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Original Contribution

AMINO ACID AND FATTY ACID CONTENT OF YOGURT SUPPLEMENTED WITH WALNUT AND HAZELNUT PIECES

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ABSTRACT
Amino and fatty acid composition of cow's milk yogurt with pieces of walnut (10%) and hazelnut (10%) added were investigated. The total amount of amino acids in the yogurt with walnut pieces was by 7.8% higher in comparison with raw milk and by 3.9% higher in comparison with natural yogurt. The amount of unsaturated fatty acids in the yogurt with walnuts and hazelnuts was by 39% higher in comparison with natural yogurt. The amount of polyunsaturated fatty acids in the yogurt with walnut pieces was almost three times higher in comparison with control yogurt.

Key words: amino acids, fatty acids, cow's milk, walnuts, hazelnuts, yogurt

INTRODUCTION

Yogurt is the most widely spread and most famous of all known dairy products in Bulgaria. It takes an important role in the diet of the Bulgarian people with its nutritional qualities. All of its components are combined in an especially beneficial ratio, which can hardly be found in other food products.

Yogurt is traditionally made from milk with no added ingredients. To improve its texture and sensory properties yogurt is supplemented with other materials such as nonfat dry milk and other dairy or plant ingredients. The traditional Bulgarian yogurt can be added by herbal extracts, seeds and nuts with proven beneficial effects on human health, which would enhance its qualities (1, 2, 3, 4, 5).

Nuts contain substantial amounts of vitamin E, which is, along with vitamin C, a strong antioxidant, i.e. it is involved in the neutralization of free radicals and thus protects tissues from being harmed, which frequently cause cancer and cardiovascular diseases (6).

Another valuable component of nuts is folic acid, which is essential for cellular division and for the formation of red blood cells. There is an increased need for folic acid during pregnancy, as it is vital for the proper growth of the foetus (7).

Walnuts, combined with honey, are recommended during recovery from severe illnesses, great physical or mental exhaustion, in cases of considerable weight loss, gastro-intestinal disorders (8), nervous and cardiovascular diseases, etc. (9, 10). Walnuts have a high nutritional value and high content of polyunsaturated fatty acids (11, 12).

Hazelnuts have a significant role in human nutrition and health (13). They contain substances, which seriously reduce the risk of cardiovascular diseases, some forms of cancer, as well as a number of physiological conditions and syndromes (14).

In people who consume hazelnuts five or more times per week, the risk of cardiovascular illness is reduced by 50%, compared to those who do not consume hazelnuts (15). Similar results have been reported in other studies (12,

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16, 27). This positive effect of hazelnuts is attributed to their fatty acid content (18).

In the last years attempts have been made to improve the quality, especially the health benefits of yogurt, but this needs to be investigated. Previous studies in our laboratory on yogurt, supplemented with nuts showed positive effect on quality characteristics of yogurt and the microbial activity (19).

The present study was designed to optimize addition of nut ingredients to produce yogurt of the better quality as functional food. The aim of this study was to estimate the amino acid and fatty acid contents of yogurt fortified with addition of walnut and hazelnut pieces.

MATERIAL AND METHODS

1. Material

1.1. Fresh milk and starter cultures

Fresh raw cow milk, obtained from local farm in Stara Zagora, and starter culture, containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* (Lactina 17, produced by Bankya, Bulgaria) ready for direct vat inoculation were used for yogurt preparation.

The yogurt was prepared in laboratory conditions.

1.2. Yogurt preparation

The milk was pasteurized (95 °C/30 min), cooled to 45 °C and inoculated with 1.5% starter culture consisting of *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* (Lactina 17, produced by Bankya, Bulgaria). The raw milk (6 l) was divided into three lots – one control (natural) and two experimental. Prior to adding the starter, 10 g kg^{-1} pieces (1-2 mm) from either walnuts or hazelnuts was added to experimental milk samples. The samples were then cultivated at 42 °C until coagulation, then cooled and stored in a refrigerator at 4-6 °C.

