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Product Quality and Safety

Fatty acid composition of yogurt supplemented with walnut extract

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Abstract. *The present study aimed to monitor the changes in fatty acid composition of yogurt supplemented with ground walnut extract and stored over 10 days. Fatty acid content of raw and pasteurized milk, of 24-hour yogurt and 10-day yogurt (stored at 4°C) was assayed. Total saturated fatty acid content of experimental samples of milk was lower while that of unsaturated fatty acids was higher as compared to control milk, 24-hour and 10-day yogurt samples. Assayed fatty acids exhibited higher differences between control and experimental samples with regard to polyunsaturated (C18:2 and C18:3) fatty acids. The total saturated fatty acid content in pasteurized milk with walnut extract decreased by 11.2% compared to raw milk, while remaining unaltered in natural pasteurized milk. Pasteurized milk with walnuts exhibited higher polyunsaturated fatty acid content – 2.6 times vs natural and almost 3 times higher than raw milk. Yogurt containing walnut extract contained by 19.25% more unsaturated fatty acids and by 2.3 times more polyunsaturated fatty acids as compared to natural yogurt. During storage of produced yogurt for 10 days, the amount of C18:3 decreased by 46.8% in the yogurt containing walnut extract and by 34.4% in natural yogurt.*

Keywords: fatty acids, yogurt, walnuts

Keywords: MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, SFA – saturated fatty acids, UFA – unsaturated fatty acids

Introduction

Milk and dairy products are an essential part of the so-called functional foods. They are aimed at satisfying all dietary needs during the entire human life. Therefore, milk is always a part of human diet and is taking part in the balance of most valuable nutrients. The dietetic and nutritive properties of yogurt make it important part of Bulgarian diet. The considerable rates of consumption of this product are not only a centennial tradition, but are mainly due to its exceptional complete dietary qualities. The functional properties of yogurt could be increased via modification and/or increase of the beneficial effects of its components on human organism. One alternative for modification of Bulgarian yogurt quality (organoleptic and physicochemical parameters) is the supplementation of fruits, seeds, nuts etc. with the purpose to increase its therapeutic, prophylactic or dietetic properties. That is why our attention has been focused on the production of Bulgarian yogurt with supplements beneficial for human health (Dimitrov et al., 2002; Boycheva et al., 2006, 2007; Boycheva et al., 2010, 2011).

During the last years, more and more attention is paid on the fat content of milk and dairy products, including the content of saturated and unsaturated fatty acids. There are two primary classes of polyunsaturated fatty acids – ώ-6 and ώ -3, determined by essential fatty acids linoleic and linolenic acids. Both linoleic and linolenic acids are essential, but they could not be synthesized in the human organism. Thus, they should be supplied with food. In previous studies of ours, we monitored the changes in the fatty acids composition of yogurt supplemented with plant extracts (Boycheva et al., 2007a), with walnuts and hazelnuts (Naydenova et al., 2008; Boycheva et al., 2011).

Health benefits of conjugated linoleic acids as anticarcinogenic (Ha et al., 1990; Ip et al., 1994; Banni et al., 1999; Parodi, 1997; Belury, 2002; Masso-Welch et al., 2002) and antiatherogenic agent (Lee et al., 1994; Pariza et al., 1996; Kritchevsky et al., 2000), immune modulator (Cook et al., 1993; MacDonald, 2000), antidiabetic agent (Schrezenmeir and Jagla, 2000) and a factor reducing the body fat depots (Pariza et al., 1996; Garnsworthy, 1997; DeLany and West, 2000; Blankson et al., 2000) have been demonstrated both *in vitro* and in animal experiments (Pariza, 1999; Hayek et al., 1999; Williams, 2000).

The aim of this research was to monitor the changes in fatty acid composition of yogurt supplemented with ground walnut extract compared to natural yogurt

Material and methods

Fresh milk and starter cultures

Fresh raw cow milk, obtained from the Experimental Farm of the Trakia University – Stara Zagora and starter culture ready for direct vat inoculation were used for yogurt preparation. The cow's yogurt was prepared in laboratory conditions.

Yogurt preparation

The milk was pasteurized (95°C/30 min), cooled to 45 °C and inoculated with 1.5% yogurt culture consisting of *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus.* The raw milk was divided into two lots – control and experimental. The

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samples were then cultivated at 42°C until coagulation, then cooled and stored in a refrigerator at 4-6°C. Walnuts (10%) were finely ground and added to milk before pasteurization. After the pasteurization, milk was filtered and they inoculated as described above.

