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Application of infrared spectroscopic analysis for quantification of free fatty acid content at palm oil mills

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Abstract. High free fatty acid (FFA) content results in low palm oil yield in refinery and 5 wt% are the limit for crude palm oil acceptance. Common method of the FFA quantification in the mills is titration. The intent of this study was to determine the FFA content in extracted palm oil by using Fourier transform infrared (FTIR) spectroscopy coupled with AOCS titration method for verification. The oil extractions took place on days 1, 5, 8, 12 and 15. The fruitlets were initially washed and autoclaved. By separating the kernel, the mesocarp was heated and pressed. After centrifugation, the oil from the top layer of extract was further analyzed. In this study, % transmittance of FFA had been measured at carboxyl band, C=O between 1730 and 1700 cm^{-1}. The stretch of carboxyl band denotes that the FFA had proportionally increased with time. The FFA content of the samples increased in sigmoidal pattern with critical rise after day 8, reaching 15 wt% equilibrium states after 15 days. A calibration model was developed via linear regression and R^2 of 0.979 indicating the results were significant.

1. Introduction

The formation of FFA was reported to be proportional with the maturity level of fresh fruit bunch (FFB) whereby the lowest FFA content was recorded by the under-ripe FFB followed by ripe FFB, overripe FFB and loose fruits [1]. Since individual fruits does not mature at the same rate (starts from top to bottom, and outer to inner layer of FFB), there are minimum number of loose fruits and particular colourations required before harvesting. The length of fruit storage, fruit bruising and light are causing the FFA content in palm oil extract to increase as well [2-5]. These are due to the presences of endogenous and exogenous lipase enzymes that induce a reaction between glycerides and water, thus forming FFA and glycerol.

Currently, acid–base titration is a well-known method to measure FFA content in palm oil [3, 6-8]. The oil sample is titrated against potassium hydroxide (KOH) in a hot 2-propanol solution, aided by phenolphthalein as an indicator, and the result will be expressed in mg KOH/g. Some of the drawbacks are laborious, time consuming and high chemicals consumption. Another method to quantify the FFA content is by using gas chromatography with flame ionization detector (GC–FID) due to its rapid analysis and high sensitivity [9, 10]. This method however is highly depending on the capillary column types and stationary phase polarity, thus FFA needs to be esterified into fatty acid methyl ester (FAME) prior to injection.

FTIR spectroscopy is a method that has rapid measurement, high accuracy and reproducibility. Specific functional groups of a sample can be identified through the spectrum of absorption bands at specific wavenumbers (cm^{-1}). FTIR spectroscopy method was applied by researchers to determine the presence of FFA through carboxyl region between 1722 and 1690 cm^{-1} in crude palm oil (CPO) and refined–bleached–deodorized (RBD) palm olein by spiking the samples with lipozyme [11]. Another study of FFA in palm olein using FTIR was also reported [12]. The palm olein was spiked with known concentrations of oleic acid and further analyzed at wavenumbers between 1728 and 1690 cm^{-1}. Other researchers had used the FTIR spectroscopy method together with multivariate calibration to evaluate the quality of frying oil [13, 14]. The method was also used to study the oxidation [15, 16] and adulteration of edible oils [17, 18]. The objective of this research was to study the application of FTIR spectroscopy method in palm oil mill for quick determination of FFA content in extracted palm oil.

2. Materials and methods

2.1. Sample preparation
FFB was picked from LCSB Lepar palm oil mill, Pahang. To obtain the average ripeness, each of fruits was manually plucked out and grouped based on three regions: top, middle and bottom as shown in Figure 1. This is due to different rate of ripening process where top region matures sooner than others. The fruits were weighed and further classified into five groups with equal weight distribution. To ensure the homogeneity of the samples, each group from the different regions was then mixed again forming five groups in total. At the end of this process, there were five batches of oil palm fruits, each to be extracted on different days.
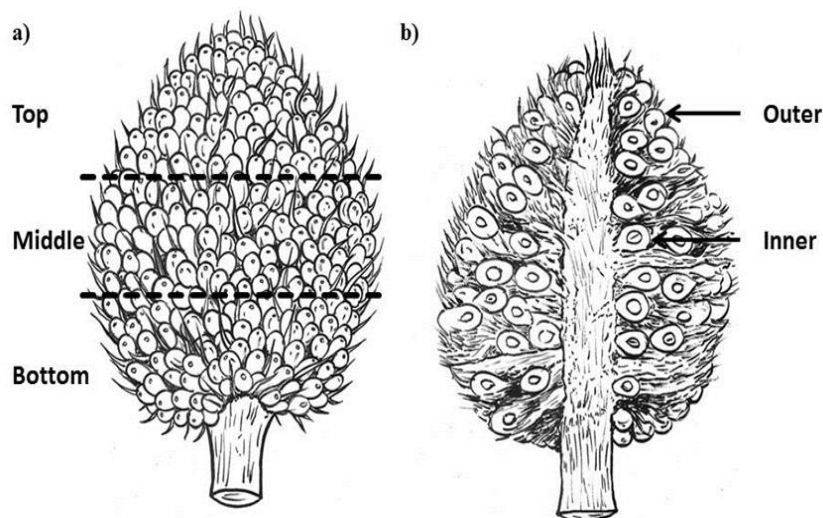

