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Ethanolysis Pre-treatment of Crude Palm Oil in High Shear Reactor

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ABSTRACT

Crude palm oil (CPO) is characterized by high carotenoid content, dissolving in the oil system and causing a difficult extraction process. To overcome this problem, transesterification of CPO with ethanol was performed to improve the carotenoid extractability using sodium hydroxide as a catalyst. Therefore, this study aimed to investigate the effect of ethanolysis parameters on the carotenoid and fatty acid ethyl ester (FAEE) content of CPO. FAEE content was quantified by thin-layer chromatography and total carotenoid content (TCC) was determined through a UV-Vis spectrophotometer. Reaction time (15-150 min), catalyst concentration (0.65-3.25%), reaction temperature (30-70 °C), and stirring speed (500-2000 rpm) were determined by one factor at a time (OFAT) design. The results showed that ethanolysis time and temperature did not affect TCC along the study range, while ethanolysis time of 45 minutes and temperature of 70 °C increased FAEE content. Additionally, catalyst concentration of 1.95% and stirring speed at 2000 rpm increased TCC and FAEE. The best ethanolysis parameters were obtained at 45 minutes, the temperature of 50 °C, catalyst concentration of 1.3%, and stirring speed of 2000 rpm, yielding FAEE 1358±16 ppm and TCC 99.9±0.2%. This showed that ethanolysis produced high FAEE content and improved carotenoids in the oil system.

Keywords: Carotenoid; crude palm oil; ethanolysis; fatty acid ethyl ester

INTRODUCTION

Indonesia is the most significant crude palm oil (CPO) producer worldwide, producing 47 million tons (Indonesian Palm Oil Association, 2020). Due to its reddish color, CPO contains a high total carotene content of 988 ppm and 1857 ppm for the *Elaeis guineensis* and *E. guineensis x E. oleifera* cultivar, respectively (Ribeiro et al., 2018). Generally, CPO carotenoid is comparable to other natural carotenoid sources, such as *Daucus carota* with 53.58±2.76 μg β-carotene /g FW (Maurer et al., 2014).

Carotenoid is widely recognized for health benefits, such as preventing diabetes (Asemi et al., 2016) and cardiovascular diseases (Meyers et al., 2013). Based on classification, carotenoid is divided into carotenes and xanthophylls, functioning as antioxidant and anticarcinogenic (Dal Prá et al., 2017). It is considered an unstable compound, which is easily degraded by heat, light, and oxygen (Gul et al., 2015) since the degradation temperature of β-carotene is 45 °C (O. Egbuna et al., 2019). Furthermore, carotenoid extraction is affected by stirring speed, reaction time (Ribeiro et al., 2012), solvent type, volume, and temperature of extraction (O. Egbuna et al., 2019). Knockaert et al. (2012) stated that conjugated system of double bonds caused carotenoid instability against isomerization and oxidation by heat, high pressure, and mechanical processing such as

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mixing or homogenizing. Meanwhile, triglyceride (TG) is classified as an ester compound with a COO functional group, which is found as the main component in the CPO, primarily consisting of palmitic acid (Japir et al., 2017). Considering the similarity of solubility, hydrophobicity, and molar mass between carotenoid and TG of CPO (Ribeiro et al., 2012), transesterification is needed to improve the extractability of carotenoids.

Transesterification or alcoholysis is a reaction between the alkyl group of esters (TG) and alcohol in the presence of the catalyst to produce mono-alkyl esters of long-chain fatty acids as the main product and glycerol as the by-product. Ethanolysis transesterification, which uses ethanol and C_2H_2 as an alkyl group, is a reaction that produces fatty acid ethyl ester (FAEE) and glycerol/glycerin.

According to Nabu et al. (2021), transesterification could convert TG at molecular weight of 807-885 g/mol into fatty acid methyl ester (FAME) of 300 g/mol, with β-carotene of 536 g/mol. This shows that carotene can be separated from FAME because of the differences in molecular size. Hoe et al. (2020) also reported that transesterification could facilitate carotenoid separation by producing a system with different hydrophobicity, viscosity, and boiling point compared to TG.

Transesterification has been applied as a pre-treatment in carotene extraction. Iftikhar et al. (2018) used transesterification, followed by molecular distillation, to produce 10% w/w carotene concentrate. Razi et al. (2022) used trans-esterified CPO in nanofiltration polyethersulfone (NF-PES) membrane technology to achieve β-carotene concentration, which was 1.14 times higher than CPO. Chiu et al. (2009) applied red palm ethyl ester in a flat sheet polymer membrane (NP10) to obtain a β-carotene rejection of 75% at 40 °C and a pressure of 2.5 MPa.

