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Fatty Acid Composition of Seed Oil from *Fremontodendron californicum*

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Abstract

The fatty acid composition of the low water-use shrub *Fremontodendron californicum* was examined by high temperature capillary gas chromatography. The ground seeds were extracted by supercritical fluid extraction (SFE) to obtain the oil (25.6% w/w) and for subsequent determination of the fatty acid composition. There are five fatty acids present at 1.0% or greater with linoleic 71% of the total. Oleic, palmitic, stearic, vaccenic plus traces of palmitoleic and linolenic comprise the remainder. The fatty acid methyl ester composition would make the oil suitable for biodiesel production.

Keywords

Oilseed, Biodiesel, Fatty Acid Analysis, Xerophyte, Firewise Plant

1. Introduction

The shrub *Fremontodendron califomicum* is a member of the plant family Sterculiaceae [1] [2], and is a member of the sub-family Fremontiaceae. The shrub is native to California and the southwestern U.S. and is commonly found in desert areas and chaparral. It is a low-water use plant and is used in xerophyte and firewise landscaping [3]. As a shrub, the plant can grow to 1 - 4 m, and as a tree, the plant can achieve heights of 10 m [1] [2].

Among the plant families, the seed oils of plants in the Sterculiaceae contain a broad array of fatty acids with uncommon functional groups, including acetylenic, cyclopropenoic and hydroxyl groups [4]. We have carried out research to evaluate oilseed crops for potential industrial uses [5] with the goal of identifying crops with unusual fatty acid composition. Such plants may be sources of genetic material for industrially useful fatty acids or, given research interest, may be developed as crops. We describe here our

findings for *F. californicum*.

2. Materials and Methods

2.1. Oil Extraction

Fremontodendron californicum seeds were obtained commercially from the Theodore Payne Foundation, Sun Valley, CA. The seeds were prepared for supercritical fluid extraction (SFE) by first processing the seed through a coffee grinder, Braun model #KSM2. The ground seeds were then extracted by SFE to obtain their oil. The supercritical extraction system consisted of the following components: 260 ml syringe pumps (Model 260D, Isco, Inc., Lincoln, NE), liquid CO_2, chilled to 5˚C with a circulating water jacket; a temperature-controlled dual-chamber extractor (IscoModel SFX 220) where cartridges containing sample were maintained at 80˚C for optimum solubilization of lipids in supercritical CO_2 [6]. A fixed volume (1.5 ml) insulated coaxially heated capillary restrictor heated to 100˚C controlled the CO_2 flow as it emerged from the extractor to prevent clogging of the restrictor. The CO_2 was vented into pre-weighed screw capped 20 × 150 mm tubes containing ethanol. Coleman-grade liquid CO_2 was pressurized to 7500 psi (517 bar) in the 260 ml syringes prior to its use. Samples were extracted for 20 minutes, with an estimated total volume of 72 ml (64 g) of supercritical CO_2 for each sample. Extracted total oil was determined after evaporating the ethanol under nitrogen. The apparatus is diagrammed in Figure 1.

2.2. Derivatization of Lipids

Fatty acid methyl esters (FAMES) were made by saponification of the oil with 0.5 N KOH in methanol at 90˚C for 6 min. After neutralization with HCl, extraction with hexane and evaporation under nitrogen, fatty acids were methylated in 14% boron trifluoride in methanol (Alltech, Deerfield, IL) for 6 min. at 90˚C. After addition of water, the FAMES were extracted with hexane and stored at −20˚C until GC analysis or for further derivatization. Pyrrolidide derivatives of the FAMES were made and purified as

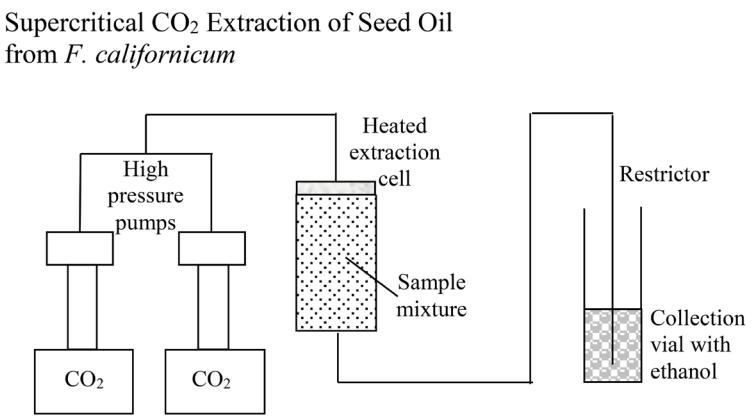

Figure 1. Diagram of the supercritical CO_2 extraction system. Seeds from *F. californicum* were ground to a powder in a coffee grinder, mixed with diatomaceous earth and placed in the extraction vial. The extracted oil was collected and treated as described in Materials and Methods.

described [7], using Superclean Envi-florisil 3 ml tubes (Supelco, Inc., Bellefonte, PA) eluted with 10 ml diethyl ether instead of hexane/diethyl ether.

