Δ5-Olefinic Acids in the Edible Seeds of Nut Pines (*Pinus cembroides edulis*) from the United States

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ABSTRACT: The fatty acid composition of the edible seeds from *Pinus cembroides edulis* (a nut pine), that might have been a commercial source of Δ5-olefinic acids in the United States, has been established. The dehulled seeds are rich in oil (64% by weight) and are characterized by high levels of both oleic and linoleic acids (*ca*. 47 and 41%, respectively), with only 10% of saturated acids. Δ5-Olefinic acids, which were not reported previously in this species, are exceptionally low as compared to most other conifer species: 5,9-18:2 acid, 0.1%; 5,9,12-18:3 acid, 0.4%; 5,11,14-20:3 acid, 0.3%. In this respect, *P. cembroides edulis* seed oil closely resembles that of *P. pinea* (a circum-Mediterranean species), which emphasizes the morphological and taxonomic relationship between the sections to which the two species belong (*Parryana* and *Pinea*, respectively).

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KEY WORDS: Nut pine seed oil, Δ5-olefinic acids, *Pinus cembroides edulis*.

Since the detailed analyses of fatty acids from Gymnosperm seed oils by Takagi and Itabashi (1), it has been known that they may contain some of a series of six Δ5-olefinic acids, depending on the family considered. These observations have been confirmed and extended more recently through several systematic studies of conifer seed oil fatty acids (2–5), and it is now recognized that Δ5-olefinic acids are characteristic components of this botanical class. They may even be used for the taxonomy of the main conifer families, and inside Pinaceae, of the main genera (5). The amount of Δ5-olefinic acids may vary from a low of *ca.* 3% [in *Pinus pinea* (2)] to a high of 33.9% [in *Larix sibirica* (5)].

Some conifer seed oils have been shown to favorably alter several lipid variables in the rat, and these effects have been reviewed recently (6,7). It was suggested that Δ5-olefinic acids could be used as new tools to explore lipid metabolism. It is thus interesting to have available such Δ5-olefinic acid-containing oils for further investigations. However, conifer seeds are seldom harvested, except for forest and ornament tree planting and sometimes, for edible purposes.

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In the United States and in Mexico, commercial edible pine seeds are those from species belonging to the subsection *Cembroides* (section *Parryana*, subgenus *Strobus*), which are called nut pines or piñons. Sagrero-Nieves (8) examined the fatty acid composition of the oil from *P. cembroides* (Mexican nut pine) seeds, and he apparently failed to detect Δ5-olefinic acids. This species might thus be an exception among *Pinus* species, and consequently, it would not be a good source of Δ5-olefinic acids. In the present study, we have investigated the fatty acid composition of the seeds from a subspecies of *P. cembroides*, *P. cembroides edulis* (two-leaved nut pine), which grows in the United States (Wyoming, Colorado, Texas) and in Mexico (Baja California, Chihuahua).

EXPERIMENTAL PROCEDURES

Seeds. *Pinus cembroides edulis* seeds collected in the United States were purchased from the Versepuy Society (Le Puy en Velay, France).

Oil extraction. The oil from the seeds (dehulled) was extracted mainly according to Folch *et al*. (9). The seeds were ground in a household electric grinder. An aliquot (10 g) of the resulting homogenate was dispersed in 50 mL methanol with an Ultra-Turrax T-25 (Janke & Kunkel GmbH and Co. KG, Staufen, Germany) equipped with an S-25N shaft. Chloroform (100 mL) was added, and the suspension was dispersed a second time. The suspension was then filtered through paper in a separatory funnel. The vessels and the residue on the filter were rinsed with several portions (total: 25 mL) of a chloroform/methanol (2:1, vol/vol) mixture. The clear filtrate was thoroughly mixed with 35 mL of a 1% (wt/vol) aqueous solution of KCl and allowed to stand for about 2 h. The lower phase was drained, the solvents were removed in a rotary evaporator at 50°C, and the oil was weighed.

Fatty acid methyl ester (FAME) preparation. FAME were prepared according to Morrison and Smith (10). Two drops of oil, introduced in a Teflon-lined screw-capping tube, were disolved in 1.5 mL of a methanolic solution of BF_3 (12%, wt/vol), and the mixture was homogenized with 1.5 mL benzene. The tubes were tightly capped, and the reaction was allowed to proceed for 1 h in a boiling waterbath. FAME were extracted twice with 2 mL hexane, with water (2 mL) being added to the mixture. The pooled upper organic phases were

dried over anhydrous Na_2SO_4. The FAME preparations were made in duplicate.

