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Variation Analysis of Cow Milk Composition Quality Depending on Year, Season and Location in Romania

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Abstract. The effects of regional environment, years and seasons on the milk technological quality (contents of fat, protein, lactose, solids-non-fat, somatic cell number and number of total germs) were studied by multivariate statistical methods. Two different counties from Romania monitored in the years 2006-2008 were used during four seasons. Data was statistically processed in accordance with the SPSS statistics package. Principal component analysis (PCA) results suggested a significant difference ($p \leq 0.01$) between fat content from the two counties. The ANOVA factorial used to evaluate the influence of the counties, years and seasons effects on milk chemical and microbiological quality show that the evaluated factors had a significant global effect ($p \leq 0.01$) on the fat, lactose and number of total germs content.

Keywords: cow milk, quality composition, seasonal influence, location variation, multivariate analysis

INTRODUCTION

In Romania the dairy sector is significant as part of the national economy because stock farming is an important and traditional occupation in rural areas, especially in mountain districts; it brings regular incomes in these areas and makes good use of the pastures and hayfields that cover about 33% of the agricultural land in Romania (NIS, 2009). The quality and the composition of milk have great importance for human health and milk processing. Obtaining high-quality dairy products depends, mainly, on the physical-chemical and microbiological milk composition. The milk composition varies according to certain factors that were the subject of several researches. So, Ozrenk and Selcuk (2008) found negative correlations between environmental temperature, fat and protein milk content. Bruhn and Franke (1997), Lacroix *et al*. (1996) have reported that percentage of fat, protein and all the nitrogen fractions have been influenced by the seasonal variations. The fat content decreased when the environmental temperature increased (Yetismeyen 2000; Sekerden 1999), Casati *et al*. (1998) states that the light-to-dark ratio can also induce marked changes in milk yield and composition. Tucker (1989) assert that a high light-to-dark ratio leads to a reduction in fat and protein contents of milk, probably as a consequence of a greater secretion of prolactin whose concentration in plasma was higher in the summer than in the winter.

Changes in milk production and its components can be influenced by cow genetic (Lucas *et al*., 2006), physiologic factors such as medical state, lactation number and stage, animal age, udder health (Matei *et al*., 2010; Othmane *et al*., 2002; Kilic and Kilic 1994; Haenlein 1996), microbiological contamination (Gaucher *et al*., 2008; Bharat 2004) and zootechnic factors-type of diet, milking conditions (Falchero *et al*., 2010; Van Nieuwenhove *et al*., 2009; Bony *et al*., 2005; Salovuo *et al*., 2005). The influence of climatic factors on milk

components can be correlated to some parameters such as region of production (Karoui *et al*., 2005), season (Van Nieuwenhove *et al*., 2009; Alomirah *et al*., 2007; Formaggioni *et al*., 2002) and year of production.

In the present study, the aim was to determine, on a large data set, by a statistical approach, the possible effects of season, location and year of production on chemical and microbiological milk characteristics. As locations it was chosen two counties in Romania, Tulcea and Suceava that are representative from the point view of the climatic area. Tulcea is a county located in the southeastern of Romania, in the lowlands, with a temperate continental climate, where annual average temperatures vary between 11° and 9° C winters are cold and summers are hot and dry. The average sum of annual precipitation quantities was between 359 mm and 455 mm. Suceava is a county in northern Romania, located in the mountains area, and has a temperate and humid climate. The average annual temperature was between values below 0° and 6° and characterize mountain climate, while climate of mountain knows an average annual temperature between 7° and 8° (with an average annual temperature of 8°C). Winters are very cold, with large amounts of snow and summers are cooler. Rainfalls are also differentiated on levels of relief with variation between 550 and 1200 mm.

In the context given above, this study was to establish the year and season variability in fat, protein, lactose, solids-non-fat, somatic cell number and number of total germs in row cow milk from the two Romania counties. We wished to determine the relationships between years, counties and seasons using ANOVA and PCA statistical testing on the technological milk quality.

MATERIALS AND METHODS

The raw cow's milk samples were supplied from different collecting points from counties Tulcea and Suceava. The research was carried out in four periods. The first period was the winter season covering the months December-January-February, the second one was the spring season covering the months March-April-May, the third was the summer season covering the months June-July-August and fourth period was the autumn season covering the months September-October-November. Collecting samples from the two counties was conducted daily, taking into account the season, during a period of three consecutive years.

