

Palm Oil Quality Determination During Fish Frying by Fourier Transform Infra Red Spectroscopy

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ABSTRACT

Determination of frying oil quality by Fourier transform infra red spectroscopy (FTIR) is rarely studied. FTIR profiles of frying oil during short term of fish frying and their free fatty acid (FFA) and peroxide values were investigated, and their relationship using multivariate statistics was determined to confirm the oil quality. Two batches of commercial palm oil were used to fry cat fishes for nine times. Each frying was done at 185 °C for 15 min. Samples were analyzed FFA and peroxide value by standard AOAC method. FTIR spectra were collected in wavenumber 400-4000 cm⁻¹ by using 32 scans and at resolution of 1.9 cm⁻¹. This analysis was done with XLSTAT 2011 software from Microsoft Excel Series. The tools used is OLS (Ordinary Least Square) analysis. The main peak positions observed in FTIR spectra mentioned the oil functional groups such as alkyl, ester, single and double bonds of carbon atoms. According to FFA and peroxide values, the worse oil quality showed the higher values. The correlation between absorbance values of peaks in FTIR spectra and FFA values exhibited a significant correlation ($R^2 = 0.955$) at $P_{value} 0.042$ obtained by multivariate analysis, whilst the correlation with peroxide values showed a

significance at $R^2 = 0.963$ with $P_{value} 0.030$. Based on these results, FTIR spectroscopy can be used to analyze the quality of palm oil after frying.

Keywords: FFA value, FTIR spectroscopy, Multivariate analysis Palm oil, Peroxide value,

INTRODUCTION

Palm oil is used as frying oil widely in Indonesia and other Southeast Asian countries. This oil has a lot of advantages compared to other oils such as higher oxidative stability, due to its high percentage (50%) of unsaturated fatty acid (Basiron, 2005). There were several parameters used for determining the quality of frying oil. Some conventional methods such as measuring free fatty acid (FFA) value and peroxide value (PV) frequently used for the determination. However, recently an instrumental method such as FTIR (Fourier Transform Infra Red) Spectroscopy has become popular in the study of edible oils and fats (Guilen and Cabo, 2000). Some researchers have used this FTIR spectroscopy to detect the adulteration in food such as Safar et al (1994) in butter, Lai et al (1994) in vegetable oils, Jaswir et al (2003) in cow and animal fat, Che Man et al (2003) in chocolate product, Vlachos et al (2006) in virgin olive oil, Rohman and Che Man (2010) in virgin coconut oil, and Rohman et al (2010) in meat ball. The other researchers have used this instrument to determine the amount of certain chemical compound such as short chain fatty acid in swiss chees (Koca et al, 2007), marine fatty acids in lard (Flatten et al, 2004), free fatty acids (FFA) in palm oil (Che Man and Setyowaty, 1998), and peroxide value (PV) in canola oil (Li et al, 2000). While the others have used it to monitor the change of chemical compound composition during processing such as the emergence of trans isomer fats and unsaturated fat grade in oils and fats during thermal oxidation

process (Moreno et al, 1998) and also the change of FFA, viscosity, and total polar compounds during frying in palm and soy bean oil (Al- Degs et al, 2011).

FTIR spectroscopy can identify molecular structures through large informations contained and assigne certain absorption bands, in which most of the peaks and shoulders in oils are attributed to the specific functional groups (Bendini et al, 2007). Moreover, it ensures the rapid measurement which take less than 5 minutes of labour per sample (Flatten et al, 2004). Researchers have used the multivariate analysis such as PLS and PCA for FTIR data correlated to those obtained from conventional analytical results such as oil viscosity, total polar compound, FFA value, and PV to assess the quality of frying oil. This analysis has been applied to various food samples during frying, including french fries (Kalogianni et al 2011), falafel (Al-Degs et al 2011), and *Catla catla* fish (Manral et al 2008). This research aims to examine the quality profile of palm oil during cat fish frying (*Clarias gariepinus*) by FTIR. The oil quality was correlated to their corresponding FFA value and PV using multivariate analysis.

