

VALIDATION OF MODIFIED MILK REFERENCE SAMPLE IN TERMS OF ITS SUITABILITY FOR INFRA-RED ANALYSIS CALIBRATION VIA EVALUATION OF PHYSICAL PROPERTIES

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Abstract

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Routine milk analyses using the efficient indirect infra-red method are important for the milk food chain quality. The reliability of the results depends on the calibration quality. It is important to use a relevant set of reference calibration samples (RCSs). RCSs with right range of values can be prepared using various methods. This paper was aimed to balance the impacts of dilution for decrease of main components in RCSs because of minimal change of matrix interference effects. Cow milk samples (MSs) were diluted (4/1) using distilled water, NaCl solution and a solution with specific composition (SC; because of disturbance in the balance of the milk matrix (NaCl 1.145; KCl 0.849; K_2HPO_4 1.8463; citric acid 1.7; urea 0.3 g/l)) for reduction in main milk components. Fat (F), crude protein (CP), lactose (L), milk freezing point (MFP), osmolality (OS) and electrical conductivity (EC) were measured in all (original as well as modified) MSs. The lowest MFP and OS were in the original milk -0.5559 °C and 274.5 mOsmol/kg. The MFP was increased to -0.4369 °C and osmolality decreased to 217.83 mOsmol/kg by the addition of water. The MFP was decreased (-0.4903 °C) and returned to original milk value by the addition of NaCl solution. MFP was -0.4788 °C due to SC addition. The decrease was less than for NaCl. The ability of other SC components (K_2HPO_4 , KCl, citric acid and urea) to MFP decrease is less than for NaCl solution. EC was highest for NaCl set 4.69 mS/cm, EC for SC was 4.48 mS/cm ($P < 0.001$). The original MSs set showed EC 4.27 mS/cm. The SC was the nearest to original MSs in terms of total mineral composition. ECs for both modifications differed ($P < 0.001$) from original MSs. The procedure is applicable for balance of interference effects of milk matrix because of relevant calibration.

milk, interference effect, composition, milk freezing point, osmolality, electrical conductivity

The results of routine milk analysis are important for improving the genetics of dairy cattle and ensuring the quality of the milk food chain. Modern methods include efficient indirect methods as infra-red (IR) analysis. This procedure is usually performed in mid IR or as near IR variant. Over the years a number of authors (Sjaunja, 1984 a, b, c; Sjaunja *et al.*, 1984; Sjaunja and Andersson, 1985; Hanuš *et al.*, 1992, 1995 a, 2002; Tsenkova *et al.*, 2000; Kukačková *et al.*, 2000; Jankovská and Šustová, 2003; Kráčmar *et al.*, 2004; Bijgaart, 2006; Broutin, 2006; Šustová *et al.*, 2006, 2007) have investigated calibration

procedures for various IR methods. This was done to measure various milk components using various indirect IR methods for result reliability and stability. The reliability of the results depends on the quality of the indirect method of calibration. It is important to use a set of reference calibration samples (RCSs) with the necessary range of values for the relevant calibration. RCSs can be prepared using various methods of modifications. These should avoid damaging the natural milk composition and changing the interference effects of basic matrix for calibration accuracy. The additions (milk fat, protein,

urea) or withdrawals (milk fat) of components or mechanical stress of milk samples (free fatty acids) are commonly used etc. (Hanuš *et al.*, 1995 b, 1997, 2003, 2008 a, b; Hering *et al.*, 2008). The addition of water or other solutions is used to achieve lower values of milk components. The required row of milk composition values in RCSs is achieved by modifications for IR analysis calibration purposes.

