Determination of Iodine Value with a Fourier Transform-Near Infrared Based Global Calibration Using Disposable Vials: An International Collaborative Study

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ABSTRACT: A method for the determination of iodine value (IV) by Fourier transform-near infrared (FT-NIR) spectroscopy was developed and evaluated in an international collaborative study. The FT-NIR analyzer employed in this work uses disposable vials for sample handling and incorporates validation protocols designed to ensure that the calibration will give accurate results from analyzer to analyzer and stability over time without any further calibration development work. The global IV calibration was developed from over 1,200 animal, marine, and vegetable oils and fats, which were obtained on a number of different instruments worldwide. The Standard Error of Cross Validation measured from a range of 0–190 IV varied from ±0.2–1.4 IV (1 sigma). The repeatability for all models was on the order of 0.1 IV, which states that most of the error was inherited from the primary methods. Finally, an international interlaboratory study was carried out with 16 samples obtained from the AOCS Smalley Laboratory Proficiency Program and an oil processor. The average reproducibility error in any one lab was better than 0.15, and the average reproducibility between labs was better than 0.33. An uncertainty of 0.45 was calculated from the average FT-NIR values obtained from the collaborative study vs. the AOCS Certified Wijs method (Cd 1d-92).

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KEY WORDS: Discriminate analysis, disposable vial sampling, fats and oils, Fourier transform-near infrared spectroscopy, global calibration, iodine value (IV), partial least squares.

Current chemical and chromatographic methods for the determination of the iodine value (IV) of fats and oils are labor-intensive, require the use of solvents and reagents, and are unsuitable for at-line analysis because of the complexity of sample handling and the long sample turnaround time. Fourier transform-near infrared (FT-NIR) spectroscopy, coupled with a sampling handling system using disposable vials, allows for rapid analysis of neat samples and thus provides an attractive alternative for edible oil analysis. In addition to greatly simplified sample handling, it reduces sample turnaround time to <2 min and eliminates the expense related to solvent use. A disadvantage associated with NIR methods is the extensive calibration effort necessary to account for all spectral variations caused by both product and analyzer variations; a popular misconception is that an NIR analyzer must be continuously calibrated to account for these variations. However, various manufacturing and diagnostic protocols can eliminate analyzer variations over time to maintain stability and minimize variation from analyzer to analyzer, making it possible to transfer calibrations between analyzers.

FT-NIR spectroscopy has been used to determine IV (1,2), *trans* content (1,3), saponification number (1), and low and high levels of hydroperoxides (4,5) in edible oils. Even though near-IR spectra show considerable variation for oils and fats of different origin, the near-IR signature for unsaturation (IV) in the second overtone region, 7,500 to 9,100 cm^{-1} (1333–1100 nm), is remarkably uniform for a wide variety of oils and fats. As part of a previous study (1), a "global IV" calibration (2) developed to determine IV over the full range from 0 to 140 IV was validated with four sets of oil formulations and shown to have good predictive accuracy.

In the present paper, we will describe the strategy for the development of a new global IV calibration, including calibration transfer principles, instrument specifications (repeatability and reproducibility), and discrimination parameters. In addition, standardization protocols for maintaining calibrations over long periods in the field will be discussed. We will also present the results obtained from an American Oil Chemists' Society (AOCS) international collaborative study for this new global IV calibration. This study involved 13 participants from 8 countries and used oil standards obtained from the Smalley Laboratory Proficiency Program and a local processor, which were all analyzed by the Official AOCS Wijs method (Cd 1d-92) (6).

MATERIALS AND METHODS

Instrumentation and sample handling. The instruments used in the design of the global IV calibration and in the international collaborative study were either Bomem model MB 154 NIR/mid-IR (spectral range 13,500–400 cm^{-1}) or Bomem model MB 160 NIR (spectral range 13,500–4,000 cm^{-1}), re-

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spectively (ABB Bomem, Quebec, Canada). Each instrument was equipped with a deuterated triglycine sulfate detector and was controlled by an IBM-compatible 486 DX-66 MHz PC running under Windows-based Bomem-Grams/386 software (Galactic Industries Co., Salem, NH). These instruments had a wavenumber repeatability of ±0.002 cm^{-1} (±2σ) and wavenumber reproducibility of ±0.04 cm^{-1} (±2σ) at 7,300 cm^{-1}, with 100% line repeatability being <0.1% from 8,000–4,500 cm^{-1}. These rigorous specifications ensure transferability of calibrations between instruments. In addition, each spectrometer was also validated in terms of absorbance using high-purity toluene relative to a composite average spectrum obtained for spectrometers coming off the production line. The absorbance difference of toluene, after baseline correction and scaling for pathlength variation, is maintained to within one milliabsorbance unit (0.001 A) over the region 4,100–6,000 cm^{-1} from all spectrometers produced. These specifications are further backed up by routine diagnostics that can be carried out in the field based on measurements made on easy-to-access water vapor bands to ensure wavelength accuracy to within 0.01 cm^{-1}.

