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ORIGINAL PAPER

Nutrition Labeling: Rapid Determination of Total *trans* Fats by Using Internal Reflection Infrared Spectroscopy and a Second Derivative Procedure

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Abstract In 2006, the US FDA mandated the declaration of the total *trans* fat content on the Nutrition Fact label of foods including dietary supplements when a product contained 0.5 or more grams of *trans* fatty acid per serving; the minimum corresponding *trans* fat content is estimated to be approximately 2% of total fat. The FDA definition is based on chemical structure and includes only fatty acids with one or more isolated double bonds in the *trans* configuration. Several issues negatively impacted the sensitivity of the current official infrared (IR) methods, thus limited the quantitation of *trans* fat to 5% of total fat. To improve sensitivity and accuracy and to meet the labeling requirement, a new internal reflection IR procedure called negative second derivative is described and evaluated for the quantitation of total *trans* fat in the present study. The enhanced spectral features of a second derivative resolved issues that traditionally limited the sensitivity of the IR methodology. Calibration standard mixtures starting at approximately 0.5% trielaidin in the total fat (tripalmitin or triarachidin) were successfully generated and used to determine the *trans* fat levels for unknown test samples with *trans* content as low as approximately 1% of total fat. Quantitative IR data were compared to those obtained by gas chromatography and were found to be in good agreement.

Keywords Fats and oils · Lipid chemistry · Lipid analysis · *Trans* fat · Food labeling · Infrared spectroscopy · Attenuated total reflection

Abbreviations

IR	Infrared
ATR	Attenuated total reflection
PHVO	Partially hydrogenated vegetable oil
TE	Trielaidin
TS	Tristearin
TL	Trilaurin
TM	Trimyristin
TP	Tripalmitin
TA	Triarachidin

Introduction

Compelling evidence based on the results of many persuasive intervention trials and cohort studies warranted the labeling of *trans* fat by the US FDA [1–4] and other regulatory agencies in Canada and around the world. Most experts from leading institutions (Institute of Medicine, National Academy of Science, the National Cholesterol Education Program, The American Heart Association), the World Health Organization, and US federal government expert panels had strongly agreed that the major adverse

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effect of *trans* fats (with respect to SFA, MUFA and PUFA) on blood lipids is on the ratio of total-cholesterol to HDL-C. In addition to increasing LDL-C, *trans* fats lower HDL-C. A lower intake of both saturated and *trans* fatty acids would reduce the risk of coronary heart disease (CHD) in the general population, particularly for those at increased risk for CHD [4]. The aim of declaring the *trans* fat content of foods and dietary supplements was to help the consumer make decisions based on sound dietary recommendations.

January 1, 2006 was the effective date set by the FDA by which all foods including dietary supplements entering interstate commerce must declare the total *trans* fat content on the Nutrition Facts labels. To provide regulatory relief and economic savings for small businesses, this date was more than two years after the FDA had issued its *trans* fat final rule on July 11, 2003 [1]. This rule for mandating the labeling of *trans* fats was the first amendment to the Nutrition Facts panel since mandatory nutrition labeling became law in 1990. The amendment was the result of a long process that started when the FDA received a citizen petition requesting the labeling of *trans* fat in 1994 [2]. In response to this petition and to assist consumers in maintaining healthy dietary practices, the FDA issued a proposed regulation in 1999 to require, in part, that the amount of *trans* fatty acids in a food or dietary supplement be declared when a product contains 0.5 g (0.2 g in Canada) or more of *trans* fatty acids *per serving,* and that the amount of *trans* fat be limited wherever saturated fat limits are placed on nutrient content claims or health claims [3]. In Denmark there is, irrespective of serving size, an upper limit of 2 g *trans*/100 g fat or oil in fat or oil products and 5 g *trans*/100 g fat or oil in food products. After publication of the FDA proposal, various comments related to claims (such as "*trans* fat free" claim) were received by the FDA and were found to express diverse and opposing views. An explanation of the FDA final rule and a critical discussion of some of these comments can be found in a recent book chapter [4]. The topics discussed include reasons for the omission of claims for "free" and "reduced" levels of *trans* fat, and for not listing a percentage Daily Value for *trans* fat on the Nutrition Fact label.

The FDA definition of *trans* fat is based on chemical structure and does not take into consideration the origin of the *trans* fat. *trans* Fat is defined as the sum of all unsaturated fatty acids that contain one or more isolated double bonds (separated by at least one methylene group) in the *trans* configuration. For the purpose of nutrition labeling, *trans* fatty acids with conjugated double bonds (not separated by a methylene group) are excluded from this definition. Conjugated *trans* fatty acid isomers, which are mostly found in dairy fat, may contain up to 20 isomers [5] collectively called conjugated linoleic acid (CLA), of which rumenic acid is the most prominent isomer. Some of the CLA isomers have been reported to have beneficial physiological activities in laboratory animals [6]. CLA isomers have been the subject of extensive research, including the commercially sold dietary CLA supplements in health food stores, which generally consist of equal mixtures of *cis*-9,*trans*-11- and *trans*-10,*cis*-12-CLA isomers.

