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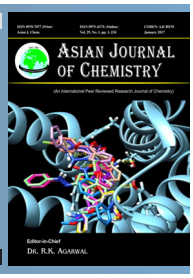

# A Novel Base Catalyzed Esterification Reaction for Spectrophotometric Determination of Free Fatty Acid in Crude Palm Oil

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In the present work, a novel spectrophotometric method has been developed for determination of free fatty acid (FFA) in palm oil based on esterification reaction of FFA in crude palm oil (CPO) for conversion of FFA to hydroxamic acid (FHA) with excess of hydroxylamine hydrochloride in the presence of base catalyst. The detection was based on the complexation of FHA with V(V) to form a blue colour complex within 5 min; reaction temperature, 70 °C and base catalyst, N-bromosuccinamide (NBS). The intensity of first derivative spectra of the complex at 402 nm was correlated with FFA content in CPO due to the overlapping between spectra of FHA-V(V) and V(V) free in the solution. This developed detection system is able to detect down to 1.5 % FFA in CPO. The detection system was also validated against standard titration method satisfactory.

**Keywords: Free fatty acid, Hydroxamic acid, Esterification, V(V), Derivative spectrophotometry.**

## INTRODUCTION

Palm fruit produce two distinct types of the oils, crude palm oil (CPO) produces from the mesocarp and palm kernel oil (PKO) produces from inside kernel [1]. Free fatty acid (FFA) contents (as palmitic acid or lauric acid) are a crucial factor in producing a good quality palm oil [2]. Free fatty acid content was determined manually using titrimetric method by titration of sample against potassium hydroxide in hot 2-propanol solution in the presence of phenolphthalein as an indicator [3]. However titrimetric method is not environmental friendly because it used extremely laborious, huge solvent and time consumption. The presence of carotene compound in palm oil itself cause inaccurate reading of end point during titration. Hence, other methods had been developed in determination of FFA with more sensitivity and accuracy. Three major groups of new developing methods in detection of FFA had been categorized: colorimetric methods, electrochemical methods and spectroscopic method [4]. In colorimetric method, various methods had been reported which are copper soap method [5,6], automated flow injection analysis system [7-10] and phenolphtalein-based method [11-15]. Free fatty acid detection using electrochemical method also imply by using voltammetric technique [16,17]. Various studies had been reported using spectroscopic method involving infrared method [18-20] and near-infrared methods [21-23].

Generally, Blatt's procedure was adopted for synthesis of hydroxamic acids [24]. This method used alkyl or aryl ester with hydroxylamine derivatives in the presence of alkali by addition of acid in appropriate quantity in cold solution. Al-Mulla *et al.* [25] reported that sodium ethoxide can be used as a catalyst in synthesis of N,N'-carbonyl difatty amides from palm oil and produced ethyl fatty ester and glycerol as by products. This reaction resulted up to 79 % N,N'-carbonyl difatty amides converted from palm oil after 8 h by using 6.2:1 molar ratio of urea to palm oil.

Hydroxamic acid derivatives had been known for their specialty as metal chelator [26]. These complexes with various metal ions had been used as reagent for gravimetric and spectrometric metal determination [27]. In this investigation we focus on determination of FFA content in crude palm oil by reaction the FHA content with V(V) ion in order to form metal complexes. The spectrum of FHA-V(V) complex has high overlapping with V(V) free in the reaction. Therefore, derivative spectrometry was used as a simple method for quantitative analysis of FFA content in CPO at wavelength of 402 nm. Several parameters were optimized including effect of base catalyst, reaction time and reaction temperature in order

to get a significant and rapid colour changes. The proposed method was validated successfully by the standard titrimetric method.

## EXPERIMENTAL

All chemicals were used without further purification and were of analytical grade. Hydroxylamine hydrochloride was purchased from Acros Organic (Malaysia). Sodium hydroxide, potassium hydroxide, N-bromosuccinamide, 1-propanol and hydrochloric acid were purchased from R & M chemical (Malaysia). Hexane and ethanol were purchased from HmBG chemical. Vanadium(V) oxide and palmitic acid were purchased from Sigma. Crude palm oil (CPO) was supplied by Sime Darby (Labu, Malaysia).

