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EFFECTS OF STORAGE ON THE MAJOR CONSTITUENTS OF RAW MILK

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ABSTRACT

Milk testing and quality control should be carried out at all stages of the dairy chain. Milk can be tested for quantity, organoleptic characteristic, compositional characteristic, physical and chemical characteristics, hygienic characteristics, adulteration or drug residues. The content of the major constituents of raw milk is important for milk payment system. Enzymes naturally present in the milk can change the chemical composition of raw milk. Also, enzymes secreted by bacteria or enzymes from somatic cells can degrade the raw milk composition. Products of these degradation reactions can have undesirable effects on milk structure, smell and taste. It is very important that farm-fresh raw milk be cooled immediately to not more than 8 °C in the case of daily collection, or not more than 6 °C if collection is not daily. During transport the cold chain must be maintained. An authorized person, properly trained in the appropriate technique, shall perform sampling of bulk milk in farm. Laboratory samples should be dispatched immediately after sampling to the dairy company and consequently to the testing laboratory. The time for dispatch of the samples to the testing laboratory should be as short as possible, preferably within 24 h. Laboratory samples shall be transported and stored at temperature 1 to 5 °C. Higher temperatures may adversely affect the composition of the laboratory sample and may cause disputes between the farmer, the dairy company and the laboratory. The effect of refrigerated storage at temperature 4 °C during 24 h on the composition of raw milk were investigated in this work, because we wanted to know how the milk composition will be changed and how the laboratory results will be affected. In many cases, the samples are not preserved with chemical preservants like azidiol, bronopol, potassium dichromate or Microtabs. We found, that the composition of raw cows' milk after 24 was changed significantly (p >0.005). We found an average decrease in the fat content of -0.04 g/100g, increase in the protein content of +0.02 g/100g, increase in the lactose content of +0.02 g/100g, increase in the solid-not-fat content of +0.02 g/100g and decrease in the total solid content of -0.02 g/100g. It is necessary to cool the raw cows' milk after the milking to decrease the changes in milk composition caused mainly due to the lipolytic activity of lipase.

Keywords: raw milk; milk composition; fat content; protein content

INTRODUCTION

The aim of this work was to investigate how composition of raw milk changes after 24 hours of storage at temperature 4 °C. According to the international standard **ISO 707 (2008)** the raw milk should be immediately transported to the laboratory at temperature 1 – 5 °C and analysed within 24 hours after collection. When sample refrigeration is not possible, sample must be preserved by appropriate means **(Kroger, 1985)**.

There are several studies, which were focused on the relationship between quality of dairy products and quality of raw milk. Very important factor is temperature during the storage **(Bachman and Wilcox, 1990; Valík et al., 2011)**. Also, contamination of raw milk before processing is an important factor **(Forsbäck et al., 2010)**.

According to the **Celestino, Iyer and Roginski, (1996)** storage of bulk raw milk resulted in increased numbers of lipolytic and proteolytic bacteria. On average, the number of psychrotrophs as a proportion of the total plate count increased from 47 to 80% after two days storage. The different trends in bacterial growth in bulk milk samples collected in three seasons suggested the importance of not only the initial load of bacteria but also of the type and activity of microflora present. Significant effects of raw milk storage on lipolysis and proteolysis were observed. The bacterial and enzyme action in the stored raw milk was greater than that in fresh raw milk and subsequently resulted in increased free fatty acids content and lower pH.

According to the **Ralyea et al., (1988)** enclosed pipeline milk systems, better sanitary design of equipment, cleaner cows, and more effective "clean in place" systems have provided the opportunity for farms to produce raw milk with less microbial contamination. Rapid cooling of raw milk before the bulk tank with inline plate coolers has reduced the growth of contaminating bacteria. Rapid cooling and refrigerated storage of raw milk has favored the growth of psychrotrophic bacteria in raw milk.

If the raw milk bacterial count is <25,000 cfu/mL, then the raw milk somatic cells count will be the most important determinant of shelf life. The influence of raw milk somatic cells count on pasteurized fluid milk quality is caused by increasing levels of heat-stable proteases and lipases originating from the cow with increasing milk somatic cells count **(Barbano, Ma and Santos, 2006)**.

