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Influence of Microbiological Quality of Raw Milk on its Technological Parameters

DIPLOMA THESIS

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Declaration

I declare that I have written my diploma thesis titled “Influence of Microbiological Quality of Raw Milk on its Technological Parameters“ alone and I have used only literature that is cited and mentioned in references. I agree with storing this thesis in the library of CULS Prague and enabling it for study use.

In Prague: 16.4. 2012

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Author's Abstract

Influence of Microbiological Quality of Raw Milk on its Technological Parameters

Diploma thesis deals with the microbiological properties of raw milk and it refers also to some other different influences on its quality and technological parameters. For the experimental work, the milk samples were collected from two stables with Holstein cattle of a private farmer from April 2011 to February 2012. First part of the milk samples came from the stable 1 with 540 cows that could not graze in the pasture. The second part of the analyzed milk samples came from the stable 2 with 110 dairy cows, which were allowed to use the pasture.

Based on the results, it can be stated that the cattle-keepers approach was one of the most important factors influencing the quality of the examined raw milk. That aspect revealed as significant for the whole farmer's herd when the cattle from the stable 2 have been moved to the stable 1; milk from that unified herd presented a significant deterioration in microbiological quality for a weeks. However, raw milk quality has been improved to the required status soon after proper care of animals and appropriate hygiene had been introduced to the whole herd.

Laboratory research of milk samples was carried out in the Dairy Research Institute - Výzkumný ústav mlékárenský s.r.o. in Prague, where microbiological, physico-chemical and technological parameters were analyzed. There were two model experiments done in that research laboratory to verify the effect of the Lactococcus culture CCDM17 (Culture Collection of Dairy Microorganisms Laktoflora CCDM 17) on the quality of the sterilized milk.

The model experiments have proven that application of Lactococcus culture CCDM 17 had improved the quality of such treated milk in terms of its increased thermostability and much better taste.

Keywords: Holstein cattle, raw milk, cattle-keepers approach, microbiological parameters, technological parameters, thermostability, sensory evaluation, dairy products

Autorský referát

Vliv mikrobiologické kvality syrového mléka na jeho technologické parametry

Diplomová práce se zabývá mikrobiologickými vlastnostmi syrového mléka a dalšími vlivy týkajícími se jeho kvality a technologických parametrů. K experimentální práci byly odebírány vzorky syrového mléka ze dvou stájí s holštýnským skotem od soukromého zemědělce v období od dubna 2011 do února 2012. První část vzorků mléka pocházela ze stáje 1 s 540 dojnicemi bez možnosti přístupu k pastvě. Další část analyzovaných vzorků mléka pocházela ze stáje 2 se 110 dojnicemi, kterým byl přístup k pastvě umožněn.

Z výsledků je možné konstatovat, že lidský činitel byl jedním z nejpodstatnějších faktorů ovlivňujících kvalitu zkoumaného syrového mléka. Tento aspekt se výrazně projevil při sloučení dojnic z obou stájí do jedné, kdy po přesunu skotu ze stáje 2 do stáje 1 bylo zaznamenáno podstatné zhoršení mikrobiální kvality syrového mléka, které se však díky správné péči o zvířata a řádné hygieně zanedlouho vrátilo do žádaného stavu.

Laboratorní vyšetření vzorků mléka probíhalo ve Výzkumném ústavu mlékárenském s.r.o. v Praze, kde byly sledovány jeho mikrobiologické, fyzikálně-chemické a technologické parametry. Laboratorně byly rovněž realizovány dva modelové experimentální pokusy za účelem ověření pozitivního vlivu přídavku laktokokové kultury CCDM 17 (Culture Collection of Dairy Microorganisms Laktoflora CCDM 17) na kvalitu sterilizovaného mléka.

V modelovém pokusu bylo prokázáno, že aplikace laktokokové kultury CCDM 17 pozitivně ovlivnila kvalitu takto ošetřeného mléka z hlediska jeho zvýšené termostability a podstatně lepší chuti.

Klíčová slova: holštýnský skot, syrové mléko, lidský faktor, mikrobiologické parametry, technologické parametry, termostabilita, senzorické hodnocení, mléčné výrobky

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List of abbreviations

FAO – Food and Agriculture Organization of the United Nations

WHO – World Health Organization

CAC – Codex Alimentarius Commission

HACCP – Hazard Analysis and Critical Control Points

TPC – Total plate count

CB – Coliform bacteria

PTM – Psychrotrophic microorganisms

TRM – Thermoresistant microorganisms

SCC – Somatic cell count

MO alk – Alkaligenic microorganisms

MO acid – Acidophilic microorganisms

CFU – Colony forming units

SH – Soxhlet-Henkel

FA – Fermentability

RA – Rennetability

RCT – Rennet coagulation time

HCT – Heat coagulation time

T – Temperature

CCDM – Culture Collection of Dairy Microorganisms

ISO – International Organization for Standardization

EEC – European Economic Community

EU – European Union

VÚM – Výzkumný ústav mlékárenský s.r.o. (Dairy Research Institute)

ČSN – Česká technická norma

1 INTRODUCTION

Milk is essential part of nutrition for all young mammals, such as for human infants. It is the first food ingested after birth and it is an important source of the diet for a significant time. The composition and physical characteristics of milk are influenced by a lot of factors including – species, breed, feeding, season, condition of animal, clime etc. But without doubt water is the main constituent of milk; it also contains varying quantities of proteins, lipids and carbohydrates, smaller quantities of minerals and other fat soluble and water soluble components.

Domestication of animals caused that animal milk became a part of the adult human diet. Since that time many animals have been used to produce milk for human consumption, such as cows, goats, sheep, buffaloes, etc. Climatic and geographic conditions influence using of a certain animal, for example in the mountains they bred goats, but in many parts of the world, cows are the most commonly used animals in milk production.

Milk and dairy products represent quite a big amount of our diet, therefore it should be important for us to know, what it contains, and if it's healthy for us or not. Regular inspection and prevention are the most significant processes in milk production, because microbiology degraded material is useless for further processing. Quality and microbiology of raw milk depend on many factors, it starts in the stable and continues in the milking parlour, its treatment, transport to the dairy plant and subsequent processing.

From microbiological point of view it is important to monitor total plate count (number of mesophilic aerobic and facultative anaerobic microorganisms), then the number of coliform bacteria, thermoresistant and psychrotrophic microorganisms.

In our country there are standards that regulate condition of milking, transport and following processing of milk. Each dairy factory has its own regulations for suppliers of milk too. So we don't have to be afraid of consuming milk and dairy products – these basic foodstuffs have to be at least harmless. But it should be important for us to know, what we use in everyday life.

2 AIMS OF THE THESIS

To confront the quality of raw milk from two different stables and to analyze the impact of various conditions.

To observe the potential changes of raw milk quality in the different period of year.

To evaluate the influence of microbiological quality of raw milk on its technological parameters.

To assess the impact of the Lactococcus culture (CCDM 17) application on the quality of sterilized milk.

Hypothesis

1. Quality of raw milk varies with the season, cow's state of health and feeding patterns

2. Technological properties correspond with the quality of milk

3. Taste and quality of milk can be influenced by the addition of Lactococcus culture

3 BIBLIOGRAPHIC RESEARCH

3.1 Milk – basic information

Milk is described by a lot ways, one of the definitions of cow's milk is: *"Milk is the lacteal secretion practically free of colostrum, obtained by complete milking of one or more healthy cows"* (Weimer, 2001).

Singh and Bennett (2002) stated another characterization of milk. It is white liquid, which is the secretion of mammary glands of mammal and almost always it is the only one source of food for the young mammal. In the first few days post parturition the milk known as colostrum, with higher content of protein, is produced. Its main role is to nourish and provide immunological protection. For human consumption milk produced by cows, sheep, goats, buffaloes, and camels is used. For most of the world's population, cow's milk represents the majority of milk processed for human consumption.

The consumption of milk, milk products and butter in the Czech Republic shows table 1, the considerable decline of butter consumption is evident from this tablethere. The changes in average annual and daily milk yield, the milk production and the average number of dairy cows in the Czech Republic during the years are represented in the table 2.

Table 1: National consumption per capita and year (kg)

Year	**1989**	**2007**	**2008**	**2009**	**2010**
Milk and milk products	259,6	244,6	242,6	249,7	243,9
Butter	9,4	4,2	4,7	5,0	4,9

(Ministerstvo zemědělství, 2011)

Table 2: Developments in the milk sector in the Czech Republic

Parameter	**Unit**	**1989**	**2007**	**2008**	**2009**	**2010**
Annual milk yield	*l / cow*	3982	6548,3	6776,2	6869,9	6903,8
Daily milk yield	*l / cow*	10,91	17,94	18,51	18,82	18,91
Milk production	*mil. l*	4892,5	2683,5	2727,7	2707,6	2612,5
Number of dairy cows	*1000 cows*	1228,5	409,8	402,5	394,1	378,4

(Ministerstvo zemědělství, 2011)

3.1.1 Milk production and utilisation

According to Varnam and Sutherland (2001) the economic importance of milk production depends greatly on the ability of area to produce grass, other factors which can influence this production are – degree of subsides provided by government, accessibility of export markets and other economic mechanisms. But as Fox (2011) says there is a great probability, that some milk and dairy products are consumed in all regions in the word. In Europe and North America milk plays a large role in the human diet, and creates about 80% of dietary calcium, 20 – 30% of dietary protein and about 15% of lipids. Without doubt calcium represents important part of our diet and adequate intake is crucial in childhood for bone development and as a prevention of osteoporosis in later life.

Milk is biological fluid used for a specific purpose – nutrition of neonatal mammals. And thanks to its suitability for production of particular dairy products and its high nutritional value, milk represents major point in human diet and for many countries it comprise a significant point in the international trade (Fox, 2011).

According to Food and Agriculture Organization (FAO) the world cow's milk production was in 2010 almost 600 million tonnes. The biggest producer in the world was USA (87 million tonnes); in EU-27 was produced 136.1 million tonnes of cow's milk. The biggest part (36,1%) of whole milk in the EU-27 was utilised for the production of cheese. Then it was applied for butter (28,7%), drinking milk (12,4) and cream (11,5%) production (Faostat, 2011; Eurostat, 2011).

Ministerstvo zemědělství (2011) reported course of the economic importance and international trade of milk and milk products from 2006 till 2010 in the Czech Republic (table 3 and 4). Milk and milk products in 2010 were exported to the 67 countries, linked to the financial value the major costumers were – Germany (32,3%), Slovakia (20,4%) and Italy (11,7%). During 2010 milk and milk products were imported from 36 countries, mainly from EU 27 (99,9%), the largest importer was Germany (41,7%), Poland (29,3%) and Slovakia (12,1%).

Table 3: Export of milk and milk products from the Czech Republic (tons)

Year	2006	2007	2008	2009	2010
Milk and cream	596305	623519	681680	681665	612169
Butter	20830	21157	14950	14105	8144
Yogurt, Kefir	51429	67591	60756	75963	68775
Cheese, curd	23663	21224	22119	25613	29117
Whey	40500	49804	52872	41436	42964

(Ministerstvo zemědělství, 2011)

Table 4: Import of milk and milk products to the Czech republic (tons)

Year	2006	2007	2008	2009	2010
Milk and cream	111244	140598	129893	117325	79978
Butter	11570	13356	15625	20149	19132
Yogurt, Kefir	33438	39520	38522	38017	42036
Cheese, curd	57162	69443	64432	74297	76629
Whey	29096	53153	37888	19910	27179

(Ministerstvo zemědělství, 2011)

3.1.2 Milk composition

According to Huppertz and Kelly (2009) the composition of milk varies between different species, but there are considerable inter-species differences too, and this applies to both qualitative and quantitative indicators. This work deals with cow's milk, but for illustration it is interesting to present the average milk compositions of other species, too (table 5).

Table 5: Approximate composition of milk (%)

Species	Fat	Protein	Lactose	Ash
Cow	3,7	3,4	4,8	0,7
Goat	4,5	2,9	4,1	0,8
Sheep	7,4	4,5	4,8	1,0
Horse	1,9	2,5	6,2	0,5
Bison	3,5	4,5	5,1	0,8

(Huppertz, Kelly, 2009)

Figure 1 shows the main components of cow´s milk. The biggest part represent water (87,4%), the dry matter content is around 12,6%. Dry matter is constituted from the fat (3,7%), lactose (4,8%), casein (2,8%) and serum protein (0,6%), smaller quantities of minerals (0,7%,), enzymes, and small intermediates of mammary synthesis (Singh, Bennett, 2002; Smith, Campbell, 2007).

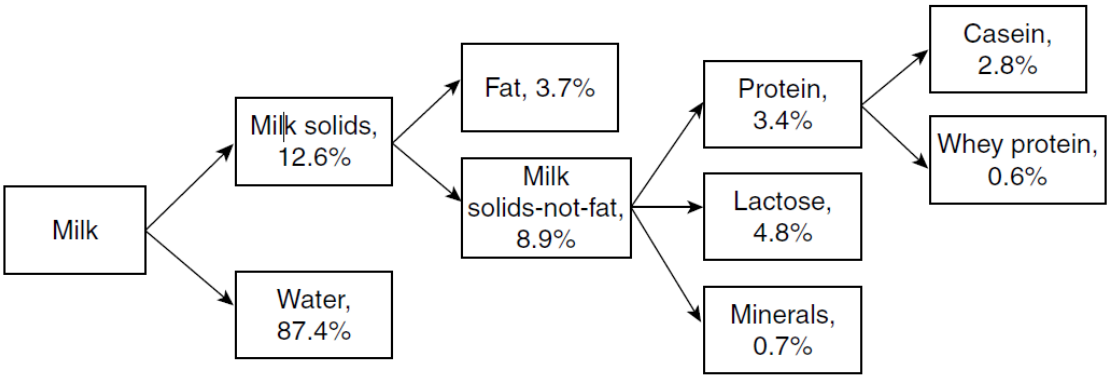


Figure 1: Major constituents of milk (Chandan, 2007)

Lipids

Triglyceride is the main component of lipid in cow's milk, it creates about 98% of milk fat. The rest of bovine milk lipids consist of phospholipids, diglycerides, cholesterol, monoglycerides, and free fatty acid, in addition there are some fat-soluble vitamins (A, D, E, K), *β*-carotene and flavoring compounds. Singh and Bennet (2002) also describe short-chain saturated fatty acids (butyric, capric acids) that may influence the flavour of milk products. Almost all of the milk fat, more than 95%, occurs in the form of globules with size about 1 – 6μm. All globules are surrounded by a slight milk fat globule membrane. The membrane serves like a natural emulsifying agent allowing the fat to stay dispersed through the aqueous phase of milk and prevent to extent flocculation and coalescence.

Lactose

According to Huppertz and Kelly (2009), lactose is a disaccharide, which presents the major carbohydrate in milk. In cow's milk there is about 4,8g lactose / 100g, other carbohydrates that are found in smaller numbers are monosaccharides (glucose and galactose) and oligosaccharides. Lactose concentration is influenced by stage of lactation

and by amount of somatic cells. If the number of somatic cells is increases, the concentration of lactose significantly decreases.

Protein

Fox (2011) describes milk proteins and divide them into two groups. Caseins – proteins which occur only in milk and are insoluble at pH 4,6 and 20 °C. And to the second group belongs protein named whey or serum protein. The ratio between caseins and whey proteins is characteristic for individual species, in bovine the caseins represent 80% of proteins and for example in human milk there is about 50% of caseins. In the species, which have high level of protein in their milk and contain more casein, the neonate growth rapidly, because caseins supply not only amino acids, but also phosphorus and calcium that are for those neonates essential.

Milk salts

Milk contains about 0,7% of salts, and because there are inorganic and organic salts too, the amount of salts is not same like the content of ash. The main salts in milk are phosphates, sulphates, citrates, chlorides, carbonates and bicarbonates of sodium, potassium, calcium and magnesium. Milk slats can be found in milk serum or in casein compounds, their composition influence a lot of factors such as feeding, species, stage of lactation and also breed of species. For example, Jersey's milk has less of sodium and chloride, but more phosphorus and calcium than milk from the other breeds (Huppertz, Kelly, 2009).

A lot of vitamins occur in milk, according to Singh and Bennett (2002) the most important are vitamins A and D, which are soluble in fat and water soluble vitamins – B_1, B_2 and C. There are present also several enzymes and somatic cells. Some of the minor components of milk represent the important function, but some could comprise the contaminants, e.g. disinfectants and antibodies.

3.2 Factors influencing milk quality and composition

Milk composition is influenced by a lot of factors, such as body condition of the animal, stage of lactation, secretion of milk, feeding of cows, milking conditions and procedures, cleaning of milking equipment and bulk tank, good handling practices during the whole process etc. Main factors influencing milk quality are presented in the following chapters.

3.2.1 Body condition

McNamara (2011) comments problems connected with the milk production and body condition that are often caused by quickly changing feed intake. There is something like a cycle which does not have a simply identifiable starting point. The problem can be caused by feed delivery, stress, weather, and diet composition, subclinical metabolic or other diseases. One or more of these reasons can generate in depressed appetite and the increasing deficit of nutrients elevates the probability of developing of calcium deficiency, ketosis or acidosis. Low feed intake with a metabolic disease decrease the ability of cows to manage other stressors, infertility, reducing production or mastitis.

Body condition has a great role in feed intake, health system and, of course, in production of milk. There is a relationship between the quantity and rate of body fat stores use and feed intake. It was found out, that cows with excessive body fat during late pregnancy habitually ate a smaller amount of feed than animals with maintained good body condition in this period. This decline of feed intake cause reduction of milk production and furthermore increase occurrence of postpartum metabolic and reproductive diseases.

A lot of studies have been done to find out more about the connection between the body condition and milk production. One of them was realized by Department of Dairy Science, by measurement of body condition scoring system. It was determined that cows with higher milk production showed no substantial increase in body condition during the lactation and although these cows had less of days open, the persistency of lactation was minor. Fewer efficient producing cows, which had considerably increased in body condition during the lactation, had more days open, but in the end of lactation they had high body condition score (Wildman et al., 2010).

3.2.2 Secretion of milk

Bylund (1995) write about secretion of milk that take place in the udder – organ, which is divided into the right and left side, furthermore each side is divided into the two quarters. Because every quarter has one teat with its own mammary gland it is possible to get milk of different quality from each teat.

Milk is secreted from molecules absorbed from the blood in the specialized cells called mammocytes. These cells are grouped and form bodies named alveoli. From the alveoli under the influence of oxytocin the milk flow towards the cistern of the udder, where is the main collecting point between milking and then it goes into the teat cistern and teat channel. Teat channel has sphincter muscle, which is closed between milking. This function is really important because it forestall the entering of the bacteria into the udder and prevents from leaking of milk. It is the reason, why the good state of animal health and appropriate hygiene of the udder is crucial in the milk production. The sectional view of the udder shows figure 2.

The interesting item that should be mention is that every day flow through the udder about 90 000 litres, for the production of one litre of cow's milk, approximately 800 – 900 l of blood has to flow through the udder (Bylund, 1995; Fox, McSweeney, 1998).

