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ORIGINAL PAPER

Trimethylsilyl Derivatization/Gas Chromatography as a Method to Determine the Free Fatty Acid Content of Vegetable Oils

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Abstract Thirty-one samples of vegetable oils were analyzed for free fatty acid (FFA) concentration by titration against sodium hydroxide and by trimethylsilyl (TMS) derivatization followed by gas chromatography (GC). In preliminary experiments, two silylation chemistries and three GC stationary phases were tested for TMS fatty acid ester formation and separation. No ideal combination of conditions was identified; however, hexamethyldisilazane with an acid catalyst and a non-polar J & W DB-5 column were chosen for comparison of the two approaches. Over the range of FFA values studied (0.04–12%), the results from the two methods were highly correlated ($R > +0.996$) and were generally in good agreement. However, values from the chromatographic method were slightly lower than values obtained by titration for the crude oil samples (which had higher FFA levels). For oils with >0.4% FFA, the GC approach was slightly less reproducible (average coefficient of variance of ~3%) compared with the titration approach (average coefficient of variance of ~1.4%). For oils with <0.4% FFA, the coefficients of variance were higher (8–9%) and comparable between the methods.

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Introduction

Free fatty acids (FFA) are formed in mature oilseeds before and after harvesting because of weathering or handling. Generally, they follow triglycerides through the oil extraction process. These acids must be removed from the crude oil either by chemical refining with caustic soda or by physical refining with vacuum steam distillation [1]. During chemical refining, the FFAs react with sodium hydroxide to form soap molecules that emulsify hydratable components in the crude oil to form soapstock. Some neutral oil is entrained in the soapstock and is considered as a processing loss. Consequently, FFA concentration affects oil recovery, and it is an element in the grading and market price of oilseeds and crude oils [2]. Accurate measurement of FFA is needed to support seed grading, to determine the amount of caustic to add during refining, and to estimate processing oil losses.

Traditionally, FFA concentration in crude vegetable oil is measured by dissolving a known amount of oil into ethanol and titrating the mixture against a solution of sodium hydroxide [3]. The method is reproducible and reliable, but lacks specificity. All acidic components of the sample are measured, including compounds that may not be derived from glyceride hydrolysis. In addition, titration generally assumes an "average" fatty acid molecular weight in converting from a molar acidity to a weight basis,

and the method provides no information on the distribution of the individual fatty acids.

Several alternatives to direct titration have been reported in recent years, including supercritical fluid chromatography [4, 5], electrochemical detection [6], and spectroscopic detection [7]. One relatively simple approach is to derivatize the acids to increase their volatility and use gas chromatography (GC) to separate and quantify the individual fatty acid derivatives. Trimethylsilylation (TMS) is a mild derivatization method that displaces the active hydrogen atoms of hydroxyl and carboxyl groups to form silyl ethers and esters that are usually more volatile than the underivatized compounds. If strong silylation reagents are used, the hydrogen atoms of primary and secondary amines can also be exchanged [8]. This chemistry, however, should not hydrolyze glycerides, and therefore allows for the analysis of fatty acids in glyceride containing materials.

Silylation of lipids in combination with GC has been used to help identify the positioning of specific fatty acids on the glycerol backbone [9], for identifying fatty acids in plasma samples [10], and for derivatizing hydroxylated fatty acid esters for mass spectral analysis [11]. In recent years, it has also been used to characterize crude vegetable oils [12, 13] and related co-products, including soapstock [14–16], acid water [17], and deodorization distillate [16, 18].

This work was undertaken to compare FFA values determined by silylation/GC with traditional titration. Optimal silylation conditions for crude oils, the most appropriate commercially available capillary column, and an efficient elution profile were determined for most common vegetable oils. Thirty-one samples of refined and crude oils covering a wide range of FFA levels were then evaluated by both techniques, and the results were compared.

Materials and Methods

Oil Samples

Except for one rice bran oil sample that was extracted in the pilot plant of our facility, all other vegetable oils were obtained from commercial sources. Thirty-one samples of ten different oil types were studied (Table 1). These included canola, coconut, corn, cottonseed, palm, peanut, high erucic acid rapeseed, rice bran, soybean, and sunflower oil samples. Samples were stored at –20 °C in replicate vials. Most samples were allowed to warm to room temperature before being thoroughly mixed and sub-sampled. The coconut and palm oils samples, however, were melted at 60 °C before mixing and sampling.

