LIPID COMPOSITION OF HAWTHORN FRUITS (*Crataegus orientalis* M.Bieb.) GROWN IN TURKEY

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The chemical composition of fruits from hawthorn (Crataegus orientalis M.Bieb.) growing in Turkey was investigated. The main components in the triacylglycerol fraction (3,9 %) were oleic (48,9%) and palmitic acids (31,9 %). α-Tocopherol (95,3 %) predominated in the tocopherol fraction, and β-sitosterol (75,8%) and campesterol (8,4 %) – in the sterol fraction.

Исследован жирнокислотной состав масел из плодов боярышника восточного (Crataegus orientalis M.Bieb.) из Турции. Основные компоненты в масле (3.9 %) олеиновая (48.9 %) и пальмитиновая кислота (31.9 %). Основные компоненты токофероловой и стероловой фракции α-токоферол (95.3 %), β-ситостерол (75.8 %) и кампестерол (8.4%), соответственно.

Key words: hawthorn, triacylglycerol fraction, tocopherols, sterols.

Introduction

Hawthorn (*Crataegus* spp.), fam. *Rosaceae*, grows in northern temperate regions such as East Asia, Europe, and eastern North America. The genus *Crataegus* consists of nearly 21 species in Turkey. All *Crataegus* species found in Turkey have long been used as a folk medicine and the fruits have been commonly used as food. The species *C. orientalis* M.Bieb. is distributed in Eastern and Central Anatolia and the surrounding areas, and its fruit is reddish-orange in color. The species prefers man-made habitats, clearing areas in the steppe, and other open places [10].

Extracts from fruits and leaves are used as they exhibit anti-inflammatory and antioxidant [8] and anti-thrombotic effects [7]. The active principles of hawthorn are probably a mixture of flavonoids, proanthocyanidins, organic acids and some amines [17].

Data about the chemical composition of the lipid fraction of hawthorn fruits are scarce. In the lipid fraction of the fruits from the common hawthorn (*Crataegus monogyna* Jacq.), growing in Russia, have been identified linoleic (41.3 %), oleic (34.2 %) and palmitic acids (14.0 %) [3]. Analyses of the lipid fraction (3.9 %) obtained from hawthorn fruits grown in Bulgaria established the presence of the three above-mentioned fatty acids but the respective amounts were 45.0 %, 37.0 % and 10.2 % [1]. In the oil from another hawthorn species (*Crataegus mordensis* Boom.), growing in Canada [6], the prevailing fatty components were linoleic (65.55 %), oleic (21.35%) and palmitic acids (5.37 %), as well as tocopherols (283,7 mg/kg), mainly β-tocopherol (164 mg/kg), and sterols (7.05 mg/kg), mainly β-sitosterol (64.0 %).

The aim of present investigation is to examine the lipid composition of hawthorn fruits from Turkey.

Materials and Methods

Samples. The fruits from hawthorn (*Crataegus orientalis* M.Bieb.) growing in Turkey were used for the investigation. The plant material was milled (0.5 mm) and its moisture content was determined by drying it up to constant weight, at 105 ^{o}C [2]. The samples were analyzed for the content of seed oil and the values are represented on the base of absolute dry weight.

Isolation of fruit oil. The oil of the fruits was extracted with n-hexane in Soxhlet for 18 h. The solvent was partly removed in a rotary vacuum evaporator, the residue was transferred to pre-weighed glass vessel and the rest of the solvent was removed under stream of nitrogen to a constant weight, in order to determine the oil content [13].

Fatty acids. The total fatty acid composition of the oil was determined by gas chromatography (GC) after transmethylation of the respective sample with 2N methanolic KOH at 50 ^{o}C according to Christie [9]. Fatty acid methyl esters (FAME) were purified by thin-layer chromatography (TLC) on 20 cm x 20 cm plates covered with 0.2 mm Silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase n-hexane:acetone, 100:8 (by volume). Determination was performed on a gas chromatograph equipped with a 30 m x 0.25 mm x 25 μm (I.D.)

capillary EC 30-Wax column (Hewlett Packard GmbH, Vienna, Austria) and a flame ionization detector. The column temperature was programmed from 130 °C (hold 4 min), at 15 °C/min to 240 °C (hold 5 min); injector and detector temperatures were 250 °C. Hydrogen was the carrier gas at a flow rate 0.8 ml/min; split was 50:1. Identification was performed by comparison of retention times with those of a standard mixture of FAME subjected to GC under identical experimental conditions [15].