Due to the elevated viscosity and semisolid properties of palm oil (Hoe et al., 2020), high shear is needed in transesterification. This high shear mixing causes intense turbulence in the small shear gap during a short residence time in a chemical reaction, producing large interphase areas to distribute heat and mass transfer in the heterogeneous multiphase reaction (Zhang et al., 2012). According to Wangi et al. (2023), a high-shear reactor (HSR) minimized the use of high temperatures in glycerolysis by increasing the contact area between oil and glycerol.

In this study, ethanol was used for transesterification due to its derivation from biological sources, low toxicity, and ability to decrease dependency on methanol consumption (Kumar & Purayil, 2019). Additionally, NaOH was used as a catalyst because of higher kinetic reaction rates and the ability to use lower reaction temperatures compared to acid or enzymatic catalysts (Atadashi et al., 2013).

According to Kumar & Purayil (2019), ethanolysis was conducted in *Arachis Hypoghea* oil at 70°C, where ethanol to oil molar ratio of 9:1 and 1% (w/w) KOH for 90 minutes, showed 95.49% yield. Noipin & Kumar (2015) reported an ethanolysis in refined palm oil at 110% ultrasonic amplitude by KOH of 1.25 wt% at 45 °C with a 9.5:1 ethanol/oil molar ratio producing 92.05% yield. The results identified reaction temperature, reaction time, catalyst concentration, and stirring speed as the most influencing process parameters that required further investigation. Despite several reports on transesterification as a pre-treatment in carotene extraction, there is still a lack of information on ethanolysis parameters' effect in both ethyl ester and carotenoid content from CPO. Therefore, the study aimed to investigate the effect of ethanolysis parameters on the ethyl ester and carotenoid content of CPO.

METHODS

Materials

The materials used were purchased from Merck (Darmstadt, Germany), including sodium hydroxide, methanol, n-hexane, ethyl acetate, glacial acetic acid, wij's solution, potassium iodide, potassium hydroxide, phenolphthalein, sodium thiosulfate, thin layer chromatography (TLC) plate (20 x 20 cm, silica gel 60 F254, TLC grade). Brilliant blue dye and β-carotene standard were purchased from Sigma-Aldrich (St. Louis, MO, USA), hydrochloride acid was obtained from Mallinckrodt (Staines-upon-Thames, UK), and ethanol of 96% purity (Indonesia). Other materials were CPO (Sumatera, Indonesia) containing 4.67% w/w of FFAs, 63.95% TG, 1156 ppm TCC, and 158 cSt viscosity at 23.5 °C.

Neutralization

Neutralization was modified by Hori et al. (2021) to achieve free fatty acid (FFA) < 2% and perform single-step ethanolysis. Initially, approximately 200 g of CPO was heated to 80 °C in a water bath (Memmert, Germany). This was followed by the addition of NaOH solution (145 g/L) to CPO and mixing with a homogenizer (IKA Ultra-turrax T25D, Germany) at 7000 rpm for 5 minutes.

The mixture was centrifuged (Damon IEC UV, USA) at 6000 rpm for 5 minutes, and the oil phase was collected and washed with distilled water (20% w/w relative to the oil). Then, it was dried at 105 °C for 10 minutes in the oven (Tokyo Rikakikai NOS-601D, Japan) to remove the water. The neutralized palm oil (NPO) was the feedstock of the transesterification reaction. The volume of added NaOH was calculated using the titration method based on stoichiometry and FFA content in CPO (Kumar & Krishna, 2014).

Effect of Ethanolysis Time on Total Carotenoid Content and Fatty Acid Ethyl Ester Content

In this study, ethanol was used as a pre-treatment to enhance carotenoid extraction. The reaction was performed in a High Shear Reactor (IKA, Eurostar 20, Germany), where approximately 120 g of NPO in the reactor flask was preheated at a temperature of 50 °C. In another flask, the catalyst was prepared by the reaction of ethanol (mol ratio of ethanol to oil was 10:1) and NaOH (0.65% w/w). The catalyst was mixed with a magnetic stirrer at 700 rpm at 50 °C until total dilution of NaOH, where the reaction temperature was maintained and controlled by a hot plate and thermocouple. The reaction started by adding the catalyst mixture to the reactor and was conducted along various reaction times of 15, 30, 45, 60, 75, 90, 105, 120, 135, and 150 minutes at 50 °C by 1500 rpm of stirring.

