การศึกษาศักยภาพของฟูเรียร์ทรานฟอร์มอินฟราเรดสเปกโทรสโกปี ในการวิเคราะห์ปริมาณเบต้าแคโรทีนในน้ำมันปาล์มโอเลอีนผ่านกรรมวิธีการทำให้บริสุทธิ์ ฟอกสี และกำจัดกลิ่น

วรรณา อัมมวรรธน์[1*]
มหาวิทยาลัยเทคโนโลยีราชมงคลล้านนา ลำปาง ต.พิชัย อ.เมือง จ.ลำปาง 52000 ประเทศไทย
และ ยาค็อบ บิน เชอ แมน[2]
มหาวิทยาลัยพูตรามาเลเซีย ประเทศมาเลเซีย

บทคัดย่อ

งานวิจัยนี้เป็นการศึกษาการใช้ฟูเรียร์ทรานฟอร์มอินฟราเรดสเปกโทรสโกปี (Fourier transforms infrared spectroscopy, FTIR) ในการวิเคราะห์ปริมาณเบต้าแคโรทีนในน้ำมันปาล์มโอเลอีนผ่านกรรมวิธีการทำให้บริสุทธิ์ ฟอกสี และกำจัดกลิ่น (Refined bleach and deodorised palm olein) ร่วมกับการใช้โซเดียมคลอไรด์วินโดว์ที่ทรานสมิชชัน 50 ไมโครเมตร ทั้งนี้เตรียมตัวอย่างน้ำมันปาล์มโอเลอีนผ่านกรรมวิธีทำให้บริสุทธิ์ ฟอกสี และกำจัดกลิ่น จำนวน 50 ตัวอย่าง จากนั้นเติมสารมาตรฐานเบต้าแคโรทีนที่ความเข้มข้นร้อยละ 95 ให้ตัวอย่างมีความเข้มข้นของเบต้าแคโรทีนต่างๆ ในช่วง 0-2,000 ส่วนต่อล้านส่วน จากนั้นแบ่งตัวอย่างเป็นสองกลุ่มคือ กลุ่มที่ใช้สำหรับการปรับเทียบ (Calibration) และกลุ่มที่ใช้สำหรับทดสอบความแม่นยำของวิธีการ (Validation) ทั้งนี้ใช้วิธีการปรับเทียบแบบสมการเชิงเส้น (Partial least square, PLS) ในการทำนายปริมาณเบต้าแคโรทีนในช่วงของเอฟทีไออาร์สเปกตรัม ซึ่งพบว่าช่วงคลื่นที่ 980-915 ต่อเซนติเมตร (ช่วงทรานส์ของพันธะ =CH) เป็นช่วงที่ดีที่สุดสำหรับการวิเคราะห์ปริมาณเบต้าแคโรทีน ด้วยค่าความเบี่ยงเบนความเที่ยงตรง (SEC) และความเบี่ยงเบนการทำนาย (SEP) ต่ำสุดเป็น 34.01 และ 47.19 ตามลำดับ ในขณะที่ได้ค่าสัมประสิทธิ์สหสัมพันธ์ (R^2) ที่สูง ในแง่ความเที่ยงตรงของวิธีการ ทดสอบโดยเปรียบเทียบผลที่ได้กับการใช้เอชพีแอลซี (High performance liquid chromatography, HPLC) ซึ่งพบว่าการใช้เอฟทีไออาร์ร่วมกับการวิเคราะห์ทางสถิติแบบสมการเชิงเส้นเป็นเครื่องมือที่มีประโยชน์ ใช้งานง่าย และรวดเร็วในการวิเคราะห์ปริมานเบต้าแคโรทีนในน้ำมันปาล์มโอเลอีนผ่านกรรมวิธีทำให้บริสุทธิ์

คำสำคัญ : เบต้าแคโรทีน / ฟูเรียร์ทรานฟอร์มอินฟราเรดสเปกโทรสโกปี / สมการเชิงเส้น / น้ำมันปาล์มโอเลอีน

---

[1] *อาจารย์, สาขาวิชาอุตสาหกรรมเกษตร คณะวิทยาศาสตร์และเทคโนโลยีการเกษตร*

[2] *ศาสตราจารย์ ภาควิชาเทคโนโลยีการอาหาร คณะวิทยาศาสตร์และเทคโนโลยีการอาหาร สถาบันวิจัยผลิตภัณฑ์ฮาลาล, อินโฟพอร์ต*

