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Analysis of Carotenoid Composition in Oil Palm Fruits (*Elaeis guineensis* Jacq.) from several Varieties: A Review

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Abstract: Basically, oil palm has three types of fruit, namely the dura, pisifera, and tenera varieties. These three varieties have different characteristics, likewise with resulting Crude Palm Oil (CPO) levels. Generally, palm oil contains 500–700 ppm of carotenoid compounds, and the amount is equivalent to 15 times the carotenoids in carrots and 300 times in tomatoes. This is a study of information about the carotenoid composition of three varieties of oil palm fruit and applying the most superior analytical methods to obtain carotenoids from CPO. The purpose of this review is to examine the carotenoid composition of three varieties of oil palm fruit and carotenoid analysis methods presented for consideration as a reference. The method used in this review is the inclusion and exclusion criteria in literary search. The results showed that the carotenoid composition of the three varieties of oil palm in the presence of 11 types of carotene and the highest percentage composition was β-carotene with a content range of 54.39–56.02%. As for the development of new methods for carotenoid analysis from CPO, namely Raman and FT-NIR spectroscopy with the advantages of being environmentally friendly, not using solvents, and fast measurement compared to methods UV-Vis Spectrophotometry, UPLC, and HPLC.

Keywords: oil palm; CPO; carotenoids; β-carotene; Raman and FT-NIR spectroscopy; UPLC; HPLC; UV-Vis spectrophotometry.

1. Introduction

Indonesia is currently the largest producer and exporter of CPO (Crude Palm Oil). According to Shi *et al.*, oil palm can produce around 4 to 6 tonnes of CPO per hectare [1]. Based on statistical data released by the Director-General of Plantation in 2019, it is estimated that oil palm plantation land in Indonesia will increase by 14.67 million hectares with total Indonesian palm oil production of 51.44 million tonnes [2].

Oil palm (*Elaeis guineensis* Jacq.) has three types of fruit, namely dura (thick shell), pisifera (without shell), and tenera (thin shell) [3,4]. The pericarp oil palm consists of three layers: exocarp (bark), mesocarp (outer pulp containing Palm Oil), and endocarp (hard shell covering the kernel) [5].

There are two types of oil derived from the fruit of the palm, namely CPO and PKO (Palm Kernel Oil) [6]. CPO and PKO are both produced from mesocarp and palm kernel. In 2019, Indonesia's total CPO production reached 42.86 million tons, and PKO was 8.57 million

tons [2]. CPO is reddish due to the presence of carotenoid pigments in the oil, giving it a yellow or red color [7]. The carotenoid content in CPO generally ranges from 500–700 ppm with the proportion of α-carotene 36.2% and β-carotene 54.4%, respectively [8,9]. Carotenoids also play an important potential role by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals [10]. Another source of antioxidants contained in oil palm is tocopherol. Carotenoid and tocopherol compounds are minor chemical components or micronutrient components in oil palm [11]. The main phytochemical analysis in oil palm includes compounds such as phenolics, terpenes, and sterols [12]. Several analytical methods have been developed to obtain carotenoids from CPO qualitatively and quantitatively, including UV-Vis spectrophotometry, Raman and FT-NIR spectroscopy, UPLC, and HPLC.

This paper aims to examine the analysis of carotenoid composition in oil palm fruit of several varieties and the use of several analytical methods to obtain carotenoids from palm oil. The analytical method recommended in this review can be used as a basis for consideration in the qualitative and quantitative analysis of carotenoid compounds in palm oil.

2. Materials and Methods

The writing of this article review thesis was prepared based on studies related to the carotenoid analysis method in palm oil. The materials used are data, namely international journals. The reference data search in the article review presented is taken from Science Direct and Google Scholar. The keywords in the literature search process used were, among others, analysis of palm oil, extraction of crude palm oil, and characterization of oil palm fruit varieties.

The inclusion criteria were adjusted to the purpose of the article review. The research articles reviewed were the research results stating the carotenoid composition analysis of oil palm varieties and the methods of carotenoid analysis on CPO, both qualitative and quantitative, used by previous researchers. At the same time, the exclusion criteria were based on research articles that did not discuss carotenoid analysis in oil palm and methods of carotenoid analysis carried out on oil palm waste.

3. Results and Discussion

3.1. Characterization of oil palm fruit varieties.

The shape of the dura fruit has a thick lignified shell and surrounds the kernel, in contrast to the pisifera, which does not have a shell. On the other hand, the shape of the tenera fruit is far superior to its parent in terms of oil mesocarp [5]. The characteristics of oil palm fruit varieties can be seen in Table 1 below.

Table 1. Characteristics of oil palm fruit varieties.

