Characterization of Triglycerides Isolated from Jojoba Oil

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ABSTRACT: Triglyceride compounds isolated from jojoba seed oil by column chromatography were composed predominantly of C_{18}, C_{20}, C_{22}, and C_{24} n-9 fatty acids with minor amounts of saturated C_{16}. Chain length and double-bond positions were determined by gas–liquid chromatography and gas–liquid chromatography–mass spectrometry of the corresponding methyl ester and picolinyl ester derivatives. Triglyceride structures were analyzed directly by ion trap mass spectrometry. The analysis of minor compounds can provide highly specific information about the identity of an oil.

Paper no. J9530 in *JAOCS 77,* 1325–1328 (December 2000).

KEY WORDS: GC–MS, ion trap MS, jojoba, triglycerides, wax ester.

Jojoba (*Simmondsia chinensis*) is a desert shrub of the American Southwest and northwest Mexico that is cultivated as a specialty oil crop for its long-chain wax esters. The jojoba seed typically contains over 50% by weight of a mixture of liquid wax esters with applications in lubricant and personal-care formulations (1,2). The major components of jojoba seed extracts have been reported (3,4). Certain minor components, such as phytosterols and fatty alcohols, have been reported more recently (5,6). Trace amounts of triglycerides have been mentioned, although these structures have not been described in detail (5). Identification of naturally occurring triglyceride compounds in jojoba oil is of fundamental interest in completing the characterization of jojoba oil. Analysis of minor compounds (triglycerides) is also of practical significance to distinguish between natural and synthetic jojoba oil. The described methodology allows also the study of the influence of different *S. chinensis* strains, and of the influence of the time of harvest or production methods on the qualitative and quantitative triglyceride composition.

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EXPERIMENTAL PROCEDURES

Materials. Jojoba oil was obtained by extraction of flaked jojoba nuts with hexane (7). Diethyl ether and hexane used in the chromatographic work were obtained from Aldrich Chemical Co. (Milwaukee, WI). Reagents used for the derivatization reactions, e.g., trifluoroacetic anhydride, 4-dimethylaminopyridine and 3-hydroxymethylpyridine, were obtained from Aldrich Chemical Co.

Isolation of jojoba oil triglycerides. Jojoba oil was fractionated as previously described to separate minor compounds from the predominant jojoba wax esters (6). Elution from an aluminum oxide column with hexane followed by acetone provided a series of fractions that were examined by thin-layer chromatography (TLC) to detect minor components.

Fractions exhibiting distinct zones by TLC were collected, concentrated, and further separated on a 90×3.5 cm i.d. glass column packed with 230–400 mesh silica. The column was eluted with hexane/diethylether, 97:3 (vol/vol), and the eluant collected in 10-mL fractions. The elution pattern was again examined by TLC. Selected fractions showing one single spot by analytical TLC were collected and used for further identification purposes.

Analytical TLC. Analytical TLC was performed on precoated silica gel plates, 40×80 mm (Polygram Sil G/UV 254; Machery-Nagel, Düren, Germany), and eluted with hexane/diethylether/formic acid, (80:20:1, by vol). Compounds were localized by spraying the plates with a mixture of concentrated sulfuric acid in ethanol (50:50, vol/vol) and heating the plates in a 110°C oven for 15 min.

Preparation of fatty acid methyl esters (FAME). Methyl esters were prepared by adding 2 mg of the isolated sample to 0.5 mL of anhydrous diethyl ether and 20 µL of methyl acetate (8). Transesterification proceeded with the addition of 50 µL 2N NaMeO in methanol. After 30 min at 20°C, the reaction was quenched by the addition of 2 µL acetic acid. The solvent was removed under a stream of nitrogen, and the residue was redissolved in 1 mL hexane, sonicated, and centrifuged at $1{,}090 \times g$ for 2 min. The supernatant was decanted, concentrated, and analyzed.

Preparation of fatty acid picolinyl esters. Picolinyl esters were prepared by adding 5 mg of the hydrolyzed triglyceride

sample to 0.5 mL trifluoroacetic anhydride (8). The mixture was sonicated for 1 min and heated to 50°C for 30 min. The reagent was evaporated under a stream of nitrogen. The cooled residue was redissolved in a solution of 20 mg 3-hydroxymethylpyridine and 4 mg dimethylaminopyridine in 0.2 mL methylene chloride. The reaction proceeded for 3 h at 20°C. The solvent was evaporated under a stream of nitrogen and the residue was redissolved in 8 mL hexane. The organic layer was washed twice with 4 mL of water, centrifuged, and concentrated for analysis. The utility of these derivatives is attributable to the fragmentation at each carbon-carbon bond under electron impact, the resulting spectra indicate both position and number of double bonds (9,10).

