Determination of Vernolic Acid Content in the Oil of *Euphorbia lagascae* by Gas and Supercritical Fluid Chromatography

Christina Borch-Jensen* and Jørgen Mollerup

Department of Chemical Engineering, Technical University of Denmark, 2800 Lyngby, Denmark

ABSTRACT: Determination of the content of vernolic acid (12,13-epoxy-9*c*-octadecenoic) in the oil of *Euphorbia lagascae* has been performed by gas chromatography of the fatty acid methyl ester derivatives of the triacylglycerols in the oil and by supercritical fluid chromatography (SFC) of the raw oil and the fatty acid derivatives of the oil. The content of vernolic acid was found to be 55 wt%. The three methods were compared, and SFC analysis of the fatty acid derivatives was found to be the most accurate method.
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KEY WORDS: Epoxy fatty acid, fatty acid methyl esters, free fatty acids, gas chromatography, supercritical fluid chromatography, vernolic acid.

The new crop seed oil, *Euphorbia lagascae* oil, contains the epoxy fatty acid (FA) vernolic acid, 12,13-epoxy-9*cis*-octadecenoic acid (1). Vernolic acid also predominates in *Vernonia* species (2). Vernolic acid-containing oils are of interest for possible use in paints and coatings. Vernolic acid-containing oils are natural sources of reactive epoxy FA and relatively low-viscosity epoxy oils. Gas chromatography (GC) of fatty acid methyl esters (FAME) (3), derived from the oil, provides the FA composition of the triacylglycerols but no information on the composition of any free FA and triacylglycerols present in the oil. The preparation of FAME of epoxy FA-containing oils should be done by base-catalyzed transmethylation because acid will disrupt the epoxy groups (3). Base-catalyzed methylation will, however, not transesterify any free FA present in the oil. Methylation of free FA should be done in a separate step by diazomethane (3), a method with many disadvantages including extremely toxic chemicals and expensive equipment. Quantitative GC analysis of the raw oil is not possible because the high temperatures needed to elute the triacylglycerols will disrupt the epoxy groups and the double bonds in the FA.

In this paper, we used supercritical fluid chromatographic (SFC) methods to analyze *E. lagascae* oil. Analysis of the raw oil gives the content of the free FA, including vernolic acid, and the triacylglycerols present in the oil. Total vernolic acid content is calculated from a mass balance and is also determined by saponification of the raw oil, followed by extraction and analysis of the FA SFC without further derivatization. The results of the two methods are compared to the result from the FAME analysis.

*To whom correspondence should be addressed at Department of Chemical Engineering, Technical University of Denmark, 2800 Lyngby, Denmark.

EXPERIMENTAL PROCEDURES

Materials. The *E. lagascae* oil was supplied by Ole Henriksen, FLS Miljø (Valby, Denmark). Ethanol, methanol, diethyl ether, tetrahydrofuran (THF), acetic acid, and hexane, all 99%, 5 M HCl, KOH (85%), $NAOCH_3$ (97%), and Na_2SO_4 (99%) were supplied by Merck (Darmstadt, Germany).

Apparatus. The SFC used in this work was an HP-SFC (Hewlett-Packard, Avondale, PA), equipped with an HP-7673 autosampler and a flame-ionization detector (FID). The GC used in this work was an HP-GC 5880A, equipped with an FID detector.

Preparation of the FA derivatives. Oil (100 mg) was placed in a screw-capped test tube, and 2 mL of 1 M KOH in 95% ethanol was added. The mixture was allowed to react for 2 min at 45°C; then 5 mL water and 5 mL diethyl ether were added; and the aquatic layer containing the saponifiables was extracted. The aquatic layer was titrated to pH 6.5 by dropwise addition of 5 M HCl. Extreme care should be taken at this point to avoid excess acidity in the mixture. The FA were extracted twice with 5-mL portions of diethyl ether. The combined extracts were dried by evaporation under nitrogen and redissolved in 1.5 mL diethyl ether. The samples were analyzed by SFC.

