A Rapid Method for the Analysis of Hydrogenated Fats by GC with IR Detection

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ABSTRACT: A rapid method for analysis of *trans* and *cis* FA in hydrogenated fats has been developed. The method is based on a single analysis by GC with IR detection. Multivariate partial least squares regression is applied on the IR spectra to predict the number of *cis* and *trans* double bonds. For each chain length the method provides information about the amount of the saturated FA, the amount of *trans* monoenes, the amount of *cis* monoenes, the amount of PUFA, and the average number of *cis* and *trans* double bonds in PUFA. The method has been validated by summing the values to a total *trans* value and total unsaturation. These sum values were compared with total *trans* unsaturation, as determined by AOCS method Cd 14-95, and iodine value, as determined by AOCS Cd 1d-92.

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KEY WORDS: Gas chromatography, hydrogenated fats, hydrogenation, infrared spectroscopy, partial least squares regression, *trans* fatty acids.

Trans double bonds (DB) in oils and fats can be quantified by IR spectroscopy by measuring the absorption at approximately 967 cm^{-1} (1,2). This principle is used in several standard procedures (3–5). Methyl elaidate (*trans* 18:1n-9) or glyceryl trielaidate is usually applied as external standard in these methods. Several reports have shown that the absorption at 967 cm^{-1} from *trans* DB in geometric isomers of linoleic acid (18:2n-6) (6–8), linolenic acid (18:3n-3) (9), and *trans* FA found in hydrogenated fats (8,10) is weaker than the absorption from the *trans* DB in elaidic acid (*trans* 18:1n-9). Underestimation of the amount of *trans* DB may therefore occur in some samples.

The IR spectra also contain information about the chain length and the number of *cis* DB. The most important signals for the determination of FA structure have been summarized by Guillén and Cabo (11). The number of *cis* DB in a FA can be calculated from the signals at 3020 and 2920 cm^{-1} (12). Iodine value (IV) also can be calculated from the ratio between the signals at 3030 and 2857 cm^{-1} (13). Van de Voort *et al*. (14) obtained good correlation between IR spectra and IV using whole spectra as input in partial least squares (PLS) regression.

Trans FA also are analyzed by GC using long polar capillary columns. The GC methods provide more detailed molecular information than IR analysis and have lower detection limits. The major drawback of the GC methods is overlap between the chromatographic zones of *cis* and *trans* FA. This overlap may lead to severe errors in quantification, especially when complex samples such as hydrogenated fats are analyzed.

Attempts have been made to combine the two techniques using GC with IR detection, often in combination with FID or MS detectors. Using a lightpipe GC-IR interface, Wahl *et al*. (15) were able to identify 31 unsaturated reference FA including both *cis* and *trans* isomers, and reported the detection limit for single isomers to be about 25 ng. GC-MS combined with GC-IR equipped with a direct deposition interface has been used to identify individual isomers in hydrogenated vegetable fats after prefractionation on silver-ion HPLC (16). The combination of silver-ion HPLC and GC-IR equipped with a matrix isolation interface has been applied for quantification of *trans* monoenes in hydrogenated fats (17). Matrix isolation GC-IR also has been used for the identification of individual isomers in hydrogenated vegetable fats (18). The direct deposition and matrix isolation interfaces have better signal-to-noise ratios than the lightpipe interface, which is a simpler and less expensive construction.

The positional and geometrical isomerization of the FA that occurs during hydrogenation of edible oils, and particularly during hydrogenation of fish oils, leads to a large number of FA isomers that are difficult to quantify by simple chromatographic methods (19). The purpose of this work was to develop a simple and rapid method for quantification of the *trans* FA content in partially hydrogenated (PH) fish oils and PH vegetable oils, using GC with a lightpipe-interfaced IR spectrophotometer as the only detector. For each chain length, we wanted to quantify the amount of saturated FA, the amount of *trans* monoenes, the amount of *cis* monoenes, the amount of PUFA, and the average *cis*/*trans* content in PUFA.

EXPERIMENTAL PROCEDURES

Preparation of reference compounds. FAME reference compounds, *cis* isomers of 14:1n-5, 16:1n-7, 18:1n-9, 19:1n-9, 20:1n-9, 22:1n-9, 24:1n-9, 18:2n-6, 19:2n-6, 20:2n-6, 22:2n-6, 18:3n-3, 18:3n-6, 20:3n-3, 22:3n-3, 20:4n-6, and 22:4n-6 (>99% pure) purchased from Nu-Chek-Prep (Elysian, MN) were isomerized with *para*-toluenesulfinic acid and fractionated on silver-ion HPLC as described elsewhere (20). Fractions of the isomerized FA that contain a specific number of *cis* and *trans* DB can easily be obtained with silver-ion HPLC

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TABLE 1
Number of *cis* and *trans* Double Bonds in the FA Isomers Used in the PLS Calibration Set[a] (after removal of outliers)

