Influence of Triacylglycerol Characteristics on the Determination of Free Fatty Acids in Vegetable Oils by Fourier Transform Infrared Spectroscopy

T. Verleyen[a,]*, R. Verhe[a], A. Cano[a], A. Huyghebaert[b], and W. De Greyt[c]

Departments of [a]Organic Chemistry, [b]Food Technology and Nutrition,
9000 Gent, Belgium, and [c]De Smet Engineering, Edegem, Belgium

ABSTRACT: A rapid and direct Fourier transform infrared (FTIR) spectroscopic method using a 25-μm NaCl transmission cell was developed for the determination of free fatty acids (FFA) in six important vegetable oils (corn, soybean, sunflower, palm, palm kernel, and coconut oils) that differ in fatty acid profile. The calibrations were established by adding either standard FFA (oleic, lauric acids) or a representative mixture of FFA obtained after saponification of the refined oils. For all oils, up to a FFA level of 6.5% for coconut oil, the best correlation coefficient was obtained by linear regression of the free carboxyl absorption at 1711 cm^{-1}. All correlation coefficients were greater than 0.993, and no significant difference between the calibration methods could be detected. Upon validation of the calibration, no significant difference ($\alpha = 0.05$) between the "actual" and the "FTIR predicted" FFA values could be observed. The calibration models developed for the six oils differed significantly and indicate the need to develop a calibration that is specific for each oil. In terms of repeatability and accuracy, the FTIR method developed was excellent. Because of its simplicity, quick analysis time of less than 2 min, and minimal use of solvents and labor, the introduction of FTIR spectroscopy into laboratory routine for FFA determination should be considered.

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A wide variety of vegetable oils and fats are important products for the food and other industrial sectors. In almost all cases general characteristics of the oil determine oil quality, which in turn strongly influences trading of this commodity. One of the specifications is the free fatty acid content (FFA), which is traditionally determined by a chemical titration.

Fourier transform infrared (FTIR) spectroscopy has received attention for use in the quantitative analysis of fats and oils (1,2). In comparison with conventional instruments, FTIR has a higher energy throughput, excellent reproducibility, and accuracy from the laser source. With the increasing use of computers, FTIR can easily manipulate spectral information, and its advanced chemometric software is equipped to handle the calibration. Liquid sample handling has been simplified by the introduction of the attenuated total reflection accessory and the flow-through transmission cell. These innovations make oils and fats ideal candidates for FTIR analysis as they are single-component systems that can be easily applied in their neat form (3).

Near-infrared and infrared spectroscopic methods have been applied to the detection of adulteration (4,5) and to the classification (6) of oils, and recently have been used in a number of quantitative lipid analysis methods, such as iodine value (7), *cis* and *trans* fatty acids (8–10), peroxide value (11,12), anisidine value (13), and phospholipids (14,15). FFA have been determined in oils such as olive (16) and palm (17).

The objective of this study was to develop an FTIR method for the determination of FFA in six vegetable oils that differ in fatty acid profile, with the aim of replacing laborious official methods, particularly when a large number of samples have to be analyzed.

EXPERIMENTAL PROCEDURES

FTIR procedure. The study was carried out on a Nicolet (Madison, WI) Impact 410 FTIR spectrophotometer capable of covering the spectral range of 4000–400 cm^{-1}. A computer operating under the program Omnic version 2.1 controlled the instrument, and Basic Quant software was used for data manipulations. All spectra were collected from 32 scans at 4 cm^{-1} resolution and a gain of 1. Before every scan, a new reference air background spectrum was taken.

The sample-handling accessory consisted of a flow cell with NaCl windows that was provided with a spacer of 25 μm pathlength. All samples (both saturated and unsaturated oils) were analyzed with the flow cell heated to 75°C. The flow cell was cleaned by flushing with *n*-hexane.

Calibration samples. Six commercially important vegetable oils (corn, soybean, sunflower, palm, coconut, and palm kernel oils) have been used in this study as the matrices for developing the calibration. Calibration curves for the different oils were obtained by regression of the FFA content determined by titration against the peak heights recorded with FTIR.

