



DEVELOPING NEW APPROACHES TO HYPERLIPIDEMIA CORRECTION TAKING INTO ACCOUNT CHANGES IN FATTY ACIDS STRUCTURE OF BLOOD SERUM

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It is still a pressing issue in contemporary medicine to examine pathogenesis mechanisms and update procedures aimed at treating atherosclerosis. Developments by domestic and foreign researchers revealed that complex molecular and cellular studies on a mechanism of impacts exerted by vegetative-based medications, produced both domestically and abroad and used to treat atherosclerosis, were of primary importance in practical medicine in terms of educating population health risks. It is assumed that disorders in formation and transfer of non-esterified fatty acids (NEFA) in blood plasma are a major reason for hypertriglyceridemia occurrence.

The article contains research data on lipid metabolism parameters taken in dynamics of experimental hypercholesterolemia development. Performed research allowed revealing hypolipidemic effects produced by a biologically active additive called Biomays. We developed theoretical grounds for recommendations that should be given to patients suffering from hyperlipidemia and not getting proper therapeutic effects from treatment with statins. We recommend a complex approach which includes a BAA (biologically active additive) Biomays made of dried wheat sprouts in order to reduce risks caused by complications related to treatment with statins.

Our research goal was to develop new approaches to correcting hyperlipidemia basing on changes in fatty acids structure of blood serum.

The experiments were performed on 30 male rabbits belonging to chinchilla breed with initial body mass equal to 2,500–3,00 grams; animals were divided into 5 groups, 6 animals in each, depending on a research goal and treatment procedures. We started a 30-day treatment of experimental animals with ultrox and Biomays in doses equal to 0.6 mg/kg and 142 mg/kg accordingly after they had been given cholesterol for 2 months. We determined fatty acids structure of blood serum with a triple quadrupole chromatato-mass-spectrometer with gas chromatographer (GC-MS/MS) TRACE 1310 TSQ 8000 and automated autosampler CTC TriPlus RSH produced by Thermo Fisher Scientific (the USA). Combined application of ultrox and Biomays led to more significant hypolipidemic effects. Use of statins and wheat sprouts had a distinct positive effect on contents of saturated and poly-unsaturated fatty acids in blood such as linoleic acid and linolenic acid.

Key words: fatty acids, water-soluble vitamins, policosanol, Biomays biologically active additive, mass-spectrometry, gas chromatography, hyperlipidemia.

Cardiovascular diseases, especially ischemic heart disease (IHD), remain the most widely spread ones all over the world; more than 50 % mortality cases occur due to these pathology. A considerable increase in cardiovascular morbidity and mortality among young people and employable population is especially alarming [1–3]. These pathologies are caused by numerous risk factors; it makes them even graver and undoubtedly requires

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correction. Therefore, activities aimed at preventing cardiovascular diseases have national significance [4–6]. Today, it is still vital to develop highly efficient prevention techniques, pre-clinic diagnostics, and therapy aimed at treating atherosclerosis; this scientific and medical-social task is hard to solve [7, 8]. At present it is obvious that hypercholesterolemia has become a global non-communicable epidemic. As per data provided by the Uzbekistan Public Healthcare Ministry, in 2014 circulatory system diseases amounted to approximately 5,800 cases per 100 thousand people and tended to grow. Ischemic heart disease (IHD) was the most frequent cause of death accounting for 22,300 deaths per year [9–11].

Hypercholesterolemia (HCS) plays an important role in atherosclerosis and IHD pathogenesis [12–14]. Prescribing medications that reduce cholesterol is a priority in IHD and HCS treatment. Inhibitors of 3-hydroxy-3-methyl-glutaril-CoA reductase are the most efficient in reducing low density lipoprotein cholesterol (CS-LDLP) and mortality caused by atherosclerosis and IHD [15–17]. Effective therapeutic daily dose of many statins causes side effects such as an increase in liver enzymes concentrations, aspartate and alanine transaminase (ALT and AST), myalgia, and myopathy with an increase in creatinine phosphokinase (CPK) concentration.

Every year there is a growth in number of research works focusing on searching for alternative hypolipidemic remedies. Leading role among them belongs to natural preparations. Over the last years, researchers have shown certain interest in wheat germ and wheat germ flour. Some data obtained by foreign researchers on effects produced by wheat germ oil and wheat germ flour on cholesterol contents in blood and liver have become a precondition for testing them in treating various cardiovascular diseases. As we can see from the data given in Figure 1, cholesterol contents in rats' blood and liver apparently goes down significantly due to use of wheat germ oil against use of cotton or soya oil [14, 15, 18–21].

