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ACER TATARICUM L. SEEDS – A NEW CONVENIENT SOURCE OF GAMMA-LINOLENIC ACID

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SUMMARY

This research was motivated by the therapeutic benefits of gamma-linolenic acid, a polyunsaturated fatty acid with limited occurrence in plants. Seeds of the tatarian maple (*Acer tataricum* L.) a widespread member of the natural Romanian flora, were analysed in a search for a new, convenient source of gamma-linolenic acid. Physicochemical study of the fatty oil obtained from the seeds established the fatty oil seed content (gravimetric method), refractive index, relative density, acidity, iodine and saponification values (official methods), and the fatty acid and phytosterol profiles (GC–MS). Tatarian maple seeds were found to contain an important amount of fatty oil (14.28–15.50%) with a high level of unsaturated fatty acids (93.89%). The essential fatty acids identified in this oil were linoleic (34.87%), gamma-linolenic (6.01%), and alpha-linolenic acids (1.04%). Stigmasterol (1.65%), beta-sitosterol (1.15%), and squalene (0.20%) were also identified. Because the not very large amount of gamma-linolenic acid in the seed oil is balanced by the natural abundance of the plant, the seeds of *Acer tataricum* could be regarded as a new convenient alternative source of this rare and valuable compound.

INTRODUCTION

Gamma-linolenic acid (GLA, 18:3*n*-6), also known as *cis*-6,*cis*-9,*cis*-12-octadecatrienoic acid, is an omega-6 polyunsaturated fatty acid of interest because of its potential health benefits. In the human body GLA is produced as a downstream metabolite of Δ6-desaturase-induced conversion from linoleic acid, an essential fatty acid supplied only by the diet. Aging, excessive alcohol consumption, smoking, nutrient deficiency, and

trans fatty acids may reduce the enzymatic activity of Δ6-desaturase, and gamma-linolenic acid may, as a result of these conditions, become essential.

GLA is metabolized to dihomo-gamma-linolenic acid (DGLA), a precursor to a variety of 3-series leukotrienes and 1-series prostaglandins, especially PGE1. Via conversion to PGE1, GLA has anti-inflammatory, antithrombotic, and antiproliferative properties. It also induces smooth muscle relaxation and vasodilatation [1,2].

GLA therapy has led to promising results in the treatment of the complications of diabetes [3–5]. In treatment of ocular diseases, GLA may reduce inflammation and produce improvements in dry eye symptoms [6]. Dietary GLA has beneficial effects in improving lipid profiles and preventing cardiovascular diseases [7–11] and a series of clinical tests has demonstrated that GLA can ameliorate atopic eczema and rheumatoid arthritis [12–16]. In a variety of therapeutic applications GLA has even shown promise in the treatment of cancer, both as a cytotoxic agent and as an adjuvant to chemotherapy [17,18].

The most studied sources of GLA are the seeds of the evening primrose (8–15% GLA), borage (14–25% GLA), and blackcurrants (12–18% GLA) [19]. The research reported in this paper was an attempt to find a new convenient vegetable source of GLA. We chose *Acer tataricum* L. (tatarian maple), an arborescent species of the *Aceraceae* family, because it is a widespread member of the natural Romanian flora with abundant seed production. According to the scientific literature, tatarian maple seed oil has not been investigated. Currently, only two species of the *Acer* genus have therapeutic uses – *Acer saccharinum* L. (sugar maple) as a source of sucrose and *Acer rubrum* L. (red maple) for the astringent effect of tannins from its bark [20,21].

EXPERIMENTAL

Chemicals and Materials

Pure reagents were supplied by Supelco and Sigma–Aldrich.

Seeds of *Acer tataricum* L. (tatarian maple), were collected in Bucharest, Romania, in August 2005.

Analytical methods

The oil content of *Acer tataricum* L. seeds was determined gravimetrically. Powdered seeds (20 g) were exhaustively extracted with *n*-he-

xane (150 mL) in a Soxhlet apparatus. The solvent was removed by distillation at low pressure and 40°C and the oily residue was weighed.

