Acta Agraria Kaposváriensis (2014) Vol 18 Supplement 1, 96-102.
Kaposvári Egyetem, Agrár- és Környezettudományi Kar, Kaposvár
Kaposvár University, Faculty of Agricultural and Environmental Sciences, Kaposvár

Variation of major mineral contents in Mediterranean buffalo milk and application of Fourier Transform Infrared spectroscopy for their indirect prediction

G. Stocco, C. Cipolat-Gotet, G. Bittante, A. Cecchinato
University of Padova, Department of Agronomy, Food, Natural resources, Animals and Environment
Viale dell'Università 16, 35020 Legnaro (PD), Italy

ABSTRACT

Minerals are important for many physiological functions and those contained in milk are actively involved in cheese manufacture. Moreover, milk minerals content is considered an indirect index of udder health in dairy cows. The aims of this study were to characterize mineral components (i.e., calcium, phosphorus, magnesium and potassium) in buffalo milk, to investigate their sources of variation and to test the effectiveness of Fourier Transform Infrared spectroscopy (FTIR) for their indirect prediction. A total of 173 buffaloes reared in five herds were milk sampled once during morning milking. Samples were analysed for calcium, phosphorus, potassium and magnesium within 3 h from collection using Inductively Coupled Plasma (ICP-OES). MilkoScan FT2 (Foss, Hillerød, Denmark) was used for the acquisition of milk spectra over the spectral range from 5000 to 900 wavenumber × cm-1179. Buffalo milk minerals (mg/L of milk) averaged 1,620, 144, 1,172 and 857 for calcium, magnesium, phosphorus and potassium, respectively. Herd and days in milk were the most important sources of variation for the former traits. Parity slightly affected only calcium and potassium. Coefficients of determination between the predicted and measured values in cross-validation (1-VR) were 0.71, 0.70, 0.72 for calcium, magnesium and phosphorus, respectively, whereas potassium exhibited a low accuracy (1-VR = 0.55). Our findings indicate that FTIR predictions could be used to assess buffalo milk components applying this rapid and non-invasive technique in the dairy industry and at the population level for breeding purposes.

(Keywords: buffalo milk, mineral content, FTIR spectroscopy)

INTRODUCTION

Buffalo milk production represents the 12% of the milk manufacture of the world, for this reason it is second after cow milk production (*Iqbal et al.*, 2011). Milk is considered an important source of minerals (*Singh and Sachan*, 2011). Considering the manufacturing of milk, calcium and phosphates have an important role in rennet coagulation of milk, in the structure of the cheese and cheese yield (*Ariota et al.*, 2007; *Lucey and Fox*, 1993). Also, the concentrations of many minerals are altered during mastitis (*Eshratkhah et al.*, 2012; *Ahmad et al.*, 2007), and these changes could be interesting to determine the manufacturing quality of the milk and for the diagnosis of subclinical mastitis. At present, one of the existing methodologies to determine mineral content in milk is Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

This method is too expensive for routine analysis of milk samples collected (*Soyeurt et al.*, 2009). The current tool used to measure the major milk components during regular milk recording is FTIR spectroscopy, that has been proposed also to predict innovative phenotypes as fatty acid profile (*Bastin et al.*, 2011), milk coagulation properties (*Bittante et al.*, 2012), lactoferrin (*Soyeurt et al.*, 2012) and mineral profile in bovine milk (*Soyeurt et al.*, 2009). Recently a study conducted by *Ferragina et al.* (2013) on FTIR predictions for cheese yield and nutrient recovery traits showed the possibility to employ this technology to help selecting cows in dairy populations. To our knowledge there are no specific FTIR spectroscopy studies on the mineral content of buffalo, as there are no studies regarding the monitoring of milk mineral salts during lactation in buffalo. Thus, the aims of this study were to assess the variation of the mineral content in buffalo milk, to estimate sources of environmental variation during lactation and to test the possibility of using FTIR spectroscopy for the indirect evaluation of the mineral content in buffalo milk.