Nanodrop 2000 spectrophotometer (Thermo Scientific) and UV-visible spectrophotometer (Perkin Elmer) were used for absorbance measurements.

**Sample preparation for validation study:** Sample preparation for validation study was carried out using a method by Ali and Abdurrahman [28] with some modification. 10 g of crude palm oil was dissolved in 100 mL of 1-propanol. This solution was used to prepare stock standard solution of palmitic acid.

Stock solution of palmitic acid was prepared by dissolving 5 g of palmitic acid in 50 mL of stock crude palm oil solution and heated to dissolve palmitic acid completely.

### Proposed method

**Synthesis of hydroxamic acid (FHA):** A conversion of FFA to FHA was prepared by using a novel method by Suhendra *et al.* [29] with some modification. Hydroxylaminolysis was carried out in the presence of hydroxylamine and hydrochloric acid (6:1 mol/mol) as follows. 138.9 mg of hydroxylamine hydrochloride dissolved in 5 mL of deionized water and mixed with 286 mg of dissolved CPO in 5 mL of hexane. The mixture was incubated in a water bath shaker for 45 min at 150 rpm shaking rate and 70 °C in the presence of 1 % of base catalyst sodium hydroxide or N-bromosuccinamide (NBS). Two layers were formed after reaction and the organic phase (FHA sample) was pipetted out while aqueous phase was discarded.

**Complexation reaction:** Vanadium solution (0.1 M) was prepared in hydrochloric acid (12.0 M) and was added in FHA sample with equal volume and agitated for about 5 min. The colour change was observed (green to blue) after complexation reaction and absorption spectra were recorded by UV-visible spectrophotometer in the wavelength range of 220 to 700 nm.

**Titrimetry (standard method):** Free fatty acid content of prepared standard palmitic acid solution was determined by using Malaysian palm oil board test method [3]. 2.5 g of prepared standard palmitic acid solution was diluted to 50 mL of neutralized 1-propanol in conical flask by adding 0.5 mL of phenolphthalein as an indicator. The sample was shaken gently while titrated against 0.1 M of KOH till first permanent pink colour appeared and persistent for 30 s. Calculation of percentage of free fatty acid is based on the number of milligrams of KOH necessary to neutralize the FFA in 1 g of sample and expressed as percent of palmitic acid.

## RESULTS AND DISCUSSION

**Derivative spectrophotometry detection of FFA based on base catalyzed hydroxylaminolysis reaction:** Esterification of FFA in CPO was synthesized using base as a catalyst to produce FHA. The hydroxamic acid is able to chelate with many metal ions. This unique property was implying for qualitative identification of FHAs in CPO by forming a stable coloured complex. Previous, we reported the formation of coloured complexes of FHA with vanadium, iron(III) and copper(II) in purple, dark red and green, respectively [29]. Fig. 1 shows the UV-Visible spectra of CPO, FHA, V(V) and FHA-V(V) complex. Crude palm oil and FHA alone were in yellow colour with maximum wavelength ($\lambda_{max}$) around 600 nm. Meanwhile V(V) alone has a maximum spectra at 394 nm and FHA-V(V) complexes in blue colour ($\lambda_{max}$ = 430 nm). As it has shown in Fig. 1(a), the FHA-V(V) and V(V) alone have overlapping completely and quantitative determination of CPO based on the amount of FHA converted using the maximum wavelength at 394 nm is impossible. Fig. 1(b) shows that first derivation spectra of FHA-V(V) complex at 402 nm has a significant value while it is negligible on the derivative spectrum of V(V). In this proposed method, we used derivative spectrophotometry for quantitative analysis of FHA/CPO content in palm oil using external calibration at 402 nm.