For each oil, two calibration lines were established by adding two types of FFA to fully refined oils. For the first calibration, laboratory-grade FFA, either oleic acid for the unsatu-

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*To whom correspondence should be addressed at Faculty of Agricultural and Applied Biological Sciences, Department of Organic Chemistry, Coupure Links 653, 9000 Gent, Belgium.
E-mail: tom.verleyen@rug.ac.be

rated oils or lauric acid for the saturated fats, was added to the refined oils. The second calibration was established by adding a mixture of FFA representative of the fatty acids present in the oil. The different oils were then saponified with NaOH, and the unsaponifiables were removed by extraction with Et_2O. The saponifiable fraction was acidified to regenerate the FFA from their salts. The FFA were extracted with *n*-hexane, dried over $MgSO_4$, and evaporated. The FFA mixture obtained after saponification was added back to its corresponding refined oil.

Chemical procedure. FFA values of the samples were determined by titration according to the AOCS reference method (18). The FFA value was expressed as oleic acid for unsaturated oils (corn, sunflower, soybean, and palm) and as lauric acid for saturated oils (coconut and palm kernel).

Validation calibration. For a calibration model to be reliable it must be representative of the system and thus include its natural variability. To ensure the selection of samples encompassing a good variability, the validation was carried out on different samples. Therefore, samples with different FFA content, originating from different countries and taken from different steps in the refining process, were obtained by Extraction De Smet (Edegem, Belgium). For every oil, the validation was based on 20 different samples.

Validation of the calibration models was done by calculating the mean difference (MD) and the standard deviation of the difference (SDD) between the actual and the predicted FFA values. A *t*-test was applied for detecting if the differences were statistically significant.

RESULTS AND DISCUSSION

The study was initiated by establishing FFA calibration curves for six different vegetable oils (corn, soy, sunflower, palm, palm kernel, and coconut). These oils were selected because they have different fatty acid profiles, as expressed by their iodine values and saponification numbers (Table 1). Different amounts of a standard fatty acid (oleic acid, lauric acid) or a representative mixture of fatty acids obtained after saponification of the oil was added to the fully refined oils. For the unsaturated oils, calibration lines were established between 0.1 and 2.5% FFA, whereas for the saturated oils calibration lines up to an FFA content of 6.5% were constructed.

Quantification of the FFA level was done by measuring the peak height of the FFA carboxyl adsorption band at 1711 cm^{-1}, which was baseline-corrected by using 1850 and 1580 cm^{-1} as anchor points. Recent research (16,17) confirmed that the only significant region for the quantification of FFA is the adsorption band at 1711 cm^{-1}.

TABLE 1
Chemical Characterization of the Different Vegetable Oils[a]

	Corn	Soybean	Sunflower	Palm	PKO	Coconut
IV	124	124	133	46	14	7.5
SN	185	185	183	196	245	250

[a] IV, iodine value; SN, saponification number; PKO, palm kernel oil.

TABLE 2
Mathematical Equations[a] **of the Calibration**[b] **Established with Standard Free Fatty Acids for Different Vegetable Oils**

Oil	Equation	r^2
Corn	$y = 0.0387x + 0.1857$	0.9999
Soybean	$y = 0.0346x + 0.1877$	0.9998
Sunflower	$y = 0.0389x + 0.1772$	0.9974
Palm	$y = 0.0301x + 0.2310$	0.9985
Palm kernel	$y = 0.0594x + 0.2345$	0.9928
Coconut	$y = 0.0548x + 0.2518$	0.9979

[a] $y = ax + b$: Fourier transform infrared (FTIR) response (y) as function of actual values (x).
[b] Calibration based on 15 samples; r^2, correlation coefficient.

Calibration lines were obtained by regression of the actual FFA (%) obtained by titration vs. the FTIR response (Tables 2 and 3). For all calibrations, up to an FFA level of 6.5% for coconut oil, the best correlation coefficient was obtained by linear regression. The correlation coefficients (r^2) were all above 0.993, making the calibrations useful for further interpretation and validation.