MATERIALS AND METHODS

Collection and analysis of milk samples

A total of 173 buffaloes were sampled once in five herds located in northern Italy from January to May 2013. Individual milk samples were collected, without preservative, during the morning milking. Samples were stored in portable refrigerators (4 °C) and transferred to the milk quality laboratory of the Department of Agronomy Food Natural resources Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Italy). Samples were analysed for mineral contents within 3 hours from collection. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Ciros Vision EOP, SPECTRO Analytical Instruments GmbH, Kleve, Germany) was used as reference method to determine milk calcium, phosphorous, potassium and magnesium.

FTIR spectral collection and calibration

Buffalo milk samples spectra were collected using a MilkoScan FT2 (Foss, Hillerod, Denmark) over the spectral range from 5000 to 900 wavenumber × cm-1179. The spectra were stored as absorbance (A) using the transformation A = log(1/T), where T is the transmittance. Two spectral acquisitions were carried out for each sample, and the results were averaged prior to data analysis. As described in detail by *Ferragina et al.* (2013), calibration models were developed using the WinISI II software (Infrasoft International LLC, State College, PA) and carried out using partial least-square regression (PLS) as the chemometric algorithm. The accuracy of the model was evaluated using R^2 according to *Williams* (2003).

Statistical analysis

Sources of variation of mineral components (Ca, Mg, P and K) and milk yield (kg/d) were investigated using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) according to the following linear model:

$$Y_{ijkl} = \mu + DIM_i + Parity_j + Herd_k + e_{ijkl,}$$

where Y_{ijkl} is the observed trait (minerals: calcium, magnesium, phosphorus and potassium; milk yield); μ is the overall intercept of the model; DIM_i is the fixed effect of the *i*th class of DIM (i=1 to 6; class 1, <30 days (40 samples); class 2, 30–60 d (24 samples); class 3, 60–120 d (28 samples); class 4, 120–180 d (27 samples); class 5, 180–240 d (59 samples); class 6, >240 d (26 samples)); $parity_j$ is the fixed effect of the *j*th parity of the buffalo (j=1 to 4 or more); $Herd_k$ is the fixed effect of the *k*th herd (k=1 to 5); e_{ijkl} is the residual random error term ~ N (0, σ^2_e).

RESULTS AND DISCUSSION

Statistics of buffalo milk mineral contents

Descriptive statistics of major mineral contents and production traits of Mediterranean buffalo milk are summarized in *Table 1*. Calcium is the predominant mineral, followed by phosphorus, potassium and magnesium. Mineral values are different from the study conducted by *Soyeurt et al.* (2009) in cow milk, where potassium was the predominant mineral, followed by calcium, phosphorus and magnesium. This dissimilarity could be attributed to the species considered and to the different approach employed to determine minerals. Nevertheless these results are in agreement with literature.

Table 1

Descriptive statistics of major mineral contents and production traits of Mediterranean buffalo milk (n=173)

Trait	Mean	P1[1]	P99[2]	CV, %
Minerals, mg/L of milk				
Calcium	1,619	909	2,378	19
Magnesium	144	82	247	25
Phosphorus	1,172	672	1,699	20
Potassium	857	453	1,203	20
Production traits				
Milk yield, kg/d	5.93	0.8	13	48
DIM, d	147.5	6	400	71

[1]P1 = 1th percentile; [2]P99 = 99th percentile

In fact buffalo milk is generally characterized by high content of calcium (180–210 mg/100 g of milk) and phosphorus (120–140 mg/100 g of milk). Calcium and phosphorus are also the main mineral milk salts mostly related with the cheese yield and rennet coagulation properties (*Ariota et al*., 2007). In particular, milk phosphorus seems to have great relevance to favour cheese yield, especially in relation to the curd hydration, while calcium is related to RCT and gel firmness (*J.A. Lucey*, 1993). Results from ANOVA (*F*-values and significance) for major mineral contents in buffalo milk and single test-day milk yield (MY) are summarized in *Table 2*.