Chemicals

Solvents examined for dissolving the oils included acetone, acetonitrile, chloroform, cyclohexane, dimethyl sulfoxide, ethyl acetate, hexane, methylene chloride, methyl ethyl ketone, pyridine, and toluene. All solvents were supplied by J. T. Baker Chemical Co. (Phillipsburg, NJ, USA). *N,O*-bis(trimethylsilyl)acetamide (BSA), hexamethyldisilazane (HMDS), and trifluoroacetic acid (TFA) were purchased from Pierce Chemical Co. (Rockford, IL, USA). Fatty acid standards and cholesteryl methyl ether (CAS# 1174-92-1) were purchased from Sigma-Aldrich (St Louis, MO, USA).

FFA Determination by Titration

The experimental procedure described in AOCS method Ca 5a-40 [3] was followed to determine the percent FFA in each sample. Briefly, oil was weighed into a flask followed by neutralized 95% ethyl ethanol and a phenolphthalein indicator. The mixture was then titrated against a sodium hydroxide solution until a permanent pink color persisted for at least 30 s. Weight percentage of FFA was calculated on either an oleic, palmitic, or lauric acid basis, depending on the type of oil being analyzed. Each sample was titrated in duplicate.

Silylation Chemistry

In preliminary experiments, several solvents and two silylation reagents were tested for compatibility and stability. To test for solvent compatibility, ~100 mg of soybean oil, 1 mL of solvent, and either 1 mL of BSA or HMDS and 100 μL of TFA was mixed and heated at 60 °C for 45 min. Trifluoroacetic acid was used as an acid catalyst with HMDS. After reaction, the solutions were allowed to cool and were observed for formation of multiple phases that might result in partitioning of the fatty acids.

To study the stability of silylated oil samples, ~100 mg of crude soybean oil was precisely weighed into a Pierce 5 mL septum-capped reaction vial, followed by addition of 1 mL of pyridine containing a known amount of cholesteryl methyl ether (as internal standard) and either 1 mL of BSA or HMDS plus 100 μL of TFA. Mixtures were heated to 60 °C for 45 min and then cooled to room temperature. Stability of the resulting silyl esters was studied by repeated injection and quantification of the fatty acids over 3 days on a DB-5 chromatography column (described below).

GC

A Hewlett-Packard gas chromatograph (model: HP5890 series 2 plus) was used for separating individual silyl fatty acid esters. Elution profiles were determined on three

Table 1 Free fatty acid concentrations by titration and by GC after silylation for a series of vegetable oils

