Rapid Determination of Oxidative Stability of Edible Oils by FTIR Spectroscopy Using Disposable IR Cards

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ABSTRACT: Disposable polytetrafluoroethylene (PTFE) polymer IR (PIR) cards were used as substrates to rapidly oxidize edible oils and simultaneously monitor the extent of oxidation by FTIR spectroscopy. Four edible oils were oxidized on PIR cards and in bulk at moderate temperature (58°C), and real-time oxidation plots were obtained by measuring changes in the IR hydroperoxide (ROOH) absorbance as a function of time. The relationship between the ROOH absorbance and PV was developed using a reference method to define absorbances corresponding to PV end points of 100 and 200 meq ROOH/kg for the oils oxidized in bulk and on cards, respectively. The real-time oxidation plots obtained for the oils oxidized in bulk and on cards were similar in appearance, but the oils on the cards reached the PV end point 20 times faster than the oils oxidized in bulk. The results indicate that the use of disposable PIR cards coupled with moderate heating and aeration provides a simple, practical, and rapid means for monitoring oxidation and determining the oxidative stability of edible oils at a normal storage temperature.

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KEY WORDS: AOM, autoxidation, edible oils, FTIR spectroscopy, polymer IR cards, OSI, OST, oxidative stability.

Most of the common methods available for monitoring the autoxidation and determining the oxidative stability of edible oils have inherent problems (1). The main problem is the use of elevated temperatures (≥98°C) to accelerate the oxidation process, which often changes the mechanism of oxidation and makes it difficult to extrapolate the data to ambient storage conditions (2). Given this limitation, there have been calls in the literature (1,3) for a rapid, low-temperature method (40–60°C) to assess oil oxidative stability.

FTIR spectroscopy has developed into a powerful tool for the analysis of edible oils, both qualitatively and quantitatively (4). FTIR spectroscopy has also been used to monitor oil oxidation, either by continuously following spectral changes related to oxidative processes as an oil undergoes oxidation on the surface of a heated attenuated total reflectance (ATR) crystal or by oxidizing an oil in bulk and periodically taking samples and recording their FTIR spectra using a transmission cell (5–9). However, the ATR approach is impractical because it monopolizes the FTIR instrumentation throughout the course of the oxidation monitoring period, whereas extended analysis times are required using the bulk oxidation approach. These practical limitations might be overcome through the use of disposable polymer IR (PIR) cards, recently shown to be effective in facilitating the rapid oxidation of model TAG under moderate heating and aeration conditions (10). This paper evaluates the possibility of monitoring edible oil oxidation using PIR cards in relation to bulk oxidation techniques and assesses the potential of this approach as a method for determining the relative oxidative stability of oils.

MATERIALS AND METHODS

Materials. Brand-name safflower, canola, sunflower, and extra virgin olive oils were purchased locally. Several samples from each bottle were transferred into vials, flushed with nitrogen, and stored at −20°C until needed. According to their labels, all the oils were additive-free and, other than olive oil, which was cold pressed, the oils were refined and deodorized. Type 2 ST-IR$^{\text{TM}}$ PIR cards were obtained from Thermo Nicolet (Madison, WI). These cards consist of a microporous polytetrafluoroethylene (PTFE) film, having a nominal pathlength of 10 µm, set in a cardboard frame that has two 1.9-cm apertures (10).

Instrumentation/data collection. Spectra were collected using a Bomem FTIR spectrometer (MB Series; ABB Bomem, Inc., Québec, Canada) equipped with a deuterated triglycine sulfate detector and operating under Bomem-Grams/386 software (Thermo Galactic, Salem, NH). The instrument was purged with dry, CO_2-free air from a Balston dryer (Balston, Lexington, MA), and spectra were collected by co-addition of 16 scans at a resolution of 4 cm^{-1} and ratioed against an open-beam background spectrum.

