Rapid Determination of Acid Value of Edible Oils via FTIR Spectroscopy Using Infrared Quartz Cuvette

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Abstract: A Fourier transform infrared (FTIR) spectroscopy with infrared quartz cuvette (IQC) as spectral accessory method was developed to determine acid value (AV) of edible oils. The absorption peak at 5680 cm$^{-1}$/5487 cm$^{-1}$ ascribed to the C–H stretching band was a substitute for the peak of an internal standard. Partial least square (PLS) regression was used for AV calibration, and samples were validated by titrated method. Results showed dilution calibration was feasible for randomly dilution among 6–13:1 (CCl$_4$: oils, v/v). PLS calibration was optimal by a spectral wavenumber (3603 cm$^{-1}$–3250 cm$^{-1}$) as the first derivative treatment. Correlation coefficient and root mean square error of calibration were 0.9967 and 0.135, respectively. Calibrated validation, blind sample validation and precision analysis presented a good correlation between IQC-FTIR and titrated methods. Based on the dilution calibration, randomly diluted oil samples can be employed by IQC-FTIR.

Key words: edible oils, acid value, determination, FTIR, infrared quartz cuvette

1 Introduction

Vegetable oils are important food ingredients and provide humans with essential unsaturated fatty acids (UFA) and numerous fat-soluble vitamins, whereas oils can oxidize during transport and storage$^{1}$. Oil oxidation is an important quality criterion for food industry because this results in off-flavor compounds and also decreases the nutritional quality of food$^{2}$. Therefore, it is necessary to monitor and determine the oil oxidation parameters, such as acid value (AV), peroxide value, iodine value, saponification value, and moisture content. During the oxidation, oils are prone to hydrolysis and produce free fatty acids (FFA) under the effects of light, oxygen, microorganisms, and enzymes$^{3}$. The American Oil Chemists’ Society (AOCS) define the degree of oil hydrolyzation as AV, which represents the content of FFA. Therefore, AV is typical considered to evaluate the oil quality in practical analyses.

AOCS has recommended a standard method for AV determination in edible oils, expressed as the number of milligrams of Potassium hydroxide required to neutralize the FFA in 1 g of sample. The titration method involves a large amount of highly toxic, carcinogenic, and environmentally unfriendly organic solvents. The method requires a simple procedure, but it is time consuming, costly, and labor intensive; moreover, the results are largely dependent on the operation of the analysts. Additionally, distinguishing accurate end points with dark oil samples is difficult. To overcome the disadvantages of titration analysis, a number of improved instrumental methods, such as automatic potentiometric titration method$^{4,5}$, colorimetric method$^{6}$, flow injection analysis$^{7,8}$, high-performance liquid chromatography$^{9}$, gas chromatography$^{10}$, liquid chromatography$^{11}$, Raman spectroscopy$^{12}$, H nuclear magnetic resonance$^{13,14}$, infrared spectroscopy, and near-infrared (NIR) spectroscopy$^{15}$, have been developed as good substitute methods. These methods have significant advantages over titrimetric methods in terms of analysis speed and reduction of harmful solvents, but require relatively expensive instruments and professionals.

Over the past few decades, Fourier transform infrared (FTIR) spectroscopy has been widely used for the qualitative and quantitative analysis of edible oils due to its property as a fingerprint technique$^{15,16}$. Various FTIR spectroscopy methods were proposed and reported to replace the traditional transmission cell which was composed of two hygroscopic and fragile halide crystals, such as KBr and NaCl containing a spacer to provide a defined pathlength: attenuated total reflection (ATR) is a feasible alternative because it is simply handled and operated, whereas its generated weak signal and short pathlength cell restrict the application; single-use, disposable spectral acquisition accessories, such as polyethylene (PE) and polytetrafluoro-
ethylene films, have been employed to collect oil spectra for semi-quantitative analysis because of the difficulty in accurately controlling the sample thickness\(^1\). According to Lambert-Beer’s law, exactly controlling the optical pathlength of the sample is very important for quantitative determination of oils\(^1\). Thus, for accuracy and precise determination, methods employed spectral marker, such as methylcyclopentadienyl manganese tricarbonyl method which can remove the diluents factors and eliminate the contribution of oil film thickness to the spectra\(^2\). To simplify this process, Dong et al.\(^6\) normalized the pathlength of spectra of oil samples to a fixed pathlength of 0.15 mm based on the characteristic band absorbance at 4334 cm\(^{-1}\) attributed to the C-H combination. Based on the procedure, AV was evaluated and determined, but sensitively and precisely determining low AV is difficult due to the short pathlength of the accessories. Indirect methods for AV determination were reported for low AV quantitative analysis, whereas they were mainly relying on extracting or transferring the FFA to salts\(^19, 20\). Because of the above downsides, a novel strategy can potentially tackle the problem. Quartz cuvette can provide with an accurate and suitable optical pathlength for UV–vis and NIR spectrophotometers for loading sample solutions. However, the complex sample preparation and routine analysis of multiple spectral parameters, which limits the practical application of IQC–FTIR technique, require to be simplified.