	0 *cis*	1 *cis*	2 *cis*	3 *cis*	4 *cis*
0 *trans*	12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0	14:1n-5[2], 16:1n-7[2], 18:1n-9[2], 19:1n-9, 20:1n-9, 22:1n-9, 24:1n-9	18:2n-6[4], 19:2n-6, 20:2n-62, 22:2n-6[2]	18:3n-6, 18:3n-3[2], 20:3n-3[2], 22:3n-3	20:4n-6, 22:4n-6[2]
1 *trans*	14:1n-5, 16:1n-7, 18:1n-9, 19:1n-9, 22:1n-9, 24:1n-9	18:2n-6[2], 19:2n-6[2], 20:2n-6[2], 22:2n-6[2]	18:3n-6[2], 18:3n-3[2], 22:3n-3[2]	22:4n-6	
2 *trans*	18:2n-6, 19:2n-6, 20:2n-6, 22:2n-6	18:3n-6, 18:3n-3, 22:3n-3	20:4n-6[2], 22:4n-6		
3 *trans*	18:3n-6, 18:3n-3, 22:3n-3	20:4n-6, 22:4n-6			
4 *trans*	22:4n-6				

[a]The *trans* isomers were prepared as described in the Experimental Procedures section. Superscript denotes the number of spectra in cases where more than one spectrum is used in the model. Abbreviation: PLS, partial least squares.

or silver-ion TLC (21–23). The purity of the fractions was examined by GC-MS as described elsewhere (20). Only samples that showed no interference from other HPLC fractions were applied in the calibration set described below.

Collection of reference spectra. The HPLC fractions were diluted with hexane, and approximately 200–2000 ng of each sample was analyzed by GC-IR. Previous studies (Mjøs, S.A., and J. Pettersen, unpublished data) indicated that from 50 to 6000 ng there is no drift or other systematic change in the spectra, and the presence of white noise can be neglected. A database of the collected GC-IR absorbance spectra was organized in Microsoft Excel (Redmond, WA). In cases where the HPLC fractions gave more than one GC peak, all peaks were included in the database. The saturated and *cis*-unsaturated (up to four DB) FAME from C_{12} to C_{24} present in the reference mixture GLC-461 (Nu-Chek-Prep) also were analyzed and included in the database. In total, the base included 73 spectra of FA with different chain lengths and different numbers of *cis* and *trans* DB. The isomers used for collection of reference spectra are given in Table 1.

Preparation of samples. Samples of PH fats with large variations in the degree of hydrogenation (Table 2) were a gift from a European producer. Four samples were PH fish oils; eight samples were PH vegetable oils. Total *trans* unsaturation was analyzed at the Scottish Crop Research Institute (Dundee, United Kingdom) according to AOCS method Cd 14-95 (3). IV was analyzed by SSF (Bergen, Norway) according to AOCS method Cd 1d-92 (3).

Solid fat samples were heated on a heating block, and three drops of fat were converted to FAME according to AOCS method Ce 1b-89 (3) but with some modifications. Reaction times of 10 min were applied both for alkali (NaOH)- and acid (BF_3)-catalyzed esterification, and 2 mL of methanolic NaOH (0.5 N) and BF_3 (12%) were applied. Approximately 1 µg of FAME was injected into the GC-IR and analyzed as described next.

GC-IR analytical conditions. The *cis*/*trans* reference compounds and the hydrogenated fats were analyzed on an HP-5890 Series II GC (Agilent Technologies, Palo Alto, CA) connected to an HP-IRD infrared detector (BioRad, Cambridge, MA). The injector was equipped with electronic pressure control. The analytical column was CP-Wax 52cb, length = 30 m, i.d. = 0.32 mm, film thickness = 0.25 µm (Varian, Middelburg, The Netherlands). Oven temperature was 60°C at injection. After 2 min the oven temperature was increased by 40°C/min to 180°C, followed by a gradient of 10°C/min to 250°C, where the temperature was held for 3 min. Total program time was 15 min.

Samples (2 µL hexane solutions) were injected in splitless mode. The split valve was opened 2 min after injection. Injector temperature was 260°C. Helium (99.996%) was used as carrier gas. Injector pressure was 40 psi at injection and was reduced to 7.6 psi after 1 min. Thereafter, the column head pressure was increased with the oven temperature to give an estimated flow of 1.4 mL/min.

