Determination of Low *trans* Levels in Refined Oils by Fourier Transform Infrared Spectroscopy

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ABSTRACT: A Nicolet 410 Fourier (FTIR) spectrophotometer, equipped with a DGTS detector and a sample cell with NaCl windows (nominal pathlength = 50 µm), was used for the development of an FTIR method for routine analysis of low *trans* levels in physically refined oils. The approach of the study differed from those previously described in that a separate calibration curve was established for each type of oil. Quantitation was established by use of Basic Quant Software® and by measuring the peak height at 967 cm^{-1} relative to a baseline drawn between 1002 and 932 cm^{-1}. The slope of the different calibration curves established in six vegetable oils (soybean, corn, sunflower, high-oleic sunflower, low-erucic rapeseed, and high-erucic rapeseed) was close to 1 (0.9942–1.0041), and correlation coefficients (r^2) were rather good (0.9990–0.9999). FTIR spectra of 20 soybean oil samples were collected and quantitated with the different calibrations. Compared to previous reported literature data, increased accuracy (mean difference = 0.05%; standard deviation of difference = 0.11%) and reproducibility (r^2 = 0.09–0.12%) were obtained when the FTIR spectra were quantitated with a calibration curve based on 10 physically refined soybean oil samples.
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KEY WORDS: Analysis, calibration, FTIR, physical refining, soybean, *trans*-fatty acids.

Several nutritional studies suggest a direct relationship between consumption of *trans* fatty acids (TFA) and an increased risk for coronary heart disease (1–3). As a consequence of this controversy, the formation of *trans* isomers during partial hydrogenation and high-temperature physical refining has become an important quality parameter. Furthermore, a trend is observed in the food industry to replace partially hydrogenated oils in margarines and shortenings by other fat sources (fully hydrogenated and/or interesterified fats, fat fractions) with a lower *trans* content (4,5).

Due to this increased attention, there is an urgent need for a rapid and accurate method for the determination of low TFA levels. Previous studies already showed that Fourier transform infrared (FTIR) spectroscopy is a useful technique for the rapid determination of isolated TFA in vegetable oils (6,7) based on the specific absorption of the *trans* CH-out-of-plane deformation at 967 cm^{-1}. However, due to the inevitable interference of triglyceride absorbance, difficulties arise when low *trans* levels present in physically refined oils have to be measured. This phenomenon of triglyceride interference was first described by Kaufmann *et al.* (8) and later confirmed by Firestone and De La Luz Villadelmar (9).

Different ways to circumvent this problem were studied. Lanser and Emken (10) developed a computer-assisted method for the estimation of isolated *trans* unsaturation by using the peak area of the *trans* absorbance band at 966 cm^{-1} from FTIR spectra of fatty acid methyl esters (FAME).

Sleeter and Matlock (11) indicated that a quadratic equation should be used to calculate *trans* unsaturation (% *trans* vs. band area). This technique allowed the analysis of FAME while eliminating the need for dilution by volatile solvents. Ulberth and Haider (6) explored the application of partial least squares (PLS) analysis for quantitation of low TFA levels in edible oils and concluded that this procedure, calibrated with background-corrected calibration standards, resulted in an excellent agreement between expected and obtained TFA.

Van de Voort *et al.* (7) developed a rapid FTIR method in which no transesterification of the oils was necessary. A general standard curve, based on 13 commercial triglycerides and applicable to all triglyceride-based oils in general over a wide range of *trans* contents (0–15%), was established with PLS as a chemometric approach. The developed method measured *trans* levels with good reproducibility and accuracy, except for samples with a high saponification number, such as palm kernel and coconut oil.

The aim of our study was to develop an FTIR method with increased reproducibility and accuracy that is useful for routine analysis of low *trans* levels in physically refined oils. The approach differed from those previously described in that a separate calibration curve was established for each type of oil which should allow analysis of neat oils and quantitation by simple linear regression.

EXPERIMENTAL PROCEDURES

Materials. Six partially refined vegetable oils [soybean, corn, sunflower, high-oleic sunflower (HO), low-erucic rapeseed (LE), and high-erucic rapeseed (HE)] with no detectable *trans* levels were obtained from De Smet Group (Edegem, Bel-

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TABLE 1
Characterization of the Different Vegetable Oils[a]

	Soybean	Corn	Sunflower	Sunflower HO	Rapeseed LE	Rapeseed HE
IV	124	124	133	88	108	103
SN	185	185	183	190	183	179

[a]IV, iodine value; SN, saponification number; HO, high-oleic; LE, low-erucic; HE, high-erucic.

gium). Iodine values and saponification numbers are shown in Table 1.

