Method for Determining Oxidation of Vegetable Oils by Near-Infrared Spectroscopy

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ABSTRACT: Use of near-infrared (NIR) transmittance spectroscopy for rapid determination of the oxidation level in soybean oils (SBO) was investigated, and calibrations were developed for quantitative determination of peroxide value (PV), conjugated diene value (CD), and anisidine value (AV) of SBO. Partial least squares (PLS) regression and forward stepwise multiple linear regression were used to develop calibration models from spectral data in log 1/*T*, first derivative and second derivative of log 1/*T* formats for both 1- and 2-mm path lengths. The models were validated by comparing NIR results from independent sample sets to the values obtained by official methods. The spectral region from 1100 to 2200 nm was best for measuring oxidation when using a 2-mm path length. PLS regression using first-derivative spectra gave the best results for PV. For the validation sets, linear relationships were obtained for PV ($r = 0.99$), and CD ($r = 0.95$), compared with accepted reference procedures. However, measurement of AV by NIR was less successful than measurement of the other two indices of oxidation, especially for an external validation sample set. Results obtained in this study indicate that NIR spectroscopy is a useful technique for measuring oxidation in soybean oil.

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KEY WORDS: Conjugated diene value, edible oil, lipid oxidation, multiple linear regression, near-infrared spectroscopy, *p*-anisidine value, peroxide value, PLS, soybean oil.

The measurement of oxidative stability and oxidation products is essential to determine shelf life, acceptability, and nutritional quality of edible oils (1,2). Lipid oxidation measurement involves a variety of techniques because the oxidation process involves several stages. The peroxide value (PV) is most commonly used to assay oxidation in oils and is applicable to the early stages of oxidation (3). A second early-stage oxidation measure is the conjugated diene (CD) value. This technique measures the conjugation that is formed as the unsaturated lipid oxidizes. Nawar (3) stated that formation of peroxides is normally coincidental with conjugated double-bond formation and oxygen uptake in polyunsaturated fatty acids. To monitor accumulated secondary oxidation products, usually carbonyl-type compounds (4), the anisidine value (AV) test is a widely accepted method. The test estimates the level of aldehydes, principally 2-alkenals, present in the oil.

Although they provide an indication of the oxidation status of an oil, these chemical methods are largely empirical in nature. In addition, these methods are time consuming, destructive to the sample, costly, and require large amounts of glassware, sample, and potentially hazardous reagents. To improve on the drawbacks of the official methods, a number of new methods have been developed over the past few years for measuring lipid oxidation.

There are several references in the literature to infrared (IR) studies of fats and oils, particularly in the areas of fatty acid composition, determination of free fatty acids (FFA), and level of unsaturation (5,6). Determination of oxidation and crystallinity and the identification of hydroxyl groups have also been achieved by mid-IR spectroscopy (7,8). Van de Voort *et al.* (9,10) reported that edible fats and oils in their neat form are ideal candidates for Fourier transform-infrared (FT-IR) analysis, in either the attenuated total reflectance or transmission mode. FT-IR spectroscopy has also been used to determine *cis* and *trans* contents (10) and FFA contents in fats and oils (11), and to quantify the PV (12). Moreover, Guillén and Cabo (13) used frequency data from the FT-IR spectra to evaluate the degree of oxidation of edible oils. Near-infrared (NIR) spectroscopy has been used for the determination of FFA (14), the iodine value of fat (15), and the fatty acid composition of fats and oils (5). NIR spectroscopy has also been used as an authenticity testing tool (16,17) and, coupled with discriminant analysis, as a means to differentiate among vegetable oil types (18,19).

NIR spectroscopy offers several advantages over conventional methods, including high speed, multiplicity of analyses from a single spectrum, nondestruction of the sample, and applicability to small amounts of sample. NIR analysis is also economical because reagents are not needed, labor requirements are low owing to minimal sample preparation, and no chemical wastes are produced. Only a few studies, however, have used NIR techniques for measuring lipid oxidation (20), and those focused primarily on the spectral properties of purified hydroperoxides from methyl oleate and linoleate.

The objectives of the present research were threefold: (i) to investigate the relationship between NIR spectra and lipid oxidation in soybean oil, (ii) to evaluate different sample-handling and data treatment techniques in order to maximize the correlation between spectral and chemical data, and (iii) to evaluate the success of the NIR analytical methods developed by testing each with an independent set of soybean oil (SBO) samples.

