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REVIEW

Detection of Free Fatty Acid in Crude Palm Oil

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Palm oil quality and price is dependent on the free fatty acids (FFA) content in palm oil. High content of free fatty acids in palm oil affect the quality of palm oil and leads to various health and environmental issues. The maximum free fatty acids content set by the Palm Oil Refiners Association of Malaysia in crude palm oil is 5 % and < 0.1 % in refined bleached deodorized oil. Due to the high demand in palm oil industry market nowadays, various works has been done to improve the quality of palm oil including the determination and reduction of free fatty acids in palm oil. The traditional method for determination of free fatty acids in palm oil is through titration of the sample against potassium hydroxide in hot 2-propanol solutions by using phenolphthalein as indicator. Several other methods have also been reported on free fatty acids determination previously for example spectroscopic, chromatography and electrochemical technique. This paper reviews all methods reported for determination of free fatty acids in palm oil.

Keywords: Palm oil, Free fatty acids.

INTRODUCTION

Oil palm (*Elaeis guineensis*) originates from Southeast Asia and Equatorial Africa[1]. Palm oil is the leading vegetable oil in the world with the highest production of 38.5 million and dominating 25 % of total global oils and fats production in the year 2007[2]. Malaysia's oil palm plantation area and crude palm oil production has been increasing gradually in the past five decades and Malaysia accounted for more than 40 % of the total world palm oil production[2]. This might be due to the suitable climate and good management arising from R&D[3]. In terms of production cost, palm oil is one of the cheapest oil in the market compared to other edible oils in the world such as soybean, rapeseed and sunflower oils[2].

Malaysian palm oil industry is one of the federal government's National Economic Key Areas (NKEAs) projects under the Economic Transformation Programme (ETP). The main purpose of this transformation programme is to generate an incremental Gross National Income (GNI) of RM 178 billion and create 42,000 new jobs by the year 2020. During the presentation of the ETP by Minister in the Prime Minister's Department, Senator Datuk Seri Idris Jala in October 2010, it was found out that the palm oil industry was the fourth largest contributor to the country's economy with an RM 1,889 GNI per capita. Malaysian palm oil production statistic in May 2013 hit the number of 1, 384, 312 tonnes and the statistic of exported palm oil in May 2013 was 1, 411, 729 tonnes which indicated that palm oil is one of the major economic contributions to the country's nowadays[4,5].

In order to achieve the country's GNI by the year 2020, improvements have to be done in palm oil industry especially in accelerating the production rate of high quality palm oil. High production rate of palm oil dependent on the methods used during laboratory works and this had been identified through eight entry point projects (EPPs) which some of it includes an accelerated replanting *via* a binding replanting policy, improving fresh fruit bunch yields, improving oil extraction rate, extraction of oil expediting growth of food and health-based segments of the industry and so forth[4]. Meanwhile, the palm oil and crude palm oil quality are dependent on the oil contents itself such as free fatty acids, phosphatides, odoriferous matter, water and impurities which can be remove through several processes such as refining process[6]. free fatty acids content is one of the important parameters in palm oil industry[7]. The free fatty acids content in palm oil indicates the level of deterioration of oil and it is responsible in dictating the price of the palm oil in industry[7,8]. Furthermore, the nutritional status of palm oil is also determined by the types of fatty acids contained in palm oil[9]. Therefore, in order to produce high quality of palm oil, it

is crucial to determine the free fatty acids content in palm oil before it is marketed.

Palm oil: Palm oil is one of the most widely used edible oils in the world because of the lower price compared to other edible oils. The palm oil can be obtained from two distinct portions of palm fruit which are from the flesh of the fruit or known as mesocarp and also from seed or kernel of the fruit[1]. High amount of oil can be obtained from the mesocarp of ripe fruits compared to the unripe one[1]. The extraction of crude palm oil (CPO) is carried out under high temperatures ranging from 90 to 140 °C[7]. Crude palm oil appears as a semisolid, deep reddish orange-coloured viscous solution at ambient temperature. The reddish orange-coloured represents the existence of carotene in crude palm oil which also known as pro-vitamin A, however carotenes are discarded during the refinery process[2,10].