After reaching the reaction time, the mixture was poured into a separating funnel to achieve two immiscible phases. The lower layer containing glycerol, ethanol, soap, and catalyst residue was decanted. Meanwhile, the upper layer containing FAEE was washed three times with 100% (v/v relative to the oil) of distilled water, packaged into air-tight amber, and stored at 4 °C until analysis, modified from Anguebes-Franseschi et al. (2016). The factors were evaluated by one factor at a time (OFAT) design, while total carotenoid content (TCC) and FAEE content as the responses were determined by UV-Vis spectrophotometer and TLC methods.

Effect of Catalyst Concentration on Total Carotenoid Content and Fatty Acid Ethyl Ester Content

The effect of catalyst concentration was evaluated by the previous ethanolysis condition at various catalyst concentrations of 0.65, 1.3, 1.95, 2.6, and 3.25% w/w, 1500 rpm of stirring speed, 50 °C for 45 minutes.

Effect of Ethanolysis Temperature on TCC and FAEE Content

The effect of reaction temperature was evaluated by the previous ethanolysis condition at various reaction temperatures of 30, 40, 50, and 60 °C, with 1500 rpm of stirring speed for 45 minutes, using 1.3% w/w of catalyst.

Effect of Stirring Speed on TCC and FAEE Content

The effect of stirring speed was performed by the previous ethanolysis condition at various stirring speeds of 500, 1000, 1500, 2000, and 2500 rpm, at 50 °C for 45 minutes, using 1.3% w/w of catalyst.

Product Characterization

Product Composition

Product and oil composition was separated on a TLC silica gel plate using hexane, ethyl acetate, and acetic acid (90:10:1, v/v) as mobile phase (Kaieda et al., 1999). The plate was lined 1 cm from the top and bottom, which was activated in the oven at 105 °C for 1 hour. The sample was spotted on the plate using a micropipette of 1 µL. The plate was developed in a TLC chamber after pre-saturation of the TLC chamber for 1 hour with mobile phase until the solvent reached 18 cm from the bottom lanes of the plate.

The plate was air-dried for 3 hours and stained by immersion in a dye solution (0.02% w/v brilliant blue diluted in distilled water, methanol, and acetic acid of 60:30:10 v/v) for 4 minutes. Furthermore, the plate was air-dried and was scanned using Camag Automatic TLC Scanner III (Camag, Swiss) at a wavelength of 629 nm, analyzed by WinCATS software to gain the area percentage of each oil composition, which was considered as oil component percentage.

The oil component appeared as a single spot, which was recognized as a single peak and classified by retention factor (R_f) value. Moreover, R_f indicates the ratio of the distance traveled by samples and solvent to the final line. This shows that higher R_f is obtained by a more hydrophobic compound since the silica gel plate is a polar stationary phase. According to Chattopadhyay et al. (2011), R_f for MG, DG, FFA, FAEE, and TG was <0.1, 0.2-0.25, 0.4-0.5, 0.8-0.9, and 0.5-0.7.

Acid value (AV) and free fatty acid (FFA)

Acid Value (AV) presents mg NaOH, which neutralizes free fatty acids in the oil. In this study, the method by the American Oil Chemists’ Society (AOCS) Ca 5a-40 (Kumar & Krishna, 2014) was used to measure oil AV and FFA.

Iodine value (IV)

The iodine value (IV) showing oil unsaturation, was measured by Wij’s method (British Standard 684: Section 2.13: 1976) (Abdullah et al., 2013).

Saponification value (SV)

The saponification value (SV) showing the mg NaOH used to saponify 1 g of oil, was measured using British Standards BS 684 2.6.1977 (Abdullah et al., 2013).

Viscosity

Viscosity was determined by a viscometer (Brookfield DV2T, USA), where 10 mL of sample was placed in the sample vessel. Subsequently, measurement was conducted using LV-02 (62) spindle at the temperature of 23.5 °C and speed of 3 rpm, expressed as cP, and converted to cSt value by dividing cP value through oil specific gravity.

Color

Color was evaluated using a chromameter (Konica Minolta CR-400, Japan), through the CIELAB method for L*, a*, and b* values, respectively. These values indicate lightness (0-100), green-red component, and blue-yellow component. Total color difference (ΔE) as just a noticeable difference (JND) indicator was calculated based on the Equation 1.

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \qquad (1)$$

A ΔE value above 3.5 shows a detectable color difference (Mokrzycki & Tatol, 2012).

