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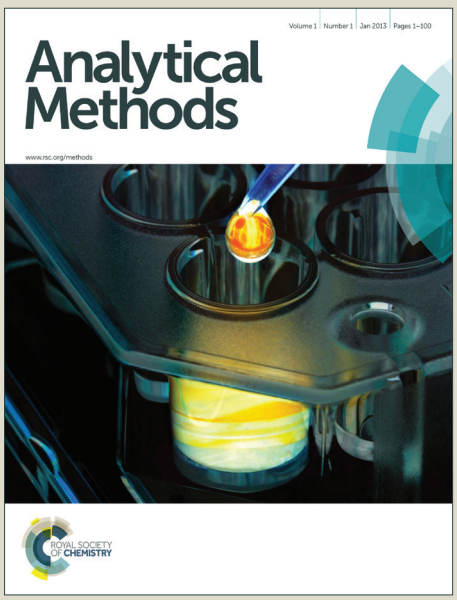

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ARTICLE

Determination of peroxide value of edible oils by FTIR spectroscopy using polyethylene film

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A new technique was developed to facilitate mid-FTIR transmission analysis of viscous edible oil using disposable polyethylene (PE) film as a sample support for the determination of peroxide value (PV) of the oils. The basis of the PV quantification is the rapid reaction of triphenylphosphine (TPP) with lipid hydroperoxides present in an oil to produce triphenylphosphine oxide (TPPO), which has a measurable absorption band at 542 cm^{-1}. Calibration standards, prepared by the gravimetric addition of a peroxide-free oil were used to develop a linear calibration equation that relates the concentration of TPPO (expressed as the equivalent PV) to the absorption value of TPPO in the normalized spectra. PV determinations on two sets of validation samples, with PV ranges of 2 to 25 and 0 to 2 mmol/kg oil, were carried out using both the American Oil Chemists' Society (AOCS) titrimetric method and our PE-based FTIR procedure. A comparison of the results of these analyses indicated that the FTIR method is more reproducible and slightly more sensitive than the conventional method.

Introduction

Edible oils are prone to oxidation during processing and storage[1]. During the initial stages of the oxidation process, lipid hydroperoxides accumulate in the oil. Hydroperoxide, known as the primary oxidation product, can degrade further into secondary oxidation products such as aldehydes, alcohols, and ketones [2,3], that give the rancid flavor. Thus, quantification of lipid hydroperoxide is necessary to determine the degree of oxidation of edible oils. Peroxide value (PV) is typically used as an index to monitor lipid oxidation, and it is one of the most frequently determined quality parameters during oil processing, storage and marketing. The accumulation of hydroperoxides is commonly monitored using the well-established iodometric procedures. For the PV test, PV is determined and expressed in milliequivalent or mmole of hydroperoxides per kilogram of oil. The determination of PV by using the standard method of the American Oil Chemists' Society (AOCS) is based on the classical redox reaction between lipid hydroperoxides in the oil sample and the excess amount of potassium iodide (KI) [4,5]. Although it is relatively simple and adequate in sensitive, titrimetric methods in general are labor-intensive and tend to consume significant

amounts of reagents and solvents that are of environmental concerns.

Due to these shortcomings, various modifications of, and alternatives to, the standard method have been proposed [6,7], including the luminal chemiluminescence PV method [8], as well as determination according to the reaction between triphenylphosphine (TPP) and oxidized oil to form triphenylphosphineoxide (TPPO) which is then analyzed by high performance liquid chromatography–UV spectroscopy [4]. A simple method used a UV spectrometer with the same reaction principle for PV determination of fried oil [9]. A flow injection analysis (FIA) method [10], and a Fourier transform-near infrared (FT-NIR) method [11], and a method of determination of changes in the electrical conductivity of the aqueous phase during the reaction of potassium iodide (KI) with the hydroperoxides presented in oils [12], have also been used for determining PV. These methods provide more information at the expense of introducing more complexity into the analyses.