The experiment was repeated four times.

2. Methods

The samples were homogenized before determination of amino and fatty acids.

2.1. Determination of amino acid

Amino acids were analyzed by technique of Moore and Stein using ion-exchange chromatography (20). The amino acid composition of raw milk and yogurt, both control and experimental, was determined by automated aminoanalyzer "T 339M:"

2.2. Determination of fatty acid composition

The extraction of milk fat was done using the method of Rose-Gottlieb using 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 (CH_3ONa) was used (21). The fatty acid composition of raw milk and yogurt, both control and experimental, was determined by gas chromatography "Pay-Unicam 304" with flame ionization detector and column EC^{TM}- WAX, 30 m, ID 0.25 mm, Film:0,25 μm.

The extraction of nuts fat was determined using the modified fatty acid methyl ester method as described by Baydar et al. (1999) (22).

3. Statistical analysis

For statistical analysis, ANOVA was performed on the three batches and the corresponding replicates, using Statistica 8.0 software (StatSoft, Inc.,). Fisher test was used for means comparison.

RESULTS AND DISCUSSION

1. Amino acid content of yogurt

During fermentation of milk, the amino acid profile changed as a result of proteolytic activity of lactic acid bacteria **(Table 1)**. The same results are observed from other authors (23, 24, 25). It was interesting to note that an increase in the total amount of amino acids could be observed in the control sample when compared to the raw cow's milk.

In both test samples, the total content of amino acids was higher compared to the control sample. The increase was higher in the sample containing walnuts – by about 4%, followed by the one containing hazelnuts – by 2.6%.

The three branched-chain amino acid leucine, valine and isoleucine support numerous metabolic processes ranging from the fundamental role as substrates for protein synthesis to metabolic roles as energy substrates (26), precursors for synthesis of alanine and glutamine and as modulators of muscle protein synthesis (27).

Table1. *Amino acid content of raw milk and yogurt, g/100 g sample, n=4*

Amino acids			yogurt					
	raw milk		control		with walnuts		with hazelnuts	
	x	Sx	x	Sx	x	Sx	x	Sx
aspartic acid	0.322	0.005	0.331	0.013	0.344	0.004	0.313	0.035
threonine	0.143	0.007	0.143	0.005	0.144	0.003	0.137	0.007
serine	0.141	0.002	0.139	0.004	0.145	0.003	0.135	0.013
glutamic acid	0.855	0.032	0.895	0.020	0.945	0.020	0.934	0.052
proline	0.340	0.002	0.354	0.007	0.354	0.009	0.352	0.014
cysteine	0.034	0.004	0.038	0.009	0.048	0.009	0.066	0.014
glycine	0.062	0.002	0.064a	0.003	0.076a	0.004	0.071	0.005
alanine	0.114b	0.008	0.129a	0.003	0.140ab	0.003	0.144	0.008
valine	0.238	0.005	0.258	0.006	0.255	0.006	0.251	0.005
methionine	0.020	0.002	0.029	0.007	0.028	0.003	0.033	0.006
isoleucine	0.189	0.008	0.196	0.009	0.198	0.004	0.190	0.008
leucine	0.336	0.001	0.356	0.009	0.364	0.005	0.356	0.018
tyrosine	0.147	0.003	0.144	0.007	0.140	0.007	0.140	0.005
phenylalanine	0.167	0.001	0.180	0.007	0.193	0.012	0.196	0.015
histidine	0.108	0.010	0.110	0.005	0.118	0.005	0.122	0.010
lysine	0.276	0.008	0.286	0.011	0.273	0.006	0.271	0.008
arginine	0.141	0.015	0.112a	0.009	0.148	0.016	0.153a	0.015
Total	3.63	0.180	3.765	0.095	3.913	0.064	3.863	0.180

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

The content of lysine was slightly higher in the control sample, compared to the raw milk and experimental samples. The percentage of lysine in the total amount of amino acids in both raw milk and natural yogurt was the same and fairly high – 7.6%. The content of lysine in the experimental samples, out of all amino acids, varied from 6.9% in the yogurt containing walnuts up to 7.01% in that containing hazelnuts.

