Stoichiometric Determination of Hydroperoxides in Fats and Oils by Fourier Transform Infrared Spectroscopy

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ABSTRACT: A primary Fourier transform infrared (FTIR) spectroscopic method for the determination of peroxide value (PV) in edible oils was developed based on the stoichiometric reaction of triphenylphosphine (TPP) with hydroperoxides to produce triphenylphosphine oxide (TPPO). Accurate quantitation of the TPPO formed in this reaction by measurement of its intense absorption band at 542 cm^{-1} provides a simple means of determining PV. A calibration was developed with TPPO as the standard; its concentration, expressed in terms of PV, covered a range of 0–15 PV. The resulting calibration was linear over the analytical range and had a standard deviation of ±0.05 PV. A standardized analytical protocol was developed, consisting of adding ~0.2 g of a 33% (w/w) stock solution of TPP in hexanol to ~30 g of melted fat or oil, shaking the sample, and scanning it in a 100-µm KCl IR transmission cell maintained at 80°C. The FTIR spectrometer was programmed in Visual Basic to automate scanning and quantitation, with the reaction/FTIR analysis taking about 2 min per sample. The method was validated by comparing the analytical results of the AOCS PV method to those of the automated FTIR procedure by using both oxidized oils and oils spiked with *tert*-butyl hydroperoxide. The two methods correlated well. The reproducibility of the FTIR method was superior (±0.18) to that of the standard chemical method (±0.89 PV). The FTIR method is a significant improvement over the standard AOCS method in terms of analytical time and effort and avoids solvent and reagent disposal problems. Based on its simple stoichiometry, rapid and complete reaction, and the singular band that characterizes the end product, the TPP/TPPO reaction coupled with a programmable FTIR spectrometer provides a rapid and efficient means of determining PV that is especially suited for routine quality control applications in the fats and oils industry.
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KEY WORDS: Fats and oils, Fourier transform infrared spectroscopy, FTIR, lipid oxidation, peroxide value, PV, triphenylphosphine, triphenylphosphine oxide.

Lipid oxidation, one of the key deteriorative reactions affecting edible oils, is initiated by a variety of agents (light, heat, metal ions, etc.) when oxygen is present. In the initial stages of the reaction, the main products are hydroperoxides, which subsequently undergo further degradation to form volatile short-chain oxygenated molecules; the latter produce a characteristic rancid off-flavor (1). Because hydroperoxides are the primary products formed as autoxidation commences and serve as precursors to the subsequent formation of secondary oxidation products, their presence and rate of change are important indicators of oil quality and potential shelf life (2). Hence, a routine quality control procedure associated with edible fats and oils is to determine the peroxide value (PV) of oils after processing, usually after deodorization, which thermally degrades residual hydroperoxides and strips out low-molecular-weight aldehydes and free fatty acids under vacuum. High-quality edible oils usually have PV values below 1.0. PV determinations are also used extensively by processors to monitor the oxidative status of bulk oils in storage, to test the efficacy of antioxidants, and, in conjunction with the Active Oxygen Method, as a means of predicting the shelf life of edible oils (3).

The standard AOCS PV determination is based on the stoichiometric release of molecular iodine by hydroperoxides when exposed to KI in an acidic environment; the reaction converts the hydroperoxides to alcohols (3). The molecular iodine released is complexed with soluble starch, which acts as an indicator, and the iodine is quantitated by titration with sodium thiosulfate. Based on the stoichiometry of the two reactions, the hydroperoxide concentration can be calculated and is commonly expressed as milliequivalents of hydroperoxide per kilogram of fat (meq/kg). The PV test, although empirical, is relatively simple, reasonably sensitive, reliable, and reproducible if carried out under standardized conditions. On the other hand, it is labor-intensive and uses reagents and solvents (KI and chloroform or isooctane) which are increasingly difficult to dispose of and considered hazardous (4). Similar limitations and concerns are associated with a variety of chemically based analytical methods for fats and oils. The McGill IR Group has worked on the development of Fourier transform infrared (FTIR) spectroscopy-based methods that allow analyses to be carried out directly on neat fats and oils and confer the advantages of analytical speed and automation (5–14). Among the methods reported to date is the FTIR determination of PV, based on the measurement of the characteristic O-H stretching absorption band of hydroperoxides (9).

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In developing a PV calibration, it was necessary to employ a multivariate calibration approach, based on the use of partial least squares (PLS) regression, to account for potential spectral interferences from other OH-containing species that may be present in oils, such as alcohols, moisture, free fatty acids, and mono- and diglycerides (9). Although this approach is conceptually sound and proved workable, the main limitation of the method developed was its lack of sensitivity, with PV values of less than 1.5 effectively being undetectable. This lack of sensitivity plus the general complexity of the calibration was considered a barrier to routine implementation of the method. In this paper, we describe a simpler FTIR PV method, based on a well-known stoichiometric reaction that overcomes the limitations of our original approach and makes FTIR spectroscopy an accurate, primary method for the determination of PV of fats and oils in their neat form.

MATERIALS AND METHODS

Materials. Canola oil (used as the base oil in the preparation of calibration standards) and a variety of other vegetable oils and fats were obtained at local retail outlets. Reagent-grade triphenylphosphine (TPP, >99%), triphenylphosphine oxide (TPPO, >99%), and *tert*-butyl hydroperoxide (TBHP; ~5.5 M in isooctane, containing 5% H_2O and 5% *tert*-butanol) were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Preparation of calibration standards. Canola oil was heated to 200°C under vacuum for 1 h to destroy any hydroperoxides and to remove any low-molecular-weight aldehydes. The oil was then passed through a column of activated silica gel to remove any remaining partially polar molecules that might be present. The cleaned canola oil was analyzed for its PV by the standard AOCS method (3), the oil being considered clean if the PV was <0.10. Calibration standards were prepared by gravimetrically adding varying amounts (0–0.2 g) of a 35% (w/w) stock solution of TPPO, dissolved in hexanol, to 30 g of clean canola oil.

