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Evaluation of Milkoscan FT2 Milk Analyzer for Determining the Freezing Point of Cow Milk under Different Analytical Conditions

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Abstract

Conservatives are widely used in quality monitoring programs and milk payment programs. Their use makes it possible to preserve the quality parameters of the milk. Therefore, the objective of this work is to evaluate Milkoscan FT2 (Foss Electric, Hillerød, Denmark) in the determination of the freezing point (FP) of cow's milk under different analytical conditions. Five conservation strategies - without preservatives, conservation with peroxide (20 and 40 µL/100 mL) and conservation with potassium dichromate (20 and 40 µL of potassium dichromate 1%/100 mL), were used to conserve the samples of milk. For each conservation strategy, eight different quantities of water were added (0 to 7% v/v). The FP was determined in duplicate for cow milk samples obtained from 25 milk samples from the Fkih Ben Salah region (Tadla-Azilal Province, Morocco), using the Milkoscan FT2 method and the Thermistor cryoscopy method (Advanced Instrument Inc., Norwood, MA). The results obtained make it possible to use the Milkoscan FT2 for the determination of the FP of the preserved cow's milk samples. For practical reasons, these 3 conservation strategies (with potassium dichromate (20 or 40 µL of potassium dichromate 1%/100 mL) and peroxide (20 µL/100 mL) can be used for the determination of the FP of cow's milk. When exceeding 3% of water added, determination of the FP with Milkoscan FT2 must be confirmed by the official method. The increase in peroxide concentration leads to somewhat higher variances between the two methods.

Keywords: Milk, freezing point, Milkoscan, thermistor cryoscope, milk preservative

1. Introduction

The freezing point (FP) of a liquid consisting of water and solutes corresponds to the temperature at which liquid water and ice are in equilibrium. The FP of milk is lower than that of water since the presence of solutes lowers the FP, This property is measured to determine whether bovine milk has been diluted with water and is employed as a legal standard [1]. Milk consists mainly of water but the FP is the most constant property of milk characteristics. This faculty is related to the physiological equilibrium existing between the osmotic pressure of the blood and that of milk. The FP of cow's milk is between -510 mºC and -530 mºC [2]. Despite this consistency, the FP may undergo small fluctuations due to variations in milk composition. But, the main cause is the dilution of milk with water by fraudulent intentions or mistakes in handling (pushes water), which reduces the concentration of the lactose considered as the main cause of the lowering of FP. The hydrolysis of the lactose results in a lowering of the freezing point of the milk and the release of the galactose.

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In fact, a linear correlation was found between the cryoscopic lowering and the amount of galactose released [3]. The acidity of the milk by the lactic fermentation causes the FP lowering; a 1 ºD lowers the FP of 0.004ºC. On the other hand, the pasteurization of the milk leads to a rise of 0.002ºC from the FP. In general, factors affecting the composition of milk also have effects on the FP [2]. It is important to note that water forms a precise hexagonal arrangement when it reaches its freezing point. This arrangement increases the volume of water and reduces its density. This feature is important in the manufacture of glacier dairy products, which can result in the formation of ice crystals [4]. The monitoring of this parameter is also important in the dairy industry. Thermistor cryoscopy is the reference method for the determination of the FP as defined by ISO5764: 2002 (IDF 108: 2002). The measurement is based on the principle of freezing milk and its crystallization by mechanical agitation. The measurement is carried out when the temperature of the frozen milk does not change for 20 s for not more than 0.5 mºC. The presence of water in the milk is determined by the FP measurement. Milk is mainly water but the presence of foreign water leads to changes in the osmotic balance of milk. The milk FP depends on the concentration of the water-soluble constituents and the lactose concentration is the main constituent that explains the variation in the FP of the milk [5].

