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Original article

SURVEY ON FATTY ACID PROFILES OF RETAIL PASTEURISED COW'S MILK AND COW'S YOGURT IN BULGARIA

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Summary

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Assessing the consumer's awareness of the diet-health relationship, this study presents the fatty acid composition of cow's milk available on Bulgarian market. Over a 5-year period, 100 samples of pasteurised cow's milk and 135 samples cow's yogurt were analysed by gas chromatography (ISO 15885:2002). The saturated fatty acids predominated – average amount 70.1% for milk and 71.1% for yogurt. The unsaturated fatty acids proportions were 29.9% and 28.9% respectively. Seasonal variations in the fatty acid composition of milk were detected, due to feeding practices. Non-milk fat was identified in 5 yogurt samples, while all pasteurised milk samples were not adulterated. This study provides the first scientific information for fatty acid composition of cow's milk on Bulgarian market with respect to dietary intake estimation.

Key words: cow's milk, cow's yogurt, fatty acid composition

INTRODUCTION

Milk is one of the most consumed foods in Bulgaria. Milk fat contains a great number of fatty acids (FAs) – approximately 400 fatty acids have been identified. Fatty acid composition of milk fat varies according to the breed, season and stage of lactation of dairy cattle. On average, 70% of fat is composed by saturated fatty acids and 30% – by unsaturated fatty acids. Milk also contains trans fatty acids (MacGibbon & Taylor, 2002).

Due to increased consumer awareness of the relationship between diet and health, information on fatty acid composition of milk becomes more and more important. There are many studies suggesting a possible negative influence of milk consumption on heart disease (Shaper *et al.*, 1991; Elwood *et al.*, 2004; 2010; Larsson *et al.*, 2009; Soedamah-Muthu *et al.*, 2011; Huth & Park, 2012). Usually, saturated fatty acids (SFAs) are associated with increased concentrations of blood lipids and low-density lipoproteins. In fact, lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids are responsible for the increase of plasma low density lipoprotein (LDL) and cholesterol levels, but their effect is neutralised by the other saturated acids in milk that increase high-density cholesterol (HDL) (Williams, 2000; Shingfield *et al.*, 2008; Parodi, 2009). Whether SFAs play a positive or negative role on human health is still debated (Lordan *et al.*, 2018). On the contrary, unsaturated fatty acids (UFAs) in milk fat, especially some monounsaturated fatty acids (MUFA) have a positive influence on human health. Short- and medium-chain fatty acids affect the energy metabolism (Schönfeld *et al.*, 2016). Oleic acid (C18:1,9c) has a protective effect against cancer (Menendez *et al.*, 2006). Poly-unsaturated fatty acids (PUFA) such as the essential linoleic acid (C18:2, 9cis, 12cis) and α-linolenic acid (C18:3,9cis, 12cis, 15cis), affect platelet aggregation and decrease the risk of cardiovascular disease (Huth *et al.*, 2006). The dietary intake of long chain PUFA and especially eicosapentaenoic acid and docosahexaenoic acids proved to be effective in reducing the risk of coronary heart disease (Lopez-Huertas, 2010).

In order to achieve a high level of health protection for consumers and to guarantee their right to stay informed, Regulation (EU) 1169/2011 requires by December 2016 mandatory nutrition declaration on the label, including the amount of saturated fatty acids. The amounts of monounsaturated and polyunsaturated fatty acids could be a supplementary information.

In the last 20 years, several studies on fatty acid composition of Bulgarian sheep's and goat's milk were published. Mihaylova *et al.* (2004) compared fatty acid content of bulk sheep milk obtained from Tsigay and Karakachan sheep. Ivanova *et al.* (2015) estimated fatty acid composition of Bulgarian Synthetic Dairy Population sheep's milk. Mihaylova & Dimov (2005) investigated the fatty acid profile in milk of White Maritza sheep in an organic farming system. In order to add information on milk nutritional quality, Pamukova *et al.* (2018) characterised the fatty acids profile and the related health lipid indices of goat's milk from different Bulgarian breeds from a private farm in the Stara Planina Mountain. Hristova (2019) has determined fatty acid composition of goat's milk in terms of modern concept of rational nutrition. However, no scientific information about fatty acid content of cow's milk, consumed by Bulgarian population for the last five years is available. Assessing the importance of the problem, the objective of the present study was to determine the fatty acid composition of cow's milk offered on the Bulgarian market.