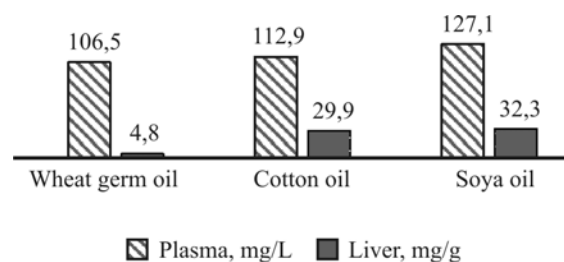


Figure 1. Effects produced by wheat germ oil on cholesterol contents in rats' blood and liver¹

Other researchers revealed that a diet with 7% wheat germ resulted in 5–10% decrease in cholesterol contents in blood against the reference group. Besides, wheat germ flour consumption didn't cause any side effects or allergic reactions. Researchers at the Therapy and Neurology Department of the Kharkov Medical Academy for Postgraduate Studies examined effects produced by wheat germ oil on patients with ischemic heart disease (angina pectoris, II and III functional category). Analysis of changes in blood biochemical structure revealed that when wheat germ oil was applied for treating patients with ischemic heart disease, it resulted in a more valid hypolipidemic effect (a decrease in lipids and β -lipoproteins contents). Positive results were also yielded as regards clinical course of the disease. Thus, when clinical picture was assessed in those patients who were additionally given wheat germ oil, it was revealed that a number of angina pectoris attacks went down by 4 times (by 2.5 times only in the reference group), and, accordingly, nitroglycerine doses also decreased. And patients from the test group endured physical loads better than their counterparts from the reference one. It was also noted that use of wheat germ oil in treatment was accompanied with a decrease in elevated coagulability that was very important from pathogenetic point of view [18–20].

Similar research performed by N.S. Radionova and O.A. Sokolova also showed that there was positive dynamics in blood lipid spectrum when a patient took wheat germ oil; it was confirmed by lower contents of chole-

¹ L.A. Shpagina. Use of wheat germ oil and Vitasar in treating internal diseases: methodical guidelines for doctors. Novosibirsk, Novosibirsk printing house Publ., 2008, 80 p. (in Russian).

terol and cholesterol in low density lipoproteins, and, more importantly, by higher contents of anti-atherogenic high density cholesterol [21, 22].

In Novosibirsk Medical Academy L.A. Shpagina supervised assessment of wheat germ oil efficiency in treating workers with cardiovascular diseases [23, 24]. The results revealed that patients who were prescribed a combined hypolipidemic diet and wheat germ oil had authentic improvement in their health, weaker clinical symptoms, and positive dynamics in blood lipids spectrum. Hypolipidemic effects were persistent and remained even 3 months after the therapy was over. Consequently, this analysis of research works that focused on examining effects produced by wheat germ oil and flour on levels and spectra of blood lipids in case of hyperlipidemia shows that these products exert apparent and persistent hypolipidemic impacts and it is a precondition for conducting further targeted studies in the sphere.

Our research goal was to develop new approaches to correcting hyperlipidemia basing on changes in fatty acid structure of blood serum.

Data and methods. Given all the above stated, we conducted an experimental study on effects produced by Biomays food additive on blood lipids spectrum and fatty acids structure in animals with modeled experimental hypercholesterolemia.

Our experiments were performed on 30 male «Chinchilla» rabbits with initial body mass being 2,500–3,000 grams; the animals were divided into 5 groups, 6 animals in each, depending on research goals and a chosen therapy. Experimental animals started to receive their treatment after 2-month cholesterol intake [25]. To comparatively assess effects produced on blood serum by Biomays vegetable preparation and ultrox statin, we determined contents of triglycerides (TG), total cholesterol (TCS), high density lipoproteins cholesterol (CS HDLP), low density lipoproteins (LDLP), very low density lipoproteins (VLDLP) with an automated biochemical analyzer (RX Daytona/Randox, Great Britain) and calculated atherogenicity coefficient (AC).

A procedure for determining fatty acids structure in blood plasma. Fatty acids structure in blood serum was determined at the scientific laboratory of the Republican Scientific and Practical Center for Sport Medicine at the Uzbekistan national Olympic Committee; to do that, we applied TRACE 1310 TSQ 8000, a triple quadrupole chromat-mass-spectrometer with a gas chromatographer (GC-MS/MS) and autosampler CTC TriPlus RSH produced by Thermo Fisher Scientific (the USA).

