

Correcting for Underlying Absorption Interferences in Fourier Transform Infrared *trans* Analysis of Edible Oils Using Two-Dimensional Correlation Techniques

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Substantive improvement in the sensitivity of the AOAC/AOCS spectral ratioing method for the determination of isolated *trans* isomers in edible oils was recently achieved by the application of a new spectral reconstitution (SR) technique that facilitates the FTIR analysis of edible oils in the transmission mode. However, the general applicability of the spectral ratioing method is still severely limited by the requirement to know the provenance of the oil to be analyzed and have on hand its *trans*-free counterpart so that the underlying triacylglycerol absorptions in the *trans* measurement region (990–945 cm⁻¹), henceforth referred to as UA_t, may be ratioed out. To eliminate the need for a *trans*-free reference oil, we have employed two-dimensional (2D) correlation spectroscopy to search for other spectral features that might correlate with and serve to estimate the UA_t. The three-dimensional contour maps obtained by 2D correlation analysis of the spectra of 10 *trans*-free oils of different oil types, recorded using the SR procedure, revealed such correlations in two spectral regions, 1700–1600 and 4500–4300 cm⁻¹, exhibiting one maximum and two maxima, respectively, with wavenumber coordinates of (968, 4407), (968, 4299), and (968, 1650). The latter two correlations, when optimized, produced excellent linear regression relationships ($r > 0.95$) with the UA_t. The spectra of five sets of trielaidin-spiked oils were corrected for the UA_t using these relationships, and their *trans* contents were predicted from the calibration equation generated for the spectral ratioing procedure. Linear regression of predicted versus added *trans* over the range of 0–1.6% *trans*, which is below the limit of quantitation of the AOAC/AOCS spectral ratioing method, yielded $r = 0.88$ – 0.90 with an SD of ~0.2% *trans*. These results indicate that the combination of the SR technique with the UA_t correction approach may provide a simple and accurate FTIR method for the analysis of the *trans* content of fats and oils that would be competitive with GC.

KEYWORDS: Transmission spectroscopy; SB-ATR; oil dilution; FTIR automation; AOCS *trans*; AOAC *trans*

INTRODUCTION

The need for a rapid, simple, sensitive, and accurate means of determining the *trans* content of edible oils and lipids extracted from foods has become increasingly important as a result of recent *trans* labeling legislation in North America, Europe, and elsewhere. A number of methods for *trans* analysis, based on either gas chromatography (GC) or infrared (IR) spectroscopy, are officially approved by AOAC International (1) and the American Oil Chemists' Society (AOCS) (2), but none of them meets all of the above criteria. The IR methods

are based simply on measurement of the CH out-of plane deformation absorption of isolated *trans* double bonds, which produces a distinctive strong band at 966 cm⁻¹. This simplicity is, however, confounded by the fact that the triacylglycerols making up food lipids exhibit weak absorptions in the 990–945 cm⁻¹ region that interfere with *trans* measurements. These underlying absorptions in the *trans* region, which will subsequently be referred to as UA_t, are dependent on the fatty acid composition of the oil and, if not accounted for, produce a bias in *trans* predictions of up to 3 percentage points (3). Over the past few decades, researchers have attempted to eliminate this source of error by various means (4). An approach developed in our laboratory involved the use of a partial least-squares (PLS) calibration to model the UA_t (5). A "universal" calibration was developed by using pure triacylglycerols as standards and was