The IR detector was equipped with a wide-band (4000–550 cm^{-1}) mercury-cadmium-telluride detector. Lightpipe and transfer line temperatures were 250°C. Transfer line A (outlet) purge pressure was 2.5 psi, and transfer line B (inlet) purge pressure was 3 psi. Nitrogen was used as purge gas. The optical resolution was 8 cm^{-1}, and the coadd factor was 4

TABLE 2
Fats Used in the Validation Experiment[a]

No.	Sample type	Avg. M.W. (g/mol)	Σ *trans* AOCS Cd 14-95[b]	IV AOCS Cd 1d-92[b]
1	FH SBO	295	1.3	1.60
2	PH PO	285	1.4	57.0
3	PH FO, m.p. 31°C	293	57.6	79.0
4	FH SBO + FH CO	252	0.0	2.20
5	PH FO, m.p. 51°C	294	21.1	32.0
6	PH SBO, m.p. 32°C	295	41.3	78.0
7	PH SBO, m.p. 41°C	295	43.4	66.0
8	FH SBO + FH CO + UH VO	270	0.2	46.0
9	PH FO, m.p. 27°C	302	27.5	78.0
10	PH CO (slightly hydrogenated)	231	1.5	5.6
11	PH FO, m.p. 39°C	294	37.9	50.0
12	PH PO, m.p. 51°C	286	24.0	37.0
13	PH PO, m.p. 41°C	284	15.3	46.0
14	PH SBO, IV ≈ 100	295	27.2	94.0

[a]FH = fully hydrogenated, PH = partially hydrogenated, UH = unhydrogenated, SBO = soybean oil, CO = coconut oil, PO = palm oil, FO = fish oil, IV = iodine value. m.p. = expected melting point, producer's value.
[b]Reference 3.

scans/spectrum. The IRD-chromatogram was calculated using the second difference spectrum from 1710 to 1790 cm^{-1}. All other settings were default values defined in the G1095A software, v.A00.02 (Agilent).

Software. Chromatograms were manually integrated using GRAMS-386 v. 2.04B software (Galactic Industries, Salem, NH). IR spectra were baseline-corrected and normalized in Microsoft Excel. PLS regressions were performed in Unscrambler 7.5 (CAMO, Oslo, Norway).

RESULTS AND DISCUSSION

GC of PH fats. GC analyzes of *trans* FA in hydrogenated fats are usually performed on very polar cyanopropyl stationary phases that give maximal resolution of *cis* from *trans* isomers, at least for monoenes where most of the *trans* isomers will elute before the first *cis* isomer.

When the average number of *cis* and *trans* DB for the FAME in each chromatographic peak can be determined from the IR spectrum, chromatographic separation between *cis* and *trans* isomers is not required for quantification. An ideal column for this application should therefore give narrow and well-resolved peaks of the saturated FA, monoenes, dienes, trienes, and others. The chromatographic separation should have maximal selectivity with respect to the number of DB in the molecule and minimal selectivity with respect to the position and geometry of the DB.

PEG (polyethyleneglycol) columns have a stationary phase of medium polarity and were found to have an acceptable selectivity. A GC-IR chromatogram of PH fish oil on a PEG capillary column is shown in Figure 1. As shown in the enlarged area of the C_{20} FAME, three regions, consisting of saturated FA, monounsaturated FA, and PUFA, can be quantified with limited or no overlap. Most monoenes elute in one sharp peak, but there is some overlap between the monoenes and dienes. There is poor separation between dienes and trienes; all PUFA were therefore quantified as one peak.

The GC-IR chromatogram is based on the strength of the carbonyl signal from 1790 to 1710 cm^{-1}. An assumption of no loss in the chromatographic system means detector responses for all FAME molecules are equal on a molar basis, which is different from an FID, where the detector responses are assumed to be approximately equal on a weight basis.

IR spectra of FAME molecules. Spectra of *cis* and *trans* unsaturated C_{20} FAME are shown in Figure 2. The signal for CH out-of-plane deformation in *trans* DB is at approximately 970 cm^{-1}. Quantification of *trans* DB is usually made by direct regression based on the area or the height of this peak.

For the quantification of *cis* DB, a single signal without interference does not exist. The strongest signal from *cis* DB is seen at approximately 3025 cm^{-1}. There may be a small shift in the peak maximum depending on the number of carbons between the DB in PUFA (18), and there is also a weak signal from *trans* DB in the same area (Fig. 2A). Since there is no strong signal from *cis* DB in the spectra that is free of interference, quantification of *cis* DB or total unsaturation must be made by multivariate regression (e.g., PLS) on the entire IR spectrum or parts of it.

PLS regression of number of DB from IR spectra. The IR spectra in the Excel database were used as independent variables (*X*-variables) in PLS regressions. PLS is a linear multivariate regression technique especially suitable in cases with a large number of *X*-variables and high covariance in the *X*-matrix, e.g., when the *X*-variables are UV, IR, or NIR spectra. The *X*-matrix is transformed to PLS components (latent variables), and regression is performed on the PLS component score values. For literature on PLS, see Reference 24.

