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RAPID QUANTIFICATION OF FREE FATTY ACIDS IN VIRGIN COCONUT OIL BY FTIR SPECTROSCOPY

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ABSTRACT

A rapid method for quantitative analysis of free fatty acids in virgin coconut oil (VCO) was determined using Fourier transform infrared (FTIR) spectroscopy. Thirty training samples of VCO were prepared by spiking lauric acid to VCO samples covering a range of 0.1% to 5% of free fatty acids. FTIR spectra between 1730 and 1690 cm^{-1} was used for quantitative determination of free fatty acids. A partial least square (PLS) model was calibrated to predict the free fatty acids content of VCO. A linear calibration curve was obtained for the actual value against FTIR predicted value which yields an equation of $y = 0.9744x + 0.0006$ and satisfactory results of root mean square error of prediction (RMSEP) of 0.1264 and correlation coefficient (R^2) of 0.9281. Thus, FTIR spectroscopy with PLS regression can serve as important tools for determining the free fatty acids in VCO because it is rapid, accurate and environmental friendly compared to the conventional chemical analyses.

ABSTRAK

Satu kaedah pantas untuk analisis pengkuantitian asid lemak bebas dalam minyak kelapa dara telah ditentukan menggunakan spektroskopi inframerah transformasi fourier (FTIR). Sebanyak tiga puluh sampel minyak kelapa dara telah disediakan dengan menambah asid laurik ke dalam sampel minyak kelapa dara untuk mendapatkan kandungan asid lemak bebas dalam julat 0.1% hingga 5%. Nilai asid lemak bebas ditentukan dengan menggunakan kaedah standard American Oil Chemists' Society (AOCS) dan kemudian dianalisa menggunakan specktroskopi FTIR. Model kalibrasi "partial least square" (PLS) telah digunakan untuk meramal kandungan asid lemak bebas dalam minyak kelapa dara. Graf kalibrasi linear telah diperolehi bagi nilai sebenar asid lemak bebas melawan nilai ramalan FTIR dengan persamaan $y = 0.9744x + 0.0006$ dan keputusan memberansangkan ralat punca min kuasa dua untuk ramalan (RMSEP) bersamaan 0.1264 dan pekali korelasi (R^2) bersamaan 0.9281 telah diperolehi. Oleh itu, spektroskopi FTIR bersama PLS regresi berpotensi menjadi alat penting dalam penentuan asid lemak bebas dalam minyak kelapa dara kerana FTIR adalah pantas, jitu dan mesra alam berbanding analisis kimia konvensional.

Key words: fats; frying, oils; virgin coconut oil; *Zea mays L*.

INTRODUCTION

Virgin coconut oil is (VCO) considered to be one of the functional food oil due to its valuable therapeutic value (Marina *et al*., 2009). Thus, its production is increasing especially in tropical countries. Though the increased in the production quantity shows a positive sign, it is important to ensure that the quality of the oil is preserve in order to obtain the full benefits from the oil.

One of the most important methods to determine the quality of VCO is to determine the free fatty acids content. Free fatty acids are the amount of acids derived from hydrolytic rancidity of triglycerides in vegetable oils (Osawa *et al*., 2007). Hydrolysis of oils could occur due to reactions between fats and water dissolves in the fat phase which leads to deterioration of VCO. VCO produced by fermentation method is more prone to have high free fatty acids due to high water contents in the oil (Dimzon *et al*., 2011). Thus, efficient method to measure the free fatty acids content in VCO is called for.

The official wet chemistry method is the standard method used to measure the free fatty acids in VCO. However, the method is time consuming and labour intensive despite being relatively simple.

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The use of infrared spectroscopy has been suggested as alternative method to determine the free fatty acids in vegetable oils. Fourier transforms infrared spectroscopy which employs the mid infrared region of electromagnet spectrum from 4000 to 400 $cm^{-1.}$ The ease feasibility of using FTIR is due to rapid technique and reduces the use of toxic solvents (Rohman *et al*., 2014). FTIR has been reported for use of quantification of various oil parameters including free fatty acids. Free fatty acids have been determined in olive oil (Bertran *et al*., 1999), bleached palm oil (Che Man *et al*., 2009), soybean oil (Al-Alawi *et al*., 2004), corn, soybean, sunflower, palm oil (Verleyen *et al*., 2001) and crude oil from damaged soybeans (Lanser *et al*., 1991). However, there is none reported for virgin coconut oil. Thus, this paper focuses on the development method to determine the free fatty acids in virgin coconut oil using FTIR with attenuated total reflectance (ATR) in conjunction with partial least square (PLS) statistical analysis.

