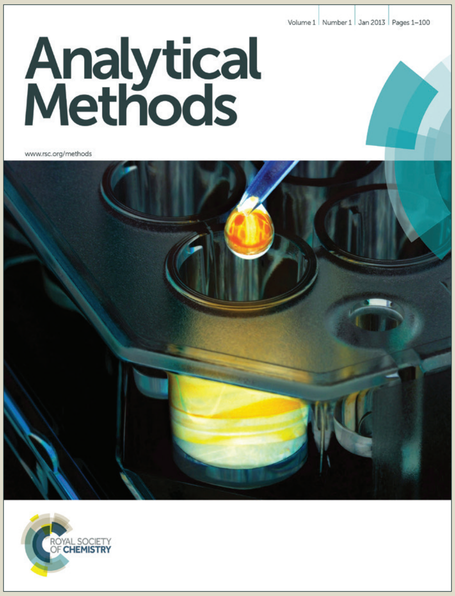
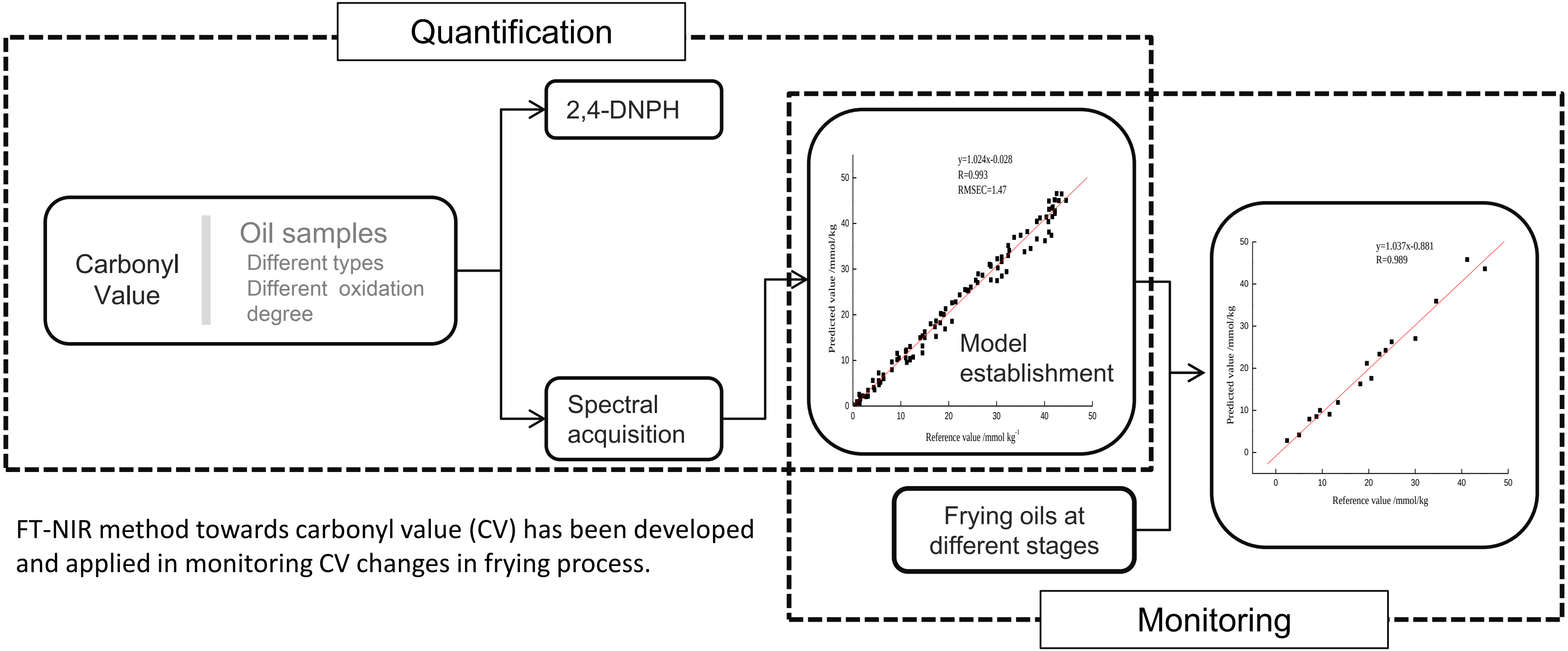
Analytical
Methods

Accepted Manuscript



ROYAL SOCIETY
OF CHEMISTRY

www.rsc.org/methods



FT-NIR method towards carbonyl value (CV) has been developed and applied in monitoring CV changes in frying process.

