

Evaluation of Factors Affecting Freezing Point of Milk

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Abstract—The freezing point of milk is an important indicator of the milk quality. The freezing point of milk is determined primarily to prove milk adulteration with water and to determine the amount of water in it. Chemical composition and properties of milk, thermal treatment and presence of any substance can influence freezing point of product. There are different substances, which can be added to milk with main purpose to prolong shelf-life of raw milk. There are detergent, preservatives, formaldehyde, hydrogen peroxide, antibiotics, sodium carbonate, and hydrogen peroxide. Therefore the aim of the present study was to determine freezing point of milk, skimmed milk, pasteurized milk and milk with different substances (formaldehyde, antibiotics, sodium carbonate, hydrogen peroxide, disinfectant, and detergent) in different concentrations. The thermal treatment and different undesirable substances presence in milk have significant influence on freezing point of it.

Keywords—Antibiotics, freezing point, milk, pH, thermal treatment.

I. INTRODUCTION

THE freezing point of milk is an important indicator of the milk quality. The freezing point of milk is determined primarily to prove milk adulteration with water and to determine the amount of water in it [1]–[5].

The osmotic pressure, or salt balance, of a cow's milk has to be in balance with that of her blood. Since the osmotic pressure of a cow's blood can only vary within narrow limits it follows that the salt balance of her milk – and hence the freezing point, dictated by salt balance – can only vary with narrow limits [3], [6].

Freezing point of milk is influenced by factors related to variation in environment, management, and breed [2], [7]. Buchberger, 2000 had mentioned more wide and deep spectrum of factors: stage of lactation, age, health status, breed, milk yield, feed quality and amount, season, region, milking time and others.

The most significant factor affecting freezing point of milk is water addition. Freezing point of a solution depends on the number of particles in the solvent (water phase of milk), rather than the kind of particles. Water without solutes will freeze at zero degrees C. The presence of any solutes will depress freezing point below zero degrees C. The freezing point of milk depends upon the concentration of water-soluble

components. As milk is more diluted, the freezing point will raise closer to zero [3].

Other researches show [1], [4], [5] that not only extraneous water presence in milk affects the freezing point of it. But there are other factors such: milk constituents that are present in genuine milk. Their concentration may be influenced by a number of factors, including dairy cow breed [8], [9], stage of lactation, subclinical mastitis, dairy cow's nutrition, water intake [6], climatic conditions [10], regional and seasonal influences, carbon dioxide concentration in milk [4], [11]. This statement is proved with data about freezing point in different countries, where the above mentioned factors are different. For example in Latvia the freezing point of milk ranged from -0.640°C till -0.494°C [1], in Germany from -0.531°C till -0.468°C [5], in Poland -0.540°C and -0.570°C [12], in Estonia -0.550°C and -0.497°C [2], in America researchers found that it ranged from -0.550°C till -0.512°C [13]. The mean freezing point of milk in Italy -0.528°C [14], in Netherlands -0.521°C [11], in Czech Republic -0.523°C [15], in Switzerland -0.526°C [16], in the UK -0.539°C [17] is different too.

Other authors exude significant factors influencing freezing point of milk – the concentration of lactose and pH of milk [1], [18], [19]. Demott (1969) and Brouwer (1981) reported that lactose content is responsible for 53.8% of the freezing point in cows, with other components contributing the following, in decreasing importance: potassium 12.7%, chloride 10.5%, sodium 7.2%, citrates 4.3%, urea 1.9% and other components 6.9%. Freezing point remains depends on the molar concentration of these soluble, low-molecular weight compounds [11].

The cooling or heating of milk causes aggregation of soluble salts and their transfer to casein micelles or fatty globules. This reaction is, however, reversible and the freezing point may therefore vary depending on the time and temperature of analysed samples [13].

According to an International Dairy Federation questionnaire the majority of responding countries use freezing point of milk as one of the quality criteria for insuring high quality milk [2]. The same situation is in Latvian market, where the competition of raw milk in as well as payment for milk detects quality of raw milk. Freezing point of milk is one of the quality parameters, which detect natural quality of milk, and it is used for water content detection in milk.

The number of compounds that can enter milk by contamination is endless. There are many concerns about compounds that may be harmful to the consumer because of their potential toxicity or mutagenicity [20].