The mandatory requirement to declare the amount of *trans* fat on the labels of food products in the US, Canada and many other countries [7] has led to an increased and urgent need for rapid analytical methods that can accurately determine the *trans* fat content of foods and facilitate compliance with government regulations.

The rapid determination of total *trans* fatty acids by infrared (IR) spectroscopy has been a widely used standard procedure [8–12]. This methodology is based on the measurement of the height of or area under the 966 cm^{-1} C–H out-of-plane deformation band, which is uniquely characteristic of isolated double bonds with *trans* configuration (Figs. 1, 2). By contrast, conjugated *trans* double bonds absorb near 985 and 945 cm^{-1} (conjugated *cis/trans*) and near 990 cm^{-1} (conjugated *trans,trans*) [13]. The isolated double bonds are found in *trans*-monoenes, and in methylene-interrupted (MI) and non-methylene-interrupted (NMI) mono-*trans*-dienes, mono-*trans*-trienes, and in trace amounts in *trans,trans*-dienes; NMI refers to the presence of more than a single methylene group between double bonds along the fatty acid chain. All these different fatty acids and their isomers exhibit the same 966 cm^{-1} absorption band irrespective of chain length or position of the isolated *trans* double bond [13, 14]. Thus measuring the intensity of the absorption of the *trans* band effectively sums up all the fatty acids containing isolated *trans* double bonds, but excluding those with conjugated *trans* double bonds. Thus the method conveniently provides a quantitative measurement of the total *trans* fat content as defined by the *trans* fat regulation.

The determination of total *trans* fats by IR spectroscopy [8] is an established methodology that has traditionally been negatively impacted, particularly at low levels (below 5%, as percent of total fat), because the sloping baseline near the *trans* absorption frequency (Figs. 1, 2) leads to greater variability in measurement. For instance, for a test sample with a reported IR mean of 0.43% trans (as percent of total fat), the repeatability and reproducibility relative standard deviation values were found to be very high, namely 11.11 and 18.35, respectively [9, 11]. In addition, we recently reported that accuracy was also compromised due to interferences by adjacent bands due to conjugated fatty acids, or overlapping bands due to saturated fats [15]. To improve both accuracy and sensitivity and overcome the presence of potential spectral interferences, a newly

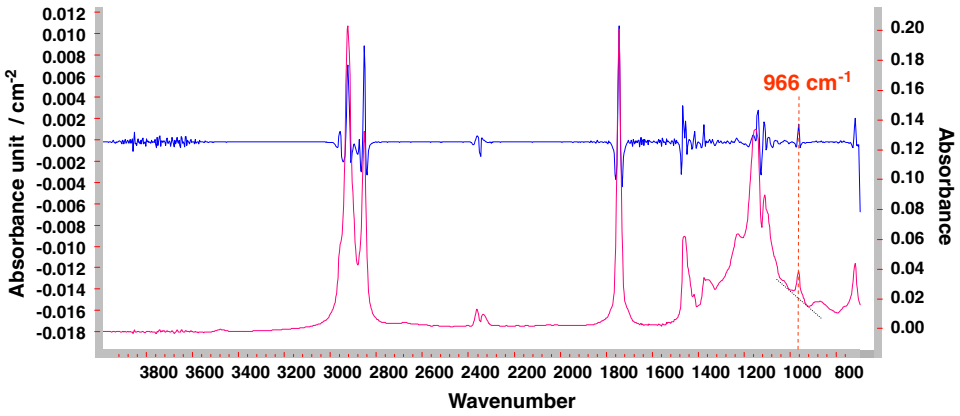

Fig. 1 Negative second derivative (*top*) and absorption (*bottom*) spectra for a test sample consisting of trielaidin in tripalmitin with a *trans* level of 12.58% (as percent of total fat). A vertical line indicates the position of the unique band due to isolated *trans* double bonds at 966 cm^{-1}. The second derivative spectrum was multiplied by −1 only to have the bands point upwards for convenience

