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Analysis

Portable FTIR analyzers for the rapid determination of total *trans* fat

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Summary

Mid-infrared spectroscopy (MIS) has been a particularly valuable, rapid analytical tool because it provides the total trans fat content of lipids in a single measurement. In recent years, portable Fourier transform infrared (FTIR) analyzers, which are most useful for field laboratories, have become commercially available. Their performance in the transmission or attenuated total reflection (ATR) modes and application to the rapid determination of total trans fat content of fats, oils and lipids extracted from fast foods were recently found to be equivalent to those of bench top FTIR spectrometers.

Introduction

The determination of total *trans* fat by mid-IR spectroscopy has been widely used for many decades [1, 2]. Its importance stems from the fact that the C–H out-of-plane deformation band observed at 966 cm^{-1} is uniquely characteristic of all isolated (non-conjugated) double bonds with *trans* configuration. The methodology currently preferred by the fats and oils industry for the determination of *trans* fats is gas chromatography (GC) because it provides detailed information on the total fatty acid composition [3]. However, GC is time-consuming and requires derivatization of the lipids to their corresponding more volatile methyl esters prior to chromatographic separation. In addition, extensive expertise is required to resolve and confirm the identity of complex mixtures of overlapping geometric and positional fatty acid methyl ester (FAME) isomers, particularly for matrices that contain partially hydrogenated vegetable oils, milk fats, and fish oils. Other challenges include the lack of availability of many *trans* fatty acid standards and the fact that official GC methods do not require a prior isolation of geometric isomers by high performance liquid chromatography (HPLC), thin layer chromatography (TLC), or solid phase extraction (SPE) techniques [4].

In contrast, mid-IR spectroscopy provides a relatively simple and rapid (<5 min) quantification of total *trans* fat concentration. An additional advantage is that this method typically involves only minimal sample preparation procedures such as heating the test sample to 65°C. The accuracy of determining *trans* fat by IR has traditionally been compromised by the fact that the band at 966 cm^{-1} occurs on a sloping baseline [1, 2]. To overcome this and other limitations and to improve sensitivity and accuracy, a new IR method was recently developed that entails the measurement of the height of the negative second derivative of this absorption band [1, 2]. The narrower bandwidth of the second derivative spectral band made it possible to recognize potential interferences [1] attributed to saturated (e.g., in cocoa butter, see **Figure 1**) and conjugated fatty acids [1] particularly in matrices with *trans* fat concentrations below 2% of total fat.

Since *trans* fat labeling requirements became mandatory in many countries, there has been a need for accurate analytical methodologies that could rapidly verify compliance with applicable regulations. The portable FTIR analyzers proved to be particularly useful for these applications and are ideally suited for field laboratories. New portable FTIR analyzers were initially developed for industrial applications and as hand-held units for emergency responders. The performance of two analyzers that were recently evaluated and applied to the determination of total *trans* fat is presented below.

Analysis of edible oils using a portable, heated, 9-reflection ATR-FTIR analyzer

To evaluate the performance of a portable, heated, 9-reflection ATR-FTIR analyzer (Agilent Technologies, Wilmington, DE), *trans* fat concentrations of up to 60% of total fat were used for calibration standards (consisting of trielaidin in triolein) and fat and oil test samples [5]. The optical bench included a Michelson interferometer with a mechanical bearing moving mirror, a potassium bromide substrate beam splitter, and a deuterated triglycine sulfate (DTGS) detector operating at room temperature (i.e., 23°C). Test portions were measured at 65°C (+/–1°C) and consisted of approximately 10 μL of neat (undiluted in any solvent) processed fats and oils including edible fats and oils purchased from local grocery stores, mixtures prepared from canola oil and partially hydrogenated canola oil obtained from a commercial supplier, and partially hydrogenated vegetable and fish oils obtained from commercial suppliers. To improve the signal-to-noise ratio, 256 scans were co-added at 4 cm^{-1} resolution and signal averaged. The reference background material used was the open beam [5].

