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OPTICAL DETECTION SYSTEM FOR FREE FATTY ACIDS IN CRUDE PALM OIL BASED ON N-BROMOSUCCINAMIDE CATALYST

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By

NORSYAMIMI BINTI CHE SULAMAN

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Free fatty acids (FFAs) content in crude palm oil (CPO) is one of parameter that dictated the price of palm oil product. In this study, an alternative optical technique system on detection of FFAs in CPO based on chemical pathway using base catalyst was investigated in order to enhance the current method that practices in industrial with more advantageous in term of using time saving, greener practices and environmentally friendly. FFAs in CPO involved a direct conversion of FFA to form fatty hydroxamic acid (FHA) with hydroxylamine in order to increase its detection prior to coloured metal complexes formation. Coloured metal complexes are an indicator in optical detection using UV-Visible (UV-Vis) spectrophotometer analysis. However, the colour changes of the FHA-V(V) complexation was observe by naked eye before characterize using the UV-Vis spectrophotometer for intensity of the complex. Before this, there was no previous study that study the detection of the FFA in CPO via chemical pathway. A rapid response time of 5 min reaction with one third of mass reactant reduce from initial mass was achieved in conversion of FFAs to FHAs in presence of N-Bromosuccinamide (NBS) as base catalyst and Vanadium (V) (V(V)) as metal ion were used. The reaction was carried out under optimize temperature at 70 °C and 150 rpm shaking. The absorptivity of FHA complexes was detected at 430 nm (range 420 nm to 440 nm) using UV-Vis spectrophotometer. From studies, it can be concluded that detection of the FFA in CPO was successful. The result from develop method was validated with standard titration method by Malaysian Palm Oil Board (MPOB) using spiked CPO sample. Optimization studies were investigated in order to achieve an optimum condition for each parameter for best monitoring system. A linear calibration plot correlation coefficients (R^2) is 0.9902 which able to detect down to 0.7813 % of FFA in CPO. A good correlation was obtained between proposed method and MPOB standard titration method (R=0.9157). Paired t-test was used to determine the significant difference between these two methods. According to paired t-test

calculation, there is no significant difference between these two methods at 95% confident level (calculated t=1.099, tabulated t=2.776).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

SISTEM PENGESANAN ASID LEMAK BEBAS DALAM MINYAK SAWIT MENTAH BERDASARKAN PEMANGKIN N-BROMOSUCCINAMIDE

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Kandungan asid lemak bebas (FFAs) dalam minyak sawit mentah (CPO) adalah salah satu parameter yang menentukan harga produk minyak sawit. Dalam kajian ini, satu sistem teknik optik alternatif untuk mengesan FFAs dalam CPO berdasarkan laluan kimia menggunakan pemangkin alkali telah disiasat untuk meningkatkan kaedah semasa yang diamalkan dalam industri dengan lebih berfaedah dari segi menggunakan penjimatan masa, amalan hijau dan mesra alam . FFAs dalam CPO melibatkan penukaran langsung FFA untuk membentuk asid hydroxamic lemak (FHA) dengan hidrokslamin untuk meningkatkan pengesanannya sebelum pembentukan kompleks logam berwarna. Kompleks logam berwarna adalah penunjuk dalam pengesanan optik menggunakan analisis spektrofotometer UV-Visible (UV-Vis). Walau bagaimanapun, perubahan warna kompleks FHA-V (V) diamati dengan mata kasar sebelum mencirikan menggunakan spektrofotometer UV-Vis untuk pengesanan kompleks. Sebelum ini, tiada lagi kajian yang mengkaji pengesanan FFA dalam CPO melalui laluan kimia. Kajian ini telah hanya memerlukan masa yang singkat iaitu selama 5 dengan menggunakan satu pertiga berat jisim dalam penukaran FFAs kepada FHAs dengan kehadiran N-Bromosuccinamide (NBS) sebagai pemangkin asas dan Vanadium (V) (V (V)) sebagai ion logam digunakan. Reaksi itu dilakukan di bawah suhu mengoptimumkan di 70 ° C dan 150 rpm goncangan. Penyerapan kompleks FHA dikesan pada 430 nm (jarak 420 nm hingga 440 nm) menggunakan spektrofotometer UV-Vis. Dari kajian, dapat disimpulkan bahawa pengesanan FFA di CPO berjaya. Hasil dari kaedah pembangunan telah disahkan dengan kaedah titrasi oleh Lembaga Minyak Sawit Malaysia (MPOB) menggunakan sampel CPO tambahan. Kajian pengoptimuman telah diselidiki untuk mencapai keadaan optimum bagi setiap parameter untuk sistem pemantauan yang terbaik. Koefisien korelasi plot penentukuran linear (R2) adalah 0.9902 yang dapat mengesan turun kepada 0.7813% FFA dalam CPO. Satu korelasi yang baik diperolehi antara kaedah

yang dicadangkan dan kaedah titrasi piawai MPOB (R = 0.9157). Ujian t-pasangan digunakan untuk menentukan perbezaan yang ketara antara kedua-dua kaedah ini. Berdasarkan pengiraan t-test berpasangan, tidak terdapat perbezaan yang signifikan antara kedua-dua kaedah pada tahap yakin 95% (dikira t = 1.099, ditala t = 2.776).