Analytical Methods RSCPublishing

ARTICLE

Application of Fourier transform near-infrared spectroscopy to the quantification and monitoring of carbonyl value in frying oils

Yage Wang, Xiuzhu Yu *, Xiumei Chen, Yandie Yang, Jingya Zhang

College of Food Science and Engineering, Northwest A&F University, 28 Xinong Road Yangling, 712100, Shaanxi, P. R. China

*Corresponding author. Tel.: +86-29-87092206; fax: +86-29-87092486. E-mail address: xiuzhuyu1004@hotmail.com.

A rapid and convenient method of determining carbonyl value (CV) in frying oil using Fourier transform near-infrared (FTNIR) spectroscopy was established. A total of 144 oil samples were subjected to CV determination by using the 2,4-dinitrophenylhydrazine (2,4-DNPH) method and FTNIR spectroscopy combined with partial least square (PLS) modelling. The PLS model worked best with pretreated spectra in the wavelength range of 9702 cm^{-1} to 4531 cm^{-1}, resulting in the root mean square error of calibration (RMSEC) of 1.47 mmol kg^{-1} and prediction (RMSEP) of 1.73 mmol kg^{-1}. Studies related to the monitoring of CV changes in a simulated frying process of the oil samples were then performed to evaluate method's feasibility for monitoring oil quality online. Results showed that the model-predicted value was close to the true value determined by the 2,4-DNPH method (R=0.989). Therefore, the FTNIR method can be used for quantifying and monitoring CV with the advantages of simple operation and no pollution.

Introduction

Special attention has been paid to the quality of frying oil because of the special conditions of its usage. The oxidation rate of frying oil increases with incensement of time that oil exposing to air at high temperature. The hydroperoxides are generated first, which subsequently decompose into aldehydes and ketones that undergo a variety of complex reactions, such as hydrolysis, isomerization, and polymerization. Thus, harmful substances become accumulated in oil and get into human body with the fried food. These substances are harmful to human health and change the color and flavor of fried food [1]. Therefore, monitoring the stability and quality changes throughout the frying process is essential. A series of parameters such as acid value (AV), peroxide value (PV), total polar compounds (TPC), and carbonyl value (CV) are used to evaluate oil quality. However, AV and PV are not considered as suitable markers for characterizing quality of the frying oil. AV has no direct relationship with the quality of frying oil [2, 3], while PV increases to a maximum level and then decreases due to the decomposition of peroxides into carbonyl compounds, conjugated dienes, and other compounds. Compared to AV and PV, CV measures the total carbonyl content, including aldehydes and ketones which increase spontaneously along with heating and are not easy to decompose [4]. Simultaneously, Farhoosh et al. reported that CV and TPC had a good linear relationship with a determination coefficient of 0.975 [5]. Therefore, CV was more appropriate for evaluating frying oil quality.

The most widely used method of CV determination is the reaction of carbonyl compounds with 2,4-dinitrophenylhydrazine (2,4-DNPH), which produced 2,4-dinitrobenzene hydrazone. The product generated quinone of wine red in alkaline conditions, which induced absorbance values at 440 nm [6, 7]. This method has the disadvantages of requiring a harmful chemical and large volume of solvent, hence a modified method that uses 2-propanol (2-PrOH) as solvent had been developed [8]. In subsequent studies, 2-PrOH was replaced by 1-butanol (1-BuOH) because the older method had the problem of excluding the carbonyl compound existing in the solvent before the reaction [9] and Farhoosh et al. recognized that it was not feasible [10]. Regardless the solvent used, all of these measurement processes were laborious and time-consuming, and required a large amount of organic solvent. Therefore, these methods are not suitable for monitoring quality of oil during the production process in industry. Efforts have been paid for developing new methods of CV determination.