Blood serum was first separated from red corpuscles via whole blood centrifuging at 2,000 turns per minute for 6 minutes. Then 0.5 ml of supernatant (blood serum) were put into 1.5-ml calibrated eppendorf vials and added with 0.4 ml acetone in order to sediment protein fraction in them. The mixture was thoroughly mixed in a vortex for 0.5–1 minute and then centrifuged at 15,000 turns per minute for 10 minutes. After that supernatants with their volume being 0.3–0.4 ml were put into new eppendorf vials and added with 0.25 ml hexane for fatty acids extraction. The mixture was again thoroughly mixed in a vortex and left for several minutes in order to achieve complete separation between water and hexane layers. Hexane layer was put into new eppendorf vials and extraction was repeated two more times in order to completely extract chemicals with lipid essence. Obtained hexane layers were evaporated in a microconcentrator until dry and then obtained sediments were dissolved in 0.5 ml hexane and put into glass vials for further GC-MS/MS analysis.

Chromatography conditions were as follows: there was a capillary column (0.2 μm · 0.25 mm · 30 m) impregnated with 5% - biphenyl-dimethylsiloxane; helium was chosen as a carrier gas with constant flow equal to 1 ml/min. Initial temperature of the column thermostat was 40 °C with 1-minute delay. Then thermostat was heated to 280 °C at a rate 20 °C/min with a 3-minute delay at 280 °C and a consequent temperature fall to its initial state during 6 minutes at a rate 40 °C per minute. Injector and mass spectrometer detector temperatures were set at 250 °C. Extract was input in a volume equal to 1 μl in a split flow mode.

Ionization was performed with an electron impact at 20 eV. A chromatographic profile was registered 3 minutes after the start in order to remove a signal belonging to a solvent. Chromatography was controlled with XCalibur program within values limits range being m/z 50–1,500. Components were identified with etalon lass-spectra taken from «NIST» library for etalon mass-spectra of natural substances.

All the obtained data were statistically processed with applied computer software for statistical analysis.

Results and discussion. Table 1 contains all the obtained data. A cholesterol diet the experiment animals were put on for 60 days resulted in apparent hypercholesterolemia

(295.0 ± 1.45 mg/dL). Treatment with ultrox and Biomays food additive led to a decrease in cholesterol contents, by 2.08 and 1.49 times accordingly ($p < 0.05$). When ultrox and Biomays were taken together, it resulted in a more apparent decrease in TCS contents.

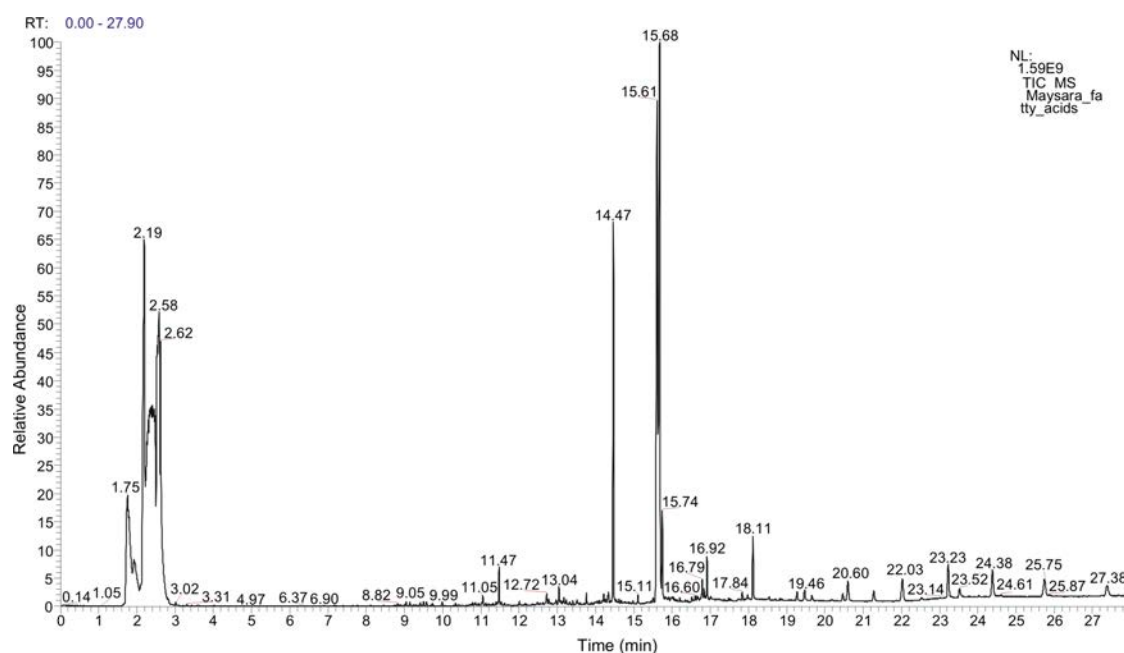
It should be noted that a decrease in TCS contents in animals that were given ultrox and Biomays were quite comparable. There was an authentic difference in TCS levels between animals from all the experimental groups and from the reference one 30 days after combined intake of two preparations, by 7.75–26.8% ($p < 0.05$); it indicates that combined intake of ultrox and Biomays is efficient in terms of cholesterol contents reduction.