Preparation of the FAME derivatives. Oil (50 mg) was placed in a screw-capped test tube, and 1 mL THF was added, followed by 2 mL of 0.5 M $NAOCH_3$ in dry methanol. The mixture was allowed to react for 10 min at 50°C, and then 0.1 mL acetic acid and 5 mL water were added. The FAME were extracted twice with 5-mL portions of hexane. The combined extracts were dried with Na_2SO_4, and the solvent was removed by evaporation under nitrogen. The FAME were redissolved in 1 mL of hexane, and the sample was analyzed by GC.

GC method for the analysis of FAME. Injection and detection temperatures were 250°C. The column used was a polar capillary HP-FFAP (Hewlett-Packard) column (25 m × i.d. 0.3 mm), and the coating thickness was 0.23 μm. The oven temperature was programmed from 140 to 220°C at a rate of 3°C/min. The final temperature of 220°C was held for 20 min. The total analysis time was 47 min.

SFC method for the analysis of FA. The oven temperature was 110°C, and the FID temperature was 350°C. The column used was a 20-m × 100-μm i.d. polar capillary DB-225 column (J&W Scientific, Folsom, CA). The density was programmed from 0.2 to 0.32 g/mL at a rate of 0.002 g/mL/min. The total analysis time was 60 min. This density program was not optimized with respect to analysis time.

SFC method for the analysis of raw oil. The same column for SFC of FA was used with an oven temperature of 170°C and the FID temperature at 350°C. The density was programmed from 0.3 to 0.5 g/mL at a rate of 0.003 g/mL/min. The final value of 0.5 g/mL was maintained for 23 min. The total analysis time was 90 min. This density program was not optimized with respect to analysis time.

Identification of triacylglycerols. Because standards of mono-, di-, and triepoxy triacylglycerols were unavailable, these components were identified by the following method. Raw oil, dissolved in *n*-heptane, was applied as a band onto a 20 × 20 cm Si60 silica TLC plate (Merck). The plate was developed in petroleum ether/diethyl ether/acetic acid (70:30:3, vol/vol/vol). This separated the oil according to the number of epoxy groups in the molecules. The normal triacylglycerols had the largest retention factor, and the triepoxy triacylglycerols the smallest. The bands containing the mono-, di-, and triepoxy triacylglycerols were recovered separately from the plate and analyzed by SFC.

RESULTS AND DISCUSSION

The chromatogram from the SFC analysis of the raw oil is shown in Figure 1. Normal free FA and free vernolic acid are separated at approximately 15 and 18 min, respectively, followed by the normal triacylglycerols (*ca.* 30–40 min) and triacylglycerols containing 1 (*ca.* 40–48 min), 2 (*ca.* 49–55 min) and 3 epoxy FA (trivernolin, *ca.* 58 min) on the glycerol backbone. The approximate vernolic acid content in the oil can be

