Portable Mid-Infrared Device and Chemometrics for the Prediction of Low (0.5%) Total Trans Fat Content in Fast Foods

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Abstract: The ruling that partially hydrogenated oils (PHO) are no longer “generally recognized as safe (GRAS),” has accelerated the replacement of PHO ingredients with fat alternatives having increasingly lower or no trans fat content. In the present study, we developed a Fourier-transform infrared (FTIR) spectroscopic procedure in conjunction with multivariate partial least squares regression (PLSR) and found it suitable for the accurate prediction of low (0.5%) total trans fat content, as percentage of total fat, measured as fatty acid methyl esters (FAME), in the lipids extracted from 24 representative fast foods. This multivariate data analysis approach is relevant because the precision of the current univariate FTIR official method (AOCS Cd 14-09) is reportedly poor below 2% of total fat, while PLSR has allowed us to accurately predict the concentration of low trans fat in fast foods. The performance of a portable FTIR device was also evaluated and compared to that of a benchtop FTIR spectrometer. For both infrared data sets, PLSR-predicted concentrations of total trans FAME, ranging from approximately 0.47% to 11.40% of total FAME, were in good agreement with those determined by a primary reference gas chromatography (GC) method ($R^2$>0.99); high prediction accuracy was also evidenced by low root mean square error of cross-validation (RMSECV) values. The lowest RMSECV error of 0.12% was obtained with the portable device. The lowest total trans FAME concentration, determined by GC to be 0.42%, was accurately predicted by the portable FTIR/PLSR procedure as 0.47% of total FAME.

Key words: low trans fat content, fast foods, portable device, infrared spectroscopy, partial least squares regression

1 Introduction

For several decades, studies have been undertaken to characterize the chemical composition and properties1 of the mono-unsaturated trans fatty acid isomers generated during the partial hydrogenation of vegetable oils and the trans fatty acid isomers of di- and tri-unsaturated fatty acids that are formed during high-temperature deodorization of vegetable oils and frying of oils at elevated temperatures. Partial hydrogenation has been used by the food industry to convert vegetable oils into semi-solid fats that are stable to oxidation for many food applications. Destaillets et al.2 have documented the detrimental health effects of consumption of trans fat on risk factors of vascular health. Consequently, in 2003, the US Food and Drug Administration (FDA) mandated the declaration of the total amount of trans fat on the nutrition facts label of packaged foods3 and, in 2015, FDA ruled that partially hydrogenated oils were no longer "generally recognized as safe (GRAS)." As a result, reformulation of processed foods has been underway, which entailed the replacement of partially hydrogenated oil ingredients with fat alternatives having increasingly lower trans fat content4. In 2011, the FDA published in the Federal Register a proposed rule for implementing the restaurant menu labeling provisions of the Affordable Care Act4. Among the proposed provisions, a restaurant that is part of a chain with 20 or more locations must provide calorie information for standard items listed on menus and, upon consumer request, written declarations of the amounts of trans fat and other food constituents. As such, there is an urgent need to develop sensitive and accurate methodologies for trans fat quantification in order to meet the challenge of measuring increasingly low trans fat levels in restaurant foods, fast foods, and processed foods4.
For decades, Fourier-transform infrared (FTIR) spectroscopy has been commonly used for the rapid quantification of total trans fat in edible fats and oils. The official attenuated total reflection (ATR)-FTIR spectroscopic method, American Oil Chemists’ Society (AOCS) Cd 14e-09\(^6\), relates to the quantitative univariate measurement of the height of the negative second derivative (NSD) of the absorbance band at 966 cm\(^{-1}\) attributed to the total isolated (non-conjugated) trans double bonds in fats and oils. Unfortunately, this official method’s reproducibility data for quantifying the height of the NSD determined in a multi-laboratory collaborative study was found to be unsatisfactory at concentrations of approximately near or below 2% total trans fat, as percentage of total fat. At these low levels, precision was calculated according to the Horwitz Ratio\(^{(a)}\) acceptance criteria, and reportedly fell outside the reference range of 0.5–2.0\(^{10}\). In a recent publication this univariate FTIR / NSD method was used to determine the total trans fat content of eight fast foods in the narrow range of 1.57% to 3.83% trans fat (as percentage of total fat)\(^{11}\). Two foods were below and four foods were near the precision threshold (2% trans fat) for the HorRat acceptance criteria\(^{a,10}\). Therefore, some of these determinations were outside the scope of the validated univariate FTIR / NSD official method\(^{a,10}\) used by these authors\(^{11}\). Previous FTIR / multivariate partial least square regression (PLSR) studies on the analysis of food matrices were satisfactory only when they entailed the analysis of test samples with relatively higher total trans fat content.