Gas chromatography–flame-ionization detection (GC–FID) of FAME and picolinyl esters. Analysis of the FAME was performed on a Chrompack 9000 gas–liquid chromatograph using a CP Sil-24 capillary column, 40 m × 0.2 mm, with a 0.33 µm film thickness (Chrompack, Antwerp, Belgium). Nitrogen was used as the carrier gas at a flow rate of 2 mL/min. The injector was set to 300°C and operated at a split ratio of 1:20. The oven was programmed for 5 min at 100°C and then increased to 295°C at 10°C/min. Column eluents were detected by FID, with the detector temperature at 300°C.

GC-mass spectrometry (MS) of FAME and picolinyl esters. Samples were analyzed on a Hewlett-Packard 5890 Series II Gas Chromatograph equipped with a 5971A Mass Selective detector and using an HP-1 capillary column, 30 m × 0.2 mm, with a 0.25 µm thick coating of methylsilicone gum. Helium was used as the carrier gas at a flow rate of 2 mL/min with the injector set to splitless mode. The oven was programmed from 50 to 100°C at 35°C/min and then from 100 to 270°C at 10°C/min. The detector was operated at 70 eV in scanning mode over the range of 50–500 AMU.

Ion trap MS. The mass spectrometric data of the isolated triglyceride fractions were acquired on a LCQ Ion Trap Mass Spectrometer (Finnigan MAT, San Jose, CA) equipped with an atmospheric pressure chemical ionization source (APCI) operated in the positive ion mode. The APCI conditions were as follows: vaporizer temperature 350°C; sheath gas flow rate 40 units; auxiliary gas flow rate 10 units; discharge current 5 µA; capillary voltage 4 V; capillary temperature 150°C; and tube lens offset +30 V. Nitrogen supplied by a Nitroprime TM membrane unit, type SNIFF (AGA, Lidingö, Sweden) was used as sheath and auxiliary gas. A Pentium II (Gateway 2000, North Sioux City, SD) equipped with the standard LCQ software package was used for instrument control, data acquisition, and processing. The solution of 5 mg sample in 1 mL of hexane (5 µg/µL) was introduced in the LCQ by direct infusion with the built-in syringe pump at a flow rate of 10 µL/min.

RESULTS AND DISCUSSION

Isolation of triglycerides from jojoba oil. A minor fraction (about 0.4%, calculated by weight) of triglycerides was obtained from jojoba oil after separation from the wax esters and other lipid-soluble components by column chromatography. This isolated fraction showed an R_f value (0.58) on TLC (silica gel: eluent hexane/diethyl ether/formic acid, 80:20:1, by vol), identical to standard corn triglycerides. Other R_f values of the isolated fraction along with the R_f values of several other classes of compounds isolated from jojoba oil are as follows: wax esters, 0.95; free fatty acids, 0.48; β-sitosterol, 0.19.

Characterization of triglycerides by ion trap MS. The mass spectrum obtained by ion trap MS from the jojoba triglyceride fraction (Fig. 1) clearly demonstrates the triglyceride structure of the compounds in the isolated fraction. The mass spectrum is characterized by the presence of different $(M + H)^+$ ions, each differing by 28 amu and representing the different triglycerides present in the isolated fraction. Those different