MATERIALS AND METHODS

One hundred samples of whole pasteurised cow's milk and 135 samples cow's milk yogurt produced by different manufacturers from all regions of Bulgaria were analysed. The samples were collected from supermarkets and provided in the laboratory by the Official control of Bulgarian Food Safety Agency, within the period 2014–2018.

Fatty acid composition of milk fat was determined by gas chromatography method described in ISO 15885:2002. The fat extraction was performed as per the ISO 14156 method. Methyl esters of the extracted fat were prepared by esterification (ISO 15884:2002).

Gas chromatographic analyses were conducted using a gas chromatograph Agilent Technologies 6890 N, FID, capillary column Supelco SP^{TM}-2560 Fused Silica: 100.0 m × 0.25 mm × 0.2 µm film thickness. Chromatographic conditions comprised temperature of the inlet 250 °C, split mode 1:100, carrier gas – nitrogen, temperature regime: 60 °C/240 °C. Identification and quantification of fatty acids were performed by CRM 47885, Supelco 37 Component FAME Mix (Traceable Certified Reference Materials).

The results are presented as % of total FAs (mean value ± SD). Statistical analysis of the results was performed using ANOVA: Single Factor (Microsoft Excel 2010); P<0.05 was considered statistically significant.

RESULTS

The data for fatty acid composition of analysed samples are presented in Tables 1 and 2. Fatty acids with amount more than 1% of total FAs were included in the tables. The results were divided in two groups, due to detected seasonal differences in the fatty acid composition of analysed samples: milk and yogurt produced during the winter (November-April) and the summer (May-October). The differences between two groups (winter/summer) were evaluated.

The fatty acid profiles of pasteurised milk and yogurt were similar. Saturated fatty acids comprised 70.14±1.70% and 71.08±1.70% of total fatty acids. The highest proportion was that of palmitic acid – 30.73±2.23% and 30.89±1.82% from all fatty acids respectively. The quantities of short- and medium-chain FAs for milk and yogurt were also similar.

Table 1. Fatty acid composition (mean±SD) of pasteurised cow's milk (% of total FAs)

Fatty acid	Milk (2014–2018) n=100	Milk produced in winter (November-April) n=56	Milk produced in summer (May-October) n=44	P value[1]
Butyric, C4:0	4.16 ± 0.30	4.32 ± 0.47	4.01 ± 0.21	NS
Caproic, C6:0	2.59 ± 0.17	2.61± 0.20	2.58 ± 0.11	NS
Caprylic, C8:0	1.45 ± 0.15	1.50 ± 0.17	1.46 ± 0.08	NS
Capric, C10:0	3.31 ± 0.46	3.62 ± 0.34	3.00 ± 0.31	<0.05
Lauric, C12:0	3.60 ± 0.54	3.87 ± 0.51	3.30 ± 0.28	<0.05
Myristic, C14:0	11.09 ± 1.08	11.33 ± 1.17	10.85 ± 0.63	<0.05
Palmitic, C16:0	30.73 ± 2.23	31.81 ± 1.62	29.73 ± 1.32	<0.05
Palmitoleic, C16:1	1.47 ± 0.21	1.57 ± 0.12	1.45 ± 0.11	NS
Stearic, C18:0	10.87 ± 1.51	10.60 ± 0.82	11.17 ± 1.01	<0.05
Oleic, C18:1, 9c	23.48 ± 1.26	22.83 ± 0.77	24.15 ± 1.14	<0.05
Elaidic, C18:1, 9t	0.30 ± 0.01	0.30 ± 0.01	0.31 ± 0.04	NS
Linolelaidic, C18:2, 9t,12t	0.20 ± 0.01	0.20 ± 0.01	0.22 ± 0.02	NS
Linoleic acid, C18:2,6c	2.56 ± 0.30	2.35 ± 0.31	2.78 ± 0.25	<0.05
Linolenic, α-C18:3	0.53 ± 0.10	0.46 ± 0.06	0.62 ± 0.07	<0.05
Total saturated fatty acids, Σ SFA	70.14 ± 1.70	71.25 ± 0.90	69.05 ± 1.12	<0.05
Total unsaturated fatty acids, Σ UFA	29.86 ± 1.70	28.75 ± 0.90	30.95 ± 1.12	<0.05

[1]Significant differences between seasons: P<0.05; NS: non-significant.