Table 2

Results from ANOVA (*F*-values and significance) for major mineral contents and single test-day milk yield (MY) in Mediterranean buffalo milk

Minerals, mg/L of milk	Herd	DIM	Parity	R^2, %	RMSE[1]
Df	4	5	3		
Calcium	11.56^{***}	2.86^{*}	3.11^{*}	47	234.25
Magnesium	5.28^{***}	8.17^{***}	1.48^{ns}	47	28.03
Phosphorus	19.88^{***}	2.18^{*}	2.25^{ns}	55	163.88
Potassium	12.91^{***}	4.48^{***}	2.96^{*}	50	127.12
MY, Kg/L	26.25^{***}	15.9^{***}	0.48^{ns}	49	2.08

[1]RMSE = root mean square error

The herd effect was relevant in explaining the variation of all minerals (P<0.001). Days in milk affected all the considered traits. Among mineral salts, Mg and K have been highly influenced (P<0.001), while Ca and P have been less affected by this factor (P<0.05). DIM considerably influenced also milk yield (P<0.001). Calcium averaged 1,713 mg/L in the first 30 days of lactation and increased of 26.50 mg/L through the period. Phosphorus increased from about 1,194 mg/L to about 1,252 mg/L in the last class of DIM, so generally it raised of 58.01 mg/L. Potassium slightly decreased from about 944 mg/L to around 816 mg/L, so it decreased of 128.18 mg/L throughout the lactation period. Magnesium raised from about 126 mg/L in the first 30 days of lactation to just over 170 mg/L in the last days, with a trend sharply opposite to the lactation curve. In fact it increased of about 46 mg/L through lactation period. Commonly, the variability in mineral content regards especially calcium and phosphates (*Ariota*, 2007). At the beginning of the lactation period, precisely around parturition, occur some physiological changes in mineral composition. Actually the calcium concentration in colostrum is much higher than that of normal milk and near the end of lactation (*Gaucheron*, 2005). Parity did not affect minerals and milk yield, except for calcium and potassium ($P < 0.05$) that tend to decrease in buffaloes with more than 4 lactations.

FTIR predictions for mineral contents in buffalo milk

Fitting statistics of predictions models for major mineral contents in Mediterranean buffalo milk is summarized in *Table 3*. For each mineral component different math treatment (e.g. MSC)/model combinations (PLS, MPLS etc.) were explored in order to retain the best calibration equation. The coefficient of determination of cross-validation (1-VR) was used as model choice criteria.

Table 3

Fitting statistics of predictions models for calcium (Ca), magnesium (Mg), phosphorus (P), and potassium (K) contents in Mediterranean buffalo milk

Trait	N[a]	#L[b]	Math[c]	SD[d]	SEC[e]	R^2 [f]	SEC_{cv} [g]	1-VR[h]	SEP(C)[i]
Ca	167	10	W,1,4,4,1	306	136	0.80	163	0.71	212
Mg	163	10	W,1,4,4,1	33	14	0.80	18	0.70	23
P	167	9	W,1,4,4,1	232	107	0.79	122	0.72	159
K	167	5	A,MSC, 0,0,1,1	167	102	0.63	112	0.55	145

[a] N = number of samples used in the calibration after removing outlier.

[b] #L = number of partial least square components.

[c] Math = mathematical treatments of the spectral data where the letters indicate the spectral range used for calibration (A= all the spectrum 5,011–930 cm^{-1}; W = spectra segments used 5,011–3,673 cm^{-1} 3,048–1,701 cm^{-1} and 1,582–930 cm^{-1}), MSC= multiplicative scatter correction, the first number is the order of the derivative, the second number is the segment length in data points over which the derivative was taken, the third and fourth numbers are the segment length for first and second smoothing respectively.