Bulk oil oxidation. Samples of the four edible oils purchased for this study, having initial PV levels of <2 meq ROOH/kg oil, were oxidized simultaneously in 500-mL Erlenmeyer flasks containing 250 mL of oil set in a water bath maintained at 58 ± 1°C. Aeration was provided by a manifold delivering room-temperature air from a pressurized laboratory air line run through the Balston drier used to purge the FTIR spectrometer. Periodically, 1-mL aliquots were removed and loaded into a 190-µm KCl transmission cell for FTIR spectral acquisition. The peak height of the ROOH absorption band at 3444 cm^{-1} was measured to obtain a spectral time-course profile of the bulk oxidation process. When substantial levels of hydroperoxides (PV > 300 meq/kg

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oil) were evident spectrally, the oxidation process was stopped, and the oils were stored at –20°C under nitrogen.

Oxidation of oils on PIR cards. Samples of the same oils as those subjected to bulk oxidation were loaded onto PIR cards by applying three drops (~0.075 g) onto the center of each of the card apertures (duplicates). The oil was then spread over the PTFE film with a synthetic fiber brush, which was rinsed with isooctane and dried after application of each sample. Any excess oil was removed from the film by blotting with an absorptive tissue. An initial spectrum was then recorded from each aperture ($t = 0$ in the oxidative process), and the card was subsequently placed in a Plexiglas^{TM} aeration chamber through which warm, dry air (58 ± 4°C) flowed over the cards in a laminar manner at a constant rate (10). Periodically, each card was removed from the chamber for FTIR spectral acquisition, with the total elapsed time being recorded.

Spectral data processing. PIR card spectra were processed through the series of steps summarized in Figure 1 to compensate for card pathlength variability as well as oil diffusion and spectral band shifts over time, as detailed in a previous paper (10). In steps 2–4, all spectra are normalized to unit height of the maximum of the ν(CH) absorption band at 2854 cm^{-1}, and a series of differential spectra are then generated for each aperture of each card by subtracting the $t = 0$ spectrum from all subsequently collected spectra. By using the resulting normalized differential spectra, it is possible to compare all the spectral data acquired using the PIR cards directly. Steps 5–9 involve the measurement of the ROOH band in the differential spectra (relative to $t = 0$) by using a peak-find algorithm (10) to locate the absorbance maximum within the range of 3550–3200 cm^{-1}, relative to a baseline at 3649 cm^{-1}. To facilitate analysis of the card spectral data, algorithms to carry out steps 2–8 automatically, illustrated in Figure 1, were implemented using UMPIRE (Universal Method Platform for Infrared Evaluation) software (Thermal-Lube Inc., Pointe Claire, PQ, Canada). The algorithms for steps 5–8 were also used in the analysis of the spectral data collected for the oils oxidized in bulk.

PV calibration/statistical analysis. Four sets of PV calibration standards were prepared by serial dilution of the oil samples collected at the end of each of the bulk oxidation experiments with their respective fresh oils. The PV of these standards were determined by the FTIR triphenylphosphine/triphenylphosphine oxide (TPP/TPPO) method (11); for this analysis, each standard was diluted with its respective fresh oil to bring its PV within the range of the method (<15 meq ROOH/kg oil). The PV of the prepared standards ranged from 0 to 250 meq ROOH/kg oil. The FTIR spectra of these standards were collected in duplicate both on PIR cards and in a 190-μm KCl transmission cell, and the ROOH absorbance at 3444 cm^{-1} was measured [in units of milliabsorbance (mAbs)]. For each set of calibration standards, these absorbance values were regressed against PV, with the regression being forced through the origin (Z-reg) to eliminate the contribution of the intercept and allow the slopes to be compared. The Z-reg slopes for the four sets of calibration standards were analyzed statistically using ANOVA with $\alpha = 0.05$ to assess whether the data could be pooled. The slope value obtained from the pooled calibration data for the transmission cell was used to calculate the time required for the oils oxidized in bulk to reach a PV end point of 100 meq ROOH/kg oil. Analogously, the slope value obtained from the pooled calibration data for the PIR cards was used to calculate the time required for the oils oxidized on cards to reach a PV end point of 200 meq ROOH/kg oil.