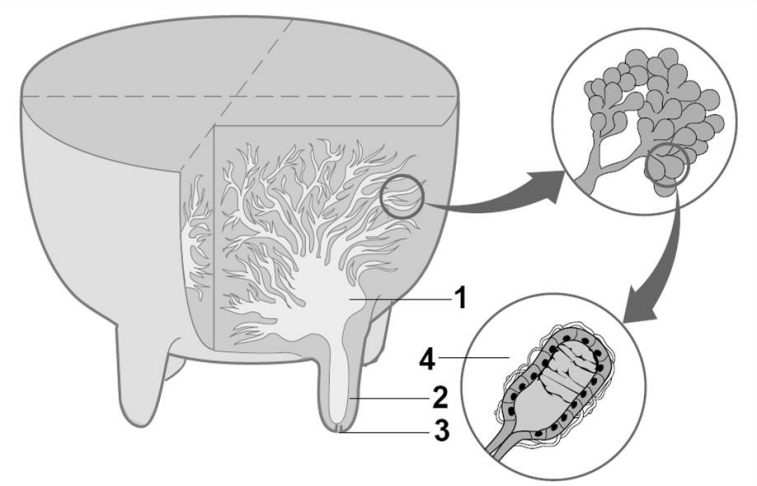


Figure 2: Sectional view of the udder (Bylund, 1995)

1.Cistern of the udder, 2.Teat cistern, 3.Teat channel, 4.Alveolus

3.2.3 Feeding of cows

According to Gillespie and Flanders (2010) the milk production is affected by a lot of factors, but one of the main aspects, that influence the amount of milk, is feeding. The nutrients needs depend on the age of the cow, body condition, stage of pregnancy, live-mass and stage of lactation. In the creation of the feeding rations it has to be taken account, that cattle are ruminants and their fibre requires are much higher than in other animals.

In practise there are different methods of feeding dairy cows – traditional, challenge (lead feeding) and feeding total mixed rations.

Traditional feeding

In this method the concentrate mix is fed in the stanchions barns in trough or during the milking in the milking parlour. This system is getting away in the modern farms because it has some disadvantages. There are, for example, difficulties to measure the quantity of eaten forage of each cow and it is complicated to balance the feeding ration with exact amount of concentrate for each cow. Other drawback is the require of grain feeding facilities and the higher dustiness in the milking parlour, the slowing dawn of milking, the problems with restlessness of cows (cows do not stand as quietly and defecate more during the milking) and the necessary of more labour – cleaning of uneated grain.

But there are some advantages too. The need of specialized milking equipment is lower and there is possibility to feed according to milk production and stage of lactation of each cow (Gillespie, Flanders, 2010).

Challenge - lead feeding

Challenge feeding is described by Ekern and Vik-Mo (2003). The goal of lead feeding is fed more concentrate in early lactation to challenge the cows and reach the maximum potential milk production. This manner of feeding permits the cows to express their yield potential during the critical period of early lactation. This method requires daily weighting of milk yield and qualitative assessment of the available roughage. Therefore the computerized systems for calculation of diet are usually applied. Challenge feeding is best used in the farms with high yielding cows and its advantage is in overall efficiency, better

use of concentrates, high forage consumption and positive effect on the infertility and ketosis.

Feeding total mixed rations

This method starts to be more and more used in modern dairy farms. In this feeding ration all required ingredients are mixed together – roughage and concentrates with balanced contain of energy, vitamin, protein, crude fiber and mineral. Then it is fed free choice to cows in individual groups. There are a lot of advantages in this system – balanced ration is delivered to each cow, effectively using of feeds and nonprotein nitrogen, rations can be modified more easily without the affecting of consumption, there is no requirement to added separately of ration some feed minerals, each cow is challenged to produce as much milk as she can, the labour for feeding is lower, cows in the milking parlour are more placid, the milking take less time and wastes of the concentrate are smaller. But there are some disadvantages too – cows with the low production of milk tend to become fat, for efficient feeding the individual groups of cows has to be done, adding of the hay to the ration is more difficult and for the mixing and weighting the rations special equipment is needed (Gillespie, Flanders, 2010).

Silage and milk products

It is used a lot of different feeds in dairy farms, but one which form the great part the feeding rations, can influence milk and therefore the milk products, should be presented in this work. It is silage, which can be described as forage conserved by fermentation.

Giffel et al. (2002) regard the silage as a considerable source of contamination of raw milk by spores. Heat resistance and PCC-RAPD fingerprinting studies of aerobic spore-formers isolated from raw milk and from maize silage proved this supposition. Reduction of the total spore content in the raw milk can be reached by prevention of outgrowth of aerobic spores in silage. It's the reason, why the silage fermentation process has to be adequately controlled. Use of the cultures of chemical additives and lactic acid bacteria could help to the right fermentation of silage and improve the aerobic stability.

No wonder that Codex Alimentarius Commission (CAC, 2007) – organ created by FAO and WHO organizations, mentioned this problem in connection with Codex Code of

Practice on Good Animal Feeding. There are standards which should be observed to prevent the introduction of contaminants through the feed or feeding practices into the raw milk. For example if the fermented feeds are used, it is necessary to prepare, store and use these feeds in a manner that will minimize the microbial contamination. Special attention has to be applied in the control of silage production, regularly checking of quality and pH of fermented feeds.

3.2.4 Seasonal effects

According to O'Brien and Guinee (2011) can be seasonality of milk easily described as a changes in the milk quality, composition and suitability for processing of dairy products during the calendar year. There are considerable variations in the concentration of fat, protein, lactose and casein related with season (Chart 1). Chart 2 shows changes in rennet coagulation time (RCT) of manufacturing milk at 31 °C and natural pH (6.6–6.7).

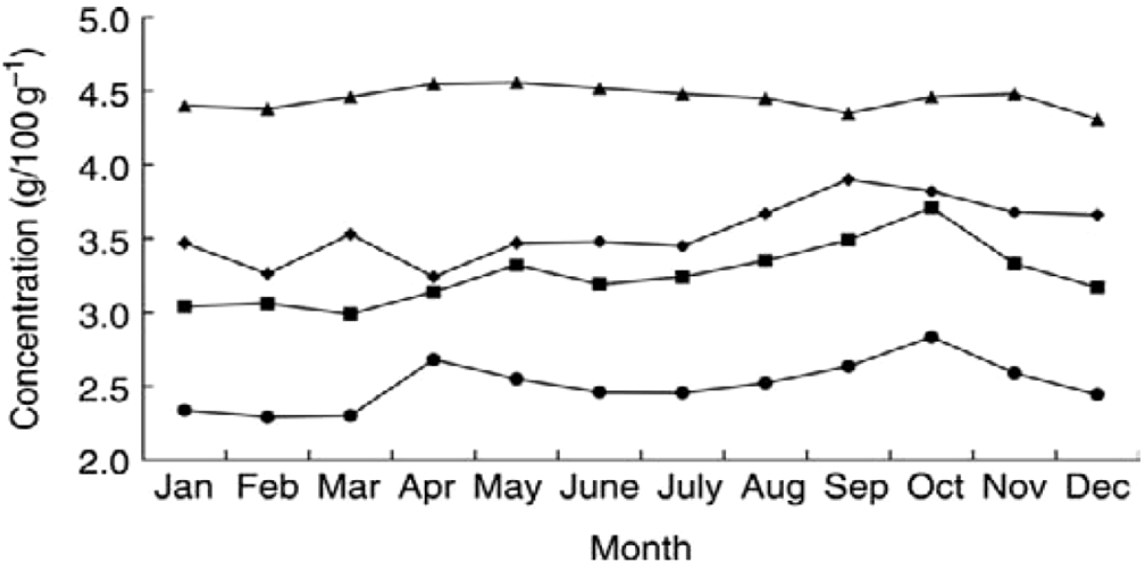


Chart 1: Seasonal variation in the concentration of fat (♦), protein (■), lactose (▲), and casein (●) in Irish manufacturing milk (O'Brien, Guinee, 2011)

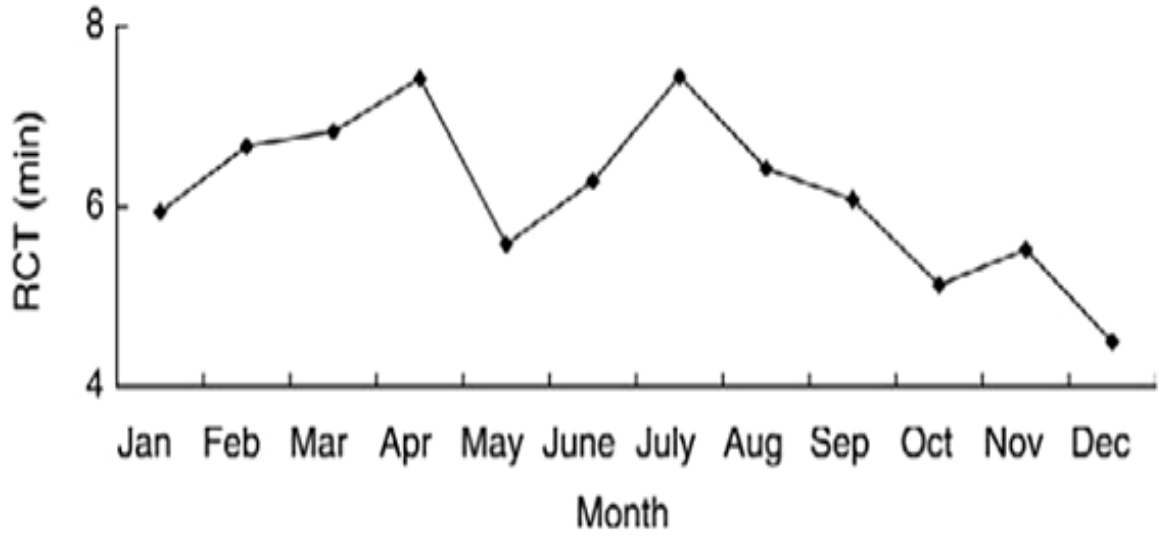


Chart 2: Seasonal changes in rennet coagulation time (RCT) of Irish manufacturing milk (O'Brien, Guinee, 2011)

The influence of season mainly relates to stage of lactation, nutritional status and changes in climate. In the beginning of lactation the yield of milk increases until it reach the peak of lactation (approximately at 6 weeks after parturition). This high yield leads to the reduction of protein, casein, total solids and fat content, but the amount of lactose increase. In comparison with late lactation, when the milk yield decrease, the level of total solids, protein, fat and casein raise, but lactose volume decrease (O'Brien, Guinee, 2011; Law, Tamime, 2010).

In Poland the research based on the Polish Holstein-Friesian cows has been done by Sitkowska and Piwczyński (2011). In the study were analysed the influences of selected factors on the milk composition and performance. Results from this experiment shows that the content of fat, protein and dry matter was the lowest in the milk obtained in summer. Amount of fat and lactose were also influenced by lactation count, the highest were in milk collected from cows in their first lactation, while in fourth lactation were the lowest.

Air temperature is another important factor connected with the season. High temperature in the summer can leads to the depression of milk production. Kunc et al. (2001) explain that the heat stress become in high yielding dairy cows at temperatures exceeding 21 °C and can cause the depression of milk production up to 25 %.

3.3 Microbiology of milk

Milk is basically sterile, if it is secreted by healthy cows. Microorganisms are introduced into the raw milk from different sources, including the exterior and interior of the udder, manure, bedding, soil, milking equipment and storage tanks (Tatini, Kauppi 2002). These possible sources of milk contamination at dairy farm are described in the figure 3.

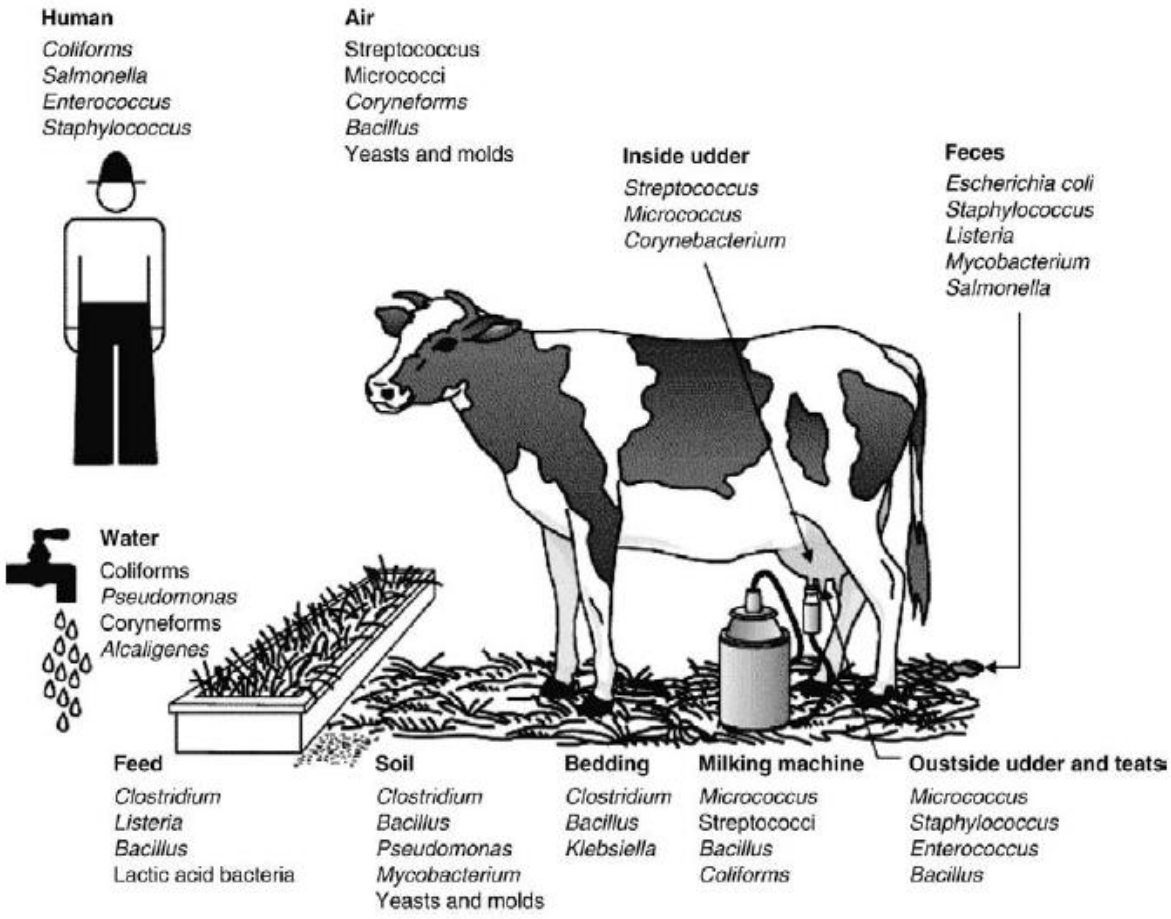


Figure 3: The sources of milk contamination (Hassan, Frank, 2011)

3.3.1 Classification of bacteria according to temperature preferences

According to Bylund (1995) the temperature is one of the most important factors, which influence growth, reproduction or death of the bacteria. For each species of bacteria exist different limits of temperature. If the temperature decreases below of this limit, the bacteria stops growing, but it will not kill the bacteria. It can happen by repeating of the freezing and thawing. However if the temperature increases above the limit, the bacteria rapidly become to die by this heat. A lot of cells are destroyed in a few seconds, when the temperature reaches 70° C. There are also bacteria which do not form spores and can survive the heating to 80° C for five minutes. Spores are normally destroyed by the treatment with steam at the temperature 120 °C for 30 minutes.

Bylund (1995) and Chambers (2002) classified the bacteria according to the temperature preferences into the 5 groups:

1. Psychrotrophic – grow in the temperature from 0 °C to 7 °C; into this group belong for example *Bacillus, Flavobacterium, Enterobacter,* or *Alcalingenes* microorganisms.

2. Psychrophilic – have the optimum temperature for growing from 10 °C to 15 °C, but can grow over the range from subzero to twenty degrees (Jay, 2000).

3. Mesophilic – the optimum temperature is between 20 °C to 44 °C.

4. Thermophilic – microorganisms grow in the temperature from 45 °C – 60 °C.

5. Thermoduric – bacteria, which survive the high temperatures – more than 70 °C, although they don't reproduce and growth, they survive at these high temperatures. To this group belong *Microbacterium*, *Micrococcus* and spores of *Bacillus* and *Clostridium*.

3.3.2 Primary sources of contamination

Fernardes (2009) describes contamination of milk from the udder, where microorganisms like *Gram-positive cocci, streptococci, staphylococci* and *micrococci,* then *lactic acid bacteria (LAB), Pseudomonas* and yeast are mostly found. If the mammary tissue is infected by inflammation known as mastitis, the number of microorganisms and somatic cells largely increases. This disease is really common in dairy cows; that infection can be caused by many bacterial species. These bacteria enter into the udder by the duct teat; the most common are bacteria like *Staphylococcus aureus, Streptococcus uberis* and *Escherichia coli.* The figure 4 shows the entrance of the bacteria through the teat channel and subsequent udder inflammation with heavily infected milk by bacteria.

Next possible source of microbial contamination can be external surface of the udder. This contamination is usually caused by faeces, bedding, soil and residues of feeds. The microorganisms like *salmonella, psychrotrophic sporoformers, enterobacteriacae, clostridia,* etc. are included in this group.

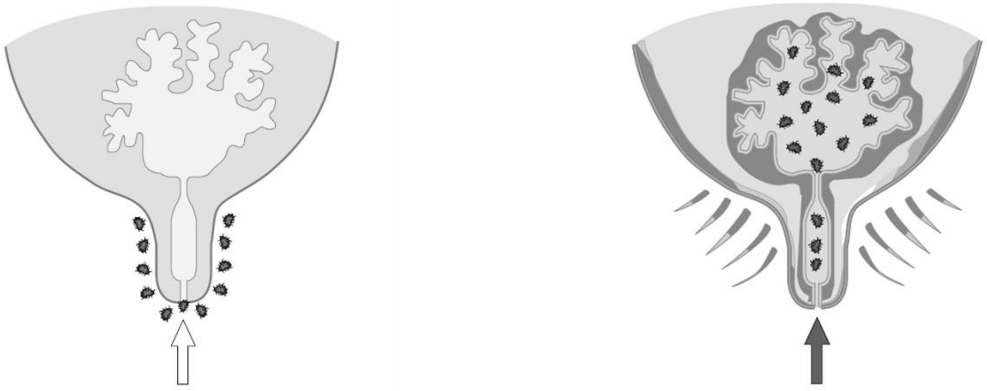


Figure 4: Entrance of the bacteria and udder inflammation (Bylund, 1995)

3.3.3 Sources of secondary contamination

Psychrotrophic microflora of raw milk can occurs because of the worse sanitised milking equipment and storage tanks, also contaminated air can be source of the contamination. If there are some residues of milk in rubber seals and joints, the numbers of psychrotrophic microorganisms could increase too. Among to this group belong, for example, *Pseudomonas, Enterobacter, Flavobacterium, Microbacterium, Micrococcus* and sporeforming *Clostridium* and *Bacillus*. The possible sources of contamination include farm workers, farm water supplies and airborne microorganisms (Fernandes, 2009).

The figure 5 shows the presence of different morphological groups of microorganisms commonly found in raw milk.

