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COMPARATIVE STUDY OF FATTY ACIDS FORMATION IN NATURAL AND GENETICALLY MODIFIED BLACKBERRY SEEDS

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The work is devoted to a comparative study of fatty acids formation in natural and genetically modified blackberry seeds (collected in the foothill landscapes of Alaverdi Town, Republic of Armenia, and those of genetically modified blackberries (*Rubus caesius* L.), grown in greenhouse conditions in Yerevan City, harvest of 2023) capable of accumulating them in large quantities in order to justify the choice of the most valuable oilseed raw material for the production of natural oils that are in high demand in the food industry and cosmetology.

It has been shown that the oil content of the studied genetically modified blackberry seeds is about two times higher than that of natural blackberry seeds. These seeds are the most promising oilseed raw material for the production of natural oils by the method of cold pressing that are in high demand in cosmetology, for example, for the production of moisturizing cream for hands and feet based on chemically pure glycerin.

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Keywords**:** natural blackberries, genetically modified blackberries without thorns, seeds, fatty acids.

Introduction. Fats play an important role in the life of living beings, including the life of plants. Every living cell always accumulates fat, but oilseed cells accumulate it in larger amounts [1–3].

With all the diversity of fatty acids (FA), in higher plants the dominant ones are mainly saturated palmitic FA (C16:0) and two unsaturated FAs – oleic (C18:1) and linoleic (C18:2). There is a scanty amount of saturated stearic FA (C18:0) in plants, and acids from C20 to C24 are not always present, and if they are, then in small amounts. A high content of palmitic FA (C16:0) in phosphatidylglycerins was also found in vacuolar membranes [4].

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Biological science and agricultural practice have accumulated sufficiently large material on ways to increase yields of oilseed raw material, however the regularities of the fat formation process that determine the relative and absolute fat content in seeds are unclear so far [5–8].

The main difficulty in solving this problem, according to the research of Prokofiev and other scientists, is associated with the accumulation of fatty acids in seeds, the content of which, under the influence of various agrotechnical factors, changes within 10–40% as distinct from the overall yield of the oil, which can also change dependening on the same factors almost 3–6 times [9, 10].

There is practically no data on the effect of genetic modification on the quantitative and qualitative parameters of oils extracted from natural and genetically modified blackberries seeds by cold pressing.

To clarify the impact of genetic modification on the quantitative and qualitative parameters of oils (seed oil content), we studied the seeds of fresh natural blackberries collected in the foothill landscapes of Alaverdi Town, Republic of Armenia, and those of genetically modified blackberries (without thorns) (*Rubus caesius* L.) grown in greenhouse conditions in Yerevan, harvest of 2023. Oils from fresh blackberry seeds were extracted by cold pressing.

The objective of this research was to study the effect of genetic modification of blackberries (without thorns) on the quantitative and qualitative parameters of oils extracted from seeds by cold pressing and gas-chromatography (GC), with preliminary derivatization according to GOST P 51486-99.

Materials and Methods.

Preliminary Preparation, Fruit Squeezing and Juice Purification. As the object of the study, ripe fruits of natural blackberries (*Rubus caesius* L.), collected in the foothill landscapes of Alaverdi Town, Republic of Armenia, and those of genetically modified blackberries grown in greenhouse conditions in Yerevan, harvest of 2023, were used.

After sanitizing the production area (class A or B) and washing fresh blackberries (accurately weighed 1 *kg*), they were squeezed using a Braun 700 juicer, which crushed the fruit sample to a particle size that passed through a sieve with a hole diameter of 0.1 *mm*.

Removing Traces of Blueberry Pulp and Water-soluble Impurities. The resulting mixture (0.4 *kg* of small blackberry seeds and water-soluble impurities) was washed several times with running water until light pink clean seeds were obtained and immediately dried first at room temperature, and then in a drying oven at a temperature of no more than 70°C for 4 *h*.

Cold Pressing and Squeezing Natural and Genetically Modified Blackberry Seed Oil. For this purpose, 0.5 *kg* of each of the studied blackberry seeds was pressed with Ölverk oil press of the OW500s-inox brand. Ölverk oil presses are designed for squeezing various oily seeds by cold and warm pressing.

Oil squeezing of blackberry seeds takes place under the action of mechanical force without chemical additives at a screw speed of no more than 40 *rpm* and a limiting nozzle at the cake outlet with a diameter of 16 *mm*. The temperature of the oil at the outlet does not exceed 45°C. The resulting samples of blackberry oils are kept for 24 *h* in a household refrigerator at 8°C and filtered with paper filters.