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There are different substances, which can be added to milk with main purpose to prolong shelf-life of raw milk on in another way. There are **detergent, preservatives, formaldehyde, hydrogen peroxide, sodium carbonate, and hydrogen peroxide.**

Antibiotics and other drugs are widely used to treat cows with mastitis or other infections or ailments. When a cow is treated, its milk is generally withheld from the bulk tank until treatment stops and milk is free of drug residues [20]. Occasionally treated cows are overlooked and antibiotic contaminated milk enters the bulk tank. Antibiotics in milk are a concern due to the risk of allergic reactions and the development of antibiotic resistant pathogens. Antibiotic may slow down the action of lactic acid bacteria used in the manufacture of fermented products, which can result in the loss of significant amounts of product and milk [20].

According to the European Union Regulations No 853 of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs [21] antibiotics presence in milk is forbidden in controlled milk samples. In Latvia still is actual problem is presence residues of disinfectants, neutralizing, cleaning and preservative substances in milk. Milk processing companies use tests for detection different forbidden substances in milk. Sometimes these methods are very expensive and time consuming. At the same time addition of some substances to milk can change chemical composition of milk and proportion of solids and water in it, as well as pH of product, as result freezing point of milk can be changed too. Milk typically consists of approximately 87% water or 13% total solids. It is noteworthy that the milk freezing point is altered when water concentration (the amount of water present) varies, which serves as an objective indicator of the presence of extraneous water in milk [4].

The detection of freezing point of milk is not expensive and it is very fast method comparing to inhibitors and other contaminants detection in milk.

Therefore the aim of the present study was to determine freezing point of milk, skimmed milk, pasteurized milk and milk with different substances (formaldehyde, antibiotics, sodium carbonate, hydrogen peroxide, disinfectant, and detergent) in different concentrations.

II. MATERIALS AND METHODS

A total 35 milk samples were analyzed. Milk samples were collected according to standard method LVS EN ISO 707:2011 Milk and milk products. Guidance on sampling. Milk samples were obtained from conventional dairy herd in Latvia. Bulk milk samples were immediately cooled to 4–8 °C and transported to the laboratory, arriving at a temperature not exceeding 8 °C. Milk samples were divided: one part of milk was pasteurized 78 °C 15-20 s, but another part of milk was added different substances: antibiotics (4% ampicillin, 4% colxacilin), disinfectants (96% etalon, 4% water), formaldehyde (60%), sodium carbonate (100%), hydrogen peroxide (30%) and detergent (surfactants 10%, methylchloroisothiazolinone, methylisothiazoline). All

substances were added in the following concentrations: 0.1%, 0.5%, and 1.0%.

Fat, protein, lactose content in milk and skimmed milk was detected with CombiFoss FT 6000 (MilkoScan Tm 6000 FT and Fossomatic Fc) according ISO 9622:1999 Whole milk. Determination of fat, protein and lactose content.

pH in milk and skimmed milk was detected according ISO 5546:2010 (IDF 115:2010) Caseins and caseinates - Determination of pH (Reference method).

The freezing point in milk and skimmed milk was determined according standard method ISO 5764/ IDF 108:2002, Milk - Determination of freezing point - Thermistor cryoscope method (Reference method).

The presence of antibiotics in milk and skimmed milk was detected by Delvotest.

The presence of formaldehyde, disinfection agent, sodium carbonate, hydrogen peroxide, and detergent in milk and skimmed milk was detected by test with resasurin.

Descriptive statistics were carried out to determine the differences of freezing point in different milk samples with and without pasteurization and different substances by Microsoft Windows for SPSS software packages.

III. RESULTS AND DISCUSSION

The freezing point of cows' milk is relatively constant as a consequence of osmotic equilibrium in milk and blood. Therefore, the freezing point of milk is widely used in dairy processing companies to detect water adding to milk. However, according to the other research results freezing point of milk has wide range. Chemical composition of milk had influence on it, still it is known, that milk typically consist of approximately 87% water or 13% total solids. It is noteworthy that the freezing point of milk is altered when water concentration varies, it mean, that if product will have higher concentration of total solids it can be changed too.

Buchberger (2000), Jonkus (2008), Navrátilová (2006), Ķedzierska-Matysek (2011) found correlation between freezing point and chemical composition of milk [1], [4]–[5], [22]. Therefore the freezing point were detected in different samples (milk and skimmed milk), the characterisation of milk samples is given in Table I. Samples with different content of fat, lactose and protein were chosen for analysis.