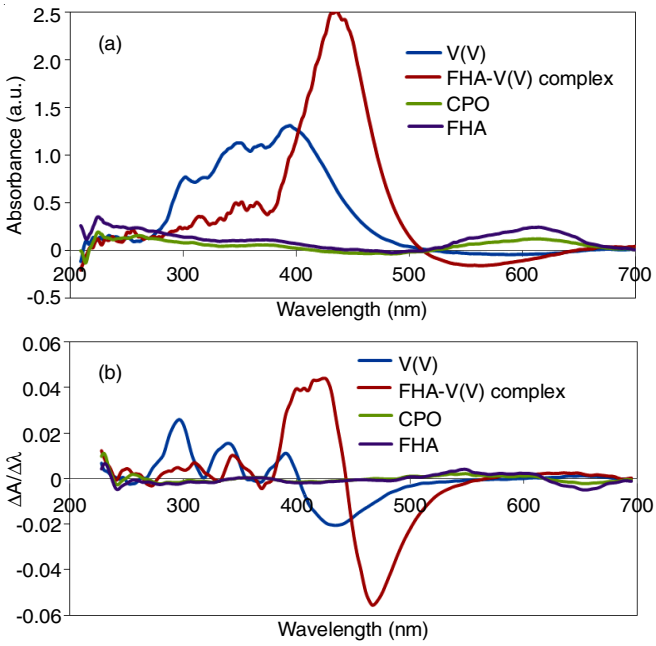


Fig. 1. (a) UV-visible spectra of CPO, FHA, V(V) and FHA-V(V) complex after reaction of FHA with V(V) at equal volume; (b) Derivative spectra of (a)

In order to determine the total content of FFA in palm oil by the proposed method, some parameters have been optimized (*e.g.*, effects of base catalyst, reaction time and reaction temperature) for enhancement of sensitivity.

**Effect of base catalyst:** Base catalysts have less corrosive than acid catalysts, so it is more favoured to use in industrial process due to faster reactions [30]. Metal alkoxides and alkaline hydroxides are common alkaline that used in esterification process. In this study, two different base catalyst including

sodium hydroxide and N-bromosuccinamide (NBS) were used as catalyst for conversion of FFA to FHA. Fig. 2 shows the solution of FHA-metal complex using NBS and NaOH as the catalysts. As it has shown there is a change of blue colour after it has been left overnight in lab. While colour changes of FHA-metal complex using NaOH catalyst was not consistent, it changes back to intermediate colour (green-blue colour) after a short time. Therefore, NBS catalyst was more favoured in this synthesis because it is inexpensive, commercially available and doing stable complexation reaction.


Fig. 2. Solution of FHA-metal complex using NBS and NaOH catalysts after left overnight. Reaction condition: reaction time 45 min, temperature 70 °C, agitation 150 rpm

**Effect of reaction time:** Previous study was reported that a maximum conversion of FHA from palm oil by enzymatic synthesis take 30 h [29], 36 h [31] and 72 h [32]. May [33] reported that recovery percentage for RBD palm oil using sodium hydroxide and potassium hydroxide are 98 % within 16 to 32 min of reaction time. In this study, the reaction time for complexation of FHA with V(V) was run in the interval of 5 to 45 min. Fig. 3 shows the changes of maximum absorbance in different reaction times. Hence, 5 min reaction was chosen as the optimum due to no difference in absorbance at longer reaction time.

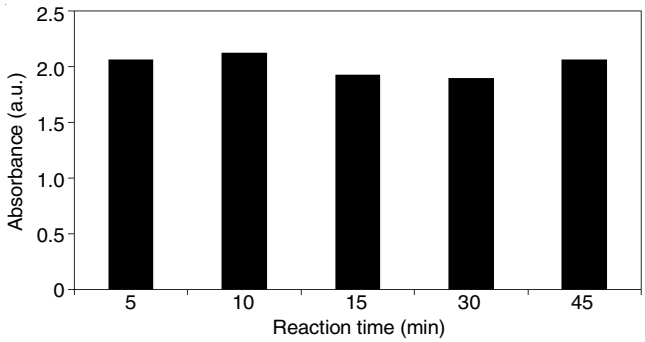


Fig. 3. Effect of complexation reaction time on the absorbance of FHA-vanadium complex at 430 nm for intervals of 5, 10, 15, 30 and 45 min. Reaction condition: 1 % NBS catalyst, temperature 70 °C, agitation 150 rpm

