The Determination of Peroxide Value by Fourier Transform Infrared Spectroscopy

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A rapid method for the quantitative determination of peroxide value (PV) of vegetable oils by Fourier transform infrared (FTIR) transmission spectroscopy is described. Calibration standards were prepared by the addition of *t*-butyl hydroperoxide to a series of vegetable oils, along with random amounts of oleic acid and water. Additional standards were derived through the addition of mono- and diglyceride spectral contributions, as well as zero PV spectra obtained from deuterated oils. A partial least squares (PLS) calibration model for the prediction of PV was developed based on the spectral range 3750–3150 cm^{-1}. Validation of the method was carried out by comparing the PV of a series of vegetable oils predicted by the PLS model to the values obtained by the American Oil Chemists' Society iodometric method. The reproducibility of the FTIR method [coefficient of variation (CV) = 5%)] was found to be better than that of the chemical method (CV = 9%), although its accuracy was limited by the reproducibility of the chemical method. The method, as structured, makes use of a 1-mm CaF_2 flow cell to allow rapid sample handling by aspiration. The spectrometer was preprogrammed in Visual Basic to guide the operator in performing the analysis so that no knowledge of FTIR spectroscopy is required to implement the method. The method would be suitable for PV determinations in the edible oil industry and takes an average of three minutes per sample.

KEY WORDS: FTIR, hydroperoxides, lipid oxidation.

The oxidation of fats and oils is an important deteriorative reaction with significant commercial implications in terms of product value. The initial oxidation products that accumulate are hydroperoxides, which may subsequently break down to form lower-molecular weight compounds such as alcohols, aldehydes, free fatty acids and ketones, leading to autoxidative rancidity (1,2). Analytically, the American Oil Chemists' Society (AOCS) peroxide value (PV) determination is the standard method used to determine hydroperoxides in the edible oil industry, either directly or in a controlled fashion *via* the active oxygen method, to determine the oxidative stability of an oil (3). Our research group has been working on the development of rapid and automatable Fourier transform infrared (FTIR) spectroscopic methods for the quantitative analysis of fats and oils, and we have recently developed such methods to determine iodine value (IV), saponification number (SN) (4) and free fatty acids (FFA) (5). As PV determinations are commonly performed in oil analysis laboratories, FTIR spectroscopy was explored as a means of automating this analysis.

It has been recognized for some time that hydroperoxide functional groups can be quantitatively determined by infrared (IR) spectroscopy. In 1972, Fukuzumi and Kobayashi (6) reported a linear relationship between the intensity of the hydroperoxide absorption band at 3550 cm^{-1} in CCl_4 and the iodometric PV value for fatty acid methyl ester hydroperoxides. This basic information has not been utilized in any practical way, largely because of the limitations associated with dispersive instrumentation available at that time and the relatively poor quantitative capabilities associated with conventional IR spectroscopy. The advent of FTIR spectroscopy, based on interferometry, has substantially enhanced the sensitivity and quantitative accuracy of IR measurements. The superior performance of FTIR instruments over traditional dispersive instruments is a result of their higher energy throughput, their multiplexing capability (i.e., simultaneous detection of all frequencies), higher signal-to-noise ratio and the use of a laser for internal wavelength calibration (7). Because FTIR systems require on-line computing capabilities, most units come equipped with powerful software-based data handling routines, providing the capability for repetitive scanning to improve the signal-to-noise ratio, macro-command language programming facilities to automate routine operations and sophisticated chemometric software for multicomponent analysis. As such, FTIR spectroscopy not only provides substantial spectral information about the functional groups present in a sample but also provides a means of data processing and automating an analysis if an appropriate means of calibration can be devised.