Table 2. Fatty acid composition (mean±SD) of cow's yogurt (% of total FAs)

Fatty acid	Yogurt (2014–2018) n=135	Yogurt produced in winter (November-April) n=84	Yogurt produced in summer (May-October) n=51	P value[1]
Butyric, C4:0	4.32 ± 0.44	4.58 ± 0.24	4.12 ± 0.25	<0.05
Caproic, C6:0	2.56 ± 0.20	2.68 ± 0.23	2.42 ± 0.16	<0.05
Caprylic, C8:0	1.39 ± 0.13	1.50 ± 0.06	1.23 ± 0.13	<0.05
Capric, C10:0	3.12 ± 0.23	3.28 ± 0.20	2.94 ± 0.21	<0.05
Lauric, C12:0	3.47 ± 0.29	3.68 ± 0,15	3.23 ± 0.33	<0.05
Myristic, C14:0	10.63 ± 0.48	10.94 ± 0.32	10.33 ± 0.24	<0.05
Palmitic, C16:0	30.89 ± 1.82	31.22 ± 0.87	30.55 ± 1.77	<0.05
Palmitoleic, C16:1	1.62 ± 0.24	1.67 ± 0.27	1.58 ± 0.07	NS
Stearic, C18:0	11.52 ± 1.29	11.06 ± 1.04	11.98 ± 1.43	<0.05
Oleic, C18:1, 9c	23.36 ± 1.56	22.15 ± 1.23	24.58 ± 2.18	<0.05
Elaidic, C18:1, 9t	0.29 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	NS
Linolelaidic, C18:2, 9t,12t	0.22 ± 0.01	0.20 ± 0.01	0.22 ± 0.03	NS
Linoleic acid, C18:2,6c	2.64 ± 0.52	2.46 ± 0.29	2.79 ± 0.46	<0.05
Linolenic, α-C18:3	0.57 ± 0.07	0.52 ± 0.12	0.59 ± 0.10	NS
Total saturated fatty acids, Σ SFA	71.08 ± 1.70	71.82 ± 0.79	70.18 ± 1.34	<0.05
Total unsaturated fatty acids, Σ UFA	28.92 ± 1.70	28.18 ± 0.79	29.82 ± 1.34	<0.05

[1]Significant differences between seasons: P<0.05; NS: non-significant.

Significant fluctuations were noticed as followed. In yogurt, the sum of C4:0, C6:0, C8:0 and C10:0 increased by 5.7% in winter and was reduced it by 6.0% during summer. Stearic acid decreased by 4.7% in winter and increased by 4.0% during summer. In milk, the sum of C12:0, C14:0, C16:0 and C16:1 was raised by 2.6% in winter and reduced by 4.2% during summer.

MUFAs were 25.25±1.26% of all fatty acids in milk and 25.27±1.56% in yogurt. Oleic acid (C18:1, 9c) was the most prevalent unsaturated acid in both milk and yogurt. The quantities of oleic acid in yogurt samples showed a large range of variation. In some samples, obtained during the summer and autumn, the oleic acid amounts reached up to 28% of total FAs. The content of oleic acid in milk and yogurt during the summer increased by 2.9% and 5.2%, respectively.

Polyunsaturated fatty acids in milk and yogurt were 3.29±0.30% and 3.43±0.52% of total FAs with highest proportion of linoleic acid. There were statistically significant differences in amount of linoleic acid (P<0.05) between milk and yogurt samples.

Trans fatty acids (C18:1, 9 trans and C18:2, 9 trans, 12 trans) in pasteurised milk and yogurt were in small amounts (about 0.50%) and did not show fluctuations.

The analysis of fatty acid composition of milk is one of the methods to detect adulteration of milk fat. Common adulteration practices in dairy products involve the replacement, partially or fully, of milk fat with other lower quality fat (vegetable or animal fat). In our study, five samples, labelled as “yogurt”, contained non-milk fat and according to the Ordinance for specific requirements for dairy products (Anonymous, 2012) they were imitation products. In one sample the content of short-chain fatty acids (unique for milk fat) was significantly reduced – butyric acid was 2.50%; caproic acid – 1.03%; caprylic acid – 0.51% and capric acid – 1.32%. The quantities of each of palmitic and oleic acids exceeded 33% of total FAs. Total saturated fatty acids content was 58.94% and unsaturated acids – 41.06% of total FAs. In the rest four samples short-chain fatty acids (C4:0÷C10:0) were not detected. The fatty acid profile of samples consisted mainly of palmitic and oleic acids. The SFA content was 50.39±2.33 % and UFA content – 49.61± 2.15 %.