For this analyzer, a single linear calibration function could not be obtained over the entire *trans* fat range of interest because the lack of fit test for a single straight line was found to be significant (p-value <0.01). This non-linear response was unexpected for an FTIR spectrometer equipped with a DTGS detector. Two calibration functions with overlapping ranges were found to give the best fit for the observed calibration data. The calibration functions consisted of a linear function for the low *trans* fat range (0.14% to 6.06%, as percent of total fat), and a quadratic function for the entire range (0.14% to 60.12%, as percent of total fat). For the linear calibration range, the slope was 0.919 (standard error = 0.007) and

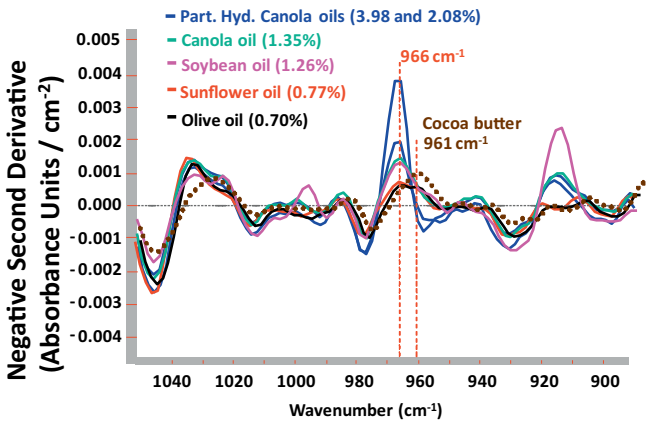

Figure 1. Expanded spectral region that exhibits the negative second derivative of the deformation band for isolated *trans* double bonds at 966 cm^{-1} for unknown test samples. consisting of partially hydrogenated or processed (refined, bleached, and deodorized) oils that contain *trans* fat below approximately 4% of total fat. A cocoa butter test sample which is high in saturated fat is shown for comparison; the feature near 961 cm^{-1} could be easily misidentified as a band for isolated *trans* double bonds. Reproduced from [5] by permission.

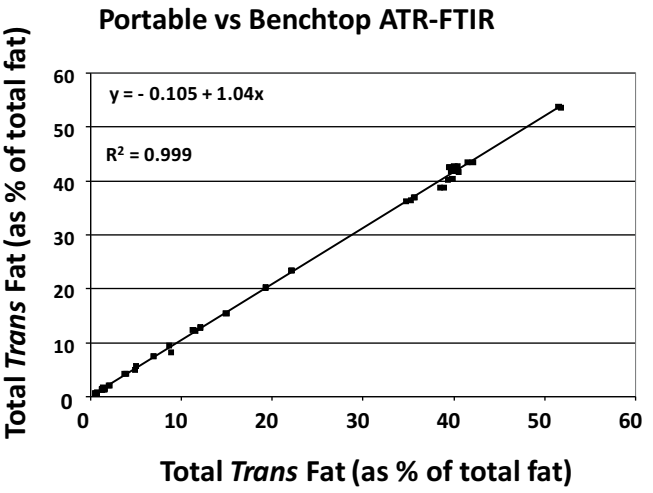

Figure 2. Comparative plot of *trans* fat determination by ATR-FTIR for the portable analyzer vs. bench top spectrometer. The figure shows data from duplicate determinations for unknown test samples. The line of best fit had a slope of 1.04 and R^2 of 0.999 that indicated good agreement between the two sets of quantitative values. Reproduced from [5] by permission.

the intercept was 0.043 (standard error = 0.020). The measure of the proportion of total variation about the mean band height described by the fitted line was given by $R^2 = 0.999$. For the quadratic calibration function the two coefficients were –0.003 (standard error <0.001) and 0.844 (standard error = 0.011), the intercept was 0.187 (standard error = 0.079), and the R^2 value was 0.999. The lower limit of quantification (LOQ) was 0.34% *trans* fat, as percent of total fat [5].

When fats and oils with *trans* fat concentrations that varied from 0.5% to 54% of total fat were measured with this FTIR analyzer (Figure 1) and, for comparison, with a bench top ATR-FTIR spectrometer (model 7000e, Agilent), good agreement was found (**Figure 2**). The line of best fit had a slope of 1.04 and an R^2 value of 0.999 [5].

Analysis of fast food lipid extracts using a portable, transmission-mode FTIR analyzer

Using 10 µL test portions, the performance of a novel, portable FTIR analyzer (Cary 630, Agilent) operating in the transmission mode with a pathlength of 30 µm was critically evaluated for the rapid determination of total *trans* FA content for the fat extracted from fast food test samples. Samples, which consisted of hamburgers (H), chicken tenders (CT), French fries (FF), and apple pies (AP), were analyzed as FAME instead of total fat extracts because the method used for total fat extraction yielded lipid extracts that were not completely transparent or free of interfering particles. While the FTIR measurement was rapid (<5 min), the time required for extraction and derivatization to FAME was not. The analyzer was equipped with a DialPath™ accessory that was factory-calibrated to three different fixed pathlengths (30, 50 and 100 µm) and a DTGS detector operated at room temperature (i.e., 23°C). The FTIR experimental conditions were the same as those specified above [6].