The European directive (Council Directive 92/46 / EC, European Community, 1992) specifies that cow's milk intended for human consumption should have a FP of not more than -520 m°C. A study showed that in most cases herd milks with a freezing point above the threshold of -0.520°C were actually wetted. Nevertheless, intakes of foreign water were detected below this value. Moreover, the possibility that a herd produces milk with an authentic freezing point greater than -0.520°C can not be ruled out [6]. However, account must be taken of the natural variations in the composition of raw milk and the inevitable addition of water to certain manipulations of milk during production and processing. For example, Regulation No. 2597 (European Community, 1997) determined that this milk should have a FP close to the average FP for raw milk from the collection area. This is especially important when the FP is part of the criteria used in milk payment programs. Analysis of a large number of milk samples involves Fourier transform infrared instruments. This automatic equipment gives complete information on the composition of the milk. The results are obtained by calibration models capable of extracting the information contained in the spectral data of the samples. Other combined instruments are capable of providing both somatic cells and milk compositions. These equipment can be fully automatic, find their interest in the programs of improvement of the dairy herds. Indeed, their high cadence makes it possible to follow the quality of the individual milk of the cows. Under certain conditions, preservatives are added to the milk samples before they are taken to the quality control laboratories. The preservation of the samples makes it possible to preserve the quality parameters during the time that separates the sampling from the realization of the analyzes. In the case of milk payment programs and dairy herd improvement, preservatives are used for the determination of somatic cells [7]. Similarly, for the determination of fat, protein and lactose by infrared analyzers [8].

The use of preservatives leads to variations in the results obtained with cow's milk [9]. A study was carried out on the effects of preservatives on the composition and somatic cells of goat milk with CombiFoss 6000 FC (Foss Electric, Hillerod, Denmark) [10]. This milk analyzer combines two devices, Fossomatic FC (Somatic Cell Counter) and Milkoscan FT 6000 (Fourier Transform Infrared Analyzer). The last device was the subject of a study on the effects of preservatives on the determination of the FP for goat's milk. Conservatives studied were Bronopol and Azidiol [11]. Factors influencing the FP determination are the preservative used, the storage temperature and the age of the milk [10]. Since preservatives are widely used in the preparation of milk samples, the effects of their different concentrations on the FP should be taken into account when verifying any addition of water. In this sense, the use of preservative supposes an addition of solute to the milk, so that its effects could be interesting for the interpretation of the results of the FP.

Milkoscan FT2 (Foss Electric) is a milk analyzer based on Fourier Transform Infrared (IR-TF) technology. It provides a multitude of parameters for quality control. The analysis carried out in 30 s gives both the fat content, proteins, lactose, dry matter (total and defatted), urea, Dornic acidity, caseins, citric acid, free fatty acids, density and lowering of the point of freezing. The latter parameter is measured by a conductivity meter incorporated into the milk pumping circuit during the analysis. The aim of this work is to study the influence of different conservation methods on the determination of the FP using the Milkoscan FT2 method and thermistor cryoscopy (reference method).

2. Materials and methods

2.1. Sampling

Twenty-five 5000 mL milk samples were obtained from a cow milk collection center (located in Fkih Ben Salah in the Tadla-Azilal region of Morocco) during the month of April 2016. Each of the samples was divided into forty 100 mL portions assigned to different analytical conditions. These analytical conditions depend on the amount of water to be added (0, 1, 2, 3, 4, 5, 6 and 7% total volume), and the used preservative: without preservative (WPR), preservation with peroxide

(20 or 40 µL/100 mL, corresponding respectively to PER20 and PER40) and preservation with potassium dichromate (20 or 40 µL of potassium dichromate 1%/100 mL respectively corresponding to BCR20 and BCR40).

A total of 40 analytical conditions were therefore examined by each method, depending on the combination of the conservation method and the amount of water added. For the preparation of 1% potassium dichromate, 1 g of potassium dichromate (Panreac, 99%) was dissolved in 100 mL of distilled water. The peroxide (Merck, 30% for analysis) was used directly in the milk sample. The physico-chemical composition of milk (fat, total protein, lactose and total solids) of the milk samples was determined using a Milkoscan FT 2 (Foss Electric) apparatus. Before passing through Milkoscan FT2, the milk samples were heated to 40°C for 20 min [12]. Table 3 shows the results obtained for raw milk samples used in this study. All milk samples were stored at 4 °C until FP analysis, which was always carried out on the day of collection. The FP values of each aliquot were determined in duplicate according to reference method [13] using the thermistor cryoscope (Advanced Cryoscope, Model 4D3, Advanced Instrument Inc.) and the Milkoscan FT2 method. The thermistor cryoscope was used in plateau-seeking mode according to IDF108: 2002 / ISO5764 [10].

Before and during the experiment, the thermistor cryoscope was calibrated using standard solutions of NaCl according to the International Standard [13]. Calibration of the Milkoscan FT2 was carried out using cow milk samples in which the reference FP values were determined by the thermistor cryoscope (range from -450 to -590 m°C). In total, 4000 FP values were finally processed. The results of the FP were expressed in millicelsius according to the international standard [13].