Therefore, the infrared quartz cuvette (IQC) as a FTIR accessory for determining AV was investigated. For more accurate and precise low AV determination, carbon tetrachloride (CCl\(_4\)) was applied to dilute the oil samples. To avoid using internal standard, an alternative dilution calibration method that involves C-H combination band calibration was proposed to allow one to prepare samples with random dilution ratio. Partial least square (PLS) regression model was developed the AV calibration, as validated by using the AOCS standard method.

2 Materials and methods

2.1 Materials

Rapeseed, linseed, cottonseed, peanut, soybean, sesame, sunflower, walnut, Silybum marianum seed oils and their blending oils used for AV calibration and validation samples were purchased from local supermarkets in Yangling, China. Ethyl alcohol, ether, tetrachloromethane, triphenylphosphine oxide (TPPO), potassium hydroxide, phenolphthalein indicator, and activated silica gel used in the experiments were of analytical grade.

2.2 Dilution calibration and its simplification

Appropriate dilution rate is able to simplify the detecting procedure and to improve determining accuracy, whereas the dilution can change the absorbance of samples, which influence the characteristic bands of specific stretch from samples. This can be solved by a model between dilution ratio and measurement of characteristic band, and the model is defined as dilution calibration in this study. The model would allow authors to prepare samples with random dilution ratio and screen target bands in further analysis.

For a pathlength determination of different dilution ratio of oil samples, CCl\(_4\) was selected as dilution solvent based on pre-experiments and TPPO as an internal standard was added in the oil samples to establish a dilution model. A precise dilution model was established by that oxidized oils with various AVs were accurately weighed and diluted with CCl\(_4\) in dilution proportions from 3:1 to 14:1 (CCl\(_4\):oils, v/v). TPPO (10 ± 0.01 mg) was added to each sample. The oil sample was then placed in the IQC (10 × 10 × 45 mm). All absorption spectra of oil samples were collected. Characteristic bands of P = O stretch from TPPO, 542 cm\(^{-1}\)/530 cm\(^{-1}\) and 3147 cm\(^{-1}\)/3253 cm\(^{-1}\), were used to analyze and to calibrate the pathlength\(^4, 21, 22\).

However, infrared spectral collection by IQC only allows spectra to have stretch from 2500 cm\(^{-1}\) because of quartz. Furthermore, extra addition of internal standard increases procedures of the sample preparation and results in additional source of errors due to the accurate weight process. Therefore, for simplification of pathlength calibration of dilution, a 2D correlation spectroscopy (written by Dr. Wang and described elsewhere\(^18\)) was applied to generalize the spectra to find the correlation bands of oil characteristic ones to dilution ratio.

