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Fatty acid composition of cow's milk: opportunities and challenges for Serbian dairy producers

Dejana Trbovic[1], Danka Spiric[1], Mirjana Lukic[1], Zoran Petrovic[1], Nenad Parunovic[1], Vesna Djordjevic, Dragan Milicevic[1]

A b s t r a c t: The objective of this study was to investigate whether there is a difference in fatty acid (FA) profile, with emphasis on C18:1cis-9 and conjugated linoleic acids (CLA), of milk obtained from dairy cows under typical farming conditions practiced in Serbia, as well as to investigate variations in fatty acid composition among retail milks labeled differently in relation to fat content (3.2%, 2.8% or 1.5%). Accelerated solvent extraction of milk lipids followed by capillary gas chromatography with flame ionization detector was used for milk FA determination. The results obtained in this study showed that in raw milk, saturated fatty acids (SFAs) accounted for up to 73.73% of the total FAs, followed by monounsaturated fatty acids ($\bar{X}$23.44 to 26.92%) and polyunsaturated fatty acids (PUFA) ($\bar{X}$2.72 to 3.92%). Very similar results were obtained in retail milks. The FA composition of raw milk did not significantly differ between the geographical regions examined, except in the contents of C18:2n-6 and PUFA, which were higher in the milk produced in South Banat than in milks produced in Central Banat and in the area of West Backa and Syrmia. In relation to commercially processed milk, the FA contents varied significantly ($p<0.05$) between the milks with different declared fat levels. However, from the nutritive point of view, all milks examined should be considered as beneficial to human health regardless of fat content, although opportunities for improvement, as related to dietary guidelines, are present.

Key words: *milk, fatty acid, conjugated linoleic acid (CLA), trans fatty acid, human health.*

Introduction

Milk is a very important source of nutrients in the human diet, providing energy, high quality protein, and essential minerals and vitamins. Besides milk protein, the level and composition of bovine milk fat is critical for milk quality with regard to its nutritive value (positive or negative effects on human health) as well as the processing and technological qualities of the milk (e.g., melting point and hardness of butter) (*Ducháček et al.,* 2014). Fat is the most variable component of milk (3.0% to 6.0%) and the fatty acid composition of milk fat is highly diverse (*MacGibbon and Taylor*, 2006; *Lock and Bauman*, 2011). This diversity is due to the presence of over 400 fatty acids (FAs) found in milk, which makes milk fat the most complex of all natural fats. FAs in bovine milk come from two sources: by *de novo* synthesis in the mammary gland or by being preformed in plasma lipids originating from absorption from the small intestine or from body stores of adipose tissue. *De novo* synthesized fatty acids include those 4 to 16 carbons in length, while preformed FAs from the circulatory system are 16 carbons in length or longer (Heck *et al.,* 2012). Furthermore, the FA composition of milk is also of interest because it might be useful as an indicator of the metabolic status of cows (*Stoop et al.,* 2009).

Although milk fat is largely composed of triacylglycerols (these comprise about 98% of the total fat; *MacGibbon and Taylor*, 2006), which have been claimed to contribute to heart disease, two other milk components considered to be beneficial for human health are the FAs conjugated linoleic acid (CLA) and butyric acid (C4:0). Biomedical studies with animal models have demonstrated a variety of effects attributed to CLA, including anticarcinogenic, antiatherogenic, antiobesity, immune system enhancement and antidiabetic properties (*Corl et al.,* 2003; *Larsson et al.,* 2005). Butyric acid, which is uniquely present in ruminant milk, has been claimed to have a role in preventing colon cancer (*Perrin et al.,* 1994). The cow's diet has a major influence on the content of CLA in milk fat, and these effects have been recently summarized (*Griinari et al.,* 2000; *Peterson et al.,* 2002; *Kelsey et al*., 2003).

