J Am Oil Chem Soc (2011) 88:1477–1483
DOI 10.1007/s11746-011-1814-z

ORIGINAL PAPER

Application of a Portable Handheld Infrared Spectrometer for Quantitation of *trans* Fat in Edible Oils

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Received: 14 July 2010 / Revised: 7 March 2011 / Accepted: 17 March 2011 / Published online: 3 April 2011

Abstract *Trans* fat poses serious health risks to consumers. In order to meet the FDA labeling requirements for *trans* fatty acids, development of fast, accurate, easy-to-use analytical methods for oils, fats and related products is desirable. Fourier transform infrared spectroscopy (FTIR) is a well-established analytical technique for quantifying *trans* fats, and the development of handheld FTIR units over the past decade presents new application opportunities. Our objective was to evaluate the performance of a handheld FTIR sensor for measuring *trans* fat content between 0.1 and 20% *trans* (w/w) in edible saturated and unsaturated oils. Calibration models were built by measuring height of the band at 966 cm^{-1} and by partial least squares regression (PLSR) using benchtop FTIR as a reference method. Predictive accuracy of the models was validated with an independent test set of commercial edible oils. Calibration models developed using PLSR and linear regression of band heights gave correlation coefficients $R^2 > 0.98$. Multivariate analysis for the handheld unit gave standard error of prediction (SEP) of approximately 1%, comparable to values obtained with benchtop systems. This study demonstrates that handheld FTIR spectroscopy coupled with chemometrics is a suitable method for quantitation of *trans* fat content.

Keywords *Trans* fat · FTIR-ATR · Handheld FTIR · Chemometrics

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Introduction

Dietary *trans* fat has been shown to have adverse effects on blood lipoprotein profiles and coronary heart disease risk impacting individuals and populations. It is recommended by the World Health Organization (WHO) that mean intake of *trans* fat should be <1% of daily energy intake and worldwide efforts from consumers and regulators are in effect to reduce TFA intake [1]. Since 2006 the FDA has required *trans* content >0.5 g/serving to be included on nutrition labeling [2].

Gas chromatographic and infrared spectroscopic methods for determining *trans* fat levels to meet FDA regulatory standards were recently reviewed by Mossoba et al. [3]. Established IR methods such as AOCS Cd 14d-99 and AOAC 2000.10 use band area at 966 cm^{-1} to measure *trans* fatty acid content, although these methods have limits of quantitation around 5% *trans*. This limitation has been overcome by a new methodology which measures the height of the negative second derivative of the *trans* absorbance band [4] which is sensitive below 2% *trans*.

Another current approach to quantitation of components in complex food matrices is chemometrics. Chemometrics offers a more robust method of spectral analysis which takes into account multiple spectral bands. FTIR-attenuated total reflectance (ATR) combined with multivariate analysis has been successfully implemented as a reliable, quick, and simple technique for analyzing food products, edible oils and lipids [5, 6]. Chemometric analysis methods, including partial least squares regression (PLSR) are well-suited to use with FTIR because they can accurately analyze data that is strongly collinear, noisy, and has numerous *x*-variables [7]. PLSR has been used in combination with FTIR-ATR in one study to quantify *trans* fat and conjugated linoleic acid in fats and oils [8].

Development of handheld portable infrared units has allowed for high quality in-field and on-site analysis of quality parameters of plums [9]. Handheld FTIR-ATR technology has also been applied to threat screening, anti-counterfeiting and toxin identification, and application in the food industry is under current investigation [10]. The compact nature of this technology could enable in-process or on-site measurements, allowing food producers to obtain real-time information, rather than performing post-production quality control tests.

Our objective was to evaluate the performance of a handheld FTIR-ATR sensor coupled with second derivative linear regression and chemometric analysis in measuring *trans* fat content of edible fats and oils.

