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ORIGINAL PAPER

Interference of Saturated Fats in the Determination of Low Levels of *trans* Fats (below 0.5%) by Infrared Spectroscopy

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Abstract The mandate to label food products with the content of total *trans* fatty acids has led to an increase in demand for sensitive and accurate methodologies for the rapid quantitation of *trans* fats. Unfortunately, the latest official infrared (IR) spectroscopic method lacks the required sensitivity. A more sensitive IR procedure that requires the measurement of the height of the second derivative (2D) of the *trans* absorption band at 966 cm^{-1} was recently proposed; however, a reported inconsistency at low *trans* levels between GC (0% of total fat) and IR (1.2% of total fat) results for a fully hydrogenated vegetable oil could not be reconciled, and triggered further investigations. For the first time, we recognize and report the presence of weak interference bands (962–956 cm^{-1}) attributed to saturated fats in the IR spectra of *trans* fats; these interference bands have an adverse impact on the sensitivity and accuracy of the IR determination at low *trans* levels (≤0.5% of total fat). Therefore, weak spectral features observed at energies below the one expected for *trans* bands (966 cm^{-1}) in test samples high in saturated fat (coconut oil and cocoa butter) must not be mistaken for *trans* bands.

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Abbreviations

IR	Infrared
HSBO	Hydrogenated soybean oil
TS	Tristearin
TL	Trilaurin
TM	Trimyristin
TP	Tripalmitin
TA	Triarachidin

Since *trans* fat labeling requirements became mandatory in the US, Canada and many other countries [1], there has been an urgent need for accurate analytical methodologies that would facilitate the verification of compliance with the various regulations.

The determination of total *trans* fatty acids by IR has been a widely used procedure [2] that has been standardized [3–6]. Its importance stems from the fact that the C-H out-of-plane deformation band observed at 966 cm^{-1} is uniquely characteristic of isolated double bonds with *trans* configuration. These double bonds are found in *trans*-monoenes, and in methylene-interrupted and non-methylene-interrupted *trans*,trans-dienes, mono-*trans*-dienes, and mono-*trans*-trienes. All these different fatty acids exhibit that same absorption band at 966 cm^{-1} regardless of chain length or position of the isolated *trans* double bond. Thus measuring the intensity of the absorption of the *trans* band effectively adds up all the different fatty acids containing isolated

trans double bonds, and conveniently provides a quantitative measurement of the total *trans* fat.

Accurate determination of *trans* fats and oils by IR spectroscopy [2] has traditionally been compromised by the presence of a sloping baseline at the *trans* absorption frequency. The most recent 2000 internal reflection IR official method [3–6] eliminated the baseline offset and slope, but was only partly successful in improving accuracy. This method required the measurement of *trans* fats relative to a *trans*-free reference fat of similar fatty acid composition. However, finding a reference fat that is absolutely *trans*-free and whose composition would closely match every unknown test sample is impossible. These factors have negatively impacted the accuracy of the IR determination at low *trans* fat levels, and limited the quantitation of *trans* fat to ≥5% of total fat [5, 6].

To improve sensitivity and accuracy, a new IR procedure that measures spectra relative to air was recently proposed [7]; it relates the amount of *trans* fat in a test sample to the height measurement of the second derivative (2D) of the *trans* absorption band. This 2D procedure is the subject of an on-going AOCS international collaborative validation study. The 2D procedure removed the baseline offset and slope of the *trans* IR band (Fig. 1), and more importantly, eliminated the need for a *trans*-free reference fat. The second derivative of an absorbance spectrum offers several advantages including the resolution enhancement of spectral bands. This advantage made it possible to detect small shifts in band position or the presence of interferences. In the present study, we report for the first time the presence of interference bands (962–956 cm^{-1}) attributed to saturated fats in the spectra observed for fats with only a trace amount (≤0.1% of total fat as determined by GC) of *trans* fat. Unless such interferences are recognized, these test samples would otherwise be erroneously determined by IR to contain approximately 1% *trans* fat (as percent of total fat).

Experimental Procedures

Lipid standards were supplied by Nu Check Prep, Inc. (Elysian, MN) and Sigma Chemical Co. (St Louis, MO). All test samples were processed (refined and deodorized) commercial products acquired locally. IR measurements were carried out on a Varian (Randolph, 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 deuterated triglycerin sulfate (DTGS) detector. FTIR spectra were collected over the wave number range of 4,000–700 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

Traditionally, the highly characteristic *trans* absorption at 966 cm^{-1} occurs on an elevated and sloping baseline in IR spectra, thus the quantitative measurement of its