2.3 Sample preparation

The oil samples were refined by silica gel column chromatography on the basis of the modification of the AOCS official method (AOCS Cd 20–91). The AV of oil samples was not detected. We used hexanoic acid to obtain the artificial blending oil (AV of the samples from 0–5 mg g\(^{-1}\)). Arbitrary selection was performed to classify 114 samples diluted randomly (among 6–13:1, CCl\(_4\):oils, v/v) into calibration, which included 88 oil samples and validation sets with 26 oil samples. For example, sample (10:1) preparation was conducted by weighting approximately 1 mL of blended oil sample and 10 mL of CCl\(_4\) into a 15 mL graduated test tube with a glass stopper. The tube was then capped with a stopper and shaken. A total of 12 different oil samples, including nine pure oils, Rapeseed, linseed, cottonseed, peanut, soybean, sesame, sunflower, walnut and Silybum marianum seed oils, and four blending oil, as blind samples were analyzed using the AOCS procedure and IQC–FTIR method in triplicate to further validate the performance of the AV-based FTIR procedure. The oil sample was placed in the IQC. All absorption spectra of the oil sample were collected.
2.4 AV determination

The AV of each sample was determined in triplicate by using the AOCS method (AOCS Official Method Cd 3a–63). The oil sample (2 ± 0.01 g) was mixed well with diethyl ether/ethanol (1/1 (v/v)) and added with 2 mL phenolphthalein in a 250 mL Erlenmeyer flask. The mixture was titrated with 0.01 M of potassium hydroxide and shaken vigorously until the color was changed (from white to pink). The color must persist for 30 s. In brief, the result was expressed as the number of milligrams of potassium hydroxide required to neutralize the FFAs in 1 g of sample.

2.5 FTIR analysis

All samples were measured in triplicate by using an FTIR spectrometer, BRUKER TENSOR 27 (German Bruker Analytical Instrument), equipped with a cuvette rack, over the region of 6000–4000 cm⁻¹ at 4 cm⁻¹ resolution over 32 scans. The samples were placed in IQCs (10 x 10 x 45 mm) (Yehui Glass Industry, China) with an optical pathlength of 10 mm at a transmission chamber with controlled temperature (20°C) and humidity (20%–30%). The IQC was cleaned with n-hexane after each scan to avoid contamination of preload. Pure carbon tetrachloride was used to collect the background spectrum.

2.6 Statistical analysis

All the data and spectra treatments were achieved using the Origin Pro 7.5 (OriginLab, Northampton, MA), OMNIC 7.3 (Thermo Electron Inc., Madison, WI), and TQ Analyst 7.2 (Nicolet Company, USA) software. The PLS method was used to establish a correlation calibration model between actual (from the AOCS method) and FTIR-predicted values. Using baseline correction, multiplicative scatter correction (MSC), spectral normalization vector (SNV), Norris derivative filter (NF), Savitzky–Golay filter (SF), first derivative, second derivative, etc. as spectral pretreatment methods can effectively increase the precision of the AV prediction model. The accuracy of the correlation model was evaluated by the root mean standard error of cross-validation (RMSECV), root mean error of calibration (RMSEC), root mean error of prediction (RMSEP), and R².

The predicted residual error sum of squares (PRESS) was used to select the optimal number of principal components. The calibration with the highest R value and the lowest RMSEC and RMSEP was appropriate for AV analysis.

3 Result and Discussion

3.1 Dilution calibration and simplification analysis

Dilution allows high absorbance samples to decline their concentration, resulting in improving detecting accuracy. Thus, a dilution calibration enables to simplify sample preparation and provide with references for further IQC spectral methods. CCl₄ was employed as dilution solvent and samples diluted with various ratios and added with TPPO as internal standard were prepared.

Figure 1 shows that normalized the IQC-based FTIR spectra of blending oil, cottonseed oil and CCl₄. The absorption band at 3535 cm⁻¹ was related to the O–H functional groups present in oils, revealing the characteristic properties specific to the components and it was related to the AV of edible oil. Figure 2 presents a typical FTIR spectrum of the sesame oil (10:1) with or without TPPO in the wavenumber range of 6000–2500 cm⁻¹. Spectral differences between oil with and without addition of TPPO occurred to several bands, as shown in Fig. 2. Among these regions, the characteristic band at 3147 cm⁻¹ is attributed to the P=O stretch from TPPO, which was confirmed by previous results. However, extra addition increase the...
sample preparation and potential errors, and therefore, simplifying dilution calibration was investigated.