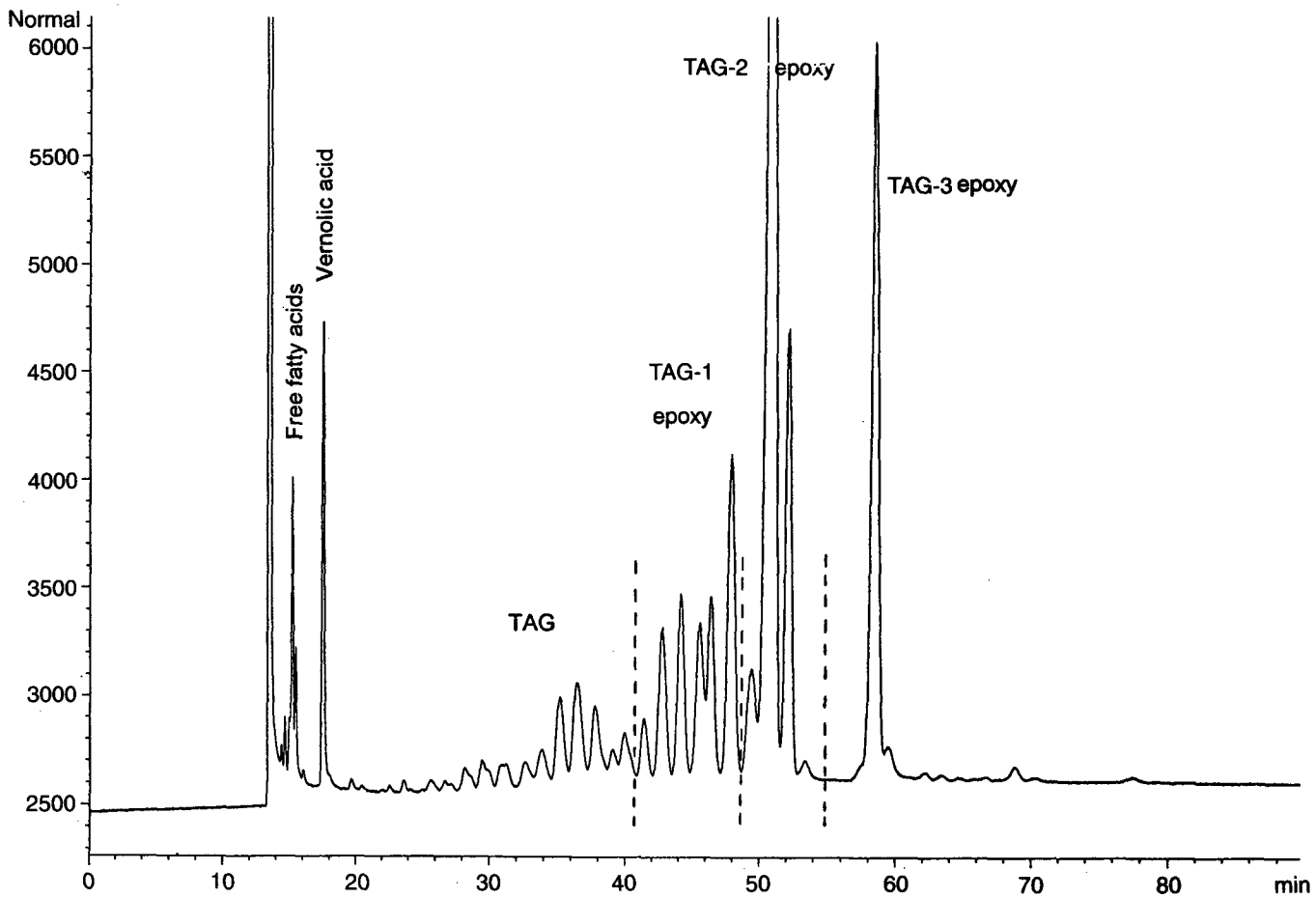

FIG. 1. Chromatogram from capillary supercritical fluid chromatography analysis of the raw oil of *Euphorbia lagascae.* TAG, normal triacylglycerols; TAG–*n* epoxy, triacylglycerols with *n* (*n* = 1–3) epoxy fatty acids.