Numerous organisms commonly found in raw milk produce degradative enzymes. Once these enzymes have been secreted, they have the potential to degrade both raw and processed milk components. Furthermore,

refrigeration conditions under which raw milk is stored selects for growth of psychrotrophs, many of which produce heat-stable enzymes. These psychrotrophs can grow and secrete heat-stable enzymes while milk waits processing **(Mottar, 1989)**. Pathogenic bacteria like *Staphylococcus aureus* can pose an elevated health hazard and have to be eliminated **(Pukáčová, Poľaková and Dudriková, 2010; Vasiľ et al., 2012; Bogdanovičová et al., 2014)**.

Ideally, microbial contamination of raw milk should be addressed primarily through preventive measures on the farm and throughout processing. However, too many contamination sources exist to prevent entry of all bacteria. Therefore, milk handling and processing strategies are designed to reduce and control bacterial numbers in processed products to protect milk quality and milk safety. The first of these measures involves efficient cooling of milk to 4 °C immediately following milking **(Marth and Steele, 2001)**.

Milk must be cooled immediately to not more than 8 °C in the case of daily collection, or not more than 6 °C if collection is not daily. During transport the cold chain must be maintained and, on arrival at the establishment of destination, the temperature of the milk must not be more than 10 °C (**Commision regulation (EC) Regulation No 1662, 2006)**.

Reduced temperatures inhibit growth of mesophils and thermophils and reduce the activity of degradative enzymes. Modern dairy farms use refrigerated bulk storage tanks which maintain milk at 4 °C or below. As bulk tank milk pick-up typically occurs daily or every other day, product from multiple milkings is frequently mixed and stored in the same tank. To prevent fresh, warm milk from the most recent milking from raising the temperature of milk already present in the bulk tank, many farms employ pretank cooling systems to reduce product temperature before addition to the tank **(Marth and Steele, 2001)**.

The presence and growth of bacteria in milk affects milk quality. Chemical components of milk can be degraded by bacterial metabolism and various enzymes secreted by bacteria. Products of these degradation reactions can have undesirable effects on milk structure, smell and taste. Fermentative metabolisms of lactose by a variety of lactic acid bacteria can occur in milk **(Cousin, 1982; Baylund, 1995; Jay, Loessner and Golden, 2005; Bezeková et al., 2012)**. *Enterococcus spp.* is the group of lactic acid bacteria, which can enter the milk from environment through milking machines **(Fabianová et al., 2010; Krebs-Artiová, Ducková and Kročko, 2013; Lačanin et al., 2015)**. Proteins can be digested by extracellular proteases. Lipase will cause break down of triglycerides. Phospholipases hydrolyze phospholipids present in fat globule membranes making interior lipids more susceptible to lipase attack **(Baylund, 1995; Cousin, 1982 and Jay, Loessner and Golden 2005)**.

MATERIAL AND METHODOLOGY

Milk samples

Raw cows' milk from morning milking was sampled from the bulk tank in farm into sterile bottles according to the standard ISO 707 (2008) and immediately transported to the laboratory at temperature 1 – 5 °C.

Instruments

To perform this research we used MilkoScan FT 120 infrared absorption analyser (FOSS, Hillerød, Denmark; distributor: Milcom servis a.s., Prague, Czech Repulic). It was calibrated quarterly with calibration samples (Actalia - Cecalait, Poligny, France) preserved with 0.02 % Bronopol.

Infrared milk analysis

Samples of fresh raw cows' milk were analysed 2 and 24 hours after milking. Each sample was analysed 10 times and the average result was calculated. Milk composition was determined in compliance with ISO 9622 (2013) and the FOSS (1998) working manual for the Milkoscan FT 120. The samples were analysed at the State Veterinary and Food Institute in Bratislava, Slovakia, at the National Reference Laboratory for Milk and Milk Products, which is accredited in accordance to the international standard ISO 17025 (2005). The experiment was replicated 10 times.

Deviation calculation

Deviations between the results of laboratory determination of milk composition were calculated following this equation:

Deviation of result of analyte (g/100g) = (A) – (B)

Where:

(A) is the result of analyte of raw cows' milk after 24 hours storage at temperature 4 °C and

(B) is the result of analyte of fresh raw cows' milk.