Table 1

 Lipid metabolism parameters in rabbits with experimental HCS, $n = 6$

Parameter	Reference group	Animals with experimental HCS	After treatment		
			Ultrox	Byomais	Both preparations combined
TCS, mg/dL	71.8 ± 0.78	295 ± 1.45	142 ± 0.66	179 ± 1.77	131 ± 1.2
TG, mg/dL	14.6 ± 0.6	28.1 ± 0.36	$29.4 \pm 0.66^*$	37.3 ± 0.54	25.6 ± 0.12
CS HDLP, mg/dL	26.7 ± 0.98	17.8 ± 0.8	29.6 ± 0.7	25.3 ± 1.08	34.8 ± 0.75
CS VLDLP mg/dL	2.92 ± 0.07	6.98 ± 0.15	5.9 ± 0.22	$7.46 \pm 0.21^*$	5.12 ± 0.68
CS LDLP mg/dL	40.78 ± 0.86	270.3 ± 2.8	106.7 ± 0.68	146 ± 1.88	91.08 ± 0.14
AC	1.37 ± 0.02	15.6 ± 0.43	3.83 ± 0.14	6.16 ± 0.12	2.76 ± 0.44

Note: * means $p < 0.05$ against the animals that were not given any treatment.



If we look at other blood lipids parameters in animals with HCS, in particular, TG contents, we can see that it was 1.92 times higher than in the reference group ($p < 0.05$). There were no authentic changes in TG contents after treatment with ultrox² [26–31].

Therefore, it seems vital to create a poly-component biologically active substance with hypolipidemic properties; it can potentially be useful not only for treating mild lipid metabolism disorders but also for combined therapy when it is taken with statins in order to decrease their doses and, consequently, reduce their side effects.

To examine impacts exerted by Biomays on fatty acids contents in blood serum, we first of all examined fatty acids structure of Biomays food additive itself. The results are given in Figure 2.

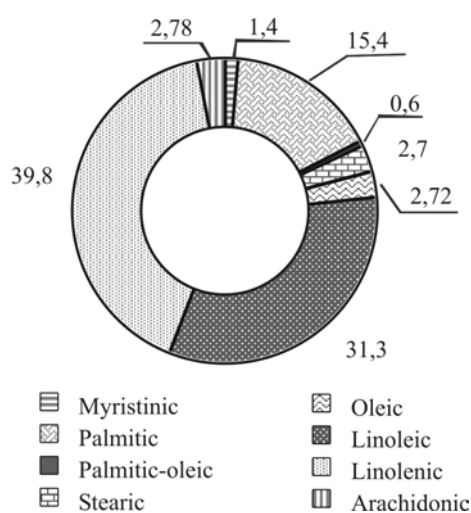


Figure 2. Biomays fatty acid structure in % per 1 mg

Saturated fatty acids in Biomays are mostly palmitic and stearic acids. At the same time, the greatest specific weight in fatty acids structure belongs to linoleic (31.3 %) and linolenic acids (39.8 %). Consequently, a substantial amount of fatty acids, including unsaturated ones, are linoleic and linolenic acids that have great physiological significance for a body [32–36].

Linoleic acid is well known to be the basic one as regards physiological effects; the acid is transformed into arachidonic acid in a body and the latter is an important component in lipid metabolism. The process occurs with vitamins A and E participating in it [37–39].

Besides, linoleic acid belongs to Omega-6 family, while linolenic acid – to Omega-3, although both families, Omega-6 and Omega-3 consist of 11 poly-unsaturated fatty acids (Table 2 and 3).