Milk samples (MSs) used in the regular calibration of analytical instruments which work on basis of IR absorption spectrophotometry are seldom purposely modified to cover the range of investigated components found in the real field. This is why distilled water is added in selected MSs of the calibration set owing to decrease in fat, protein and lactose contents. Of course, such addition can disturb the whole milk composition (milk matrix) to a certain extent in particular in terms of soluble mineral composition. The mineral matters or salts of some inorganic and organic acids in milk can be considered as agent of interference effects for measurement via IR analyzer (Hanuš *et al.*, 1992). There are above all chlorides, sodium, potassium, citric acid, urea, free fatty acids etc. (Kerkhof Mogot *et al.*, 1982; Sjaunja, 1984 c). The dilution of milk with distilled water alone leads to decrease in the fat, protein and lactose contents and a change in concentration of minerals and other soluble matter at the same time. This can cause a change in interference effects in measured MS and consequently also error measurement in the IR analysis. The calibrated instrument automatically compensates mean interference effects on the results of measurement in the current (average) MSs. However, this could be different in modified milk. MS was diluted using NaCl solution at a concentration that was approximately equal to the total milk mineralization, instead of plain water to avoid possible unfavourable effects such as change of normal interference effect in milk matrix. However, the NaCl solution could cause different interference effect to the above mentioned whole specific composition (SC; chlorides, sodium, potassium, citric acid and urea). For this reason the SC water solution which approaches the modified MS to final current matrix composition after its addition to milk as much as possible, was created. This leads to desired decrease in fat, protein and lactose concentrations in modified MS. The other composition (minerals, organic acids) should approach the composition of original milk. The change of interference effects for IR analysis should be minimal. For instance the measurable signals and in this way also possible interference effects of minority milk components such as urea and citric acid on infrared milk analysis are marked in results more papers (Grappin, 1987; Hanuš *et al.*, 1995 b, 2001, 2008 b, 2009 a, b; Hering *et al.*, 2008).

The measurement results of milk composition by the indirect method of IR spectrophotometry can be influenced by interference effects. Average interference effects are automatically compensated by the technical solution of the method. These in-

terference effects are caused by mutual interactions of milk components in simultaneous measurement. The changes in interference effects can be caused by milk components which have been manipulated. These effects are known but missing is detailed information about their size or dependence on quantity of manipulated components.

The hypothesis tested was that it was possible to increase the calibration quality of indirect analytical method (IR analysis) for reliability of measurement of concrete milk components via compensation of possible negative interference effect caused by modification changes in milk matrix during the preparation of RCSs. This could be successfully done by verifying targeted method for modification of milk RCSs in terms of composition. Hence the goal of this study was to verify and balance the impact of specific dilution for reduction in main milk components in RCSs, which are intended for IR analysis calibration, into milk matrix via manipulation in the soluble composition of cow milk.

MATERIAL AND METHODS

Experimental sample preparation

Cow milk RCS was prepared with targeted decreased fat, protein and lactose content for calibration of IR analyzer. The milk dilution was carried out using: distilled water; NaCl solution; solution of other matter composition (SC). This was based on the rationale of approximating the percentage of main mineral and other minority non-protein nitrogen matter and organic acids in milk as much as possible (Tab. I). The replacement of distilled water by NaCl solution was made to balance the decrease in mineral matter caused due to sample dilution. The total mineral concentration approximated the original concentration after sample treatment although the composition was a little different. The effort to minimize the interference effect changes and maximize the analytical improvement of method led consequently to proposed SC which should correspond to original milk as much as possible.

A row of six MSs was prepared with original fat content from 3.61 to 6.94%. This row was marked as set 1 (original set). Three further sets (Tab. II) were prepared from this original row as follows: – set 2 (water): six MSs of original set were diluted by distilled water in the ratio milk/water 4/1; – set 3 (NaCl): six MSs of set 1 was diluted by NaCl solution (4 g/l

I: Specific composition (SC) used for preparation of reference milk sample (MS) set 4

Component	Mass [g/l]
NaCl	1.1450
KCl	0.8490
K ₂ HPO ₄	1.8463
Citric acid (anhydride)	1.7000
Urea	0.3000

