

Towards Carotene-Retentive and Low-FFA Palm Oil: Evaluating Process Variables in Hybrid Acid-Enzymatic Degumming

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Abstract. The hybrid acid-enzymatic (HAE) degumming is an alternative method that offers a solution by reducing free fatty acid (FFA) formation and enhancing phospholipid removal. This study aims to assess the effect of HAE operating conditions on carotene content and FFA through complete randomized design (CRD). The variation of operating conditions included temperature (55, 75, 95, and 115°C), phosphoric acid concentration (1.3, 1.8, 2.2, and 2.7%v/v) and lipase enzyme concentration (0.5, 1, 1.5, and 2%v/v). *The hybrid acid-enzymatic (HAE) degumming is an alternative method that offers a solution* The results showed that increasing the temperature caused a decrease in carotene content 454.958 ppm and FFA content 0.723%. On increasing phosphoric acid in degumming, there was decreasing in carotene content to 453.855 ppm though FFA content was increased at 1.044%. The carotene degradation was minimal at 453.462 ppm, and the FFA content was also reduced to 0.5%. The kinetic studies revealed that β -carotene degradation and FFA removal during HAE degumming follow first-order kinetics. β -carotene demonstrated higher temperature sensitivity (E_a 19.6 kJ/mol) compared to FFA (E_a 12.0 kJ/mol), indicating that carotene stability is key factor of process optimization. Future research should focus on optimizing the HAE process through kinetic and thermodynamic analysis to enhance carotene retention while minimizing FFA.

1 Introduction

Indonesia stands as the principal global producer of palm oil, with 15.9 million hectares allocated for plantations and an annual production of 47 million tons of crude palm oil. East Kalimantan demonstrated significant growth in palm oil production, with an increase in land area of 14.6% to 1.49 million hectares and a rise in CPO production of 35.7% to 3.8 million tons, thereby securing its position as Indonesia's third most productive province [1] in 2023. This elevated productivity has catalysed research focused on local palm oil innovations, particularly in enhancing CPO processing for improved quality of derivative products.

CPO is composed mainly of triglycerides (94–96%), along with diglycerides (2–4%), FFA (3–5%), and a minor fraction (1%) of bioactive compounds like tocopherols and carotenoids [2]. The key carotenoids observed in CPO are β -carotene (56%) and α -carotene (35%), which play a vital role in the oil's reddish appearance [3]. The supplementation of palm oil, abundant in vitamins A and E—renowned antioxidants—has been effective in diminishing stunting rates among Indonesian children [4]. Nonetheless, for CPO to be utilized in various products, it requires refinement to eliminate impurities such as phospholipids and non-triglyceride compounds that may affect oil quality, ensuring compliance with edible oil standards.

The cooking oil sector necessitates that refined oil demonstrates transparency, the absence of any discernible odor, and neutral flavour profile. The traditional bleaching earth method employed to attain these qualities frequently diminishes natural antioxidant like carotenoids, tocopherols, and tocotrienols [5]. Mitigating this concern, advancements in CPO refining are essential to efficiently eliminate impurities while retaining carotenoid levels—potentially via chemical degumming synthesis.

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Degumming is defined as the operation of extracting gums from CPO, subsequently producing Degummed Red Palm Oil (DRPO). This can be achieved via physical or chemical methods. Chemical degumming, utilizing strong acids or enzymes, is the most prevalent and effective method. Acid degumming typically employs phosphoric and citric acids as reducing agents. However, acid degumming often leaves residual gums, fails to effectively reduce FFA levels, and diminishes carotenoid content. Therefore, process optimization is necessary. [6]. Conversely, enzymatic degumming utilizes enzymes like phospholipases and lipases as biocatalysts to facilitate the breakdown of FFA into gum.

Prior research demonstrates that enzymatic degumming employing phospholipase surpasses lipase in the reduction of impurities, achieving efficiencies as high as 89.42% [7]. However, phospholipase is challenged by factors such as increased manufacturing costs and lowered stability in operational temperature ranges. As a result, scholarly investigations have transitioned towards more economically viable and thermally stable enzyme substitutes, such as lipase, which also exhibits potential for degumming. Lipase-based degumming may mitigate oil degradation resulting from excessive heat [8]. Improving impurity extraction in CPO, a hybrid approach that integrates acid and enzymatic treatments can yield red palm oil (RPO) with reduced contaminants. The impact of phosphoric acid and enzyme incorporation in the degumming process is assessed utilizing a Completely Randomized Design (CRD).

The CRD was chosen to effectively assess the effects of temperature, acid concentration, and enzyme concentration on oil quality. This study aims to evaluate RPO quality post-degumming according to the Indonesian National Standard (SNI), investigate the effects of the three variables on carotenoid content and FFA level via CRD analysis, and evaluate the significance of the Hybrid Acid-Enzymatic Degumming method on carotenoid retention through ANOVA using Minitab 18.

2 Material & Methods

The research was conducted in Process Technology Laboratory and Characterization Laboratory Institut Teknologi Kalimantan. The main raw material used was CPO procured via e-commerce platforms. The independent variables in this study were lipase enzyme powder KDN 99% 100.000 U/G Cas Number 9001-62-1 from China with concentrations of 0.5, 1.0, 1.5, and 2.0% v/v, and food-grade phosphoric acid (H_3PO_4 , 85%) at concentrations of 1.3, 1.8, 2.2, and 2.7% v/v. Supporting chemicals included sodium hydroxide (NaOH), n-hexane (C_6H_{14}) PA, propanol (C_3H_8O) PA, ethanol, and distilled water. Most chemical reagent from supplier in East Kalimantan.