Figure 1. Oil palm fruits sketches: (a) Three regions are top, middle and bottom (b) Outer and inner layers [19].

A batch of fruits was first washed using water to remove any dirt. To mimic the sterilization process in palm oil mills, the fruits were autoclaved at temperature of 121°C for 20 min. This process was aiming to soften the palm mesocarp and to inhibit the lipolytic activities which can cause further increase of FFA content [20, 21]. The fruits were then manually cut to separate the mesocarp and the kernel. To enhance the oil cell breakdown process, the mesocarp was preheated in 80°C water bath for 2 hours prior to extraction [22, 23]. The CPO was then extracted by pressing the mesocarp using a hydraulic jack press. The collected CPO was heated in 80°C water bath followed by centrifugation process at 4,000 rpm which forming three different layers: oil, water and non-oily solid (NOS). The oil layer was

separated and collected by using micropipette for further analysis. The extraction process took place on day 1, 5, 8, 12 and 15, by taking different batches of samples that were equally divided beforehand.

2.2. AOCS titration method

For wet chemical analysis, the American Oil Chemists' Society (AOCS) titration method had been applied [7]. 5 g of CPO was added into a conical flask and diluted with 50 mL isopropanol. 10 drops of phenolphthalein was then added as an indicator. The solution was heated until the bubbles formed at the bottom of the flask. It was further titrated with 0.1 M sodium hydroxide (NaOH) and slowly swirled until the colour changed from colourless to pink. The volume of NaOH was recorded and used to calculate the FFA content in the oil sample as in Equation (1):

$$\% \text{ FFA as palmitic acid } = \frac{M \times V \times 25.6}{m} \tag{1}$$

where M is the molarity of standard NaOH solution (mol L^{-1}), V is the volume of the standard NaOH solution used (mL), 25.6 is the equivalence factor for palmitic acid and m is the weight of the oil sample (g). The analysis for each sample was repeated twice to obtain an accurate reading.

2.3. FTIR spectroscopy analysis

A Nicolet™ iS™ 5 FTIR spectrometer was used in this study. For each run, the crystal surface of the attenuated total reflectance (ATR) system was thoroughly cleaned with 70% ethanol solution and dried with a lens tissue. The background spectrum was calibrated, followed by placing the oil sample on the diamond crystal. All spectra were collected from the range of 4000 to 400 cm^{-1} by using 32 scans. The spectra were analysed using Omnic software (Thermo Scientific USA).

2.4. Calibration model

Each of CPO samples was used as calibration sets for FTIR analysis. FFA content was determined according to carbonyl stretch C=O of a carboxylic acid at band range between 1730–1700 cm^{-1}. The intensity of the band was measured as % transmittance and converted into absorbance as in Equation (2). The FTIR results were then correlated with the results from AOCS titration method by using linear regression method.

$$A = 2 - \log(\% T) \tag{2}$$

where A is the absorbance and T is the transmittance.