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validated against *trans* analytical data obtained by GC. However, as the method relies on advanced chemometrics with which many analysts are not adequately familiar, it has largely been considered impractical for routine implementation. The two approaches that have been incorporated in official methods adopted by AOAC International and the AOCS are (a) conversion of triacylglycerols to their respective fatty acid methyl esters (FAME) and (b) spectral ratioing against a *trans*-free reference oil. The first of these is neither simple nor highly accurate, as it requires substantial sample preparation (i.e., saponification of the oil and methylation as well as quantitative dissolution of the FAME in CS₂ for IR analysis) and has been reported to underestimate *trans* content by 1.5–3 percentage points (3). This approach was originally developed before the advent of FTIR spectrometers, which provided spectral ratioing capabilities as well as the possibility of employing the attenuated total reflectance (ATR) technique to record the spectra of oils in their neat form. In the AOAC/AOCS spectral ratioing procedure for the determination of *trans* content (6, 7), the ATR spectrum of the sample is ratioed against that of a reference (*trans*-free) oil of the same type, thereby eliminating the spectral features common to the sample and the reference (including the UA_{*t*}) such that the resulting differential spectrum contains only the *trans* spectral contributions (8). A weakness of this method, however, is that accurate results can be obtained only if a certified *trans*-free reference oil corresponding to the type of oil being analyzed is available. Should the fatty acid composition of the reference oil not match that of the sample, the UA_{*t*} will not be properly ratioed out, resulting in either a positive or a negative bias (4). Moreover, the sensitivity of the method is restricted by the inherently short effective path length of ATR measurements (~4–40 μm at 966 cm⁻¹, depending on the type of ATR accessory), and lower limits for quantitation of 5% and 2% *trans* have been specified by AOAC International (6) and the AOCS (7), respectively.

The limited sensitivity of this ATR method can be overcome by turning to transmission spectroscopy (9); however, the difficulty of loading viscous edible oils into a transmission cell makes this option problematic. Recently, this sample handling problem has been overcome through the use of a procedure termed spectral reconstitution (SR), which generates an excellent facsimile of a neat oil spectrum from the spectrum of a sample that has been diluted with odorless mineral spirits (OMS) to reduce its viscosity, allowing it to be readily loaded into a 200 μm transmission cell (10). In this procedure, the absorption of a spectral marker (chromium hexacarbonyl) added to the diluent is used to calculate the precise dilution ratio, so that the spectral contributions of the diluent can be mathematically stripped out and the resulting spectrum corrected for dilution. Application of this SR procedure to *trans* analysis by the spectral ratioing method yielded an ~20-fold increase in sensitivity by comparison with the ATR method [utilizing a single-bounce ATR (SB-ATR) accessory], and excellent quantitative accuracy was achieved, the *trans* content of oils being predicted to within ±0.05% *trans* (11).

With the enhanced sensitivity provided by the SR procedure, the only remaining limitation to FTIR *trans* analysis is the requirement for an appropriate *trans*-free reference oil, which is highly problematic in the case of oils of unknown provenance, blends, or lipids extracted from foods. This paper presents an alternative means of compensating for the UA_{*t*} that eliminates this limitation by utilizing absorption bands in the spectrum of the sample itself to estimate the contribution of the UA_{*t*} to the measurement of the *trans* absorption and correct for the bias it



Figure 1. COAT FTIR system designed for the automated analysis of oils and lubricants.

produces. The development of this approach through the application of generalized 2D correlation spectroscopy (12) to locate absorptions in the IR spectra of *trans*-free oils that correlate strongly with the UA_{*t*} is described. The implementation of this approach, in conjunction with the SR procedure, is presented, and the results obtained for the analysis of various types of oils spiked with known amounts of trielaidin are compared with those obtained by the spectral ratioing procedure.

MATERIALS AND METHODS

Reagents and Oils. Trielaidin (>99%) and chromium hexacarbonyl were acquired from Sigma-Aldrich, and odorless mineral oil spirits (OMS) were obtained from Thermal-Lube Inc. (Pointe-Claire, QC, Canada). Chromium hexacarbonyl was dissolved in OMS at a concentration of ~0.03% (w/w) to serve as a spectral marker. This solution was used to dilute samples prior to FTIR analysis. A variety of unhydrogenated edible oils (almond, canola, grapeseed, mustard, palm, poppyseed, safflower, sesame, and two brands of sunflower) were purchased locally and determined to contain <0.5% *trans* by GC analysis, performed in accordance with AOCS Method Ce 1f-96 (1).