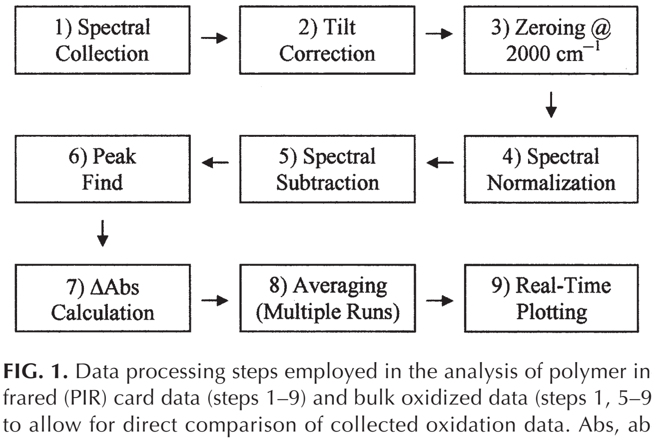


FIG. 1. Data processing steps employed in the analysis of polymer infrared (PIR) card data (steps 1–9) and bulk oxidized data (steps 1, 5–9) to allow for direct comparison of collected oxidation data. Abs, absorbance.

RESULTS AND DISCUSSION

Forced oxidation of edible oils at temperatures ≥98°C is rapid but not necessarily representative of the oxidative processes taking place in oils maintained at room or normal storage temperatures. For this reason, all oxidation experiments in this study were performed at a moderate temperature (~58°C). The same temperature was utilized for oil oxidation both in bulk and on PIR cards to facilitate direct comparison of the oxidative processes. In both cases, the time course of oil oxidation was monitored by following the growth of the ROOH absorption band, which is observed at 3444 cm^{-1} in the spectra of low-PV oils but which shifts progressively to lower wavenumbers as oxidation proceeds, making it necessary to use a peak-find algorithm (10). In addition, normalization of the spectral data collected from the cards is required to obtain quantitative data because of variability in the thickness of the PTFE film among different cards as well as variability in sample loading and diffusion over time (10). The PTFE film substrate of the PIR card has absorptions over the 1270–1100 cm^{-1} and 660–460 cm^{-1} regions, neither of which interferes with the measurement of the ROOH absorption band nor with that of the CH absorption (2854 cm^{-1}) used to normalize the spectral data. For illustrative purposes, typical differential spectra showing the increase in the ROOH band over time for safflower oil oxidizing on an IR card are presented in Figure 2.

Real-time oxidation plots. Figures 3A and 3B present real-time oxidation plots, showing the change in the ROOH absorbance value (ΔAbs) vs. time for the bulk oxidation of four edible oils and for the oxidation of the same oils on PIR cards. In all cases, classic oxidation profiles are observed, with each

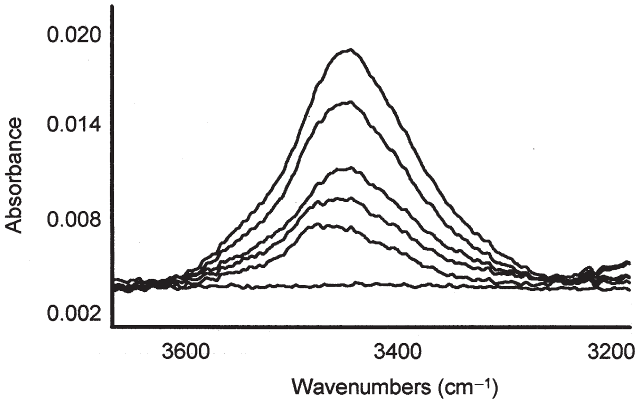


FIG. 2. Time-course differential spectra of safflower oil undergoing oxidation on an IR card, illustrating the development of hydroperoxides (~3444 cm^{-1}) over time (0–15 h).

