A review of free fatty acid determination methods for palm cooking oil

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Abstract. Free fatty acids (FFA) is one of the main content of palm cooking oil. High percentage of FFA in palm oil can affect human health and the quality of palm oil. Based on quality standards specification set by Malaysia Palm Oil Board (MPOB), the allowable content of free fatty acid (FFA) for crude palm oil (CPO) must not exceed 5%, meanwhile less than 0.1% of FFA in Refined Bleached Deodorized Oil (RBDO). There are many researches have been carried out to determine the level of FFA. However, they are using different type of cooking oil. A few researches are also reported regarding the palm cooking oil but they are using different method such as titration, spectroscopic, gas chromatography, reflection method and other type of detectors. All methods to determine the amount of free fatty acids (FFA) in palm cooking oil are reviewed in this paper.

Keywords— optical sensor, crude palm oil, fatty acids, FFA, titration, gas chromatography

1. Introduction

South East Asia and Africa is the countries that widely produce palm oil. As of now, land in Malaysia cover almost 4.49 million hectares of oil palm. With that size of land, Malaysia can produce more than 17 million tonnes of palm oil and 2 tonnes of kernel palm oil. Malaysia also one of the countries that produce and export palm oil in the world. Where it produced and export 11% and 27% respectively of the world's oils & fats. There are two types of oil that can be produce from palm oil which is crude palm oil and kernel palm oil. The mesocarp or fruit flesh is use to produce crude palm oil and the seed or kernel use to generate palm kernel oil.

Palm oil is the great choice as a frying oil because it can withstand to oxidation and heat at higher temperatures. In fact, it has been used as a replacement for other type of oils such as canola oil, olive oil and soybean oil. The quality of palm oil and crude palm oil is measured based on fatty acids, phosphatides, odoriferous matter, water and impurities contents. The fatty acids composition of palm oil is mainly Myristic C14, Palmitic C16, Stearic C18, Oleic C18:1, and Linoleic C18:2. Details of the concentration can refer to Fig. 1. The amount of free fatty acids or acid value (AV) will affect the oil quality. Oil with high content of free fatty acid will have a poor quality of oil.
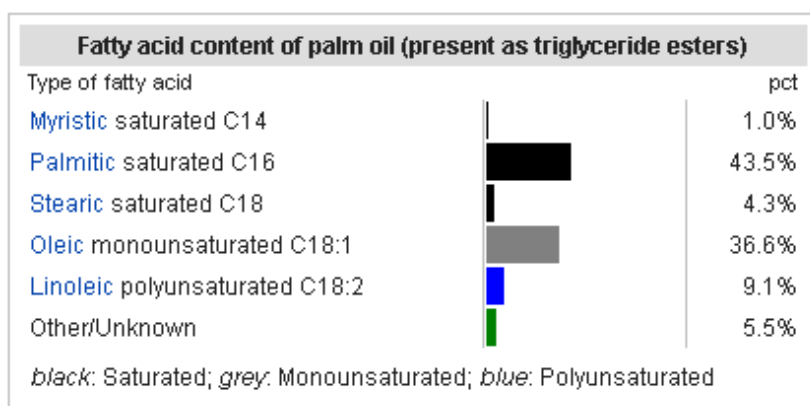
Figure 1: Fatty acid content of palm oil

Based on quality standards specification set by Malaysia Palm Oil Board (MPOB), the allowable content of free fatty acid (FFA) CPO must not exceed 5%, meanwhile less 0.1% of FFA in RBDO. Due to lower ability of palm oil on enzymeic and microbial lipase reactions will produce the high level of FFAs in CPO. The oil quality also can be affected if the CPO store in high temperature and high moisture.

Basically, hydrolysis process can produce free fatty acids (FFA) in palm cooking oil during the palm oil processing [1]. Other than that, FFA also can be released naturally in CPO by microbial lipases process and also during storage when the oil response with eater [2].

The techniques to measure the amount of free fatty acid in other oils is similar with the process used in palm cooking oil. However, the methods applied previously is time consuming and required a lot of manual operation. There are various methods used for FFA determination in palm oils by using chemical equipment which is titration, high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and Gas Chromatography (GC) analysis. Extraction of palm cooking oil by using analytical separation such as liquid-liquid extraction (LLE) technique, or solid phase extraction [3]. All methods mentioned have been discovered due to their advantages and disadvantages.