Modern instrumental analytical techniques, such as Fourier-transform near-infrared (FTNIR) spectroscopy and gas chromatography (GC) are now used for the determination of carbonyl compounds in oil samples [11]. FTNIR spectra of overtones and combination bands (X-H, X=C, O, or N) will vary when the oil compositions change. This technique is rapid and convenient, which provides the possibility of monitoring oil quality online. Moreover, it is nondestructive, and does not require hazardous reagents and complex sample pretreatment. Besides FTNIR, GC is also commonly employed in CV measurement. Yiannis et al. developed a GC method for the determination of 10 characteristic carbonyls in biological and oil samples based on the in-drop formation of hydrazones by using 2,4,6-trichlorophenylhydrazine (TCPH)[11]. However, the method requires expensive instrument and complicated sample treatment procedure. Compared with the GC method, using FTNIR spectroscopy can be more affordable and easier for food industries. In recent years, a large amount of studies regarding rapid determination of oil oxidation have been done. A PLS model was created to study the relationship between physicochemical parameters and near infrared spectroscopic data of frying oils. Ng. et al. used both forward stepwise multiple linear regression (FSMLR) and PLS to predict TPC of soy-based frying oils and reported that performance of FSMLR and PLS were related to wavelength range [12]. They also studied FTNIR spectroscopic methods for measuring TPC and FFA in soy-based frying oil used for frying various foods. Their findings indicated that the quality of oil used for frying different foods could be measured with a single model. PLS models gave better prediction results than FSMLR models [13]. In addition to these two parameters, peroxide value, viscosity, and smoke point were also studied, and good correlation between spectroscopic and traditional methods was achieved [12-21]. Contrary to the above conclusion, Szabo et al. reported less favorable results for the determination of CV in porcine subcutaneous fats during frying by FTNIR, probably because of very low concentration of carbonyl compounds in the samples. However, to our best knowledge, few contributions exist on the use of FTNIR spectroscopy for determining and monitoring CV of frying oil in the frying process. The present work describes a rapid FTNIR method for CV measurement and demonstrates the feasibility of applying it for monitoring frying oil quality online.

Materials and methods

Materials and reagents

Potatoes were obtained from a local supermarket. Soybean oil, rapeseed oil, peanut oil, blend oil, olive oil, maize oil, and two batches of frying oils were purchased from local market. Potassium hydroxide, 95% ethanol, benzene, 2,4-DNPH, and trichloroacetic acid were of analytical grade.

Sample preparation

Potatoes were washed, peeled, and cut into pieces, then submerged in water to minimize browning. Because frying oil is not a single type of oil but a mixture of several oils, it was prepared by mixing two or three oils in a mass ratio of 1:1 (two oils) and 1:1:1 (three oils), respectively. One batch of 90 samples was generated by accelerating oxidation at 105 °C in an oven through a certain period of time. The other batch of 54 samples was collected from three types of mixed oil used to fry potatoes at 180 ± 5 °C. Continuous frying lasted for 6 h per day for 3 consecutive days. A 100 ml volume of the filtered oil sample was taken every hour [5]. No oil was replenished during the frying process. Arbitrary selection was used for dividing 144 samples into calibration set and validation set with the purpose of even distribution of different oxidation degrees from two batches. All of the samples were sealed, wrapped with aluminum foil, and kept at 4 °C in a refrigerator. Another series of frying oil samples was used for evaluating the feasibility of FTNIR spectroscopy in monitoring process.

Chemical determination

CV of the oil samples was measured using the 2,4-DNPH method. A 0.5 g sample was dissolved in a 25 ml refined benzene solution. A 5 ml volume from this solution was placed in a stoppered 25-ml test tube, then 3 ml trichloroacetic acid and 5 ml 2,4-DNPH were added. The test tube was vigorously shaken. After that, the solution was incubated at 60 °C for 30 min in a water bath, and then 10 ml of KOH-CH_3OH was slowly added along the tube wall after cooling. The solution was then shaken and allowed to stand for 10 min. The absorption was measured at a wavelength of 440 nm through a 1-cm cuvette against a blank that contained all the reagents except the oil using a UV-1240 spectrophotometer (Shimadzu Corporation, Japan) [6]. Chemical analysis was performed in duplicate for each sample.

Spectral acquisition

A MPA-TM FTNIR (Bruker Optics, Germany) system with InGaAs detector and a liquid chamber (tube diameter = 5 mm) was used for collecting transmission spectra. The wavelength rang was from 12000 cm^{-1} to 4000 cm^{-1}. The co-addition was 32 scans and the resolution was 4 cm^{-1}. To reduce the temperature effect, the oil sample was maintained at 60 °C in a water bath before acquiring the spectra [22].