Statistical analysis

The statistical analysis was performed using statistical program Tanagra 1.4 (Lumière University, Lyon, France) according to **Rakotomalala (2005)**. To evaluate the results, data was classified into two groups representing the composition of raw cows' milk and the composition of raw cows' milk after 24 hours. Subsequently, the Principal Components Analysis (PCA) was performed with the Hierarchical Clustering Procedure (HAC). To evaluate the difference between the results with paired samples of fresh raw cows' milk and raw cows' milk after 24 hours storage at temperature 4 °C, the Student's t-test was used and the *p*-value was calculated.

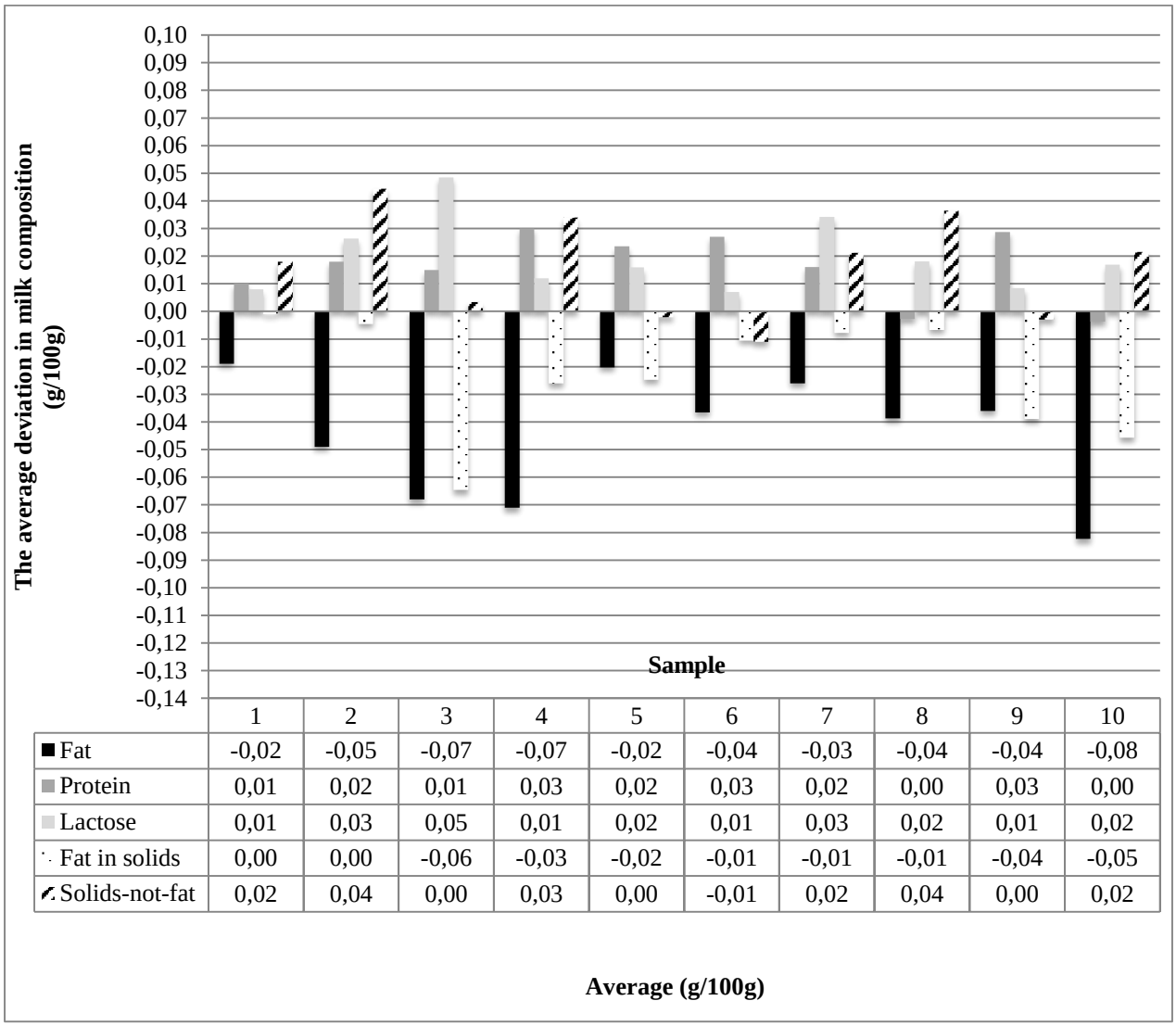
RESULTS AND DISCUSSION

The composition of the fresh raw cow's milk used in experiments is presented in Table 1. The effect of 24 h storage at temperature 4 °C on milk composition is presented in Figure 1. Figure 2 represents the Principal Component Analysis of data of fresh (▲) and stored (●) raw cow's milk. The data do not overlap. It means the composition of raw cow's milk after 24 was changed significantly (p >0.005). We found an average decrease in the fat content of -0.04 g/100g, increase in the protein content of +0.02 g/100g, increase in the lactose content of +0.02 g/100g, decrease in the total solid content of -0.02 g/100g andincrease in the solids-not-fat content of +0.02 g/100g.

Table 1 The composition of fresh raw cows' milk.

		Composition of raw cows' milk (g/100g) [a]					Composition of raw cows' milk after 24 hours stored at 4 °C (g/100g)				
Experiment No.		**Fat**	**Protein**	**Lactose**	**Total solid**	**Solids-not-fat**	**Fat**	**Protein**	**Lactose**	**Total solid**	**Solids-not-fat**
1	Average (g/100g)	3.82	3.22	4.65	12.37	8.54	3.80	3.23	4.66	12.36	8.56
	Cv (%)	0.39	0.35	0.21	0.17	0.20	0.17	0.33	0.10	0.12	0.12