2. Colorimetric Methods

Colorimetric analysis is a method of determined the concentration of chemical compound in a solution by using a colour reagent. The concentration of an acid or based solution is determined by neutralized the unknown acid or base concentration with the solution that known the concentration. At the end of the titration, the colour or pH changes indicate the neutralization reaction has occurred. The equipment that required in this analysis is a colorimeter, cuvettes and a suitable reagent. Acid-based titration analysis and flow injection analysis (FIA) are two colorimetric methods that commonly used in determining free fatty acids (FFA).

3. Titration Analysis

Acid-base titration method is where the oil titrated hot 2-propanol solution against potassium hydroxide in. In this paper, phenolphthalein solution is used as a colour changes indicator [4]. The value of neutralized acid obtained in the solution represents the sample of acid number whereas the acid number is proportionate to the free fatty acid (FFA) content [1].

There another research done by using paper-based colorimetric sensing method to inspection fatty acids. The principle of this method, some specific reagents will develop colour and/or hue intensity

relates with the concentration and type of analytes [5]. In the research, they combined the selective binding ability of silver ion (Ag+) to olefinic bonds of fatty acids and Ag+ mediated colour variation of 3,3',5,5', tetramethylbenzidine (TMB) [6]. Ag+-FAs complexes are formed by mixing Ag+ with fatty acid in a solution, and free Ag+ ions will remnant. Then free Ag+ ions oxidized with TMB and blue colour developed. The amount of free Ag+ is based on the type of fatty acids and its concentration. Thus, five types fatty acid model with subtle structure difference successfully identified.

The analysis of different unsaturation degrees and olefinic bonds configuration on five fatty acids including stearic acids (SFAs), oleic acids (mono-CUFAs), linoleic acids (poly-CUFAs), linolenic acids (poly-CUFAs) and elaidic acid (TUFAs) were done (Fig. 2a). Fig. 2b and Fig. 2c is the photo result of Ag+-TMB identifying towards the five type of FAs above and the corresponding UV-visible absorption spectra recorded using spectrophotometer respectively. Fig. 2d is test papers towards five FAs.
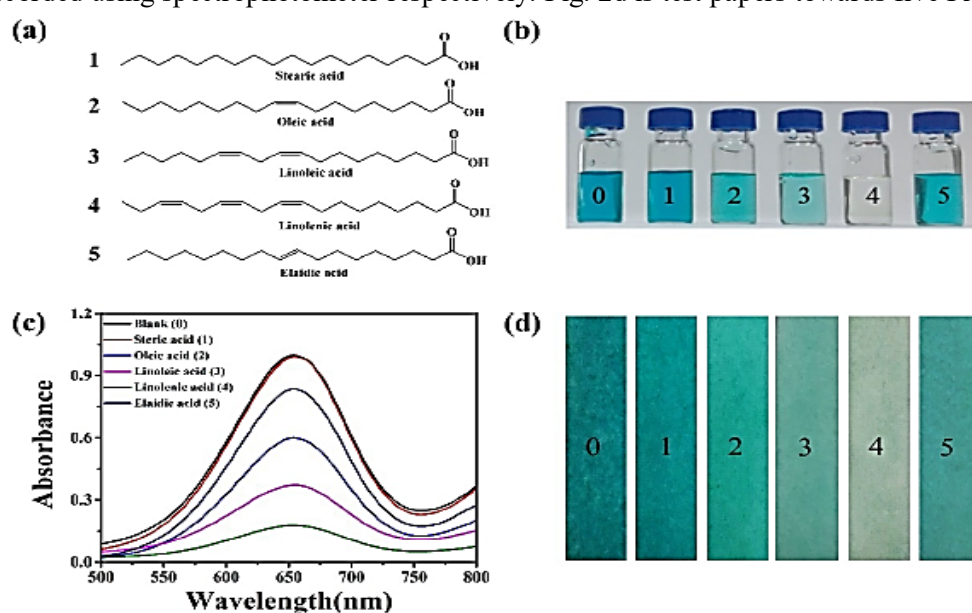

Figure 2. (a) Molecular structure of five FAs; (b) photo of FAs in bottle; (c) absorbance spectra of five FAs; (d) test papers of five FAs. Each numbers 0-5 represent blank, steric acid, oleic acid, linoleic acid, linolenic acid and elaidic acid respectively.

Although titration analysis is simple, analysis at room temperature, facile and economic method [7] but it is sluggish, consume high amount of reagents and solvents and laborious [8]. Furthermore, this method lack of accuracy due to other substance, which can react with KOH, might be neutralize too [1]. Another limitation is, it does not classify the profile of FFAs even though it measures an overall titration acids level [8].