In preliminary development work carried out on the use of FTIR spectroscopy as a method for the determination of PV, a relatively simple calibration approach was attempted first. It was based on the use of *t*-butyl hydroperoxide (TBHP) as a standard, added to peroxide-free canola oil, and ratioing the spectrum of the spiked oil against that of the peroxide-free oil to obtain a clearly resolved hydroperoxide band. This approach, with a simple dual wavelength calibration (3444/2225 cm^{-1}), produced excellent calibration curves and predictions for that base oil. However, predictions for oils other than the base oil did not always correspond well with the chemical PV values. Based on a careful analysis of the FTIR spectra of other oils, relative to the base canola oil used (8), it became apparent that variable amounts of other OH-containing components present in each oil, i.e., alcohols, FFAs and water, all of which exhibit absorptions that overlap with the hydroperoxide band, interfere with the hydroperoxide determination. Variations in the triglyceride composition of the oils are also possible sources of spectral variability. With so many potential sources of interference and variability affecting the PV determination, more sophisticated chemometric techniques were called for. For situations of this nature, one of the most powerful chemometric approaches available is the partial least squares (PLS) method (9), which is capable of accounting for interactions, underlying absorptions, overlapping bands and other factors that may affect the spectra as the concentrations of all components change. PLS is a powerful tool in such circumstances and has been used successfully to develop a robust calibration for the direct prediction of IV and SN of edible fats and oils (4). This paper describes the development of a practical, automated FTIR method to determine PV, illustrating the utility of PLS for multivariate analysis of FTIR data.

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EXPERIMENTAL PROCEDURES

Instrumentation/sample handling. IR spectroscopy was carried out with a Nicolet (Madison, WI) "Impact 400" FTIR spectrometer, interfaced to a 486/33MHz PC operating under Windows-based Nicolet Omnic 1.1 software. The instrument and sample compartment were purged with dry air produced by a Balston dryer (Balston, Lexington, MA) to minimize water vapor and CO_2 interferences. Sample handling involved the aspiration of oil samples through a 1022-μm pathlength CaF_2 flow cell *via* 1/16″ i.d. silicone tubing, allowing the rapid filling and emptying of the cell. A valve was used to control the vacuum, thereby regulating the filling of the cell and facilitating the sequential analysis of samples. The cell was purged of the previous sample by passing ≈3 mL of the next sample through the cell, and a trap was used to collect spent oil. The cell was rinsed with hexane every ≈5 samples to avoid oil build-up on the cell windows. Spectra were recorded by co-adding 128 scans at a resolution of 4 cm^{-1} and were ratioed against a 512-scan background spectrum recorded from the clean, empty cell.

Reagents/standards. A 3M solution of TBHP in isooctane was obtained from Aldrich Chemical Company (Milwaukee, WI) and used for the preparation of calibration standards. This solution was diluted with isooctane, and its PV was determined in triplicate by the AOCS iodometric method (3). Six commercial edible oils (olive, corn, soy, sunflower, canola and peanut) were purchased locally and analyzed in duplicate for their PV. A set of 30 primary calibration standards were prepared by adding TBHP, oleic acid and water in random amounts (w/w) to the six base oils. The PV of the standards was calculated from the PV of the base oil plus the PV contributed by the added TBHP. A series of fractionally distilled monoglycerides derived from vegetable oils was provided by Eastman Chemical Co. (Rochester, NY), and diglycerides (1,3-dilinolein and 1,3-dielaidin) were obtained from Sigma Chemical Co. (St. Louis, MO). These mono- and diglycerides were added to canola oil at a ≈1% level, and the FTIR spectra of the mono- and diglyceride-spiked oils and that of unspiked canola oil were recorded. The spectra of the spiked oils were ratioed against that of the unspiked canola oil to obtain difference spectra representative of mono- and diglycerides in an oil. The primary calibration set was further expanded through the technique of spectral addition and subtraction (4), incorporating contributions of the mono- and diglycerides and the inclusion of overtone bands representative of the six calibration oils. To obtain overtone spectra (free of hydroperoxides, FFA and alcohols) 500 μL D_2O was added to 5 mL of each of the base oils. After centrifugation to remove excess D_2O, the spectra of the deuterated samples were recorded, all the OH absorptions having been shifted to lower frequencies due to H–D exchange. These spectra were also multiplied by a scaling factor (0.8–1.2) to provide variability in the intensity of the overtone band to account for potential variability in SN. In total, the final calibration set was composed of 81 spectra (standards).

