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QUALITATIVE CHARACTERISTICS OF THE FAT FRACTION OF SHEEP YOGHURT AND A LOCAL PRODUCT "KATAK" FROM KARAKACHAN SHEEP REARED IN THE MIDDLE BALKAN MOUNTAINS REGION

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Abstract

The studies were pooled sheep milks obtained from Karakachan sheep, pasture-raised without nutrition in the farm of the Research Institute of Mountain Stockbreading and Agriculture (RIMSA)-Troyan, Bulgaria. Samples of raw sheep milk for analysis were taken during the the months of April, May and June. Sheep yoghurt and local product "Katak" were produced from the pooled samples and the fatty acid content of the products was analysed by gas chromatography. Saturated fatty acids were found to decrease from 63.01 g/100 g in raw sheep's milk to 62.28 g/100 g in yoghurt on day 10. Monounsaturated fatty acids and polyunsaturated fatty acids predominated in the local product "Katak" - 29.54 g/100 g;7.0 g/100 g. The ratio of essential omega-6/omega-3 fatty acids is less than 5 according to health recommendations. The atherogenic index (AI) of milk fat is in the range-1.75-1.83, with a decrease from raw milk to dairy products, indicating that the products are healthier in terms of lipid content. Low trans fatty acid content was reported from 0.45 g/100 g in raw milk to 0.73 g/100 g product in sheep's yoghurt at day 10 and 0.92 g/100 g product in local product "katak".

***Key words**: fatty acids, lipid indices, local product-katak, sheep's yoghurt.*

INTRODUCTION

The dairy industry is one of the most important and traditional industries in our country. Sheep breeding in Bulgaria has a centuries-old tradition. In the mountain regions, where climatic conditions are harsh and fodder resources are limited, sheep's milk processing is a basic livelihood and its irreplaceable nutritional value is of paramount importance for maintaining the nutritional balance of the population. The short shelf-life of sheep's milk means that it can be incorporated into fermented products such as sheep's yoghurt and local product "Katak'' to extend its shelf-life and nutritional potential. The excellent taste of sheep's milk dairy products makes them an essential part of the Bulgarian table. Compared to other types of milk (cow, goat, human), sheep milk is dominated by biologically active substances - protein, essential amino acids, monounsaturated and essential fatty acids, macro and microelements, vitamins B6, B12, etc. (Valchkov et al., 2016). Milk fat as a component of dairy products is characterised by controversial opinions due to its high content of saturated fatty acids (SFAs), but according to Simopoulos (2002) only a few of them are fatty acids with harmful effects on human health. Lauric, myristic and palmitic affect cholesterol, but only C16:0 has been shown to affect the cardiovascular system. Trans fatty acids are also important for a healthy diet as they have a negative effect on the ratio of low-density lipoprotein (LDL) cholesterol to high-density lipoprotein (HDL) cholesterol (Mihaylova et al., 2007). Milk also contains unsaturated fatty acids including conjugated linoleic acid (CLA), vaccenic acid (VA) which lowers total blood cholesterol and acts anticarcinogenic and immunostimulant (Mills et al., 2011). In order to determine the quality of milk fat, the amount of saturated and unsaturated fatty acids can be compared using Ulbright and Southgate's (1991) atherogenic index, and the omega-6/omega-3 ratio can be determined in relation to their impact on human health. The grading of sheep's milk as a health product can also be done by the lipid preventive score (LPS) proposed by Richard and Charbonier (1994). A balanced fatty acids composition is obtained when the saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) content is such that the total lipid content is equal to the theoretically calculated LPS (Ivanova et al., 2011).

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Studies on the fatty acid composition of milk fat in sheep milk have been reported by many authors over the years (Gerchev & Mihaylova, 2009; Mihaylova et al., 2012; Angelov et al., 2017; Gerchev et al., 2018). Ivanova et al. (2017), reported the content of BMD in raw sheep milk during the grazing period as 65.54-66.74 g/100 g fat, MUFAs as 25.28-26.15 g/100 g fat and PUFAs as 7.39-8.31 g/100 g. Dimitrova et al. (2021) found values for SFA-55.75 g/100 g, MUFA-37.84 g/100g and PUFA-6.86 g/100 g in Karakachan sheep milk.

