ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(6): 945-952
doi: 10.25083/rbl/24.6/945.952

Received for publication, March, 4, 2018
Accepted, November, 5, 2018

Original paper

Chemometric guidelines for assessment of Fatty Acid Content in Cow Milk from different farming system

DENIS KUČEVIĆ[1]*, STRAHINJA KOVAČEVIĆ[2], MILICA KARADŽIĆ[2], LIDIJA JEVRIĆ[2], KSENIJA ČOBANOVIĆ[1], VESNA GANTNER[3], SANJA PODUNAVAC-KUZMANOVIĆ[2]

[1]University of Novi Sad, Faculty of Agriculture, Trg D. Obradović 8, 21000 Novi Sad, R. Serbia
[2]University of Novi Sad, Faculty of Technology Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia
[3]Josip JurajStrossmayer University of Osijek, Faculty of Agriculture, VladimiraPreloga, 31 000 Osijek, Croatia

Abstract In order to determine potential differences among the fatty-acid content in raw cow's milk in terms of organic and conventional dairy farming, various approaches of chemometric tools were applied. Furthermore, reliable regression models for prediction of unsaturated and saturated fatty acids contents in milk based on milk fat content was used. During 12 months research (four seasons) were collected a total of 7.978 samples of raw milk. The results included dendrograms (as results of cluster analysis) and univariate linear models. The cluster analysis covered the annual saturated and unsaturated fatty acid content values. Linear models present the dependence between milk fat and saturated fatty acids content, and milk fat and unsaturated fatty acids content. For all established statistical models, adequate validation procedures were conducted as an important step in testing the performance of the obtained models. Established models can be applied for the purpose of prediction of saturated and unsaturated fatty acid on the basis of milk fat, which was confirmed by internal and external validation parameters (R^2 in some models was higher than 0.90). Also, results of correlation between milk fat and fatty acids content provide very useful information for ~~a~~ breeding organizations and milk producers.

Keywords Chemometrics, Fatty acid, Milk, Cluster analysis, Regression analysis.

To cite this article: KUČEVIĆ D, KOVAČEVIĆ S, KARADŽIĆ M, JEVRIĆ L, ČOBANOVIĆ K, GANTNER V, PODUNAVAC-KUZMANOVIĆ S. Chemometric guidelines for assessment of Fatty Acid Content in Cow Milk from different farming system. *Rom Biotechnol Lett*. 2019; 24(6): 945-952. DOI: 10.25083/rbl/24.6/945.952

*Corresponding author: DENIS KUČEVIĆ, University of Novi Sad, Faculty of Agriculture, Trg D. Obradović 8, 21000 Novi Sad, R. Serbia; Tel: +381 21 485 3474
E-mail: denis.kucevic@stocarstvo.edu.rs

Introduction

A plenty of non-genetic factors (e.g. stage of lactation, feed, climate, method of milking, health status of the cow, parity, season etc.) and genetic factors (e.g. breed and individual) are known to influence the composition of raw milk (TAMIME [1]; JENKINS & al [2]; KUČEVIĆ & al [3]). Diets of cow and breed have a major influence on milk fat fluctuation and fat content (JENKINS & al [2]; KELSEY & al [4]). Factors such as lactation stage, nutrition and seasonality, significantly affects the fatty acid profile. Milk produced on an organic dairy farm has a more favorable fatty-acid composition because it contains more unsaturated FA, especially a group of polyunsaturated FA with omega-3 (ELLIS & al [5]; BALTUŠNIKIENE & al [6]; PRANDINI & al [7]). A number of studies show that nutrition of dairy cows, which is based on use of pasture (fresh grazing, or usage of specific silage types), as well as type of farming (organic vs conventional), have the highest effect on fatty-acid content of milk, as it increases the content of polyunsaturated fatty acids, especially omega-3 and conjugated linoleic acid (ELLIS & al [5]; KUČEVIĆ & al [3]; POPOVIĆ-VRANJEŠ & al [8]). The group of unsaturated FA (especially polyunsaturated) has positive effects on human health. Chemometrics is considered to be a very practical tool for application not only in chemistry, but also in many other fields, such as pharmaceutical engineering, microbiology, food technology, biotechnology, forensics (KOVAČEVIĆ & al [9]; KOVAČEVIĆ & al [10]; DODIĆ & al [11]). Recently, chemometrics has been used in agricultural researches for prediction of antioxidant activity of *Lactuca sativa* L. varieties on the basis of the content of different phytochemicals (JEVRIĆ & al [12]), for the selection ofcultivation conditions that influence the antioxidant potential of different beetroot extracts (KOVAČEVIĆ & al [13]), for identification of apple varieties (FERNÁNDEZ-GONZÁLEZ & al [14]). In the present study, chemometrics was used in order to determine potential differences among the analyzed samples and farms, as well as to establish reliable regression models for prediction of unsaturated fatty acid (UFA) and saturated fatty acids (SFA) contents in milk, obtained from organic dairy farm (OF) and conventional dairy farm (CF), on the basis of milk fat content (F). Also, the analysis of similarities between milk samples obtained from two different farms (OF and CF) was carried out applying the analysis of variance (ANOVA). The Hierarchical Cluster Analysis (HCA) was conducted in order to reveal the similarities/dissimilarities among the milk samples obtained in different seasons on the basis of fat acids content.

