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Modelling Relationships Between Raw Milk Quality Parameters and Climatic Conditions – The Case Study of a 3-years Survey in Serbia

Ilija Djekic[a*], Jelena Miočionović[b], Marija Bojčevski[c], Nada Šmigić[a], and Igor Tomašević[b]

[a] Department of Food Safety and Quality Management, University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Republic of Serbia
[b] Department of Animal Origin Products Technology, University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Republic of Serbia
[c] AD Imlek, Industrijsko naselje bb, 11213 Padinska Skela, Belgrade, Republic of Serbia
[*]Corresponding author
idjekic@agrif.bg.ac.rs
Tel: +381-11-441-3427

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Abstract

This work examined the relationships between quality characteristics of raw milk and climatic conditions. Over a period of three years, a total of 5,065 samples were collected encompassing two types of farms. The quality characteristics analysed were titratable acidity (TA), total plate count (TPC) and somatic cells count (SCC). Climatic conditions were evaluated in respect to the outdoor air temperature, pressure, humidity and precipitation.
Big farms showed a stronger correlation between TA and climatic conditions as opposed to SCC and climatic conditions. TPC was out of limit in big farms when the outdoor air temperature was higher than 19.8 ^{o}C ($p<0.05$) and during periods with accumulated precipitation over 4.2 mm ($p>0.05$). Small farms showed a stronger correlation between SCC and climatic conditions as opposed to TA. In these farms, occurrence of acidity out of limit was detected in less than 7.2% of samples. Samples with TA out of limit were observed when air temperature was higher than 18.4 ^{o}C ($p<0.05$) and accumulated precipitation was below 3.1 mm ($p>0.05$). These results can be used to improve good agricultural practices in respect to climatic conditions and size of farms.

Keywords: Raw milk; Quality characteristics; Climatic conditions; Farm size

1 Introduction

Dairy products are considered as very important in human diet due to their uniqueness and nutritional value (Djekic, Miocinovic, Tomasevic, Smigic, & Tomic, 2014). The quality and safety of these products are of highest importance and represent one of the main goals throughout the milk chain (Djekic, Miocinovic, Pisinov, Ivanovic, & Smigic, 2013). Therefore, production and distribution of high quality raw milk is necessary for achieving high quality dairy products (Smigic, Djekic, Tomasevic, Miocinovic, & Gvozdenovic, 2012). Due to its specific composition and characteristics, milk is a good environment

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for the growth of both spoilage and pathogenic microorganisms (Nsofor & Frank, 2013). Although milk is sterile in the mammary gland, different bacteria might contaminate raw milk as a result of direct contact with soil, air, workers hands, faeces, grass and excretion from the udder of an infected animal (Lejeune & Rajala-Schultz, 2009), but also with contaminated surfaces during storage and transport of raw milk (Millogo, Sjaunja, Ouedraogo, & Agenas, 2010) and occasionally by milking of mastitic cows (Hayes et al., 2001; Pantoja, Reinemann, & Ruegg, 2009).
Microbiological quality of raw milk is assessed by total plate count (TPC) and somatic cell count (SCC), and these parameters are used for the comparison and estimation of milk quality (Piepers, Zrimsek, Passchyn, & De Vliegher, 2014; Smigic et al., 2012). SCC is an important measure of milk quality, reflecting the health status of the mammary gland, the increased risk of non-physiological changes to milk composition and reduced milk yield (More, Clegg, Lynch, & O'Grady, 2013). Titratable acidity (TA) is a quality parameter that is normally used to estimate the freshness of milk and to monitor the production of lactic acid during fermentation (McCarthy & Singh, 2009).
The microbiological quality indicators of raw milk may depend on the climate conditions, as increased outdoor air temperature allows faster increase in environmental bacterial population (Elmoslemany et al., 2010), better survival, proliferation and increase of total bacterial load in animal reservoirs. On the other hand, higher precipitation might allow development of environmentally mediated bacteria transmission pathways (Lal, Hales, French, & Baker, 2012) and consequently greater TPC.
Relationship between various hygienic indicators and climatic conditions, mostly temperature and precipitation, have been confirmed in various studies (Djekic et al., 2016). It has been shown that climatic conditions have an impact on food safety as well as on the prevalence of foodborne diseases under certain circumstances (Bezirtzoglou, Dekas, & Charvalos, 2011; Jacxsens et al., 2010). The majority of research on farms has focused on the contamination pathway of pathogens. Parker, McIntyre, and Noble (2010) investigated the effects between animals on farms and climatic conditions by analyzing pathways from manure at livestock farms and from grazing pastures. Effects of climate change on agriculture include variations in the seasons, modifications of the areas suitable for growing crops, grazing of livestock, production efficiency of livestock and changes in plant pests (Miraglia et al., 2009). Consequently, they can have an influence on the raw milk quality and indirectly on the quality and safety of dairy products. This brings to the attention the importance of managing certain decisions related to agricultural practices on farms caused by seasonal climatic variability as suggested by (McCown, Carberry, Dalgliesh, Foale, & Hochman, 2012).
Studies analyzing the influence of seasonal variation on the raw milk quality are usually of small scale, below 1,000 samples and over a short period of time, mostly covering a one-year period (Auldist, Walsh, & Thomson, 1998; Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009; Jahreis, Fritsche, & Steinhart, 1996; Lock & Garnsworthy, 2003). Studies investigating long term relations between climatic conditions and quality of raw milk have not been in the focus of research, and this was identified as a research gap by the authors of this paper. The objective of this study was to examine possible correlations between selected quality characteristics of raw milk and climatic conditions, primarily outdoor air temperature and precipitation during a period of three years in respect to the size of farms.