MATERIALS ANS METHODS

Materials

Virgin coconut oil was obtained from Kelantan Biotech Corporation. All chemicals and reagents used were of analytical grade.

Chemical analysis

Thirty samples of training set was prepared by spiking lauric acid to the virgin coconut oil samples in known amount covering a range of 0.1 to 5% of free fatty acids content. The conventional analysis of free fatty acids was done according to standard method of American Oil Chemists' Society (AOCS, 5a-40, 1996). The results were expressed as the number of mg of potassium hydroxide necessary to neutralize the free fatty acids in 1g of sample. Analyses were carried out in triplicate.

Spectra collection

The FTIR measurement was done using Bruker Tensor 27 spectrometer (BRUKER, Karlsruhe, Germany) equipped with flow through horizontal ATR accessory. Drops of oil samples were placed in contact with the ATR. Single beam spectra in the range of 4000 to 650 cm^{-1} were obtained and corrected against the background to present spectra in absorbance unit. Spectra were obtained using 32 scans at 4cm resolution. Opus software (Bruker, Karlsruhe, Germany) version 5.5 was used to operate the FTIR spectrometer and collect the spectra. The spectra interpretation was done using Essential FTIR v3.10.004 software (Operant LLC). The partial least square (PLS) calibration model that correlate between actual and predicted values of free fatty acids was performed. The 'leave one out' cross validation was used to access the predictive accuracy of the calibration model. The correlation coefficient (R^2) was used to measure the strength of the linear relationship between the predicted and actual values.

RESULTS AND DISCUSSION

Figure 1 shows the FTIR spectrum of virgin coconut oil. One of the main feature of the spectrum is in the region of 3050 to 2800 cm^{-1} which was attributed to the absorbance of CH stretching vibrations (cis- CH=CH-, $-CH_2-$, CH_3, CH_2/CH_3) (MOH *et al*., 1999). The region between 1400 to 900 cm^{-1} is due to the stretching of C-O-C and CH bending (Marina *et al*., 2007). The –C=O stretching absorption of the triacylglycerol ester linkage can be observed in the region from 1800 to 1650 cm^{-1}. The region of 1500 to 1000 cm^{-1} is known as fingerprint region. The carbonyl absorption of the triacylglyceol linkage is observable at 1744 to 1739 cm^{-1} (Guillen and Cabo, 1997; Rohman and Che Man, 2010). A band near 1238 cm^{-1} and 1163 cm^{-1} are related to the proportion of saturated acyl groups (Guillen and Cabo, 1999).

Figure 2 shows the overlay spectra of virgin coconut oil at region 1900 to 1600 cm^{-1}. The spectra show the increasing in absorbance at 1711 cm^{-1} as free fatty acid content is increased. The peak at 1711 cm^{-1} corresponds to free fatty acids carboxyl adsorption (Verleyen *et al*., 2001; Sim and Ting, 2012). The peak at 1711 cm^{-1} was used as the significant region for quantification of free fatty acids by Che Man *et al*. (2009), and Bertran *et al*. (1999). Thus, the region between 1730 and 1690 cm^{-1} was used in this study as spectral region that correlates with the free fatty acids content in virgin coconut oil for calibration purposes.

The ability of PLS to mathematically correlate spectral changes to changes in the concentration of component of interest is being utilize in this study. Figure 3 shows the PLS calibration model of the actual and free fatty acid value determined by chemical method versus the FTIR predicted value in virgin coconut oil. The slope indicates that there is linear relationship between FTIR predicted and chemically determined values with slope approaching to 1. The regression lines equation obtained was $y = 0.9744x + 0.0006$ with coefficient of determination (R^2) equals to 0.9281. The mean difference (MD) and standard deviation of the difference (SDD) for accuracy were 0.015 and 0.247 respectively. Validation was done by removing one standard at a time and satisfactory result of root mean error of prediction (RMSEP) of 0.1264 was obtained.