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EXPERIMENTAL PROCEDURES

Selection of samples. Eight SBO samples were purchased from local supermarkets in Lincoln, Nebraska. To achieve variation in the oils, samples were selected from seven commercial brands. Care was taken to avoid duplication of samples. This was done by tracking "sell by" dates and batch codes, if available, from packaging. It was assumed that packages from the same batch would not have different "sell by" dates. Three additional samples were purchased at a later date and used to prepare an external validation set.

Oxidation of SBO. To generate different levels of oxidation, 100-g subsamples from each of the eight samples were placed under a fluorescent light source (4200 lux) as described by Hall (21). During light exposure, samples for chemical and spectroscopic analysis were taken every 12 h until a PV of 20 meq/kg was reached for each treatment. PV, CD, and AV were determined using the AOCS official methods (22). After generation, all samples were placed in brown glass bottles under nitrogen gas and then kept in a freezer (–20°C) until further analysis.

Reference chemical methods. All chemical analyses were carried out in duplicate for each sample under the same conditions, and the means were calculated. All solvents and reagents used were of analytical grade. The oxidation levels of oil samples were analyzed using the following standard methods: PV (AOCS Official Method Cd 8-53) (22), CD value (AOCS Official Method Ti 1a-64) (22), *p*-AV (AOCS Official Method Cd 18-90) (22).

The absorbances at 233 nm for CD and at 350 nm for AV were measured by using a Beckman DU 520 General-Purpose UV/VIS spectrophotometer (Beckman Instrument, Inc., Fullerton, CA).

NIR spectroscopy. Spectroscopic data were collected by a Foss NIRSystems Model 6500 scanning spectrophotometer (NIRSystems Division of Foss Electric, Silver Springs, MD). Instrument control, data collection, and statistical analysis were carried out with the Near Infrared Spectral Analysis Software (NSAS) package (version 3.53; Foss NIRSystems, Inc.). The single-beam instrument was configured for direct transmission measurements with standard 1- and 2-mm quartz cuvettes. Transmittance spectra were recorded in log $1/T$ format at 2-nm intervals from 400–2500 nm, using ambient air as the reference.

Collection of spectral data and sampling. All samples were allowed to reach room temperature (25 ± 2°C) prior to collection of spectra. The sample transport temperature control unit was set at 26°C. For collecting the transmittance spectrum of each oil sample, the oil sample was first placed into a quartz cuvette and then into the cuvette holder. Each spectrum was then obtained by collecting and averaging 32 individual spectral scans. The cuvette was then removed and rinsed three times with the next oil sample.

Different cleaning procedures were investigated for their ability to clean cuvettes after completing the analysis, including rinsing with acetone, ethanol, and hexane. The best results were obtained when cuvettes were rinsed with methanol followed by washing with Micro-90 detergent (International Products Corporation, Burlington, NJ). For most applications, a 1–2% detergent solution produced good results at 66°C. A thorough rinsing step with distilled water followed cleaning.

Calibration development and validation. The 119 samples representing eight oils oxidized to various degrees were divided into two sets for calibration and validation, with 75 samples in the calibration set and the remaining 44 samples in the prediction set. A subroutine of the NSAS software identified calibration samples by random selection. The calibration samples selected were inspected to ensure that the extremes of PV, CD, and AV values were included in the set. The validation set was composed of those samples not used for calibration. The same grouping of calibration and validation samples was used for both the 1- and 2-mm path length spectra. An external (independent) validation set, consisting of 30 samples of different oxidation levels prepared from the three SBO purchased at a later date, was also prepared.

To select wavelengths and relate log $1/T$ values to PV, CD, and AV values from each set of spectra, multivariate regression was used. Optimal NIR calibration equations were explored using both modified partial least squares (PLS) regression (23) and forward stepwise multiple linear (FSML) regression techniques (24). These regression techniques were applied to the log $1/T$ spectra, as well as first and second derivatives of log $1/T$ at two path lengths. The optimal number of wavelengths or PLS factors to include in a calibration was evaluated by comparing correlation coefficients (R) and standard errors of calibration (SEC), and also standard errors of cross-validation (SECV) for PLS calibrations. The use of an optimal number of PLS factors in the calibration minimized the SECV. Additionally, these statistical parameters were used to select the optimal segment and gap values for calculating the derivatives (25). Each optimized calibration was tested with the set of 44 validation samples. The r values, standard errors of prediction (SEP), and slope values were compared to evaluate which calibrations were most successful at measuring oxidation in SBO. Moreover, prediction was also carried out using the external validation set.

