



# Analysis of $\beta$ -Carotene Content in Crude Palm Oil from PT. REA Kaltim using Transesterification and Esterification Methods with UV-Vis Spectrophotometry

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## Abstract

Crude Palm Oil (CPO) is a major source of vegetable oil containing bioactive compounds such as beta-carotene, which significantly influences the quality and nutritional value of palm oil. The beta-carotene content may change during chemical processes such as esterification and transesterification, commonly applied in biodiesel production. This study examines the effect of these processes on the beta-carotene content of CPO from PT. REA Kaltim. The esterification process utilized 0.225% sulfuric acid catalyst based on oil volume, while transesterification employed 80% sodium methoxide catalyst. Parameters observed include acid value, saponification value, and beta-carotene concentration determined using UV-Vis spectrophotometry. The analysis provides insights into the effectiveness of both chemical processes on beta-carotene stability and supports efforts to enhance the quality and added value of palm oil derivatives in the vegetable oil industry.

**Keywords:**  *$\beta$ -carotene, Crude Palm Oil, Esterification, Transesterification, UV-Vis Spectrophotometry.*

## 1. Introduction

Crude Palm Oil (CPO) is one of the primary products of the palm oil industry and plays a vital role in Indonesia's national economy. As the world's largest producer and exporter of Crude Palm Oil, Indonesia contributes approximately 58% of total global production [1]. In addition to its high economic value, Crude Palm Oil contains various bioactive minor components, one of which is  $\beta$ -carotene, a natural pigment responsible for the reddish-orange color of palm oil and a precursor of vitamin A.

$\beta$ -carotene possesses important physiological functions, acting as a strong antioxidant and preventing vitamin A deficiency in humans. However,  $\beta$ -carotene is highly sensitive to heat, oxygen, and light, making it prone to degradation during oil refining processes such as bleaching and deodorization. Therefore, quantitative analysis of  $\beta$ -carotene content in Crude Palm Oil is essential to ensure the product's quality and stability.

Palm oil is among the most widely produced and consumed vegetable oils globally and contains significant biomolecules such as carotenoids, tocopherols, and phytosterols that play essential roles in human nutrition and health. These bioactive compounds, including  $\beta$ -carotene, act as antioxidants and precursors of vitamin A, contributing to the therapeutic and nutritional value of palm oil [2].

A study conducted at the Indonesian Oil Palm Research Center (PPKS) in Medan reported that  $\beta$ -carotene concentrations in several Crude Palm Oil samples ranged from 330 to 549 ppm using UV-Visible spectrophotometry based on the Malaysian Palm Oil Board (MPOB) standard method [3]. The findings revealed significant variation in  $\beta$ -carotene levels depending on processing conditions, with some samples showing concentrations below the Codex Alimentarius standard range of 500–2000 ppm, indicating potential quality issues in locally produced Crude Palm Oil.

The carotenoid content in Crude Palm Oil generally ranges between 500–800 ppm, with  $\beta$ -carotene accounting for approximately 90% of the total carotenoids. UV-Visible spectrophotometric analysis at a wavelength of 446 nm showed  $\beta$ -carotene concentrations as high as 867.98 ppm and as low as 542 ppm. These variations are influenced by factors such as heating temperature, processing duration, and the



ripeness of the fresh fruit bunches (FFB). Higher processing temperatures increase the likelihood of  $\beta$ -carotene degradation due to isomerization from the trans- to the cis-form, which results in a decrease in concentration [4].

$\beta$ -carotene levels in Crude Palm Oil ranged from 337.33 ppm to 653.19 ppm [5]. The ripeness of the fruit, processing temperature, and storage conditions were identified as major factors affecting  $\beta$ -carotene stability. Higher  $\beta$ -carotene content indicates that optimized processing conditions can maintain palm oil quality within the Special Prime Bleach (SPB) category.

Furthermore, Popang et al. reported that the olein fraction of Crude Palm Oil contains a relatively high concentration of  $\beta$ -carotene, with an average value of 592.00 ppm after vacuum heating treatment at 70°C. The high  $\beta$ -carotene content correlates with the reddish color of the olein fraction and demonstrates significant antioxidant activity. Heating temperature plays a crucial role in  $\beta$ -carotene stability, as excessive heat accelerates its degradation. Therefore, temperature control during oil separation and refining processes is a key factor in maintaining Crude Palm Oil quality [6].