[*] *ผู้ติดต่อประสานงานตามที่อยู่ เบอร์โทรศัพท์: +6654-342548 แฟกซ์: +6654-342549, อีเมลล์: wannam14@hotmail.com*

Potential of Fourier Transform Infrared Spectroscopy for Quantitative Analysis of β-Carotene in Refined, Bleached and Deodorised (RBD) Palm Olein

Wanna Ammawath[1*]

Rajamangala University of Technology Lanna Lampang, Pichai, Muang, Lampang 52000 Thailand

and Yaakob Bin Che Man[2]

Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Abstract

Fourier transform infrared (FTIR) spectroscopy was used to determine β-carotene in refined, bleached and deodorised (RBD) palm olein. The method used sodium chloride windows with a 50 μm transmission path. Fifty RBD palm olein samples spiked by known amount of standard (95%) β-carotene to produce a wide range of concentration up to 2,000 ppm were used. The samples were separated into two groups for the calibration and validation. Partial least square (PLS) calibration models were employed for predicting the β-carotene content in the FTIR spectral region. It was found that FTIR spectral regions of 980-915 cm^{-1} (trans =CH region) were the best for determining the β-carotene content with the lowest SEC (34.01) and SEP (47.19) and high R^2 values. The accuracy of the method was comparable to that of the high-performance liquid chromatography (HPLC). FTIR in conjunction with PLS has thus proved to be a useful analytical tool for simple and rapid determination of β-carotene in RBD palm olein for routine analysis.

Keywords : β-carotene / Fourier Transform Infrared Spectroscopy / Partial Least Square / Palm Olein

1 *Lecturer, Department of Agro-industry, Faculty of Science and Agriculture Technology*

2 *Professor, Department of Food Technology, Faculty of Food Science and Technology. Products Research Institute.*

* *To whom correspondence should be addressed. Tel: +6654-342548 fax: +6654-342549; E-mail: wannam14@hotmail.com*

1. Introduction

One of the major deteriorative reactions of cooking oil is lipid oxidation. It is well known that lipid oxidation can lead to changes in functional, sensory, nutritive values and even the safety of fried foods. Antioxidants are added to frying oil to retard lipid oxidation and extend the frying life of the oil. Appropriate antioxidants that should be used are determined by various factors including legislations, effectiveness, safety and cost [1].

Nowadays, commercial red palm olein, which contains high carotenoids content is available in the market. Carotenoids have been reported to play a role as antioxidants in lipid phase [2]. By far the most common carotenoids that are found in plant tissue and have been intensively studied is β-carotene [3, 4]. Food and pharmaceutical industries are interested in isolating β-carotene from biological matrices because of its strong antioxidant and pro-vitamin A activity [5]. It is recognized that β-carotene is an effective quencher of singlet oxygen, and hence acts as an antioxidant by preventing the formation of hydroperoxides in the presence of singlet oxygen [6]. β-carotene has also been shown to act as a pro-oxidant during lipid oxidation, both in the light and in the dark at higher concentrations [7]. β-carotene content is normally determined using high performance liquid chromatography (HPLC), which is unfortunately time-consuming, expensive and often involves environmentally unfriendly reagents [8, 9].

Fourier transform infrared (FTIR) spectroscopy is a very powerful technique for investigating the structure of food compositions and for monitoring changes of chemical constituents in food [10]. Mirghani et al. [11], for example, employed a simple horizontal ATR-FTIR spectroscopic method for quantitative determination of alflatoxins in groundnut and groundnut cake using horizontal ATR. More recently, FTIR spectroscopy has also been reported as a fast and accurate method to determine malondialdehyde, which is a secondary oxidation product, in palm olein system [12], α-tocopherol in RBD palm olein [13], butylated hydroxytoluene (BHT) content in RBD palm olein and RBD palm oil [14]. FTIR spectroscopy is based on the overall spectrum data to determine the concentration of a chemical constituent by factoring the signal of all wavenumbers into a resulting predictive equation. By balancing the spectral information and related concentrations, the method reduces the impact of large but irrelevant variations in the spectra [15]. However, in complex reaction mixtures the technique may be impracticable because serious spectral interferences (or overlapped signals) may result in non-linear correlations [16]. Multivariate analysis such as partial least square regression analysis (PLS) is required to discrete wavelengths in a sample spectrum to construct a linear model that correlates to a particular analysis concentration [14].