Spectral baseline drift was corrected by subtracting the linear baseline estimated by regression on wavenumbers with no absorbance in FAME molecules: 775, 1550, 2100, 2550, 3275, and 3750 cm^{-1}. Baseline corrected spectra were normalized to the maximum of the carbonyl signal at 1746 cm^{-1}, i.e., all spectra are expressed on a scale where the carbonyl maximum has the value 1 (Fig. 2B). The spectral region from

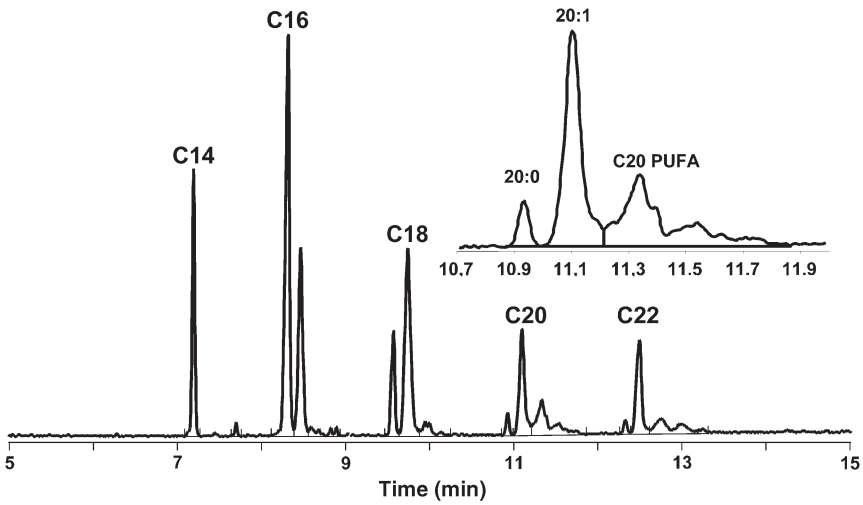

FIG. 1. GC-IR chromatogram of hydrogenated fish oil (sample 3). Chromatographic response is based on the carbonyl peak (1710–1790 cm^{-1}).

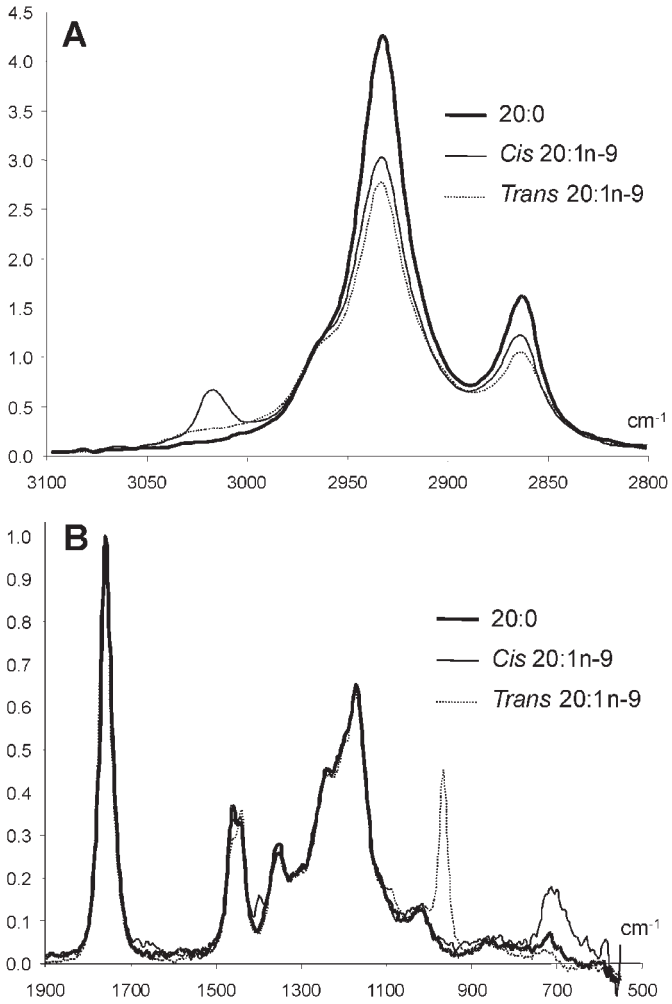

FIG. 2. GC-IR spectra of 20:0, *cis* 20:1n-9, and *trans* 20:1n-9. The absorption scale is normalized to the carbonyl maximum (value = 1). (A) 3100–2800 cm^{-1}, (B) 1900–500 cm^{-1}.

2800 to 1850 cm^{-1} contains no signals from the FAME molecule; neither is there any signal above 3100 cm^{-1}. For this reason, only the regions from 3200 to 2700 cm^{-1} and 1900 to 550 cm^{-1} were used in the PLS regression models.

In PLS regressions, outliers in the calibration set can be detected in three ways: (i) by having extreme score values on any of the significant PLS components, (ii) by having large residual variance, i.e., the spectrum is poorly explained by the PLS model, (iii) by having a large deviation between the predicted and real *Y* value when the PLS model is applied for prediction of *Y*. Objects that looked suspicious by any of these criteria were excluded from the calibration set if the spectra showed large amounts of random noise (amounts injected were too low) or showed baseline drift. Some tetraene fractions were also excluded because the GC-MS procedure (20) could not exclude the possibility of contamination.