Chemometric analysis

OMNIC 7.3 and TQ Analyst 7.2 (Nicolet Company, U.S.A.) were used for PLS model development. Multivariate calibration was performed by PLS regression to study the relationship between the reference and the FTNIR spectra[15, 16, 23]. Predicted residual sum of squares (PRESS) by cross validation was used for determining the optimum number of principal components. Correlation coefficient (R), root mean square error of calibration (RMSEC), and root mean square error of prediction (RMSEP) were used for model evaluation. Paired t-test [24] was used to determine whether or not FTNIR and 2,4-DNPH methods are equivalent. The average relative error and relative standard deviation (RSD) were used to prove the accuracy and precision of the CV model. Relevant indicators were calculated as follows:

$$\text{Average relative error}=\frac{1}{\text{n}}\times\sum_{i=1}^{n}\left|\frac{y_i'-y_i}{y_i}\right|\times100\%$$

$$\text{RSD}=\frac{1}{\bar{\text{y}}'}\sqrt{\frac{\sum_{i=1}^{n}(y_i'-\bar{y}')^2}{n-1}}\times100\%$$

Where y_i is CV determined by the 2,4-DNPH method, y_i' represents CV determined by the NIR method, $\bar{y}'$ represents the average CV determined by the NIR method .

Frying process monitoring

A series of frying oil samples was used for evaluating the feasibility of FTNIR spectroscopy in monitoring the quality of oil during the frying process. A sample of 100 ml was collected every hour, and was cooled to 60 °C. CV was measured using FTNIR spectroscopy and the 2,4-DNPH method to investigate the correlation of results between these two techniques. Frying was conducted for 6 h every day, and was not stopped until CV exceeded 43.5 mmol kg^{-1} according to previous studies[5]. The process did not involve replenishment with new oil.

Results and discussion

CV determination

The accuracy of the model was closely linked to the reference method used. Although some modified methods has been developed, the 2,4-DNPH method is still the most acceptable for CV measurement. The reference values obtained by the 2,4-DNPH method for calibration and validation sets are presented in Table 1.

Table 1 CV for calibration and validation sets determined by 2,4-DNPH method

NO. of samples		Average (mmol kg^{-1})	Range (mmol kg^{-1})
Oven oxidation	60	10.16	0.53–44.51
Frying oxidation	44		
Oven oxidation	30	12.48	0.94–43.65
Frying oxidation	10		

The CV ranged from 0.53 to 44.51 mmol kg^{-1}. Relative error and standard deviation of the results obtained by this analytical method ranged from 0.9% to 3.2% and 0.8% to 1.5%, respectively. Previous studies have reported that the total amount of polar material (carbonyl compounds such as ketones and other compounds) increased as duration of heating increased [12, 13]. The frying process generates hydroperoxides and other polar compounds through hydrolysis, isomerization, and polymerization. The amount of these compounds is related to oil and food types, frying conditions and others[25]. The samples collected represented various levels of oil oxidation and were evenly distributed, and were desirable for creating a good model. These samples were divided into calibration and validation sets with arbitrary selection for the purpose of even distribution of different oxidation degrees of the two batches.

Spectroscopy analysis

Total amount of 144 spectra were obtained by subjecting samples to FTNIR. Fig. 1 showed spectra of the two batches. These similar spectra of two batches indicated that the oxidation process of frying oxidation and oven oxidation were similar. (The absorbance of spectra for oven oxidation was increased by 0.5 in order to more clearly discriminate the spectra of the two batches).

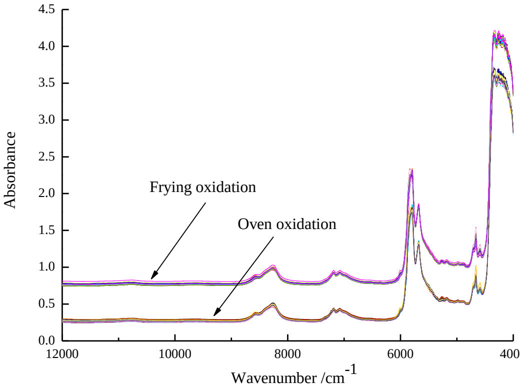


Fig. 1 Spectra of calibration and validation sets.