Materials and Methods

1. Animals management

The experiment was conducted at the two Dairy Farms located in the Province of Vojvodina. The first farm was operated in accordance with the organic principles of farming (OF). Six hundred sixty-five multi and primiparous Holstein dairy cows (Body Weight: 605.0 ± 42.0 kg, Day in Milking 136 ± 76 d; parity: 2.8 ± 1.2; milk yield: 24.4 ± 9.3 kg/d), were selected for research. The second farm was followed by conventional principles of dairy farming (CF). Five hundred and fifty multi and primiparous Holstein dairy cows (Body Weight: 655.0 ± 22.0 kg, Days in Milking: 116 ± 46 d; parity: 2.2 ± 1.0; milk yield: 26.5± 10.3 kg/d) were selected for the research. Dairy cows were housed in free stall barns in both farming systems and were milked twice per day (around at 6 h and 18 h). During the trial, the cows had free access to water (ad libitum) and they were fed twice a day. Each cows group on the both of farms received a total mixed ration (TMR) based on maize and lucerne silage, lucerne hay, concentrates feed (home-grown grains) and mineral supplements. During the pasture season, cows in organic farming additionally had access to the natural pastures. Chemical compositions of diets were formulated according to NRS standard and adjusted to cow's lactation groups (assigned to milk yield).

2. Sample collection and instrumental analysis

A total of 7.978 samples of fresh milk during 12 months research were collected from certified organic (OF) and conventional (CF) dairy farming systems. The collection of samples was carried out in compliance with the regulations of the International Committee for Animal Recording *(ICAR- AT_4)*. Individual milk samples (40 mL/each) from the morning milking were collected from each cow to be analyzed for milk composition and FA profile. Collected samples were preserved with potassium dichromate. The milk analysis was performed in the international accredited Laboratory for Quality Control of Milk *(ISO 17025)*, the Faculty of Agriculture, University of Novi Sad, using a CombiFoss™FT+ analyzer for routine compositional raw milk analysis employing Fourier Transform Infrared "FTIR". This device is a combination instrument consisting of the MilkoScan™ FT+ and the Fossomatic™ FC, techniques comply with: *ISO 9622 / IDF 141:2013* and the *AOAC* official method 972.16.

3. Chemometric tools and modeling

*Initial modeling.*The chemometric modeling cannot start until the data set is cleaned up for outliers and is internally consistent (ESBENSEN & al [15]). The whole data set, containing the information about total milk fat content, saturated and unsaturated fatty acids contents, was previously checked in order to detect potential misspelled numbers and outliers. The data screening revealed some potential outliers which were not taken into consideration in the further chemometric modeling. *Hierarchical cluster analysis (HCA)*. It is used for simple detection of similar objects (samples) by forming clusters (MILLER & al [16]). It finds the objects closely positioned to each other in the space of variables used for the similarity detection. This method calculates the distances between two objects and the smallest distances is the objects are more similar. The result of HCA analysis is represented graphically by a diagram well-known as a dendrogram. *Analysis of variance*

(ANOVA). ANOVA is another similarity detection method. It is a powerful statistical method which can be applied for separation and estimation of the different causes of variation (ERIKSSON & al [17]). The ANOVA can be used to compare two means and to make comparisons of more than two population means (ESBENSEN & al [15]; ERIKSSON & al [17]). Also, it can be used to determine the effects of various factors in more complex experimental designs (ERIKSSON & al [17]). In the present study, the ANOVA (Univariate Analysis of Variance – full factorial) was used in order to reveal if there was a statistically significant difference among the milk samples obtained from two different farms, organic and conventional. *Regression analysis*. In the present study, simple linear regression analysis or so-called univariate regression ($y \mid x$) was carried out in order to determine the dependence between milk fat and saturated fatty acids content, and milk fat and unsaturated fatty acids content. It is one of the most exploited regression methods. It is simple and if the best models are obtained applying univariate regression, there is no need for the application of more complex regression methods. *Validation of regression models* is a very important step in testing the performance of a regression model. It was carried out applying internal and external validation procedures. The cross-validation was used as an internal validation method, while the external validation was conducted by forming the external test set. The models were validated on the basis of the following statistical parameters: Pearson's correlation coefficient (R), determination coefficient (R^2), adjusted determination coefficient (R^2_{adj}), leave-one-out cross-validation determination coefficient (R^2_{cv}), root mean square error (RMSE), variation coefficient (CV%), Fisher's test (F), significance level (p), predicted residual error sum of squares (PRESS), total sum of squares (TSS) and PRESS/TSS ratio. The external validation was done by applying the randomly selected external test set which contained 110–121 samples. The prediction based on the external test set is described by the aforementioned statistical parameters, as well as by the graphical comparison of the concurrence of the training and external test sets taking into account the intercept and slope of the linear regression of the training and external test sets (ESBENSEN & al [15]). The comparison of the training and test results can be done by prediction of errors, calculation of residual variances and quantification of accuracy and precision of predicted values (ESBENSEN & al [15]). Also, the external validation was done by calculation of the concordance correlation coefficient (CCC) which verified the concurrence of experimental and predicted data (CHIRICO & al [18]). It is calculated on the basis of the following equation (CHIRICO & al [18]):