The sample handling accessory was a temperature-controlled multivial holding block capable of accepting 8-mm (o.d.) transparent glass vials (Kimble Glass Inc., Vineland, NJ), having a volume of ~1 mL. The dimensional variation in the vial diameter was not more than ±0.25 mm i.d. and ±0.4 mm o.d. Figure 1 illustrates the sample accessory installed in the spectrometer. The temperature of the sample-handling accessory was held at 75°C with constant ambient temperature. In the design of the global IV calibration, some samples were intentionally varied by ±1°C in order to model some tolerance to temperature in the models. This is to permit unforeseen temperature variation, such as operators not waiting for temperature equilibrium prior to scanning or significant changes in ambient temperature. For sample analysis, vials were filled with 0.5–0.7 mL of oil or pre-melted fat and scanned over the range of 12,000–4,500 cm^{-1}.

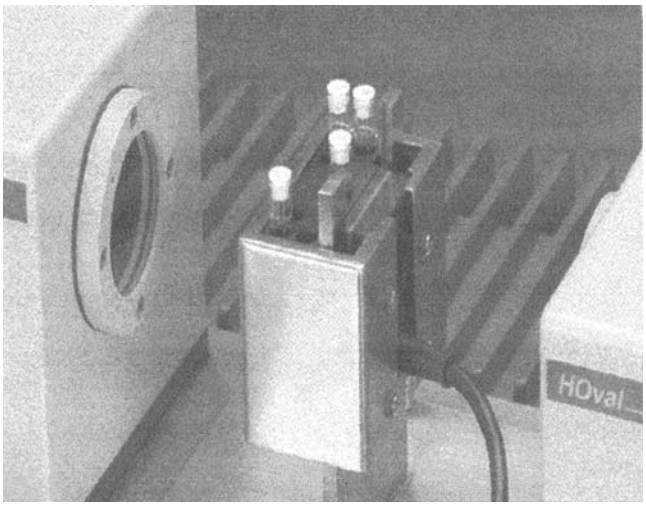

FIG. 1. Heated vial sampling accessory.

All sample and background spectra were recorded by co-adding 128 scans at a resolution of 16 cm^{-1}. This gives a total collection time of approximately 2 min. For the design of the global IV calibration, air background spectra were collected every 30 to 120 min with the vial holder in the IR beam. The reference scan waiting period was dependent upon temperature and humidity fluctuations. For the collaborative study, one reference was collected prior to scanning all 16 samples in duplicate. All sample spectra were ratioed against the most recently collected air background spectrum. The ratioed spectra were subsequently normalized to account for inherent variations in the vial pathlength by using a normalization routine. This routine involved normalizing the spectral area between 9,100 to 7,560 cm^{-1} to unit area after baseline correction.

Software. NIR IV calibrations were developed using the partial least squares (PLS)/Plus chemometrics program (Galactic Industries Co.). The predicted residual error sum of squares, standard error of cross validation (SECV), and F-ratios, which are associated with the cross validation of the calibrations tested, were used to select the optimal number of factors for each PLS method. A user interface created using AIRS (Advanced Infrared Software), a software package designed to facilitate routine laboratory implementation of quantitative IR analytical methods, provided the participants in the collaborative study with instructions to collect background and sample spectra, selected the appropriate global IV calibration model for each sample based on the discrimination criteria established during calibration development, and output the IV predicted from that calibration model.

Calibration samples. Over 1,200 neat, processed, and blended oil samples representing a wide variety of oil types were collected from over 20 oil processors worldwide (canola, cocoa butter, sunflower, corn, milk-fat, coconut, lard, olive, beef tallow, cottonseed, palm kernel, palm stearin, palm olein, crude palm, fish, soybean, castor, linseed, margarine blend, walnut, almond, safflower, soy, and emulsified shortening); these were collected at random over various production periods to ensure that normal process variation was represented. All samples were pre-analyzed in duplicate by the Wijs (Cd 1d-92) primary method (6). The reproducibility of the analyses carried out by each participating processor was evaluated by calculating the standard deviation of the differences (SDD) between duplicates. Samples for which the SDD was greater than one standard deviation from the mean SDD for all samples analyzed (±0.2 for 0–10 IV, ±0.5 for 10–80 and ±1.0 for 80–200 IV) were rejected. The remaining samples were employed as calibration standards and were assigned to calibration sets on the basis of their IV.