2.1. Statistical Analysis

The results obtained by the two methods under the various analytical conditions were examined by comparing means, standard deviations of repeatability and regression analysis. Means were analyzed using the GLM procedure of SAS V9.1.3 software (SAS Institute Inc., Cary, NC, USA). The model used to integrate the effects of the 40 analytical conditions, the 2 analytical methods and the effect of the sample (n=25) and a residual effect related to the duplicate determination of the FP performed for both methods.

The standard deviation of repeatability (Sr) and its relative value (Sr%) was calculated for each analytical condition according to IDF 128A [14] with Equations 1 and 2:

$$Sr = \sqrt{\left(\frac{1}{2q}\sum_{i=1}^{q} Wi^2\right)} \quad (1)$$

$$Sr\,\% = Sr * 100/\bar{x} \quad (2)$$

where q is the number of samples, Wi is the absolute difference between duplicate results, and $\bar{x}$ is the arithmetic mean of the FP.

To establish the relationship between the reference method and the Milkoscan FT2 method, linear regression was performed for the 40 analytical conditions studied. Linear regression was performed using the REG procedure of SAS V9.1.3 software. The module allowed estimation of the coefficients of determination (R^2), the regression coefficient (a) and the intercept (b). The GLM procedure of the SAS V9.1.3 software was also used to study the effects of different variables. The effect of the addition of water (8 classes: 0 to 7%), the effect of the preservative (5 classes: WPR, BCR20, BCR40, PER20 and PER40), the analytical method (2 classes: Reference method and Milkoscan FT2 method), the effect of the sample (n = 25) and the factorial interactions of these effects at n = 2 and n = 3.

3. Results and Discussion

Table 1 compares the mean FP values obtained by the Milkoscan FT2 method and the reference method under the analytical conditions studied. The results obtained with the Milkoscan FT2 method (-525 to -477 m°C) were lower than those obtained with the reference method (-523 to -476 m°C), and the absolute value of the mean deviation between the values of FP obtained with both methods was 3.12 m°C. Taking the averages of the deviations recorded by conservation strategy, the differences recorded were 1.38 m°C, 1.72 m°C, 2.26 m°C, 4.27 m°C and 5.99 m°C corresponding to WPR, BCR20, BCR40, PER20, and PER40, respectively. The highest variances were recorded with the two strategies PER20 and PER40. The mean deviation of the BCR20 and BCR40 samples under the analytical conditions was 1.99 m°C compared to 5.13 m°C recorded with PER20 and PER40 samples.

Taking the averages of the deviations recorded by percent water addition, the recorded deviations were 2.8 m°C, 2.7 m°C, 3.15 m°C, 2.69 m°C, 3.32 m°C, 3.44 m°C, 3.03 m°C and 3.84 m°C corresponding to 0, 1, 2, 3, 4, 5, 6 and 7%, respectively. The average difference between the two methods increases slightly with the addition of water. The lowest difference with the BCR20 (0.27 m°C) samples and the highest deviation with the PER40 samples (10.29 m°C) was recorded

at 5% addition of water. In general, the recorded deviations show the low effect of the addition of water on the values of the FP. However, the use of peroxide in the preservation of the samples leads to very high values compared to the reference method. The differences recorded with the samples conserved with potassium dichromate recorded slightly greater deviations from those without preservatives (WPR).

Table 1. Least Squares Means of the Freezing Point (m°C) and Standard Deviations of Repeatability (Sr and Sr%) for each of the 40 Analytical Conditions Examined with the Instrumental (IM) and Reference (RM) Methods