High purity oils with yellowish tint are obtained. Furthermore, the yield of oil from genetically modified blackberry seeds (0.40 *kg*) was almost twice as high as that obtained from natural blackberry seeds (0.22 *kg*).

Determination of FA by GC. In GC experiments with the studied blackberry oils, a Thermo Scientific TRACE 1300 gas chromatograph (USA) with a flame ionization detector (FID) was used. To separate the components, a Thermo Scientific TR 5MS column with dimensions of 30 *m* 0.25 *mm* (inner diameter), 0.25 *m* (film thickness) was used. N_2 was used as carrier gas at a flow rate of 1.2 *mL*/*min* in constant flow mode. A sample volume of 2 μL was injected in "*splitless*" mode. The injection port was set to 270°C, and the oven temperature was initially set to 40°C for 1 *min*. The oven temperature was increased to 70°C at a rate of 5°C/*min* for 5 *min*, to 140°C at 5°C/*min* for 5 *min*, to 200°C at 5°C/*min* for 5 *min*, to 250°C at 5°C/*min* for 5 *min*, and finally to 270°C at a rate of 5°C/*min* for 5 *min*. The maximum oven temperature was set to 270°C.

Preparation of Oil Samples. To prepare samples, 10 μL of each oil was dissolved in 1 *mL* methanol/water solution at a ratio of 4:1 (v/v), stored at 4°C, and used as needed.

Preparation of Standard Solutions. Standard solution of the esters of the following acids – myristic, palmitic, linoleic, oleic, stearic, and that of a tocopherol mixture were prepared by weighing 10 μg of each standard and dissolving it in 50 *mL* of methanol/water solution at a ratio of 4:1 [11].

Results and Discussion. The qualitative content of FA contained in the studied samples of natural and genetically modified blackberries is depicted in Figs. 1 and 2. The results of gas chiral analysis (Apex retention time (RT), Start RT, End RT, Area and quantitative content of FA esters) are presented in Tabs. 1 and 2.

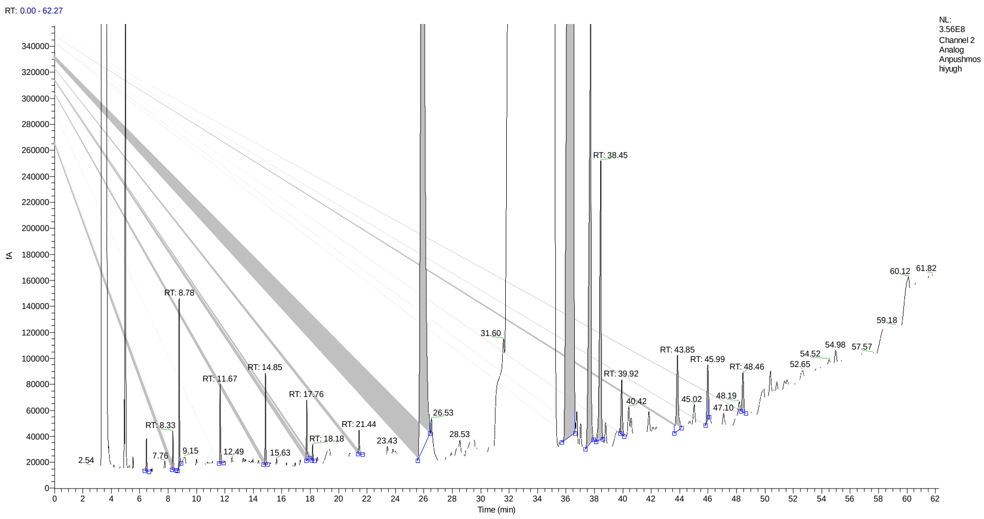

Fig. 1. GC chromatogram of the studied sample of natural blackberries.

As can be seen from Tabs. 1 and 2, both seeds of natural and genetically modified blackberries contain methyl hexocanoate (C6:0), methyl undecanoate (C11:0) and methyl tridecanoate (C13:0) (three identical FAs). Moreover, the amount of methylhexanoate (C6:0) in genetically modified blackberry seeds is 9 times greater than in wild blackberry seeds.

