, 2007). Other two important factors influencing microbial status of raw milk are rapid cooling and storage time.

Rapid cooling and refrigerated storage of raw milk has favoured the growth of psychrotrophic bacteria. Nonspore forming psychrotrophic bacteria, particularly *Pseudomonas* spp. are killed by high-temperature-short-time pasteurization. *Pseudomonas* spp. would need to grow to relatively high number ($1x10^6$ cfu/mL) in raw milk before pasteurization to produce an off-flavour directly. But bigger problem than high bacterial counts of *Pseudomonas* spp. is their production of heat stable proteases and lipases that may produce off-flavours later during the shelf life of pasteurized milk (Barbano et al., 2006). When growing in aerated milk at 4 °C many strains of *Pseudomonas* spp. can produce sufficient proteinases to hydrolyze all of the available casein into soluble peptides (Sorhaug and Stepaniak, 1991). The adverse effect on the quality of dairy products due to the growth of psychrotrophs in raw milk before heat treatment is presented in Table 1, adopted by Sorhaug and Stepaniak (1997).

Since bacterial spoilage still causes significant losses for the food industry there is constant need for monitoring the microbiological quality of raw material such as the presence of particular members inside the groups of organisms with potentially spoilage activities. Complex raw milk and raw milk cheeses microbiota make effective prevention of spoilage difficult. Although many reports describe microbial diversity in raw milk, only few are applying reliable molecular methods for bacterial identification. Some of the latest reports based on molecular techniques describe identification of psychrotrophic isolates from raw milk by using 16S rRNA gene sequencing (Hantsis-Zacharov and Halpern, 2007), RAPD-PCR and 16S rRNA sequencing (Ercolini et al., 2009) and PCR method based on *apr*X gene (Marchand et al., 2009).

Table 1. Effect on the quality of dairy products due to the growth of psychrotrophs in raw milk before heat treatment

| Product type | Log cfu/mL psychrotrophs in raw milk | Effect on quality |
|---|---|---|
| UHT milk | 5.9 | Gelation not earlier than after 20 weeks |
| | 6.9-7.2 | Gelation after 2-10 weeks; gradual development of lack of freshness; slightly stale, unclean, bitter flavour |
| Milk powder, freeze dried milk | 6.3-7.0 | Reduced heat stability; increased foaming capacity of reconstituted milks |
| Pasteurised milk | 5.5 | Inferior flavour compared with that of pasteurized milk produced from fresh milk |
| | 7.8 | Shorter shelf life; increased fouling in heat exchanger |
| Hard cheeses | 6.5-7.5 | Rancidity |
| | 7.5-8.3 | Different flavour defects, predominantly rancidity and soapy taste; reduced cheese yield |
| Cottage cheese | 5-7.8 | Significant correlation between psychrotrophic counts in raw milk and bitter taste |
| Butter | ND | Faster development of rancidity in butter made from cold-stored milk than in that made from fresh milk; lipase from *Pseudomonas* was active in frozen butter |
| Yoghurt | 7.6-7.8 | Bitter, unclean or fruity flavours, depending on the specific microflora |

In Slovenia, it is estimated that two-day collection of milk evidently increases the counts of psychrotrophs. The aim of the present study was to evaluate the dynamics of culturable psychrotrophs and accompanying microbiota in raw milk through 10 consecutive cycles of two-day collection of milk in village milk collection facility by the use of classical plating methods and PCR molecular method.