Sample Preparation. Samples were prepared for analysis by filling 20 mL plastic autosampler vials approximately one-quarter full of oil and dispensing 10 mL of OMS diluent containing the marker with a repipet so as to attain an oil/diluent ratio of ~1:1.5. The vials containing the diluted samples were placed in autosampler racks, each holding 14 vials. Each rack was covered with a rubberized plate and inverted several times to thoroughly mix the oil and diluent in all vials simultaneously, and the racks were then loaded into a four-rack autosampler tray. The first two vials in the tray were reserved for diluent, the first being used to rinse and condition the cell and the second to collect the spectrum of the diluent required by the SR procedure (see below).

FTIR Spectroscopy. FTIR measurements were carried out on a COAT FTIR system (Thermal-Lube Inc., Pointe-Claire, PQ, Canada), consisting of a Bomem WorkIR spectrometer integrated with a Model 223 Gilson autosampler (Gilson, Inc., Middleton, WI), using a positive displacement micropump to load samples from the autosampler vials into a 200 μm KCl transmission cell (Figure 1). The COAT system

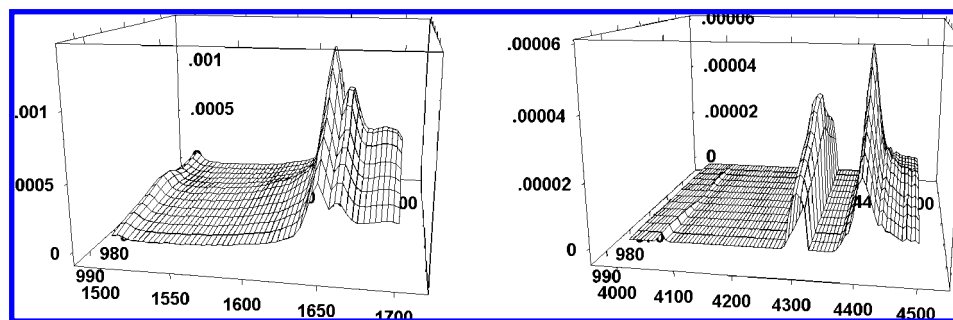


Figure 2. 3D contour maps for the two spectral regions showing correlations with the 990–945 cm^{-1} UA_t region: (a, left) 1700–1600 cm^{-1} ; (b, right) 4500–4300 cm^{-1} .

was operated and controlled using UMPIRE Pro (Universal Method Platform for Infrared Evaluation), a proprietary Windows-based software package developed by Thermal-Lube. Samples were pumped into the IR cell from vials placed in the autosampler tray under software control. All spectra were collected by coaddition of 16 scans at a resolution of 8 cm^{-1} (acquisition time of ~ 30 s). The COAT system was programmed to carry out the mathematical procedures associated with the SR procedure and predict *trans* content from the calibration equation reported previously (11).

SR Procedure. Spectral reconstitution involved measuring the amount of diluent present in each sample using the ratio (r) of the height of the marker band in the sample spectrum relative to that in the diluent spectrum obtained from the second vial in the autosampler tray. The spectrum of the neat oil was reconstituted in two steps. First, the spectrum of the diluent scaled by r was subtracted from the spectrum of the diluted oil to remove the OMS spectral contributions. The resulting spectrum was then multiplied by $(1 - r)^{-1}$ to correct for dilution, so as to reconstitute the neat oil spectrum, and normalized to a path length of 100 μm , the multiplication factor for normalization being determined using the path length determined from the spectrum of the diluent as described previously (13).

2D Correlation Analysis. 2D correlation analysis was performed using the KG2D program [written by Dr. Y. Wang and described elsewhere (14)], which was implemented within GRAMS/386 (Galactic Industries Co., Salem, NH). The spectra of 10 *trans*-free oils (listed above in Reagents and Oils), obtained by the SR procedure, were employed for the 2D correlation analysis. Synchronous 2D correlation plots were generated with the UA_t region (990–945 cm^{-1}) on one axis and the balance of the on-scale portions of the spectra, including the near-IR (NIR) portion commonly accessible with mid-IR spectrometers (6000–4000 cm^{-1}), on the other. To facilitate visual interpretation of the 2D correlation plots, they were displayed as 3D contour maps, which could be rotated in three-dimensional space, with the coordinates associated with any point being obtained using the cursor.

