Determination of Peroxide Value by Fourier Transform Near-Infrared Spectroscopy

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ABSTRACT: A Fourier transform-near infrared (FT-NIR) method originally designed to determine the peroxide value (PV) of triacylglycerols at levels of 10–100 PV was improved upon to allow for the analysis of PV between 0 and 10 PV, a range of interest to the edible oil industry. The FT-NIR method uses convenient disposable glass vials for sample handling, and PV is determined by spectroscopically measuring the conversion of triphenylphosphine (TPP) to triphenylphosphine oxide (TPPO) when reacted with hydroperoxides. A partial-least-squares calibration was developed for 8 mm o.d. vials by preparing randomized mixtures of TPP and TPPO in a zero-PV oil. The method was validated with samples prepared by gravimetric dilution of oxidized oil with a zero-PV oil. It was shown that the American Oil Chemists' Society primary reference method was quite reproducible (±0.5 PV), but relatively insensitive to PV differences at lower (0–2) PV. The FT-NIR method on the other hand was shown to be more accurate overall in tracking PV, but slightly less reproducible (0.9 PV) due to working close to the limit of detection. The sensitivity and reproducibility of the FT-NIR method could be improved upon through the use of larger-diameter vials combined with a detector having a wider dynamic range. The proposed FT-NIR PV method is simple to calibrate and implement and can be automated to allow for routine quality control analysis of edible fats and oils.

Paper no. J9221 of *JAOCS 77,* 137–142 (February 2000).

KEY WORDS: Fourier transform infrared spectroscopy, FT-IR, FT-NIR, oil analysis, peroxide value.

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 in triacylglycerols are hydroperoxides, which may subsequently break down to form lower-molecular weight compounds, such as alcohols, aldehydes, free fatty acids, and ketones, ultimately leading to rancid product. There are two standard chemical methods (Cd 8b-90 and Cd 8-53) approved by the American Oil Chemists' Society (AOCS) for the determination of hydroperoxides (1). Both are iodometric procedures for determining peroxide value (PV), differing only in the solvent used. Although claimed to be relatively simple, reasonably sensitive, reliable, and reproducible, the iodometric method is labor-intensive and uses significant amounts of reagents and solvents of environmental concern.

The McGill IR Group has worked on the development of methods for the edible oil industry based on Fourier transform infrared (FTIR) and near-infrared (FT-NIR) spectroscopy that allow quantitative analyses to be carried out directly on neat fats and oils, conferring the advantages of analytical speed and automation (2). In terms of PV methodology development, the first FTIR method developed was based on the measurement of the characteristic O-H stretching absorption band of hydroperoxides in the mid-IR (3). Subsequently, a simpler and more accurate mid-FTIR method (4) was developed, based on the well-characterized stoichiometric reaction of triphenylphosphine (TPP) with hydroperoxides to form triphenylphosphine oxide (TPPO). This reaction (Fig. 1) is rapid and complete when an excess of TPP is present. Accurate quantitation of the TPPO is readily achieved by measuring the intensity of the unique and intense mid-IR absorption band of TPPO at 542 cm^{-1}. The mid-IR method developed was accurate, reproducible, and very sensitive, capable of measuring PV down to ~0.2 PV (4,5).

Subsequent work related to monitoring the progress of oxidation in rapeseed lubricants led to the development of an FT-NIR method for PV determination (6). Based on the same concepts as the mid-IR method, the FT-NIR approach provided access to a simpler and more convenient sample handling system, making use of readily available glass vials. Because the objective was to monitor oxidative stress, in a manner analogous to the active oxygen method, the FT-NIR method was originally devised to measure PV trends over a broad range of PV (0–100). In this paper, we describe the upgrading of the FT-NIR PV method for the determination of PV over the range of 0–10 PV.