	SD (± g/100g)	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.00	0.01	0.01
2	Average (g/100g)	3.40	3.25	4.68	12.01	8.60	3.35	3.27	4.70	12.00	8.65
	Cv (%)	0.19	0.13	0.15	0.08	0.10	0.14	0.16	0.10	0.08	0.07
	SD (± g/100g)	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.01
3	Average (g/100g)	4.26	3.36	4.80	13.10	8.84	4.20	3.38	4.85	13.04	8.84
	Cv (%)	0.19	0.42	0.52	0.18	0.34	0.19	0.00	0.22	0.14	0.12
	SD (± g/100g)	0.01	0.01	0.03	0.02	0.03	0.01	0.00	0.01	0.02	0.01
4	Average (g/100g)	4.00	3.30	4.71	12.68	8.69	3.93	3.33	4.73	12.65	8.72
	Cv (%)	0.13	0.20	0.51	0.12	0.27	0.12	0.15	0.53	0.13	0.19
	SD (± g/100g)	0.01	0.01	0.02	0.01	0.02	0.00	0.00	0.03	0.02	0.02
5	Average (g/100g)	3.43	3.24	4.73	12.07	8.63	3.41	3.27	4.75	12.04	8.63
	Cv (%)	0.24	0.57	0.18	0.17	0.30	0.09	0.15	0.18	0.07	0.10
	SD (± g/100g)	0.01	0.02	0.01	0.02	0.03	0.00	0.00	0.01	0.01	0.01
6	Average (g/100g)	4.00	3.28	4.78	12.69	8.73	3.96	3.31	4.79	12.67	8.72
	Cv (%)	0.32	0.10	0.37	0.44	0.30	0.18	0.13	0.10	0.07	0.06
	SD (± g/100g)	0.01	0.00	0.02	0.06	0.03	0.01	0.00	0.00	0.01	0.01
7	Average (g/100g)	3.50	3.27	4.78	12.20	8.70	3.48	3.29	4.81	12.20	8.72
	Cv (%)	0.09	0.17	0.24	0.06	0.14	0.20	0.20	0.15	0.11	0.13
	SD (± g/100g)	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
8	Average (g/100g)	3.72	3.29	4.74	12.41	8.69	3.68	3.29	4.76	12.40	8.72
	Cv (%)	0.29	0.24	0.42	0.13	0.23	0.21	0.22	0.33	0.15	0.21
	SD (± g/100g)	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02
9	Average (g/100g)	3.44	3.25	4.73	12.10	8.66	3.40	3.28	4.74	12.06	8.66
	Cv (%)	2.87	0.22	0.12	0.80	0.12	0.14	0.13	0.17	0.08	0.08
	SD (± g/100g)	0.10	0.01	0.01	0.10	0.01	0.00	0.00	0.01	0.01	0.01
10	Average (g/100g)	4.10	3.38	4.80	12.91	8.83	4.01	3.37	4.81	12.87	8.85
	Cv (%)	0.13	0.28	0.28	0.10	0.18	0.21	0.12	0.15	0.07	0.07
	SD (± g/100g)	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01