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Best Regards
Norsyamimi Che Sulaman

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LIST OF ABBREVIATIONS

AOCS	American Oil Chemist Society
BPO	Bleached Palm Oil
CPO	Crude Palm Oil
DAG	Diacylglyceride
DBPO	Distilled Bleach Palm Oil
DG	Diglyceride
ECD	Electron Capture Detector
FFA	Free Fatty Acid
FHA	Fatty Hydroxamic Acid
FID	Flame Ionization Detector
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionisation Detection
GCMS	Gas Chromatography Mass Spectroscopy
GLC	Gas Liquid Chromatography
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
KOH	Potassium Hydroxide
MAG	Monoacylgliceride
MG	Monoglyceride
MIR	Mid-Infrared Region

MLR	Multiple Linear Regression
MPOB	Malaysian Palm Oil Board
MS	Mass Spectrometry
NaOH	Sodium Hydroxide
NBS	N-Bromosuccinamide
NIR	Near-Infrared Region
NPD	Nitrogen Phosphorus Detector
PCR	Principal Component Regression
PID	Photo Ionisation Detector
PKO	Palm Kernel Oil
PLS	Partial Least Square
R^2	Correlation Coefficient
RBD	Refluxed Bleached Deodorize
RBPO	Refluxed Bleach Palm Oil
RSD	Relative Standard Deviation
SE	Standard Error
SEC	Standard Error of Calibration
TAG	Triacylglyceride
TG	Triglyceride
TLC	Thin Layer Chromatography
TOTOX	Total Oxidation Value
UHPLC	ULTRA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY
UV-Vis	UV-Visible

CHAPTER 1

INTRODUCTION

1.1 Background

Palm oil industry was mainly focusing on such as cultivating of fruit bunches, processing fresh fruit bunches (FFB) in mills for CPO and PKO, producing refined palm oil (RPO) from CPO, and fractionating palm oil to obtain liquid olein and solid stearin fraction as well as oleochemical products (Choo, 2014). In September 2010, Malaysian government was introduced the Economic Transformation Program (ETP) under the 12 National Key Economic Area (NKEA) to drive the nation's economy. The ETP is a comprehensive effort that outlines a 10-year economic roadmap to reinforce Malaysia toward becoming a high-income nation by 2020. The palm oil industry was one of the industries that been affected positively by this implementation of ETP. The palm oil sector NKEA is aimed at changing the traditional approach by improving upstream productivity, increasing downstream expansion and sustainable development of the oil palm industry (Choo, 2014).

Over decades of the existence of oil palm tree, palm oil industry continues to thrive to produce high-quality edible oil compared to other vegetable oils. Palm oil was first introduced as decorative crops that later regain a lot of benefits to consumer in terms of its nutrition (Basiron, 2007). Palm oil had a deep rich red colour which derived from its rich carotenoid contents, which also known as a pigment that often found in plants and animals. The appearance of palm oil was in viscous semi-solid and solid fat in temperate climate due to a saturated palmitic acid is a major component of its glycerides. Crude palm oil (CPO) and palm kernel oil (PKO) were a major type of palm oil production. Thus, high yield of oil extraction rate (OER) and CPO quality had become an interest to millers. However, there was many hurdles need to overcome to produce good quality of CPO.

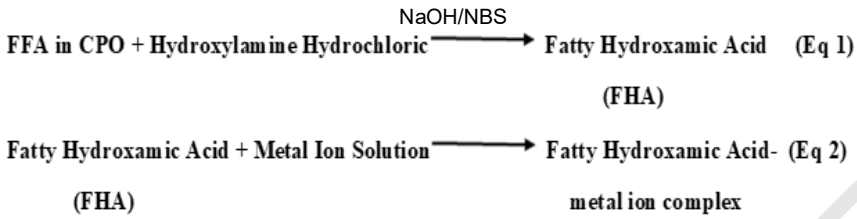
A variety of physicochemical oil quality parameters were determined including free fatty acids (FFA), moisture, peroxide value, iodine value, and saponification number. However, FFA was considered as the most influential parameter that indicates a quality of CPO and other vegetable oil; its bad or good as well as its economic value indices. FFA was naturally released in palm oil which is caused by hydrolysis and can be increased by microbial activity (Che Man *et al.,* 1999). During the refining process, removal of FFA as a contaminant in edible oils was indeed, since high contents of FFA will lead to rancidity of the oil and will lower quality of flavour (Keurentjes *et al.,* 1991). According to standard specification of FFA content by Palm Oil Refiner Association of Malaysia, FFA content must not exceed 5% for crude palm oil (CPO) and not less than 0.1% of refined-bleached-

deodorised oil (RBDO) (Che Man *et al.,* 1999). The level content of FFA must be analysing first under this regulation for trading purpose.

