Monitoring PV in Corn and Soybean Oils by NIR Spectroscopy

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ABSTRACT: NIR spectroscopy was used successfully in our laboratory to monitor oxidation levels in vegetable oils. Calibration models were developed to measure PV in both soy and corn oils, using partial least squares (PLS) regression and forward stepwise multiple linear regression, from NIR transmission spectra. PV can be measured successfully in both corn and soy oils using a single calibration. The most successful calibration was based on PLS regression of first derivative spectra. When this calibration was applied to validation sample sets containing equal numbers of corn and soy oil samples, with PV ranging from 0 to 20 meq/kg, a correlation coefficient of 0.99 between titration and NIR values was obtained, with a standard error of prediction equal to 0.72 meq/kg. For both types of oil, changes occurred in the 2068 nm region of the NIR spectra as oxidation levels increased. These changes appear to be associated with the formation of hydroperoxides during oxidation of the oils.

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KEY WORDS: Corn oil, edible oil, forward stepwise multiple linear (FSML) regression, lipid oxidation, multiple linear regression, near-infrared spectroscopy, partial least squares (PLS) regression, peroxide value, soy oil.

Existing analytical procedures for measuring the oxidation level in vegetable oils are time consuming, destructive to the sample, and costly, and they require large amounts of glassware, lipids, and potentially hazardous reagents. NIR spectroscopy, which can overcome many of these drawbacks, has many applications in the food industry, including fat and oil quality evaluation. In the recent past, a number of new spectroscopic methods have been developed for measuring lipid oxidation (1–5). These methods are simple, fast, and safe, and they dramatically reduce the labor and solvent needed. Previous work in our laboratory demonstrated that soybean oil oxidation, expressed as PV, can be determined successfully using NIR transmission spectroscopy as an alternative to the official AOCS iodometric titration method, and satisfactory repeatability was obtained by both methods (6).

In the present study, NIR spectroscopy was examined as a possible alternative to the conventional titration method for determining oxidation levels in corn oils, and calibrations were developed for quantitative determination of PV of corn oils. Additionally, the potential of using a single calibration to measure oxidation in oils from multiple sources was evaluated. Therefore, the objectives of the present research were threefold: (i) to investigate the relationship between NIR spectra and lipid oxidation in corn oil; (ii) to evaluate the success of the NIR models developed by testing each with an independent set of corn oil samples; and (iii) to create and evaluate calibrations encompassing both soybean oil and corn oil.

EXPERIMENTAL PROCEDURES

Selection of corn oil samples. Commercial corn oils (CO) were used for calibration development and validation. To achieve variation in the oils, five CO representing four commercial brands with different "sell by" dates and batch codes were purchased from local supermarkets in Lincoln, Nebraska. Samples with a range of oxidation levels as measured by PV were then prepared by subjecting each corn oil to fluorescent light for varying lengths of time, as previously described (6). The PV data from a comparable number of soy oils (SO) were also obtained from a previous study (6) and used to prepare a combined CO and SO set. Three additional CO samples were purchased at a later date and used to prepare an external validation set. Prediction was also carried out using an external validation set containing 24 samples from three additional SO prepared at different times and obtained from a previous study (6).

Reference procedure. The oxidation levels of these oil samples were determined by using the standard PV method (AOCS Method Cd 8-53) (7). 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.

NIR spectroscopy. Spectroscopic data were collected using a Foss NIRSystems Model 6500 scanning spectrophotometer (NIRSystems Division of Foss Electric, Silver Spring, MD) in the transmittance mode. Spectra were recorded in log 1/T format at 2-nm intervals from 400 to 2500 nm. Based on the best results from our previous study (6), a 2-mm pathlength quartz cuvette was used throughout this study. The Near Infrared Spectral Analysis Software (NSAS) package (version 3.53; Foss NIRSystems, Inc.) running under MS-DOS was used to collect, store, and manipulate data and to perform statistical analyses.

Collection of spectral data. Data collection was the same as in previous work (6) in that all samples were allowed to reach room temperature (25 ± 2°C) prior to collection of spectra because of the effect of temperature on spectral response (8). The spectrum of each individual oil sample was then

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TABLE 1
Characteristics of Reference Data for Calibration and Prediction Sets of Soybean Oils and Corn Oils

		Calibration set		Prediction set	
Oil	Constituent	n^a	Range (meq/kg)	n	Range (meq/kg)
Corn oil	PV	45	1.40–22.29	31	2.11–21.37
Soybean oil[b]	PV	75	0.30–20.85	44	0.20–23.45
Corn and soybean oil	PV	88	0.30–22.29	60	0.30–21.60

[a] *n*, number of samples.
[b] Yildiz *et al.* (6).

obtained by collecting and averaging 32 individual spectral scans of each sample.

