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Characterization of Flax and Soybean Soapstocks, and Soybean Deodorizer Distillate by GC-FID

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Abstract Gas chromatography with a flame ionization detector has been used for the characterization of flax and soybean soapstocks and soybean deodorizer distillate. Derivatization by silylation of the samples prior to their injection into a medium polar column enabled a peak resolution of the different components, which led to unambiguous identification of the majority of the components of the samples. The identification and quantification of the fatty acids and sterols in soapstocks and of the fatty acids and tocopherols in deodorizer distillate were successfully achieved. The system has been sensitive enough to characterize 87.5% (mass basis) of the flax soapstock, 95.4% (mass basis) of the soybean soapstock and 83.8% (mass basis) of the soybean deodorizer distillates composition. The method can be safely used for the identification and quantification of most of the components of any type of soapstock and deodorizer distillates.

Keywords Silylation · GC-FID · Flax soapstock · Soybean soapstock · Soybean deodorizer distillates

Introduction

Chemical refining is the most widely used vegetable oil refining process. It has several advantages such as a successful reduction of free fatty acid levels and is not restricted by the types of oil to be refined. However, the process creates

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significant amounts of undesirable by-products (soapstocks and deodorizer distillates). These by-products are a source of valuable components such as free fatty acids, monoacylglycerols (MAGs), diacylglycerols (DAGs), triacylglycerols (TAGs), sterols and tocopherols. The extraction of these components from soapstock and deodorizer distillates and their conversion into valuable products has been the subject of several investigations. For example, fatty acids present in soapstock, traditionally used as a supplement in animal feeds, have been considered for the production of biodiesel [1–4]. Of interest are also the tocopherols (vitamin E) present in deodorizer distillates [5–9]. The evaluation of the quantity of each class of components present in these wastes is therefore an important factor for a good estimation of their economical suitability for conversion into valuable products.

The gas chromatography (GC) characterization of these by-products is a challenging task because soapstocks and deodorizer distillates are composed of several classes of components that have different polarities. Waliszewski's [10] work was the first attempt to analyze soapstock using GC but his method did not separate the GC peaks of important soapstocks' components. Dowd [11, 12] increased significantly the efficiency of GC characterization of soapstock by introducing silylation as a derivatization method. This derivatization technique displaced the sodium counterions of the fatty acids, producing peaks that elute at the retention time of its corresponding fatty acid, simplifying its identification. It also allowed the identification of MAGs, DAGs, TAGs, sterols and tocopherols. Dowd's method showed some limitations, the most important being its failure to separate linoleic and linolenic acid peaks. Previous studies of deodorizer distillates focused on the tocopherols and sterols content because of their important commercial value and neglected the fatty acids [13–15].

More recently, our group employed the silylation method for derivatization and used a GC-MS with a medium-polar column for the characterization of canola soapstock and deodorizer distillates [16]. With the medium-polar column, the separation of every class of components was greatly improved. Furthermore, all the components of the same class that had similar retention times including linoleic and linolenic acids were separated. The method was successful in the complete characterization of canola oil soapstock and deodorizer distillate.

In the present study a gas chromatography flame ionization detector (GC-FID) instead of a GC-MS has been used with the silylation method employed in our previous study [16] to analyze flax and soybean soapstocks and soybean deodorizer distillate which have never been totally characterized before. The technique has been evaluated and accurate quantification data presented.

Materials and Methods

Chemicals and Materials

Flax soapstock, soybean soapstock and soybean deodorizer distillate were provided by Bunge Oils, Danville, Illinois, USA and shipped from their Ontario (Canada) plant in sealed plastic containers. Hexamethyldisilazane (HMDS) 99%, trifluoroacetic acid (TFA) 98% and standards of cholesterol, dodecanoic (12:0), tetradecanoic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1, n-9), linoleic (18:2, n-6), linolenic (18:3, n-3), eicosanoic (20:0), docosanoic (22:0) and tetracosanoic acid (24:0), monostearin, distearin, α- and δ-tocopherol, campesterol, stigmasterol and sitosterol were purchased from Sigma-Aldrich, (St-Louis, MO, USA). β- and γ-tocopherol standards were purchased from Matreya LLC (PA, USA). Analytical grade chloroform was purchased from Caledon (Georgetown, Ont. Canada).