Refractive index, relative density, iodine, saponification, and acid values of tatarian maple seed oil were established in accordance with methods in the European Pharmacopoeia.

The fatty acid (free and esterified) and phytosterol profiles of tatarian maple seed oil were established by gas chromatography–mass spectrometry [22,23] using an Agilent Technologies 6890N gas chromatograph coupled to an Agilent Technologies 5973 mass spectrometer. Because of the high level of fatty acids and its complex composition, tatarian maple seed oil was separately derivatized with two reagents – sodium hydroxide and bis-trimethylsilyltrifluoracetamide (BSTFA). The fatty acids were identified and quantified in a sample of oil derivatized with sodium hydroxide in methanol after transformation of the acids to the methyl esters. The oil (0.5 g) was mixed with sodium hydroxide solution (60 g L^{-1}, 0.2 mL) and 10 mL anhydrous methanol and the mixture was boiled for 15 min. After cooling, saturated sodium chloride solution (10 mL) was added and the methyl esters of the fatty acids were extracted with 4 mL *n*-heptane. The extract was diluted 1:5 (*v*/*v*) with *n*-heptane and 1 μL of this solution was analysed by GC–MS using a 30 m × 0.32 mm capillary column coated with a 0.15-μm film of the poly(ethylene glycol) DB-Wax. Helium for chromatography was used as carrier gas at a flow rate of 1.5 mL min^{-1}. The split ratio was 1:50.

Another sample of the oil was derivatized with BSTFA, and free fatty acids and phytosterols were transformed into more volatile trimethylsilylether derivatives. The oil (0.5 g) was heated with 0.5 mL BSTFA at 90°C for 1 h then extracted with 0.5 mL *n*-heptane. This sample (1 μL) was analysed by GC–MS using a 60 m × 0.25 mm capillary column coated with a 0.25 μm film of the polyphenylsiloxane DB5-MD. Helium for chromatography was used as carrier gas at a flow rate of 1.2 mL min^{-1}. A split ratio of 1:10 gave the best results. In this sample of the oil, squalene was also identified.

In GC–MS analysis the injector, interface, ion source, and quadrupole temperatures were 250°C, 280°C, 230°C, and 150°C, respectively. Compounds in the oil were identified by comparison of their mass spectra with those in the NIST mass spectra data base, 1989 edition, using Chemstation software. Quantitative evaluation of chromatograms was performed on the basis of direct proportionality of peak area (normalization procedure). The percentages of fatty acids, phytosterols, and squalene were determined from total identified compounds in the oil.

RESULTS

The fatty oil content of tatarian maple seeds and the physicochemical properties of the oil are listed in Table I. Results from fatty acid, phytosterol, and squalene analysis are summarized in Tables II and III. Figures 1 and 2 show gas chromatograms obtained from the fatty acid methyl esters and from the trimethylsilylether derivatives of free fatty acids, phytosterols, and squalene, respectively.

Table I

Physicochemical properties of tatarian maple seed fatty oil

Fatty oil content of seeds	14.28–15.50% (*w*/*w*)
Refractive index, n_D^{20}	1.4812
Relative density, d_{20}	0.9411
Acid value, I_A	10.01
Iodine value, I_I	99.51
Saponification value, I_S	180.81

Table II

Results from fatty acid analysis

No.	Fatty acid		Retention time of methyl ester (min)	Percentage of total fatty acids
1	Palmitic acid	C16:0	15.22	2.81
2	7-Hexadecenoic acid	C16:1	15.35	0.04
3	Palmitoleic acid	C16:1	15.42	0.03
4	Heptadecanoic acid	C17:0	16.41	0.05
5	Stearic acid	C18:0	17.67	2.04
6	Oleic acid	C18:1	17.83	18.39
7	11-Octadecenoic acid	C18:1	17.90	1.49
8	Linoleic acid	C18:2	18.37	34.87
9	gamma-Linolenic acid	C18:3	18.66	6.01
10	alpha-Linolenic acid	C18:3	19.12	1.04
11	Arachic acid	C20:0	20.58	0.21
12	11-Eicosenoic acid	C20:1	20.78	6.45
13	Behenic acid	C22:0	24.10	0.83
14	Erucic acid	C22:1	24.36	16.55
15	Nervonic acid	C24:1	28.48	8.65