Total Carotenoid Content (TCC)

TCC was measured by the PORIM method (Abd Rashid et al., 2019), where a sample of 40 μg was dissolved in 10 mL n-hexane, followed by absorbance measurement at 450 nm using UV-Vis spectrophotometer (Genesys 10S UV–Vis Thermo Fisher Scientific, China). The equation from the absorbance of standard curves with a concentration range of 0-10 ppm was used to determine TCC. The calibration curves equation was $y=0.1024x + 0.0313$, $R^2=0.9975$ (Equation 2).

Statistical Analysis

All experiments conducted in this study were repeated two times, while analyses were conducted three times. The results were expressed as means ± standard deviations and analyzed using one-way ANOVA as the data difference test and Duncan Multiple Range Test as the post-hoc test.

RESULTS AND DISCUSSION

Effect of Neutralization

The neutralization process reduced FFA to 18.68 times lower than the previous value, as shown in Table 1. Therefore, it met the requirement for single-step alkali-catalyzed transesterification at FFA less than 2% (Kumar & Purayil, 2019). Based on the neutralization, CPO containing 63.95±1.24 of TG had increased NPO to 78.74±1.75%, which could be converted to ethyl ester.

Table 1. Composition of crude palm oil (CPO) and neutralized palm oil (NPO)

Components	CPO	NPO
MG (%)	8.17±0.14^{b}	1.02±0.05^{a}
DG (%)	15.71±0.61^{b}	9.06±0.58^{a}
FFA (%)	4.67±0.08^{b}	0.25±0.00^{a}
TG (%)	63.95±1.24^{b}	78.74±1.75^{a}
TCC (ppm)	1156±6^{a}	1179±5^{b}

Notes: The results are followed by different letters, indicating a significant difference. The initial letter shows the lowest value ($p < 0.05$); MG = monoglyceride, DG = diglyceride, FFA = free fatty acid, TG = triglyceride, TCC = total carotenoid content.

CPO, which was used as oil feedstock in neutralization, contained 1165±7 ppm of carotenoid. As the feedstock in the transesterification reaction, NPO contained 1138±43 ppm of carotenoid (Table 1). This result was higher than the value obtained by Ribeiro et al. (2018) and Cardenas-Toro et al. (2014), which reported 988-1857 ppm of carotene content in palm oil and 800 ppm in pressed palm fiber oils. The variation showed that the intrinsic characteristics of the raw materials, processing method (thermal treatment durations), and post-harvesting handling in this study were proper to obtain high carotenoid content in feedstock and product.

Effect of Ethanolysis Time

TCC was stable and not significantly different ($p < 0.05$) during ethanolysis, as 1160±19 ppm at first 15 minutes until 1165±15 ppm at 150 minutes (Figure 1). This showed that ethanolysis could maintain carotenoid content in the oil phase due to the production of different hydrophobicity by converting triglycerides in palm oil into fatty acid alkyl ester, leading to the separation of carotenoids (Hoe et al., 2020). Iftikhar et al. (2018) also obtained similar results using transesterification followed by molecular distillation to produce 10% w/w carotene concentrate. Moreover, Razi et al. (2022) used trans-esterified CPO in NF-PES membrane technology to get β-carotene concentration, which was 1.14 times higher than CPO.

FAEE increased along the first 45 minutes reaction time to 42±5% and decreased until the lowest content of 15±2% at 150 minutes, as shown in Figure 1. This suggested that a longer ethanolysis time to optimal value could allow ethanol, catalyst, and the oil system to react and produce higher fatty acid alkyl ester (Abo El-Enin et al., 2013).

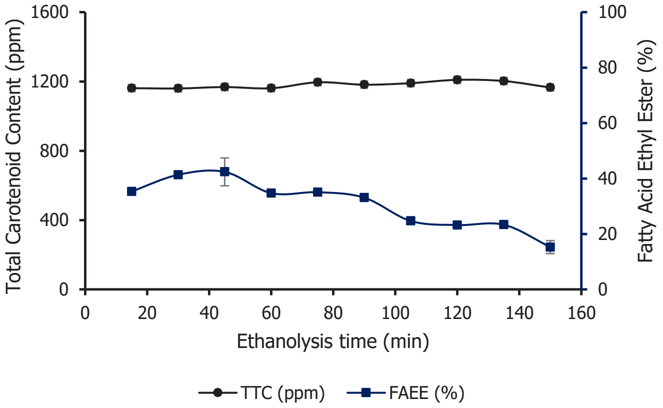

Figure 1. Effect of Ethanolysis Time on Total Carotenoid Content and Fatty Acid Ethyl Ester.