MATERIALS AND METHODS

Frying Experiments

Two batches of palm oil from the same commercial palm oil were used as the samples. These samples are then subjected to cat fishes frying experiment. The cat fishes were marinated for 30 minutes by a mix of spices (10% w/w of fresh cat fishes) consisted of 25% spices blend from Dramaga market, 25% salt, and 50% vinegar 7.5%. marinated cat fish using deep fat frying method. Each frying requires 1 kilogram of cat fishes and 10 litres of palm frying oil. The frying was conducted for nine times with the substitution of cat fish and without any addition or disposal of

palm frying oil. The temperature is set to 180°C and each frying was done for 15 minutes. The interval time between frying is 15 minutes. The oils from 1st, 3rd, 5th, 7th, and 9th) were then taken as samples. The fresh oil before frying was used as a control sample. All of the samples were frozen prior to the analysis.

Each frozen samples were divided into two parts. The thawed frozen samples were then analyzed titrimetrically to determine their FFA value and PV. While those for spectrometric analysis, the thawed sample should be centrifuged at 3000 rpm for 20 minutes in order to precipitate the residues prior to analysis. The residues were then discarded.

FFA value analysis

The FFA value analysis was performed using AOCS Ca 5a-40 method

PV Analysis

The PV analysis was performed using AOCS Cd 8-53 method

FTIR spectroscopy analysis (Al-Degs et al 2011)

The FTIR instrument used was IR-Prestige 21 from Shimadzu Corporation, Japan. This instrument was equipped with high sensitivity DLAGTS detector, KBr disk, and IR solution software for data interpretation.

The disk oil samples were prepared as follows: The oil sample is dropped on the KBr disk and then closed with another KBr disk so that they formed KBr disk sandwich. The sample is then measured with wavenumber between 400-4000 cm^{-1} at resolution 1.9. Before the measurement, the KBr disk should be cleaned and rinsed with pure n-hexane (PA) and then dried with lens tissue to ensure that no residues were left.

Multivariate statistical analysis

Prior to the multivariate analysis calculation, the data of absorbance intensity obtained from FTIR measurement should be converted to percentage of absorbance intensity with this equation:

$$\% IA_x = \frac{IA_x}{IA_{total}}$$

Where: %IA_x = Percentage of absorbance intensity at certain wavenumber

IA_x = Absorbance intensity at certain wavenumber

IA total = Total of absorbance intensity

This analysis was done with XLSTAT 2011 software from Microsoft Excel Series. The tools used is OLS (Ordinary Least Square) analysis.

RESULT AND DISCUSSION

FTIR profile

The FTIR absorbance spectrum is measured through 24 samples within the wavenumber between 400-4000 cm⁻¹. This region is similar with the region chosen by other researchers such as Vlachos et al (2006) and Al Degs et al (2011). The FTIR absorbance spectrum is shown on the Figure 3. From the Figure 3, certain wavenumbers contribute to specific functional group. Referring to Guillen dan Cabo (1997), Al-Degs *et al*(2011), Proctor *et al* (1996), Lerma- Garcia *et al* (2011), Guillen and Cabo (2002), and Navarra *et al* (2010), the FTIR peaks and wavenumbers which are shown here indicate the functional groups of alkene double bond (-C=C-) both cis and trans, C=O ester, CH methyl, and secondary oxidation compound.

