Gas Chromatographic Analysis of Simmondsins and Simmondsin Ferulates in Jojoba Meal

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A capillary gas chromatographic method was developed for the simultaneous determination of simmondsins and simmondsin ferulates in jojoba meal, in detoxified jojoba meal, in jojoba meal extracts, and in animal food mixtures.

Keywords: *Simmondsins; simmondsin ferulates; jojoba meal; gas chromatography*

INTRODUCTION

The seeds of the jojoba plant (*Simmondsia chinensis*), an evergreen shrub native to the Sonoran desert, produce a liquid wax commonly referred to as jojoba oil. The seed meals, remaining after isolation of the oil, contain about 30% protein. However, when used as an animal food ingredient, the meal causes food intake reduction and growth retardation. The inhibitory effect on food intake is mainly due to the presence of simmondsin and simmondsin 2′-ferulate as described by Booth et al. (1973) and Cokelaere et al. (1995). Verbiscar et al. (1978) and Van Boven et al. (1993, 1994a,b, 1995, 2000) describe the presence of other simmondsins and simmondsin ferulates in jojoba meal.

For use as an animal feed ingredient the mentioned appetite suppressants have to be eliminated from the meal. For that reason different authors describe methods for the inactivation or elimination of simmondsins and simmondsin ferulates from jojoba meal. They can be inactivated by a chemical method, exposing the meal to ammonia for several days as patented by Elliger et al. (1974). The use of ammonia and hydrogen peroxide resulted in the same effect but in a shorter period of time as described by Baningan and Verbiscar (1980). A patent to Sodoni et al. (1979) describes an extraction procedure. Cotageorge et al. (1979) examined "detoxification" by extraction, enzymatic hydrolysis, and germination procedures. Abbott et al. (1999) and Holser et al. (1999) have considered solvent extraction to remove simmondsins from defatted jojoba meal. Verbiscar et al. (1978, 1980, 1981, 1991) describe detoxification procedures by solvent extraction, heat treatment, and chemical and microbiological methods.

The first aim of the present work was to develop a specific and sensitive method for monitoring the detoxification procedures of jojoba meal as a substitute for the HPLC methods described by Abbott et al. (1988) and Van Boven et al. (1996). The method was also developed for the analysis of feed mixtures containing either jojoba meal either individual simmondsins or simmondsin ferulates used for animal experiments with the prepared feed mixtures.

MATERIALS AND METHODS

Materials and Reagents. Jojoba meal was obtained from EMEC Agro Industries (Antwerp, Belgium). Simmondsin [2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl β-D-glucopyranoside], 5-demethylsimmondsin [2-(cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl β-D-glucopyranoside], 4-demethylsimmondsin [2-(cyanomethylene)-3,4-dihydroxy-5-methoxycyclohexyl β-D-glucopyranoside], didemethylsimmondsin [2-(cyanomethylene)-3,4,5-trihydroxy-cyclohexyl β-D-glucopyranoside], simmondsin 2′-*trans*-ferulate [2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl β-D-glucopyranoside 2′-*trans*-ferulate], simmondsin 3′-*trans*-ferulate [2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl β-D-glucopyranoside 3′-*trans*-ferulate], 5-demethylsimmondsin 2′-*trans*-ferulate [2-(cyanomethylene)-3,5-dihydroxy-4-methoxycyclohexyl β-D-glucopyranoside 2′-*trans*-ferulate], and 4-demethylsimmondin 2′-*trans*-ferulate [2-(cyanomethylene)-3,4-dihydroxy-5-methoxycyclohexyl β-D-glucopyranoside 2′-*trans*-ferulate] used as references were isolated from jojoba meal as described by Van Boven et al. (1993, 1994a,b, 1995, 2000).