Analytical condition[1]	RM[2] LSM[4] (m°C)	IM[3] LSM[4] (m°C)	*P*	RM Sr (m°C)	RM Sr (%)	IM Sr (m°C)	IM Sr (%)
0-WPR	-518	-516	<0.001	1.26	0.24	1.63	0.32
0-BCR20	-521	-519	<0.001	1.52	0.29	1.53	0.29
0-BCR40	-523	-521	<0.001	0.96	0.18	1.31	0.25
0-PER20	-518	-521	<0.001	0.88	0.17	1.25	0.24
0-PER40	-520	-525	<0.001	1.23	0.24	1.15	0.22
1-WPR	-512	-510	<0.001	0.85	0.17	1.12	0.22
1-BCR20	-515	-513	<0.001	1.57	0.31	1.07	0.21
1-BCR40	-517	-513	NS	1.36	0.26	1.17	0.23
1-PER20	-512	-514	<0.05	0.89	0.17	1.29	0.25
1-PER40	-514	-518	<0.001	0.93	0.18	1.50	0.29
2-WPR	-506	-507	<0.001	1.49	0.29	1.33	0.26
2-BCR20	-510	-507	<0.001	1.60	0.31	1.18	0.23
2-BCR40	-511	-508	<0.001	1.59	0.31	1.10	0.22
2-PER20	-508	-512	<0.001	0.94	0.19	1.51	0.29
2-PER40	-509	-514	<0.001	1.48	0.29	1.08	0.21
3-WPR	-501	-500	<0.01	1.52	0.30	1.55	0.31
3-BCR20	-503	-501	NS	1.49	0.30	1.53	0.31
3-BCR40	-505	-502	<0.001	0.95	0.19	1.60	0.32
3-PER20	-501	-503	<0.001	1.13	0.23	1.39	0.28
3-PER40	-503	-508	<0.001	1.32	0.26	1.06	0.21
4-WPR	-495	-493	<0.001	1.25	0.25	1.45	0.29
4-BCR20	-498	-496	<0.001	0.98	0.20	1.53	0.31
4-BCR40	-499	-496	<0.001	0.89	0.18	1.06	0.21
4-PER20	-494	-499	<0.001	0.86	0.17	1.68	0.34
4-PER40	-497	-503	NS	0.89	0.18	1.48	0.29
5-WPR	-489	-488	<0.001	1.35	0.28	1.43	0.29
5-BCR20	-492	-492	<0.001	0.85	0.17	1.03	0.21
5-BCR40	-493	-491	<0.001	1.30	0.26	0.87	0.18
5-PER20	-489	-492	<0.001	1.71	0.35	0.82	0.17
5-PER40	-491	-501	<0.001	1.42	0.29	0.72	0.14
6-WPR	-482	-484	<0.01	1.63	0.34	1.03	0.21
6-BCR20	-487	-486	NS	0.97	0.20	1.53	0.31
6-BCR40	-489	-487	NS	1.23	0.25	1.04	0.21
6-PER20	-484	-489	<0.001	1.49	0.31	1.16	0.24
6-PER40	-485	-490	<0.001	0.84	0.17	1.48	0.30
7-WPR	-476	-477	<0.001	0.99	0.21	1.67	0.35
7-BCR20	-480	-478	<0.001	1.48	0.31	1.35	0.28
7-BCR40	-481	-480	<0.001	1.13	0.23	1.66	0.35
7-PER20	-477	-486	<0.001	0.98	0.21	1.09	0.22
7-PER40	-480	-486	NS	0.83	0.17	1.01	0.21

[1]0–7 = percentage of added water (total volume); WPR = Without preservative; BCR20 = Potassium dichromate with 20µL of potassium dichromate 1%/100 mL; BCR40 = Potassium dichromate with 40 µL of potassium dichromate 1%/100 mL; PER20 = Peroxide with 20 µL/100 mL; PER40 = Peroxide with 40 µL/100 mL
[2]Thermistor cryoscope (Advanced Cryoscope, model 4D3, Advanced Instrument Inc., Norwood, MA)
[3]MilkoScan FT2 (Foss Electric, Hillerød, Denmark)
[4]SE of LSM = 1,317

The Milkoscan FT2 method showed an average relative Sr of 0.26% slightly higher than that shown in Annex A of IDF108: 2002 / ISO5764 [13] and also slightly higher than that obtained with the method of reference (0.24%). The results obtained in terms of Sr% are consistent with the results of Sànchez et al. [11] who had a Sr% of 0.2% for the official method and Milkoscan FT6000. The maximum Sr% obtained with the reference method was 0.35% with the PER20 samples with 5% added water and that obtained with the Milkoscan FT2 method (0.35%) was obtained with 7% in the BCR40 samples and samples without preservatives (WPR). The small differences observed in Sr under the various analytical conditions suggest that this parameter was not modified by the addition of water or preservatives in both methods.

