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SHORT COMMUNICATION

Fatty Acid Composition of Seeds From Wild and Cultivated Ginseng (*Panax ginseng* Meyer): Occurrence of a High Level of Petroselinic Acid

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Abstract The lipid content and fatty acid (FA) composition of seeds from the Asian ginseng *Panax ginseng* growing naturally in taiga forests of the Russian Far East and seeds from cultivated ginseng were studied in this work. The total lipid content of seeds from both wild and cultivated plants was 9–12 % of fresh weight. FA were analyzed as isopropyl esters on a polar capillary column BD-225, which allows good separation of petroselinic and oleic acids. The structure of FAs was confirmed using GC–MS of fatty acid methyl ester (FAME) and 4,4-dimethyloxazoline derivatives. In all the seed samples, the major FA was petroselinic acid 18:1(n-12) which comprised more than 60 %; the contents of oleic and linoleic acids were lower (15–17 and 15–16 %, respectively). Earlier, a higher level (>80 %) of oleic acid had been reported for ginseng seeds. This discrepancy can be explained by an insufficient separation of these acids on standard columns used for GC of FAME. In general, seeds of wild and cultivated ginseng are very similar in lipid content and FA composition.

Keywords *Panax ginseng* Meyer · Lipids · Fatty acids · Petroselinic · Oleic

Introduction

Ginseng (*Panax ginseng* C.A. Meyer) is a very valuable relict plant, which has been traditionally used in Asian medicine for thousands of years. In the Russian Far East, ginseng grows naturally in taiga forests and is enlisted in the Red Data Book of the Russian Federation. In many countries, ginseng is cultivated as a medicinal plant. Recent pharmacological investigations confirm the healing effects of ginseng on human health, which are associated mainly with the action of glycosides (ginsenosides) localized in roots [1]. Furthermore, many other numerous compounds such as phytosterols, polyphenols, polyacetylenes, alkaloids, lipids, and polysaccharides have also been identified in ginseng plants [1]. The information on chemical composition of ginseng seeds and berries is still limited. As is reported in some publications, ginseng seeds contain a substantial amount of oil (10–26 %) [2–7]. The major FAs of ginseng seeds are oleic 18:1(n-9), *cis*-vaccenic 18:1(n-7), and linoleic 18:2(n-6) [3–5, 7]. The oleic acid content of oil from the American ginseng (*Panax quinquefolius* L.) reaches 87 % of total FA [4, 5]. In our work, we compared both total lipid content and FA composition between seeds collected from wild and cultivated Asian ginseng (*Panax ginseng* C.A. Meyer). We paid special attention to FA analysis, because, according to our data, the main FA in ginseng seeds is petroselinic 18:1(n-12) acid rather than the oleic one, as was reported earlier [3–5, 7]. Petroselinic acid is known to have a specific biosynthesis pathway and a particular physiological activity [8, 9].

Materials and Methods

Ginseng Seeds

Mature seeds of wild ginseng were collected from plants found in three forest (taiga) localities of Spassky District (Primorsky Krai, Russia) in 2011 and 2012. The exact

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ages of the plants are unknown. Seed samples of cultivated ginseng were obtained from an experimental nursery, where the plants had been grown from roots of wild ginseng collected in different parts of Primorsky Krai and transplanted to the nursery plantation. In this experiment, we used seeds of the second-generation progeny of wild ginseng plants found in Spassky and Dalnegorsk districts. Seeds from red and yellow berries of Korean ginseng were collected from the plants cultivated under laboratory conditions. Roots of Korean ginseng were transplanted from the experimental nursery to the laboratory greenhouse in May, where the plants were cultivated in pots with forest soil until full ripening of berries at an air temperature of 25 °C and illuminance of 15,000 lx. Originally, seeds of these plants were a generous gift from the Korean Ginseng and Tobacco Research Institute, Daejon, Republic of Korea. All seeds of cultivated ginseng were collected from 5-year-old plants.