Grouping of calibration and validation samples. Oxidized CO samples were randomly divided by the software into separate calibration and validation sets as shown in Table 1. The selected calibration samples were inspected to ensure that the extremes of PV were included in the set. The validation set was composed of those samples not used for calibration. The spectra of CO and SO samples were also combined into a single set ($n = 148$), which was then divided into calibration and validation sets in the same manner (Table 1). Moreover, prediction was also carried out using an external validation set prepared from three additional CO and SO samples representing three brands of each oil type, with different sell by dates and batch codes, purchased at a different time.

Calibration development and validation. Calibrations specific for CO samples and calibrations applicable to both SO and CO samples were developed. To select wavelengths and relate log 1/T values to PV from each set of spectra, multivariate regression was used. NIR calibration equations were developed using both modified partial least squares (PLS) regression (9) and forward stepwise multiple linear (FSML) regression techniques (10). In the present research, these regression techniques were applied to the log 1/T spectra, as well as first and second derivatives of raw log 1/T spectra, and the prediction results obtained from each mathematical treatment were compared to select the best one. 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 (11). Each optimized CO calibration was tested with the set of 31 validation samples. Also, each optimized calibration that included both SO and CO was tested with 60 validation samples including a comparable number of SO and CO. Correlation coefficients, standard errors of prediction (SEP), and slope values were compared to evaluate which calibrations were most successful at measuring PV in vegetable oil. Moreover, prediction was also carried out using the external validation set of CO ($n = 34$) and SO ($n = 24$).

RESULTS AND DISCUSSION

Preparation of calibration and validation sample sets. Five commercial CO were used to prepare a calibration set containing a range of PV (Table 1). Before exposure to UV light, the PV of these samples ranged from 1.40 to 2.51 meq/kg. 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 (12). Thus, the range of PV values selected for this study covered oxidation levels that may be routinely encountered in commercial samples.

NIR spectroscopy. Spectra of all oil samples were collected under the same NIR operating conditions. Strong correlations between NIR-predicted values and reference data for PV in both the calibration and validation CO sample sets were obtained (Table 2). From Table 2, it can be seen that *N*-point smoothing, first derivative, and second derivative mathematical treatments performed satisfactorily for measuring PV in CO. Calculating derivatives of spectra can remove scattering and other spectral differences that result in both slope differences and offsets among spectra. To obtain quality FSML results, the choice of appropriate wavelengths is important. The wavelengths selected by the computer that produced calibration equations with the lowest SEC and highest *R* are given in Table 2. Table 2 shows that of all the FSML calibrations tested, the log 1/T mathematical treatment gave the highest *r*-value (0.991) and lowest SEP (0.76 meq/kg) for the samples in the prediction set.

For FSML, a maximum of four wavelengths was used in the calibrations to prevent overfitting. It can be seen in Table 2 that 2064, 2022, and 1738 nm are the key wavelengths for measuring lipid peroxide when using log 1/T. A wavelength in the 2064–2068 nm region appears to be highly significant. Takamura *et al.* (2) found a similar result: 2084 nm was the key wavelength for lipid peroxides 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, a single wavelength approach can be complicated by a large number of potential spectral interferences from other OH-containing components, such as alcohols, FFA, water, and MG present in oil, all of which exhibit absorptions that overlap with the hydroperoxide band and interfere with the hydroperoxide determination (1). Use of a multiwavelength approach helps to eliminate interferences from other absorbing substances. Cho *et al.* (5) coupled measurements at 2080 nm with those at 2020 nm to estimate PV in soybean oil.