**Effect of reaction temperature:** The effect of reaction temperature was also investigated on the complexation reaction of FHA with V(V). In this study, reaction temperature was varied in the range of 40 to 90 °C. Feuge and Gros [34] reported that 50.5 °C was optimum temperature for ethanolysis of peanut oil with sodium hydroxide (molar ratio 6:1) as catalyst. As it has shown in Figs. 4 and 5, the complexation reaction at 70 to 90 °C gave an intense colour change before and after

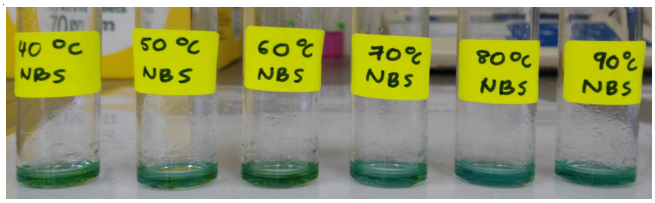


Fig. 4. Fatty hydroxamic acid complexation after 30 s mixed with V(V) solution. Reaction condition: 1 % NBS catalyst, temperature 40 to 90 °C, agitation 150 rpm

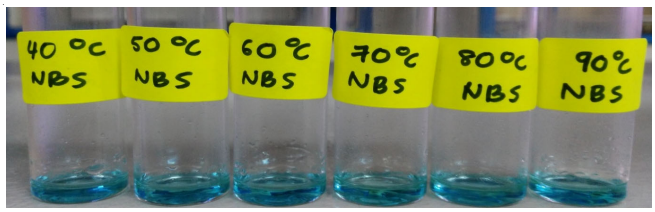


Fig. 5. Fatty hydroxamic acid complexation after left overnight. Reaction condition:1 % NBS catalyst, temperature 40 to 90 °C, agitation 150 rpm

being left overnight. The absorbance of solution after complexation at different temperatures has been shown in Fig. 6. The figure shows a maximum absorption at 70 °C compare to other reaction temperatures. Hence, 70 °C was chosen as the optimum reaction temperature for complexation reaction of FHA with V(V).

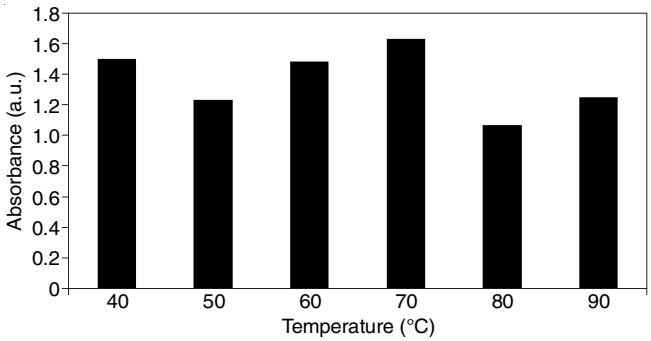


Fig. 6. Effect of complexation reaction temperature on the absorbance of FHA-V(V) complex in the range of 40-90 °C. Reaction condition: 1 % NBS catalyst, temperature 70 °C, agitation 150 rpm

Table-1 shows optimized parameters for spectrophotometric determination of FFA content in CPO based on catalytic hydroxylaminolysis of crude palm oil and complexation with V(V). This developed method uses less solvent instead of titrimetry method which consumes large volume of solvent. Hence, this developed method is an environmental friendly and greener method.