Total Lipid Extraction

Total lipids were extracted from air-dried and ground seeds (0.3–0.7 g) with 20 mL chloroform–methanol 2:1 (v/v) on a magnet stirrer for 2 h, according to Folch et al. [10]. After filtration, the extract was supplemented with 4 mL 0.9 % NaCl, and total lipids were recovered in the chloroform layer. The solvent was removed on a rotary vacuum evaporator at 40 °C. The amount of total lipids was measured gravimetrically, and their weight percentage was calculated based on the fresh weight of seeds. The extracted total lipids were stored at −20 °C under argon.

Synthesis of Methyl and Isopropyl Esters and 4,4-Dimethyloxazoline Derivatives of Fatty Acids

Methyl esters (FAME) were prepared from 3 to 5 mg of total lipids using the two-step methylation method (1 % $NaOCH_3$/MeOH followed by 5 % HCl/MeOH); both steps were performed at 60 °C, 20 min [11]. FAME were extracted with hexane and purified by thin layer chromatography (TLC) on silica gel plates in hexane-diethyl ether 9:1 (v/v). Isopropyl esters (FAIPE) were prepared by incubation of 3–5 mg of total lipids with isopropanol (1.8 mL) and sulphuric acid (0.25 mL) at 100 °C, 1 h [12]. After extraction with hexane, FAIPE, the same as methyl esters, were purified by TLC.

The 4,4-dimethyloxazoline (DMOX) derivatives were prepared from FAME according to the procedure described earlier [13]. In brief, FAME (2–5 mg) were incubated with 60 μL of 50 % 2-amino-2-methylpropanol in benzene and 10 μL of 1 % $NaOCH_3$ as catalyst in MeOH at room temperature overnight. The obtained acyl-2-methylpropanol amides were isolated by partition between hexane-diethyl ether 9:1 (v/v) and water. After solvent evaporation, acyl-2-methylpropanol amides were converted into DMOX by incubation with 150 μL of trifluoroacetic anhydride at 50 °C for 45 min. The reagent was evaporated under an argon stream. The derivatives were dissolved in hexane and washed with water to remove the residues generated during the reactions. All operations were done under argon.

GC and GC–MS Analysis

The FAME and FAIPE were analyzed on a gas chromatograph (GC) Shimadzu GC 2010 with a flame ionization detector. Separations were conducted on a capillary columns SUPELCOWAX 10 (Supelco, USA) and DB-225 ms (J&W Scientific, USA); all columns were 30 m × 0.25 mm ID, 0.25 μm film. The FAME and FAIPE derivatives were analyzed at 200 and 205 °C, respectively. The injector and detector temperatures were set at 250 °C. Fatty acids were identified using the standards (methyl esters of palmitic, stearic, oleic, petroselinic, linoleic, and linolenic acids; all produced by Sigma, USA), equivalent chain length values [14], and MS spectrometry data. The DMOX derivatives were analyzed on a gas chromatograph–mass spectrometer (GC-MS) Shimadzu GCMS-QP5050A (Japan) equipped with a DB-225 MS column. The temperature ramp was from 190 to 230 °C, 1 °C/min; the injector and detector temperatures were set at 250 and 240 °C, respectively. Helium was used as the carrier gas at a linear velocity of 30 cm/s. Electronic impact mass spectra were recorded at 70 eV. Spectra were compared with the NIST library and the FA mass spectra archive [15].

All GC analyses of the fatty acids were carried out in triplicate, and the results were expressed as the mean value ± standard deviation (STD).