3. Results and discussion

3.1. AOCS titration method

Figure 2 shows the FFA content in CPO for five days extraction. From day 1 to day 8, the FFA content increases steadily from 0.919% to 4.073%. On day 12, the graph displays a significant increase from 4.073% to 10.534%. The formation of FFA in CPO is particularly related with mesocarp and microbial lipolytic activities [24, 25]. The mesocarp lipase however was found to be a dominant factor in rising FFA content immediately after harvest [26].
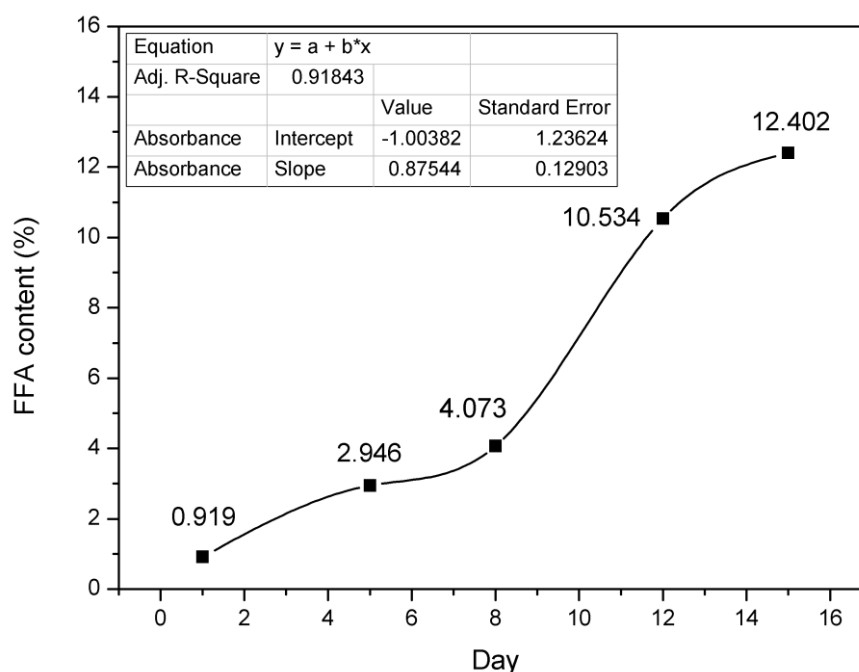
Figure 2. Effect of day of extraction on FFA content measured using AOCS titration method.

The presence of lipases caused hydrolysis reaction of triglyceride (TG) thus forming monoglyceride (MG), diglyceride (DG), glycerol and molecules of FFA. Factors that induced the lipolytic activities include the bruising and moisture content of the fruitlets. The loose fruits as in this study; tend to have higher FFA content compared to others because of softening and bruising pericarp [1]. This study was supported by other findings whereby an adverse bruised fruit showed a significant FFA content than a less bruised fruit [3, 4]. This was due to the disruption of the oil cell bearing which consequently released the mesocarp lipase, inducing the hydrolysis reaction. The soft pericarp also might easily be attacked by microorganisms, thus increases the microbial lipolytic activity.

The result obtained in this study is in accordance with a previous study whereby an increment trend of FFA was recorded after 2 hours storage prior to extraction [3]. Another study concluded that regardless of oil palm fruit types (*dura*, *pisifera* and *tenera*), there was a notable increase of FFA from day 0 to day 7 of fruit storage [2].

3.2. FTIR spectroscopy analysis
Figure 3 shows the FTIR spectrogram of different batches of CPO sample. The sharp stretches at both 2920 and 2850 cm^{-1} signify the presence of alkane, aliphatic C–H bond. Meanwhile, 1743 and 1711 cm^{-1} bands denote the carbonyl ester, C=O and carboxyl group, C=O respectively.
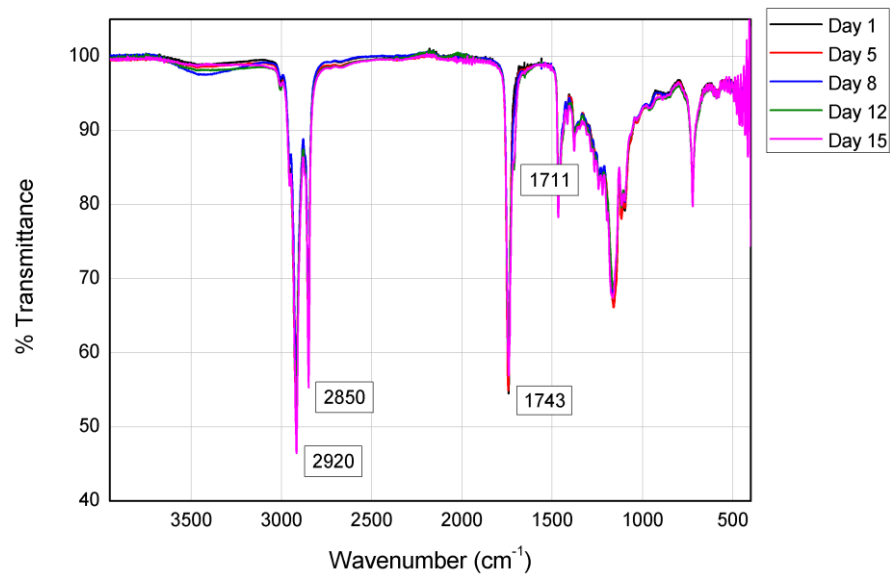
Figure 3. Effect of day of extraction on FFA content measured using FTIR spectroscopy.