The lipase enzyme activity was not independently assayed prior to experimentation and was based on the supplier's Certificate of Analysis (100,000 U g^{-1}). The enzyme was stored and handled according to the manufacturer's recommendations to minimize potential activity loss. The adequacy and consistency of enzymatic performance were evaluated indirectly through process outcomes, particularly free fatty acid (FFA) reduction and carotene retention, which were assessed in accordance with SNI 7709:2019 for palm cooking oil. The equipment utilized included 250–500 mL Iwaki beakers, 200 mL Iwaki Erlenmeyer flasks, dropper pipettes, 10 mL and 25 mL Iwaki volumetric pipettes, suction bulbs, watch glasses, cloth filters, 15 mL centrifuge tubes, a hot plate, analytical balance, alcohol thermometer (110°C), aluminum foil, tabletop centrifuge, 25 mL and 100 mL Iwaki volumetric flasks, 5 cm stirrer bars, 10 mL Pyrex pycnometers, cuvettes, 50 mL Iwaki burettes, clamps, stands, UV-Vis spectrophotometer single beam, universal pH paper, and other supporting laboratory instruments.

2.1 Raw Material Preparation

The initial treatment involved heating the CPO to achieve a homogenous liquid phase, as CPO is typically solidified at room temperature with a melting point of (21–24°C). A 100 mL sample of CPO was heated at 60°C for 20 minutes until no solid residues remained. Once the CPO was fully homogenized, the degumming process could proceed.

2.2 Acid Degumming Process

This procedure entails the elimination of impurities, specifically phosphatides, via a methodical approach involving heating, stirring, and the integration of phosphoric acid at concentrations between (1.3–2.7 %v/v) based on established parameters. Four distinct treatment conditions were formulated to ascertain the most effective degumming scenario. The consistent parameters for this procedure included a stirring velocity of 250 rpm for a duration of 30 minutes, with heating performed under aluminium foil to avert contamination.

The acid degumming process, an advanced purification technique, began with the incorporation of food-grade 85% phosphoric acid (H_3PO_4) into the crude palm oil (CPO). The composition was agitated for 30 minutes at the designated temperature and acid concentration, facilitating the binding of phosphoric acid with the phosphatides present in the oil. Upon completion of the 30 minutes agitation, the gum settled at the bottom of the container. Subsequently, the mixture was permitted to cool for 3 minutes, gum was separated then transferred into enzymatic flask process.

2.3 Neutralization and Enzymatic Degumming Procedure

The enzymatic degumming process utilizing lipase enzyme encompassed several systematic procedures. It commenced with a neutralization phase, intended to eliminate FFA through a reaction with a base to produce soap. Sodium hydroxide (NaOH) was incorporated at 10% of the sample's weight to achieve a pH of 5. This neutralization phase not only optimized the pH but also aided in FFA elimination via saponification, resulting in the precipitation of soap alongside phospholipids and other impurities [9].

Upon attaining pH 5, the enzymatic degumming was executed by the introduction of lipase enzyme and heating of the NaOH-treated CPO. The concentrations of the enzyme and temperature were adjusted following the specified experimental design. The mixture was agitated at 250 rpm for 30 minutes to improve phosphatide binding efficacy. The separation of oil and precipitated phosphatides was accomplished through centrifugation. Subsequent to enzymatic treatment, the sample was allowed to cool for 10 minutes prior to centrifugation at 3000 rpm for 30 minutes. This operation produced two fractions: lighter fraction comprising RPO and a heavier fraction containing gum and soap [10]. The RPO was subsequently transferred to a sterile container for further examination.

2.4 Carotenoid Content Analysis

The carotenoid content was assessed utilizing the adsorption method outlined in the MPOB Test Method p.2.6 [11] with a UV-Visible spectrophotometer. A precise measurement of 0.1 grams of RPO was conducted and subsequently placed into a 25 mL volumetric flask. The flask was subsequently filled to the calibration line with n-hexane and thoroughly mixed. The absorbance of the resultant solution was meticulously recorded at a wavelength of 446 nm utilizing a UV-Vis spectrophotometer. The carotenoid concentration (in ppm) was determined employing the specified equation:

$$\beta - \text{Carotene (ppm)} = \frac{25 \times A \times 383}{W \times 100} \quad (1)$$

2.5 FFA Determination

The FFA content was assessed via a modified titration method [12]. A 5-gram sample of RPO was prepared by heating to 50°C. This sample was subsequently mixed with 50 mL of propanol, to which three drops of phenolphthalein indicator were included. The mixture underwent titration with a 0.1026 N NaOH solution until the endpoint was accomplished. The FFA content was expressed as palmitic acid equivalent, utilizing a molecular weight of 256. The FFA percentage was calculated through a specific equation:

$$FFA (\%) = \frac{V \times N \times Mw \times 100}{W \times 1000} \times 100\% \quad (2)$$

3 Result and Discussion

3.1 Functional Analysis and Effectiveness Hybrid Acid-Enzymatic (HAE) Degumming CPO

The results of the initial analysis of untreated CPO are presented in control **Table 1**. The high concentration of bioactive compounds, especially carotenoids, imparts the characteristic orange hue of CPO and serves as a natural antioxidant beneficial to human health. However, during processing, carotenoid content often decreases, in some cases falling below the minimum threshold defined by the Indonesian National Standard (SNI) [13] for edible oil.