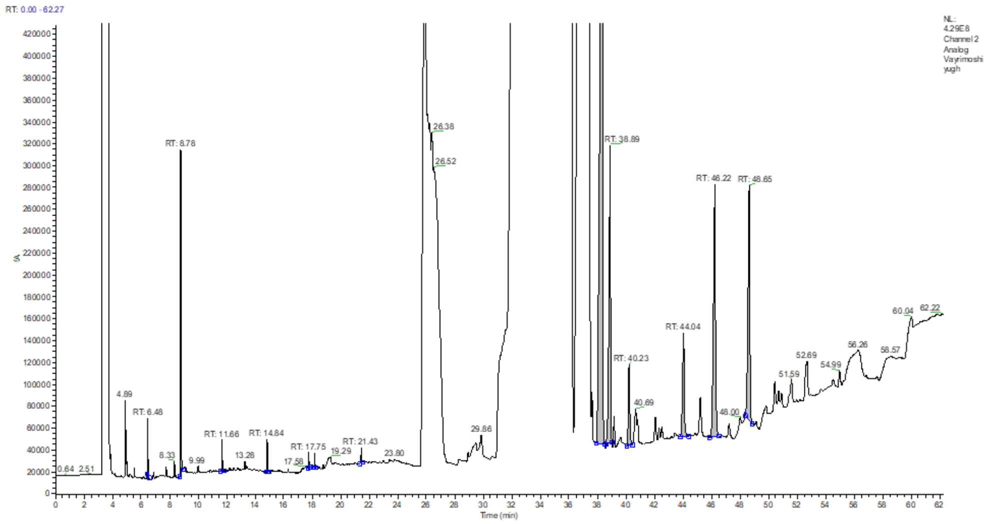

Fig. 2. GC chromatogram of the studied sample of genetically modified blackberries.

Table 1

Quantitative content of fatty acids in the studied sample of natural blackberries

Name of FA esters	Apex RT	Start RT	End RT	Area	% Area	Amount, *μg/mL* ±5%
Undetermined substance	6.48	6.34	6.65	71841.59	0.14	
Methyl hexanoate (C6:0)	8.33	8.27	8.56	89073.14	0.18	9048
Undetermined substance	8.78	8.64	8.92	357401.3	0.71	
	11.67	11.55	11.89	189316	0.38	
	14.85	14.7	14.99	199649.6	0.4	
	17.76	17.7	17.92	139362.5	0.28	
Methyl undecanoate (C11:0)	18.18	18.09	18.3	33239.94	0.07	63
Methyl tridecanoate (C13:0)	21.44	21.36	21.66	71684.15	0.14	94
Undetermined substance	25.95	25.55	26.41	12384541	24.74	
	36.52	35.72	36.63	30337477	60.61	
	37.74	37.36	37.94	3194031	6.38	
	38.45	38.12	38.59	1682634	3.36	
Methyl cis-11-eicosanoate (C20:1)	39.92	39.8	40.09	287013.3	0.57	308
Methyl behenate (C22:0)	43.85	43.62	44.12	541719.1	1.08	1176
Undetermined substance	45.99	45.81	46.05	256172.4	0.51	

Natural blackberry seeds contain methyl cis-11-eicosanoate (C20:1) and methyl behenate (C22:0), while genetically modified blackberry seeds are rich in methyl linolenate (C18:3) and methyl tricosanoate (C23:0), presumably due to changes in the mechanism of FA synthesis.

Cold-pressed oil, rich in a mixture of above mentioned FAs, can be recommended for the production of moisturizing cream for hands and feet based on chemically pure glycerin.

Table 2

Quantitative content of fatty acids in the studied sample of genetically modified blackberries

Name of FA esters	Apex RT	Start RT	End RT	Area	% Area	Amount, *μg/mL* ± 5%
Undetermined substance	6.48	6.43	6.55	125017.7	0.76	
Methyl hexanoate (C6:0)	8.78	8.7	8.94	820555.6	4.97	83350
Undetermined substance	11.66	11.58	11.78	86185.7	0.52	
	14.84	14.77	14.96	82545.91	0.5	
	17.75	17.67	17.84	36028.96	0.22	
Methyl undecanoate (C11:0)	18.17	18.12	18.27	35356.76	0.21	67
Methyl tridecanoate (C13:0)	21.43	21.31	21.54	48363.94	0.29	63
Undetermined substance	38.33	37.91	38.49	7619755	46.2	
	38.89	38.61	39.01	2071701	12.56	
Methyl linolenate (C18:3)	40.23	40.05	40.45	525735.2	3.19	854
Undetermined substance	44.04	43.78	44.37	786603.9	4.77	
Methyl tricosanoate (C23:0)	46.22	45.84	46.5	2299334	13.94	4793
Undetermined substance	48.65	48.38	48.84	1956751	11.86	

Conclusion.