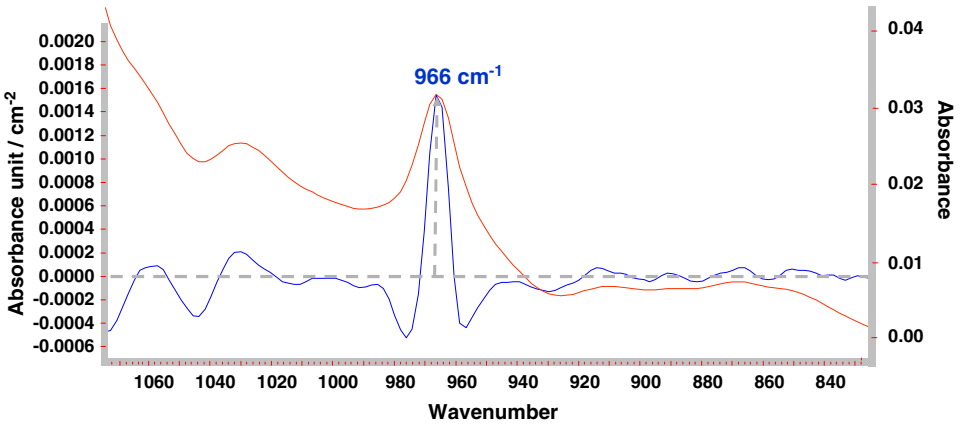

Fig. 2 The region of the spectra that exhibit the deformation band for isolated *trans* double bonds at 966 cm^{-1} is expanded for the negative second derivative and absorption spectra for a test sample consisting of trielaidin in tripalmitin with a *trans* level of 12.58% (as percent of total fat). The height of the negative second derivative band, as indicated by the vertical arrow, can be accurately measured for any test sample from the *same* horizontal baseline (Y = Zero). It is noted that several weak bands observed in the same spectral region are also more pronounced in the second derivative spectrum (narrower bandwidths) than in the absorption spectrum

developed IR procedure is presented in this study. It entails the measurement of the height of the second derivative of the *trans* absorption band at 966 cm^{-1} (Figs. 1, 2) and the use of attenuated total reflection (ATR)—Fourier transform (FT) IR spectroscopy. A preliminary communication was previously published [16].

Experimental Procedures

Lipid standards were supplied by Nu Check Prep, Inc. (Elysian, MN) and Sigma Chemical Co. (St. Louis, MO). A total of nine test samples that covered the range of 1-12% *trans* fat (as percent of total fat) were quantified in the present study. Three (hydrogenated lard, lard, and margarine oil) of these nine test samples had been collaboratively studied by GC as methyl ester derivatives for the validation of official method AOCS Ce 1h-05 [19]. They had reported [19] GC mean *trans* levels of 1.00% (hydrogenated lard), 0.90% (lard), and 11.62% (margarine oil). The remaining six test samples determined in the present study were processed (refined and deodorized) specific mixtures of canola oil and partially hydrogenated canola oil that were acquired commercially. It is noted that among the test samples analyzed in the Ce 1h-05 GC validation study [19], four had reported mean *trans* values of 0.17 (sunflower oil), 0.10 (coconut oil), 0.06 (cocoa butter), and 0.11% (coconut oil), and their *trans* levels could not be quantified in this IR study because of poor IR sensitivity at this very low *trans* level. However, these four test samples were also qualitatively measured in the present study; those that were high in saturated fat content were investigated because they exhibited an interference band near 960 cm^{-1} [15] that could be mistaken for a *trans* band (see "Results and discussion" section).

A Hewlett Packard Model 5890 Series II GC equipped with a flame ionization detector, an autosampler (HP Model 7673), and a 100-m CP-Sil 88 fused capillary column (Varian Inc., Mississauga, ON) was used. Hydrogen was used as the carrier gas at a flow rate of 1 mL/min. The *trans* fatty acids were analyzed using two complementary GC temperature programs [17]. Briefly, the two temperature programs were: 45 °C held for 4 min, increased at 13 °C/min to 175 °C and held for 27 min, then increased at 4 °C/min to 215 °C and held for 35 min. The second program was: 45 °C held for 4 min, increased at 13 °C/min to 150 °C and held for 47 min, then increased at 4 °C/min to 215 °C and held for 35 min. Details regarding *trans* fatty acid identification and determination by GC were previously described [17].

IR measurements were carried out on a Varian (Lexington, MA) FTS 7000e IR spectrometer operating under Resolution Pro software in the attenuated total reflection (ATR) mode. The optical bench included a Michelson interferometer with an air bearing moving mirror, a potassium bromide substrate beam splitter, and a linearized mercury cadmium telluride (MCT) detector. FTIR spectra were collected over the wavenumber range of 4,000 cm^{-1} to 700 cm^{-1} at a resolution of 4 cm^{-1}. To enhance the signal-to-noise ratio, 256 scans were co-added and signal averaged. The reference background material used was air. To enhance spectral features, the negative second derivatives of absorption spectra were generated. A heated single-reflection diamond internal reflection cell with a capacity of approximately a single microliter was used. The cell was warmed to about 65 °C in order for test samples, which consisted of *neat* fats and oils, to remain melted during analysis.