The spectra showed strong absorption peaks at 8280, 7074, 5801, 5795, and 4663 cm^{-1}. The small bumps at 8280 and 7074 cm^{-1} revealed the second overtone of the C–H, stretching vibration of –CH=CH– and the combination of C–H and –CH$_2$, respectively. The major peaks at about 5801 and 5795 cm^{-1} were mostly correlated with the first overtone of the C–H, stretching vibration of –CH=CH– and –CH$_3$. The peak at 4663 cm^{-1} represented the combination of C–H, stretching vibration of –CH=CH– [26]. Osborne et al. reported that the absorption bands obtained between 5000 and 4545 cm^{-1} were connected to the degree of oxidation in oils [27].

In order to improve the performance of the model, several pretreatments such as second derivative, multiplicative scatter correction (MSC) and standard normal variate (SNV) in different regions have been employed. The derivative can strengthen the band characteristics by removing background, eliminating baseline drift, increasing spectral resolution. However, noise is also amplified when this pretreatment is used[28]. In contrast with derivative, the effect of smoothing is to reduce random noise and improve the signal to noise ratio[29]. The comparison of the results obtained by different preprocessing methods was displayed in table 2. The results obtained in 9702–4531 cm^{-1} apparently had better performance compared with other spectra regions. This spectral range included the absorption bands (4513–5369 cm^{-1}) of the second overtone of the C=O stretching vibration, which were related to carbonyl compounds [20, 30]. The preprocessing method of the combination of SNV and second derivative worked better than others, and resulted highest *R* (0.993) and lowest RMSEC (1.47 mmol kg^{-1}).

Table 2 Selection of spectral range and pretreatments for CV in oils

Spectral region (cm^{-1})	Pretreatments	PCs	RMSECV (mmol kg^{-1})	*R*	RMSEC (mmol kg^{-1})
8343–6826	Second derivative	6	2.76	0.962	4.56
10803–4531	Second derivative	8	2.66	0.960	4.66
9702–4531	Second derivative	7	2.70	0.981	3.2
9702–4531	NF(15,10)[b]+ Second derivative	8	2.69	0.951	3.14
9702–4531	SF(9,6)[a] + Second derivative	7	2.68	0.980	2.30
9702–4531	MSC+ Second derivative	8	2.68	0.983	2.06
9702–4531	SNV(9702-4531)+ Second derivative	9	2.40	0.993	1.47

a SF (9,6) represents the smoothing under 9 data points and the 6th polynomial;
b NF (15,10) represents the Norris derivative smoothing under segment length is 15 and the gap between segments is 10.

Model development and validation

PLS models were established using the reference data and spectra obtained in selected wavelength range (Figs 2 and 3). The appropriate number of PCs was determined by the leave-one-out cross validation method. Minimum root mean square error of cross validation (RMSECV) of 2.40 mmol kg^{-1} was achieved by increasing the number of PCs to 9 (data not shown). The larger number of PCs was probably associated with the variety of oil types and seasonal variations in oils.

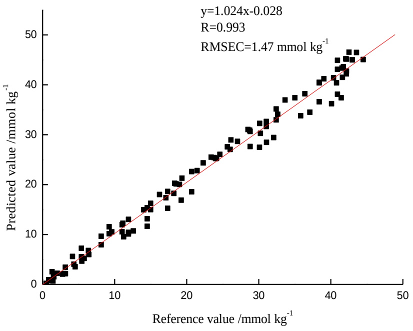


Fig. 2 Predicted and reference values of CV in calibration set.

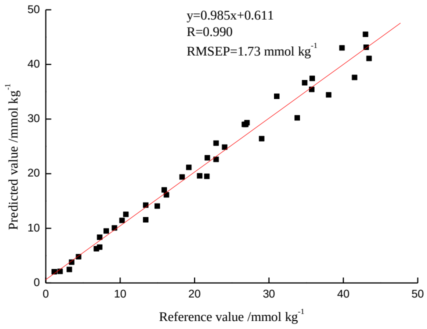


Fig. 3 Predicted and reference values of CV in validation set.

There was a strong correlation between the actual CV and the one predicted by FTNIR. The R value was 0.993 for the calibration set, and a RMSEC of 1.47 mmol kg^{-1} was achieved. Meanwhile, the validation set resulted in R of 0.990 and RMSEP of 1.73 mmol kg^{-1}. These results demonstrated that FTNIR spectroscopy method can accurately predict CV and be useful for quality-control applications. Szabo et al. reported less favorable results for the determination of CV in porcine subcutaneous fats during frying by FTNIR[31]. In this study, the calibration samples included frying oils consisting of many different types of vegetable oils, but the method proposed was still able to give accurate predictions of CV.