II: Means of indicators of MS sets and differences modified set – original set

SS	1	2	3	4	TVDSs
F (%)	5.35 ± 1.108	4.35 ± 0.892	4.34 ± 0.891	4.38 ± 0.885	4.31
CP (%)	3.203 ± 0.009	2.58 ± 0.011	2.58 ± 0.007	2.60 ± 0.007	2.58
L (%)	4.675 ± 0.109	3.71 ± 0.100	3.72 ± 0.094	3.69 ± 0.091	3.76
SNF (%)	8.43 ± 0.099	6.83 ± 0.087	6.84 ± 0.087	6.83 ± 0.089	
SCC (ths./ml)	652.5 ± 243.505	681.83 ± 264.073	719.67 ± 266.450	738.00 ± 278.409	
log SCC	2.781 ± 0.1754	2.7900 ± 0.1929	2.8179 ± 0.1989	2.8400 ± 0.1932	
EC (mS/cm)	4.25 ± 0.024	3.75 ± 0.052	4.69 ± 0.053	4.48 ± 0.039	
MFP (°C)	-0.5559 ± 0.0119	-0.4369 ± 0.0098	-0.4903 ± 0.0121	-0.4788 ± 0.0111	
OS (mOsmol/kg)	274.5 ± 2.930	217.83 ± 2.267	245.33 ± 2.687	237.83 ± 2.911	
OS 2 (mOsmol/kg)	290.4 ± 6.277	240.6 ± 5.155	268.6 ± 6.361	262.5 ± 5.841	
difference modified – original					
F (%)		-1.00 ± 0.216	-1.01 ± 0.217	-0.97 ± 0.223	
CP (%)		-0.62 ± 0.002	-0.62 ± 0.002	-0.60 ± 0.002	
L (%)		-0.97 ± 0.009	-0.96 ± 0.015	-0.99 ± 0.018	
SNF (%)		-1.60 ± 0.012	-1.59 ± 0.012	-1.60 ± 0.010	
SCC (ths./ml)		29.33 ± 20.568	67.17 ± 22.945	85.50 ± 34.904	
log SCC		0.01 ± 0.0175	0.04 ± 0.0235	0.06 ± 0.0178	
EC (mS/cm)		-0.50 ± 0.029	0.44 ± 0.030	0.23 ± 0.016	
MFP (°C)		0.1190 ± 0.0021	0.0656 ± 0.0002	0.0771 ± 0.0008	
OS (mOsmol/kg)		-56.67 ± 0.663	-29.17 ± 0.243	-36.67 ± 0.019	
OS 2 (mOsmol/kg)		-49.80 ± 1.122	-21.80 ± 0.084	-27.90 ± 0.436	
pair t-test: original versus modified					
F (%)		***	***	***	
CP (%)		***	***	***	
L (%)		***	***	***	
SNF (%)		***	***	***	
SCC (ths./ml)		ns	ns	*	
log SCC		ns	ns	*	
EC (mS/cm)		***	***	***	
MFP (°C)		***	***	***	
OS (mOsmol/kg)		***	***	***	
OS 2 (mOsmol/kg)		***	***	***	

Set 1: original set, six cow milk samples. Set 2 (water): six MSs of set 1 were diluted by distilled water in the ratio milk/water 4/1. Set 3: (NaCl): six MSs of set 1 was diluted by NaCl solution (4 g/l of distilled water) in the ratio 4/1. Set 4 (specific composition; SC): six MSs of set 1 was diluted by salt solution (NaCl, KCl, K_2HPO_4), citric acid (anhydride) and urea (Tab. I) in the ratio 4/1. Statistical significance: ns = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$. SS = sample set. TVDSs = theoretical values for diluted samples, calculated according to dilution ratio from original values; $\bar{x} \pm sd$ = arithmetical mean \pm standard deviation; difference $d \pm sd$ = mean difference \pm standard deviation; F = fat; CP = crude protein; L = lactose monohydrate; SNF = solids non fat; SCC = somatic cell count; log = decimal logarithm; EC = electrical conductivity; MFP = milk freezing point; OS = osmolality; OS 2 = calculated osmolality; used abbreviations are valid for all the tables and figures.