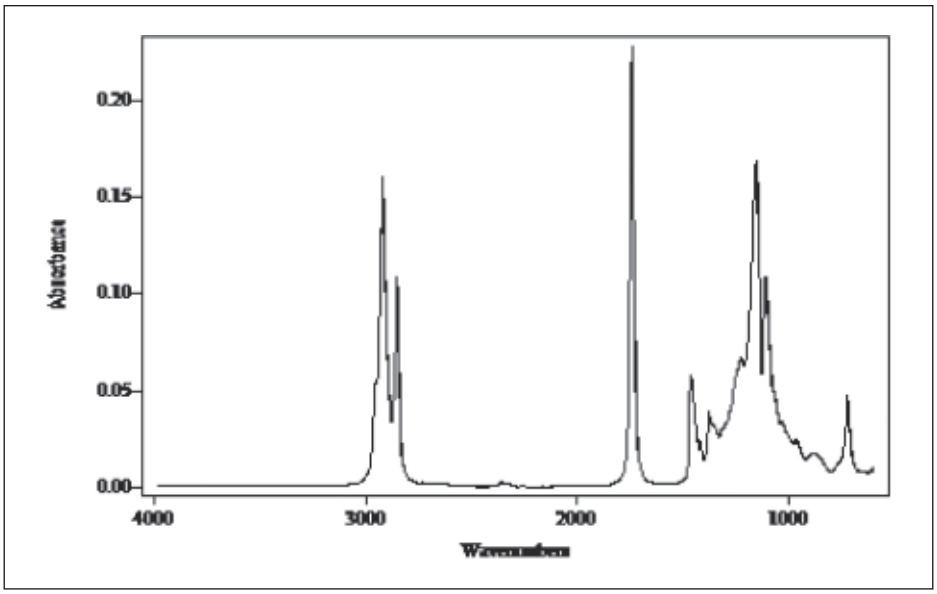

Fig. 1. FTIR absorption spectra of virgin coconut oil.