Carboxylic acid has a wide range of spectrum which can be represented by C–H, O–H, C=O and C–O stretch. It was reported that the carboxyl band is the most appropriate region to quantify the FFA content in palm oil sample due to significant variance compared to others [11]. In this study, FFA had been measured at the C=O stretch between 1730 and 1700 cm^{-1} [11, 12]. The intensity of the band was converted from % transmittance to absorbance due to flexibility. According to Beer's Law, the absorbance is directly proportional to the concentration thus, as C=O stretch increased, the absorbance also increased indicating higher FFA content in the oil sample. Figure 4 shows a graph of absorbance which focusing on the wavenumbers between 2000 and 1500 cm^{-1}.
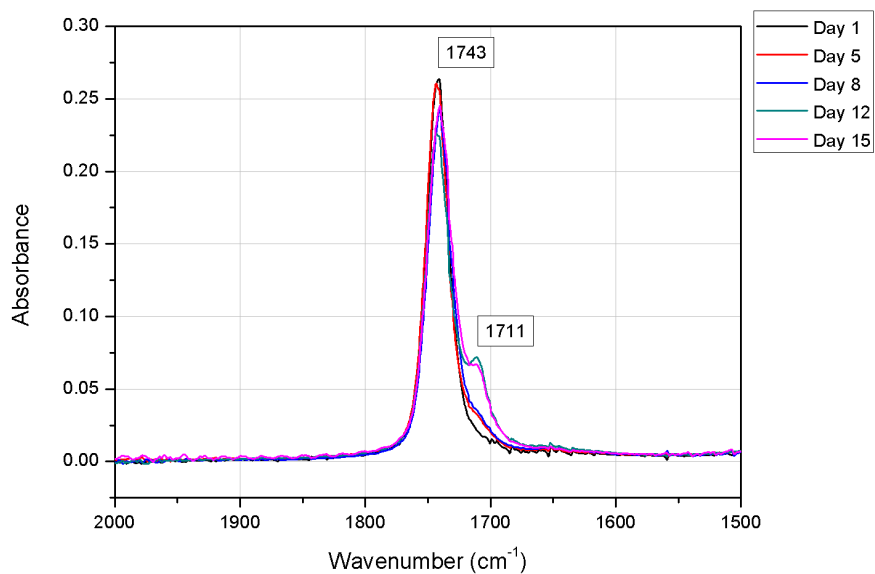

Figure 4. Absorbance of oil samples between 2000 and 1500 cm^{-1}.

From the spectrogram, FFA content was analysed through the height and area of the absorbance. The band was particularly observed at wavenumber 1711 cm^{-1}. For height method, it can be seen from *Figure 4*, the absorbance increases as the day increases from day 1 to day 15. This increment is in accordance with previous result measured by using AOCS titration method.

For area under the curve method, deconvolution of the graphs was initially performed due to the overlapping of the stretching band 1711 and 1743 cm^{-1} of the ester carbonyl functional group. As the oxidation occurs, the maximum absorbance was in the region between 1700 and 1730 cm^{-1} and this consequently caused the 1743 cm^{-1} band to broaden to lower wavenumbers [15]. The data obtained from height and area under the curve methods are tabulated as shown in Table 1.

Table 1. Height and area under the curve retrieved from absorbance versus wavenumber at 1711 cm^{-1}.

Day	Height	Area
1	0.03930	0
5	0.04516	0
8	0.05298	0.92755
12	0.07073	1.74640
15	0.07484	1.76423