TABLE-1
OPTIMUM CONDITIONS OF HYDROXYLAMINOLYSIS REACTION OF FFA TO FHA AND COMPLEXATION REACTION WITH V(V)

| Parameters | Condition |
|---|---|
| Base catalyst | N-Bromosuccinamide (NBS) |
| Reaction time | 5 min |
| Reaction temperature | 70 °C |
| Metal ion | V(V) |

Fig. 7 shows a calibration curve based on the derivate spectrum of FHA-V(V) complex against percentage of FFA content in CPO under optimum conditions. The calibration equation is linear in the range of 1.23 to 6.47 % of FFA in CPO with the equation of $y = 0.00223x + 0.0245$ ($R^2 = 0.969$). This developed method is able to detect below 1.4979 % of FFA in CPO.

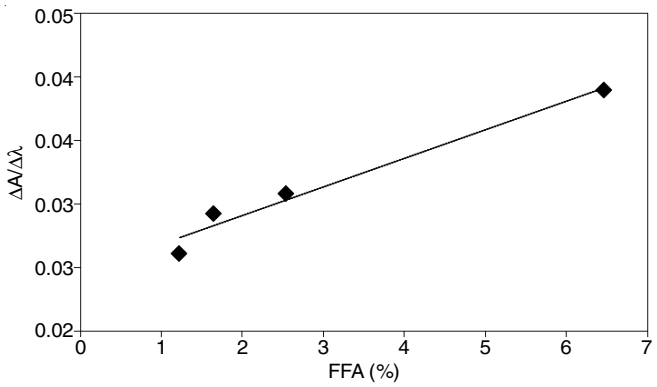


Fig. 7. Calibration curve based on the derivative spectrophotometric at wavelength of 402 nm

**Validation study for the proposed method:** Fig. 8 shows a good correlation between developed method and standard method (manual titration) with correlation coefficient of 0.994. Paired t-test was used to determine the significance difference between these two methods. According to paired t-test calculation, there is no significant difference between two methods at 95 % confidence level (Table-2) [35].

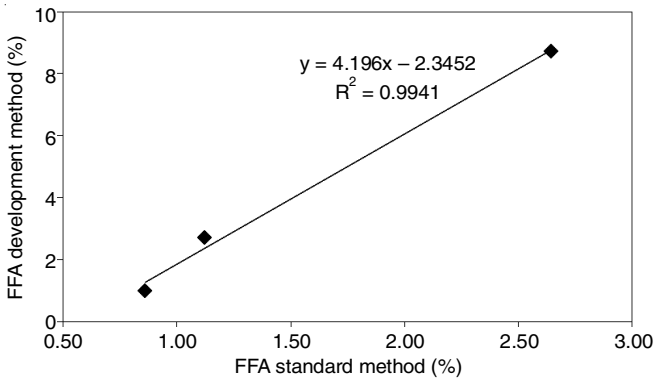


Fig. 8. Correlation between developed method and standard method (titration method) for determination of FFA in CPO

TABLE-2
PAIRED t-TEST ANALYSIS FOR COMPARATIVE STUDY OF PROPOSED METHOD

| Method | Correlation between develop method with standard method |
|---|---|
| Regression equation | Y = 4.196x – 2.345 |
| Correlation coefficient, | 0.994 |
| Paired t-test | |
| t-calculated | 1.447 |
| t-tabulated (degree with freedom: 2) | 4.303 |
| Conclusion | $t_{calc} < t_{table}$, hence there is no significant difference between two methods at 95 % confidence level |

## Conclusion

A simple FHA synthesis using base catalyst has been developed for spectrophotometric determination of FFA in crude palm oil based on formation of metal complex between FHA and V(V). The overlapping spectra of FHA-V(V) and V(V) alone is completely resolved by first derivative method at 402 nm. All important parameters for FHA synthesis had been successfully optimized (reaction time of 5 min at 70 °C in the presence of N-bromosuccinamide catalyst by shaking the mixture at 150 rpm). This developed method is found suitable for higher acidity value of CPO samples. A reasonable good correlation was found between developed method with standard method in determination of FFA in CPO.

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