In one study\(^{12}\) the accuracy of the total trans fat content in ground cereals was reportedly satisfactory only at, or above, 0.9% of total fat. In another study, the lowest accurately predicted total trans fat content in cereal-based foods was reported to be 2.5% of total fat\(^{13}\).

To conveniently apply FTIR spectroscopy to the measurement of trans fat in fast food matrices that include protein, carbohydrate, lipids, and unsaponifiable matter, extraction of total lipids was first required. However, to avoid and eliminate the diffuse reflection of infrared light by any residual solid materials potentially present in lipid extracts, which would interfere with spectral measurements, fatty acid methyl ester (FAME) derivatives were prepared from the total lipid extracts. An advantage of measuring FAME derivatives by FTIR was that it allowed direct comparison of total trans FAME concentrations with those determined by using a primary reference gas chromatographic (GC) method\(^{14}\), which also successfully allowed the accurate prediction of low (0.5%) trans fat content.

In the present study, we evaluated the performance of a portable, transmission-mode FTIR device in conjunction with PLSR as a potential substitute for the univariate official method, AOCS Cd 14e-09\(^6\). This chemometric procedure was successfully used for the accurate quantitative prediction of low total trans FAME content for a set of 24 representative fast foods from U.S.-based restaurants at concentrations found to be as low as approximately 0.5%, as percentage of total FAME.

### 2 Materials and Methods

#### 2.1 Fast foods

Twenty-four fast foods\(n=24\) belonging to four categories were purchased from 11 fast food restaurants in Prince George’s County, MD, USA\(^{14,15}\). These were French fries (FF, \(n=7\)), hamburgers (H, \(n=4\)), chicken tenders/ nuggets (CT, \(n=7\)), and apple pie/turnovers (AP, \(n=6\)). For each fast food, duplicate servings were acquired “as served” without the addition of toppings or sauces. Each food serving was weighed, frozen at \(-75^\circ\)C overnight, homogenized by using a GrindoMix GM 300 Knife Mill (Retsch, Newton, PA), and the resulting powder or paste was stored at \(-75^\circ\)C until it was analyzed.

#### 2.2 Extracts of total lipids from fast foods and preparation of FAME

To extract the total lipids in the homogenized fast foods (in duplicate), the Official Method 996.06 of the AOAC INTERNATIONAL\(^{16}\) was used as previously described\(^{17}\). FAME derivatives for both the GC and FTIR analyses were prepared from a 400 mg portion of each of the extracted fast food lipids by using AOCS Official Method Ce 2-66\(^{17}\).

#### 2.3 Gas chromatography (GC)

The determination of FAME by GC-flame ionization detector (FID) was carried out according to AOCS Official Method Ce 1j-07\(^{18}\). A 6890N gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with an SP-2560 column (100 m \(\times\) 0.25 mm i.d., 0.20 \(\mu\)m film; Supelco, Bellefonte, PA) was used. Determination of the concentration of each FAME component was performed using theoretical correction factors\(^{19}\). For ease of comparison with FTIR data, the total trans fat content determined by GC were reported as total trans FAME, as percentage of total FAME\(^{14}\).