The results in Tables 2 and 3 indicate that for the different oils nearly similar mathematical equations were obtained for the addition of standard FFA (oleic, lauric acids) or the representative FFA mixture obtained after saponification. However, between the different types of oil, calibration lines having a distinctively different slope and intercept were obtained. An overlay plot of the actual FFA value determined by titration vs. the FTIR response is represented in Figure 1. A clear trend relating the oil saturation and the position of the calibration line can be distinguished. The unsaturated oils (corn, soybean, and sunflower) have similar iodine values and saponification numbers, resulting in calibration lines that are very similar to each other in this graph. As the degree of saturation increases to palm oil and then to palm kernel oil/coconut oil, the intercept and the slope of the calibration lines are shifted upward. These differences can probably be attributed to the chain length and the degree of unsaturation of the fatty acids. Previous studies (19,20) on *trans* absorbency also concluded that the chain length and the degree of unsaturation of the fatty acids strongly affected the FTIR response. Therefore, it is necessary to build several specific calibration models.

Validation of the calibrations. For the different oils, sev-

TABLE 3
Mathematical Equations[a] **of the Calibration**[b] **Established with a Mixture of Free Fatty Acids Obtained After Saponification for Different Vegetable Oils**

Oil	Equation	r^2
Corn	$y = 0.0387x + 0.1854$	0.9998
Soybean	$y = 0.0344x + 0.1878$	0.9999
Sunflower	$y = 0.0385x + 0.1774$	0.9971
Palm	$y = 0.0301x + 0.2309$	0.9939
Palm kernel	$y = 0.0572x + 0.2406$	0.9955
Coconut	$y = 0.0577x + 0.2505$	0.9961

[a] $y = ax + b$: FTIR response (y) as function of actual values (x).
[b] Calibration based on 15 samples; r^2, correlation coefficient; for other abbreviation see Table 2.

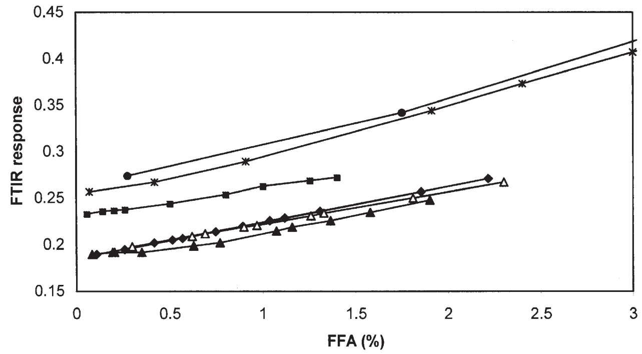

FIG. 1. Overlay plot of the Fourier transform infrared (FTIR) response vs. free fatty acid (FFA) content (%) for the different oils: corn (◆), soybean (△), sunflower (▲), palm (■), palm kernel (*), and coconut (●).

TABLE 4
Calibration[a] Lines of Actual vs. Predicted FFA Values Using the Calibration Model of the Same Oil

Oil	[b]	Equation[c]	MD	SDD
Corn oil	1	$y = 1.000x - 0.008$	0.01	0.04
	2	$y = 1.000x - 0.002$	0.00	0.04
Soybean	1	$y = 0.997x + 0.006$	0.00	0.14
	2	$y = 1.002x + 0.003$	0.01	0.14
Sunflower	1	$y = 1.047x - 0.081$	0.06	0.11
	2	$y = 1.058x - 0.073$	0.08	0.12
Palm	1	$y = 1.054x - 0.228$	–0.06	0.09
	2	$y = 1.054x - 0.224$	–0.06	0.09
Coconut	1	$y = 1.080x - 0.536$	–0.34	0.14
	2	$y = 1.026x - 0.487$	–0.42	0.06

[a] $y = ax + b$: Predicted free fatty acid (FFA) value (y) as a function of actual FFA values (x).
[b] 1: Calculated according to calibration line established for standard fatty acids; 2: calculated according to calibration line established for representative mixture of fatty acids obtained after saponification.
[c] Validation of calibration based on 20 samples. MD, mean difference; SDD, standard deviation of the difference.