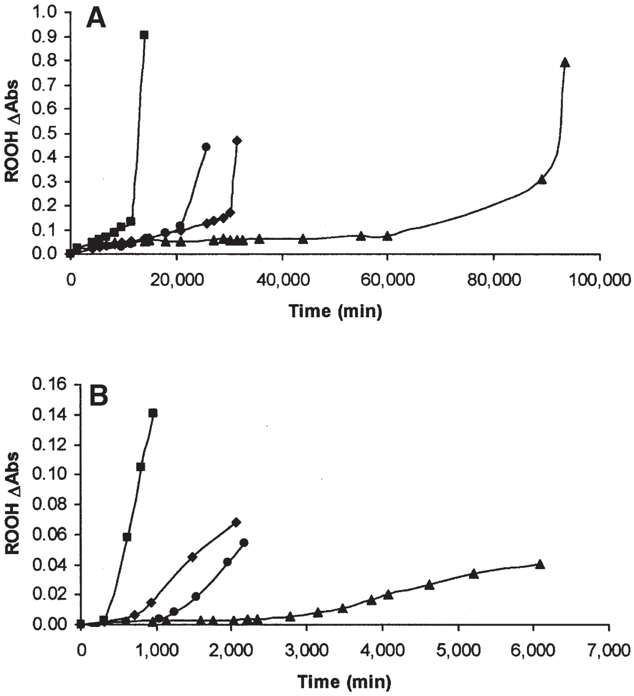
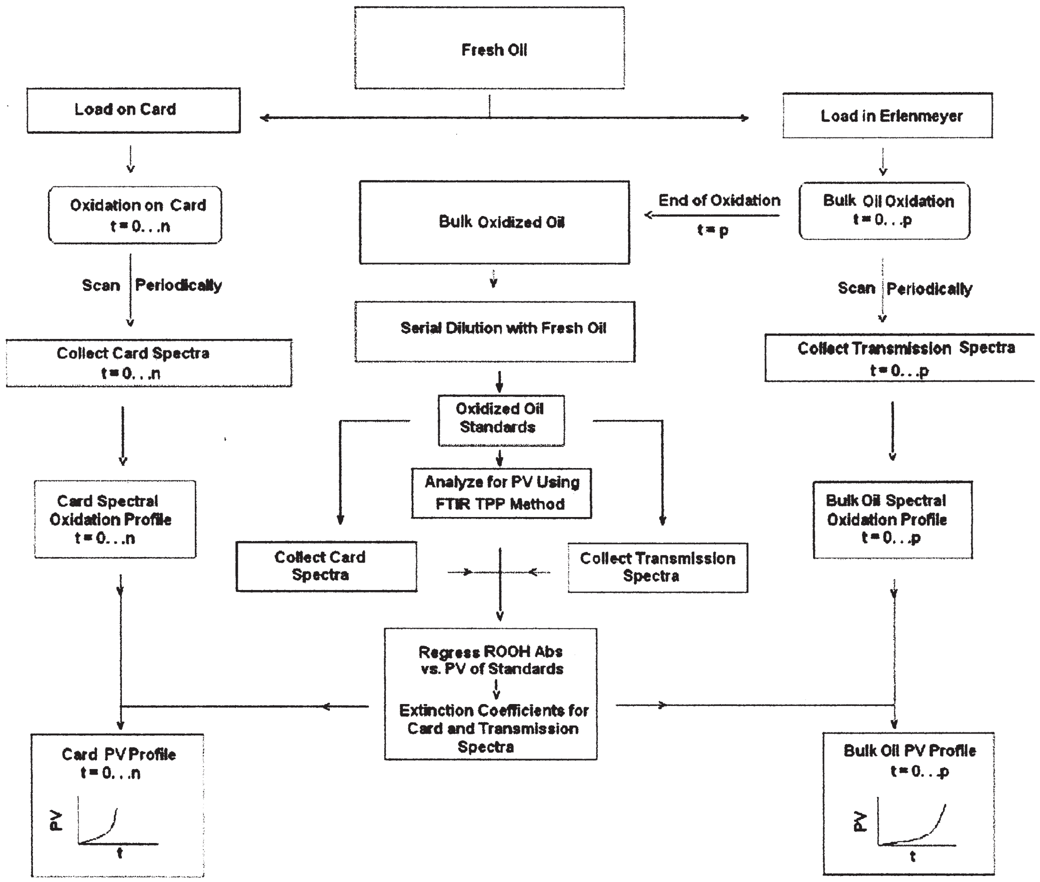