2 Materials and Methods

2.1 Sampling

A total of 5,065 raw milk samples were analyzed on a daily basis at the reception of a dairy plant during a period of three years (from 2012 until 2014). Raw milk was transported to the plant from two different sources: (i) big farms and (ii) centres for collecting raw milk from small farms and households. All farms were situated in the vicinity of Belgrade, capital of Serbia, and have good agricultural practices in place. Transportation of raw milk from big and small farms was organized in vehicles equipped with temperature control units to provide an adequate cold-chain and to satisfy high hygienic criteria.

2.2 Analytical methods

Samples of raw milk were analyzed for titratable acidity (TA), total plate count (TPC) and somatic cells count (SCC). TPC was determined according to ISO 4833:2003. TA of milk was analyzed by titratable method and expressed in Soxhlet-Henkel degrees (^{o}SH). SCC was determined using a Fossomatic Minor dairy analyser (Foss, Denmark).

Data processing and statistical methods

Based on TPC, raw milk was classified in three classes (E, I and II) in line with current legislation in Serbia (Serbia, 2009). Extra class (E) is classified as raw milk with TPC not exceeding 100,000 CFU/ml ($\leq$5 $\log_{10}$), while I and II classes are defined as groups with TPC between 100,001 (5 $\log_{10}$) and 400,000 (5.6 $\log_{10}$) CFU/ml and TPC $\geq$ 400,000 ($\geq$5.6 $\log_{10}$) CFU/ml, respectively. Samples with TPC greater than 1,000,000 ($\geq$ 6 $\log_{10}$) CFU/ml were considered as nonconforming raw milk according to the internal specification of the dairy plant. Requirements for the somatic cell count were always the same: less than 400,000 cells/ml. The percentage of raw milk which belonged to a specific category was calculated as the amount in the total quantity of received raw milk.
The limits for TA were set between 5.5 and 7.0 ^{o}SH. Samples that had a TA out of this range were considered as nonconforming milk. The percentage of these samples was calculated as the quantity of non-conforming raw milk in the total quantity of received raw milk.
The climatic parameters used in statistical analysis during the three year period were the mean outdoor air temperature, the mean pressure, the mean humidity and accumulative precipitation calculated from the daily data as reported from the nearest local weather station (RHSS, 2013, 2014, 2015).
Classes of raw milk were expressed as percentages. The Chi-Square test for association was used in analyzing possible relationships between raw milk classes (based on TPC) and types of farms.
Pearson's rank order correlation coefficients were calculated for selected raw milk quality parameters, namely TPC, SCC and TA and the climatic parameters temperature, pressure, humidity and precipitation. This was performed separately for the big and the small farms.
Binomial logistic regression was employed to determine the probability of the occurrence of out of limit raw milk quality parameters with respect to climatic parameters. If normality or equality of variance could not be assumed, the Mann Whitney U test was used to determine the difference between the climate parameters and raw milk quality parameters.
The level of statistical significance was set at 0.05. Statistical processing was performed using Microsoft Excel 2010 and SPSS Statistics 17.0.