Soapstock and Deodorizer Distillate Derivatization

Soapstock samples were kept at −20 °C and deodorizer distillates were kept at −10 °C until use. All samples were vigorously stirred before sampling for homogeneity. The water content of both soapstock and deodorizer distillate was determined by drying them at 110 °C for 24 h. The detailed silylation procedure prior to chromatographic analysis was carried out according to a protocol detailed in our previous publication [16]. Both soapstocks and deodorizer distillate samples were analyzed by GC within the same day of their derivatization and each sample was injected three times.

GC FID

The GC apparatus used for this study was a Hewlett–Packard 3500. A split injection 1:50 was used for 1 μl of the derivatized samples. Helium was used as the carrier gas and the flow rate was 1 mL/min. The initial oven temperature was held at 80 °C for 3 min, rising to 150 °C at 10 °C/min, to 250 °C at 5 °C/min, and then to 340 °C at 10 °C/min where it was held for 20 min. The universal flame ionization detector had a range of 0–10 V. A CP-TAP (Chormo Pack-Triglycerides Analysis Phase) column (25 m × 25 mm i.d. × 0.1 μm film thickness) (Varian, USA) was selected.

Standard Solutions

Standard solutions were prepared by dissolving the standards (listed in section "Chemicals and Materials") in chloroform. Standards of fatty acids were dissolved together prior to derivatization. Standards of tocopherol, monoolein, distearin and sterols were dissolved separately. All the standard solutions had a concentration of 10 mg mL^{-1}, except linolenic acid, which had a concentration of 100 mg mL^{-1}. All the standard solutions were injected with the internal standard. The choice of cholesterol as an internal standard was justified by its absence in the soapstock and deodorizer distillate samples. Furthermore, the retention time of sterols present in the samples do not interfere with the internal standard, which strengthened the choice of cholesterol as an internal standard. Note that the standards were silylated prior to their injection with standard mixtures. Standard calibration curves were generated by the analysis of a series of standard solutions over appropriate concentration ranges. The fatty acid standards have been injected within the concentration range of 0.1–2.5 mg mL^{-1}, stigmasterol standards within the concentration range of 0.05–2.5 mg mL^{-1} and tocopherols standards within the concentration range of 0.05–1 mg mL^{-1}. All calibration concentration points were run in triplicate.

All the reported values are the averages of the measured values of the different runs and their subsequent standard deviations.

Results and Discussion

For the identification of the compounds, the relative retention time (RRT) to the internal standard of the compound is compared with that of the standard. The complete list of RRT and tolerances are presented in Table 1. As can be seen, very good values for the tolerances were obtained.

Table 1 Components of soapstock and deodorizer distillate samples identified by GC-FID with their respective relative retention times (RRT), standard deviation and tolerance

Component	RRT (min)	Standard deviation[a] (%)	Tolerance[b] (%)
Tetradecanoic acid	19.13	1.28	0.52
Palmitic acid	16.39	1.91	0.88
Oleic acid	13.23	1.92	1.34
Linoleic acid	12.66	1.76	3.24
Linolenic acid	12.48	2.02	1.09
Eicosanoic acid	10.63	0.04	0.45
Docosanoic acid	7.76	0.12	2.64
Tetracosanoic acid	5.02	1.82	3.65
Squalene	3.37	0.06	3.37
δ-Tocopherol	2.77	0.03	3.17
γ-Tocopherol	1.46	0.01	4.10
Stigmasterol	−1.53	1.22	3.86
Sitosterol	−2.02	1.02	3.79