Sterols. Unsaponifiables were determined by weight after saponification of the glyceride oil and extraction with hexane [16]. The unsaponifiable matter (100 mg, precisely measured) was applied on 20 cm x 20 cm glass plates (ca. 1 mm thick Silica gel G layer) and developed with n-hexane:acetone, 100:8 (by volume). Free sterols (R_f = 0.4) were detected under UV light by spraying the edges of each plate with 2′,7′-dichlorofluorescein, they were then scraped, transferred to small glass columns and eluted with diethyl ether. The solvent was evaporated under a stream of nitrogen and the residue was weighed in small glass containers to a constant weight. Sterol composition was determined by GC using HP 5890 gas chromatograph (Hewlett Packard GmbH, Vienna, Austria) equipped with a 25 m x 0.25 mm DB-5 capillary column (Agilent Technologies, Santa Clara CA, USA) and a flame ionization detector. Temperature gradient was from 90 °C (hold 2 min) up to 290 °C at a rate 15 °C/min and then up to 310 °C at a rate of 4 °C/min (hold 10 min); the injector temperature was 300 °C and the detector temperature was 320 °C. Hydrogen was used as carrier gas at a flow rate 0.8 ml/min; split 50:1. Identification was confirmed by comparison of retention times with those of a standard mixture of sterols [14].

Tocopherols. Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) by a Merck-Hitachi (Merck, Darmstadt, Germany) unit equipped with a 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and a fluorescent detector Merck-Hitachi F 1000. The operating conditions were as follows:mobile phase n-hexane:dioxan, 96:4 (by volume), flow rate 1.0 ml/min, excitation 295 nm, emission 330 nm. 20 μkl 1 % solution of crude oil were injected. Tocopherols were identified by comparing the retention times to those of authentic individual pure tocopherols. The tocopherol content was calculated on the base of tocopherol peak areas in the sample vs. tocopherol peak area of the standard tocopherol solution [12].

Results and discussion

The seeds contain 3.9 % glyceride oil, which complies well with reference data [1, 19].

The fatty acid composition is presented in Table 1 and the data show that 13 fatty acids were determined, constituting 100 % of the total oil content. The main fatty acids in the triacylglycerol fraction were oleic (48.9 %) and palmitic (31.9 %). In the work by Angelova-Romova et al. [1] three fatty acids are specified as predominating – linoleic (45.0 %), oleic (37.0 %) and palmitic (10.2 %), a difference that could be explained by the influence of the investigated species.

Table 1 – Fatty acid composition of seed oil

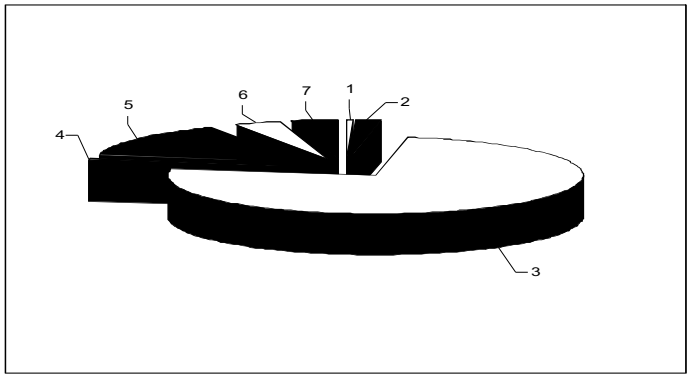
№	Fatty acids		Content, % (w/w)
1	$C_{12:0}$	Lauric	0.2
2	$C_{14:0}$	Miristic	0.9
3	$C_{16:0}$	Palmitic	31.9
4	$C_{16:1}$	Palmitoleic	0.3
5	$C_{17:0}$	Margaric	0.3
6	$C_{18:0}$	Stearic	6.2
7	$C_{18:1}$	Oleic	48.9
8	$C_{18:2}$	Linoleic	2.6
9	$C_{18:3}$	Linolenic	0.5
10	$C_{20:0}$	Arachidic	1.6
11	$C_{20:1}$	Gadoleic	4.3
12	$C_{20:2}$	Eicosadienoic	0.8
13	$C_{22:0}$	Behenic	1.5