3 Results and discussion

3.1 Raw milk quality and type of farms

The results indicated that there was a statistically significant association between the raw milk classes based on the TPC and types of farms, Table 1 (χ^2 = 3,074,385; $p < 0.05$). It was shown that small scale farms delivered inferior quality of raw milk compared to big farms. The production of raw milk with low bacterial counts is influenced by many factors related to good agricultural practices on dairy farms (Elmoslemany et al., 2010). Several studies confirm that milking, equipment hygiene, sanitizing procedure (Elmoslemany et al., 2010), and milk storage conditions, are the crucial factors influencing the variability in bacterial counts and overall microbial load. Also, herd health management, transition and feeding management, or housing, which are known to affect udder health, are also identified as important for achieving good quality of raw milk (Piepers et al., 2014).
Big farms included in this study were characterized as intensive production system with clearly defined management procedures at all levels compared to small scale farmers. Educational level of farmers and milking methods differed between

the two farm types but both had good agricultural practices in place. During the last few years, there has been a higher demand in terms of quality and microbiological integrity of raw milk in Serbia and hence small farmers have shifted to commercial family farms with increased production and improved agricultural practices to enable more profitability and economically sustainable production (Bogdanovic & Petrovic, 2015).

3.2 Big farms

Pearson's rank correlation was conducted using all data gathered during the survey. Regarding the subset of raw milk samples from big farms (Table 2), two quality characteristics, TA and SCC were significantly correlated with each other ($p < 0.05$) showing a negative correlation (-0.113). Strong significant correlations were observed between SCC and outdoor air temperature, atmospheric pressure and humidity ($p < 0.05$) while TA positively correlated only with precipitation (below 0.10). Results from Brazil confirm a positive and significant correlation between the outdoor air temperature and SCC, while rainfall and humidity showed no correlation (Vargas et al., 2014).
Seasonal effects on the occurrence of TPC and TA out of limit obtained from big farms are presented in Figures 1a and 1b. Most frequently TPC was out of limit in July, which is the time of the year when the outdoor air temperature was the highest. This was in agreement with other reports, which noted a positive association between summer temperature and bacterial levels in raw milk (Elmoslemany et al., 2010; Van Schaik, Lotem, & Schukken, 2002). On the contrary, Piepers et al. (2014) found an opposite trend. The exact reasons were not found but results might be related to the temperature and precipitation in different regions. Climate condition in some regions influences the animal housing and exposure of udder and teats and humid weather conditions can contribute to the high bacterial levels, increasing the risk for contamination (Piepers et al., 2014). Occurrence of TPC out of limit ranged from 0.0% (January, March, September, October and November) up to 1.7% (July). It is of note that the highest temperatures occurred in July (Fig. 1c).
Comparing TPC results and outdoor air temperature, it was noted that the average temperature (19.8 ^{o}C) was significantly higher than the temperature (14.0 ^{o}C) in the subset of samples showing result out of and within limits respectively (Mann–Whitney U test, $p < 0.05$), Figure 2a.
A higher, but not significant, accumulated precipitation (4.3 mm) was noted when TPC was out of limit compared to the value (4.2 mm) when TPC was within limits. In the rainy period, it is more difficult to perform good agricultural practices, as more dirt and mudis present in the environment and consequently may be present on the udder and different contact surfaces. In contrast, TPC results were out of limit in periods with lower humidity (55.0% compared to 65.5%), Figure 2a.
The greatest number of raw milk samples with TA out of limit was detected in August (1.8%), July (1.7%) and December (1.7%). During the observation period no samples with TA out of limit were detected in January, September, October and November. Samples with TA out of limit were reported when temperature was significantly higher than in the cases when TA was within limits (17.0 ^{o}C compared to 14.0 ^{o}C). A higher (not significant) accumulated precipitation (4.9 mm) was noted when TA was out of limit compared to 4.2 mm when TA was within limits. In contrast, TA was out of limit in periods with lower (not significant) humidity (60.3% compared to 65.6%), Figure 2b.
Regression modelling was performed to ascertain the effects of outdoor air temperature, pressure, precipitation, humidity and SCC on the likelihood that TPC or TA were out of limit. Results obtained for raw milk samples delivered from big farms, were not statistically significant.