## Materials and methods

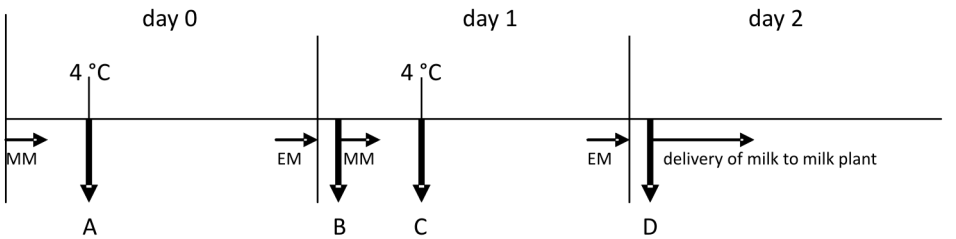
### *Milk collection and milk sampling*

The model of research was a village milk collection facility in the north-eastern part of Slovenia where 6 local farmers bring their milk. In collection tank, milk was cooled down to 4 °C and on every second day transported to the dairy plant. The milk collection regime of one cycle of every second day collection was performed as follows:

day 0: 7.45-8.00: collection of morning milk
20.00-20.15: collection of evening milk
day 1: 7.45-8.00: collection of morning milk
20.00-20.15: collection of evening milk
day 2: 5.30: delivery of collected milk by milk tanker into the dairy plant and cleaning of the collection tank

During milk collection the temperature of milk as well as the ambient temperature of milk collection facility was measured. Three independent sensors, submerged into milk, measured the temperature of milk while the fourth sensor measured the ambient temperature of milk collection facility. The values of measured temperatures were recorded every 5 minutes with the accuracy of ±0.4 °C.

This study started on July 15th and ended on August 8th, 2009. In this period, milk of ten independent cycles of two-day collection was analyzed. Milk from each cycle was aseptically sampled 4 times according to the following regime:




MM = morning milk
EM = evening milk
sample A: sampled immediately after morning milk on the day 0 in cooling tank (immediately after milk in cooling time was cooled down to 4 °C ) reached desired temperature of 4 °C
sample B: sampled just before addition of the morning milk on day 1
sample C: sampled immediately after milk collected from 3 milking in cooling tank reached desired temperature of 4 °C (day 1)
sample D: sampled just before delivery of collected milk to milk plant (day 2)

Milk samples were kept on ice and transported to the laboratory as quick s possible for further analyses.

### *Microbiological analyses*

Milk sampling resulted in total of 40 milk samples that were further subjected to microbiological analyses (IDF 100B, 1991). Briefly, one milliliter of each milk sample was mixed with 9 mL of quarter strength Ringer solution (Merck, Darmstadt, Germany) and serially diluted. One milliliter aliquots of appropriate dilutions were analyses by the use of media as follows: PCA (plate count agar, Merck) supplemented with 1 % skim milk powder (Merck) was used to determine the total counts of mesophilic aerobic bacteria, proteolytic, psychrotrophic and psychrotrophic proteolytic bacteria. Lactobacilli and cocci (lactococci/enterococci) manipulations were performed with MRS and M17 agar, respectively, according to the producer instructions (Merck). Incubation

Table 2. Incubation regimes for individual group of microorganisms

| Group of bacteria | Temperature °C | Time day | Oxygen requirement | Media |
|---|---|---|---|---|
| Mesophilic aerobic Proteolytic | 30 | 2 | aerobic | PCA with skim milk powder |
| Psychrotrophic Psychrotrophic proteolytic | 7 | 7 | aerobic | PCA with skim milk powder |
| Lactobacilli | 37 | 2 | aerobic | MRS |
| Lactococci/enterococci | 37 | 2 | aerobic | M17 |

regimes are listed in Table 2. After visible growth, the counts of individual microbial groups were expressed as cfu/ml of milk.

*DNA extraction and PCR manipulation*

The DNA from bacterial consortia from milk samples grown on different media was examined to establish the presence of the individual group of microbes. The protocols for microbial consortia preparation and DNA extraction used were as previously described by Trmčić et al. (2008). Briefly, for DNA extraction from consortia of strains, approximately 300 colonies were rinsed from the surface of PCA, MRS, and M17 agar plates with 2 mL of quarter strength Ringer solution. Further, DNA was isolated from 1 mL of each suspension using the Maxwell 16 Cell DNA Purification Kit and Maxwell 16 System (Promega, Madison, WI), and finally resuspended in 300 $\mu$L of elution buffer.

PCR primers for presence detection of bacterial groups are listed in Table 3.

