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Chemical composition and biological activity of seed oil of amaranth varieties

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Abstract

The work is devoted to study of seed oil composition of amaranth varieties: Kharkov, Lera, Andijan and Helios, acclimatized in Uzbekistan. We demonstrated the possibility of using reversed-phase HPLC using a refractometric detector, which allows simultaneous determination of squalene and triacylglycerides in plant seeds and determining the authenticity of amaranth oils. Established seed oiliness ranged from 6.39 to 7.81 % of the initial mass. Amaranth oil samples contained quite large amount of unsaturated fatty acids 72.72 – 73.28 %, 1.17 % of which is omega-3-alpha-linolenic acid. The squalene content in the seeds ranged from 0.35 % to 0.55 %. It was established that the squalene content in oils obtained by extraction is greater than the one obtained by cold pressing. In the triacylglyceride composition of the investigated cold-pressed and extracted oils, no significant differences were found.

Introduction

Amaranth oil is a rich source of unsaturated fatty acids and thus is widely used as antioxidant means. Antioxidative and related properties of amaranth oil are often linked with squalene, which is acyclic polyunsaturated hydrocarbon of triterpene nature ($C_{30}H_{50}$) containing 12 double bonds. In a purified form, squalene is a colourless, almost tasteless, transparent liquid with no significant odour. The name squalene came from the Latin *Squalus* a shark, the liver of which is a rich source of this compound. In addition to shark liver, squalene is found in olive and amaranth oils, as well as in oils from wheat germ and rice bran (Bondioli *et al.* 1993; Makeev *et al.* 1999; Jeleznov *et al.* 2009).

Squalene is an intermediate in biosynthesis of cholesterol, although 10 % of it is converted to cholesterol (Govind Rao and Acaya 1968). Besides, squalene is a part of the secretion mass of sebaceous glands of human skin (up to 12 – 14 %), due to which it is easily absorbed and penetrates into the body. Moreover, it accelerates the penetration of substances dissolved in it. In human body, squalene is synthesized as a result of complex biochemical reactions leading to the formation of vital substances, such as coenzyme Q10, cholesterol, bile acids, vitamin D, sex and other steroid hormones. When the formation processes of these biologically active substances are violated, metabolic disorders leading to the development of atherosclerosis, cardiovascular and other diseases are in progress (Berg *et al.* 2002; Kuntz and Kuntz 2008).

In recent literature, recommendations on the use of squalene in medicine, cosmetology, gerantology

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have widely been presented (Kamimura *et al*. 1992; Chernenko *et al*. 1997; Tokhtaboev 1999; Chernenko and Glushenkova 1998; Abdurakhimov and Makhmudov 2014). Squalene hasa unique ability to saturate cells with oxygen. Allegedly, this is due to the fact that squalene as an unsaturated compound is unstable, and for the saturation of double bonds and transition to a more stable states it needs 12 hydrogen atoms, the best source of which are water molecules. Authors consider that squalene easily reacts with water, resulting in the formation of molecular oxygen, which saturates all cells (Jeleznov *et al*. 2009).

Most squalene is contained in amaranth seed oil (up to 8 %), which makes it possible to recommend the plant to produce squalene as another alternative to shark liver. Squalene accumulates in olive oil, in rice bran, in germs of wheat seeds during their germination and its significance for the plant metabolism is not clear yet. It is assumed that the seeds of these plants are deficient in oxygen upon germination, and squalene, as an antihypoxic compound, performs a protective function. Like other antioxidant triterpenes, squalene is involved in antioxidative processes. Well-known antioxidants, such as vitamins E and A, beta-carotene are terpenes, or contain isoprene groups characteristic for terpenes (Huang *et al.* 2009).

The ability of amaranth seeds, rich in squalene, to maintain high germination for dozens of years, possibly, is due to its extraordinary antioxidant properties. Oil of most wild plants and cultivars are among very valuable medicines used for a long time in medical practice. Amaranth oil which is obtained from the plant seeds has a very special place among organic vegetable oils. Its oil content reaches 6.3 – 8.0%, which is 1.5-times greater than the oil content of rice and wheat seeds (Jeleznov *et al*. 2009).