The fundamental issues in producing high-quality palm oil were dictated by the high level content of FFA in palm oil. Various strategies and development in the detection of FFA had already established such as using spectrophotometric, amperometric, colorimetric, flow injection methods and others. However, those approaches often employ highly cost maintenance when involving high-performance instrumentation analysis even though in a shorter time, it performed a good analysis. Conventionally, FFA content was determined using manual titration method which carried out by titrating the palm oil sample against potassium hydroxide (KOH) by using phenolphthalein as an indicator (Ainie *et al.,* 2004). The limitations for this method include time-consuming, laborious usage, high solvent usage (isopropanol) as well as easily interrupted prone to human error. Technically, conventional method is needed for a skilful and competence employee in order to get an accurate result for end point determination which may lead to over titration due to the presence of orange colour of carotene compound (Saad *et al.,* 2007) . Thus, new development method in indicating FFA content had to be investigated using simpler way, greener and practical. Direct detection of FFA in vegetable oils especially palm oils based on sensor is not developed yet.

1.2 Problem Statement

During refining process, there are some parameter that need to remove in order to get a good quality of crude palm oil such as the amount of free fatty acid (FFA), phosphatides, odoriferous matter, water and impurities that meets industry standards (Junior *et al.,* 2012).Free fatty acid (FFAs) was the most importance factor that indicates the quality of the palm oil (Corley and Tinker, 2003). Thus, the amount of FFAs in palm oil need to be determined before distribute to the market. With all kinds of reasons and problems arise in the detection of FFA; an alternative method based on the chemical sensor using optical technique was designed using colourimetric detection. Chemical sensors were promising rapid detection, high specification, and sensitivity and selectivity, inexpensive, and easy for users as per requirements. Generally, the FFAs concentration in palm oil was based on the optical observation of the formation of the fatty hydroxamic acid-vanadium (V) (FHA-V(V)) complex, whereby the FFAs were converted to FHAs prior to complexation via aminolysis. Later, the complex characterized using a spectrophotometric method. The principle of the detection of FFAs content in CPO can be summarized as follows in **Scheme 1.1:**



Scheme 1.1 : Principle of detection of free fatty acids (FFAs) content in crude palm oil (CPO)

The equation 1 shows the aminolysis reaction involved in the conversion of FFAs to FHAs and the equation 2 show the complexation step between fatty hydroxamic acid with metal ions will form coloured FHA-metal complex. Rapid detection system was required in this study; thus, all parameter involves during aminolysis reaction were a crucial factor prior the development of the detection system. The parameter studies were included varying base catalyst, time reaction, temperature reaction and metal ions. By considering a sensitivity and selectivity of this chemical sensor, detection of FFA in palm oils is promising.

1.3 Justification

Titrimetric method was done over decades in determination of FFA in crude palm oil. This method was done by titrating the CPO in isopropanol against the KOH solution in presence of phenolphthalein as indicator. The disadvantage of this method was skilful and competence labour needed, high solvent usage and tedious procedure. This is because the CPO contain orange coloured carotene compound in which lead a difficulty in determine the end point of titration and may cause over titration. Basically, for normal titration which the colourless acid against the KOH solution resulted a pale pink coloured as the end point of the analysis. Thus, it is easy to determine the end point of titration. The crucial factor in detection of FFA in CPO by manual titrimetric was complicated due to difficulty to determine the end point of sample.

An alternative method was approach to detect the FFA in more rapid and simpler way to improve the current method on detection of FFA in CPO that had applied in industrial. This study involved aminolysis of the FFA in CPO with hydroxylamine hydrochloric acid convert FFA to FHA. FHAs was reacted with metal ion solution to form coloured FHA-metal complex. Then, coloured FHA-metal complex was observed by naked eyes and characterized using UV-Visible Spectrophotometer to determine the FFA concentration contain based on absorption intensity of FHA-metal complex.

1.4 Objective of Study

1.4.1 General Objective

The general objective of the study was to develop a colourimetric detection for free fatty acids (FFAs) in crude palm oil (CPO) samples utilizing base catalyst via optical technique.

1.4.2 Specific Objectives

The objectives of this research were:

1. To optimize the response time for the conversion of free fatty acids (FFAs) to fatty hydroxamic acids (FHAS) prior the colorimetric detection.
2. To investigate the effect of different base catalyst, effect of reaction temperature, effect of different metal ions and effect of mole ratio of reactant involved on conversion of free fatty acids (FFAs) to fatty hydroxamic acids (FHAs) to obtain the best monitoring.
3. To correlate the optimize parameters with MPOB standard titration method for validation to obtain the best monitoring system.

1.5 Scope of Work

The scope of study was focused to investigate free fatty acids content in crude palm oil (CPO) sample using optical technique utilizing base catalyst. The aim of study is to improve the response time in order to get a rapid analysis toward conversion of FFAs to fatty hydroxamic acids (FHA) in CPO instead of 24 hours reaction by previous synthesis. Other parameters were optimized in order to get a good conversion of FHA. Proposed method was validated with MPOB standard titrimetric method.

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PUBLICATION

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