Table 1. HAE Technology Running Tabulation vs Functional Analysis Result

| Run | Factor | | | Average Triplicate | |
|----------------|-----------------|----------------------------|------------------------------|-------------------------|------------------|
| | Temperature (C) | Acid Concentration (% v/v) | Enzyme Concentration (% v/v) | β -carotene (ppm) | FFA (%) |
| 1 | 55 | 1.3 | 0.5 | 516.65 \pm 3.95b | 1.35 \pm 0.01a |
| 2 | 75 | 1.3 | 0.5 | 496.01 \pm 4.72b | 1.22 \pm 0.07a |
| 3 | 95 | 1.3 | 0.5 | 465.69 \pm 3.69b | 1.07 \pm 0.03a |
| 4 | 115 | 1.3 | 0.5 | 454.96 \pm -a | 0.84 \pm 0.16a |
| 5 | | 1.3 | 0.5 | 455.24 \pm 0.15a | 0.84 \pm 0.16a |
| 6 | | 1.8 | 0.5 | 454.59 \pm 0.14a | 0.89 \pm 0.1a |
| 7 | | 2.2 | 0.5 | 454.46 \pm 0.12a | 0.89 \pm 0.03a |
| 8 | Best | 2.7 | 0.5 | 454.15 \pm 0.42a | 0.98 \pm 0.1a |
| 9 | Temperature | | 0.5 | 454.15 \pm 0.49b | 0.95 \pm 0.13b |
| 10 | | Best Acid | 1 | 454.12 \pm 0.53b | 0.79 \pm 0.1b |
| 11 | | Concentration | 1.5 | 453.96 \pm 0.59b | 0.61 \pm 0.1b |
| 12 | | | 2 | 453.87 \pm 0.58b | 0.52 \pm 0.03b |
| Control Sample | | | | 552.158 | 2.506 |

*Data are expressed as means \pm standard deviation from three independent trials. Means "a" letter in the same column reflect significant differences at $p < 0.05$.

CPO comprises elevated levels of FFA and acid number, which may result in oil degradation and a diminished shelf life [14]. FFA, as a non-triglyceride constituent, is susceptible to autooxidation at ambient temperatures. The interaction of oxygen with free fatty acids results in the formation of intricate oxidation products, leading to undesirable flavours and rancidity. Thus, efficient refining is crucial to adhere to industry standards and preserve valuable bioactive compounds.

The hybrid degumming process employs various techniques to improve refining efficiency. This research amalgamated acid and enzymatic degumming approaches. **Table 1** presents the analysis of CPO composition pre- and post-treatment. The carotenoid content is a vital metric of CPO quality, attributed to its pigmentation and antioxidant properties. It processes proved highly effective in maintaining carotenoid levels. The two-stage purification, executed at moderate temperatures, mitigated substantial carotenoid loss while efficiently eliminating phospholipids. Following degumming, carotenoid concentrations reduced from 584.075 ppm to 519.444–453.462 ppm. SNI the required minimum carotenoid content in edible palm oil is 30 ppm, indicating a yellow-orange hue. Consequently, the oil post-treatment significantly exceeds the stipulated standard.

Hybrid degumming effectively reduces carotenoid loss and removes non-triglyceride components through a dual-step method. The presence of FFA, a key impurity, critically impacts oil quality for cooking. Thus, it's essential to remove FFA efficiently. This research indicated an FFA reduction from 2.5% to between (1.359–0.5%) after applying combined acid and enzymatic degumming. While FFA levels remained above the 0.3% limit set by SNI [13] for edible palm oil, the hybrid method achieved better results than traditional techniques. [15] study using conventional acid degumming with (0.5–2%) phosphoric acid lowered FFA from 1.42% to 0.7% over two hours at 120°C. Increased acid concentrations and temperatures enhanced FFA removal but led to greater carotenoid loss. Conversely, this study's hybrid degumming process reached FFA levels of 0.5% with less than 2% acid and temperatures under 120°C, preserving more carotenoids.

Analysis after treatment showed changes in oil quality in three areas, as detailed in Table 2. The degumming process led to 17.8% decrease in carotenoid content. Despite this drop, final carotenoid levels remained significantly above the SNI limit. FFA levels also declined but were still marginally above regulatory standards. To comply with regulations, an additional refining step, like deodorization, is suggested to further reduce FFA.

Table 2. Comparison of Oil Quality Before and Post HAE Treatment

| Parameter | Before | After | SNI |
|------------------|---------|---------|-------|
| β-carotene (ppm) | 552.158 | 453.462 | > 30 |
| FFA (%) | 2.506 | 0.5 | < 0.3 |

3.2 Analysis of the Effect Temperature, Acid and Enzyme Concentration on Degummed Red Palm Oil (DRPO) Carotene Content from the HAE Technology

Three primary factors affecting carotene content in CPO were heating temperature, phosphoric acid concentration, and lipase enzyme dosage during degumming. The impact of each variable is detailed below.