The composition of milk and its standardization are essential to guarantee the quality of dairy

[1]*Institute of Meat Hygiene and Technology, Kacanskog 13, 11 000 Belgrade, Republic of Serbia.*

Corresponding author: Dragan Milicevic, *dragan.milicevic@inmes.rs*

products/ingredients and so have great importance for the dairy industries (*Swensson and Lindmark-Mansson,* 2007); therefore, it is necessary to monitor milk quality (*Demment and Allen*, 2003). The dairy industry in Serbia is economically important for the country. According to official statistics, total milk production in Serbia is around 1500 million t year^{-1} (3477 t per milked cow) (*Statistical Office of the Republic of Serbia*, 2016).

The objective of this study was to investigate whether there is a difference in FA profile, with emphasis on C18:1cis-9 and CLA, of milk obtained from dairy farms under standard farming conditions as practiced in the north of Serbia, as well as to investigate variations in mean FA composition among differently labeled retail milks in relation to fat content (3.2%, 2.8% or 1.5%). In addition, this study provides information relevant for consumers on possible impacts on the risk of developing cardiovascular disease due to consumption of milks with different fat contents.

Materials and methods

Milks studied

In October and December 2016, using a stratified random sampling method, 24 raw cow's milks were collected from four different municipalities in Vojvodina Province, northern Serbia: South Backa District, South and Central Banat Districts, and West Backa and Syrmia. Two breeds of cattle form the basis of the cattle industry in Serbia: Simmental cattle or domestic spotted Simmental type cattle, which are most common in rural areas on family smallholdings, and Black and Red Holstein-Friesian cattle (dairy type cattle), which are mainly present in the organized manufacturing farm production that supplies raw milk to the dairy industry (*Petrovic et al.,* 2013). Feeding regimens were based on total mixed rations directly formulated on-farm by farmers or their technicians. The ingredient composition of the diet corresponded to the current level of individual daily milk yields, and feeding rations were completely balanced for energy, protein, and fat as well as mineral and vitamin content. In addition, a total of 15 commercially processed milks (sterilized) with different declared fat contents (3.2%, 2.8% or 1.5%) were purchased in a retail supermarket.

FA analysis by capillary gas chromatography

The total lipids were extracted from the milks by accelerated solvent extraction (ASE), (ASE 200, Dionex, Sunnyvale, CA, USA) with petroleum ether and isopropanol mixture (60:40, v/v) (as proposed by *Dionex Application Note No. 345*) at 100°C over three static cycles under nitrogen at 12 MPa. The solvent from the collected extracts was removed under a stream of nitrogen (Dionex Solvent evaporator 500) at 50°C until dry.

Fatty acid methyl esters (FAMEs) in the extracted lipids were prepared by esterification using 0.5 M sodium methoxide in anhydrous methanol as proposed by *Christie et al*. (2001). FAMEs were determined by gas-liquid chromatography (Shimadzu 2010, Kyoto, Japan) equipped with a flame ionization detector and capillary HP-88 column (length 100m, i.d. 0.25 mm, film thickness 0.20 μm). Injector and detector temperature were 250ºC and 280ºC, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.87 mL min^{-1}. The injector split ratio was set at 1:50. A programmed column oven temperature starting at 50ºC and ending at 230ºC was applied. Total analysis time was 66.5 min. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, PA) and a standard mixture of methyl esters of *cis*-9,11 and *trans*-10,12 isomers of conjugated linoleic acid (CLA) (O5632 ≥99%, Sigma-Aldrich, USA). Each sample was analyzed in duplicate. Results were expressed as mass of FA (g) in 100 g of FAs.

Statistical analysis

Statistical analysis of the data was performed using JMP software (SAS Institute Inc., version 10.0). Differences in individual FAs in milks from the different regions in Serbia or in retail milks with different declared fat contents were assessed using one-way ANOVA and Tukey's HSD post-hoc test. Differences were considered statistically significant at the levels of $p<0.05$, $p<0.01$ and $p<0.001$. The data structure and descriptive statistics relating to the milks studied are presented in Tables 1 and 2.