The dark-orange coloured liquid has specific taste, without bitterness. The fatty acids composition of amaranth oil obtained from the seeds of various species differed slightly. The sum of saturated fatty acids is 24.58 – 24.84 % with dominance of palmitic acid (20.34 – 20.68 %). Linoleic (43.0 – 43.21 %) and oleic acids (22.57 – 22.39 %) are predominant among unsaturated fatty acids which makes up 66.75 – 66.85 % of oil. Seeds of some varieties contain elaidic acid (1.07 – 1.15 %), a trans-isomer of oleic acid, which is very rare in nature. In addition, amaranth oil contains phospholipids (up to 10 %), tocopherols (2 %) and phytosterols (up to 2 %) that possess medicinal effects on the animal and human organism (Govind Rao and Acaya 1968).

Scientists of Uzbekistan have been conducting research on the adaptation of various varieties of plants of the genus Amaranthus in different climatic zones of Uzbekistan and obtaining cold pressed oils from amaranth seeds over recent years (Chernenko *et al*. 1997; Chernenko and Glushenkova 1998; Tokhtaboev 1999; Abdurakhimov and Maxmudov 2014).

The aim of the work is investigating the fatty acid and triacylglyceride composition and determining the quantity of squalene in Amaranth seeds acclimatized in Uzbekistan. The seeds of amaranth varieties *Amaranthus hypochondriacus* – Kharkov, Lera and *Amaranthus cruentus* – Andijan and Helios, grown in Andijan region, were used in this work.

Experimental

Isolation of squalene from seeds

The sample of the seeds (0.20 – 0.35 g) was ground in a porcelain mortar with 0.2 – 0.3 g of quartz sand sieved through a hole with diameter of 0.25 mm to the visual homogeneity of the powder. The resulting powder was transferred to a 10 mL flask and insisted in acetone by periodical stirring for 30 min. After settling the main part of the solid phase, the solution was filtered through a TFE-FG-3 Teflon filter in a filter cartridge and chromatographed in 2 μL portions.

Determination of triacylglyceride composition and quantity of squalene in amaranth oil

Crude oil from the seeds of amaranth was extracted by two methods: cold pressing and extracting with organic solvents in Soxhlet apparatus with subsequent removal of the solvent and drying. In order to determine triacylglycerides, 100 μL of the testing oil samples were dissolved in 900 μL of absolute acetone. The resulting solutions were

centrifuged at 6,000 rpm. The clear supernatant is chromatographed by 2 μL in HPLC.

Chromatography

A liquid chromatograph Agilent Technologies 1260 with a refractometer was used. Chromatographic column 4.6 x 250 mm, Agilent Eclipse XDB - C18, 5 μm was included. For the preparation of mobile phases acetone (imp., REAHIM) and acetonitrile (Sigma) were used. Chromatograms were registered and processed using the Open Lab program (Agilent Technologies). Mobile phase: 25 mL of acetonitrile is added to a 250 mL volumetric flask and the solution was brought to the mark with acetone, stirring. Feed rate: 1 mL/min., to calibrate the response of the detector, squalene solutions were prepared in the eluent in concentrations of 0.075 – 0.200 mg/mL. Squalene (97 %, Alfa Aestar, Lancaster) was used as a standard sample. Obtained solutions were chromatographed, as well as the test samples.

GC-MS Chromatography

HP 9010 Column (50 m capillary) was used with a stationary phase based on PEG. Chromatography conditions were: injector: 180 °C; detector 250 °C; the program for the column thermostat was: the beginning of 100 °C (0.5 min), by 10 °C in a minute until 230 °C, delay at 230 °C for 5 min. Helium at the rate of 1.7 mL/min was used as carrier gas. Parameters of the mass-selective detector were: scan mode from 40 to 1,000 amu; ionization by electron impact 70 eB. The used mass spectral libraries were the Database/W9N11.L and Database/RTLPEST3.L.

Fatty acids composition

Investigation on the fatty acid composition of amaranth oil was carried in accordance with state standards 30418-96 (P 51483-99 2017). The method was based on conversion of fatty acids' triglycerides into methyl (ethyl) ester of fatty acids with subsequent GC-analysis. The method is applicable in the range of 0.1 – 100 % of mass portions of fatty acids. For the conversion of triglycerides of fatty acids into their methyl esters, 0.2 mL of each tested oils sample is dissolved in 2 mL of diethyl ether. Then 0.1 mL of 10 % KOH solution in methanol is added to the samples and incubated at 100 °C for 3 min. Further 0.2 mL of hexane and 1 mL of water were gradually added to the samples. After phases were separated, organic phase was analysed in a volume of 2 μL in GC-MS.