Source	Type	FFA, titration (n = 2)			FFA, GC (n = 3)		ΔFFA
		Acidity basis	Ave. ± SD (%)	CV	Ave. ± SD (%)	CV	(Tit. – GC) (%)
Palm Kernel	RBD	Lauric	0.04 ± 0.01	22.2	0.045 ± 0.003	6.7	–0.005
Palm Kernel	RBD	Lauric	0.05 ± 0.00	0.0	0.053 ± 0.002	3.8	–0.003
Cottonseed	R	Oleic	0.12 ± 0.01	8.3	0.076 ± 0.001	1.3	0.044
Palm	RBD	Palmitic	0.05 ± 0.00	0.0	0.07 ± 0.01	16.9	–0.02
Soybean	R	Oleic	0.04 ± 0.00	0.0	0.08 ± 0.02	31.6	–0.04
Cottonseed	RB	Oleic	0.11 ± 0.03	27.3	0.089 ± 0.009	10.1	0.021
Cottonseed	RB	Oleic	0.12 ± 0.01	8.0	0.14 ± 0.01	8.1	–0.02
Palm Olein	RBD	Palmitic	0.14 ± 0.01	7.4	0.137 ± 0.001	0.7	0.003
Cottonseed	RBD	Oleic	0.06 ± 0.01	18.2	0.137 ± 0.007	5.1	–0.077
Canola	RB	Oleic	0.12 ± 0.01	8.0	0.140 ± 0.008	5.7	–0.020
Rapeseed	RB	Oleic	0.12 ± 0.01	8.3	0.141 ± 0.004	2.8	–0.021
Cottonseed	R	Oleic	0.26 ± 0.01	3.8	0.2 ± 0.2	84.9	0.06
Soybean	DC	Oleic	0.42 ± 0.00	0.0	0.41 ± 0.01	2.9	0.01
Canola	C	Oleic	0.57 ± 0.01	1.8	0.43 ± 0.02	3.9	0.14
Soybean	DC	Oleic	0.52 ± 0.01	1.9	0.46 ± 0.02	5.2	0.06
Rapeseed	DC	Oleic	0.61 ± 0.01	1.6	0.48 ± 0.02	4.5	0.13
Canola	C	Oleic	0.94 ± 0.03	3.2	0.78 ± 0.02	2.3	0.16
Cottonseed	C	Oleic	1.28 ± 0.02	1.6	1.22 ± 0.09	7.5	0.06
Cottonseed	C	Oleic	1.48 ± 0.08	5.4	1.24 ± 0.03	2.2	0.24
Sunflower	C	Oleic	1.58 ± 0.01	0.6	1.43 ± 0.01	0.6	0.15
Peanut	C	Oleic	1.84 ± 0.02	1.1	1.79 ± 0.02	1.3	0.05
Sunflower	C	Oleic	2.48 ± 0.04	1.6	2.38 ± 0.06	2.5	0.10
Peanut	C	Oleic	2.78 ± 0.02	0.7	2.62 ± 0.07	2.8	0.16
Corn	C	Oleic	3.16 ± 0.02	0.6	2.97 ± 0.04	1.3	0.19
Cottonseed	C	Oleic	3.16 ± 0.03	0.9	3.03 ± 0.08	2.5	0.13
Coconut	C	Lauric	3.36 ± 0.02	0.6	3.1 ± 0.2	5.8	0.26
Coconut	C	Lauric	3.7 ± 0.1	2.9	3.2 ± 0.1	3.8	0.50
Rice Bran	C	Oleic	4.40 ± 0.01	0.2	3.80 ± 0.05	1.4	0.60
Palm	C	Palmitic	4.64 ± 0.00	0.0	4.21 ± 0.09	2.1	0.43
Rice Bran	C	Oleic	7.84 ± 0.02	0.3	7.0 ± 0.2	3.2	0.84
Cottonseed	C	Oleic	12.6 ± 0.2	1.4	12.2 ± 0.1	0.9	0.40

R refined; *RB* refined and bleached; *RBD* refined, bleached, and deodorized; *DC* degummed crude; *C* crude

commercially available stationary phases. DB-5HT and DB-17HT columns (both 15 m × 0.25 mm id. × 0.15 μm film thickness) were purchased from J & W Scientific (Folsom, CA, USA). (These columns are currently available from Agilent Technologies, Santa Clara, CA, USA.) An UltiMetal CP-TAP-CB column (25 m × 0.25 mm × 0.1 μm film thickness) was purchased from Chrompack International (Raritan, NJ, USA). Derivatized fatty acids were separated on each stationary phase under similar GC conditions. In each case, the carrier gas was helium flowing at a linear velocity of ~30 cm^3/s. Injector and detector temperatures were set at 360 °C, and the injector split ratio was set to 1:50. For testing the separation of individual TMS fatty acid esters, the column temperature was held at 75 °C for 3 min, then increased to 150 °C at 10 °C/min, then increased to 250 °C at 5 °C/min, then increased to 300 °C at 10 °C/min. Inlet pressure control was used to maintain the carrier gas flow rate as the column temperature was increased.

GC Determination of FFAs

Oil samples and fatty acid standards were derivatized as described above for soybean oil with pyridine as the solvent and HMDS/TFA as the silylation reagent. Oil samples were analyzed on the DB-5 column. The same GC conditions were used for these analyses, except that the column temperature was extended to elute triglycerides. For these

samples, the final temperature ramp was from 250 °C at 10 °C/min to 370 °C (instead of 300 °C) and the column was held at 370 °C for 10 min. TMS fatty acid esters were identified by comparing elution times with the elution times of standards. Each oil sample studied was silylated and analyzed in triplicate.

Relative response factors were developed for each fatty acid of interest. Pyridine solutions of each fatty acid and cholesteryl methyl ether were prepared in known concentrations. A series of solutions was then prepared by weighing known amounts of the fatty acid and internal standard solutions. The mixtures were silylated (as described above) and subjected to analysis by GC. Relative response factors were calculated as described by Kaiser and Debbrecht [19].