Fourier transform infrared (FTIR) technique has been developed into a powerful tool for the analysis of edible oils, both qualitatively and quantitatively. It has also been used to monitor oil oxidation [13,14,15,16,17]. However, the attenuated total reflection (ATR) approach is impractical as it has a short pathlength cell and a weak signal, thus, it is less accurate. van de Voort and colleagues have worked toward developing instrumental methods for the quantitative analysis of edible oils based on FTIR spectroscopy [18,19]. Initially, they used a chemometric approach based on the measurement of the characteristic O–H stretching absorption band of hydroperoxides at 3444 cm^{-1} [18]. Subsequently, they developed a much simpler and more accurate method utilizing the well-characterized stoichiometric reaction of TPP with hydroperoxides to form TPPO [20]. Altogether, the sample handling difficulties associated with the mid-infrared (IR) analysis of neat oils in the transmission mode limits its practical application [19, 21]. For overcoming handling difficulty of the viscous oil samples, Ma et al. (1997; 1998) investigated quantifying hydroperoxides by using disposable polyethylene infrared cards (e.g. 3M IR cards) in edible oils [20,22]. Another technique so called spectral reconstitution (SR), was applied to simplify and automate the FTIR method for determining the PV of edible oil. It used the SR procedure to eliminate cell loading problems [23]. Recently, our research group has developed a new technique to facilitate mid-FTIR transmission quantification of free fatty acid (FFA) content in edible oils using disposable polyethylene (PE) films [24]. In the PE procedure, the spectra of the oil films were collected and collected against a PE film background spectrum. The pathlength of oil films was determined by a CH combination band calibration and was normalized to a fixed pathlength of 0.1 mm. This study evaluates this new technique in determining the PV of edible oils using the PE film for for FTIR spectroscopy.

Material and methods

Materials and reagents

Several edible oils were obtained from local retail outlets. Among these, refined rapeseed oil was used as the base oil for the preparation of calibration standards; the other oils were blended with an oxidized rapeseed oil to produce samples of having various PV. When peroxide-free oils were required, the oils were passed through a column of microwave-activated silica gel to remove any polar oxygenated molecules and then were verified to be peroxide-free using the standard AOCS Cd

8b-90 titrimetric PV procedure [5]. The peroxide-free oils were kept in 4 ℃ refrigerator with nitrogen. Reagent-grade TPP (>99%) and TPPO (>99%) were obtained from Sigma Aldrich (St. Louis, MO), and both the TPP and TPPO were ground with a mortar and pestle and passed through an 80-mesh sieve to facilitate their dissolution in oils. Transparent food wrap polyethylene (PE) film (Food grade), about 0.025 mm thick, was obtained from a local food market.

Instrumentation

A Bruker VERTEX 70 series FTIR spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector was used for this study. PE film was mounted on the transmission cell holder. All spectra were collected by the co-addition of 16 scans at a resolution of 4 cm^{-1} with PE film as background.

PE sample preparation procedure

For the PE sample preparation, the viscosity of oil samples was reduced by mixing them with hexane which allows them to be readily deposited onto PE films to form oil films after the solvent evaporated. To estimate the pathlengths of the oil films, a pathlength calibration technique was developed. Sample of 500 μL oil dissolving 100 μL hexane was deposited onto the central surface of the films of about 1 cm^2 areas. The oil film spectra were collected and corrected against a PE film background spectrum. The pathlength of oil films were determined by a CH combination band calibration and then normalized to a fixed pathlength of 0.1 mm [24]. Different types of oils were loaded into the transmission cells of varying pathlength (0.015-1.00 mm, as determined by the fringe count) and the spectra were collected. The pathlength calibration was obtained by correlating with the absorbance at 4334 cm^{-1}, relative to a baseline at 3850cm^{-1} (4334cm^{-1}/3850 cm^{-1}) [25].

$$y=0.8982x+0.0029;\ R=0.9998;\ SD=0.0064 \quad (1)$$

(Where y is pathlength, mm; x is absorbance at 4334cm^{-1}/3850 cm^{-1}.)