RESULTS

Development of the global IV model. Figure 2 shows the second C-H overtone region (9,100–7,560 cm^{-1}) of three normalized FT-NIR spectra of neat vegetable oils having low, medium, and high IV obtained from 8-mm (o.d.) high-performance liquid chromatography (HPLC) vials, which are appropriate for measurements in this region. These spectra clearly

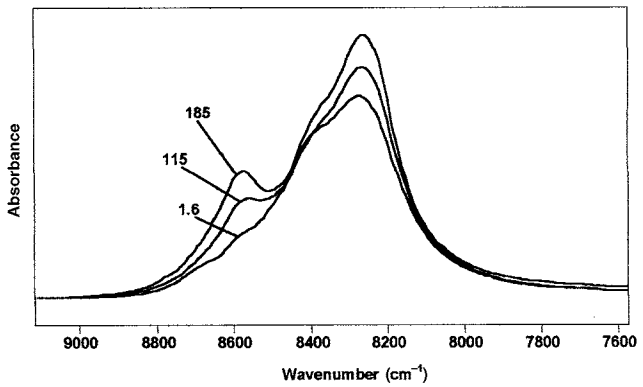

FIG. 2. Fourier transform-near infrared (FT-NIR) spectral region displaying spectral correlation with 1.6, 115, and 185 iodine value (IV).

show a spectral response related to changes in IV, and this spectral region was used to develop NIR IV calibrations. To compensate for scattering effects due to sample vial curvature, as well as the inherent pathlength variation among vials, all spectra were mean-centered, baseline-corrected, and normalized to unit area over the wavenumber range of 9,100 to 7,560 cm^{-1}.

Although a generally satisfactory global IV calibration could be obtained when all 1,200 calibration standards were combined into a single training set, it was found that subdividing the calibration samples into smaller IV groups was a better approach. Breaking up the training set produced better cross-validation results and reduced the number of factors required to describe the calibration model. In addition, the reproducibility of the primary method tends to be better at lower IV such that the development of calibrations for distinct IV ranges reduces the relative error contribution associated with higher IV samples. This approach also allows individual discrimination thresholds (IV range, spectral residuals, and PLS factor scores) to be set for each grouping and optimized. Overall, subdividing the calibration samples into IV ranges has the effect of tightening the accept/reject criteria employed to identify samples that are not adequately represented by the samples used in the design of the global calibration.

Table 1 summarizes the internal validation results for each IV calibration developed, which cover a range of 0–190 IV in roughly 30 IV increments. Figure 3A and Figure 3B provide representative cross-validation plots for two of the six PLS calibrations. Table 1 also provides the number of samples used for each calibration, the number of PLS factors, and their linear regression equations and statistics. The SECV indicates that IV can be predicted to within ±0.23 IV for IV of <10, to within ±0.5 IV for IV between 10 and 60, and to 0.8–1.4 IV for IV > 60. The trend toward generally higher variability at higher IV is normal as reference method reproducibility deteriorates and there is more risk of substantive changes in IV due to oxidation/degradation over time.

Analytical repeatability. The repeatability of the FT-NIR IV predictions was assessed by selecting a midrange sample from each of the IV calibration sets and measuring the root mean standard deviation (RMSD) for 10 consecutive measurements on the same or different vials of the same sample. The repeatability for measurements on a single vial was ≤ 0.15 IV units for all of the calibrations (Table 1), indicating that the contribution of instrumental error to the overall SECV is minor (~10%). The RMSD for the samples analyzed 10 consecutive times in different vials was not determined to be significantly different, providing evidence that the small pathlength differences due to variations in vial size are compensated for by the normalization routine, and that sample handling has little effect on the repeatability of the method. Accordingly, most of the SECV can be attributed to errors associated with modeling imperfections and/or errors in the reference values obtained from the primary method. Robustness (i.e., long-term stability of the instrument response) was evaluated by scanning a sample having an IV of 75.8 every other day for 2 wk, and the resulting RMSD of 0.05 IV is indicative of very good instrumental stability.