Results and Discussion

Identification of Petroselinic Acid

The GC chromatogram of ginseng seed FAME, separated on a Supecowax 10 column, shows a large peak, which corresponds to the standard of oleic acid. At the same time, this peak is slightly skewed in shape. It is well known that seeds of some members of the family Araliaceae contain petroselinic acid [16, 17]. We assumed presence of this acid in ginseng seed lipids. There are several methods for separation of petroselinic and oleic acids. The most suitable method for the petroselinic–oleic acid pair separation is the use of isopropyl esters and a high polarity capillary column [12]. Figure 1 shows separation of a mixture of standard isopropyl esters of petroselinic and oleic acids (A) and FAIPE of seeds from wild ginseng (B) on a DB-225

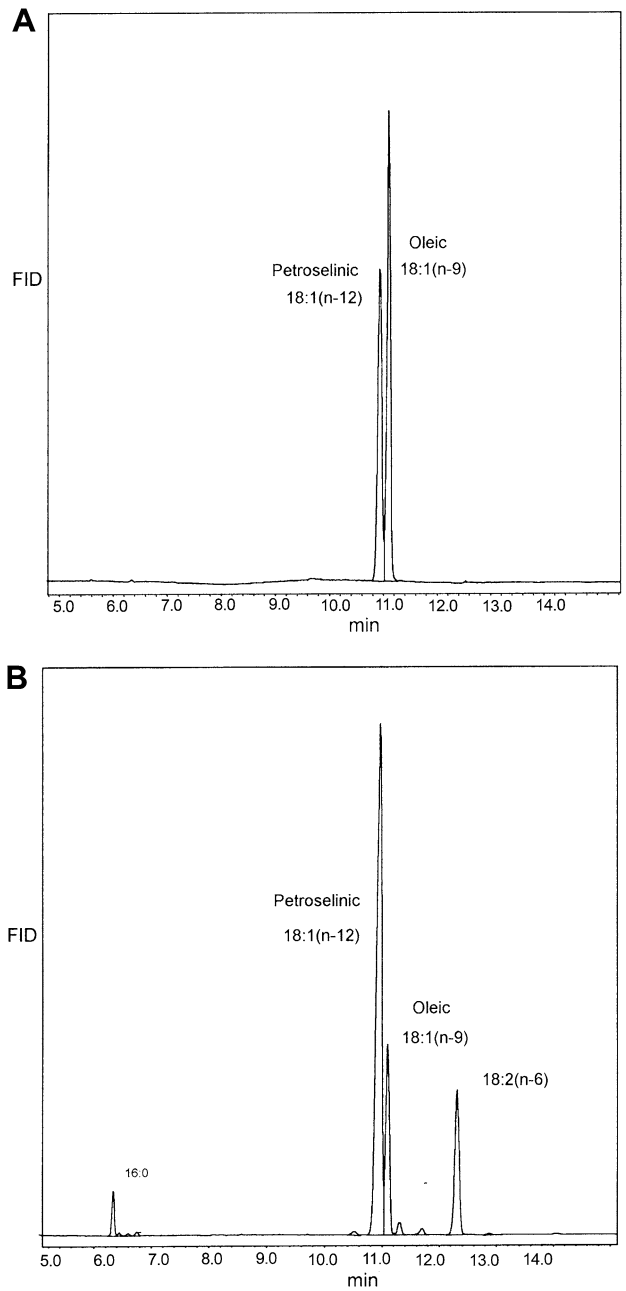

Fig. 1 **a** GC chromatogram of the standard mixture of petroselinic and oleic isopropyl esters and **b** FA isopropyl esters of seeds of wild ginseng from Spassky District (sample 6 in Table 1), separated on a DB-225 capillary column at 205 °C. *FID* flame ionization detector response

capillary column. A similar separation was achieved for DMOX derivatives of these acids. This separation provided mass spectra suitable for identification of the FA in seeds. The MS spectrum of the DMOX derivative of petroselinic acid is shown in Fig. 2. The fingerprint at *m/z* 167 is responsible for the location of a double bond at the Δ6 or n-12 position [15]. Consequently, the main FA in ginseng seed lipids was identified as petroselinic 18:1(n-12) acid. In all the studied samples, petroselinic acid was always accompanied by a lower concentration of oleic and *cis*-vaccenic acids. It looks surprising that petroselinic acid was not found in seeds of this very valuable medicinal plant previously. A number of articles, reviews, and the Internet database, providing information on the FA composition of seed oil indicate nearly ten species of the family Araliaceae that contain a significantly high level of petroselinic acid [16–18]. The main cause of misidentification of petroselinic and oleic acids can be their close retention times on common capillary columns for GC FAME analysis [14].