FIG. 3. Real-time oxidation plots of the changes in ROOH absorbance in the FTIR spectra of edible oils as they are (A) oxidized in bulk and (B) oxidized on PIR cards, as a function of time; (■) safflower, (●) canola, (◆) sunflower, and (▲) olive oils. For abbreviation see Figure 1.

oil exhibiting an initial induction period followed by an accelerated rate of ROOH formation. However, the times associated with these changes differ substantially between Figures 3A (bulk oxidation) and 3B (oxidation on cards). The ΔAbs values in Figures 3A and 3B cannot be compared directly because they are dependent on the pathlengths of the transmission cell and the PIR cards, which differ by more than an order of magnitude. Therefore, to compare the relative rates of oil oxidation in bulk and on cards in a meaningful way, the FTIR absorbance data were converted into the corresponding PV data. This data conversion also allows the results of the FTIR oxidation monitoring experiments to be expressed in terms of the time required to attain a specified PV (e.g., 100 meq/kg oil), as is customary in oxidative stability tests such as the Active Oxygen Method (AOM) and the Oven Stability Test (OST).

Scheme 1 summarizes the experimental procedures leading to the comparison of the PV vs. time profiles of oils oxidized on cards relative to oils oxidized in bulk. Samples of the four edible oils collected at the end of the bulk oxidation experiments provided high-PV stocks from which to prepare standards to determine absorbance/PV relationships using PV data obtained by a reference method. An FTIR method based on the stoichiometric reaction of ROOH with TPP, which produces a strong and distinct IR absorption band at 542 cm^{-1} owing to the formation of TPPO, was selected as the reference method, since this simpler alternative to the official AOCS PV method has been shown in extensive validation studies to be highly reproducible and accurate (11). For this FTIR method, a calibration can be devised simply by adding known amounts of TPPO to an oil to represent the amounts that would be formed by addition of TPP to oils of known PV based on the stoichiometry of the reaction. The PV of the standards were determined from the calibration equation obtained in this manner, and the spectra of these standards were then recorded both on PIR cards and in the transmission cell used in monitoring bulk oil oxidation to obtain relationships between ROOH absorbance changes and PV (Table 1). All the regression plots were linear, with coefficients of determination (R^2) ranging from 0.995 to 0.999, and had intercept values only slightly different from zero. Because the slopes of these plots were proportional to the ROOH extinction coefficients of the respective oils, which were not necessarily the same, ANOVA analysis was performed to assess whether the data for the different oils could be pooled. The results of this analysis indicated that the Z-reg slopes for the four sets of calibration standards were statistically different in the case of both the cards and the transmission cell. However, given the similarity of the slope values among the oils, with the exception of olive oil in the case of the transmission cell data, the use of the slopes obtained from the pooled data was considered a reasonable approximation from a practical standpoint.