of distilled water) in the ratio 4/1; – set 4 (specific composition; SC): six MSs of set 1 was diluted by salt solution (NaCl, KCl, K_2HPO_4), citric acid (anhydride) and urea (Tab. I) in the ratio 4/1. The followed indicators were measured and compared to characterization of modified MSs in terms of mineral composition: milk freezing point (MFP, °C); osmolality (OS, mOsmol/kg); electrical conductivity (EC, mS/cm).

Analytical methods

The following milk indicators were investigated in the MSs sets: somatic cell count (SCC, thousand/ml); fat (F, %); crude protein (CP, %); lactose monohydrate (L, %); solid non fat (SNF, %). The SCC was determined by Fossomatic 90 (Foss Electric, Denmark) according to ČSN EN ISO 13366-3. The extensive combined result uncertainty (UN) was $\pm 9.3\%$ for SCC < 900 thousands/ml. F, CP, L and SNF were measured via instrument MilkoScan 133B (Foss Electric, Denmark), which was regularly calibrated accord-

III: The extensive combined uncertainties (UNs) of measurement results of individual indicators for the Milkoscan 133B apparatus (95% of probability level)

Component	Fat	Crude protein	Lactose
UNs (%) relative	± 2.77	± 2.59	± 2.37
UNs (%) absolute	± 0.101	± 0.085	± 0.115

IV: Differences between averages of set 4 (composition) and set 2 and set 3

SS	2 Water	3 NaCl
F (%)	*	***
CP (%)	**	***
L (%)	*	***

(F = fat; CP = crude protein; L = lactose monohydrate; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$)

ing to the reference method results (ČSN 57 0536; the Röse-Gottlieb's method for fat content, Kjeldahl's method for crude protein content and polarimetric and gravimetric methods for lactose and SNF contents (ČSN 57 0530)). The UNs are shown in Tab. III. OS was investigated using a Roebbling osmometer with thermistor probe principle (Hermann Roebbling Messtechnik, Berlin, Germany). This was calibrated according to relevant solutions. Further the osmolality values were calculated on the basis of MFP according to relevant recalculation equation freezing point of concentration row of urea solutions with known molality. These were marked as osmolality (OS) 2. MFP values were analysed using the cryoscopic instrument Cryo-Star automatic (Funke-Gerber, Germany). The selected measurement mood was Plateau Search (with parameters: interval = 23 seconds and $\Delta t = 0.4 \text{ m}^\circ\text{C}$). Instrument was regularly calibrated using standard NaCl solutions. The UN was $\pm 0.00608 \text{ }^\circ\text{C}$, it means $\pm 1.18\%$. EC was measured using OK 102/1 (Radelkis, Hungary) conductometer at $20 \text{ }^\circ\text{C}$ (in $\text{mS} \times \text{cm}^{-1}$) with help of the geometrical exactly defined bell glass electrode with the platinum ring contacts. The instrument was calibrated by the relevant salt (KCl) solution ($10.2 \text{ mS} \times \text{cm}^{-1}$) at the measurement of each milk sample set.

Statistical evaluation

The average values of investigated indicators were calculated for each set much like the differences between values of the original set and modified sets. These values of modified sets were tested used the paired t-test against original set of MSs (Tab. II). The difference in values of individual sets and original set were used for characterization of single sets and their mutual comparison to determine whether the added NaCl solution or SC solution influenced the properties of single sample sets. The paired t-test was carried out at SC versus sets 2 and 3 for F, CP and L contents.