3.3 Small scale farms

Regarding the samples of raw milk obtained from small scale farms (Table 3), TPC and SCC were significantly and positively correlated with each other ($p < 0.05$,) 0.406). In some studies, correlations between high SCC and high TPC have also been reported (D'Amico & Donnelly, 2010).

Table 1: Quantity and classes of received raw milk based on TPC by type of farm

Quantity (L)	2012	2013	2014	Total (L)
Farms „A“	10,085,155	9,849,975	8,999,927	28,935,057
Farms „B“	14,866,760	10,561,929	8,468,923	33,897,612
Total (L)	24,951,915	20,411,904	17,468,850	62,832,669
n (%)	Class E	Class I	Class II	Total
Farms „A“	1,796 (65,43%)	860 (31,33%)	89 (3,24%)	2,745 (100%)
Farms „B“	84 (3,62%)	533 (22,98%)	1,702 (73,39%)	2,319 (100%)
χ^2 = 3074,385; $p < 0.05$				

(n) represents the number of samples of raw milk during the observed period; (%) represents their share in the sample of that group of farms

Note: Items denoted with different letters are significantly different at the level of 5%.

Legend: Class E ($\leq 5 \log_{10}$ CFU/ml); Class I (results between 5 $\log_{10}$ CFU/ml and 5.6 $\log_{10}$ CFU/ml); Class II (results $\geq 5.6 \log_{10}$ CFU/ml)

Table 2: Pearson's Rho correlation coefficient between quality parameters of raw milk samples from big farms and climatic conditions

		Pressure	Temperature	Humidity	Precipitation	TA	TPC	SCC
Pressure	Coefficient		-0.232	-0.049	-0.107			-0.055
	N		2,745	2,745	1,317			2,745
Temperature	Coefficient	-0.232		-0.619				0.240
	N	2,745		2,745				2,745
Humidity	Coefficient	-0.049	-0.619		0.240			-0.071
	N	2,745	2,745		1,317			2,745
Precipitation	Coefficient	-0.107		0.240		0.063		
	N	1,317		1,317		1,317		
TA	Coefficient				0.063			-0.113
	N				1,317			2,745
TPC	Coefficient							
	N							
SCC	Coefficient	-0.055	0.240	-0.071		-0.113		
	N	2,745	2,745	2,745		2,745		

[a] TA - titratable acidity

[b] TPC – total plate counts

[c] SCC – somatic cells count

[d] N: amount of samples

Results in tables present the combinations which showed significant correlations ($p < 0.05$)

Table 3: Pearson's Rho correlation coefficient between quality parameters of raw milk samples from small scale farms and climatic conditions

		Pressure	Temperature	Humidity	Precipitation	TA	TPC	SCC
Pressure	Coefficient		-0.237		-0.109		-0.068	-0.132
	N		2,319		1,091		2,319	2,319
Temperature	Coefficient	-0.237		-0.626			-0.050	0.236
	N	2,319		2,319			2,319	2,319
Humidity	Coefficient		-0.626		0.240	0.044		-0.076
	N		2,319		1,091	2,319		2,319
Precipitation	Coefficient	-0.109		0.240				
	N	1,091		1,091				
TA	Coefficient			0.044				
	N			2,319				
TPC	Coefficient	-0.068	-0.050					0.406
	N	2,319	2,319					2,319
SCC	Coefficient	-0.132	0.236	-0.076			0.406	
	N	2,319	2,319	2,319			2,319	

[a]TA - titratable acidity

[b]TPC – total plate counts

[c]SCC – somatic cells count

[d]N: amount of samples

Results in tables present the combinations which showed significant correlations ($p < 0.05$)