Results and Discussion

The improved sensitivity of the negative second derivative IR procedure allowed for the qualitative detection of trielaidin (TE, *trans*-18:1) in commercially available triolein (TO, *cis*-18:1) [15], as subsequently quantitatively determined by GC on the corresponding FAME to be approximately 0.5% of total fat. Therefore, an alternative to TO had to be found in order to prepare calibration standards that traditionally consisted of mixtures of TE in TO. After screening various saturated fats, specifically trilaurin (TL, 12:0), trimyristin (TM, 14:0), tripalmitin (TP, 16:0), tristearin (TS, 18:0), and triarachidin (TA, 20:0), we recently found [15] that the two least interfering saturated triacylglycerols with the lowest absorptivities, and whose band maxima were farthest from the *trans* absorption at 966 cm^{-1}, were TP (956 cm^{-1}) and TA (958 cm^{-1}). Hence, to prepare calibration standard mixtures in the present study, TP and TA were each evaluated as a potential substitute for TO. The TE content had to be greater than the limit of detection of 0.5% (as percent of total fat). This is because below 0.5%, the TE band appears as a shoulder on the leading edge of the increasingly visible TP or TA band, which prevented the measurement of the height of the *trans* TE band at 966 cm^{-1}.

Calibration standards were prepared gravimetrically from TE in TP at *trans* levels of 0.63, 1.05, 1.19, 1.66, 1.87, 4.97, 5.01, 10.20, 13.08, 14.88, 17.13, 18.00, and 19.80% (as percentage of total fat). They were measured (in four replicates) by IR and the heights of the second derivative absorption bands were recorded. A calibration plot of height versus *trans* level was generated and a regression fit (solid line in Fig. 3) was obtained. The regression parameters of the fitted line were the slope = 0.1179 (standard error = 0.0127) and intercept = 0.0768 (standard error = 0.0012). The measure of the proportion of total variation about the mean band height described by the fitted line was given by $R^2 = 0.995$. The 95% confidence interval (CI) of the true mean of band height values (dotted lines) and the 95% prediction interval (PI) of a single future value (dashed lines) are also given in Fig. 3. The 95% CI of the regression line indicates that this interval will contain the true mean of band height values at a given *trans* level. The 95% PI of the regression line indicates that this interval will contain an individual future observation at a given *trans* level with 95% confidence. Scattered data points in a calibration plot underscore the importance of having calibration standards accurately weighed; a less than optimal gravimetric determination of calibration standards is potentially a major source of quantitative error.

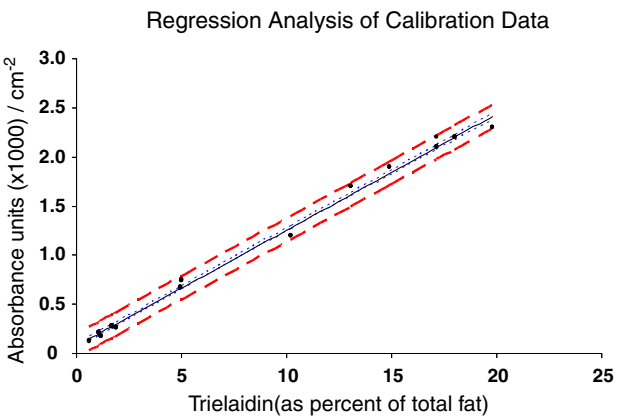

Fig. 3 Calibration scatter plot of second derivative band heights versus *trans* levels. *Solid line* linear least-square regression line. *Inner dotted lines* 95% confidence interval (CI) of the true mean of band height values. *Outer dashed lines* 95% prediction interval (PI) of a single future value (see text)

An additional set consisting of TE in TA calibration mixtures in the 0.5–20% range was independently prepared by a second IR analyst and compared in this study. Similar calibration data and correlation coefficients were obtained for both TE in TP and TE in TA sets, and indicated that either TP or TA could be used to prepare *trans* fat calibration mixtures.

The noise level observed for the baseline for a second derivative spectrum was found to be 0.01 milliabsorbance unit/cm^{-2}, while the *trans* fat negative second derivative band height observed for the lowest (0.63%) TE calibration standard was 0.13 mAU/cm^{-2}, thus yielding a signal-to-noise ratio of (0.13/0.01) or 13:1. While the type of FTIR detector used is only one factor that can impact sensitivity, the required SNR can be achieved by using either a DTGS, an MCT or a linearized MCT detector.