Milk quality tests were accomplished according to Romanian standard methods for fat content (SR EN ISO 1211:2010), somatic cell number (SR EN ISO 13366-3:2001) and solids-non-fat content (SR ISO 6731:1996). Protein and lactose content were analyzed using a infrared milk analyzer unit Bentley 150 (Bentley Instruments Inc., Chaska, MN, USA) and number of total germs were done by flow cytometry count analyzer unit BactoCount IBC 50 (Bentley Instruments Inc., Chaska, MN, USA).

All determinations were performed at least in triplicate. Values of the parameters are expressed as the mean ± standard deviation to a confidence interval of 95%. Treatment of data and statistical test were analyzed using SPSS software, version 16.0; a $p<0.05$ was considered statistically significant. Comparisons between the treatment groups were performed in two ways. In the first stage, was tested the difference between averages characteristics of the milk, specific to the two counties using the independent sample Student t-test. Multivariate exploratory technique, Principal Component Analysis (PCA) was performed to compare the results characteristics of raw cow milk distinguish. This statistical multivariate analysis was used to observe similarities among characteristics of the milk from two different locations, reducing the dimension to two PCs, while keeping most of the original information found in the data. Only PCs with eigenvalue larger than one were retained for further analyses. In the second stage, to highlight the degree of influence of different factors: season, location, year

and interactions between them on chemical and microbiological characteristics of the milk, were applied factorial Analysis of Variance (ANOVA) in the conditions specified (Tabachnick and Fidell 2007). The three-way ANOVA was used to evaluate the overall effect (of all independent variables taken together), the main effects (one for each independent variable included in the analysis) and effect of the variables interaction (three double interaction effects and a triple interaction effect). The research design includes three independent variables and its type 4x2x3 because: the first independent variable - the season, has four levels, the second independent variable -the area has two levels and the third independent variable - the year, has three levels. Dependent variables are represented by their biochemical characteristics - the contents of fat, protein, lactose, solids-non-fat and by their microbiological characteristics of milk -somatic cell number and number of total germs. In the post-hoc analysis was used the Bonferroni test (α=0.05) to test possible differences between the averages of the group analyzed.

RESULTS AND DISCUSSION

Mean results content of fat, protein, lactose, solids-non-fat, somatic cell number and number of total germs of milk samples from Suceava and Tulcea counties, during three consecutive years. From the point of view of mean value of data obtained during three consecutive years from chemical and microbiological analysis of the milk composition, from the two counties in Romania (Tab. 1), were obtained lower values for fat, lactose and solids-non-fat content than the mean values specified by Walstra *et al.* (2006) for these parameters and approximately the same values for the protein content. In terms of comparison, between the two counties, significant differences ($p \leq 0.01$) have been obtained only for milk fat content, which reached the higher values in Suceava region, probably due to more favorable pedoclimatic parameters. This result corresponds with the information provided by Collomb *et al.* (2008). From the point of view of mean values recorded for SCN and NTG, NTG does not fit the standard accepted by the European Union.

Tab. 1

The composition of raw cow milk (mean value of three consecutive years ± SD) obtained from two counties

Variable type	County	Variable abbreviation	Mean value	Standard deviation	Significance
Fat, %	Tulcea	F_TL	3.52	0.08	**
	Suceava	F_SV	3.87	0.12	
Protein, %	Tulcea	P_TL	3.32	0.15	ns
	Suceava	P_SV	3.36	0.13	
Lactose, %	Tulcea	L_TL	4.32	0.24	*
	Suceava	L_SV	4.24	0.08	
Solids non fat, %	Tulcea	SNF_TL	8.45	0.16	ns
	Suceava	SNF_SV	8.43	0.10	
Somatic cell number, cells·ml^{-1}	Tulcea	SCN_TL	385.50 x 10^3	90.63 x 10^3	*
	Suceava	SCN_SV	339.25 x 10^3	100.58 x 10^3	
Number of total germs, no·ml^{-1}	Tulcea	NTG_TL	289.19 x 10^3	42.74 x 10^3	*
	Suceava	NTG_SV	265.39 x 10^3	41.15 x 10^3	

^{ns}No significant differences; *Significant differences at $p \leq 0.05$; **Significant differences at $p \leq 0.01$