Undetected components in soapstocks and deodorizer distillate

Component	RRT (min)	Component	RRT (min)
Dodecanoic acid	21.38	β-Tocopherol	1.67
Palmitoleic acid	16.26	α-Tocopherol	−0.47
Stearic acid	13.55	Brassicasterol	−0.63

[a] SD, $N = 3$ replicates

[b] Tolerance (in %) is calculated for the relative retention times (RRT) when compared to that of the standard

Calibration curves were used for the quantification. For each analyte, the linear dynamic range was fitted to a least squares regression line. The equations and correlation coefficients (R^2) are presented in Table 2. The correlation coefficients R^2 were greater than 0.99 for all the components except for squalene and stigmasterol where $R^2 \approx 0.98$. Note that the standard deviation percentage for individual concentrations was less than 10% in all measurements.

Flax and Soybean Soapstock

The chromatograms of flax and soybean soapstocks are presented in Figs. 1 and 2, respectively. The mass % of individual components in flax and soybean soapstocks is presented in Tables 3 and 4, respectively. The chromatograms in Figs. 1 and 2 show that all the analytes were successfully separated. The chromatograms (Figs. 1 and 2, respectively) show two unidentified peaks at ∼3.1 and 4.6 min (see peaks 1 and 2 from each figure). These peaks could be due to phosphoric acid or a type of phosphate [12]. The soybean soapstock chromatogram (Fig. 2)

Table 2 Calibration curves least squares regression line parameters and correlation coefficients (R^2)

Component	m	b	R^2
Dodecanoic acid	6E-06	0.0308	0.9959
Tetradecanoic acid	9E-06	0.0363	0.9958
Palmitic acid	8E-06	0.0555	0.9940
Stearic acid	8E-06	0.0034	0.9919
Oleic acid	7E-06	−0.011	0.9911
Linoleic acid	9E-06	0.0876	0.9945
Linolenic acid	8E-06	−0.0284	0.9947
Eicosanoic acid	1E-05	0.0228	0.9918
Docosanoic acid	1E-05;	0.0471	0.9913
Tetracosanoic acid	7E-06	0.1212	0.9905
Squalene	8E-07	−0.0325	0.9779
δ-Tocopherol	1E-05	−0.0233	0.9960
Stigmasterol	9E-07	0.129	0.9831

$y = mx + b$ where y is the concentration in mg mL^{-1} and x is the area of the chromatogram peak
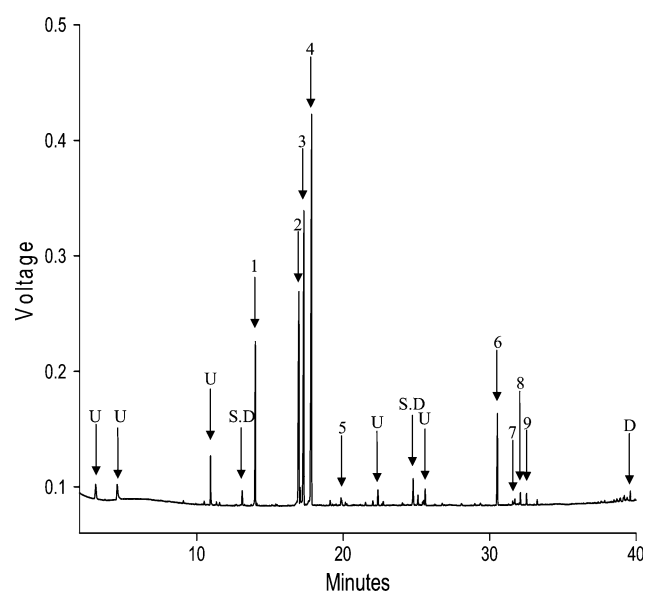
U – Unknown;

D - Derivatization peak agent;