Materials and Methods

Sample Preparation

Pure trielaidin was weighed gravimetrically into tripalmitin (TP) and triolein (TO) (>99%, Nu-Chek Prep, Elysian, MN, USA) to create calibration standards of 0.1–20% *trans* (w/w). Validation samples were prepared in the same manner by weighing trielaidin into edible oils (peanut, safflower, corn, or coconut) obtained from local grocery stores.

Infrared Spectroscopy

Samples were heated in an oven to 65 °C prior to measurement. Spectra of all standards and test samples were measured in duplicate with the TruDefender^{TM} FT infrared handheld spectrometer (Ahura Scientific, Wilmington, MA, USA) equipped with a diamond ATR crystal. The crystal was heated by heat gun to 65 ± 5 °C to prevent sample solidification during measurement. Spectra were collected by co-adding four scans at a resolution of 4 cm^{-1}. Resolution was a fixed parameter on this instrument. Reference spectra were also collected using an FTS 3500GX Fourier-transform infrared spectrometer (Varian, Palo Alto, CA, USA) in combination with a KBr beamsplitter and deuterated triglycine sulfate (DTGS) detector. Samples were pipetted directly onto a Pike MIRacle^{TM} triple-bounce ZnSe ATR crystal (Pike Technologies, Madison, WI, USA) that had been heated with a heat gun to 65 ± 5 °C to prevent fat solidification and a FatIR^{TM} temperature-controlled single bounce ZnSe crystal (Harrick Scientific, Pleasantville, NY, USA) set to 65 °C. Spectra were collected over a range of 4,000–700 cm^{-1} at 4 cm^{-1} resolution, and an interferogram of 64 scans was co-added.

Data Analysis

Second Derivative Linear Regression

Spectra were analyzed with Resolutions Pro software (Varian Inc., Palo Alto, CA, USA). Regression models were constructed using trielaidin in tripalmitin standards due to a higher purity than triolein [3]. The second derivative of the spectrum was taken and height of the band at 966 cm^{-1}, associated with the *trans* CH out-of-plane deformation vibration, was measured [11]. Taking the second derivative of the spectrum allows for resolution of overlapping bands and extraction of useful spectral information [12, 13]. A standard curve relating band height to percentage *trans* content was constructed. Partial Least Squares Regression (PLSR): spectra were imported into Pirouette software (Infometrix, Bothell, WA, USA). The region of 930–1,400 cm^{-1} was analyzed by PLSR by mean-centering, normalizing, and taking the second derivative of each spectrum. Models were constructed using both tripalmitin and triolein standards in order to account for spectral differences based on matrix. Models were cross-validated using a leave-one-out approach. Key bands were identified and standard error of cross validation (SECV) was calculated in order to evaluate model fitness.

The predictive accuracy of models was validated with an independent test set of oils spiked with known amounts of trielaidin. Predictive ability of second derivative and PLSR models was evaluated by calculating mean and standard deviation for the predictions of the test sets. Standard error of prediction (SEP) was also calculated for the PLSR models.

Results and Discussion

Determination of *trans* fat level in oils, fats, and food products by infrared spectroscopy has been extensively investigated [3, 11]. In this study, we evaluated the feasibility of using a portable handheld FTIR-ATR unit to quantify *trans* levels with no sample preparation except heating to 65 °C. The handheld spectrometer collected a spectrum that was highly similar to benchtop instruments, although it showed higher noise levels (Fig. 1). Noise was due to co-adding of fewer scans per sample due to the need to maintain a constant sample temperature throughout measurement and to keep a uniform effective path length [14].

The spectral profiles collected (Fig. 1) show characteristic bands of lipids. The region from 900 to 1,200 cm^{-1} is considered to be the "fingerprint region" and is rich with spectral information. It contains the band at 966 cm^{-1} that is associated with C=H out-of-plane deformation