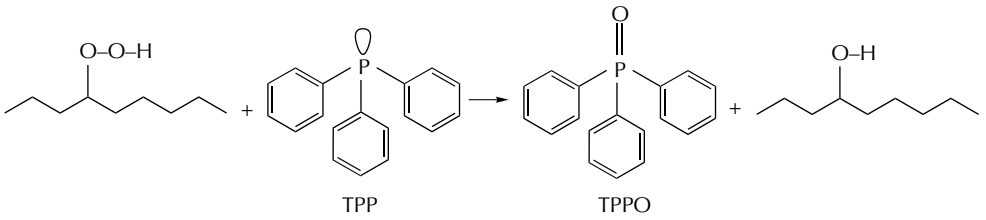
Instrumentation/spectral acquisition. The instrument used for this work was a Magna 550 FTIR spectrometer (Nicolet Instrument Corp., Madison, WI), controlled by a 486 MHz personal computer that was run under Nicolet OMNIC 2.1 software. To minimize water vapor and carbon dioxide interferences, the instrument and its sample compartment were continuously purged with CO_2-free dry air supplied by a Balston dryer (Balston, Lexington, MA). The instrument was equipped with a custom-designed heated sample-handling accessory (8) set to 80°C, capable of handling both fats and oils (Dwight Analytical, Toronto, Ontario, Canada). The sample-handling accessory housed a transmission flow cell with KCl windows and a pathlength of 100 μm. Prior to analysis, mineral oil was passed through the system to clean the cell and transfer lines. All standards and samples were preheated for ~1 min to 80°C in a water bath prior to being loaded into the cell by aspiration. For the calibration standards prepared in canola oil, spectra were collected by coadding 256 scans at a resolution of 4 cm^{-1} and a gain of 1.0 and were ratioed against an open-beam background spectrum to produce absorbance spectra that were stored to disk for subsequent chemometric analysis. Samples were run under the same instrumental conditions as the calibration standards, except the number of scans was reduced to 64 scans to minimize analysis time.

Analytical protocol/validation. Validation samples consisted of a variety of fats and oils, some spiked with *tert*-butyl hydroperoxide (TBHP) to increase their PV. Chemical PV determinations were carried out on these samples by the standard AOCS method (3). For validation studies of the FTIR PV method, a standardized protocol was devised to maintain a relatively consistent TPP reagent/oil ratio for the analysis of oils within the range of 0.1–15 PV. To match the calibration curve based on the serial dilution of a 35% (w/w) TPPO stock solution, an equimolar stock solution of TPP (33% w/w) in hexanol was prepared, to be added to the oils to be analyzed to provide a reactant reservoir. The analytical protocol consisted of weighing ~30 g of oil into a tared 50-mL, wide-mouth plastic vial and adding 10 drops of TPP/hexanol stock solution (representing ~0.2 g) from a 60-mL dispensing plastic dropper bottle, which was kept warm on a Radio Shack coffee cup heater (~80°C) to keep the TPP soluble. After addition of the TPP stock solution to the oil, the vial was capped and shaken, and the sample was then presented to the input spigot of the sample-handling accessory of the FTIR spectrometer.

RESULTS AND DISCUSSION

Analytical concept/spectroscopy. In considering an alternative approach to the determination of PV by FTIR spectroscopy, our efforts focused on developing an FTIR method that effectively had characteristics similar to those of the primary AOCS chemical method, i.e., a method based on a stoichiometric reaction involving hydroperoxides. One such well-characterized reaction is that of TPP, which reacts stoichiometrically with hydroperoxides to form their respective alcohols, TPP being converted to TPPO according to Scheme 1. The TPP/TPPO reaction has been employed in a microassay for lipid hydroperoxides in biological samples. The assay uses a combination of high-performance liquid chromatography and ultraviolet detection and is capable of detecting PV as low as 1 in 10-mg lipid samples (15). We have also made effective use of this reaction as a means of eliminating hydroperoxides from oil samples, the reaction being rapid and complete when an excess of TPP was present (9). It was our hypothesis that the relatively strong phenyl bands in the IR spectrum of TPP would be perturbed sufficiently upon the addition of oxygen to its molecular structure to allow for accurate quantitation of TPPO in the presence of unreacted TPP by FTIR spectroscopy.

For preliminary FTIR investigations of the feasibility of this approach, mineral oil was spiked with TPP/hexanol, TPPO/hexanol, and TBHP; mineral oil was chosen as the base oil for this work because its viscosity and flow characteristics



SCHEME 1

are similar to those of edible oils but it provides a clearer spectral window; and hexanol was used as a carrier to facilitate dissolution of TPP and TPPO in the oil. "Differential spectra," which allow one more readily to visualize and isolate spectral features that otherwise might be difficult to detect (13), were generated by ratioing the single-beam spectra of the spiked oils against the single-beam spectrum of the unspiked oil. Figure 1A–C illustrates the differential spectra of TPP/hexanol, TPPO/hexanol, and TBHP, respectively, over the range of 1225–475 cm^{-1}. A variety of absorption bands is evident in the TPP and TPPO spectra, including our target bands, located in the 775–675 cm^{-1} region, which are among the strongest absorptions associated with the phenyl groups. Two bands in these spectra—the relatively weak, broad TPP band at 505 cm^{-1} and the sharp, strong band at 542 cm^{-1} in the spectrum of TPPO, which is roughly double the intensity of the bands in the 775–675 cm^{-1} region—were unexpected, as the Aldrich spectral library reference (16) for TPP and TPPO only spanned the spectral range of 4000–625 cm^{-1}. In a subsequent search of the literature, a study by Deacon and Green (17) provided a detailed enumeration and description of the vibrational spectra of TPP and TPPO, including these lower-frequency vibrations. Some of the more pertinent band assignments for TPP mulls and TPPO in the solid phase are presented in Table 1 and compared with the band positions observed in mineral oil.