4. Flow Injection Analysis (FIA)

In mid-1970s, flow injection analysis (FIA) is developed and Ruzicka, J et al reported FIA is an efficient method to do automated sample analysis [9]. With this method, fast and sequential analysis can be done on unlimited number of samples. Figure 3 below showed the component and the basic diagram of flow injection analyzer. The reagent is placed in a reservoir which serving as a carrier. Then, a propelling unit keeps the flow of the carrier stream constant through a tubing system. The sample injected straight away into the flowing carrier stream. Then it goes to mixing and reaction zones before it reached the detector.
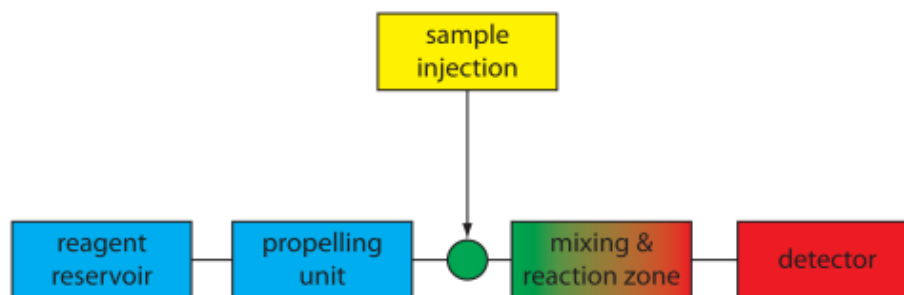
Figure 3. Schematic diagram of a simple flow injection analyser.

Saad et al. [10] developed a simple, low cost and automated FIA for determining FFA in palm oil. In this paper, the single-line manifold technique and two-line manifold FIA methods is applied. The changes of phenolphthalein (PHP and bromothymol blue absorbance when react with FFA is being monitor. In order to determine FFAs in higher concentration and acidity in higher than 0.4, PHP solution is recommended to be used in single-line manifold method. Meanwhile, two-line manifold used BTB solution to determining the lower acidity in palm oil. The sample throughputs observed in this study were 35-74 samples/h for single-line manifolds and 21-46 samples/h for two-line manifolds. This shows that two-line manifold required larger amounts of reagents but resulted in lower sample throughput. However, both methods are good to recover oil sample spike and also have a good sensitivity [10].

The research study by A.S.M Ali et al. [3] determined the FFA in palm oil by using two-line manifold FIA method. The research shows colour change between acidic and basic medium based on the synthesized hydrazone reagent. The synthesized compound which salicyladhyde-2,4-dinitrophenyllhydrazone (SDPH) as an indicator to find FFA in palm oil. The schematic of FIA manifold used in this study as shown in Fig. 4. The oil samples containing FFA injected in the carrier stream whereby 1-propanol solution used as a carrier solution. Carrier solution mixed with titrant solution which is potassium hydroxide and SDPH used as indicator. Then, the sample and carrier stream mixed and reacts in the mixing chamber. As a result, the acidity along the sample zone is increase, and the SDPH colour is changed from red-brownish to yellow. This method consumes lesser amounts of reagent and resulting a good throughput. However, the mixing chamber used in this study is four times larger than chamber used by Saad et al, on his FIA study using PHT as indicator. Thus, two-line manifold technique by using SDPH as indicator is recommend to determine FFA of the sample (> 0.5 a.d.).
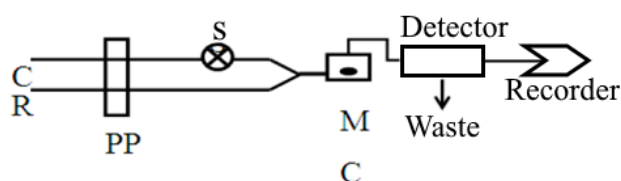

Figure 4. Two-line Flow Injection Analysis (FIA) manifold. C: Carrier Stream; S: Sample Injector; PP: Peristaltic Pump; R: Reagent system; MC: Mixing Chamber; and W: Waste

