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Extraction of oil, carotenes and tocochromanols from oil palm (*Elaeis guineensis*) fruit with subcritical propane

H. Phan-Tai[a,✉] and G. Brunner[b]

[a]Faculty of Chemical Engineering and Food Technology, Nong Lam University, Ho Chi Minh City 700000, Vietnam.

[b]Institute of Thermal Separation Processes, Hamburg University of Technology, Eissendorfer Strasse 38, D-21073 Hamburg, Germany.
✉Corresponding author: pthuan@hcmuaf.edu.vn

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SUMMARY: This work aims to screen the extraction of oil and bioactive compounds including carotenes and tocochromanols from oil palm fruit with subcritical propane and without using a cosolvent. The overall extraction curves of palm oil with subcritical propane were studied and compared to those extracted with supercritical carbon dioxide. Carotenes and tocochromanols were evaluated not only in the extracted oil, but also in the oil of residual fiber in order to calculate the efficiency to recover these valuable compounds. The experimental results showed that oil yield of up to 70 % could be obtained within 120 minutes with subcritical propane at 50 bar and a flow rate of 35 $kg \cdot h^{-1} \cdot kg^{-1}$. It was also shown that compressed propane is an excellent solvent for the extraction of oil enriched in carotenes and tocochromanols. Subcritical propane extraction can be used as an alternative process for the simultaneous recovery of these valuable minor components from palm fruit.

KEYWORDS: *Carotenoids; Palm oil; Subcritical propane extraction; Tocochromanols.*

RESUMEN: ***Extracción de aceite, carotenos y tococromanoles del fruto de palma aceitera (*****Elaeis guineensis*****) con propano subcrítico.*** Este trabajo tiene como objetivo evaluar la extracción de aceite y compuestos bioactivos, incluidos los carotenos y tococromanoles, del fruto de la palma aceitera mediante propano subcrítico sin usar codisolventes. Se estudiaron las curvas generales de extracción de aceite de palma con propano subcrítico y se compararon con las extraídas con dióxido de carbono supercrítico. Se evaluaron carotenos y tococromanoles no solo en el aceite extraído, sino también en el aceite de fibra residual para calcular la eficiencia de recuperación de estos valiosos compuestos. Los resultados experimentales mostraron que se podía obtener un rendimiento de aceite de hasta el 70 % en 120 minutos con propano subcrítico a 50 bares y un caudal de 35 $kg \cdot h^{-1} \cdot kg^{-1}$. También se demostró que el propano comprimido es un excelente solvente para la extracción de aceite enriquecido en carotenos y tococromanoles. La extracción con propano subcrítico se puede utilizar como un proceso alternativo para la recuperación simultánea de estos valiosos componentes menores del fruto de la palma.

PALABRAS CLAVE: *Aceite de palma; Carotenoides; Extracción subcrítica con propano; Tococromanoles.*

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1. INTRODUCTION

Palm oil is a liquid which is extracted from the fleshy mesocarp of the fruits of the palm tree, *Elaeis guineensis,* which typically contain 45 to 55% oil (Tan and Nehdi, 2012). According to Phoon *et al.* (2018), **c**rude palm oil contains carotenoids, ca. 500–700 $mg \cdot kg^{-1}$, mainly in the form of alpha- and beta-carotenes, and ca. 1000–1200 $mg \cdot kg^{-1}$ tocopherols and tocotrienols (the whole group called tocochromanols). Carotenoids and tocochromanols are interesting valuable bioactive minor compounds. Many studies have reported that carotenes can provide support for the prevention and control of diseases caused by vitamin A deficiency (Strobel *et al.*, 2007). Alpha-tocopherol, known as vitamin E, and gamma-tocotrienol are strong antioxidants. The combined effects of the properties of carotenes and tocochromanols give palm oil a higher natural oxidative stability compared to many other edible oils. Therefore, palm oil has become the starting material to produce natural carotenes and tocochromanols (Abu-Fayyad and Nazzal, 2017; Ghazali *et al.*, 2022; Hoe *et al.*, 2020; Iftikhar *et al.*, 2017).