After the spectra were generalized by 2D correlation spectroscopy\(^{18}\), the absorbance of the spectra measured at 5680 cm\(^{-1}\)/5487 cm\(^{-1}\) and 3147 cm\(^{-1}\)/3253 cm\(^{-1}\) were linearly related at a certain dilution range, as Fig. 3 shows, with a correlation coefficient (R) of 0.9807 and standard deviation (SD) of 0.1700. In addition, the absorbance of samples measured at 5680 cm\(^{-1}\)/5487 cm\(^{-1}\) avoided influencing the analysis of other parameters such as AV. Therefore, absorbance peak at 5680 cm\(^{-1}\)/5487 cm\(^{-1}\) was substitute for the peak of an internal standard (TPPO). Absorbance and dilution ratios of samples were linearly related at a certain dilution range by analyzing the spectral absorbance at 5680 cm\(^{-1}\) ascribed to the C–H stretching band\(^4\). Hence, band of 5680 cm\(^{-1}\)/5487 cm\(^{-1}\) serves the dilution calibration.

Based on 5680 cm\(^{-1}\)/5487 cm\(^{-1}\), spectra of samples with dilution ratios were collected (Fig. 4). Spectral absorption of oil samples overflowed at low dilution ratios (1–5) due to the completed absorption of infrared radiation, while samples exhibited a weak and inaccurate signal at high dilution ratios (>13). This suggests that dilution ratios below 5 or over 13 could not be analyzed. In accordance with Lambert–Beer’s law, the absorbance of samples is inversely proportional to the dilution ratios. A linear equation (R, 0.9885 and SD, 0.0077) between dilution ratios of 6–13 and their corresponding absorbance was calibrated (Fig. 5a). The absorbance peak at 5680 cm\(^{-1}\)/5487 cm\(^{-1}\) decreased as the dilution ratios increased. Extra dilution samples were to investigate feasibility and practicability of the dilution model. Figure 5b displayed the calculated dilution ratios, figures calculated by calibration, versus actual dilution ratios, which were from operation. The linear equation described an R value of 0.9956 and SD of 0.0937. This demonstrates that the determined dilution ratios were practical.

3.2 The AV application by IQC–FTIR

For accurate AV determination, IQC–FTIR can serve the purpose, from comprehensive analysis and the theory that
FFAs in the dilute solution would exist as monomers\(^{25}\). Therefore, spectra of all samples with random dilution were collected and were calibrated to the dilution ratio at 10:1 by dilution calibration model. Among these bands, several regions were reported to succeed to determine AV of oils. In the proposed method, spectral region from 4000–2500 cm\(^{-1}\) was to be investigated.

### 3.3 Calibration optimization

To avoid interference, such as the spectral fluctuation, different spectra preprocessing methods were developed to improve the accuracy. PLS regression calibrations were developed in effective characteristic bands. PRESS values are an indicator of the correlation of calibration data and are used to specify the optimal number of factors. The best model includes the fewest number of factors such that the PRESS for that model is not significantly greater than the minimum PRESS value\(^{26}\). The accuracy of the results corresponds to degree of agreement between actual and predicted values using the PLS model upon RMSEC, RMSEP, and RMSECV\(^{27}\).

Table 1 shows that the first derivative of the original spectrum was considered as optimal to establish the prediction and validation of determining AV, as maximum R value (0.9955) and minimum RMSEC value (0.158). Furthermore, the principal component number (PCs) was 7. The characteristic bands screened by the interval PLS algorithm contained spectral intervals, which still contain wavelength variables unrelated to the absorption of infrared spectra of the AV. The wide selection of spectral region reduces the proportion of the valid information; on the contrary, the narrow selection of spectral region may have omitted valid information. Therefore, the spectral region should be comprehensively screened and optimized. Table 2 shows that the wavenumbers from 3603 cm\(^{-1}\) to 3250 cm\(^{-1}\) were used for AV analysis as optimal calibration conditions. Thus, high R value (0.9967) and low PRESS (0.3086), RMSEC (0.135), RMSEP (0.182), and RMSECV (0.1862) were acceptable.