TABLE I
 CHEMICAL COMPOSITION OF DIFFERENT SAMPLES

Samples	Content, %		
	Fat	Protein	Lactose
raw milk	4.21±0.010	4.03±0.010	5.51±0.010
raw skimmed milk	0.10±0.001	4.12±0.010	4.50±0.010

The skimmed milk was with the lowest fat (0.10%), but with highest protein content (4.12%).

Freezing point of raw milk was –0.533 °C, similar results – 0.535 °C – presented researches form Latvia [1]. In all analysed samples freezing point of milk was according to the Latvian Regulation [23]. The freezing point discrimination limit for cow's raw milk is set – 0.520 °C.

Comparing freezing point of raw skimmed milk and milk was detected, that lowest temperature was in milk ($-0,533\text{ }^{\circ}\text{C}$) ($p<0.05$), in a product with a higher fat content (4.21%), the same tendency were in the another research with goat milk. Genčurova et al (2008) found a negative correlation between freezing point of milk and fat content [24].

Freezing point of raw milk and skimmed milk before and after pasteurization is given in Table II.

TABLE II
FREEZING POINT AND pH IN DIFFERENT SAMPLES

Samples	Freezing point, $^{\circ}\text{C}$	pH
raw milk	-0.533 ± 0.008	6.747 ± 0.030
raw skimmed milk	-0.525 ± 0.007	6.742 ± 0.030
pasteurized milk	-0.525 ± 0.007	6.737 ± 0.030
pasteurized skimmed milk	-0.520 ± 0.007	6.730 ± 0.030

After thermal treatment freezing temperature increase as in milk (for $0.008\text{ }^{\circ}\text{C}$) as in skimmed milk (for $0.009\text{ }^{\circ}\text{C}$), and the tendency still was the same – lower freezing temperature was in pasteurized milk. The significant difference was not established ($p>0.05$). The same results were established in another research with cow milk. Singhal (1997) reported the same elevations of the milk freezing point after pasteurization ($0.006\text{--}0.009\text{ }^{\circ}\text{C}$) [25]. But data on the effects of the heat treatment on the freezing point in milk vary, Kessler (1984) found that freezing point of milk after pasteurization at $74\text{ }^{\circ}\text{C}$ for 30 s was not changed, but pasteurization at $85\text{ }^{\circ}\text{C}$ for 3 s increased freezing point by $0.002\text{ }^{\circ}\text{C}$, but prolonged pasteurization at $95\text{ }^{\circ}\text{C}$ for 30s increased the freezing point by $0.001\text{ }^{\circ}\text{C}$ [26]. Janštová (2007) reported about significant difference between freezing point values of raw and pasteurized milk [27].

During pasteurization the chemical composition of milk is affected as result freezing point of milk can be affected too. It can be explained with change inside the calcium phosphate complex and a change in the pressure of carbon dioxide [28]. Carbon dioxide affects the freezing point of raw and pasteurized milk in the processing chain because of its current decrease due heating. Carbon dioxide volumetric content decreases approx. from 7% down to 2% [29]. Moreover the lactose isomerisation and degradation to lactulose and organic acid influence pH of product – it decreases [20].

In milk is of dubious microbiological quality there might be a temptation to use an antimicrobial agent to act as a preservative. Different substances: formalin, sodium carbonate, hydrogen peroxide and other can be used as preservatives. Antibiotics, used for animal treatment, can be found in milk too.

Still it is known, that chemical composition and properties of milk can influence freezing point of milk, and presence of any substance can affect it. The milk samples with different undesirable substances in milk were analysed in the current research (see Table III). The most popular substances detected in raw milk in Latvia were chosen for the research.

TABLE III
FREEZING POINT AND pH IN MILK WITH DIFFERENT SUBSTANCES 0.1% CONCENTRATION

Substances	Freezing point, $^{\circ}\text{C}$	pH
control	-0.525 ± 0.015	6.730 ± 0.030
formaldehyde	-0.585 ± 0.015	6.730 ± 0.030
antibiotic	-0.539 ± 0.015	6.700 ± 0.030
disinfection agent	-0.570 ± 0.010	6.755 ± 0.030
sodium carbonate	-0.574 ± 0.010	7.032 ± 0.030
hydrogen peroxide	-0.535 ± 0.010	6.788 ± 0.030
detergent	-0.545 ± 0.015	6.765 ± 0.030