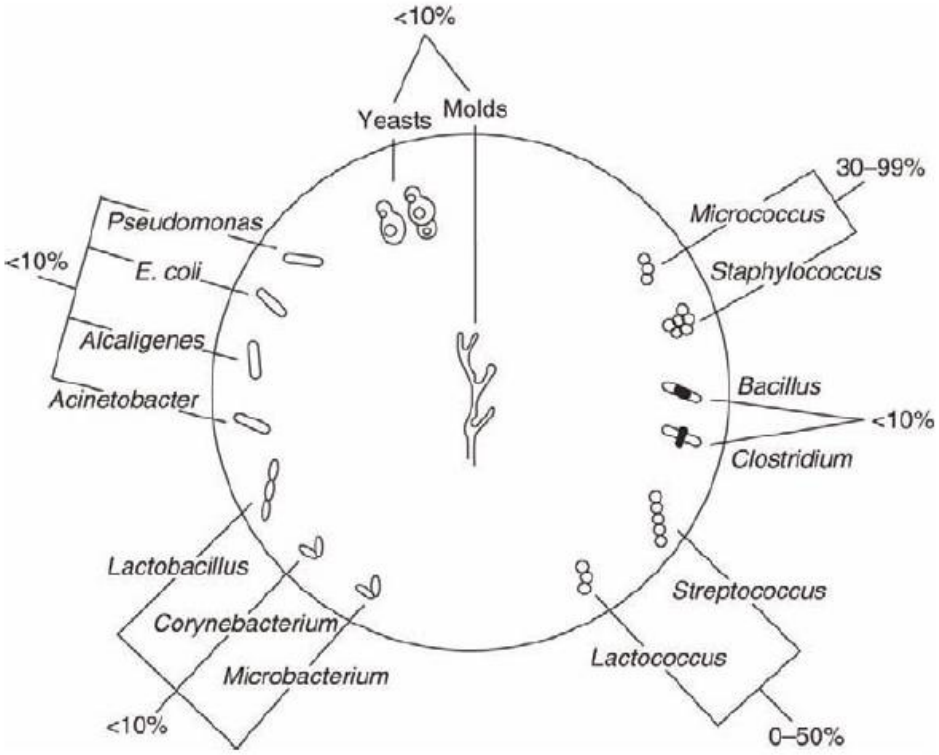


Figure 5: Microorganisms in raw milk (Hassan, Frank, 2011)

3.4 Hygiene on the farm

Microbiology of milk and sources of contaminations are greatly connected with the hygiene on the farm. Saran (1995) says that the goal of each dairy farmer should be to produce the high quality milk. That can be reached by implementation of good hygiene on the farm – using of appropriate techniques to disinfect and clean milking equipment and milking parlour. According to Frye and Kilara (2008) different stables and milking machine can be used – milking is done in the milking barn, stable or parlour. Cows are usually milked twice a day, but sometimes it can be realized 3 times a day in intervals of approximately 8 hours.

If milking is done by hand, bacteria can enter into the milk throw the milker, the litter, the animal or the air. The bacterial contaminations depend greatly on the proficiency and hygiene knowledge of the milker. A lot of these sources of contamination are eliminated by using the milking machine. But if the milking equipment is not cleaned appropriately, the bacteria can easily enter into the milk also by this way (Bylund, 1995).

Microbiological contamination can be reduced by cleaning and disinfection of the udder, post and pre milking disinfection and by cleaning and disinfection of the equipment.

3.4.1 Cleaning and disinfection of the udder

Slaghuis et al. (2011) show a lot of sources which could pollute the udder between milking. It is, for example, mud, faeces, straw, sawdust, etc. Therefore it has to be taken in account that for hygienic milking, good hygiene not only in stables, but also outdoor the stable must be provide. There are differences between the housed cows and cows on the pasture – it was found out, that on the pasture is the contamination of teat lower, while in the housed cows is the udder polluted by feedstuffs and bedding material. Frye (2006) describe different recommendations, which should be accomplished in the farms for the observance of good hygiene. The parlour, stable or milking barn has to be appropriately constructed with resistant and easily cleaned floors, smooth and painted ceiling and walls to decrease the dustiness. To prevent the condensation and excess of odours, there must be also good air circulation.

Although the post milking disinfection of the teat is required because it helps to decrease the amount of udder's diseases (mastitis), precise pre milking disinfection of teat is significant in minimizing of the bacteria count on the teat skin. Cleaning of the udder is carried out by spraying the disinfectant on the teats – automatically or by hand, or by dipping the teat into the special solution. The most used disinfectants are based on the chlorhexidine gluconate solutions or iodine solutions in different concentrations commonly mixed with glycerine. Same authors (Slaghuis et al., 2011) described the different ways of the removing of the visible dirt and preparation of the udder, there are 4 methods:

1. Cleaning with a dry textile or paper towel
2. Cleaning with wet towel, which is put in to the disinfectant between each cows
3. Washing with water and left wet / dry with towel
4. Washing with water including disinfectant and left it wet / dry it with towel

The fourth method with the drying by the towel is the most used way and can reached a maximum reduction (90%) of total bacterial and spore count from the teat. But in practise there is a problem with the short time for drying of the teat, therefore the teats are sometimes left wet and it can lead to opposite effect – the increasing of bacteria. Thus the cleaning and mainly the drying of teat have to be done really carefully. To the preparation of the udder before milking belongs also milk letdown – stimulation is made by massage of udder.

3.4.2 Cleaning and disinfection of the equipment

Many different types of equipment are used in the farms, but usually the milk from each cow is pumped under the vacuum throw the tube into the storage tank (figure 6). Fry and Kilara (2008) define the temperature of milk directly after milking at about 38° C that's the reason, why a lot of mesophilic microorganisms can grow if the temperature is not quickly lowered. The cooling is usually reached by mixing in the refrigerated bulk storage tank or by plate heat exchangers.

These processes require a lot of equipments, which should be cleaned properly. The basic demands are to use the equipments with smooth and cleanable surface; habitually control

and change the rubber parts, apply good detergents to eliminate milk residues and utilize clean water and disinfection to destroy microorganisms.

The method of cleaning of the milking parlour is influenced by national regulations, but it depends also on local habits, costs of heating and chemicals. In Europe a lot of countries use this system: it start with water rinsing (heated on 35 – 45 °C) directly after milking – to eliminate most of the remaining milk in the equipment, then it is cleaned about 10 minutes with an alkaline detergent and disinfectant to clear away organic soils, milk proteins and fat. In the end the cold water rinse, that takes away residues from the milking machine, is performed. Sometimes to remove milk stone the acid solution rinse is used; the necessary frequency depends on the quality of water (Vissers, Driehuis, 2009; Slaghuis et al., 2011).

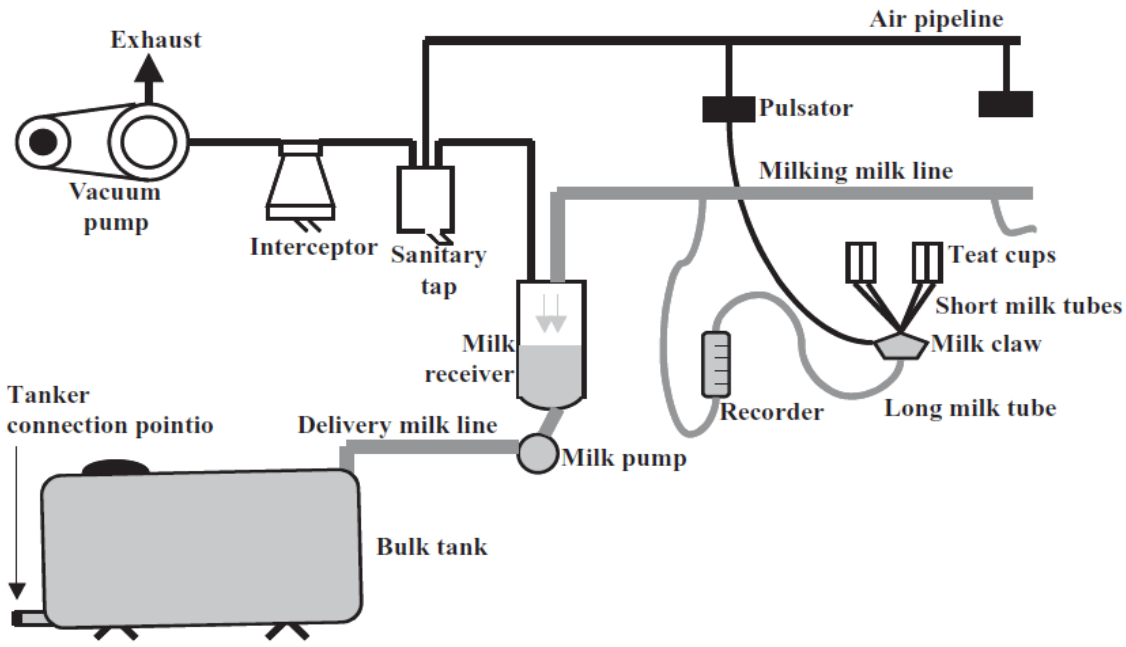


Figure 6: Scheme of milking equipments (Vissers, Driehuis, 2009)

3.5 Requirements for the transport of raw milk

As Czech standard 57 0529 (1998) stated, the cooling of milk must become immediately after beginning of milking and if the milk is not transported until two hours after the end of milking, it has to be cooled down on the temperature 4 °C – 8 °C in daily transportation. If the milk is transported every other day, the temperature of milk must be maintained at 4 °C – 6 °C until the transportation to the dairy plant.

In European Directive 92/46 EEC (1992), there are some differences. If milk is transported daily, it must be cooled to temperature 8 °C or lower. If collection is not performed daily the temperature cannot exceed 6 °C. It is important to observe these rules, to minimize microbial growth of mesophilic microorganisms, because many of them can growth in the high temperature which has got the milk directly after milking (Kilara, 2011).

Another requirement concern with temperature of milk during transportation – that temperature cannot exceed 10 °C. All the equipment which comes in contact with milk has to be made from resist corrosion, easily washed and disinfected materials. Tankers are usually made from sanitary stainless steel and milk is getting from the farm bulk milk tank into the transport tanker by pump with a volumetric meter. After emptying of the farm bulk tank, the pump is turned off to prevent mixing of the air with milk in the tanker. Milk is habitually collected from numerous farms, therefore the tanker driver has to acquire samples of milk from each farm at the time of collection. These samples are fundamental in the determination of quality of milk and subsequent payment based on the milk composition (Kilara, 2011; Bylund, 1995).

In Bohušovice dairy plant (that is related to this thesis) is applied that practise: milk samples are collected each day from all farms to determinate the fat and dry matter content. First, after arriving to the dairy plant, the test for the potential occurrence of residues of inhibitory substances has to be done, after that milk is pumped into the tankers in dairy plant and amount of milk from each tanker is measured. Afterwards the tank is cleaned by water then by sodium hydroxide (heated on 78 °C) and subsequently again by water, this process takes about 30 minutes and is made every day, once a week is used also nitric acid (Matějka, 2011). Every dairy plant has its own rules, but one of the most important factors is to observe the principles of The Hazard Analysis and Critical Control Points (HACCP).

3.6 Legislative basis for milk quality evaluation

3.6.1 Microbiological parameters

One of the most important factors, which show the quality of milk are the somatic cell count (SCC) and total plate count (TPC) – number of mesophilic aerobic and facultative anaerobic microorganisms. These parameters are divided into the 4 grades of quality according the number of microorganisms in 1 ml of milk – table 6.

Table 6: Qualitative grades of milk

Parameters	**Q**	**I**	**II**	**III**
SCC/ml				
Till 31.12.1994	300 000	400 000	500 000	500 000
From 1.1.1995	300 000	400 000	400 000	400 000
TPC/ml				
Till 31.12.1994	100 000	300 000	800 000	2000 000
From 1.1.1995	50 000	100 000	300 000	800 000

(ČSN 57 0529, 1998)

Further microbiological parameters according to standard ČSN 57 0529 (1998) are:

- Number of psychrotrophic microorganisms – up to 50 000/ml
- Number of thermoresistant microorganisms – up to 2000/ml
- Number of coliform bacteria – up to 1000/ml

In determination of milk price these parameters are significant; nevertheless each dairy plant has moreover its own rules for payment. For example, dairy plant Bohušovice, in which is supplied milk that deal with the thesis, pay extra for the milk that meets the parameters from Q qualitative grade of milk, furthermore for the higher fat (> 3,7%) and protein (> 3,4%) content. Deduction are performed for the milk with lower fat (< 3,5%) and protein content (< 3,2%) and for milk with worse values than are in I qualitative grade.

3.6.2 Physico - chemical parameters

In the Czech Republic the standard ČSN 57 0529 (1998) determine the qualitative characteristics of the raw milk. For example, the content of fat must be at least 33,0 g/l. In one litre of milk has to be minimally 28,0 grams of protein and non-fat solids contents must be at least 8,50%. Test of inhibitory substances must be negative.

Remarkable fact is that the freezing point of milk according to Czech standard has to be less than minus 0,515 °C (till 1994 it was just less than – 0,510), but European Directive 92/46 EEC (1992) define it to not be higher than minus 0,520. Determination of freezing point is done at least once a month and the main reason of doing this test is to detect the possible addition of water. Although this value is relatively constant, it can vary a little between breeds. Bhandari and Singh (2011) declare that Holstein milk has generally the lowest freezing point

Titratable acidity measured by the method of Soxhlet-Henkel (°SH) should be between 6,2 to 7,8. As Bylund (1995) describes, the result in this method is obtained by titrating 100 ml of milk with 0,25 M NaOH, as a indicator phenolphthalein is used. Then the colour of milk is monitored, until it is changed from colourless into the pink one. The titratable acidity can be expressed also in other values - Thörner degrees (°Th), Dornic degrees (°D) and per cent lactic acid (% l.a.) Titratable acidity is used to characterize milk and furthermore to determine the freshness and the amount of lactic acid formed in milk by fermentation (Pritchard, Kailasapathy 2011; McCarthy, 2011)

Active acidity – pH can be specified like the negative logarithm of the hydronium ion concentration (pH = – log [H+]). Active acidity of milk at 25 °C is usually located between 6,5 – 6,7. The most is influenced by temperature, but it can vary depending on the stage of lactation and health condition of cow (mastitis leads to increasing of pH). Value of active acidity may also indicate bacterial spoilage of milk. It is possible to use the pH of milk for the separation of the whey and casein proteins, because casein precipitates at pH 4,6 (Pritchard, Kailasapathy 2011; Fox, McSweeney 1998).

3.6.3 Taste and flavour

Clean and pleasantly sweet flavour of milk is one of the most important properties for each consumer. Milk has its typical flavour because of the equilibrium in sweet taste from lactose and salty taste from chloride. But as the same author says (Chandan, 2006) this taste could be changed due to a lot of factors, because the fat globules tend to absorb aromatic odours easily. Table 7 shows some origins and off-flavours in milk, but any off-flavours shouldn't be present in raw milk received by dairy plant. In the Czech Republic according to standard (ČSN 57 0529, 1998) taste of milk has to be clean and without any off-flavours or bed odour. To the sensory characteristics belong also colour and consistency of milk. Consistency of the milk should be homogenous without flakes and any coarse dirt.

Table 7: Off-flavours in milk

Origin	Off - flavour	Description	Potential causes
Chemical/ biochemical	Rancid, lipolytic	Soapy, bitter, unclean, blue - cheese - aroma	Raw milk homogenization, delay in pasteurization after homogenization
	Oxidized, light - induced	Tallow, burnt, medicinal, chemical taste	Milk exposed to UV light (sunlight/fluorescent light in dairy cabinet)
Micro-biological	Malty	Grape nut flavour, burnt, caramel	Equipment not properly sanitized, milk not cooled directly to less than 10 ° C
	Acid/sour	Tingling sensation on tongue	Milk stored warm for prolonged period
	Fermented/	Vinegar, apple or other fruity odour	Old, refrigerated pasteurized milk, raw milk stored for prolonged time
	Bitter/unclean	Bitter/unclean	Dirty equipment
Absorbed during milk production	Feed	Aromatic, onion, garlic, reminiscent of feed	Feeding cows 0.5 to 3 hours before milking
	Barn - like	Aroma of poorly maintained barn, unclean aftertaste	Poor barn ventilation and accumulated aromatic odours in barn
	Cow - like	Reminiscent of cow breath odour, medicinal aftertaste	Cows afflicted with ketosis

(Chandan, 2011)

3.7 Technological properties of milk

3.7.1 Heat stability

The heat stability represents further significant parameter of milk quality. It is the ability of milk (casein) to resist the intensive heat treatment and to maintain the initial colloid property without coagulation or thickening (Bylund, 1995). Today, the heat treatment is an important step in the processing of dairy products and almost all milk is subjected to at least one heat treatment. Milk is, compared to other food systems, extremely stable on heating, but when it is concentrated, it may not be enough stable to withstand sterilization. Milk heat stability can be determined by different ways. Same authors (O'Connell, Fox, 2011) write about the most habitual method, which is evaluated in an oil bath maintained at the required temperature (140 °C for normal milk) and then the time at which start coagulation or flocculation of milk proteins is measured – so called heat coagulation time (HCT). Heat stability is influenced by season, stage of lactation and manure of feeding and by protein composition (ratio between k-casein and β-lactoglobulin), mineral balance (ratio between cations and anions) and concentration of milk solids. Great role plays also the pH of milk (Chandan, 2006). Effect of pH on the heat coagulation time, at 140 °C, shows figure 7, the maximum heat stability of normal milk exhibits at pH around 6,7 and the minimum at pH about 6,9.

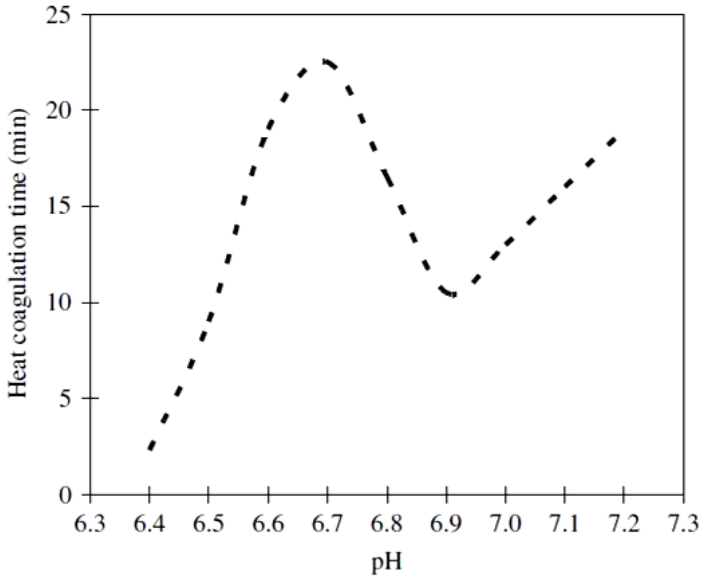


Figure 7: Heat coagulation time - pH curve (Deeth, Hartanto, 2009)

3.7.2 Fermentability

Pešek (1997) describes this parameter as the ability of milk to provide a good condition for increasing of needed microorganisms, especially lactic acid bacteria. The valid standard (ČSN 57 0529, 1998) determines that the fermentability expressed by method Soxhlet-Henkel has to be at least 25. This factor is significant in determination of residues of inhibitory substances, but as the name indicates, it is the crucial indicator mainly for the fermented products. Fermentation of milk is reached by the addition of special culture, for yogurt production RX culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) is used and by subsequently incubation at 42 – 43 °C for about 3 – 4 hours. This process involve the metabolism of lactose to lactic acid and increasing of acidity (pH about 4-5). It leads to the coagulation of milk proteins (denaturation) and forming of solid mass – curd. The low pH also contributes to extend the shelf life of product. It was already mentioned that inhibitory substances, for example, antibiotics and chemical disinfectants, may forestall fermentation. But fermentability could be worsened also by higher somatic cell count in the milk (mastitis, older cows), by unbalanced nutrition of cows or by the occurrence of mycotoxins in the forage, which may be cause by the poor conservation or storage of feeds (Yildiz, 2010; Surono, Hosono, 2011).