Fatty acid composition

The extraction of milk fat was done using the method of Rose-Gottlieb using a diethyl ether and petroleum ether (Methodenbuch, Bd. VI VDLUFA-Verlag, Darmstadt, 1985). After that the solvents were evaporated on a vacuum-rotary evaporator. For obtaining methyl esters of the fatty acids sodium methylate (CHONa) was used (Jahreis et al., 1997). The fatty acid composition of yogurt, both control and experimental, was determined by gas chromatography "Pay-Unicam 304" with flame ionization detector and column EC™-WAX, 30 m, ID 0.25 mm, Film:0,25 µm. The content of the different fatty acid groups and their ratios were calculated. The atherogenic index was calculated according to a formula on the basis of medium-chain fatty acids content – C12:0, C14:0 and C16:0, monounsaturated and polyunsaturated fatty acids (Chilliard et al., 2003).

Statistical analysis

For statistical analysis, ANOVA was perform on the two batches and the corresponding replicates, using a statistical software (Statistica 6.0).

Results and discussion

Data about the fatty acid content of raw and pasteurized milk – natural and supplemented with walnut extract (Table 1) showed that saturated caproic, carpylic and capric acids decreased in both walnut extract supplemented (by 18.73%, 23.08% and 38.9%, respectively) and in natural pasteurized milk (by 17.31%, 17.31% and 23.24% respectively) compared to raw milk. In a previous study of ours (Boycheva et al., 2007) the concentrations of C8:0 and C10:0 were also lower in pasteurized vs natural milk, but C6:0 content was increased. The experimental batch of pasteurized milk exhibited also reduction in saturated medium-chain fatty acids C12:0, C14:0 and C16:0 as compared to control pasteurized milk. In previous experiments with linseed (Boycheva et al., 2007), lower C12:0 and C14:0 contents in pasteurized milk were also reported.

The content of linoleic acid, an n-6 fatty acid, was almost 3 times higher in pasteurized milk with walnut extract compared to raw milk and 2.4 times higher than that of natural milk. The polyunsaturated linolenic acid was 3 times and 2.7 times higher, respectively, in pasteurized milk with walnut extract compared to natural pasteurized and raw milk. These data are not consistent with previous reports (Mihaylova, 2007) regarding the changes in C18:2, where a reduction of this acid's content has been established.

It is acknowledged that ώ-6 and ώ-3 fatty acids are precursors of hormone-like substances – eicosanoids, with a primary regulatory role. These molecules possess signalling functions improving the relationships between cells in the nervous and immune systems. They regulate cell growth, inflammatory events, the development of thrombosis, allergic reactions, hypertension etc. (Sarau et al., 1999; Evans et al., 2000; Boyce, 2005). Their effect in most cases is contradictory, that puts an emphasis on the substantial importance of balanced and healthy nutrition.

The total amount of short-chain fatty acids after pasteurization of milk decreased by 23.42% in milk with walnut extract and by 13.69% in natural milk. The total content of saturated fatty acids (SFA) in control milk samples was almost unchanged after the pasteurization, while in pasteurized milk with walnut extract it was by

Table 1. Fatty acid content of raw and pasteurized milk

Fatty acids	Raw milk		Pasteurized milk			
			Natural		With walnut extract	
	Mean	SEM	Mean	SEM	Mean	SEM
C 4:0	2.56	0.920	2.73	0.595	2.53	0.112
C 6:0	2.83[a]	0.441	2.34[a]	0.243	2.30[a]	0.375
C 8:0	1.56	0.196	1.29	0.226	1.20	0.211
C 10:0	3.83[ab]	0.269	2.94[a]	0.551	2.34[b]	0.413
C10:1	0.33[c]	0.036	0.28[c]	0.065	0.14[c]	0.045
C 12:0	3.56[a]	0.389	3.11	0.674	2.58[a]	0.433
C 14:0	10.26	0.284	10.51	2.362	9.30	1.157
C 14:1	1.07	0.370	0.71[c]	0.014	1.18[c]	0.053
C 15 ai	0.40[a]	0.025	0.51[a]	0.099	0.45	0.188
C 15:0	0.60	0.184	1.15	0.684	0.48	0.078
C 16:0	33.01[b]	0.166	33.82[c]	1.109	27.17[bc]	2.880
C 18:0	8.52[b]	1.680	8.85[b]	0.723	11.26[b]	0.595
C 18:1	27.06	0.724	26.93	1.712	26.62	1.048
C 18:2	2.75	0.331	3.32	0.742	7.88	2.435
C 18:3	1.67	0.642	1.53	0.325	4.58	1.560