Table 2. Coefficients of Regression (b), Intercept (a), and R^2 Values based on Regression Analyses for Freezing Point Values (m°C) Obtained by the Reference and Milkoscan FT2 Methods under Different Analytical Conditions

Analytical condition[1]	b	SE	a	SE	R^2
0-WPR	0.580***	0.096	-215.476***	49.763	0.612
0-BCR20	0.826***	0.046	-88.294**	23.912	0.933
0-BCR40	0.843***	0.076	-80.409^{NS}	39.450	0.844
0-PER20	1.002***	0.147	-2.052^{NS}	76.715	0.668
0-PER40	1.343***	0.162	174.240^{NS}	85.049	0.749
1-WPR	0.586***	0.059	-208.736***	29.962	0.812
1-BCR20	0.803***	0.043	-99.239***	21.924	0.939
1-BCR40	0.818***	0.045	-89.520***	23.244	0.934
1-PER20	0.960***	0.144	-22.655^{NS}	73.900	0.660
1-PER40	1.348***	0.090	176.838***	46.436	0.908
2-WPR	1.229***	0.151	115.063^{NS}	76.525	0.742
2-BCR20	0.887***	0.052	-53.889^{NS}	26.328	0.927
2-BCR40	0.704***	0.033	-147.849***	16.763	0.952
2-PER20	1.293***	0.125	145.883*	63.834	0.824
2-PER40	1.391***	0.151	195.917*	77.478	0.788
3-WPR	0.702***	0.047	-148.056***	23.685	0.905
3-BCR20	0.708***	0.070	-144.549***	35.271	0.815
3-BCR40	0.839***	0.034	-78.155***	16.998	0.964
3-PER20	1.033***	0.129	14.159^{NS}	65.149	0.735
3-PER40	1.384***	0.150	189.636*	76.226	0.787
4-WPR	0.541***	0.072	-224.975***	35.755	0.708
4-BCR20	0.775***	0.059	-110.070**	29.190	0.883
4-BCR40	0.589***	0.069	-201.384***	34.028	0.763
4-PER20	1.445***	0.178	216.873**	88.867	0.741
4-PER40	1.456***	0.168	223.220*	84.350	0.767
5-WPR	0.702***	0.035	-144.149***	17.165	0.945
5-BCR20	1.251***	0.139	123.264^{NS}	68.521	0.778
5-BCR40	0.573***	0.024	-207.069***	11.847	0.961
5-PER20	0.977***	0.176	-14.522^{NS}	86.620	0.573
5-PER40	1.773***	0.076	376.713***	38.293	0.959
6-WPR	1.251***	0.083	119.963**	39.931	0.909
6-BCR20	0.735***	0.032	-127.469***	15.415	0.959
6-BCR40	0.751***	0.066	-119.424**	32.129	0.849
6-PER20	1.372***	0.161	176.313*	78.537	0.760
6-PER40	1.235***	0.157	110.458^{NS}	76.954	0.729
7-WPR	1.255***	0.113	120.326*	54.021	0.843
7-BCR20	0.697***	0.049	-143.639***	23.402	0.898
7-BCR40	0.819***	0.042	-85.753***	20.180	0.943
7-PER20	1.525***	0.102	246.592***	49.421	0.907
7-PER40	1.585***	0.138	277.849***	66.980	0.852

[1]0–7 = percentage of added water (total volume); WPR = Without preservative; BCR20 = Potassium dichromate with 20 µL of potassium dichromate 1%/100 mL; BCR40 = Potassium dichromate with 40 µL of potassium dichromate 1%/100 mL; PER20 = Peroxide with 20 µL/100 mL; PER40 = Peroxide with 40 µL/100 mL.
*P < 0.05; **P < 0.01; ***P < 0001.

Table 2 shows the results of the regression analysis between the reference method and the Milkoscan FT2 method for the analytical conditions studied. The highest correlation coefficient was recorded with the BCR40 samples with 3% water (r=0.981) and with 5% water (r=0.981). For samples of 0 to 1% water added, the slope and intercept were respectively different ($p < 0.05$) of 1.00 and 0.00 for BCR 20 and without preservative (WPR) samples, respectively. By exceeding 3% of water added, the same remark was made for the other conservation strategies WPR and BCR40.

The best regression coefficients were recorded with BCR20 samples for 0-2% water percentages and BCR40 and PER20 samples for percentages 0%, 1% and 3%. The greatest accuracy was obtained with samples containing less than 3% water. Based on the results of the regression analysis, the determination of the water content added by the Milkoscan FT2 method must be confirmed by the reference method, especially where high levels are suspected. However, in a study of Milkoscan FT 6000, the use of Bronopol and Azidiol gives the greatest accuracy in samples containing more than 3% water. In addition, the samples used in this work (Table 1), have high FPs compared to the samples used by Sánchez et al. [11].