Three separate PLS calibration models were created with the number of *cis* DB, *trans* DB, and total number of DB in the molecules as dependent variables (*Y*-variables). Full cross-validation (19) was used for evaluation of the models. For all three models, the standard errors of prediction (SEP) decreased significantly until four components were included in the models; inclusion of more components led to only minor reduction in SEP. The predicted vs. real numbers for the three models using four PLS components are displayed in Figure 3.

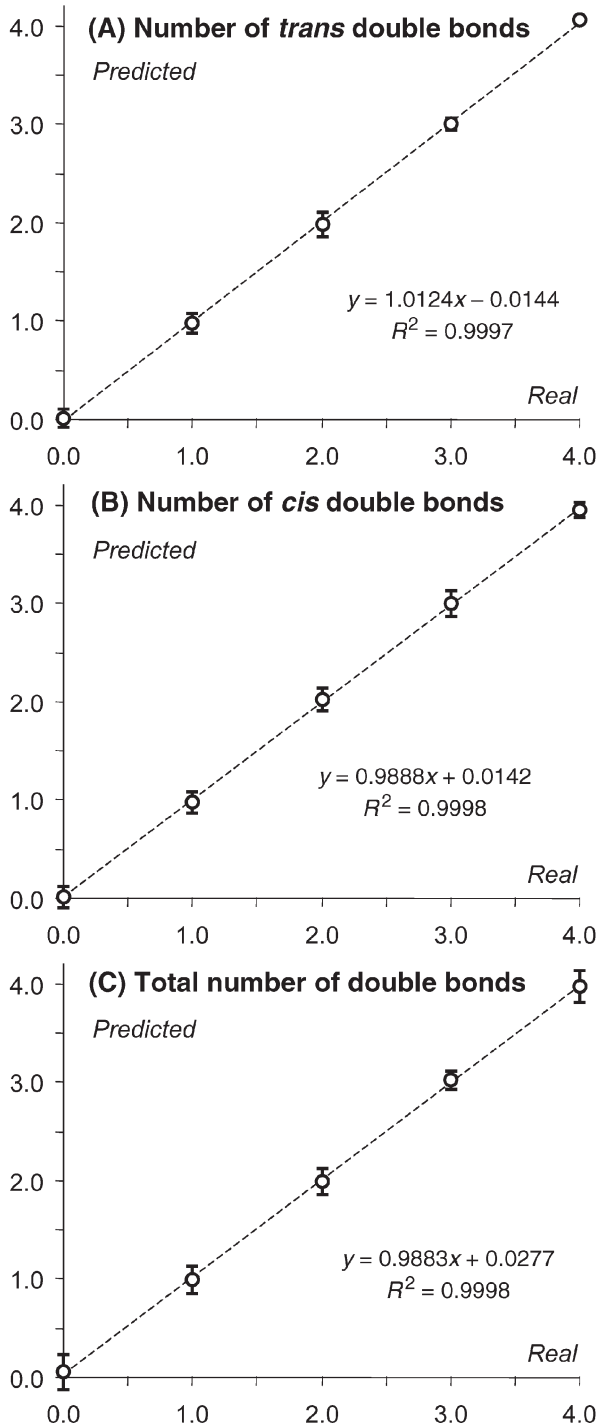

FIG. 3. Predicted vs. measured values for four-component PLS regressions (cross validation results) of the number of *cis* and *trans* DB and the total number of DB in analyzed FAME reference compounds. IR spectra from 3200 to 2700 cm^{-1} and from 1900 to 550 cm^{-1} were used in the regression models as independent variables. Symbols refer to mean ± 2 SD. PLS, partial least squares; DB, double bond.

SEP for the four-component models of *trans* DB, *cis* DB, and total DB were 0.052, 0.059, and 0.072, respectively.

Predictions. Samples of 14 hydrogenated fats were analyzed by GC-IR as described in the Experimental Procedures section. The chromatographic peaks were integrated as illustrated in Figure 1. The spectra of each chromatographic peak were baseline corrected and normalized in the same way as the reference compounds. The PLS models based on the reference compounds were then used to predict the average number of *cis* and *trans* DB in each peak; any negative predictions were set to zero. By combining the areas from the chromatograms with the results from the PLS predictions, the amounts of *trans* monoenes, *cis* monoenes, and the *cis* and *trans* unsaturation in the PUFA peaks were calculated.

Table 3 lists the results obtained for sample 3 (Table 2). The *cis* and *trans* values of a chromatographic peak are calculated by multiplication of the peak's area percentage by the peak's average number of *cis* and *trans* DB. Values for the total unsaturation can be calculated by summing the values in the *cis* and *trans* columns or from the direct PLS prediction of total DB. The sum of the *trans* values was 62.9; the corresponding value analyzed by AOCS Cd 14-95 was 57.6 (Table 2), a 9% overestimation by the GC-IR method relative to Cd 14-95.