Supercritical extraction has been proven to be a modern separation technique applied in edible oil processing. Supercritical carbon dioxide ($SCCO_2$) is the most commonly studied fluid. However, propane is also an interesting fluid because it is non-toxic with low critical pressure (P_c = 42.5 bar). Because the critical temperature of propane is rather high (T_c = 96.7 °C), this fluid is preferably used at subcritical conditions (Brunner, 1994). It was reported that subcritical propane has been successfully used to extract oil from pequi (*Caryocar coriaceum*) pulp (Pessoa *et al.*, 2015), inajá (*Maximiliana maripa*) pulp (Turola Barbi *et al.*, 2019), baru (*Dipteryx alata vogel*) seeds (Fetzer *et al.*, 2018), foxtail millet bran (Shi *et al.*, 2015), kiwi fruit seeds (Coelho *et al.*, 2016), macauba pulp (Trentini *et al.*, 2017), flaxseed (Piva *et al.*, 2018), pumpkin seeds and peel (Cuco *et al.*, 2019). With the palm fruit (*Elaeis guineensis*), the subcritical propane extraction of oil using ethanol as cosolvent had been performed in a few studies (da Silva *et al.*, 2018; Jesus *et al.*, 2013). However, the determination of oil, carotenes, and tocochromanol contents in the pure subcritical propane extract and the residual fibers of palm fruit within a single run has not been reported.

In this context, the study aimed at extracting oil from the palm mesocarp by means of pure subcritical propane and compared with $SCCO_2$ extraction. In addition, samples of extracted oil and the oil of residual fibers were analyzed for their contents in carotenes and tocochromanols to evaluate the efficiency of using the compressed propane to recover these valuable minor compounds.

2. MATERIALS AND METHODS

2.1. Materials

Palmitic acid (> 99%) and squalene (GC grade) came from Merck (Germany). Monopalmitin (99%), dipalmitin (99%) and tetradecane (99%) were supplied by Sigma (USA). Pyridine (99.8%), hexane (> 95%), acetone (> 99.8%), acetonitrile (HPLC grade) and N-methyl-N-trimethylsilyl-trifluoroacetamide were purchased from Fluka (Switzerland), Lab-Scan (Ireland), Riedel-de Häen (Germany), Prolabo (France), and Macherey-Nagel (Germany), respectively.

Ripe palm fruits (*Elaeis guineensis*) were from Carotech (Malaysia). The fruits were separated into skin, mesocarp (pulp), and kernel. The yellow part of the mesocarp was investigated in this work. The average particle size of the pulp ready for extraction was about 1 mm x 2 mm x 6 mm (Phan Tai and Brunner, 2019).

2.2 Equipment and experimental procedure

A standardized supercritical extraction system developed at the Institute of Thermal Separation Processes, Hamburg University of Technology was used as described in previous research (Phan Tai and Brunner, 2019). Fluid, propane or carbon dioxide (99.95% purity) was delivered from the reservoir tank by a Maximator pump (max. 600 bar) to the 100 mL steel extractor cell, which was loaded with 14.5 g of palm mesocarp for each run. A specific flow rate of 35 $kg \cdot h^{-1}$ of gas per kg of sample was used and the pressure and temperature were monitored. The extracts were collected continuously in 10-mL glass vials, used as sample collectors at atmospheric pressure. Duplicate runs were carried out for each experimental condition with a reproducibility of ± 5%.

2.3. Analytical method

2.3.1. High-performance liquid chromatography (HPLC) analysis

A Gynkotek HPLC system equipped with a RF 1002 Fluorescent detector was used for the analysis. Tocopherols and tocotrienols in the oil samples were separated on a LiChrosorb Diol 5 μm column (250 mm x 4.6 mm). The mobile phase was hexane (96%) and butyl-methyl-ether (4%) at a flow rate of 1300 $\mu L.min^{-1}$. The injection volume was 20 μL. External standard curves were used to determine tocochromanol contents in the oil samples.

2.3.2. Gas chromatography (GC) analysis

A capillary gas chromatograph system (Hewlett Packard HP 5890A) was used to analyze monoacylglycerols (MAGs) and diacylglycerols (DAGs). The stationary phase was a J & W Scientific fused silica (DB-5ht) column (30m×0.25mm i.d. with 0.1-μm coating). The carrier gas was nitrogen (2 $L.min^{-1}$). The oven temperature was programmed as followed: 120 °C, 2 min constant; 10 $°C·min^{-1}$ to 220 °C; 5 $°C·min^{-1}$ to 360 °C; 360 °C, 10 min constant. Injection volume was 1 μL at a split ratio of 1:20. For a better peak recording, sample compounds were silylated with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA). For the quantification of MAGs and DAGs, monopalmitin and dipalmitin were used as reference standards, respectively.

2.3.3. Soxhlet extraction

A Soxhlet method was used to determine the oil contents in fresh palm mesocarp and its fibers after extraction. Hexane was used as extraction solvent. The extraction time was 8 hours (Phan Tai and Brunner, 2019).