In this study, analysis of β-carotene in RBD palm olein by FTIR spectroscopy was conducted. PLS regression analysis was used for statistical data evaluation.

2. Materials and methods

RBD palm olein with no added antioxidant was purchased from a local palm oil refinery (Selangor, Malaysia). β-Carotene (95%) standard was purchased from Sigma-Aldrich (Selangor, Malaysia). All reagents were of analytical grade. RBD palm olein samples were spiked by known amounts of standard of β-carotene to concentrations up to 2,000 mg/kg (ppm). Spiked samples were separated into two groups for the calibration

and validation models.

3. Instrumental analysis

3.1 HPLC analysis

HPLC was used to determine the β-carotene content of the spiked samples of RBD palm olein. The HPLC system is equipped with UV visible detector (Shimadzu, SPD-10 AV, Kyoto, Japan), which operated at 446 nm; HPLC pump (LC-10AT); column oven (CTO-10A) and a TSK gel ODS-80TS column (4.6 mm ID x 25 cm). Isocratic mode was used in this study. The mobile phase consisted of acetonitrile/dichloromethane at 80:20 (v/v). The mobile phase was filtered through a 0.45 μm membrane, and degassed ultrasonically prior to its use. The flow rate of mobile phase was at 1 mL/min.

3.2 FTIR analysis

The instrument used was a Perkin-Elmer FTIR Spectrum BX (Perkin-Elmer, Norwalk, CT), with a room temperature deuterated triglycine sulfate (DTGS) detector. The spectrometer was connected to a computer running Perkin-Elmer Spectrum windows software to manipulate the spectra. The instrument was maintained with two automatic dehumidifiers to minimize interference from water vapor.

Each drop of sample was placed between the sodium chloride (NaCl) windows; the transmission path was fixed at 50 μm by adjusting the polytetrafluoroethylene (PTFE) spacer. The cell was placed in a cell holder; scanning was then made. After each sample scanning, the NaCl windows of the transmission cell were rinsed three times with acetone and then dried with soft tissue.

3.3 Multivariate statistical analysis

All experiments and measurements were done in duplicate. The relationships between each FTIR spectrum parameter and data from HPLC analysis were determined using Nicolet Turbo Quant IR-Calibration and Prediction Package, Version 1.1 (Nicolet Instrument Co., Madison, WI). Fifty samples were used, with 38 samples in the calibration set and the remaining 12 samples in the prediction set. The calibration standards were designed to obtain the data for the PLS regression, The HPLC and spectral data were correlated and correlation coefficients (R^2), standard errors of calibration (SEC) and standard errors of prediction (SEP) were taken as estimates of the factor scores which were then used as regressors to model both the spectral and chemical data.

4. Results and Discussion

4.1 Development of calibration and validation models

HPLC analysis was used to determine the initial β-carotene content in oil. The result showed that no β-carotene was presented in fresh RBD palm olein from the local palm oil refinery. These results are in close agreement with those of May [17], Ammawath and Che Man [18]. The crude palm oils must have been refined by a physical refining method, which includes three major processes: degumming, bleaching and deodorization to remove undesirable components in which all carotenoids are destroyed in the refining process [19].

4.2 Spectral analysis

Fifty RBD palm olein samples were spiked by standard (95%) β-carotene for the FTIR calibration and validation. HPLC analysis and the data were used to estimate the calibration range up to 2,000 ppm. Figure 1 illustrates FTIR spectra of standard (95%) β-carotene. Ammawath et al.

[18] found that the exhibited spectrum peaks of β-carotene are at 962 cm^{-1} for trans conjugated alkene -CH=CH- out-of-plane deformation mode (as quoted in Moh et al. [20]), 1,033 cm^{-1} for in plane -CH- (as quoted in Nakanishi and Solomon [21]), 1,360 cm^{-1} for splitting due to dimethyl group 1,445 cm^{-1} for CH_2 scissoring 2,862 cm^{-1} and 2,922 cm^{-1} for asymmetric and symmetric stretching vibrations of the CH_2 and CH_3 as reported by Guillen and Cabo [22]. The wavelength regions around the exhibited spectra peaks were used to build the calibration model for β-carotene determination.