Makahleh et al. [11] reported a FIA method using C^4D to identify the free fatty acid (FFA) in vegetable oils. Before injected sample into the FIA system, the sample is extracted by using liquid-liquid extraction (LLE) method. Two type of different solvents used as extraction solvent which is sodium methanoate and methanol. These two solvents don't have any different but some samples that contained

high FFAs showed a reaction when extract using sodium methanoate solvent. Makahleh et al. [11] used single-line manifold FIA system combined with C^4D. To develop the best FIA system, the carrier stream conductivity should be low as possible and analytes should be in the form of charged. In this paper, the mixture of Methanol/1.5mM and sodium acetate (pH8) is choosing as a carrier stream. Sodium acetate used in this mixture because of it behaviour that able to ionize all the analytes and provide suitable PH environment. The flow rate of 1mL min^{-1} is use in the carrier stream. The proposed method which used C^4D detector shows better performance compared with standard titration method which used optical detector. In addition, the proposed method offers high sampling rate 40-60 sample/h and consider as low cost automated system that required minimum human involvement. However, a suspected of FA excessive dispersion by the carrier stream led to the pre-concentrator column combination between the injector and the detector.

5. Chromatography Methods

Chromatography is a method to separate a mixture of solution by distribute it components between stationary phase, and mobile phase. Stationary phase is a substance that remained inside the column while the mobile phase carries the solvent being used through the column. The components in the mixture which adhere more to the stationary phase will move slower compare to the component that has weaker adhesion. The factor that makes mobile phase and stationary phase difference is the solubility of certain components phase and the strength of their attractions respectively. For example, in the stationary phase some components mixture stays longer, and move slowly, while rapidly pass through into mobile phase, and faster leaving the system [12].

5.1 Gas Chromatography (GC)

Tsvett Early 1900s, Gas chromatography (GC) is discovered by Mikhail Semenovich as shown in Fig. 5. This method used to do a separation technique to separate compounds [13]. In this GC method, stationary phase is a column that contains substance is located in the device. The gases filled in carrier phase meanwhile mobile phase is an inert gas, which went through a column under the high pressure. The sample that needs to analyse have to be heated to produce gas before it go to gaseous mobile phase.
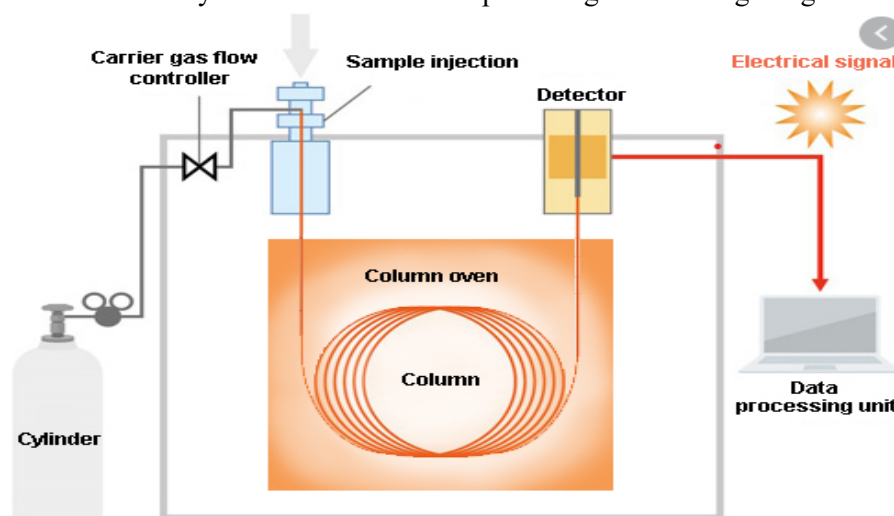

Figure 5. Schematic of the Gas Chromatography

Gas chromatograph analysis commonly used because it is simple, multifaceted and highly sensitive as reported by Ozlem Coskun [14]. Other than that, this method is very convenience, accurate and small budget needed to do an analysis [15]. Normally, GC chromatogram results showed sharp and symmetric peaks. If the stationary phase is being used, the interpreted resolution of GC is highly rely on the column length and polarity.

There are two type of detectors that used to determine FFAs which is Mass spectrometry detector (MS) and flame ionization detector (FID). However, as reported by Azeman et al., flame ionization detector (FID) is one of detector that widely and commonly used as a detector to detect existence of FFA [1]. FID is the most appropriate compared to other detector because of its sensitivity and resolution and because it reliable and relatively easy to use. In addition, various compounds can to be detected by using FID [1]. Meanwhile, mass spectrometry detectors tend to thermally degrade the samples before it can be detected. As the result; the entire sample is destroyed by fragmentation as reported by Scott RPW [16]. The schematics of mass spectrometry and flame ionization detector are shown in Fig.6 and Fig.7 respectively.