Results and discussion

The fatty acid profile of milk obtained from bovine dairy farms under the farming conditions practiced in northern Serbia is presented in Table 1, while fatty acid composition of milk with different declared fat contents from the Serbian retail market (3.2%, 2.8% or 1.5%) is presented in Table 2.

Short-chain saturated FA (SCSFA) were represented by butyric (C4:0), caproic (C6:0) caprylic (C8:0), capric (C10:0) and lauric (C12:0)

acid in the milks, and in general, their levels were lower than other saturated (SFA) and unsaturated (UFA) FAs. This SCSFA are de novo FA, synthesized in the mammary gland and are derived from acetic and butyric acids that are generated in the rumen by fermentation of feed components. The SCSFA (C6–10) do not appear to affect human health, whereas it has been claimed that butyric acid (C4:0) could perhaps prevent colon cancer (*Parodi*, 2001).

The levels of major medium and long-chain saturated FAs (LCSFA) (C14:0, C16:0 and C18:0) showed the highest concentrations among the different FAs in the milks studied (Table 1). These results agree with those reported by *Palmquist et al.* (1993), *Jensen* (2002) *Lock & Garnsworthy* (2011) for the FA composition of cow's milk. In the current study, 46% of the FAs were medium length (14–16), while long-chain FAs (≥18:0) and short-chain (<14:0) FAs corresponded to approximately 12% of total fat. Palmitic acid (C16:0) has a lower genetic effect and a higher herd effect compared with other even-chain *de novo* synthesized FA (*Garnsworthy et al*., 2006; *Craninx et al*., 2008; *Moate et al*., 2008). Therefore, C16:0 is considered to be partly synthesized *de novo* and partly derived from the blood (*Garnsworthy et al*., 2006; *Craninx et al.,* 2008; *Moate et al.,* 2008). The remaining SFAs and long-chain UFAs originate from dietary lipids and from lipolysis of adipose tissue triacylglycerols.

The medium-chain saturated FAs, lauric (C12:0) myristic (14:0) and palmitic (16:0) acids, and some *trans* FAs (TFAs), are nutritionally undesirable because they adversely affect plasma cholesterol levels. They decrease the level of blood low density lipids – LDL – which are thought to be associated with an increased risk of coronary heart disease. Approximately 2.7% of the FAs in milk are TFAs with one or more *trans*-double bonds (*Precht and Molkentin,* 1995). TFAs are not synthesized by the human body and are not required in the human diet.

TFAs are produced during biohydrogenation of polyunsaturated FA (PUFA) and isomerization of monounsaturated FA (MUFA) in the rumen. The major TFA is vaccenic acid (VA) (18:1, 11t), although small amounts of several other TFAs, including those with *trans* double bonds in positions 4-16 are also observed in milk fat *(MacGibbon and Taylor,* 2006). TFAs have a significant impact on dairy economics, causing important losses to dairy producers (*Lock et al.,* 2005**)**. According to *Hulshof et al.* (1999), in many European countries, approximately 50% of TFAs in the human diet come from dairy fat and they have adverse effects on blood lipids and lipoproteins similar to those from industrial sources when consumed in equal amounts. The American Heart Association recommended in 2006 that <20% of total energy content should be consumed in the form of MUFA. Overall, it is important for human health to reduce them in milk fat (*Cicero et al.,* 2012; *Imamura et al.,* 2012; *Lefevre et al.,* 2012).

In contrast to saturated fats, polyunsaturated fats, particularly those in the n-3 series have been shown to be very beneficial to human health. UFAs detected in the milks in the current study with the highest percentages were oleic (C18:1cis-9), linoleic (C18:2n-6) and VA (18:1, 11t) (25.40, 2.68 and 2.40%, respectively; Table 1). The level of linoleic acid (C18:2n-6) in the milks studied varied significantly ($p<0.05$) in the range 1.54 to 2.68%. The significant differences in PUFA levels observed ($p<0.05$) were a direct result of differences in linoleic acid content between the groups of milks. This was probably due to differences in the feeding during the year. The percentages of SFA and UFA in the milks ranged from 69.87 to 73.83 % and 25.16 to 30.11%, respectively (Table 1).