Legend: a – p<0.05, b – p<0.01, c- p<0.001

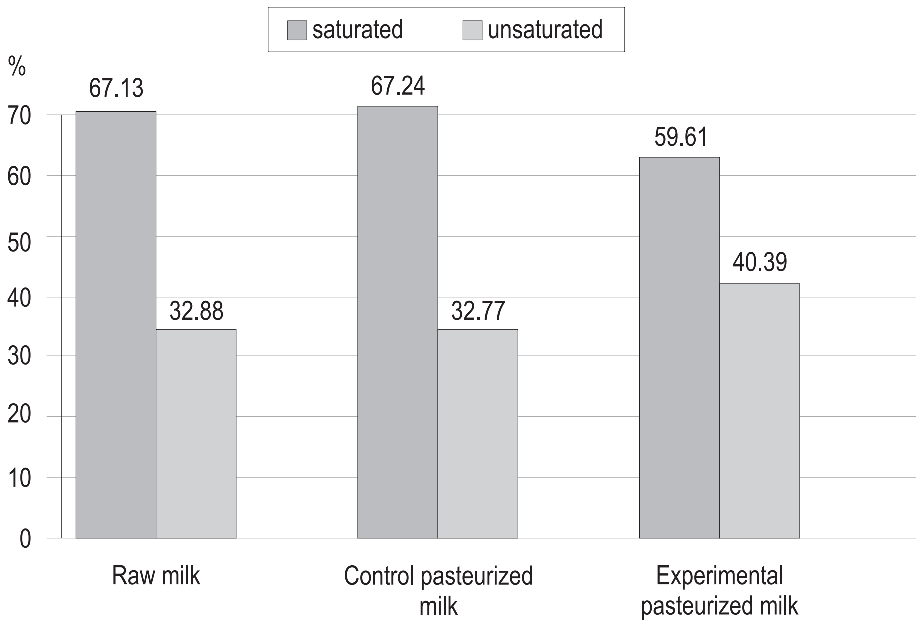

Figure 1. Dynamics of the saturated and unsaturated fatty acids in raw and pasteurized milk

11.2% lower (Figure 1). Total unsaturated fatty acid content (UFA) in pasteurized milk supplemented with walnut extract was by 22.84% higher than that of raw milk and fairly similar to that of natural pasteurized milk. The sums of SFA and UFA in pasteurized milk reported in a previous study were similar (Boycheva et al., 2007).

The sum of monounsaturated fatty acids (MUFA) decreased slightly after the pasteurization of milk in both experimental and control samples, whereas the total content of polyunsaturated fatty acids (PUFA) increased in natural milk samples and was almost 3 times higher in pasteurized milk with walnut extract. The PUFA/SFA ratio was almost equal in raw and natural pasteurized milk (0.06 and 0.07, respectively), whereas in pasteurized milk supplemented with walnut extract it was higher – 0.21 by reason of the higher UFA concentration. The ώ-6/ ώ-3 ratio was balanced from the point of view of healthy nutrition – from 1.65 to 2.17, in raw and pasteurized milk samples, respectively. The atherogenic index was the lowest in experimental pasteurized milk – 1.66 vs 2.36–2.41 for raw and pasteurized natural milk.

The fatty acid composition of the product is presented in Table 2. The content of the individual short-chain fatty acids changed slightly

Table 2. Fatty acids in yogurt

Fatty acids	24-hour yogurt			
	Natural		With walnut extract	
	Mean	SEM	Mean	SEM
C 4:0	2.69	0.380	2.68	0.012
C 6:0	2.57	0.279	2.30	0.226
C 8:0	1.22	0.031	1.18	0.078
C 10:0	2.79	0.384	2.85	0.042
C 12:0	2.94	0.205	2.66	0.057
C 13:0	0.18	0.070	-	-
C 14:0	10.75	0.951	9.91	0.559
C 14:1	0.69^{c}	0.094	1.16^{c}	0.018
C 15:0	1.02^{c}	0.045	0.38^{c}	0.003
C 16:0	34.94^{b}	1.998	29.08^{b}	3.401
C 18:0	8.23	0.857	10.00	0.276
C 18:1	26.62	3.435	25.60	0.969
C 18:2	3.55	1.697	7.56	0.231
C 18:3	1.83^{b}	0.585	4.66^{b}	0.488