$$CH_3\ CH_2OH \text{ (ethanol)} + NaOH \text{ (catalyst)} \rightarrow CH_3\ CH_2ONa + H_2O \text{ (moisture content)} \quad (3)$$

The ethyl ester content remained constant and ended up with content reduction because of the reverse transesterification reaction, which was triggered by the moisture content. According to previous studies, moisture content could hydrolyze the TG to form FFA, which reacted with the base catalyst to form soap, leading to catalyst and ester loss (Thoai et al., 2019). Moisture content could come from the raw materials or formed during the reaction (Equation 3), often influenced by several factors such as ethanol to oil molar ratio, catalyst concentration, and reaction temperature.

Shahla et al. (2012) obtained similar results, where palm oil conversion increased to the optimal ethyl ester of 97.5±0.5% and further remained constant. Therefore, 45 minutes was selected as the optimal time to achieve both the best content of TCC at 1168±16 ppm and FAEE at 42±5% in this study, considering the stable TCC over time and the decreasing value of FAEE after 45 minutes.

The low results of FAEE in this reaction were caused by a decreased catalyst concentration of 0.65%. According to Surya Abadi Ginting et al. (2012), the produced esters were around 20, 80, and 90% at NaOH of 0.5, 1, and 1.5% (wt), respectively, using 20 gr of *Jatropha curcas* kernels in 140 mL ethanol at 70°C, 600 rpm, for 2 hours. Veličković et al. (2013) reported a similar trend, where yield increased along with a significant rise in the catalyst concentration. FAEE purities of 88.49, 90.24, and 96.46% were achieved at 0.75, 1, and 1.25% wt NaOH, respectively (9:1 ethanol/oil molar ratio, 50 °C, for 5 minutes). Therefore, a higher concentration (1.3-3.25%) was further evaluated to study the catalyst concentration effect in this study.

Effect of Catalyst Concentration

Catalyst concentration increased TCC and FAEE until an optimum condition was achieved. TCC was 1168±16 ppm at 0.65%, which further increased to the highest of 1512±15 ppm using 1.95% of catalyst, as shown in Figure 2. A higher concentration of catalyst was observed to have a negative effect on TCC. The value of TCC followed the FAEE content because ethanolysis produced different molecule hydrophobicities by converting triglycerides into fatty acid alkyl ester (Nabu et al., 2021). The difference in molecule hydrophobicities led to carotenoid separation from the oil system. Therefore, the more FAEE content was achieved, the higher the TCC.

Higher TCC was obtained in the greater content of FAEE, and vice versa. Since the catalyst could enhance the production of FAEE, the optimal catalyst concentration was evaluated to obtain the highest value of both TCC and FAEE. Based on the result, the catalyst of 0.65% formed 42±5% FAEE, which increased to 93±1% at an increase in catalyst to 1.95%, as shown in Figure 2. Shahla et al. (2012), also reported that a higher catalyst concentration of 1.4% produced a greater ethyl ester of 98.4% compared to 0.5% catalyst, which formed 92.3% at 7 minutes.

Abo El-Enin et al. (2013) stated that catalyst could speed up the reaction rate of ethanolysis. However, excessive catalyst concentration would lower ethyl ester content because of the emulsion formation. This occurred due to the saponification reaction, which increased product viscosity, formed gels, and hindered the separation of glycerol and ester. Consequently, the appropriate catalyst concentration should be selected to achieve high TCC and FAEE as well as avoid hard separation in the washing process. So 1.3% was chosen as gaining 1353±45 ppm of TCC and 96±1% of FAEE.

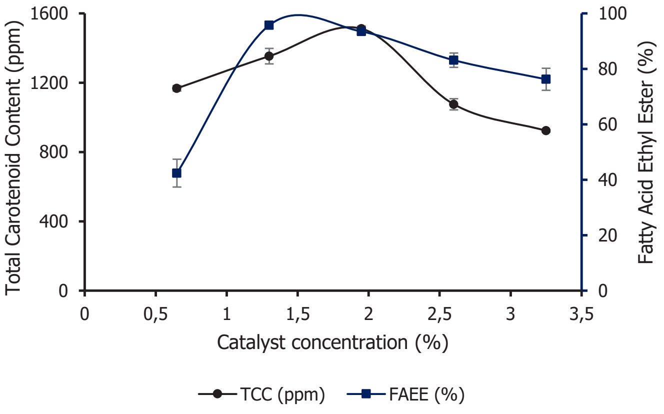

Figure 2. Effect of Catalyst Concentration on Total Carotenoid Content and Fatty Acid Ethyl Ester.