The correlation saturated:unsaturated fatty acids was 42.6:57.4, which differs from data by Angelova-Romova et al. [1]. The distribution of fatty acids is presented on figures 1 and 2. Palmitic (31.9 %) and stearic acids (6.2 %) predominated in the fraction of saturated fatty acids, representing 74.9 % and 14.6 % of their total content, respectively. Oleic (48.9 %) and gadoleic acids (4.3 %) were predominant among the unsaturated acids,

representing 85.2 % and 7.5 %, respectively, of their total content. The ratio between the two main acids, typical of the *Rosaceae* family representatives – oleic and linoleic, varies among species, and in our investigation it was in favor of oleic acid. In common hawthorn (*Crataegus monogyna* Jacq.) it is 1:1 [1], in almond, apricot and plum – 3:1, while in morello – 2:1 [4, 5].

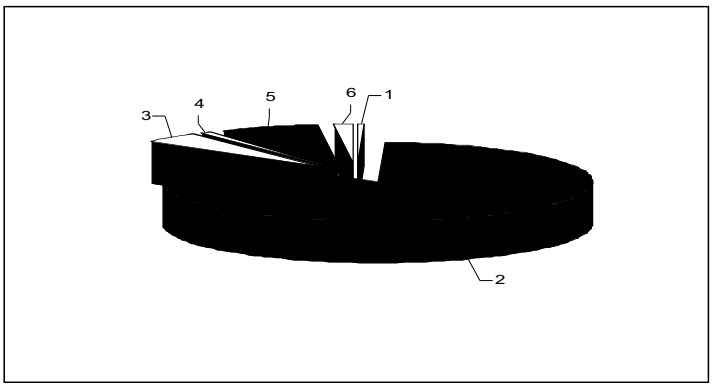
The results showed that regarding their oil content, hawthorn fruits were similar to other nontraditional oil-bearing materials such as grape seeds, watermelon, melon, tobacco, as well as to fruits from the *Lamiacea* family [5, 19].

Regarding the individual presence of oleic and linoleic acid, the oil from hawthorn fruits was similar to the oils from other nontraditional materials such as grape seeds, watermelon, tobacco and poppy seeds [4, 5]. The general fatty acid profile was like that of the edible sunflower oil. It was found to contain very high amounts of the undesirable saturated palmitic acid, in comparison to the widely used olive and peanut oils [11].



1 – Lauric, 2 – Miristic, 3 – Palmitic, 4 – Margaric, 5 – Stearic, 6 – Arachidic, 7 – Behenic

Fig. 1 – Distribution of saturated fatty acids



1 – Palmitoleic, 2 – Oleic, 3 – Linoleic, 4 – Linolenic, 5 – Gadoleic, 6 – Eicosadienoic

Fig. 2 – Distribution of unsaturated fatty acids

In the lipid fraction non-saponificated part was 9.5 % and sterols (7.2 %) are the major important component. Their total content in the investigated hawthorn fruits oil was found to be 0.7 %. The individual composition is presented in Table 2. The most significant contribution to the total content of sterols was by β-sitosterol (75.8 %), followed by campesterol (8.4 %). It is obvious from the data, that regarding its sterol content and composition, was similar to the findings by Angelova-Romova et al. [1] for hawthorn (*Crataegus monogyna*), by Zlatanov and Ivanov [18] for fruits from the *Apiaceae* family and by Gunstone et al. [11] for sunflower oil.

The total content of tocopherols in the lipid fraction was 290 mg/kg, and was dominated by α-tocopherol (95.3 %), followed by γ-tocopherol (4.5 %).