$$(Y_{i,PRED} - \overline{Y_{PRED}})^2 + {}^{n}EXT$$

where ^{n}EXT is the number of samples in the external test set, Y_i is the experimental value, $\bar{Y}$ is the average of the experimental values, $Y_{i,PRED}$ is the predictedvalue and $Y_{PRED}$is the average of the predicted values. The values of CCC higher than 0.85 describe a model as highly predictive (CHIRICO & al [18]).

Results and Discussion

1. ANOVA

The ANOVA (Univariate Analysis of Variance – full factorial) was used in order to reveal if there was a statistically significant difference among the milk samples obtained from the different system of farming during the year. The fixed effect of the system of farming (OF vs CF) and season (winter, spring, summer and fall) have shown a high statistical significance ($P < 0.01$) on examined fatty acid parameters in row milk (Table 1). According to the results, it is evident that there was a highly statistically significant difference between the content of fatty acid (SFA vs UFA) in different dairy farming.

Table 1. The effect of system of farming and season on examined fatty acid parameters

Source of variation	Dependent variable	Sum of Squares	d.f.	Mean Square	F- value
System	SFA	25.036	1	25.036	125.48*
	UFA	6.932	1	6.932	91.56*
Season	SFA	34.652	3	11.551	57.89*
	UFA	14.692	3	4.897	64.69
System x Season	SFA	12.959	3	4.320	21.65*
	UFA	10.059	3	3.353	44.29*
Error	SFA	1351.508	6774	0.200	
	UFA	512.788	6774	0.076	

*indicate significant differences at the level $P < 0.01$; d.f. – degree of freedom

2. Hierarchical Cluster Analysis

HCA was carried out on the basis of the average values of SFA and UFA contents obtained for every season. Therefore, the cluster analysis covered the annual SFA and UFA content values. The clustering was done on the basis of Single linkage algorithm and Euclidean distances. The obtained results are given in Figure 1. The dendrograms given in Figure 1A/1B, regarding the concentration of UFA, presents the clustering of the samples collected in OF in CF. On the basis of the presented dendrograms, it can be seen that the clustering of UFA in OF was much more uniform compared to clustering of UFA in CF. It can be noted in the Figure 1A that spring stands out from the other months during the year. The same display can be seen for a conventional production on 1B, whereby winter stands out from the other months. These results could be expected because the composition of FA content is greatly influenced by the season and nutrition of dairy cows during the year (ELLIS & al [5];POPOVIĆ-VRANJEŠ & al [8]). During the pasture season, cows in organic farming additionally had access to the natural pastures or received fresh grass-clover products, which leads to changes in the composition of the FA. In this regard, there is a significant difference in the diet of dairy cows in OF vs CF from early spring until early winter. Additionally, milk produced in organic dairy farming has a significantly more concentration of UFA, especially polyunsaturated fatty acid (PUFA) than in conventional farming (KUČEVIĆ & al [3];POPOVIĆ-VRANJEŠ & al [8]). Effects of season, access to fresh grazing, or usage of specific silage types could be used by producers to enhance the content of beneficial FA in milk.

3. Linear univariate regression modeling

The results of the linear univariate regression modeling where milk fat content (F) was the independent variable and SFA and UFA were dependent variables are given in Figure 2.