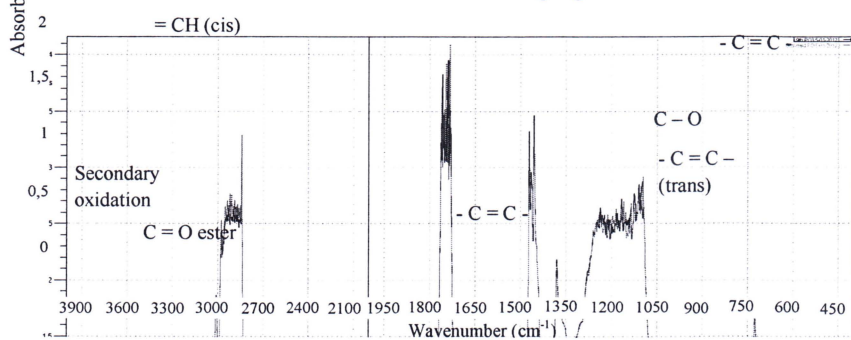


Table 2 Comparison of IR Absorbance Spectra Profile of the frying oils

Researcher	Type of Frying Oil	Wavenumber	Functional Group
Che Man and Selyowaty (1998)	Palm Oil	3550	OH
		3473	Triglyceride ester
		3006	C = C
		2900 - 2.800; 1465; and 1377	CH ₃ and CH ₂
		2677	ester C = O
Vlachos et al (2006)	Olive Oil	723	C = O
		3009; 2925; 2854; 1377; 723	C = C
		2962; 2872; and 1654	CH ₃
		1746	ester C = O
		1700	Free fatty acid
		1465	CH ₃ and CH ₂
		1418 and 1397	cis C = C
1238 and 1163	C = O in ester		
Mossoba et al (2007)	Hydrogenated Soy Bean Oil	966	trans C = C
Rohman A et al (2010)	Virgin coconut oil	2954 and 1377	CH ₃
		2924; 2852; and 1465	CH ₂
		1.743	ester C = O
		1417 and 721	cis C = C
		1228 and 1155	C - O
		962	trans C = C
		872	C = C
Al Degs et al (2011)	Palm oil	3491.2	OH in carboxylic
		3005.1; 2974.1; 2837.1; 1452.4; 1379.1; and 1234.6	C - H
		1762.9 and 1753.2; 1192.2 and 1118.7	C - O and C=O
		721.4	C = O

Hocevar et al (2011)	Soy bean oil, palm oil and hydrogenated oil	2915 and 2845 1741 1154	C – H C = O in ester C – O and CH ₂ cis C = C
Kahfi J (2012)	Palm oil	722; 872; 912.5; 1650; 1402; 1418; and 3005.54 966 1032; 1091; 1130; and 1729 2974 3474.91 3536	trans C = C C – O in ester C – H C = O in ester Secondary oxidation product (alcohol, aldehyde, keton)

From the Table 2, it can be seen that there is a slight difference of frying oil's wavenumber obtained by each researcher. This is due to the difference of frying oil sample used. However, some detected functional groups are still in a common since those are the main functional groups found on vegetable oil such as ester, aldehyde, keton, carboxylic acid, and unsaturated hydrocarbon.

Wavenumbers of 722cm^{-1} , 872cm^{-1} , 912.5cm^{-1} , 966cm^{-1} , 1654cm^{-1} , 1418cm^{-1} , 1402cm^{-1} and $3005,54\text{cm}^{-1}$ contribute to the double bond of alkene (-C=C-) (Guillen and Cabo, 1997 and Lerma-Garcia *et al*, 2011). Alkene is a common functional group found on vegetable oil which has a lot of unsaturated fatty acids composition. Therefore, Moreno et al (1998) have used the wavenumber around 3006cm^{-1} to determine the saturation level on vegetable oil.

Furthermore, the occurrence of wavenumber 966cm^{-1} shows the existence of trans fatty acid on the frying oil. This can be caused by the heating process during frying. According to Sartika (2007), the frying process can turn the cis unsaturated fatty acid into trans unsaturated fatty acid. This increasing number of trans fatty acid is in accordance with the decreasing number of cis fatty acid (oleic fatty acid). The

oxidation of oleic fatty acid (C 18:1 cis) will turn it into trans elaidic fatty acid. While the oxidation of linoleic fatty acid (C 18:2) will produce a mixture conjugation between 9- and 13- hydroperoxy diene which undergoes geometric isomerization to form its isomeric trans which is linoleidic trans fatty acid (C 18:2 trans).

The wavenumbers 1032 cm^{-1} , 1091 cm^{-1} , 1130 cm^{-1} , and 1729 cm^{-1} contribute to the interaction of C – O in ester (Guillen and Cabo, 1997; Lerma-Garcia et al, 2011; and Proctor et al, 1996). The ester bond indicates that the fatty acid is still inbound with the ester. This functional group is mostly found in monoglyceride, diglyceride, and triglyceride. Palm oil is consisted mostly of triglyceride while the mono- and diglyceride only exists within small number (Basiron, 2005). According to Sundram (2004), around 95% of palm oil is consisted of triglyceride. About 7-10% of its total triglyceride is consisted of all saturated triglyceride, mostly palmitic fatty acid. About 6-12% is consisted of all unsaturated triglyceride. Generally, the Sn-2 position of triglyceride is filled with unsaturated fatty acid. More than 85% of unsaturated fatty acid forms ester bond with glycerol on that position.

The wavenumber 2974 cm^{-1} contributes to the C-H bond (Al-Degs et al, 2011). This bond is mostly found on hydrocarbon. The wavenumber 3474.91 cm^{-1} shows the interaction of C = O ester (Guillen and Cabo, 1997). This ester indicates the presence of triglyceride. Lastly, the detection of wavenumber 3536 cm^{-1} indicates the presence of secondary oxidation compound such as alcohol, aldehyde, and ketone (Guillen and Cabo, 2002 and Navarra et al 2010).