Stoichiometric reaction

An initial assessment was carried out to determine the quantification of conversion the TPP to TPPO by hydroperoxides in the oil samples. A set of test samples was prepared by serial dilution of high-PV oil with fresh peroxide-free oil, then the oils were reacted with excess TPP and the spectra were collected through the PE procedures. The spectra of these samples were recorded in ~5 min after the start of the reaction.

Preparation of calibration standards and validation samples

A stock solution of TPPO in rapeseed oil was prepared at a concentration corresponding to that produced by the reaction of TPP with all the hydroperoxides in an oil having a PV of about 18 mmol/kg. This was done by adding about 0.066 g of ground and sieved TPPO to 10 g of oil and heating this oil in a microwave oven for about 60 s to facilitate the dissolution of the TPPO. This stock oil dipersion was then gravimetrically diluted with peroxide-free rapeseed oil to produce a series of PV calibration standards, which were subsequently used to develop a calibration.

Curve validation samples were prepared by blending an oxidized peanut oil (PV of 75 mmol/kg) with two peroxide-free validation sets; one having a broad range of PVs (2–25 mmol/kg) and the other having a much narrower PV range (0–2 mmol/kg). The calibration equation relating PV standards to absorbance at 542 cm^{-1} (relative to a baseline value at 530

cm^{-1}) was programmed using the TQ analysis software. The spectra of the validation samples were processed through this calibration equation, and the reported PV results were compared with the values obtained by using the AOCS titrimetric PV method immediately prior to FTIR analysis.

Instrumental procedures

An approximately 600 µL sample was deposited onto the surface of the PE film using a micropipette and the oil was then spread uniformly with the tip of the micropipette. The viscosity of oil samples is reduced by mixing them with hexane which allows them to be readily deposited onto PE films. The oil films were formed after the solvent was evaporated. Films prepared in this manner were then placed in the instrument and maintained in a horizontal position and the spectra of the oil films were collected using the PE film background spectrum. The effective pathlength of oil film spectra were calculated by using Eq. 1 and normalized to a fixed pathlength of 0.1 mm. Spectral data processing and statistical analysis were carried out using TQ Analyst 7.2 (Thermo Electron Inc., Madison, WI) and Origin Pro7.5 (Originlab, Northhampton, MA).

Blind oil samples validation

To further validate the performance of the PE-based FTIR procedures, 10 different edible oil samples, purchased from the local market, were immediately analyzed using the AOCS titrimetric PV method in triplicate and using the PE-based FTIR procedures. The results obtained were compared to each other.

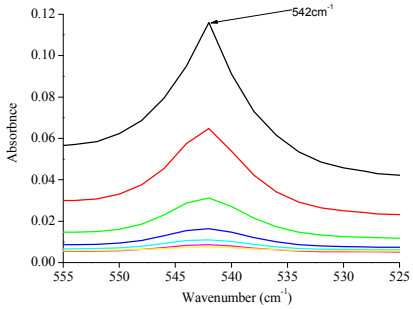
Statistical analysis

All analyses were performed in triplicate and the mean values were used to express the results. The figures were plotted using Origin Pro 7.5 and the correlation coefficients and standard deviations were used to evaluate the correlations and deviations.

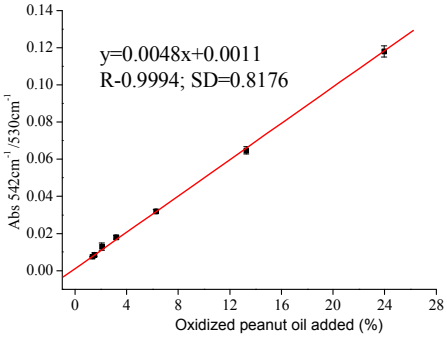
Results and Discussion

Stoichiometric reaction

The spectra of these samples were recorded ~5 min after addition, which showed a progressive increase with different amount of PV present (Fig. 1) indicating that the desired TPP-TPPO conversion had taken place. The TPP TPPO conversion is rapid and complete when excess of TPP is present [20]. Accurate quantification of TPPO is achieved by measuring the intensity of the unique and intense mid-IR absorption band TPPO at 542cm^{-1} [23].