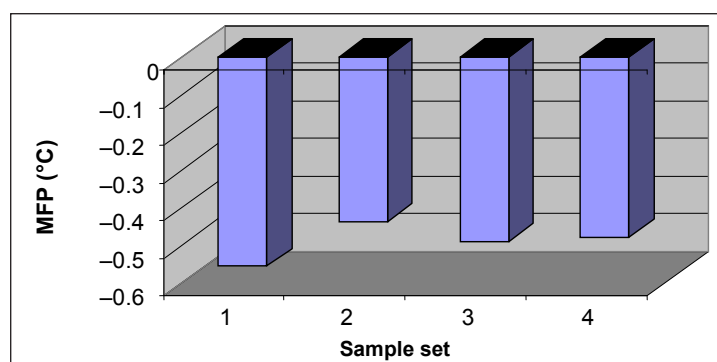
RESULTS AND DISCUSSION

Basic comparison of milk indicators of sample sets

Characteristics of milk composition and properties of single sample sets are shown in Tab. II. The mutual comparison of results of modified RCSs via MFP, OS and EC determination was used for investigation the importance of all changes from the original milk matrix. The goal was not to cause deviations in mentioned measurements by manipulations. It is presumed that the changes in possible interference effects on IR analysis were minimal. Results of MFP and closely related OS and EC are tight connected to main mineral and other minority non-protein nitrogen matters and organic acids (buffer system) in milk (Freeman and Bucy, 1967; Demott, 1969; Eisses and Zee, 1980; Brouwer, 1981; Walstra and Jenness, 1984; Koops *et al.*, 1989; Bauch *et al.*, 1993; Wiedemann *et al.*, 1993; Buchberger, 1990, 1994). The average differences between original set and sets 2 and 3 were insignificant for SCC and log SCC values. The SCC and log SCC difference between original set (1) and set 4 (SC) was significant ($P < 0.05$). The values were significantly different ($P < 0.001$) for EC, OS, MFP and also for other indicators as F, CP, L and SNF. The evaluation shows the result of manipulating the design in MSs. In set 3 (NaCl) that values F, CP and L were different ($P < 0.001$) from values of set 4 while in set 2 (water) were different ($P < 0.05$) for F and L and also for CP ($P < 0.01$; Tab. III).

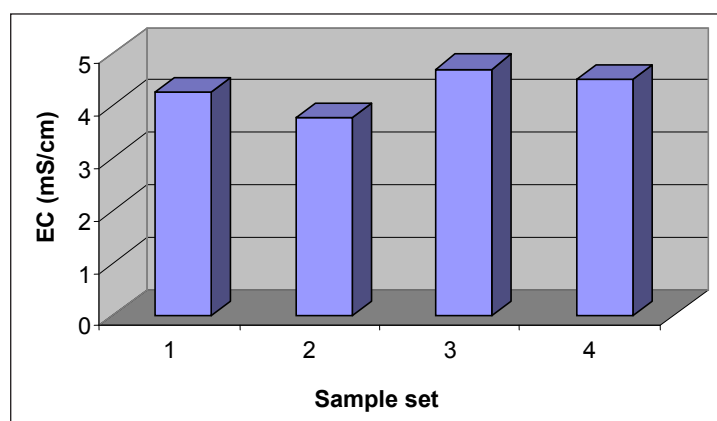
The comparison of MFP, OS and EC values as most important characteristics in terms of mineral sample composition between single sets

Set 1 showed the lowest values of MFP and OS $-0.5559 \text{ }^\circ\text{C}$ and 274.5 mOsmol/kg (Tab. II, Fig. 1, 3). The MFP value increased to $-0.4369 \text{ }^\circ\text{C}$ and OS value decreased to 217.83 mOsmol/kg due to addition of distilled water in set 2. The MFP value decreased again ($-0.4903 \text{ }^\circ\text{C}$) and approached the value of MS (set 1) by NaCl solution addition (set 3) in lieu of water. However, the original value was not reached because L in sample was decreased. The L causes MFP depression by 54% in contrast to NaCl which together with other mineral salts cause 30.5% (Walstra and Jenness, 1984). The MFP was only $-0.4788 \text{ }^\circ\text{C}$ in set 4 due to SC addition. The decrease is smaller than for NaCl. It is possible to explain this by the fact that NaCl concentration was lower in the SC. However other salts (Tab. I), citric acid and urea were present. For instance the quantity of supplied NaCl is equal to 68.4 mmol/l in set 3 while in SC set the total quan-