The band at 542 cm^{-1} in the spectrum of TPPO was assigned by Deacon and Green (17) to an X-substituent-sensitive phenyl vibration. The corresponding band in the spectrum of TPP is part of a broad feature (512–489 cm^{-1}) that is composed of several bands, as we have confirmed by deconvolution. According to Deacon and Green, the addition of an oxygen to the phosphorus shifts this band to higher frequencies; however, they provided no explanation for this effect, because this band was expected to shift to lower frequencies with an increase in the mass of the substituent on the phenyl ring. Given the 40-cm^{-1} shift of this band on going from TPP to TPPO, as well as its strong intensity for TPPO, this band provides a unique means of quantitating the conversion of TPP to TPPO upon its reaction with ROOH. This is confirmed in Figure 1D, which shows the strong TPPO band at 542 cm^{-1} in the differential spectrum obtained by ratioing the

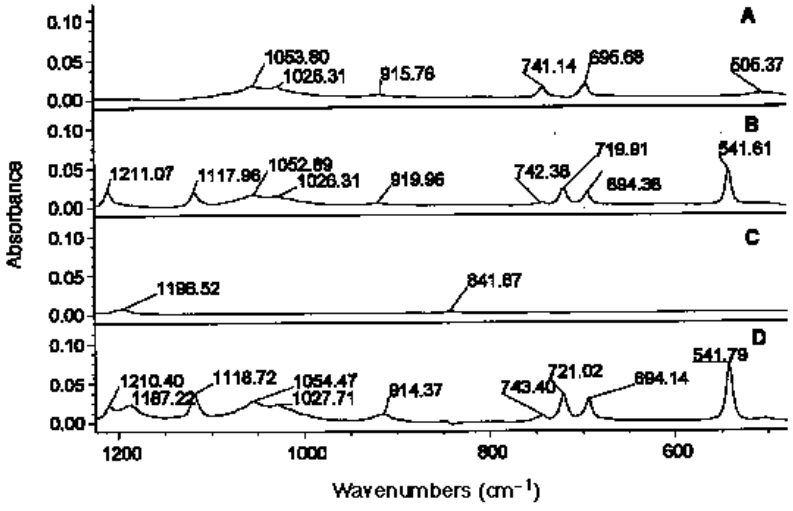


FIG. 1. Differential spectra of triphenylphosphine (TPP)/hexanol (A), triphenylphosphine oxide (TPPO)/hexanol (B), *tert*-butyl hydroperoxide (TBHP) (C), and reacted TBHP and TPP/hexanol (D) in mineral oil over the spectral range of 1225–475 cm^{-1}. Spectra A–C have been ratioed against the spectrum of mineral oil; spectrum D has been ratioed against the spectrum of TBHP in mineral oil. The unique TPPO band at 542 cm^{-1} (B) is clearly seen in the spectrum of the reaction mixture (D).

TABLE 1
Assignment of Relevant Triphenylphosphine (TPP) and Triphenylphosphine Oxide (TPPO) Infrared Absorptions

Assignment[a]	Band position (cm^{-1})	
	TPP (mull)[b]	TPP in mineral oil[c]
d β(CH) (b_2)	1068–1065 w	1053
b β(CH) (a_1)	1028 m	1026
f γ(CH) (b_1)	754–741 vs	742
ν φ(CC) (b_1)	692 vs	695
y X-sens (b_1)	512–489 s	505
	TPPO (solid)[b]	TPPO in mineral oil[c]
P=O Stretch	1193 vs	1211
q X-sens. (a_1)	1122 vs	1117
d β(CH) (b_2)	1074 m	1052
b β(CH) (b_2)	1028 m	1026
f γ(CH) (b_1)	755 m	742
r X-sens. (a_1)	721 vs	719
ν φ(CC) (b_1)	698 s	694
y X-sens. (b_1)	542 vs	542

[a]Assignments are given in the notation of Whiffen (18), as described by Deacon and Green (17).
[b]These data reported by Deacon and Green (17). Abbreviations: w: wide; m, medium; s, sharp; vs, very sharp.
[c]Data reported in the present study.

spectrum recorded after reaction of TPP with TBHP in canola oil against the spectrum recorded before the addition of TPP. Although other spectral changes occur when TPP is converted to TPPO, particularly in the 775–675 cm^{-1} region, in which the appearance of a new band at ~720 cm^{-1} is observed, the formation of the strong 542-cm^{-1} band potentially provides a unique and sensitive means of quantitation. The spectrum of TBHP (Fig. 1C) also indicates that there are no significant underlying absorptions due to hydroperoxides *per se* that might interfere with measurements in this portion of the spectrum.

Figure 2A–D illustrates the same spectra as in Figure 1 but focuses on the spectral region between 3750 and 3350 cm^{-1}. The main band in Figure 2A and B is due to hexanol, its absorption maximum being at ~3642 cm^{-1}. Figure 2C illustrates the ROOH absorption band of TBHP at 3562 cm^{-1}, the hexanol band being absent as the use of hexanol as a carrier was not required for TBHP. Figure 2D, which illustrates only the spectral changes associated with the TPP/TBHP reaction, shows a new alcohol band and a negative hydroperoxide band, reflecting the formation of alcohol and the consumption of hydroperoxide. Figures 1 and 2 provide spectral confirmation of the reaction of TPP with hydroperoxides (TBHP) to form TPPO and an alcohol as per the reaction mechanism. It is also clear from these spectra that the alcohol carrier, the alcohols formed, TBHP, and TPP do not exhibit absorptions in the 550–530 cm^{-1} region. Thus, the 542 cm^{-1} band has excellent potential as an indirect measure of hydroperoxides.