Table 2 – Sterol composition of seed oil

№	Sterols	Content, % (wt/wt)
1	Cholesterol	0.4
2	Brasicasterol	2.8
3	Campesterol	8.4
4	Stigmasterol	4.0
5	Δ^7- Campesterol	4.5
6	β- Sitosterol	75.8
7	Δ^5- Avenasterol	2.4
8	Δ^7- Stigmasterol	0.5
9	Δ^7- Avenasterol	1.2

Conclusion

The hawthorn fruits (*C. orientalis*) can be used as a non-traditional material for producing oil rich in biologically active substances such as sterols and tocopherols for nutritive purposes, as well as for an additive in fodder mixtures in order to enrich them with valuable nutrients.

References

1. Ангелова-Ромова М., М. Стоянова, Г. Антова, П. Денев, Н. Петкова, А. Стоянова. Липиден състав на плодове от глог (*Crataegus monogyna* Jacq.) // Научни трудове СУ. Пловдив. – 2010. – v. 10. – С. 116-120.
2. Государственная фармакопея СССР. XI. Москва: Медицина. – 1990.
3. Зинченко Л. Изучение химического состава липофильной фракции, полученной из шрота плодов боярышника //Научный журнал Современные проблемы науки и образования. – 2008. – № 3. – С. 1-3.
4. Попов А., П. Илинов. Химия на липидите. София: Наука и изкуство. – 1986.
5. Щербаков В. Биохимия и товароведение масличного сырья. Москва: Пищепромиздат. – 1963.
6. Anwar F., R. Przybylski, M. Rudzinska, E. Gruczynska, J. Bain. Fatty acid, tocopherol and sterol composition of Canadian prairie fruit seed lipids // Journal of American Oil Chemistry and Society. – 2008. – v. 85. – P. 953-969.
7. Arslan R., Z. Bor, N. Bektaş, A. Meriçli, Y. Öztürk. Antithrombotic effects of ethanol extract of *Crataegus orientalis* in the carrageenan-indiced mice tail thrombosis model//Thrombosis Research. - 2011. - v. 127, Issue 3. - P.210-213.
8. 8. Bor Z., R. Arslan, N. Bektaş, S. Pırıldar, A. Dönmez. Antinoceptive, antiiflammatory, and antioxidant activities of the ethanol extract of *Crataegus orientalis* // Turkish Journal of Medical Sciences. – 2012. – v. 42, – № 2. – P. 315-324.
9. Christie W. Lipid Analysis. The Oily Press: Bridgwater (3rd Edition). England. – 2003.
10. Dönmez A. The genus *Crataegus* L. (*Rosaceae*) with special reference to hybridisation and biodiversity in Turkey // Turkish Journal of Botany. – 2004. – v. 28. – P. 29-37.
11. Gunstone F., J. Harwood, A. Dijkstra. The Lipid Handbook. CRC Press: Boca Raton (3rd Edition). London: New York. – 2007.
12. ISO 9936. Animal and vegetable fat and oils. Determination of tocopherols and tocotrienol contents (HPLC method). – 1997.
13. ISO 659. Oilseeds. Determination of hexane extract (or light petroleum extract), called «oil content». – 1988
14. ISO 12228. Animal and vegetable fat and oils. Determination of individual and total sterols contents (Gas chromatographic method) – 1999.
15. ISO 5508. Animal and vegetable fat and oils. Analysis by gas chromatography of methyl esters of fatty acids. – 1990.
16. ISO 18609. Animal and vegetable fat and oils. Determination of unsaponifiable matter (Metod using hexane extraction). – 2000.
17. Özcan M., H. Haciseferoğulları, T. Marakoğlu, D. Arslan. Hawthorn (*Crataegus* spp.) fruit: some physical and chemical properties // Journal of Food Engineering. – 2005. – v. 222, – № 4. – P. 409-413.
18. Zlatanov M., S. Ivanov Untersuchungen über die Sterinzusammensetzung der Samenöle einiger Vertreter der Familie *Apiaceae* // Feet Wissenschaft Technologie. – 1995. – v. 97, – № 10. – P. 381-383.
19. Zlatanov M., G. Antova Composition of biologically active lipids of *Lamiacea* seed oils // Grasas y Aceites. – 2004. – v. 55, – № 2. – P. 143-147.