Character	Dura	Pisfera	Tenera	Ref.
Shell thickness (mm)	2–8	–	0.5–4	[13]
Shell thickness (%)	25–65	–	1–30	
Mesocarp content (%)	33–55	96–100	60–95	
Kernel (%)	5–25	0–4	2–15	
Present of ring of fiber	No	Yes	Yes	
Seed/nut size (cm)	2–3	–	< 2	
Seed/nut weight (g)	4–13	–	2	
Ultility	Mother palm	Pollen parent (mostly female sterile)	Commercial Planting material (Cross between Dura x Pisifera)	

Previous studies stated that dura varieties have healthier nutritional components compared to varieties of pisifera and tenera [14]. The relative proportions of moisture, fat, and kernels in the three varieties of *Elaeis guineensis Jacq*. are shown in Table 2.

Table 2. Relative proportions of moisture, fat, and kernel in three varieties of *Elaeis guineensis* Jacq.

Parameter	Dura	Pisifera	Tenera	Ref.
Moisture (%)	0.34 ± .01	0.32 ± .05	0.76 ± .04	[14]
Oil yield (%)	23.57 ± 1.00	55.24 ± 2.64	30.88 ± 1.18	
Kernel content (%)	65.52 ± 1.45	12.44 ± .26	14.85 ± 1.08	

Based on the table presented above, it can be seen that the characteristics of the moisture, fat, and kernel content have different values. However, the reason is quite clear because it is influenced by the shape of the seeds in the oil palm fruit. The table explains that pisifera has the highest oil yield and the least kernel content. On the other hand, dura has the highest kernel content and produces the least amount of oil. This is supported by previous theories, which states that dura produces lower oil than others [15]. However, dura seeds have a thick seed shell that prevents water and gas from entering the seeds [16].

Pisifera has a higher oil content, but the disadvantage is that the pisifera seed coat is very thin, so it is susceptible to dryness and microbial contamination. Pisifera, has low fertility and produces relatively little fruit, so it is not used commercially in oil palm plantations except to provide pollen to produce tenera hybrid offspring [17]. Previous literature revealed that the highest yielding varieties of palm oil were yield tenera a cross between dura and pisifera, so that tenera seeds are mostly cultivated [18]. Hence, the tenera variety forms the basis of commercial palm oil production worldwide [19].

3.2. Carotenoid profile in palm oil extract.

Based on the table presented above, the carotenoid content in palm oil is quite high, especially in the dura variety followed by tenera. Carotenoids are a class of tetraterpenoids that play an important role in plants and animals [21].

Table 3. Composition of carotenoids contained in Malaysian palm oil extract.

		Carotene Composition (%)			
	Chemical Strucure	**Dura**	**Pisifera**	**Tenera**	**Ref.**
Phytoene	Acyclic carotene	2.49	1.68	1.27	[20]
Cis-β-carotene	Cyclic carotene	0.15	0.10	0.68	
Phytofluene	Acyclic carotene	1.24	0.90	0.06	
β-carotene	Cyclic carotene	56.02	54.39	56.02	
α-carotene	Cyclic carotene	54.35	33.11	35.16	
Cis-α-carotene	Cyclic carotene	0.86	1.64	2.49	
ζ-carotene	Acyclic carotene	2.31	1.12	0.69	
γ-carotene	Cyclic carotene	1.10	0.48	0.33	
δ-carotene	Cyclic carotene	2.00	0.27	0.83	
Neurosporene	Acyclic carotene	0.77	0.63	0.29	
β-zeacar otene	Cyclic carotene	0.56	0.97	0.74	
α-zeacarotene	Cyclic carotene	0.30	0.21	0.23	
Lycopene	Acyclic carotene	7.81	4.50	1.30	
Total carotene (ppm)		997	428	673	

Carotenoids have various functions in human health, such as antioxidant effects, eye health, heart health, improved cognitive function, and prevent certain types of cancer [22]. The carotenoid composition that is often used is β-carotene. β-carotene, the main dietary source of provitamin A, is necessary for maintaining optimal human health [23].

The process of analyzing the carotenoid composition above has been investigated by Chee *et al.* The first thing is done by extracting the mesocarp using the method Soxhlet and hexane solvent for 5 hours. The extract obtained was added with 5 mL of 50% ethanolic KOH for the saponification process, then the sample was added with 50 mL of petroleum ether until the resulting supernatant became colored. The combination of the extract and petroleum ether was washed with distilled water and dried over sodium sulfate. The next step is to analyze the sample using an HPLC (High-Performance Liquid Chromatography instrument) with a UV detector. The results obtained from this study were that many carotenes are 11 types had been identified, the largest components being α-carotene and β-carotene with a proportion of 90% of the total carotene [24].

Table 4. Carotenoid content in palm oil from several countries.