2.3. Fatty Acidmethylester Identification

The fatty acid methyl esters from the SFE extraction were tentatively identified with a Hewlett Packard 6890 gas chromatograph equipped with a flame ionization detector and split/splitless capillary injection system (model 8835B; Hewlett Packard). A fused silica capillary column (30 m × 0.25 mm) coated with Stabilwax CW20M (df = 0.25 μm; Restex) was used. Detector and injector temperature were 320˚C and 260˚C, respectively. Initially, oven temperature was 60˚C and was held for 1 min, then increased by 25˚C/min to 200˚C. The ramp was slowed to 2˚C/min until 250˚C and held for 10 min. Hydrogen (10 psi) was the carrier gas, and splitless injection was used.

All fatty acid methyl esters and pyrrolidide derivative identifications were confirmed by mass spectrometry with a Hewlett-Packard 5970A quadrupole-based mass selective detector (MSD) coupled to a 5890 Series II gas chromatograph with a capillary direct interface operated at 280˚C. A fused silica capillary column (20 m × 0.18 mm I.D.) Coated with DB-WAX (0.3 μm; J & W Scientific) was used to separate both derivatives using different temperature programs. Helium was the carrier gas with EPC set at constant flow and splitless injection (260˚C). In the temperature program used for FAMES, helium flow was (0.6 ml/min) and the oven temperature was initially held at 70˚C for one minute, then ramped to 220˚C at 25˚/min, then 5˚C/min to 250˚C and held for 30 min. The temperature program for pyrrolidide derivatives was initially 60˚C for one minute, followed by increase to 240˚C at 25˚C/min, then 2˚C/min to 260˚C where the temperature was held for 20 min. The MSD was operated in the scan mode. Ionization voltage was fixed at 70 eV, with the ion source operated at the fixed nominal temperature of 250˚C.

3. Results and Discussion

The amount of total oil extracted from *F. califomicum* seed as shown in **Table 1** was 25.6% (w/w). There are five fatty acids present at 1.0% percent or greater. The fatty acid complement did not include any fatty acids with uncommon functional groups. *Cis*-vaccenate, (octadec-*cis*-11-enoate) (l.6%) is present as a trace component in many plant oils and is present in a few oils, e.g. milkweed, at higher levels [8]. The linoleic acid content (71.2%) is high, but similar to levels observed in corn, safflower and sunflower oil [9].

Table 1. Fatty acid composition of *F. californicum* per cent.

Palmitate 16:0	Palmitoleate $16:1\Delta^{9}$	Stearate 18:0	Oleate $18:1\Delta^{9}$	Vaccenate $18:1\Delta^{11}$	Linoleate $18:2\Delta^{9,12}$	Linolenate $18:3\Delta^{9,12,15}$
6.9	Trace	3.3	16.3	1.6	71.2	Trace

Fatty acid analysis was carried out as described in Materials and Methods. Results are from duplicate determinations with separate seed samples and were in agreement ±2%. Oil content of the seed was 25.6% ± 0.4%.

Since there were no uncommon fatty acids present in the oil of *F. californicum*, it does not appear to be a source of potentially useful genes for producing industrial-use fatty acids. However, the fatty acid composition is consistent with use in producing biodiesel after methanolysis of the triacylglycerols. Although high polyunsaturated fatty acid content is not desirable from the standpoint of cetane number, such fatty acids do improve the cold-flow properties of biodiesel [10]. Fatty acid methyl esters derived from *F. californicum* oil could therefore be of value as biodiesel additives. Conversion to fatty acid methyl esters can be carried out conventionally using acid or base catalyzed methanolysis or, as we have previously demonstrated, using SFE combining CO_2 and MeOH with immobilized lipase to produce methyl esters [8]. Use of SFE coupled with immobilized lipase-catalyzed transesterification is an especially valuable method for FAME production from oils containing reactive functional groups.

F. californicum is useful in preventing erosion on hillsides, in designing fire safe landscapes and in drought-tolerant landscaping. It produces large quantities of seed [2] and if the seed could be suitably harvested, the plant would provide the added benefit of an oil that could serve as a feedstock for biodiesel.

4. Conclusion

The shrub *F. californicum* is useful as a freeway median strip planting in areas with very limited water supply, such as the California Central Valley and desert areas. Although usually grown as a decorative, its low to no requirement for irrigation and its low flammability make it an ideal plant for such areas. Moreover, further development of the plant seed as a source of oil could make it a productive plant on these areas that are currently unutilized for productive agriculture. The oil obtained from these seeds would provide a useful source of biodiesel if the plant can be bred into a productive crop.

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