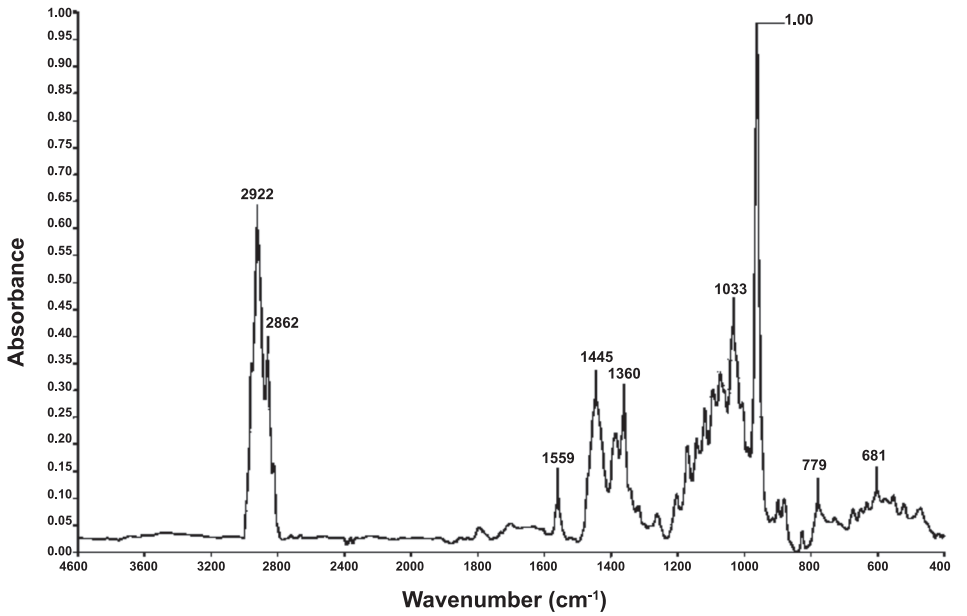

Fig. 1 FITR spectra of standard (95 %) β-carotene.

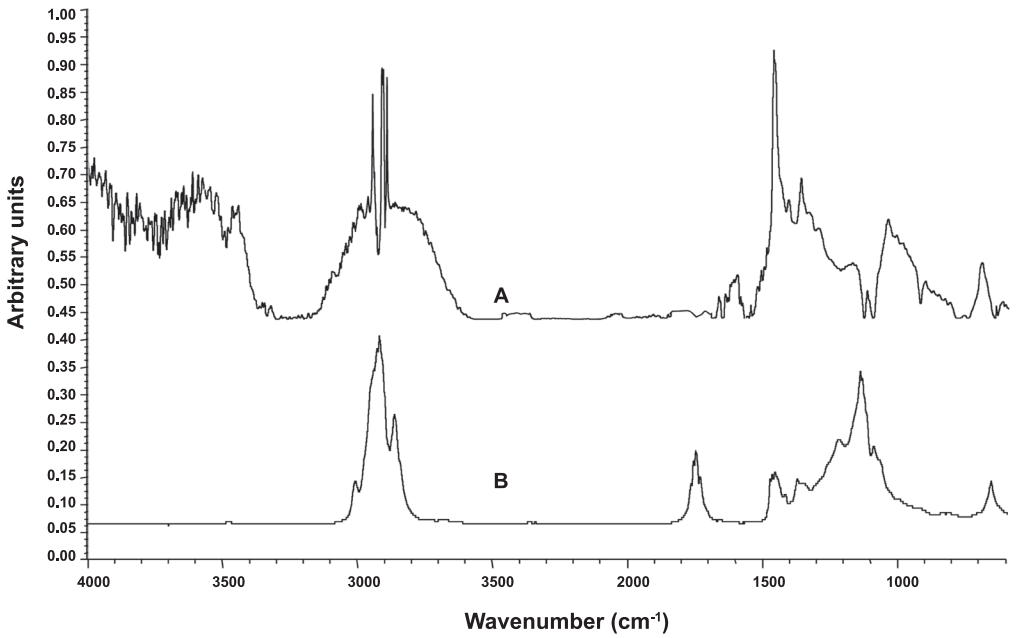

Fig. 2 FITR spectra; (A) Correlation spectra obtained from a calibration set (B) Mean spectrum of RBD palm olein sample spiked by standard (95%) β-carotene with different concentrations.