Origin	Fruit Type	Fruit Color Type	Range (ppm)	Ref.
Indonesia	Dura, tenera	Virescens	155–1246	[25]
Malaysia	Unknown	Unknown	500–700	[26,27]
Thailand	Unknown	Unknown	554.7 –731.5	[28]
Nigeria	Dura, tenera	Virescens, nigrescens, and albescens	100–1000	[29]
Brazil	Unknown	Unknown	422.1 –584.2	[30]
America	Unknown	Unknown	4600	[24,27]

The study of the table above provides information on the range of carotenoid levels in palm oil from parts of America, Africa, and Asia, which are the distribution areas of oil palm trees. The information obtained from the table shows that carotenoid levels in various countries are different (Table 4). This can be influenced by several factors, such as differences in species, varieties, and growing locations [27]. Also besides, the resulting carotene content will differ according to the variety and fruit maturity [31]. Judging from the varieties of fruit types and fruit color types, the highest carotenoid producers were tenera (fruit type varieties) and virescens, nigrescens was the color type variety of the highest carotenoid-producing fruit types.

These varieties of fruit color types have their respective advantages. The palm oil of the fruit color type albescens provides a selective advantage for the industry because it has the lowest carotene content compared to others. According to research by Obibuzor *et al.*, palm oil of the fruit color type albescens only requires 5% adsorbent for the process of bleaching 500-1000 ppm oil [29].

Based on the study of the table above, there are several unknown varieties of fruit types. The related literature also does not explain the varieties of fruit types that have been analyzed in detail. However, the varieties of these fruit types may be dura and tenera, as they are the most widely cultivated varieties.

3.3. Method of carotenoid analysis in palm oil.

Some methods of carotenoid analysis in palm oil are presented in table 5.

Table 5. Some methods of carotenoid analysis in palm oil.

Method of Analysis	Extraction Method	Optimized extraction conditions	Results		Tahun	Ref.
			Qualitative	Quantitative		
UPLC	Solvent Extraction	• Solvent: hexane (soaked using hexane solvent for 24 hours, then filtered and evaporated)	There are 11 types of carotenoids that have been identified	–	2016	[32]

Method of Analysis	Extraction Method	Optimized extraction conditions	Results		Tahun	Ref.
			Qualitative	Quantitative		
HPLC	Extraction by saponification reaction	• Solvent: petroleum ether • Addition of 30 mL in 60% KOH (w/v) • Washed with distilled water	–	832–3575 μg g^{-1}	2009	[33]
	Solvent extraction, mixer-settler system	• Solvent: EL and Etol • Temperature (°C) 20 • Mixer-settler: CPO /EL/Ethol mixture (48%: 31.2%: 20.8%) • Time (min) 10	–	11.3%	2018	[34]
UV-Vis spectrophotometry	Ultrasound-assisted extraction	• Solvent: ethanol • Temperature (°C) 20 ± 2 • Comparison of sample to solvent (10 g / 100 mL) • Time (hour) • Rotation (rpm) 150 • Ultrasound intensity: 120 $W.cm^{-2}$	–	2.53– 4.07 mg/g	2017	[35]
	Accelerated Solvent Extractor (ASE)	• Solvent: petroleum ether • Temperature (°C) 70 • Time (min) 5 • The filtrate is evaporated at 35 °C, and the extract is stored at –20 °C	–	>1500 mg Kg^{-1}	2018	[36]
	Solvent extraction	• Solvent: hexane • Comparison of CPO with hexane (1: 5) • Divortex for 10 minutes	–	510 μg mL^{-1}	2019	[37]
	Soxhlet, Supercritical carbon dioxide	• Solvent: CO2 and hexane • (hexane solvent to extract total oil for 8 hours using soxhlet) • Temperature (°C) • Pressure (bar) 40 • Time (min) 120	–	90%	2019	[38]
Raman and FT-NIR spectroscopy	Solvent extraction	• Solvent: hexane (palm fruit samples were extracted using hexane solvent and evaporated in a vacuum evaporator to produce CPO)	Raman analysis: 1100-1500 cm-1 (number wave) FT-NIR analysis: 5276 cm-1 (wave number)	539.79 ppm	2019	[39]

EL: ethyl lactate, Etol: ethanol, FT-NIR: Fourier transform near-infrared.

3.3.1. UPLC.

Research Ng *et al.* developed a new method for qualitative analysis of carotenoids in CPO using UPLC (Ultra Performance Liquid Chromatography) with a PDA detector. UPLC refers to ultra-performance liquid chromatography, which improves in three ways: speed, resolution, and sensitivity. The reason for choosing this method is because it offers a more efficient and time-saving analysis compared to the HPLC method. Where, this system uses fine particles (less than 2.5 μm), thereby reducing column length, saving time, and reducing solvent usage. The results obtained from this qualitative analysis study revealed that 11 types of carotenes had been identified from CPO but were not included from the cis/trans carotene isomer [32,40].