Valine, in terms of percentage of the total amount of amino acids, was also in a relatively high amount in the control yogurt sample – 6.85%, whereas in the experimental samples it was about 6.5%, with the variations being statistically insignificant (p>0.05).

The amount of isoleucine varied from 4.92% of the total amount of amino acids in the yogurt containing hazelnut pieces up to 5.21% in yogurt without additives.

Sulfur amino acids and their metabolites are of major importance in health and disease. Methionine is classified as nutritionally essential. Cysteine is classified as semi essential due to the variable capacity of body for its production from methionine (28).

Methionine, which has an effect on the functioning of the liver and the thyroid gland, and is beneficial in cases of osteoporosis, varied from 0.55% in raw cow's milk up to 0.86% of the total amino acid content in yogurt with hazelnuts.

Cysteine content was higher in yogurt compared to raw milk, with the increase being the largest in the sample containing hazelnuts.

Of the non-essential amino acids, the highest content was that of glutamic acid, which is responsible for protection from cardiovascular diseases – with variations from 23.56% of the total amount of amino acids in raw milk up to 24.18% in the sample with hazelnuts, followed by proline. The differences between these two amino acids were insignificant ($p>0.05$).

Significant variations were observed in the amounts of the essential amino acids glycine and alanine between control yogurt and yogurt with walnuts ($p<0.05$), in alanine content between raw milk and yogurt with walnuts ($p<0.01$), as well as in arginine content between control yogurt and yogurt containing hazelnuts ($p<0.05$).

The amounts of non-essential amino acids had increased in all yogurt samples, compared to raw milk, with the highest increase being in the yogurt with walnuts, followed by the one with hazelnuts **(Figure 1)**.

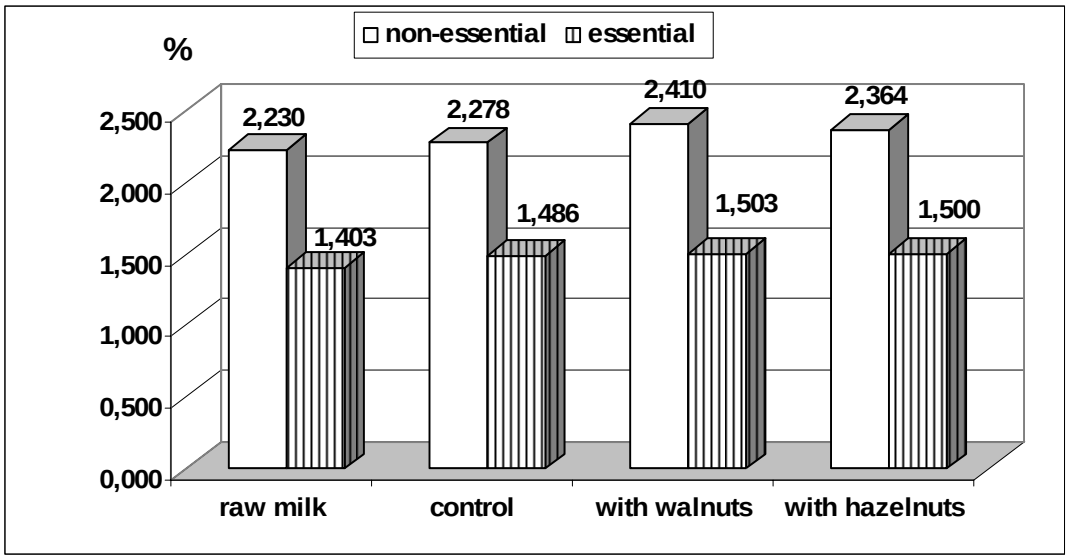

Figure 1. Content of non-essential and essential amino acids in yogurt

The amount of essential amino acids was also increased during the process of coagulation of milk and attained, from 1.4 mg/g in raw milk, up to 1.5 mg/g in samples with added walnuts and hazelnuts.