Total Lipid Content and Fatty Acid Composition

Table 1 presents data on the FA composition and total lipid content of seed samples from wild and cultivated ginseng, including two samples from the Korean plants with red and yellow berries. The mean total lipid content of seeds from three wild and five cultivated plants was 10.42 and 11.01 %, respectively. A similar value (10.2 %) was reported by Lee et al. [3]. Some articles mention even higher lipid contents, up to 26.6 % [4–7]. The significant variations in the total lipid content may be linked with the different presentation of data (for fresh or oven-dried seeds) or the special conditions used for the extraction procedure.

All the analyzed samples had petroselinic, oleic, and linoleic acids as the major components of ginseng seed lipids. The total content of unsaturated FA was higher than 90 %. Saturated acids were represented mostly by palmitic acid, 2.2–2.6 %. The level of stearic acid did not exceed 0.5 %. The combined content of all isomers of monoenoic acids, 18:1(n-12), 18:1(n-9), and 18:1(n-7) was close to 80 %. Polyenoic acids were represented mainly by linoleic acid 18:2(n-6) [15–16 %], as well as by 18:2(n-9) and 18:3(n-6) detected at low concentrations. Structures of these acids were confirmed by GC–MS of DMOX derivatives. The MS spectrum of 18:2(n-6) acids had gaps 12 amu at *m/z* 196–208, and 236–248, which are characteristic for double bonds at the Δ9 and Δ12 positions. The spectrum of 18:2(n-9) acid had a peak at *m/z* 167, typical for a double bond at the Δ6 position, and a gap 12 amu at *m/z* 194–206 characteristic for a Δ9 double bond. FA 18:3(n-6), having a level of about 0.1 %, was identified using GC–MS of methyl ester (molecular ion and peak at *m/z* 150, characteristic for polyenoic (n-6) acids) [15].

Linolenic acid 18:3(n-3), being very common in plants, was present only as a trace component (lower than 0.1 %), and for this reason, it was not included in Table 1. In general, our data on the FA compositions are very similar to those published in literature [3–5, 7]. The only distinction is the occurrence of petroselinic acid, which was considered in these articles as oleic acid. Oil from seeds of the American ginseng *P. quinquefolius* is distinguished from a

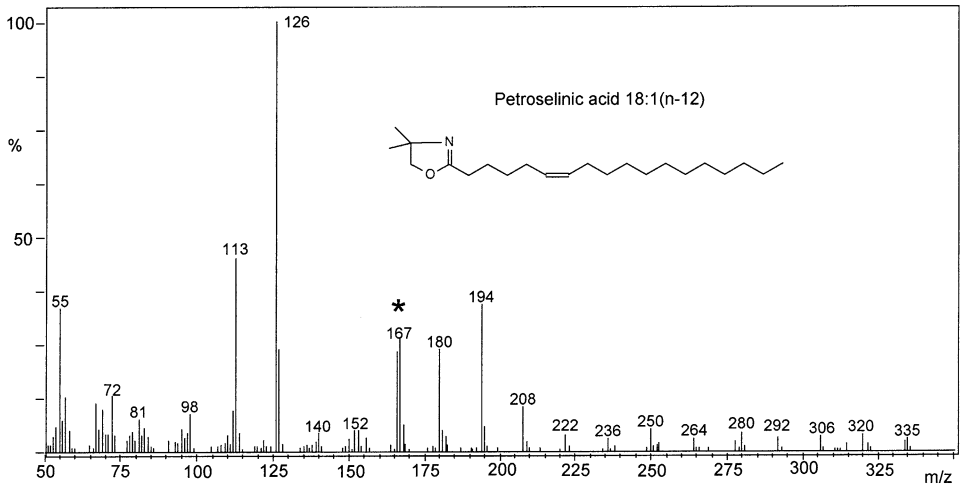

Fig. 2 Mass spectrum of the 4,4-dimethyloxazoline derivative of petroselinic acid from ginseng seed lipids. The peak at *m/z* 167 indicates the position of a double bond at the six carbon atom