There is no issue for GC analysis to deal with volatile samples as compare to the non-volatile sample. Thus, before injected FFA into the gas chromatography column, it has to be esterified.
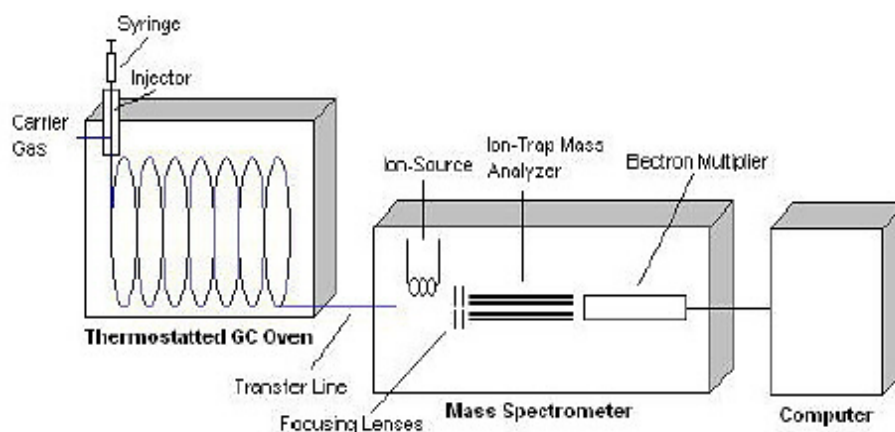

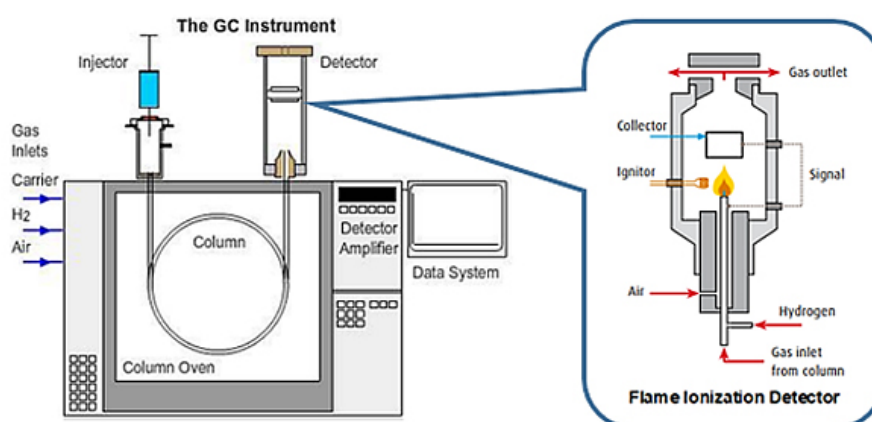**Figure 6.** Schematic of GC-MS systems

Figure 7. Schematic of GC-FID systems

J. Song et al. [15] used GC-FID to analyze trans-fat contents in six vegetables oil and corn oil with cooking process effect including baking, stir and frying. By mixing FA with BF3/MeOH (14% boron triflouride), fatty acids were derivative to fatty acid methyl esters (FAME). FAME Hewlett-Packard 6890 GC with FID is a device that used as a FAME analyser. Fatty acids result of standard mixture and corn oil in Fig. 8 shown corn oil has 0.25g trans-fat/100g but no trans-fat content detected in other type of oils. Trans-fat content in corn oil is increased due to the stir-frying process while other cooking process did not make any changes.

Sample	Fatty acid										Trans fatty acid	
	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	C18:1n9t	C18:2n6t
Corn oil	12.65 ± 0.15[1]	0.11 ± 0.00	2.24 ± 0.02	31.32 ± 0.34	50.28 ± 0.61	0.93 ± 0.02	0.52 ± 0.00	0.23 ± 0.00	0.18 ± 0.14	N.D.[2]	N.D.	0.25 ± 0.01
Rapeseed oil	5.69 ± 0.13	N.D.	2.28 ± 0.01	68.20 ± 0.24	15.38 ± 0.12	7.70 ± 0.08	N.D.	0.75 ± 0.08	N.D.	N.D.	N.D.	N.D.
Soybean oil	16.05 ± 0.02	N.D.	5.59 ± 0.04	27.03 ± 0.01	45.23 ± 0.08	6.09 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Olive oil	13.65 ± 0.01	0.75 ± 0.00	3.38 ± 0.02	78.11 ± 0.05	3.61 ± 0.02	0.49 ± 0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Perilla seed oil	6.87 ± 0.08	0.22 ± 0.00	2.13 ± 0.02	19.71 ± 0.15	10.71 ± 0.14	57.78 ± 0.27	0.16 ± 0.00	0.12 ± 0.00	N.D.	N.D.	N.D.	N.D.
Sesame oil	10.58 ± 0.60	N.D.	6.18 ± 0.39	41.41 ± 2.94	33.83 ± 1.73	N.D.	0.64 ± 0.03	N.D.	N.D.	0.08 ± 0.14	N.D.	N.D.