2. Fatty acid profile

Data on the fatty acid content of raw milk and the used walnuts and hazelnuts are presented in Table 2. Compared to the data by Mihaylova (2007) (29) for cow's milk from the Stara Zagora region, the present study established lower values of C4:0, C6:0, C8:0, C10:0, C16:0, C18:0 and C18:2 fatty acids. The total amount of saturated (SFA) and unsaturated fatty acids (USFA) in raw cow's milk was 68.15% and 31.85% respectively, values close to what was found out by other authors (29, 30).

Data show that the majority of the fatty acids in hazelnuts are unsaturated (93.14%), while saturated fatty acids comprised only 6.86% **(Table 2).** Koksal et al. (2006) (31) reported that unsaturated fatty acid contents of Turkish hazelnuts had been 92.8%, lower than our results.

The total unsaturated fatty acid content of the walnuts was 85.61% that is lower than the data from Ozkan and Koyuncu (2005) (32).

Table 2. *Fatty acid content of initial products, % , n=4*

Fatty acids	Raw milk	Walnuts	Hazelnuts
C 4:0	3.46	-	-
C 6:0	1.527	-	-
C 8:0	1.019	0.63	0.092
C 10:0	2.549	0.155	-
C 10:1	0.249	-	-
C 12:0	3.176	0.103	-
C 12:1	0.072	-	-
C 12:2	0.061	-	-
C 13:0	0.082	-	-
C 14 i	0.155	-	-
C 14:0	11.538	0.406	0.196
C 14:1	0.972	-	-
C 14:2	0.231	-	-
C15ai	0.535	-	-
C 15:0	0.944	0.064	-
C 16 i	0.377	-	-
C 16:0	30.0	7.543	6.215
C 16:1	0.184	0.059	0.15
C16:2	1.631	0.112	-
C 17:0	0.548	2.503	-
C 17:1	0.269	-	-
C 18:0	9.253	2.987	-
C 18:1	22.931	16.613	85.172
C 18:2	4.155	57.18	7.819
C 18:3	1.093	11.645	-
C 20:0	0.267	-	0.356
Saturated	68.152	14.391	6.859
Unsaturated	31.848	85.609	93.141
Mono-unsaturated	24.677	16.672	85.322
Poly-unsaturated	7.171	68.937	7.819
C18:2/C18:3	1.99	4.91	-
PUSFA/SFA	0.10	4.79	1.14

Unsaturated fatty acids (USFA) play an important role in human nutrition and health. The fatty acid profile of nuts, high in unsaturated fatty acids such as oleic acid and low in saturated fatty acids, contributes to cholesterol lowering and hence coronary heart disease risk reduction by increasing the high density lipoprotein (HDL) in blood (33, 34).

The ratios of PUFA/SFA and USFA/SFA of hazelnuts and walnuts were found to be 1.14 v/s 4.79 and 13.58 v/s 5.95 respectively. These results were close to the ones found by Koksal et al. (2006) for hazelnuts and lower than those of Ozkan and Koyuncu (2005) for walnuts.

Data on the fatty acid content of the milk fat in the produced yogurt with walnut or hazelnut pieces added are presented in **Table 3.**

The fatty acid content of the control yogurt sample was similar to that of raw milk. In the experimental samples, containing walnut and hazelnut pieces, the amounts of nearly all saturated fatty acids were lower than those in the control sample (natural yogurt). The differences between the control sample and the yogurt with added hazelnuts were significant with regard to C6:0 ($p<0.05$).

The biggest reduction was found in C14:0 – by about 2% for all experimental samples and in C16:0 – by 4.36% and 5.27% in samples with walnuts and hazelnuts respectively, which represented, respectively, 14.5% and 17.51% of the control sample value. The differences were significant for C14:0 between the control sample and the yogurt with added hazelnuts ($p<0.05$).