Six accuracy standards prepared gravimetrically from trielaidin in tripalmitin were also measured (in four replicates) by IR and their *trans* levels calculated from the observed negative second derivative band height and the calibration linear regression equation. Based on the gravimetric data, these standards had *trans* levels of 0.65, 1.30, 1.99, 4.86, 10.10, and 18.35% (as percent of total fat). When a two-sided *t*-test was applied to determine whether the calculated values determined by IR were different from the corresponding gravimetric (true) values (Fig. 4), the mean of differences (Table 1) was not statistically significant, p-value > 0.05.

In the present ATR–FTIR study, several of the unknown test samples analyzed (Fig. 5) had been determined by GC in an AOCS collaborative study among 12 laboratories in 2005 [19]; they were hydrogenated lard, margarine oil, lard, sunflower oil, coconut oil, and cocoa butter. In addition, specific mixtures of canola oil and partially hydrogenated canola oil were also analyzed by ATR–FTIR and by GC for comparison. The total *trans* content for all the test samples was calculated from the observed height of the negative second derivative of the 966 cm^{-1} IR band and by using the calibration linear regression equation based on TE in TP calibration mixtures. There was good agreement between ATR–FTIR and GC determinations (Fig. 6). The *trans* levels were found to fall between approximately 1 and 12%, as percent of total fat. The *trans* fat levels for several test samples fell outside the 0.5–20% calibration range (such as sunflower oil whose *trans* level was reported [19] to be 0.17% of total fat (determined by GC as FAMEs), and were therefore not quantified by ATR–FTIR.

Test samples that exhibited no band at 966 cm^{-1} but maxima at slightly lower frequencies, namely in the 962–956 cm^{-1} range (Fig. 7), were interpreted to be due to having high levels of saturated fatty acids [15]. They had been found to have negligible *trans* levels by GC [19], namely 0.1% (coconut oil) and 0.06% (cocoa butter) of total fat. Due to the close proximity of these IR bands to 966 cm^{-1} they could have been erroneously attributed to isolated *trans* fatty acids. However, the narrower bandwidth of the second derivative bands allowed for the discrimination between these adjacent band positions. Therefore, the negative second derivative procedure eliminated or minimized the possibility of erroneously reporting a high IR bias (relative to GC) of approximately 1.5% *trans* fat (Fig. 7) for highly saturated test samples that contain only a trace amounts (approximately 0.1%) of *trans* fatty acids [15].

Fats, oils, or oil blends that are coincidentally high in saturated fat and contain low *trans* fatty acid levels, for instance near 1% (as percent of total fat) would erroneously lead to significantly more intense *trans* bands with broader band widths and possibly asymmetric band shapes. The full width at half height of such a band would probably be significantly greater by up to 50%. This is due to the contribution from the broad bands of the saturated

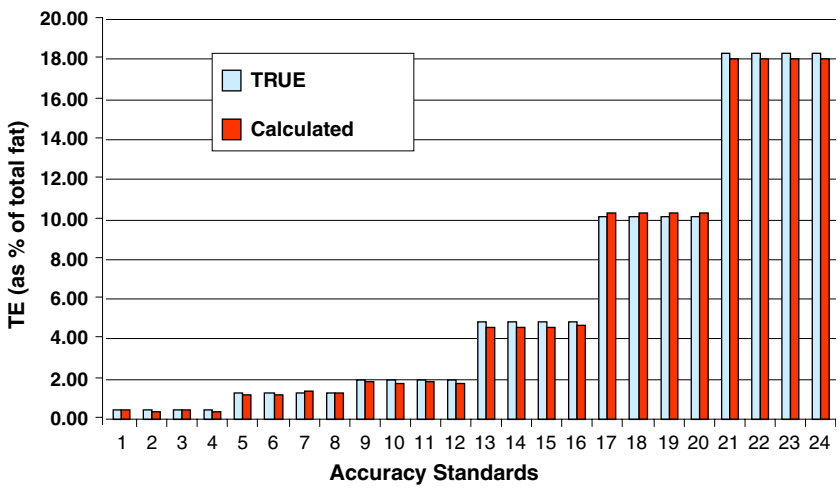

Fig. 4 Comparison of the gravimetric (TRUE) values for six accuracy standards and those calculated from the observed IR measurements (in 4 replicates) and the linear regression equation previously determined by the calibration plot (see Table 1)

Table 1 Statistical analysis of accuracy standards plotted in Fig. 4

N observations	Mean of differences*	Standard deviation	Variance	*t*-value**
24	−0.081	0.200	0.040	−1.98

* The mean of differences was not statistically significant at the significance level of 0.05