[1] Mean ± standard deviation (n = 3).
[2] Not detected.

Figure 8. Major fatty acids profiles and trans-fat content in vegetable oil (g/110g)

Fatty acid profiles in deep-frying process free fatty acids (FFA) has been developed by Bazina N. [8] by using gas chromatography (GC) with liquid-liquid extraction (LLE) method. To do separation of FFA from the oil, liquid-liquid extraction (LLE) technique has been used. In Shah and Venkatesan [17] paper, aqueous isopropyl alcohol used as a solvent meanwhile Rodriques and Meirelles [18] used water and ethanol as a solvent. In this research, a mixture of 0.02M phosphate buffer at pH 12 and acetonitrile at solvent: buffer ratio larger than 2:1 was used to extract FFAs b using LLE method. This method shows accurate result on oil samples medium rancidity and capable to produce a precise fatty acid profile. While other methods of FFA extraction used by Paik et al which is solid phase extraction (SPE) [19], and thin layer chromatography (TLC) stated by Samples and Pickova [20] were relatively complicated and expensive.

The research carried out by Bazina N. [8] on FFAs of rapeseed oil extracted by using LLE technique. Observed a positive correlation between different rancidity level of three types of rapeseed oil with increased frying time. The comparison of FFA profiles between medium rancidity rapeseed oil (MRRO), fresh rapeseed oil (FRO), and high rancidity rapeseed oil (HRRO) shows oleic acid concentration in HRRO 74% higher compared to MRRO. GC-LLE method allowed non-FFA attached to glycerol while in extraction process. The other advantages of this technique are cost effective, simple and not time consuming. The drawback of this method is limitation of the instrument because it required larger quantity of FFA. The other disadvantage is the precision of FFA quantification across samples and fatty acids also not consistence.

5.2 High Performance Liquid Chromatography Analysis (HPLC)

HPLC was developed to solve some of the limitations of classic liquid chromatography as a separation method. In order to FFA analysis using this method, the researcher need to consider the size of column packing. Separation could be faster if smaller and narrow columns being used. Due to smaller and narrow column, high pressure is compulsory to force the mobile phase and sample. In addition, by using high pressure in narrow column also can shorten the separation time if compared with previous liquid chromatography method.

The main difference between HPLC and GC is the type of mobile phase used on these two methods. Gas mobile phase used in GC and HPLC is using liquid mobile phase. HPLC has better in selectivity and capable to measure high diversity of analytes [1]. That why it widely used to determine FFA in palm oil and other vegetable oils. Due to high quality equipment required, the HPLC analysis is very expensive and it generates more waste.

In HPLC has two different techniques that is normal phase (NP-HPLC) and reverse phase (RP-HPLC). Usually silica has being used as stationary phase (polar) and for mobile phase (non-polar) usually being hexane or chloroform. Meanwhile in RP-HPLC, bonded silica being used as a stationary phase (non-polar) and mobile phase (polar), usually being water or polar organic solvent as shown in Fig. 9. A.M. Tarola et al come out with a research by using reverse-phase HPLC to determine fatty acid composition in drying oil such as walnut, linseed and poppy seed [21]. This research intended to do derivative of fatty acids by using enzymatic hydrolysis method instead of acid hydrolysis. Highly versatile enzyme like lipase is use because it has been labelled as the cheap and versatile compound.
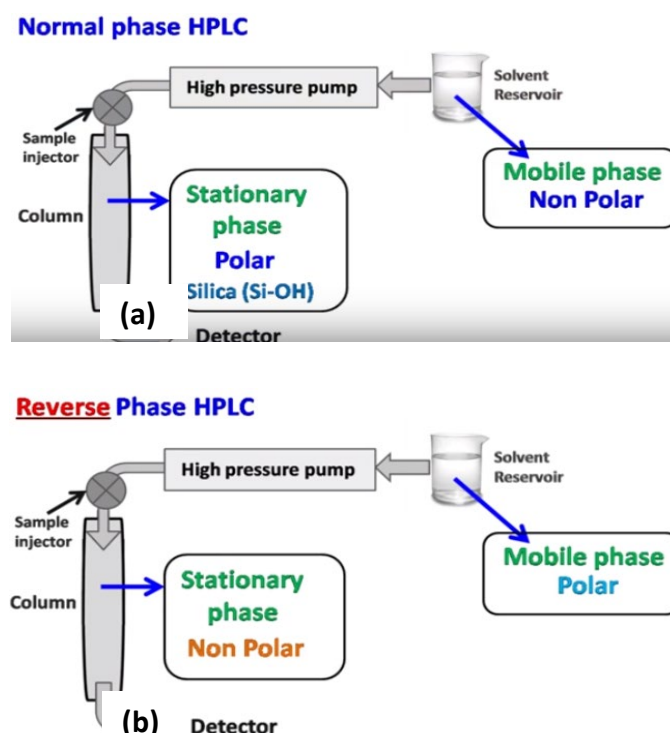