The *cis* values can be evaluated by comparing the IV with the sum of *cis* and *trans* values. The sum of these values is 86.06 for this sample. The IV is by definition the weight of iodine in centigrams absorbed by 1 g of fat. When the average M.W. of the sample is close to the M.W. of oleic acid, a correction factor of 0.86 can be applied, as in AOCS recommended practice Cd 1c-85 (3). This gives an estimated IV for the GC-IR method of 74.0, which is 6% lower than the value according to Cd 1d-92 (Table 2).

The figures in Table 3 show that the average numbers of *cis* and *trans* DB in the monoene peaks sum to approximately one. This indicates correct predictions for the monoenes. A similar verification cannot be made for the polyenes since the number of DB is not known for these peaks. Except for C_{16} the average number of DB in PUFA peaks is slightly above 2. The results for C_{16} PUFA show the average number of DB of 0 *cis* and 1.15 *trans*. A closer inspection of the spectra showed no *trans* signal at 970 cm^{-1}. This error is probably caused by the presence of branched C_{17} FA, which are present in small amounts in fish oil and elute with similar retention times as C_{16} PUFA. Branched FA were not included in the PLS regression models, and their presence in prediction spectra may therefore lead to inaccurate results. Because of the small area of this peak, the error has a limited influence on the sum values.

The sums of *cis* DB, *trans* DB, and total unsaturation were calculated for each sample, and the results were used for validation of the method by comparison with the total *trans* value (AOCS Cd 14-95) and the IV (AOCS Cd 1d-92). Calculated sums for the 14 fat samples are compared with the reference methods in Figure 4. To simplify the interpretation, regression lines with intercepts of zero were applied. The plot of the *trans* values (Fig. 4A) shows a regression line with a slope of about 1.10, which corresponds to an overestimation relative to Cd 14-95 of approximately 10%. The two ways of calculating total unsaturation, either by summing the *cis* and *trans* values or by directly predicting the number of DB, gave slopes of 1.06 (Fig. 4B) and 1.05 (Fig. 4C). By using the conversion factor of 0.86 between total unsaturation and IV, the slope should in theory be 1.16 (i.e., 0.86^{-1}); thus, the GC-IR method underestimates the IV by approximately 9%.

It has been observed that the IR absorption per *trans* DB

TABLE 3
Average Amounts of Double Bonds in Each Peak and the Corresponding Values That Are Obtained by Multiplication with the Area Percentage from Results Obtained for a Hydrogenated FO[a]

	Ret. time	Area	*cis*		*trans*		*cis* + *trans*[d]		Total DB[e]	
	(min)	(%)	Avg[b]	Value[c]	Avg[b]	Value[c]	Avg[b]	Value[c]	Avg[b]	Value[c]
14:0	7.21	10.33	0.00	0.00	0.01	0.15	0.01	0.15	0.00	0.00
15:0	7.71	0.60	0.09	0.06	0.00	0.00	0.09	0.06	0.03	0.02
16:0	8.33	22.27	0.03	0.71	0.00	0.04	0.03	0.75	0.03	0.74
16:1	8.47	12.94	0.28	3.68	0.70	9.11	0.99	12.79	0.99	12.80
16-PUFA	8.75	2.53	0.00	0.00	1.15	2.91	1.15	2.91	0.91	2.30
18:0	9.58	4.45	0.05	0.20	0.00	0.00	0.05	0.20	0.03	0.14
18:1	9.75	14.56	0.31	4.53	0.72	10.44	1.03	14.97	1.03	15.04
18-PUFA	9.95	2.61	0.47	1.21	1.55	4.05	2.02	5.27	2.05	5.35
20:0	10.93	0.98	0.00	0.00	0.09	0.09	0.09	0.09	0.00	0.00
20:1	11.11	7.15	0.27	1.91	0.74	5.32	1.01	7.23	1.01	7.22
20-PUFA	11.34	8.47	0.36	3.04	1.82	15.39	2.18	18.43	2.17	18.36
22:0	12.33	0.66	0.41	0.27	0.26	0.17	0.67	0.44	0.73	0.48
22:1	12.50	6.12	0.32	1.98	0.70	4.27	1.02	6.25	1.03	6.29
22-PUFA	12.76	6.34	0.89	5.62	1.72	10.91	2.61	16.53	2.64	16.70
Sum		100.00		23.20		62.85		86.06		85.44

[a]Corresponding chromatogram is shown in Figure 1. All PLS predictions were based on four PLS components. For abbreviations see Tables 1 and 2.
[b]Average number of double bonds per molecule calculated from infrared spectra.
[c]Double bond value, calculated by multiplication of the average value of each peak and the peak area.
[d]Total number of double bonds calculated by summing the average number of *cis* and *trans* double bonds in each peak.
[e]Total number of double bonds calculated from the direct calibration of the double bond numbers.