The aim of our study was to qualitatively characterise the fat fraction of sheep's yoghurt and local product “Katak” from Karakachan sheep from the Middle Balkan Mountains Region.

MATERIALS AND METHODS

The study was carried out on pooled sheep milk obtained from Karakachan sheep from the RIMSA-Troyan flock, pasture-raised in the Middle Balkan Mountains Region. Samples were taken during the lactation period, in the months of April, May and June, from morning milking, for yoghurt production and pooled raw milk samples for the production of local product “Katak” in August. Several batches of sheep's yoghurt were produced under laboratory conditions, using classical technology (Peichevski & Chomakov, 1988) and several batches of the local product “Katak” using technology typical for the area of the town of Apriltsi.

Full-fat milks were used. For this purpose, raw sheep's milk was pasteurized at 92-95˚C with a holding time of 10-15 min, cooled to a souring temperature of 42-46˚C, fermented with 1% (0.5-1.5%) starter of *L. bulgaricus* and *Str. thermophilus* - 1:4-1:6, cut at 41-42˚C, thermostat at 42-43˚C, then coagulate for 2-2.5 hours to 75-80˚T. Cool 1-2 hours to 20˚C and 3-4 hours to 8-10˚C and refrigerate at 0-4˚C. Samples of yoghurt were analysed at the 24th hour and 10th day of the storage process as well as samples of local product “Katak” and the lactation period data are presented averaged.

Extraction of total lipids was carried out by Roese-Gottlieb method, using diethyl and petroleum ether and subsequent methylation with sodium methylate (CH_3ONa, Merck, Darmstadt) and drying with $NaHSO_4.H_2O$. The fatty acid methyl esters/FAME/ were analyzed using a Shimadzu-2010 gas chromatograph (Kioto, Japan) equipped with a flame ionization detector and an automatic injection system (AOC-2010i). The assay was performed on a capillary column CP 7420 (100 m x 0.25 mm i.d., 0.2 µm film, Varian Inc., Palo Alto, CA). Hydrogen was used as the carrier gas and nitrogen was used as the make-up gas. The four-step furnace mode was programmed – the initial column temperature is 80ºC/min, which was maintained for 15 minutes, then increases by 12ºC/min to 170ºC and maintained for 20 minutes, followed by a new increase of 4ºC/min to 186ºC for 19 min and up to 220ºC with 4ºC/min until the process is complete. The qualitative assessment of the fat fraction includes the following indicators: lipid preventive score (LPS), atherogenic (AI) and thrombogenic index (TI) (Ulbricht & Southgate, 1991), the ratio of hyper- and hypocholesterolemic (h/H) fatty acids, trans fatty acids (TFAs) and the amount of saturated fatty acids (SFAs) (Regulation (EC) No1924/2006).

LPS=FAT+2xSFA-MUFA-0.5PUFA

AI=12:0+4x14:0+16:0/[ΣMUFAs+PUFAn-6+PUFAn-3]

TI=(14:0+16:0+18:0)/[0.5xΣMUFAs+0.5x PUFAn-6+3xPUFAn-3+PUFAn-3/PUFAn-6]

h/H = (C18:1n-9 + C18:1n-7 + C18:2n-6 + C18:3n-3 + C18:3n-6 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3)/ (C14:0 + C16:0)

The data were processed using the variation statistics methods using the statistical package of the EXCEL 2013 computer program.

RESULTS AND DISCUSSIONS

Table 1 summarises the main groups of fatty acids in raw sheep's milk, yoghurt and the local product “Katuk”. Current aspects related to milk fat composition concern the content of conjugated linoleic acid (CLA), which can reduce the risk of various diseases- overweight, various forms of cancer, atherosclerosis and whose content changes during the seasons depending on animal nutrition (Chilliard et al., 2005). In raw sheep's milk, its concentrations are 1.89 g/100 g fat, and its content decreases slightly to 1.81 g/100 g fat in sheep's yoghurt on the 10th day of storage. Lower than our results indicated by Markova & Slavov (2019) for milk of the Koprishchenska breed of sheep CLA - 1.62% and for Srednostaroplanska CLA -1.20%, which is probably due to the breed and phase of pasture vegetation.