Degummed, neutralized soybean oil samples were refined under different conditions in a lab-scale deodorizer to obtain varying levels of TFA. Trielaïdin (18:1*trans*) was purchased from Sigma Chemical (Bornem, Belgium). Potassium hydroxide and p.a.-grade solvents were purchased from Janssens Chimica (Geel, Belgium).

FTIR spectroscopy. A Nicolet Impact 410 FTIR Spectrophotometer (Thermogroup, Breda, The Netherlands), equipped with a DTGS detector (Thermogroup) and connected to a 486-based IBM/AT-compatible PC, which operated under Omnic version 2.1, was used for recording the infrared (IR) spectra. Basic Quant® Software (Thermogroup) was used for data manipulations. The region of 4000 to 650 cm^{-1} was scanned with a resolution of 4.00 cm^{-1} (32 scans/sample). Neat oil samples without transesterification were analyzed.

Sample cell. A cell with NaCl windows and a nominal pathlength of 50 µm was placed in the cell insert. The cell was cleaned with acetone at the end of each analytical run.

Quantitation. Separate calibration mixtures were prepared for the different oils by adding accurately known amounts of trielaidin to the partially refined oils. TFA concentration of the standards ranged from 0.0 to 4.5%. Additionally, a calibration mixture, based on 10 physically refined soybean oil samples, was prepared. All series were analyzed by measuring the peak height at 967 cm^{-1} relative to a baseline drawn between 1002 and 932 cm^{-1}.

Gas–liquid chromatography (GLC) of FAME. Analyses of FAME by GLC were carried out on a Carlo Erba 4130 chromatograph, equipped with a flame-ionization detector and a split injector (Carlo Erba, Milan, Italy). A fused-silica capillary column, coated with 100% cyanopropyl polysiloxane, (CP™ Sil 88, 50 m × 0.25 mm internal diameter; 0.20 µm film thickness; Chrompack, The Netherlands) was used with helium as a carrier gas. Temperature programming started at 150°C up to 200°C at a rate of 1.3°C/min and was held at 200°C until completion of the analysis. Injector and detector were maintained at 250°C. Quantitative analyses were performed with a Shimadzu RC3A integrator (Kyoto, Japan).

RESULTS AND DISCUSSION

At first, separate calibration curves were established for six vegetable oils (soybean, corn, sunflower, sunflower HO, rapeseed LE, and rapeseed HE). Different standard mixtures were prepared by adding accurately weighed amounts of trielaidin to the *trans*-free oils. Quantitation of *trans* levels was performed by measuring the peak heights at 967 cm^{-1} relative to a baseline drawn between 1002 and 932 cm^{-1} using background corrected absorptivity (12). Calibration was established by simple linear regression of predicted vs. actual *trans* levels. Resulting mathematical equations appeared to be similar for the different oils (Table 2). The slope of the different calibration curves was close to 1 (0.9942–1.0041), and correlation coefficients (r^2) were rather good (0.9990–0.9999) (Table 2), which made the calibrations useful for further quantitation.

However, TFA present in refined vegetable oils are not elaidic acid but mainly a mixture of C18:2 and C18:3 polyunsaturated fatty acids with one double bond in the *trans* configuration (Δ9*c*,12*t* C18:2; Δ9*t*,12*c* C18:2; Δ9*c*,12*c*,15*t* C18:3; and Δ9*t*,12*c*,15*c* C18:3 fatty acids). Therefore, an additional calibration curve was established, based on 10 physically refined soybean oil samples (Table 2). TFA levels determined by GLC ranged between 0.15 and 6.03% and were considered as "actual" *trans* values. Subsequently, FTIR spectra were collected from 20 soybean oil samples, physically refined under different process conditions in a lab-scale deodorizer. Each FTIR spectrum was quantitated by use of the seven described calibration curves (Table 2), resulting thus in seven different FTIR *trans* values for each soybean oil sample. Mathematical equations of TFA by FTIR vs. TFA by GLC are shown in Table 3. Accuracies of the different FTIR quantitations were compared with the GLC data by use of the mean difference (MD) and standard deviation of difference (SDD) (Table 3). Excellent agreement with GLC data could be achieved when soybean calibrations were applied to the FTIR spectra. Quantitation with the calibration based on the refined soybean oil samples resulted in the best accuracy (MD = 0.05%; SDD = 0.11%). Application of the calibrations, established in corn and sunflower oil, to the FTIR spectra of the soybean oil samples resulted in a lower but still acceptable agreement with the GLC data (MD = 0.24–0.32%; SDD