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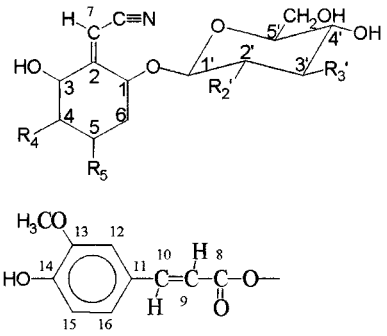

trans ferulic acid moiety

$R_{2'}$ = OH	$R_{3'}$ = OH	R_4 = OCH_3	R_5 = OCH_3	simmondsin
$R_{2'}$ = OH	$R_{3'}$ = OH	R_4 =OH	R_5 = OCH_3	4-demethylsimmondsin
$R_{2'}$ = OH	$R_{3'}$ = OH	R_4 = OCH_3	R_5 = OH	5-demethylsimmondsin
$R_{2'}$ = OH	$R_{3'}$ = OH	R_4 = OH	R_5 = OH	didemethylsimmondsin
$R_{2'}$ = fer. ac. moiety	$R_{3'}$ = OH	R_4 = OCH_3	R_5 = OCH_3	simmondsin 2'-ferulate
$R_{3'}$ = fer. ac. moiety	$R_{2'}$ = OH	R_4 = OCH_3	R_5 = OCH_3	simmondsin 3'-ferulate
$R_{2'}$ = fer. ac. moiety	$R_{3'}$ = OH	R_4 = OCH_3	R_5 = OH	5-demethylsimmondsin 2'-ferulate
$R_{2'}$ = fer. ac. moiety	$R_{3'}$ = OH	R_4 = OH	R_5 = OCH_3	4-demethylsimmondsin 2'-ferulate

Figure 1. Structure of simmondsins and simmondsin ferulates.

The corresponding simmondsin *cis*-ferulates were prepared as described by Van Boven et al. (1996). The structures of the isolated substances are presented in Figure 1. Blank jojoba samples were prepared by Soxhlet extraction of jojoba meal with acetone for 15 days. Phenyl β-D-glucopyranoside was obtained from Aldrich (Bornem, Belgium), and Tri SIL Z, from Pierce (Rockford, IL). All solvents used were of analytical grade.

A rotary mixer (Labin, Belgium) was used for the extraction of jojoba meal by means of 150 mL glass extraction tubes. Extracts were concentrated on a SC110 SpeedVac concentrator (Savant Instrument Inc., Hicksville, NY).

Gas Chromatography. A Chrompack 9000 gas chromatograph equipped with a flame ionization detector was used for the analysis. Separations were made by means of a 50 m × 0.25 mm i.d. glass capillary column with a chemically bonded phase (Chrompack, Middelburg, The Netherlands) of phenyl (50%) dimethylpolysiloxane, CP-Sil 24 (0.25 μm film). Samples of 1 μL were injected by means of a split injector (1/100). To protect the column a special insert glass liner (Chrompack Cat. No. 729814) was used. Injector and detector temperatures were set at 325 °C. Hydrogen was used as carrier gas at 25 cm/s (at 60 °C). Samples were injected at 100 °C. The oven temperature was kept at 100 °C for 2 min, programmed to 250 °C at 5 °C/min, kept at 250 °C for 10 min, and then programmed to 320 °C at 10 °C/min. The temperature was held at 320 °C for 20 min. A Merck-Hitachi 2500 Chromato integrator was used for measuring the chromatographic parameters.

Preparation of Standard Solutions. Stock solutions of simmondsins and simmondsin ferulates were prepared by dissolving equivalents of 100 mg of simmondsins or simmondsin ferulates in 100 mL of methanol. These solutions were kept in the freezer at −20 °C. Stock solutions for the simmondsin ferulates were protected from daylight. Phenyl-β-D-galactopyranoside (100 mg), which was used as an internal standard, was also dissolved in 100 mL of methanol. Aliquots of 100 mg of jojoba meal extracts were dissolved in 100 mL of methanol.

Silylation Procedure. The residues containing 1−10 mg of product were dissolved in 1 mL of Tri SIL Z reagent, and after 60 min reaction time at ambient temperature, 1 μL aliquots were injected in the gas chromatograph.

