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ASSESMENT OF BYOLOGICALY ACTIVE AND TRANS FATTY ACIDS IN FAT FRACTION ON THE COW'S YOGURT

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Abstract

Natural sources of trans isomers of fatty acids are primarily the milk fat and other fats from animal origin. The study was conducted with yogurt containing 2, 3 and 4.5% for the determination of biologically active and trans fatty acids and qualitative assessment of the fat fraction. Yogurt with 4.5% fat have a highest content of saturated fatty acids SFA- 3.13 g/100 g product, monounsaturated fatty acids MUFA- 1.18 g/100 g product, polyunsaturated fatty acids PUFA- 0.15 g/100 g product, oleic- 0.98 g/100 g product, linoleic-0.08 g/100 g product, trans fatty acids- 0.14 g/100 g product, conjugated linoleic acid CLA- 0.03 g/100 g product as long as 2% yogurt is the poor of biologically active fatty acids- oleic acid- 0.43 g/100 g product, linoleic- 0.04 g/100 g product, trans fatty acids- 0.09 g/100 g product, CLA-0.01 g/100 g product. Lipid preventive score, index of atherogenicity and thrombogenicity is highest at 4.5% yogurt- 9.50 g/100 g product, 3.02 and 2.82. The analyzed yoghurts are characterized as a food product with a low content of trans fatty acids- 0.06 to 0.14 g/100 g product and a low content of saturated fatty acids in the yogurt with 2% fat- 1.45.

Key words*: conjugated linoleic acid (CLA), trans fatty acids, yogurt.*

INTRODUCTION

Yogurt was a dairy product produced by bacterial fermentation of milk using *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. It was a widespread food product on the Balkans and the Middle East and was produced from cows, sheep's, goat's, buffalo's milk or a mixture and is one of the healthiest and most nutritious foods (Serafeimidou et al., 2012; Gahruie et al., 2015). Florence et al. (2012) found that the use of organic cow's milk compared to conventional cow's milk production has more health benefits because the content of saturated fatty acids was lower due to an increase in biologically active trans fatty acids: trans octadecene acid-1.6 times, CLA-1.4 times and alpha linolenic-1.6 times compared to conventional yogurt. Serafeimidou et al. (2013), in their research on the shelf life of yogurt from cows and sheep, found that saturated fatty acids in cow's yogurt increase, while in sheep decrease, omega-3 fatty acids increase the both types in yogurt at the end of the shelf life, while the conjugated linoleic acid decreases with the storage of cow's yoghurt, but increases with sheep's. Diets rich by saturated fatty acids such as lauric (C12:0), myristic (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) were strongly associated with an increased risk of atherosclerosis, obesity and coronary heart disease (Pilarczyk et al., 2015). According to the indices proposed by Ulbricht and Southgate (1991), lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) were atherogenic, and myristic (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) had a thrombogenic by nature, while omega-3, omega-6 and monounsaturated fatty acids were antiatherogenic and antithrombogenic pattern. De Souza et al. (2015) were found in cow's milk atherogenic index- 4.10 and thrombogenic index- 5.17. The ratio between hyper- and hypocholesterolemic fatty acids, found by Fernandez et al. (2007) in Iberian ham below 2.5, which was defined as favorable compared to the other types of ham studied by them. Tonial et al. (2014), in two species of fish, received values for AI-0.55-0.60; TI- 0.82-0.87 and hH- 1.56-1.63.

The daily intake of trans fatty acids should not exceed 0.5% of energy intake. According to EU and Council Regulation (EC) No 1924/2006 on 20 December 2006, the content of saturated

fatty acids and trans fatty acids in solid products shall not exceed 1.5 g/100 g product or 0.75 g/100 ml liquid, as in both cases the content of saturated fatty acids and trans fatty acids does not exceed 10% of the daily energy intake and these foods were labeled as foods with low SFA content. The claim that a food does not contain SFA may be indicated only if the content of SFA and TFA does not exceed 0.1 g/100 g product or 0.1 g/100 ml liquid (Regulation (EC) No 1924/2006).

The study was conducted with yogurt containing 2, 3 and 4.5% for the determination of biologically active and trans fatty acids and qualitative assessment of the fat fraction.