2.3.4. Spectrometer

A UV-Vis spectrometer (UV-120-02 from Shimadzu) was used to determine the concentrations of carotenes in the analyzed samples. For each measurement, an amount of 10 to 20 mg oil sample was diluted with a 2-mL mixture of acetone and hexane (30:70 by Vol. %). The absorbance was recorded at the wavelength of 450 nm and compared to the standard curve prepared by the same treatment of a series of known amounts of β-carotene.

2.4. Statistical analysis

Statistical analysis was performed with JMP® version 10 software (SAS, USA). Data were expressed as the mean of triplicate measurements. One-way analysis of variance (ANOVA) and Tukey test ($P < 0.05$) were carried out to test any significant differences between means.

3. RESULTS AND DISCUSSION

3.1. Characteristics of palm mesocarp

The composition of mesocarp varies with the size and age of the palm fruit. Table 1 presents the average composition of palm mesocarp used as material input for the extraction in this study. The results show that palm mesocarp is a good source to extract oil and valuable minor compounds like carotenes or tocochromanols. The concentrations of these components are in agreement with those reported by Phoon *et al.* (2018). However, the total mono and diacylglycerol contents in the studied palm fruit is rather high compared to 5% reported elsewhere (Tan and Nehdi, 2012). This can be attributed to the enzymatic hydrolysis of the oil under the influence of an endogenous lipase in the pulp (Doye R. Abigor, 1985) after long transportation and preservation of the palm fruit. The difference may be also due to the analysis method, maturation stage, and environmental growth variation of the palm fruit.

Table 1. Composition of palm fruits used in this work

Component	Concentration*
Total oil	45.1 ± 0.9%
Carotenes	$450 \pm 14\ mg·kg^{-1}$
Tocochromanols	$800 \pm 40\ mg·kg^{-1}$
Monoacylglycerols	3.0 ± 0.1%
Diacylglycerols	7.0 ± 0.2%

*: Average values of triplicate analyses ± standard deviation.

3.2. Course of extraction of palm oil

The course of a solid extraction of oil can be represented by an overall extraction curve, in which the amount of extract accumulated during the course of the extraction is plotted as a function of time. Figure 1 shows the extraction curves of palm oil with subcritical propane in comparison with $SCCO_2$. According to Brunner (1994), the first part of the overall extraction curve is linear, corresponding to a constant extraction rate. The gradient of this part may represent the equilibrium solubility of the extract in supercritical fluid. However, the straight line of the overall extraction curve could correspond to a constant mass transfer resistance. In the second part, the extraction rate is declining and the graph approaches a limiting value where all the extractible substances are removed from the input material.

The results show that free oil was more soluble in subcritical propane than in $SCCO_2$. However, extraction with $SCCO_2$ gave better total oil yields after 45 minutes when the available oil near the palm surface was depleted. Within the study conditions, palm oil could be recovered by up to 80% after 120 minutes with $SCCO_2$ at 400 bar and 70% with subcritical propane at 50 bar. The difference in oil recovery can be attributed to the structural change in palm fibers during the process. It was proven that $SCCO_2$ can affect the cellulose structure by increasing the accessible surface area of the cellulosic substrates (Kim and Hong, 2001; Putrino *et al.*, 2020). Lau *et al.* (2006) reported a palm oil yield of 77.3% obtained with $SCCO_2$ at 300 bar and 80 °C for 8h. In another research, it was shown that flaxseed oil extraction yields using subcritical propane were lower compared to the result from using $SCCO_2$ (Piva *et al.*, 2018).

3.3. Solubility of palm oil in subcritical propane and $SCCO_2$

The loading of solvent during the extraction can also be obtained from the first part of palm oil extraction curves. This value is commonly considered as apparent solubility and calculated from the extraction when palm oil is easily accessible throughout the fixed bed (at constant extraction rate). In the case of palm oil, $SCCO_2$ at 400 bar only had a loading capacity of 1.7 - 2.7%, while subcritical propane at 50 bar can reach an oil loading of up to 4.5% depending on the extraction condition as described in Figure 2. The same phenomena were also reported by Zanqui *et al.* (2016) in which the extraction of Sacha inchi (*Plukenetia volubilis* L.) oil using subcritical propane was faster compared to $SCCO_2$ due to the higher solubility of lipids in propane.