MATERIALS AND METHODS

Oil samples. Canola oil, used as the base oil, was obtained locally. The oil was passed through a column of microwave-activated silica gel to remove partially polar oxygenated molecules, in particular, any residual hydroperoxides. The silica

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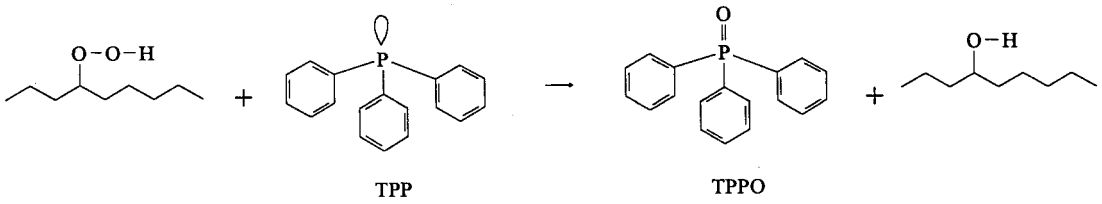

FIG. 1. Reaction of triphenylphosphine (TPP) with hydroperoxides to form triphenylphosphine oxide (TPPO) and alcohol.

gel-treated canola oil was verified to be peroxide-free by the AOCS standard method (Cd 8b-90) (1). Reagent-grade TPP (>99%) and TPPO (>99%) were obtained from Aldrich Chemicals (Milwaukee, WI), and separate concentrated stock solutions (40%, w/w) of TPP and TPPO were prepared in chloroform.

Instrumentation and sample handling. The instrument used for this study was a Bomem FT-NIR spectrometer (MB-Series, Bomem Inc., Quebec, Canada) equipped with a deuterated triglycine sulfate detector capable of scanning the spectral range of 12,000–2000 cm^{-1}. The spectrometer was controlled by an IBM-compatible Pentium 200-MHz PC running under Windows-based Bomem-Grams/32 software (Galactic Industries Co., Salem, NH). The sample handling accessory used in this study was a temperature-controllable multivial holding block (Bomem Inc.) maintained at 30°C, capable of accepting 8 mm o.d. transparent glass vials (Kimble Glass Inc., Vineland, NJ) which had a volume of ~1 mL. For sample analysis, vials were filled with ~0.7–1.0 mL of oil and scanned over the spectral range of 12,000–4000 cm^{-1}. All sample and background spectra were recorded by co-adding 128 scans at a resolution of 4 cm^{-1}. Background spectra were collected every 30 min through an empty vial placed in the vial holder in the IR beam, and each sample spectrum was ratioed against the most recently collected background spectrum (Fig. 2).

Calibration standards and validation samples. Stock solutions (0.5 g) of TPP and TPPO (40% in chloroform) were sep-