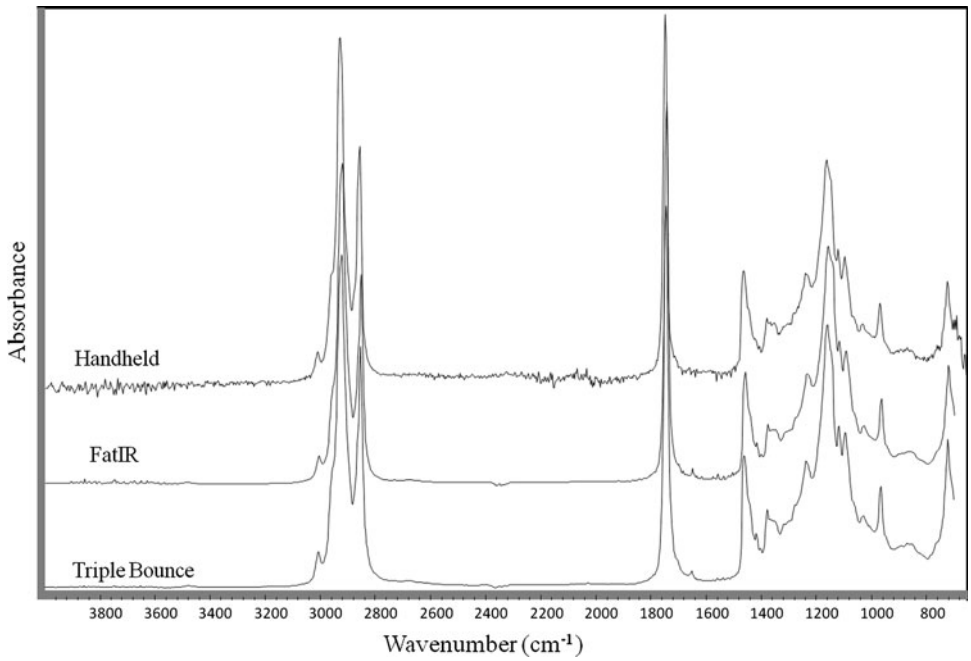


Fig. 1 Comparison of spectral quality and shape of handheld and benchtop spectrometers

vibration of *trans* double bonds [15] and the region 1,050–1,200 cm^{-1}, associated with several C–O stretching vibrations [16].

Overlaying sample spectra at varying *trans* levels from the calibration set (Fig. 2) shows that the intensity of the band at 966 cm^{-1} in the second derivative spectra varies with *trans* content. A comparison of all three figures reveals that the triple bounce and temperature-controlled accessories show equally good separation of bands for different *trans* levels. All three systems have bands which are indistinguishable between 0 and 0.5%. Figure 2 begins to suggest that the limit of quantification is around 1%, since separation of bands cannot be easily distinguished for levels below 1% *trans*. This is most likely due to interferences from surrounding bands such as saturated fat [3], which are most evident at lowest *trans* concentrations.

Models

Second derivative linear regression models showed excellent linear correlation between 1 and 20% *trans* content in tripalmitin standards and band height at 966 cm^{-1} (Fig. 3). Infrared calibration models developed using linear regression of band height gave $R^2 > 0.995$ for benchtop triple bounce and temperature-controlled systems. The triple bounce accessory regressions had much higher slope due to additional internal reflections in the crystal, which yields a stronger signal. Signal is improved by an increased the effective path length, which is caused by increasing the number of reflections within the ATR crystal [17]. Handheld correlation coefficient was $R^2 = 0.992$, indicating a strong linear relationship between band height and *trans* fat content.