3.4. Extraction of carotenes and tocochromanols

Palm oil is a very good source of carotenes and tocochromanols, which are interesting valuable minor compounds in supercritical fluid extraction. The concentrations of carotenes and tocochromanols in extracted palm oil by compressed propane at 50 bar are presented in Figure 3. It was observed that the concentration of these compounds varied moderately during extraction time with the subcritical propane. Using subcritical propane can co-extract these minor compounds with concentrations in the same range as a normal pressed palm oil. This is in agreement with

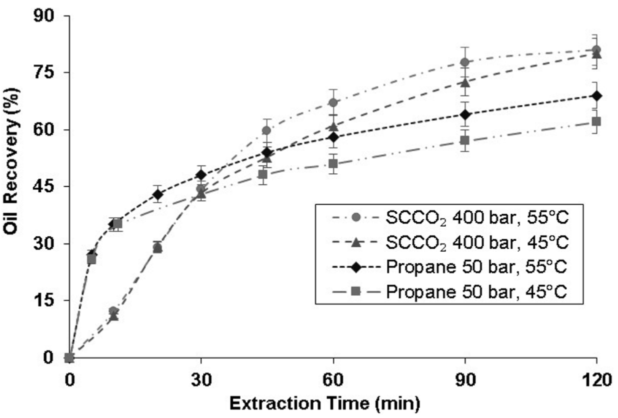

FIGURE 1. Extraction of mesocarp with subcritical propane and $SCCO_2$ at 35 $kg \cdot h^{-1} \cdot kg^{-1}$. Bars represent the experimental standard deviation of duplicates.

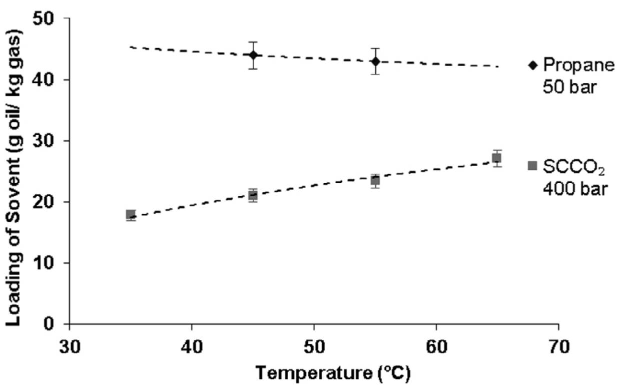

FIGURE 2. Palm oil loading capacity of subcritical propane and $SCCO_2$. Data points represent mean values and standard deviation (n=2).

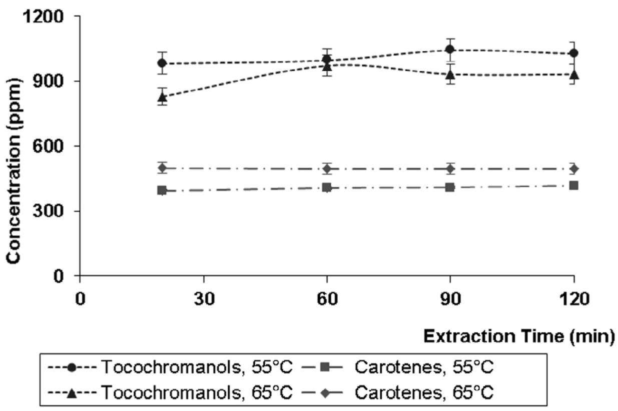

FIGURE 3. Carotenes and tocochromanols as extracted with propane at 50 bar and 35 kg·h^{-1}·kg^{-1}. Bars represent the experimental standard deviation of triplicates.

Trentini *et al.* (2017) regarding extraction from macauba pulp, who assert that propane is more efficient to extract active compounds like tocopherols and carotenoids compared to CO_2. In the extraction of oil from perilla, a higher concentration in tocopherols was determined in oil resulting from compressed propane extraction compared to the classical Soxhlet method (Silva *et al.*, 2015).

Palm oil is a complex product consisting of many components as presented in Table 1. Therefore, the concentration in carotenes and tocochromanols of extracted palm oil depends not only on their solubilities in the solvent but also on the solubilities of other compounds like mono, di- or triglycerols at the same time. A decrease in tocochromanol concentration was observed when the temperature was increased from 55 to 65 °C. A rather high temperature may decrease the content in tocochromanols because these compounds are sensitive to temperature. In contrast, it was observed that the concentration of carotenes increased with an increase in temperature. The condition of less solubility of tocochromanols in extracted palm oil can be more favourable for the solubility of other compounds like carotenes. As a result, the concentration in carotenes increased from ca 400 mg·kg^{-1} to 500 mg·kg^{-1} when temperature increased from 55 to 65 °C. Zanqui *et al.* (2016) also showed that temperature can influence the lipid composition of Sacha inchi oil extracted by subcritical propane. The subcritical propane of *Maximiliana maripa* pulp at 40 °C and 60 bar also provided fast extractions and high yields of oil enriched in beta-carotene (Turola Barbi *et al.*, 2019).