### 3.4 Calibration

The 88 samples in the calibration set were prepared at random dilution ratios, and spectra of samples were collected by IQC–FTIR. The spectra were then calibrated to the dilution ratio at 10:1 by dilution calibration model. After the optimization process, Fig. 6 displays the actual (determined by AOCS titration method) versus predicted (determined by IQC-FTIR method) values. As it shows, the equation with an R value of 0.9968 and SD of 0.1343 AV units reveals an excellent linear relationship between AOCS titration and IQC-FTIR methods in measuring the AV of samples with the slope and R value being close to 1. The reduction of the number of calibration samples reduced the predictive ability of all equations. In general, the increased R and RMSEC values indicate excellent predictive ability of a calibration\(^{28}\). Therefore, the IQC-FTIR

| Spectral preprocessing | PCs | PRESS | R     | RMSEC |
|------------------------|-----|-------|-------|-------|
| Original spectrum      | 5   | 5.0064| 0.9929| 0.197 |
| First derivative       | 7   | 5.1005| 0.9955| 0.158 |
| Second derivative      | 9   | 27.5741| 0.9922| 0.207 |
| SF + original spectrum | 5   | 5.0065| 0.9930| 0.197 |
| NF + original spectrum | 8   | 4.4947| 0.9949| 0.168 |
| SNV + original spectrum| 5  | 12.8600| 0.9797| 0.334 |
| MSC + original spectrum| 6  | 5.7011| 0.9932| 0.195 |
| SNV + first derivative | 7   | 17.2508| 0.9827| 0.309 |
| SF + first derivative  | 7   | 5.1147| 0.9954| 0.191 |
| SNV + first derivative +NF | 6  | 16.1493| 0.9784| 0.159 |

| Spectral range       | PCs | PRESS | R     | RMSEC | RMSEP | RMSECV |
|----------------------|-----|-------|-------|-------|-------|--------|
| 4000–2500 cm\(^{-1}\) | 8   | 6.2218| 0.9954| 0.160 | 0.194 | 6.2218 |
| 3250–2500 cm\(^{-1}\) | 9   | 17.3042| 0.9875| 0.263 | 0.319 | 0.4409 |
| 3603–3250 cm\(^{-1}\) | 7   | 3.0856| 0.9667| 0.135 | 0.182 | 0.1862 |
| 4000–3603 cm\(^{-1}\) | 6   | 70.0673| 0.9169| 0.665 | 0.585 | 0.8873 |
| 3600–3500 cm\(^{-1}\) | 1   | 11.9534| 0.9793| 0.337 | 0.321 | 0.3665 |
method is theoretically considered to determine the AV of edible oil.

3.5 Validation
In validation set, samples were prepared and analyzed as the same as the calibration set: AOCS and IQC-FTIR methods measured 26 oil samples with various dilution ratios processed similarly with the calibration set for an effective investigation.

The regression equation with an R value of 0.9913 and SD of 0.1867 AV units demonstrates the concurrence of both methods. Therefore, the developed PLS models are capable of determining the AV of edible oil.

3.6 Blind sample validation
The AV of 12 oil samples, including eight pure oils and four blend oils, with random dilution as the blind sample set was determined using the AOCS and IQC-FTIR methods to further evaluate the accuracy of the developed calibration method.

Figure 8 confirms the overall ability of quantitatively and rapidly determining the accurate and reproducible AV of edible oil by using the IQC-FTIR method. The regression relationship between AVs determined by both methods describes a slope value close to 1 (1.0012) and an intercept close to 0 (0.0114) with an R value of 0.9987 and SD of 0.0444. The results indicated that the values from the two methods were close to the measured values. Hence, the proposed method is a good alternative for the titrimetric method.

3.7 Precision analysis
To establish a practical quantitative analysis method, a high predicted accuracy indicates a good repeatability and reliability of the developed model. Precision primarily reflecting the magnitude of random errors in an analytical method was applied. The AV of an oil sample randomly selected was repeatedly measured six times by using the AOCS and IQC-FTIR method to validate the precision of the quantitative calibration.

Table 3 shows that no significant difference between the AOCS and IQC-FTIR methods was observed. Low SD (0.0024) and coefficient of variation (CV; 1.29) values, as the evaluation indicators, reveal the excellent accuracy of the IQC-FTIR and official methods. In addition, the plot of the measurement results and average value was used to validate the stability of the IQC-FTIR method. Stability was significantly improved because SD and CV values of the IQC-FTIR method were less than those of the official method. Thus, FTIR spectroscopy based on IQC can achieve a rapid AV determination of edible oil.