Antiatherosclerotic property

Experimental studies on antiatherosclerotic effect of amaranth oil were carried on 40 white mongrel male rats with an initial body weight of 250 – 300 g. A tween model of hyperlipidemia was used by intraperitoneal administration of a single detergent Tween-80 at a dose of 200 mg/kg. Animals under hyperlipidemia treatment and prophylactic regime were administered orally 5 mL/kg of amaranth oil and an aqueous solution of latitude in a dose of 200 mg/kg once a day. The duration of the experiment was 5 days. On the sixth day, blood was taken from the iliac artery, the material was frozen until the day of the biochemical analysis. The following lipid profile indices were studied: total lipids, triglycerides, low-density lipoproteins, very low-density lipoproteins, atherogenic coefficient. To assess the antioxidant properties, the level of lipid peroxidation products in plasma was measured.

Determination of malonedialdehyde

Malonedialdehyde levels in samples were determined by using molar extinction coefficient (Ishimov *et al*. 2016).

Table 1. Oiliness and squalene percentage of amaranth seeds (M±m; *n* = 5).

Cultivar	Kharkov	Lera	Andijan	Helios
Oiliness [%]	7.81±0.17	7.55±0.23	6.39±0.06	7.68±0.16
Squalene [%]	0.55 ±0.02	0.48±0.03	0.35±0.02	0.44±0.02

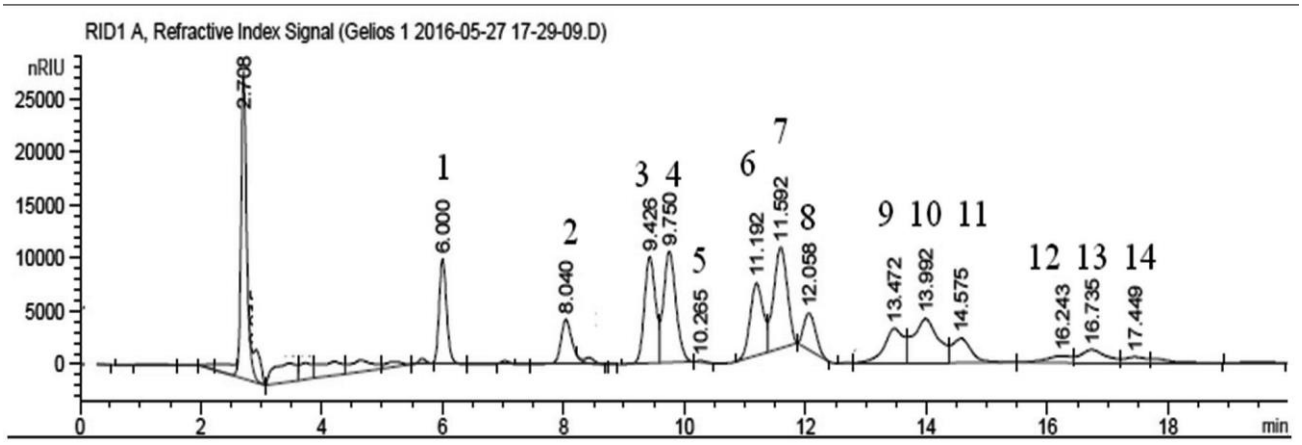

Fig. 1. HPLC analysis of amaranth oil, determination of quantity of squalene and triacylglyceride compositions (the numbers and the names of the identified substances are given in Table 2). ± means indicate relative standard deviation.

Results and Discussion

The oiliness of the investigated amaranth varieties was in the range of 6.39 – 7.81 % of the initial mass of the seeds. The highest oil content was determined in the variety Kharkov (7.81±0.17 %), and the lowest is in Andijan (6.39±0.06 %). The oil contents of Lera and Helios varieties were 7.55±0.23 % and 7.68±0.16 %, respectively (Table 1). Exact methods for the quantification of squalene in amaranth seeds have been developed and processed in products (Sulpice and Ferezou 1984; Brunner *et al.* 1991; Lehmann 1996; Grieveson *et al.* 1997; Hagiwara *et al.* 1998; Bruni 2001; Spanggord *et al.* 2002; Spanggord *et al.* 2006; Grigoriadoua *et al.* 2007).

In this work we used simultaneous quantitative determination of squalene and triacylglycerides in one cycle of chromatographic separation. The advantage of the method is simplicity of sample preparation and availability of chemical reagents. The essence of the proposed method is separation

Table 2. Squalene content and triacylglyceride composition of amaranth seed oils (RSD≤4 %; *n*=5).