Research on red palm oil by Marliyati and Harjanti also revealed that  $\beta$ -carotene content could reach up to 214.13 mg/kg, contributing to its deep red color and strong antioxidant activity. The variation in  $\beta$ -carotene concentration among different palm oil types highlights the importance of process parameter control to preserve this active compound during production and storage stages [7].

The accuracy and reproducibility of  $\beta$ -carotene quantification are highly dependent on the sample preparation technique used. Two commonly applied approaches are esterification and transesterification, which serve to separate carotenoid compounds from the triglyceride fraction using solvents such as ethanol. The selection of an appropriate preparation method is essential for obtaining accurate analytical results, as it directly affects the stability of  $\beta$ -carotene during the analysis.

PT REA Kaltim Plantations is one of the major palm oil companies in East Kalimantan, Indonesia, that has implemented sustainable plantation management practices certified by the Roundtable on Sustainable Palm Oil (RSPO) and the Indonesian Sustainable Palm Oil (ISPO) schemes. The company manages an area of 69,071 hectares, of which 35,873 hectares are cultivated, and 31,729 hectares consist of mature, actively producing oil palm trees. Its annual production of Crude Palm Oil and Crude Palm Kernel Oil (CPKO) exceeds 252,000 tons, supported by palm oil mills with a processing capacity of 80 tons of Fresh Fruit Bunches (FFB) per hour [8].



**Fig 1.** Crude Palm Oil from PT REA Kaltim

This study aims to analyze the  $\beta$ -carotene content in Crude Palm Oil produced by PT REA Kaltim using a combination of transesterification and esterification methods, followed by quantification through UV-Visible spectrophotometry. The objective of this research is to determine the most efficient and accurate analytical method, which can serve as a reference for the palm oil industry in Crude Palm Oil quality control and contribute to enhancing the nutritional value of its derivative products.

## 2. Methodology

### 2.1 Data Collection and Raw Material Analysis

The raw material utilized in this research was Crude Palm Oil (CPO) obtained from PT. REA Kaltim. The Crude Palm Oil was refrigerated until solidification occurred. The solidified Crude Palm Oil was then pressed using a hydraulic press to separate it into olein (liquid fraction) and stearin (solid fraction), both of which were subsequently analyzed. Additional chemicals used included ethanol, methanol, chloroform, potassium hydroxide (KOH), sulfuric acid ( $H_2SO_4$ ), hydrochloric acid (HCl), and n-hexane.

### 2.2 Equipment and Experimental Site

The equipment employed in this study comprised a spectrophotometer (model MLR-ECG-100, 100 W power), a hydraulic press, a DLAB MS-H280-Pro hot plate magnetic stirrer, an Ohaus Pioneer analytical balance, a distillation apparatus, a thermometer, a separatory funnel, cuvettes, beakers, burettes, stirring rods, spatulas, graduated cylinders, dropper pipettes, volumetric pipettes, volumetric flasks, and Petri dishes. The entire experimental work was conducted in the Chemical Engineering Laboratory, Faculty of Engineering, Mulawarman University.

## 2.3 Research Variables

The independent variables in this study included the concentration of sulfuric acid catalyst, set at 0.225% of the oil volume during the esterification stage, and the sodium methoxide catalyst added during the initial transesterification stage. These parameters were varied to evaluate their influence on the acid value and saponification value of the resulting biodiesel. The dependent variable observed was the  $\beta$ -carotene content in the Crude Palm Oil (CPO).