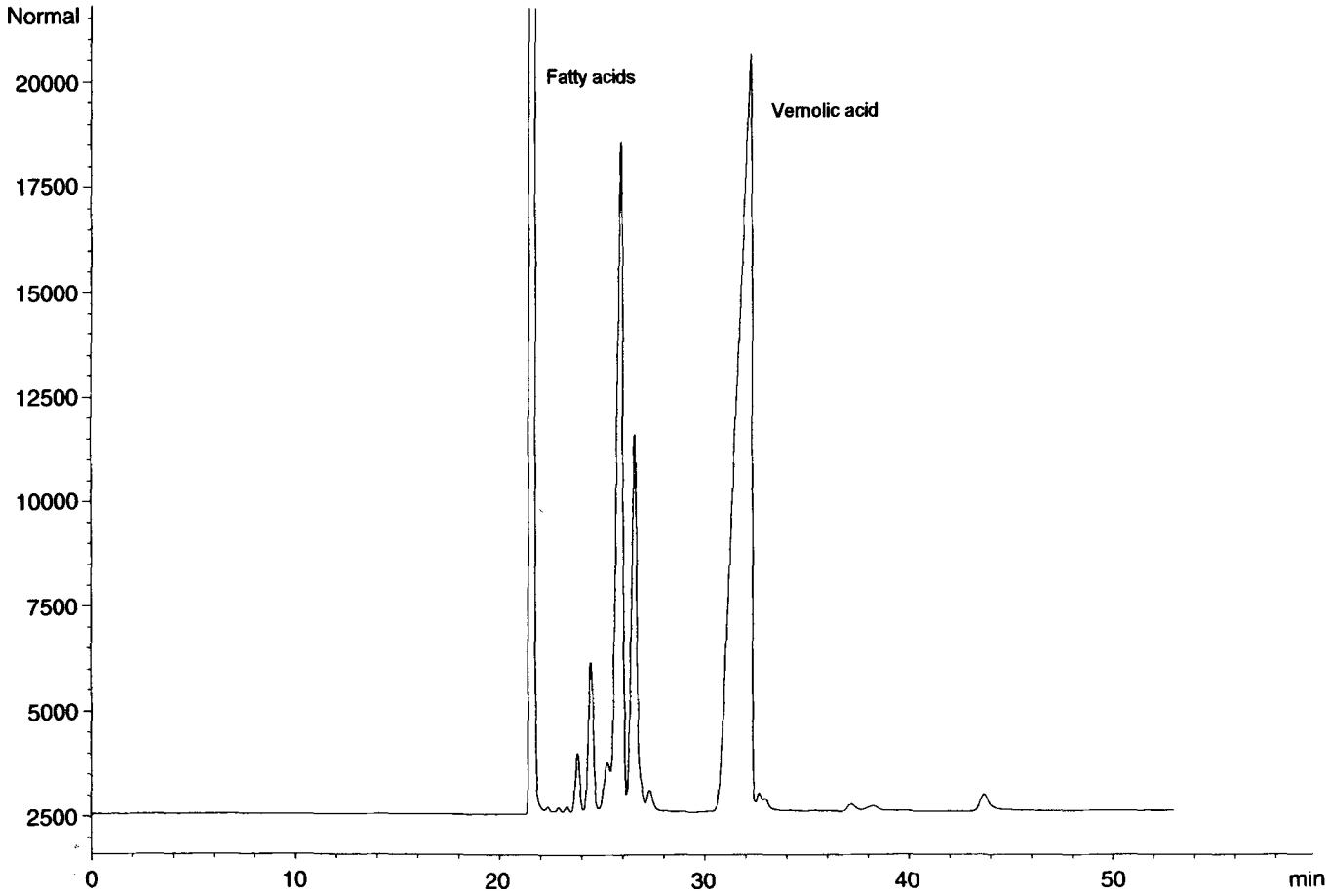

FIG. 2. Chromatogram from capillary supercritical fluid chromatography analysis of the total fatty acids of *Euphorbia lagascae* oil.

calculated by adding the contributions of vernolic acid in the free FA and in the triacylglycerols that contain 1, 2, and 3 vernolic acids on the glycerol backbone.

Figure 2 presents the FA analysis by SFC. The normal FA elute first in the chromatogram, while vernolic acid, the major peak, elutes as the last peak. The content of vernolic acid is found directly from the area of the major peak.

In Table 1, the results from the three analyses, GC of the FAME, SFC of the raw oil, and SFC of the FA, are presented. The results from the two SFC methods agree, although the SFC result of raw oil is only approximate. The vernolic acid content from the GC analysis of the FAME is lower than the two other results, because the free FA present in the oil were not derivatized. The percentage of free vernolic acid found by SFC of the raw oil is 2 wt%, and adding this to the vernolic acid content obtained by GC analysis of the FAME results in agreement among the three results. Muuse *et al.* (1) have reported a vernolic acid content (GC of FAME) in the oil of *E. lagascae* of 64 wt%. This high level may be due to the fact that available *E. lagascae* selections do not yet have uniform vernolic acid contents. *Euphorbia* breeding is actively being pursued.

The SFC analysis of the raw oil gives the overall composition of the oil from which an approximate vernolic acid content can be calculated. The SFC analysis of the total FA derived from the oil probably gives a more accurate vernolic acid content. The GC method for FAME gives only the vernolic acid content in the triacylglycerols, and has erratic results if the oil has a high free FA content. The two SFC methods give both the free FA and triacylglycerol composition and the total vernolic acid content.

TABLE 1
Vernolic Acid Content of *Euphorbia lagascae* Seed Oil as Found by GC of FAME, SFC of Raw Oil, and SFC of FA[a]

Method	% Vernolic acid
GC FAME	53.5[b]
SFC Raw oil	54.9
SFC FFA	55.3

[a]GC, gas chromatography; FAME, fatty acid methyl ester; SFC, supercritical fluid chromatography; FA, fatty acid.
[b]Does not include any free fatty acids present in the raw oil.

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