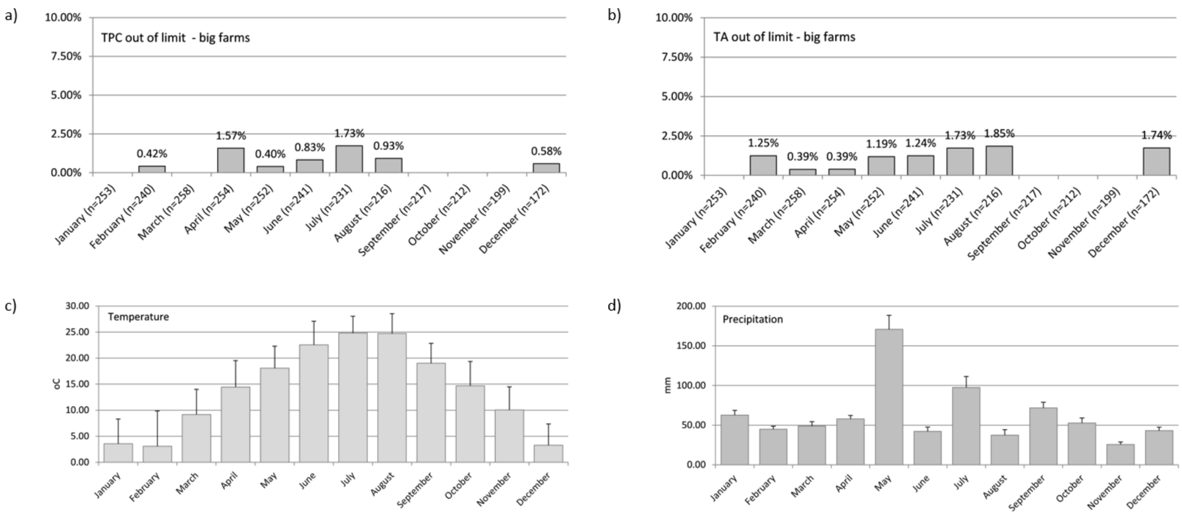

Figure 1: Seasonality of raw milk results out of limits and characteristics of climatic conditions by month. a: TPC out of limit (n = 15); b: TA out of limit (n = 22); c: Outdoor air temperature (3 years); and d: Precipitation (3 years). Bars are the 95% confidence intervals and n = the amount of samples. The outdoor air temperature and precipitation included is the mean temperature and accumulative precipitation calculated from the daily data of temperature and precipitation collected from the national hydrometeorological service

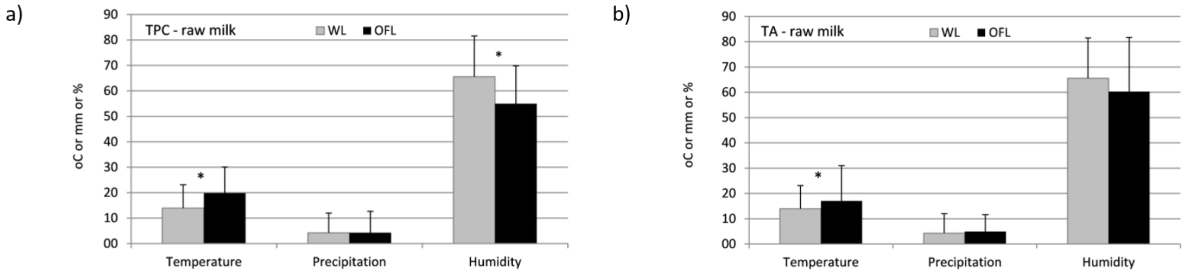

Figure 2: The potential impact of the climatic parameters (outdoor air temperature, precipitation and humidity) on the occurrence of samples out of limits in the raw milk samples from big farms a: TPC (Temperature WL n = 2,730 and OFL n = 15; Precipitation WL n = 1,311 and OFL n = 6; Humidity WL n = 2,730 and OFL n = 15); b: TA (Temperature WL n = 2,733 and OFL n = 22; Precipitation WL n = 1,309 and OFL n = 8; Humidity WL n = 2,733 and OFL n = 22). WL – samples within limits; OFL – samples out of limits Mann–Whitney U test performed to indicate significant difference ($p < 0.05$). Bars are 95% confidence interval; (*) significant difference

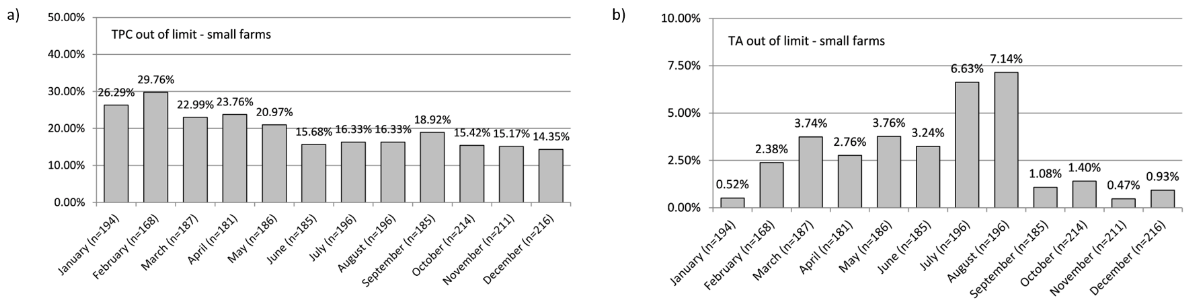

Figure 3: Seasonality of raw milk results out of limits and characteristics of climatic conditions iby month. a: TPC out of limit (n = 450); b: TA out of limit (n = 65); Bars are the 95% confidence intervals and n = the amount of samples