Relationships between chemical and microbiological raw milk parameters collected over a period of three consecutive years in the Tulcea and Suceava counties during the four seasons. Principal Component Analysis (PCA) was used to study attribute-sample

relationships. According to PCA, a Tulcea and Suceava counties space was created where samples were positioned in the attribute-sample space according to their chemical and microbiological characteristic attributes. For the attribute -sample PCA (Fig.1), the first two PCs explain 88.27% of the total variance in the data (PC1=60.98% and PC2=27.29%). The principal component (PC1) was characterized mainly by milk properties of Tulcea County and the second principal component PC2 mostly by milk properties of Suceava County. Some variables display positive loadings, that is to say positive coefficients in the linear combinations, while others have negative loadings. In respect to the first principal component PC1 a good correlation was obtained between the content of fat (F_TL), somatic cell number (SCN_TL) and number of total germs (NTG_TL) of cow milk from Tulcea county. The closeness of the variable protein content from the two counties (P_SV, respectively P_TL), have a reduced contribution to describe the differences between the characteristics of the milk collected from two counties. The second principal component, PC2 underlined a clear opposition between some characteristics of the milk from Tulcea County like the content of solids-non-fat (SNF_TL) respectively lactose (L_TL) and somatic cell number (SCN_TL), between these parameters, obtaining significant correlations. Using PCA significant direct correlations (p=0.01) between lactose content (L_TL) and solids-non-fat content (SNF_TL) (r=0.876) on the one hand and indirect correlations between lactose content (L_TL) and somatic cell number (SCN_TL) (r=-0.519), somatic cell number (SCN_TL) and solids-non-fat (SNF _TL) (r=-0.580) on the other hand were obtained for Tulcea county. Regarding Suceava county, significant direct correlations were obtained (p=0.01) between the number of total germs (NTG _SV) and somatic cell number (SCN_SV) (r=0.562), between protein content (P_SV) and solids-non-fat content (SNF _SV) (r=0.464) and indirect significant, at p=0.05, between fat content (F_SV) and somatic cell number (SCN_SV) (r=-0.420), and between lactose content (L_SV) and somatic cell number (SCN_SV) (r=-0.334).

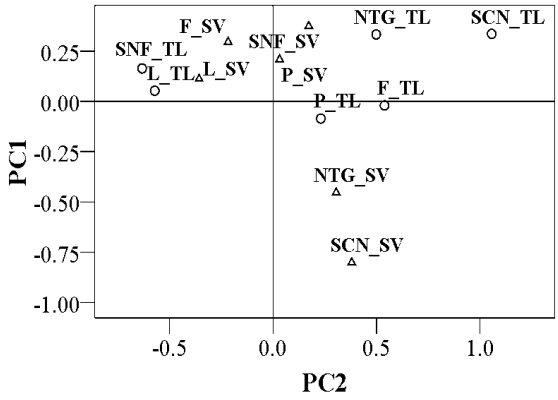


Fig. 1. PC1 and PC2 score for the chemical characteristics and microbiological characteristics from raw cow milk samples from Tulcea and Suceava Counties

The effect of season, counties and year of collection on the milk characteristics. The evaluation results of the main effects of season, county and the collecting year on the milk technological characteristics (fat, protein, lactose, solids non fat, somatic cell number and total number of germs) on the one hand and the interaction effect between the factors (season, county, year), on the other hand, are presented in the following paragraphs.

The effect of season, counties and year of collection on the fat content. Analysis of variance with "three way" revealed significant main effects for season, county and year on fat content and statistically significant interaction effects, at different levels, between factors. Significant main effect of the three factors and significant interaction effects between them give a significant global effect, F=31.63, p<0.001 also the effect size, explaining 93% of