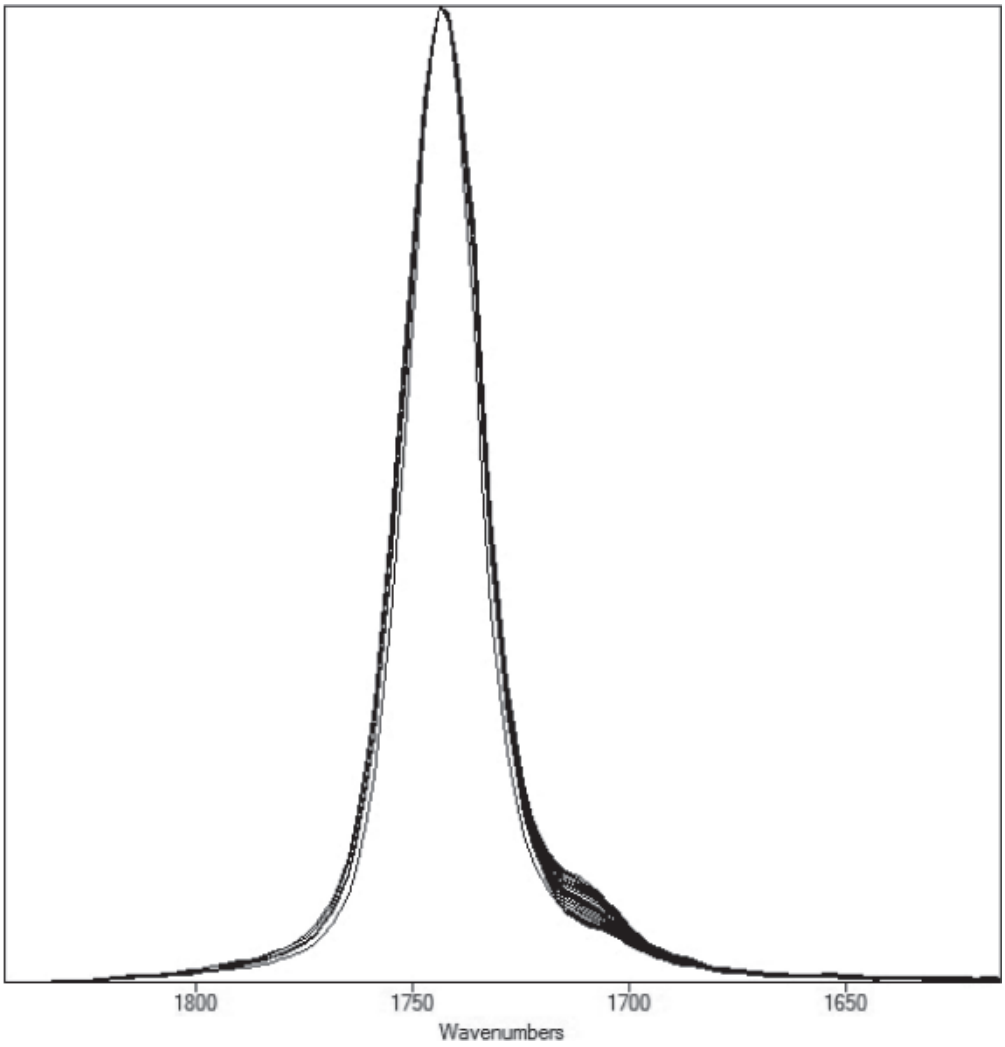

Fig. 2. Overlay spectra of virgin coconut oil.

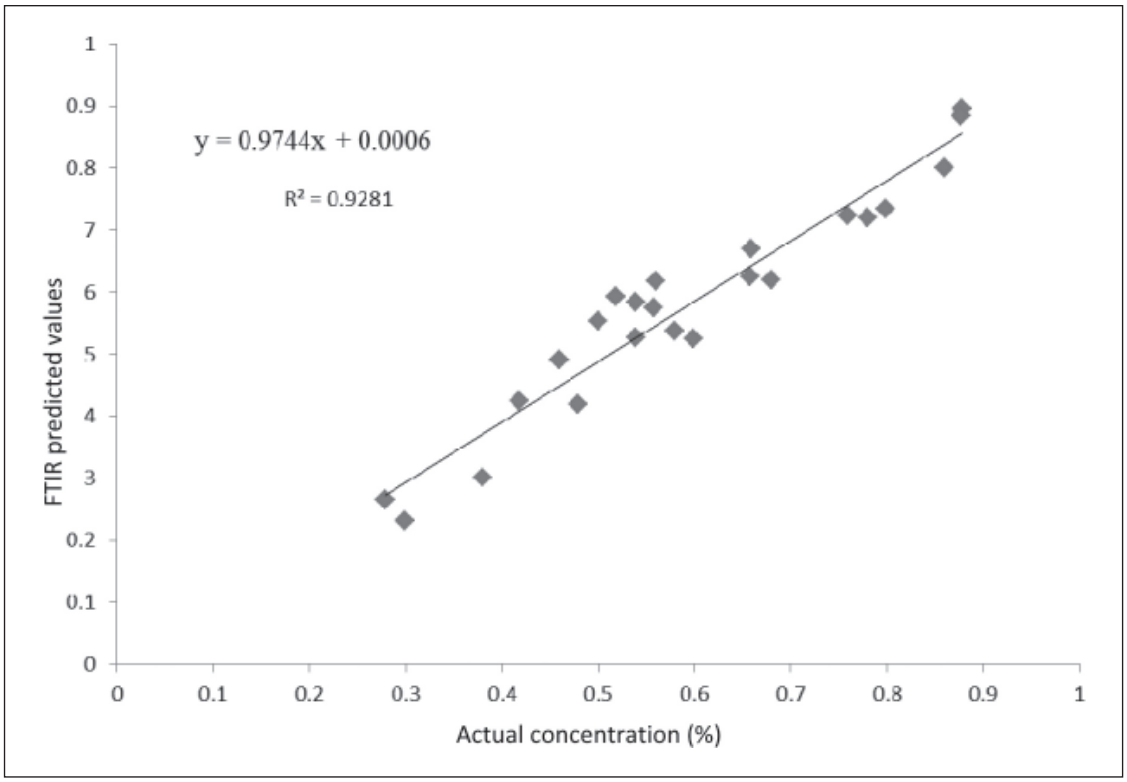

Fig. 3. PLS calibration model of actual versus predicted FTIR free fatty acid values.