It was reported that a high amount of carotenes and tocochromanols remained in the palm residue from extraction with $SCCO_2$ or screw pressing (Birtigh *et al.*, 1995). However, subcritical propane stands out as a good solvent to recover these valuable compounds. As shown in Figure 4, there is a slight difference in tocochromanol and carotene concentrations in the oil extracted from palm mesocarp by subcritical propane and those of oil in the palm residue of extraction. To objectively evaluate the efficiency of recovery of tocochromanols and carotenes, a relative comparison of the concentrations of these compounds in the extracted oil and residue oil was used. Enrichment factor K of a component, as described in a previous study (Phan Tai and Brunner, 2019), is defined as the following equation (Eq. 1). As a result, extraction with a higher value of K(X) provides better potential to recover component X.

$$K(X) = \frac{\text{Concentration of component X in the extracted oil}}{\text{Concentration of component X in the residue oil}} \quad \text{Eq. 1}$$

The results in Table 2 show that carotenes and tocochromanols can be recovered effectively by sub-

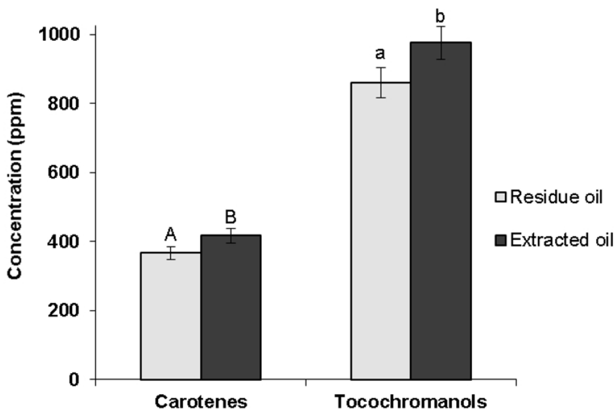

FIGURE 4. Concentration in carotenes and tocochromanols in extracted and residue oils by subcritical propane extraction at 50 bar, 55 °C, 35 kg·h^{-1}·kg^{-1}. Bars with different lower-case and upper-case letters are significantly different by the Tukey test ($P < 0.05$) within the same group (n=3).

TABLE 2. Enrichment factors of carotenes and tocochromanols with subcritical propane.

Temperature	K (carotenes)	K (tocochromanols)
45 °C	1.24	0.79
55 °C	1.14	1.13
65 °C	1.27	1.03

critical propane. The recovery efficiency of these minor compounds by using subcritical propane was better compared to other extraction methods by using $SCCO_2$ or traditional crew pressing. Phan Tai and Brunner (2019) reported that enrichment factors K(carotenoids) and K(tocochromanols) of palm oil extracted by $SCCO_2$ at 400 bar and temperature of 45–65 °C was only around 0.90–1.19 and 0.54–0.86, respectively. This also agrees with a previous study which confirmed that compressed propane has higher solvating power compared to $SCCO_2$, which results in a reduction in the consumption of solvent, higher efficiency and shorter extraction time (Silva *et al.*, 2015). It was also reported that subcritical propane extraction was a suitable and selective method for the extraction of the foxtail millet bran oil in view of smaller times and lower pressures employed compared to $SCCO_2$ and revealed the possible high content in carotenoids and highest tocopherol content obtained (Shi *et al.*, 2015).

Moreover, it was reported that the extraction of minor compounds from the palm-pressed fiber is not very practical (Chuang and Brunner, 2006). Therefore, the results of this study prove that using subcritical propane as an extraction solvent will bring more benefit because of better recovery of these valuable compounds. The subcritical propane extraction of palm oil, simultaneously recovering its high contents in carotenes and tocochromanols from the palm fruits appears as a promising alternative separation technique for palm oil processing.

4. CONCLUSIONS

Subcritical propane extraction has been proven as an alternative separation technique for palm oil processing. The preliminary study shows that it is possible to extract palm mesocarp directly by subcritical propane without using cosolvent with the aim of recovering valuable minor compounds like carotenes and tocochromanols. Compressed propane at a pressure of 50 bar and flow rate of 35 $kg \cdot h^{-1} \cdot kg^{-1}$ can be used to recover up to 70% palm oil after 120 minutes. Carotene and tocochromanol concentrations in the extracted oil reached the same levels as in commercial palm oil. Moreover, recovery efficiencies of carotenes and tocochromanols were much higher in the case of extraction with subcritical propane than with $SCCO_2$.

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