	Amaranthus hypochondriacus				***Amaranthus cruentus***			
	Kharkov		**Lera**		**Andijan**		**Helios**	
	C/p	Ext.	C/p	Ext.	C/p	Ext.	C/p	Ext.
Squalene [%]	6.79	7.12	6.05	6.87	5.29	5.88	5.53	6.25
Triacyl-glycerides				*Quantity [molar %]*				
L_3	4.96	4.37	5.90	5.47	7.22	7.68	5.42	5.88
L_2O	12.98	11.85	13.60	12.90	12.69	12.15	11.94	13.06
L_2P	15.68	13.87	16.00	15.61	15.26	15.39	13.34	15.15
LPS	0.35	0.09	0.13	0.11	0.17	0.15	0.12	0.09
LO_2	11.63	12.88	10.79	11.93	11.62	11.36	12.09	11.77
L_2C+ LOP	15.58	19.09	16.60	19.54	18.12	17.72	18.12	16.10
LP_2	4.60	7.61	5.10	8.45	7.91	7.96	7.35	4.72
O_3	8.09	8.98	7.47	6.05	6.91	7.50	8.16	9.95
LOS	11.00	10.86	12.66	9.49	11.20	9.74	11.62	13.17
O_2P	3.99	4.07	5.30	4.12	4.48	4.45	5.08	5.57
O_2S	3.99	1.70	1.83	2.03	1.85	2.21	1.71	1.64
OP_2	2.76	3.28	3.77	3.08	2.07	2.73	2.90	2.16
OPS	0.38	1.34	1.37	1.23	0.50	0.95	2.15	0.74

Designations: C/p – cold pressed oil; Ext. – Extracted oil; L – Linoleic acid; O – Oleic acid; P – Palmitic acid; S – Stearic acid; where LP_2 stands for two acyls of linoleic acid and one acyl of palmitic acid.

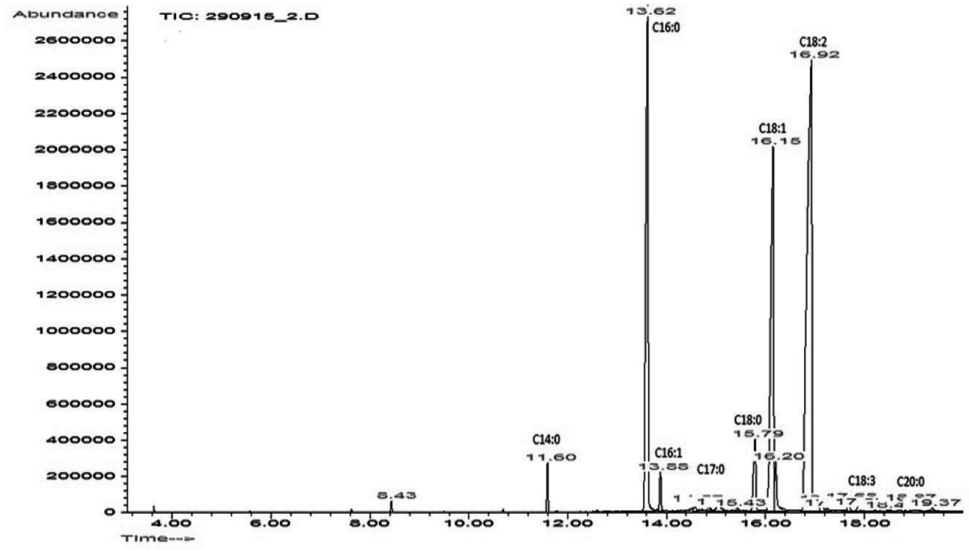

Fig. 2. GC – chromatogram of fatty acids of amaranth oil.

of amaranth oil components in a chromatographic column by polarity and their registration by refractometric detector.

A typical chromatogram of amaranth oil, obtained from Kharkov variety, is shown in Fig. 1 and corresponding results for the oiliness and squalene content are given in Table 1. The seed oiliness degree of these four amaranth varieties, ranging from 6.39 to 7.81 % (Table 1), can be considered as high oil content. The highest oiliness degree among 104 amaranth genotypes has been established to be 8.7 %, whereas the lowest compiled 1.9 % (He and Corke 2003).