In prior work [14, 15] where quantification of different types of compounds was required, we used cholesteryl methyl ether as an internal standard. This compound does not co-elute with other oil components (including expected phytosterols); its detector response is similar to fatty acid esters (i.e., relative response factors near 1); and it is available in pure crystalline form. Of course, other standards, e.g., odd carbon number fatty acids, would be equally suitable.

Statistics

The experimental design was a randomized complete block with two treatments (FFA concentration by GC and titration), where the treatments were blocked by oil type. As the distribution of FFA values was skewed toward low values, the FFA means were log transformed. Analysis of variance was performed on the log transformed data, and the treatments were compared by least significant difference (LSD) ($P \leq 0.05$).

Results and Discussion

Crude soybean oil was used to study silylation conditions. Pyridine, methylene chloride and chloroform were found to be acceptable solvents for silylating soybean oil with BSA, and pyridine and chloroform were found to be suitable when silylating with HMDS/TFA. All other solvents yielded multiple phases after reaction. Both the BSA and HMDS/TFA silylation chemistries yielded similar fatty acid results, but when BSA was used as the agent, the concentration of fatty acids tended to rise slowly over time. Because BSA is considered a strong silylation reagent, there was some concern that this change (~1% increase over several hours and ~3–5% increase over 2–3 days) might have resulted from unwanted side reactions. The concentrations obtained with HMDS/TFA were more stable over several hours. From these tests, pyridine was chosen as the solvent and HMDS/TFA was chosen as the derivatization agent for further work.

Pure fatty acid standards were used to assess the elution pattern and the resolution of the derivatized fatty acids for each of the three tested columns. None of the columns provided baseline separation for all of the fatty acid esters of interest. The DB-17HT column gave good separation for palmitic and palmitoleic acid esters, and linoleic and α-linolenic acid esters, but the silyl esters of stearic and oleic acids co-eluted. Another concern for this column was that its upper temperature limit was 350 °C, which might not permit elution of triglycerides in a timely fashion. The ultimetal CP-TAP-CB column provided acceptable separation of the silyl derivatives of palmitic and palmitoleic acids. However, as for the DB-17HT column, silyl esters of stearic and oleic acids were not separated. Durant et al. [16] have recently reported GC results with the silica version of this column on canola oil by-products. Stearic acid was not discussed in this work. Because this acid is present in canola oil at concentrations of ~1–3% [20], these authors may have also had difficulty with the separation of this fatty acid ester. On the DB-5HT column, most of the TMS fatty acid esters were well resolved with the exception of the oleic and α-linolenic esters, which co-eluted. Although none of the three columns provided an ideal separation for all the silyl fatty acids of interest, the DB-5HT column was able to separate most of the FFA esters found in common vegetable oils and was used for the remaining experiments in this study. However, for work on oils containing higher levels of α-linolenic acid than stearic acid, e.g., canola and soybean oils, the TAP column might be preferable.

Gas chromatographic conditions and temperature programs (described in the "Methods" section) were chosen to separate both low molecular weight fatty acids (as would be expected in palm kernel and coconut oils) and high molecular weight fatty acids (as would be expected in high erucic acid rapeseed oil). To achieve the best separation of fatty acids and elution of the triglycerides, the temperature program can be modified as needed. Relative response factors for most of the TMS derivatized fatty acids were similar and varied by less than 5% from 1.0, with the exception of the low molecular weight fatty acids (e.g., caproic and caprylic acids), which showed response factors closer to 0.9. In this regard, the relative response factors for oleic and α-linolenic acids were essentially the same. Hence, it is reasonable to use the co-eluted chromatographic peak to estimate the sum of these components.

Chromatograms for soybean, palm, and coconut oils on the DB-5 stationary phase illustrate the elution patterns of the TMS silylated FFAs, TMS silylated diglycerides, sterols, and triglycerides (Fig. 1). Total concentration of free fatty acids was determined from the integrated peak