Figure 5 shows a correlation between FFA content measured through height and area under the curve, versus day of extraction. Both methods show an increment trend of FFA content over time. However, on day 1 and day 5, there is no FFA being detected by area under the curve method. This was due to the very small peak at 1711 cm^{-1} even after the deconvolution. In accordance with previous study, FTIR spectroscopy was reported to only detect the presence of C=O as a flat line rather than a band when the FFA is too low [12]. The data was then fitted to a linear model and summarized as shown in Table 2.
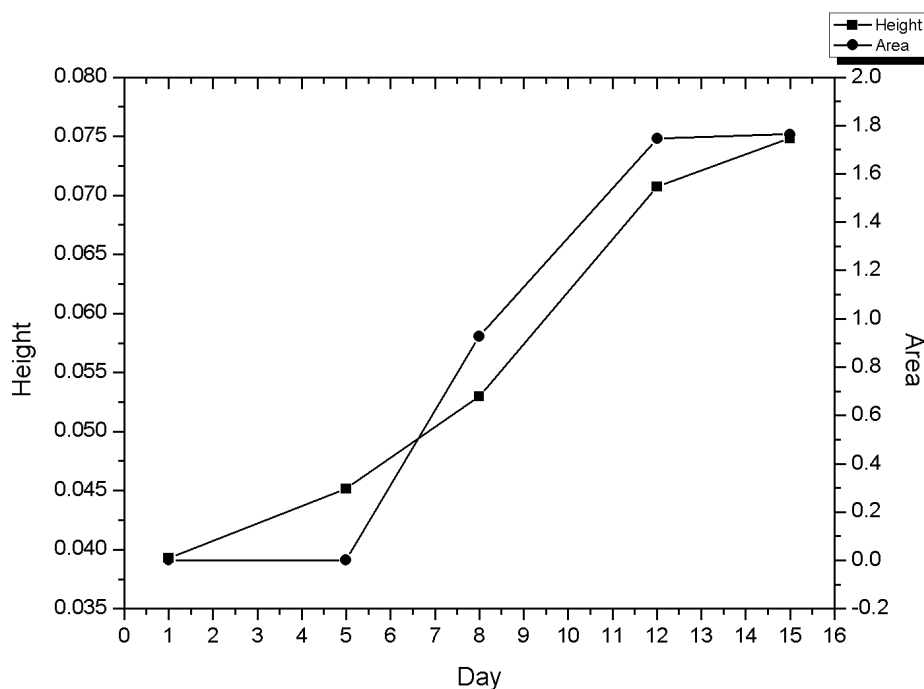

Figure 5. Relationship between FFA content and day based on height and area under the curve.

Table 2. Linear models for height and area of the samples.

	Intercept		Slope		Statistics
	Value	Standard error	Value	Standard error	R^2
Height	0.03392	0.00301	0.00277	3.14198E-4	0.95030
Area under the curve	-0.34420	0.27883	0.15022	0.02910	0.86508

Figure 6 shows a calibration curve generated by using known concentrations of oleic acid in RBD palm oil, from 0 to 14 % v/v. The absorbance by height method increases steadily over concentration. Meanwhile, for 0 and 2 % v/v oleic acid, due to very small peaks at 1711 cm^{-1}, the areas remain zero. The graph then sharply increases at 4 % v/v followed by steady increases afterwards. By fitting a linear model to the data, FFA content measured by height gave significant R^2 value of 0.96167 compared to area under the curve with only 0.88471. The results are summarized and indicated in Table 3.
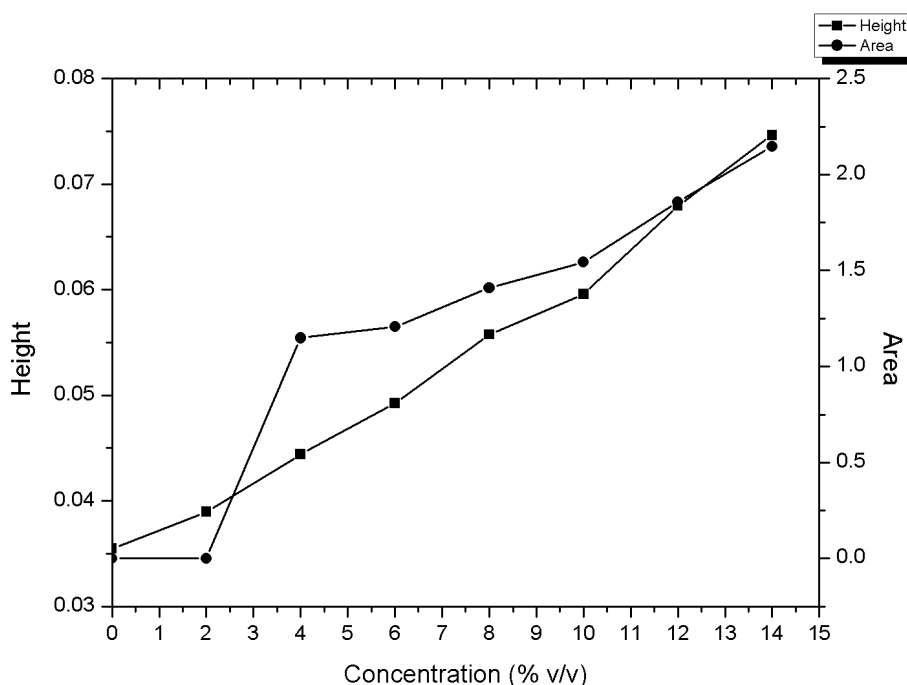