## 2.4 Determination of Acid Value and Free Fatty Acid Content

The acid value is defined as the amount of potassium hydroxide (KOH), in milligrams, required to neutralize the free fatty acids present in one gram of oil. The analytical procedure was as follows:

- Approximately 5 g of olein was weighed into a 250 mL Erlenmeyer flask.
- A total of 100 mL of a previously neutralized solvent mixture was added to the flask.
- While stirring vigorously, the mixture was titrated with alcoholic KOH solution until the pink coloration reappeared, matching the color intensity of the neutralized solvent mixture. The pink color was maintained for at least 15 seconds.
- The titrant volume (V mL) was recorded.
- The acid value (AV) was calculated using Equation (1):

$$AV = \frac{56.1 \times V \times N}{W} \text{ mg } \frac{\text{KOH}}{\text{g}} \text{ oil} \quad (1)$$

- The free fatty acid (FFA) content was determined using Equation (2):

$$\%FFA = \frac{(V \times N) \times 256 \times 100\%}{\text{sample weight} \times 1000} \quad (2)$$

Where:

V = Volume of alcoholic KOH titrant (mL)

N = Exact normality of KOH solution

W = Mass of olein sample (g)

## 2.5 Esterification Process

Since the feedstock exhibited an acid value greater than 3 mg KOH/g oil, a pre-treatment step via esterification was required. The esterification process was performed as follows:

- The oil sample was weighed and its acid value (W oil) was recorded.
- The oil was placed in the reactor, and a condenser was attached.
- Methanol was added, and the mixture was heated with continuous stirring to 50°C.
- Concentrated sulfuric acid catalyst was introduced, and the reaction time was recorded.
- The reaction was maintained at 65°C for 3 hours.
- The reaction mixture was transferred to a separatory funnel and allowed to stand until two layers formed. The upper layer contained methanol, while the lower layer contained biodiesel (or vice versa).
- The biodiesel layer was separated and reanalyzed for its acid value before proceeding to the transesterification stage.

## 2.6 Transesterification Process

The transesterification process was carried out when the feedstock's acid value was below 3 mg KOH/g oil. The procedure was as follows:

- The oil volume (V oil) was measured.
- The oil was placed into the biodiesel reactor, and a condenser was attached.
- The mixture was heated and stirred at 60°C.
- The methoxide catalyst (80%) was added, marking the start of the first transesterification stage (Trans 1).
- The reaction was maintained at 60°C for 1 hour.
- The mixture was transferred to a separatory funnel to form two distinct layers: the upper layer containing biodiesel and the lower layer containing glycerol.
- The biodiesel phase was reheated to 60°C.
- The remaining 20% methoxide catalyst was added, marking the second transesterification stage (Trans 2).
- The reaction was maintained for another hour at 60°C.
- The mixture was again separated into two layers; the upper layer contained biodiesel and the lower layer glycerol, which was subsequently removed.
- The biodiesel was washed several times with distilled water until the wash water reached a neutral pH (pH 7).
- The purified biodiesel was then dried under vacuum at 50–60°C until a clear appearance was obtained.

## 2.7 Determination of Saponification Value

The saponification value represents the number of milligrams of KOH required to saponify one gram of olein. The analytical procedure was as follows:

1. Weigh approximately  $4-5 \pm 0.005$  g of the alkyl ester biodiesel sample into a 250 mL Erlenmeyer flask. Add 50 mL of alcoholic KOH solution and allow it to drain naturally.
2. Connect the flask to an air-cooled condenser and gently boil the mixture until complete saponification occurs (approximately 1 hour). The resulting solution should be clear and homogeneous; if not, continue boiling until completion.
3. Once the solution has cooled slightly (but before gel formation), rinse the inner walls of the condenser with a small amount of distilled water. Remove the condenser, add 1 mL of phenolphthalein indicator, and titrate the contents with 0.5 N HCl until the pink colour disappears. Record the titration volume.

The saponification value (SV) was calculated using Equation (3):

$$SV = \frac{56.1(B-C)N}{W} \text{ mg } \frac{\text{KOH}}{\text{g}} \text{ biodiesel} \quad (3)$$

Where:

B = Volume of 0.5 N HCl used in the blank titration (mL)

C = Volume of 0.5 N HCl used in the sample titration (mL)

N = Exact normality of 0.5 N HCl solution

W = Mass of the biodiesel sample (g)

## 2.8 $\beta$ -Carotene Analysis Using UV-Vis Spectrophotometry

The  $\beta$ -carotene concentration was analyzed using UV-Visible (UV-Vis) spectrophotometry. UV-Vis spectroscopy is an analytical technique that utilizes ultraviolet and visible electromagnetic radiation to determine molecular absorption characteristics. The principle of UV-VIS spectrophotometry is based on the absorption of ultraviolet or visible light by molecules, resulting in electronic excitation from a lower to a higher energy level. The UV-Vis spectrophotometer measures light absorbance in the ultraviolet region (100–200 nm) and the visible region (200–700 nm), allowing quantitative analysis of  $\beta$ -carotene in the sample.