Reference strains used as positive controls in PCR reactions in presented study are outlined in Table 4. Total DNA from reference strains was extracted by the use of Wizard® Genomic DNA Purification Kit (Promega).

A typical PCR reaction mixture (20 $\mu$L) for lactobacilli, lactococci, pseudomonas and *Str. thermophilus* contained 4 $\mu$L of 5X Green GoTaq reaction buffer (with $MgCl_2$ at 7.5 mM; final conc. 1.5 mM; Promega), 0.16 $\mu$L of 25 mM dNTP master mix (final conc. 0.2 mM of each dNTP; Fermentas, Germany) and 1 $\mu$M of each primer (Invitro-

Table 3. Genus/species specific primers, used for detection of bacterial groups

| Genus/species | PCR primer | target | Length of amplicon | Reference |
|---|---|---|---|---|
| *Lactobacillus* spp. | LbLMA1-rev<br>R16-1 | 16S/23S rRNA<br>16S rRNA | 250 bp | Dubernet et al. (2002) |
| *Enterococcus* spp. | E1<br>E2 | 632-646*<br>1353-1369* | 737 bp | Deasy et al. (2000) |
| *Lactococcus lactis* | 27f<br>Lla | 8-27*<br>72-91* | 87 bp | Barakat et al. (2000) |
| Proteolytic psychrotrophs (*Pseudomonas*) | SM2F<br>SM3R | *aprX* gene | 800 bp | Marchand et al. (2009) |
| *Streptococcus thermophilus* | Th I<br>Th II | 16S rRNA<br>23S rRNA | 259 bp | Tilsala-Timisjärviand Alatossava (1997) |

* E. coli numbering system

Table 4. Reference strain, used in this study

| Genus/species | Reference strain |
|---|---|
| *Lactobacillus* | *Lb. paracasei* DSMZ 5622 |
| *Enterococcus* | *E. faecalis* F4 IM 270 |
| *Lactococcus lactis* | *Lc. lactis ssp. lactis* DSMZ 20481$^T$ |
| Proteolytic psychrotrophs (*Pseudomonas*) | *P. fluorescens* LMG 1794$^T$ |
| *Streptococcus thermophilus* | *Str. thermophilus* CCM 7711 |

DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany
IM: Chair of Dairy Science, University of Ljubljana, Domžale, Slovenia
LMG: Laboratorium voor Microbiologie, Universiteit Gent, Belgium
CCM: Czech Collection of Microorganisms, Brno, Czech Republic

gen, USA). Two microliters of DNA (extracted from consortia or reference strains) was found to be sufficient for each reaction. Finally, 0.1 $\mu$L of 5U/$\mu$L GoTaq® DNA polymerase (final conc. 1.25 U; Promega) was added. The DNA for enterococci was amplified in a volume of 50 $\mu$L containing 10 $\mu$L 5X Green GoTaq reaction buffer (without $MgCl_2$) and 10 $\mu$L 25 mM $MgCl_2$ (final conc. 5 mM). All other components were added up to same final concentrations.

Amplifications were performed in thermal cycler (Mastercycler Gradient, Eppendorf, Hamburg, Germany) under the conditions previously reported by Dubernet et al. (2002), Deasy et al. (2000), Barakat et al. (2000), Marchand et al. (2009) and Tilsala-Timisjärvi and Alatossava, (1997). Ten microliters of the PCR product was electrophoresed on a 1.8 % (w/v) agarose gel using 1X TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.2). Gels were stained with Sybr Safe™ DNA Gel Stain (10 $\mu$L/ 100 ml; Invitrogen) and visualised under UV light.