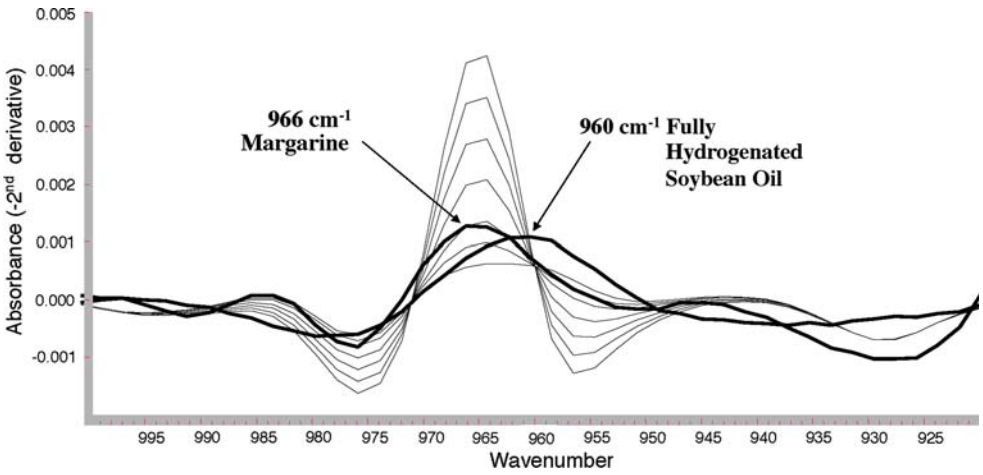

Fig. 1 Negative second derivative (–2D) spectra for trielaidin (TE) calibration standard mixtures, as well as for margarine and fully hydrogenated soybean oil test samples. The second derivative (2D) spectra were multiplied by –1 only to have the bands point upwards for convenience

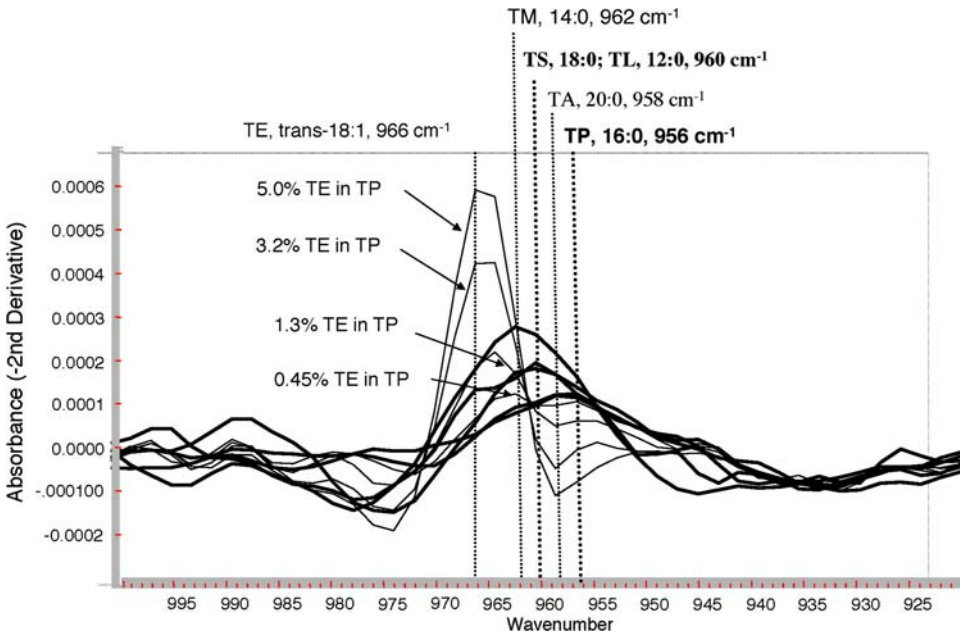

Fig. 2 Negative second derivative (–2D) spectra for several mixtures of trielaidin (*TE*) in tripalmitin (*TP*) at 0.45, 1.3, 3.2 and 5.0% that absorbed at 966 cm^{-1}, compared to those spectra of pure trimyristin (*TM* 14:0), tristearin (*TS* 18:0), trilaurin (*TL* 12:0), triarachidin (*TA* 20:0) and *TP* (16:0) that absorbed at 962, 960, 960, 958 and 956 cm^{-1}, respectively

height or area becomes increasingly less accurate as the *trans* levels decrease [2]. The experimental aspects of the ATR mode are far less complex than those involving transmission measurements [2], and ATR was used to develop validated official methods [2–6] and other procedures [2, 7]. Moreover, the proposal to generate the second derivative of an absorbance spectrum eliminated the baseline problems and offered several qualitative and quantitative advantages [7]. If a linear relationship between absorbance and concentration exists for an absorbance spectrum, then it would also exist for the second or any higher derivatives of that spectrum. A second derivative shows all the peaks that are present in the original spectrum, and improves the resolution of shoulders and overlapping peaks. Thus small shifts in IR band position or the presence of interferences would be more easily detected.