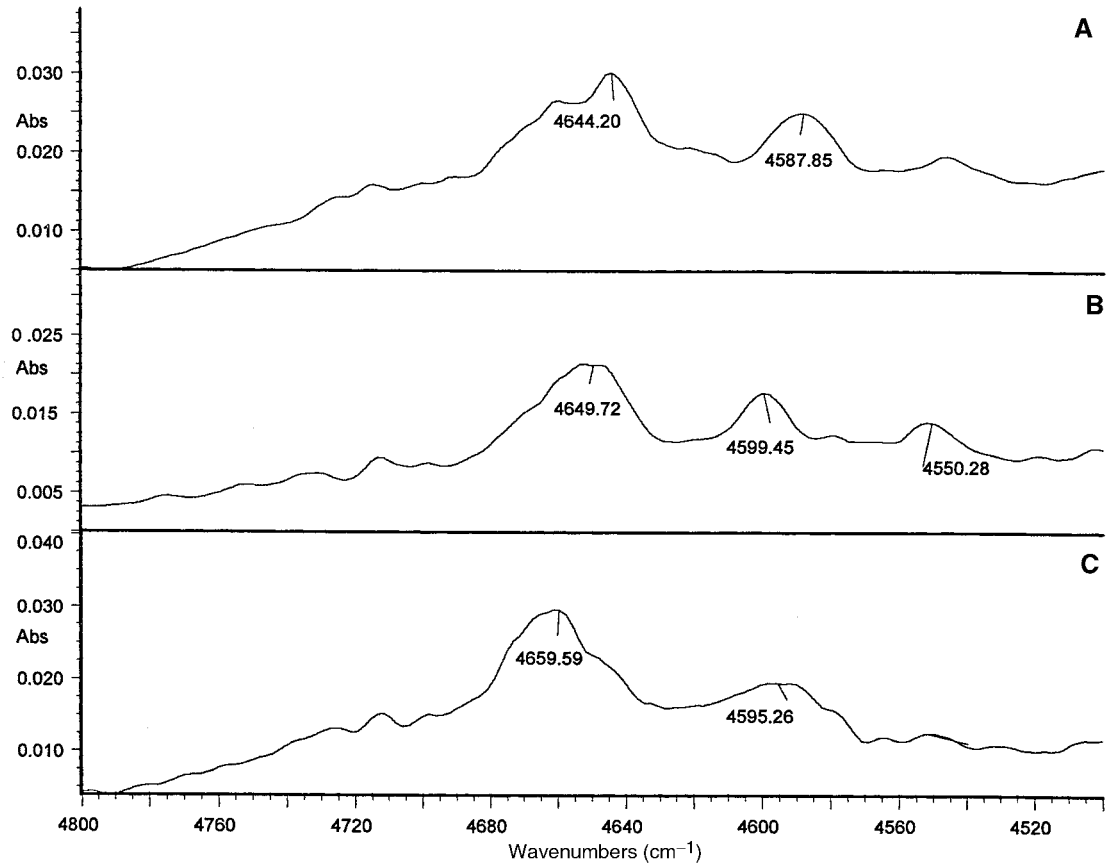

FIG. 2. Differential spectra of (A) TPP, (B) TPPO, and (C) TPP + TPPO in canola oil produced by ratioing out the spectral contributions of canola oil. See Figure 1 for abbreviations.

arately incorporated into 100 g of zero-PV canola oil to produce base oils which contained 15 PV equivalents of TPP or TPPO, respectively. Calibration standards (~8 g) having randomized TPP/TPPO ratios were then prepared by gravimetrically mixing various amounts of zero-PV canola oil with the two base oils (Table 1). Randomization was visually verified by plotting the concentrations of TPP and TPPO against each other to ensure that the two-dimensional space of possible combinations of TPP/TPPO concentrations was fairly uniformly spanned (Fig. 3). Of each standard, ~0.7 mL was transferred to an 8-mm NIR sample vial and scanned as described above. The spectral data were normalized to unit area over the region of 9100–7560 cm^{-1} and then stored to disk for subsequent development of a partial-least-squares (PLS) calibration model using Omnic TQ Analyst chemometric software (Nicolet Instrument Co., Madison, WI). Correlation and variance spectra were generated to determine where most of the spectral changes in the calibration set took place. These regions were then explored for calibration development and refinement. Each calibration was assessed by using the leave-one-out cross-validation procedure and optimized in terms of the appropriate number of factors by minimizing the predicted residual error sum of squares. Validation samples were prepared by gravimetrically mixing oxidized canola oil (PV ~13) and zero-PV canola oil to produce samples having PV values within the range of 0 to 10. Two additional sets of validation samples were prepared in the same manner utilizing olive or sunflower oil, respectively, instead of canola oil.

TABLE 1
Calibration Matrix of Oil Containing TPP and TPPO for Developing a Partial-Least-Squares PV Calibration[a]

Std. no.	Pure oil (g)	15 PV TPP oil stock(g)	15 PV TPPO oil stock (g)	PV TPP	PV TPPO
1	0	8	0	15.13	0
2	7.1772	0.6184	0.2701	1.16	0.53
3	6.0437	0.706	1.2777	1.33	2.52
4	6.0861	1.2208	0.6934	2.31	1.37
5	5.1475	1.7925	1.2974	3.29	2.49
6	6.5499	0.1468	1.3255	0.28	2.62
7	3.5888	3.4366	1.0816	6.41	2.11
8	5.1104	2.2196	0.6419	4.21	1.27
9	4.7146	1.1785	2.1124	2.23	4.18
10	4.7901	0.6036	2.6548	1.13	5.22
11	3.7153	0.4532	3.9275	0.85	7.68
12	4.3947	2.2084	1.5682	4.09	3.04
13	4.0141	0.86	3.226	1.61	6.3
14	3.209	1.276	3.5474	2.4	6.99
15	3.1966	2.181	2.52	4.18	5.05
16	2.8168	0.6746	4.5317	1.27	8.94
17	2.718	0.1146	5.1514	0.22	10.21
18	2.8407	3.1268	1.9246	5.99	3.86
19	2.9425	4.7076	0.3127	8.94	0.62

[a]PV = peroxide value; the amounts of triphenylphosphine oxide (TPP) (molecular weight = 262.28) and triphenylphosphine (TPPO) (molecular weight = 278.29) added to zero-PV canola oil are expressed in terms of PV equivalents, based on the stoichiometry of the reaction between TPP and hydroperoxides to form TPPO. Oil containing 1 PV unit of ROOH as determined by the standard iodometric reaction would react with 0.5 mmol TPP/kg oil, producing 0.5 mmol TPPO/kg oil. Hence, 1 PV equivalent of TPP = 0.1311 g TPP/kg oil and 1 PV equivalent of TPPO = 0.1391 g TPPO/kg oil