3.3 Effect of Heating Temperature on DRPO Carotene Content

Temperature variation was utilized to assess the impact of heating on the degumming of CPO. The temperatures examined were 55, 75, 95, and 115°C, with phosphoric acid and lipase enzyme concentrations held constant at 1.3% and 0.5% v/v, respectively. Heating significantly contributed to the decrease in carotene levels in CPO. The specific effects of temperature are illustrated in Figure 1.

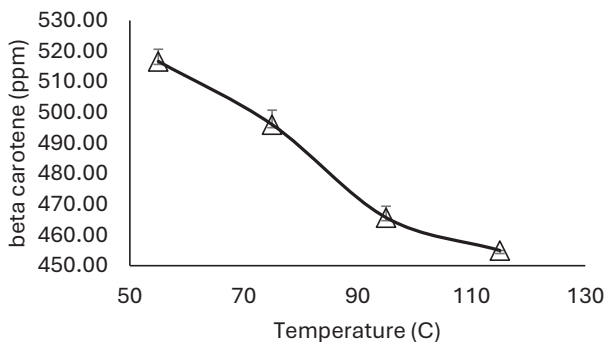


Figure 1 HAE Heating Temperature Data Trend vs Carotene Content

High-temperature heating during degumming may cause oxidation and generate free radicals, leading to a significant decline in antioxidant levels [16]. Carotenoids and various phytonutrients contain reactive conjugated double bonds, thus making them liable to oxidation and degradation in the presence of oxygen, light, and heat [3]. Consequently, the reduction of carotene content during heating adversely affects the overall quality of CPO.

Carotene degradation increases with temperature during degumming. Significant degradation occurs at 55°C and 75°C, ranging from (5.9–10.7%). Degumming below 80°C typically results in a carotene loss of 5–15%. Pratiwi (2020) documented a 12% degradation at 60°C. At 95°C and 115°C, degradation escalates to (15–17.6%). Heating beyond 80°C can lead to a (25–50%) degradation rate. [10] found that heating CPO at 90°C for 20 minutes resulted in a 32% loss of carotene. The least degradation occurred at 55°C, demonstrating that lower temperatures enhance carotene retention. Carotene degradation correlates directly with temperature increases, as elevated temperatures expedite reaction rates. Carotene's multiple conjugated double bonds render it inherently unstable

and susceptible to auto-oxidation [17]. Interestingly, degradation at 95°C and 115°C was still less than previous studies. The reduced degradation may be due to the formation of stable carotene structures. Enzymes promote interactions between lipase and bioactive compounds, creating stable complexes that safeguard carotene. The enzyme-carotene binding serves as a barrier, preserving carotenoid structure [16]. Furthermore, enzymes may act as natural antioxidants that prevent carotene oxidation. Although higher temperatures generally increase degradation, they may also stabilize other oil components. [3] noted that heating at 150°C for 30 minutes led to only a 20% decrease in carotene content.

Heating at 55°C and 75°C effectively maintains carotene stability within an acceptable degradation range. This is consistent with prior research indicating that heating below 100°C for a maximum of 120 minutes does not considerably impair carotene, while heating at 200°C may result in a 59% loss of carotene, causing significant reductions. Despite the general observation that heating above 90°C leads to substantial carotene degradation, this pattern was not observed in the current study. The heat treatments at 95°C and 115°C resulted in only (10–17.6%) degradation, which remains within the carotene content criteria established by the Indonesian National Standard [18] for cooking oil.

Assessing the impact of temperature variation on carotene content, ANOVA was conducted. The results produced an F-value of 123.6 and p-value 0, demonstrating that the F-value surpasses the critical threshold ($p < 0.05$). Consequently, it is concluded that temperature variation during the degumming process significantly influences carotene content in CPO.

3.4 Effect of Phosphoric Acid Concentration on DRPO Carotene Content

The variation in phosphoric acid concentration was intended to determine the extent to which this factor influences the carotenoid content in CPO during the degumming process. To evaluate this effect, various phosphoric acid concentrations were applied 1.3, 1.8, 2.2, and 2.7 (%v/v)—at a constant temperature of 115°C and an enzyme concentration of 1.3%. The relationship between phosphoric acid concentration and carotenoid content is illustrated in Figure 2.

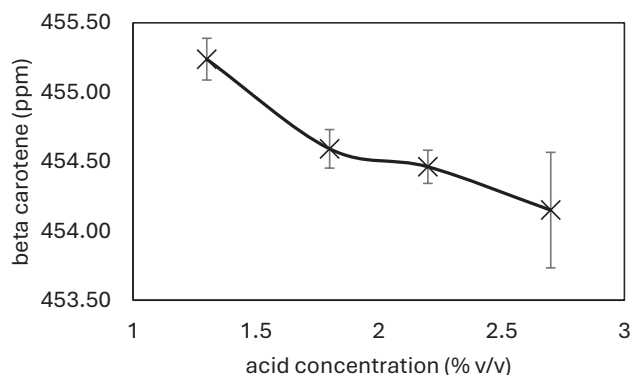


Figure 2 HAE Acid Concentration Data Trend vs Carotene Content

Phosphoric acid is utilized in the degumming process to transform phospholipids into separable gums. While its primary purpose is to lower phospholipid content, it may also influence carotenoid levels in CPO. According to Table 4.6, increased phosphoric acid concentration correlates with a decrease in carotenoid content, although not statistically significant. Rising 0.5% in phosphoric acid concentration leads to (0.3–0.11%) decline in total carotenoid content. At high concentrations (>1.5%), phosphoric acid may reduce carotenoid content by (15–20%). Phosphoric acid facilitates the protonation of carotenoid molecules by generating H⁺ ions, which compromise the carotenoid structure. As phosphoric acid concentration escalates, the quantity of H⁺ ions in the mixture increases. This rising in H⁺ ions initiates the protonation of carotenoids, evidenced by the cleavage of conjugated double bonds in their molecular structure [19].