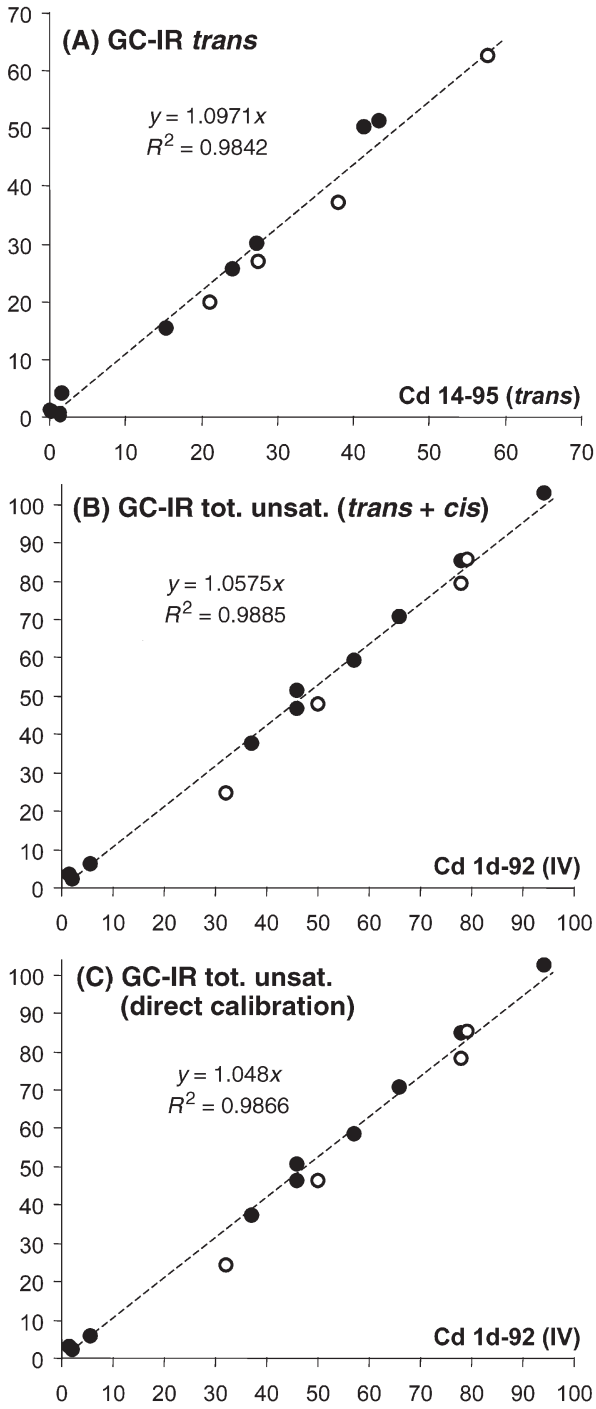

FIG. 4. (A) Sum of *trans* DB calculated from GC-IR results vs. values obtained by Cd 14-95 (Ref. 3). (B) Sum of *trans* and *cis* DB calculated from GC-IR results compared with iodine values by Cd 1d-92 (Ref. 3). (C) Sum of DB (direct calibration) calculated from GC-IR results compared with iodine values. Open circles are partially hydrogenated fish oil samples. Closed circles are partially hydrogenated vegetable oils. For abbreviation see Figure 3.

at 967 cm^{-1} is lower for PUFA than for elaidic acid, which is applied as external standard in AOCS Cd 14-95 (6–10). Consequently, an underestimation of the *trans* content in samples high in PUFA must be expected. For *trans* isomers of 18:2n-6 the response relative to elaidic acid has been reported to be about 85% (6,7,10). When the height of the *trans* peak at 967 cm^{-1} was measured with the baseline drawn according to Cd 14-95, the GC-IR spectra gave a response of 92% for the *trans* DB in all-*trans*-18:2n-6. The average response for the two isomers with one *trans* DB was 79%. This may explain the discrepancy between the two methods. The PLS calibration uses the entire spectrum as variables and a large number of isomers with 0–4 *trans* DB as objects. The cross-validation results (Fig. 3A) show no underestimation of *trans* DB for PUFA. It is therefore probable that the GC-IR approach provides the most accurate figures for samples with high PUFA content.

A PLS calibration of *trans* DB based on only the *trans* peak (1030–900 cm^{-1}) in our calibration spectra gave an SEP of 0.109, twice as large as the SEP for the calibration based on the whole spectrum (0.052). The difference in SEP between the two models indicates that there are response differences in the *trans* signal (1030–900 cm^{-1}) that are corrected for by inclusion of larger parts of the spectrum. When the PLS model based on the *trans* peak was applied for quantification of the fats in Table 2, the plot corresponding to Figure 4A showed better agreement between the two methods of quantification; the regression slope was 0.96 and R^2 was 0.986. The explanation for the better agreement is probably that both the PLS regression on 1030–900 cm^{-1} and AOCS Cd 14-95 are based on the *trans* peak only and that the differences in response in the *trans* peak are not corrected by any of the methods. Which of the two approaches to choose—the regression that best explains the FA in the calibration set, or the regression that shows best agreement with the AOCS method—may be application dependent.