#### 2.4 FTIR spectral acquisition

Absorbance spectra of FAME from fast food lipid extracts were collected by using a portable FTIR device operating in the transmission mode (Cary 630, Agilent Technologies, Danbury, CT). Data were collected at three different factory-calibrated fixed pathlengths (30, 50, and 100 \(\mu\)m) using a DialPath\(^TM\) accessory. The optical bench was equipped with a deuterated triglycerine sulfate (DTGS)/detector operating at room temperature. To enhance the signal to noise ratio (SNR), 256 scans were collected in approximately 2 min at 4 cm\(^{-1}\) resolution. Absorbance spectra were also collected using a benchtop attenuated...
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2.5 Multivariate data analysis

The relationships between the actual concentrations of trans FAME in a test sample, as determined by a primary reference GC method, and the corresponding observed FTIR spectra were established by applying PLSR analysis performed using the multivariable Pirouette software (Version 4.5; Infometrix, Bothell, WA). PLSR calibration models were developed and optimized for data collected on each FTIR instrument and for spectral data collected with each of the three different pathlengths available with the portable FTIR device. Models were developed by using spectra for all the test samples in the calibration set and were validated by applying a stepwise, leave-four-out cross-validation procedure to find the optimum number of PLS factors for each of the calibration models and to verify method performance. In this approach, a calibration model was developed using the spectra of all the test samples within a dataset, except for one, and subsequently used to predict the concentrations of total trans FAME for the left-out sample. In this cross-validation step, the four (out of a total of 96) spectra that were left-out at a time were, as stated above, those that represented duplicate FTIR spectral measurements of two replicate lipid extracts for a single fast food item. This calibration and the subsequent prediction procedure are repeated so that every test sample is left out and predicted once. The prediction accuracy of the PLSR model was evaluated by the root mean square error of cross-validation (RMSECV)

\[
\text{RMSECV} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (y_i - \hat{y}_i)^2}
\]

Where, \(y_i\) is the measured value for total trans FAME by the reference GC method, \(\hat{y}_i\) is the value predicted by PLSR of \(i^{th}\) test sample which was left out of the data set during calibration, and \(N\) is the number of test samples in the calibration set.

To find the optimum number of factors for each of calibration model, the RMSECV values were plotted against the number of PLS factors. The number of factors that yielded a minimal RMSECV value was considered to be optimal and subsequently used to build a calibration model. Including too many factors in a model or overfitting would deteriorate the prediction ability for the test samples in the test set. Generally, the optimal number of factors should be the lowest one that would yield the minimum prediction error\(^{21}\). The percentages of spectral variance explained by each PLS factor were also examined. The regression vector was evaluated to confirm that the optimal number of latent factors selected was based upon chemical variance attributed to the total trans FAME concentration rather than random noise or false correlations\(^{22}\). Several PLSR calibration models were developed using different data pre-processing techniques and for different spectral regions that included the total trans FAME spectral feature for isolated (non-conjugated) double bonds at 966 cm\(^{-1}\). Different spectral pre-processing techniques were evaluated to correct for the baseline effects (sloping and shifted) usually found around 966 cm\(^{-1}\), and possibly for the presence of potentially overlapping weak interference bands, such as those for saturated or conjugated fatty acids\(^{14, 20, 23, 24}\). The spectral pre-processing techniques used were Savitzky-Golay second or first derivatives, standard normal variate (SNV), or the combination of SNV and Savitzky-Golay second or first derivatives. The optimal spectral pre-processing method and spectral region combination were selected based on the minimum RMSECV value.

2.6 Statistical analysis

Statistical analysis was performed using JMP (version 11.2.0, SAS Institute, Cary, NC). The mean differences between the total trans FAME predictions for fast foods using both a portable transmission FTIR device and a benchtop ATR-FTIR spectrometer combined with PLSR and the GC reference data were statistically evaluated via one-tailed \(t\)-tests at the 95% confidence level. Before the \(t\)-test, the assumption of normality was evaluated by using the Shapiro-Wilk test\(^{14, 25}\). If significant non-normality was observed, then the Wilcoxon signed-rank test\(^{14, 25}\) was used to test the null hypothesis that the mean differences between the reference GC determinations and FTIR/PLSR predictions were equal to zero.