TABLE 2
Mathematical Equations of the Calibrations Established in Different Vegetable Oils

Matrix	Mathematical equation	r^2
Corn	$y = 0.9976x + 0.0020$	0.9990
Rapeseed LE	$y = 1.0041x + 0.0022$	0.9999
Rapeseed HE	$y = 0.9988x + 0.0021$	0.9993
Sunflower	$y = 0.9990x + 0.0130$	0.9997
Sunflower HO	$y = 0.9942x + 0.0110$	0.9992
Soybean	$y = 0.9998x + 0.0028$	0.9999
Soybean[b]	$y = 0.9998x + 0.0028$	0.9992

[a]$y = ax + b$. Predicted values (y) as a function of actual values (x).
[b]Calibration based on 10 refined soybean oil samples; r^2, correlation coefficient.

TABLE 3
***Trans* Fatty Acid (TFA) Content of Refined Soybean Oil Samples[a]**

Calibration	Equation	r^2	MD[b]	SDD[c]
Soybean	$y = 0.9837x–0.10$	0.9951	0.13	0.11
Soybean[d]	$y = 1.0045x–0.05$	0.9953	0.05	0.11
Corn	$y = 1.0488x–0.30$	0.9952	0.24	0.12
Sunflower	$y = 1.0309x–0.36$	0.9963	0.32	0.11
Sunflower HO	$y = 1.1877x+0.31$	0.9776	–0.59	0.40
Rapeseed LE	$y = 1.0142x+0.71$	0.9953	–0.73	0.11
Rapeseed HE	$y = 1.1302x+0.23$	0.9809	–0.43	0.31

[a]Mathematical equations: $y = ax + b$. % *trans* by Fourier transform infrared spectroscopy (FTIR), according to different calibrations (y) as a function of % *trans* by gas–liquid chromatography (GLC) (x).
[b]Mean difference.
[c]Standard deviation of difference.
[d]Additional calibration based on (10) physically refined soybean oil samples. See Tables 1 and 2 for other abbreviations.

TABLE 4
Comparison of the Reproducibility of the GLC and FTIR Method for the Determination of *trans* Fatty Acids

	FTIR			GLC		
Sample	Mean	SD[a]	r^b	Mean	SD	r
1	3.29	0.03	0.09	3.05	0.05	0.15
2	1.09	0.03	0.09	0.94	0.05	0.15
3	0.63	0.04	0.12	0.52	0.06	0.17

[a]Standard deviation.
[b]Reproducibility. See Table 3 for abbreviations.

0.11–0.12%). On the other hand, sunflower HO, rapeseed LE, and HE calibrations resulted in poor accuracy, which was illustrated by higher MD (–0.43––0.73%) and SDD (0.31–0.41%).

Characterization of the different oils (Table 1) indicates that their saponification number (SN) is similar but that the degree of unsaturation, expressed by the iodine value (IV), is somewhat different. Sunflower HO, rapeseed LE and HE mainly consist of monounsaturated fatty acids (oleic and erucic acid) and are thus less unsaturated than the other oils (soybean, corn, and sunflower), which mainly consist of polyunsaturated fatty acids (linoleic and linolenic acids). This difference in degree of unsaturation is probably at the origin of the observed differences in accuracy when applying the different calibrations to the FTIR spectra. Earlier studies, conducted by Firestone and De La Luz Villadelmar (9) and Firestone and Labouliere (12), showed that triglyceride interference in the region of *trans* absorbance depended not only on the chainlength but also on the degree of unsaturation of the fatty acids.

Reproducibility was studied with the selected calibration curve based on the refined soybean oil samples. Three samples that contained low *trans* levels were analyzed tenfold by GLC and FTIR (Table 4). Compared to the GLC results, excellent standard deviations (0.03–0.04%) and reproducibilities (0.09–0.12%) were obtained by FTIR, even for the sample with the lowest *trans* content.

In general it can be stated that, compared to previous reported literature data (4,5,8,9), increased accuracy and reproducibility can be obtained when FTIR spectra of vegetable oils are quantitated with a calibration curve established in the oil under study. The fact that this approach is only applicable when the oil under study is known forms no limitation for its application in quality-control laboratories of refineries. Most oil refiners are only dealing with a limited number of oil types and can, therefore, perfectly introduce the described method for the rapid and accurate determination of low *trans* levels in physically refined oils.

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