The spectral characteristics of TPP/hexanol, TPPO/hexanol, and TBHP in vegetable oils were subsequently investigated because the medium in which components are dissolved can have a strong influence on their spectral characteristics. Figure 3A–C shows the spectra of TPP/hexanol, TPPO/hexa-

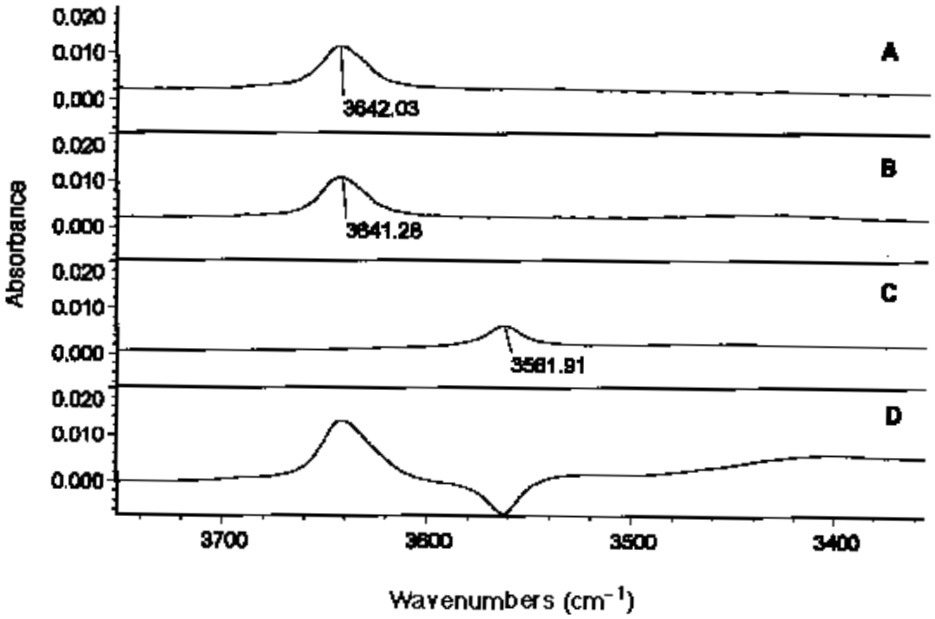


FIG. 2. Differential spectra of TPP/hexanol (A), TPPO/hexanol (B), TBHP (C), and reacted TBHP and TPP/hexanol (D) in mineral oil over the spectral range of 3750–3350 cm^{-1}. Spectra A–C have been ratioed against the spectrum of mineral oil; spectrum D has been ratioed against the spectrum of TBHP in mineral oil. The TBHP band at 3561 cm^{-1} (C) is negative in the differential spectrum of the reaction mixture (D), indicating the loss of hydroperoxides, while the OH absorption at 3642 cm^{-1} is positive, owing to both the addition of hexanol to the sample as a carrier for the TPP and the formation of alcohol in the reaction of TPP with TBHP. See Figure 1 for abbreviations.

nol, and TBHP in canola oil in the same spectral regions as in Figure 2; the absorptions of canola oil have been ratioed out of these spectra. The bands in these spectra are much broader than in the spectra of the same constituents dissolved in mineral oil. This result is attributable to hydrogen bonding, there being little opportunity for hexanol or hydroperoxide to hydrogen-bond in a highly hydrophobic solvent, such as mineral oil, other than through hydrogen bonding with themselves, an effect which only manifests itself at high concentrations. In triglyceride oils, the oxygens of the triglyceride ester linkages provide many hydrogen bonding sites for alcohols and hydroperoxides. This results in a combination of band broadening and a shift of absorption maxima toward lower frequencies. These effects are evident when Figures 2 and 3 are compared. Figure 3D shows the differential spectrum obtained by ratioing the spectrum of TBHP in canola oil after reaction with TPP against the spectrum recorded before addition of TPP. Even with the superposition of hydrogen bonding, the spectral changes associated with the reaction of TPP with ROOH, i.e., the formation of a new hydroxyl band and the loss of ROOH intensity, are evident.

Figure 4 parallels Figure 1, except the spectral region displayed is reduced to 775–475 cm^{-1} because the strong fingerprint absorptions of canola oil in the 100-μm pathlength cell do not ratio out adequately in the 1225–775 cm^{-1} region to allow spectral detail to be discerned. The spectra in Figure 4 are similar to those observed in mineral oil, and, as in mineral oil, the TPP/ROOH reaction results in the appearance of a strong TPPO absorption band at 542 cm^{-1}, indicating that this band can serve as a means for quantitating PV in edible oils.

Reaction stoichiometry/calibration. PV is defined as milliequivalents (meq) of peroxide/kg of oil, the reactions for the AOCS method being:

$$ROOH + 2\ I^- + 2H^+ \rightarrow I_2 + ROH + H_2O \quad [1]$$

$$2\ Na_2S_2O_3 + I_2 \rightarrow 2\ NaI + Na_2S_4O_6 \quad [2]$$

An equivalent refers to combining proportions of substances by weight relative to hydrogen as a standard. In the AOCS chemical method, two hydrogen ions are consumed to convert one ROOH to ROH and two iodide ions to I_2 with the concomitant formation of H_2O. Reaction 2 uses similar stoichiometry to quantitate the molecular iodine formed. Hence, in the AOCS method, the loss of one ROOH requires two equivalents of hydrogen or two moles of the iodide ion to convert one mole of ROOH to ROH.