3 Results and Discussion

3.1 FTIR spectra and PLSR model optimization for prediction of total trans fat content

Exhibited in Fig. 1 are representative FTIR spectra in the fingerprint region for the four fast food categories investigated with examples of total trans FAME concentrations, as percentage of total FAME, in the very low (0.42-0.89%), low (1.65%), medium (4.52%) and high (10.80%) ranges. The optimized regression model parameters for

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each of the benchtop and portable instruments in terms of the number of PLS factors, spectral region and spectral preprocessing methods, were selected based on the minimum RMSECV and highest $R^2$ values (Table 1). For the portable device, PLSR models were developed for the data collected in transmission mode with pathlengths of 30, 50 and 100 μm. Ideally, the PLSR models should account for all possible matrix variations present in fast food lipid extracts after conversion to FAME to accurately predict the total trans FAME content of a new test sample. Therefore, a representative set of fast foods were purchased from restaurants that had posted the composition of frying oils used for food preparation on their websites or had reported this information in their nutritional documents 14, 15. The 24 fast foods from four categories (FF, AP, H, and CT) that were investigated covered the total trans FAME concentration range from approximately 0.42 to 11.40 % of total FAME, as determined by a reference GC method (Table 2). This large difference between the concentration of total trans FAME (as % of total FAME) in fast food lipid extracts, such as FF1 (0.66) & FF4 (11.40) and CT1 (0.70) & CT4 (10.80) (Table 2), may be explained as follows. These fast food items were purchased from different restaurants and were not replicate test samples of the same item acquired from a single restaurant. The large difference in trans fat content for a given food item may potentially be attributed to different factors including those used to prepare that food in various installations. For instance, frying procedures may entail differences due to frying temperature, length of frying time, nature of the frying oil (cottonseed, peanut, palm, sunflower, etc.), oxidation, time required for the addition of make-up oil during frying operations, etc. 27

Five factors were found to be optimal as evidenced by the minimal RMSECV for the data collected using the portable device with each of the three different pre-selected pathlengths. Four PLS factors were found to be optimal for the benchtop ATR-FTIR spectral data. The regression coefficients provided insights into the spectral variables that influenced the PLSR model. With respect to the spectral features relating to fast foods, the regression coefficients

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**Table 1** Optimized parameters for PLSR models developed for predicting total trans FAME content in fast food lipid extracts using a portable FTIR device, in the transmission mode at three different optical pathlengths (30, 50 and 100 μm), and a benchtop ATR-FTIR spectrometer.

| FTIR mode       | Spectral range, cm$^{-1}$ | Spectral preprocessing          | Cross-Validation |
|-----------------|---------------------------|---------------------------------|-----------------|
|                 |                           |                                 | PLS Factors | RMSECV | $R^2$ |
| Transmission FTIR (Portable) |               |                                 | 5 | 0.12  | 0.999 |
| 30 μm           | 900-1230                  | First Derivatives               | 5 | 0.16  | 0.997 |
| 50 μm           | 900-1230                  | First derivatives               | 5 | 0.20  | 0.996 |
| 100 μm          | 900-1001                  | SNV + First derivatives         | 5 | 0.16  | 0.997 |
| ATR-FTIR (Benchtop) | 900-1230                | SNV + First derivatives         | 4 | 0.16  | 0.997 |
plots for the optimized models developed for each of FTIR data sets collected with the portable device and the benchtop spectrometer indicated that PLSR predictions were strongly dependent on variables that corresponded with the observed spectral feature attributed to the total trans FAME band near 966 cm$^{-1}$, rather than with noise or other artefacts (Fig. 2).

Individual or a combination of spectral pre-processing methods yielded the lowest RMSECV values, namely: (1) SNV + first derivatives ($2^{\text{nd}}$ order polynomial, 9 points) for benchtop ATR-FTIR and portable FTIR data (100 μm); and (2) first derivatives ($2^{\text{nd}}$ order polynomial, 9 points) for the portable FTIR data (30 μm and 50 μm). For all four models, good predictive accuracies were observed with low RMSECV values of 0.16% for the benchtop spectrometer and 0.12, 0.16, and 0.20% for the portable device with 30, 50 and 100 μm pathlengths, respectively, when the stepwise leave-four-out cross-validation was used (Table 1).

Therefore, the highest accuracy was found for the PLSR model developed using the spectral data collected with the 30 μm-pathlength portable device. These data suggest that increasing the transmission-mode pathlengths to 50 and 100 μm slightly degraded model performance in terms of prediction accuracy as indicated by the modest increase in RMSECV values (Table 1); this may be attributed to an increase in baseline variations in the fingerprint region.