FFA Value Profile and Correlation with FTIR Profile



Kalapathy and Proctor <i>et al</i> (1996)	Soy bean oil	10	0.66	0.66	0.81	0.80	0.01	Decreasing
Kahfi (2012)	Palm oil	15	2.25	0.25	0.57	0.21	0.36	Decreasing

This different result can be caused due to the difference time of frying sample. From the Table 1, Tyagi and Vasishta (1996) and Abdulkarim *et al* (2007) used long frying time. Tyagi and Vasishta (1996) used 6 hours interval time for each sampling with total frying time 70 hours. While Abdulkarim *et al* did the sampling every 6 hours and fried with total time 30 hours. In contrast, this research has used much less sampling and frying time. Sampling interval is done for every 15 minutes with total frying time 2.25 hours. Consequently, this different frying time produces different FFA value trends.

Other researchers such as Kress-Rolgers *et al* (1990), Manral *et al* (2008), and Kalapathy and Proctor *et al* (1996) also have shown that the FFA value has decreasing trend during frying. Kress-Rolgers *et al* (1990) used interval sampling time 4 minutes each with total frying time 13.5 hours and did first sampling after 30 minutes. Manral *et al* (2008) fried the sample with total 14 hours, interval frying time 6 minutes, and took the first sample after 2 hours. While Kalapathy and Proctor *et al* (1996) did their first sampling after 40 minutes of frying. These three researches, which use shorter frying time and shorter sampling interval time, have shown a decreasing trend of FFA value.

The decreasing of FFA value is caused by the oxidation reaction of the free fatty acid. According to Ketaren (1986), the forming of peroxide can help the oxidation of small number free fatty acid. This reaction occurs by the interaction between free fatty acid and oxygen which is triggered by the high heat during frying. The oxidation reaction on this free fatty acid is faster than glycerol bonded fatty acid (Velasco *et al*,

0 (control)

Figure 1. Plot of FFA value profile during frying

The FFA profile obtained during the short term frying process has showed a decreasing trend. This result seems in contrast with other results from several researchers. Abdulkarim et al (2007) and Tyagi and Vasishta (1996) have found that the FFA value is increased along with the duration of frying oil. According to Lalas (2009), the FFA value tends to increase in accordance with the increasing frying time. Theoretically, the existing water in the food sample will react with triglyceride in the oil and cause the hydrolytic reaction. This reaction will break the ester bond on the triglyceride and produce free fatty acid and glycerol.

Table 1. Comparison of FFA value during frying from different researches

Researcher	Type of Frying Oil	Time per Frying (minute)	Total Frying Time (hour)	Time of First Sample Taken (hour)	FFA value of control sample (% oleic acid)	FFA value of first sample (% oleic acid)	Interval (% oleic acid)	Trend
Tyagi and Vasishta (1996)	Vanashpati oil	30	70	6.00	0.12	0.25	0.13	Increasing
Abdulkarim et al (2007)	<i>Moringa olifeira</i> oil	3	30	6.00	0.19	0.25	0.06	Increasing
Manral et al (2008)	Sunflower oil	6	14	2.00	0.50	0.10	0.40	Decreasing
Kress-Rogers et al (1990)	Partially hydrogenated vegetable oil	4	13.5	0.50	0.20	0.10	0.10	Decreasing

2009). During the early phase of frying, the rate of this oxidation reaction is faster than that of hydrolytic reaction. Therefore, the FFA value shows decreasing trend on the first stage of frying.

Palm frying oil contains saturated fatty acid such as oleic acid (C 18:1) and linoleic acid (C 18:2). The oleic acid composition reaches 38.7% and the linoleic acid composition reaches 10.5% of the total fatty acid (Rival, 2010). According to this, as much as 49.2% of palm oil's total fatty acid is consisted of unsaturated fatty acid which is vulnerable to oxidation reaction. Moreover, the oxidized free fatty acid may undergo another series of reaction. The oxidized fatty acid can make a bond with protein function and produce carboxyl compound. This carboxyl compound is an insoluble macromolecule which is hard to be detected (Pokorny, 1999).