## Results and discussion

Milk collection has been performed fluently according to the schedule previously described. The amount of delivered milk varied from 3 up to 76 liters per milking per farmer, with average of 32 liters. The temperature of delivered milk varied within the range of 26.3 up to 35.9 °C (average 32.3 °C) indicating that milk was not cooled prior delivery. At every single delivery all 6 farmers together averagely collected 180 liters of milk which resulted in total of 680 up to 750 liters of milk during two-day collec-

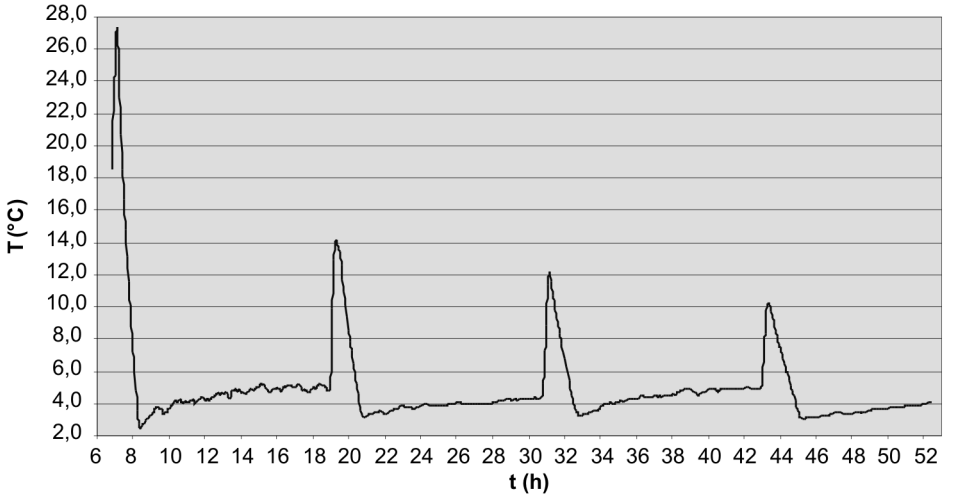



Figure 1. Average oscillation of milk temperatures in collection tank during two-day collection

tion of milk (4 milking). Each refueling of milk from new milking rose the temperature of milk in collection tank above desired 4 °C. After, collection tank cooled the total quantity of milk down to desired 4 °C which took a certain time, which was dependent on the quantity of milk in the tank, increased temperature of milk and ambient temperature. Collection tank has satisfactorily performed its task, since despite the high outdoor temperatures (up to 33.7 °C) and high ambient temperatures (up to 44.4 °C) cooled the milk below 4 °C within two hours at most. However, as shown in Figure 1, after each delivery into collection tank, for certain period of time, milk was exposed to temperatures higher than desired (4 °C), which has facilitated the development of micro-organisms, especially psychrotrophs.

Total counts of mesophilic aerobic bacteria were determined at 30 °C on PCA medium supplemented with skim milk powder, so that proteolytic microbes could have been counted as well. At first total counts of mesophilic aerobes were determined only in samples A, but in the last three cycles of two-day collection of milk total counts of mesophilic aerobes were determined in samples B, C and D as well. Results of total counts of mesophilic aerobic bacteria are presented in Table 5.

Mesophilic areobic bacteria analyzed in samples A reached counts from 164.000 cfu/mL up to 580.000 cfu/mL indicating diverse initial contamination of milk. Therefore, with average population of 270.000 cfu of mesophilic aerobic bacteria /mL of milk a high contamination of milk according to the requirements of Pravilnik o veterinarsko-sanitarnem nadzoru (1999) was concluded. However, a high count of mesophilic aerobic bacteria in itself is not problematic. Of the last three cycles (8, 9 and 10), beside sample A we also analysed samples B, C and D in order to determine changes in the number of microorganisms during the two-day collection of milk. A slight trend of increase of the total mesophilic aerobic bacteria in all samples of the last three cycles of milk collection has been detected. In the research performed in 2006, Godič Torkar and Golc Teger (2008) investigated the microbiological quality of 203 raw milk samples. They reported 32.000 cfu/mL of mesophilic aerobes in pooled raw milk samples which is lower compared to these results of 270.000 cfu/mL indicating almost 10 times heavier initial contamination of milk. But the high total counts of mesophilic aerobes solely cannot be sufficient information on microbiological quality of raw milk. More important is the actual composition of raw milk microbiota. When lactic acid bacteria are predominant population of raw milk microbiota this represents no major threat for production of cheeses and other fermented dairy product, but the technological problems appear when the shift of microbial composition turns towards psychrotrophic bacteria. From the analysis of samples B, C and D of last three cycles the slight increase of factor 1.4 in mesophilic aerobic counts due to two-day collection of milk was noted.