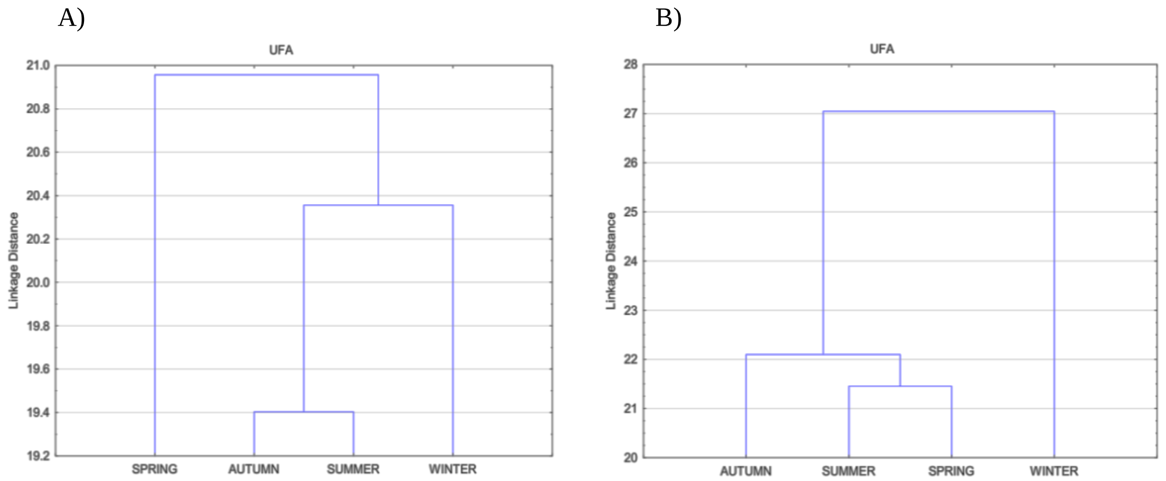

Figure 1. The dendrograms of the cluster analysis of the milk samples obtained from organic farm – OF (A) and conventional farm – CF (B) based on UFA content

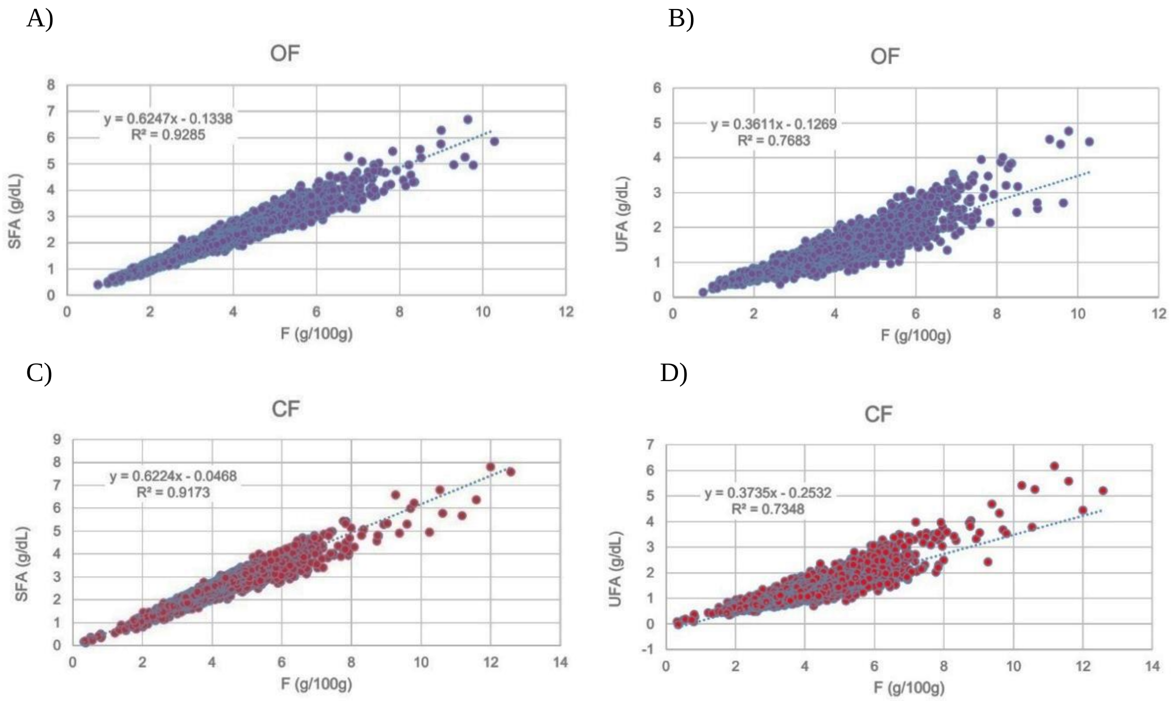

Figure 2. Linear univariate regression models of the correlations between F and SFA for the samples originating from OF: OF-SFA model (A), the correlations between F and UFA for the samples originating from OF: OF-UFA (B), the correlations between F and SFA for the samples originating from CF: CF-SFA (C) and the correlations between F and SFA for the samples originating from CF: CF-UFA model (D). The samples used in the present models are collected during the whole year

The aforementioned statistical models provide very useful information for a breeding organizations and milk producers. Namely, national (central) milk laboratories play an important role in the context of Dairy herd improvement (DHI) program. Within this program is being implemented individual milk recording too. For this purpose, laboratories use a quick Fourier transform infrared spectroscopy method (FTIR) to analyze the most important components of raw cow's milk (fat, protein, lactose, total solids and other). Additional and reliable information about the correlation between milk fat and fatty acids are important from the point of several aspects. Primarily due to the successful management of dairy cow's feeding in all stages of production. Managing nutrition, as well as a choice of farming system, can contribute to the control of fatty acids content in milk during the year in all seasons (ELLIS & al [5]; KUČEVIĆ & al [3]; POPOVIĆ-VRANJEŠ & al [8]). Useful information about FA content is one of the best approaches to improving a milk quality. Hereby, managing of FA content is an essential component to farm profitability and it can positively affect the health of consumers (CONNOR & al [19]; CONTRERAS & al [20]).