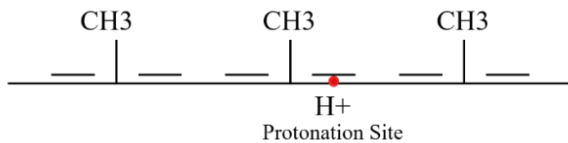


Figure 3 Degradation H⁺ ion

Figure 3 depicts the elongated carbon chain structure of carotenoids, characterized by conjugated double bonds and methyl groups. The introduction of phosphoric acid leads to H⁺ ions attacking the double bonds, resulting in structural instability due to the disruption of the conjugated system and the generation of smaller molecular fragments [3]. This protonation process converts carotenoids into aldehyde and ketone derivatives.

Assessing the statistical significance of phosphoric acid concentration on the carotenoid content in CPO, an ANOVA was performed. The analysis produced an F-value of 7.294, surpassing the p-value, thereby indicating that the employed variable model significantly affects the results, consistent with the trends observed in the graphical data.

3.5 Effect of Lipase Enzyme Concentration on DRPO Carotene Content

The study investigated the influence of lipase enzyme concentrations on carotenoid levels in CPO. Lipase acts as a biocatalyst to decrease FFA levels by transforming non-triglyceride compounds into more hydratable forms. While enzyme addition reduces FFA levels, it also aids in maintaining carotenoid retention in CPO. Figure 4 illustrates that higher enzyme concentrations led to noticeable carotenoid degradation.

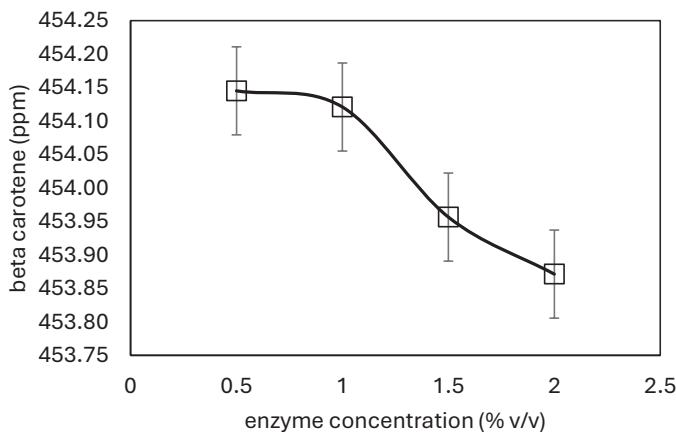


Figure 4 HAE Enzyme Concentration Data Trend vs Carotene Content

Enzymatic treatment is superior in preserving carotenoids compared to chemical methods, as enzymes effectively break down phospholipids without harming carotenoid molecules. Previous studies have reported that enzymatic degumming minimizes carotenoid loss by reducing the severity of thermal and oxidative stress compared to conventional chemical refining [9]. The results showed a minimal degradation of carotenoids at around 0.07%. Although lipase enzymes support carotenoid retention, degradation may still arise from oxidative conditions or enzymatic activity of naturally occurring enzymes in the oil. Carotenoid cleavage oxygenases (CCO), a category of enzymes present in CPO, exemplify this phenomenon [20]. CCO have subfamilies such as NCED (9-cis-epoxycarotenoid dioxygenases) and CCD (carotenoid cleavage dioxygenases), which can cleave

carotenoids, yielding various apocarotenoid compounds like β -citral, β -cyclocitral, and β -ionone [17]. Increasing enzyme concentration during degumming improves the conversion of phospholipids into FFA and promotes FFA binding while minimally affecting carotenoid content.

Evaluating enzyme concentration's impact on carotenoid retention, an ANOVA test was conducted. The results demonstrated p-value surpassing 0.05, indicating an absence of statistically significant impact of enzyme concentration on carotenoid degradation. This result aligns with [20], who asserted that the addition of enzymes does not directly degrade carotenoids. Rather, any observed degradation during degumming may result from the biosynthesis of endogenous enzymes within the crude palm oil itself.

3.6 Analysis of the Effect Temperature, Acid and Enzyme Concentration on Degummed Red Palm Oil (DRPO) FFA Content from the HAE Technology

FFA levels in RPO were assessed using an acid-base titration method based on colorimetric changes. The FFA content was expressed as palmitic acid [21]. FFA serves as an indicator of triglyceride hydrolysis, which evaluates the quality of CPO post-degumming. Per the Indonesian National Standard [13], an FFA content above 0.5% in oil can result in rancid odors and hasten oil degradation. This study seeks to examine the impacts of temperature, acid concentration, and enzyme concentration on FFA levels utilizing completely randomized design (CRD).

3.7 Effect of Heating Temperature on DRPO FFA Content

Based on the variable design using CRD, the effect of increasing heating temperature on FFA content was analyzed using a consistent combination of acid and enzyme concentrations across experiments.