Simultaneously with counting of mesophilic aerobic bacteria in samples A the counts of proteolytic bacteria were recorded as well. Results are summarized in Table 6.

In average, counts of proteolytic bacteria represented 10 % of total mesophilic aerobic counts. Identification of proteolytic bacteria from agar plates was occasionally rendered difficult due to the size of halos of proteolysis and their covering over.

Identification of psychrotrophic bacteria was performed at 7 °C by the use of PCA medium supplemented with skim milk powder, which enabled

Table 5. Results of total counts of mesophilic aerobic bacteria in samples A through 10 cycles of two-day collection of milk

| Cycle | x10$^3$ cfu/mL | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| A | 245 | 234 | 263 | 580 | 220 | 264 | 277 | 249 | 164 | 205 |
| B | | | | | | | | 248 | 239 | 211 |
| C | | | | | | | | / | 246 | 224 |
| D | | | | | | | | 340 | 261 | 314 |

/ no result due to media contamination

analysis was not performed

Table 6. Counts of proteolytic bacteria in samples A through 10 cycles of two-day collection of milk

| Cycle / Sample | x10³ cfu/mL | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| A | / | / | / | 23 | 26 | 38.5 | 33 | 32 | 19 | 22.5 |

/ no result due to bacterial overgrowth

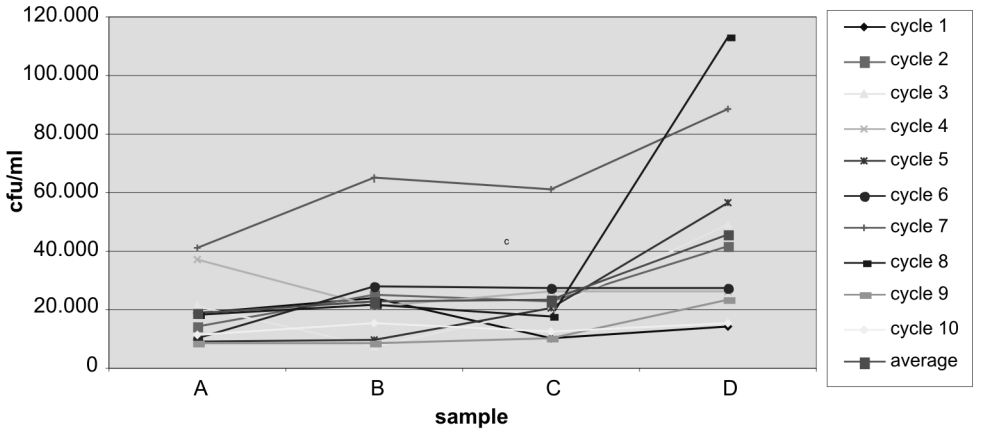



Figure 2. Counts of psychrotrophic bacteria in samples A, B, C and D of each cycle of two-day collection of milk

simultaneous detection of proteolytic psychrotrophic bacteria. The presence of psychrotrophs was determined in all samples from all 10 cycles of two-day collection of milk (Figure 2). The initial contamination (samples A) of milk with psychrotrophic bacteria varied in range from 8.300 cfu/mL up to 41.000 cfu/mL (average 18.800 cfu/mL) representing from 3.8 up to 15 % (average 7 %) of total mesophilic aerobic bacteria. In none of the samples A except in cycle 7, the portion of psychrotrophs did not exceed 10 % of the mesophilic aerobic counts which means that milk has been obtained in satisfactory hygienic conditions (Rogelj and Perko, 2003; Hantsis-Zacharov and Halpern, 2007).