Fig. 2 (A) shows the correlation spectrum, which is calculated by multiplying the difference between each standard spectrum and the mean spectrum at each wavelength by difference between the corresponding property concentration and the mean property concentration, and summing overall the standards. Peaks that do not correlate with the change in concentration are summed to zero, producing a spectrum that highlights the peaks that change with the change in concentration [12]. Fig. 2 (B) shows the mean spectrum of samples RBD palm olein spiked by standard β-carotene with different concentrations of the calibration set which were the spectral interferences of complex mixture. From Fig. 2 (A) and Fig. 2 (B), noticeable differences were found in the wavelength region of 980-915 cm^{-1} and correlated well with the change in β-carotene concentration without interference from RBD palm olein spectral. Fig. 3 illustrates 5 overlay FTIR absorbance with significant changes at the region of 980-915 cm^{-1} for RBD palm olein samples containing different β-carotene concentrations.

The absorption changes at 980-915 cm^{-1} as trans =CH stretching band with the changes in concentration of β-carotene (A) 0 ppm, (B) 500 ppm, (C) 1,000 ppm, (D) 1,500 ppm, (E) 2,000 ppm. Seven spectral regions 3,050-2,775 cm^{-1}, 1,490-1,406 cm^{-1}, 1,406-1,296 cm^{-1}, 1,222-980 cm^{-1}, 980-915 cm^{-1}, 1,490-1,296 and 1,222-915 were used to build the calibration models for β-carotene determination.

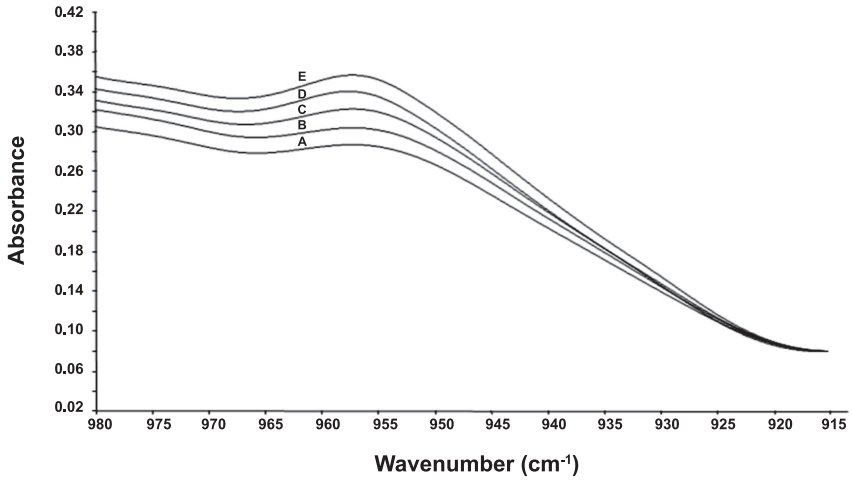

Fig. 3 Absorption changes at 980-915 cm^{-1} with different concentrations of β-carotene (ppm) (A) 0, (B) 500 (C) 1000 (D) 1500 and (G) 2000.

Table 1 Comparison different wavelength regions of FTIR spectra and β-carotene content from HPLC analysis for development of calibration model of standard β-carotene content in RBD palm oleina[a]

Region No.	Wavelength Region (cm^{-1})	Standard β-carotene		
		R^2	SEC	SEP
2	3050-2775	0.1358	491.0	569.6
2	1490-1406	0.5285	401.9	427.8
3	1406-1296	0.3340	445.0	488.1
4	1222-980	0.1484	486.5	600.3
5	980-915	0.9971	34.01	47.19
2+3	1490-1296	0.1347	491.1	562.4
4+5	1222-915	0.1464	487.2	597.2

[a]R^2 = Coefficient of determination; SEC = standard errors of calibration; SEP = standard errors of prediction.

The calibration standards were designed to obtain data for the PLS regression. Table 1 shows the results obtained from the PLS calibration in terms of the coefficient of determination (R^2), the standard errors of calibration (SEC) and the standard errors of prediction (SEP) of standard β-carotene used in choosing the best regions for determining β-carotene in RBD palm olein. It was found that FTIR spectral regions 980-915 cm^{-1} (trans =CH region) were the best for determining standard β-carotene with lowest SEC (34.01) and SEP (47.19) and highest R^2 (0.9971). These results are in close agreement with Moh et al. [20], Ammawath and Che Man [18].