[a] Raw cows' milk from morning milking was sampled from the bulk tank immediately after the end of milking. Composition of raw cows' milk was analysed two hours after sampling.



	1	2	3	4	5	6	7	8	9	10
Fat	-0,02	-0,05	-0,07	-0,07	-0,02	-0,04	-0,03	-0,04	-0,04	-0,08
Protein	0,01	0,02	0,01	0,03	0,02	0,03	0,02	0,00	0,03	0,00
Lactose	0,01	0,03	0,05	0,01	0,02	0,01	0,03	0,02	0,01	0,02
Fat in solids	0,00	0,00	-0,06	-0,03	-0,02	-0,01	-0,01	-0,01	-0,04	-0,05
Solids-not-fat	0,02	0,04	0,00	0,03	0,00	-0,01	0,02	0,04	0,00	0,02

Figure 1 The effect of 24 h storage at temperature 4 °C on milk composition. Each sample (n = 30).

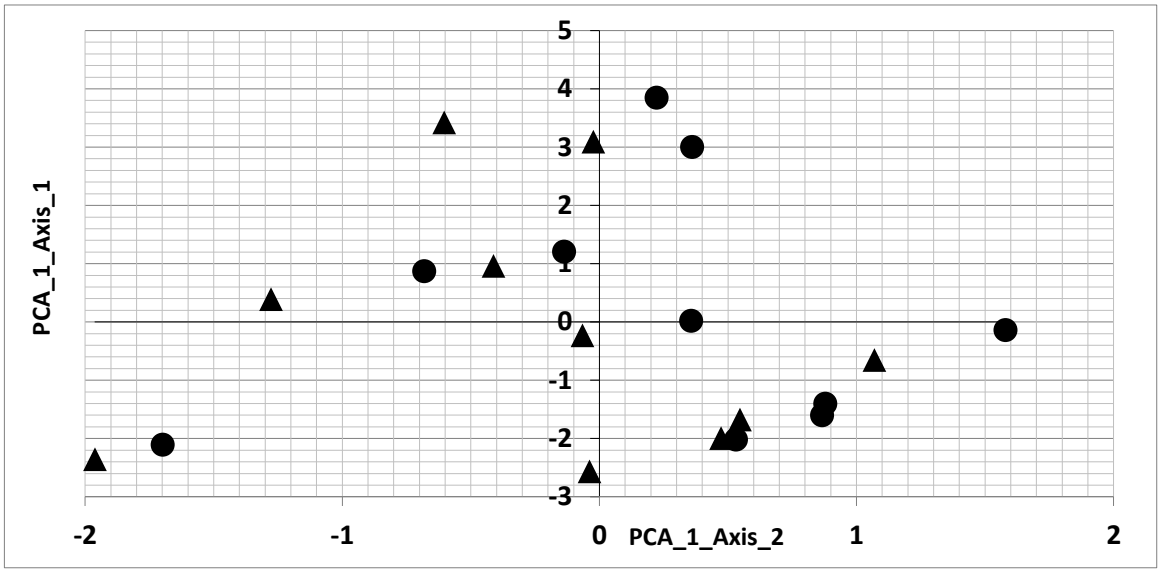


Figure 2 The Principal Component Analysis of the composition of (▲) fresh raw cows' milk versus (●) raw cows' milk stored 24 hours at temperature 4 °C. The PCA_1_Axis_1 and PCA_1_Axis_2 represent the results of fat, protein, lactose, total solids and solids-not-fat content.