(a)



(b)

Fig.1 PE-FTIR spectral response at 542 cm^{-1} obtained after the addition of TPP in oils with different PV contents.

Calibration

The PV calibration standards consisted of a peroxide-free oil containing varying amounts of TPPO, representing a PV range of 0 to 18 mmol/kg. The absorbance of TPPO of the normalized spectra is presented in Figure 2.

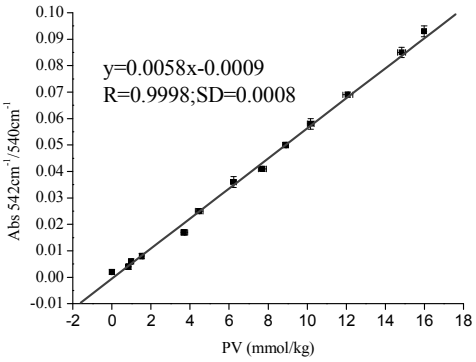


Fig. 2 Calibration curve of absorbance of TPPO *vs.* PV, measured at 542 cm^{-1} and referenced to a baseline at 530 cm^{-1}, obtained from the corresponding spectra of rapeseed oil spiked with varying amounts of TPPO.

Figure 2 shows that standard curve was obtained when using oil films standardized at the same pathlength (0.10 mm). The corresponding linear regression equation is present below. The results can be predicted by the following equation:

$$y = 0.0058x - 0.0009;\ R = 0.9998;\ SD = 0.0008 \qquad (2)$$

(Where y is absorbance at 542cm^{-1}/530 cm^{-1}; x is PV, mmol/kg.)

This regression equation states that PV can theoretically be measured to within ±0.14 mmol/kg over the range of 0 to 18 mmol/kg.

Validation

This FTIR PV method was validated using two sets of oils, one having a PV range from 2 to 25 mmol/kg and the other from 0 to 2 mmol/kg. Both sets of oils were analyzed in duplicate by the AOCS titrimetric method and by the PE-based FTIR analysis.

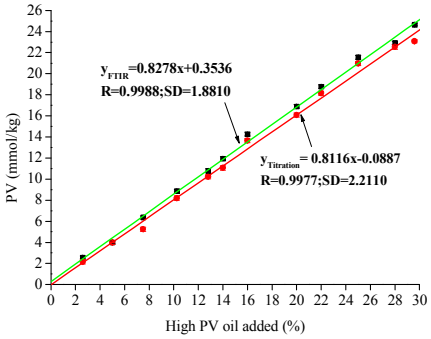


Fig. 3 Relationship determined by the FTIR and titration of oils with high amount of added oxidized peanut oil

As shown in Fig.3, the PE-based FTIR (predicted from Eq. 2) and AOCS PV related well. The two lines had similar slopes and intercepts, but the PE-based equation has a lower SD than the equation from the titration procedure. It indicates that the proposed PE-based method has better reproducibility than AOCS chemical method. Therefore, the PE-based FTIR can be used as alterative PV method.

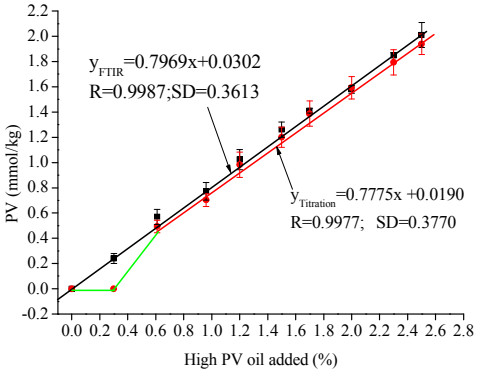


Fig.4 Relationship determined by the FTIR and titration of oils with low amount of added oxidized peanut oil

To test the relative sensitivity of the two methods, the second validation set, covering a low PV range (0 to 2 mmol/kg) was assessed. Fig. 4 compares the PE-based and titrimetric PV results obtained for this series of samples. As shown, the results from the two methods paralleled each other at the upper end of the range. Although standard AOCS method is quite simple and reproducible but it seemed to be relatively less sensitive at lower PV range compared to the PE-based method. The titration method was also found to start to fail at PV values below 0.50 mmol/kg [23], while the PE-based method responds linearly down to 0.50 mmol/kg with lower SD value, as shown by our results.