The sum of saturated fatty acids (SFAs), which are risky for health, in the studied samples is high, but decreases in the yoghurt at the 24th hour and 10th day compared to the starting raw material (63.01-62.28 g/100g fat), while for the local product “Katak” we report a slight increase - 63.25 g/100 g fat. Close to our results were found by Gerchev & Mihaylova (2009), for Tsigai sheep during the grazing period- ΣSFA of 57.20- 65.98%.

Table 1. Groups of fatty acids in raw sheep milk, yoghurt and local product “Katak”, g/100 g fat, n = 4

Groups of fatty acids	raw sheep milk	sheep yoghurt - 24 h	sheep yoghurt - 10th day	local product “Katak”
	x±Sx	x±Sx	x±Sx	x±Sx
ΣCLA	1.89±0.124	1.86±0.081	1.81±0.0111	2.02±0.0112
ΣC-18:1 trans forms	5.72±0.550	8.55±0.328	8.88±0.434	7.64±0.234
Σ C-18:1 cis forms	20.45±2.020	17.45±1.399	17.03±1.255	18.80±1.755
ΣSFA	63.01±2.302*	62.52±2.149*	62.28±2.132*	63.25±2.132
ΣMUFA	28.77±1.811*	28.41±1.501*	29.36±1.543*	29.54±1.421*
ΣPUFA	6.76±0.902	6.84±1.011	6.52±0.455	7.00±0.955
Σ n-3	2.01±0.087	2.07±0.062	1.98±0.080	2.09±0.083
Σ n-6	3.05±0.378	2.98±0.820	2.80±0.754	3.10±0.259
Σn-6/Σn-3	1.52±0.123	1.44±0.352	1.41±0.521	1.48±0.423
ΣMCT(C-10>C14)	16.81±0.015	16.66±0.145	16.61±0.760	17.19±0.123
ΣSCT(C-4>C8)	8.06±0.091	8.0±0.312	8.57±0.560	8.35±0.150
ΣBranched fatty acids	2.39±0.188	2.28±0.105	2.44±0.230	2.42±0.238

Note: * P<0.05

The higher data for saturated fatty acids corresponded to lower results for monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), with values increasing from raw milk -28.77 g/100 g fat; 6.76 g/100 g fat to 29.54 g/100 g fat and 7.0 g/100 g fat in the „local product katak”. Gerchev & Mihaylova (2012) found lower values than ours for ΣMUFA (23.41-25.04 g/100 g fat) and ΣPUFA (4.19-4.38 g/100 g fat) in yoghurt from the Srednostaroplaninska and Tetevenska sheep breeds, probably due to breed differences in the animals. C-18:2 and C-18:3 have been shown to perform a number of important functions, and in the presence of vitamin B_6 in the body, C-18:2, which has high biological activity, is converted to the essential arachidonic acid (Gladkii & Fedakina, 2006).

Sheep's milks are characterised by low levels of omega-3 fatty acids, ranging from 1.98 g/100 g fat in sheep's yoghurt on day 10 to 2.09 g/100 g fat in a local product “Katak”, with unreliable results. The omega-3/omega-6 ratio decreases from raw milk to products and remains below 5, indicating that the samples studied are of low health risk to consumers. Ivanova (2017) found values for this ratio of 1.62-1.93, respectively for milk from sheep of the Karakachan breed reared in the Middle Rhodopes region, and Gerchev et al. (2018) - 1.98 for milk from Karakachan sheep from the RIMSA-Troyan.