MATERIALS AND METHODS

Yogurts produced by Research Institute of Mountain Stockbreeding and Agriculture-Smolyan with a milk fat content, respectively 2% (4 pieces), 3% (4 pieces) and 4.5% (4 pieces) for fatty acid composition and for establishment of the content of trans fatty acids, biologically active and anticancer substances in the fatty fraction were studied. The extraction of total lipids was carried out by the Roese-Gottlieb method, using diethyl ether and petroleum ether and subsequent methylation with sodium methylate (CH_3ONa, Merck, Darmstadt) and drying with $NaHSO4.H_2O$. 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 analysis was performed on a CP 7420 capillary column (100 m x 0.25 mm i.d., 0.2 μm film, Varian Inc., Palo Alto, CA). Hydrogen is used as the carrier gas, and as a make-up gas - nitrogen. Four-step furnace mode is programmed - the column's initial temperature is 80°C/min, maintained for 15 minutes, then increased by 12ºC/min to 170ºC and maintained for 20 minutes, followed by a further increase of 4ºC/min to 186ºC for 19 minutes and up to 220ºC with 4ºC/min until the process is complete.

The qualitative evaluation of the fat fraction of the yoghurts obtained includes the following indicators: lipid preventive score, atherogenic and thrombogenic index (Ulbricht & Southgate, 1991), the ratio of hyper- and hypocholesterolemic fatty acids (Ivanova & Hadzhinikolova, 2015), trans fatty acids and the amount of saturated fatty acids (Regulation (EC) No 1924/2006).

LPS= FAT +2x SFA- MUFA- 0.5 PUFA

AI= 12:0+ 4×14:0 +16:0 /[ΣMUFAs+PUFA n6+PUFA n3]

TI = (14:0+16:0+18:0)/[0.5×ΣMUFAs+0.5× ×PUFAn6+3×PUFAn−3+PUFA n3/ PUFA n6]

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)

where:
LPS - lipopolysaccharide;
AI - atherogenic index;
TI - thrombogenic index;
h/H - hypocholesterolemic/ hypercholesterolemic ratio.

The data were processed using the variation statistics methods using the statistical package of the EXCEL 2013 computer program. The reliability of the differences between the analyzed milks was established by Student's t-test.

RESULTS AND DISCUSSIONS

The fatty acid composition was an important characteristic of the fat fraction for determining the content of trans fatty acids and biologically active components. Milk fat contains mainly saturated fatty acids. The amount of saturated fatty acids in the studied yoghurts varies from 68.54 to 69.85 g/100 g fat, monounsaturated fatty acids range from 26.30 to 27.43 g/100 g fat and polyunsaturated from 3.12 to 3. 29 g/100 g fat. The biologically active fatty acids in yoghurt were from 2.86 to 3.01 for trans fatty acids, from 0.43 to 0.47 g/100 g fat for ω-3, from 2.39 to 2.50 g/100 g fat for ω-6 and from 0.49 to 0.56 g/100g fat by conjugated linoleic acid. The ratio between ω-6 and ω-3 fatty acids was from 5.19 to 5.54 in the analyzed yogurts (Table 1). Yogurt produced

from cow, sheep and goat milk yogurt contain 0.128-1.501, 0.405-1.250 and 0.433-0.976 g conjugated linoleic acid or CLA per 100 g fat, respectively (Serafeimidou et al., 2012; Serafeimidou et al., 2013; Sumarmono et al., 2015; Kalinova, 2020). Determined that the trans fatty acids in pasteurised milk and yogurt were in small amounts (about 0.50%) and did not show fluctuations. Paszczyk et al. (2020) was established the storage effect in yogurt and changes in fatty acids, especially CLA and trans isomers in cow milk yogurts which decrease by shelf life. Gutiérrez (2016) establishes CLA content in cow's milk varies between 2 and 37 mg/g fat and is mainly influenced by the diet offered to animals, as technological processes may cause slight changes in CLA concentration, but the mechanisms leading to these changes have not yet been established, but the increase of the concentration of CLA by lactic fermentation depends on the strain due to the different linoleate isomerase activity of the species.