** *t*-value with 23 degrees of freedom

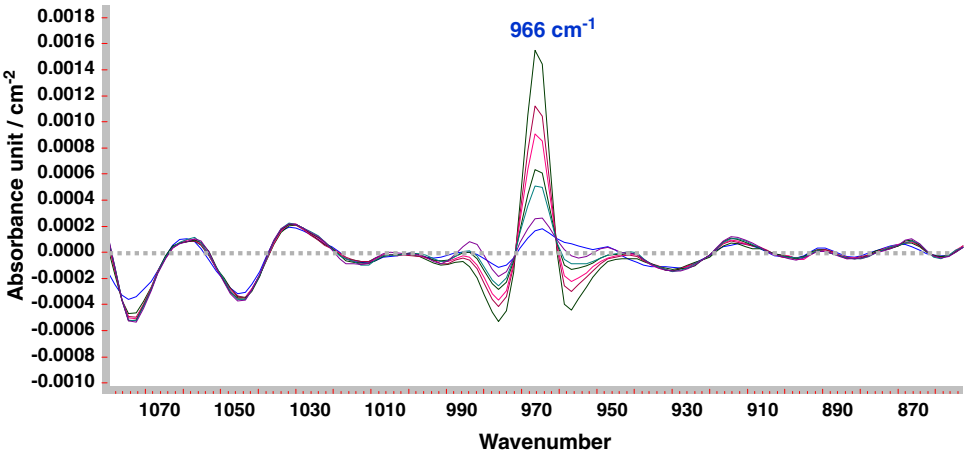

Fig. 5 Expanded spectral region that exhibits the negative second derivative of the deformation band for isolated *trans* double bonds at 966 cm^{-1} for test samples covering approximately the 1–12% range and containing *trans* fat levels determined by IR to be for lard 1.21%, and Canola oil mixtures 2.21; 4.20; 4.72; 7.35; 9.11; and 12.62%, as percent of total fat. Values used are means of two determinations. The height of the negative second derivative band can be easily measured from the horizontal baseline (*dotted line*)

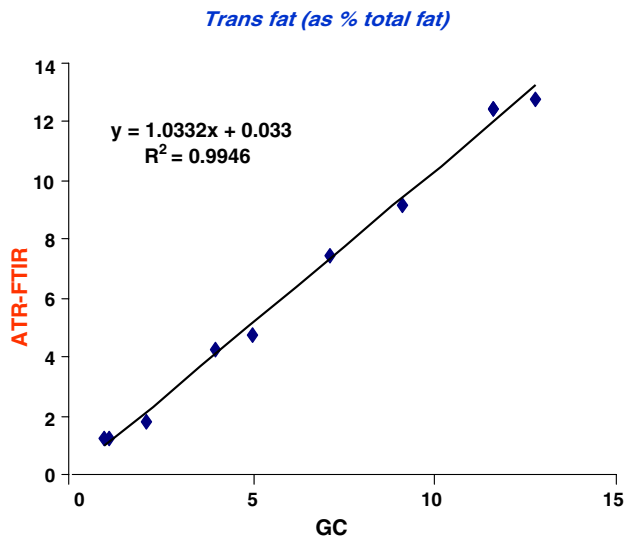

Fig. 6 Plot of nine unknown test samples determined by ATR–FTIR and GC indicates good agreement between the two techniques. Values used are means of two determinations. The *trans* levels were found by IR (and GC) to be for lard 1.21% (0.90%, [19]); Canola oil mixtures 2.21% (2.05%); 4.20% (3.97%); 4.72% (4.97%); 7.35% (7.11%); 9.11% (9.08%); and 12.62% (12.79%); and margarine oil 12.35% (11.62%, [19]), as percent of total fat

components. Therefore, such material would not be accurately quantified under the current experimental conditions used. Analysts should be able to recognize this limitation from the broad bandwidth and asymmetric band shape (as well as position) of the observed band. However, an enhancement in resolution (from 4 to 2 wavenumbers) should provide a better indication of the presence of anomalous band shapes, and diminish and better characterize the overlap between bands attributed to *trans* fat and saturated fat components.

Conjugated dienes could also have interfered with the accurate determination of the 966 cm^{-1} band. However, the narrow bandwidth of the second derivative bands once again allowed the spectral resolution of the 966 cm^{-1} band from weak absorption bands at 990 cm^{-1} *(trans/trans)* and the doublet at 985 and 945 cm^{-1} (*cis/trans*) due to conjugated fatty acids [13, 20]. Hence, the negative second derivative procedure should improve IR accuracy at low *trans* levels particularly for matrices, such as dairy fat or processed vegetable oils, containing small amounts (below 5% of total fat) of conjugated fatty acids.