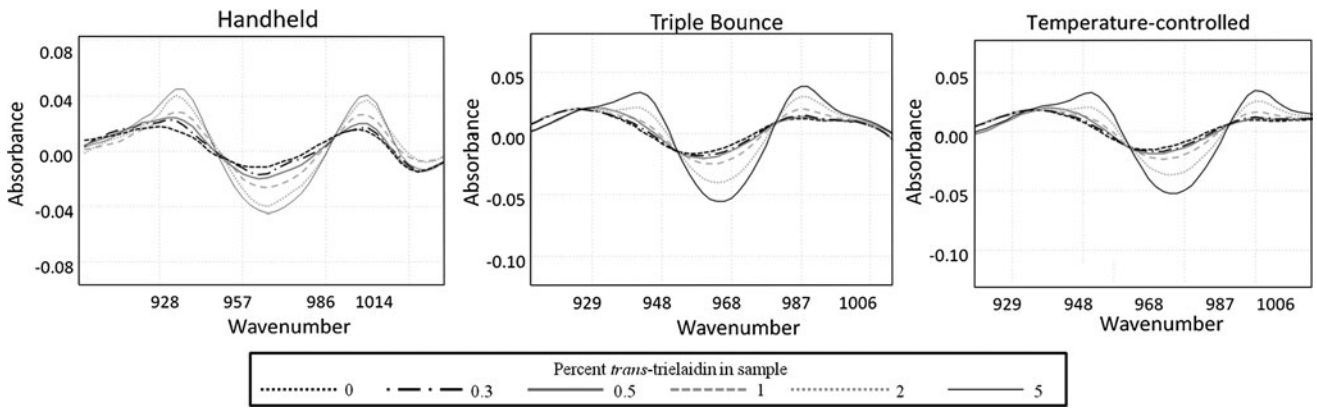
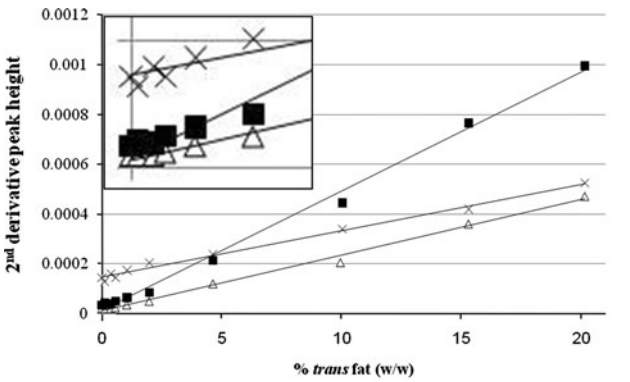


Fig. 2 Overlaid spectra after pre-processing shows the change in absorbance at 966 cm^{-1} with change in *trans* fat level



Instrument	Regression Equation	Correlation Coefficient
X Handheld	y=0.000023x+0.000143	R^2 = 0.992
■ Triple Bounce	y=0.000038x+0.000029	R^2 = 0.995
△ Temperature-controlled	y=0.000022x+0.000014	R^2 = 0.995

Fig. 3 Comparison of linear regression calibration model for tripalmitin spiked with trielaidin

PLSR calibration models are shown in Fig. 4. Models were constructed using only standards between 0 and 5%, in order to focus on model sensitivity at lower *trans* levels. It was necessary to build PLSR models with both tripalmitin and triolein calibration standards in order to obtain accurate predictions. A PLSR model was originally constructed using only tripalmitin standards due to concerns over the purity of triolein [11]. While the model showed good linearity (SECV ~0.5) and could accurately predict saturated validation samples (SEP ~1%), error was unacceptably high for the unsaturated validation set, SEP ~5%. Spectral differences between saturated and unsaturated fat needed to be built into the models in order to accurately predict both types of samples. Models were then created using both tripalmitin and triolein standards. Although this introduces some error based on the inherent *trans* fat content on triolein, it was necessary for the construction of models robust enough to predict the validation oils. Model improvements are reflected in the lower SEP values presented here.

It can be clearly seen that the benchtop systems yielded the best calibration and that the handheld spectrometer showed a lower degree of linearity. PLSR multivariate analysis gave standard error of cross-validation (SECV) of ≤1% for the handheld and benchtop systems (Table 1). *R* of validation (R_{val}) was >0.96 for all models, indicating good model linearity. Loadings (Fig. 5) indicated which spectral bands were important in model construction and prediction. The *trans* marker band at 966 cm^{-1} explained ~80% of the variance in the models, although other spectral factors were used to increase model robustness and performance. These additional bands were distinct for saturated and unsaturated fat. Bands at 1,084, 1,144 and 1,182 cm^{-1}, related to C–O stretching were the most important loadings [15].