In regard to the FA profile of commercially processed sterilized retail milks with different declared fat contents (3.2%, 2.8% or 1.5%), processing raw milk into sterilized commercial milk did not seem to result in any changes in the FA profiles of the milk. In all retail products, the percentage of SFAs was higher than the percentage of UFAs (Table 2). However, the FA content was reduced according to the fat content (Table 2). When FAs in sterilized milk are expressed as mg of FAs per 100 g (*Greenfield & Southgate*, 2003) (Table 2), the obtained data are more important for consumers and demonstrate the nutritional value of the milk consumed per specific milk serving.

Several national and international organizations and authorities have formulated Dietary Reference Values or recommendations for the intakes of total fat, FAs, and cholesterol. The WHO/FAO (2003) set population nutrient intake goals for SFA at <10% of energy intake (E%), and for n-6 PUFA at 5 to 8 E% with a total intake of PUFA of 6 to 10 E%. In a report on health risks and benefits of TFAs in food, the European Food Safety Authority states that daily consumption of total TFAs higher than 2 E% gives rise to a significant increase in the risk of cardiovascular disease. Therefore, they recommended this value as a consumption level that should not be exceeded (*EFSA*, 2010).

The FA composition of cow's milk is influenced by many factors, both internal (cattle breed, age, stage of lactation, etc.) (*White et al.,* 2001; *Kelsey et al.*, 2003; *Auldist et al.*, 2004; *Kay et al.*, 2005; *Soyeurt et al.*, 2006) and external (feeding systems,

Table 1 Fatty acid composition ($\bar{X}$±SD for % of total fatty acids) in 24 raw cow milks from four regions in northern Serbia

Fatty acids	South Backa	South Banat	Central Banat	West Backa and Syrmia
C4:0	2.97±0.06	2.71±0.19	2.71±0.06	2.66±0.08
C6:0	2.15±0.18	2.01±0.22	1.97±0.02	1.98±0.10
C8:0	1.35±0.19	1.28±0.20	1.18±0.01	1.24±0.08
C10:0	2.98±0.49	3.04±0.61	2.65±0.06	2.93±0.28
C12:0	3.35±0.54	3.53±0.73	3.08±0.11	3.45±0.43
Total	**12.8±0.29**	**12.57±0.39**	**11.59±0.05**	**12.26±0.19**
C14:0	11.34±1.49	12.58±1.18	13.47±0.61	12.95±0.82
C15:0	1.04±0.06	1.08±0.19	1.12±0.04	1.18±0.17
C16:0	33.83±2.52	34.10±1.72	36.17±2.44	36.90±2.17
C17:0	0.51±0.06	0.54±0.07	0.59±0.03	0.57±0.14
C18:0	11.40±0.61	10.25±1.88	9.78±1.41	9.84±1.32
C22:0	0.08±0.04	0.07±0.02	0.09±0.02	0.06±0.02
C24:0	0.08±0.02	0.06±0.02	0.08±0.03	0.06±0.01
Total	**58.28±0.68**	**58.68±0.72**	**61.3±0.65**	**61.56±0.66**
C16:1	1.52±0.53	1.45±0.12	1.56±0.13	1.47±0.19
C18:1trans-9	2.09±0.39	2.40±0.65	2.10±0.50	1.60±0.25
C18:1cis-9	25.40±1.10	23.77±2.20	22.77±1.64	21.97±2.36
Total	29.01±0.67	27.62±1.0	26.43±0.75	25.04±0.93
C18:2n-6	2.10±0.69^{ab}	2.68±0.34^{a}	1.54±0.36^{b}	1.77±0.21^{b}
C20:0+C18:3n-6	0.24±0.12	0.23±0.04	0.25±0.05	0.24±0.03
C18:3n-3	0.26±0.15	0.34±0.06	0.28±0.09	0.22±0.04
c9. t11CLA	0.51±0.03	0.62±0.09	0.70±0.19	0.46±0.06
C20:3n-6	0.32±0.03	0.28±0.09	0.23±0.06	0.26±0.06
Total	3.43±0.20	4.15±0.12	3.0±0.15	2.95±0.08
SFA	69.87±1.46	70.88±2.05	72.91±1.79	73.83±2.43
MUFA	26.92±1.25	25.20±2.15	24.33±1.51	23.44±2.24
PUFA	3.19±1.22^{ab}	3.92±0.42^{a}	2.75±0.37^{b}	2.72±0.25^{b}
Lipids %	3.66±1.41	3.82±0.98	2.60±0.41	3.36±0.87