Calibration/validation. Calibration development was carried out by using the Nicolet QuantIR® Calibration and Prediction Package (10), which includes a PLS calibration routine. The optimum number of spectral factors to be included in the calibration model was selected on the basis of significant changes based on the F-statistic in the predicted residual error sum of squares (PRESS) test. The "leave one out" cross validation routine was used to assess the predictive accuracy of the calibration model. Further validation of the calibration model developed was carried out by chemically analyzing 25 vegetable oils in varying states of oxidation for their PV and comparing the FTIR-predicted results to those obtained by the chemical method.

RESULTS

General spectroscopy. Hydroperoxide moieties exhibit characteristic absorption bands between 3600 and 3400 cm^{-1} due to their -OO-H stretching vibrations, with the peak maximum being a function of the polarity of the medium and the extent of hydrogen bonding (11). Figure 1 (bottom panel) illustrates the 3800–3200 cm^{-1} region of the spectrum of a sample of canola oil with a PV of ≈80 recorded in a 100-μm CaF_2 cell. The band observed at 3473 cm^{-1} is the overtone of the triglyceride ester carbonyl absorption (1748 cm^{-1}), being approximately double its frequency. No apparent hydroperoxide absorption band is discernible in this spectrum. One of the strengths of FTIR spectroscopy is its ability to ratio out common features in the spectra of two samples that differ in a constituent. Upon ratioing the oxidized canola oil against the same oil free of hydroperoxides, a band centered at 3444 cm^{-1} appears [Fig. 1 (center panel)]. The low intensity of the hydroperoxide absorption band in Figure 1 (center panel) indicates that a longer pathlength cell is required to measure PV at the levels found in processed oils (PV < 10). Accordingly, a 1022-μm CaF_2 cell was assembled to maximize the hydroperoxide absorbance without losing detector linearity. Figure 1 (top panel) is a spectrum of TBHP spiked oil in the 1022-μm cell ratioed against the spectrum of a peroxide-free oil, illustrating the absorption band of TBHP, which is similar to the "natural hydroperoxide" band in the spectrum of the oxidized oil. The TBHP band is centered at 3444 cm^{-1}, confirming the assignment of this band in the spectra of oils to the -OO-H stretching vibration of hydroperoxides. This simple TBHP spiking and ratioing approach was used to develop our original dual-wavelength ratio calibration procedure, mentioned briefly in the introduction.

Although TBHP is not representative of lipid hydroperoxides in its chemical behavior (it is quite stable), its spectral behavior is similar to that of hydroperoxides formed in oxidizing oils, as illustrated in Figure 1. In addition, the extinction coefficient determined for the hydroperoxide band of TBHP by serial dilution in oils was not significantly different from the extinction coefficient of hydroperoxides formed under accelerated oxidative conditions in any of the six base oils. As such, TBHP appears to be spectroscopically representative of lipid hydroperoxides in general and can therefore be used as a convenient, stable standard. Preparing calibration standards by adding known weights of TBHP to oils avoids the extensive analytical effort usually exerted in calibrating a secondary method, such as FTIR, against a primary chemical method. As noted in the introduction, although excellent calibrations were obtained by the ratioing technique, with an accuracy of ±0.65 PV relative to the calculated value obtained from the amount spiked and a reproducibility