Figure 6. Calibration curve of oleic acid measured using FTIR spectroscopy.

Table 3. Linear models for height and area of the calibration curve.

	Intercept		Slope		Statistics
	Value	Standard error	Value	Standard error	R^2
Height	0.03363	9.53386E-4	0.00280	1.13951E-4	0.98856
Area under the curve	0.09329	0.17303	0.15297	0.02068	0.88471

3.3. Calibration model
A calibration model was constructed via linear regression model based on the results from AOCS titration method and FTIR spectroscopy as shown in *Figure 7*. R^2 value of 0.979 indicates the results were significant.

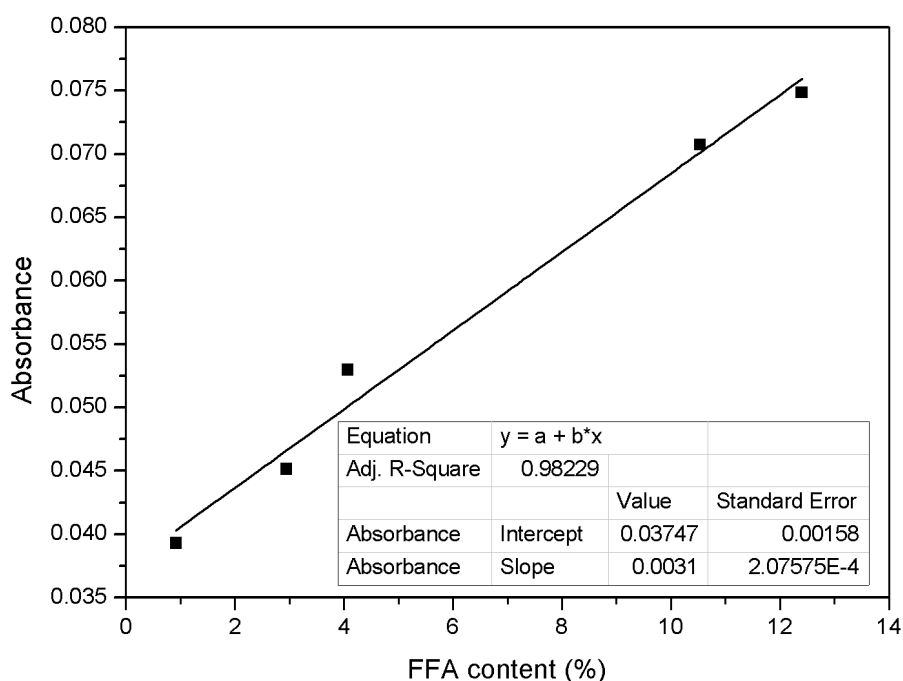| Equation	y = a + b*x		
Adj. R-Square	0.98229		
		Value	Standard Error
Absorbance	Intercept	0.03747	0.00158
Absorbance	Slope	0.0031	2.07575E-4

Figure 7. Linear regression model constructed based on AOCS titration and FTIR spectroscopy results.

These were similar to other studies of AOCS titration method and FTIR spectroscopy whereby a calibration using partial least square (PLS) regression in determining FFA in CPO and RBD palm olein had been found significant with both R^2 values of 0.998 [11]. A calibration model of FFA determination in palm olein via PLS was also comparable with R^2 of 0.997 [12]. Ordinary least square (OLS) regression was applied to determine the quality of palm oil used for frying and the result was significant with R^2 of 0.955 [13]. Other study on quality of frying oil concluded the suitable of FTIR spectroscopy as a substitution for titration method via multivariate calibration [14].

4. Conclusion
From the linear regression model, R^2 of 0.979 indicates that FTIR spectroscopy is a reliable method to measure the FFA content in the extracted palm oil. This method is practical to be implemented at palm oil mills due to its rapid measurement, less chemical consumption, accuracy and repeatability.

Acknowledgements

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