Estimation of UA_t . The y -coordinates of the maxima on the 3D contour maps were selected as starting points in the iterative process of developing equations to estimate the UA_t , which was carried out using TQ Analyst (Version 7.2; Thermo Electron Corp., Madison, WI). A linear relationship between absorbance at each of the selected wavenumbers (referenced to an appropriate baseline) and the area of the UA_t , obtained by integrating between 990 and 945 cm^{-1} , was assumed. Each of the resulting equations was optimized by identifying the measurement area/baseline combination that provided the best fit, as assessed by simple linear regression of the predicted versus the actual UA_t . Only the equations that yielded $r > 0.95$ were retained.

Testing and Validation. Samples for testing and validation of the UA_t equations were prepared by spiking the 10 *trans*-free oils employed in the 2D correlation analysis with various amounts of trielaidin within the range of 0.07–1.6 g/100 g of oil. To test for invariance of the predicted UA_t in the presence of *trans* isomers, the predicted UA_t of four spiked samples of each oil were compared with that of the unspiked oil. For validation, the unspiked and spiked samples of five of the oils (namely, almond, grapeseed, safflower, sesame, and sunflower) were employed, as these samples were concurrently being used in an evaluation of the spectral reconstitution technique as applied to *trans* analysis by the spectral ratioing method, the results of which have been

Table 1. Optimized Calibration Equations for the Prediction of the UA_t Area (990–945 cm^{-1}) Based on Peak Area Measurements in the Vicinity of 2D Correlation Peaks with Their Associated Linear Regression Parameters and Statistics

eq	peak area	baseline	intercept	slope	SD	R
1	1654–1596	1666–1635	−4.104	3.451	0.173	0.961
2	4335–4289	4366–4289	11.413	−6.503	0.151	−0.970
3	4408–4400	4435–4365	−1.762	50.083	0.290	0.886

reported in a previous publication (11). For each of the validation samples, the predicted UA_t was subtracted from the measured area of the *trans* band (990–945 cm^{-1}) prior to determination of the *trans* content from the calibration equation developed for the spectral ratioing method (11). The corrected *trans* values were then compared to the values obtained by the ratioing method with the use of the appropriate reference oil as well as those obtained by using a single reference oil (11).

RESULTS AND DISCUSSION

The concept being evaluated is that 2D correlation spectroscopy might reveal triacylglycerol absorptions that are linearly related to the UA_t and that measurement of these absorptions would provide a good estimate of the UA_t , thus allowing their contribution to the area of the *trans* band to be eliminated. This approach would make accurate FTIR *trans* analysis possible without the requirement of having a *trans*-free oil available for spectral ratioing. To investigate this possibility, the transmission spectra of 10 *trans*-free samples of nine diverse oil types were acquired by the SR procedure and examined by 2D correlation analysis. Correlations with the 990–945 cm^{-1} UA_t region were observed in two spectral regions, 1700–1600 and 4500–4300 cm^{-1} , the latter falling within the combination band region of the NIR spectrum. **Figure 2** presents the corresponding 3D contour maps, which exhibit one maximum and two maxima, respectively, with wavenumber coordinates of (968, 1650), (968, 4299), and (968, 4407). These results show that there are correlations between the UA_t and bands in other regions of the spectrum. To investigate whether these correlations are sufficient to allow for the accurate prediction of the UA_t , a systematic examination of various measurement area/baseline combinations in the vicinity of 1650, 4299, and 4407 cm^{-1} , corresponding to the maxima observed on the 3D contour maps, was carried out using the TQ Analyst software package. In effect, this process involved the iterative optimization of simple linear calibration equations for the prediction of the UA_t area (990–945 cm^{-1}). **Table 1** presents a summary of the optimized peak area measurements and their respective linear regression equation parameters and statistics. Good linear relationships ($r > 0.95$) between the UA_t area and the optimized measures associated with the 3D contour map maxima of 1650 and 4299 cm^{-1} were obtained, whereas measures in the vicinity of the 4407 cm^{-1} maximum could not be optimized to