RESULTS AND DISCUSSION

Sample distribution. Eight SBO were purchased. Before exposure to light, PV of the eight SBO samples ranged from 0.20 to 1.49 meq/kg (mean 0.63 meq/kg), with CD values ranging from 0.20 to 0.26% (mean 0.22%), and AV ranging from 0.53 to 1.8 (mean 1.11).

Reference procedure analysis. Reference methods were performed in duplicate. The data obtained by the above methods for the calibration and prediction sets are presented in Table 1. Seventy-five samples were randomly selected for inclusion in the calibration set. The mean and standard deviation values of PV, CD, and AV contents in the SBO samples can be seen in Table 1. Two samples were omitted from the CD calibration set because of reference values that were well

TABLE 1
Characteristics of Reference Data for Calibration and Prediction Sets

	Calibration set			Prediction set		
Constituent	*n*[a]	Mean	SD[b]	*n*	Mean	SD
PV*	75	9.60	6.18	44	10.23	6.32
CD**	73	0.31	0.09	43	0.31	0.11
AV	75	1.72	0.85	44	1.85	0.91

[a] *n,* number of samples.
[b] Standard deviation of mean for all samples. *Values are given in meq/kg, **values are given in %. PV, peroxide value; AV, anisidine value; CD, conjugated diene value.

above the highest value of the other samples in the calibration set. One sample was deleted from the CD validation set owing to a reference value that was well above the range of the calibration samples.

As the exposure time under light increased from 0 to 180 h, the PV of the SBO reached ~23 meq/kg oil. Published data have reported PV ranges for oxidized vegetable oils, including soy, sunflower, and canola, to be 3–5 for low oxidation, 10–12 for moderate oxidation, and 16–18 for high oxidation (26).

NIR spectra. Figure 1 shows the NIR mean spectra of SBO samples from 400 to 2500 nm for 1- and 2-mm path lengths, respectively. In the 2-mm path length spectrum, some nonlinearity in the strongly absorbing region above 2200 nm can be observed. This nonlinearity is reduced with the shorter path length, but sensitivity is also lost in the shorter-wavelength region where absorption bands are weaker.

NIR and PV. Strong correlations between NIR-predicted values and reference data for PV in both the calibration and validation sample sets for 1- and 2- mm path lengths were obtained. Log 1/*T*, first-derivative, and second-derivative mathematical treatments performed satisfactorily for measuring PV in SBO. Calculating derivatives of spectra can remove scattering and other spectral differences that result in both slope differences and offsets among spectra. When using FSML regression to develop calibrations for PV, the first-derivative mathematical treatment provided better results than either log 1/*T* or second-derivative treatments for both 1- and 2-mm path lengths. For the 1-mm path length, first derivative gave the highest *r*-value (0.981) and lowest SEP (1.22 meq/kg), and for the 2-mm path length, first derivative mathematical processing also gave the highest *r* value (0.981) and lowest SEP (1.25 meq/kg).

To obtain quality FSML results, the choice of appropriate wavelengths is important. For FSML, a maximum of four wavelengths was used in the calibrations to prevent overfitting. Selected as the key wavelengths, 2070, 2036, 1746, and 1400 nm were used for measuring lipid peroxide when using first derivative and a 1-mm path length. When using first derivative and a 2-mm path length, 2068, 2016, 1612, and 1242 nm were shown as the key wavelengths. In both cases, a wavelength in the 2070 nm region appears to be highly significant. Similarly, Takamura *et al.* (20) reported that 2084 nm was the key wavelength for lipid peroxide in the spectra of purified hydroperoxides of methyl oleate and methyl linoleate and could be used for the determination of lipid oxidation in edible oils. However, this single wavelength approach was complicated by a large number of potential spectral interferences from other OH-containing components such as alcohols, FFA, water, and monoglycerides, all of which exhibit absorptions that overlap with the hydroperoxide band and interfere with the hydroperoxide determination (10). Use of a multiwavelength approach helps to eliminate interferences from other absorbing substances.