Of the different spectral regions investigated, the narrow range from 1230 to 900 cm$^{-1}$ was found to be optimal for all instrumental configurations, except for the portable device when used with a 100 μm pathlength. In this case, the spectral range of 1001-900 cm$^{-1}$ was found to be optimal.

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**Table 2** Comparison of PLSR-predicted total trans FAME (as % of total FAME) in fast food lipid extracts using FTIR data collected with a portable transmission-mode FTIR device at 30 μm optical pathlength and a benchtop ATR-FTIR spectrometer in combination with stepwise leave-four-out cross-validation and reference concentrations determined by GC.

| Fast Food FAME$^a$ | Reference GC | Benchtop$^b$ | Benchtop-(GC) | Portable$^b$ | Portable-(GC) |
|-------------------|--------------|--------------|---------------|--------------|---------------|
| FF1               | 0.66 ± 0.02  | 0.80 ± 0.00  | 0.14          | 0.74 ± 0.02  | 0.08          |
| FF2               | 0.66 ± 0.01  | 0.66 ± 0.01  | 0.00          | 0.73 ± 0.01  | 0.07          |
| FF3               | 0.54 ± 0.00  | 0.61 ± 0.01  | 0.07          | 0.56 ± 0.02  | 0.02          |
| FF4               | 11.40 ± 0.04 | 11.35 ± 0.04 | -0.05         | 11.40 ± 0.01 | 0.00          |
| FF5               | 1.22 ± 0.01  | 1.32 ± 0.01  | 0.10          | 1.28 ± 0.05  | 0.06          |
| FF6               | 0.89 ± 0.00  | 0.90 ± 0.02  | 0.01          | 0.98 ± 0.01  | 0.09          |
| FF7               | 6.34 ± 0.02  | 6.48 ± 0.04  | 0.14          | 6.47 ± 0.10  | 0.13          |
| AP1               | 0.42 ± 0.01  | 0.38 ± 0.01  | -0.04         | 0.47 ± 0.02  | 0.05          |
| AP2               | 0.84 ± 0.03  | 0.61 ± 0.03  | -0.23         | 0.80 ± 0.07  | -0.04         |
| AP3               | 1.36 ± 0.01  | 1.37 ± 0.01  | 0.01          | 1.36 ± 0.14  | 0.00          |
| AP4               | 1.73 ± 0.00  | 1.64 ± 0.01  | -0.09         | 1.75 ± 0.00  | 0.02          |
| AP5               | 0.83 ± 0.01  | 0.64 ± 0.01  | -0.19         | 0.65 ± 0.01  | -0.18         |
| AP6               | 4.66 ± 0.01  | 4.68 ± 0.11  | 0.02          | 4.73 ± 0.08  | 0.07          |
| H1                | 4.69 ± 0.00  | 4.89 ± 0.07  | 0.20          | 4.74 ± 0.01  | 0.05          |
| H2                | 5.30 ± 0.03  | 4.97 ± 0.06  | -0.33         | 5.52 ± 0.20  | 0.22          |
| H3                | 4.52 ± 0.00  | 4.42 ± 0.01  | -0.10         | 4.36 ± 0.04  | -0.16         |
| H5                | 2.84 ± 0.00  | 3.31 ± 0.01  | 0.47          | 2.83 ± 0.02  | -0.01         |
| CT1               | 0.70 ± 0.01  | 0.68 ± 0.02  | -0.02         | 0.70 ± 0.03  | 0.00          |
| CT2               | 1.65 ± 0.00  | 1.77 ± 0.10  | 0.12          | 1.60 ± 0.11  | -0.05         |
| CT3               | 0.81 ± 0.01  | 0.76 ± 0.02  | -0.05         | 0.87 ± 0.02  | 0.06          |
| CT4               | 10.80 ± 0.01 | 10.60 ± 0.00 | -0.20         | 10.62 ± 0.01 | -0.18         |
| CT5               | 0.81 ± 0.00  | 0.74 ± 0.01  | -0.07         | 0.76 ± 0.02  | -0.05         |
| CT6               | 1.08 ± 0.00  | 1.02 ± 0.05  | -0.06         | 0.94 ± 0.01  | -0.14         |
| CT7               | 5.88 ± 0.00  | 5.81 ± 0.00  | -0.07         | 5.79 ± 0.05  | -0.11         |

$^a$ Abbreviation: FF, French fries; AP, apple pies; H, hamburgers, CT, chicken tenders.