Legend: $^{a, b, c}$ Means with different letters within the same row are significantly different (p≤0.05)

seasonal changes, milking frequency and milking system, etc.) (*Jensen,* 2002; *Kalac* and *Samková*, 2010; *Morales-Almaráz et al.*, 2010). Another important factor is geographical, which determines the edible plant variety underlying the feeding of ruminants (*Frelich et al.*, 2009; *Rutkowska and Adamska,* 2011).

It is well established that the FA composition of milk can be modified by manipulating the cow's diet. However, the lipid profile in bovine milk does not exactly match the lipid profile of the cow's diet because of the biohydrogenation of most of the dietary PUFA (*Jenkins et al.,* 2007). Ruminants consume a large proportion of UFAs. These UFAs are,

Table 2 Fatty acid composition of 15 commercially processed sterilized milks from retail with different declared fat contents (3.2%, 2.8% or 1.5%) ($\overline{X}$±SD for % of total fatty acids and; mg 10^{-2} g)

Fatty acids	milk 3.2% fat		milk 2.8% fat		milk 1.5% fat	
	(%)	(mg 10^{-2} g)	(%)	(mg 10^{-2} g)	(%)	(mg 10^{-2} g)
C4:0	2.62±0.03	79.23^{x}	2.68±0.11	71.04^{y}	2.66±0.07	37.70^{z}
C6:0	1.85±0.01	56.09^{x}	1.94±0.07	51.46^{x}	1.97±0.06	27.93^{y}
C8:0	1.16±0.02	35.08^{x}	1.20±0.04	31.75^{y}	1.20±0.03	17.08^{z}
C10:0	2.80±0.02	84.82^{x}	2.87±0.06	76.07^{y}	2.85±0.06	40.40^{z}
C12:0	3.34±0.02	101.15^{x}	3.37±0.02	89.30^{y}	3.35±0.03	47.56^{z}
Total	**11.77±0.02**	**356.37**	**12.06±0.06**	**319.62**	**12.03±0.05**	**170.67**
C14:0	13.78±0.05^{a}	416.86^{x}	13.36±0.16^{ab}	353.64^{y}	13.00±0.07^{b}	184.27^{z}
C15:0	1.10±0.01^{a}	33.26^{x}	1.04±0.01^{b}	27.65^{y}	1.06±0.01^{b}	15.02^{z}
C16:0	36.78±0.82	1112.23^{x}	35.82±0.19	947.80^{y}	34.91±0.07	494.85^{z}
C16:1	1.44±0.01	43.55^{x}	1.38±0.02	36.65^{y}	1.40±0.01	19.91^{z}
C17:0	0.57±0.01^{a}	17.24^{x}	0.53±0.01^{b}	14.02^{y}	0.53±0.01^{b}	7.58^{z}
C18:0	10.14±0.19	306.78^{x}	10.27±0.16	271.88^{y}	10.57±0.01	149.83^{z}
C22:0	0.07±0.01^{ab}	2.27^{x}	0.06±0.01^{b}	1.45^{y}	0.09±0.01^{a}	1.35^{y}
C24:0	nd	1.06^{x}	nd	0.93^{x}	nd	nd
Total	**63.88±0.84**	**1933.25**	**192.46±0.08**	**1654.13**	**61.56±0.03**	**872.81**
C18:1trans-9	2.07±0.02^{b}	62.75^{x}	2.09±0.03^{b}	55.30^{y}	2.44±0.03^{a}	34.66^{z}
C18:1cis-9	22.61±0.53	683.88^{x}	22.41±0.15	593.10^{y}	23.15±0.38	328.15^{z}
Total	**24.68±0.27**	**746.63**	**24.5±0.09**	**648.40**	**25.60±0.20**	**362.81**
C18:2n-6	2.04±0.05^{b}	61.69^{x}	2.09±0.04^{b}	55.30^{y}	2.30±0.01^{a}	32.67^{z}
C20:0+C18:3n-6	0.22±0.02	6.65^{x}	0.22±0.01	5.95^{x}	0.20±0.01	2.83^{y}
C18:3n-3	0.30±0.02	9.22^{x}	0.31±0.01	8.20^{y}	0.33±0.01	4.75^{z}