3.7.3 Rennetability

Rennetability can be explained like the suitability of raw milk to coagulate and to create coagulating form with the adequate solidity, or like the fitness of milk for cheese-making technology (Pešek, 1997). In the determination can be monitored also curd firmness, but in general the rennet coagulation time – RCT is observed. This factor considered to be a relevant economical point in the production of cheese and can vary significantly depending on the temperature of milk during processing. Renneting time may also be different in milk obtained from individual cows; it is caused primarily by Ca^{2+} activity and partly by variation of casein content. RCT is expressed like the time required for the visible enzymatic coagulation of milk maintained at the temperature around 30 – 35 °C. The most important enzyme used in cheese-making is the specific proteolytic enzyme chymosin, isolated from the abomasums of calves. But it is good to know, that coagulation of cheese may be caused by different coagulants include rennet and other clotting enzymes of animal, plant, or microbial origin (Walstra et al., 2006; Kapoor, Chandan, 2011).

4 MATERIALS AND METHODS

4.1 Field research

Samples of raw milk were collected from two stables of the private farmer. In the first stable (S1) were 540 dairy cows and were only fed. To the cattle from the second stable was allowed access to the pasture and number of dairy cows was 110. Table 8 shows that from both stables was taken altogether 72 samples of raw milk (S1=42; S2=30). The research was carried out for 11 month (April 2011 – February 2012). Data on somatic cell count (SCC) were obtained from Laboratory for analyses of milk Buštěhrad, the number of results was 36 (S1=21; S2=15). Other factors were also monitored during the research, such as the diet composition, hygiene of the udder, milk storage conditions and meteorological parameters (temperature). Samples of raw milk were collected twice a month for microbiological analysis (S1=126; S=90) and monitoring of physico-chemical (S1=42; S2=30) and technological parameters of milk (S1=42; S2=30) in the laboratory of Dairy Research Institute (VÚM) in Prague. Total of 360 samples were analyzed in the laboratory (S1=210; S2=150). Table 8 shows the design of the entire research.

Table 8: Research – evaluation of the raw milk quality

	Field research		Laboratory research (Research institute)			
	SCC (Buštěhrad)	Farm samples	Parameters	N° of samples	Analyses	Total N° of analyzes
Stable 1	21	42	Physico - chemical	42	SH pH	210
			Techno - logical	42	Ferment ability Rennet ability	
			Microbio - logical	126	TPC MO alk MO acid CB PTM TRM	
Stable 2	15	30	Physico - chemical	30	SH pH	150
			Techno - logical	30	Ferment ability Rennet ability	
			Microbio - logical	90	TPC MO alk MO acid CB PTM TRM	
Total	**36**	**72**		**360**		**360**

In order to use data on the somatic cell count (SCC) from the laboratory Buštěhrad, it was agreed that each month the author will be informed about sampling days of both stables. These analyzes were ordered by dairy plant Bohušovice, where the milk from both stables (S1, S2) was delivered. The author adapted to these sampling dates, and collected the samples of raw milk in the same day, to reach the harmonizing of results.

In the field research there was handled with samples of raw milk obtained from private farmer in two separate stables (S1, S2). Milk samples were collected by the sterile ladle from the cooling tanks into the sterile bucket with a capacity of 10 litres. Afterwards entire quantity of milk was mixed (to achieve the greatest objectivity) and subsequently transferred from the bucket into the sample container with volume of 500 ml for determination of the technological and physico-chemical parameters.

For the microbiological analyzes it was collected about 25 ml of raw milk into the small sterile sample containers containing preservative agent (Heschen's agent). In the cool box milk samples were transported to the Dairy Research Institute (VÚM) and after that immediately analyzed in the laboratory. The external air temperature was also recorded on all sampling days.

4.2 Laboratory research

In the field research was already mentioned that the samples of raw milk were transported from the stables into the accredited laboratory (Certificate of Accreditation – appendix 3) of the Dairy Research Institute (VÚM) and in this place subsequently analyzed (figure 8).

Dairy Research Institute (VÚM) is an institution with an important tradition; it was founded in 1952 as institute serving to the entire dairy industry of Czechoslovakia and later the Czech Republic. After a certain time the central control of dairy industry was cancelled and it caused establishment of VÚM s.r.o. as a subsidiary of the company MILCOM a.s.

Research activity of the VÚM results from the research projects of National Agency for Agricultural Research, Grant Agency of the Czech Republic, projects from the scope of programs supporting research EU PHARE, Copernicus and EU framework programs.

As stated above, analyzes of collected milk, which was kept at 6 °C, started immediately after arrival to the VÚM. Raw milk samples were analyzed from the physico-chemical, technological and microbiological point of view.

Figure 8: Author in the laboratory (photo Ing. Peroutková, 2011)

4.2.1 Monitoring of the qualitative parameters of raw milk

Active acidity (pH) and titratable acidity (SH) were measured from the **physico-chemical parameters**.

Active acidity (pH) of raw milk was measured by pH meter Sension 1 (Hach) – figure 9, which was calibrated using the buffers at pH 4,0 and 7,0. Electrode and temperature probe of pH meter were rinsed by distilled water, dried and immersed in the sample of raw milk. After that the measurement was initiated and the resulting value was recorded (determination according to the instructions for pH meter).

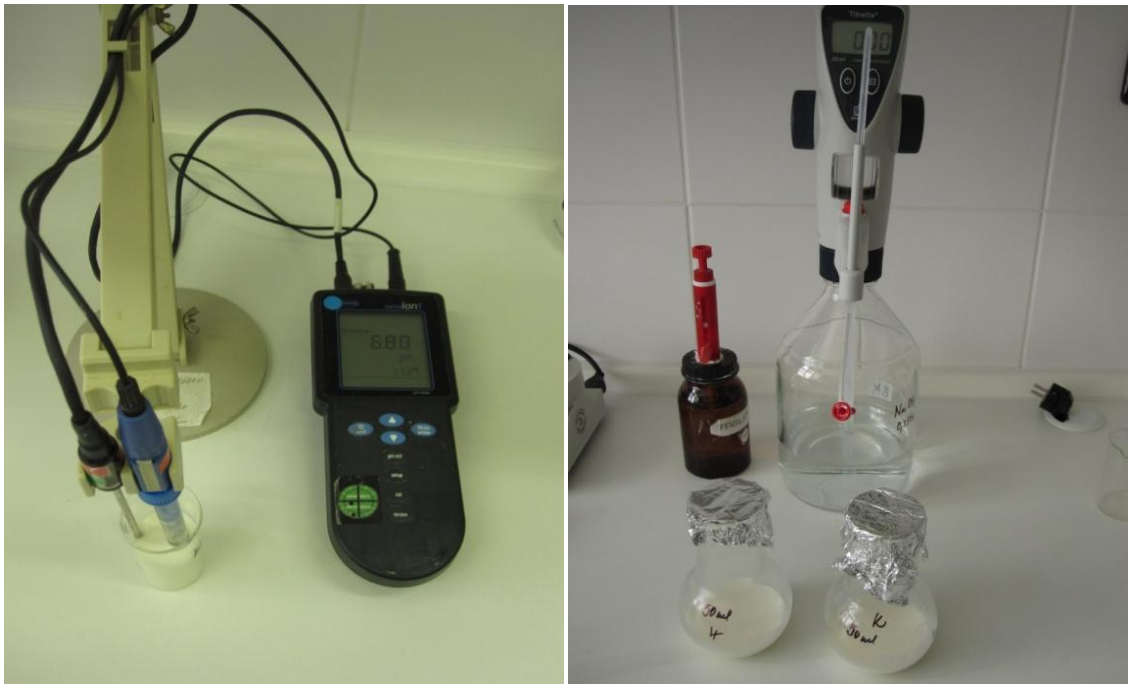


Figure 9: pH meter Sension 1 and digital burette Titrette (photo author)

Furthermore the **titratable activity (SH)** of raw milk was observed (according to Černá, Mergl, 1971). In determination of titratable acidity it was measured 50 ml of raw milk. Then were added 2 ml of 2% solution of phenolphthalein and subsequently by using of digital burette Titrette (Brand) – figure 9, there was titrated 0,25 M NaOH until the detection of a light pink colour. Titratable acidity was expressed in the SH per 100 ml of sample, therefore the obtained result was multiplied by two. According to the standard (ČSN 57 0529) titratable acidity of milk, measured by the methods of Soxhlet-Henkel, should be SH = 6,2 – 7,8.

From **technological parameters** of raw milk were evaluated fermentability and rennetability; in the model experiment was also observed heat stability of milk.

For determination of **fermentability** by the yogurt test there were measured 50 ml of raw milk. Milk was pasteurized at 85 °C for 5 minutes (figure 10), then it was cooled dawn to 42 °C, inoculated by 2% of yogurt culture RX (Milcom, a.s.; mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) and cultivated for 3,5 hours at 42 °C. After the cultivation, titratable acidity was measured and expressed in the SH per 100 ml of sample – multiplied by two (measured according to ČSN ISO 1211, 57 0534).

Figure 10: Pasteurization of milk (photo author)

Rennetability of raw milk was determined according to the method described by Černá and Mergl (1971), 100 ml of raw milk was warmed up to 35 °C, water bath TW 12 (Julabo) – figure 11, was used to maintain this temperature. For the experiment it was applied 1 ml of 1% solution of microbial rennet MICROCLERICI 1:60 000 (Caglificio Clerici) with coagulating activity of 100 000. Rennet solution was pipetted to the milk samples and in the same time the measuring of needed time was initiated. Flask with milk was maintained at 35 °C ± 0,5 °C and the time needed for the coagulation of milk was monitored and recorded at the first sign of coagulation (figure 12).

Figure 11: Measuring of rennetability at 35 °C – water bath TW 12 (photo author)

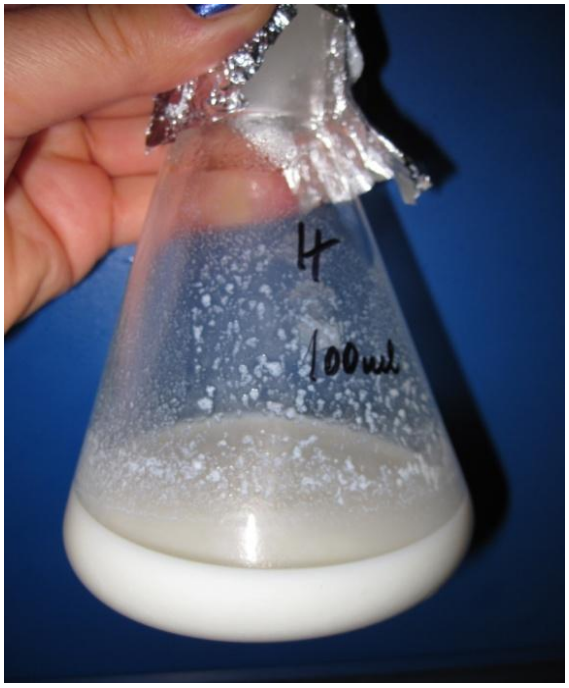


Figure 12: Coagulation of milk (photo author)

Heat stability was measured according to the method described by Patrovský et al. (1987) in the oil bath (Mlékárenský průmysl vývojová dílna Chotyně) with silicone oil heated to 140 °C. First it was pipetted 2,5 ml of milk sample to the special glass tubes sealed with rubber stoppers on both sides. Subsequently the tubes with milk were placed into the moving holders which swayed with the samples in the oil bath (figure 13). Afterwards the moment of the first flocculation (coagulation) of milk, so called Heat coagulation time (HCT), was monitored. Milk suitable for the production of sterilized products should have HCT at least 5 minutes.

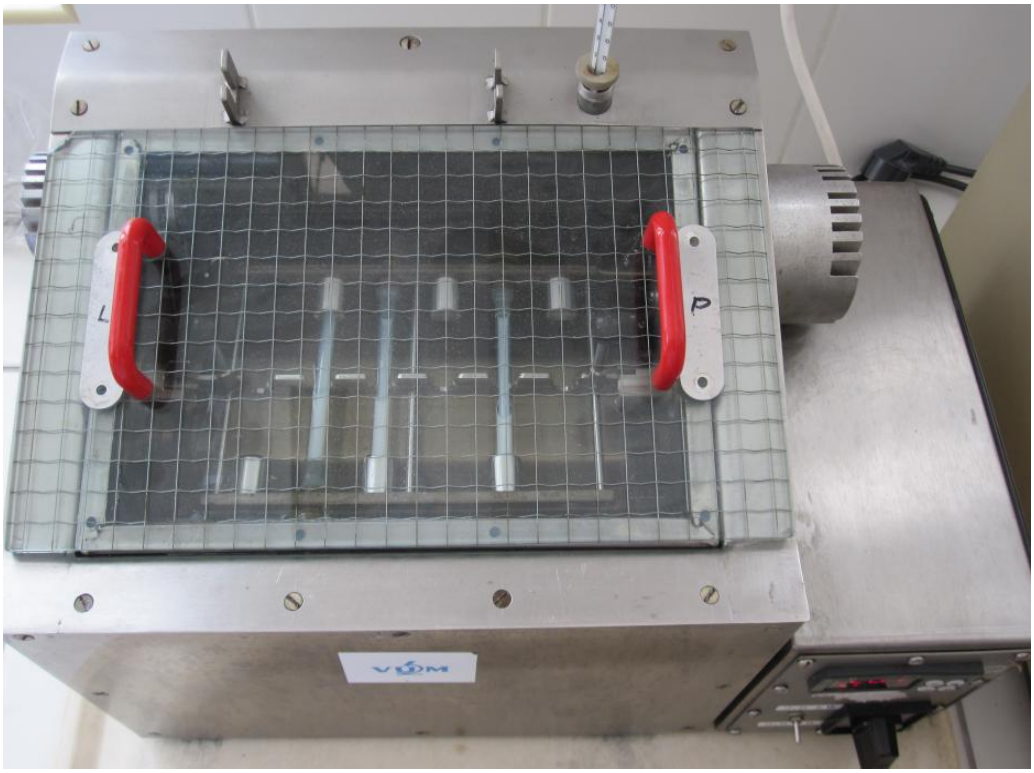


Figure 13: Oil bath with the milk samples (photo author)

From the **microbiological parameters** of milk were determined: total plate count (TPC), coliform bacteria (CB), psychrotrophic (PTM) and thermoresistant microorganisms (TRM). Dilution of milk samples was performed using saline solution with the peptone, Petri dishes were filled with the agars PCA, PCA AB and VRBL – figure14. The biological thermostat BT 120 M (Laboratorní přístroje Praha) was used for the cultivation. Numbers of individual microorganisms were counted using the device for the counting colonies ColonyStar (Funke Gerber) – appendix 4.

Total plate count (TPC) – number of mesophilic aerobic and facultative anaerobic microorganisms, was determined according to the standard (ČSN EN ISO 4833, 560083), with the difference of the use of the PCA AB – Plate Count Agar Anilin Blue, for the identification of acidophilic (blue) and alkaligenic (yellow) colonies – appendix 5. Samples of raw milk were diluted with the peptone saline solution to the second, third and fourth dilution (figure 15). After 72 hours of cultivation at 30 °C, using the device for the counting colonies ColonyStar (Funke Gerber) the numbers of alkaligenic and acidophilic microorganisms were counted and by the summation the number of total plate count was obtained.

Coliform bacteria (CB) were determined according to the standard (ČSN ISO 4832, 560085). Individual dilutions of raw milk were poured by VRBL – Violet Red Bile Agar. After solidification of agar the Petri dishes were put to the thermostat and there were cultivated 24 hours at 37 °C. Counting of microorganisms was carried out at zero, first and second dilution.

Psychrotrophic microorganisms (PTM) were also determined according to the standard (ČSN ISO 8552, 570548). First, second, and third dilution of milk samples were poured by PCA – Plate Count Agar. Number of psychrotrophic microorganisms was established after 25 hours of cultivation at 21 °C.

Thermoresistant microorganisms (TRM) were measured after the inactivation of the milk sample at 85 °C for 10 minutes. Then the milk samples were cooled dawn and subsequently zero, first and second dilution of milk was applied to the Petri dishes. In the establishing of thermoresistant microorganism it was used PCA – Plate Count Agar. Cultivation took place for 3 days at 30 °C (determined according to the standard ČSN ISO 4833, 560083; inactivation according to the ČSN 57 0101).

Figure 14: Agars – PCA, PCA AB and VRBL (photo author)

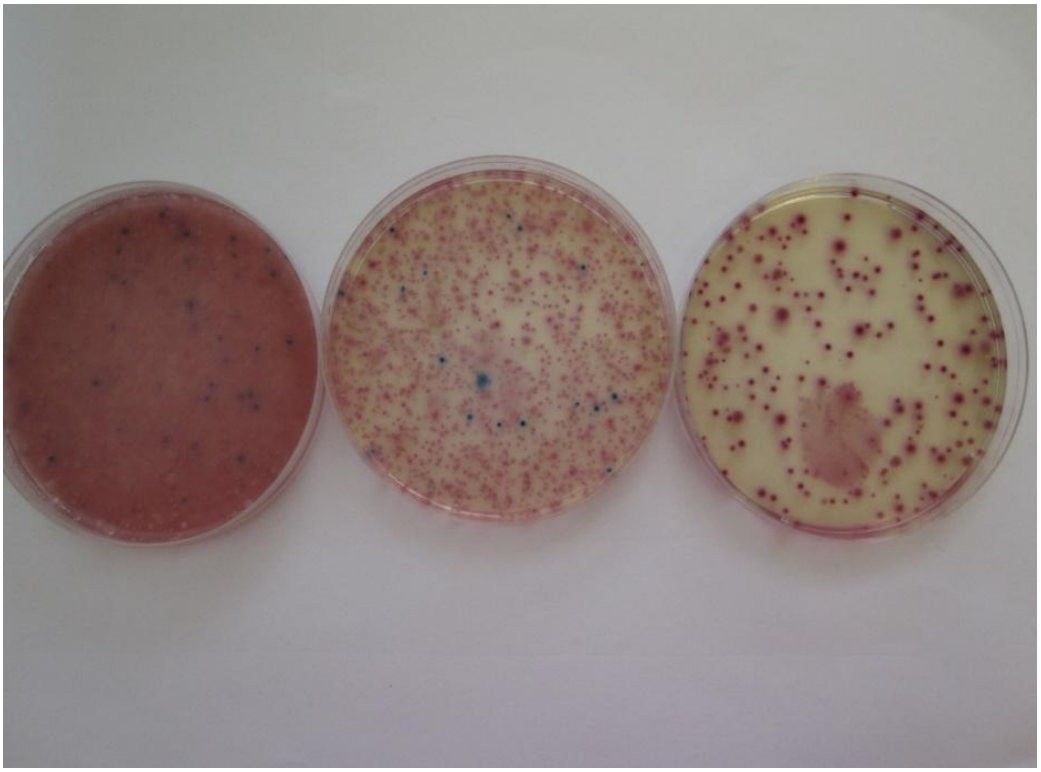


Figure 15: Growing of the microorganisms according to different dilution (photo author)

To determine the number of microorganisms, by accredited laboratories commonly used formula according to the standard (ČSN 7218) was applied:

$$\mathbf{N} = \frac{\sum \mathbf{C}}{\mathbf{V} * \mathbf{1,1} * \mathbf{d}}$$

In this formula **N** expresses the final number of microorganisms, **Σ C** sum of all colonies counted in the selected plates, **V** the volume of the inoculums used in the plate, and **d** represent the factor of the first for counting used dilution. The formula may be explained in the fallowing example:

$$\mathrm{N} = \frac{280 + 29}{\mathrm{V} * 1{,}1 * \mathrm{d}} = \frac{309}{1 * 1{,}1 * 10^{-4}} = \frac{309}{0{,}00011} = 2809090 = 2{,}9 * 10^{6}$$

In the 4th dilution of milk the number of colonies was 280; in the 5th dilution it was 29.