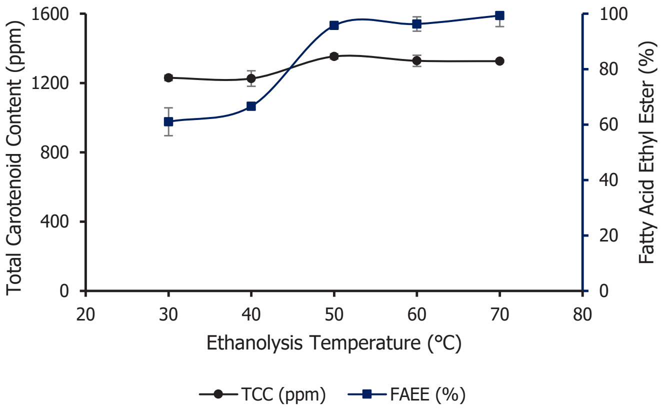

Figure 3. The Effect of Reaction Temperature on Total Carotenoid Content and Fatty Acid Ethyl Ester.

Effect of Ethanolysis Temperature

TCC was stable at various ethanolysis temperatures, ranging from 1229±23 ppm at 30 °C to 1326±32 ppm at 70 °C, as shown in Figure 3. This study was in line with the previous report, where carotenoid was retained during the thermal process since the activation energy for carotene degradation was high at 71±5 kJ/mol in virgin palm oil (Mba et al., 2017), and 109.4 kJ/mol in palm oil (Sampaio et al., 2013). Egbuna et al. (2019) found that β-carotene concentration increased as the temperature rose from 35 °C to 50 °C in extraction using ethanol. This occurred because temperature facilitated the cell wall breakdown of the oil system and raised mass transfer rate (Kaur et al., 2012), thereby producing higher β-carotene content.

FAEE increased with a rise in the temperature, ranging from 61±0% at 30 °C to 99±0% at 70 °C, as shown in Figure 3. Similarly, Shahla et al. (2012) found that higher temperatures led to greater yield in palm oil ethanolysis, as 25 °C, 35 °C, 45 °C, and 55 °C produced 94.16%, 96.01%, 96.82%, and 97.19% of ethyl ester, respectively. These values were obtained at 10 minutes of reaction by 1% wt. of sodium ethoxide and 12:1 ethanol/oil molar ratio at 55 °C and 600 rpm.

According to Qu et al. (2021), the optimum temperature for methanolized palm oil was 65 °C by 7.5% wt of calcium-modified Zn-Ce/Al_20_3 catalyst at 18:1 methanol/oil molar ratio. This temperature achieved 98.96% of FAME, which was close to the boiling point of methanol at 64.7 °C. Generally, optimum reaction temperature was found to be closely related to the boiling point of the alcohol in alcoholysis to prevent losses from the oil system due to evaporation, which was 78.37 °C for ethanolysis.

Higher reaction temperature could decrease oil viscosity (Enyoh et al., 2017), causing an increase in the reactant's solubility and a faster reaction rate. Therefore, regarding the high response of FAEE and preventing the thermal degradation of carotenoid, 50 °C was selected as the optimum temperature for achieving 1353±45 ppm of TCC and 96±1% of FAEE.

Effect of Stirring Speed

Stirring speed in the HSR affected TCC and FAEE positively. Faster stirring speed produced higher TCC, as 1194±15 ppm by 500 rpm increased to 1358±16 ppm by 2000 rpm (Figure 4). Compared to a slightly different process, Nekkaa et al. (2021) found that yield of extraction increased with the stirring speed until 650 rpm for reaching the maximal amount of 203.0 mg GAE/g and 108.7 mg QE/g of total polyphenol and flavonoid content, respectively. This showed that stirring speed could increase ethanol distribution into the oil system, thereby allowing the release of carotenoid.