Figure 9. a) Schematic of Normal Phase HPLC and b) Reverse Phase HPLC

As reported by Tarola et al., at temperature below 40°C, this method can completely hydrolysis of fats to fatty acids and glycerols [21]. Correspond to the catalytic activity, the experimental conditions of the hydrolysis reaction are pH7 and 30°C.

The small quantities of samples from 0.1 to 0.5 mg being used in this experiment which helps to solve the issue to identifying the nature of oils due to small quantity of sample. This method also can to reduce utilization of mobile phase and produced analytes separation efficiently. Yet, enzymatic hydrolysis with lipase, denature rapidly at higher temperature.

6. Spectroscopic Analysis

Spectroscopic analysis is an interaction atom or compound with electromagnetic radiation at specific wavelength. Spectroscopy normally used to identify the substance through the spectrum which emitted or absorbed by them.

6.1 Fourier Transform Infrared Spectroscopy Analysis (FTIR)

FTIR is a technique used to obtain an infrared spectrum (frequency range) of absorption or emission of a solid, liquid or gas. FTIR can be used to analyse total composition, including protein, fatty acids, carbohydrates, nucleic acids, lipopolysaccrides. A few studies have been done by using FTIR method to determine free fatty acids in palm oils and other types of vegetable oils. [1], [22] & [6].

Che Man et al. [23] did a research on bleached palm oil (BPO), distilled BPO and refluxed BPO free fatty acids whereby those oils are from crude palm oil. The researched to determine FFA was done by using FTIR spectroscopy method. This researched objective is to develop a technique which is fast, efficient and precise method to determine the FFA content. The FFA content of palm oil samples were predicted by using partial least square (PLS) model. It used spectral range from 1,729.89-1,694.48/cm. The results obtained from the PLS calibration were correlated with the value found from the official American Oil Chemist's Society (AOCS) method. The results for PLS calibrations were linear and the cross-validation root mean standard error was 0.063 with the correlation coefficient (R^2) of 0.9898. This showing that the determination of FFA using FTIR method is successful.

In other study, Faridah D N et al [24] performed an analysis to determine free fatty acid (FFA) and peroxide value (PV) in palm oil frying activities using FTIR method. Two batches of commercial palm oil were repeatedly used for nine times to fry fish at 180°C for 15min. FTIR spectra collected at wavelength range 400-40000 cm^{-1} by using 32 scans at resolution of 1.9 cm^{-1}. The correlation result between FTIR and the conventional analytical was performed using Ordinary Least Square (OLS) multivariate analysis with XLSTAT 2011 software. The FFA profile obtained in this study showed a decreasing trend which contrast with previous researchers results whereby the FFA value increased in accordance with the increasing frying time. The decrease of FFA value as shown in this study due to short frying time and also oxidation reaction of the FFA. From the calculation of OLS with multivariate analysis, the coefficient of correlation (R) is 0.9777 and P number (Pr>F) of 0.042 at significant level 95%. The P number less than 0.05 shows there is a significant relationship between the percentage of absorbance of main wavenumber and FFA value. There is a correlation between the analysis of palm oil by using FTIR and conventional methods. Therefore, the formula derived from this study can used as a rapid analysis the quality palm oil from a food processing.

6.2 Near Infrared Spectroscopy Analysis

Near Infra-Red (FT-NIR) technology offers many advantages over classical wet-chemical and chromatographic analysis. It is safe, quick, and cost effective since no hazardous chemicals are used. One of the parameters can be analysed using this method is free fatty acids (FFA).