c9. t11CLA	0.54±0.06	16.48^{x}	0.52±0.02	13.76^{x}	0.51±0.06	7.30^{b}
C20:3n-6	0.08±0.01	2.42^{x}	0.07±0.01	1.85^{x}	0.08±0.01	1.20^{x}
Total	**3.18±0.03**	**96.46**	**3.21±0.02**	**85.06**	**3.42±0.02**	**48.75**
SFA	72.98±0.71	2206.92^{x}	73.18±0.27	1936.34^{y}	72.20±0.44	1023.51^{z}
MUFA	24.05±0.54	727.42^{x}	23.79±0.16	629.62^{y}	24.55±0.36	348.07^{z}
PUFA	3.18±0.06	89.66^{x}	3.21±0.09	79.91^{x}	3.24±0.08	46.00^{y}

Legend: $^{a, b, c}$ Means with different letters within the same row are significantly different (p≤0.05); $^{x, y, z}$ Means with different let ters within the same row are significantly different (p≤0.01); nd Not detected

to a large part, biohydrogenated by the rumen bacteria to SFAs (long-chain), and during this process, several intermediates, mainly TFAs, are formed (*Jenkins et al.,* 2008). Therefore, milk contains mostly SFAs. A small proportion of the feed UFAs and a small proportion of the biohydrogenation intermediates are not completely biohydrogenated to SFA (C18:0) and are secreted into milk. Under the farming conditions practiced in Serbia, the nutrition of cows is based mainly on corn silage and hay supplemented with grains. Those fodders contain much more SFA than do green fodder materials, and so their consumption results in correspondingly high contents of long-chain SFAs in milk.

Increasing the overall total PUFAs in the diet is considered beneficial to human health with respect to reducing cardiovascular disease; additionally, cancer research has mainly focused on the benefits of increasing these FAs as well. However, the high level of unsaturation increases lipid oxidation that could affect the quality of dairy products due to the development of off-flavors (*Hu and Willett*, 2002).

Conclusion

Variability in milk composition is a result of genetic, physiological, nutritional, and environmental factors. This study showed that the major FAs in Serbian cow's milk were palmitic (C16:0), oleic (C18:1cis-9), myristic (C14:0), and stearic (C18:0) acids. These results will help to explain to farmers and dairy product manufacturers the importance of raw milk quality factors that fluctuate depending on dairy cow feeding practices and dairy plant management. In the future, this study needs to be repeated on a larger scale in order to achieve statistical analysis relevant to the bovine dairy herds over the entire country, and to enable the factors (feeding regime, breeding etc.) that could affect the FA composition of milk fat to be further studied. Furthermore, detailed work is essential to understand the regional and seasonal effects on milk fat composition.

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