FAME standards used for calibration consisted of methyl elaidate (ME) in methyl oleate (MO) in the range of interest of 0.52–13.96% ME, as % of total FAME. Parameters for the fitted linear regression function for the calibration plot were as follows: the slope was 1.1512 (standard error = 0.0020), the intercept was –0.5430 (standard error = 0.0123), and $R^2 = 0.999$. The LOQ was 0.58% *trans* FAME, as percent of total FAME. One proposed explanation for the relatively large magnitude of the negative inter-

Table 1. Concentration of total *trans* FAME (as % total FAME) in the fat extracts of fast food test samples. Values represent the means ±SD of duplicate determinations of each of two separate extractions for each fast food sample. One-way ANOVA was used to determine differences between the three analytical approaches on the content of total *trans* FAME (as % of total FAME). Mean comparisons were performed using the Student's t-test at p = 0.05. Different superscript letters within a column indicate significant differences. H, hamburgers; CT, chicken tenders; FF, French fries; AP, apple pies.

Extract	Benchtop FTIR	Portable FTIR	GC	P
H1	5.14 ± 0.01^{B}	5.36 ± 0.02^{A}	4.69 ± 0.00^{C}	0.0002
H2	5.69 ± 0.00^{B}	6.16 ± 0.02^{A}	5.30 ± 0.03^{C}	0.001
H3	5.03 ± 0.02^{B}	5.39 ± 0.04^{A}	4.52 ± 0.00^{C}	0.0009
H5	3.39 ± 0.01^{A}	2.84 ± 0.04^{B}	2.84 ± 0.00^{B}	0.003
CT2	1.37 ± 0.02^{C}	1.52 ± 0.01^{B}	1.65 ± 0.00^{A}	0.005
CT3	0.58 ± 0.00^{C}	0.68 ± 0.01^{B}	0.81 ± 0.01^{A}	0.001
CT4	9.86 ± 0.02^{C}	9.95 ± 0.00^{B}	10.80 ± 0.01^{A}	<0.0001
CT5	0.47 ± 0.00^{C}	0.68 ± 0.01^{B}	0.81 ± 0.00^{A}	0.0003
CT6	0.87 ± 0.01^{C}	0.99 ± 0.01^{B}	1.08 ± 0.00^{A}	0.005
CT7	6.19 ± 0.01^{A}	6.04 ± 0.01^{B}	5.88 ± 0.00^{C}	0.0008
FF4	10.62 ± 0.01^{B}	10.82 ± 0.04^{B}	11.40 ± 0.04^{A}	0.003
FF5	1.06 ± 0.00^{C}	1.16 ± 0.01^{B}	1.22 ± 0.01^{A}	0.002
FF6	0.65 ± 0.00^{C}	0.78 ± 0.00^{B}	0.89 ± 0.00^{A}	<0.0001
FF7	6.83 ± 0.05^{A}	6.70 ± 0.07^{A}	6.34 ± 0.02^{B}	0.03
AP2	0.45 ± 0.00^{C}	0.66 ± 0.02^{B}	0.84 ± 0.03^{A}	0.005
AP3	1.30 ± 0.04	1.39 ± 0.06	1.36 ± 0.01	NS
AP4	1.67 ± 0.08	1.78 ± 0.09	1.72 ± 0.00	NS
AP5	0.47 ± 0.00^{C}	0.57 ± 0.00^{B}	0.83 ± 0.01^{A}	<0.0001
AP6	4.90 ± 0.03^{A}	4.72 ± 0.01^{B}	4.66 ± 0.01^{B}	0.01

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cept and the higher than expected LOQ may be related to the possibility that, in the absence of any test sample, the collected reference background single-beam spectrum (open beam) was not a true blank because of difficulties in reaching the infrared transmission element of the DialPath™ accessory due to space limitations and the possibility that the accessory was not thoroughly cleaned after measurement of each new test portion [6].