For TPP, the reaction is:

$$ROOH + TPP \rightarrow TPP{=}O + ROH \quad [3]$$

In this reaction, one mole of TPP (MW = 262.28) is required to convert one mole of ROOH to ROH. Expressing the conversion of TPP to TPPO (MW = 278.29) in terms of the AOCS PV definition, one milliequivalent of peroxide reacts with half a millimole of TPP, with each millimole of TPPO formed representing a PV of 2. As such, an oil with one PV

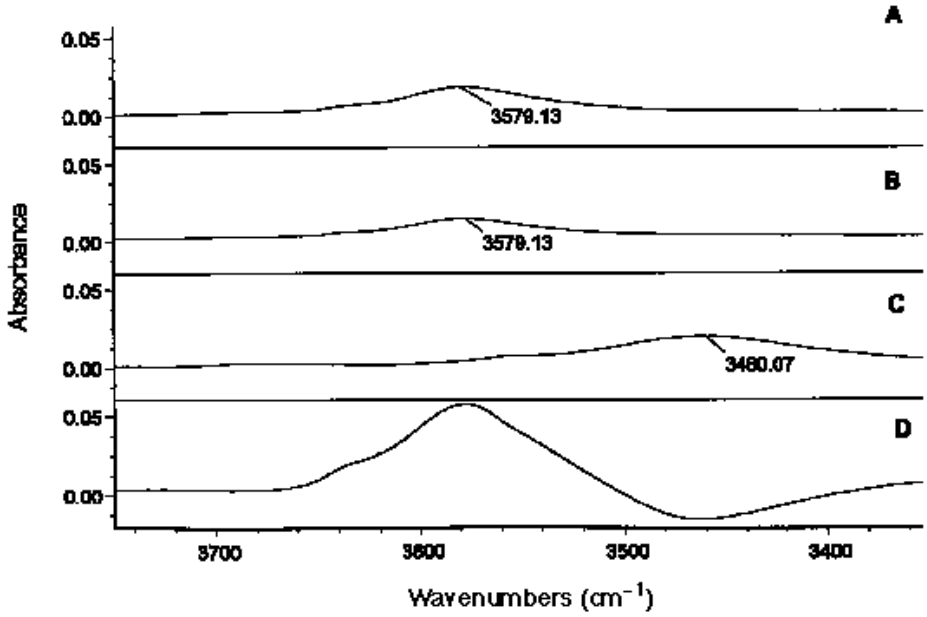


FIG. 3. Differential spectra of TPP/hexanol (A), TPPO/hexanol (B), TBHP (C), and reacted TBHP and TPP/hexanol (D) in canola oil over the spectral range of 3750–3350 cm^{-1}. Spectra A–C have been ratioed against the spectrum of canola oil; spectrum D has been ratioed against the spectrum of TBHP in canola oil. Owing to hydrogen bonding in triglyceride-based oils, the OH absorptions are shifted from their positions in mineral oil (Fig. 2), the hexanol solvent band appearing at 3579 cm^{-1}. In the differential spectrum of the reaction mixture (D), the TBHP band at 3460 cm^{-1} (C) is negative, confirming the reaction of TBHP with TPP. See Figure 1 for abbreviations.

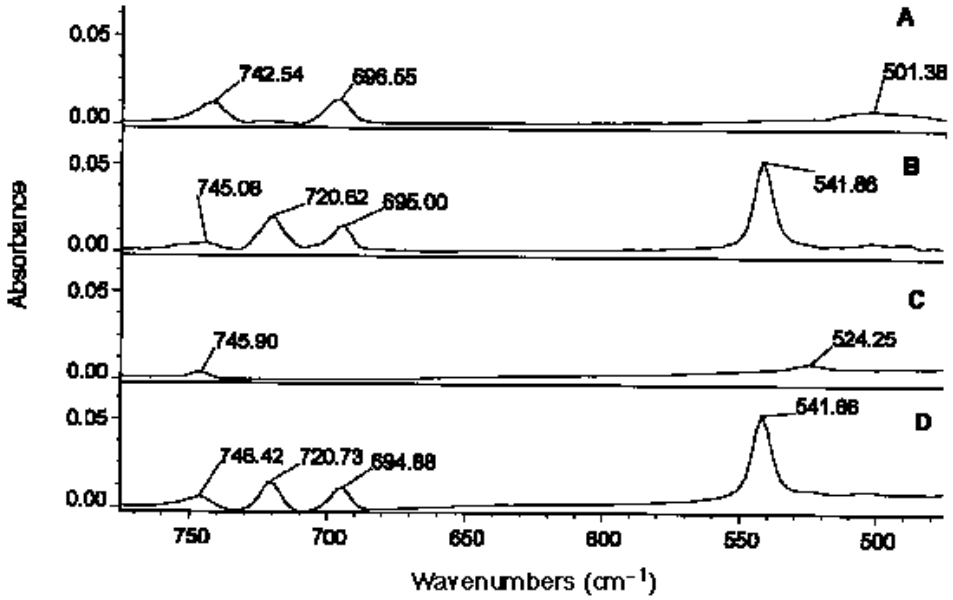


FIG. 4. Differential spectra of TPP/hexanol (A), TPPO/hexanol (B), TBHP (C), and reacted TBHP and TPP/hexanol (D) in canola oil over the spectral range of 775–475 cm^{-1}. Spectra A–C have been ratioed against the spectrum of canola oil; spectrum D has been ratioed against the spectrum of TBHP in canola oil and shows the formation of the band at 542 cm^{-1} due to TPPO formed by the reaction of TPP with TBHP.

unit of ROOH, as determined by the standard iodometric reaction, would require 0.5 mmol of TPP or 0.1311 g of TPP/kg oil to produce 0.5 mmol of TPPO or 0.1391 g of TPPO/kg oil.

The AOCS standard PV method is considered applicable for measurement of PV between 0 and 150. The method recommends the use of 5 g of lipid and 0.1 N thiosulfate as long as the thiosulfate titer is >0.5 mL, with PV calculated according to the following formula:

$$PV = (S_t - B_t)(N)(1000)/S_w \quad [4]$$

where PV = meq hydroperoxide/kg oil, S_t = sample titer, B_t = blank titer, S_w = weight of fat, and N = thiosulfate normality.