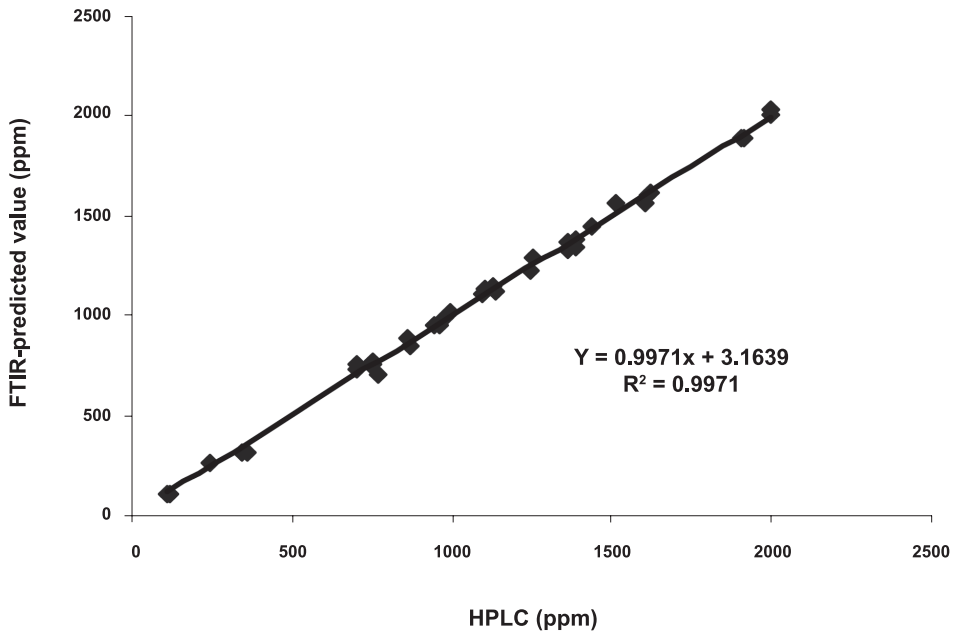

Fig. 4 Calibration plot of actual data from HPLC versus PLS FTIR predicted values.

A correlation plot of standard β-carotene as shown in Fig. 4 presents a correlation plot established in building the model using region 980-915 cm^{-1} by using the actual data from HPLC analysis that give the coefficient of determination (R^2) of 0.9971 and the equation of Y = 0.9971x + 3.1639. Fig. 5 shows a validation plot of standard β-carotene in RBD palm olein with R^2 of 0.9921 that test the calibration validity. Table 2 compares these data in terms of mean and standard deviation (SD) for reproducibility between duplicates of the HPLC method versus FTIR and the coefficient of variation (CV) of standard β-carotene content in RBD palm olein sample sets obtained from both the calibration and validation models. The mean and SD of the data obtained from calibration and validation sets of standard β-carotene by HPLC analysis were 1,077.70, 16.38 and 951.51, 10.63, respectively, while for the FTIR, the values were 1,076.54, 14.28 and 943.40, 13.73, respectively. The coefficient of variation (CV) obtained using the PLS statistical method to predict FTIR results from the HPLC method were 1.34% and validation sets were 1.46%.