For all fast food extracts, the total *trans* FAME concentrations varied from approximately 0.5% to 11% of total FAME. Determinations of total *trans* FAME content were consistent with those obtained for comparison with a bench top ATR-FTIR and by GC using official methods AOCS Cd 14e-09 [2] and AOCS Ce 1j 07 [3], respectively (**Table 1**). Statistically significant differences among the three methods for the determination of total *trans* FAME concentration were observed for all but two of the test samples (Table 1). These differences could be attributed to the negligible standard deviations found for each set of replicate measurements obtained by each of the three techniques. In general, samples with *trans* FAME concentrations below 2% of total FAME had slightly larger values when determined by use of the portable FTIR compared with the bench top FTIR system (**Figure 3**), while those determined by GC were slightly greater than the ones found by either FTIR system (Table 1, Figure 3). For the 10 fat extracts with total *trans* FAME concentrations below 2% of total FAME (CT2, CT3, CT5, CT6, FF5, FF6, AP2, AP3, AP4, and AP5), eight of the test samples had higher GC than FTIR values. In contrast, samples which showed total *trans* FAME contents that exceeded 2% of total FAME generally had lower GC values than those found by FTIR with the exception of CT4 and FF4 (**Table 1**, **Figure 3**) [6]. However, statistical evaluation of the data using the Bland-Altman analysis, which may be used to compare the performance of analytical methods, indicated satisfactory agreement between the two FTIR systems and between each FTIR method and GC.

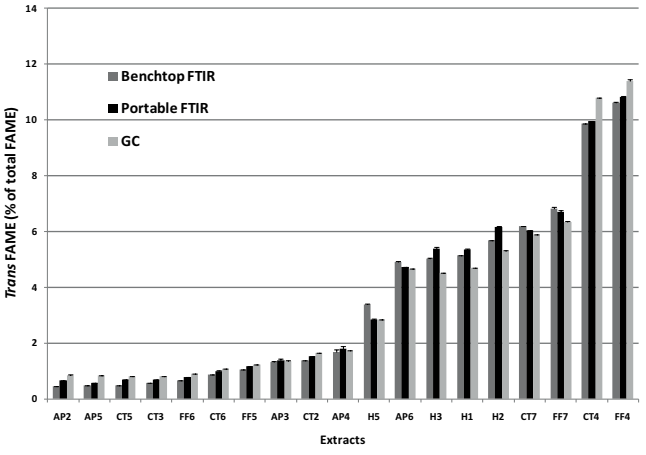

Figure 3. Comparison of *trans* FAME concentration (as % of total FAME) in fat extracted from fast foods obtained by using a portable transmission-mode FTIR analyzer and a bench top ATR-FTIR spectrometer, and by GC. One-way ANOVA was used to determine pairwise comparisons of the mean *trans* FAME content. Negligible standard deviation values were found (see Table 1) for each set of replicate measurements. H, hamburgers; CT, chicken tenders; FF, French fries; AP, apple pies. Reproduced from [6] by permission.

Based on the total fat concentration expressed in grams per serving determined by GC and the mean *trans* FAME concentrations determined by FTIR, the corresponding *trans* fat contents (means ± SD) expressed in grams per serving for various types of fast food test samples were calculated as follows: Hamburgers, FTIR 1.00 ±0.42, GC 0.86 ± 0.38; chicken tenders, FTIR 0.67 ± 0.78, GC 0.73 ± 0.84; French fries, FTIR 1.00 ± 1.24, GC 1.07 ± 1.39; apple pies, FTIR 0.27 ± 0.23, GC 0.26 ± 0.22. The corresponding ranges for the mean values of *trans* fat (as grams per serving) for the extracts analyzed by FTIR were as follows: Hamburgers, FTIR 0.6–1.5, GC 0.5–1.3; chicken tenders, FTIR 0.1–2.0, GC 0.2–2.3; French fries, FTIR 0.1–2.8, GC 0.1–3.1; apple pies, FTIR 0.1–0.6, GC 0.0–0.5. Overall, the means ±SD for *trans* fat contents and the corresponding ranges for the mean values of *trans* fat contents (in g/serving) obtained by FTIR were consistent with those independently determined by GC.

Conclusions

New portable FTIR analyzers operating in the transmission or ATR modes have been commercially developed in recent years and successfully applied to meet an increasing demand for rapid (<5 min) and accurate quantification of the total *trans* FA content of lipids. The performance of two portable FTIR analyzers was evaluated and shown to be as equally satisfactory as that of a bench top ATR-FTIR spectrometer for the determination of total *trans* fat concentrations. The mean *trans* FAME contents (g/serving) determined for fast food lipid extracts by FTIR were found to be comparable to those obtained by an official GC method. These results indicate that portable FTIR analyzers are suitable for the rapid and routine quantification of total *trans* fat in lipid matrices.

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