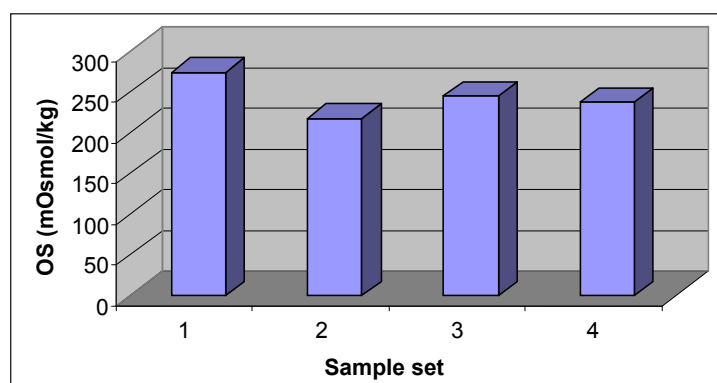
1: Milk freezing point (MFP) averages of original and modified MS sets

MS sets (x axis): set 1 (original milk); 2 (milk diluted by distilled water); 3 (milk diluted by NaCl solution); 4 (milk diluted by solution of specific composition (SC) of mineral salts, urea and citric acid (Tab. I)).



2: Electrical conductivity (EC) averages of original and modified MS sets

MS sets (x axis): set 1 (original milk); 2 (milk diluted by distilled water); 3 (milk diluted by NaCl solution); 4 (milk diluted by solution of specific composition (SC) of mineral salts, urea and citric acid (Tab. I)).



3: Osmolality (OS) averages of original and modified MS sets

MS sets (x axis): set 1 (original milk); 2 (milk diluted by distilled water); 3 (milk diluted by NaCl solution); 4 (milk diluted by solution of specific composition (SC) of mineral salts, urea and citric acid (Tab. I)).

tity of mineral salts, citric acid and urea was equal to 55.44 mmol/l. The NaCl matter amount in SC was only 19.6 mmol/l. The ability of other components of composition (K_2HPO_4 , KCl, citric acid and urea) to reduce MFP was less than for alone NaCl alone. The EC was highest in set 3 (4.69 mS/cm; Tab. II,

Fig. 2) and for set 4 was lower (4.48 mS/cm), the difference was significant ($P < 0.001$). Set 1 reached EC in about 4.27 mS/cm. In total from mineral quantity point of view set 4 was nearest to the original MS. The values of both solutions were different ($P < 0.001$) from original MS.

The theoretical calculation fat, protein and lactose in sample diluted by distilled water (set 2)

Theoretical values of F, CP and L in set 2 were calculated to discover the interference effects of added (missing) minerals in the reference milk sample. The calculation was carried out on the basis of known composition of original sample set and exact volume (ratio milk/water) of added distilled water into MSs in set 2. Knowledge of the theoretical F, CP and L contents in set 2 allows us to discover the inaccuracies in infraanalyzer measurement which could be caused by possible changed interference effects. These could be determined by comparison with real measured values and also by comparison with values which were measured in sets 2 and 3, since due to addition of NaCl or eventually SC solution the amounts of F, CP and L are not changed in these sets. In this way it is possible to determine the possible effects of selected minerals, citric acid and urea on measurement accuracy.