Table 1. Fatty acid composition of yoghurt with different milk fat content (g/100 g fat)

FA	2%		3%		4.5%	
	x	sd	x	sd	X	Sd
SFA	**68.54**	1.86	**69.85**	1.77	**69.58**	3.62
MUFA	**27.43**	1.73	**26.21**	0.93	**26.30**	2.65
PUFA	**3.28**	0.23	**3.12**	0.34	**3.29**	0.57
Σ *trans* FA	**2.97**	0.16	**2.86**	0.91	**3.01**	1.07
Σ *cis* FA	**23.55**	1.61	**22.41**	0.05	**22.38**	1.66
Σ CLA	**0.56**	0.04	**0.54**	0.12	**0.60**	0.15
C-16:0/C-18:1cis9	**1.37**	0.14	**1.47**	0.08	**1.48**	0.22
C-16:0/C-18:1total	**1.18**	0.12	**1.26**	0.12	**1.27**	0.23
Σ n-3	**0.45**	0.04	**0.43**	0.02	**0.47**	0.06
Σ n-6	**2.50**	0.15	**2.39**	0.33	**2.46**	0.48
Σ n-6/ Σ n-3	**5.54**	0.10	**5.61**	0.37	**5.19**	0.27
Σ SCT (C-4>C-8)	**7.10**	0.22	**6.95**	1.21	**6.60**	1.00
Σ MCT (C-10>C-14)	**16.23**	1.14	**17.04**	0.03	**17.80**	1.11
CLA 9c,11t	**0.51**	0.03	**0.49**	0.12	**0.56**	0.15

Table 2. Fatty acid composition of yoghurt with different fat content (g/100 g product)

	2%		3%		4.5%	
FA	x	sd	x	sd	x	sd
C12:0	**0.06**	0.02	**0.09**	0.03	**0.14**	0.05
C14:0	**0.22**	0.03	**0.32**	0.05	**0.49**	0.07
C16:0	**0.62**	0.06	**0.93**	0.09	**1.40**	0.14
C18:0	**0.23**	0.04	**0.34**	0.05	**0.51**	0.08
C18:1n-9	**0.43**	0.06	**0.65**	0.09	**0.98**	0.14
C18:2n-6	**0.04**	0.01	**0.05**	0.01	**0.08**	0.02
SFA	**1.39**	0.10	**2.09**	0.15	**3.13**	0.23
MUFA	**0.53**	0.08	**0.79**	0.13	**1.18**	0.19
PUFA	**0.07**	0.01	**0.10**	0.02	**0.15**	0.02
TFA	**0.06**	0.02	**0.09**	0.03	**0.14**	0.05
ω-3	**0.01**	0.00	**0.01**	0.00	**0.02**	0.00
ω-6	**0.05**	0.01	**0.07**	0.02	**0.11**	0.02
CLA 9c,11t	**0.01**	0.00	**0.02**	0.00	**0.03**	0.00

Based on the obtained fatty acid profile the amount of saturated fatty acids in the final product was highest at 4.5% fat content and represents 3.13 g/100 g product, while in low-fat 2% yogurt they were 1.39 g/100 g product. The content of mono- and polyunsaturated fatty acids, 0.53 and 0.07 g/100 g product in 2% yoghurt and 1.18 and 0.15 g/100 g of product in 4.5% yoghurt, respectively (Table 2).

The main representatives of saturated fatty acids that were related to human nutrition were lauric (C12:0), myristic (C14:0) acid, palmitic (C16:0) and stearic acid (C18:0). Yogurt with 4.5% fat content has the highest content of these four representatives, respectively 0.14, 0.49, 1.40 and 0.51 g/100 g product. The oleic acid in the tested samples was in the highest concentration by 4.5%- 0.98 g/100 g products, the lowest by 2% yogurt- 0.43 g/100 g product. The linoleic acid in the tested yoghurts was range from 0.04 to 0.08 g/100 g product.

Serhan et al. (2016) in the production of concentrated yoghurt from goats, has a fat content of 9.25%, protein 9.12%, ash 1.16%, saturated fatty acids 69.1 g/100 g fat, polyunsaturated 27.2 g/100 g fat and monounsaturated fatty acids 3.4 g/100 g fat and atherogenic index 2.84. TFA was an indicator of food safety, because it has a negative effect on many vital functions (Chen & Liu, 2020).