Fatty acids within Biomays structure (analysis):

- 9.73 min; methyl ether of decanoic acid;
- 11.05 min; eicosan;
- 13.04 min; tetra-decanoic acid;
- 13.76 min; penta-decanoic acid;
- 14.34 min; 9-hexanoic acid;
- 14.47 min; palmitic acid;
- 15.61 min; linoleic acid;
- 15.68 min; linolenic acid (α -form) (Omega-3);
- 15.74 min; stearic acid;
- 16.52 min; 9-cis, 11-trans, 13-trans-octadecatrienoic acid;
- 16.79 min; 10,13-eicosadienic acid;
- 16.84 min; 6,9,12,15-docosatetraenoic acid;
- 16.92 min; eicosanoic acid.

Our study on fatty acid structure of Biomays food additive revealed that some other poly-unsaturated fatty acids from Omega-3 and Omega-6 families could be found there, for example docosatetraenoic (arachidonic) acid (Omega-6), eicosapentadienic acid (Omega-3), and octadecatrienoic acid (Omega-6). But still, it should be noted that the greatest specific weights among them belong to linoleic and linolenic acids. These polyunsaturated fatty acids as components in Biomays food additive have certain physiological significance for a body. Indeed, polyunsaturated fatty acids produce positive effects, first of all, on fat metabolism as they accelerate lipid oxidation intensity [40, 41]. Besides, they participate in detoxification of a body, support immunity

² Diagnostics and correction of lipid metabolism disorders aimed at preventing and treating atherosclerosis: national recommendations. In: V.V. Kukharchuk, G.A. Konovalov [et al.] eds. Moscow, 2009, 50 p. (in Russian).

Table 2

Structure of Omega-6 family containing poly-unsaturated fatty acids

№	Poly-unsaturated fatty acids	Chemical structure
1	Linoleic acid	18: 2 ω 6, cys,cys-9,12-octadecadienoic acid
2	γ -linolenic acid	18: 3 ω 6, cys, cys, cys-6,9,12-octadecatrienoic acid
3	Calendic acid	18: 3 ω 6, 8-trans,10-trans,12-cys- octadecatrienoic acid
4	Eicosadienic acid	20: 2 ω 6, cys,cys-11,14-eicosadienic acid
5	Dihomo- γ -linolenic кислота	20: 3 ω 6, cys,cys,cys-8,11,14-eicosatrienic acid
6	Arachidonic acid	20: 4 ω 6, cys,cys,cys,cys-6,9,12,15- eicosatrienic acid
7	Docosadienic acid	22: 2 ω 6, cys,cys-13,16-docosadienic acid
8	Adrenic acid	22: 4 ω 6, cys,cys,cys,cys-7,10,13,16- docosadienic acid
9	Docosapentaenic acid	22: 5 ω 6, cys,cys,cys,cys,cys-4,7,10,13,16-docosapentaenic acid
10	Tetracosatetraenic acid	24: 5 ω 6, cys,cys,cys,cys,cys-6,9,12,15,18-tetracosapentaenic acid
11	Tetracosapentaenic acid	24: 5 ω 6, cys,cys,cys,cys,cys-6,9,12,15,18- tetracosapentaenic acid

Table 3

Structure of Omega-3 family containing poly-unsaturated fatty acids

№	Poly-unsaturated fatty acids	Chemical structure
1	Hexadecatrienic acid	16: 3 ω 3, cys,cys,cys-7,10,13-hexadecatrienic acid
2	α -linoleic acid	18: 3 ω 3, cys,cys,cys-9,12,15-octadecatrienic acid
3	Stearic (stioridic) acid	18: 4 ω 3, cys,cys,cys,cys-6,9,12,15-octadecatetraenic acid
4	Eicosatrienic acid	20: 3 ω 3, cys,cys,cys-11,14,17-eicosatrienic acid
5	Eicosatetraenic acid	20: 4 ω 3, cys,cys,cys-8,11,14,17-eicosatetraenic acid
6	Eicosapentaenic acid	20: 5 ω 3, cys,cys,cys,cys,cys-5,8,11,14,17-eicosapentaenic acid
7	Geneicosapentaenic acid	21: 5 ω 3, cys,cys,cys,cys,cys-6,9,12,15,18- geneicosapentaenic acid
8	Docosapentaenic acid	22: 5 ω 3, clupanodonic acid, cys,cys,cys,cys,cys-7,10,13,16,19-docosapentaenic acid
9	Docosahexaenic acid	22: 6 ω 3, cys,cys,cys,cys,cys,cys-4,7,10,13,16,19- docosahexaenic acid
10	Tetracosapentaenic acid	24: 5 ω 3, cys,cys,cys,cys,cys-9,12,15,18,21- docosahexaenic acid
11.	Tetracosahexaenic acid	24: 6 ω 3, cys,cys,cys,cys,cys,cys-6,9,12,15,18,21- tetracosahexaenic acid