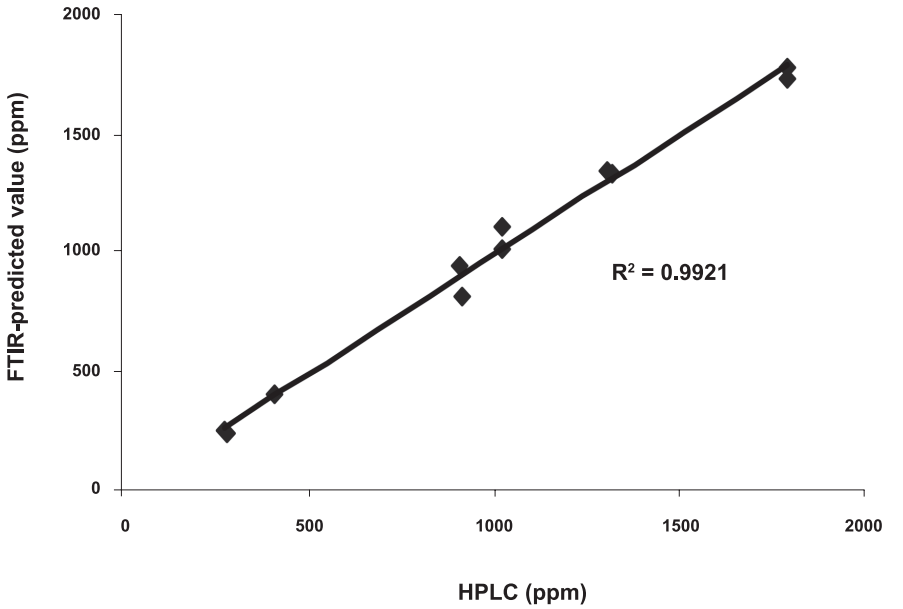

Fig. 5 Validation plot of actual data from HPLC versus PLS FTIR predicted values.

Table 2 Calibration and validation statistics for standard β-carotene in RBD palm olein as determined by HPLC and FTIR spectroscopic method using PLS of wavelength regions 980-915 (cm^{-1})[a]

Data Set	HPLC			FTIR		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Calibration	1077.70	16.38	1.52	1076.54	14.28	1.34
Validation	951.51	10.63	1.12	943.40	13.73	1.46

The results of statistical analysis (Table 3) show the assessment of accuracy (MD_a), the mean difference for repeatability (MD_r), the standard deviation of difference for repeatability (SDD_r) and the standard deviation of difference for accuracy (SDD_a) of the HPLC and FTIR methods for determining standard β-carotene content in RBD palm olein samples. The MD_r value was very low for HPLC and FTIR methods in standard β-carotene sample sets, implying that there was little difference between them in the two series of analyses. The results in terms of SDD_r, SDD_a and MD_a were also very low for both FTIR and HPLC methods.

Table 3 Calibrations statistics of standard β-carotene in RBD palm olein from data obtained by HPLC and FTIR spectroscopy[a]

Statistic	Standard β-carotene	
	HPLC	FTIR
MD_r	2.84	1.51
SDD_r	3.59	2.81
Min. value	109.51	106.90
Max value	1998.88	2029.00
MD_a	1.16	
SDD_a	0.31	

[a] MD, mean difference; SDD, standard deviation of difference; r, repeability; a, accuracy; FTIR, Fourier transform infrared spectroscopy; all the data were the means from two replicates.

5. Conclusion

Predictions of standard (95%) β-carotene in RBD palm olein were realized by FTIR spectroscopy. PLS was used to determine β-carotene content with a good correlation of the FTIR spectral regions at 980-915 cm^{-1}. The accuracy of the method was comparable to that of HPLC method, with an R^2 of 0.9971 from calibration samples of RBD palm olein. The analysis is rapid and requires only minimal sample of less than 1 mL.

6. Acknowledgements

The authors express their sincere appreciation to the Universiti Putra Malaysia for providing funding for this research under the Intensification of Research in Priority Areas Program awarded to Professor Dr. Yaakob bin Che Man. We also would like to acknowledge for moral supporting publication under Hands-on research and development project of Rajamangala University of Technology Lanna.

7. References

1. Ammawath, W., Che Man, Y.B., Yusof, S., and Rahman, R.B., 2002, "Effects of type of packaging materials on physicochemical and sensory characteristics of deep-fat-fried banana chips", *Journal of the Science of Food and Agriculture*, Vol. 82, pp. 1621-1627.

2. Anguelova, T. and Warthesen, J., 2000, "Degradation of lycopene, α-carotene and β-carotene during lipid peroxidation", *Journal of Food Chemistry and Toxicology*, Vol. 6, pp. 71-75.

3. Johnson, I.T., 2001, *Antioxidants and Antitumour Properties, in J. Pokorn, N. Yanishlieva and M. Gordon (Eds.). Antioxidants in Food Practical Applications*, Woodhead Publishing, Cambrige.

4. Von Elbe, J.H. and Schwartz, S.J., 1996, *Colorants, in O.R. Fennema (Ed.) Food Chemistry*, Marcel Dekker, New York.

5. Araus, K.A., Canales, R.I., del Valle, J.M., and de la Fuente, J.C., 2011, "Solubility of β-carotene in ethanol- and triolein-modified CO_2",

The Journal of Chemical Thermodynamics, Vol. 43, pp. 1991-2001.