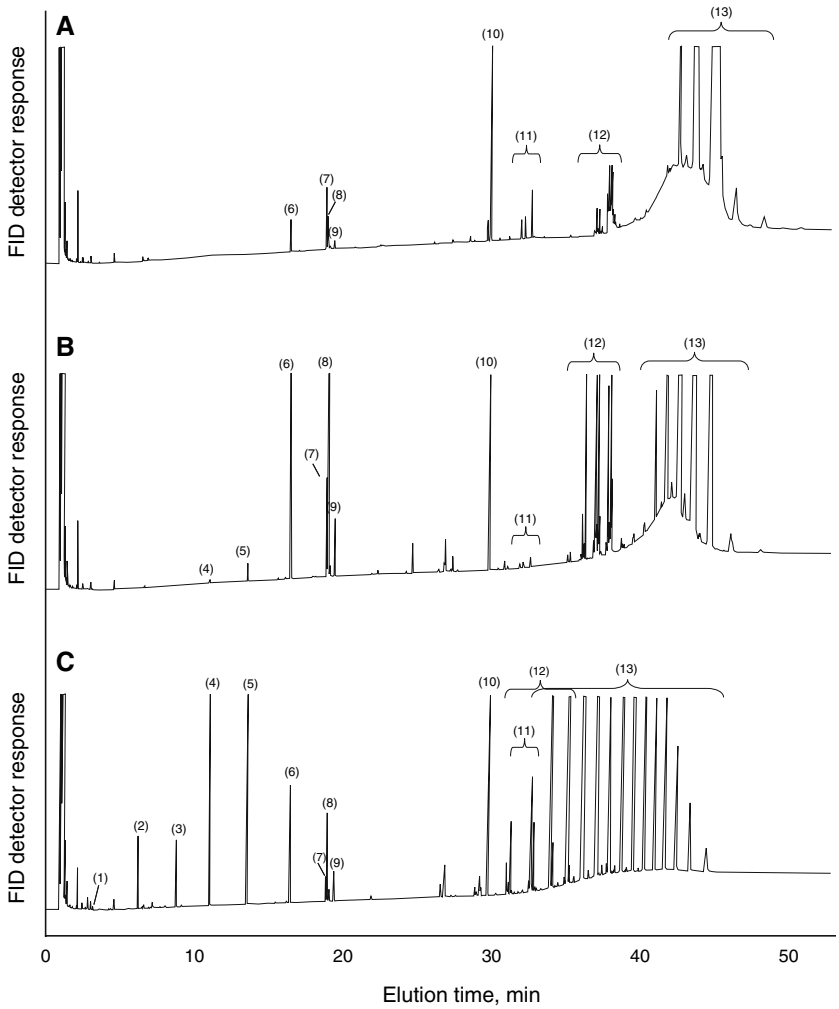

Fig. 1 Chromatograms of **a** Silylated degummed soybean oil. **b** Silylated crude palm oil. **c** Silylated crude coconut oil on a J & W Scientific DB-5 capillary column. The identified compounds are trimethylsilyl (TMS) esters of (*1*) caproic acid, (*2*) caprylic acid, (*3*) capric acid, (*4*) lauric acid, (*5*) myristic acid, (*6*) palmitic acid, (*7*) linoleic acid, (*8*) oleic acid, and (*9*) stearic acid. Other identified components include (*11*) TMS derivatives of phytosterols, (*12*) TMS derivatives of diglycerides and (*13*) triglycerides. The latter two components tend to separate in groups based on carbon number. The internal standard was (*10*) cholesteryl methyl ether

areas correcting for response factor differences (Table 1). A strong correlation (R = +0.996) was found between FFA levels determined by silylation/chromatography and titration (Fig. 2). Generally, the distribution of free fatty acids determined by silylation/GC resembled the distributions of the total fatty acids of the corresponding oil (Table 2), but a few pronounced differences existed. Several factors can influence the FFA distribution. Lipases tend to act on the terminal positions of glycerides and glycerides have preferentially positioning of fatty acids, which will affect the distribution of free fatty acids. Extraction conditions are also known to affect both the amount and composition of the FFAs in the resulting crude oil [12]. Because we specifically asked for oils with variable levels of fatty acids (i.e., problem oils), we also expect that a few of our samples were either improperly stored or were otherwise contaminated and that these factors contributed to some of the distributional differences observed.