and hormonal balance in it thus exerting favorable influence on functions performed by many organs and systems, for example, the digestive, cardiovascular, endocrine, and nervous systems etc. Moreover, polyunsaturated fatty acids become involved into energy formation and turn out to be basic energy suppliers for a body just as any other fatty acids. The promote decrease in total cholesterol contents in blood, increase in high density lipoproteins contents, and decrease in low density lipoproteins contents [42–44]. It is probably due to this mechanism that positive shifts in lipid blood spectrum were revealed in our research. Together with hypolipidemic effects Omega-3 fatty acids also produce positive effects on co-

agulation via reducing thrombocytes aggregation as well as increase oxygen inflow to tissue and reduce arterial hypertension [45].

Omega-6 polyunsaturated fatty acids, just as Omega-3 ones, have a lot of benign physiological properties. Their derivatives accelerate regeneration in tissues, participate in immune system regulation and, above all, reduce cholesterol contents in blood that makes for a reduced risk of atherosclerosis. It is probably these effects that predetermined positive dynamics in blood lipids spectrum that we obtained for animals with experimental hypercholesterolemia.

Biomays food additive, apart from comparatively large amounts of polyunsaturated

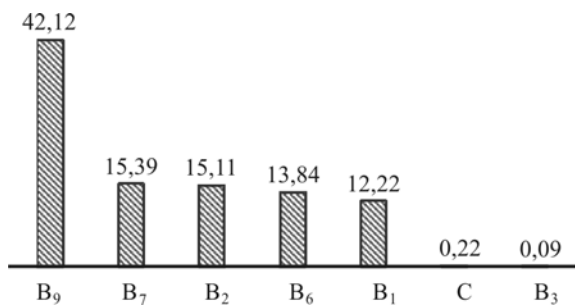


Figure 3. Structure and contents (in %) of water-soluble vitamins in Biomays food additive

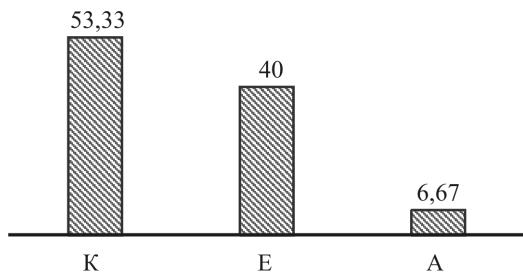


Figure 4. Structure and contents (in %) of fat-soluble vitamins in Biomays food additive

fatty acids, also contains many water-soluble and fat-soluble vitamins. Their contents and structure are given in Figures 3 and 4. As we can see, Biomays food additive contains quite a significant number of different vitamins. The greatest share among water-soluble vitamins belongs to B₉ or folic acid, namely 2/5 of all the water-soluble vitamins. At the same time,

other vitamins from group B have almost identical shares. Thus, specific weight of biotin (B₇) amounts to 15.39 %, specific weight of B₂, 15.11 %; B₆ and B₁, 13.84 % and 12.22 % accordingly. So, water-soluble vitamins structure is quite diverse in this food additive and it is good for physiological processes in a body. At the same time, fat-soluble vitamins are mostly K, E and A, and 90 % belong to vitamins K and E (Figure 4). Rather high specific weight of vitamin E is necessary for transforming linoleic acid into arachidonic one. Still, vitamin structure of the examined food additive indicates it is quite good for a body.

Vitamin B₉ or folic acid has the biggest share among water-soluble vitamins and takes active part in synthesis of many biologically active substances that are important for vital activities performed by cells and tissues [46].

Results of our research revealed effects produced by Biomays food additive on fatty acids structure of blood taken from animals with hypercholesterolemia. The chromatograms show that treatment with this product results in considerable shifts in fatty acid spectrum in experimental animals against the initial one (Figure 5). It becomes especially apparent by the end of the 2nd month after treatment started (Figure 2).