The external statistical validation of the linear univariate regression models of the correlations between F and SFA/UFA confirmed the significance of the established models, as well as the strong dependence between the analyzed variables (Figure 3 and Figure 4). The concurrence between the experimental and predicted data in the case of the models which predict SFA is outstanding, while in the case of the models aimed for prediction of UFA it is considered to be quite satisfactory. *The statistical quality of the established models has been confirmed by the statistical parameters given in Table 2. In both cases (OF and CF) the models trained for prediction of SFA have R^2 of training and external test sets higher than 0.90. Besides, the internal validation parameters, presented in Table 3, confirm the predictivity of the established models. However, the models which predict UFA have slightly worse statistical quality than the models aimed for prediction of SFA in the case of both OF and CF, but still acceptable for prediction of the mentioned parameters. Also, the models based on the data obtained from OF are slightly statistically better than the models established on the basis of the data originating from CF.

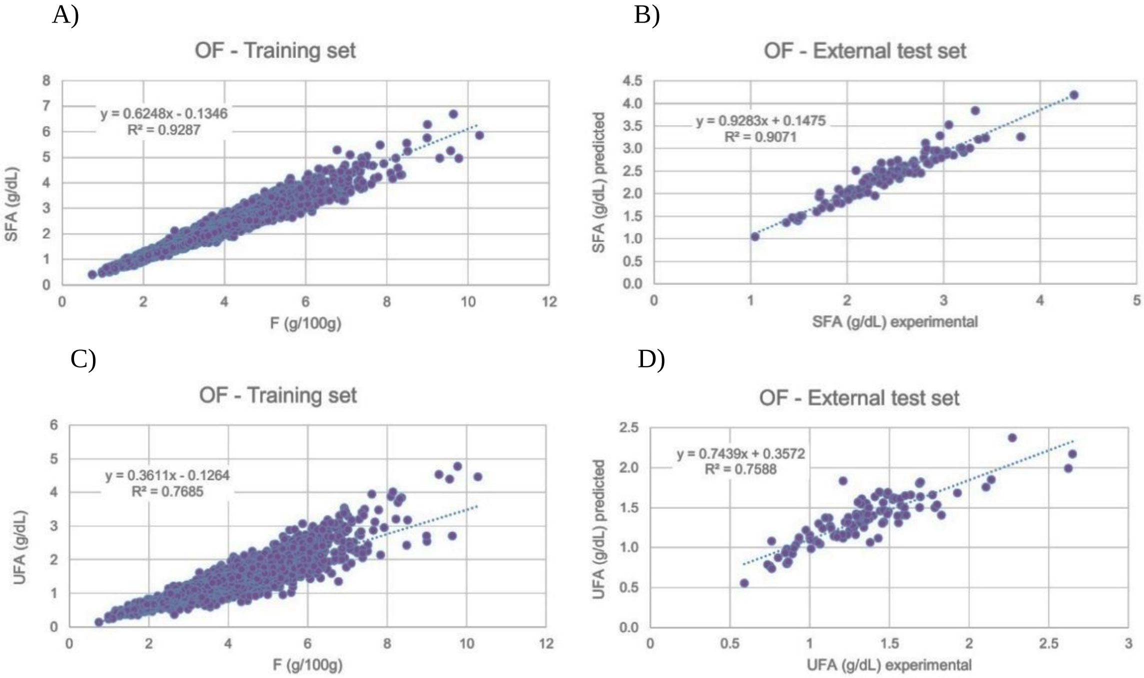

Figure 3. The external validation of the linear univariate regression models of the correlations between F and SFA/UFA for the samples originating from OF. A) The regression model established for prediction of SFA; B) Fitting of the external data set where the predicted values of SFA were obtained applying the established regression model; C) The regression model established for prediction of UFA; D) Fitting of the external data set where the predicted values of UFA were obtained applying the established regression model.

The external validation and the analysis of the residuals showed that the established models can be applied for the purpose of prediction of SFA and UFA on the basis of F for the samples originating from OF and CF. The residuals are randomly distributed around the $y = 0$ axis and have an acceptable amplitude (Figure 5). Also, the values of CCC, given in Table 3, describe the models as high-predictive, since their values are higher than 0.85. The comparison of the experimental and predicted SFA and UFA data (Figure 4B and 4D) indicate a very good fitting, particularly in the case of CF. The intercept of this dependence is very close to zero, while the intercept is close to 1. In the case of CF, these values are slightly worse, but still good to consider this model statistically relevant and predictive.

Generally, all the established models have a quite good predictivity, which is confirmed by internal and external validation parameters (R^2_{cv}> 0.60, R^2_{adj}> 0.70, CV% < 20%, very high values of F-test, very small RMSE values, and PRESS/TSS ratio < 0.40).