eral samples were analyzed by FTIR, and the predicted FFA values were calculated by the previously established calibration lines. Results of the MD and SDD between the actual and the predicted FFA value for the different calibration lines are listed in Table 4. Regression lines between the FFA values obtained by titration and the predicted FFA value obtained by FTIR were calculated. The slopes of the regression lines are close to 1, indicating a perfect linear relationship between FTIR and chemically determined FFA values. For four oils (corn, soybean, sunflower, and palm), a *t*-test between the actual and the predicted values confirmed the absence of significant differences (t_{exp} was smaller than t_{critical} at a significance level of $\alpha = 0.05$). However, for coconut oil, a higher MD (–0.34), a higher SDD (0.14), and a significant difference were found. Thus, satisfactory calibrations based on the addition of FFA to a refined oil were established for corn, soybean, sunflower, and palm oils, but the calibration did not fit for coconut oil. Building a new calibration for coconut oil based on more samples will be necessary.

Because the calibration models for corn, soybean, and sunflower oils were rather similar, the possibility that their calibrations could be used interchangeably was checked. The predicted FFA values were calculated by using the calibration model of another oil, which resulted in six possibilities (Table 5). In all cases, the MD and the SDD of the validation were considerably higher than the results obtained by using the calibration model specific for each oil. The calibration lines for corn oil and soybean oil were found not to be significantly different ($\alpha = 0.05$), whereas the calibration model of sunflower oil was significantly different from the model developed for corn and soybean oil.

Reproducibility and accuracy. Replicate instrumental analyses for corn and soybean oils were carried out to check repeatability and accuracy of the method (Table 6). In terms of repeatability ($n = 10$) excellent values for MD_r and SDD_r were obtained which were, respectively, lower than 0.010 and 0.020% FFA. The accuracy of the FTIR method was excellent as well with an MD_a of 0.020% and a SDD_a ranging between 0.015 and 0.030% FFA.

Results from this study indicate that FTIR spectroscopy coupled with a flow-through transmission cell is a useful technique for the determination of FFA in vegetable oils. Accurate and reproducible results can be obtained after calibration of the FTIR instrument with either standard FFA or a representative FFA mixture of every oil. Calibration lines seemed to be strongly dependent on the oil under study. For each oil, the best validation results were obtained by using the calibration

TABLE 5
Calibration Lines of Actual vs. Predicted FFA Values Using the Calibration of a Different Oil

Oil	Equation[a]	MD	SDD	St[b]
Corn oil equation				
Soybean	$y = 1.119x - 0.067$	0.04	0.08	A
Sunflower	$y = 0.995x + 0.211$	0.21	0.04	B
Soybean oil equation				
Corn	$y = 0.891x + 0.057$	–0.05	0.08	A
Sunflower	$y = 0.886x + 0.275$	0.15	0.16	B
Sunflower oil equation				
Corn	$y = 1.153x - 0.288$	–0.12	0.11	B
Soybean	$y = 1.289x - 0.380$	–0.10	0.19	B

[a] $y = ax + b$: Predicted FFA value (y) as a function of actual FFA values (x) calculated by using the oleic acid calibration model.
[b] A, values are not significantly different, B, values are significantly different. For abbreviations see Table 4.

TABLE 6
Reproducibility and Repeatability of the FTIR Method for the Determination of FFA in Corn and Soybean Oil[a]

Oil	FFA (%)	FFA cal. (%)	MD_r	SDD_r	MD_a	SDD_a
Corn	1.21	1.20[b]	−0.006	0.023	−0.018	0.021
Corn	1.63	1.62[a]	−0.007	0.014	0.012	0.014
Soybean	1.00	0.99[a]	−0.009	0.016	0.011	0.029

[a] MD_r, mean difference of repeatability; SDD_r, standard deviation of the difference with respect to repeatability; MD_a, mean difference of accuracy; SDD_a, standard deviation of the difference with respect to accuracy; for other abbreviation see Table 4.

line established for each specific oil. Therefore, it seems necessary to provide a calibration for each oil. This approach is only applicable when the separate oil under study is known, which provides no barrier to application of the method for quality control laboratories or refineries. Most laboratories only deal with a restricted number of oils and can therefore easily apply the described method.

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