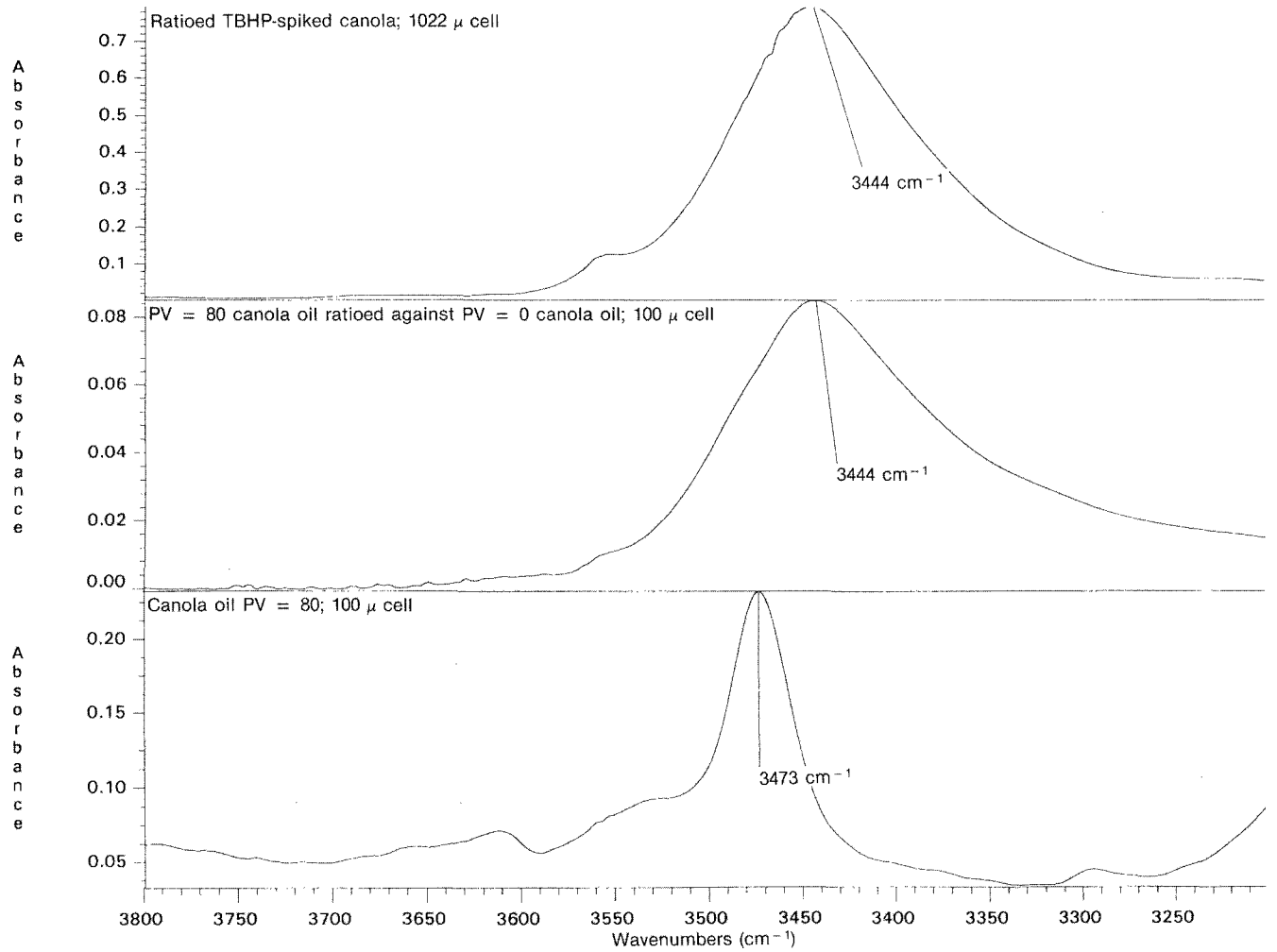

FIG. 1. Bottom panel: Fourier transform infrared spectrum of the 3800–3200 cm^{-1} region of canola oil [peroxide value (PV) = 80] in a 100-μm CaF_2 transmission cell. Middle panel: spectrum of the same sample as in the bottom panel, ratioed against the spectrum of peroxide-free canola oil. Top panel: spectrum of *t*-butyl hydroperoxide (TBHP)-spiked canola oil in a 1022-μm cell ratioed against the spectrum of peroxide-free canola oil.

of ±0.35 PV spectroscopically, this approach failed because real oils contained varying amounts of other OH-containing constituents (water, FFA and alcohols), which threw off the FTIR predictions to such an extent that the method was unworkable. For these reasons we had to abandon this relatively simplistic approach and make use of a more sophisticated chemometric method, PLS.