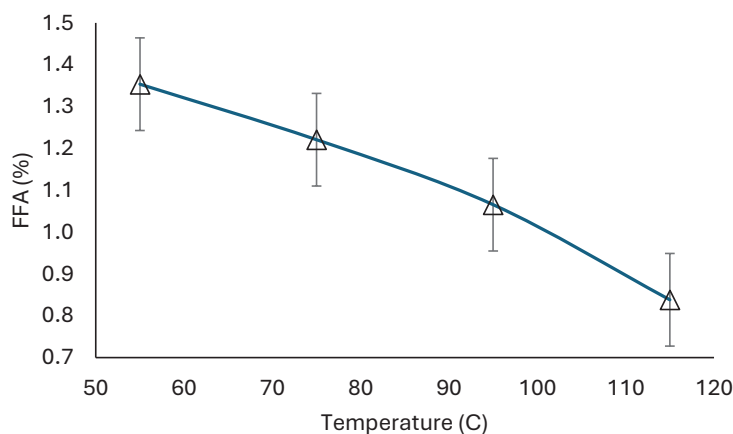


Figure 5 HAE Heating Temperature Data Trend vs FFA Value

As illustrated in Figure 5, heating temperature significantly influenced FFA levels in palm oil during the degumming process. The graph shows that a temperature of 55°C yielded the highest FFA content at 1.359%, whereas the highest temperature of 115°C resulted in the lowest FFA content at 0.723%. Chemically, increasing temperature during degumming accelerates the breakdown of complex bonds between phospholipids and FFA. At elevated temperatures, phospholipids decompose and bind with water molecules, facilitating their separation from the oil. [7] reported that degumming at 50°C reduced FFA from 1.42% to 1.00%, while at 80°C it decreased further to 0.76%. However, excessively high temperatures can also increase FFA content due to lipid hydrolysis [10].

3.8 Effect of Phosphoric Acid Concentration on DRPO FFA Content

During the degumming process, varying phosphoric acid concentrations were applied to analyze their effect on CPO FFA content. The results are shown in Figure 6.

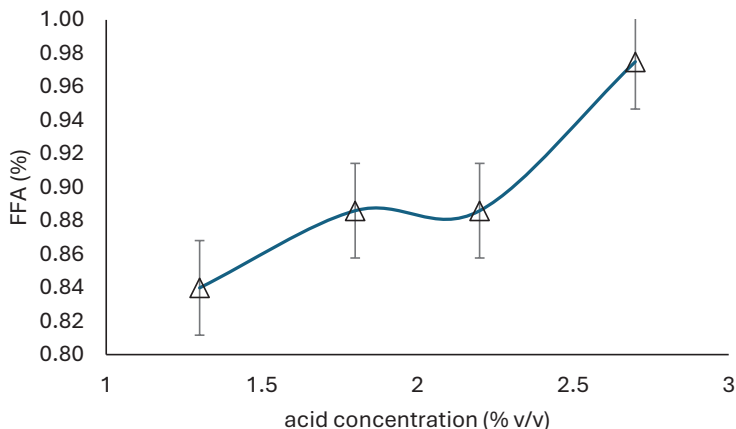


Figure 6 HAE Acid Concentration Data Trend vs FFA Value

The graph demonstrates that elevated phosphoric acid concentrations correlate with rising FFA levels in oil. At 1.3% phosphoric acid, the FFA content recorded was 0.955%, which increased to 1.044% at 2.7%. Degumming primarily focuses on gum removal but does not effectively eliminate FFA. The use of phosphoric acid in degumming is associated with increased FFA levels. Similar observations were made by [22], noting that 2% citric acid induced lipid hydrolysis and elevated FFA levels. [6] stated that FFA formation arises from hydrolysis reactions involving enzymes, water, and acid, yielding one glycerol molecule and three FFA. Additionally, free fatty acids may arise from the interplay of phospholipids and water, resulting in their release into the oil [8] noted that 0.1% phospholipids could yield 0.036% FFA. Phosphoric acid facilitates the binding of phospholipids, known for causing odors in oil, converting them into gums as illustrated by the subsequent reaction:



Phospholipids are intricate lipids comprising phosphate residues, fatty acids, and glycerol (alcohol) [8]. Adding acid raises FFA levels while also facilitating the elimination of phospholipids, consequently resulting in better oil quality and extended longevity. Over time, oil may absorb moisture and deteriorate due to proteins and other contaminants, resulting in further FFA production. Consequently, inadequate degumming exacerbates FFA accumulation during storage. Advanced processes such as neutralization and enzymatic degumming are essential for improved FFA and phospholipid removal [5]. In enzymatic degumming, preliminary phospholipid reduction is crucial for optimizing enzyme effectiveness.

Though degumming can eliminate gums and impurities, it is not the foremost technique for FFA reduction. The combination of acid degumming with neutralization is imperative. In neutralization, NaOH interacts with FFA to generate soap (NaCOO⁻), which can be easily separated from the oil. Nonetheless, this study noted a rise in FFA levels despite neutralization, likely due to inadequate NaOH concentration in relation to phosphoric acid. [23] exhibited that employing 1% acid and 1% NaOH resulted in 21.32% FFA, whereas increasing the acid concentration to 1.3% without a corresponding rise in NaOH elevated FFA to 21.87%. Simultaneously augmenting both acid and NaOH concentrations decreased FFA to 20.3%. The reaction is followed:



3.9 Effect of Lipase Enzyme Concentration on DRPO FFA Content

Various lipase enzyme concentrations were tested to assess their effect on CPO FFA content. As shown in Figure 7, enzyme application effectively reduced FFA content.