In contrast to this, Rysanek and Babak (2005) considered that SCC data do not sufficiently reflect the hygiene status of herds because of low correlation coefficients between bulk tank milk somatic cell score and log bulk tank total bacterial count. Microbiological quality of raw milk is more influenced by hygiene and environmental conditions than the mastitis frequency in dairy herds (Souto et al., 2008). The positive correlation between TPC and SCC may have indicated that producers were effectively controlling good agricultural practice (reflected in low TPC) and have also implemented good herd health management practices (reflected in low SCC) (Borneman & Ingham, 2014).

The strongest positive correlation between quality characteristics and climatic parameters was observed between SCC and outdoor air temperature (0.236). SCC was correlated with pressure, temperature and humidity, TPC with pressure and temperature while TA was correlated with humidity. There was no significant correlation between precipitation and raw milk quality parameters.

A study in the USA indicated that during hot and humid summer dairy farms produce less milk

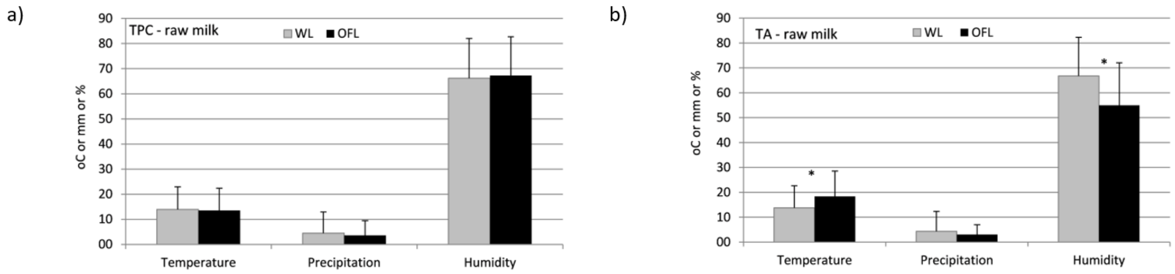

Figure 4: The potential impact of the climatic parameters (outdoor air temperature, precipitation and humidity) on the occurrence of samples out of limits in the raw milk samples from small farms a: TPC (Temperature WL n = 1,869 and OFL n = 450; Precipitation WL n = 840 and OFL n = 251; Humidity WL n = 1,869 and OFL n = 450); b: TA (Temperature WL n = 2,254 and OFL n = 65; Precipitation WL n = 1,066 and OFL n = 25; Humidity WL n = 2,254 and OFL n = 65). WL – samples within limits; OFL – samples out of limits Mann–Whitney U test performed to indicate significant difference ($p < 0.05$). Bars are 95% confidence interval; (*) significant difference

and milk with higher SCC (Ferreira & De Vries, 2015). The lowest level of SCC was observed in February, March, and April, while the highest was reported during August, September, and October. In these months it is necessary to introduce programmes for improving milk quality. Similar data were presented by Shock et al. (2015) analyzing data on farms in Ontario, Canada . Since the elevation of SCC is a response to an insult to the mammary gland and is modulated by inflammatory mediators, Harmon (1994) points that the major factor influencing SCC is infection status and that few factors other than infection status may have a significant impact on milk SCC. Several authors emphasize seasonal variation of SCC (Bernabucci et al., 2015; Paula, Ribas, Monardes, Arce, & Andrade, 2004; Roma Júnior, Montoya, T. Martins, Cassoli, & Machado, 2009; Simioni et al., 2014).