Our results revealed that the content of squalene in the seeds of the amaranth varieties ranged from 0.35 to 0.55 % of the initial mass of the seeds or 5.48 – 7.81 % of the oil (Table 1). The higher content of squalene, like oil content, was determined in Kharkov variety (0.55±0.02 %), and the lowest in Andijan (0.35±0.02 %). There were no significant differences in varieties Lera and Helios with squalene contents 0.48±0.03 % and 0.44±0.02 %, respectively. In comparison to squalene content in amaranth oil of up to 104 genotypes (1.04 to 7.3 %), seed oils of these four amaranth varieties can be expected as a rich squalene source (He and Corke 2003). HPLC results, obtained by the analysis of the contents of squalene and triacylglycerides in amaranth seed oils, are represented in Table 2.

The content of squalene in the cold pressed seed oils of the investigated amaranth varieties was in the range from 5.29 to 6.79 %. The highest percentage was found in varieties Kharkov (6.79 %) and Lera (6.05 %). In Andijan (5.29 %) and Helios (5.53 %) the squalene content was much lower. These data are also confirmed in experiments to determine the amount of squalene in extracted oils: Kharkov – 7.12 %, Lera – 6.87 %, Andijan – 5.88 % and Helios – 6.25 %. Besides, obtained results indicate insignificant differences among triacylglyceride compositions in cold pressed and extracted oils of the investigated amaranth oils (Table 2).

The sum of saturated fatty acids made up 26.72 – 27.28 % with the dominance of palmitic acid from 22.6 % to 23.19 %; unsaturated fatty acids compiled 72.72 – 73.28 % with a predominance of linoleic acids (40.16 – 47.31 %) and oleic acids (24.67 – 31.31 %). Three high intensity peaks in Fig. 2 demonstrate palmitic, oleic and linoleic acids. In the oils of the studied amaranth varieties, omega-3-alpha-linolenic acid was found in the range of 1.17% – 1.65 %. Earlier has been established a positive correlation between amaranth seed oil and oleic acid contents (Hlinková *et al.* 2013). Similar results were found in our work since Kharkov and Helios varieties had higher oiliness degree and oleic acid content. Besides, the negative correlation between oleic acid and linoleic acid quantities corresponds to results in 10 amaranth samples of *A. cruentus* and *A. hypochondriacus* (Hlinková *et al.* 2013).

Molar ratios of predominant unsaturated oleic (C 18:1) and linoleic (C 18:2) acids correspond to previous results in 5 *Amaranthus* accessions

Table 3. Chemical compositions of fatty acids of amaranth oil of investigated varieties (n = 5).

Fatty acids	*Quantity [Molar %] [±0.05 %]*			
	Kharkov	***Lera***	***Andijan***	***Helios***
C 14 : 0 Myristic	0.27	0.28	0.27	0.24
C 16 : 0 Palmitic	23.19	22.60	22.27	22.80
C 18 : 0 Stearic	3.16	3.57	3.59	2.99
C 18 : 1 Oleic	31.31	24.67	24.52	28.80
C 18 : 2 Linoleic	40.16	47.01	47.31	42.83
C 18 : 3 Linolenic	1.23	1.17	1.26	1.65
C 20 : 0 Arachidic	0.66	0.69	0.78	0.69
Saturated	27.28	27.14	26.91	26.72
Unsaturated	72.72	72.86	73.00	73.28

± means indicate relative standard deviation

representing two species (oleic acid compiled 22.8 – 31.5 % and linoleic acid 39.4 – 49.1 %, respectively) (Table 3). The results obtained with the predominant saturated palmitic acid differed from 21.4 to 22.2 %, which correspond to previous studies (Jahaniaval *et al.* 2000). Comparative review of fatty acid compositions of *Amaranthus* species demonstrate that palmitic (C 16:0), oleic (C 18:1) and linoleic (C 18:2) acids have been established as predominant in several studies (Gamel *et al*. 2006; Venskutonis and Kraujalis 2013).

In our work the molar ratio of saturated and unsaturated fatty acids was established ~ 27:73 among the four amaranth seed oils. Comparable results (23.7:76.3 and 22:78, respectively) were demonstrated in *A. hypochondriacus* and *A. cruentus* (El Gendy *et al.* 2018).