PLS calibration. The power of PLS is based on its ability to mathematically correlate spectral changes to changes in the concentration of a component of interest while simultaneously accounting for all other significant spectral factors that perturb the spectrum (9). As such, any calibration to be devised must account for all the variability that may be encountered in the samples to be analyzed. Based on detailed spectral investigations of oils undergoing oxidation under various conditions (8), the main sources of variation that can be expected to interfere with the determination of hydroperoxides were found to be moisture, FFAs and alcohols (mainly mono- and diglycerides) and variability in the overtone band, which is dependent upon the SN of the oils being investigated. Accordingly, a global set of calibration standards was devised with TBHP to model the spectral contribution of lipid hydroperoxides by including water, FFAs, mono- and diglycerides and overtone variations in random amounts. The calibration standards were designed to meet the two requirements for a valid PLS calibration: (i) that no correlation exists between the concentrations of the interfering components and the component of interest; and (ii) that the concentration range of such components encountered in the samples to be analyzed is adequately spanned. For this calibration we chose a FFA range of 0–2%, mono- and diglycerides from 0–1.5% and water from zero to saturation (≈1%), which covered the ranges encountered in the samples we originally had problems with in our dual-wavelength PV method.

Table 1 summarizes the 81 calibration standards prepared and used to develop the PLS calibration. Standards 1–30 are the primary calibration standards, physically prepared by adding increasing amounts of TBHP and random amounts of water and FFA to the six base oils. Additional standards were generated by adding or subtracting spectral contributions from a series of mono- and diglycerides. For the spectral contributions of the mono- and

TABLE 1

Calibration Mixture for Partial Least Squares Calibration[a]

S#	Oil + Component	PV	S#	Oil + Component	PV	S#	Oil + Component	PV
1	Can + W + O	3.58	28	Sun + O + T	18.20	55	Pnt OVT	0.00
2	Can + W + O + T	13.97	29	Sun + T	28.56	56	Soy OVT	0.00
3	Can + T	23.74	30	Sun + W + O	39.40	57	Sun OVT	0.00
4	Can + T	32.92	31	S02 + M	13.97	58	S03 + D	23.74
5	Can + O + T	42.74	32	S07 + M	4.72	59	S05 + D	42.74
6	Crn + O	1.54	33	S10 + M	34.07	60	S07 + D	4.72
7	Crn + T	4.72	34	S12 + M	23.12	61	S09 + D	24.97
8	Crn + T	14.49	35	S16 + M	6.10	62	S11 + D	11.63
9	Crn + W + O + T	24.97	36	S18 + M	26.32	63	S13 + D	32.25
10	Crn + W + O + T	34.07	37	S20 + M	47.23	64	S16 + D	6.10
11	Olv + W + O	11.63	38	S22 + M	11.91	65	S18 + D	26.32
12	Olv + O + T	23.12	39	S25 + M	41.25	66	S21 + D	1.67
13	Olv + O + T	32.25	40	S28 + M	18.20	67	S24 + D	31.11
14	Olv + O + T	40.50	41	S30 + M	39.40	68	S27 + D	9.18
15	Olv + O	51.59	42	S01 − M	3.58	69	S28 + D	28.56
16	Pnt + W + O	6.10	43	S09 − M	24.96	70	Can VOVT	0.00
17	Pnt + T	16.62	44	S12 − M	23.12	71	Can VOVT	0.00
18	Pnt + T	26.32	45	S14 − M	40.50	72	Crn VOVT	0.00
19	Pnt + O + T	35.00	46	S17 − M	16.62	73	Crn VOVT	0.00
20	Pnt + O + T	47.23	47	S19 − M	35.00	74	Olv VOVT	0.00
21	Soy + W + O	1.67	48	S20 − M	47.23	75	Olv VOVT	0.00
22	Soy + O + T	11.91	49	S22 − M	11.91	76	Pnt VOVT	0.00
23	Soy + O + T	21.87	50	S26 − M	3.50	77	Pnt VOVT	0.00
24	Soy + O + T	31.11	51	S30 − M	39.40	78	Soy VOVT	0.00
25	Soy + W + T	41.25	52	Can OVT	0.00	79	Soy VOVT	0.00
26	Sun + O	3.50	53	Crn OVT	0.00	80	Sun VOVT	0.00
27	Sun + W + T	9.81	54	Olv OVT	0.00	81	Sun VOVT	0.00