Further statistical analysis indicated that the results by the 2,4-DNPH method and FTNIR method were comparable. A paired *t* test for comparing the results of the two methods resulted a *t* value of 1.935, which was less than the critical value of $t_{39,\ 0.05}$ (i.e., 2.023), indicating that the FTNIR method was suitable for rapid CV determination. A careful assessment of precision and stability of the model was conducted using 10 randomly selected samples. Each sample was measured 5 times. The relative error ranged from 1.2% to 4.6% and RSD of CV ranged from 1.43% to 1.89%, indicating that the model was robust. Many researches have been done to investigate the potential of analyzing frying oils through FTNIR spectroscopy, but only a few papers have used statistical criteria to evaluate the accuracy and precision of FTNIR spectroscopy methods. Similar FTNIR methods with similar performances were reported for the determination of TPC and AV[12-21]. This study added the model for the determination of CV in frying oils and further extended the application of the software.

Frying process monitoring

To evaluate the feasibility of FTNIR spectroscopy in monitoring oil quality online and 17 samples were collected along the frying process and then analyzed by both standard method and FTNIR.

According to Fig. 4, it demonstrated that the model can be used for quality monitoring of frying oil in frying process. In practical applications, after 13 h frying, CV significantly exceeded 43.5 mmol kg^{-1}, hence, frying was stopped. Both methods showed that CV linearly increased with frying time. The results showed that the FTNIR method can be used for monitoring the CV content in real time. Moreover, FTNIR spectroscopy can be combined with side linear automatic sampling to form an analysis system for oil companies to control oil quality online.

The monitoring of an additional set of oil samples using this model indicated that the model was not specific to a certain type of oil. Hence, it could be applied for the analysis of CV in a more variable frying oil samples. On the other hand, the model provided the possibility of monitoring oil in real time. CV in frying oils can be quantitatively measured by FTNIR in less than 1 min. Therefore, FTNIR can immediately determine whether the oil is deteriorated. Its rapid feedback on the quality of oil can be useful for food enterprises and poor quality oil can be replaced in time. Obviously, traditional methods have failed to achieve this target. An FTNIR spectroscopy instrument, in combination with automatic sampling equipment over the line and with the results recorded through a computer, could provide real-time information on frying oil. FTNIR spectroscopy has the advantages of requiring no sample pretreatment and no chemicals. Its easy operation makes it even more applicable in the detection domain.

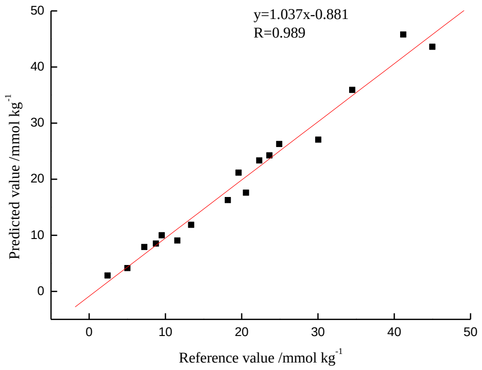


Fig. 4 Correlation of predicted and reference CV in frying oils.

Conclusions

The 2,4-DNPH method is reliable and accurate for the determination of CV in various frying oil samples. However, the test is time consuming and the chemicals used are harmful to laboratory personnel. FTNIR method appears to be a good alternative with the advantages of simple operation, rapid determination and no hazardous chemicals requirement. A FTNIR-based method for the determination of CV in frying oil was successfully developed. Good correlation was found between FTNIR predicted values and the reference data. Our results were favorable compared to those in the literatures for the determination of TPC, AV, and PV by using FTNIR[12-21]. The FTNIR-based method worked well for monitoring oil quality online in a simulated frying process of the oil samples (R= 0.989).

FTNIR spectroscopy has the unique ability of simultaneously determining multiple indicators. Additional research is currently ongoing, and further researches should involve constructing a comprehensive index to fully reflect the degree of oxidation. This degree shall be expressed through commonly used indicators of oxidation, such as AV, CV, and PV, and their weights.

Acknowledgements

The authors would like thank the Central University for supporting this work through the Fundamental Research Funds (QN 2013057).