6. Gordon, M.H., 1990, *The Mechanism of Antioxidant Action in Vitro, in B.J.F. Hudson (Ed.) Food Antioxidants*, Elsevier Science Publishers, Essex.

7. Che Man, Y.B. and Tan, C.P., 2003, *Carotenoids*, The Oily Press, Bridgwater, pp 25-46.

8. Baharin, B.S., Abdul Rahman, K., Abdul Karim, M.I., Oyaizu, T., Tanaka, Y., and Takagi, S. 1998, "Separation of palm carotene from crude palm oil by adsorption chromatography with a synthetic polymer adsorbent", *Journal of the American Oil Chemists' Society*, Vol. 75, pp. 399-404.

9. Van de Voort, F.R., Ismail, A.A., Sedman, J., Dubois, J., and Nicodemo, T., 1994, "The determination of peroxide value by Fourier transform infrared spectroscopy", J*ournal of the American Oil Chemists' Society*, Vol. 71, pp. 921-926.

10. Dupuy, N., Duponchel, L., Huvenne, J.P., Sombret, B., and Legrand, P., 1996, "Classification of edible fats and oils by principal component analysis of Fourier transform infrared spectra", *Food Chemistry*, Vol. 57, pp. 245-251.

11. Mirghani, M.E.S., Che Man, Y.B., Jinap, S., Baharin, B.S., and Bakar, J., 2001, "A new method for determining aflatoxins in groundnut and groundnut cake using FTIR spectroscopy with attenuated total reflectance technique", *Journal of the American Oil Chemists' Society*, Vol. 78, pp. 985-992.

12. Mirghani, M.E.S, Che Man, Y.B., Jinap S., Baharin, B.S., and Bakar, J., 2002, "Rapid method for determining malondialdehyde as secondary oxidation product in palm olein system by Fourier transform infrared spectroscopy", *Journal of Phytochemical Analysis*, Vol. 13, pp. 195-201.

13. Che Man, Y.B., Ammawath, W., and Mirghani, M.E.S., 2005, "Determining α-tocopherol in refined bleached and deodorized palm olein by Fourier transform infrared spectroscopy", *Food Chemistry*, Vol. 90, pp. 323-327.

14. Ammawath, W., Che Man, Y.B., Abdul Rahman, R.B., and Baharin, B.S., 2006, "Development of rapid Fourier transform infrared spectroscopic method for quantitative analysis of butylated hydroxytoluene in RBD palm olein and RBD palm oil", *Journal of the American Oil Chemists' Society*, Vol. 83, pp. 187-191.

15. Dupuy, N., Meurens, M., Sombret, B., Legrand, P., and Huvenne, J.P., 1992, "Multivariate determination of sugar powers by attenuated total reflectance infrared spectroscopy", *Applied Spectroscopy*, Vol. 47, pp. 452-457.

16. Zagonel, G.F., Peralta-Zamora, P., and Ramos, L.P., 2004, "Multivariate monitoring of soybean oil ethanolysis by FTIR", *Talanta*, Vol. 63, pp. 1021–1025.

17. May, C.Y., 1996, "Antioxidant in red palm oil", *Malaysian Oil Science and Technology*, Vol. 5, pp. 15-16.

18. Ammawath, W., and Che Man, Y.B., 2010, "A rapid method for determination of commercial β-carotene in RBD palm olein by Fourier transform infrared spectroscopy", *Asian Journal of Food and Agro-Industry*, Vol. 3, pp. 443-452.

19. Szydlowska-Czerniak, A., Trokowski, K., Karlovits, G., and Szlyk, E., 2011, "Effect of refining process on antioxidant capacity, total contents of phenolics and carotenoids in palm oils", *Food Chemistry*, Vol. 129, pp. 1187-1192.

20. Moh, M.H., Che Man, Y.B., Badlishah, B.S, Jinap, S., Saad, M.S., and Abdullah, W.J.W., 1999, "Quantitative analysis of palm carotene using Fourier transform infrared and near

infrared spectroscopy", *Journal of the American Oil Chemists' Society*, Vol. 76, pp. 249-254.

21. Nakanishi, K. and Solomon, P.H., 1977, *Infrared Absorption Spectroscopy* (2nd Ed). In Holden-Day, San Francisco.

22. Guillen, M.D. and Cabo, N., 1997, "Infrared spectroscopy in the study of edible oils and fats", *Journal of the Science of Food and Agriculture*, Vol. 75, pp. 1-11.