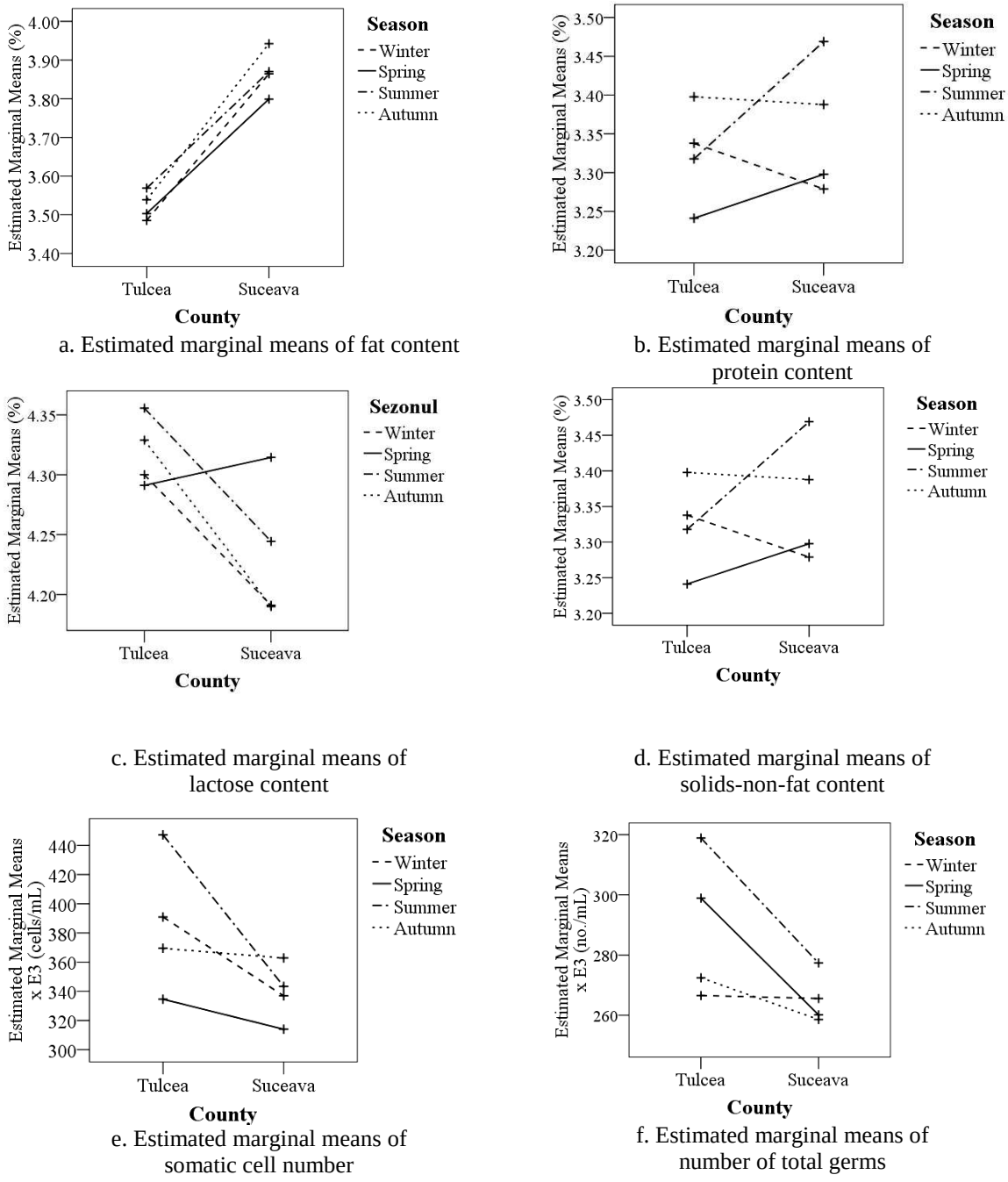
variance of variable fat content. The fat content in winter was significantly lower than in autumn season and also in the spring season than in summer season, respectively in autumn season, the mean difference was significant at $p<0.05$, results that are consistent with those set by Bruhn and Franke (1997). The percentage of milk fat presented higher values in autumn and summer seasons than in spring and winter seasons. These variations could be affected by the feeding and lactation (Mendia *et al*. 2000). The absence of interaction tells that it's reasonable to believe that the difference in mean fat content between seasons spring and summer, autumn and winter was the same for each of the counties. In Suceava County, the lowest mean fat value was obtained in spring season and the highest mean value in autumn. In Tulcea County, the lowest mean fat value was obtained in spring season and the highest mean value in summer season. Multiple comparisons indicate that in the year 2008 the mean value of the fat milk content was higher, than in the year 2006, respectively in the year 2007, in Suceava County. In Tulcea County, the highest mean value of the fat content was recorded in the summer of year 2006 when in Suceava County was recorded the lowest mean value of this parameter.

The effect of season, counties and year of collection on the protein content. Analysis of variance results indicates that significant global effect, which explains 45% of the variance of variable protein content, comes from the significant main effect of the seasonal factor. The factors county and year and the interactions between factors have no effect on milk protein content. In spring is lower protein content than in summer and autumn, the mean difference being significant at $p<0.05$. Seasonal variations are more pronounced in the case of the protein content than in the case of the fat content of milk collected from the two counties. In Suceava County the protein content was much lower during winter than during summer, probably due to the hay-based feed in winter, compared with outdoor grazing in summer. Another reason could be a higher secretion of prolactin whose concentration in plasma was higher during summer than during winter (Sevi *et al*. 2004). The mean value of protein content was highest in the 2008 year during summer season than in 2006 and 2007 respectively. In Tulcea County was recorded the lowest protein content during the spring season; the highest protein content was obtained in Suceava county during the summer season (Fig. 2b).