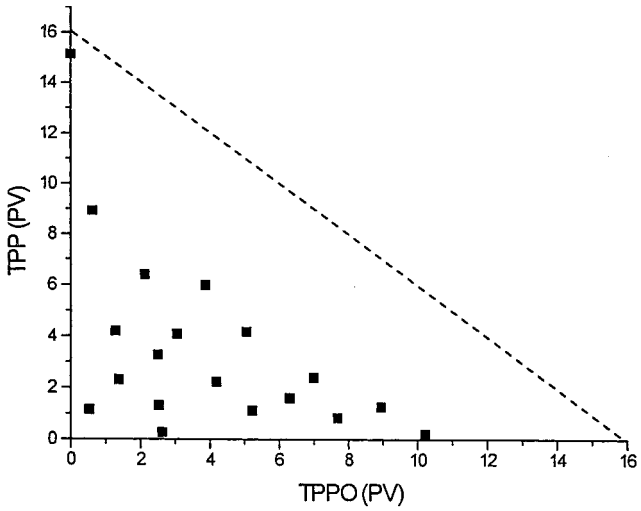

FIG. 3. A plot illustrating the relative concentrations of TPP and TPPO in the partial-least-squares calibration standards. The concentrations of TPP and TPPO are expressed in terms of PV equivalents, in accordance with the stoichiometry of the reaction between TPP and hydroperoxides to form TPPO (1 PV equivalent of TPP = 0.1311 g TPP/kg oil and 1 PV equivalent of TPPO = 0.1391 g TPP/kg oil). The two-dimensional space of possible combinations of TPP/TPPO concentrations is represented by the area below the dashed line. See Figure 1 for abbreviations.

Sample analytical protocol. For sample analysis, the protocol consisted of adding 50 µL of 40% stock TPP–chloroform solution with a precalibrated repipette to 15 g of the sample, mixing for 20 s on a Vortex mixer and then transferring *ca*. 0.7 mL to an 8-mm (o.d.) NIR vial. The amount of TPP added was sufficient to react with all the hydroperoxides in an oil having a PV of ~15, well in excess of the measurement range considered (0–10 PV). After scanning the sample, the PV was predicted from the PLS TPP/TPPO calibration. The validation samples were also analyzed in parallel by the AOCS chemical PV method (Cd 86-90) (1).

RESULTS AND DISCUSSION

General spectroscopy. The NIR spectral characteristics of TPP and TPPO in canola oil at low concentrations were investigated by adding each component individually to zero-PV canola oil, recording the FT-NIR spectra of the spiked oils, and ratioing out the spectral contributions of the canola oil to produce "differential spectra." Figure 2 shows the differential spectra of TPP, TPPO, and a mixture of TPP + TPPO between 4800 and 4500 cm^{-1}. TPPO has similar spectral features to TPP, but the major bands are shifted toward shorter wavelengths. When TPP reacts with hydroperoxides in oil to produce TPPO, both TPP and TPPO are simultaneously present in the oil, and the bands of these two components will overlap (Fig. 2C). Quantitation under these circumstances requires the use of more sophisticated chemometric techniques such as PLS regression (7).

PLS calibration. Table 1 presents the calibration standard matrix used for the PLS calibration. The FT-NIR determination of PV requires that the amount of TPPO formed by the reaction between hydroperoxides and TPP be quantitated in the presence of unreacted TPP, thus the calibration must be performed with standards containing both TPP and TPPO. The concentrations of TPPO and TPP in the standards, expressed in terms of PV equivalents, are plotted against each other in Figure 3 to illustrate their lack of correlation. This is in accordance to PLS, requiring that the calibration standards contain randomly varying amounts of potentially interfering components in the samples to be analyzed. A PLS calibration model using five factors was developed to predict PV based on the quantitation of TPPO using the region 4695–4553 cm^{-1} referenced to a single baseline point at 9143 cm^{-1}. Figure 4 presents a plot of predicted vs. actual TPPO concentration, expressed in PV equivalents, obtained from cross-validation of the PLS calibration. The linear regression equation for this plot was $y = 0.390 + 0.904x$, having a correlation coefficient of 0.98 and an SD of ±0.55 PV.