Based on the titer limitation, the PV analytical range is ~10–150 for 0.1 N thiosulfate, dropping to 1–10 and 0.1–1.0 PV for subsequent 10-fold reductions in thiosulfate normality. Theoretically, the lower limit of sensitivity of this method is ~0.2 PV; however, from a practical standpoint, it is ~1 PV because the starch indicator end point becomes difficult to assess accurately.

In terms of developing a TPP/TPPO method, given the straightforward stoichiometry of the reaction and the presence of a clean, strong TPPO band at 542 cm^{-1}, a standard curve can be prepared by the gravimetric addition of TPPO to an oil to represent selected PV values. In practical terms, consideration has to be given to ensuring uniform dispersion of TPP in the oil so that the reaction with hydroperoxides is rapid and complete. To assist this process, hexanol was selected as a carrier because it is able to dissolve both TPP and TPPO in relatively high concentrations (>30%) while also being miscible with fats and oils. Hence, calibration standards were prepared by gravimetrically adding varying amounts of TPPO/hexanol (35% w/w) to canola oil. The TPPO concentrations used correspond to those that would result from the complete reaction between TPP and the hydroperoxides in oils with PV in the range of 0.1–15. Because neither hexanol nor vegetable oils have absorptions in the region of interest, calibrations were developed by the dual-wavelength peak height method (541.9 cm^{-1}/550 cm^{-1}). Figure 5 illustrates the linear regression plot for the peak height calibration, and the regression equation for this plot is:

$$Y = -0.01295 + 210.355X \qquad R = 0.9999 \quad SD = 0.056\ PV \quad [5]$$

where R = correlation coefficient and SD = standard deviation.

A second calibration subsequently developed for PV values up to 100 was also linear but had a slightly higher SD (0.25 PV). The calibration errors for both these calibrations represent a substantive improvement over that for our previous FTIR hydroperoxide method (9), which had an overall standard error of calibration of 1.3 PV. For purposes of comparison, a calibration, based on peak area (560–520 cm^{-1}) and a PLS calibration (using the 560–520 cm^{-1} region and referenced to a single-point baseline at 560 cm^{-1}) were also developed. The performance of both these calibrations was similar to that of the peak height calibration.

As noted in our previous papers, FTIR analysis can be automated by the use of an appropriate sample-handling system, combined with programming of the spectrometer, allowing

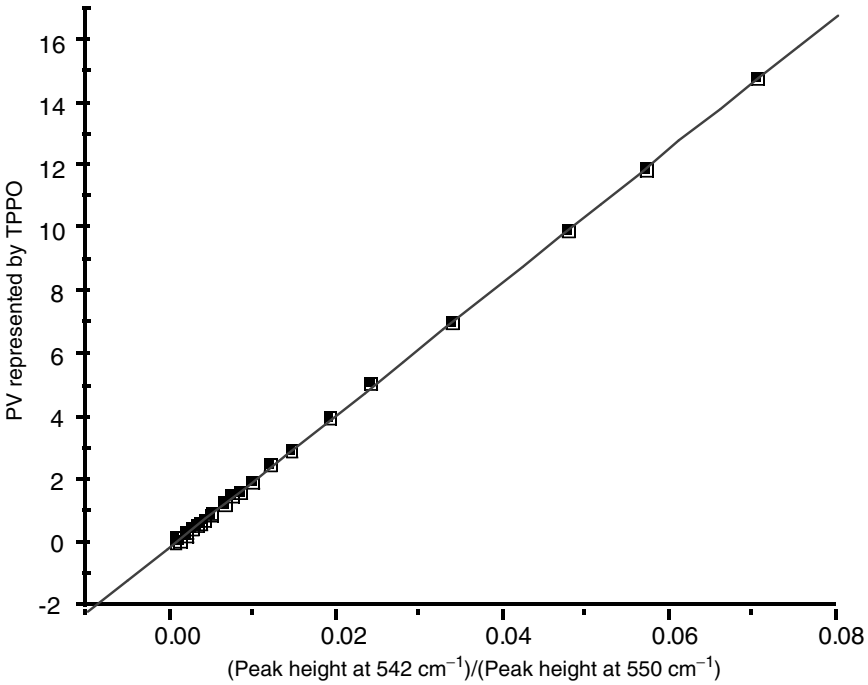


FIG. 5. Calibration plot for the determination of peroxide value (PV) by the TPP-based Fourier transform infrared (FTIR) method. Calibration standards were prepared by gravimetrically adding varying amounts of TPPO/hexanol (35% w/w) to canola oil, the TPPO concentrations used corresponding to those that would result from the complete reaction between TPP and the hydroperoxides in oils having a PV in the range of 0.1–15. The concentration of TPPO, expressed in terms of the equivalent PV, is plotted vs. absorbance at 542 cm^{-1}, measured relative to a single-point baseline at 550 cm^{-1}. For abbreviations see Figure 1.

an FTIR spectrometer to be used as a routine quality control tool without requiring any expertise in FTIR spectroscopy. In our methodology development work, we employ Nicolet Macros\Pro and Microsoft Visual Basic to develop programs that automate spectral acquisition, data processing, and data output as well as to create a user-friendly interface for the operator. For validation studies of the PV method, the FTIR spectrometer was programmed as described, leaving the operator only to weigh the oil, add the TPP, shake the sample, and present the reacted sample at the input spigot of the sample-handling accessory of the FTIR instrument. Validation was based on a direct comparison of duplicate FTIR PV predictions to duplicate analyses obtained by the official AOCS method. These validation trials were carried out by serially diluting rancid, high-PV oils with fresh oil and by spiking fresh oils with TBHP. Figures 6 and 7 present representative plots of duplicate FTIR and iodometric analyses of a rancid oil that was sequentially diluted with a fresh oil. Both plots are linear; however, it is apparent that the precision of the FTIR method is substantially better than that of the iodometric method. The respective SD values were 0.18 and 0.83 PV, with the individual data points for duplicate analyses being clearly discernible on the plot for the iodometric method. Figure 8 presents a plot of the mean iodometric PV (IOPV) vs. the mean FTIR-predicted PV for duplicate analyses of a variety of TBHP-spiked vegetable oils (canola, olive, soybean, safflower); the linear regression equation for the best-fit line was:

$$\text{FTIR PV} = -0.048 + 1.04\ \text{IOPV} \qquad \text{SD} = 0.628 \quad R = 0.9896 \quad [6]$$