[d] SD = standard deviation.

[e] SEC = standard error of calibration.

[f] R^2 = coefficient of determination of calibration.

[g] SEC_{cv} = standard error of cross-validation.

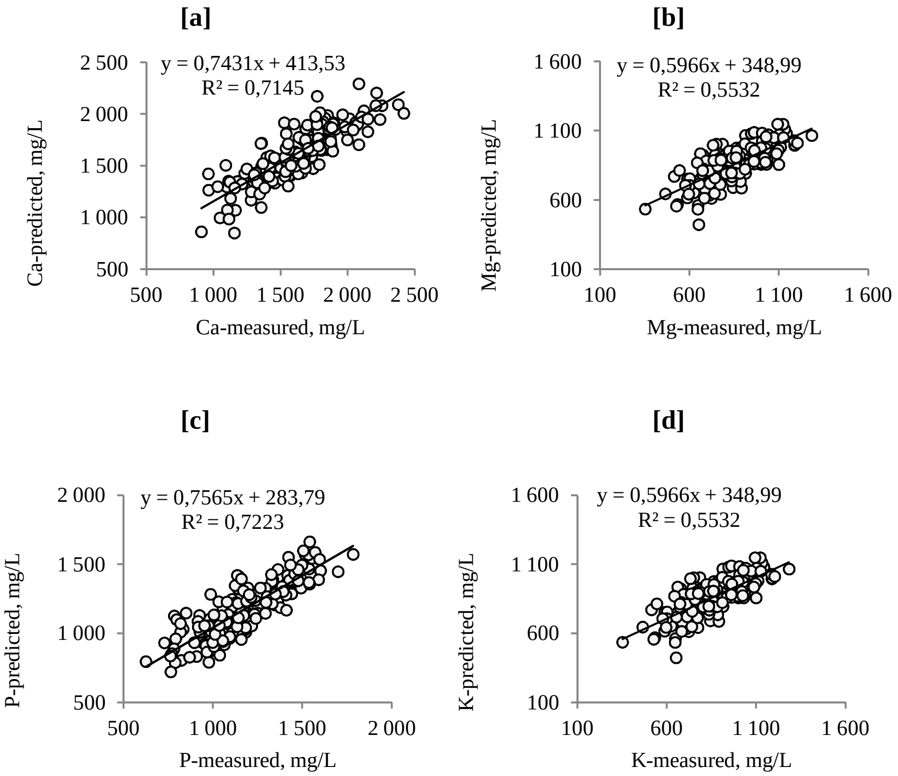
[h] 1-VR = coefficient of determination of cross-validation.

[i] SEP(C) = standard error of prediction corrected for the bias.

The coefficient of determination of cross-validation (1-VR) of the predicted versus measured values of mineral contents was good for calcium, magnesium, and phosphorus, and low for potassium (*Figure 1*). *Soyeurt et al.* (2009) found 0.97 R^2 for calcium and 0.88 R^2 for phosphorus in bovine milk. This could be due to the differences in terms of number of samples used for building the calibrations equations, sampling conditions and milk composition (buffalo vs cow).

Figure 1

Scatter plots of predicted vs measured values of calcium (Ca) [a], magnesium (Mg) [b], phosphorus (P) [c] and potassium (K) [d] contents (mg/L of milk) in buffalo milk



CONCLUSIONS

The present study investigated the variation of the mineral content in buffalo milk, the sources of environmental variation during buffalo lactation and the using of FTIR spectroscopy for the indirect evaluation of minerals in buffalo milk. According to the literature, calcium was the predominant mineral, followed by phosphorous, potassium and magnesium. Herd and DIM were the most important sources of variation for the considered traits, as expected. Parity slightly affected only calcium and potassium. R^2 for cross-validation of the predicted versus measured values of mineral contents was good for calcium, magnesium and phosphorus. Potassium revealed the worst fitting value. Ca, Mg and P predictions could therefore be useful for the efficient monitoring of health status of dairy populations, besides being a rapid and cheap tool for improving the nutritional quality of milk. Future work is necessary to examine the economic importance of these traits and their improvement in dairy populations.