Table III

Results from analysis of phytosterols, free fatty acids, and squalene

No.	Compound	Retention time of trimethylsilylether derivative (min)	Percentage of total identified compounds
1	Stigmasterol	40.25	1.65
2	beta-Sitosterol	42.25	1.15
3	3-beta-Stigmastadienol	42.71	0.05
4	3-beta,24*R*-Ergost-5-en-3-ol	44.46	0.82
5	Myristic acid	16.81	0.15
6	Palmitic acid	18.79	6.75
7	gamma-Linolenic acid	20.88	2.82
8	Linoleic acid	21.18	28.90
9	Oleic acid	21.27	15.64
10	alpha-Linolenic acid	21.32	0.07
11	11-Octadecenoic acid	21.37	1.93
12	Stearic acid	21.67	2.28
13	11-Eicosenoic acid	24.89	2.79
14	Squalene	30.29	0.20

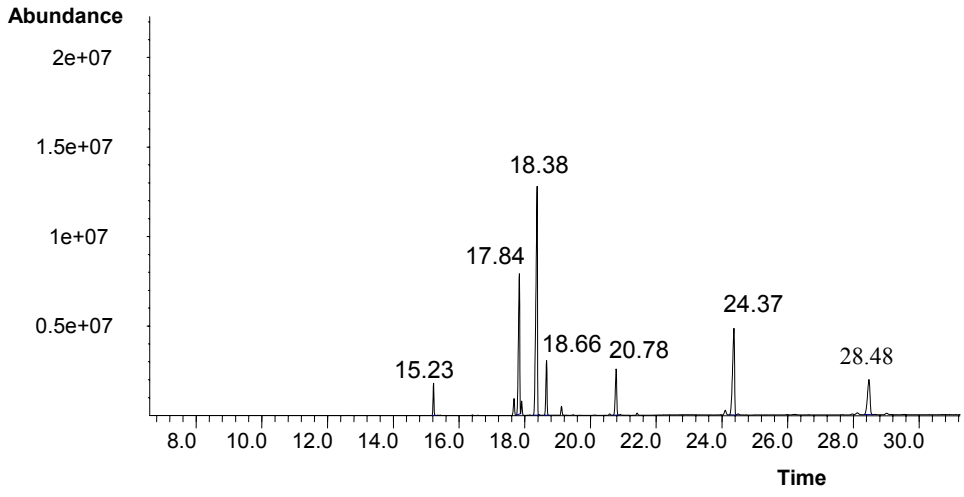

Fig. 1

Gas chromatogram obtained from the methyl esters

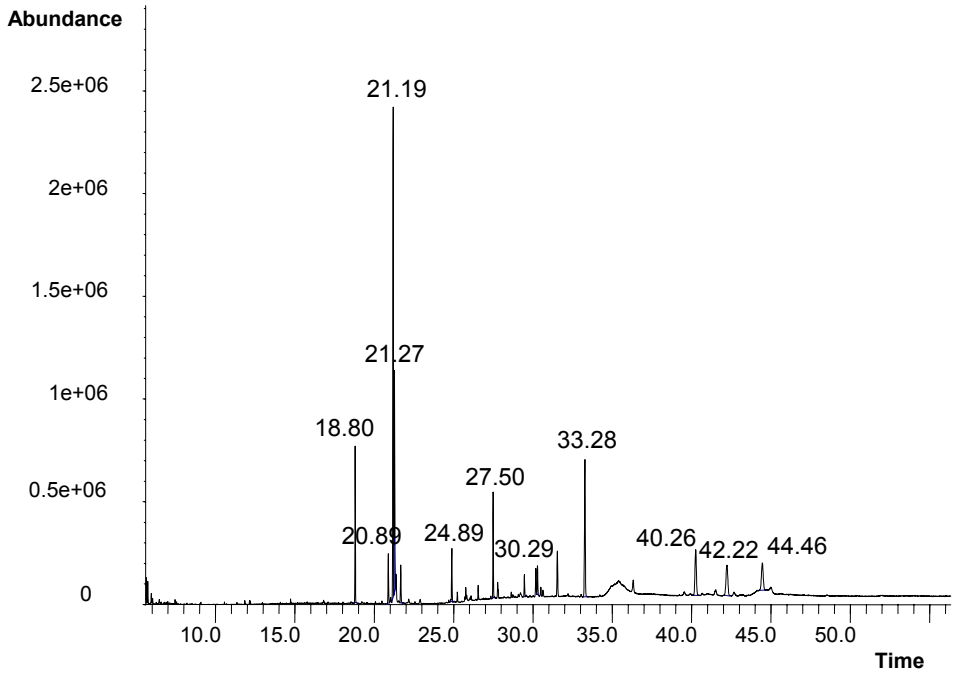

Fig. 2

Gas chromatogram obtained from the trimethylsilylether derivatives

DISCUSSION

The fatty oil content of tatarian maple seeds was substantial – 14.28 to 15.50% (*w*/*w*). The oil was yellow–green in colour with an oily nonspecific taste and no smell. The physical properties (refractive index and relative density) were typical of plant seed fatty oils with high unsaturation. The acid, iodine, and saponification values of the oil were normal for vegetable oils in relation to the chemical composition, high degree of unsaturation, and solvent extraction technique used.

Tatarian maple seed fatty oil was found to contain a large proportion of unsaturated fatty acids – nearly 94% of total fatty acids. Linoleic acid (34.87%) was the main constituent of the essential fatty acid (EFA) fraction. The most valuable component, gamma-linolenic acid, was found to be present in a significant amounts (6.01%). The level of alpha-linoleic acid was low (1.04%). Seven monounsaturated fatty acids were identified – oleic (18.39%), erucic (16.55%), nervonic (8.65%), 11-eicosenoic (6.45%), 11-octadecenoic (1.49%), palmitoleic, (0.03%), and 7-hexadecenoic (0.04%).