During the prolonged storage the counts of psychrotrophs increased from an average of 18.800 cfu/mL (samples A) to 45.300 cfu/mL (samples D), representing an increase by a factor of 2.4. The most evident increase was detected between collection of samples C and D, which is illustrated in Figure 2. If the counts of psychroptrophs in samples C (day 1) were still rather moderate (average 22.700 cfu/mL) their average counts doubled by the day 2 (samples D).

The presence of proteolytic psychrotrophic bacteria was analyzed in all samples from all 10 cycles of two-day collection of milk but results from cycles 1-4 are missing due to their identification difficulties from agar plates (Figure 3).

During two-day collection of milk the counts of proteolytic psychrotrophs increased from an initial average value of 1.180 cfu/mL to 4.920 cfu/mL representing an increase by a factor of 4.2. Moreover, the portion of proteolytic psychrotrophs inside the total population of psychrotrophs increased from 6.2 % to 10.8 %. These results strongly indicate that lower temperatures are even more favourable for better growth of proteolytic psychrotrophs. Similarly as with psychrotrophs, the average counts of proteolytic psychrotrophs in samples C (day 1) were rather low (1.520 cfu/mL) but more than tripled by the day 2 (samples D) (Figure 3). Proteolytic psychrotrophs are extremely undesired milk contaminants because of their proteolytic and lipolytic activities, but these results clearly demonstrate that prolonged storage of milk promote growth especially of this group of microorganisms.

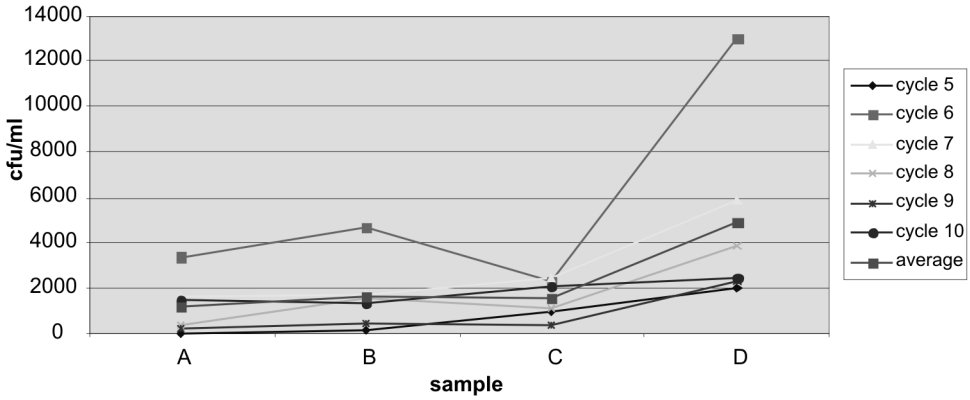



Figure 3. Counts of proteolytic psychrotrophic bacteria in samples A, B, C and D in cycles 5-10 of two-day collection of milk

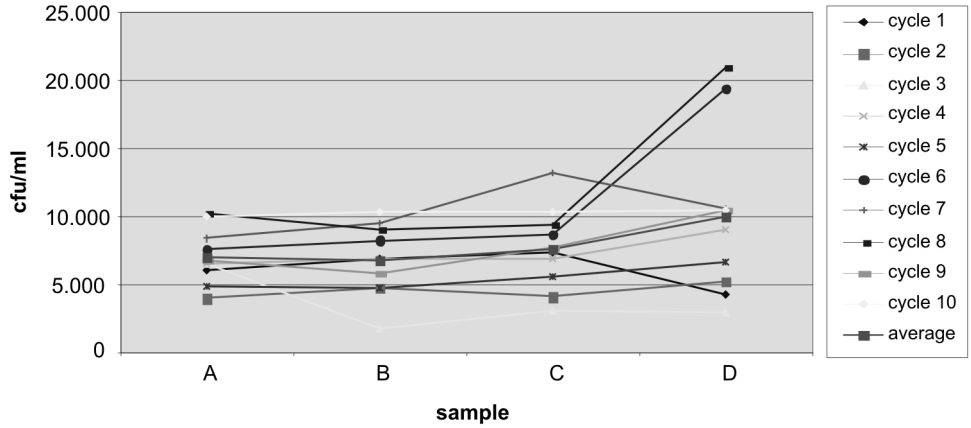



Figure 4. Counts of lactobacilli in samples A, B, C and D of each cycle of two-day collection of milk