In order to meet the *trans* fat labeling requirements, in particular the claim of zero gram *trans* fat per serving, it

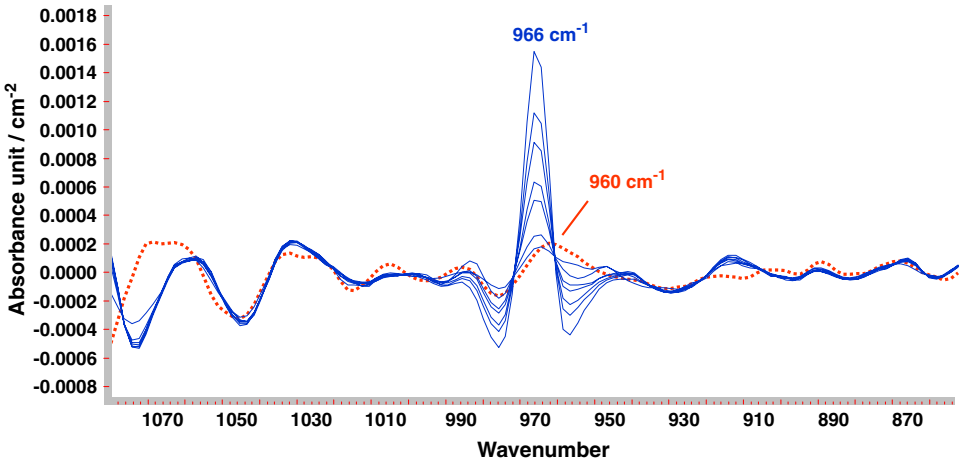

Fig. 7 Expanded spectral region that exhibits the negative second derivative of the deformation band for isolated *trans* double bonds at 966 cm^{-1} for several test samples containing *trans* fat, as well as for coconut oil that is high in saturated fat and contain only a trace (approximately 0.1%) of *trans* fat [19]. The latter test sample exhibited a spectral feature at slightly lower wavenumbers, near 960 cm^{-1}, which is easy to misidentify as a band for isolated *trans* double bonds [15]

was necessary to develop a methodology with a sensitivity that is capable of measuring 0.5 g *trans* fat per serving in the US or other limits specified by different countries, for example 0.2 g *trans* fat per serving (Canada), and 1 g *trans* fat /100 g fat in food products (Denmark). A recently published compositional database on the *trans* fat content of a wide range of foods [18] was used to estimate the *trans* fat content, as percent of total fat. For a food product that consists mostly of fat or oil, such as Ranch dressing with 27.6 g total fat per 30 g serving size (total fat 92%) [18], it is possible to calculate the percentage *trans* level to meet the *trans*-fat-free labeling requirement in the US (i.e., less than 0.5 g per serving size). The corresponding value for the *trans* fat content, as percent of total fat, would be ((0.5 g/27.6 g) × 100 = 1.8%) approximately 2% of total fat. This is the minimum level of *trans* fat in a product that should be measured with confidence to meet the declaration requirement of zero *trans* fat on the Nutrition Fact label. See reference [18] for other food products with a high total fat content per serving. For food products containing less total fat, the level of *trans* fat in the total fat of the product would be higher than 2% and would still meet the zero *trans* definition of 0.5 g *trans* fat per serving. For example, for food products with a low total fat content per serving, typically 3 g per 28 g serving size (total fat 11%), a *trans* fat level of 0.5 g per serving corresponds to a *trans* fat content of (0.5 g/3 g) × 100 = approximately 17%, as percentage of total fat. Actual examples of food products with low levels of total fat, such as cereal bars, are also given in reference [18]. These calculations are necessary because the IR determination provides *trans* fat levels as the percentage of total fat, irrespective of serving size. Therefore, the required methodology should have a lower limit of quantitation that is approximately equal to (or lower than) 2.0%, as percentage of total fat. For other countries such as Canada and Denmark the corresponding *trans* fat content limits are 0.7% and 1% of total fat in food products, respectively.

Traditional IR absorption spectra recorded in the transmission mode for fats and oils exhibit the highly characteristic *trans* band at 966 cm^{-1}. However, the accuracy of measuring the height or area of this band is poor particularly at lower *trans* levels because the absorption band occurs on an elevated and sloping baseline (Figs. 1, 2). As a result, there have been many reported attempts at improving the accuracy of this measurement. The most recent 2000 IR official method [8–12] successfully eliminated the baseline offset and slope by measuring *trans* fat test samples relative to a *trans*-free reference fat. The result was a symmetric *trans* band on a horizontal baseline. However, for some matrices the accuracy of the method was still inadequate, particularly for fat products containing less than 5% *trans* fat. This is due to the fact that the fatty acid composition of the *trans*-free reference fat should closely match that of every unknown test sample, and this ideal condition has often been hard to meet. Consequently, the quantitation of *trans* fat was limited to ≥5% of total fat [11, 12].