Validation. The PLS calibration model was validated by analyzing a series of samples prepared by gravimetric dilution of an oxidized canola oil with a zero-PV canola oil. Duplicate analyses were performed 2 d apart and the mean difference for reproducibility (MD_r) and the SD of the differences (SDD_r) for the NIR duplicate predictions were 0.27 PV and *ca.* ±0.90 PV units, respectively. These validation samples as well as the oxidized oil used to prepare them were also chemically analyzed by the AOCS PV method (Cd 86-90) (1). In addition, the PV of the validation samples were calculated from the chemical PV of the base-oxidized oil and the gravimetric dilution factors. Figure 5 illustrates the validation plot obtained by plotting the FT-NIR-predicted PV for the validation samples vs. their calculated PV, the linear regression equation for this plot being $y = 0.036 + 0.875x$, with a correlation coefficient of 0.964 and an SD of ±0.95 PV. The mean difference for accuracy (MD_a) and the SD of the differences (SDD_a) for the NIR duplicate predictions were –1.0 PV and *ca.* ±0.67 PV units, respectively. A plot of FT-NIR-predicted PV vs. actual chemical PV (Fig. 6) indicates some curvature, its linear regression equation being $y = 1.672 + 0.861x$, having a correlation coefficient of 0.93 and an SD of 1.35 with MD_a and SDD_a being –0.95 and ±1.41 PV, respectively

A careful assessment of duplicate analyses by the AOCS chemical method indicated that the reproducibility of the chemical method was very good, producing a mean difference for reproducibility (MD_r) of 0.21 PV and an SDD_r of ±0.54 PV. A plot of the duplicates against each other (Fig. 7) was linear ($r = 0.994$), had a slope close to 1 (1.09), with an intercept close to zero (–0.26 PV) and an SD of ±0.44 PV. These data for the chemical method clearly indicate that one can

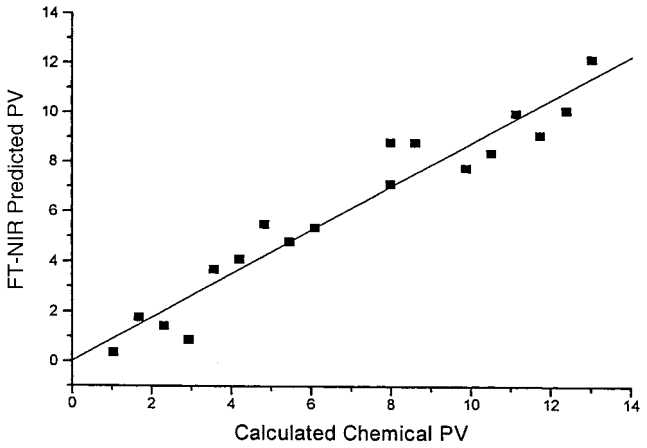

FIG. 5. Plot of Fourier transform near infrared (FT-NIR)-predicted PV vs. calculated PV for gravimetrically diluted oxidized canola oil samples. See Figure 4 for other abbreviations.

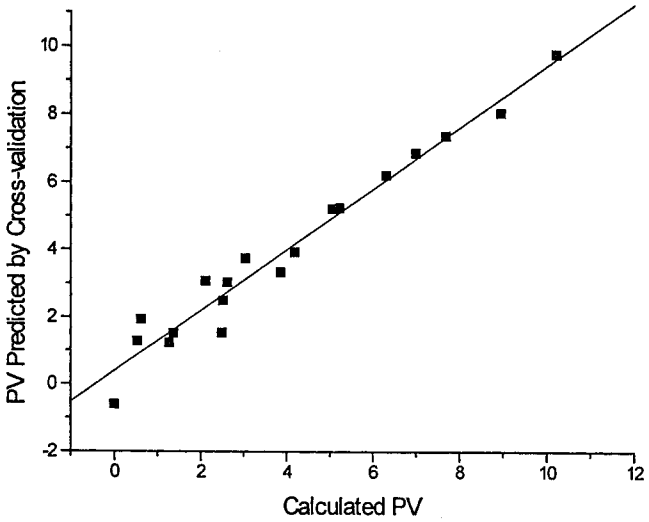

FIG. 4. A cross-validation plot of predicted peroxide value (PV) vs. calculated PV based on the gravimetric addition of TPPO. See Figure 1 for other abbreviations.