High percentage of squalene and unsaturated fatty acids in amaranth oil makes it possible for use in medicine. Therefore, we studied the effects of amaranth oil on antioxidative system in rats. Coronary heart disease and atherosclerosis are on the first places by morbidity and mortality in economically developed countries. The role of diet therapy in the prevention and treatment of atherosclerosis is significant. From this point of view, modifying the fat composition of the diet, in particular reducing the content of saturated fatty acids and increasing unsaturated fatty acids, exerts a pronounced influence. Analyses on the antiatherosclerotic effect of oil from seeds of amaranth plants (Helios variety), acclimatized in Uzbekistan, on the tween model of hyperlipidemia was carried. The results showed, that tween model in rats was accompanied by a number of metabolic changes (Table 4), among which significant changes in the plasma lipoprotein spectrum: the relative content of fractions of plasma lipoproteins of very low density increased by 1.9-times and low density lipoproteins 2.6-times. There was a decreasing level of high-density lipoproteins, that 1.9-times lower degree was observed in experimental animals as tween was introduced. Total cholesterol values increased by 68 % and triglycerides by 77 %. All these changes led to more than 5-times greater coefficient of atherogenicity, increasing from 0.793±0.12 (outcome) to 4.47±0.30 (control). Thus, feeding with amaranth oil (Helios variety) significantly reduced the concentration of atherogenic lipid profile of the blood plasma in animals, in particular the total cholesterol for 27.0 %, triglycerides 9 % and low-density lipoproteins 38 %. High-density lipoproteins in basal conditions are considered as anti-inflammatory factor, capable of destroying oxidized lipids forming an inflammatory response.

Amaranth oil authentically increased the level of anti-atherogenic high-density lipoproteins cholesterol by 64 % compared to control. All these changes led to decrease in the coefficient of atherogenicity, respectively 2.4- and 1.9-times, which dropped from 4.47±0.30 (control) to 1.9±0.14. Simultaneously, by processes of increasing lipid peroxidation and as a result of interaction with the antioxidative system, a significant decrease in antioxidant activity occurs. Results show, in rats under the tween model, activation of lipo-oxidation processes occurred comparing to indicators in intact animals. Thus, the concentration of malonedialdehyde increased by 51 %, and conjugated dienes by 26 %. As intensity of lipid peroxidation lowered, antioxidant and catalase activities degrees decreased by 39 %. These results correspond to studies on patients, which demonstrated dose-dependent reduction of high-density lipoproteins, reversely proportional to squalene concentration, compiled 3.33 % in amaranth oil composition. While the reductions of low-density lipoproteins were fluctuating, the concentration of very low-density lipoproteins increased (Martirosyan *et al.* 2007). On the 5th day of the treatment with

Table 4. Effects of amaranth oil of Helios variety on lipids' profile of rats under hyperlipidemia tween model (M±m; *n* = 10).

Groups, Doses	Total cholesterol [mg/100 mL]	Triglycerides [mg/100 mL]	High-density lipoproteins [mg/100 mL]	Very low-density lipoproteins. [mg/100 mL]	Low-density lipoproteins [mg/100 mL]	Atherogenicity coefficient
Intact animal	52.0±0.3	60.0±0.6	31.0±0.5	11.0±0.3	12.0±0.4	0.793±0.12
Control (HLP+H_2O)	87.5±0.3	106.0±0.4	16.0±0.5	21.2±0.5	31.5±0.3	4.470±0.30
HLP+amaranth oil, 5 mL/kg	64.0±0.3*	80.0±0.6*	26.7±0.5*	17.5±0.3*	19.5±0.4*	1.900±0.14*

*HLP – hyperlipidemia condition

amaranth oil, a significant reduction in the level of peroxidation was observed; since the MDA level decreased aby 20 % (from 4.03±0.25 μM/mL.min. to 3.25±0.19 μM/mL. Simultaneously, conjugated dienes decreased by 19 % (from 7.17±0.18 units to 5.80±0.16). Previously, decreased levels of MDA were also observed in patients with heart diseases that consumed 6 – 12 g of amaranth oil possessing 3.33 % squalene (Martirosyan *et al.* 2007). Obtained results enable us to conclude that the amaranth oil have a pronounced atherogenic effect, and can be applied in the form of biologically active food additives.

Conclusions

In this work we studied chemical compositions of Amaranth seed oil, acclimatized in Uzbekistan and its oiliness degree. We established that amaranth oil contains amounts of unsaturated fatty acids and squalene comparable with amaranth oils obtained in other regions. Our results showed that its biological activity resemble lowering the level of lipoproteins rats in agreement with previous reports. Thus, seed oil of acclimatized amaranth variety studied in this work can be considered as a source of biologically active substances. A detailed study of the biochemical composition of amaranth seeds of various species and products of their processing, in order to obtain biologically active substances and food additives, can serve as basis for further research.

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