By using the slope obtained from the pooled data for the transmission cell, the absorbance corresponding to a PV end point of 100 meq ROOH/kg oil was determined to be readily measurable (>100 mAbs) for the oils oxidized in bulk (Table 2). On taking into account the 95% confidence interval for the slope, the end point for the bulk oils represents a PV range of 97–104 meq ROOH/kg. Owing to the very short pathlength of the cards (~9.5 μm), the absorbance value corresponding to a PV of 100 for the oils oxidized on cards is only on the order of 2 mAbs, which would be barely detectable above the background noise. As a consequence, a more measurable and reliable absorbance end point corresponding to a PV of 200, with a 95% confidence interval of PV 180–220, was chosen for the card method. The larger PV range in the latter case is mainly due to the much shorter pathlength of the card as compared to the transmission cell. This limitation could be overcome with the use of 100-μm 3M (St. Paul, MN) “quant” PIR cards, but



SCHEME 1

TABLE 1
Absorbance/PV Regression Results for PV Calibration Standards Scanned in a 190-μm KCl Transmission Cell and on PIR Cards[a]

	Transmission cell		PIR cards	
Oil	Z-Reg slopes (mAbs/PV)	R^2	Z-Reg slopes (mAbs/PV)	R^2
Safflower	1.057	0.998	0.057	0.996
Canola	1.074	0.999	0.051	0.999
Sunflower	1.016	0.999	0.048	0.995
Olive	1.329	0.999	0.053	0.998
Pooled	1.073	0.993	0.054	0.993

[a] PV in units of meq ROOH/kg oil; PIR, polymer IR. mAbs, milliabsorbance units; Z-reg slope, a slope that is mathematically forced through the origin.

production of these cards was discontinued by the original manufacturer. However, given that variations of up to 13.4% are considered acceptable for the AOM (12), the confidence interval for the present PIR card method is also quite acceptable. It may also be noted in Tables 1 and 2 that the confidence intervals for the PV end points derived from the slopes for the individual oils are all very similar to those derived from the pooled data, supporting the validity of using the ROOH ΔAbs/PV relationships derived from the pooled data.

By using the real-time oxidation plots in Figures 3A and 3B, the times required for the oils oxidized in bulk and on cards to reach the corresponding PV end points were determined. Polynomial regression was used to obtain the best fit through the

TABLE 2
Absorbance Values at PV End Points for a 190-μm KCl Transmission Cell and PIR Cards and Corresponding PV End Point Confidence Intervals Derived from the Regression Slopes in Table 1 and Their Confidence Intervals[a]

	Transmission cell			PIR cards		
Oil	Abs at PV 100	LCL 95% (PV)	UCL 95% (PV)	Abs at PV 200	LCL 95% (PV)	UCL 95% (PV)
Safflower	0.106	95	104	0.011	182	218
Canola	0.107	98	103	0.010	180	222
Sunflower	0.102	97	102	0.010	180	222
Olive	0.133	96	104	0.011	182	218
Pooled	0.107	97	104	0.011	180	222

[a] LCL, lower confidence limit; UCL, upper confidence limit. See Table 1 for other abbreviations.

TABLE 3
Time to PV 100 for Oils Oxidized in Bulk and Time to PV 200 for Oils Oxidized on PIR Cards[a]

Oil sample	Bulk oil time to PV 100 (h)	Oil on card time to PV 200 (h)
Safflower	172.4	5.32
Canola	338.2	21.7
Sunflower	365.8	12.2
Olive	1124.9	53.7

[a]See Table 1 for abbreviation.

absorbance/time curve around the specified end point, and the time corresponding to the end point was calculated using the regression equation. Table 3 lists the times (in hours) required for each of the four oils studied to reach the PV end points of 100 and 200 for the bulk oxidation and card methods, respectively, and Figure 4 presents a plot of these data. Linear regression indicated that the results from the two methods were strongly correlated ($R^2 > 0.95$), supporting the validity of the higher PV end point used in the card method. The regression slope indicated that at the temperature used in these experiments (58°C), the oils oxidized on cards reached the PV end point ~20 times faster than the oils oxidized in bulk, despite the fact that the end point for the card method is set at twice that used in the bulk oxidation method. The two methods agree on the ranking of safflower and olive oil as the least and most stable of the oils tested; however, their ranking of canola and sunflower oils in terms of relative oxidative stability is reversed. Inadequate information is available to resolve which ranking is correct; however, similar discrepancies were encountered in other studies in which different oxidative stability methods were compared (13).