AOCS collaborative study. To provide tangible evidence that this preprogrammed and precalibrated FT-NIR method is reliable and accurate in the hands of general users, and hence suitable as a tentative AOCS method, an international collaborative study was undertaken. Substantial care was taken in designing the collaborative validation phase of the FT-NIR IV system by co-opting 13 industrial and research collaborators from eight countries to provide as broad a range of analytical situations and experience as possible. Most of the participants were unfamiliar with FT-NIR technology and the method, but were involved because of the potential benefits this analytical methodology could bring to their day-to-day operations. Each participant was provided with a procedure for scanning 16 blind, coded samples in the format of an AOCS method.

The blind samples comprised 11 fats and oils obtained from the AOCS Smalley Laboratory Proficiency Program plus five

TABLE 1
PLS Cross-Validation Statistics and Repeatability for All Calibrations[a]

Calibration by IV range	*n*	PLS factors	SECV	R^2	IV for repeatability study	Repeatability RMSD same vial	Repeatability RMSD, different vials
0.2–10.05	324	7	0.234	0.99	4.4	0.07	0.08
9.5–30.5	55	6	0.443	0.99	23.2	0.08	0.08
30.4–60	290	5	0.523	0.99	50.9	0.09	0.15
60–90.1	296	6	0.808	0.99	77.6	0.07	0.09
90.2–126.1	134	6	1.4	0.98	123.5	0.10	0.16
126.1–190.3	64	4	0.83	0.99	180.2	0.10	0.1

[a]PLS, partial least squares; IV, iodine value; SECV, standard error of cross validation; RMSD, root mean standard deviation.

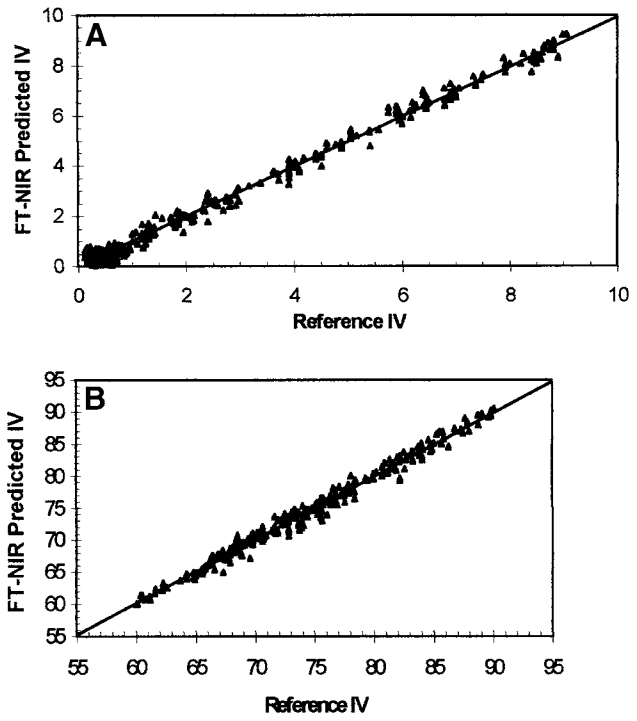

FIG. 3. Scatter plots for cross-validation results for two calibrations for the FT-NIR predicted IV vs. AOCS Certified Methods. (A) 0–10 IV, (B) 60–90 IV. See Figure 2 for abbreviations.

additional pre-analyzed samples obtained from a processor, the processor samples being included to provide a boxcar distribution of samples over the range 0–144 IV. The samples were split into 13 lots and distributed in 8-mm o.d. HPLC vials to each of the study participants.

The FT-NIR reproducibility of each of the laboratories participating in the round robin was evaluated with SDD criteria. For this, each collaborator scanned the validation standards in duplicate. The statistical results obtained from all participants are summed in Table 2. These results indicate that the analysis for IV by FT-NIR with disposable vials is highly repeatable (r) and reproducible (R) from 0–144 IV. The average repeatability error within any one lab was less than 0.15, and the average reproducibility between labs was less than 0.33.

Figure 4 displays the scatter plot for the average IV prediction obtained from the interlaboratory study and the known IV values. All of the samples were analyzed by the Official AOCS Wijs method Cd 1d-92 (6). The average Wijs values from over 37 participant labs that participated in the Smalley Laboratory Proficiency Program were used as reference values against which the standard error of prediction (SEP) was determined. The average duplicate values from the five samples provided by the processor were also used to determine the SEP. The SEP of the calibration is 0.45 with an R^2 of 0.99 from 0.2 to 140 IV. These results are very good considering they are based on full range analysis.