Results obtained for small scale farms were complemented by the binary logistic regression (odds ratios) between TPC, TA, SCC and climatic conditions (data not shown). A logistic regression was performed to ascertain the effects of outdoor air temperature, precipitation, pressure, humidity, TA and SCC on the likelihood that TPC was out of limit. The logistic regression model was statistically significant, $\chi^2 = 192{,}306$; $p < 0.005$. Results suggested a significant association between TPC and climatic parameters (odds ratio >0.99). The model explained 24.5% of the variance in TPC and correctly classified 80.0% of cases. It is 2.7 times more likely to exhibit TPC out of limit when TA is out of limit. Increasing temperature, pressure, humidity and accumulated precipitation as well as TA were associated with an increased likelihood of exhibiting TPC out of limit, but increasing SCC was associated with a reduction in the likelihood of exhibiting TPC out of limit. The results indicated that outdoor air temperature, pressure, humidity, TA and SCC were significant predictor variables in the regression model.

Seasonal effects of occurrence of TPC and TA out of limit in raw milk samples obtained from small scale farms are presented in Figures 3a and 3b. Occurrence of TPC out of limit ranged from 14.3% (December) up to 29.8% (February). Raw milk samples containing TPC within limit was found when the outdoor air temperature (13.6 ^{o}C) was lower than the outdoor air temperature (14.0 ^{o}C) in the subset of samples showing result out of limit (Mann–Whitney U test, $p < 0.05$), Figure 4a. Lower accumulated precipitation (3.6 mm) was noted when TPC was out of limit compared to 4.5 mm when it was within limits. In contrast, TPC was out of limit in periods with higher humidity (67.3% compared to 66.2%), Figure 4a. However, none of the results

were statistically significant (p>0.05).
The significant presence of raw milk samples showing TA out of limit was detected in August (7.1%), and July (6.6%), while in January, November and December they were below 1%. Samples with TA out of limit were detected when average outdoor air temperature was significantly higher than in the cases of samples with TA within limits (18.4 ^{o}C compared to 13.8 ^{o}C). A lower (not significant) accumulated precipitation (3.1 mm) was noted when TA was out of limit compared to 4.4 mm when TA was within limits. In contrast, TA was out of limit in periods with lower humidity (54.9% compared to 66.2%), Figure 4b. Research from Iran stresses that milk obtained in the winter and spring seasons has the lowest (although not necessarily significant) acidity levels compared to those collected in the summer and autumn seasons (Najafi, Mortazavi, Koocheki, Khorami, & Rekik, 2009).
Same regression model on the likelihood that TA was out of limit was performed to ascertain the effects of outdoor air temperature, pressure, humidity, precipitation, TPC and SCC (data no shown). The logistic regression model was not statistically significant, χ^2 = 10,038, p > 0.005.

4 Conclusions

This study contributes to the literature by providing another perspective into the possible nature of raw milk quality parameters out of limit originating from different farm types and affected by climate parameters. It brings to the attention the necessity of analysing various climatic conditions influencing the raw milk quality.
In big farms, a negative correlation was observed between TA and SCC. A stronger correlation was observed between TA and climatic conditions opposed to SCC. The occurrence of TPC out of limit was below 2.0%, with the highest share during the hottest period of the year. In contrast, TPC was out of limit in periods with lower humidity. Samples with TA out of limit were detected when temperature was significantly higher than in the cases of samples with TA within limits.
In small farms, a positive correlation was observed between TPC and SCC. A stronger correlation was observed between SCC and climatic conditions opposed to TA. Logistic regression confirmed that increasing temperature, pressure, humidity and accumulated precipitation were associated with an increased likelihood of exhibiting TPC out of limit. Occurrence of TPC ranged from 14.3% up to 29.8%. TA out of limit was detected in less than 7.2% of all samples during periods when temperature was significantly higher and when humidity was lower.
Our results provide practical implications for both food technologists and farmers. This bottom-up approach in analyzing raw milk samples from a climate perspective provides an added value regarding analysis of the current practices in farms. The scientific value of this approach is that results confirmed that the temperature and precipitation are two climatic conditions that have an effect on the quality of raw milk.
A limitation of this research is the fact that the authors did not include knowledge of the employees working on farms. Also, good veterinary practices at farms, namely animal health and adequate usage of medicine for treating the animals, animal welfare and animal feeding were not analysed.
These results can be used as a basis for discussion in order to improve good agricultural practices in respect to climatic conditions and size of farms. Application of the similar method to the results of raw milk in other regions could offer a better insight into effects of climatic conditions globally in order to enhance milk quality along the chain.

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