When calibrations developed using PLS regression were used to predict samples in the validation set, *r* range was 0.986–0.989 with the 1-mm cuvette, and 0.993–0.994 with the 2-mm cuvette. With a 1-mm cuvette, SEP range was 0.938 to 1.06 meq/kg, and with a 2-mm cuvette, SEP range was 0.72–0.757 meq/kg. Although all the PLS calibration equations worked well, equations using *N*-point smoothing of log 1/*T* generally gave the lowest SEP with a 1-mm cuvette, while using the first derivative of log 1/*T* generally gave the lowest SEP with a 2-mm path length. Overall, use of the PLS

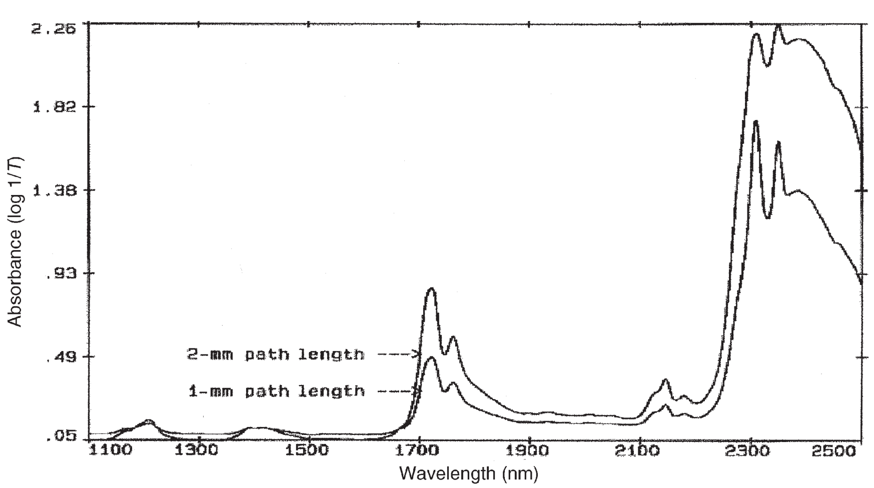

FIG. 1. Near-infrared spectra of soybean oil obtained with 1- and 2-mm path lengths.

TABLE 2
Best Calibration and Prediction Results from PLS Models for PV, CD Value, and AV[a]

Partial least squares				Calibration			Prediction			Prediction for the external validation set		
Constituent	Path length (mm)	Treatment	Number of factors	Range (nm)	Multiple *R*	SEC*	Correlation (*r*)	SEP	Slope adjustment	Correlation (*r*)	SEP*	Slope adjustment
PV	1	Log 1/*T*	13	1100–2200	0.997	0.540	0.989	0.938	0.971	0.993	1.640	1.018
	2	First-derivative Segment 5 Gap 5	13	1100–2200	0.996	0.557	0.994	0.720	1.011	0.997	0.760	0.997
CD	1	First-derivative Segment 10 Gap 5	9	1100–2200	0.926	0.022	0.916	0.025	0.917	0.955	0.021	0.779
	2	First-derivative Segment 5 Gap 5	12	1100–2200	0.963	0.016	0.945	0.020	0.970	0.926	0.021	0.864
AV	1	First-derivative Segment 10 Gap 5	12	1100–2300	0.940	0.302	0.924	0.350	1.000	0.881	0.590**	0.405
	2	First-derivative Segment 10 Gap 5	15	1100–2200	0.966	0.232	0.938	0.328	0.950	0.949	0.920**	0.338

[a] Values are given in meq/kg for PV and in percentage for CD. **Values shown have been bias-corrected. PLS, partial least squares; SEC, standard error of calibration; SEP, standard error of prediction. See Table 1 for other abbreviations.

calibration technique gave better prediction results than the calibration equations developed using multiple linear regression. Results from the best PLS calibrations for 1- and 2-mm path lengths are shown in Table 2. More PLS factors can be used in a calibration than discrete wavelengths without overfitting, since the factors are not correlated. Use of a 2-mm path length lowered the SEP compared to a 1-mm path length. Plots of NIR predicted vs. reference data were linear and had slopes close to unity (Figs. 2 and 3). Statistical analysis indicates that chemical and instrumental results have comparable means. In order to compare the repeatability of the NIR and titration methods, 10 replicate measurements of one oxidized oil sample were made by each method. The coefficients of variation were 1.97 and 1.52% for the NIR and titration methods, respectively. Satisfactory repeatability was obtained by both methods.