1. It has been established that genetic modification leads to both quantitative and qualitative changes in the content of fatty acids in cold pressed oils. In particular, due to this modification, the synthesis of methyl hexanoate (C6:0) increased almost tenfold, and the presence of methyl linolenate (C18:3) and methyl tricosanoate (C23:0) instead of methyl cis-11-eicosanoate (C20:1) and methyl behenate (C22:0) probably occurred as a result of the change in the mechanism of FA biosynthesis.

2. It is shown that the oil content in the studied genetically modified blackberry seeds is about two times higher than the oil content in natural blackberry seeds.

3. Consequently, genetically modified blackberries (without thorns) are the most promising oilseed raw material for the production of natural oils that are in high demand in cosmetology, for example, for producing moisturizing cream for hands and feet based on chemically pure glycerin.

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ԲՆԱԿԱՆ ԵՎ ԳԵՆԵՏԻԿՈՐԵՆ ՄՈԴԻՖԻԿԱՑՎԱԾ ՄՈՇԻ ՍԵՐՄԵՐՈՒՄ ՃԱՐՊԱԹԹՈՒՆԵՐԻ ԱՌԱՋԱՑՄԱՆ ՀԱՄԵՄԱՏԱԿԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Աշխատանքը նվիրված է բնական և գենետիկորեն մոդիֆիկացված մոշի սերմերի համեմատական հետազոտմանը՝ ըստ ճարպաթթուների առաջացման և առավել բարձր քանակներով կուտակման, հետագայում՝ սննդարդյունաբերության և գեղարարության մեջ մեծ պահանջարկ ունեցող արժեքավոր յուղի ստացման համար լավագույն հումքի ընտրության նպատակով:

Հետազոտության առարկա են հանդիսացել Հայաստանի Հանրապետության Ալավերդի քաղաքի նախալեռնային լանդշաֆտներից հավաքված բնական մոշի (*Rubus caesius* L.) և Երևանում ջերմոցային պայմաններում աճեցված, գենետիկորեն մոդիֆիկացված անփուշ մոշի թարմ պտուղները (2023 թ. բերքահավաք): Հետազոտության նպատակն էր ուսումնասիրել սառը մամլման եղանակով ստացված յուղերի որակական և քանակական ցուցանիշների փոփոխությունը՝ գենետիկական մոդիֆիկացիայի արդյունքում:

Ցույց է տրվել, որ գենետիկորեն մոդիֆիկացված մոշի սերմերի յուղայնությունը մոտավորապես 2 անգամ գերազանցում է բնական մոշի սերմերի յուղայնությանը և հեռանկարային հումք է գեղարարության մեջ մեծ պահանջարկ ունեցող բնական յուղերի արտադրության համար, օրինակ՝ քիմիապես մաքուր գլիցերինի հիման վրա ձեռքերի և ոտքերի խնամքի խոնավեցնող քսուքի արտադրության համար:

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С. Г. КАЗАРЯН, М. А. ШЕЙРАНЯН, А. С. ДАДАЯН

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ОБРАЗОВАНИЯ ЖИРНЫХ КИСЛОТ В НАТУРАЛЬНЫХ И ГЕНЕТИЧЕСКИ МОДИФИЦИРОВАННЫХ СЕМЕНАХ ЕЖЕВИКИ

Работа посвящена сравнительному исследованию образования жирных кислот в семенах натуральной и генетически модифицированной ежевики, способных накапливать их в больших количествах, с целью обоснования выбора наиболее ценного масличного сырья для производства натуральных масел, имеющих повышенный спрос в пищевой промышленности и косметологии.

Объектом исследования являлись семена свежих натуральных и генетически модифицированных плодов черной ежевики (*Rubus caesius* L.) собранных на предгорных ландшафтах г. Алаверди (Республика Армения) и выращенных в тепличных условиях г. Еревана, урожая 2023 г. Целью исследования являлось изучение влияния генетической модификации растения ежевики (без шипов) на количественные и качественные показатели масел, выделенных из семян методом холодного прессования.

Показано, что масличность исследованных семян генетически модифицированной ежевики примерно на 2 раза больше масличности семян натуральной ежевики, а значит они являются наиболее перспективным масличным сырьем для производства натуральных масел, имеющих повышенный спрос в косметологии, например для производства увлажняющего крема для рук и ног на основе химически чистого глицерина.