An assessment of FT-NIR capability is prepared by N.H.H.A, Bakar et al in-screening crude palm oil genuine and quality [25]. In this study, the palm oil needs to replicate the effect of frying oil same as in food industry. As of that, a few of oil samples were fried with beef, fish and mutton. Then, the frying oils mixed with CPO and analyse with infrared spectrum. Near infrared spectrometer with heated transmission accessory was used to obtain spectra of the oil samples in the wavenumber range of 10000 cm^{-1} to 4000 cm^{-1}. The heated transmission accessory was used to maintain the samples temperature at 40°C because once the temperature drop, it will affect the spectrum obtained. The spectrum obtained in absorbance mode at a resolution of 4 cm^{-1} after 32 scans.

From the spectra result shown in Fig. 10, the absorption band around 5330 cm^{-1} to 5303 cm^{-1} is attributed to the C=O stretching second overtone. Since the spectra had an extensive overlapping of bands, chemometric analysis is an integral part which ensures that the spectroscopic data makes sense due to its power to detect the slightest differences in each sample spectrum obtained by the NIR instrument. These rapid screening techniques, which is near infrared spectroscopy have been proven to be useful for the authentication of edible palm oil.
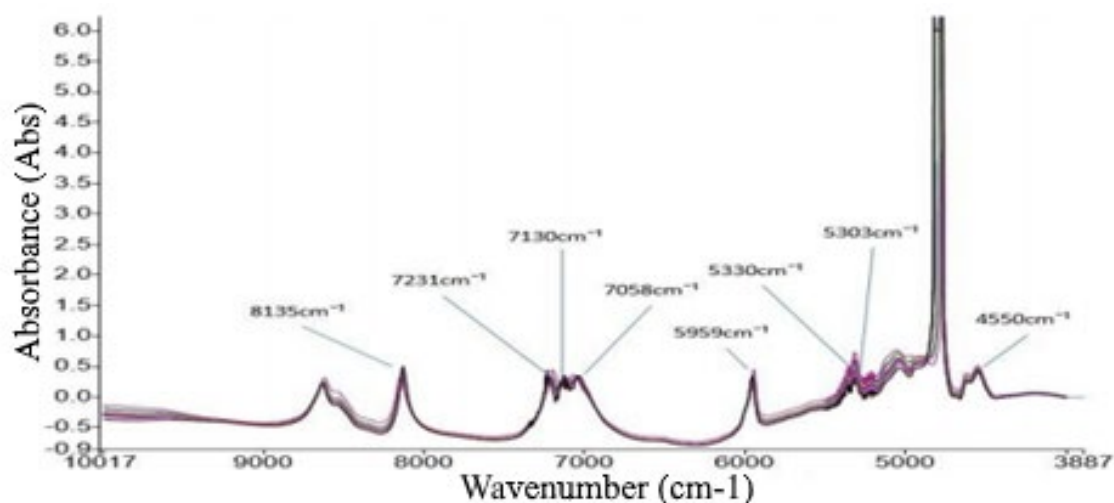

Figure 10. FT-NIR spectra overlay of typical used frying oil

Che Man et al [23] has developed a near-infrared spectroscopy (NIRS) calibration to determine of free fatty acids (FFA) in crude palm oil, refined-bleached-deodorized palm oil and refined bleached deodorized-palm-olein using NIR reflectance approach. 400 g of palm oil sample was spiking with 0.15% w/w enzyme, and then incubated at 60C and 200 rpm. Che Man et al determine FFA in palm oil by using this method and it was based on the C=O stretching bands in the region of 1850-2050 nm. The absorption obtained from NIR is correlate with standard AOCS titration method.

Calibration using multiple linear regressions (MLR) analysis was used due to the chemical interactions of the absorbing species and molecules. A good calibration result is shown. R2 reading for crude palm oil is 0.994, refined bleached deodorized-palm-olein is 0.961 and refined-bleached-deodorized palm oil is 0.971. This NIR analysis proved that it able to test hundred samples daily using less amount of solvent and shorter analysis time as compared to conventional wet chemical method.

7. Conclusion

The analytical methods described in this paper commonly used to determined free fatty acid (FFA) in cooking oil. The benefits and drawbacks of each sensor are discovered. These methods have been used by researchers to determined FFA. The selection of method for FFA determination is depends on the availability of the equipment, budget, skilled worker, the purpose of study, and the number of samples.

8. Acknowledgment

9. References

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