After addition different substances (0.1% concentration) freezing temperature decreases in all samples. The smallest changes were detected in sample with **hydrogen peroxide** ($0.010\text{ }^{\circ}\text{C}$), **antibiotic** ($0.014\text{ }^{\circ}\text{C}$), and **detergent** ($0.020\text{ }^{\circ}\text{C}$). Significant difference ($p<0.05$) was established in milk samples with **formaldehyde** ($0.060\text{ }^{\circ}\text{C}$), **disinfection agent** ($0.055\text{ }^{\circ}\text{C}$), **sodium carbonate** ($0.049\text{ }^{\circ}\text{C}$). Mean freezing point values of analyzed samples were according to the data from literature $-0.520\text{ }^{\circ}\text{C}$ to $-0.560\text{ }^{\circ}\text{C}$ [30]. It is mean, that not only highest, but the lowest freezing point of milk can be a signal about milk adulteration.

Lower freezing point values in adulterated samples compared to control sample are in agreement with goat milk studies of Sánchez (2005), Sánchez (2007), Radeljević (2010), Pintić-Pukec (2011) [31–34]. The results are in agreement also with the investigation deducted by Williams (2007) that showed a direct, measurable correlation between concentration of preservatives and freezing point depression in samples [35]. Formaldehyde, disinfection agent, sodium carbonate can be added with aim to preserve milk.

Still it is known, that presence of any substance in milk can influence pH it was necessary to control it (see Table III).

In all samples, excluding antibiotics and formaldehyde, pH increase, and significant difference ($p<0.05$) was established in the following samples: with hydrogen peroxide and detergent, it raised accordingly for 0.058 and for 0.035 units.

Increasing concentration of different substances in raw milk till 0.5% freezing point continue to change (see Table IV).

TABLE IV
FREEZING POINT AND pH IN MILK WITH DIFFERENT SUBSTANCES 0.5% CONCENTRATION

Substances	Freezing point, $^{\circ}\text{C}$	pH
control	-0.525 ± 0.015	6.730 ± 0.030
formaldehyde	-0.685 ± 0.015	6.567 ± 0.030
antibiotic	-0.541 ± 0.015	6.524 ± 0.030
disinfection agent	-0.699 ± 0.010	6.758 ± 0.030
sodium carbonate	-0.705 ± 0.010	7.358 ± 0.030
hydrogen peroxide	-0.540 ± 0.010	6.793 ± 0.030
detergent	-0.558 ± 0.015	6.775 ± 0.030

In the case with **antibiotics** freezing point significantly decreases for $0.016\text{ }^{\circ}\text{C}$ comparing to control sample ($p<0.05$). In milk samples with antibiotics pH significantly decreased ($p>0.05$) accordingly for 0.206 units.

Further increasing concentration of **formaldehyde** (0.5%) promoted decrease of freezing temperature for $0.160\text{ }^{\circ}\text{C}$, comparing with control sample, the difference was significant

($p < 0.05$). pH of the same sample significantly decrease ($p < 0.05$) for 0.163 units.

The similar situation was with **detergent**, freezing point decrease for 0.033 °C, significantly ($p < 0.05$). Detergent has very low freezing point (-114.3°C) as result it decrease in milk too. At the same time, pH of milk significantly increase ($p < 0.05$).

After addition 0.5% of **disinfection agent** freezing temperature of milk significantly decrease ($p < 0.05$) for 0.174 °C, but pH increases insignificantly ($p > 0.05$) for 0.028.

In the case with **sodium carbonate** freezing point significantly decreases ($p < 0.05$) for 0.180 °C comparing to control sample. In the same milk samples pH significantly decreased ($p > 0.05$) accordingly for 0.628 units.

Significant lower freezing temperature ($p < 0.05$) was established in milk sample with **hydrogen peroxide**, it decreased for (0.015), but pH significantly increased ($p < 0.05$) for 0.063 units.

It is mean that even at 0.5% concentration of all in the current research used substances (antibiotics, formaldehyde, detergent, disinfection agent, sodium carbonate, hydrogen peroxide) significantly influence ($p < 0.05$) freezing point of milk. The freezing point of raw milk can be as indicator of milk falsification not only with water, but with other substances too. After detection freezing point of milk is possible to predict presence of undesirable substances in milk.

Freezing point of milk samples with 1.0% of different substances is given in Table V.