The quality of milk fat can be assessed using indicators such as the lipid preventive score, the atherogenic and thrombogenic index, and the ratio of hyper- to hypocholesterolemic fatty acids (Figures 1, 2, 3). The lipid preventive score, calculated on the basis of the total fat content and the main fatty acid groups in 100 g of product (Richard & Charbonnier, 1994), is lowest in raw sheep's milk - 15.23 g/100 g of milk - and increases with the dairy products, with the highest results in the „local product katak" - 25.45 g/100 g of product. Close to our data are those of Mihaylova, (2006), or LPS - 15.8 g/100 g product in sheep milk of the Karakachan breed from the Rhodope Mountains. Studying milk from four species, the same author found that the difference between fat and LPS content was lowest in cow's milk, followed by goat's milk, and highest in sheep's and buffalo's milk (Mihaylova, 2007). The atherogenic index (AI) of milk fat in the samples studied ranged from 1.75 to 1.83, with a decrease from raw milk to dairy products, indicating that the products were healthier in terms of lipid content. Close to our results, Gerchev et al. (2018) for milk from Karakachan sheep from RIMSA-Troyan and Mihaylova (2007) for raw sheep milk from the Balkan Mountains region, respectively, found AI in raw milk - 1.92, in yoghurt - 1.72 and in "dairy delicacy" - 2.06. The thrombogenic index (TI) varied irregularly and within a narrow range of 1.88-1.92 in the samples studied, and the cholesterolemic index was low, below 1.0.

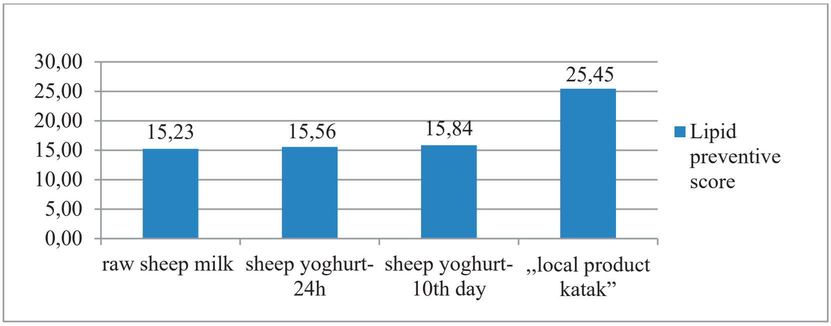

Figure 1. Lipid preventive score (LPS) in sheep milk and dairy products, g/100 g product

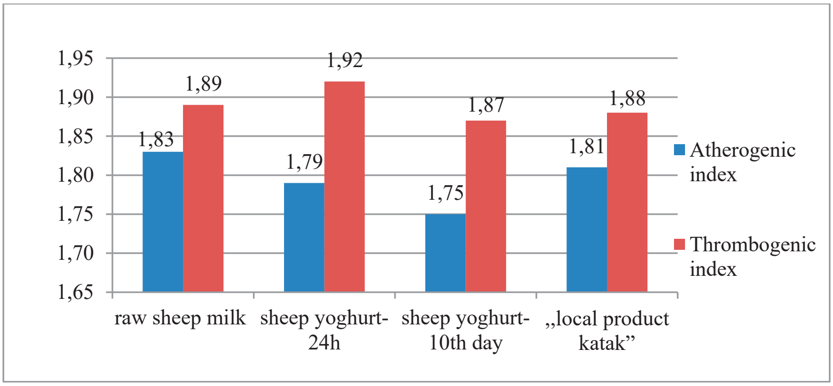

Figure 2. Atherogenic (AI) and Thrombogenic index (TI) in sheep milk and dairy products