Palmitic acid and oleic acid are the main fatty acids which contain in the palm oil. Palm oil also possess antioxidant, anti-cancer and cholesterol lowering effect properties due to the existence of tocopherols and tocotrienols (pro-vitamin E) in the palm oil compositions[2,10]. The palm oil flavours usually are contributed by the combination of volatile components especially contribution by unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid together with terpenes[10]. Palm oil also has been used as the staple oil in some of the countries in the world which includes Malaysia, Indonesia, Papua New Guinea and Nigeria[2].

Free fatty acids

Free fatty acids in palm oil: The free fatty acids amount or also known as the acid value (AV) content of the palm oil determines the quality of the palm oil itself[11]. Oils which are high in free fatty acids content have poor quality of oil and suffer significant losses during refining process[11]. The maximum standard specifications set by the Palm Oil Refiners Association of Malaysia for the free fatty acids content (as palmitic acid) in crude palm oil (CPO) is 5 % and should be lower than 0.1 % in Refined Bleached Deodorized Oil (RBDO)[12]. Low free fatty acids content in crude palm oil produced good physico-chemical properties of crude palm oil products and could be useful for industrial applications[1].

Basically, the production of free fatty acids in palm oil is performed through the hydrolysis of fatty acid during the palm oil processing[13]. Apart from that, free fatty acids is also released naturally in crude palm oil and can be produced due to the action of enzyme in the palm fruits, by microbial lipases and by the reaction of oil with water during storage[12]. Moreover, the damaged palm fruits[14] and lengthy storage of palm fruits[15] may also increase the free fatty acids content, thus affect the quality of palm oil. According to Arzamendi and co-workers[16], fatty acids can be produced directly through transesterification and hydrolysis process of fats and oil. The transesterification process is very sensitive to free fatty acids, thus may lead to the undesirable saponification, low product yields and complication in the next separation processing steps[17]. The free fatty acids also normally present in downstream by-products of edible oil processing[16].

Based on previous study, it was found out there was an endogenous lipase which also known as triacylglycerol acylhydrolase found in oil palm fruits[1,18,19]. The action of lipase may increase the free fatty acids level in crude palm oil[1]. The contamination of fungi in palm oil may lead to the hydrolysis of glycerides and free fatty acids formation, thus affect the quality of palm oil[11,20-22]. Short chain fatty acids in palm oil can be converted into a series of methyl ketones by certain xerophilic fungi and this phenomenon is known as ketonic rancidity[20,23-25].

Current method for determination of free fatty acids in palm oil: Most of the methods for determination of free fatty acids in palm oil usually are the same with the determination of free fatty acids in other edible oils. free fatty acids determination in palm oil had been studied previously. However, most of the methods applied during the studies involved manual operation and time consuming. Basically the oil has to be extracted prior to the free fatty acids analysis by using chemical equipment. There are various ways of determining free fatty acids in palm oil which includes titration[26], Gas chromatography (GC), high performance liquid chromatography (HPLC)[8] and capillary electrophoresis (CE)[27] analysis. The palm oil is extracted prior to the analytical separation either through supercritical fluid extraction[28], soxhlet extraction[28], liquid-liquid extraction and solid-phase extraction methods[8].

Acid-base titration analysis: The traditional way of determining free fatty acids in palm oil is through the acid-base titration method by titrating the sample against potassium hydroxide in hot 2-propanol solution, using phenolphthalein as an indicator[26] and the result is expressed in mg KOH g^{-1} oil[29]. Theoretically, the acids contain in the solution are neutralized during the titration and the value obtained represents the acid number of the sample which is proportional to free fatty acids content[30]. Although this method is simple, it is sluggish, laborious and lack of accuracy[31]. On top of that, during the neutralisation process, apart from free fatty acids, other substances which can react with KOH might be neutralized as well, thus leads to the inaccurate calculation of acid number value[30]. Moreover, high amount of reagents and solvents are also consumed during the operation[31-34]. Various researches has also been reported for determination of free fatty acids in various types of palm oil and other vegetable oils by using American Oil Chemists' Society (AOCS) standard titration method[13,35,36].

Gas chromatography analysis: Gas liquid chromatography has been widely used and among the popular choice for half of century. The advantages of using gas chromatography are because it offers rapid analysis, high sensitivity and provide good reproducible analysis. It also relatively low cost in terms of analysis and convenience. Principally, fatty acids are separated by the interaction between stationary phase and mobile phase along the column before being detected by the detector. Flame ionization detector (FID) is the most common and widely used detector in chromatographic analysis for free fatty acids detection. On top of that, FID detector also able to detect various compounds as small as pictogram level concentration. Normally, gas chromatography chromatogram shows sharp and symmetric peaks. Nonetheless, the interpreted resolution of gas chromatography is highly dependent on the column length and polarity of stationary phase used. Basically, the choices of capillary column types, stationary phase polarity

and column length are crucial in order to obtain good resolution and separation in gas chromatography analysis.