The total content of trans fatty acids in the analyses yoghurts was from 0.09 g/100 g product in 2% yoghurt to 0.14 g/100 g product in 4.5% yoghurt. Omega-3 fatty acids in yogurt with a fat content of 2 and 3% were 0.01 g/ 100 g product, while in 4.5%- 0.02 g/100 g product. Omega-6 fatty acids range from 0.05 to 0.11 g/100 g product. Another important biologically active component of milk fat was CLA, whose content was highest in yogurt with 4.5% fat content- 0.03 g/100 g product. The statistical reliability of the results for fatty acids is presented in Table 3.

Table 3. Statistical reliability of the results for the fatty acid composition of yoghurt with fat content of 2, 3 and 4.5%

Fatty acids	2% / 3%	2% / 4.5%	3% / 4.5%
C12:0		*	
C14:0	**	**	**
C16:0	**	***	**
C18:0	**	**	**
C18:1n-9	**	***	**
C18:2n-6	*	**	*
SFA	***	***	***
MUFA	*	**	**
PUFA	**	***	**
TFA		*	
ω-3	***	***	***
ω-6	*	**	*
CLA 9c,11t	***	***	***

P< 0.001- ***; P< 0.01- **; P< 0.05- *

Table 4. Qualitative indicators of the fat fraction in yoghurt with different fat content (g/100 g product)

Показател	2%		3%		4.5%	
	X	SD	X	SD	X	SD
LPS	4.22 a***,b***,c***	0.29	6.34	0.44	9.50	0.66
AI	2.72	0.88	2.43	0.88	3.02	0.88
TI	2.61	0.64	2.41	0.64	2.82	0.64
h/H	0.59	0.14	0.62	0.14	0.56	0.14
TFA	0.06	0.02	0.09	0.03	0.14	0.05
SFA+TFA	1.45 a***,b***,c***	0.08	2.18	0.12	3.27	0.18

a-2%/ 3% yogurt, b- 2%/ 4.5% yogurt, c- 3%/4.5% yogurt, *P<0.05, ** P<0.01, ***P<0.001

High reliability of the results was found for saturated fatty acids and conjugated linoleic acid in the three types of yoghurt.

The qualitative assessment of the fat fraction was made on the basis of the following indicators: lipid preventive score, atherogenic and thrombogenic index and the ratio between hyper- and hypocholesterolemic fatty acids (Table 4). All indices have their advantages and disadvantages; therefore, the rational choice to

be used is crucial. Of these nutritional indices, IA and IT are the most commonly used to assess the composition of fatty acids as they outline significant implications and provide clear evidence (Chen & Liu, 2020).

The lipid preventive score in yogurts with different fat content was the highest at 4.5% and reaches a value of 9.50 g/100 g product and was lowest at 2%- 4.22 g/100 g product. The atherogenic index gives the relationship between the sum of basic saturated fatty acids and unsaturated fatty acids, the former being considered pro-atherogenic (favoring the adhesion of lipids in immune and circulatory cells) and the latter anti-atherogenic (inhibiting plaque aggregation and reducing plaque aggregation and levels of esterified fatty acids, cholesterol, and phospholipids, thus preventing the onset of micro- and macrocoronary heart disease). The thrombogenic index gives the tendency to form clots in blood vessels and is defined as the ratio between prothrombogenic (saturated fatty acids) and antithrombogenic (monounsaturated and polyunsaturated omega-3 and omega-6 fatty acids) fatty acids (Gahruie et al., 2015). The atherogenic index was the lowest in yogurt 3% - 2.43, while in 4.5% it reaches - 3.02, the results were similar for the thrombogenic index- the lowest in 3% yogurt - 2.41 and the most-high at 4.5% - 2.82. The studied yoghurts were characterized as a food product with low content of trans fatty acids from 0.06 to 0.14 g/100g product and low content of saturated fatty acids in yogurt with 2% fat content- 1.45, while in 3 and 4.5% fat content of yoghurt, the content of saturated and trans fatty acids exceeds 1.5 g/100 g product, therefore exceeding 10% of the daily energy intake was defined as a food with a high content of saturated fatty acids.

CONCLUSIONS

Of the studied yoghurts with different fat content, the poorest of biologically active fatty acids was 2% yoghurt, but according to quality indicators for assessment of the fat fraction it was most suitable for healthy human nutrition and with the lowest content of saturated and trans fatty acids, while 4.5% yogurt was richest in natural biologically active fatty acids.

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