Table 3. *Fatty acid content of yogurt, %, n=4*

Fatty acids	Yogurt					
	control		with walnuts		with hazelnuts	
C4:0	2.037c	0.002	1.189cd	0.035	2.051d	0.046
C 6:0	2.091a	0.118	1.647	0.467	1.508a	0.162
C 8:0	1.364a	0.094	1.052	0.239	1.027a	0.084
C 10:0	3.069a	0.178	2.406	0.355	2.429a	0.158
C 10:1	0.302	0.019	0.238	0.044	0.247	0.019
C 12:0	3.515a	0.258	2.791	0.295	2.806a	0.150
C 12:1	0.075	0.014	0.101	0.061	0.112	0.051
C 12:2	0.163	0.091	0.076	0.010	0.057	0.00
C 13:0	0.090	0.008	0.092b	0.005	0.071b	0.002
C 14 i	0.177	0.042	0.127	0.009	0.131	0.009
C 14:0	11.849a	0.855	9.568	0.699	9.495a	0.537
C 14:1	1.133	0.276	0.972	0.097	0.986	0.062
C 15 i	0.224	0.014	0.191	0.028	0.320	0.069
C15ai	0.543a	0.040	0.430	0.041	0.445a	0.014
C 15:0	1.101	0.289	0.764	0.062	0.796	0.014
C 16 i	0.389	0.049	0.283	0.015	0.296	0.027
C 16:0	30.097a	1.538	25.734a	0.745	24.827a	1.640
C 16:1	1.826c	0.180	0.405cd	0.041	0.152d	0.016
C 17 i	0.509b	0.150	1.236b	0.057	1.404b	0.161
C17 ai	2.353a	0.717	2.687b	0.553	0.241ab	0.171
C 17:0	0.542b	0.217	0.333e	0.073	2.551be	0.511
C 17:1	0.376c	0.021	0.329	0.126	0.540c	0.021
C 18 i	0.200c	0.005	0.211d	0.010	0.129cd	0.011
C 18:0	9.535	0.712	8.400	0.832	8.449	0.371
C 18:1	21.401b	1.971	24.791	6.744	31.999b	1.526
C 18:2	3.512	1.035	11.173	4.649	5.067	1.765
C 18:3	2.356c	0.112	2.507d	0.153	1.514cd	0.116
C 20:0	0.182	0.095	0.267	0.075	0.355	0.051
C18:2/C18:3	1.49	-	4.46	-	3.35	-
PUSFA/SFA	0.07	-	0.23	-	0.11	-

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

The contents of most important fatty acids for human health – C18:1, C18:2 and C18:3 – were significantly higher in the yogurt from experimental samples than in natural yogurt. A significant increase was observed in C18:1 in the yogurt sample containing hazelnut pieces – by 49.5% compared to the control sample ($p<0.01$), vs. 15.84% in the sample containing walnuts. An increase in oleic acid was found in yogurt supplemented with linseed and sesame (1).

The increase in C18:2 was the greatest in yogurt with walnuts – by 7.66%, which is 3 times more than the amount in the control sample. A significant difference was found in the content of the polyunsaturated fatty acid C18:3 between control yogurt and yogurt with hazelnuts ($p<0.001$) and between the two experimental lots with added nuts ($p<0.001$).
The C18:2/C18:3 ratio was reduced in natural yogurt compared to raw milk (1.99 to 1.49, respectively) yet it increased up to 3.35 in yogurt with added hazelnuts and to 4.46 in yogurt with added walnuts.

The contents of short-chain fatty acids (from C4 to C10) in the control yogurt sample were insignificantly higher as compared to raw milk but reduced by about 2% in the experimental samples **(Figure 2)**. The amount of medium-chain fatty acids (from C11 to C17) decreased during the process of milk coagulation, compared to both raw milk and the control yogurt. The highest reduction was observed in the sample containing hazelnut pieces – 17.7%. The total amount of long-chain fatty acids (above C18) in the experimental yogurt samples was higher compared to the amounts in raw milk and the control sample, with the greatest increase occurring in the sample containing hazelnuts – by 26% and 27.7%, respectively.