Forcing the regression through the origin to eliminate the small constant produced Equation 7, which allows one to obtain an unambiguous measure of the slope:

$$\text{FTIR PV} = 0.992\ \text{IOPV} \qquad \text{SD} = 0.614 \quad R = 0.9919 \quad [7]$$

The slope obtained is close to the ideal value of 1.0, indicating a direct concurrence between the iodometric and FTIR PV methods. Most of the uncertainty associated with this relationship is due to the greater variability of the iodometric method. Table 2 summarizes all data for these experiments in terms of mean differences (MD) and standard deviations of the differences (SDD) for reproducibility (subscript *r*) and accuracy (subscript *a*) to compare the overall reproducibility of the two methods and the accuracy of the secondary FTIR method relative to the primary iodometric method (19). In this statistical analysis, data for vegetable oils that had undergone oxidation over time and those for samples spiked with TBHP were separated.

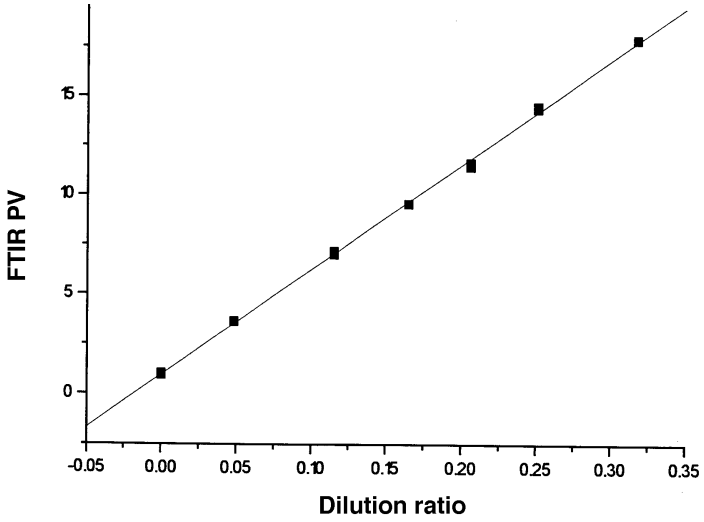


FIG. 6. Plot of FTIR PV data obtained as an oxidized oil is proportionally diluted with an unoxidized oil. The results of duplicate analyses for each sample are plotted as individual points. For abbreviations see Figure 5.

Ideally, the MD_r should be close to zero, implying that the overall differences are negligible in a series of analyses, while the SDD_r provides a measure of the variability around the MD. Table 2 clearly shows the superior reproducibility of the FTIR method, the SDD_r values being a factor of ~7–10 times smaller than those for the iodometric method. While there is no evidence that the reproducibility is affected by the type of hydroperoxides measured (TBHP vs. naturally formed hydroperoxides), the accuracy data show a significant difference between the MD_a values for the natural and the synthetic hydroperoxide samples, whereas the SDD_a values are quite similar. Although there is good agreement between the FTIR-predicted and the iodometric values for the TBHP-spiked samples, the FTIR-predicted values for the naturally oxidized samples are higher on average than the iodometric values. Linear regression of the FTIR-predicted vs. the iodometric PV data for naturally oxidized oils yielded a slope of ~1.15, outside the SE specifications derived from validation with TBHP-spiked oils. Perhaps TPP may react with peroxides as well as hydroperoxides, and this additional response may account for the slightly different slope and the bias associated with the MD_a. Nakamura and Maeda (15) have reported good agreement over the range of ~1–70 PV between the results obtained for various oxidized oils by the iodometric method and by their HPLC-based PV microassay, which is also based on the reaction between TPP and hydroperoxides; however, they did not present any statistical analysis of the data. The same authors also monitored the autoxidation of methyl linoleate under accelerated oxidative conditions by the two methods and found good agreement between them up to ~300 PV, after which the iodometrically determined PV showed a faster rate of change. They attributed the discrepancy for PV > 300 to the stronger reducing power of KI in the acidic $CHCl_3$/acetic acid medium vs. TPP in cyclohexane, the former being capable of attacking peroxidized products formed from methyl linoleate, including monocyclic and bicyclic endoperoxides and polymers.

TABLE 2
Reproducibility and Accuracy Data for Peroxide Value Determinations by the Fourier Transform Infrared (FTIR) and Iodometric Methods for Oxidized and TBHP-Spiked Oils

	Naturally oxidized oils		TBHP-spiked oils	
Statistic	FTIR	Iodometric	FTIR	Iodometric
MD_r	–0.017	–0.540	0.008	0.490
SDD_r	0.116	0.823	0.124	1.170
MD_a	–1.290		0.149	
SDD_a	1.10		0.847	

[a]Abbreviations: TBHP, *tert*-butyl hydroperoxide; MD, mean differences; SDD, standard deviation of differences; subscript *r*, reproducibility; subscript *a*, accuracy.

Nakamura and Maeda (15) also conducted time course studies with methyl 13-hydroperoxyoctadecadienoate and reported that the reaction with TPP was 95% complete within 5 min, whereas our results indicated that the reaction between hydroperoxides and TPP was 99.7% complete within 1.5 min after addition of the reagent. This apparent difference in reactivity may be attributed to the more uniform dispersal of TPP in the sample through the use of hexanol as a carrier for the TPP.