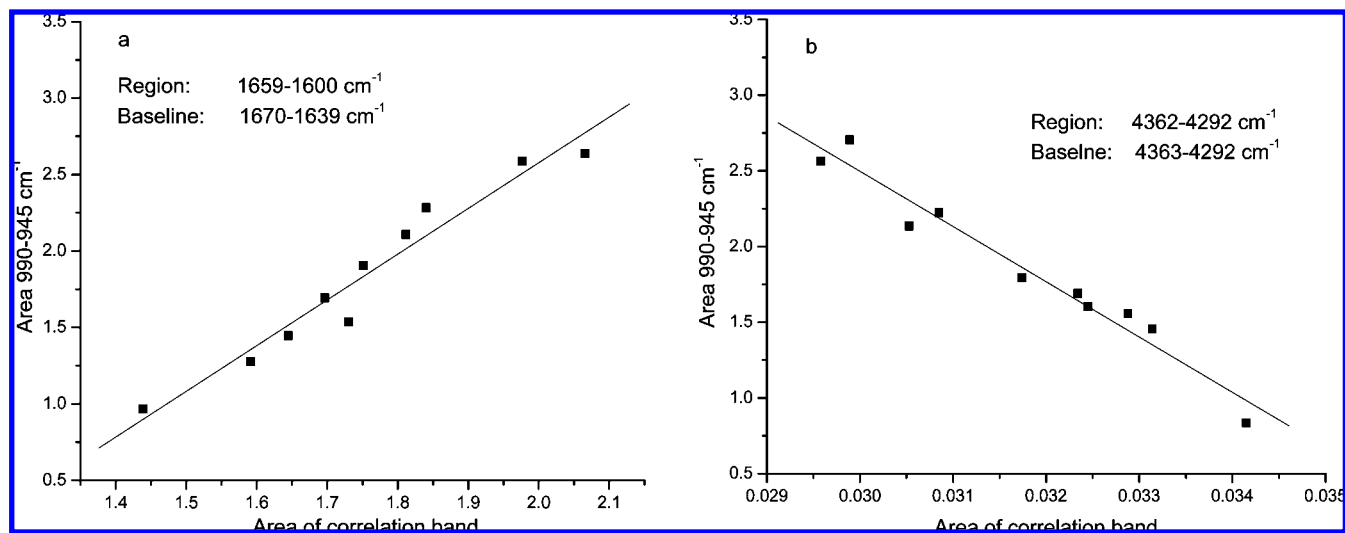


Figure 3. Relationships between the UA_t absorptions (measured as the area between 990 and 945 cm^{-1}) in the spectra of *trans*-free oils and the optimized area measurements of the correlating bands at 1650 cm^{-1} (a) and 4299 cm^{-1} (b) on the 3D contour maps in **Figure 2**.