Legend: a – $p<0.05$, b – $p<0.01$, c- $p<0.001$

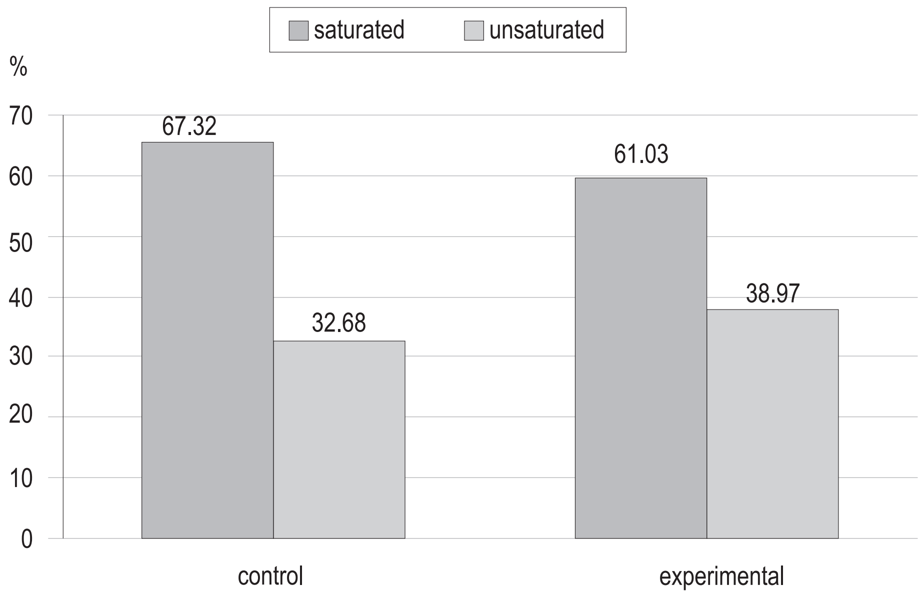

Figure 2. Dynamics of saturated and unsaturated fatty acids in control and experimental yogurt at 24 h

after the lactic acid fermentation while the concentrations of some medium- and long-chain fatty acids in yogurt supplemented with walnut extract exhibited various trends is the natural yogurt. The caproic acid in walnut yogurt was by 10.5% lower compared to control yogurt. The changes in caprylic and capric acids were insignificant. The contents of long-chain fatty acids – C18:2 and C18:3 were higher in milk with walnut extract compared to natural yogurt, unlike the concentration of monounsaturated C18:1. Lower oleic acid content in experimental samples was probably due to lactic acid bacteria and more specifically, to lactobacilli which need this acid and whose counts in this yogurt sample were higher, as also shown in previous studies (Naydenova et al., 2008). The amounts of essential linoleic and linolenic acid in yogurt supplemented with walnut extract were significantly higher (2.13 and 2.55 times respectively) than in control samples.

Yogurt with walnut extract exhibited the same tendency as pasteurized milk – increased total PUFA amount (2.3 times) and slightly lower MUFA content. The UFA amount was higher (by 19.25%) whereas SFA decreased by 9.34% in yogurt produced from walnut extract-supplemented milk (Figure 2). The PUFA/SFA ratio in walnut yogurt was 2.5 times higher than that of natural yogurt. The atherogenic index and the n-6/n-3 ratio were lower.

Table 3. Fatty acids in yogurt after storage for 10 days

Fatty acids	10-day yogurt			
	Natural		With walnut extract	
	Mean	SEM	Mean	SEM
C 4:0	2.29	0.135	2.40	0.226
C 6:0	2.48	0.138	2.47	0.035
C 8:0	1.39	0.106	1.37	0.071
C 10:0	3.23	0.333	2.99	0.318
C10:1	0.32[c]	0.008	3.08[c]	0.332
C 12:0	3.30	0.255	2.89	0.339
C 12:1	0.08[c]	0.001	0.66[c]	0.063
C 14:0	10.74	0.485	9.78	0.297
C 14:1	0.76	0.020	1.01	0.085
C 15:0	0.91	0.123	0.94	0.057
C 16:0	35.23[c]	0.361	27.41[c]	0.940
C 18:0	8.24	1.134	10.03	0.827
C 18:1	26.35	5.408	25.20	2.135
C 18:2	3.48[c]	0.418	7.32[c]	0.204
C 18:3	1.2[b]	0.002	2.48[b]	0.025