PLS regression allows the whole spectrum in a selected region to be investigated simultaneously to establish correlations between spectral and compositional data. The wavelength region was optimized along with the number of factors used in the calibrations. It was demonstrated that the NIR spectral region range 1100–2200 nm was the best region for

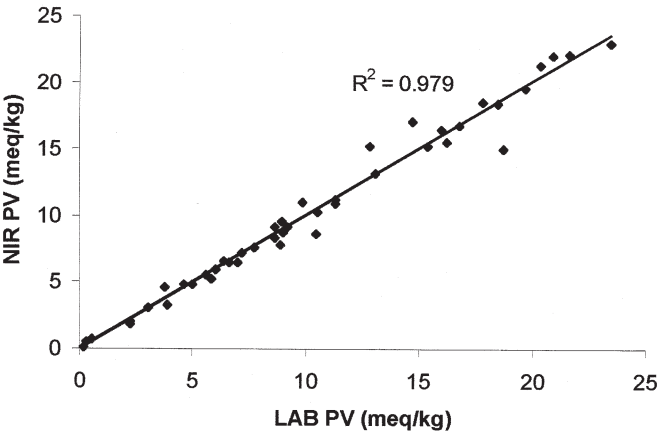

FIG. 2. Scatter plot diagram comparing peroxide value (PV) of soybean oil validation samples using a 1-mm cell. Ordinate values are the PV of validation samples as determined by first derivative of log 1/*T* and partial least squares (PLS) calibration. Abscissa values are PV as determined by the reference procedure. NIR, near infrared; LAB, laboratory.

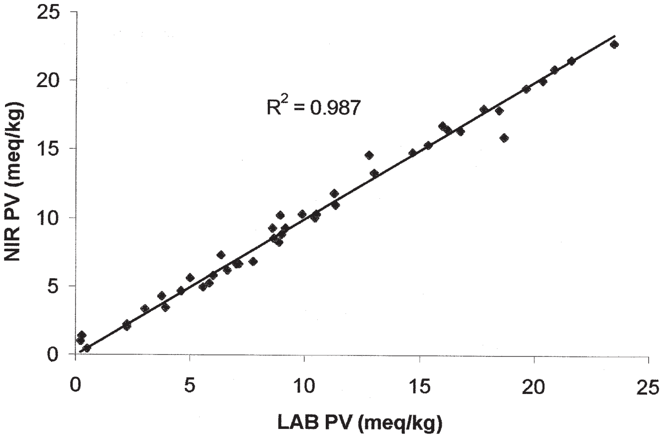

FIG. 3. Scatter plot diagram comparing PV of soybean oil validation samples using a 2-mm cell. Ordinate values are the PV of validation samples as determined by first derivative of log 1/*T* and PLS calibration. Abcissa values are PV as determined by the reference procedure. See Figure 2 for other abbreviations.

predicting PV, as eliminating the wavelength region >2200 nm lowered the SEP. Removing a region that may not contain useful information about the constituent of interest can improve the prediction results. Also, using an excessive number of PLS factors actually makes the prediction results worse due to overfitting. The PLS loadings show structure in the same regions as the wavelengths selected by FSML. The PLS loadings reveal visible differences among samples between 1200 and 1400 nm, around 1700 nm, and between 2000 and 2200 nm. The best wavelengths for measuring PV contents can be interpreted in terms of the chemical characteristics of different types of oxidation compounds in SBO, and by reference to previously known chemical assignments (27). Absorptions around 2070 nm are probably caused by –OH combination bands, while 1200–1400 nm likely represents first overtones of O–H stretching vibrations and overtones and combinations of –CH stretches. The region around 1700 nm shows absorption as a result of first overtones of the C–H stretching vibrations (28).

Prediction was also carried out using an external validation set containing 30 samples from the three oils prepared at different times. Strong correlations between NIR-predicted values and reference data for PV in the external validation sample set for each path length were obtained, as well. When using FSML and a 1-mm path length, a first-derivative treatment gave the highest *r*-value (0.992) and lowest SEP (2.37 meq/kg), and for the 2-mm path length, first-derivative mathematical processing also gave the highest *r* value (0.998) and lowest SEP (1.43 meq/kg). Overall, use of the PLS calibration technique gave better prediction results than the calibration equations developed using multiple linear regression. Results from the best PLS calibrations for 1- and 2-mm path lengths are shown in Table 2. With the 2-mm path length, SEP values for both validation sets were very similar and were lower than those obtained with the 1-mm path length.