Predictions

Predictions were made using the second derivative and PLSR methods on an independent test set of unsaturated and saturated oils spiked with trielaidin measured with all three FTIR-ATR systems. Although calibration models were made from spiked triglycerides, models were validated using commercial oils spiked with TE. Since infrared spectra are highly matrix-dependent, it is expected that predictions will lose some accuracy due to spectral differences caused by switching matrices for validation [8]. Thus, models were validated on varying matrices (peanut, safflower, corn, or coconut oils) in order to account for the matrices' effects on model performance. Validation samples were constrained to *trans* content <5% in order focus on model sensitivity at lower levels.

It has been shown in the literature that non-hydrogenated commercial oils can contain very low levels of *trans* fat [3], mainly the mono-*trans*-dienes. The *trans* content of oils used in validation samples was evaluated using the

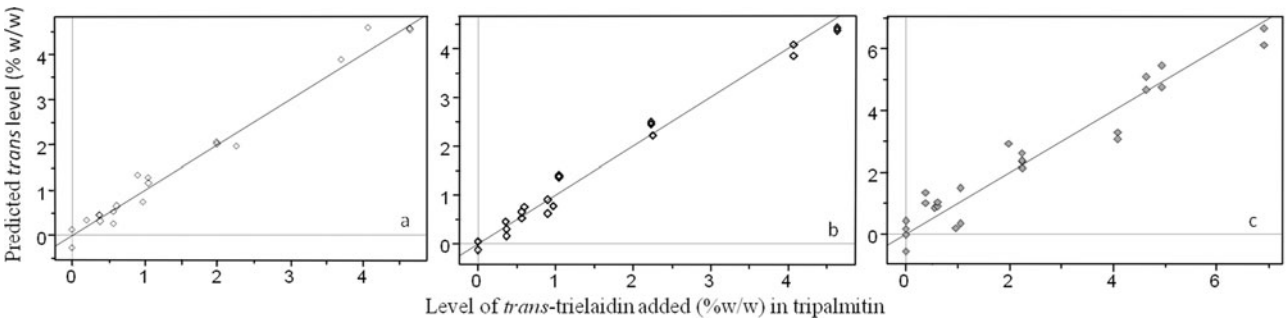


Fig. 4 PLSR calibration models for trielaidin in saturated tripalmitin using **a** triple bounce ATR, **b** temperature controlled ATR accessory and **c** handheld spectrometer

Table 1 Comparison of PLSR model performance evaluation statistics for calibration curves

	Temperature-controlled	Triple bounce	Handheld
Factors	3	4	4
SECV	0.19	0.22	0.54
R_{val}	0.992	0.991	0.968
SEP	0.56	0.28	0.62

SECV standard error of cross-validation, R_{val} *R* of validation, *SEP* standard error of prediction calculated from predictions with an independent test set

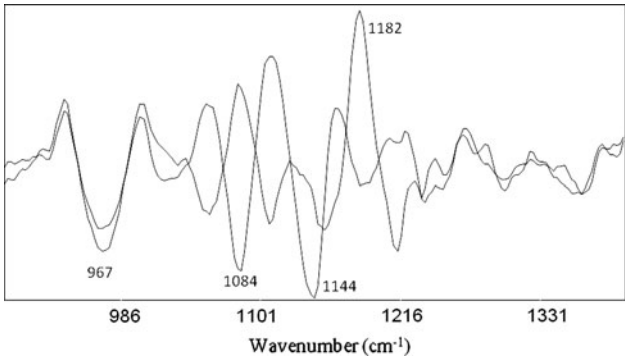


Fig. 5 Loadings of the first two factors for PLSR calibration model using the handheld spectrometer

temperature-controlled system and the second derivative procedure prior to spiking with trielaidin. Very low levels were found, <0.3% in unsaturated oils and <0.5% in saturated oil. Therefore, the values given for the validation samples are the sum of *trans* fat values from both the oil, determined using FTIR, and the trielaidin, determined gravimetrically.