It follows that the sum of all colonies (Σ C) from the plate of 4th and 5th was 309. The volume of the inoculums (V) was 1 ml. First dilution used for counting (figure 16) was the fourth one (d = 10^{-4}). Based on the formula the final result was obtained – in this example was the number of microorganisms 2,9 *10^{6}.

Figure 16: Author counting the number of microorganisms (photo Ing. Peroutková, 2011)

4.2.2 Model experiment with the application of the Lactococcus culture

Apart observing of the quality of raw milk, two model experiments were realized during the research. In the model experiments, there was the goal to monitor the effects of the Lactococcus culture CCDM 17 on the quality of milk. To capture the influence of the season (weather conditions, feeding, etc.) the samples from both stables (S1, S2) were first collected in the summer (11. 7. 2011 – 11. 1. 2012) and subsequently in the autumn (1. 10. 2011 – 10. 3. 2012).

Design of the project (tab. 9) for the model experiments shows that after the counting of the samples from both stables (S1 + S2), from physico-chemical parameters there was analyzed 72 samples (pH = 20; SH = 52). In the assessing of the technological parameters it was performed 52 analyzes (fermentability = 20; rennetability = 20; heat stability = 12). Further 104 samples from the microbiological parameters was analyzed (TPC = 52; MO alk = 20; MO acid = 20, CB = 4; PTM = 4; TRM = 4). By evaluation of the sensory properties it was obtained 64 analyzes (consistency = 32; taste = 32). In both experiments from both stables (S1 + S2) altogether 292 analyzes was performed (raw milk = 44; milk without culture = 124, milk with culture = 124).

Table 9: Model experiments with the Lactococcus culture

	Parameters	Total N° of samples	Analyzes	Raw milk	Without culture			With culture			N°of analyzes
					24h (6°C)	48h (6°C)	6 mths (20°C)	24h (6°C)	48h (6°C)	6 mths (20°C)	
2 Model experiments (S1 + S2)	Physico - chemical	72	SH	4	4	4		4	4		20
			pH	4	4	4	16	4	4	16	52
	Techno-logical	52	Ferm. ability	4	4	4		4	4		20
			Rennet ability	4	4	4		4	4		20
			Heat stability	4		4			4		12
	Microbio - logical	104	TPC	4	4	4	16	4	4	16	52
			MO alk	4	4	4		4	4		20
			MO acid	4	4	4		4	4		20
			CB	4							4
			PTM	4							4
			TRM	4							4
	Sensory properties	64	Consistency				16			16	32
			Taste				16			16	32
Total		292		44	124			124			292

In the model experiments, raw milk samples were collected in the same way as it was in the case of the previous research. After transportation of the milk samples to the laboratory of VÚM, first analyzes of the raw milk from both stables (S1 + S2) were performed (tab 9. – altogether 44 analyzes), in this experiment heat stability was also determined.

Part of the milk samples was left unchanged and were designated as the control samples (without culture – 124 analyzes). In the case of the experimental samples (with culture – 124 analyzes) the method of the standard application of the selected Lactococcus culture (Culuture Collection of Dairy Microorganisms Laktoflora, mixture of *Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. diacetilactis,* producer Milcom, a.s.) was performed at a maximum density 50 000 CFU/ml.

Both parts of the samples (with + without culture) were stored for 48 hours at +6 °C. After 24 and 48 hours, the active acidity (pH), titratable acidity (SH), rennetability and total plate count (TPC) was analyzed. Moreover after 48 hours of storage the heat stability of milk was measured. Subsequently the milk samples were poured into the bottles and sterilized in the autoclave 2540EL (Tuttnauer) at 117 °C for 18 minutes (technological parameters of sterilization in the dairy plant Bohušovice).

These sterilized milk samples were stored for 6 months at 20 °C. After that the milk samples were evaluated; the total plate count (TPC) and active acidity (pH) was analyzed and also the sensory evaluation of milk (consistency, taste) was accomplished.

4.3 Statistical evaluation

For the statistical evaluation there were used programs SPSS 19 and STATISTICA 10. For determination of individual dependences the regression and correlation analyzes were carried out. Regression analyzes represented the course of the dependence and the correlation analyzes measured the strength of the dependence between variables. To verify and compare the results with other measurements it was used parametric two sample tests, in our case F test and two – sample T test. It was assessed the dependence of the psychrotrophic microorganisms (PTM) on the total plate count (TPC), dependence of the fermentability and rennetability on the PTM and CPM, further dependence of the TPC on the measured temperature; the authors results from TPC and results from the TPC of laboratory Buštěhrad was also compared.

5 RESULTS

5.1 Monitoring of the qualitative parameters of raw milk

After the analyzing of raw milk samples from both stables (S1, S2) of private farmer, evaluation of their results was made. Data about somatic cell count (SCC) were obtained from the laboratory Buštěhrad.

Research focused on the monitoring of the quality of raw milk took place from the 14.4. 2011 to 22.2. 2012. During the research it was analyzed physico-chemical (pH, SH), technological (FA, RA) and microbiological parameters of raw milk from (S1, S2).

From the meteorological parameters there were recorded the temperatures of the air in the day of collection.

Table 10 describes the results obtained from the samples of raw milk from the first **stable (S1)**. At this stable the **somatic cell count (SCC)** from all milk samples met the standard limit (up to 400 000/ml) and only at 7 samples (SCC – 312000, 282000, 330000, 281000, 272000, 298000, 363000) from total 21 milk samples were found the SCC value above 250 000/ml, which corresponds to the limit value for the good technological milk processing (according to the practice of VÚM).

At **physico-chemical parameters** (active acidity – pH, titratable acidity – SH) were not observed considerable differences; titratable acidity values determined by the method of Soxhlet-Henkel were found in the standard range (6,2 – 7,8).

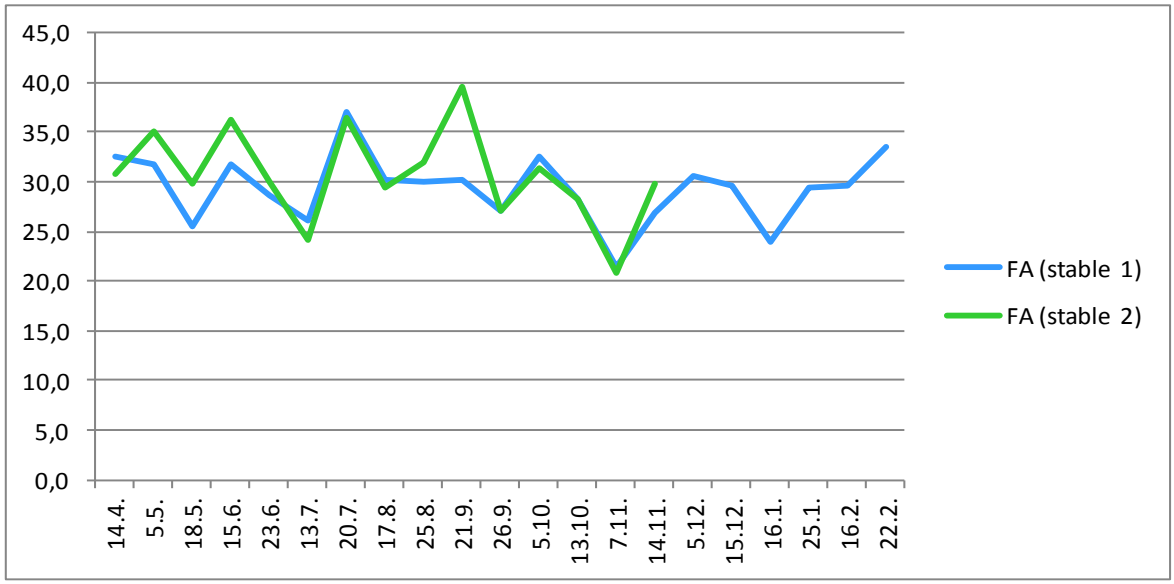
Fermentability of milk below the set standard (25) was recorded only at one sample from 7.11. 2011 (21,5); in all of the other samples were values measured with the probability for trouble-free production of fermented dairy products (chart 3).

Rennetability of milk, an important indicator for production of cheese and quark, was good in all observed milk samples (optimal rennet coagulation time is 5 – 7 minutes).

Table 10: Somatic cell count (SCC), SH, pH and technological properties in stable 1

Date	SCC/ml	SH	pH	Ferment ability	Rennet ability
14.4.2011	312000	6,8	6,79	32,6	6 min 40 s
5.5.2011	229000	6,8	6,73	31,8	6 min 45 s
18.5.2011	220000	7,1	6,81	25,6	5 min 38 s
15.6.2011	227000	7,0	6,75	31,8	5 min 56 s
23.6.2011	234000	7,2	6,85	28,6	7 min 29 s
13.7.2011	282000	7,4	6,75	26,2	7 min 14 s
20.7.2011	330000	7,1	6,72	37,0	7 min 09 s
17.8.2011	235000	7,5	6,73	30,2	6 min 45 s
25.8.2011	229000	6,4	6,73	30,1	7 min 33 s
21.9.2011	245000	7,2	6,71	30,2	5 min 04 s
26.9.2011	281000	7,0	6,84	27,0	7 min 07 s
5.10.2011	272000	7,0	6,78	32,6	7 min 10 s
13.10.2011	205000	6,8	6,80	28,2	6 min 10 s
7.11.2011	298000	7,5	6,85	21,5	7 min 16 s
14.11.2011	220000	7,0	6,82	26,8	5 min 52 s
5.12.2011	363000	6,7	6,73	30,6	6 min 11 s
15.12.2011	250000	7,1	6,72	29,7	7 min 12 s
16.1.2012	216000	7,3	6,77	23,9	6 min 25 s
25.1.2012	201000	7,2	6,79	29,5	6 min 56 s
16.2.2012	233000	6,8	6,79	29,5	7 min 21 s
22.2.2012	245000	7,3	6,86	33,6	7 min 23 s

Chart 3: Fermentability (FA) of stable 1 and stable 2



According to the chart 3, the **fermentability** of milk was in samples from both stables relatively equal, only at one sampling day were recorded lower values in the samples from both stables. Given that the residues of inhibitory substances in milk were negative (data obtained from laboratory Buštěhrad), this deviation was probably caused by abnormally performing test (wrong inoculation or accidentally lower virulence of yogurt culture).

At the **second stable (S2)** the results of **somatic cell count (SCC)** were significantly worse (tab. 11). In the 3 samples (SCC – 447000, 481000, 402000) from the total 15 evaluated, there were observed values of SCC exceeding the limit set by standard (up to 400 000/ml) and only in two samples of 7.11. 2011 (SCC – 227000) and 14.11. 2011 (SCC – 235000) the SCC was lower than 250 000/ml, which is the limit value corresponding to the good technological processing of milk (according to the practice of VÚM).

In the measured **physico-chemical parameters** (pH, SH) were not found significant differences in the milk from stable 2.

As in the previous stable (S1), in the stable 2 (S2) were also observed **fermentability** of milk complying the limit set by standard (above 25) in all samples except one from 7.11. 2011 (20,8) – chart 3.

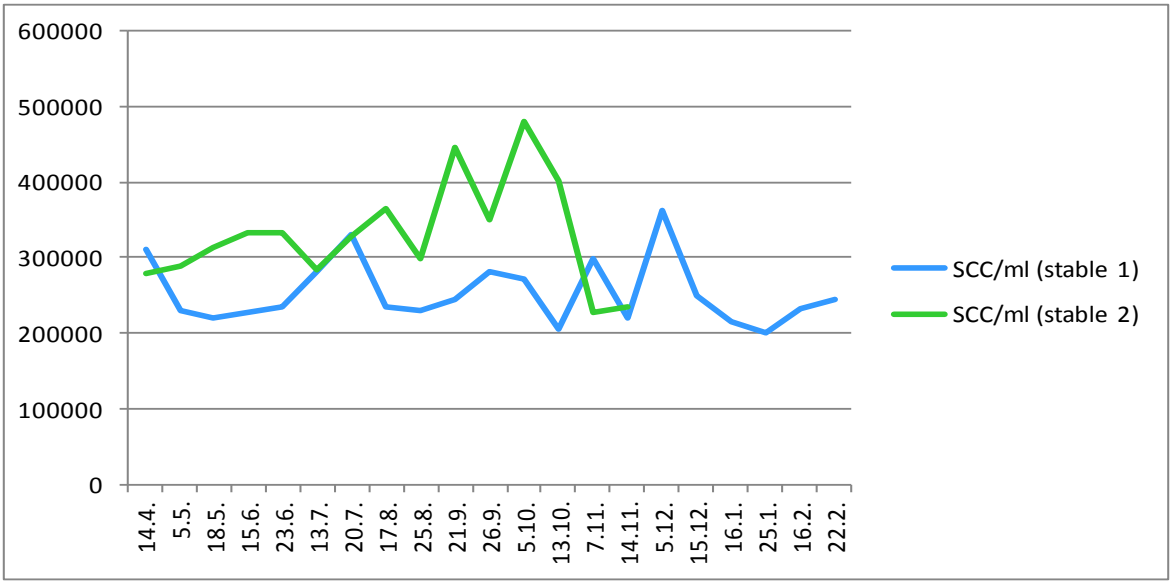
In the **rennetability** of milk, which markedly influences the production of quark and cheese, was determined good rennet coagulation time – RCT (optimal time for milk clotting is 5 – 7 minutes) in all samples, only at first observed sample from 14. 4. 2011 were RCT slightly higher (8 minutes).

Graphical representation of the **somatic cell count (SCC)** obviously reflects worse (higher) values founded in the milk samples from stable 2. Chart 4 also show negative effect of cattle movement from stable 2 (S2) to the stable 1 (S1), which occured on the 15. 11. 2011. The increased number of somatic cell count (SCC) after this date was probably caused by the unification of all animals, which led to the psychological stress and deterioration of animal state of health. According to chart 4, the quality of milk again equalized from the date 16.1. 2011, perhaps due to proper treatment, hygienic obtaining of milk and good care of animals.

Table 11: Somatic cell count (SCC), SH, pH and technological properties in stable 2

Date	SCC/ml	SH	pH	Ferment ability	Rennet ability
14.4.2011	280000	6,8	6,80	30,8	8 min 00 s
5.5.2011	290000	7,0	6,81	35,0	7 min 00 s
18.5.2011	313000	6,9	6,79	29,8	6 min 17 s
15.6.2011	332000	7,1	6,72	36,2	6 min 48 s
23.6.2011	334000	7,0	6,82	30,0	6 min 52 s
13.7.2011	283000	7,0	6,71	25,2	6 min 36 s
20.7.2011	327000	7,0	6,74	36,5	5 min 31 s
17.8.2011	364000	6,2	6,76	29,4	5 min 52 s
25.8.2011	299000	6,6	6,75	31,9	6 min 35 s
21.9.2011	447000	7,8	6,76	39,6	6 min 18 s
26.9.2011	351000	6,6	6,90	27,0	6 min 29 s
5.10.2011	481000	7,2	6,76	31,4	6 min 35 s
13.10.2011	402000	6,9	6,80	28,2	6 min 15 s
7.11.2011	227000	7,3	6,84	20,8	7 min 10 s
14.11.2011	235000	7,2	6,79	29,8	6 min 08 s

Chart 4: Somatic cell count (SCC) in stable 1 and stable 2



From the microbiological values (tab. 12) measured in the milk samples from **stable 1 (S1)** follow that the number of **total plate count (TPC)** in milk exceeded the limit stated for the qualitative grade Q (up to 50 000 CFU/ml) only in two samples from total 21, namely at collecting days 17.7. 2011 and 15.12. 2011 (TPC – 110200, 1130000). Higher proportion from the total plate count represented desirable acidophilic microorganisms (MO acid) forming blue colonies. While undesirable alkaligenic microorganisms (MO alk), forming white colonies, represented lower proportion of TPC.

At **coliform bacteria (CB)** there was exceeded the value stated by standard (up to 1000 CFU/ml) also only in two samples (from total 21), which were analyzed 14.11. 2011 and 22.2. 2012 (CB – 2500, 7700)

Numbers of **psychrotrophic microorganisms – PTM** (up to 50 000 CFU/ml) and **thermoresistant microorganisms – TRM** (up to 2000 CFU/ml) did not exceed the standard limits.

In the microbiological parameters of milk from the **stable 2 (S2)** were usually found worse results than in the milk from stable 1 (S1). Table 13 shows that the numbers of **total plate count (TPC)** were higher than the limit stated for qualitative grade 1 (up to 100 000 CFU/ml) in the six samples (TPC – 120000, 170000, 105000, 128000, 370000, 220097) from 15 analyzed. In this case numbers of alkaligenic microorganisms (MO alk) exceeded over the numbers of acidophilic microorganisms (MO acid).

These results also correspond with other measured values, like the number of **psychrotrophic microorganism – PTM**, where 6 samples (68000, 70000, 59000, 93000, 60000, 108000) exceeded limit stated by the standard (up to 50 000 CFU/ml). Numbers of **coliform bacteria** were found above the limit (up to 1000 CFU/ml) also in the 6 samples (CB – 2000, 8200, 1700, 9800, 22300, 5700) from the total 15 milk samples.

In the **thermoresistant microorganisms (TRM)** there was detected value above the limit of the standard (up to 2000 CFU/ml) only in one sample – 20.7. 2011 (TRM – 15000).

Statistical dependence of the total plate count on the measured temperature (T) was not proven even in the first stable, even in the case of the second stable (see 5.3 statistical research).