Blind oil samples validation

As seen in Eq. 2, a plot of TPPO concentration (expressed as PV) versus absorbance at 542 cm^{-1} has a non-zero intercept, which was due to a minor contribution of the oil to the absorbance at this wavenumber. This non-zero intercept raised the possibility that the PV values obtained by the PE-based procedure would be affected by the type of oil being analyzed. To investigate this possibility, a variety of oils were obtained from the retail outlets and analyzed by both the PE-based and the AOCS titration procedures. The PE-based FTIR and AOCS PV results agreed well (Fig. 5), with a R of 0.9998 and a slope of 0.995. Therefore, oil types does not appear affect the PE-based FTIR method.

In this study, we have developed an effective and efficient PE film based FTIR method for the accurate determination of PV in fats and oils.

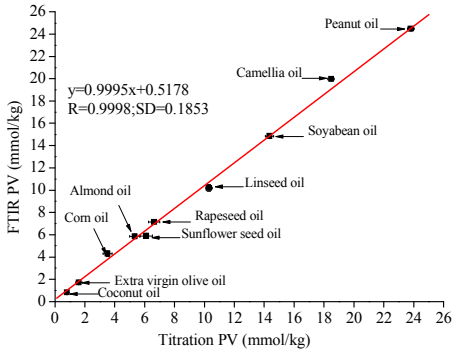


Fig. 5 Plot of PV determined by PE-based FTIR and the AOCS titrimetric procedure for 10 different oil samples

This method is easy to execute and is independent of the problems associated with the viscosity of oils were avoided. The film material was used in the study is transparent and not affected by the different sources. The method used the absorbed at 4334cm^{-1} as a marker for pathlength determination, which locates in near-infrared region and can also avoid interference the other absorption bands of edible oils. This result was comparable to the method developed by Ma et al [20,23]. They used the ester linkage carbonyl overtone band at 3475 cm^{-1} as marker, and this gave an overall effective pathlength variation of ±5%, and the band was easily interfered by hydroperoxides and moisture.

The effective pathlength of oil films using our method was 0.020-0.050 mm, which can generate enough signals for the PV determination. The effective pathlength of the SR method was ~0.15 mm [23,25]. It was shown that SR method was more accurate and sensitive, but the conventional transmission cells are not easy to handle in the routine analysis, and the cells are more expensive than the PE film. Recently, our research group has successfully developed a mid-FTIR transmission quantification of FFA content in edible oils using PE films [21].

The results were comparable to that from AOCS standard method from the standpoint of accuracy. The method was particularly well suited to process control in laboratories where routine high-volume of fats and oils for FFA content analysis was required. PE based FTIR method is currently under way to further investigations that involve other quality parameters for edible oils, such as carbonyl value, total polar materials content.

Conclusions

In conventional FTIR analysis, the oil samples are difficult to be loaded into the narrow flow-transmission cells, especially for viscous oil. In addition, cells are difficult to clean and easy to be cross-contaminated. This complex and time-consuming process also uses massive amounts of cleaning solvent thus restricts the general application of conventional FTIR spectroscopy. In contrast, our results show that PE-based FTIR procedure is practical and is without any complicated operation. Comparison of the results of PV measurement by the conventional AOCS method and our method indicates that the PE based FTIR method is more reproducible and slightly more sensitive. Consequently, this new FTIR method could serve as the basis for developing a new AOCS method for quantifying PV in edible oils. It is a significant contribution to PV quantification of edible oils for in-plant quality control, as well as for centralized commercial laboratories requiring automated, high-throughput analytical capability.

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