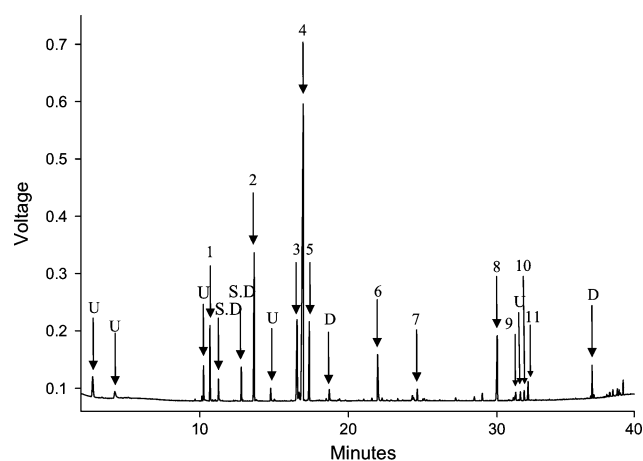S.D – Silyl Derivative of fatty acid;

1- Palmitic acid; 2- Oleic acid; 3-Linoleic acid; 4- Linolenic acid; 5- Eicosanoic acid; 6- Cholesterol (I.S.); 7- Campesterol; 8- Stigmasterol; 9- Sitosterol

Fig. 1 Chromatogram of flax soapstock

showed an unidentified peak within the sterols area, between campesterol (peak 8) and stigmasterol (peak 9). However, there is no sterol that could fit this particular retention time. The flax and soybean soapstock chromatograms presented other unknown peaks that emerged before

D- Derivatization peak;

U- Unknowns;

S.D – Silyl Derivative of fatty acid;

1- Tetradecanoic acid; 2- Palmitic acid; 3- Oleic acid; 4- Linoleic

acid; 5- Linolenic acid; 6- Docosanoic acid; 7- Tetracosanoic acid;

8- Cholesterol; 9- Campesterol; 10- Stigmasterol; 11- Sitosterol

Fig. 2 Chromatogram of soybean soapstock

Table 3 Components of flax soapstock

Components	Mass content (%)	Standard deviation (%)
Moisture	44.2	–
Palmitic acid	4.6	1.9
Oleic acid	6.9	7.1
Linoleic acid	11.4	3.0
Linolenic acid	13.2	2.8
Eicosanoic acid	0.6	17.2
Cholesterol (I.S.)	1.2	–
Campesterol	1.8	0.1
Stigmasterol	1.8	0.1
Sitosterol	1.8	0.1

the internal standard peak. Their relative areas were comparable to the sterols' peaks relative areas. Their identification using the literature [11, 12, 16] and the relative retention times available including those of monoolein and distearin has been unsuccessful.

Entities in the MAGs and DAGs region can be observed. However, quantification limits based on the injection of monostearin and distearin standards did not allow the quantification of these components. Since they have been detected and identified with GC MS (our previous study [16]), it is obvious that the FID detector has an inherent weakness for the characterization of small quantities of MAGs and DAGs.

Table 4 Components of soybean soapstock

Components	Content (mass %)	Standard deviation (%)
Moisture	31.8	–
Tetradecanoic acid	3.6	6.8
Palmitic acid	8.5	3.7
Oleic acid	4.6	8.3
Linoleic acid	30.8	3.7
Linolenic acid	3.2	4.6
Docosanoic acid	4.5	2.8
Tetracosanoic acid	1.8	0.8
Cholesterol (I.S.)	1.2	–
Campesterol	1.8	0.7
Stigmasterol	1.8	1.2
Sitosterol	1.9	0.5