DISCUSSION

Requirements for global calibrations. There are three essential elements required for the development of stable global calibrations. The first is a protocol for maintaining and validating the standard spectral response of the analyzer. While there are several certified wavelength and intensity standards utilized for this purpose, these standards are not compatible with the high degree of repeatability that is routinely achieved with FT-NIR. We propose routine analyzer validation protocols that provide more stringent control of both intensity and wavelength scales. One of the protocols ensures that the reproducibility of a stable test sample is maintained to better than 0.1% root mean square of its nominal at all relevant wavelengths. Another protocol

TABLE 2
Statistical Results (evaluated in accordance with ISO 5725-1994) from an International Interlaboratory Study Completed by the AOCS Edible Oil Committee in 2000[a]

	Sample number[b]															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Labs after outliers	13	12	11	13	12	13	11	12	13	11	13	13	12	12	12	12
Labs removed	0	1	2	0	1	0	2	1	0	2	0	0	1	1	1	1
Mean value (IV)	105.12	69.27	79.15	75.23	51.11	57.58	141.26	126.20	142.11	95.12	46.92	24.18	8.83	1.15	4.42	0.36
Known value (IV)	104.30 ± 1.40	69.12 ± 1.00	78.33 ± 1.57	74.8 ± 1.00	51.21 ± 0.46	57.45 ± 0.52	140.99 ± 0.73	126.08 ± 0.77	142.37 ± 1.74	96.100 ± 0.3	46.550 ± 0.3	24.500 ± 0.2	8.750 ± 0.1	0.800 ± 0.06	4.400 ± 0.1	0.200 ± 0.06
S_r	0.30	0.18	0.19	0.18	0.19	0.21	0.04	0.10	0.08	0.11	0.12	0.18	0.13	0.13	0.09	0.09
RSD_r	0.28	0.26	0.24	0.24	0.37	0.36	0.03	0.08	0.06	0.12	0.25	0.76	1.42	11.77	2.02	23.89
$r = (2.8 \times S_r)$	0.83	0.50	0.53	0.50	0.53	0.59	0.12	0.29	0.22	0.32	0.32	0.51	0.35	0.38	0.25	0.24
S_R	1.02	0.24	0.21	0.31	0.34	0.31	0.19	0.34	0.34	0.44	0.30	0.33	0.16	0.44	0.16	0.19
RSD_R	0.97	0.35	0.27	0.42	0.67	0.54	0.14	0.27	0.24	0.46	0.64	1.34	1.76	37.98	3.66	52.35
$R = (2.8 \times S_R)$	2.87	0.68	0.59	0.88	0.96	0.87	0.54	0.95	0.96	1.23	0.84	0.91	0.43	1.22	0.45	0.53

[a]Thirteen laboratories participated, each obtaining two test results for each sample analyzed by FT-NIR at 75°C with disposable glass sample vials. Abbreviations: FT-NIR, Fourier transform-near infrared spectroscopy; r, repeatability; R, reproducibility; S_r, within-laboratory standard deviation; S_R, among-laboratory standard deviation; RSD_R, among laboratories relative standard deviation.

[b]1,2,3, and 4 = AOCS Smalley sample edible fat; 5 and 6 = AOCS Smalley sample palm oil; 7 = AOCS Smalley sample crude safflower oil; 8 and 9 = AOCS Smalley sample crude corn oil; 10 = private lab oleic safflower oil; 11 = private lab hydrogenated palm; 12 = private lab palm kernel olein; 13 = private lab coconut oil; 14 = private lab hydrogenated walnut; 15 = private lab hydrogenated soybean oil; 16 = private lab hydrogenated palm kernel oil.