$^b$ Values represent the means ± SD of two measurements of each of duplicate lipid extracts for each fast food item.
3.2 PLSR prediction of total trans FAME in fast food lipid extracts

The total trans FAME concentrations for the representative set of 24 fast food samples analyzed by the reference GC method were found to fall in the range 0.42-11.40%, as percentage of total FAME (Table 2). All the test samples measured in the transmission or ATR modes were accurately predicted when a leave-four-out cross-validation was performed. A comparison is presented in Fig. 3 for the total trans FAME predictions for the data collected with the portable device with all three optical pathlengths and those determined by the reference GC method. Overall all the methods yielded a fairly similar performance. However, with the 100 μm pathlength, the FTIR device exhibited a comparatively less satisfactory performance (higher RMSECV, Table 1), particularly at lower concentrations. Among the three optical pathlengths available, the 30 μm accessory yielded the highest prediction accuracy (lowest RMSECV, Table 1), hence, only the predictions for that configuration will be further discussed in the following sections.

A similar comparison is exhibited in Fig. 4 for total trans FAME predictions for data collected with the portable device at 30 μm pathlength to those obtained by ATR-FTIR and a reference GC method. The benchtop spectrometer data yielded slightly less accurate predictions for some test samples by comparison to the GC determinations (Table 2).

The lowest concentration of total trans FAME in the 24 fast food lipid extracts determined by GC was 0.42% of total FAME for AP1. The corresponding values obtained with the portable and benchtop instruments for the same test sample were 0.47 and 0.38% of total FAME, respectively, corresponding to satisfactory absolute differences, relative to GC, of 0.05 and 0.04%, respectively. The lowest total trans FAME concentrations, as percentage of total FAME, found by GC for each of the other three categories were 0.54% (FF3), 2.84% (H5) and 0.70% (CT1), respectively. The corresponding results for these test samples were satisfactorily predicted with the ATR-FTIR data as 0.61, 3.31 and 0.68% of the total FAME, respectively. However, much better accuracy (0.56, 2.83 and 0.70% of total FAME) was obtained with data collected with the portable device (30 μm). All the test samples with total trans FAME contents below 1% of total FAME (i.e. FF1, FF2, FF3, FF6, FF7, FF8, FF9, FF10, FF11, FF12, FF13, FF14, FF15, FF16, FF17, FF18, FF19, FF20, FF21, FF22, FF23, FF24) were also satisfactorily predicted with the portable FTIR device at 30 μm pathlength (RMSECV = 0.18%).

3.3 Regression analysis of NMR spectra

NMR spectra were analyzed using the MestReNMR 3.0 software package. The lineshape analysis was performed using the INOVA Option of MestReNMR 3.0 software package. The peak areas were integrated to determine the amount of each component. The coupling constants were determined using the DIPolar Option of MestReNMR 3.0 software package.
AP1, AP2, AP5, CT1, CT3 and CT5) were predicted accurately with data from both the benchtop and portable FTIR systems. However, higher accuracy was obtained with the portable device relative to the benchtop spectrometer, which may be attributed to the long pathlength (30 μm) provided by the transmission accessory of the former. A similar level of satisfactory performance was found for all the other fast food test samples that were analyzed with the benchtop instrument, except for three, H2, H5 and AP2, which yielded under/over predictions of total trans FAME with over 0.20% absolute difference relative to their corresponding GC determinations (Table 2). With the portable device, only the H2 test sample had an absolute difference above 0.20% when compared with the reference GC value. All the remaining test samples with total trans FAME concentrations, ranging from 0.42 to 11.40 % of total FAME as determined by GC, were accurately predicted with data from the portable device with a maximum absolute difference below 0.20%, and for the majority of the test samples, below 0.10 %. A higher degree of repeatability was observed for the four replicate FTIR measurements (two measurements for duplicate extracts of each fast food item) as the coefficient of variation (CV) value was below 5% for all the test samples except for five (portable device: AP2, CT2, AP3 and AP1) and (benchtop: CT2).