Standard error of prediction (SEP) is an estimate of the predictive ability of a chemometric model. SEP was calculated for each prediction set using PLSR prediction analysis (Table 1). The benchtop systems showed lower SEP than the handheld system, which is expected due to differences in system components and performance. SEP was ~0.6% for the handheld spectrometer, therefore the limit of this technology for quantification was estimated at 1% *trans* by weight of fat. In a high fat product, 1% *trans* (w/w) is equal to 0.25 g per serving [3]. It is hypothesized that this limit is due to interference from saturated fat, which absorbs in a similar region as *trans* fat [11].

Using band height measurement, the handheld unit showed poor predictive ability (Table 2). Prediction were inaccurate by as much as 6% (w/w), indicating spectral differences between triglyceride calibration standards and commercial oil validation samples were too great and spectra were too noisy. However, these results confirmed what as has been shown in numerous studies, that benchtop systems were able to accurately predict validation samples [3, 11].

Using PLSR analysis, the handheld performed on par with the benchtop systems for oil predictions (Table 2). Samples with *trans* content >1% were accurately predicted by the handheld system to within 0.3% of gravimetrically determined values, although lower level sample predictions were less accurate. Samples <1% *trans* (0.62, 0.68, 0.52 and 0.75%) were not accurately predicted, samples above 1% could be accurately predicted by the handheld spectrometer combined with PLSR analysis. It should be noted that predictions made using both benchtop systems had a lower standard deviation than the handheld spectrometer. This indicates that while the handheld was able to predict *trans* levels to a suitable level, it lacks some of the precision and consistency of the benchtop systems.

Overall, the handheld spectrometer coupled with the second derivative band height measurement method greatly over-predicted the level of *trans* fat in this study. PLSR analysis, however, generally gave *trans* values closer to the actual amount present in the sample. Handheld ATR has been demonstrated in this study to be useful for the quantitation of *trans* fat at levels >1% with an SEP of 0.6%.

A variety of spectroscopic methods have been used in combination with chemometric analysis for determining *trans* fat with varying levels of accuracy (Table 2). FTIR has been extensively studied, and a limit of quantitation for the band height measurement technique has been estimated at ~1% [3]. FTIR coupled with PLS analysis gave an SEP of 0.9% [8]. Other forms of spectrometry have also been investigated for application to *trans* quantitation. A study using Raman spectroscopy coupled with PCA [18] to investigate partially hydrogenated oils could predict levels with an SEP ~2%, while SEP ~0.2% was found using NIR coupled with PLS [19] on fats and oils (Table 2). Each method provides unique information and detection method selected depends on available resources and analytical needs. Raman, NIR and handheld spectrometry need to be validated via collaborative study to assess repeatability, reproducibility and established limits of quantitation (Table 3).

Combining the portable handheld FTIR spectrometer with PLSR analysis offers three distinct benefits for *trans* fat analysis. First, a handheld unit is portable allowing for flexible sampling; spectra can be collected when samples are available and analyzed at a later time. Second, PLSR analysis is advantageous because it can simultaneously use several spectral bands to quantify the component (Fig. 5). Multiple loadings are useful in creating robust models that give reproducible results. While increasing the number of loadings offers greater model calibration, it is important to evaluate the loadings so as not to include noise in

