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Determination of the freezing point in cow milk samples preserved with azidiol

Nataša Pintić-Pukec, Zdravko Barać, Ana Dakić, Danijela Stručić, Davorka Blažek*

Croatian Agricultural Agency, Poljana Križevačka 185, Križevci, Croatia

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Summary

The study involved determination of the freezing point of cow milk by a reference (thermistor cryoscopy) and an instrumental (infrared spectrometry) method. The aim of the study was to evaluate the possibility of milk freezing point determination in milk samples preserved with azidiol by using a reference and an instrumental method of analysis. Five hundred cow milk samples were analysed during three research periods. Samples were taken at milk collection points in north-western Croatia. Samples preserved with azidiol (0.3 mL azidiol/40 mL; 0.011 g sodium azide/40 mL) and without preservatives (control samples) were analysed. The freezing point of milk was determined in duplicate. Average freezing point results of azidiol preserved samples were lower compared to control samples. A statistically significant difference between the means of the results obtained for azidiol preserved and control samples was determined ($P<0.05$; $P<0.01$) in all research periods. The results revealed a significant influence of the preservative azidiol on milk freezing point determination regardless of the method of analysis applied, which could lead to wrong interpretation of the results.

Key words: cow milk, freezing point, preservative, thermistor cryoscopy, infrared spectrometry

Introduction

Freezing point is a physical property of milk that is determined as a parameter in order to detect milk adulteration. Freezing point determination is one of the most important methods of milk quality evaluation (Golc Teger et al., 2005). The increasingly used alternative, fast methods of testing raw milk samples in quality control laboratories enable the use of preservatives that ensure produced milk to remain liquid and unchanged until analysis. Preservatives are widely used in quality control laboratories, and the effect of preservative concentration on milk freezing point should be taken into account in freezing point determination (Sánchez et al., 2007). Good reproducibility and reliable accuracy of the results achieved in determination of goat milk freezing point with a MilcoScan FT 6000 milk analyser suggest that this method can be used as the screening method of choice, though it was found that an increase in sodium azide concentration may cause freezing point depression (Sánchez et al., 2007). Studies of milk freezing point determination by infrared spectrometry and thermistor cryoscopy methods (Pintić Pukec et al., 2009) showed that infrared spectrometry can be used as the screening method of choice for unpreserved cow milk samples.

Thermistor cryoscopy method is the reference method for milk freezing point determination, HRN EN ISO 5764 (2002). In addition to the basic milk quality indicators (milk fat, protein, lactose...), MilcoScan FT 6000 (Foss) instruments offer the possibility of freezing point determination by the routine method (instrumental), infrared spectrometry, HRN ISO 9622 (2001).

The research goal was to explore the possibility of freezing point determination in preserved cow milk samples, and to find out whether the preservative azidiol, regularly used for sample preservation

*Corresponding author/Dopisni autor: Phone/Tel.: + 385 49 279 050; E-mail: npintic@hpa.hr

in the Central Laboratory for Milk Quality Control, affects the freezing point of milk as determined by two methods, thermistor cryoscopy and infrared spectrometry.

Material and methods

Research was performed in the Central Laboratory for Milk Quality Control of the Croatian Agricultural Agency in the period from 2007 to 2009, further referred to as research periods I, II and III. Sampling was done once a week at milk collection points in north-western Croatia. Samples were preserved with azidiol (0.3 mL azidiol/40 mL; 0.011 g sodium azide/40 mL) in the Central Laboratory for Milk Quality Control using the standard procedure of sample preservation for regular monthly quality control. Sample duplicates were used as control samples (no azidiol added). To simplify record keeping and statistical processing, azidiol preserved samples were marked AZ, and those without preservative bore the mark BK. Freezing points of samples were determined within 24 hours upon their delivery to the laboratory. Freezing points were determined by the reference, thermistor cryoscopy, method using a Cryoscope 4C3 analyser (Advanced Instruments, Inc. USA) according to HRN EN ISO 5764 and by the instrumental method, infrared spectrometry, using MilcoScan FT 6000 analysers (Foss Electric, Denmark) according to HRN EN ISO 9622 and the manufacturer's instructions. Each sample was analysed in duplicate. Five hundred samples of cow milk were analysed in all.

Research data were processed using the statistical analysis software Statgraphics centurion XV (2006). Major statistical indicators are presented in the paper: arithmetic mean value, standard deviation, standard error and coefficient of variation. Significance of the differences between different analytical conditions (preserved samples and samples without preservative, two testing methods) was investigated. Significance of the differences between arithmetic mean values was further tested by Fisher's least significant difference method (LSD).