In our opinion, lipolysis of milk can be initiated both by indigenous milk lipases and by microbial lipases, which could change the milk composition.

The liberation of fatty acids by the action of lipases can change the instrument's readings. Increasing the lipolysis index by 1 milliequivalent per 100 g of fat changes the instrument's signal for fat by -0.022% and signal for protein by +0.013% **(ISO 9622, 2013)**. Bovine milk contains a lipoprotein lipase that accounts for most, if not all, of its lipolytic activity. The total lipase activity in raw milk is sufficient to cause rapid hydrolysis of a large proportion of the fat. Physical damage to milk fat globule membrane in raw milk initiates lipolysis. Furthermore, simply cooling milks soon after secretion can initiate the so-called spontaneous lipolysis **(Deeth, 2006)**. Raw milk stored at 4 °C enables the growth of lipolytic psychrotrophic bacteria **(Fonseca et al., 2013)**. Extracellular microbial lipolytic and proteolytic enzymes may cause spoilage problems **(Baur et al., 2015)**. Also, **Leitner et al., (2011)** described the negative effect of bacterial infection on milk composition. **Barbano, Ma & Santos (2006)** expect the activity of various enzymes in milk. The microbial count and somatic cell count determine the load of heat-resistant enzymes in milk and these enzymes reducing the shelf life of the milk. Proteolysis can occur during 4 °C storage of preserved milk samples **(Santos et al., 2003)**. Proteolysis in milk during storage at 4 °C for six days points to the greater importance of microbial proteinases than plasmin activity. Plasmin activities decreased during the six days of storage at 4 °C **(Guinot-Thomas et al., 1995)**. Temperature during cold storage can have a significant influence on plasmin levels and thus contribute to the subsequent proteolysis rate in milk **(Schroeder, Nielsen & Hayes, 2008)**. **Marino et al. (2005)** stated that the proteolytic activity associated with somatic cells in milk could affect milk composition. **Verdi & Barbano (1991)** were observed casein proteolysis of milk by enzymes isolated from somatic cells. The higher protease activity may be present due to the higher concentrations of activated macrophages. Different somatic cells counts and milk composition during the lactation, activities of cathepsin D, cysteine proteases and another unidentified milk proteinase in milk were fluctuate during lactation **(Larsen et al., 2006)**. Fifteen per-cent of 19,830 samples analysed for total bacterial count and twenty-six per-cent of 13,037 samples analysed for somatic cells count didn't meet the legal requirements. It means the enzymatic activity due to the presence of microorganisms and somatic cells in bulk tank milk have to be expected **(Zajác et al., 2012)**. The activity of these enzymes can lead to the laboratory results deviations when unpreserved laboratory samples or improper lower concentrations of preservants are used. As shown in Figure 2, only the lipolysis of fat content occurred after 24 hours storage at temperature 4 °C. In contrast, the protein and lactose content was slightly increased. Because, the fat content was changed, the calculation of the other milk components by instrument was affected. According to **Kaylegian et al., (2007)**, the proteolytic activity in milk increased only about 1% after 8 days storage at temperature 4 °C. The activity of lipases can decrease the fat content during infrared readings and increase other milk components **(ISO 9622, 2013)**. It is then necessary to analyse the milk samples as soon as possible after collection; otherwise, they must be preserved by appropriate means and stored in the temperature 5 °C **(ISO 707, 2008)** to eliminate changes in milk composition. The application of milk preservatives can extend the shelf life of the sample as well **(Chalermsan et al., 2004)**.

CONCLUSION

The composition of raw cows' milk after 24 was changed significantly (p >0.005). We found an average decrease in the fat content of -0.04 g/100g due to the lipolitic activity of lipase. We found increase in the protein content of +0.02 g/100g, increase in the lactose content of +0.02 g/100g, increase in the solid-not-fat content of +0.02 and decrease in the total solid content of -0.02 g/100g. Increased content of these milk components was caused due to the instrument's readings, because the fat content was decreased, subsequent calculation of other components was affected. It is necessary to cool the raw cows' milk after the milking to decrease the changes in milk composition. Also, it is necessary to analyse the milk samples and process the milk as soon as possible, preferably within 24 hours.

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