Analysis of Jojoba Meal. Samples of 1.0 g of jojoba meal or 5.0 g of food mixtures were brought into glass extraction tubes and sonicated with 100 mL of 95% methanol containing 25 mg of internal standard for 5 min and then extracted by means of a rotary mixer. After 30 min on the rotary mixer, the solvent was separated from the jojoba meal or food mixtures by centrifugation. The extraction tubes were protected from daylight by means of aluminum foil.

Analysis of Jojoba Meal Extracts Obtained during Detoxification Procedures. Extracts obtained from jojoba meal following different extraction procedures used to eliminate simmondsins and simmondsin ferulates from jojoba meal were submitted to quantitative analysis. After evaporation of the extraction solvent, residues of about 10.0 mg were dissolved in 10.0 mL of methanol containing 2.5 mg of internal standard. Aliquots (1 mL) of this mixture were concentrated by means of the vacuum concentrator and then dissolved in 1 mL of Tri SIL Z as already described; after 60 min reaction at ambient temperature, 1 μL samples were injected in the gas chromatograph.

Calibration. Standard linearity was verified for simmondsin, 5-demethylsimmondsin, didemethylsimmondsin, simmondsin 2′-*trans*-ferulate, simmondsin 3′-*trans*-ferulate, 4-demethylsimmondsin 2′-*trans*-ferulate, and 5-demethylsimmondsin 2′-*trans*-ferulate. For this purpose, amounts of 0.1, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mg of each reference standard along with 2.5 mg of internal standard were added to blank jojoba meal (100 mg) samples, extracted with 10 mL of 95% methanol, derivatized, and injected as described above. Calibration graphs were obtained by plotting the ratios of the peak areas between the reference standards and the internal standard against quantities of the individual substances.

Recovery. For measuring the recoveries of the different standards, the extraction was performed on blank jojoba samples to which concentrations of 0.5 and 2.5% of simmondsin, 5-demethylsimmondsin, didemethylsimmondsin and simmondsin 2′-*trans*-ferulate were added; the internal standard was added after the extraction. The obtained ratios between references and internal standard are compared to the ratios obtained by adding the internal standard to the described mixtures of references without extraction.

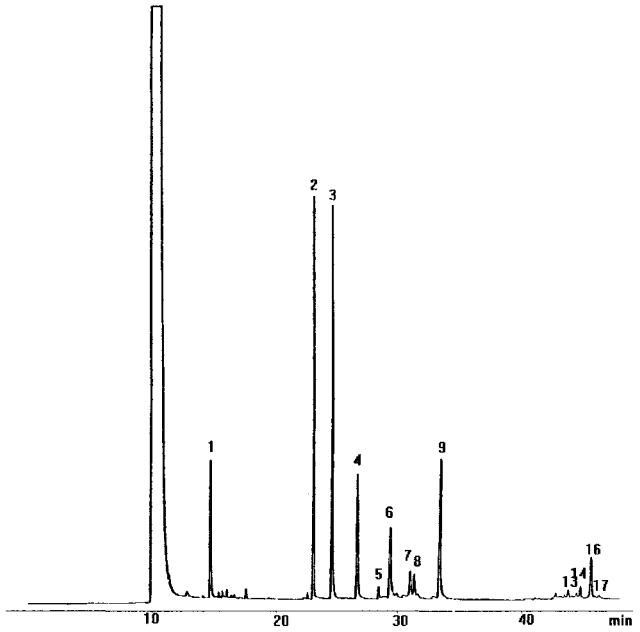

Figure 2. Gas chromatogram of the trimethylsilyl ether derivatives of a jojoba meal extract. The numbers of the peaks correspond to the numbers represented in Table 1.