Prediction of PV for the validation samples using PLS calibrations indicated the applicability of the obtained equations (Table 2). Simple correlation coefficients (*r*) ranged from 0.984 to 0.991. SEP ranged from 0.75 to 1.03 meq/kg. These high correlation coefficients demonstrate that the NIR transmittance procedure is an accurate method for the determination of oxidation level in CO. A PLS equation using five factors and first derivative mathematics resulted in an *R* of 0.987

TABLE 2
Results of Selected Forward Stepwise Multiple LInear (FSML) and Partial Least Squares (PLS) Models for Measuring PV of Corn Oil Samples

FSML regression	λ (nm)	Calibration Multiple *R*	Calibration SEC (meq/kg)	Prediction Correlation (*r*)	Prediction SEP (meq/kg)	Prediction Slope adj.
Log 1/T *N*-point Smooth	2064 1738 2022	0.983	1.06	0.991	0.76	1.025
1st derivative Segment 5 Gap 5	2068 1242 1612 2016	0.989	0.93	0.981	1.25	1.033
2nd derivative Segment 10 Gap 5	2080 2200 1384 1446	0.981	1.14	0.991	0.83	1.007

PLS regression	# of factors	Calibration Multiple *R*	Calibration SEC (meq/kg)	Prediction Correlation (*r*)	Prediction SEP (meq/kg)	Prediction Slope adj.
Log 1/T *N*-point Smooth	7[a]	0.993	0.71	0.986	0.94	1.016
1st derivative Segment 5 Gap 5	5[b]	0.987	0.95	0.991	0.75	1.032
2nd derivative Segment 10 Gap 5	6[b]	0.993	0.70	0.984	1.03	1.041

[a] Spectral range = 1100–2100 nm.

[b] Spectral range = 1100–2300 nm. Abbreviations: SEC, standard error of calibration; SEP, standard error of prediction.

and SEC of 0.95 meq/kg. More PLS factors can be used in a calibration than discrete wavelengths without overfitting because the factors are not correlated. Prediction of 31 validation samples using this calibration model resulted in an *r*-value of 0.991 and an SEP of 0.75 meq/kg (Table 2); the plot was linear (Fig. 1) and had a slope close to unity. In our previous study investigating NIR measurement of PV in SO, PLS regression gave better validation results than FSML regression (6). However, in this study, FSML methods worked as well as PLS regression for predicting PV in CO.

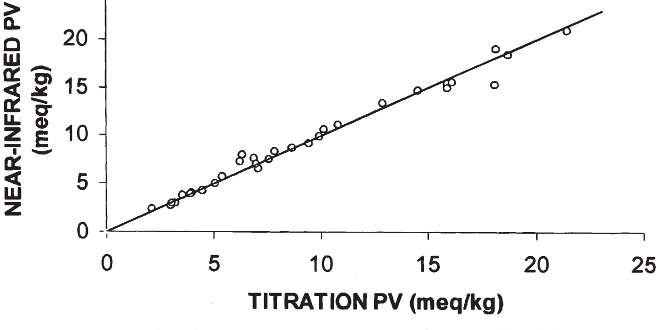

FIG. 1. Scatter plot diagram comparing PV of corn oil validation samples. Abscissa values are the PV of validation samples as determined by the reference procedure. Ordinate values are the PV as determined by NIR using first derivative of log 1/T and partial least squares calibration.

PLS 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 for PV determination, along with the number of factors used in the PLS calibrations; it was demonstrated that the NIR spectral region from 1100 to 2300 nm was the best region for predicting PV. Eliminating the wavelength region >2300 nm improved the results. Removing such 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. Observation of the PLS loadings for the CO showed structure in the same regions as the wavelengths selected by FSML. The PLS loadings revealed visible differences around 1200 to 1400 nm, 1700 nm, and 2000 to 2200 nm. Most influential wavelengths for measuring PV contents (Table 2) can be interpreted in terms of the chemical characteristics of different types of oxidized compounds in CO and by reference to previously known chemical assignments (13). Absorptions around 2060–2080 and 1400 nm are likely first overtones and combinations of O–H stretching vibrations. The region around 1700 nm shows

TABLE 3
Best Calibration and Prediction Results for PV of Soybean and Corn Oils from Single and Combined Oil Calibrations[a]

				Calibration		Prediction		
Oil type	Constituent	Math treatment	# of PLS factors	Multiple *R*	SEC (meq/kg)	Correlation (*r*)	SEP (meq/kg)	Slope adj.
Corn oil	PV	1st derivative Segment 5 Gap 5	5[b]	0.987	0.95	0.991	0.75	1.032
Soybean oil[c]	PV	1st derivative Segment 5 Gap 5	13[d]	0.996	0.56	0.994	0.72	1.011
Corn and soybean[e] oils	PV	1st derivative Segment 10 Gap 5	12[d]	0.993	0.75	0.992	0.72	1.008

[a] PLS, partial least squares; see Table 2 for other abbreviations.
[b] Spectral range = 1100–2300 nm.
[c] Yildiz *et al.* (6).
[d] Spectral range = 1100–2200 nm.
[e] Equal numbers of corn and soybean oils.

absorption due to the first overtones of the C–H stretching vibrations and some O–H stretch first overtones. Osborne *et al.* (14) also indicated that the 1200–1400 nm region included –CH stretch second overtones, some –CH stretch and deformation combination bands, and –OH first overtones.