The problem with gas chromatography analysis is it might face difficulty while dealing with non-volatile samples as compared to the volatile one. In order to improve this limitation, free fatty acids has to be esterified before being injected into the gas chromatography column. In GC/FID, comparison of retention time between standard and sample is used to identify free fatty acid. However, complexity of fatty acid composition and limitation of fatty acid standard is clearly a major difficulty in order to identify some peaks with conventional FID detector. Identification by using GC/FID may also reflect by the presence of contaminants or coeluting compounds. Besides, using GC/FID requires adequate standards and standards are not available for some fatty acids especially for the complicated fatty acid compounds like polyunsaturated fatty acids. Therefore, fatty acid analysis can be done by converting free fatty acids into fatty acid methyl ester (FAME) before injecting into the gas chromatography column. The combination of GC/FID and GC/Mass spectrometry (GC/MS) can be applied in order to ensure the accurate identification of a peak[37,38]. Several works had been reported in literature for determination of free fatty acids in various kind of palm oils and vegetable oils[39-41].

High performance liquid chromatography analysis: High performance liquid chromatography is one of the methods that is widely used for determination of free fatty acids either in palm oil or other vegetable oils. HPLC method has been widely chosen for determination of free fatty acids nowadays due to the fact that it has excellent selectivity and it ables to quantify high diversity of analytes[42,43]. The main difference between HPLC and gas chromatography method lies on types of the mobile phase used where liquid mobile phase is used in HPLC while gas mobile phase is used in gas chromatography[42]. The free fatty acids is widely analyzed by using RP-HPLC. According to Christie[44], the separation of free fatty acidss is based on the chain length and degree of unsaturation. Octadecylsilyl (ODS) is normally used as stationary phase and acetonitrile or methanol in water is used as mobile phase for free fatty acids analysis[42]. The free fatty acids is detected by the UV detector between the wavelength of 205 and 210 nm[42].

Determination of underivated fatty acids in palm oil and other vegetable oils by RP-HPLC has been carried out by Hein and Isengard[45]. This research intended to improve the previous method by skipping the derivatization process which used high amount of chemicals and also time consuming. Three different alternative RP-HPLC methods were introduced where different detectors and different mobile phases were performed. A good resolution relies on the pH value of mobile phase. It was observed that the optimum pH for the separation of fatty acid was in the range of 3.0-3.5. On top of that, based on this research it was proven that the vegetable oils can be differentiated based on the characteristic of fatty acid pattern in oils. By applying HPLC method for determination of fatty acids in oils, it was also possible to determine the amount of free fatty acids in the oil by calculating the acid number. The amount of free fatty acids content in oil was based on the concentration of free oleic acid determined from the vegetable oils[45].

Determination of free fatty acids, partial acylglycerols and tocols in palm oil products using HPLC with evaporative light scattering detector (ELSD) was performed by Moh and coworkers[46]. Different mobile phases were used which are heptanes and 2-propanol with the flow rate of 1 mL min^{-1}. The prepared samples were dissolved in dichloromethane (DCM) before being injected into the HPLC column. It was found out that the total free fatty acids content in crude palm oil and crude palm olein is high (3.80-5.30 %) but low in the refined-bleached-deodorized palm olein (< 0.1 %). The results obtained from HPLC analysis were compared with the results obtained from GC/FID analysis for validation[46]. Although the steps of derivatization was eliminated in HPLC analysis, however high consumption of solvents are still being used as mobile phase and HPLC analysis also possess long time of analysis.

Fourier transform infrared spectroscopy analysis: Free fatty acids in palm oil can be determined by using fourier transform infrared spectroscopy method[12,47]. FTIR is a physico-chemical method based on measurement of vibration molecule excited by IR radiation at specific wavelength range. FTIR can be used to analyze total composition, including proteins, fatty acids, carbohydrates, nucleic acids and lipopolysaccharides.