A higher rapid stirring speed caused an increase in FAEE content, which ranged from 91.4±1.4% at 500 rpm to 99±0.2% at 2000 rpm, as shown in Figure 4. Similarly, Kabbashi (2015) reported that increasing speed of stirring from 100 to 400 rpm raised FAME yield to approximately 96% in the esterification of hydrolyzed *Jatropha curcas* oil by 4% wt of a calcinated niobic acid catalyst, using 5:1 methanol to FFA ratio at 60 °C for 6 hours. This occurred because stirring speed could increase the frequency of molecule collision in the system. A higher stirring speed maximally distributed all the reactants and the oil, leading to higher ethyl ester content. However, over-stirring speed might cause evaporation loss of the ethanol and limit mass transfer in the system. Considering the high response of FAEE and TCC, 2000 rpm was selected as the

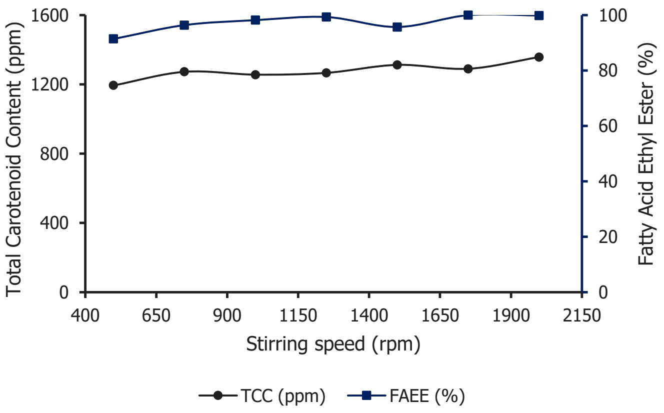

Figure 4. The effect of stirring speed on Total Carotenoid Content and Fatty Acid Ethyl Ester.

optimum value of stirring, yielding 1358±16 ppm of TCC and 99±0.2% of FAEE. Stirring speed more than 2000 rpm had not been studied the threshold produced high FAEE content.

Product Characterization

Several parameters of FAEE are shown in Table 2 and compared to CPO and FAME quality standards in the left and right columns, respectively. The FFA, SV, Color, and TCC were compared only between CPO and FAEE.

As shown in Table 2, CPO had 63.95±1.2% of TG, 15.71±0.6% of diglycerides (DG), and 8.17±0.1% of monoglycerides (MG), which was converted to FAEE containing 99.9±0.2% of esters. The composition of FAEE was found within the standard value, showing that the ethanolysis was in complete reaction. Li et al. (2013) stated that ethanolysis consisted of three sequentially reversible reactions. The first reaction was the conversion of TG to DG, followed by DG to MG, and MG to form glycerol, resulting in one ester molecule from each glyceride. Therefore, the glycerides (TG, DG, MG) in this reaction were all converted to form FAEE. This showed that high TCC was obtained in the elevated content of FAEE since transesterification could facilitate carotenoid separation by producing a system with different hydrophobicity, viscosity, and boiling point compared to TG (Hoe et al., 2020).

Relatively low viscosity was required to maintain oil lubricity, fuel atomization, complete combustion, and avoid carbon deposition on the injector. This study showed that ethanolysis could reduce the product viscosity of CPO and FAEE to 137±6 and 23±4 cSt at 23.5 °C, respectively. Similarly, Encinar et al. (2007) found that reducing viscosity from 14.75 cSt to 6.06 cSt increased ethyl ester from 46.8% to 74.2% at 20 °C. The reduced viscosity of FAEE in this study was due to ethanolysis and could be lower at temperatures higher than 40 °C to meet the standard. Moreover, lower viscosity facilitated carotenoid release to achieve higher TCC.

The IV showed double bonds of fatty acid as indicators of unsaturated fatty acid, which depended on the oil's origin. In this study, CPO and FAEE had close IV lower than the standard, which were 51.08±0.56 and 54.88±2.34 g iodine/100 g sample, respectively. This condition led to the deposit gum formation or deterioration of the lubricating oil, thereby the low IV of raw materials was preferable. Bolonio et al. (2019)

Table 2. Characteristic of FAEE produced by optimal ethanolysis conditions (2000 rpm, 1.3% catalyst (w/w), 45 minutes, 50 °C, 10:1 ethanol: oil molar ratio)

Parameter	CPO	FAEE	Alkyl ester standard value
Product composition:			
Esters (FAEE) (%)	n.d.	99.9±0.2	96.5 min*
Triglyceride (%)	63.95±1.2	n.d.	0.2 max*
Diglyceride (%)	15.71±0.6	n.d.	0.2 max*
Monoglyceride (%)	8.17±0.1	n.d.	0.8 max*
Viscosity at 23.5 °C (cSt)	158±6^b	27±5^a	3.5-5 at 40°C*
Iodine value (g iodine/100 g)	51.08±0.56^a	54.88±2.34^b	115 max**
Acid value (mg KOH/g)	10.22±0.16^b	0.65±0.07^a	0.8 max**
Free fatty acid (%)	4.67±0.08^b	0.29±0.03^a	
Saponification value (mg KOH/g)	139.31±1.44^b	131.76±4.07^a	
Color:			
L*	26.6±0.4^b	24.9±0.3^a	
a*	8.8±0.2^a	12.1±0.2^b	
b*	15.5±0.5^a	16.5±0.5^b	
ΔE	3.9±0.7		
Total Carotenoid Content (ppm)	1156±6^a	1358±16^b	