Table 1. Retention Times from Jojoba Constituents Determined as the Trimethylsilyl Ether Derivatives

no.	compd	R_t (min) (CP-Sil 24)
1	pinitol	14.71
2	internal standard	23.07
3	sucrose	24.61
4	unknown I	26.66
5	unknown II	28.44
6	didemethyl-simmondsin	29.38
7	4-demethylsimmondsin	31.05
8	5-demethylsimmondsin	31.38
9	simmondsin	33.46
10	4-demethysimmondsin 2′-*cis*-ferulate	43.34
11	5-demethylsimmondsin 2′-*cis*-ferulate	43.74
12	simmondsin 2′-*cis*-ferulate	44.26
13	4-demethysimmondsin 2′-*trans*-ferulate	44.59
14	5-demethylsimmondsin 2′-*trans*-ferulate	45.34
15	simmondsin 3′-*cis*-ferulate	45.43
16	simmondsin 2′-*trans*-ferulate	46.10
17	simmondsin 3′-*trans*-ferulate	46.96

Accuracy and Precision. To evaluate intraday and interday precision, blank jojoba samples were spiked with 1% simmondsin, 5-demethylsimmondsin, didemethylsimmondsin, and simmondsin 2′-*trans*-ferulate standards and analyzed three times on three different days.

RESULTS AND DISCUSSION

Capillary Gas Chromatography. Capillary gas chromatography was used to separate and quantify individual simmondsins and simmondsin ferulates. Retention times of the different simmondsins (simmondsin, 4-demethylsimmondsin, 5-demethylsimmondsin, and didemethylsimmondsin) and simmondsin *trans*-ferulates (simmondsin 2′-*trans*-ferulate, simmondsin 3′-*trans*-ferulate, 5-demethylsimmondsin 2′-*trans*-ferulate, and 4-demethylsimmondsin 2′-*trans*-ferulate), the corresponding *cis*-ferulates, and the internal standard as the trimethylsilyl ether derivatives are presented in Table 1. Figure 2 shows a typical gas chromatogram of the trimethylsilyl ether derivatives of a jojoba meal extract obtained by the CP-Sil 24 column; the simmondsins and the different simmondsin ferulates are very well separated. The present column allows baseline separation of the jojoba meal constituents mentioned in Table 1 and also the separation of the *trans*-ferulates from the *cis*-ferulates present as artifacts if the extracts are not protected from daylight. Because of this baseline separation of the simmondsins and simmondsin *trans*- and *cis*-ferulates by the mentioned CP-Sil 24 column, the gas chromatographic method is superior to the described HPLC methods which need two systems for the determination of all the mentioned isomers.

The chromatogram shows also the presence of pinitol and sucrose as well as the presence of two galactopinitols, in Table 1 mentioned as unknowns I and II because some details about their stereochemistry have still to be elucidated.

The method is suitable for the analysis of samples from defatted jojoba meal, detoxified jojoba meal, jojoba meal extracts, and jojoba/feed mixtures. The analysis of the feed used for preparing the mixtures did not shown the presence of compounds interfering with the determination of simmondsins or simmondsin ferulates.

Recovery. Quantitative recoveries (98 ± 3.5%) were obtained for each of the reference compounds added to jojoba meal by using an excess of 95% methanol (1/100, w/v) and rotating the mixture for at least 30 min following 5 min of sonication.

Linearity, Precision, and Reproducibility. The ratios of the areas of the different simmondsins and simmondsin ferulates to the area of the internal standard were plotted against the concentrations of the different simmondsins and simmondsin ferulates. Linear regression was applied to these data to produce calibration graphs. The resulting calibration curves were linear and reproducible; *r* values for simmondsin, 5-demethylsimmondsin, and didemethylsimmondsin were 0.9999, and for the different simmondsin ferulates *r* values were between 0.998 and 0.996. To evaluate the precision and reproducibility, jojoba samples were extracted three times on three different days and injected three times for each extraction. The raw data were subjected to analysis of variance. Intraday repeatability was good with a mean relative standard deviation (RSD) < 2%. Mean RSD values for interday determinations were <3%. Concentrations of 0.1% for the simmondsins and simmondsin ferulates could be determined without any interference from other jojoba components or feed components in jojoba/feed mixtures.

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