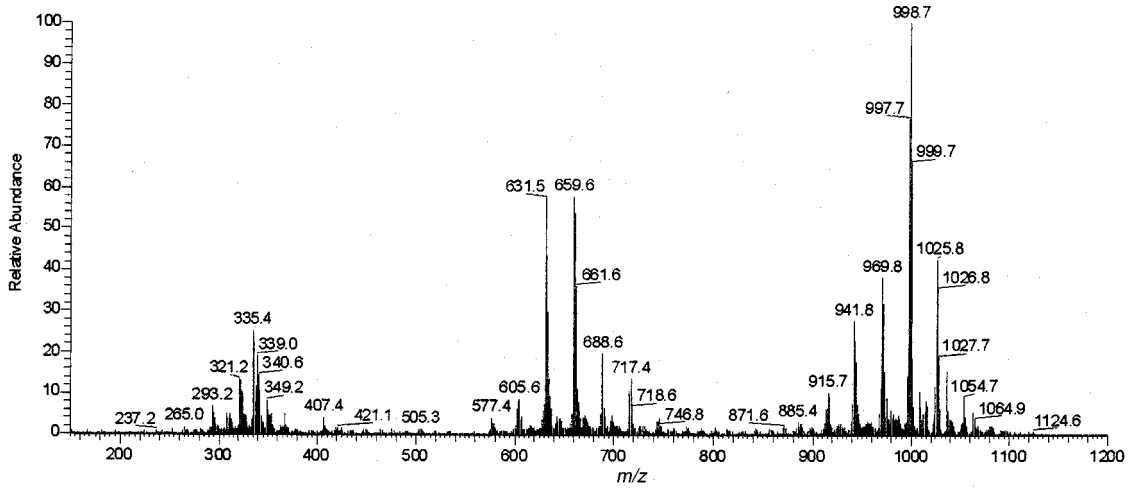

FIG. 1. Mass spectrum of jojoba triglycerides, obtained with an ion trap mass spectrometer equipped with an atmospheric pressure chemical ionization source.

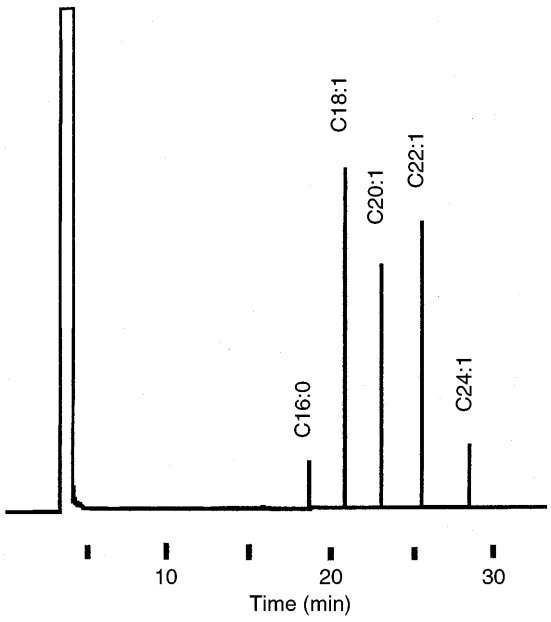

FIG. 2. Gas chromatogram of the fatty acid picolinyl esters from jojoba triglycerides. Conditions: CP Sil-24 column, 40 m × 0.2 mm; injection at 100°C; temperature programmed to 295°C at 10°C min^{-1}.

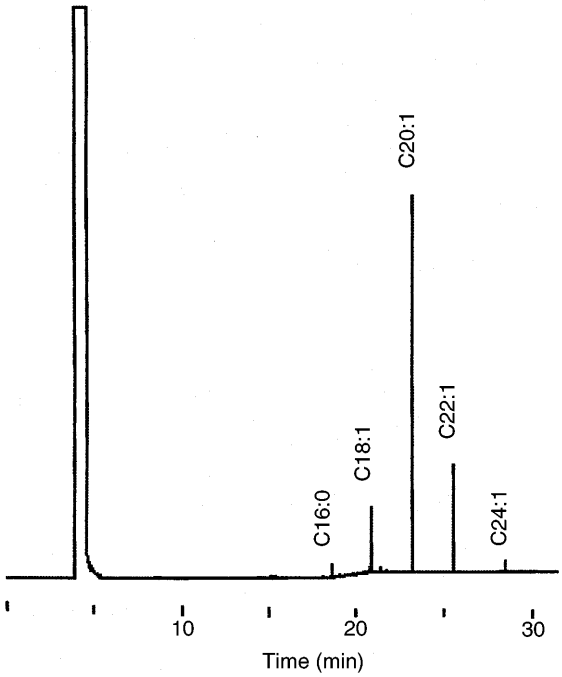

FIG. 3. Gas chromatogram of the fatty acid methyl esters from jojoba wax esters. Conditions as for Figure 2.

ions are seen at *m/z* 941, 969, 997, 1025, and 1053, corresponding to the different combinations of the mono-unsaturated C_{18}, C_{20}, C_{22}, and C_{24} fatty acids. The fragments at *m/z* 915, 943, 971, and 999 can indicate the presence of the saturated C_{16} acid in some triglycerides. Loss of the RCOO– fragments (281, 309, 337, and 365, respectively) gives rise to the important fragment ions $(M - RCOO)^+$ at *m/z* 631, 659, 689, and 717.

Characterization of the fatty acids of the isolated triglycerides. Capillary GC of the FAME prepared by the described transesterification procedure indicated the presence of five fatty acids with the relative composition shown by the gas chromatogram in Figure 2. This separation is based on chain length and polarity and allows the resolution of unsaturated fatty acids from the saturated analogs. The relative composition of fatty acids in the isolated triglyceride fraction is very different from the composition of the fatty acids present in jojoba wax esters and in the traces of free fatty acids present in jojoba oil (3,5). These fractions contain the same C_{16}, C_{18}, C_{20}, C_{22}, and C_{24} fatty acids, although in different proportions. The fatty acid profile from the isolated triglyceride fraction, presented in Figure 2, may be compared to that for jojoba waxes presented in Figure 3.