We did not attempt to quantitate oils with PV values <0.2 meq/kg. The iodometric titration procedure used as the reference method may be unreliable for very low oxidation levels owing to interference by molecular oxygen, the reaction of liberated iodine with other components, and uncertainty in the titration endpoint (29). Because of the lack of reliable reference data, the ability of NIR to quantitate very low PV levels in freshly refined oils remains unknown.

TABLE 3
Regression Analysis and Prediction Results for FSML Models Measuring CD Value[a]

		Calibration		Prediction			Prediction for the external validation set		
	λ (nm)	Multiple *R*	SEC (%)	Correlation (*r*)	SEP (%)	Slope adjustment	Correlation (*r*)	SEP (%)	Slope adjustment
CD value for 1-mm path length									
Log 1/*T*	2390 2318 2480	0.345	0.053	0.358	0.055	1.147			
First derivative Segment 10 Gap 5	2064 2430 2350 1398	0.915	0.023	0.925	0.023	0.970	0.949	0.017	0.822
Second derivative Segment 5 Gap 5	2080 1890 1450 2020	0.905	0.024	0.884	0.029	1.052			
CD value for 2-mm path length									
Log 1/*T*	1728 1900 2080 2048	0.837	0.031	0.904	0.027	1.168	0.936	0.019	0.908
First derivative Segment 5 Gap 5	2070 1164 1396 1406	0.908	0.024	0.930	0.022	0.973	0.939	0.018	0.737
Second derivative Segment 5 Gap 15	2054 1940 1368 1698	0.904	0.024	0.899	0.026	0.985			

[a] FSML, forward stepwise multiple linear regression. See Tables 1 and 2 for other abbreviations.

CD value. As storage time under light increased, the CD content continually increased in the SBO, which indicated an increase in the oxidation of the SBO. The increase in absorption at 233 nm is due to formation of conjugated double bonds from polyunsaturated fatty acids such as linoleic and linolenic acids. Formation of peroxides is normally coincidental with conjugated double-bond formation in polyunsaturated fatty acids, and with oxygen uptake. The pattern of changes in CD contents for the samples was similar to the changes in their PV, although increases were relatively small in magnitude.

NIR and CD value. Strong correlations between NIR-predicted values and reference data for CD in both the calibration and validation sample sets for 1- and 2- mm path lengths were obtained. When using FSML regression to develop calibrations for CD, the first-derivative mathematical treatment provided better results than *N*-point smoothing of log 1/*T* for both 1- and 2-mm path lengths (Table 3). For FSML, a maximum of four wavelengths was used in the calibrations to prevent overfitting, and these key wavelengths can be seen in Table 3. There is no similar study in the literature with which to compare results. Note that for the 2-mm path length, wavelengths >2200 nm are no longer chosen, probably because nonlinearities are observed in this strongly absorbing region at longer path lengths.

Prediction of CD values for the validation samples using these calibrations indicated applicability of the equations obtained. Although r values are lower than those for PV, the high r, except for $r = 0.358$ for log 1/*T* with a 1-mm path length (Table 3), demonstrate the NIR transmittance procedure to be a useful method for determining oxidation level in SBO as estimated by CD.

When using PLS regression, the wavelength region was optimized along with the number of factors used in the calibrations; it was found that the NIR spectral region 1100–2200 nm was the best region for predicting CD with a 2-mm path length (Table 2). However, eliminating the wavelength region >2200 nm did not improve the results when using a 1-mm cuvette. Removing a spectral region that may contain useful information about the constituent of interest can degrade the prediction results; however, including spectral regions that do not contain useful information can also increase prediction errors. Previously, FSML regression found that the most influential wavelengths for measuring CD contents with a 2-mm path length are similar to the best wavelengths for the PV value at both 1- and 2-mm path lengths; however, key wavelengths for CD content when using a 1-mm cuvette ranged from 2300 to 2500 nm. Osborne *et al.* (28) indicated that the 2200–2400 nm region represents combination bands of –CH stretching motions and deformations, along with a few –OH combination bands.