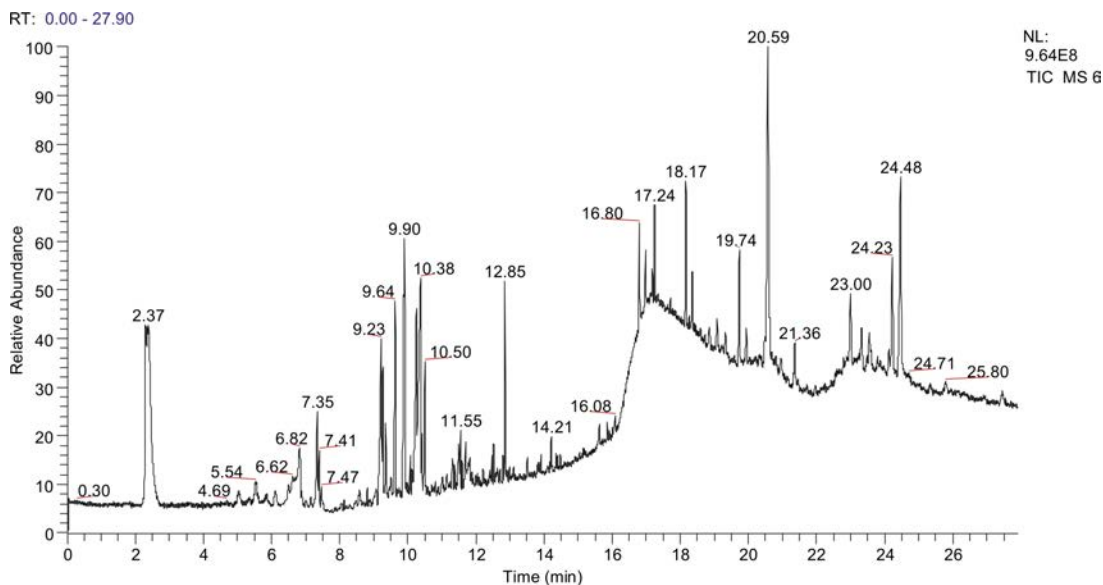


Figure 5. Fatty acids structure of blood taken from experimental animals with hypercholesterolemia prior to preparations intake

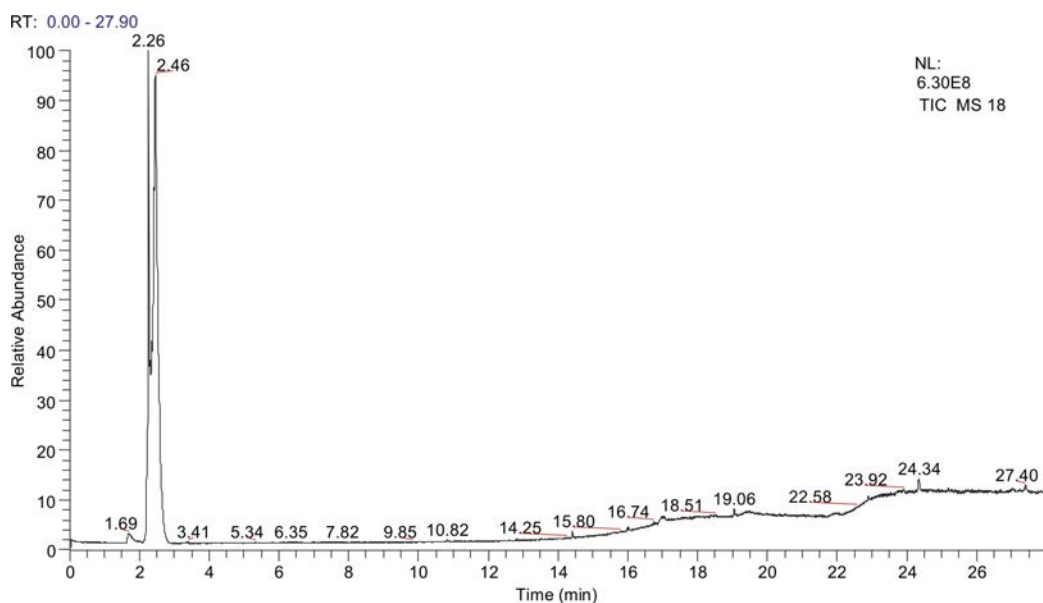


Figure 6. Fatty acids in blood of experimental animals with hypercholesterolemia after 1 month of Biomays intake

We quantitatively analyzed certain fatty acids in animals with hypercholesterolemia that were given Biomays food additive and compared the results with animals that weren't given it (Figure 7). The analysis revealed that contents of such saturated and monounsaturated fatty acids as palmitic, palmitic-oleic, stearic, and oleic acid went down by 46.0 %, 64.0 %, 37.7 %, and 20.0 % accordingly in animals that received Biomays against animals that didn't. At the same time concentrations of such polyunsaturated fatty acids as linoleic and linolenic on the contrary increased by 29 % and 141 % accordingly. Hence, treating animals with hyperlipidemia with Biomays food additive promoted a considerable decrease in concentrations of saturated fatty acids in blood and an increase in polyunsaturated ones such as linoleic and linolenic acid. Bearing in mind that these acids belong to Omega-3 and Omega-6 fatty acids, it becomes obvious that they play an important role in metabolism in a body as a whole and cholesterol metabolism in particular. Shift which we revealed in fatty acids structure of blood taken from experimental animals with hypercholesterolemia underlie positive shifts in spectra of low and high density lipoproteins against Biomays food additive intake.