Che Man *et al.*[12] determined the free fatty acids content in crude palm oil (CPO) and refined-bleached-deodorized (RBD) palm olein by using a rapid direct FTIR spectroscopic method. The main objective of this research was to develop a technique which is rapid, low cost and using fewer amounts of solvents. The sample was prepared by hydrolizing the oil with enzyme in an incubator at 60 °C and 200 rpm and analyzed by using FTIR. The FTIR spectra shows the carboxyl (C=O) absorption band in 1722-1690 cm^{-1} region which indicates the presence of free fatty acids in palm oil samples. The results obtained from the FTIR study were correlated with the standard titration method adopted by the American Oil Chemists' Society (AOCS) by constructing partial least square (PLS) model analysis.

The purpose of hydrolizing the sample prior to develop a PLS calibration model for the prediction of free fatty acids content in palm oil, was to overcome the difficulty of obtaining a wide range of free fatty acids concentration in production plant. The hydrolysis step may prevent the formation of oxidative products during the reaction[12]. It was found out that the FTIR method offers more advantages over the wet chemical method where it is much simpler, rapid, highly sensitive, consistent and also accurate. Based on the optimal PLS calibration models constructed, the correlation coefficient (R^2) and standard error of calibration (SEC) for crude palm oil are 0.992 and 0.08 respectively. Meanwhile, refined-bleached-deodorized palm olein possessed an R^2 of 0.994 and SEC of 0.01. Both crude palm oil and refined-bleached-deodorized palm olein show good linear relationship between FTIR method and AOCS method. The results obtained proved that FTIR spectroscopy coupled with a flow-through transmission cell was successfully applied in determining free fatty acids in palm oil[12].

In other publication, Che Man and Setiowaty[47] performed a simple and rapid method for quantitative determination of free fatty acids contents in palm olein by using Fourier

transform infrared (FTIR). A set of palm olein samples was prepared by spiking increasing amounts of oleic acid into a series of palm oleins which covers about 0.08-1.04 % range of free fatty acids and this set was used as the calibration set. The sample was analyzed by using the FTIR instruments and the spectral range was found to be 1728-1662 cm^{-1}. The free fatty acids content then was correlated with AOCS standard titration method by developing the partial least square (PLS) calibration model based on the obtained spectral range. The R^2 and standard error of palm olein were 0.997 and 0.017 % of free fatty acids unit, respectively. The model was tested by cross-validation steps in order to minimize standard error of the model. The overall reproducibility for both methods proved that FTIR gave good results over wet chemical method. The implementation of FTIR method may eliminate the use and disposal of hazardous solvents and it is also able to be performed in rapid way which is less than 2 min per sample[47].

Near-infrared reflectance spectroscopy (NIRS) analysis: Several studies had also been conducted for determination of free fatty acids by using near-infrared reflectance spectroscopy (NIRS)[48]. Che Man and Moh[48] developed an near-infrared reflectance spectroscopy calibration for determination of free fatty acids in crude palm oil, refined-bleached-deodorized palm olein and refined-bleached-deodorized palm oil using the NIR reflectance approach in order to replace the current wet chemical methods which is using a lot of hazardous solvents[48]. The palm oil sample was prepared by spiking a 400 g of sample with 0.15 % w/w enzyme and was incubated at 60 °C and 200 rpm. The sample was then analyzed by using NIR spectroscopic instrument and was performed in a Dutch cup. The determination of free fatty acids in palm oil by NIR was based on the C=O stretching bands in the region of 1850-2050 nm. The absorption obtained from the NIR was correlated with standard AOCS titration method to validate the results.

Multiple linear regression (MLR) was carried out due to the chemical interactions of the absorbing species and molecules. This interaction will interfere with the linearity of the relationship between the absorbance and concentration[48]. Good calibration is shown in MLR analysis and the R^2 for crude palm oil, refined-bleached-deodorized palm olein and refined-bleached-deodorized palm oil are 0.994, 0.961 and 0.971 respectively[48]. The total analysis time is short as compared to the conventional wet chemical method which is less than 5 min per sample. On top of that, NIR analysis also able to test hundred samples daily without using much solvents[48].