Legend: a – p<0.05; b – p<0.01; c- p<0.001

During storage in a refrigerator for 10 days (Table 3), no significant changes in individual fatty acids have occurred in either natural or walnut extract supplemented yogurt with the exception of C16:0 and C18:3 contents in experimental samples. The amount of C18:3 decreased by 46.8% during storage in experimental samples and by 34.4% in control samples as compared to respective levels in 24-hour yogurt. The ratio C18:2/C18:3 increased in both yogurts to almost equal levels – 2.9 in natural yogurt and 2.95 in walnut yogurt. The PUFA/SFA ratios decreased after 10 days of storage compared to 24-hour storage to 0.07 in control and 1.16 in experimental samples. The total PUFA content decreased during the storage. The 10-day storage of yogurt containing walnut extract resulted in slight reduction of saturated fatty acids and increased unsaturated fatty acids, mainly on account of monounsaturated fatty acids (Figure 3.).

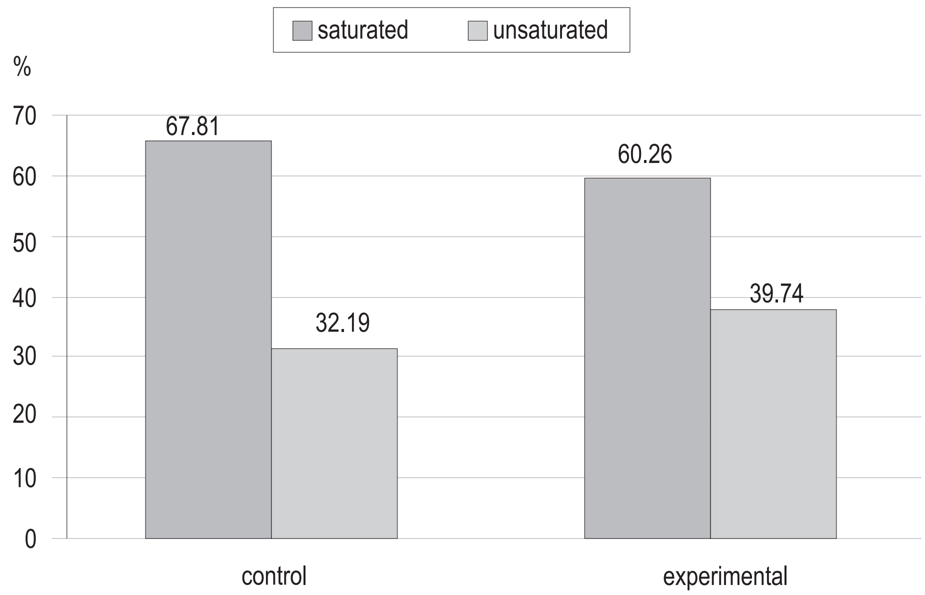

Figure 3. Dynamics of the saturated and unsaturated fatty acids in control and experimental yogurt at 10 days

Conclusion

The total saturated fatty acid content in pasteurized milk with walnut extract decreased by 11.2% compared to raw milk, while remaining unaltered in natural pasteurized milk. Pasteurized milk with walnuts exhibited higher polyunsaturated fatty acid content – 2.6 times vs natural and almost 3 times higher than raw milk. Yogurt containing walnut extract contained by 19.25% more unsaturated fatty acids and by 2.3 times more polyunsaturated fatty acids as compared to natural yogurt. During storage of produced yogurt for 10 days, the amount of C18:3 decreased by 46.8% in the yogurt containing walnut extract and by 34.4% in natural yogurt.

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The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods, Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

Names and affiliation of authors The names of the authors should be presented from the initials of first names followed by the family names. The complete address and name of the institution should be stated next. The affiliation of authors are designated by different signs. For the author who is going to be corresponding by the editorial board and readers, an E-mail address and telephone number should be presented as footnote on the first page. Corresponding author is indicated with *.

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Keywords: Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

The introduction must answer the following questions: What is known and what is new on the studied issue? What necessitated the research problem, described in the paper? What is your hypothesis and goal ?

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Results are presented in understandable tables and figures, accompanied by the statistical parameters needed for the evaluation. Data from tables and figures should not be repeated in the text.

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Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

Conclusion: The most important consequences for the science and practice resulting from the conducted research should be summarized in a few sentences. The conclusions shouldn't be numbered and no new paragraphs be used. Contributions are the core of conclusions.

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Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows, IX th International Conference on Production Diseases in Farm Animals, Sept.11 – 14, Berlin, Germany, p. 302 (Abstr.).

Thesis:

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