Coefficient of variance (CV) was determined for each FFA value measured by both procedures (Table 1). Repeatability of the titration method is limited to the

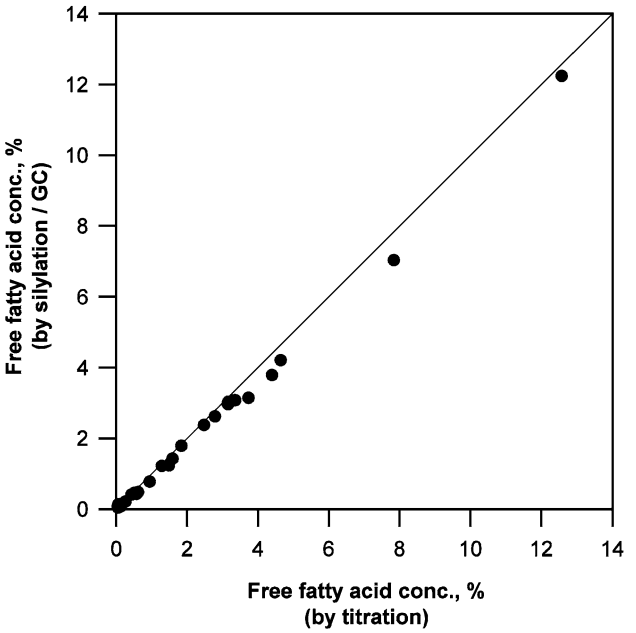

Fig. 2 Correlation of FFA values of vegetable oils obtained by titration and GC

Table 2 Distribution range of measured free fatty acids (FFAs) determined by trimethylsilylation/GC compared with CODEX (or equivalent) overall fatty acid distributions of the same oils

Oil type		C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1 + C18:3	C18:2	C20:0	C22:0	C22:1	C24:0
Canola	CODEX[a]	–	–	–	<0.2	3.3–6.0	0.1–0.6	1.1–2.5	58.4–81.0	16.1–24.8	0.1–3.4	<2.0	<2.0	<0.2
	FFA (n = 3)					10.1–13.2	–	5.0–6.4	62.5–64.9	16.6–22.4	–	–	–	–
Coconut	CODEX	4.6–9.4	5.5–7.8	45.1–50.3	16.8–20.6	7.7–10.2	–	2.3–3.5	5.4–8.3	1.0–2.1	<0.2	<0.2	–	<0.2
	FFA (n = 2)	4.6–5.0	4.2–4.4	45.0–45.2	19.3–19.4	10.7–11.6		3.1–3.4	8.7–9.0	2.5–3.2	–	–	–	–
Corn	CODEX	–	–	<0.3	<0.3	8.6–16.5	<0.4	1.0–3.3	20.5–43.7	39.4–62.5	0.3–0.6	<0.5	<0.1	<0.1
	FFA (n = 1)	–	–	–	–	16.0	0.1	2.9	29.4	49.9	0.8	0.3	–	0.6
Cottonseed	CODEX	–	–	<0.2	0.6–1.0	21.4–26.4	<1.2	3.0–6.0	14.7–21.7	46.7–58.2	0.2–0.5	<0.6	<0.3	<0.1
	FFA (n = 9)	–	–	–	0.0–0.9	25.3–48.0	0.0–1.0	0.0–6.2	14.8–21.1	31.1–53.7	0.0–0.4	0.0–0.2	–	0.0–0.2
Palm	CODEX	–	–	<0.4	0.5–2.0	40.1–47.5	<0.6	3.5–6.0	36.0–44.5	6.5–12.0	–	–	–	–
	FFA (n = 2)	–	–	0.0–0.2	0.0–1.6	47.3–57.5	0.0–0.2	4.6–5.3	23.3–36.7	0.0–9.6	–	–	–	–
Palm kernel	CODEX	2.4–6.2	2.6–5.0	41.0–55.0	14.0–18.0	6.5–10.0	–	1.3–3.0	12.0–19.0	1.0–3.5	–	–	–	–
	FFA (n = 2)	–	–	32.2–36.1	15.9–17.8	20.0–22.4	–	–	27.6–28.0	–	–	–	–	–
Peanut	CODEX	–	–	<0.1	<0.1	8.3–14.0	<0.2	1.9–4.4	36.4–67.2	14.0–43.0	1.1–1.7	2.1–4.4	<0.3	1.1–2.2
	FFA (n = 2)	–	–	–	–	13.2–14.9	–	2.8–3.5	42.7–48.2	28.6–38.3	0.6–0.9	0.6–0.9	–	0.5–1.9
Rapeseed	CODEX	–	–	–	0.2	1.5–6.0	0.0–3.0	0.5–3.1	13.0–73.0	11.0–23.0	<3.0	<2.0	5.0–60.0	<2.0
	FFA (n = 2)	–	–	–	–	9.4–11.4	–	0.0–6.3	40.7–41.5	23.2–27.4	0.0–1.8	2.7–3.4	8.5–14.1	–
Rice bran	See footnote[b]	–	–	–	0.4	16.4	–	1.8	43.6	37.1	0.4	–	–	–
	FFA (n = 2)	–	–	–	0.4–0.6	18.3–21.0	0.2	2.0–2.6	38.4–40.3	31.6–38.3	0.4–0.8	0.1–0.3	–	0.2–0.9
Soybean	CODEX	–	–	<0.1	<0.2	8.0–13.3	<0.2	2.4–5.4	23.2–35.6	49.8–57.1	0.1–0.6	0.3–0.7	<0.3	<0.4
	FFA (n = 3)	–	–	–	–	21.4–27.5	–	6.6–7.9	24.0–27.2	37.4–48.0	–	–	–	–
Sunflower	CODEX	–	–	<0.1	<0.2	5.6–7.6	<0.3	2.7–6.5	14.0–39.6	48.3–74.0	0.2–0.4	0.5–1.3	<0.2	0.2–0.3
	FFA (n = 2)	–	–	–	–	8.3–8.8	0.1–0.7	3.7–4.7	19.9–25.5	60.9–65.9	0.0–0.2	0.0–0.7	–	0.0–0.5