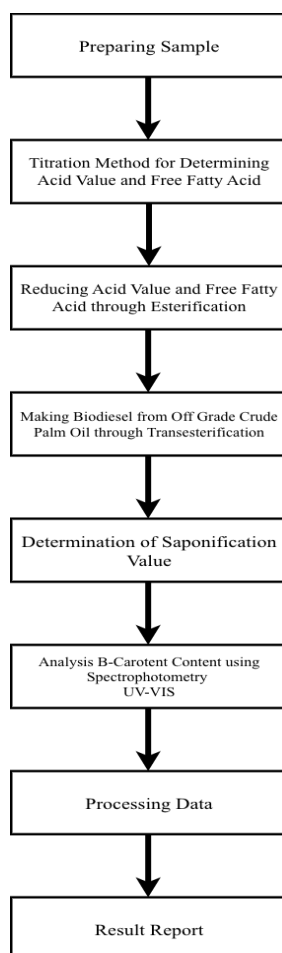


Fig 2. Research Flow Diagram



The Free Fatty Acid (FFA) content in Crude Palm Oil from PT REA East Kalimantan is 4.61%. This value indicates that the analyzed Crude Palm Oil is classified as Off Grade. The acid value of Crude Palm Oil from PT REA East Kalimantan is 10.1057 mg KOH/g biodiesel, which is still too high. Therefore, esterification is necessary to reduce the Free Fatty Acid level.

### 3.2. Reducing Acid Value and Free Fatty Acid Content through Esterification

The esterification reaction is a chemical process between a carboxylic acid and an alcohol to form an ester by converting free fatty acids contained in triglycerides into methyl esters, with water produced as a by-product. This by-product can be mitigated by using excess methanol, as the water formed will dissolve in methanol and will not inhibit the reaction. In biodiesel production, the addition of methanol shifts the reaction equilibrium toward the product side, resulting in a more complete conversion. The remaining methanol from each biodiesel production stage can be recovered and recycled to minimize operating costs and reduce environmental impact [15]. Therefore, the Free Fatty Acid content and acid value in Off-Grade Crude Palm Oil can be significantly reduced. The esterification method can lower the Free Fatty Acid content to below 5%. The key variables influencing Free Fatty Acid reduction and acid value in this evaluation are reaction time, methanol-to-oil ratio, and catalyst usage.

A temperature of 60 °C is considered efficient for biodiesel esterification, as temperature affects the equilibrium rate of the reaction. The optimal reaction time also contributes to the stability of the process. The esterification reaction using methanol as a reactant is deemed optimal, while sulfuric acid serves as an effective catalyst with good catalytic performance [13].

Esterification of Off-Grade Crude Palm Oil was conducted at a temperature of 50–65 °C for three hours. The catalyst used was sulfuric acid, with a methanol-to-oil ratio of 1:7.8 mL. The acid value decreased from 10.1057 mg KOH/g biodiesel to 1.3735 mg KOH/g biodiesel. The Free Fatty Acid content dropped from 4.61% to 0.63%. This reduction did not reach 5% because the amount of oil used in the esterification process was greater than that of methanol. Although the acid value and Free Fatty Acid percentage decreased, the reduction was not substantial since the molar ratio of oil/fatty acid to alcohol is a critical parameter that directly affects biodiesel yield and production cost. Stoichiometrically, one mole of alcohol reacts with one mole of fatty acid in esterification; however, to drive the reaction toward product formation, an excess amount of alcohol is required [16]. A lower acid value indicates an increase in  $\beta$ -carotene content, as the oil becomes purer and more stable after the free fatty acids are converted into esters [14].