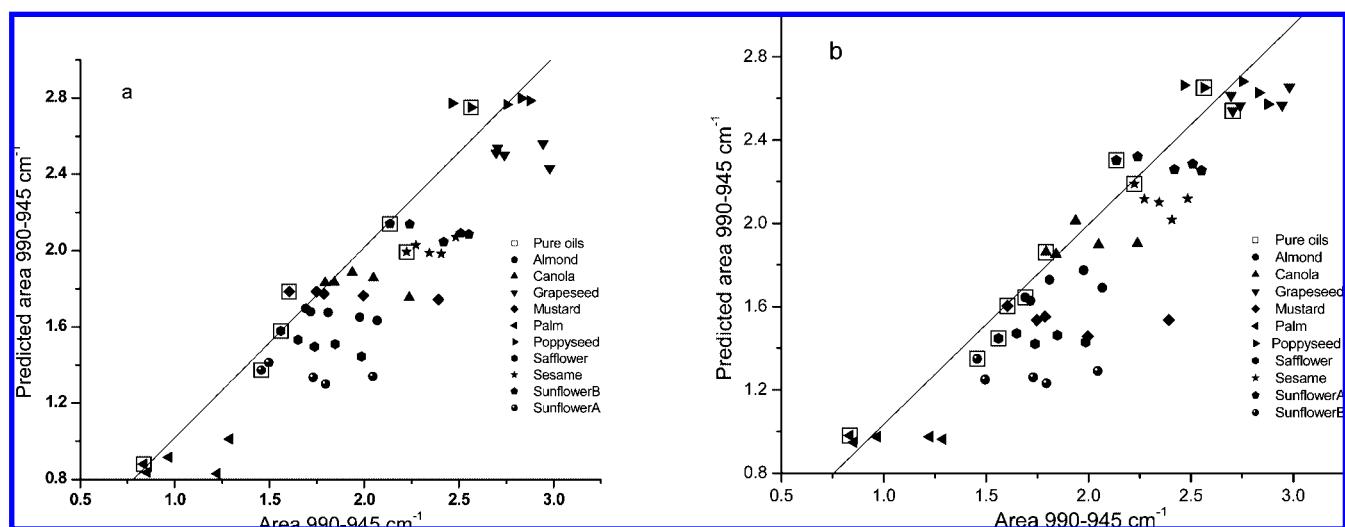


Figure 4. Relationships between the measured area between 990 and 945 cm^{-1} in the spectra of *trans*-free oils (\square) and trielaidin-spiked oils (\bullet) and the corresponding area predicted from the UA_t correction equations: (a) eq 1; (b) eq 2. The solid lines represent the regression lines for the pure oil data.

Table 2. Regression Equations Relating Predicted *trans* to Added *trans*

type of correction	intercept	slope	SD	<i>R</i>
UA_t estimated from eq 1	0.246	1.038	0.23	0.889
UA_t estimated from eq 2	0.214	1.041	0.21	0.909
ratioing against spectrum of matching unspiked oil	-0.034	1.022	0.05	0.994

the same extent. **Figure 3** graphically illustrates the respective “optimal” relationships derived from the search around the 1650 and 4299 cm^{-1} maxima. It may be noted that the 1654–1596 cm^{-1} area is positively correlated with the UA_t , while the 4335–4289 cm^{-1} area is negatively correlated, with a slightly lower SD.

The potential utility of these relationships as a basis for compensating for the contribution of the UA_t in *trans* analysis is clearly dependent on the assumption that *trans* double bonds do not contribute to the peak area measurements listed in **Table 1**. The validity of this assumption was tested by spiking the *trans*-free oils used to derive these relationships with four levels of trielaidin (C18:1*t*) and predicting the contribution of the UA_t to the area of the *trans* band (990–945 cm^{-1}) from eqs 1 and

2 in **Table 1**. The results for each of the *trans*-free oils and the corresponding set of four spiked oils are plotted in **Figure 4** for eqs 1 and 2, respectively. Both plots illustrate that for each oil the predicted UA_t contributions to the 990–945 cm^{-1} area measurements (on the y-axis) are basically invariant as the *trans* content, and hence the actual value of this area (on the x-axis), increases. Accordingly, it may be concluded that the measurements made to estimate the UA_t are largely unaffected by *trans* content and hence may serve to correct for the UA_t in *trans* analysis.