Contents of F, CP and L in original sample were: 5.35; 3.20 and 4.68% (g/100g). Water addition corresponded to 4/1 in the volume ratio milk/water. The concentrations of components in diluted milk were: F 4.18%; CP 2.50%; L 3.65%. The theoretical values were compared with real measured values of F, CP and L contents in other sets (Tab. II). The calculated values (Tab. II) were lower than measured values. F theoretical value was 4.18% while for set 2 (water) and set 3 (NaCl) they were 4.35 and 4.34%. The average F 4.38% which is the value mostly distant from calculated value was measured in the SC. CP values are more balanced between sets. The SC addition showed again the higher value (2.60%) than water (2.58), NaCl (2.58) and theoretical value (2.50%). On the contrary it is for L where the SC result (set 4) 3.69% was the nearest to theoretical value (3.65%). The set 2 and 3 showed a little higher values (3.71 and 3.72%). The theoretical calculation did not

confirm the harmony of calculated values with set 4. On the contrary set 4 values deviated from theoretical values in the cases of F and CP. However, the differences between compared sets were very small (in the order of hundredths of %). However, it is also necessary to take the uncertainties of instrument measurement (UNs) into account in comparison (Tab. III). UNs were higher than the mentioned differences.

Main attention was paid to differences between original sample (set 1) and modified sample in SC (set 4). Theoretically these procedures should approximate their values as much as possible. As it was shown by MFP and OS values the set 2 (NaCl solution) was near to original milk. However, it is possible to explain the fact that NaCl, which was contained in higher amount in set 3 than in set 4, participated in the MFP depression and in OS increase much more than other mineral matter contained in the SC. This is why the EC is a property which is considered the most important indirect indicator of milk mineral composition. EC values measured in set 4 were the nearest to original milk. We confirmed that SC approaches in composition and properties the composition of original milk in terms of appreciation of main soluble components.

CONCLUSIONS

The procedure described for balancing the interference effects of milk matrix at manipulation with milk components in RCSs for IR analysis calibration is applicable. The reasons for application of procedure in RCS set preparation are two: 1) preclusion of deviation of mean interference effects of milk matrix in the framework of multicomponent simultaneous milk IR analysis; 2) stability assurance of buffering system and capacity and also shelf-life of relevant RCSs for obtaining low values of calibration curves. More research and experiments are necessary.

SUMMARY

The knowledge about main milk composition is essential for cattle breeding and also for dairy plant technology and information of consumers. Routine milk analyses using the efficient indirect infrared method are important for the milk food chain control and quality. The reliability of the results depends on the quality of calibration. It is important to use a set of reference calibration samples (RCSs) with necessary range of values of measured milk components. RCSs can be prepared using various methods of modification that avoid damaging the natural milk composition and changing the interference effects of the basic matrix for calibration accuracy securing. Additions and removal of major and minor milk components are used often. The addition of water can be used because of lower composition value. This paper was aimed to balance the impacts of milk dilution for decrease of concentration of main components in RCSs because of achievement of minimal changes of matrix interference effects. Cow milk samples (MSs) were diluted (4/1) using distilled water, NaCl solution and a solution with specific composition (SC; because of disturbance in the balance of the milk matrix (NaCl 1.145; KCl 0.849; K_2HPO_4 1.8463; citric acid 1.7; urea 0.3 g/l)) for reduction in main milk components. Fat (F), crude protein (CP), lactose (L), milk freezing point (MFP), osmolality (OS) and electrical conductivity (EC) were measured in all (original as well as modified) MSs. The lowest MFP and OS were in the original milk -0.5559 °C and 274.5 mOsmol/kg. The MFP was increased to -0.4369 °C and osmolality decreased to 217.83 mOsmol/kg by the addition of water. The MFP was decreased (-0.4903 °C) and re-

turned to original milk value by the addition of NaCl solution. MFP was -0.4788 °C due to SC addition. The decrease was less than for NaCl. The ability of other SC components (K_2HPO_4 , KCl, citric acid and urea) to MFP decrease is less than for NaCl solution. EC was highest for NaCl set 4.69 mS/cm, EC for SC was 4.48 mS/cm ($P < 0.001$). The original MSs set showed EC 4.27 mS/cm. The SC was the nearest to original value of MSs in terms of total mineral composition. EC values for both modifications differed ($P < 0.001$) from original MSs. The mentioned procedure is applicable for balance of interference effects of milk matrix because of relevant calibration of infra-red method with using of modified milk samples. The method is important for laboratories which analyse raw milk in system of milk recording and milk quality control.