The factors of water addition, conservation, analytical method, milk sample effect and their interactions contributed significantly to the observed FP variation (Table 4). The addition of water was the main factor affecting the FP. The FP mean values range from -528 m°C to -466 m°C in samples with 0 and 7% water, with an increase of 6 m°C for each percentage point. The results obtained are consistent with the mathematical estimates made for goat milk [15] and allow detection of the fraudulent addition of water. Hence, the value of this parameter in the monitoring of milking and cleaning systems, while taking into account that automatic milking systems increase the FP of cow's milk [16-17]. These results do not

agree with the results of Pomies and Bony (2000) who did not detect differences between farms using a conventional milking parlour and a milking robot [18].

Table 3. Composition of Bulk Tank Milk Samples

Item	N	Minimum	*Maximum*	Mean	Std. Deviation
Fat (g/l)	25	31	41.1	38	2.29
Protein (g/kg)	25	29	33	31	1.00
Nonfat DM (g/l)	25	89	95.4	92	1.63
FP[1] (m°C)	25	-515	-528	-520	3.93
Lactose (g/l)	25	44	47.8	46	0.77

[1]Reference method

Table 1. Analysis of Variance of Variations in Freezing Point

Source of variation	df	F	Sig.
Watter added	7	113393,544	<.0001
Preservative	4	5544,416	<.0001
Analytical Method	1	1787,677	<.0001
Sample	24	9946,542	<.0001
Sample x Preservative	96	44,099	<.0001
Sample x Watter added	168	8,066	<.0001
Sample x Analytical Method	24	104,572	<.0001
Preservative x Watter added	28	61,332	<.0001
Preservative x Analytical Method	4	4,461	<,001
Watter added x Analytical Method	7	5,431	<.0001
Sample x Preservative x Watter added	672	10,351	<.0001
Sample x Preservative x Analytical Method	96	0,888	NS
Sample x Watter added x Analytical Method	168	1,375	<0.05
Preservative x Watter added x Analytical Method	28	6,915	<.0001

df: degree of freedom; F: F Ratio; Sig.: Significance value; NS: non-significant

The effects of the different conservation strategies studied on the FP were different (P <0.001). The smallest differences were recorded with the WPR, BCR20 and BCR40 samples. The most reliable results were recorded with the BCR20 samples. The lower FPs of the conserved samples are explained by changes in the osmotic balance of milk due to the increase in the water-soluble constituents of the samples, this effect is similar in both analytical methods (Table 5). The differences between the two methods are related to changes in the electrical conductivity of the milk. Indeed, the measurement of the FP of milk by Milkoscan FT2 is carried out by a conductivity meter integrated into the milk pumping circuit during the analysis.

Table 2. Least Squares Means of the Freezing Point According to the Interaction of Preservative × Analytical Method

Analytical Method	Preservative[3]	LSM[4] FP (m°C)
Reference method[1]	WPR	-497
	BCR20	-501
	BCR40	-502
	PER20	-498
	PER40	-500
Instrumental method[2]	WPR	-497
	BCR20	-499
	BCR40	-500
	PER20	-502
	PER40	-506

[1]Thermistor cryoscope (Advanced Cryoscope, model 4D3, Advanced Instrument Inc., Norwood, MA)
[2]MilkoScan FT2 (Foss Electric, Hillerød, Denmark)
[3]WPR = Without preservative; BCR20 = Potassium dichromate with 20 µL of potassium dichromate 1% / 100 mL; BCR40 = Potassium dichromate with 40 µL of potassium dichromate 1% / 100 mL; PER20 = Peroxide with 20 µL / 100 mL; PER40 = Peroxide with 40 µL / 100 mL
[4]SE of LSM = 0.466

Hydrogen peroxide (H_2O_2) is the traditional preservative to inhibit microbial growth and deterioration of milk. One milliliter of a 23% solution of H_2O_2 in 300 mL of raw milk is able to maintain a reduced number of bacteria for 6 days at 8 to 10°C [19]. The effect of peroxide on milk is linked to its decomposition by the peroxidases present in raw milk, these enzymes degrade the peroxide by releasing atomic oxygen capable of causing certain particular oxidation (for example: quinone oxidation of certain polyphenols with formations of colored substances). In addition to releasing atomic oxygen, these reactions release water [20].