The effect of season, counties and year of collection on lactose content. The results indicates the existence of a significant global effect $F=50.85$, $p<0.001$. This comes from the significant main effect ($p<0.001$) of the factors: season, county, and year respectively from the significant interactions ($p<0.001$) between them. In the 2008 year, the value of lactose content was higher than in 2006 and 2007 respectively. Lower lactose content was recorded in the winter season than during the spring and summer seasons and during autumn season than during the spring season, the mean difference being significant at $p<0.05$. These results correspond with the information provided by Bruhn and Franke (1997). The highest content of lactose was recorded in the milk collected from Tulcea County during the summer season and from Suceava County during the spring season (Fig. 2c). From this figure we can see that for the milk collected in Tulcea County, there is an intersection for lactose content encountered in winter season with the spring season, probably due to specific climatic conditions offered by the area.

The effect of season, counties and year of collection on solids-non-fat content. Factorial analysis of variance results indicated the significant global effect $F=9.08$, $p<0.001$. The effect size explains 81% of the total variance of the variable solids-non-fat content and is given by the significant main effect of season, year factor and by significant interactions county * year factors. Higher content of solids-non-fat has been recorded in summer than winter and autumn seasons, the mean difference being significant at $p<0.05$. In the 2008 year the solids-non-fat content value was

higher than in 2006 and 2007 respectively. The highest content of solids-non-fat was recorded in the summer season in Suceava and during autumn in Tulcea (Fig. 2d).



a. Estimated marginal means of fat content

b. Estimated marginal means of protein content

c. Estimated marginal means of lactose content

d. Estimated marginal means of solids-non-fat content

e. Estimated marginal means of somatic cell number

f. Estimated marginal means of number of total germs

Fig. 2. Estimated marginal means of: a) fat content; b) protein content; c) lactose content; d) solids-non-fat content; e) somatic cell number; f) number of total germs.

The effect of season, counties and year of collection on somatic cell number. The results indicates a significant global effect on the variable SCN, given by the significant main effect, at different levels, of the year, county and season factors and by significant interaction effect of county * year factors. Significant global effect size explains 70% of the total variance of the SCN variable. In 2006 year the SCN was higher than in 2007 and 2008,

respectively; the mean difference being significant at $p<0.001$. Lower value of SCN was obtained during spring than during summer, the mean difference being significant at $p<0.05$. The maximum SCN value was obtained during the summer season, in Tulcea county and during the autumn season in Suceava County, the value being much lower than that obtained in Tulcea County as we ca see from *Figure 2e*.

The effect of season, counties and year of collection on number of total germs. The results obtained indicate that significant main effect of season, year and county factors and significant interaction effects between them contribute to the global significantly effect, whose size explains 78% of the total variance of the NTG variable. Higher value of NTG was obtained in summer than in winter and autumn seasons, the mean difference being significant at $p<0.05$. In 2006 year, NTG was higher than in 2007 and 2008, respectively; the mean difference being significant at $p<0.001$. A higher value of NTG was obtained in the milk collected in summer season in both counties. In Suceava County NTG was much lower than in Tulcea County (Fig. 2f).

CONCLUSIONS

From the analysis performed, the cow's milk used as a raw material in the dairy industry of Romania can be concluded that has a relatively good chemical composition, but bad microbiological quality. The microbiological quality was only marginally acceptable with respect to the somatic cell count for 2008 year. For all chemical and microbiological row milk characteristics, difference among counties, season and year were significant. Tulcea County produced milk with less fat ($p\leq0.01$) than Suceava County. While difference between fat content was very significant, counties variation for lactose and solids-non-fat differences were not as great, on average, Tulcea County recorded higher values for these parameters than in Suceava County. Regarding season variations we found that all chemical and microbiological characteristics presented higher values during summer than during winter. Increasing the environmental temperature leads mainly to an increase of microbiological load of milk for all variants taking count. Compared to the years analyzed, the lowest microbiological values were recorded in milk samples collected from 2008, which show an improvement hygiene conditions in milk collecting and of temperature from the milk storage tank after milking.

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