The presence of unsaturated compounds in the unsaponifiable fractions of the fats is a possible explanation for the 9% underestimation of the IV. These will influence the IV, but not the DB content determined by the GC-IR method. Some of the analyzed fats had a strong yellow color, which may be caused by the presence of carotenoids or other highly unsaturated compounds.

Application of correction factors. There is a difference by definition in the way the results from the GC-IR methods and the results from the AOCS reference methods are reported. The GC-IR method reports the results as the number of DB relative to the number of FA molecules. This is different from *trans* values and IV determined by Cd 14-95 and Cd 1d-92 where the results are reported on a weight basis. The discrepancies between the methods can be corrected for by converting the GC-IR chromatographic areas to weight percentages, and converting the average number of DB per molecule to the average number of DB per gram. In most cases these corrections have limited influence on the results. Application of suitable correction factors changed the slopes of the regression lines in Figure 4A–C from 1.10, 1.06, and 1.05 to 1.12, 1.08, and 1.07, respectively. This corresponds to an increase in the GC-IR results of 2–3% when correction factors are applied. In special cases the discrepancy between amounts given on a molar basis and amounts given on weight basis

may be larger, and application of correction factors may be more appropriate. In theory, pure brassidic acid methyl ester (*trans* 22:1n-9) will be reported as 100% *trans* FA when analyzed by the GC-IR method. For the same compound, method Cd 14-95 should give a *trans* value of only 84%; relative to the weight of the molecules, brassidic acid methyl ester contains 84% as many *trans* DB as the elaidic acid used as calibration standard in the AOCS method.

As already explained, the IV and the total *trans* value analyzed by AOCS Cd 14-95 have clear limitations for validation of the GC-IR data. Silver-ion HPLC, possibly in combination with GC or GC-MS, could be an alternative for samples rich in monoenes. We have evaluated this as an alternative but were not able to obtain sufficient fractionation of dienes and trienes on the basis of the DB geometries. A possible explanation is the large influence on retention by DB position in nonmethylene-interrupted dienes and trienes (25).

Reduced models. With some instruments, an increased signal-to-noise ratio can be achieved by scanning over fewer wavelengths. In addition to the wide-band (WB) detector (4000–550 cm^{-1}) applied in this study, the HP-IRD can also be equipped with a narrow-band (NB) detector scanning from 4000 to 750 cm^{-1}. The NB detector has increased sensitivity relative to the WB detector (15). It was therefore of interest to find out whether spectra without the region from 550 to 750 cm^{-1} could be used in the PLS regressions without loss of prediction accuracy. When the results were reanalyzed without this region, cross-validation results produced SEP for the PLS models close to the results obtained with WB spectra, with SEP for the predictions of *trans* DB, *cis* DB, and total DB of 0.054, 0.069, and 0.078, respectively. When the models were applied for predictions, R^2 for the regression lines corresponding to those seen in Figure 4A–C were 0.979, 0.984, and 0.987, respectively. The slopes of the regression lines changed by less than 3% and were 1.07, 1.08, and 1.06, respectively. This indicates that the NB detector gives the same amount of information about the structure of the FA molecule as the WB detector. The small increase in SEP relative to the WB spectra may be more than compensated for by the increased sensitivity of a NB detector.

When working with PLS models, an important question is how many PLS components should be used for prediction. The number of components was selected on the basis of the R^2 values when the calculated values from GC-IR were compared with AOCS Cd 14-95 or Cd 1d-92 as illustrated in Figure 4. Increasing numbers of PLS components in the models gave better R^2 values up to a certain number, where the R^2 were stable or weakly decreasing. The number of components necessary to reach this plateau was used in the models. The number of components determined by the PLS software was usually one or two components lower and gave models with a positive bias for the prediction of both *cis* and *trans* DB for the saturated FA. Inclusion of a few more components removed this problem.

In this work we focused on differences between different types of hydrogenated fats. There was a very large variation in the samples selected for validation. The method was not tested for the ability to distinguish between very similar samples (e.g., batch-to-batch variations of the same product). Nor were limits of detection, limits of quantification, or precision figures thoroughly investigated. Optimization of the method toward each type of fat with respect to amounts injected, temperature program, and the like would probably give better precision than achieved with the more general method that was applied in this work. Replicate analyses showed that differences between replicates were below 10% when the values of total *trans*, total *cis,* and total unsaturation were considered (average differences were 4, 5, and 3%, respectively).

A method for quantification of geometrical isomerism in hydrogenated fats has been developed. It is possible to use a single GC-IR analysis of FAME. For each chain length, the amount of the saturated FA, the amount of *trans* monoenes, the amount of *cis* monoenes, the amount of PUFA, and the average number of *cis* and *trans* DB in PUFA can be found. The data support previous findings that the response of the *trans* DB of elaidic acid at 970 cm^{-1} is higher than the average response of the *trans* DB in hydrogenated fats. The results suggest that the method can be used with a NB detector (4000–750 cm^{-1}) instead of a WB detector (4000–550 cm^{-1}) without loss of accuracy.

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