Results and discussion

Mean values of the results of freezing point analyses, obtained by the reference and instrumental methods, for azidiol preserved samples and control samples, are shown in Table 1 per research period. Results of freezing point analyses are lower for azidiol preserved samples (AZ), ranging from -529.66 to -544.62 m°C, compared to control samples (BK), the values of which ranged from -515.89 to -527.47 m°C, regardless of the method of analysis in all research periods (Table 1, Fig. 1). Obtained mean freezing point values -522.65 m°C of control sam-

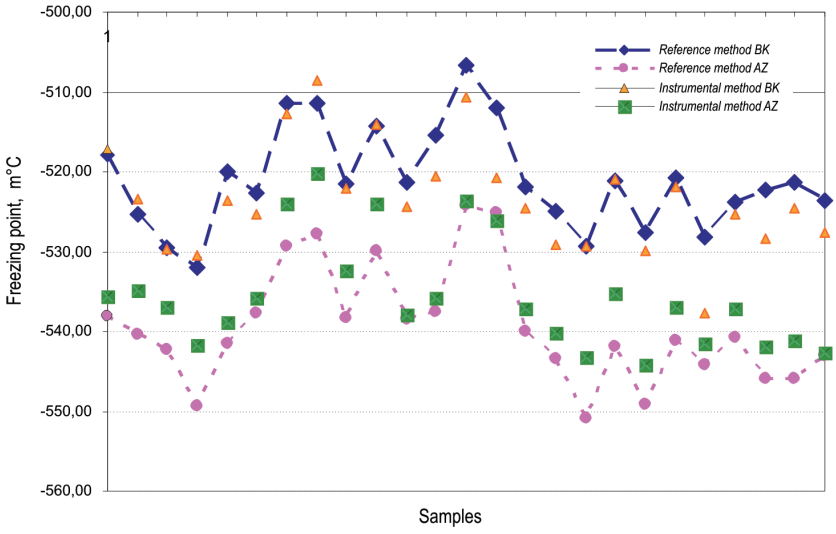

Figure 1. Freezing points of control and azidiol preserved samples determined by the reference and instrumental methods (BK, AZ)

samples: BK - no preservative added; AZ - with azidiol preservative

Table 1. Statistical indicators for milk freezing points determined by two analytical methods

Analytical method	Preservative[3]	Statistical indicators	FP[4], m°C / research period[5]		
			I. (n=100)	II. (n=200)	III. (n=200)
Instrumental[1]	BK	$\bar{x}$	-524.92^{Cc}	-518.36^{Cc}	-527.47^{Cc}
		sd	13.33	23.23	11.42
		s_r	1.33	1.64	0.81
		C	2.54	4.48	2.16
	AZ	$\bar{x}$	-537.56^{Bb}	-529.66^{ABab}	-540.42^{Bb}
		sd	10.46	23.24	10.42
		s_r	1.05	1.64	0.74
		C	1.95	4.39	1.93
Reference[2]	BK	$\bar{x}$	-524.98^{Cc}	-515.89^{Cc}	-524.28^{Dd}
		sd	13.67	23.20	10.73
		s_r	1.37	1.64	0.76
		C	5.12	4.50	2.05
	AZ	$\bar{x}$	-542.30^{Aa}	-532.82^{Aa}	-544.62^{Aa}
		sd	10.56	23.15	10.55
		s_r	1.06	1.64	0.75
		C	4.65	4.34	1.94

*A, B, ... P<0.05 Values within the same column marked by different letters differ significantly
a, b, ... P<0.01 Values within the same column marked with different letters differ significantly
[1]Instrumental method=infrared spectroscopy
[2]Reference method=thermistor cryoscopy method
[3]Preservative: BK= no preservative; AZ= azidiol with 0.011 g of sodium azide/40 mL
[4]FP= freezing point; [5]research period= I, II, III (n=number of tested samples)
$\bar{x}$ - mean value; sd - standard deviation; s_r - standard error; C - coefficient of variation

ples are similar to results given in the investigations of Slaghuis (2001) where the mean freezing point was -520.9 m°C with a standard deviation of 4.7 m°C and -527.0 m°C in the survey of Golc Teger et al. (2005). Lower freezing point values in azidiol preserved samples compared to samples without preservatives are in agreement with the studies of Sánchez et al. (2005); Sánchez et al. (2007) and Radeljević et al. (2010).

The results are in agreement also with the investigation deducted by Williams et al. (2007) that showed a direct, measurable correlation between concentration of preservative-bronopol and freezing point depression of the samples.