Table 12: Stable 1 – number of microorganisms/ml of raw milk and air temperature

Date	TPC	MO alk	MO acid	CB	PTM	TRM	T (°C)
14.4.2011	27000	4000	23000	40	17000	1800	8
5.5.2011	3200	400	2800	12	8100	10	13
18.5.2011	27000	2000	25000	20	4600	30	24
15.6.2011	2300	500	1800	11	480	8	26
23.6.2011	3500	1400	2100	19	1300	20	29
13.7. 2011	110200	200	110000	72	8500	16	28
20.7.2011	29000	1000	28000	52	24600	200	19
17.8.2011	26000	4000	22000	630	3500	20	26
25.8.2011	22700	20000	2700	250	35000	20	28
21.9.2011	2500	1300	1200	60	9100	< 100	19
26.9.2011	4100	2200	1900	20	200	< 100	23
5.10.2011	2600	600	2000	16	280	80	18
13.10.2011	4800	2600	2200	490	500	100	10
7.11.2011	48000	1000	47000	30	260	< 100	13
14.11.2011	3200	400	2800	2500	3400	20	-1,5
5.12.2011	36100	100	36000	11	1200	10	5
15.12.2011	1130000	230000	900000	9	12000	40	4
16.1.2012	7200	3500	3700	820	2300	< 1000	-2
25.1.2012	3500	1000	2500	12	300	30	0
16.2.2012	4100	1800	2300	43	630	200	-1
22.2.2012	45000	3000	42000	7700	13000	10	2,4

Table 13: Stable 2 – number of microorganisms/ml of raw milk and air temperature

Date	TPC	MO	MO acid	CB	PTM	TRM	T (°C)
14.4.2011	47000	30000	17000	50	1400	400	8
5.5.2011	33000	10000	23000	490	2900	91	13
18.5.2011	120000	56000	70000	980	39000	100	24
15.6.2011	17000	9000	8000	50	3200	40	26
23.6.2011	66000	1000	65000	440	35000	40	29
13.7.2011	170000	120000	50000	2000	68000	100	28
20.7.2011	105000	36000	69000	8200	38000	15000	19
17.8.2011	95000	65000	30000	800	70000	500	26
25.8.2011	128000	11000	18000	460	59000	< 1000	28
21.9.2011	98000	59000	39000	100	14500	90	19
26.9.2011	370000	160000	210000	1700	93000	< 100	23
5.10.2011	96000	20000	76000	9800	60000	30	18
13.10.2011	220097	220000	97	22300	108000	600	10
7.11.2011	39000	14000	25000	550	7500	100	13
14.11. 2011	74000	45000	29000	5700	32000	20	-1,5

Chart 5 shows that in the milk samples from stable 2 were observed much higher values of **total plate counts (TPC)** than in the samples from stable 1. Sudden increase of TPC in milk from stable 1 was probably caused by stress of cows induced by movement of cattle.

Worse microbiological quality of raw milk in the stable 2 is also obvious from the chart 6, numbers of **psychrotrophic microorganisms (PTM)** were much higher than in the milk from stable 1.

Chart 5: Total plate count (TPC) in milk

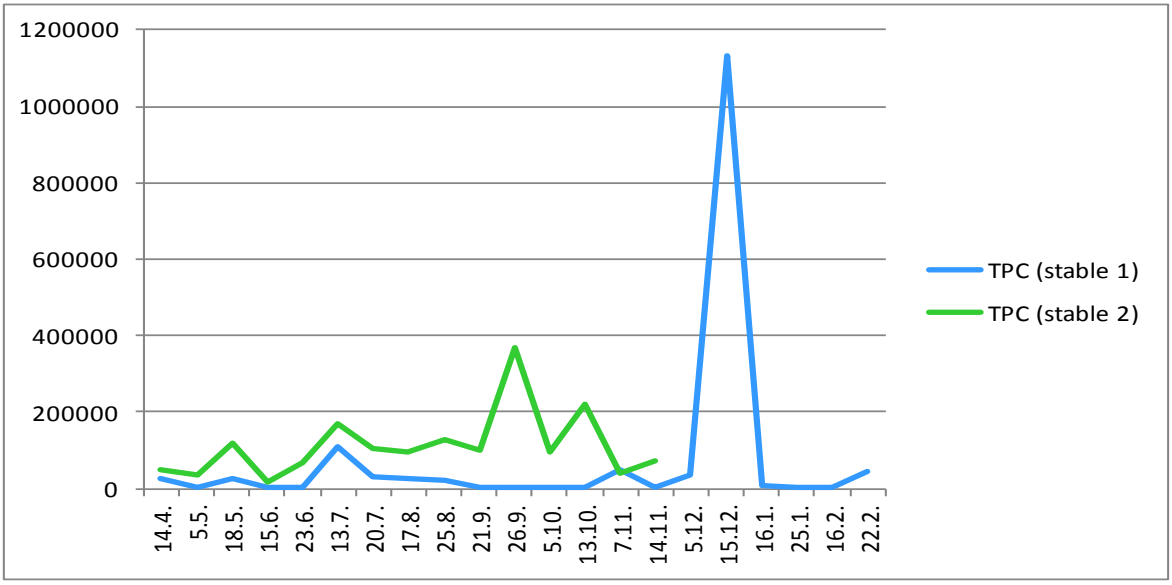
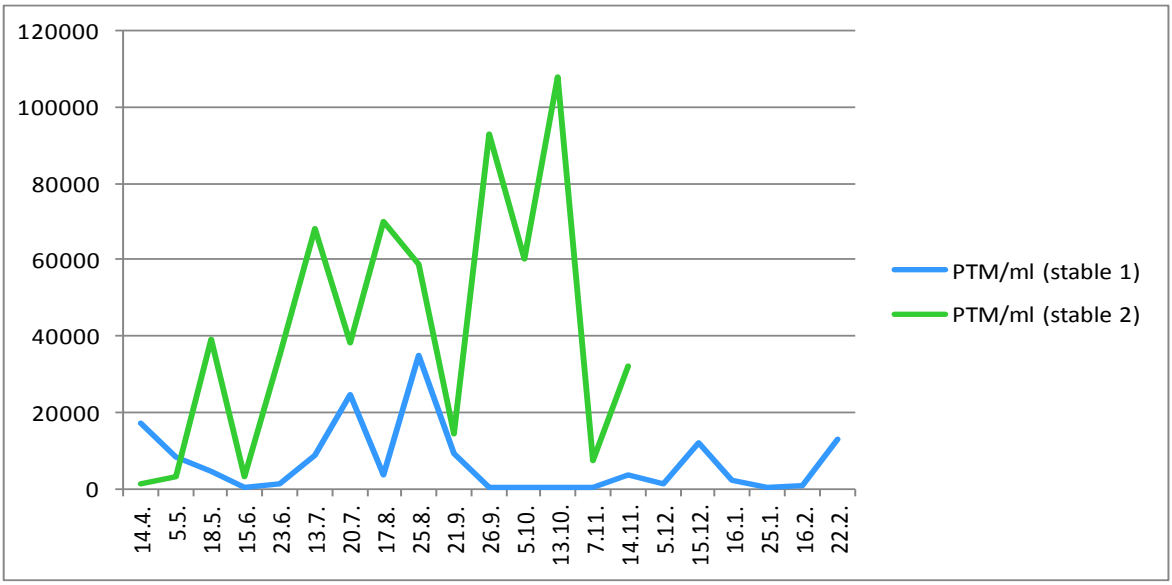


Chart 6: Psychrotrophic microorganisms (PTM) in milk



5.2 Model experiment with the application of the Lactococcus culture

In the course of monitoring the quality of raw milk, two model experiments were also carried out. First experiment was initiated in the summer (15. 7. 2011 – 15. 1. 2012) and the second one started in the autumn (1. 10. 2011 – 10. 3. 2012) in order to monitor the season effect on the technological parameters of milk. At the same time in the model experiments the influence of the application of the special Lactococcus culture on the quality of sterilized products was observed. In the research the samples of storage milk with and without application of the Lactococcus culture were evaluated.

The observation was performed at two different stables. While animals in the first stable (S1) did not have access to the pasture, to the cattle from second stable (S2) was allowed to graze in the pasture.

From the table 14 can be stated, that application of Lactococcus culture (CCDM 17) did not adversely affect any of the monitored parameters. In the first and also second model experiment **physico-chemical** values (pH, SH) of raw milk were comparable in both stables (S1, S2). Significant variations were not evident even after 24 hours of storage at 6 °C. After 48 hours of storage (6 °C) slight increasing of the titratable acidity (SH – 7,6) was already monitored, but the limit value for the technological processing of milk on the pasteurizer was not exceeded (8,5 SH).

In the tables 15 and 16, the **technological parameters** of milk samples from stable 1 and stable 2 from both experiments are shown. Even there the application of Lactococcus culture did not negatively affect the monitored parameters. On the contrary, after 48 hours of storage better **heat stability** (S1 – 9 minutes 18 seconds; S2 – 5 minutes 58 seconds) in the experimental samples with the culture were measured compared to the samples without culture (S1 – 6 minutes 2 seconds; S2 – 4 minutes 0 seconds). In general heat stability of milk from the stable 1 was considerable higher (S1 – 6 minutes 2 seconds; 9 minutes 18 seconds), implying that for the production of sterilized dairy products that milk would be more suitable. Lower heat stability in the milk samples from the stable 2 (S2 – 4 minutes 0 seconds; 5 minutes 58 seconds) was probably caused by the higher number of psychrotrophic microorganisms with the possible undesirable production of proteolytic and lipolytic enzymes, that may deteriorate the heat stability.

Table 14: Physico-chemical parameters in the milk – 1. + 2. Experiment

Physico-chemical parameters		FIRST EXPERIMENT					SECOND EXPERIMENT				
		Raw milk	Without culture		With culture		Raw milk	Without culture		With culture	
			24h (6°C)	48h (6°C)	24h (6°C)	48h (6°C)		24h (6°C)	48h (6°C)	24h (6°C)	48h (6°C)
Stable 1	SH	7,1	7,1	7,6	7,1	7,6	7,0	7,4	7,4	7,4	7,6
	pH	6,7	6,7	6,8	6,7	6,7	6,8	6,7	6,7	6,7	6,7
Stable 2	SH	7,0	7,1	7,4	7,4	7,6	7,2	7,2	7,2	7,2	7,4
	pH	6,7	6,7	6,7	6,7	6,7	6,8	6,8	6,7	6,7	6,7

Table 15: Technological parameters of milk – 1. Experiment

Technological parameters		FIRST EXPERIMENT				
		Raw milk	Without culture		With culture	
			24h (6°C)	48h (6°C)	24h (6°C)	48h (6°C)
Stable 1	Fermentability (SH)	37,0	34,8	36,0	35,0	34,8
	Rennetability	6 min 40 s	6 min 12 s	6 min 22 s	6 min 42 s	6 min 10 s
	Heat stability	8 min 19 s	-	6 min 02 s	-	9 min 18 s
Stable 2	Fermentability ((SH)	36,0	36,4	36,4	34,8	35,0
	Rennetability	5 min 18 s	5 min 58 s	6 min 52 s	6 min 58 s	7 min 03 s
	Heat stability	4 min 58 s	-	4 min 00 s	-	5 min 58 s

Table 16: Technological parameters of milk – 2. Experiment

Technological parameters		SECOND EXPERIMENT				
		Raw milk	Without culture		With culture	
			24h (6°C)	48h (6°C)	24h (6°C)	48h (6°C)
Stable 1	Fermentability (SH)	31,4	32,0	31,8	31,8	32,0
	Rennetability	5 min 04 s	4 min 56 s	5 min 18 s	5 min 48 s	4 min 51 s
	Heat stability	8 min 12 s	-	6 min 49 s	-	8 min 12 s
Stable 2	Fermentability (SH)	32,6	34,0	34,0	32,0	32,6
	Rennetability	6 min 05 s	5 min 09 s	6 min 08 s	6 min 12 s	7 min 00 s
	Heat stability	4 min 56 s	-	3 min 59 s	-	5 min 03 s

Tables 17 and 18 show that the results of the microbiological parameters in milk samples from the stable 1 (S1) were much better than in the stable 2 (S2). The number of **total plate count – TPC** in milk samples from stable 1 was lower as in the first experiment (S1 – 29000; S2 – 105000), as in the second one (S1 – 2600; S2 – 96000).

In the first experiment (tab. 17) number of **psychrotrophic microorganisms,** which may produce undesirable thermostable proteolytic enzymes, was in the stable 1 (S1) much lower than in samples of raw milk from the stable 2 (S1 – 200; S2 30000).

In the second experiment even worse microbiological results (tab. 18) were detected in the stable 2. In addition the number of psychrotrophic microorganisms (PTM) was two times higher (PTM – 60000) compared with the first model experiment (PTM – 30000). These parameters probably caused worse taste of milk from the stable 2, which was detected in the sensory evaluation of sterilized milk.

In the first model experiment after 48 hours of storage (6 °C) and before technological processing, the number of **total plate count (TPC)** did not exceed the value stated by regulation on TPC density (Veterinary requirements for milk and milk products – max. density 300 000 CFU/ml) in the samples without culture (S1 – 143000; S2 – 180000) and even in the samples with application of Lactococcus culture (S1 – 180000; S2 – 238000).

In the second experiment that regulation on the number of TPC before technological processing was also complied in both samples – without culture (S1 – 121000; S2 – 190000) and with culture (S1 – 203000; S2 – 263000).

Table 17: Number of microorganisms/ml of milk – 1. Experiment

Microbiological parameters		First experiment				
		Raw milk	Without culture		With culture	
			24h (6°C)	48h (6°C)	24h (6°C)	48h (6°C)
Stable 1	TPC	29 000	72 000	143 000	129 000	180 000
	MO alk	1 000	18 000	62 000	29 000	40 000
	MO acid	28 000	54 000	81 000	100 000	140 000
	CB	120	-	-	-	-
	PTM	200	-	-	-	-
	TRM	200	-	-	-	-
Stable 2	TPC	105 000	143 000	180 000	223 000	238 000
	MO alk	36 000	56 000	80 000	43 000	78 000
	MO acid	69 000	87 000	100 000	180 000	160 000
	CB	1 800	-	-	-	-
	PTM	30 000	-	-	-	-
	TRM	15 000	-	-	-	-

Table 18: Number of microorganisms/ml of milk – 2. Experiment

Microbiological parameters		Second experiment				
		Raw milk	Without culture		With culture	
			24h (6°C)	48h (6°C)	24h (6°C)	48h (6°C)
Stable 1	TPC	2 600	9 590	121 000	4 910	203 000
	MO alk	600	590	31 000	810	53 000
	MO acid	2 000	9 000	90 000	4 100	150 000
	CB	160	-	-	-	-
	PTM	280	-	-	-	-
	TRM	30	-	-	-	-
Stable 2	TPC	96 000	96 400	190 000	111 000	263 000
	MO alk	20 000	6 400	100 000	14 000	53 000
	MO acid	76 000	90 000	90 000	97 000	210 000
	CB	9 800	-	-	-	-
	PTM	60 000	-	-	-	-
	TRM	80	-	-	-	-

From the table 19 it is obvious, that in all milk samples after sterilization and subsequent storage for 6 months at room temperature (20 °C) the parameters of the commercial sterility for the **total plate count** – TPC (max. density TPC 100 CFU/ml) were met. After sterilization and storage of milk, the decrease at maximum 0,2 pH units were found in all samples.

Table 20 shows that between samples without culture and with culture, even between the collecting places – stables (S1, S2) were not found noticeable differences in the **consistency** of milk.

However during the commissional assessment of the **taste properties**, significant difference was detected by all evaluators – samples with Lactococcus culture CCDM 17 had clean taste. Samples from the milk of the first stable (S1) were very tasty, in the first experiment, samples of milk from the second stable (S2) were also without any defects.

In the second experiment, during the commissional assessment **slightly bitter and unclean taste** was found in the samples without culture from the stable 2 (S2), which was probably caused by negative influence of the season (link to the diet) in combination with the high numbers of the psychrotrophic microorganisms (PTM) analyzed in the initial raw milk (the excepted effect of the proteolytic and lipolytic enzymes).

The crucial was the finding that in the samples from the same stable (S2), but with the application of Lactococcus culture, the taste of the milk was clean and only gently cooked.

Due to the heating above 110 °C the taste of all milk samples was slightly caramelized. That effect is connected with so called Maillard reaction, in which lactose reacts with the free amino acids and that leads to the non-enzymatic browning. Finally the change of the colour and taste is founded in all sterilized products.

Table 19: pH and total plate count in milk after 6 months (20 °C)

		First experiment		Second experiment	
		Without culture	With culture	Without culture	With culture
Stable 1	pH_1	6,36	6,35	6,39	6,39
	pH_2	6,36	6,37	6,40	6,40
	pH_3	6,38	6,36	6,41	6,38
	pH_4	-	-	6,38	6,41
	pH_5	-	-	6,39	6,40
	TPC_1	40	20	10	neg/0,1ml
	TPC_2	50	10	neg/0,1ml	neg/0,1ml
	TPC_3	30	20	30	10
	TPC_4	-	-	10	20
	TPC_5	-	-	10	20
Stable 2	pH_1	6,39	6,40	6,39	6,41
	pH_2	6,37	6,35	6,41	6,39
	pH_3	6,35	6,35	6,41	6,39
	pH_4	-	-	6,40	6,39
	pH_5	-	-	6,39	6,39
	TPC_1	20	neg/0,1ml	neg/0,1ml	10
	TPC_2	10	10	neg/0,1ml	20
	TPC_3	neg/0,1ml	neg/0,1ml	neg/0,1ml	10
	TPC_4	-	-	neg/0,1ml	neg/0,1ml
	TPC_5	-	-	neg/0,1ml	neg/0,1ml

Table 20: Sensory evaluation after 6 months (20 °C)

Sensory properties		First experiment		Second experiment	
		Without culture	With culture	Without culture	With culture
Stable 1	**Consistency**	Unchanged	Unchanged	**Unchanged**	**Unchanged**
	Taste	Clean and slightly cooked	Clean, **tasty** and slightly cooked	**Clean and slightly cooked**	**Clean, tasty and slightly cooked**
Stable 2	**Consistency**	Unchanged	Unchanged	**Unchanged**	**Unchanged**
	Taste	Slightly unclean and cooked	Clean, **tasty** and slightly cooked	**Slightly bitter, unclean and cooked**	**Clean and slightly cooked**

5.3 Statistical evaluation

During the research the dependence between the psychrotrophic microorganisms (PTM) on the total plate count (TPC) by the regression analyses was determined. In the stable 1 (S1) there was found small dependence, while in the second stable (S2) there was found high dependence (chart 7).

Dependence of the PTM on the TPC in the stable 2 (chart 7):

High dependence: Correlation coefficient = 0,819; Coefficient of determination = 0,670

Regression line: y´= 7529,941 + 0,309

Testing of the regression coefficient β:

H0: $\beta = 0$; H1: $\beta \neq 0$; α = 0,05 and 0,01; test criterion = 5,138; p value = 0,000

0,000 < 0,05 → p < α = reject H0 on the significance level of 0,05.

0,000 < 0,01 → p < α = reject H0 on the significance level of 0,01.

The regression coefficient was statistically significant.

Confidence interval for regression coefficient: P (0.179 <β <0.429) = 0.95.