### 3.3. Biodiesel from Off Grade Crude Palm Oil through Transesterification

Biodiesel is an alkyl ester compound produced through the transesterification process between triglycerides and alcohol in the presence of a basic catalyst, yielding alkyl esters and glycerol. The transesterification reaction of vegetable and animal oils is a reversible reaction [15]. The transesterification process is carried out when the acid value of the feedstock is less than 3 mg KOH/g biodiesel. However, transesterification tends to reduce the  $\beta$ -carotene content since this compound is easily decomposed by basic catalysts [17].

Several variables affect the biodiesel yield, including reaction time, temperature, catalyst concentration, and methanol-to-oil ratio. In this study, transesterification was conducted for one hour at 60 °C, using a methanol-to-oil ratio of 0.2:1 and alcoholic KOH catalysts of 20% and 80%. The resulting biodiesel yield was 37%.

The optimal reaction time for biodiesel formation through transesterification ranges from 1–2 hours, and the optimal temperature is around 65 °C. However, excessive use of basic catalysts in biodiesel production has drawbacks, as soap formation may occur during the reaction, and water produced as a by-product can reduce the biodiesel yield [18]. The stoichiometric ratio of methanol to triglycerides that provides optimal transesterification results is 12:1 [19]. In this study, however, less methanol was used, causing incomplete formation of alkyl esters and consequently a lower biodiesel yield.

### 3.4. Saponification Value Analysis

The saponification value is defined as the number of milligrams of KOH required to saponify one gram of oil. The lower the saponification value, the higher the average molecular weight of triglycerides present in the oil. Consequently, carotenoids in biodiesel are more prone to degradation.

In this study, the saponification value obtained was 108.834 mg KOH/g biodiesel. The standard saponification value for Crude Palm Oil ranges from 195–205 mg KOH/g biodiesel [20]. Based on this result, it can be concluded that the saponification value in this study is relatively low, indicating that the carotenoid content is reduced due to heating processes that break down  $\beta$ -carotene molecules and cause pigment degradation.

**Table 2.** Analysis of Beta Carotene Content in Crude Palm Oil

Treatment	B-carotene Content (PPM)	Reference
Fresh Crude Palm Oil (acid value 10,1057 mg KOH/gr oil)	531,8228	[14]
Esterification (acid value 1,3735 mg KOH/gr oil)	650,2792	[14]
Transesterification	522,9317	[17]
Saponification Value	236,3657	[20]

## 4. Conclusion

Fresh Crude Palm Oil (CPO) from PT REA East Kalimantan is classified as Off Grade, as it exhibits an acid value of 10.1057 mg KOH/g oil and a Free Fatty Acid (FFA) content of 4.61%. Its  $\beta$ -carotene content is also relatively low, at 531.8228 PPM. To increase the  $\beta$ -carotene content, it is necessary first to reduce the acid value and Free Fatty Acid percentage through the esterification process. After esterification, the acid value decreased to 1.3735 mg KOH/g oil and the Free Fatty Acid content to 0.63%. These reductions resulted in an increased  $\beta$ -carotene concentration of 650.2792 PPM.

Transesterification was carried out to observe the formation of alkyl esters. However, this process tends to reduce  $\beta$ -carotene levels because the compound is easily decomposed by heat and basic catalysts. The saponification value obtained in this study was 108.834 mg KOH/g biodiesel, which is lower than the standard Crude Palm Oil range of 195–200 mg KOH/g biodiesel. Consequently, the  $\beta$ -carotene content decreased after the saponification analysis due to heating, which breaks down carotenoid pigments.

The analysis of variance indicated that all examined parameters significantly influenced biodiesel conversion. Nevertheless, sulfuric acid used as the esterification catalyst could not be directly reused because its concentration dropped substantially after the initial cycle. Notably, the addition of fresh methanol to the catalyst maintained a conversion rate comparable to that of the first reaction. Comparative studies demonstrated that acid-catalyzed esterification of high-FFA feedstocks assisted by a homogenizer can be effectively performed at ambient temperature within a short reaction period.

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