To examine the efficacy of the UA_t correction, the predicted UA_t contributions plotted on the y-axis in **Figure 4** were subtracted from the corresponding 990–945 cm^{-1} area measurements on the x-axis. The residual values of the 990–945 cm^{-1} area, considered to be corrected for the UA_t , were then processed through the *trans* calibration equation previously derived for the SR-based spectral ratioing method (11):

$$\% \text{trans}_{\text{SR-FTIR}} = -0.036 + 2.6416(\text{Abs}_{990-945 \text{ cm}^{-1}}) \quad (4)$$

where $r = 0.9996$ and $\text{SD} = 0.032$. The results were then compared to those reported in our evaluation of the spectral

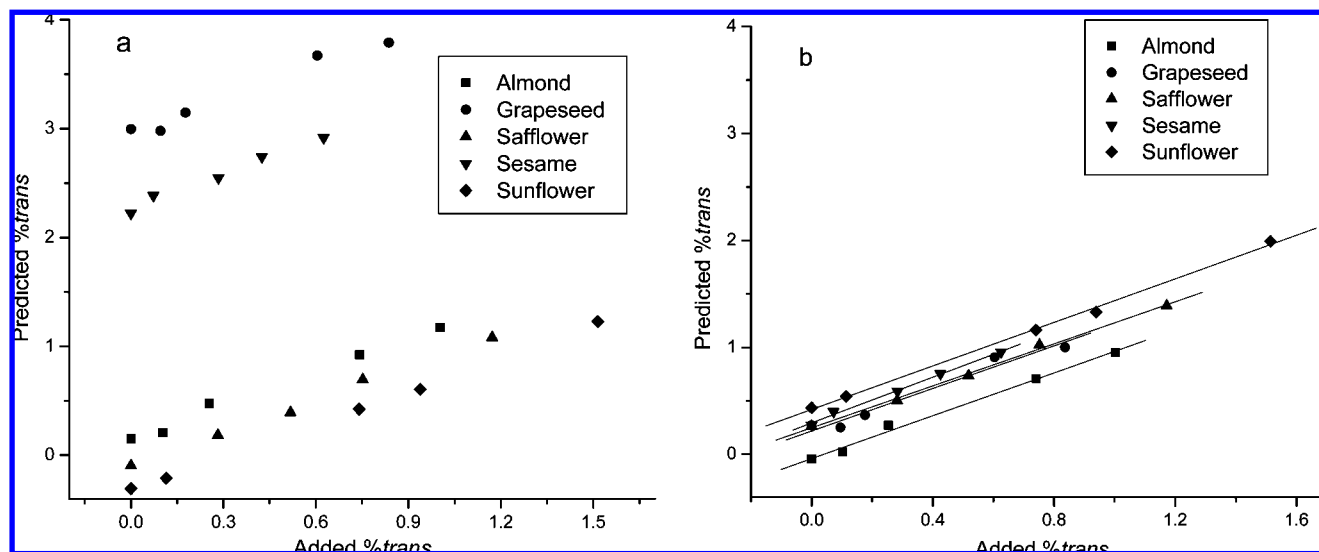


Figure 5. Plots of predicted *trans* from eq 4 vs added *trans* for trielaidin-spiked oils obtained after (a) ratioing the spectrum of each oil against the spectrum of extra virgin olive oil instead of its matching unspiked oil and (b) correcting for the contribution of the UA_{*i*} by using eq 2.

reconstitution technique as applied to *trans* analysis by the spectral ratioing method (11), which was restricted to a subset of the trielaidin-spiked samples employed in the present study (comprising 5 of the 10 oils). The relationships between the predicted *trans* values, obtained after the *trans* peak areas were corrected using eq 1 or eq 2, and added *trans* are presented in **Table 2** for this subset, together with the corresponding relationship for the ideal situation in which the UA_{*i*} in the spectra of the spiked oils were eliminated by ratioing against the spectrum of the corresponding unspiked oil (11). The latter relationship serves as a benchmark, as the accuracy of the *trans* predictions are limited only by instrumental noise and the calibration error (which is minimal; see eq 4). Accordingly, comparison of the data in the first two rows of **Table 2** with those in the bottom row provides a means of evaluating the efficacy of the UA_{*i*} correction approach. Such a comparison reveals 4-fold higher SDs as well as larger deviations of the intercept and slope from the ideal values of 0 and 1, with eq 2 yielding marginally better accuracy than eq 1. It is important to note, however, that the spectral ratioing results in **Table 2** were obtained by using the SR procedure; the official AOAC/AOCS spectral ratioing method, which utilizes ATR-FTIR spectroscopy, yielded a correlation coefficient of <0.5 because the levels of *trans* isomers in the sample set considered here are below the limit of quantitation of the method owing to the limited sensitivity of ATR (11). Moreover, the high level of accuracy of the spectral ratioing method indicated by the data in **Table 2** was attained by ratioing the spectrum of each spiked oil against that of the matching unspiked oil, corresponding to the ideal “reference oil”. In practice, such an ideal situation seldom, if ever, occurs, and in many circumstances the requirement of the spectral ratioing method for a *trans*-free reference oil that matches the triacylglycerol composition of the sample simply cannot be met. As indicated at the outset, the purpose of the UA_{*i*} correction approach is to overcome this key limitation of the spectral ratioing method, the effects of which were illustrated in our previous paper (11) by ratioing the spectra of trielaidin-spiked oils against that of a single *trans*-free olive oil rather than their *trans*-free counterparts. As shown in **Figure 5a**, significant biases relative to the actual *trans* added were observed for some oil types because the UA_{*i*} are not fully