Small and roughly equal freezing point variability was determined for the studied methods, ranging from 2.05 to 5.12 m°C for control samples and 1.93 to 4.65 for azidiol preserved samples (Table 1). The infrared spectroscopy methods for extraneous water or freezing point depression determination are valuable supplemental determinations which can suitably be performed using the same overall type of instruments which is used for the routine determinations of the concentration of milk components, cite Arnvidarson et al. (1998).

Analysis of variance revealed statistically significant differences (P<0.05; P<0.01) between the mean values of azidiol preserved samples and control samples in all research periods regardless of the method of analysis (Table 1). Sánchez et al. (2007) recorded that the increase in the concentration of sodium azide in the azidiol formula contributed to an important reduction in the freezing point.

The mean difference between the freezing point values of azidiol preserved samples and control samples was 18.20 m°C for the reference method, and 12.31 m°C for the instrumental method (Table 2). Lower freezing point values recorded by the instrumental method compared to the reference method are in agreement with the investigation of Sánchez

Table 2. Mean differences in freezing points between and within methods

Analytical method Samples	Difference, m°C		
	$\bar{x}$AZ-BK	$\bar{x}$BK	$\bar{x}$AZ
Reference	18.20	-	-
Instrumental	12.31	-	-
Instrumental-Reference	-	3.90	4.61

et al. (2007) where mean difference of 1.5 m°C was obtained between analytical methods. The mean difference in deviation of the results between instrumental and reference methods for control samples was 3.90 m°C for all research periods. A slightly greater difference (4.61 m°C) was determined for azidiol preserved samples (Table 2). A statistically significant difference ($P<0.05$; $P<0.01$) between the mean values of the results obtained by different methods (reference, instrumental) was determined in the third research period for control samples, whereas there were no significant differences in the first two periods ($P>0.05$).

Statistically significant differences ($P<0.05$; $P<0.01$) between the two methods were determined in the first and third research periods for azidiol preserved samples, whereas there were no significant differences in the second period ($P>0.05$), (Table 1).

Conclusions

The obtained research results point to the conclusion that the mean freezing points of cow milk samples preserved with azidiol calculated by the reference method are on average 18.20 m°C lower and those obtained by the instrumental method 12.31 m°C lower compared to the control samples. The mean difference in the result deviation of 3.90 m°C was determined between the instrumental and reference methods for control samples, and 4.61 m°C for azidiol preserved samples.

Statistically significant difference ($P<0.05$; $P<0.01$) was determined between mean freezing point values of azidiol preserved samples and control samples in all research periods regardless of the method of analysis.

The obtained research results point to the conclusion that the preservative azidiol had a significant influence on the determination of cow milk freezing point regardless of the method of analysis. Freezing point determination should not be performed on milk samples preserved with azidiol since this could lead to wrong interpretation of the results.

Određivanje točke ledišta u uzorcima kravljeg mlijeka konzerviranog azidiolom

Sažetak

Provedeno je istraživanje određivanja točke ledišta kravljeg mlijeka referentnom (termistor krioskopskom) i instrumentalnom metodom (infracrvena spektrometrija). Cilj istraživanja bio je utvrditi mogućnost određivanja točke ledišta kravljeg mlijeka u uzorcima konzerviranim azidiolom referentnom i instrumentalnom metodom. Tijekom triju istraživanih razdoblja ukupno je analizirano 500 uzoraka kravljeg mlijeka. Uzimani su na sabiralištima sjeverozapadne Hrvatske. Ispitani su nakon konzerviranja azidiolom (0,3 mL azidiola/40 mL; 0,011 g natrij azida/40 mL) i bez konzervansa (kontrolni). Točka ledišta određivana je u duplikatu. Utvrđeno je da su prosječni rezultati analiza točke ledišta uzoraka kravljeg mlijeka konzerviranog azidiolom niži u usporedbi s kontrolnim. Utvrđena je statistički značajna razlika ($P<0,05$; $P<0,01$) između prosječnih vrijednosti analiza uzoraka konzerviranih azidiolom i kontrolnih uzoraka u svim razdobljima istraživanja. Na temelju rezultata istraživanja zaključuje se da konzervans azidiol značajno utječe na određivanje točke ledišta kravljeg mlijeka neovisno o metodi ispitivanja, što može dovesti do krivog tumačenja rezultata analiza.

Ključne riječi: kravlje mlijeko, točka ledišta, konzervans, termistor krioskopska metoda, infracrvena spektrometrija

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