Notes and references

1. G. Billek, *Annals of Nutrition and Metabolism*, 1980, **24**, 200-210.
2. H. Pardun, J. Blass and E. Kroll, *Fette, Seifen, Anstrichmittel*, 1974, **76**, 151-158.
3. Z. Pazola, J. Gawecki, M. Buchowski, J. Korczak, J. Jankun and B. Grześkowiak, *Fette, Seifen, Anstrichmittel*, 1985, **87**, 190-193.
4. F. B. Gonzaga and C. Pasquini, *Analytica Chimica Acta*, 2006, **570**, 129-135.
5. R. Farhoosh and S. M. R. Moosavi, *Analytica chimica acta*, 2008, **617**, 18-21.
6. A. Henick, M. Benca and J. Mitchell Jr, *Journal of the American Oil Chemists' Society*, 1954, **31**, 88-91.
7. H. Kumazawa and T. Oyama, *J. Jpn. Oil Chem. Soc*, 1965, **14**, 167-171.
8. Y. Endo, C. M. Li, M. Tagiri-Endo and K. Fujimoto, *Journal of the American Oil Chemists' Society*, 2001, **78**, 1021-1024.

9. Y. Endo, M. Tominaga, M. Tagiriendo, K. Kumozaki, H. Kouzui, H. Shiramasa and K. MiyakoshiI, *Journal of oleo science*, 2003, **52**, 353-358.

10. R. Farhoosh and S. M. R. Moosavi, *Journal of Food Lipids*, 2006, **13**, 298-305.

11. Y. C. Fiamegos and C. D. Stalikas, *Analytica chimica acta*, 2008, **609**, 175-183.

12. C. L. Ng, R. L. Wehling and S. L. Cuppett, *Journal of agricultural and food chemistry*, 2007, **55**, 593-597.

13. C. L. Ng, R. L. Wehling and S. L. Cuppett, *Journal of agricultural and food chemistry*, 2011, **59**, 12286-12290.

14. M. Hein, H. Henning and H.-D. Isengard, *Talanta*, 1998, **47**, 447-454.

15. M. Öğütcü, B. Aydeniz, M. B. Büyükcan and E. Yılmaz, *Journal of the American Oil Chemists' Society*, 2012, **89**, 1823-1830.

16. J. A. Gerde, C. L. Hardy, C. R. Hurburgh Jr and P. J. White, *Journal of the American Oil Chemists' Society*, 2007, **84**, 519-522.

17. S. B. Engelsen, *Journal of the American Oil Chemists' Society*, 1997, **74**, 1495-1508.

18. S. Kazemi, N. Wang, M. Ngadi and S. O. Prasher, *Journal of food science*, 2005.

19. R. Du, K. Lai, Z. Xiao, Y. Shen, X. Wang and Y. Huang, *Journal of food science*, 2012, **77**, C261-C266.

20. S. Armenta, S. Garrigues and M. De la Guardia, *Analytica chimica acta*, 2007, **596**, 330-337.

21. A. A. Yavari, M. Hamedi and S. Haghbin, *Journal of the American Oil Chemists' Society*, 2009, **86**, 941-947.

22. R. Wehling and M. Pierce, *Journal-Association of Official Analytical Chemists*, 1988, **71**, 571.

23. S. Kazemi, N. Wang, M. Ngadi and S. O. Prasher, 2005.

24. D. L. Massart, B. Vandeginste, S. Deming, Y. Michotte and L. Kaufman, 1988.

25. E. Kalogianni, T. Karapantsios and R. Miller, *Journal of Food Engineering*, 2011, **105**, 169-179.

26. P. Hourant, V. Baeten, M. T. Morales, M. Meurens and R. Aparicio, *Applied Spectroscopy*, 2000, **54**, 1168-1174.

27. B. Osborne, T. Fearn and P. Hindle, *Practical NIR spectroscopy with applications in food and beverage analysis. Eds. Browning, D., Longman Scientific & Technical, London*, 1993, 145-159.

28. H. Cen and Y. He, *Trends in Food Science & Technology*, 2007, **18**, 72-83.

29. A. Savitzky and M. J. Golay, *Analytical chemistry*, 1964, **36**, 1627-1639.

30. A. A. Christy and P. K. Egeberg, *Chemometrics and intelligent laboratory systems*, 2006, **82**, 130-136.

31. A. Szabó, G. B'azár, G. Andrássy-Baka, L. Locsmándi and R. Romvári, *Acta Alimentaria*, 2009, **38**, 97-106.