Gas–liquid chromatography (GLC). FAME were analyzed by GLC in a Carlo Erba 4130 chromatograph (Carlo Erba, Milano, Italy), equipped with a fused-silica DB Wax capillary column (30 m × 0.32 mm i. d., 0.5 μm film; J&W Scientific, Folsom, CA). The oven temperature was 190°C, and the inlet pressure of helium was 140 kPa. The injector (split mode) and the flame-ionization detector were maintained at 250°C. Quantitative data were calculated by an SP 4290 integrator (Spectra Physics, San Jose, CA).

Peak identification. Δ5-Olefinic acids were identified *via* their equivalent chainlengths (ECL) according to Wolff *et al*. (3). The use of ECL for identification was supported by identification through GLC–mass spectrometry of appropriate fatty acid derivatives (11).

RESULTS AND DISCUSSION

The oil content of the dehulled seeds was 64% on a weight basis. Its fatty acid composition is shown in Table 1. The main component was oleic acid (46.9% of total fatty acids), followed by linoleic acid (40.7%). Saturated acids (totaling *ca.* 10%) were 16:0, 18:0 (major), 20:0 (minor), with minute amounts (≤ 0.05%) of 14:0, 17:0, 22:0, and a branched acid tentatively identified as 14-methyl-16:0. We failed to observe lauric and myristic acids in amounts of 4.8 and 9.1%, respectively, as reported by Sagrero-Nieves (8) for *P. cembroides* seeds collected in Mexico. Δ5-Olefinic acids that could be identified on the basis of their ECL were 5,9-18:2, 5,9,12-18:3, and 5,11,14-20:3 acids, which are habitual in the seeds of all *Pinus* species analyzed until now (1–6). However, their contents were quite low: 0.1, 0.4, and 0.3%, respectively. It is not surprising that Sagrero-Nieves (8) failed to detect fatty acids in such a small amount in *P. cembroides* seed lipids. Consequently, *P. cembroides edulis* seed oil is not a good potential source of Δ5-olefinic acids.

Nevertheless, our analyses show that this species, like any other pine or conifer species, actually contains Δ5-olefinic acids, and is not an exception among Gymnosperms. To our knowledge, *P. cembroides edulis* presents the lowest level of Δ5-olefinic acids ever found in pine or conifer seed oils (1–6). In this respect, it resembles *P. pinea*, which grows in several countries around the Mediterranean sea, from Portugal to Syria. In the seed oil from this species, the contents of 5,9-18:2, 5,9,12-18:3, and 5,11,14-20:3 acids, are 0.1, 0.4, and 2.5%, respectively (2). This resemblance is noteworthy, because the two sections to which *P. cembroides edulis* and *P. pinea* belong (*Parryana* and *Pinea*, respectively) are considered, based on morphological criteria, as closely related (12). This is a supplementary example of the usefulness of the fatty acid compositions of seed oils as a complementary chemometric means for the taxonomy of conifers (4,5).

TABLE 1
Fatty Acid Composition of the Oil from *Pinus cembroides edulis* Seeds Collected in the United States

Fatty acid	ECL[a]	Weight %[b]
14:0	14.00	0.05
16:0	16.00	7.05
16:1[c]	16.26	0.18
br-17:0[d]	16.68	0.02
17:0	17.02	0.03
18:0	18.00	2.27
18:1Δ9	18.22	46.90
18:1Δ11	18.32	0.61
18:2Δ9,12	18.70	40.73
18:3Δ9,12,15	19.37	0.19
20:0	20.00	0.47
20:1Δ11	20.20	0.53
20:2Δ11,14	20.69	0.24
22:0	21.99	trace[e]
18:2Δ5,9	18.44	0.14
18:3Δ5,9,12	18.91	0.36
20:3Δ5,11,14	20.83	0.29

[a]Equivalent chainlength, calculated according to Reference 3, with 16:0, 18:0, and 20:0 acid methyl esters as standards.
[b]Data are the means of analyses of two fatty acid methyl ester preparations.
[c]Sum of two isomers. The ECL is that of the first-eluting isomer.
[d]Tentatively identified as 14-methylhexadecanoic acid by co-injection with an authentic standard.
[e]Trace amounts (peak visible on the chromatogram, but not taken into account by the integrator).

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