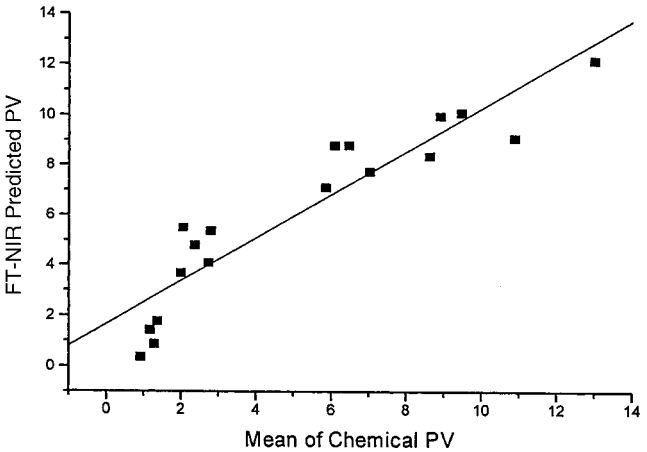

FIG. 6. Plot of FT-NIR-predicted PV vs. chemical PV for gravimetrically diluted oxidized canola oil samples. See Figures 1 and 4 for abbreviations.

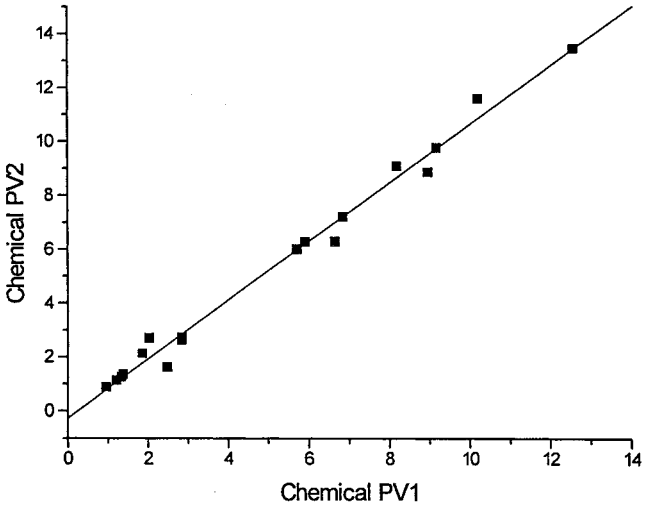

FIG. 7. Plot of PV data obtained from duplicate chemical analyses of the gravimetrically diluted oxidized canola oil samples. See Figure 4 for abbreviations.

routinely reproduce PV results to within *ca.* ±0.50, well within the general expectation of the method of ±1.0 PV. However, a plot of the individual chemical PV results vs. calculated PV for the gravimetrically diluted oxidized canola oil samples (Fig. 8) reveals that the relationship is curvilinear, fitting a quadratic relationship quite well and, on this basis, having an SD of *ca.* ±0.72 PV. These results indicate that the chemical PV method, although quite reproducible, does not necessarily respond linearly, being relatively insensitive to changes in PV at lower PV values. Without this more careful analysis, it would have been reasonable to conclude that the nonlinearity between instrumental and chemical results (Fig. 6) is due to the secondary method rather than the primary reference method, but this is clearly not the case. Based on our experience, the reproducibility and sensitivity of the reference method tend to be the limiting factors.

To investigate the scope of applicability of the PV calibration devised in this work, validation sets were prepared with olive and sunflower oils and their PV predicted from the canola oil calibration. Linear regression of the predicted PV against the chemically determined values yielded the following equations for olive and sunflower oils, respectively:

$$\text{FT-NIR–PV} = -29.18 + 0.899\ \text{PV} \quad r = 0.97 \quad \text{SD} = 0.50 \quad [1]$$

$$\text{FT-NIR–PV} = 13.13 + 1.123\ \text{PV} \quad r = 0.99 \quad \text{SD} = 0.38 \quad [2]$$