To improve sensitivity and accuracy and meet the labeling requirement, a new ATR–FTIR procedure called negative second derivative was developed and evaluated in the present study. A second derivative is traditionally used to enhance spectral features. Advantages of measuring the second derivative of the *trans* absorption band (Figs. 1, 2) include: (a) problems associated with the baseline offset and slope no longer exist since the height of the second derivative is directly proportional to the amount of total *trans* fat in a test sample; (b) the need for a *trans*-free or any reference background oil is eliminated; and (c) a second derivative has a narrower bandwidth than an absorption band, and therefore allows the detection of interference bands that are adjacent to the 966 cm^{-1} band of interest. In addition,

this IR procedure requires that measurements be carried out in the ATR mode rather than the conventional transmission mode. The ATR technique is advantageous in many respects. The effective pathlength in ATR is inherently precise because it depends solely on the number of internal reflections, the angle of incidence, the wavelength, and the refractive indices of the ATR crystal used and the test sample investigated at a given temperature. The ATR technique allows for the measurement of a single microliter of neat oils (without solvent) or melted fats (at approximately 65 °C) without the time-consuming requirement of having to quantitatively prepare solutions in a volatile and toxic carbon disulfide solvent. Finally, the ATR–FTIR measurement is rapid (5 min), and the negative second derivative procedure does not require any derivatization of the oil or fat test material to its corresponding FAMEs. However, this procedure does not provide information on fatty acid composition. The ATR mode makes it easy to handle test samples, but is more susceptible to cross-contamination than the transmission mode particularly for small amounts of *trans* fat. In addition, this procedure assumes that all the *trans* fat and oil components of test samples consist of TE. This approximation is valid as long as the levels of mono-*trans* dienes and trienes constituents and their positional isomers in a test sample are not major components; this is because these compounds may not necessarily have absorptivity values identical to that of TE.

By contrast, the identification and quantitation of *trans* fatty acids by GC is challenging because of the large number of *trans* mono-, di-, and triunsaturated fatty acid isomers present in partially hydrogenated vegetable oils PHVO [21, 22], milk [17, 23], or other matrices, and optimized separation conditions must be used to minimize peak overlap. Most *trans* fat in foods are due to the inclusion of PHVO into food products, while small amounts of *trans* fatty acids are derived from naturally occurring *trans* fatty acids present in dairy and meat products of ruminants. Unresolved and overlapping GC peaks and the lack of commercial standards are unresolved issues. Complementary fractionation techniques such as silver ion- TLC, silver ion-HPLC, silver ion-SPE, and reversed phase-HPLC have been used to resolve and identify all possible *trans* fatty acids. However, official GC methods involve the use of only a single chromatographic analysis [19], without prior fractionation. While GC methods are time consuming, and unlike IR methods, require conversion of fat test samples into volatile fatty acid methyl esters before separation using very long (100 m) capillary columns coated with highly polar stationary phases, they provide valuable information about fatty acid composition. It is noted that several of the test samples that were used to validate the latest GC official method Ce 1h-05 [19] were determined to have a *trans* level of 1% or less of total fat. For all these low-level test samples, the corresponding HORRAT parameters, which are a measure of precision among laboratories, were outside the expected range of 0.5–2.0 values [19]. As a result, this GC official method AOCS Ce 1h-05 did *not* specify a lower limit of quantitation [19].

In conclusion, the distinction between the band at 966 cm^{-1} due to isolated *trans* double bonds and those at only slightly higher frequency due to saturated fatty acids was possible only because of the narrower bandwidth of second derivative bands. Similarly the second derivative eliminated the overlap between (a) the leading and tailing edges of the 966 cm^{-1} absorption band due to isolated *trans* double bonds, and (b) the bands near 945, 985, and 990 cm^{-1}attributed to conjugated *cis*/*trans* or *trans*,*trans* double bonds, and allowed the accurate measurement of band height. It is expected that once validated, the negative second derivative method will provide better sensitivity and accuracy than the current official IR methods, and a lower limit of quantitation from 5% [11, 12] to approximately 1% of total fat (a 5-fold improvement over the current official IR method). This will facilitate compliance with the *trans* fats nutrition labeling of most food products and dietary supplements. This methodology should also allow for the rapid verification of declarations of zero *trans* fat for servings with less than 0.5 g *trans* fat (approximately 2% of total fat in the US), since this limit, as well as those specified by most countries, would probably be near or above the expected lower limit of quantitation.

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