We also performed a study on effects produced by Biomays food additive on prac-

tically healthy people; the results also revealed quite a similar fatty acids structure in blood.

As we can see from Figure 8, quantitative analysis of certain fatty acids in blood of practically healthy people who were given Biomays food additive revealed that palmitic acid contents decreased by almost 2 times after 2 months of Biomays intake. At the same time, contents of polyunsaturated fatty acids, such as linoleic and linolenic acid, increased by 3.5 and 2.6 times accordingly. Arachidonic acid concentration also grew (Figure 8), but this growth was less apparent.

Therefore, our research revealed that the examined food additive, when given to practically healthy people, promoted an increase in polyunsaturated fatty acids in blood, in particular linoleic, linolenic, and arachidonic acid; it again confirms that Biomays produces positive effects on lipid metabolism.

Therefore, our research results indicate that both hypolipidemic medication ultrox and Biomays food additive made of wheat germ produced obvious positive effects on high and low density lipoproteins spectra in animals with hypercholesterolemia; their combined intake promoted more apparent hypolipidemic effects.

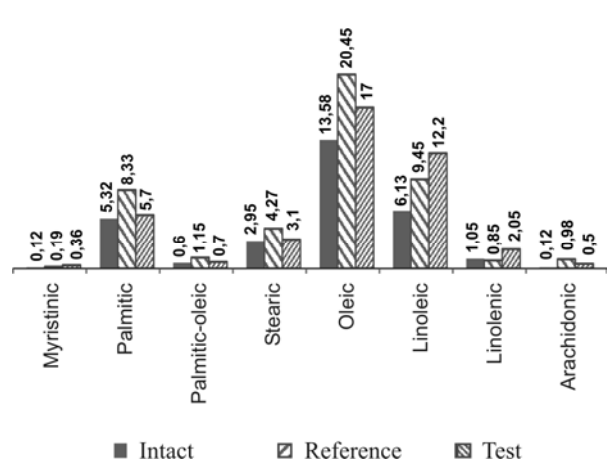


Figure 7. Comparative contents of certain fatty acids in blood of experimental animals with hypercholesterolemia after 1 month of Biomays intake

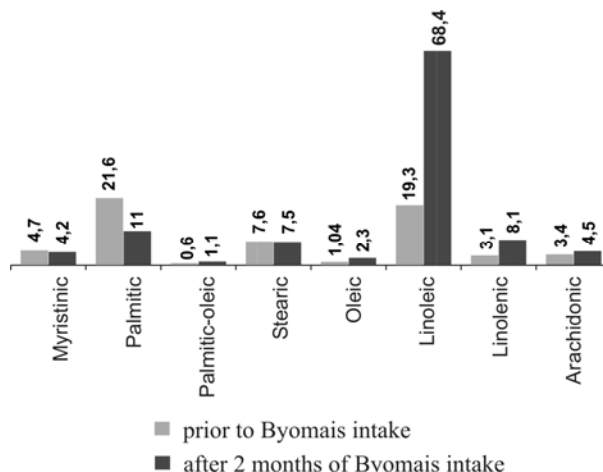


Figure 8. Comparative contents of certain fatty acids in blood of practically healthy people after 2 month of Biomays intake

Conclusion. Experimentally induced hyperlipidemia resulted in higher concentrations of atherogenic very low density and low density lipoproteins (VLDLP and LDLP) and a decrease in concentration of anti-atherogenic high density lipoproteins in blood plasma of rabbits from the test group against intact animals. Mono-therapy with ultrox in a dose equal to 0.5 mg/kg and Biomays statistically significantly reduced TCS and LDLP contents in comparison with animals that were not given any treatment. When both preparations were taken together,

there was an authentic decrease in LDLP and VLDLP contents.

Basing on the accomplished research, we developed theoretical grounds for recommending treatment with Biomays food additive to patients receiving anti-hyperlipidemia therapy who had troubles with statins. Application of this biologically active additive helps reducing statins doses.

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Conflict of interests. The authors declare there is no any conflict of interests.

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