Good linear correlation between the PLSR-predicted total trans FAME values for data acquired with the two FTIR spectrometers and the reference GC method was achieved as the coefficients of determination (R²) was close to one (Fig. 5a and 5b) and the slopes and intercepts were not significantly different from 1 and zero, respectively (p > 0.05). The relative differences, which were defined as: (PLSR predicted value–GC determined value)/GC determined value, were non-normally distributed for both the portable device and benchtop spectrometer. Therefore, the Wilcoxon signed-rank test was applied to test the null hypothesis. The mean relative differences between the reference GC determinations and the FTIR/PLSR-predicted total trans FAME concentrations by both the portable device and the benchtop spectrometer were not significant, and the expected value of 0 was observed (p > 0.05).
As previously determined in our laboratory\textsuperscript{14}, the same transmission-mode (30 μm) portable FTIR device in conjunction with the univariate negative second derivative (NSD) data analysis method was used to determine the concentration of total \textit{trans} FAME for only 19 of the same set of 24 fast food lipid extracts used in the present study\textsuperscript{14}. The total \textit{trans} FAME concentration for five (FF1, FF2, FF3, AP1, and CT1), out of the 24 test samples, were not determined by the NSD method\textsuperscript{14} due to its lack of sensitivity particularly at concentrations below 1% of total FAME. As demonstrated in Fig. 6, for all the remaining 19 test samples, the quantitative determination by the univariate NSD method yielded inaccurate and unreliable results\textsuperscript{14}, by comparison to the reference GC determinations. By contrast, the quantitative prediction of total \textit{trans} FAME concentrations for all 24 fast food items, down to approximately 0.5% of total FAME (Table 2), were accurately predicted by the multivariate PLSR data analysis in the present study (Fig. 6). This may be attributed to the fact that, unlike the univariate NSD method, the multivariate PLSR approach allowed for the quantification of total \textit{trans} FAME in a complex lipid matrix while implicitly modeling other sources of variances.

## 4 Conclusions

The performance of a portable, transmission-mode FTIR device was evaluated and compared to that of a benchtop ATR-FTIR spectrometer for the determination of low (approximately 0.5%) total \textit{trans} FAME concentrations in fast food lipid extracts. To overcome the complexity of food matrices and the presence of interfering soluble and/or insoluble components in the lipid extracts and to yield accurate prediction of total \textit{trans} fat content, extracts were analysed by FTIR as FAME which permitted direct comparison of PLSR predictions to the reference GC determinations. Of the various FTIR measurement modes investigated, the FTIR portable device equipped with a factory-calibrated transmission-mode optical pathlength of 30 μm, yielded the highest accuracy for predicting total \textit{trans} FAME concentrations relative to those determined by the reference GC method. High accuracy and linear correlation with reference GC data were evidenced by the low error in cross-validation (RMSECV) and a high R\textsuperscript{2} value close to 1. Regarding FTIR data analysis, multivariate PLSR analysis was found to yield superior accuracy and sensitivity when compared to the univariate NSD method. The portable FTIR device combined with PLSR was successfully used to predict total \textit{trans} FAME concentrations as low as 0.42% of total FAME (as determined by GC) for a representative set of 24 fast food lipid extracts. An advantage offered by FTIR spectroscopy has been the ability to simultaneously measure the total content of all the \textit{trans} mono-, di-, and tri-unsaturated fatty acid positional isomers. As the contribution from the \textit{trans} 18:1 fatty acid isomers will be minimized in fast foods and processed foods, as a result of re-formulation without the use of partially hydrogenated oils, quantification of the total \textit{trans} 18:2 and 18:3 fatty acid isomers will become increasingly important, and could be
accurately achieved in a single spectroscopic measurement by FTIR. The application of PLSR is convenient for routine data analysis of total trans fat content.

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Compliance with Ethical Standards:

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