[a]Base oils: canola (Can), corn (Crn), olive (Olv), peanut (Pnt), soybean (Soy) and sunflower (Sun). Other abbreviations: W, water; O, oleic acid; T, *t*-butyl hydroperoxide; S, selected samples; M, monoglycerides; D, diglycerides; OVT, overtone bands of each oil; VOVT, variations of OVT; PV, peroxide value.

diglycerides to be representative of real samples, the spectra of the individual mono- and diglycerides dissolved in an oil were recorded (the spectrum of the oil itself ratioed out being 1) to ensure that any hydrogen-bonding effects and other interactions were represented in their spectra. The remaining standards are simply the spectra of deuterated base oils, where deuteration clears the OH stretching region and leaves only the overtone band. The overtone spectra set was generated by multiplying standards 52–57 by scaling factors in the range of 0.8–1.2 to account for any variability in the overtone band between oils resulting from differences in SN. These overtone spectra also served as definitive spectra for zero PV. This technique of generating additional calibration standards by spectral co-addition was used successfully in developing PLS calibrations for the determination of IV and SN (4), minimizes the actual number of samples that must be prepared and provides an efficient means of introducing additional variability to the calibration set. Figure 2 shows the overlaid spectra of the 81 standards and illustrates the spectral variability associated with the various components present in the standards.

With this global calibration matrix, an optimal PLS calibration was developed to predict the PV of oils by using the spectral range of 3750–3150 cm^{-1}, with 3750 cm^{-1} as a single point baseline. The selection and optimization of the wavelength range, the type of baseline configuration, number of factors and assessment of the validations is an iterative process, guided by the interpretation of the spectra and statistical parameters such as the PRESS test (10).

Figure 3 illustrates the calibration plot obtained from the PLS calibration model in terms of predicted vs. actual PV for the 81 standards, with an overall mean error of 0.85 PV and a standard deviation of 1.3 PV. The "leave one out" cross validation procedure, designed as a preliminary test of the accuracy of the calibration, indicated that the predictions should be accurate to within ± 1.2 PV. Examination of the validation plot did not reveal any obvious outliers in the calibration set.

Analysis of oils for PV. To verify the ability of the calibration to perform adequately with real unknown samples, 25 pre-analyzed vegetable oils that covered a wide range of PV were predicted by FTIR. Figure 4 presents a plot of the predicted vs. chemical PVs for these samples, which produced an overall standard error of prediction of 2.66 PV. Based on an average PV of 23.9 for these samples, an SE of 2.66 PV represents a coefficient of variation (CV) of ≈11.1%, compared to an overall CV of 9.0% for duplicate chemical analyses of these samples. In terms of reproducibility of the FTIR method, duplicate analyses had a standard deviation of the difference for reproducibility (SDD_r) of ≈ 1.3 PV, compared to an SDD_r of 2.15 PV for duplicate chemical analyses. As such, the overall reproducibility of the FTIR method is better than that of the chemical method;