The results show that, for practical reasons, the three concentrations of BCR20, BCR40 and PER20 can be used in the determination of the cow's milk FP using the two methods: Reference Method and Milkoscan FT2 Method. On the other hand, the increase in the peroxide concentration (added directly to the milk samples) leads to very high differences between the two FP determination methods and leads to a significant reduction of the FP. At high concentration, the use of the peroxide as a preservative will be conditioned by a bias correction on the Milkoscan FT2 prior to FP determination. This is to limit a misinterpretation of the FP values.

For both methods, the effects of the two preservatives were not very similar, with different concentrations of water added (Figure 1 and 2). Therefore, the type of preservative used and its concentration should be taken into account when interpreting FP results. This is to avoid considering samples retained as being defrauded by the addition of water.

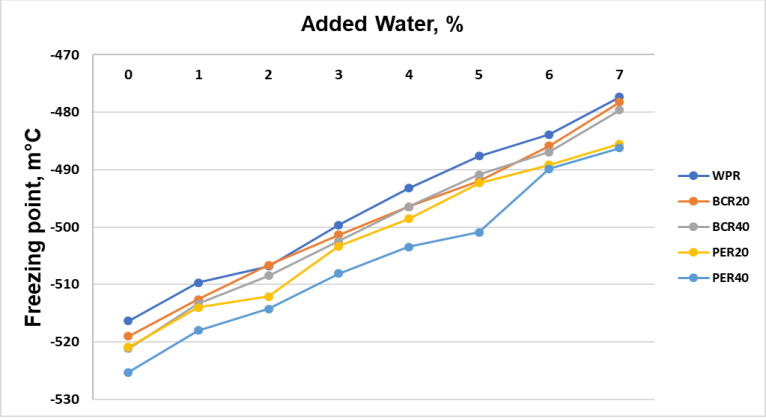

Figure 1. Least Squares Means of Freezing Points (milliCelsius) for the Milkoscan FT2 Method According to the Interaction of Added Water x Preservative (WPR = Without preservative; BCR20 = Potassium dichromate with 20 µL of potassium dichromate 1%/100 mL; BCR40 = Potassium dichromate with 40 µL of potassium dichromate 1%/100 mL; PER20 = Peroxide with 20 µL/100 mL; PER40 = Peroxide with 40 µL/100 mL)

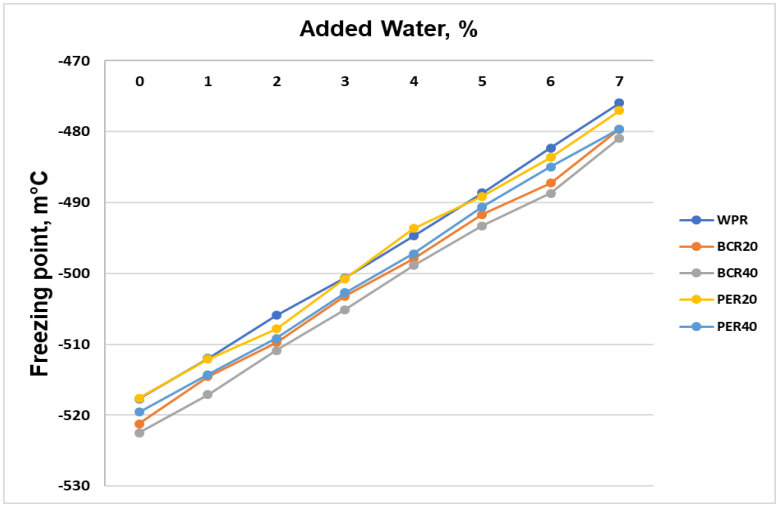

Figure 2. Least Squares Means of Freezing Points (milliCelsius) for the Reference Method According to the Interaction of Added Water x Preservative (WPR = Without preservative; BCR20 = Potassium dichromate with 20 µL of potassium dichromate 1%/100 mL; BCR40 = Potassium dichromate with 40 µL of potassium dichromate 1%/100 mL; PER20 = Peroxide with 20 µL/100 mL; PER40 = Peroxide with 40 µL/100 mL)

4. Conclusion

The precision obtained under the various analytical conditions suggests that Milkoscan FT2 can be used for the determination of the FP of cow's milk with added preservative. According to the regression analysis, the best analytical condition for FP determination when using Milkoscan FT2 involves preservation of the samples with potassium dichromate (at 20 and 40µl of potassium dichromate 1%/100 mL) and peroxide (at 20 µL/100 mL). The increase in the peroxide concentration leads to somewhat removed differences between the two methods: Milkoscan FT2 method and thermistor cryoscopy. For each method of preservation used, the determination of the FP must take into account the type of preservative and the concentration used. By neglecting these parameters, the interpretation of the FP values may be erroneous in terms of water

content.