TABLE V
 FREEZING POINT AND pH IN MILK WITH DIFFERENT ADDED SUBSTANCES
 1.0% CONCENTRATION

Substances	Freezing point, °C	pH
control	-0.525±0.015	6.730±0.030
formaldehyde	-0.949±0.015	6.436±0.030
antibiotic	-0.543±0.015	6.434±0.030
disinfection agent	-0.836±0.010	6.765±0.030
sodium carbonate	-0.859±0.010	7.421±0.030
hydrogen peroxide	-0.545±0.010	6.796±0.030
detergent	-0.568±0.015	6.803±0.030

Sodium carbonate can get into the milk as by carelessness of farmer as deliberately for decreasing acidity of milk.

After addition the highest concentration (1.0%) of different substances freezing point of milk significantly decrease ($p < 0.05$) in all milk samples: with **sodium carbonate** for 0.334 °C, with **disinfection agent** for 0.311 °C, with **detergent** for 0.043 °C, with **antibiotic** for 0.018 °C, and with **hydrogen peroxide** for 0.020 °C. with **formaldehyde** for 0.424 °C,

In all samples pH continues to change, significant increase ($p < 0.05$) was established in the following samples: with **sodium carbonate** (0.691), with **detergent** (0.073) with **hydrogen peroxide** (0.066) and with **disinfection agent** (0.035).

In samples with **formaldehyde** and **antibiotics**, pH significantly decrease ($p < 0.05$) accordingly for 0.294 and 0.296 units.

In all analyzed milk samples freezing temperature was not higher than -0.520 °C, it mean that all analyzed milk samples were according to the EU Regulation and Latvian Regulation requirements [21], [23]. At the same time freezing point of separately milk samples (with formaldehyde, with disinfection agent, with sodium carbonate, with detergent) did not range only from -0.520 °C to -0.560 °C.

The presence of antibiotics is strictly controlled in milk, but still is detected in some milk samples in Latvia. The increasing concentration of **antibiotics (1.0%)** even diminish freezing point of milk. It can be explained with antibiotic chemical composition and properties, which have higher solids content and different pH [36].

Freezing point of raw milk with 1.0% **formaldehyde** was the lowest comparing with other samples. Significantly low ($p < 0.05$) freezing temperature can be explained with freezing point of formaldehyde -118 °C [37]. pH of sample with 1.0% formaldehyde concentration continue to decrease and achieved the minimal value. It can be explained with formaldehyde pH (~3.5) value, which decrease pH of analyzed milk sample [37].

Addition of **disinfection agent** in concentration 1.0 % significantly decreases ($p < 0.05$) freezing point of analyzed sample. At the same time pH of sample continue to increase and achieved maximal value. These results can be explained with property of main component of disinfection agent – ethanol, freezing point - 114°C and pH ~7, [38], as result it can influence freezing point of analyzed sample and it decrease.

The significant lower freezing point and significant higher ($p < 0.05$) pH was established in sample with 1.0% of **sodium carbonate**. As in previous cases it can be explained with properties of sodium carbonate: freezing point 97 °C and pH 11.6 [39].

Sodium carbonate very often is component of detergents, or it can be added deliberately to milk, or deliberately for increasing pH.

Addition of **detergent** at concentration 1.0% significant difference was established ($p < 0.05$) as in a case with freezing point of milk as in a case with pH. Still it was detected, that pH of detergent was ~ 7.5, it can influence the pH of analysed milk sample, as well the freezing point of it [40].

Detergents are usually only found in milk as result of accidental addition of cleaning agents. This is usually easily detected by the extraneous water test, as the detergent itself does not significantly affect the freezing point. Strong detergents usually impart an odd odour and taste to the milk.

Hydrogen peroxide even at the higher concentration (1%) did not change freezing point of analysed milk more, and it still was according to the data from literature -0.520 °C to -0.560 °C [30].

Summarizing current research results, the main conclusion is, that not only the higher value of freezing point (-0.520 °C), but the lowest freezing temperature can be an indicator about not corresponding milk quality. Unfortunately freezing point cannot be used for controlling presence of different substances in milk.

IV. CONCLUSIONS

The thermal treatment of milk has significant influence of freezing point of milk.

The presence of different substances in milk has different influence on freezing point and pH of milk.

The freezing point of milk can be as indicator for controlling undesirable substances presence in milk.

The presence of formaldehyde, disinfection agent and sodium carbonate can be forecasted by freezing point of milk. The research results show, that even contaminated in a low concentration (0.1%) with formaldehyde, disinfection agent and sodium carbonate freezing point of milk significantly decrease ($p < 0.05$) comparing to the unadulterated milk samples.

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