The FAME showed mass spectra with molecular ions at *m/z* 270, 296, 324, 352, and 380, corresponding to the retention times of 15.2, 17.02, 18.9, 20.7, and 23.09 min. These times indicated the presence of a C_{16} saturated fatty acid and the C_{18}, C_{20}, C_{22}, and C_{24} mono-unsaturated fatty acids, respectively. The results of the GC–MS analysis of the FAME and the fatty acid picolinyl derivatives from jojoba triglycerides are given in Table 1.

The spectra obtained for the FAME provide information on chain length and degree of saturation but do not allow the precise localization of double bonds. Location of the double bonds in the respective fatty acids was obtained by the analysis of the corresponding fatty acid picolinyl derivatives.

Capillary GC of the picolinyl esters also showed the presence of five peaks as in the gas chromatogram from the FAME, and in the same relative proportions. GC–MS analysis of this fraction showed molecular ions for all of the picolinyl esters, indicating the presence of the saturated fatty acid ($C_{16:0}$) and the mono-unsaturated fatty acids ($C_{18:1}$, $C_{20:1}$, $C_{22:1}$, and $C_{24:1}$). The fragmentation pattern also allowed us to locate the exact position of the double bonds in the unsaturated fatty acids because of the fragmentation at each carbon-carbon bond under electron impact ionization.

The mass spectrum of the $C_{18:1}$ picolinyl ester exhibited $(M + H)^+$ at *m/z* 373 (monounsaturated) and a base peak at *m/z* 92 containing the pyridine ring. The fragment *m/z* 344

TABLE 1
Gas Chromatographic Retention Times (Rt) and Molecular Weights[a] of Fatty Acid Methyl Esters (FAME) and Fatty Acid Picolinyl Esters from Jojoba Triglycerides

	FAME		Picolinyl esters	
Compound	Rt (min)	M^+ (amu)	Rt (min)	M^+ (amu)
16:0	15.21	270	22.45	347
18:1n-9	17.06	296	25.61	373
20:1n-9	18.91	324	30.45	401
22:1n-9	20.76	352	38.57	429
24:1n-9	23.09	380	48.08	457

[a]The results were obtained by the gas chromatography–mass spectrometry method described in the Experimental Procedures section.

corresponds to M – 29. Additional fragments occurred, each time separated by 14 amu ($–CH_2$), up to the fragment at *m/z* 260 and starting again at *m/z* 234 (–26 msu). This change in fragmentation pattern indicates the location of the double bond, in the present case, at n-9. For the C_{20} monoene with $(M + H)^+$ at *m/z* 401, the fragmentation pattern is analogous. The consecutive $–CH_2$ fragmentations stop at *m/z* 288 and start again at *m/z* 262, demonstrating again the location of the double bond at n-9. The spectrum of the fourth peak shows $(M + H)^+$ at *m/z* 429 corresponding to the picolinyl ester of the C_{22} monoene fatty acid with a pattern of consecutive –14 fragmentations up to *m/z* 316 and then starting again from *m/z* 290. Similarly for the $C_{24:1}$ picolinyl ester, the spectrum shows $(M + H)^+$ at *m/z* 457 and the same fragmentation pattern up to *m/z* 344, and then starting again at *m/z* 318. The mass spectrometric data obtained clearly prove the n-9 nature of the four monounsaturated fatty acids present in the isolated triglyceride fraction. The spectrum of the C_{16} saturated derivative displayed $(M + H)^+$ at *m/z* 343 and only consecutive fragments separated by 14 amu as expected for a saturated fatty acid.

The fatty acid profile of the isolated triglyceride fraction obtained is completely different from the profiles from any known fat or oil (11) and allows for the differentiation between natural oil and synthetic jojoba oil, containing no triglycerides, or synthetic jojoba oil adulterated with a low quantity of a common triglyceride oil. The described method for the analysis of triglycerides in jojoba oil is also suited for the study of the triglyceride composition in different batches of jojoba oil or for the study of the influence of different production methods, time of harvest, or jojoba strain on the jojoba oil triglyceride composition.

ACKNOWLEDGMENTS

This work was supported by research grants from the Research Board of the Katholieke Universiteit Leuven, Project OT/35/99.

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[Received February 7, 2000; accepted August 28, 2000]