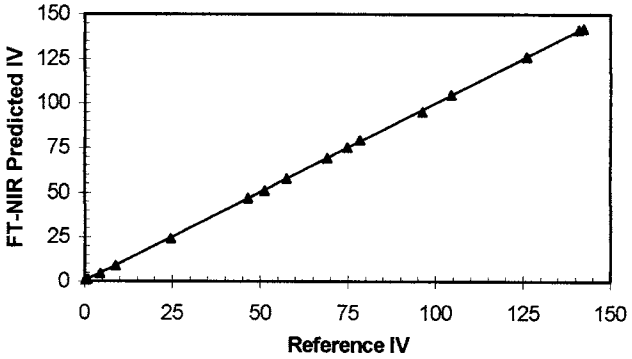

FIG. 4. Scatter plot for external validation statistics showing the mean IV predicted on each sample vs. the average known IV from Wijs (CD 1d-92) (6). See Figure 2 for abbreviations.

tests the wavelength position of bands produced by water vapor in the air path of the spectrometer. Protocols also exist for tests for linearity of response and stray light. Thanks to the continuous internal calibration by means of a laser built into the FT-NIR, there is essentially no drift. Nevertheless, easy-to-apply instrument test codes certify that the analyzer is within specifications.

A second requirement for developing global calibrations is that the primary method for which the model is based upon must be a certified method and give reproducible results. The uncertainty estimated from an FT-NIR calibration is highly dependent on the accuracy and precision of the reference values used in the calibration. The repeatability and reproducibility values are included in official AOCS methods. In this case, it is only necessary to demonstrate that the reference measurement is being practiced in accordance with the procedure described in the official AOCS method, and that the repeatability obtained is statistically comparable to that published in the method. Data from established quality control procedures are used to demonstrate that the repeatability of the reference method is within AOCS specifications. If archived data are not available, then repeatability should be obtained on at least 10 samples in duplicate across the concentration range of interest. The standard deviation should be calculated and compared to that expected based on the published repeatability.

Finally, the spectral characteristics of the material under investigation must be fully evaluated. Several pattern recognition techniques are available for this. Pattern recognition of the spectral signatures can be used as a blueprint for separating distinct populations into their respective families. Moreover, unique spectral regions can be identified for many materials that are attributed to specific functional group behavior. Although the location for uniform spectral signatures can be a real asset for global calibration development, several discrimination functions can be used to further strengthen the stability of the model. Three discriminate functions used by these authors for IV analysis (7) have been described previously. Each function performs a specific task for discrimination. The same functions and principles are used for the design and routine use of the global IV model described in this paper. They are concentration residuals, spectral residuals, and PLS factor scores.

Simplified sampling. A novel sampling strategy was introduced. It utilized low-cost and disposable HPLC autosampler vials inserted into a heated sample block at 75°C. The vials measure 8 mm in outside diameter and 50 mm in length. Spectral measurement is done in transmission. The NIR beam passes transversely through the lower part of the vial. Less than 1 mL of sample is required to perform an analysis. To eliminate analysis errors due to variations in vial dimensions, the chemometrics performs a normalization step that makes the analysis independent of absorption pathlength. The vials eliminate traditional flow cells and their plumbing, which must be continually heated and rinsed to prevent leaching effects and clogging from high melting-point samples. In addition, the vials lower the probability of down time due to excess moving parts.

Collaborative study. Based on the results obtained in the collaborative validation study, the FT-NIR IV method using disposable vial sampling is clearly capable of providing highly accurate, repeatable, and reproducible IV. It is particularly noteworthy that these results were obtained using 13 different instruments located in eight different countries with a variety of industrial environments, conditions, and operators. In using this system, IV results are available from neat oils in less than 2 min, without any sample preparation, making the FT-NIR IV method ideal for at-line measurements where rapid sample turnaround time is required. Although great care was taken to ensure that consistent and high-quality primary data were obtained for calibrating the system, even primary, AOCS approved methods can produce variable results between laboratories. Hence from the standpoint of implementation it is important that the IV method in use (iodometric or gas chromatographic) be vigorously evaluated by collecting reproducibility data to ensure a consistent database to which the FT-NIR results can be compared. Thus, the FT-NIR predictions obtained using the global calibration set can be regressed against the results of the method in use so that, if there is not a direct match numerically, the data can be trended and corrected to give the results the processor is accustomed to working with. Should the oil samples routinely being analyzed by a processor be particular or unique, and not adequately represented by a global calibration, the calibration can be supplemented with such samples to further broaden its application and accuracy.

This paper has outlined the basic elements associated with a new FT-NIR IV method, based on an integrated instrument, software and sample handling system, that provides IV values within 2 min on neat oil samples. The system, which is proprietary, provides a means for industry to rapidly obtain IV data from a broad range of samples as well as to fine-tune the system for custom samples with particular properties. The system has been evaluated in a carefully structured collaborative study. The results of this study indicate that the method is both reproducible and accurate in the hands of 13 different laboratories in eight countries.

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