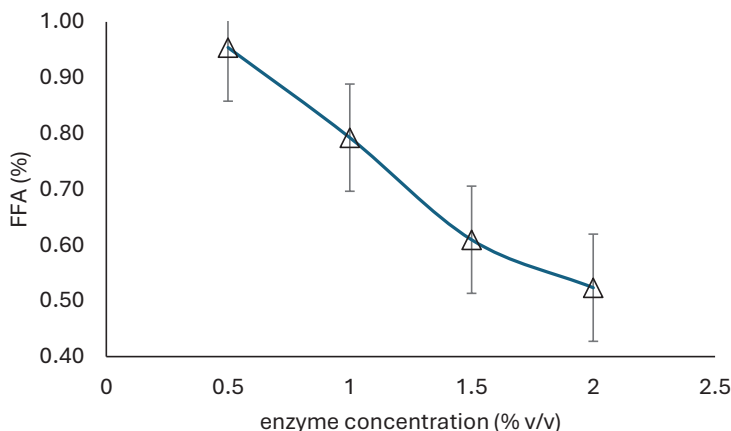


Figure 7 HAE Enzyme Concentration Data Trend vs FFA Value

Increasing enzyme concentration decreased FFA yield. The maximum concentration 2 (%v/v) lowered FFA to 0.5%. Enzymes function as biocatalysts, typically enhancing FFA production via lipid hydrolysis [8]. As reported by [24] boosting on lipase activity corresponded with increased FFA amounts in CPO. The fatty acids produced are depicted in Figure 8.

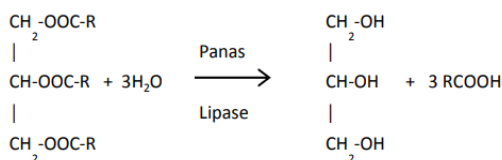


Figure 8 Fatty Acid Formation Reaction at Lipase Catalysis

[25] research illustrated how lipase enzymes effectively facilitate transesterification processes involving fatty acids in crude palm oil. Through their function, these enzymes enable hydrolysis reactions that yield diminished FFA amounts [26]. Specifically, 10% enzyme addition lowered FFA to 6.33%, while a 25% addition reduced it further to 6.06%. During hydrolysis, lipase enzymes convert triglycerides into FFA, which become trapped in a colloidal system formed during degumming, allowing for easier separation from the oil [27]. This layout results from the correlations among the enzyme, phospholipids, water, and the redundant phosphoric acid. Enzymatic functions could be impaired because phospholipids and gums cling to their surfaces. Thus, pretreatment with acid is essential to eliminate gums prior to enzymatic degumming. Although enzymatic degumming diminishes FFA, its effectiveness is limited as the process primarily targets phospholipids. For optimal FFA reduction, neutralization is more effective, with NaOH effectively binding FFA.

3.10 Kinetic Studies Heating Temperature on Degradation DRPO Carotene and FFA Value

The kinetic estimates by using heating temperature (55, 75, 95, 115°C) within β-carotene (516.65, 496.01, 465.69, 454.96 ppm) and FFA data (1.359, 1.220, 1.070, 0.723%). The β-carotene and FFA control sample C₀ 552.158 ppm and 2.606%, respectively. This data give the fitted Arrhenius parameters. All degumming runs used 30

minutes heating step ($t = 30$ min) as described in methods with single fixed treatment. First order degradation of β -carotene and FFA rising value with respect to its concentration where $R = 8.314$ J/mol.K:

$$\ln \frac{C}{C_0} = -k \cdot t; C = \text{Considerable between } \beta - \text{carotene and FFA} \tag{5}$$

$$k = -\frac{1}{t} \ln \frac{C}{C_0}; \text{ then } k = Ae^{\left(-\frac{E_a}{RT}\right)} \tag{6}$$

Table 3 Calculated Kinetic Heating Temperature Studies vs FFA and β -carotene level

| Parameter Temperature (K) | β -carotene | | | FFA | | |
|------------------------------|-------------------|------------------------|-----------|-----------------|------------------------|-----------|
| | degradation (%) | k (min ⁻¹) | t ½ (min) | degradation (%) | k (min ⁻¹) | t ½ (min) |
| 328.15 | 6.43 | 0.0022 | 312.85 | 45.77 | 0.0204 | 33.98 |
| 348.15 | 10.17 | 0.0036 | 193.91 | 51.32 | 0.0240 | 28.89 |
| 368.15 | 15.66 | 0.0057 | 122.09 | 57.30 | 0.0284 | 24.43 |
| 388.15 | 17.60 | 0.0065 | 107.40 | 71.15 | 0.0414 | 16.73 |

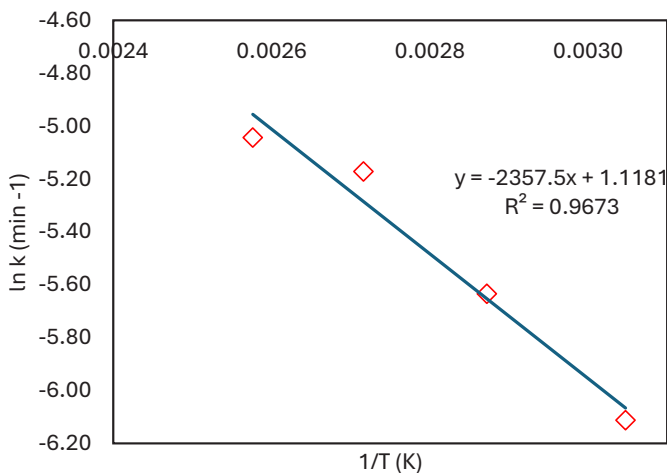


Figure 9 Kinetic Plot Carotene Degradation

Linear regression first order of $\ln(k)$ vs $1/T$ gives carotene degradation activation energy (E_a) 19.6 kJ/mol and pre-exponential factor (A) 3.06/min.