All tested samples pasteurised cow’s milk were without adulteration of the fat.

DISCUSSION

The data for fatty acid composition of pasteurised cow’s milk in the present study were very similar to the results of cow’s milk (3.2% fat) purchased from supermarkets, commonly consumed by the Serbian population, (Arsic *et al.*, 2009). SFAs of Serbian milk were 70.15±2.43% of total FAs, palmitic acid predominated with the highest amount (35.9±1.64%). The MUFA percentage was 26.17±2.04% with oleic acid being the main fatty acid – 23.97±0.95%. PUFA content was 3.60± 0.57%, linoleic acid: 2.92% and linolenic acid: 1.02%.

Our results corresponded with the fatty acids content of UK whole cow’s milk, available in supermarkets (Butler *et al.*, 2010). The determined SFA in milk from the UK ranged within 68.2–72.5% of total FAs, MUFA were 24.3–28.0% and PUFA varied from 3.28% to 3.76%.

An investigation of fatty acid composition of milk from the Bulgarian Rhodopes cattle breed showed similar range of SFAs (68.95÷75.32 g/100 g fat), palmitic acid varied from 28.52 to 34.72 g/100 g fat, MUFAs were 21.78÷27.27 g/100 g fat and PUFAs were 2.87÷4.98 g/100 g fat (Ivanova *et al.*, 2012). Authors observed a decrease in palmitic acid (28.52 g/100 g fat) and total SFAs up to 69.66 g/100 g fat in the milk after the transition to free pasture rearing in July. The quantities of stearic (8.66 g/100 g fat) and oleic (16.87 g/100 g fat) acids were also elevated the same month.

The fatty acid composition of milk in this study showed variations in the content of 6 fatty acids (C10:0, C12:0, C14:0, C16:0, C18:0 and C18:1, 9c). The determined fluctuations could be attributed to the effect of season. Summer samples showed bigger concentrations of stearic and oleic acids, because cows were grazing fresh forage, rich in polyunsaturated fatty acids. During winter, cows are housed and received conserved forage that increased the levels of SFAs, especially the palmitic acid concentration. Similar seasonal fluctuations in fatty acid content of UK retail milk were reported by Kliem *et al.* (2013). They found the highest concentration of C16:0 and C14:0 in the winter samples (34.1 and 11.0 g/100 g fatty acids respectively) and lowest concentrations in samples from May and July (30.0 and 10.0 g/100 g fatty acids). The milk sold during summer contained fewer total saturated fatty acid

(SFA; 67 vs 72 g/100 g fatty acids) and had higher cis-monounsaturated fatty acid content (MUFA; 23 vs 21 g/100 g fatty acids).

The observed changes in FAs content of investigated yogurt could be attributed to seasonal feeding practices. There were more significant variations in fatty acid concentrations of yogurt than in those of pasteurised milk. Noticeable fluctuations in the contents of short-chain FAs (C4:0÷ C10:0) compared to pasteurised milk were identified.

The fatty acid composition reported for Greek cow's yogurts was very close to that established in our study (Serafeimidou *et al.*, 2012): SFA 71.21±0.87%, MUFA 25.23±1.84% and PUFA 2.86± 1.62%.

Naydenova *et al.* (2014) examined five brands of yogurt, commercially available in Bulgaria. The content of short chain FAs (8.81÷11.15%), palmitic acid (25.94÷30.06%) and SFAs (58.41%÷ 62.91%) were lower, compared with our results. The quantities of UFAs (37.10÷ 41.59%) were higher, due to elevated oleic acid content (30.11÷34.62%). The results for fatty acid composition of yogurt were explained by the authors with the milk used for the production.

The number of samples with substituted milk fat for the investigated period was small. Nevertheless, unregulated milk fat substitution, without informing the consumer, is a breach of the current legislation.

CONCLUSIONS

For first time, this survey provided scientific information for fatty acid composition of cow's milk, offered on Bulgarian market. The milk fat had an average of 70% saturated fatty acids and 30% unsaturated fatty acids. The fatty acid profile of milk was slightly influenced by seasonal feeding. The information could be useful for estimation of dietary intake.

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