[a] CODEX values taken from [21]

[b] Values taken from [22]

increments of the buret even when a low normality of alkali is used. For low acid samples, this results in a fairly coarse gradation of the CV (Table 2). Regarding the precision of the titration method, AOCS method Ca 5a-40 notes an inter-laboratory CV for titrated fatty acid levels in the 0–0.05% range of 34% and in the 0.05–0.1% range of 12.7%. Even for a FFA range of 0.1–1.0%, the expected CV is 9.9%. Although our analysis is limited by having conducted only duplicates, our results tended to be a little more reproducible, which we believe is because of the experience of our operator. At low FFA levels (<0.4%), titration yielded an average CV of 9.3%. At higher FFA levels (>0.4%), titration yielded an average CV of 1.4%. Estimation of the repeatability of the GC results was less coarse, as the samples were analyzed in triplicate and the peak area measurement of the flame ionization detector is much finer. For samples below 0.4% FFA, the average CV was 8.4% by chromatography. (One sample was excluded from this average as its CV was strangely high and suggestive of an error in sample labeling.) For FFA levels greater than 0.4%, the average CV was 3% by chromatography (Table 2). The results suggest that the repeatability of the determination is comparable between the two approaches when the acid level is low and slightly better by titration when the acid level is high. Regardless of the differences, both methods are precise enough for most applications.

Analysis of the LSD over the complete dataset indicated that there was no significant difference between the two techniques. However, the same analysis conducted on only the crude oil samples (which had higher FFA levels) indicated a significant difference in the measurement techniques, with the values obtained by derivatization/chromatography being slightly lower than values obtained by titration. This difference appears to result from the presence of small concentrations of low molecular weight acids and minor fatty acids that were measured by titration but were excluded in the GC analyses and a small systematic difference caused by the assumption of a single fatty acid in converting titrated acidity to a weight basis. On this point, the comparative study by Lau et al. [13] on crude palm oil samples differs. In this report, silylation/chromatography yielded slightly higher levels of fatty acids than did titration. This difference may be related to the silylation chemistry, as their reagent, *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), is stronger than the HMDS/TFA used in this work. It is possible that the same drift in the results that we observed with BSA could also occur with BSTFA, which may contribute to the higher FFA levels obtained by this method compared with titration.

In summary, none of the stationary phases tested yielded baseline-to-baseline separation of all the TMS fatty acid esters that would be expected to occur in silylated vegetable oils. The DB-5 stationary phase provided the best separation for most cases, co-eluting only oleic and α-linolenic acids. As an analytical method, silylation followed by GC yielded FFA values that are comparable to those obtained by titration over a wide range of acid levels. The GC method discussed in this report can be recommended as an alternative approach to titration for determining FFA levels in most vegetable oils. Because the technique also provides information on the distribution of FFAs, it may also be useful as a research tool for agricultural researchers studying oilseed chemistry.

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