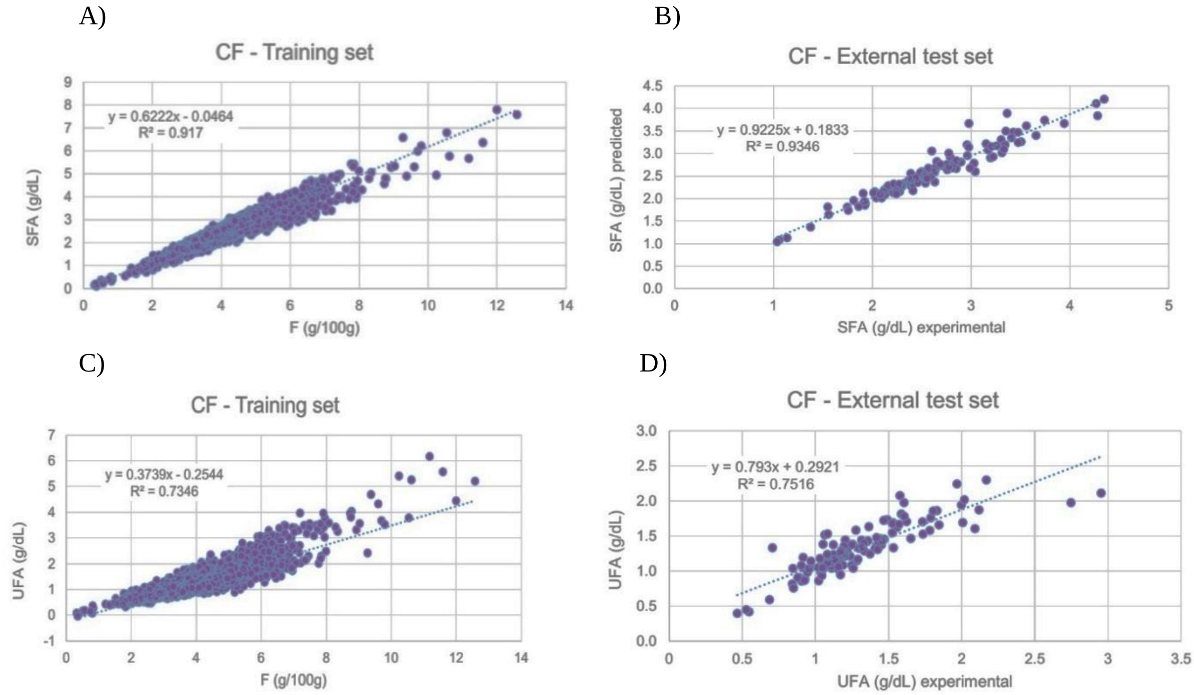

Figure 4. The external validation of the linear univariate regression models of the correlations between F and SFA/UFA for the samples originating from CF. A) The regression model established for prediction of SFA; B) Fitting of the external data set where the predicted values of SFA were obtained applying the established regression model; C) The regression model established for prediction of UFA; D) Fitting of the external data set where the predicted values of UFA were obtained applying the established regression model.