TPC of author (TPC – A) and TPC of Buštěhrad (TPC – B) – stable 2, chart 8:

High dependence: Correlation coefficient = 0,786; Coefficient of determination = 0,617

Regression line: y ´ = 12390,788 + 0,153

Testing of the regression coefficient β:

H0: $\beta = 0$; H1: $\beta \neq 0$; α = 0,05 and 0,01; test criterion = 4,579; p value = 0,001

0,001 < 0,05 → p < α = reject H0 on the significance level of 0,05.

0,001 < 0,01 → p < α = reject H0 on the significance level of 0,01.

The regression coefficient was statistically significant.

Confidence interval for regression coefficient: P (0,081< β < 0,225) = 0,95.

F test = analysis of variance

H_0: $\sigma_1^2 = \sigma_2^2$; H_1: $\sigma_1^2 \neq \sigma_2^2$; α: 0,05 and 0,01; $S_1^2 >$; S_2^2

Test criterion = 26,30937; p value = 0,000

0,000 < 0,05 → p < α = reject H0 on the significance level of 0,05.

0,000 < 0,01 → p < α = reject H0 on the significance level of 0,01.

Between the variances was proven the statistically significant difference on the significance level of 0,05 and 0,01.

T test = comparison of the averages (based on the results from the F test)

H_0: $\mu_1 = \mu_2$; H_1: $\mu_1 \neq \mu_2$; α: 0,05 a 0,01

Test criterion = - 3,49088; p value = 0,001614

0,001614 < 0,05 → p < α = reject H0 on the significance level of 0,05.

0,001614 < 0,01 → p < α = reject H0 on the significance level of 0,01.

Between the averages was proven the statistically significant difference on the significance level of 0,05 and 0,01.

Dependence of the TPC and PTM on the temperature:

Dependence of the TPC on the temperature:

Stable 1: Pearson Correlation Coefficient = - 0,180

Stable 2: Pearson Correlation Coefficient = 0,176

Dependence of the PTM on the temperature:

Stable 1: Pearson Correlation Coefficient = 0,206

Stable 2: Pearson Correlation Coefficient = 0,219

Dependence of the number of total plate count (TPC) and the number of psychrotrophic microorganisms (PTM) on the measured air temperature (T) was not statistically proven, that result was probably caused by too big differences in the measured temperatures.

Dependence of the technological parameters (RA, FA) on the TPC and PTM:

From the statistical examination any significant correlation was proven, thus in the case of the higher number of psychrotrophic microorganisms (PTM) or total plate count (TPC) it cannot be clearly deduced deterioration of technological parameters (rennetability, fermentability) of milk.

Chart 7: Dependence of PTM and TPC

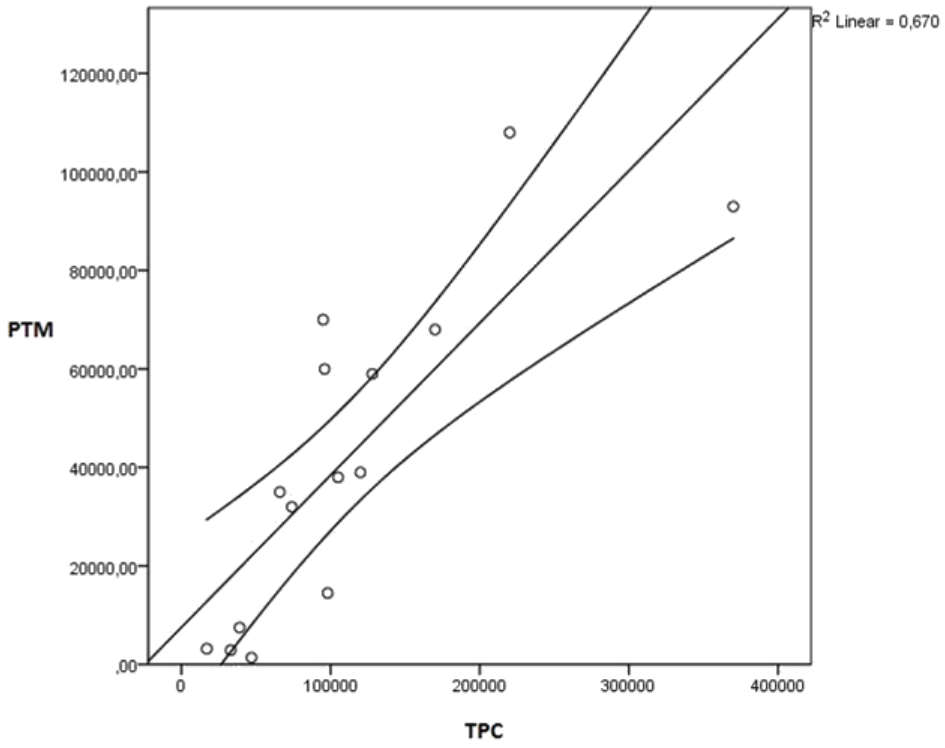
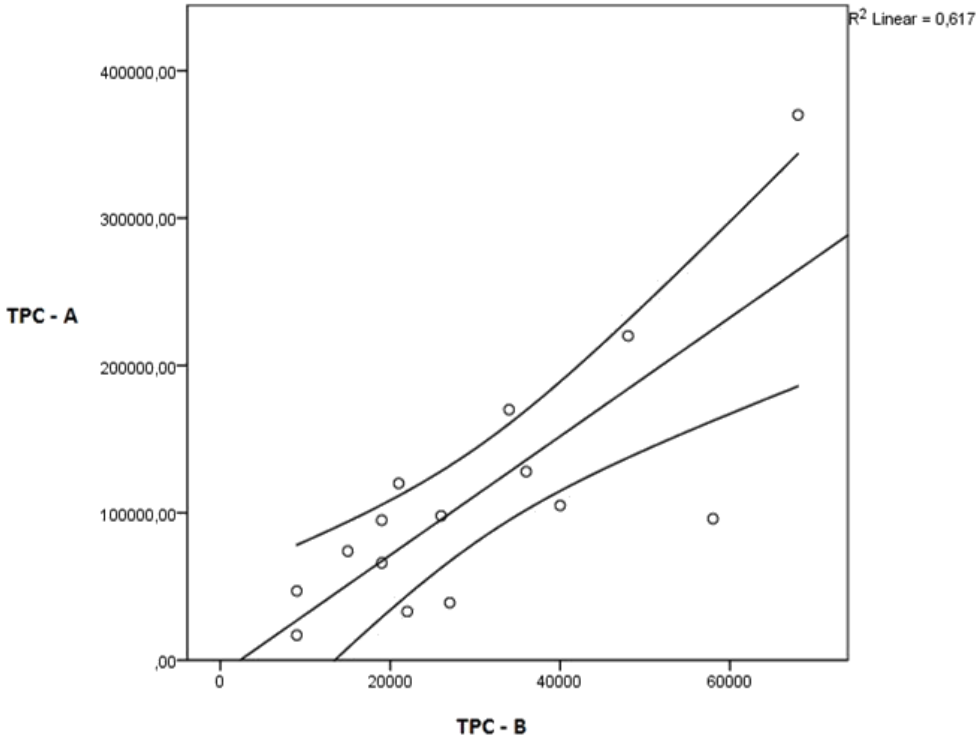


Chart 8: Dependence of author's TPC (–A) and Buštěhrad TPC (–B)



6 DISCUSSIONS

Diploma thesis was focused on the monitoring of the raw milk quality and the influence of the various indicators on its technological parameters. Monitoring the quality of raw milk plays an important role in the primary agricultural production; it includes cow's state of health, quality of milking equipment sanitation, economic aspect, etc. At the same time, it is a significant indicator for the manufacturing entity – dairy plant.

To monitor the changing raw milk quality is for farmers important especially in connection with the dairy cow's health control. The increased number of somatic cell count (SCC) is one of the key indicators that may suggest the beginning udder inflammation (mastitis), the cows stress inflicted by different effects (environment, human factor) or nutritionally unbalanced food quality. Yildiz (2010) writes about the increased number of SCC due to the unbalanced diet and the occurrence of the mycotoxins in poorly preserved feeds. In contradiction another author (Fernandes, 2009) describes the emergence of mastitis due to the udder contamination by various microorganisms, which pass through the teat duct to the milk and cause a noticeable increase in the number of SCC.

During the research, the somatic cell count data were obtained from the laboratory Buštěhrad. From these results, it is evident that the quality of milk is significantly influenced by the human factor and the method of milk extraction, too. Comparing the situation of the observed stables (1 and 2), it was found that the amount of SCC in the individual stables varied considerably. While the number of SCC of all samples from the first stable met the limit stated by the valid standard (up to 400 000/ml), the results from the second stable were considerably worse. In three samples out of fifteen, there were recorded values exceeding the standard limit and only in two samples the number of SCC was lower than the limit value (up to 250 000/ml) for the good technological processing of milk (Pechačová, 2011).

Yildiz (2010) also describes that limit; according to this author, it is necessary to achieve the number of SCC in the raw milk lower than the mentioned 250 000/ml of milk in order to obtain yogurt of high sensory quality. If the SCC is higher than 400 000/ml, it can reduce the metabolic activity of yogurt starter bacteria and if SCC exceeds the 1000 000/ml of milk, it leads to the complete inhibition of starter bacteria.

As a consequence of the non-standard care of the animals and level of technical support in the stable 2 (S2), the economical parameters of the milk production became worse in this facility. The results confirmed that the inevitable movement of the cattle from the stable 2 (S2) to the stable 1 (S1), it means a unification of the two stables, which were performed on 15.11. 2011, had a distinct negative impact on the quality of milk in that unified stable (S1). The increased number of somatic cell count after this date was probably caused by the putting together of two herds of animals, which perhaps led to the psychological stress and deterioration of the animal health. In the following period of a few weeks, thanks to the right treatment, hygienic milk extraction and proper care of the animals, equalization of this deviation was recorded.

For the dairy plant, the number of somatic cell count is the crucial factor influencing technolgical processing of the milk, with an impact on determining the purchase price.

Fox and McSweeney (1998) evaluated the physico-chemical parameters of milk, which represent another indicator of the milk quality. The active acidity (pH) of milk at 25 °C usually occurred in the range of pH = 6,5 – 6,7. Increased value of the milk active acidity may indicate inflammation of the udder in dairy cows, lower pH often occurs in the milk of early lactation, when the pH of "colostrum" is about pH = 6,0. The values of the titratable acidity (SH) are used to determine the freshness of milk; according to the standard (ČSN 57 0529), the titratable acidity established by the method of Soxhlet-Henkel should be in the range of SH = 6,2 – 7,8.

While observing the physico-chemical parameters (titratable acidity – SH and active acidity – pH,), noticeable differences were not found in raw milk; all analyzed samples occurred in the range for the titratable acidity stated by the standard.

Milk from both stables (S1 and S2) was delivered to the dairy plant Bohušovice (Litoměřice ditrict), where dairy products like yogurt, sterilized milk, cream cheese etc. were produced (appendix 6 and 7). Therefore all the basic technological parameters, which could affect technological processing and quality of the final products, were monitored – i.e. fermentability, rennetability and heat stability.

Fermentability could be depicted as an ability of milk to create good conditions for growing of the needed microorganisms, especially lactic acid bacteria. According to the

standard (ČSN 57 0529, 1998) fermentability, as per Soxhlet-Henkela method, should have the minimum value of 25.

The fermentability is important primarily for the production of ferment products and it is used as well as the proof of the residues of inhibitory substances (RIL). The outcomes of the samples were more or less identical, just on one collecting day lower values in the samples from the both stables (S1, S2) were registered. With regard to the negative occurrence of the residues of inhibitory substances, according to data obtained from the laboratory Buštěhrad, this difference was probably caused by incorrect inoculation of the milk or contamination of the yogurt culture from the air. The registered values from the rest of the samples complied with parameters for production of ferment products.

Term rennetability can be depicted as a suitability of raw milk for enzymatic coagulation or suitability of milk for cheese processing (Pešek, 1997). Rennetability is usually measured and expressed in the rennet coagulation time (RCT). As per O'Brien and Guinee (2011), in Irish dairy production the RCT was significantly influenced by season, that variability is related primarily to changes of fat, casein, protein and lactose concentrations in the milk during the year. Similar experiment was made in Poland, where Sitkowska and Piwczyński (2011) observed milk from Polish Holstein cows. Their results show that the lowest concentration of fat, protein and dry matter was noticed in the summer period.

In the milk samples taken from the both stables (S1, S2) any considerable variation in the course of the year (2011/2012) was noticed in the rennetability. Almost all samples complied with the optimal RCT (5 – 7 min.). Just during the first measuring, the RCT was higher (8 min.) - that was probably caused by a little author's experience in this measuring.

Among the technological parameters belongs also thermostability of milk, which shows the ability of milk to resist the intensive heat treatment and to maintain the initial colloid property without coagulation or thickening, thus it is used to choose the appropriate milk for the production of sterilized products. O'Connell and Fox (2011) describe the commonly used method of thermostability measuring, where the time of the first coagulation of the milk sample in the oil bath is observed – so called heat coagulation time (HCT). Milk suitable for the production of sterilized products should have the heat coagulation time 5 minutes minimally.

Although statistical examination did not ascertain dependence of the rennetability and fermetability on the TPC and PTM, it has been proven that microbiological quality of milk can be related to its heat stability. Thermostability was measured in the milk samples from the model experiments. Milk from the first stable S1 had considerably higher thermostability and therefore it is more suitable for production of the sterilized milk products. Lower thermostability in the samples from the second stable S2 was probably caused by the higher number of psychrotrophic microorganisms, which may produce undesirable proteolytic enzymes, that deteriorate the thermostability of milk. This model experiment showed that the application of the Lactococcus culture had a positive effect on the thermostability of milk – the experimental milk samples compared with the control samples showed significantly much better thermostability. The Lactococcus culture was added to raw milk in purpose to modify the proportion of acidophilic and alkaligenic microorganisms, to the benefit of the first one. To preserve the biochemical processes which accompany fermentation, the inoculated milk was kept at max. temperature + 6 °C. Reduced portion of alkaligenic microflora with the significant enzymatic activity leads to the increasing of HCT – that finding offers to be widely used in dairy production practice.

Among to the observed microbiological parameters belong total plate count (TPC) and number of the coliform, psychrotrophic and thermoresistant microorganisms. Limit given by the standard for the total plate count (TPC) is for the third grade quality up to the 800 000 CFU/ml. This limit has not been exceeded neither at analyzed samples from the first nor from the second stable.

However at the first stable (S1), the milk samples showed much better quality, where milk complied the limit given for the quality grade Q (up to 50 000 CFU/ml) at 19 samples (from total 21) and share of the desirable acidophilic microorganisms, was higher than share of the alkaligenic microorganisms. In the second stable (S2), just 4 samples (from total 15) achieved the quality grade Q and in addition, the limit given for the first quality grade (up to 100 000 CFU/ml) complied just 6 samples from 15 analysed. In this case, the amount of the undesirable alkaligenic microorganisms predominated over the amount of acidophilic microorganisms. Černá and Mergl (1971) similarly stated that when milk contains a low amount of acidophilic microorganisms, that result show a good hygiene of milk obtaining and early cooling down, in this case the alkaligenic microflora almost does not occur. On the contrary, an increased number of alkaligenic microorganisms shows

unsanitary milk obtaining, testifies of a low degree of hygiene applied in the second stable (S2) and high influence of human factor on the milk quality. The lower quality of milk was probably amplified by potential occurrence of mastitis in the subclinical form.

Results stated above are also related with the higher amount of the coliform bacteria at the second stable S2, which is usually found when the inadequate hygiene of the udder is provided. While at the first stable (S1) the number of coliform bacteria was over the limit (up to 1000 CFU/ml) just at one sample (from total amount 21 analysed), at the second stable (S2) the limit was exceeded by six samples (from total 15).

Bylund (1995) says that thermoresistant microorganisms are those, which can survive the temperatures above +70 °C, but are not able to grow in those high temperatures. Other author (Zelenka, 2006) also states that monitoring the thermoresistant microorganisms is important mainly because of the survival of their spores in the course of the primary thermal processing. Their increased amount is usually caused by unsuitable composition of the feeding ration and by the occurrence of the sporeforming microorganisms in low-grade silages. This fact is confirmed by Giffel et al. (2002), these authors mention the low-quality silages issues in their study too. According that study, by the application of the lactic acid bacteria and the control of the fermentation process in the silage, it is possible to reduce occurrence of spores in milk.

The value of thermoresistant microorganisms exceeded the limit standard (2000 CFU/ml) just in one case in the milk from the second stable (S2). This finding shows the high quality of feeds served to the cattle in the both stables.

Psychrotrophic microorganisms (PTM) belong to the serious group of the udder pathogens, mainly because of their ability to grow and multiply in temperatures between 0 °C to 7 °C, it means temperatures in which milk is usually stored (Bylund, 1995). The process of multiplying of the psychrotrophic microorganisms causes significant organoleptic changes of milk, e.g. taste, smell and other, which is inflicted by the production of microbiological proteolytic and lipolytic enzymes. Fernardes (2009) explains reasons for the increased presence of psychrotrophic microorganisms primarily by worse sanitation of milking equipment (remains of milk in the folds and rubber seals). The source can be found also in the bedding litter, vegetation, or water.

Analysed milk samples from the first stable (S1) did not exceeded the given limit for the number of psychrotrophic microorganisms (up to 50 000/ml). However in the second stable (S2) was the given limit exceeded at 6 samples (from total 15 analyzed). This could have a negative impact on the quality of the final dairy product.

During the research, the statistical evaluation was also performed. Results showed on the linear relationship between the total plate counts – TPC and PTM counts, with the correlation coefficient 0,819; and then among results of the TPC determined by the author of this diploma thesis and those found by the laboratory Buštěhrad, with the correlation coefficient 0,786.

Although the cattle from the second stable (S2), in comparison to the cattle from the first stable (S1), could graze on the pasture, the owner of the private farm decided to close the stable 2 (S2) and to move all the dairy cows to the stable 1 (S1). This arrangement was done due to the constantly negative results of the microbiological quality of milk from the stable 2. Most likely the mentioned problems were caused by the human factor and relatively lower technical level of the milking equipment (milking parlours of stable 1 and 2 – appendix 1 and 2). Unification of the cattle from both stables had to be made not only with regard to the health of the dairy cows, but also because of the economic aspect. If the number of the somatic cell count (SCC) in milk exceeded the limit of 400 000/ml, the dairy plant in which the observed milk was delivered, penalized suppliers by 0,30 CZK per one litter of milk. In the case of the higher number of the total plate count then 100 000 CFU/ml of milk, the deduction represented 0,70 CZK per one litter of milk. Unfortunately, both of these parameters were often exceeded in milk from the second stable (S2), which led to its further liquidation.

After the unification of the animals from the stable S1 and S2, significantly worse microbiological quality of milk was initially recorded. That was probably caused by the psychological stress of the dairy cows and initial personnel disorganization, because the staff had to deal with a new situation. Thanks to the proper care of animals, adaptation of the dairy cows, hygienically obtaining of the milk and personal settlement, the quality of milk returned to the common state.