Moreover, prediction was also carried out using an external validation set containing 30 samples from the three oils prepared at a different time. Results from the best PLS calibrations for 1- and 2-mm path lengths are shown in Table 2, and the best results from FSML models are shown in Table 3. Strong correlations between NIR-predicted values and reference data for CD in the external validation sample set were obtained for each path length. Overall, use of the PLS calibration technique did not give better prediction results than the calibration equations developed using FSML regression. Correlations between NIR-predicted CD values and those obtained by wet chemical analysis were not as strong as the correlations obtained for PV. When NIR and wet chemical results were compared, use of a 2-mm path length gave slope values closer to unity.

p-*AV.* The results showed that as storage time under light increased, the AV also continually increased in the SBO, which indicated an increase in oxidation. The increase in absorption at 350 nm resulted from reaction in an acetic acid solution of the aldehydic compounds in an oil and *p*-anisidine. 2-Alkenals especially will contribute substantially to the value found. The *p*-AV is defined by convention as 100 times the optical density measured in a 1-cm cell of a solution containing 1.00 g oil in 100 mL of a mixture of solvent and reagent (AOCS Method 18-90) (22). The pattern of changes in the AV contents for the samples was similar to the changes in their PV and CD values.

NIR and p-*AV.* Relatively strong correlations between NIR-predicted values and reference data for AV in both the calibration and validation sample sets for 1- and 2-mm path lengths were obtained. Calibrations were established using PLS and FSML regression methods. When using FSML and a 1-mm path length, a second derivative gave the highest r-value (0.858) and lowest SEP (0.481), but for the 2-mm path length, the first-derivative mathematical treatment gave the highest r value (0.635) and lowest SEP (0.709).

For FSML, a maximum of four wavelengths was used in the calibrations to prevent overfitting. The selected wavelengths 2452, 2398, 1554, and 1344 nm were used for measuring the AV content when using a second derivative and 1-mm path length. The selected wavelengths 2066, 2004, 1408, and 1398 nm were used for the AV content when using a first derivative and a 2-mm path length. Again note that at the longer path length, the nonlinear region >2200 nm is not used. There is no similar study in the literature with which to compare the results.

Compared to FSML regression, PLS regression gave higher r overall (Table 2). The best results were obtained when the PLS calibration method was used with a path length of 1-mm and wavelength range of 1100–2300 nm, or a 2-mm path length with wavelength range of 1100–2200 nm, respectively (Table 2). The PLS calibration equation using first derivative of log 1/*T* gave the lowest SEP with a 1-mm cuvette, and using the second derivative of log 1/*T* gave the lowest SEP with a 2-mm path length. Both calibrations gave good linearity with slope values approaching unity (Table 2). Overall, use of the PLS calibration technique gave much improved prediction results compared to calibration equations developed using multiple linear regression.

Additionally, prediction was also carried out using an external validation set containing 30 samples from the three oils prepared at a different time. Strong correlations between NIR-predicted values and reference data for AV in the external validation sample set were obtained for each path length, as well. Results from the best PLS calibration for 1- and 2-mm path lengths are shown in Table 2. However, a large systematic bias error occurred when the calibrations were applied to the external validation sample set. Even when a bias correction was applied, the SEP values were still relatively large compared to the range of AV values in the sample set. A comparison of NIR and wet chemical results yielded slope values far from unity. The results indicate that, although it appears possible to develop successful AV calibrations for narrowly defined sample sets, global calibrations applicable to a wide range of samples may be more difficult to achieve. The AV procedure involves the reaction of *p*-anisidine with carbonyl compounds in the oil, especially 2-alkenals. However, carbonyl groups do not have absorption bands in the NIR region. In general, only hydrogen-containing functional groups have overtone or combination bands that fall in the NIR region. Therefore, NIR correlations with AV are most likely secondary correlations, rather than direct measurements of carbonyl compounds, resulting in the problematic nature of predicting AV by NIR spectroscopy.

In summary, PV was successfully determined in SBO by NIR spectroscopy. Calibrations developed using PLS regression and a 2-mm path length resulted in the lowest SEP for both validation sets. Wavelengths in the 1100–2200 nm region were found to be the most useful for prediction. CD values were also successfully predicted in both validation sets, with FSML and PLS calibrations giving similar performance. Measurement of AV by NIR was less successful than measurement of the other two indices of oxidation.

Results obtained in this study indicate that NIR spectroscopy is a useful technique for measuring oxidation in SBO. The developed near-IR method is simple, fast, nondestructive, and safe, and by applying this technique, the amount of hazardous solvents as well as the cost of labor can be reduced dramatically. In addition, the potential of automatic sampling using a continuous-flow cell exists, which could improve convenience and sample throughput. The methods described provide a simple and direct means of determining the oxidation level of oils, giving results comparable to the AOCS reference methods.

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