These results indicate that the calibration tracks the PV changes quite well; however, the values are biased in an oil-dependent manner. This effect is likely due to a combination of factors, i.e., the presence of interfering absorptions of the oil in the measurement region as well as due to area normalization attributing any changes in area to a pathlength change, whereas these changes may arise from differences in fatty acid composition between oil types. The regression errors above indicate that PV calibrations are transferable between oil types. However, if absolute rather than relative PV values are required, regression equations of the types given above need to be developed. In contrast, the mid-IR method previously developed (4) is oil-independent and thus universally applicable. Somewhat better reproducibility also was obtained in the mid-IR method which uses a fixed pathlength transmission cell. However, the use of disposable vials in the NIR method confers advantages of lower cost and simpler sample handling while still providing satisfactory reproducibility, provided that the spectra are normalized to compensate for the variability between and within vial lots. Based on the results obtained in this study, it is clear that one can readily determine the PV of oil samples by FT-NIR spectroscopy over a PV range of 1–10, with a reproducibility of *ca.* ±1.0 PV.

From the standpoint of analysis, the method is quite straightforward, requiring only the weighing of the sample into the vial, adding a fixed amount of excess TPP, mixing gently, and scanning the spectrum of the sample. Weighing can be eliminated if accurate and reproducible repipettes are used and the method standardized. The use of disposable glass vials, being particularly attractive from the standpoint of convenience, is an added benefit. Modern FTIR systems, being programmable, effectively allow one to automate a method by developing a user-friendly interface and building the PLS calibration into the system so that a PV value is presented directly to the user after scanning the sample. An FT-NIR instrument configured and calibrated in the manner described in this paper would be a useful tool for the routine quality control analysis of finished and stored oil products.

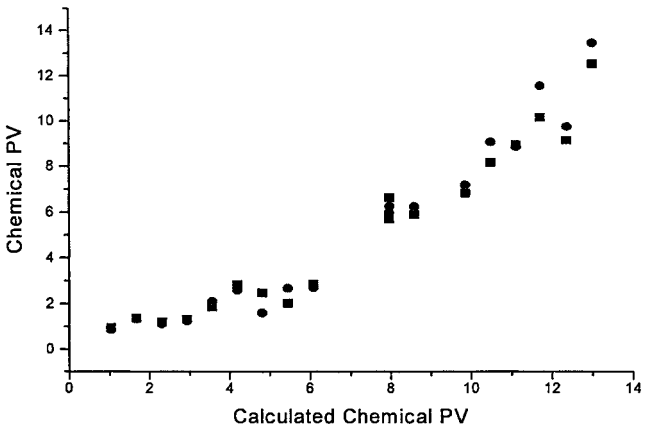

FIG. 8. Plot of duplicate (■,●) chemical vs. calculated PV values of gravimetrically diluted oxidized canola oil. See Figure 4 for abbreviations.

ACKNOWLEDGMENTS

The authors would like to thank Bomem Inc. for the FT-NIR spectrometer and the Natural Sciences and Engineering Research Council of Canada (NSERC) University/Industry program for their financial support of this research. The technical and chemometric advice of Jacqueline Sedman is gratefully acknowledged.

REFERENCES

1. American Oil Chemists' Society, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., American Oil Chemists' Society, Champaign, IL, 1989.
2. van de Voort, F.R., J. Sedman, and A.A. Ismail. Edible Oil Analysis by FTIR Spectroscopy, *Lab. Robot. Autom. 8*:205–121 (1996).
3. van de Voort, F.R., A.A. Ismail, J. Sedman, J. Dubois, and T. Nicodemo, The Determination of Peroxide Value by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 71*:921–926 (1994).
4. Ma, K., F.R. van de Voort, J. Sedman, and A.A. Ismail, Stoichiometric Determination of Hydroperoxides in Fats and Oils by Fourier Transform Infrared Spectroscopy, *Ibid. 74*:897–906 (1997).
5. Dong, Jun, K. Ma, F.R. van de Voort, and A.A. Ismail, Stoichiometric Determination of Hydroperoxides in Oils by Fourier Transform Near-Infrared Spectroscopy. *JAOAC Int. 80*:345–352 (1997).
6. Ma, K., F.R. van de Voort, A.A. Ismail, and J. Sedman, Quantitative Determination of Hydroperoxide Formation by Fourier Transform Infrared Spectroscopy Using a Disposable Infrared Card, *J. Am. Oil Chem. Soc. 71*:1095–1101 (1998).
7. van de Voort, F.R., FTIR Spectroscopy in Edible Oil Analysis, *INFORM 5*:1038–1042 (1994).

[Received April 28, 1999; accepted October 10, 1999]