The worse microbiological quality of milk from the stable 2 was evident also in the model experiments with the Lactococcus culture CCDM 17. Despite to the bad microbiological

parameters, the Lactococcus culture was able to increase the amount of the desirable acidophilic microflora. In the second model experiment (autumn 2011), there was probably reflected the impact of the season, when the higher number of the psychrotrophic microorganisms, which produce undesirable thermostable proteolytic and lipolytic enzymes and can affect the organoleptic properties of milk, was found in milk,. O'Brien and Guinee (2011) stated that seasonality of milk can be easily described as changes in the quality, composition and technological suitability of milk for the processing of dairy products during the calendar year.

The statistical examination did not ascertain dependence of the rennetability and fermentability on TPC and PTM counts, but according to the laboratory research, the microbiological quality of raw milk can be related to its heat stability. The increased number of the PTM in the second stable (S2) probably caused lower heat stability of milk, which was measured in the model experiment. After the application of the Lactococcus culture CCDM 17 and subsequent storage (48 hours, at + 6 °C), the thermostability of the milk samples was improved in milk from the both stables. The results from the model experiments (in the summer and also in the autumn) did not show any difference in the consistency between the control samples (e.i. without Lactococcus culture) and experimental samples (e.i. with the Lactococcus culture) and even not between the collecting places, e.i. stables (S1 and S2).

On the contrary, the sterilized milk samples from the second stable (S2), with addition of Lactococcus culture CCDM 17, had after six months of storage demonstrably better taste in the comparison with the control samples (without culture). Those samples had unclean and almost bitter taste.

The achieved results in this diploma thesis confirmed the importance of the connection between the raw milk quality and its technological properties, which are significant mainly for processing of fermented and long shelf life dairy products.

The economical indicators of the milk production depend also on the technological facilities, which in our study, on the observed farm, supported the decision for the farming of dairy cattle in the stable with much better hygiene and animal care.

7 CONCLUSIONS

Based on the obtained results, it can be stated that the goals of the diploma thesis have been fulfilled. The difference between stables (1 and 2) was evident due to increased number of the somatic cell counts in the milk from the stable 2. Similarly, the observed microbiological parameters (prevailing alkaligenic microflora, increased numbers of coliform and psychrotrophic microorganisms – PTM) of the analyzed milk from the same stable (S2) indicated the bad cattle-keeper approach and poor animal hygiene in that stable.

Counts of thermoresistant microorganisms, which usually correspond with the quality of served feed, were determined in milk from both stables in the range stated by the valid legislative standard. Thus, that possible negative impact of feeding on the milk quality has not been confirmed.

In spite of the fact, the cattle from the second stable (S2), on the contrary to the cattle from the first stable (S1), could graze in the pasture, the above mentioned unfavourable raw milk parameters has led the owner of that private farm to the liquidation of the stable 2. Cattle from that stable had been transported and the new increased and unified animal herd has been created. The relatively lower technical level of the milking equipment and the approach of the staff working in the stable 2 contributed to this decision, too. Once the unified herd had been formed, significantly worse microbiological quality of milk was recorded, but that status improved within a few weeks.

Values of the analysed physical-chemical, microbiological and technological parameters did not demonstrate any noticeable deviations during the year, resp. at the transition to the summer and winter diet.

Statistical evaluation showed a linear relationship between the total plate counts – TPC and PTM counts, with the correlation coefficient 0,819; and then among results of the TPC determined by the author of this diploma thesis and those found by the laboratory Buštěhrad, with the correlation coefficient 0,786.

The statistical examination didn’t ascertain dependence of rennetability and fermentability on TPC and PTM counts, but according to the laboratory research the microbiological quality of raw milk can be related to its heat stability. While milk from the stable 1 was characterized by the higher thermostability and good quality, the lower thermostability of

milk from the stable 2 was very probably caused by the higher number of PTM, which can produce proteolytic enzymes deteriorating also the heat stability of milk.

The both model experiments confirmed that the application of the Lactococcus culture had improved the observed heat stability. In addition, those experiments established the positive effect of the Lactococcus culture on the organoleptic properties of the sterilized milk. In the first phase of the experiment in the summer of 2011, the lower values of PTM counts were analysed and the taste of milk was not changed. In the second experiment in autumn 2011, the number of those microorganisms increased and a significant organoleptic change in the taste of the samples without addition of Lactococcus culture was observed. Those milk samples were characterized by the unclean or even bitter taste, but the samples with addition of Lactococcus culture had clean and delicious taste.

The achieved results revealed that the quality of milk in the monitored stables had been influenced mainly by the attitude of the cattle-keepers and by the way of milking and its primary treatment. From the point of view of the first hypothesis, no significant variations in the quality and technological properties of milk were found during the season. Nevertheless the results from stable 2 demonstrated the connection of the improper milking conditions and cattle-keepers approach with impaired microbiological parameters of milk.

The second hypothesis has been proven by the results of the model experiment with the Lactococcus culture. The influence of microbiological quality of raw milk on its technological parameters, in that case on the heat stability of milk, was found in laboratory conditions. Milk from the stable 1 with the lower number of microorganisms had the heat stability verifiably higher than the milk obtained from the stable 2.

Application of the Lactococcus culture had important influence on the milk quality; it improved not only the heat stability, but also the taste of the analyzed milk. Thus, the third hypothesis has been also confirmed. That finding is important mainly for the production of the sterilized milk, where the long shelf life is required and the negative effect of the undesirable enzymatic changes may occur.

The obtained results could serve as a basis for a further diploma thesis focused on improving of the milk quality by the addition of selected dairy cultures and on their effect on the organoleptic properties of milk and dairy products in the course of their shelf life.

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APPENDICES

List of the Appendices:

APPENDIX 1: MILKING PARLOUR IN THE STABLE 1 II
APPENDIX 2: MILKING PARLOUR IN THE STABLE 2 II
APPENDIX 3: CERTIFICATE OF ACCREDITATION – LABORATORY OF VÚM III
APPENDIX 4: DETERMINATION OF BACTERIA COUNT IV
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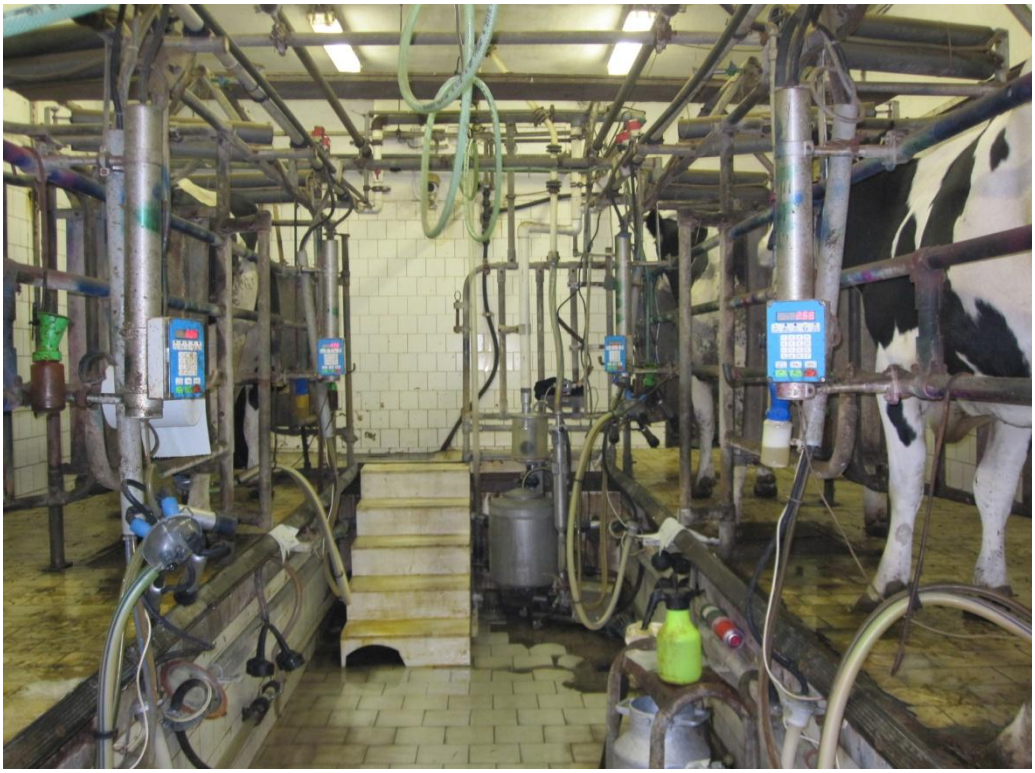
APPENDIX 6: FILLING OF THE YOGURTS IN DAIRY PLANT BOHUŠOVICE V
APPENDIX 7: TYPICAL PRODUCT OF DAIRY PLANT BOHUŠOVICE V

Appendix 1: Milking parlour in the stable 1 (photo Kamila Nováková, 2011)



Appendix 2: Milking parlour in the stable 2 (photo Kamila Nováková, 2011)

Český institut pro akreditaci,
obecně prospěšná společnost
110 00 Praha 1 - Nové Město, Opletalova 41

vydává

OSVĚDČENÍ O AKREDITACI

č. **503 / 2009**

pro

zkušební laboratoř č. **1319**

MILCOM a.s.
(IČ 16193296)
Zkušební laboratoř MILCOM a.s. VÚM
Ke Dvoru 12a, 160 00 Praha 6

Předmět akreditace:

Chemické analýzy složení mléka a mléčných výrobků a stanovení bodu mrznutí mléka. Mikrobiologické zkoušky mléka, mléčných výrobků a potravin v rozsahu uvedeném v příloze tohoto osvědčení.

Jménem akreditované zkušební laboratoře jedná Ing. Petr Roubal, CSc. a za správnost protokolů odpovídají Ing. Jitka Peroutková a Ing. Ondřej Elich.

Toto osvědčení o akreditaci vydal Český institut pro akreditaci, o.p.s. na základě posouzení splnění akreditačních kriterií podle

ČSN EN ISO/IEC 17025:2005

a po zjištění, že zkušební laboratoř je odborně způsobilá objektivně a nezávisle vykonávat činnosti uvedené v rozsahu předmětu akreditace.

Adresát tohoto osvědčení je oprávněn používat při své činnosti v rozsahu tohoto osvědčení a po dobu jeho platnosti vedle svého názvu označení „akreditovaná zkušební laboratoř č. 1319", pokud dodržuje veškeré příslušné předpisy vztahující se k činnosti akreditované zkušební laboratoře, včetně předpisů vydaných Českým institutem pro akreditaci, o.p.s.

Prokáže-li se, že adresát tohoto osvědčení nesplní akreditační kriteria rozhodná pro jeho vydání a závazky podmiňující akreditaci, může Český institut pro akreditaci, o.p.s. účinnost tohoto osvědčení pozastavit nebo osvědčení o akreditaci zrušit nebo změnit.

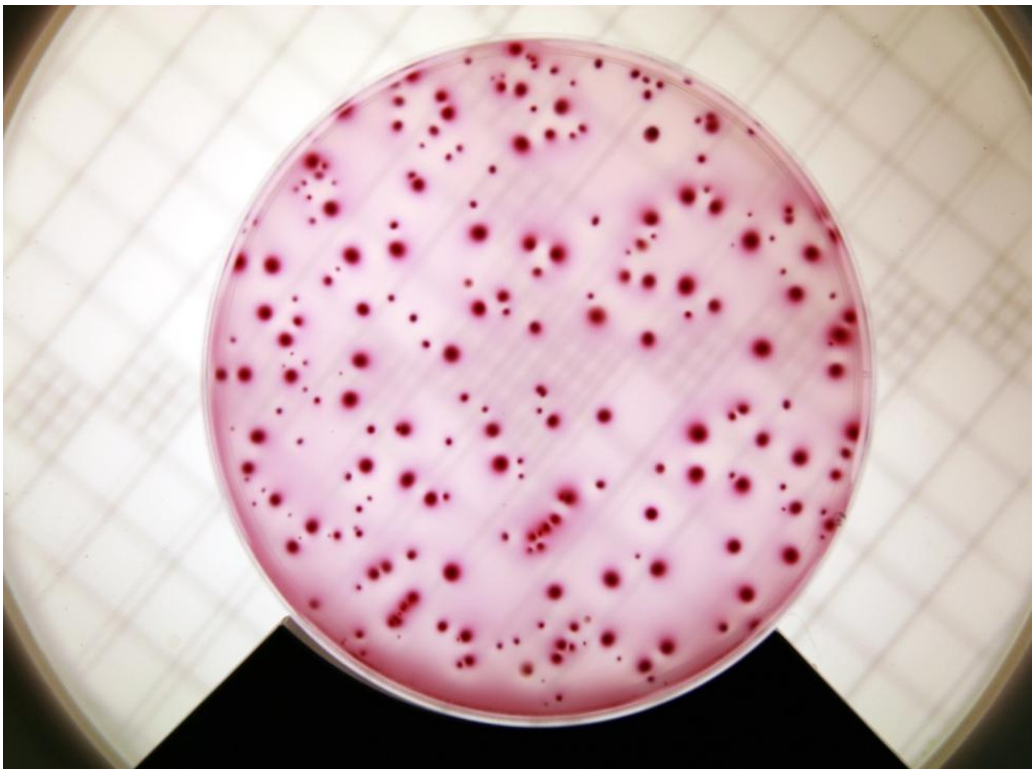
Toto osvědčení platí do: **10.08.2014**

V Praze dne: 31.08.2009

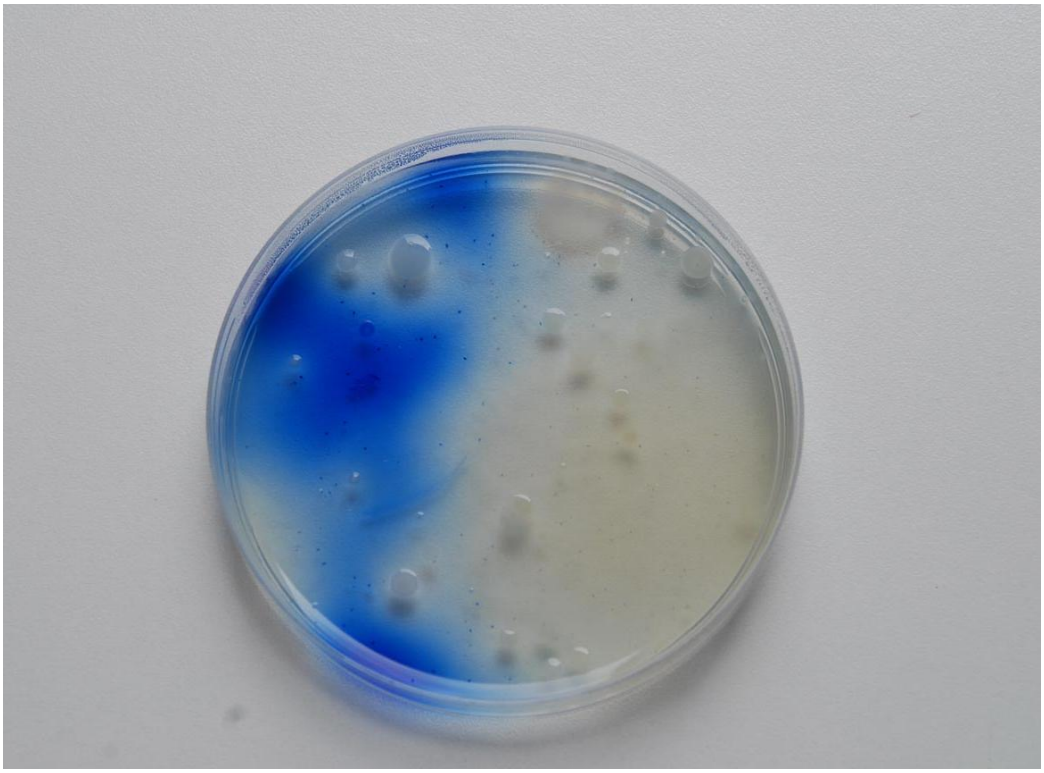
Ing. Jiří Růžička, MBA
ředitel
Českého institutu pro akreditaci, o.p.s.

Poučení:
Proti tomuto osvědčení, pokud jde o rozsah předmětu akreditace, má adresát možnost podat písemné námitky do 10 dnů od jeho převzetí. Námitky nemají odkladný účinek.

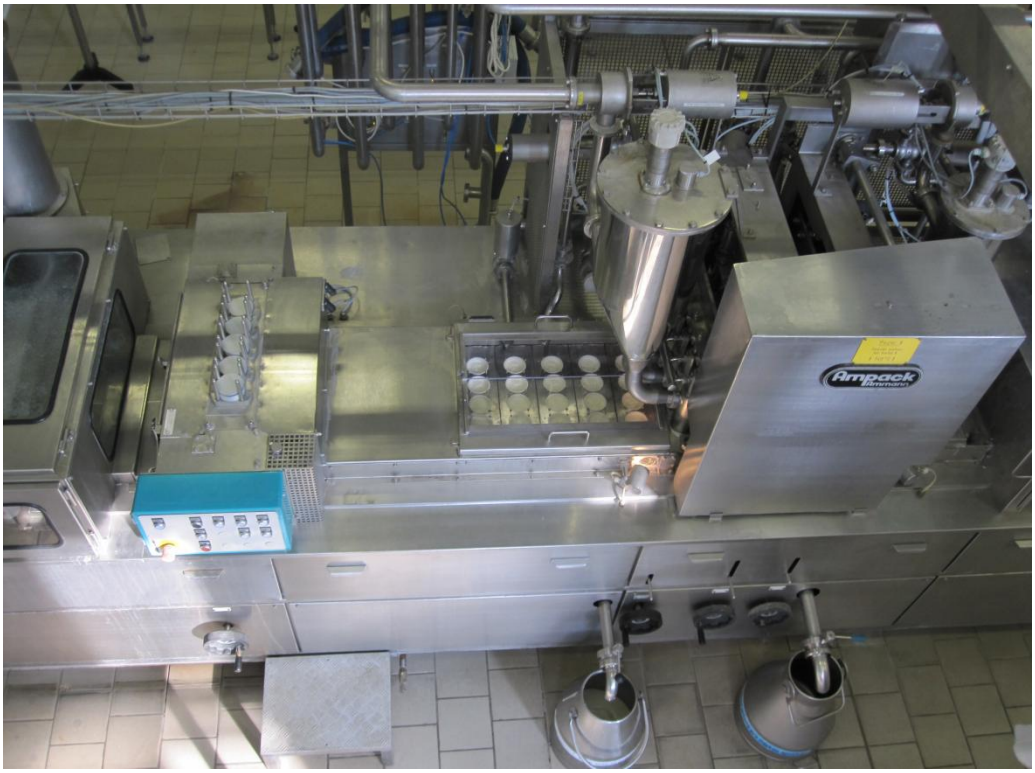
Appendix 3: Certificate of Accreditation – laboratory of VÚM (MILCOM a.s., 2011)



Appendix 4: Determination of bacteria count (photo Kamila Nováková, 2011)



Appendix 5: Acidophilic and alkaligenic MO (photo Kamila Nováková, 2011)



Appendix 6: Filling of the yogurts in dairy plant Bohušovice (photo Kamila Nováková, 2011)

Appendix 7: Typical product of dairy plant Bohušovice (Dairy plant Bohušovice, 2011)