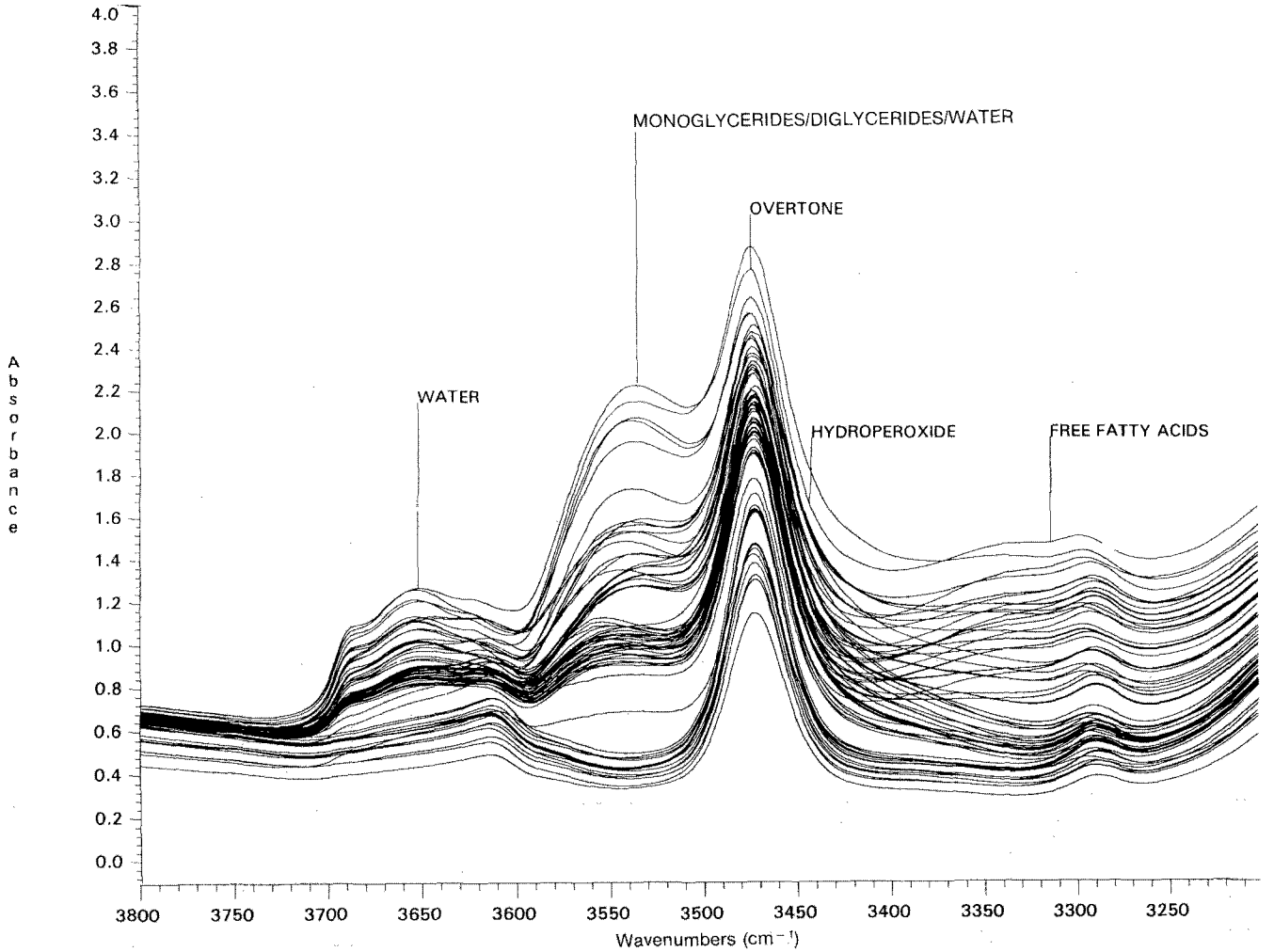

FIG. 2. Overlaid Fourier transform infrared spectra in the 3800–3200 cm^{-1} region of the 81 standards used for the partial least squares calibration, indicating the key sources of variability in the spectra.

however, the accuracy of the FTIR method is inherently limited by the reproducibility of the chemical method against which it is calibrated.

DISCUSSION

In our initial approach to the development of an FTIR PV method, we had hoped to obtain a relatively simple calibration based on ratioed spectra with TBHP as a standard. It was found that spectrally the situation was much more complicated due to interferences being caused by other OH-containing components, including alcohols, FFAs and water, which may be present in variable amounts. PLS provided a means of accounting for such variability and producing a robust calibration. However, development of such a calibration requires a detailed understanding of the interfering spectral contributions, their magnitude and the means by which this variability could be incorporated. As such, the development of a PV calibration is a fairly complex undertaking, and would be difficult for anyone not familiar with FTIR spectroscopy and PLS.