compensated for by the spectral ratioing procedure (11). These biases, ranging from 2.8% to −1% *trans*, encompass more than the full analytical range covered (0–1.6% *trans*). **Figure 5b** presents the corresponding plots obtained when the UA_{*i*} corrections determined from eq 2 were employed as an alternative to spectral ratioing. The plots of predicted *trans* vs added *trans* for the five oils in **Figure 5b** are much closer together than those in **Figure 5a**, demonstrating the efficacy of the corrections in minimizing the biases resulting from the oil-dependent variability of the UA_{*i*}. Furthermore, the plot obtained by pooling the data for the five oil types yields the relationship already presented in **Table 2**, with an overall SD of 0.2% *trans*.

In our previous work (11), automated *trans* analysis at a rate of ~120 samples/h was achieved by implementing the SR procedure on the COAT FTIR system, with its attendant autosampler and software, and it was shown that *trans* levels well below the limit of quantitation of the ATR-based AOAC/AOCS spectral ratioing method (variously stated as 2% or 5% *trans*) could be determined accurately. The main remaining limitation of FTIR *trans* analysis, namely, the need for a *trans*-free reference oil to eliminate the contribution of the UA_{*i*} to absorption in the *trans* measurement region, was addressed in the present study by hypothesizing that the spectrum of the sample itself contains the information required to compensate for the UA_{*i*}. The application of 2D correlation spectroscopy has afforded a means of investigating this possibility, and the results presented above indicate that correction of measured *trans* peak areas for the UA_{*i*} contribution, as estimated from absorptions elsewhere in the spectrum, is a viable alternative to the spectral ratioing procedure. The key benefit of this approach is that it allows a sample to serve effectively as its own reference when an appropriate *trans*-free reference oil is not available, as is frequently the case. Implementation of this methodology does require calibration with a small set of *trans*-free oils, preferably three to five samples of different oil types, to adjust the coefficients of eq 1 or eq 2; in principle, it should not be necessary to repeat the 2D correlation analysis, although further studies are required to verify the general applicability of the UA_{*i*} correction equations presented in this paper. Extensive validation studies to evaluate the accuracy of FTIR *trans* analysis based on the use of these equations are also needed, as our

limited study with trielaidin-spiked oils is only intended to serve as proof of concept.

As a final note, it should be mentioned that the UA_t correction equations devised in this paper cannot be employed in conjunction with ATR spectra owing to the dependence of the effective path length on wavelength, which results in a poor signal-to-noise ratio at 1650 cm^{-1} and precludes measurements at wavelengths in the near-IR region. Application of 2D correlation analysis to ATR spectra might reveal correlations with the UA_t at other spectral locations that could be used to derive appropriate correction equations. However, given that FTIR *trans* analysis in the transmission mode can now be performed conveniently with the use of the SR procedure and with higher sensitivity than in the ATR mode, this possibility did not warrant investigation.

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