The Ordinary Least Square (OLS) method is used to find the correlation between spectrum profile and the FFA value. The selection of wavenumbers that highly correlated with FFA value is based on the fittest model with high degree of R^2 and the most significant P number ($P > F$) in the ANNOVA. The 14 wavenumbers that meet this criterias are 722 cm^{-1} , 872 cm^{-1} , 912.5 cm^{-1} , 1032 cm^{-1} , 1091 cm^{-1} , 1130 cm^{-1} , 1400.5 cm^{-1} , 1418 cm^{-1} , 1654 cm^{-1} , 1729 cm^{-1} , 2974.36 cm^{-1} , 3005.54 cm^{-1} , 3474.91 cm^{-1} , and 3530 cm^{-1} . These wavenumbers are related with functional group alkene and esther. Alkene can be found in unsaturated free fatty acid while esther is correlated inversely with free fatty acid since it indicates the glycerol bonded free fatty acid. The more esther means lesser free fatty acid and vice versa.

Several previous researches have used different wavenumbers. Al-Degs et al (2011) has used wavenumbers between $1109.1\text{-}1240.2\text{ cm}^{-1}$, $1703.1\text{-}1724.4\text{ cm}^{-1}$, 1749.4 cm^{-1} , and 2837.3 cm^{-1} . While Che Man and Setyowaty (1998) have used wavenumbers between $1662\text{-}1728\text{ cm}^{-1}$ and Lanser et al (1991) have used

wavenumbers between 1600-2000 cm^{-1} . The difference of wavenumbers is due to the difference of frying oil sampling on each research. Al Degs (2011) has taken the sample for every 3 days of frying. While Che Man and Setyowaty (1998) have used oleic fatty acid standard.

From the calculation of OLS with mutivariate analysis, the correlation between wavenumber's percent of absorbance and FFA value is shown on this formula : FFA value = $-161.34 + 0.74x(\%A_{722 \text{ cm}^{-1}}) + 7.02x(\%A_{872 \text{ cm}^{-1}}) + 3.50x(\%A_{912.5 \text{ cm}^{-1}}) + 1.11x(\%A_{1091 \text{ cm}^{-1}}) + 2.30x(\%A_{1130 \text{ cm}^{-1}}) + 1.44x(\%A_{1400.5 \text{ cm}^{-1}}) + 1.49x(\%A_{1418 \text{ cm}^{-1}}) + 1.01x(\%A_{1654 \text{ cm}^{-1}}) + 1.59x(\%A_{1729 \text{ cm}^{-1}}) + 1.9x(\%A_{2974.36 \text{ cm}^{-1}}) + 1.09x(\%A_{3005.54 \text{ cm}^{-1}}) + 4.25x(\%A_{3474.91 \text{ cm}^{-1}}) - 2.14x(\%A_{3530 \text{ cm}^{-1}})$. This formula has coefficient of correlation (R^2) 0.955 point and P number ($\text{Pr}>\text{F}$) 0.042 point at significance level 95%. P number which is less than 0.05 shows that there is a significance relationship between the variable of wavenumber's percent of absorbance and FFA value. This formula is can only be applied under the condition of frying 10 litres of palm oil with 1 kilogram of cat fish for each frying under temperature 180° C and maximum frying time 2.25 hours. The plot of OLS predicted FFA value and actual FFA value is shown on Figure 4.

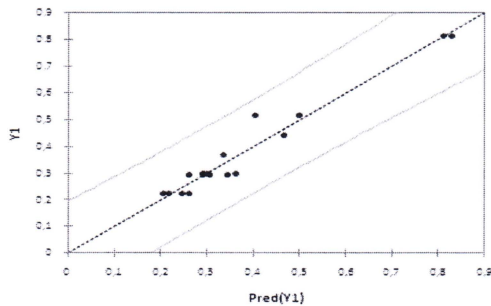


Figure 4. Plot of OLS predicted FFA value with actual FFA value

Peroxide Value Profile and Correlation with FTIR Profile



Sample 1

Sample 2

0 (control)

Figure 2. Plot of peroxide value during frying

From the figure 2, it can be seen that the trend of peroxide value is increasing on the first frying and declining after reaching its maximal value. This trend of peroxide value profile during frying meets the basic theory on the literature. According to Chatzilazarou et al (2006) and Tsaknis et al (1998), the peroxide value will be increased during the first stage of frying. Later on, this number will gradually decreased at prolonged frying at 180°C due to the decomposition of peroxide into secondary oxidation compound.