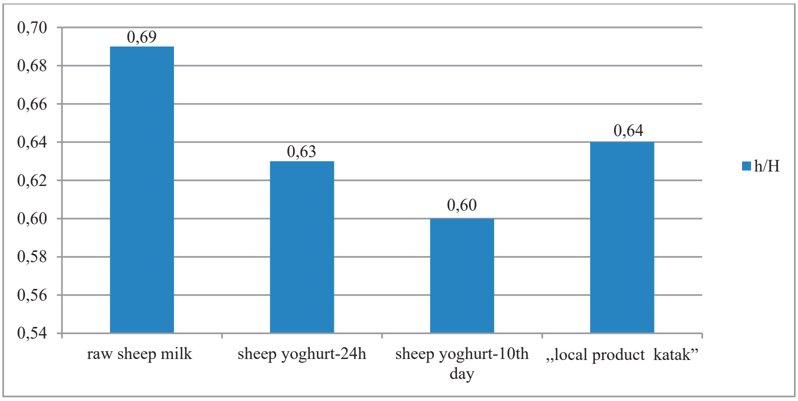

Figure 3. The ratio of hyper and hypocholesterolemic (h/H) fatty acids

Trans fatty acids (TFAs) play an important role in human nutrition, and there is widespread concern about their negative effects on health, as they have been shown to increase LDL-cholesterol levels in the same way as saturated fatty acids. Studies have shown that TFAs are associated with coronary heart disease and that it is the type of fat, not the amount, that increases the risk of this disease (Becker, 2003). According to the European Union Regulation №1924/2006, the saturated fatty acid and trans fatty acid content of solid products should not be more than 1.5 g/100 g product or 0.75 g/100 ml liquid. In both cases the content should not exceed 10% of the daily energy intake, then these foods are designated as low saturated fatty acid foods (Dimitrova et al., 2017).

The saturated fatty acid/trans fatty acid ratio in our study (Figure 4) was lowest in raw sheep's milk - 5.40 g/100 g milk, and highest in sheep's yoghurt on day 10 - 5.88 g/100 g product and in local product “Katak” - 9.24 g/100 g product, from which we conclude that our samples are characterized by a high saturated fatty acid content and low trans fatty acid concentrations from 0.45 g/100 g milk to 0.73 g/100 g product in yoghurt on day 10 and 0.92 g/100 g product in local product “Katak”.

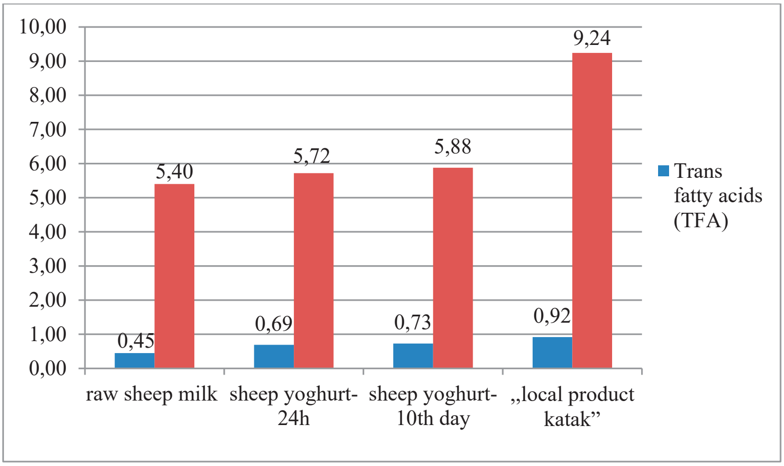

Figure 4. Trans fatty acids (TFA) and the amount of Saturated fatty acids (SFA), g/100 g product

CONCLUSIONS

The sum of saturated fatty acids (SFAs) decreased from raw sheep's milk to yoghurt at 24 hours and 10 days of storage. The atherogenic index (AI) of milk fat decreased from -1.83 for raw milk to 1.75 for sheep's yoghurt and 1.81 for local product “Katak” on the 10th day of storage, indicating that the products are healthier in terms of lipid content. The ratio of omega-6 to omega-3 fatty acids is below 5, in accordance with health recommend-dations. The samples tested were high in saturated fatty acids ranging from 5.40 g/100 g in raw sheep's milk to 9.24 g/100 g product in local product “Katak” and low in trans-fatty acids ranging from 0.45 g/100 g in raw sheep's milk to 0.92 g/ 100 g product in local product “Katak” according to EU Regulation 1924/2006.

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