The identification of the analytes revealed that the fatty acids and sterols are the two main components found within flax and soybean soapstock. Fatty acids and sterols represent 42.1% (wet basis) and 57.0% (wet basis) of the total mass of flax and soybean soapstock, respectively. The results are consistent with previous studies [11, 12, 16], which indicated that fatty acids were the main class of components in soapstocks. The amounts of linolenic acid and linoleic acid in flax and soybean soapstock are higher than the other fatty acids. This is reasonable because they are the main fatty acids found in their respective oils. Interestingly, stearic acid has not been detected neither in flax soapstock (Fig. 1) nor in soybean oil (Fig. 2) even if the literature indicates that it can be found in a amounts between 3 and 4% in flax oil, and 2.8–24.7% in soybean oil [17]. As can be seen in Figs. 1 and 2, no peak was detected between palmitic and oleic acid, where stearic acid should have eluted. The peak of stearic acid has not been confounded with the oleic acid peak as demonstrated by their respective RRT and tolerance (see Table 1).

The amount of sterols in both soapstocks was found to be approximately 2% (mass basis). Overall, almost all of the components within these soapstocks have been identified. The unidentified components in flax soapstock were approximately 12.5% (mass basis) and those in soybean soapstock were approximately 4.6% (mass basis).

Soybean Deodorizer Distillate

The chromatogram of soybean deodorizer distillates is presented in Fig. 3 and the quantification results are listed in Table 5. The separation between the different classes of compounds (fatty acids, tocopherols, squalene and sterols) has been successfully achieved and their components have been easily identified (see Table 2). Like in soybean

Fig. 3 Chromatogram of soybean deodorizer distillate
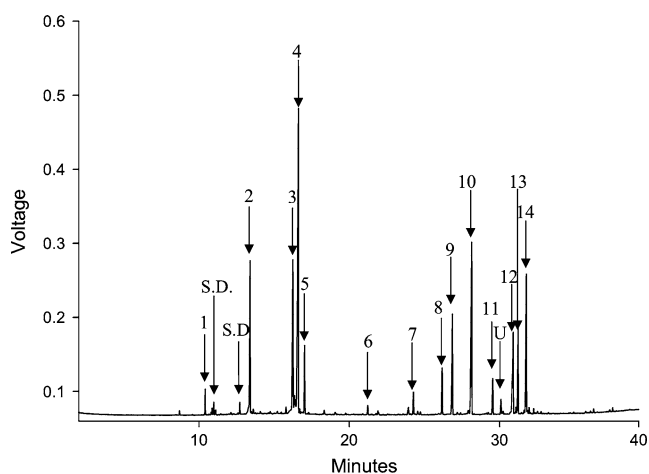
U – Unknown;

S.D – Silyl Derivative of fatty acid;

1- Tetradecanoic acid; 2- Palmitic acid; 3- Oleic acid; 4- Linoleic acid;

5- Linolenic acid; 6- Docosanoic acid; 7- Tetracosanoic acid; 8-

Squalene; 9-δ-Tocopherol; 10- γ-Tocopherol; 11- Cholesterol; 12-

Campesterol, 13- Stigmasterol, 14- Sitosterol

Table 5 Components of soybean deodorizer distillate

Component	Content (mass %)	Standard deviation (%)
Moisture	7.4	–
Tetradecanoic acid	1.3	11.1
Palmitic acid	7.1	12.2
Oleic acid	7.1	17.8
Linoleic acid	22.6	14.4
Linolenic acid	2.5	18.1
Docosanoic acid	0.9	5.2
Tetracosanoic acid	2.6	4.8
Squalene	5.5	7.0
δ-Tocopherol	6.1	17.4
γ-Tocopherol	12.5	18.3
Cholesterol (I.S.)	1.2	–
Campesterol	2.4	4.9
Stigmasterol	2.1	10.7
Sitosterol	2.5	9.7

soapstock, no stearic acid has been detected in the soybean deodorizer distillate. Furthermore, linoleic acid has been found to be the main constituent of soybean deodorizer distillate (22.6% mass basis). Two types of tocopherols (δ and γ) have been found with a concentration of approximately 19.1% on a mass basis. The high mass percentage of tocopherols in soybean deodorizer distillates has already been reported by Verleyen et al. [15].

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