CONCLUSIONS

Free fatty acids test serves as one of the most important quality parameter in virgin coconut oil. The conventional method to determine the free fatty acids involve the use of solvents and time consuming. However, FTIR can greatly simplify the method and highly recommended for routine quality control for virgin coconut oil. The present study shows that reliable prediction ability is obtained for determination of free fatty acids in virgin coconut oil using PLS which is applied in the range of 1730 and 1690cm^{-1}. This indicates that FTIR combines with PLS can serves as rapid powerful technique to determine the free fatty acids in virgin coconut oil than can be achieved using conventional methods.

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REFERENCES

Al-Alawi, A., van de Voort, F.R. and Sedman, J. 2004. New FTIR method for the determination of FFA in oils. *Journal of the Americam Oil Chemists' Society*, **81**: 441-446.

AOCS. 1996. Official Methods and Recommended Practice of the American Oil Chemists' Society (1996). *AOCS Press, Champaign*, vol 5.

Bertran, E., Blanco, M., Coello, J., Iturriaga, H., Maspoch, S. and Montoliu, I. 1999. Determination of olive oil free fatty acids by fourier transform infrared spectroscopy. *Journal of the American Oil Chemists' Society,* **76**: 611-616.

Che Man, Y.B., Aye, W.W., Tan, C.P. and AbdulKarim, S.M. 2009. Determination of free fatty acids in crude palm oil, bleached palm oil and bleached deacidified palm oil by fourier transform infrared spectroscopy. *Journal of Food Lipids,* **16**: 475-483.

Dimzon, I.K.D., Valde, M.F., Santos, J.E.R., Garrovillas, M.J.M., Dejarme, H.M., Remollo, J.M.W. and Dayrit, F.M. 2011. Physico-chemical and microbiolological parameters in the deterioration of virgin coconut oil. *Philippine Journal of Science,* **140**: 89-103.

Guillen, M.D. and Cabo, N. 1997. Characterization of edible oils and lard by fourier transform infrared spectroscopy. Relationship between composition and frequency of concrete bands in the fingerprint region. *Journal of the American Oil Chemists' Society,* **74**: 1281-1286.

Guillen, M.D. and Cabo, N. 1999. Usefulness of the frequency data of the fourier transform infrared spectra to evaluate the degree of oxidation of edible oils. *Journal of the Agricultural and Food Chemistry,* **47**: 709-719.

Lanser, A.C., List, G.R., Holloway, R.K. and Mounts, T.L. 1991. FTIR estimation of free fatty acid content in crude oils extracted from damaged soybeans. *Journal of the American Oil Chemists' Society*, **68**: 448-449.

Marina, A.M., Che Man, Y.B., Nazimah, S.A.H., Amin, I. and Syahariza, Z.A. 2007. Analysis of adulteration of virgin coconut oil by palm kernel olein using fourier transform infrared spectroscopy. *Journal of Food Lipids*, **14**: 111-121.

Marina, A.M., Che Man, Y.B. and Amin, I. 2009. Virgin coconut oil: emerging functional food oil. *Trends in Food Science and Technology*, **20**: 481-487.

Moh, M.H., Tang, T.S., Che Man, Y.B. and Lai, O.M. 1999. Rapid determination of peroxide value in crude palm oil products using fourier transform infrared spectroscopy. *Journal of Food Lipids,* **6**: 261-270.

Osawa, C.C., Goncalves, L.A.G. and Ragazzi, S. 2007. Correlation between free fatty acids of vegetable oils by rapid tests and by the official method. *Journal of Food Composition Analysis*, **20**: 523-528.

Rohman, A. and Che Man, Y.B. 2010. Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive adulterated with palm oil. *Food Research International,* **43**: 886-892.

Rohman, A., Riyanto, S., Sasi, A.M. and Yusof, F.M. 2014. The use of FTIR spectroscopy in combination with chemometrics for the authentication of red fruit (*Pandanus conoidues* Lam) oil from sunflower and palm oils. *Food Bioscience,* **7**: 64-70.

Sim, S.F. and Ting, W. 2012. An automated approach for analysis of Fourier transform infrared (FTIR) spectra of edible oils. *Talanta,* **88**: 537-543.