It is evident from this work that monitoring oil oxidation by FTIR spectroscopy with the use of PIR cards provides a practical means of comparing the oxidative stability of oils. By comparison with the AOM and the Oil Stability Index (OSI), this approach allows for rapid oil oxidation at temperatures more representative of ambient storage conditions (1,3). This study was conducted at 58°C, which is the temperature used in the OST (14), but oxidation is greatly accelerated relative to the OST, as well as to oxidation of oils in bulk with forced aeration, because of the high surface-to-volume ratio provided by the porous PIR card, with laminar air flow over the card further accelerating the oxidative process. The FTIR absorbance data collected as oils undergo oxidation on cards can be transformed into "apparent" PV, values that are well-understood in the industry as a measure of the oxidative status of oils. Although the extinction coefficient of the ROOH absorption (and hence, the slope factor relating absorbance to PV) could, in principle, vary among different oils, the use of a global slope factor for the four oils studied in this work proved satisfactory and is considered to be adequate in general, facilitating the routine implementation of the method. Regardless of how the progress of oxidation is measured, a time corresponding to a specified end point has to be obtained from the real-time oxidation plots to have a relative measure of oxidative stability. This can be done either by graphical interpolation or by using more sophisticated data-fitting techniques to provide a more refined estimate, much like the approach used in the OSI procedure (15). Using the PIR card provides the distinct advantage of allowing one to obtain many data points with little effort and hence provides smooth and largely continuous plots that clearly delineate the oxidation process.

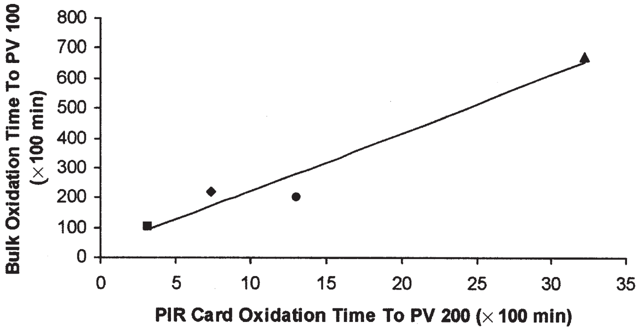


FIG. 4. Relationship between the time to PV 100 for oils oxidized in bulk and the time to PV 200 for the same oils oxidized on PIR cards; (■) safflower, (●) canola, (◆) sunflower, and (▲) olive oils. For abbreviation see Figure 1.

This study indicates that PIR cards allow oils exposed to mild heating and aeration conditions to oxidize rapidly, and FTIR spectroscopy facilitates the dynamic monitoring and quantification of the oxidative stability of oils in a convenient manner, avoiding wet chemical procedures commonly associated with such evaluations. The utility of the method would be further enhanced if PIR cards similar to the discontinued 3M "quant" cards become available again. These cards were ideally suited for the present application owing to their long pathlengths (on the order of 100 μm) as well as the nonporous annular ring around the circumference of the aperture, which limited sample diffusion during the oxidation monitoring period. However, with the ST-IR cards currently available, the latter problem can be compensated for through the application of data-processing algorithms such as those developed for this work using UMPIRE software (Scheme 1). In general terms, the use of such data-processing algorithms is a prerequisite for the routine implementation of this method so that reliable absorbance and/or PV data can be obtained and the interpretation of the spectral data can be performed automatically. In addition, the conditions of oxidizing oils on the PIR cards would require standardization, much as in the case of the OSI (15), since temperature, air-flow rate, and other variables must be carefully controlled to obtain consistent and comparative data.

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