Primary methods, such as the iodometric method, represent an analytical reference method to which other methods are compared, usually because they have a sound analytical basis, in this case, a relatively straightforward stoichiometric reaction. In our assessment, the rationale for considering the AOCS chemical method as a primary method becomes prob-

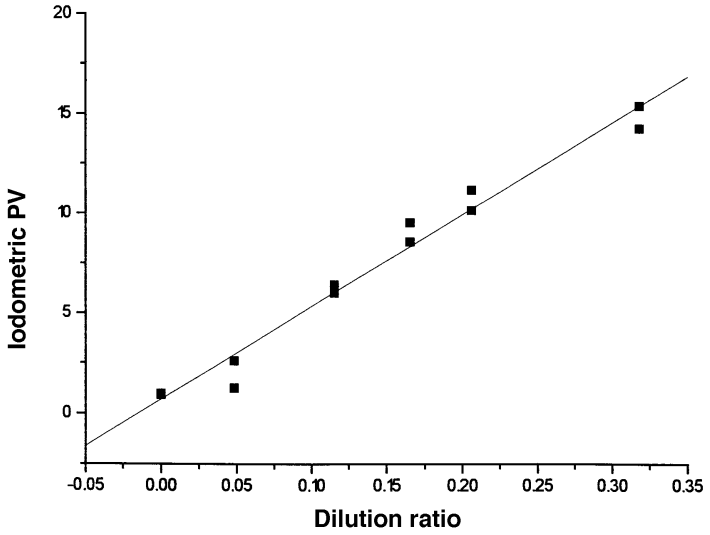


FIG. 7. Plot of iodometric PV data obtained for the same samples as shown in Figure 6. The results of duplicate analyses for each sample are plotted as individual points. For abbreviation see Figure 5.

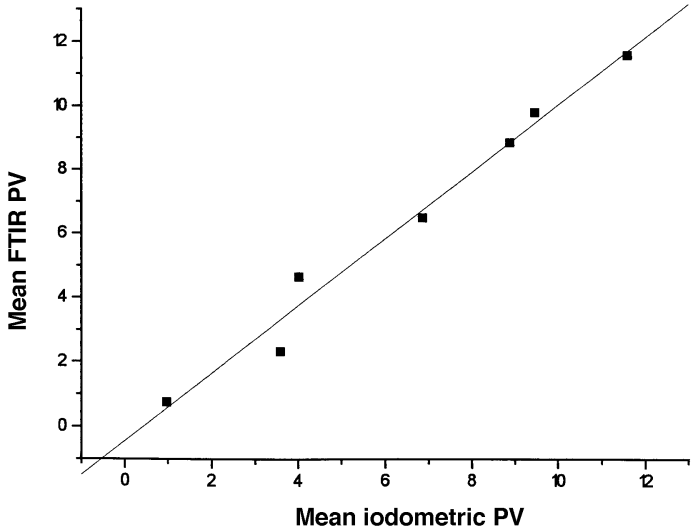


FIG. 8. Plot of the mean FTIR-determined PV vs. the mean iodometric PV from duplicate analyses of TBHP-spiked oils. For abbreviations see Figures 1 and 5.

lematic because it is not so reproducible as the FTIR approach. Because both methods involve well-defined stoichiometric reactions, the superior performance of the FTIR method indicates that it would better serve as a primary method than the iodometric procedure. In considering the variables and manipulations associated with the iodometric method, specifically the need for accurate preparation of reagents, the effects of adequate mixing during titration, and the human error associated with the determination of the end point, it is not surprising that the FTIR method can outperform the iodometric method. Beyond these factors, the iodometric method is highly empirical, and its results depend strongly on the standardization of all aspects of the procedure; as such, it is difficult to establish an absolute relationship to other methods. In general, the results obtained through the study of naturally oxidized oils, as well as of oils spiked with TBHP, confirm the overall ability to monitor PV changes quantitatively and rapidly by FTIR determination of TPPO formed by the reaction of TPP with hydroperoxides.

Although we were able to use the unique, strong 542 cm^{-1}

TPPO absorption band, we also followed up on the original concept of using the phenyl absorption bands (~750–675 cm^{-1}) to complete this study. The PLS regression technique was used to develop a two-component (TPP/TPPO) calibration, which had a detection limit of ~0.80 ± 0.25 PV in a 100-µm cell. Although this more complex calibration approach did work quite well, measurement of the 542 cm^{-1} band is definitely a superior approach from the standpoint of calibration simplicity and sensitivity because it avoids complications associated with small underlying absorptions owing to triglycerides and hexanol, which need to be taken into account in the phenyl band calibration approach. The TPP/TPPO method developed and described in this paper is substantially simpler than our original FTIR approach. It is more accurate and sensitive and may be improved up to fivefold in sensitivity if the cell pathlength is expanded to ~500 µm. For routine quality control analyses, a detection limit of 0.5 PV is adequate for most purposes, a degree of sensitivity which can be obtained with a cell pathlength of 25 µm. This makes the PV analysis compatible with some of our other FTIR analytical packages (e.g., IV/SN/*cis*/*trans* and Solid Fat Index methods), and, as such, PV analysis can be incorporated as a convenient add-on method.

As structured, the FTIR approach could effectively become a primary method because it is based on a simple, well-defined stoichiometric reaction and has better reproducibility than the recommended AOCS method. As noted earlier, the FTIR spectrometer has been programmed to automate the method. The program incorporates internal standardization procedures to compensate for instrument baseline drift and changes in cell pathlength over time (e.g., owing to window wear) and provides for automatic recalibration without rerunning standards, making the calibration transferable to other instruments without loss of performance. As configured, the TPP/TPPO approach to the FTIR determination of PV provides a robust, rapid, and accurate method for PV determination, eliminating the key drawbacks associated with the AOCS chemical method and reducing analytical time, manpower, and reagent disposal problems.

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