REFERENCES

Ahmad T., Bilal, M.Q., Uallah, S., Rahman, Z., Muhammad, G. (2007). Impact of mastitis severity on mineral contents of Buffalo milk. Pak. Journal of Agricolture Science, 44(1). 176-178.

Ariota, B., Campanile, G., Potena, A., Napolano, R., Gasparrini, B., Neglia, G. L., Di Palo, R. (2007). Ca and P in buffalo milk: curd yield and milk clotting parameters. Italian Journal of Animal Science, 6(1). 497–499.

Bastin, C., Gengler, N., Soyeurt, H. (2011). Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. Journal of Dairy Science, 94. 4152–4163.

Bittante, G., Penasa, M., Cecchinato, A. (2012). Invited review: Genetics and modeling of milk coagulation properties. Journal of Dairy Science, 95. 6843-6870.

Eshratkhah, B., Beheshti, R., Shayegh, J. (2012). Variation of some minerals values in subclinical mastitic milk of Buffalo during different ages and lactation stages. Global Veterinaria, 8(4). 333-337.

Ferragina, A., Cipolat-Gotet, C., Cecchinato, A., Bittante, G. (2013). The use of Fourier-transform infrared spectroscopy to predict cheese yield and nutrient recovery/whey loss traits from unprocessed bovine milk samples. Journal of Dairy Science, 96. 7980-7990.

Gaucheron, F. (2005). The minerals of milk. Reproduction. Nutrition Development, 45. 473-483.

Iqbal, A., Khan, B.B., Kausar, A., Aftab, M.J. (2011). Buffalo-milk production potential and its comparative milk qualities. International symposium on Sheep, Goat and other non-Cow Milk. International Dairy Federation (1201).

Lucey, J. A., Fox, P. F. (1993). Importance of Calcium and Phosphate in cheese manufacture: a review. Journal of Dairy Science, 76(6). 1714-1724.

Singh, V.P., Sachan, N. (2011). Nutraceutical properties of milk and milk products: A review. American Journal of Food Technology, 6(10). 864-869.

Soyeurt, H., Colinet , F.G., Arnould, V. R., Dardenne, P., Bertozzi, C., Renaville, R., Portetelle D., Gengler, N. (2007). Genetic variability of lactoferrin content estimated by mid-infrared spectrometry in bovine milk. Journal of Dairy Science, 90(9). 4443-4450.

Soyeurt, H., Bruwier, D., Romnee, J.M., Gengler, N., Bertozzi, C., Veselko, D., Dardenne, P. (2009). Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry. Journal of Dairy Science, 92. 2444-2454.

Soyeurt, H., Bastin, C., Colinet, F.G., Arnould, V.M.R., Berry, D.P., Wall, E., Dehareng, F., Nguyen, H.N., Dardenne, P., Schefers, J., Vandenplas, J., Weigel, K., Coffey, M., Theron, L., Detilleux, L.J., Reding, E., Gengler, N., McParland, S. (2012). Mid-infrared prediction of lactoferrin content in bovine milk: potential indicator of mastitis. Animal, 6.1830-1838.

Williams, P.C. (2003). Near-infrared technology getting the best out of light. A Short Course in the Practical Implementation of Near Infrared Spectroscopy for the User. PDK Projects, Nanaimo, Canada.

Corresponding author:

Giorgia Stocco
Department of Agronomy, Food, Natural resources, Animals and Environment
University of Padova, Agripolis
Viale dell’Università 16, 35020 Legnaro (PD), Italy
E-mail: giorgia.stocco.1@studenti.unipd.it