Notes: The results are followed by different letters indicating a significant difference; the initial letter shows the lowest value ($p < 0.05$); n.d. = not detected;* EN 14214 (Knothe, 2006), ** SNI 04-7182-2006 (BSN, 2006).

reported a higher IV estimation of 113 in grape seed FAEE, which contained elevated unsaturated fatty acids.

As shown in Table 2, FAEE had a lower AV of 0.65±0.07 and free fatty acid (FFA) of 0.29±0.03 in comparison to CPO, which had a 10.22±0.16 and 4.67±0.08, respectively. This result showed that FFA content in CPO was high, indicating the need for neutralization to decrease the FFA to <2% (Kumar & Purayil, 2019). The result showed that AV and FFA of FAEE met the standard, although a low level of acidity was needed since high acid content could form deposits that damage the engine filter. Lower AV and FFA indicated high FAEE content, which facilitated the separation of carotenoid due to different hydrophobicity, viscosity, and boiling points of FAEE compared to TG (Hoe et al., 2020).

The SV was attributed to the mean molecular weight of the sample. In this study, the SV value of FAEE was 131.76±4.07, lower than CPO, which had 139.31±1.44. This showed a reduction of the mean molecular weight of CPO in the FAEE conversion according to the simpler structure. The chemical bond structure of FAEE was ($RCOOR_1$) with R as the alkyl group from ethanol (C_2H_5). Therefore, the structure became $C_2H_5COOR_1$, compared to TG (CH_2COOR_1- CH_2COOR_2- CH_2COOR_3), with R_1, R_2, and R_3 being palmitic acid.

Compared to CPO color, FAEE had a lower L*, a higher a*, and a higher b* value, which showed lighter (ΔL^* was 1.7±0.5), redder (Δa^* was 3.3±0.4), and more yellow color (Δb^* was 1.0±0.7), respectively. Moreover, the total color difference (ΔE) between CPO and FAEE was 3.9±0.7, showing a detectable color difference.

According to Abdullah et al. (2013), the red color of the oil was associated with the presence of highly colored materials such as carotenoid, which had at least seven conjugated double bonds (c.d.b.). Since the lower number of double bonds led to colorlessness, and the higher number of double bonds caused shifting maximum absorbance to the longer wavelength, the hue of carotenoid became redder. Gurak et al. (2014) stated that the color of carotenoid depended on its chemical structure, concentration, and interaction with other molecules.

Meléndez-Martínez et al. (2007) found that carotenoid color changed from red to yellow at the decreasing value of carotenoid content. In this study, the increasing redness value was more intense than the yellow value, showing the increase of carotenoids due to the release of carotenoids from the oil system by ethanolysis. The rise in carotenoid was proven by TCC of FAEE as 1358±16 ppm, which was higher than CPO as 1156±6 ppm. Ethanolysis could release carotenoid from the FAEE system, which had different hydrophobicity compared to CPO, thereby facilitating the extraction process.

CONCLUSION

In conclusion, this study investigated the effect of ethanolysis parameters in CPO using NaOH and ethanol using OFAT design. The results showed that catalyst concentration of 1.95% and stirring speed at 2000 rpm increased TCC and FAEE. Although ethanolysis time of 45 minutes and temperature of 70 °C improved FAEE content, there was no significant effect on TCC. The selected parameters were obtained to achieve high TCC and FAEE contents by considering some conditions. Therefore, a time of 45 minutes was selected by considering the stable TCC over time and the decreasing content of FAEE. A catalyst concentration of 1.3% was preferable to avoid hard separation in the washing process. A temperature of 50 °C was selected to prevent the thermal degradation of carotenoids. A stirring speed of 2000 rpm was used to enhance carotenoid release. By selecting the optimal conditions, ethanolysis product had a high FAEE of 99.9±0.2% and TCC of 1358±16 ppm, compared to non-ethanolysis CPO which contained only 1156±6 ppm of TCC. This showed that ethanolysis as a pre-treatment could effectively enhance carotenoid content in CPO.

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