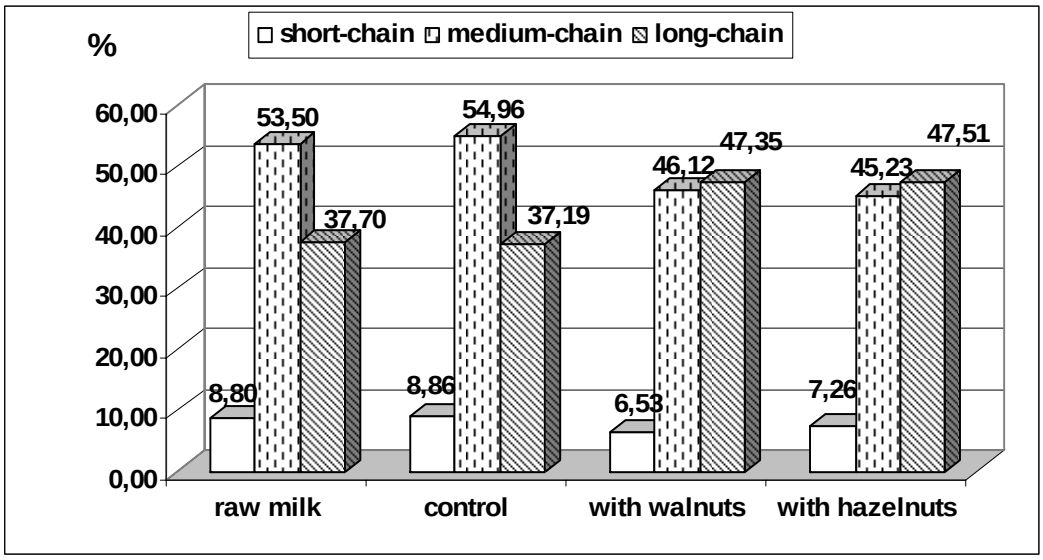

Figure 2. Content of short-, medium- and long-chain fatty acids in raw milk and yogurt.

During fermentation, the total amount of saturated fatty acids decreased significantly in experimental samples in comparison to raw milk and control yogurt –by 13% and 15.15% for the samples containing walnuts and hazelnuts, respectively (Figure 3). The tendency for unsaturated fatty acids was inverse. Their content was the lowest in control yogurt (without additions) and higher in the experimental samples. In the sample containing walnut pieces, the amount of unsaturated fatty acids was increased the most – 35% compared to natural yogurt, nearly 28% compared to raw milk, and 27.69% compared to the hazelnut-containing sample.