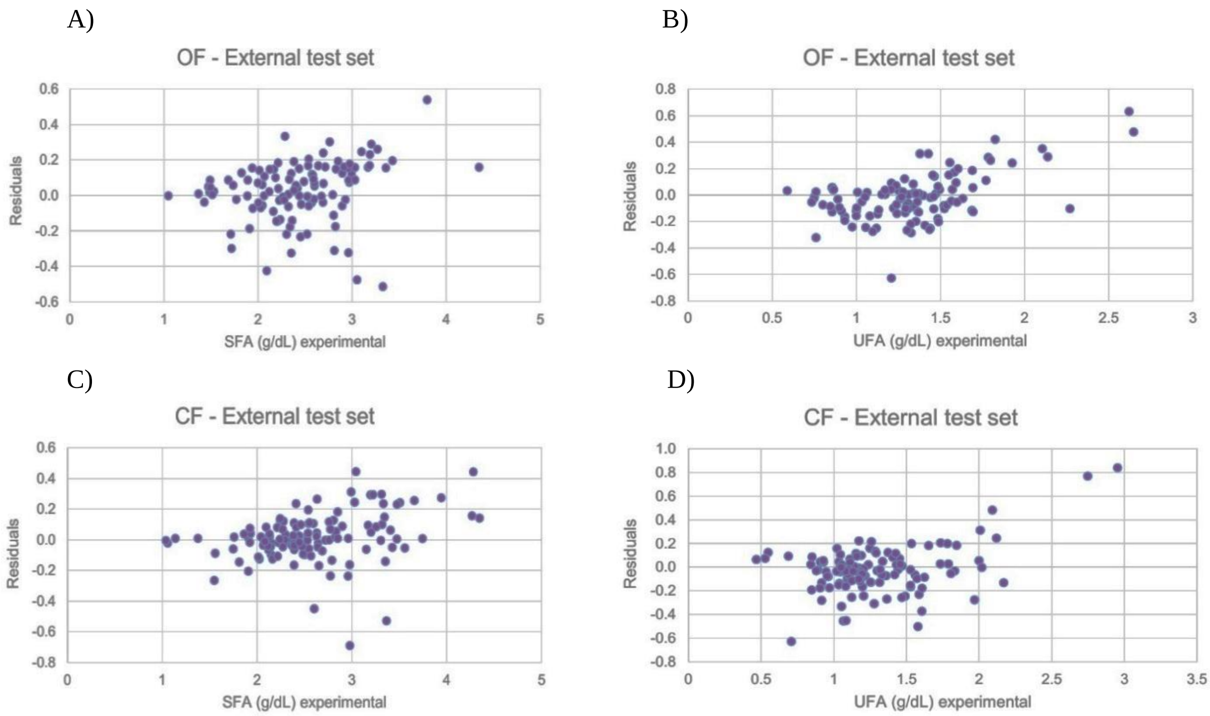

Figure 5. The graphical analysis of the randomness of the residuals distribution for the external test sets used in regression modeling for the models based on the samples collected on OF and CF.

Table 2. Statistical values of the internal validation parameters of the linear univariate regression models aimed for prediction of SFA and UFA contents in the milk samples originating from OF and CF

Parameter	Regression model			
	OF-SFA	OF-UFA	CF-SFA	CF-UFA
R	0.9636	0.8765	0.9578	0.8572
R^2	0.9285	0.7683	0.9173	0.7348
R^2_{adj}	0.9284	0.7683	0.9173	0.7347
R^2_{cv}	0.9284	0.7681	0.9172	0.7342
RMSE	0.1622	0.1855	0.1719	0.2064
CV%	6.89	14.14	6.75	15.84
p-value	0.000000	0.000000	0.000000	0.000000
F-test	105131	26868	74806	18679
PRESS	213.39	279.11	199.61	287.96
TSS	2979.94	1203.53	2410.38	1083.49
PRESS/TSS	0.07	0.23	0.08	0.27

Table 3. The external validation parameters of the linear univariate regression models aimed for prediction of SFA and UFA contents in the milk samples originating from OF and CF

Parameter	Regression model							
	OF-SFA-TR	OF-SFA-EXT	OF-UFA-TR	OF-UFA-EXT	CF-SFA-TR	CF-SFA-EXT	CF-UFA-TR	CF-UFA-EXT
R	0.9637	0.9524	0.8766	0.8711	0.9576	0.9668	0.8571	0.8669
R^2	0.9287	0.9070	0.7685	0.7589	0.9170	0.9347	0.7346	0.7516
R^2_{adj}	0.9287	0.9062	0.7685	0.7566	0.9170	0.9341	0.7345	0.7495
R^2_{cv}	0.9286	0.9030	0.7682	0.7463	0.9169	0.9324	0.7340	0.7310
RMSE	0.1621	0.1636	0.1856	0.1523	0.1721	0.1525	0.2066	0.1787
CV%	6.89	6.80	14.15	11.35	6.76	5.94	15.85	13.58
p-value	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
F-test	104101	1054	26526	340	73146	1702	18326	360
PRESS	210.26	3.02	275.64	2.64	196.53	2.87	283.31	4.12
TSS	2946.53	31.11	1189.27	10.39	2363.68	42.37	1065.20	15.30
PRESS/TSS	0.07	0.10	0.23	0.25	0.08	0.07	0.27	0.27
CCC	-	0.9509	-	0.8591	-	0.9653	-	0.8616

Conclusion

The conducted study resulted in the univariate linear models that can be used for prediction of unsaturated and saturated fatty acids contents in raw milk based on milk fat content in the samples originating from organic and conventional dairy farming. Internal and external validation procedures confirmed the significance of the established models on the basis of the numerous statistical parameters, as well as the strong dependence between the analyzed variables. Obtained results, particularly the correlation between milk fat and fatty acids contents, provide very useful information for breeding organizations and milk producers, primarily due to the successful management of dairy cow's feeding and nutrition in all stages of milk production during the year as well as achoice of farming system. Managing of fatty acids contents during the production can positively affect the farm profitability as well as the health of milk consumers.

References

1. Y.A. TAMIME, Milk Processing and Quality Management, Blackwell Publishing Ltd., 978-1-405-14530-5 (2009).
2. T.C. JENKINS, M.A. MCGUIRE, Major advances in nutrition: Impact on milk composition. *Journal of Dairy Science* 89: 4, 1302-1310 (2006).
3. D. KUČEVIĆ, S. TRIVUNOVIĆ, V. BOGDANOVIĆ, K. ČOBANOVIĆ, D. JANKOVIĆ, D. STANOJEVIĆ, Composition of Raw Milk from Conventional and Organic Dairy Farming. *Biotechnology in Animal Husbandry*, 32: 2,133-143 (2016).