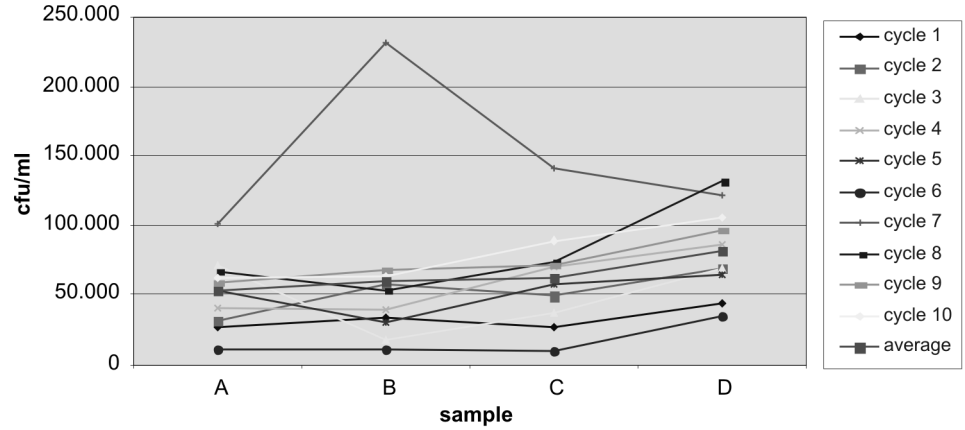



Figure 5. Counts of cocci (lactococci and enterococci) in samples A, B, C and D of each cycle of two-day collection of milk

Moreover, proteolytic, as well as lipolytic enzymes are heat resistant so none of the heat treatments that are normally used in milk processing are effective in destroying them. Although vegetative cells of proteolytic psychrotrophs are thermally destroyed, enzymes remain active and spoil fermented dairy products, which is especially problematic in cheese production.

The counts of lactic acid bacteria were monitored as well. Identification of lactobacilli and cocci was performed at 37 °C by the use of MRS and M17 media, respectively. As evident from Figure 4, during two-day collection of milk the counts of lactobacilli increased from average initial value of 7.130 cfu/mL to 10.030 cfu/mL, representing an increase by a factor of 1.4, which is a small increase. The major increase in lactobacilli counts was observed on the second day of two-day collection of milk.

Moreover, during two-day collection of milk the counts of cocci (lactococci and enterococci) increased from average initial value of 52.400 cfu/mL to 82.600 cfu/mL, representing an increase by a factor of 1.6, which again is a small increase (Figure 5).

However, the presence and the counts increase of lactobacilli and cocci in milk are not problematic, since these groups are members of lactic acid bacteria which are technologically relevant microbes in dairy industry. After plate count analysis of milk samples was accomplished, bacterial consortia from selected samples were PCR examined to establish the presence of the individual group of microbes. Results are summarized in Table 7.

As evident from Table 7, members of cocci group were detected in all (*Lactococcus lactis*) and in 5/6 (*Enterococcus* spp.) consortia. Lactobacilli were confirmed in 3/6 and pseudomonas in 2/6 consortia. Members of *Streptococcus thermophilus* species were not detected. The ubiquitous nature of cocci was confirmed with their presence in microbial consortia obtained also from atypical media for growth of cocci such as MRS and PCA with milk powder (30 and 7 °C, respectively). The growth of cocci to a lesser extent on other media beside M17 is not very surprising since the media used in our study were not defined as strictly selective but rather "promoting" ones. Lactobacilli presence was PCR detected in microbial consortia obtained from MRS, PCA with milk powder (30 °C) and M17 agar plates. Members of *Pseudomonas* spp. were PCR detected in microbial consortia from PCA with milk powder at 30 °C and 7 °C, respectively. The temperature span for pseudomonas was also observed by Ercolini et al. (2009). As already published on many occasions, plate counts solely are not discriminatory enough to describe the composition of milk microbiota. One must be aware that methods based on plate counts should be supported by the molecular based methods. Nevertheless, these results show some useful perspectives in psychrotrophs detection in manner to prevent their detrimental activities in raw milk and raw milk cheeses.