In a 2D ATR-IR study reported previously [7], test samples were also measured by GC. A hydrogenated soybean oil (HSBO) sample was found to have no *trans* fat by GC, but was reported by IR to have a low and significant *trans* level of 1.2% of total fat. In the present study, this discrepancy in accuracy observed at low *trans* levels by IR spectroscopy was addressed further.

A closer inspection of the IR spectrum observed for the HSBO test sample [7] indicated that the band position shifted to 960 cm^{-1} from the expected position of the *trans* band absorption at 966 cm^{-1} (Fig. 1). This difference was initially overlooked [7] probably because the band intensity was relatively weak. However, since this test sample was fully hydrogenated, the weak band at 960 cm^{-1} should have been attributed to tristearin (TS, 18:0), the major product of this fully hydrogenated fat. An identical weak band at 960 cm^{-1} was observed for a reference sample of TS (Fig. 2) in the present study.

Another finding reported in the 2D study [7] was the presence of 0.5% (determined by GC) trielaidin (TE, *trans*–18:1) contaminant in the commercially available triolein (TO, *cis*–18:1). Since standard mixtures of TE in TO were used to generate the IR *trans* fat calibration data, commercially available TO should no longer be used to prepare calibration standard mixtures. Several saturated fats, in addition to tristearin (TS), were screened for possible interferences with the *trans* band at 966 cm^{-1}. The saturated fats trilaurin (TL, 12:0), trimyristin (TM, 14:0), tripalmitin (TP, 16:0), and triarachidin (TA, 20:0) were measured by IR and found to exhibit similar weak absorption bands with varying degrees of interferences (Fig. 2). The least interfering saturated fat with the lowest absorptivity was TP that absorbed at 956 cm^{-1}, farthest from the *trans* absorption at 966 cm^{-1}. TP was therefore used as a substitute for TO to prepare calibration standard mixtures of TE in TP as low as approximately 0.1% of total fat; see Fig. 3.

In general, the very weak TP feature does not cause interferences except at *trans* levels near or below 0.5% of total fat. For TE in TP calibration standards at these very low *trans* levels, the TE band at 966 cm^{-1} shifts to lower values, close to 963 cm^{-1}, due to the predominance of TP (≥99.5%) in the mixture. At about 0.5% TE in TP, two very weak overlapping bands are

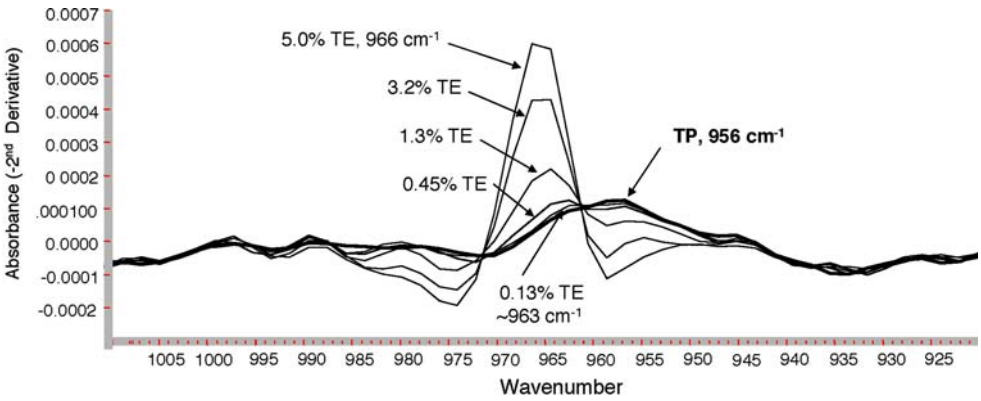

Fig. 3 Negative second derivative (–2D) spectra of four calibration standard mixtures of trielaidin (*TE*) in tripalmitin (*TP*) at 0.13, 0.45, 1.3, 3.2 and 5.0% TE that absorbed at 963–966 cm^{-1}, and TP by itself at 956 cm^{-1}

observed: the 963 cm^{-1} band attributed to TE and the 956 cm^{-1} band due to TP. Once validated in the ongoing international collaborative study, the *trans* fat lower limit of quantitation of this 2D procedure will be determined; even if as expected, it is found to be near 1% of total fat, it would still then be five-fold lower than that of the latest official IR method which was set at 5% of total fat [5, 6].

These results should serve as a caution when analyzing the *trans* content of fats and oils with a high content of saturated fats and only a trace amount (≤0.1% of total fat as determined by GC) of *trans* fat, such as coconut oil and cocoa butter. Therefore, the weak bands observed at energies slightly *lower* than 966 cm^{-1} must not be mistaken for, and erroneously reported as, *trans* bands.

The recognition of potential interferences from saturated fats would result in the correct interpretation of IR spectra, improve the accuracy of the IR determination at low *trans* levels (particularly ≤1% of total fat), and make this relatively sensitive 2D IR procedure suitable for the rapid determination of total *trans* fats for food labeling purposes.

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