4. J.A. KELSEY, B.A. CORL, R.J. COLLIER, D.E. BAUMAN, The Effect of Breed, Parity, and Stage of Lactation on Conjugated Linoleic Acid (CLA) in Milk Fat from Dairy Cows. *Journal of Dairy Science* 86: 8, 2588-2597 (2003).
5. K.A. ELLIS, G. INNOCENT, D. GROVE-WHITE, P. CRIPPS, W.G., MCLEAN, C.V. HOWARD, Comparing the fatty acid composition of organic and conventional milk. *Journal of Dairy Science* 89: 6, 1938-1950 (2006).
6. A. BALTUŠNIKIENE, Z. BARTKEVIČIÛTE, J. ČERNAUSKIENE, Fatty acids content and composition of milk fat from cows consuming pasture and total mixed ration. *Veterinarija IR Zootechnika* 42: 64, 28-33 (2008).
7. A. PRANDINI, S. SIGOLO, G. PIVA, Conjugated linoleic acid (CLA) and fatty acid composition of milk, curd and Grana Padano cheese in conventional and organic farming systems. *Journal of Dairy Research*, 76: 3, 278-282 (2009).
8. A. POPOVIĆ-VRANJEŠ, M. SAVIĆ, R. PEJANOVIĆ, G. JOVANOVIĆ, M. KRAJINOVIĆ, The effect of organic milk production on certain milk quality parameters. *Acta Veterinaria* 61: 4, 415-421 (2011).
9. S.Z. KOVAČEVIĆ, S.O. PODUNAVAC KUZMANOVIĆ, L.R. JEVRIĆ, Multivariate regression modelling of antifungal activity of some benzoxazole and oxazolo [4,5-b] pyridine derivatives. *Acta Chimica Slovenica*, 60: 4, 756-762 (2013).
10. S.Z. KOVAČEVIĆ, S.O. PODUNAVAC-KUZMANOVIĆ, L.R. JEVRIĆ, V.R. VUKIĆ, M.P. SAVIĆ, E.A. DJURENDIĆ, Preselection of A- and B- modified D-homo lactone and D-secoandrostane derivatives as potent compounds with antiproliferative activity against breast and prostate cancer cells – QSAR approach and molecular docking analysis. *European Journal of Pharmaceutical Sciences*, 93, 107-113 (2016).
11. J. DODIĆ, J. GRAHOVAC, N. KALAJDŽIJA, S. KOVAČEVIĆ, L. JEVRIĆ, S. PODUNAVAC KUZMANOVIĆ, Chemometric approach to prediction of antibacterial agents production by Streptomyces hygroscopicus. *Applied Biochemistry and Biotechnology* 174,534-541 (2014).
12. L.R. JEVRIĆ, M.Ž. KARADŽIĆ, S. PODUNAVAC-KUZMANOVIĆ, A.N. TEPIĆ HORECKI, S.Z. KOVAČEVIĆ, S.S. VIDOVIĆ, Z.M. ŠUMIĆ, Ž.M. ILIN, New guidelines for prediction of antioxidant activity of Lactuca sativa L. varieties based on phytochemicals content and multivariate chemometrics. *Journal of Food Processing and Preservation* DOI: 10.1111/jfpp. 13355 (2017).
13. S.Z. KOVAČEVIĆ, A.N. TEPIĆ, L.R. JEVRIĆ, S.O. PODUNAVAC-KUZMANOVIĆ, S.S. VIDOVIĆ, Z.M. ŠUMIĆ, Ž.M ILIN, Chemometric guidelines for selection of cultivation conditions influencing the antioxidant potential of beetroot extracts. *Computers and Electronics in Agriculture*, 118, 332-339 (2015).
14. A. FERNÁNDEZ-GONZÁLEZ, J.M. MONTEJO-BERNARDOB, C. CASTAÑO-MONLLORC, R. BADÍA-LAÍÑOB, M.E. DÍAZ-GARCÍAB, Easy-to-use analytical approach based on ATR–FTIR and chemometrics to identify apple varieties under Protected Designation of Origin (PDO), *Computers and Electronics in Agriculture*, 108,166-172 (2014).
15. K.H. ESBENSEN, Multivariate Data Analysis – In Practice. 5th ed. Oslo: CAMO Software AS (2009).
16. J.N., MILLER, J.C MILLER, Statistics and chemometrics for analytical chemistry. Pearson Education Limited, Harlow, England (2010).
17. L. ERIKSSON, E. JOHANSSON, N. KETTANEH-WOLD, S. WIKSTRÖM WOLD, Design of Experiments, Principles and Applications, Umeå: Umetrics Academy (2008).
18. N. CHIRICO P. GRAMATICA, Real External Predictivity of QSAR Models: How to Evaluate It? Comparison of Different Validation Criteria and Proposal of Using the Concordance Correlation Coefficien. *Journal of Chemical Information and Modeling* 51: 9, 2320-2335 (2011).
19. W.E. CONNOR, Importance of n-3 fatty acids in health and disease. *The American Journal of Clinical Nutrition* 71,171S-175S (2009).
20. M.A. CONTRERAS, S.I. RAPOPORT, Recent studies on interactions between n-3 and n-6 polyunsaturated fatty acids in brain and other tissues. *Current opinion in lipidology* 13:3,267-272 (2002).