Table 2 Validation set sample predictions using peak height and linear models

	PLSR predicted values				Second derivative predicted values			
	Actual	Handheld (SD)	Temperature-controlled (SD)	Triple bounce (SD)	Actual	Handheld (SD)	Temperature-controlled (SD)	Triple bounce (SD)
Unsaturated								
1	0.82	0.25 (0.39)	0.86 (0.00)	0.65 (0.01)	0.82	3.13 (0.31)	0.73 (0.00)	0.82 (0.00)
2	0.88	0.04 (0.02)	0.94 (0.03)	0.78 (0.08)	0.88	2.48 (0.00)	0.73 (0.00)	0.95 (0.19)
3	1.30	0.73 (0.74)	1.70 (0.00)	0.90 (0.05)	1.30	4.87 (1.54)	0.95 (0.32)	1.21 (0.19)
4	1.59	1.00 (0.31)	1.89 (0.03)	1.57 (0.17)	1.59	6.39 (1.84)	1.18 (0.00)	1.61 (0.00)
5	2.05	1.74 (0.37)	2.12 (0.06)	1.37 (0.01)	2.05	5.96 (0.61)	1.64 (0.00)	1.87 (0.00)
6	2.12	1.65 (0.03)	1.58 (0.08)	2.31 (0.06)	2.12	6.17 (2.15)	1.18 (0.00)	2.00 (0.19)
7	4.38	3.81 (0.28)	4.01 (0.00)	4.16 (0.03)	4.38	9.87 (2.46)	3.00 (0.00)	4.11 (0.19)
Saturated								
8	1.02	1.27 (0.44)	1.33 (0.01)	1.03 (0.04)	1.02	1.83 (0.31)	0.73 (0.00)	0.68 (0.19)
9	1.25	1.38 (0.02)	1.17 (0.02)	1.03 (0.08)	1.25	2.70 (0.92)	0.95 (0.32)	0.68 (0.19)
10	1.48	0.94 (0.01)	1.45 (0.03)	1.38 (0.06)	1.48	2.70 (0.31)	0.73 (0.00)	1.08 (0.00)
11	2.18	1.58 (0.06)	2.27 (0.05)	1.88 (0.01)	2.18	2.48 (1.84)	1.41 (0.32)	1.61 (0.00)
12	2.79	2.35 (0.45)	2.82 (0.00)	2.58 (0.01)	2.79	5.52 (0.61)	1.64 (0.00)	2.26 (0.19)
13	4.20	2.68 (0.28)	3.68 (0.00)	3.62 (0.01)	4.20	5.09 (0.61)	2.55 (0.00)	3.32 (0.19)

SD standard deviation

Table 3 Error estimation of spectroscopic studies on *trans* fat determination

Instrument	Limit of quantitation or SEP (%)
FTIR-ATR [3, 8]	1[a], 0.9[b]
NIR [19]	0.2[b]
Raman [18]	2.1[b]
Handheld FTIR-ATR	0.6[b]

[a] Represents value of limit of quantitation

[b] Represents value of SEP

calibration models [20]. PLSR also allows for quantitation of components in a more complex matrix and can be useful when a component is not correlated with a specific wavenumber. Third, as this study has demonstrated, analysis can be semi matrix-independent. This aids in ease of analysis, as a new calibration model would not necessarily need to be created for each new oil matrix. Overall, the combination of portable FTIR and PLSR offers greater flexibility and ease of analysis in measuring *trans* fat.

The implementation of handheld FTIR technology has both benefits and tradeoffs. ATR technology allows for minimal sample preparation, measurements are very quick, and the portable nature of the device would allow it to be used in situ in receiving and processing of edible oils. However, the compact and portable nature of the handheld spectrometer necessitates some compromises and limitations in signal strength, spectral quality, and interface features. Modifications such as the addition of heat control and a triple bounce crystal could improve the performance and applicability of the handheld portable unit. So while the handheld FTIR unit was found suitable for quantifying levels >1% of *trans* fat in edible oils, it may be advantageous to use benchtop FTIR or GC analysis in other situations.

Conclusion

This study found that a portable handheld infrared spectrometer is a suitable method for the detection and quantitation of *trans* fat. Chemometrics was determined as a more accurate and robust technique than measuring height of the *trans* band at 966 cm^{-1}. Using chemometrics, levels >1% *trans* were accurately measured in edible oils with no sample preparation. In summary, FTIR-ATR coupled with PLSR has shown to be a simple, quick and robust technique for *trans* fat quantitation that will give food manufacturers real-time, field-based measurements to assess the nutritional quality of foods.

Acknowledgments The authors wish to acknowledge the Ohio Agricultural Research and Development Center and Ahura Scientific for their financial support of this research.

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