The total saturated fatty acid content of the oil was 5.89% only. Four acids were identified – palmitic (2.81%), stearic (2.04%), arachic (0.21%) and behenic (0.83%).

The principal free fatty acids identified in tatarian seed fatty oil, after conversion into their trimethylsilylether derivatives, were unsaturated – linoleic, the major constituent (28.90%), oleic (15.64%), gamma-linolenic (2.82%), alpha-linolenic (0.07%), 11-octadecenoic (1.93%), 11-eicosenoic (2.79%), and myristic (0.15%) acids.

Four phytosterols were found in the unsaponifiable fraction of the oil – stigmasterol (1.65%), beta-sitosterol (1.15%), 3-beta-stigmastadienol (0.05%), and 3-beta, 24*R*-ergost-5-en-3-ol (0.82%). Two of these, beta-sitosterol and stigmasterol, are normal components of plant seed oils and the levels found are typical. Squalene (0.20%) was also identified in the unsaponifiable fraction of tatarian maple oil. Squalene is a rare unsaturated compound in plant seed oils and has appreciable antioxidant properties.

Tatarian maple seed oil was found to have a lower gamma-linolenic acid content than evening primrose and borage oil – 6.01% compared with 8–15% and 14–25%, respectively – but could be obtained more easily in larger quantities, because *Acer tataricum* is a common species with prolific seed production.

CONCLUSION

For the first time the seeds of *Acer tataricum* L. (tatarian maple) a native Romanian plant, have been analysed and found to contain a large amount of fatty oil (14.28–15.50% *w*/*w*) with a substantial essential fatty acid content (41.91%).

Abundant in nature, tatarian maple seeds could be a valuable source of gamma-linolenic acid and an alternative to evening primrose or borage seeds which are currently used in phytotherapy as dietary supplements rich in essential fatty acids.

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