REFERENCES

[1] Neville M.C., Jensen R.G., 1995. E - The Physical Properties of Human and Bovine Milks BT - Handbook of Milk Composition, in: Food Science and Technology. Academic Press, San Diego, p. 81-85.

[2] Pointurier H., 2003. La gestion matières dans l'industrie laitière. Éditions Tec & Doc, pp 388.

[3] Ramet P.J., Novak G., Evers A.P., Nijpels H.H., 1979. Application de la cryométrie à la mesure de l'hydrolyse enzymatique du lactose. Lait, 59(581-582), 46-55.

[4] Lapointe-Vignola C., 2002. Science et technologie du lait: transformation du lait. Presses inter Polytechnique, pp 600.

[5] Wheelock J.V., Rook J.A.F., Dodd F.H., 1965. The relationship in the cow between the osmotic pressure of milk and of blood. J. Dairy Res., 32, 79-88.

[6] Corrot G., Parguel P., Sauvée O., 1994. Variations du point de congélation et principales causes du mouillage du lait de vache. Renc. Rech. Ruminants., 1, 129-132.

[7] IDF (International Dairy Federation), 2006. Milk-Enumeration of somatic cells. Part 2: Guidance on the operation of fluo-ro-optoelectronic counters. FIL-IDF Standard no. 148-2 (E), IDF, Brussels, Belgium.

[8] IDF (International Dairy Federation), 2000. Whole milk, determination of milkfat, protein and lactose content. Guidance on the operation of mid-infrared instruments. FIL-IDF Standard no. 141C, IDF, Brussels, Belgium.

[9] Bertrand J.A., 1996. Influence of shipping container, preservative, and breed on analysis of milk components of shipped samples. J. Dairy Sci., 79,145-148.

[10] Sánchez A., Sierra D., Luengo C., Corrales J.C., Morales C.T., Contreras A., Gonzalo C., 2005. Influence of storage and preservation on fossomatic cell count and composition of goat milk. J. Dairy Sci., 88, 3095-3100.

[11] Sánchez A., Sierra D., Luengo C., Corrales J.C., de la Fe C., Morales C.T., Contreras A., Gonzalo C., 2007. Evaluation of the MilkoScan FT 6000 milk analyzer for determining the freezing point of goat's milk under different analytical conditions. J. Dairy Sci., 90, 3153-3161.

[12] Sierra D., Sánchez A., Luengo C., Corrales J.C., Morales C.T., Contreras A., Gonzalo C., 2006. Temperature effects on Fossomatic cell counts in goats milk. Int. Dairy J., 16, 385-387.

[13] IDF (International Dairy Federation), 2002. Milk. Determination of freezing point. Thermistor cryoscope method (reference method). FIL-IDF Standard no. 108:2002/ISO 5764, IDF, Brussels, Belgium.

[14] IDF (International Dairy Federation), 1999. Definition and evaluation of the overall accuracy of indirect methods of milk analysis application to calibration procedure and quality control in the dairy laboratory. FIL-IDF Standard no. 128A, IDF, Brussels, Belgium.

[15] Szijarto L., van de Voort F.R., 1983. Determination of added water and bovine milk to caprine Milk, J. Dairy Sci., 66, 620-623.

[16] Rasmussen M.D., Bjerring M., Justesen P., Jepsen L., Zonnenveld R.T.F.-V., 2002. Milk quality on Danish farms with automatic milking systems. J. Dairy Sci., 85, 2869-2878.

[17] Klungel G. H., Slaghuis B. A., Hogeveen H., 2000. The Effect of the Introduction of Automatic Milking Systems on Milk Quality. Journal of Dairy Science, 83 (9), 1998-2003

[18] Pomiès D., Bony J., 2000. Comparison of hygienic quality of milk collected with a milking robot vs. with a conventional milking parlour. Robotic milking, Procedings of the international symposium held in Lelystad, The Netherlands. Ed. Wageningen Pers., 122-123.

[19] Kroger M.,1985. Milk sample preservation. J. Dairy Sci., 68, 783-787.

[20] Pien J., Désirant J., Lafontaine D. M., 1954. La recherche de l'eau oxygénée dans le lait, Lait., 34, 133-145.