The Ordinary Least Square (OLS) method is also used to find the correlation between spectrum profile and the peroxide value. The selection of wavenumbers that highly correlated with FFA value is based on the same criterias like in the FFA value. The 13 wavenumbers that meet the criterias are 722 cm⁻¹, 872 cm⁻¹, 912.5 cm⁻¹, 966 cm⁻¹, 1091 cm⁻¹, 1130 cm⁻¹, 1400.5 cm⁻¹, 1418 cm⁻¹, 1654 cm⁻¹, 1729 cm⁻¹, 2974.36 cm⁻¹, 3005.54 cm⁻¹, and 3474.91 cm⁻¹. These wavenumbers belong to carbonyl and alkene functional group which are mostly affected by oxidation reaction. According to Lerma-Garcia et al (2011), the functional group of = C – H (trans and cis), ester C –

O, and double bond =CH₂ have been detected by FTIR spectroscopy measurement and these groups are vulnerable to oxidation reaction.

On the previous researches, Russin et al (2004) correlated wavenumbers and peroxide value using region 3444 cm⁻¹, 2854 cm⁻¹, 1100-1270 cm⁻¹, and 460-660 cm⁻¹. Guillen and Cabo (2002) used wavenumbers 3470 cm⁻¹, 3006 cm⁻¹, 1238 cm⁻¹, 1746 cm⁻¹, 1728 cm⁻¹, 1163 cm⁻¹, and 1118 cm⁻¹. The difference of the wavenumbers chosen is caused by the difference of treatment and sample used. Russin et al (2004) used mixture oil of canola, sunflower, and VCO. Guillen and Cabo (2002) used sunflower oil.

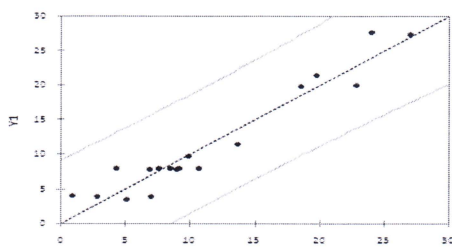


Figure 5. Plot of OLS predicted peroxide value with actual peroxide value

Figure 5 shows the plot of OLS predicted peroxide value and actual peroxide value. From the calculation of OLS with multivariate analysis, the correlation between wavenumber's percent of absorbance and peroxide value is shown on this formula:

$$\begin{aligned} \text{Peroxide Value} = & 3284.74 - 37.89x(\%A_{722 \text{ cm}^{-1}}) + 136.08x(\%A_{872 \text{ cm}^{-1}}) - \\ & 62.67x(\%A_{912.5 \text{ cm}^{-1}}) - 183.24x(\%A_{966 \text{ cm}^{-1}}) - 17.61x(\%A_{1091 \text{ cm}^{-1}}) - \\ & 32.70x(\%A_{1130 \text{ cm}^{-1}}) - 27.97x(\%A_{1400.5 \text{ cm}^{-1}}) - 53.34x(\%A_{1418 \text{ cm}^{-1}}) + \\ & 30.66x(\%A_{1654 \text{ cm}^{-1}}) - 30.22x(\%A_{1729 \text{ cm}^{-1}}) - 40.09x(\%A_{2974.36 \text{ cm}^{-1}}) - \end{aligned}$$

$33.56 \times (\%A_{3005.54 \text{ cm}^{-1}}) - 111.92 \times (\%A_{3474.91 \text{ cm}^{-1}})$. This formula has coefficient of correlation (R^2) 0.963 point and P number ($Pr > F$) 0.030 point at significance level 95%. P number which is less than 0.05 shows that there is a significance relationship between the variable of wavenumber's percent of absorbance and peroxide value.

CONCLUSION

The measurement of spectrum profile by FTIR spectroscopy shows that the FTIR wavenumbers and profiles of palm oil indicate the presence of palm oil's functional group such as alkyl, ester, saturated and unsaturated fatty acid. Quality profile of palm frying oil is determined by the frequency and the duration of frying. On the early hours of frying, the FFA value shows decreasing trend while the peroxide value shows increasing and then followed by decreasing trend after it has reached its peak.

From the OLS calculation with multivariate of analysis, the correlation formula of wavenumber's percent of absorbance and FFA value has coefficient of correlation (R^2) 0.955 point and P number ($Pr > F$) 0.042 point at significance level 95%. The correlation formula of wavenumber's percent of absorbance and peroxide value has coefficient of correlation (R^2) 0.963 point and P number ($Pr > F$) 0.030 point at significance level 95%. P number which is less than 0.05 shows that there is a significance relationship between the variable of wavenumber's percent of absorbance and peroxide value. This study have suggested that instrument FTIR spectroscopy can be used to determine the quality of palm oil with respect to the FFA value and PV.

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