SOUHRN

Ověření modifikovaného referenčního vzorku mléka ve smyslu jeho vhodnosti pro kalibraci metody infračervené analýzy pomocí hodnocení fyzikálních vlastností

Znalost základního složení mléka je významná pro šlechtění skotu a také pro technologii v mlékárně a informaci spotřebitelů mléka. Rutinní mléčné analýzy za použití výkonné nepřímé infračervené metody jsou důležité pro řízení a kvalitu mléčného potravinového řetězce. Věrohodnost výsledků závisí na kvalitě kalibrace. Ke kalibraci je důležité použít sadu referenčních kalibračních vzorků (RCSs) s nezbytným oborem hodnot měřených mléčných ukazatelů. RCSs může být připravena prostřednictvím použití četných metod modifikací, které se vyvarují poškození přirozeného složení mléka a změn interferenčních vlivů základní mléčné matrice pro zajištění správnosti kalibrace. Často jsou použity přídavky nebo odejmutí hlavních nebo minoritních složek mléka. Přídavek vody může být použit pro dosažení nižších složkových hodnot. Cílem této práce bylo vyvážit vlivy ředění mléka pro snížení koncentrace hlavních složek v RCSs pro dosažení minimálních změn interferenčních vlivů matrice. Vzorky kravského mléka (MSs) byly ředěny (4/1) za použití destilované vody, roztoku NaCl a roztoku se specifickým složením (SC; kvůli porušení v rovnováze v mléčné matrici ($NaCl$ $1,145$; KCl $0,849$; K_2HPO_4 $1,8463$; kyselina citronová $1,7$; močovina $0,3$ g/l)) pro snížení hlavních mléčných složek. Ve všech (původních i modifikovaných) MSs byly měřeny tuk (F), hrubé bílkoviny (CP), laktóza (L), bod mrznutí mléka (MFP), osmolalita (OS) a elektrická vodivost (EC). Nejnížší MFP a OS byly v původním mléce $-0,5559$ °C a $274,5$ mOsmol/kg. MFP byl zvýšen na $-0,4369$ °C a osmolalita snížena na $217,83$ mOsmol/kg prostřednictvím přídavku vody. MFP byl snížen ($-0,4903$ °C) a navrácen k původní hodnotě mléka pomocí přídavku roztoku NaCl. MFP byl $-0,4788$ °C v důsledku přídavku roztoku SC. Pokles byl menší než pro roztok NaCl. Schopnost ostatních složek SC (K_2HPO_4 , KCl, kyselina citronová a močovina) k poklesu MFP je menší než pro roztok NaCl. EC byla nejvyšší pro soubor NaCl $4,69$ mS/cm, EC pro SC byla $4,48$ mS/cm ($P < 0,001$). Původní sada MSs ukázala EC $4,27$ mS/cm. Roztok SC byl nejbližší k původní hodnotě MSs ve smyslu celkového minerálního složení. Hodnoty EC se lišily od původních MSs pro obě modifikace ($P < 0,001$). Uvedený postup je použitelný pro vyvážení interferenčních vlivů mléčné matrice pro správnou kalibraci infračervené metody s použitím modifikovaných vzorků mléka. Metoda je důležitá pro laboratoře analyzující syrové mléko v kontrole užitečnosti a kontrole kvality mléka.

mléko, interferenční vliv, složení, bod mrznutí mléka, osmolalita, elektrická vodivost

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