Table 1 Lipid content (as a % of fresh seed weight) and FA composition (as % of total FAs) of seeds from cultivated and wild ginseng *P. ginseng*

FA	Cultivated ginseng seeds						Wild ginseng seeds			
	1	2	3	4	5	Mean ± STD	6	7	8	Mean ± STD
16:0	2.50	2.38	2.51	2.22	2.42	2.40 ± 0.12	2.31	2.22	2.17	2.23 ± 0.06
16:1(n-9)	0.19	0.17	0.16	0.16	0.14	0.16 ± 0.02	0.20	0.18	0.17	0.18 ± 0.01
16:1(n-7)	0.17	0.11	0.12	0.10	0.12	0.12 ± 0.03	0.12	0.09	0.12	0.11 ± 0.01
16:1(n-5)	0.27	0.24	0.22	0.25	0.31	0.26 ± 0.04	0.20	0.23	0.20	0.21 ± 0.01
18:0	0.42	0.33	0.39	0.30	0.39	0.36 ± 0.05	0.32	0.32	0.37	0.34 ± 0.02
18:1(n-12)	62.19	62.85	62.44	61.03	60.29	61.76 ± 1.06	62.38	59.61	61.64	61.21 ± 1.17
18:1(n-9)	16.10	15.56	16.52	16.96	17.43	16.51 ± 0.73	16.33	17.50	17.26	17.03 ± 0.51
18:1(n-7)	1.27	1.25	1.12	1.48	1.58	1.34 ± 0.19	1.08	1.64	1.03	1.25 ± 0.28
18:2(n-9)	0.71	0.70	0.67	0.72	0.67	0.69 ± 0.02	0.60	0.63	0.46	0.56 ± 0.08
18:2(n-6)	15.24	15.49	15.10	15.94	15.53	15.46 ± 0.32	15.69	16.89	15.58	16.05 ± 0.59
18:3(n-6)	0.14	0.14	0.13	0.13	0.12	0.13 ± 0.01	0.14	0.10	0.10	0.11 ± 0.02
20:0	0.27	0.12	0.04	0.54	0.53	0.30 ± 0.23	0.04	0.05	0.55	0.21 ± 0.24
20:1(n-9)	0.53	0.66	0.58	0.16	0.47	0.48 ± 0.19	0.60	0.54	0.38	0.51 ± 0.10
Sum 18:1 isomers	79.56	79.67	80.08	79.47	79.30	79.62 ± 0.29	79.79	78.75	79.93	79.49 ± 0.52
TL as % of fresh wt	11.53	11.74	9.32	10.50	8.98	10.42 ± 1.25	9.69	11.88	11.45	11.01 ± 0.95

(1) Seeds from red berries of Korean ginseng (laboratory greenhouse, 2012); (2) seeds from yellow berries of Korean ginseng (laboratory greenhouse, 2012); (3) Spassky District population, experimental nursery, 2011; (4) Spassky District population, experimental nursery, 2012; (5) Dalnegorsk District population, experimental nursery, 2012; (6) Spassky District, wild ginseng, 2011; (7) Spassky District, wild ginseng, 2012; (8) Spassky District, wild ginseng, 2012. Samples 6–8 were collected from different localities of taiga forest

P. ginseng seed oil by higher values of oleic acid (87 %) [5, 7]. It is very probable that these closely related species have similar FA compositions and both include petroselinic acid as the major monoenoic acid.

Petroselinic acid has been found in members of the families Umbelliferae (or Apiaceae), Araliaceae, Garryaceae, Aceraceae, and Geraniaceae [16–19]. This acid has a specific pathway of biosynthesis [8].

Conclusions

Our study demonstrates that petroselinic acid is a major component in lipids of ginseng seeds, as well as the similarity between seeds from wild and cultivated plants both in lipid content and fatty acid composition.

Compliance with Ethical Standards

Conflict of interest The authors don't have any conflicts of interest.

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