3.8 Discussions
FTIR spectroscopy has been widely apply to determine
quality of fats and oils, because it requires little sample preparation and minimal hazardous solvents. A number of quantitative calibration technique based on FTIR has been proposed and provided analysts with precision and accuracy. Numerous quality parameters, such peroxide value, trans fatty acid, iodine value and saponification number, could be predicted by models established through the relationship between the chemical parameters and spectral data of oils, using a variety of chemometric methods. These detection methods relied on spectral acquisition accessory, such as ATR and PE film, to collect the sample spectra. However, the short pathlength and weak signal of the accessories limit the methods to detect low AV.

Various methods have been undertaken as direct or indirect AV determination methods. The direct method is based on the characteristic absorption of C = O of the carboxylic acid group at 1711 cm\(^{-1}\) and the AV determination of oil is further achieved by modeling. Yu and co-workers developed a method for direct AV determination by using the developed spectral reconstitution technique. Other methods to facilitate mid-FTIR transmission analysis of viscous edible oil samples by using disposable polyethylene (PE) as spectral acquisition accessory were established. However, sensitively and precisely low AV determination is difficult due to the short pathlength of the PE film and the measurement result is also influenced by triglyceride and substrate effects.

Indirect FTIR spectroscopic methods were also developed for AV analysis. Potassium hydroxide/methanol(1%) is used to extract the FFAs and convert them to their potassium salts. The carboxylate COO\(^{-}\) of potassium salts has a characteristic absorption at 1570 cm\(^{-1}\), which in turn enables the indirect determination of FFA in edible oils. Other indirect FTIR spectroscopic methods were developed based on a transmission spectroscopic method using potassium phthalimide as a weak base to convert the FFAs in oils to their carboxylate salts without causing oil saponification. An AV analysis method for determining FFA products converted to carboxylate salts with a characteristic absorption at 1573 cm\(^{-1}\), by using a portable variable filter array IR spectrometer equipped with a transmission flow cell.

In the study, suitable optical pathlength of IQC allows authors to establish an accurate and robust calibration between spectra and actual AV. Comparing with the results of several studies based on IR, such as diffuse reflectance IR spectroscopy combining chemometrics(detection limit, 0.2 mg g\(^{-1}\)), ATR-FTIR spectroscopy (0.2 mg g\(^{-1}\)) and FTIR method by mixing methanol with oils (0.2 mg g\(^{-1}\)), the proposed AV determination shown a lower detection limit is about 0.16 mg g\(^{-1}\). According to the proposed method, samples can be randomly diluted and the spectra can be calibrated by a dilution model, which simplify sample preparation and improve quantitative accuracy.

### 4 Conclusion

For a rapid and accurate AV determination, dilution calibration and IQC-FTIR method were developed. A characteristic band at 5680 cm\(^{-1}\) attributed to the C–H stretch, due to the tedious operation of dissolving TPPO, generalized by 2D correlation analysis, was used to establish the dilution calibration method. Eighty-eight oil samples diluted randomly (among 6–13:1, CCl\(_4\): oils, v/v) with various AVs were prepared to calibrate the IQC-FTIR method. Effective pathlength of each spectrum requires to be determined and then the spectra were normalized to a fixed pathlength (dilution ratio of 10) to allow quantitative comparison of the results obtained from different dilution ratio. The first derivation and 3603–3250 cm\(^{-1}\) were selected to establish the PLS AV model. Twenty-six validation samples showed that the PLS model was robust and reliable. To compare with the standard AOCS method by using blind sample validation and precision analysis, repeatability and reliability of the proposed method were demonstrated.

Replacing the internal standard of pathlength establishment by a correlation absorption band simplified the sample preparation and AV determination procedure. Results demonstrated that the IQC-FTIR method served a rapid and precise AV determination purpose. Furthermore, IQC is a robust and durable FTIR accessory, which extends the application of FTIR spectroscopy in quantitative analysis.

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Conflict of Interest
The authors have declared no conflicts of interest.

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