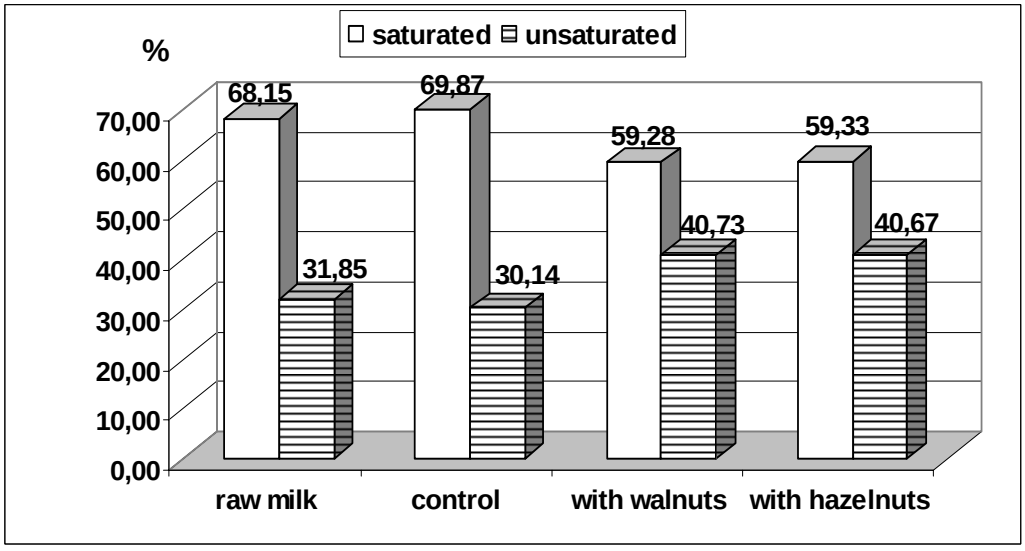

Figure 3. Content of the saturated and unsaturated fatty acids in raw milk and yogurt containing walnuts or hazelnuts.

The content of monounsaturated fatty acids was higher in the test samples, compared to both raw milk and the control sample (Figure 4). The greatest increase was observed for the yogurt with added hazelnuts – 43.75% compared to raw milk and 35.56% compared to control yogurt. As a result of milk's coagulation, the amounts of polyunsaturated fatty acids (PUFA) in yogurt containing walnut pieces has increased 1.7 times compared to raw milk and 2.7 times compared to control yogurt.

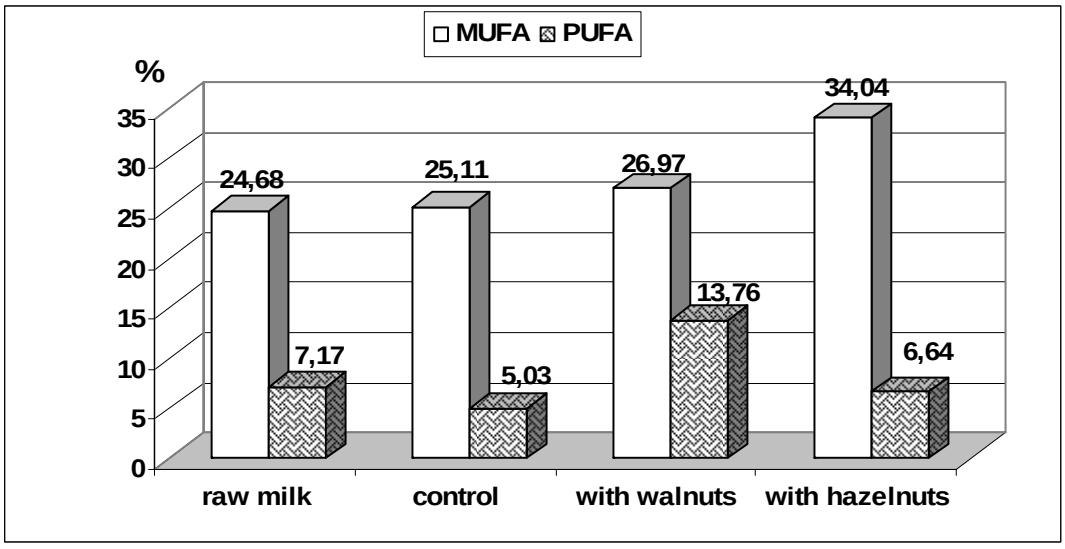

Figure 4. Content of the mono- and polyunsaturated fatty acids in raw milk and yogurt containing walnuts or hazelnuts.

The PUFA/SFA ratio was the lowest in natural yogurt – 0.07, followed by yogurt with hazelnuts – 0.11, and yogurt with walnuts – 0.23, which is significantly lower than what was established for the nuts.

CONCLUSIONS

The addition of walnut pieces to yogurt increased its total amino acids content by 7.8% compared to raw milk and by 3.9% compared to natural yogurt.

The amount of unsaturated fatty acids in yogurt supplemented with walnut or hazelnut pieces was by 35% higher than that in control yogurt. The amounts of saturated fatty acids were lower by 15%.

The amount of polyunsaturated fatty acids in yogurt with added walnut pieces has increased nearly 3 times, compared to control yogurt.

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