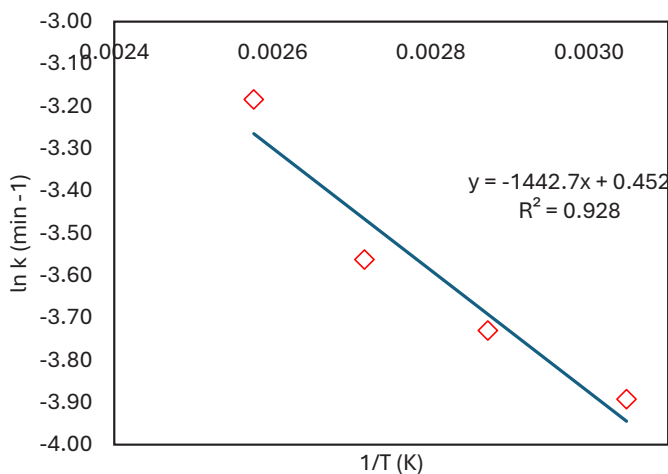


Figure 10 Kinetic Plot FFA Degradation

Linear regression first order of $\ln k$ vs $1/T$ gives FFA degradation activation energy (E_a) 11.99 kJ/mol and pre-exponential factor (A) 1.57/min. Kinetic analysis established that β -carotene degradation during the HAE process adhered to first-order kinetics, with rate constants increasing from 0.0022/min at 55°C to 0.0065/min at 115°C. Half-lives decreased from 312.85 to 107.40 min, signifying increased carotene instability at higher temperatures. The Arrhenius plot indicated an activation energy (E_a) of 19.6 kJ·mol⁻¹ ($R^2 = 0.967$) and a pre-exponential factor (A) of 3.06 min⁻¹, demonstrating considerable sensitivity to temperature variations. Nonetheless, carotene losses were confined within 6.4–17.6%, maintaining over 80% retention and conforming to SNI. These findings are in accordance with prior studies [24], [28], [29] and imply a protective function of lipase through potential enzyme–carotene complex formation [30].

Conversely, FFA levels notably diminished with rising temperature, from 1.36% at 55°C to 0.72% at 115°C. First-order kinetic modeling indicated k values increasing from 0.0204 to 0.0414/min, with half-lives reducing from 33.98 to 16.73 min. The Arrhenius analysis yielded a lower E_a of 12.0 kJ·mol⁻¹ ($R^2 = 0.928$), suggesting that FFA reduction exhibits less temperature dependency than carotene degradation. These observations align with previous research [31], [32] which indicated enhanced phospholipid breakdown and separation under elevated temperatures.

Collectively, the results illustrate a distinct process trade-off: elevated temperatures expedite FFA removal but exacerbate β -carotene loss. Given that carotene degradation is more susceptible to temperature changes, it serves as the limiting factor in process optimization. Consequently, the optimal HAE operation should strive to reconcile these outcomes by maintaining moderate temperatures (85–95°C) and brief exposure durations, thus ensuring carotene retention above 85% while achieving over 50% FFA reduction. Alternatively, augmenting lipase activity at lower temperatures may further enhance FFA reduction without jeopardizing carotene stability. Such strategies facilitate the production of carotene-rich, low-FFA red palm oil that satisfies nutritional and physicochemical criteria for functional food applications.

4 Conclusions

The Hybrid Acid–Enzymatic (HAE) degumming process effectively decreased FFA and preserved carotene in crude palm oil. Carotene levels decreased by 17.8% from 552 to 453 ppm, remaining significantly above the SNI minimum of 30 ppm, while FFA reduced from 2.51% to 0.50%. Temperature was the primary factor affecting both parameters, with higher temperatures hastening degradation and removal. Acid concentration exacerbated

carotene loss and increased FFA, whereas lipase addition lowered FFA to 0.5% with negligible impact on carotene. Despite FFA levels being slightly above the SNI limit (0.3%), HAE shows significant promise as an alternative refining technique that maintains carotene retention while reducing FFA. Further refinement in deodorization and acid–enzyme formulation will be necessary to comply with full edible oil standards. Previous studies have shown that additional downstream treatments such as optimized alkali neutralization, vacuum deodorization, or adsorption-based deacidification are required. The kinetic studies indicated that both β -carotene degradation and FFA removal during HAE degumming adhere to first-order kinetics. β -carotene exhibited greater temperature sensitivity (E_a 19.6 kJ/mol) than FFA (E_a 12.0 kJ/mol), suggesting that carotene stability is the critical constraint in process optimization. Therefore, moderate temperatures (85–95°C) with brief residence times are advised to effectively reduce FFA while ensuring carotene retention exceeds 85%.

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