In this paper, we have succeeded in our primary objective of developing a workable calibration that allows one to directly predict the PV of an oil from its FTIR spectrum. Several additional objectives must be met to implement this method for routine analysis. First, the instrument must be pre-programmed so that the operator does not require any knowledge of FTIR spectroscopy and is guided through the analysis by prompts/instructions from the PC interfaced to the spectrometer. This has been accomplished by programming the analytical procedure through the use of Microsoft Visual Basic within the Macros/Pro programming environment of the Nicolet Omnic software (10) that drives the spectrometer. A PV analysis program has been developed that prompts the operator to aspirate the sample into the cell, scans the oil and predicts PV by means of the PLS calibration model developed. The second requirement for the practical application of this technology is to achieve transferability of the calibration so that the method can be implemented on different instruments without the need for recalibration. We have described the basis for such a calibration transfer

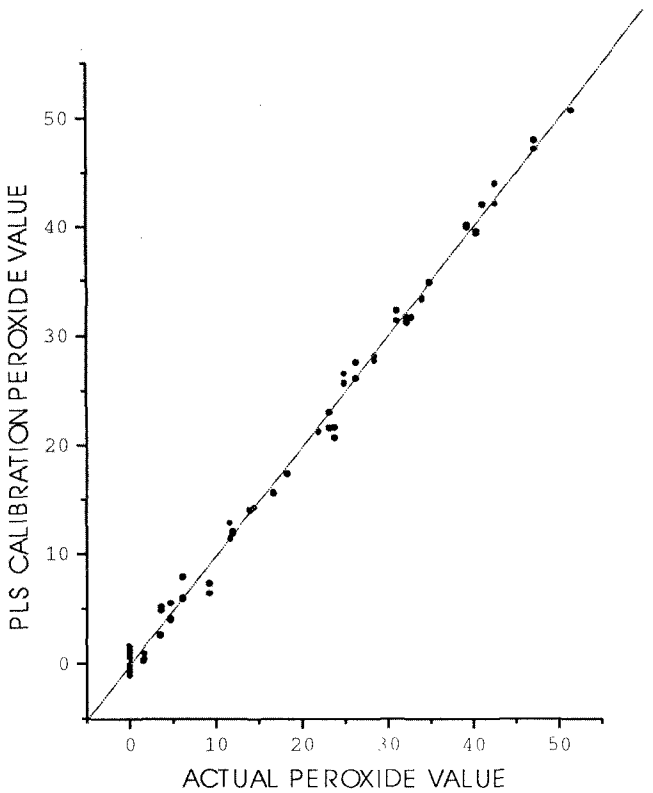

FIG. 3. Plot of the predicted peroxide value vs. the actual peroxide value for the 81 calibration standards as derived from the partial least squares calibration (PLS).

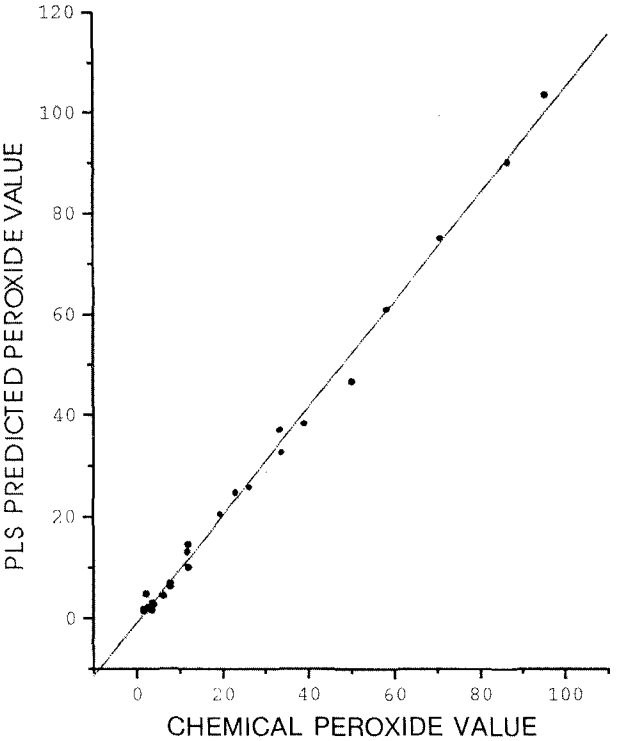

FIG. 4. Plot of the peroxide value predicted by the PLS calibration for 26 vegetable oils vs. their chemical peroxide value. See Figure 3 for abbreviation.

technique, based on the use of triolein as a calibration transfer standard (4). We are currently studying the applicability of this approach to the PV calibration, which we will report in a subsequent paper.

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