Calibrations combining both SO and CO into the same model were also developed. Results for the best PLS calibration encompassing both CO and SO, along with results from the best single oil calibrations, are shown in Table 3. Calibrations specific to CO required fewer PLS factors than those including SO (Table 3). Oxidation in CO causes a greater spectral change in the 1100–1500 nm region than is observed in SO. These relatively large spectral changes occurring in CO allow PV to be predicted with a minimum number of PLS terms. When SO is included in the calibrations, smaller and more subtle changes that occur in the 2068 nm region in both CO and SO may become more important to the prediction, thus requiring more terms in the PLS model. Again, eliminating wavelengths >2200 nm from the PLS model improved the prediction results (Table 3). NIR and wet chemical results for PV are graphically compared in Figure 2 for the validation samples. For the validation set, a linear relationship was obtained for PV ($r = 0.99$) when compared with the accepted reference procedure. The plot of NIR predicted vs. reference data was linear and had a slope close to unity (Fig. 2, Table 3). Statistical analysis indicated that chemical and instrumental results have comparable means. A ratio error range (RER) value was also calculated. The RER is the ratio of the range in reference values of the validation samples divided by the SEP. An RER value of 29.6 was obtained for the samples in the validation set. NIR methods with RER values ranging from 21 to 30 are considered to have good performance and are useful for quality control applications (11). Since the mixed calibration for SO and CO samples yielded results comparable to single oil calibrations, specific calibrations for CO and SO should not be needed. However, further investigation will be needed to create a universal calibration that includes other vegetable oils.

Additionally, prediction using the combined calibration was carried out using an external validation set prepared from three CO and three SO obtained at a different time. Strong correlations between NIR-predicted values and reference data for PV were obtained in the external CO validation sample set ($r = 0.995$ and SEP = 0.77 meq/kg) and in the external SO validation sample set ($r = 0.999$ and SEP = 0.52 meq/kg).

Results obtained in this study indicate that NIR transmittance spectroscopy is an effective, reproducible, and rapid alternative method capable of replacing the standard titration method currently in use for measuring PV as an index of oxidation in both SO and CO. Moreover, a mixed calibration for both SO and CO samples has been successfully developed.

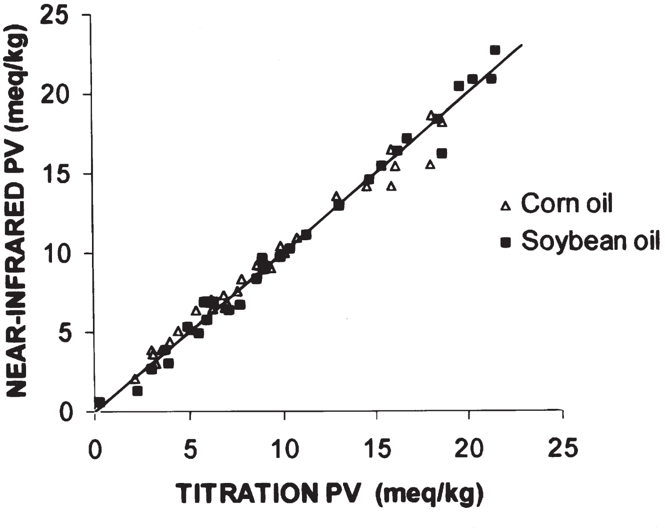

FIG. 2. Scatter plot diagram comparing NIR and reference PV values of mixed corn and soybean oil validation samples. NIR predicted values were determined using one calibration encompassing both soybean and corn oil samples.

Once the NIR instrument is precalibrated, the total analysis takes less than 3 min. The NIR 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. Any scanning or Fourier-transform NIR instrument capable of transmission measurements in the 1100–2500 nm region should be adaptable to this measurement. In addition, the method should be adaptable to an automated, continuous-flow sampling system. Further investigation may enable establishment of a universal calibration that includes other vegetable oils in addition to SO and CO.

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