Houmøller and co-workers[49] had conducted an experiment to determine the solid fat content (SFC) and Free fatty acids in blends of palm stearin, coconut oil and rapeseed oil with the presence of *Thermomyces lanuginosa* lipase at 70 °C. The edible oils were then analyzed by using near infrared spectroscopy (NIRS). According to Houmøller *et al.*[49] near-infrared reflectance spectroscopy analysis is rapid and does not requires any sample pre-treatment. They used the partial least squares regression (PLSR) method to calibrate the quantitative determination of solid fat content and free fatty acids at various temperatures ranging from 10 to 40 °C. From the data obtained, it was proven that the near-infrared reflectance spectroscopy could be used to replace the traditional methods for determining free fatty acids and solid fat content in vegetable oils. This method can also be applied in quality control, process control and optimization purposes. The activity of the immobilized enzyme for esterification of margarine oils can also be monitored through the prediction of equivalent reaction time in a batch reactor from NIR spectra. Determination of free fatty acids in other edible oils by using FT-NIR spectroscopy had also been reported previously[31].

Flow injection analysis: Saad and co-workers[8] developed a flow injection analysis (FIA) system for determining free fatty acids in palm oil which is simple, low cost and automated. The method applied in this research based on observing the baseline absorbance of indicators, namely phenolphthalein and bromothymol blue when reacted with free fatty acids with the existence of KOH in the reaction. The results obtained from the system were validated with PORIM standard titration method. Two types of FIA manifolds were used in this research study, single-line and two-line manifolds. In single-line manifolds study, the sample needs to be diluted off-line prior to injection; nonetheless the sample can be injected directly into the stream without dilution in the two-line manifold method since the sample and the carrier can be mixed on-line during the reaction occur in flow injection analysis[8].

The single-line manifold using phenolphthalein as indicator was found out to be more suitable for determination of free fatty acids in higher concentration and the two-line manifold using bromothymol blue was more suitable to be used for the determination of lower acidity degree in palm oil sample. Although the two-line manifold resulted in lower sample throughput and consumes larger amounts of reagents, both of the manifold methods gave good sensitivity and reasonable recoveries for the spiked oil sample[8].

Makahleh and Saad[50] developed an FIA system for detection of free fatty acids by using capacitively coupled contactless conductivity detection (C^4D) in various vegetable oils. Sixteen vegetable oils were analyzed in this research which includes palm olein, crude palm oil, refined-bleached-deodorized palm oil, soybean oil, corn oil, extra virgin olive oil, rice bran oil and walnut oil[50]. Liquid-liquid extraction (LLE) method was used for the sample preparation before being injected into the FIA system. Two different solvents were used as extraction solvents which are sodium methanoate and methanol. It was observed that there was no significant difference between these two solvents. However, some of the samples which have high free fatty acids content shows saponification when sodium methanoate used as solvent extraction[50].

Makahleh and Saad (2011)[50] used a single line FIA manifold. Several FIA parameters were optimized which includes the carrier stream and also the charged of the analyte. It was found out that, in order to develop the best FIA system, the conductivity of the carrier stream should be as low as possible and the analyte also should be in a charged form. Since sodium acetate was able to ionize all the analytes and provide suitable pH environment, methanol/1.5 mM sodium acetate (pH 8) was chosen as a carrier stream with the flow rate of 1 mL min^{-1}. The calibration curve was also constructed and good correlation was obtained between the standard non-aqueous titrimetry methods with the proposed method for detection of free fatty acids in vegetable oils. It was also revealed that the C^4D detector shown better performance over the other optical flow-through

detectors. The background colour and viscosity changes of the oil sample also not affecting the performance of C^4D detector[50].

Response surface methodology analysis: Tan *et al.*[1] applied the response surface methodology method in order to optimize the pre-treatment of oil palm (*Elaeis guineensis*) fruit spikelets before oil extraction. They employed the central composite design for studying the responses which are percentage of free fatty acids and oil yield. The optimum conditions for producing minimum free fatty acids and maximum oil yield were identified from the contour plots. They concluded that the low content of free fatty acids in crude palm oil exhibits good physicochemical properties and can be used in many industrial applications.

Conclusion

In summary, palm oil is one of the highest contributors to Malaysian's economic industry. Thus, improvements for determining the free fatty acids in palm oil which is rapid and environmentally friendly have to be done since free fatty acids play an important role in dictating the palm oil quality and price. Enzymatic reaction is one of the alternative methods which can be applied in this area. Although enzymes are expensive nowadays, by immobilizing the enzyme it is able to improve the performance of enzyme where it can be used at high temperature and high pH condition. Furthermore, it can be used repeatedly in the reaction. It is hoped that this kind of research will be able to give huge impact towards the palm oil industry in future.

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