Table 7. PCR identification of individual groups of microbes in bacterial consortia

| Consortia: / Organism: | psychrotrophs sample D cycle 10 | lactobacilli sample D cycle 10 | MAB* sample D cycle 9 | MAB* sample D cycle 10 | psychrotrophs sample D cycle 8 | cocci sample D cycle 10 |
|---|---|---|---|---|---|---|
| *Lactobacillus* spp. | - | + | - | + | - | + |
| *Lactococcus lactis* | + | + | + | + | + | + |
| *Enterococcus* spp. | - | + | + | + | + | + |
| *Streptococcus thermophilus* | - | - | - | - | - | - |
| *Pseudomonas* spp. | - | - | - | + | + | - |

*MAB: mesophilic aerobic bacteria

## Conclusions

Results of this study showed clear and unequivocal effect of prolonged storage on microbiological quality of raw milk. The uniqueness of conditions (small village collection facility with over 30 years old collection tank, no air conditioning, milk from 6 local farmers, two-day collection system) do not allow generalization of obtained results but are limited to and of invaluable importance for local farmers and dairy plant to which collected milk is delivered. The most burning effect of two-day collection of milk is increase of psychrotrophic and especially proteolytic psychrotrophs that probably is valid for around the world but the level of increase depends on local conditions of milk collection. The big dilemma of two-day collection of milk stays open and requires thorough investigation regarding the purposes of further milk processing. For production of fermented milk products, especially hard type cheeses daily collection system from the microbiological point of view is of crucial importance, but lowering of costs reached by the two-day collection of milk is not negligible as well. Only the compromise between milk produces (farmers), milk processors (dairy plants) and consumers/market demands will provide optimal solution regarding the effect of prolonged storage on microbiological quality of milk.

## *Utjecaj produženog skladištenja na mikrobiološku kvalitetu sirovog mlijeka*


### Sažetak

Cilj ovog rada bio je utvrditi promjene u mikrobnoj populaciji skupnog sirovog mlijeka nakon dva dana pohrane u hladnim uvjetima čuvanja. Za svaki od 10 neovisnih ciklusa dvodnevnog sakupljanja mlijeka analizirana su 4 uzorka. PCA medij s dodatkom obranog mlijeka u prahu korišten je za određivanje ukupnog broja mezofilnih aerobnih bakterija, proteolitičkih, psihrotrofnih i proteolitičkih psihrotrofnih bakterija. MRS i M17 mediji korišteni su za određivanje laktobacila i koka (laktokoki i enterokoki). Nakon dvodnevne pohrane mlijeka, ukupan broj svih istraživanih mikrobnih skupina se povećao u odnosu na prvi dan pohrane. Međutim, utvrđena je značajna razlika između pojedinih skupina u njihovom udjelu u ukupnoj mikrobnoj populaciji. Najznačajnije promjene utvrđene su za psihrotrofne proteolitičke bakterije koje su od posebne važnosti za mljekarsku industriju. Proteolitički enzimi te skupine bakterija hidroliziraju bjelančevine mlijeka, a konvencionalna toplinska obrada mlijeka ih ne inaktivira. Zastupljenost pojedinih mikrobnih skupina utvrđena je PCR metodom. Rezultati potvrđuju da je mikrobiološka kvaliteta mlijeka kod dvodnevnog prikupljanja značajno lošija u usporedbi s mikrobiološkom kvalitetom mlijeka koje se prikuplja svaki dan.


*Ključne riječi:* mezofilne aerobne bakterije, psihrotrofne bakterije, proteolitičke bakterije, PCR identifikacija, dvodnevno prikupljanje mlijeka

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