3.3.2. HPLC.

Carotenoid analysis often uses HPLC because it can distinguish carotenoids from other compounds with a geometric structure similar to carotenoids [41]. HPLC is an analytical separation technique and is suitable for the separation of macromolecules [42]. The information study of the carotenoid analysis method using HPLC (Table 5) shows that the extraction methods used by the two researchers are very different, namely solvent extraction and saponification reactions.

Research by *Kua et al.* stated that ethyl lactate and ethanol solvents are safe methods for extracting phytonutrients such as carotenes and tocols from CPO. The choice of these two solvents has been considered by him because most of the carotene extract and tocol are used as food additives. Thus, the solvent used must-have criteria such as non-toxic, non-corrosive, and non-carcinogenic so that it is safe for consumption. The use of a mixer-settler system in this study is based on the stability of carotenoids, where carotenoids are thermolabile compounds [43]. Also besides, temperature, heating duration, and type of solvent have been reported to affect the structural effect of carotene [44]. Meanwhile, Monderesearch *et al.* used the extraction method with the saponification reaction technique. The goal is to separate the fatty acids that are bound to carotene. The fatty acids are expected to form soap compounds with the addition of bases so that they can be released from carotene.

3.3.3. UV-Vis Spectrophotometry.

According to The United States Pharmacopeia 38, the analysis of carotenoid compounds, especially β-carotene, can use visible spectrophotometric instruments [45]. The use of this instrument shows the potential for analyzing β-carotene levels, where the pigment can absorb radiation in the area of visible 400–600 nm [46]. Also besides, the β-carotene structure has alternating double and single bonds, so this method is more specific for β-carotene analysis.

The extraction methods used by some of these researchers are very diverse, ranging from solvent extraction, accelerated solvent extractor, soybean, and supercritical carbon dioxide. The use of organic solvents in food processing negatively affects people's thinking about safety. One alternative extraction method for this problem is technology supercritical fluid extraction (SFE) [47]. To obtain carotenoids or natural oil products that are residue and solvent-free, the use of SFE is highly considered. One of the supercritical fluids that are often used is carbon dioxide because it has the advantages of being non-toxic, non-flammable, economical, and has high purity [48].

Research by Tai *et al.* has carried out carotenoid extraction using the method supercritical carbon dioxide to recover minor compounds contained in CPO [38]. Supercritical CO_2 has been applied in the extraction, purification, and fractionation of CPO [49]. This pretreatment to remove the oil fraction using SC-CO_2 plays an important role in the enrichment of β-carotene from lower concentrates to higher concentrates [50].

On the other hand, ultrasonic-assisted extraction is an environmentally friendly, efficient technique because the solvent used is reduced during sample preparation and the extraction time is shorter than conventional extraction methods [51].

3.3.4. Raman and FT-NIR spectroscopy.

The research of Nokkaew *et al.* has carried out the extraction process of carotenoids using solvent extraction. The analytical methods used were Raman and FT-NIR spectroscopy. The basic consideration for choosing these two methods is considered to have advantages, namely environmentally friendly, not using solvents, and fast measurement compared to the UV-Vis and HPLC spectrophotometric methods, where the method must use solvents and spend a lot of time during the analysis process. These studies showed that Raman spectrophotometry was better than FT-NIR for carotenoid determination in CPO [39].

The study above includes the consideration of the analytical method used to obtain carotenoids in CPO. Based on Table 5, the most commonly used method is UV-Vis Spectrophotometry. However, previous researchers also used other analysis methods such as HPLC and UPLC, which are analytical techniques with the advantage of being able to separate the analyte from the matrix. Also besides, there is a process of developing new analytical methods, namely Raman and FT-NIR spectroscopy.

Based on the study of the table above, it can be seen that the levels of carotenoids obtained from CPO are different. The difference in levels of a compound can be influenced by several factors, such as internal and external factors. The internal factors include differences in species, varieties, and growing locations, while the external factors themselves include sample preparation, differences in solvent use, and the methods used at the time of analysis, both for the extraction method and the analysis method.

4. Conclusions

Carotenoid compounds are phytonutrients contained in CPO with the main composition of α-carotene and β-carotene. Based on the information presented in this paper, it can be seen that the carotenoid composition of three varieties of oil palm fruit is in the presence of 11 types of carotene and the highest percentage composition is β-carotene with a content range of 54.39–56.02%. To obtain carotenoid compounds from CPO, various analytical methods have been used, as presented above. As for the development of new methods for carotenoid analysis from CPO, namely Raman and FT-NIR spectroscopy with the advantages of being environmentally friendly, not using solvents, and fast measurement compared to methods spectrophotometric UV-Vis, UPLC, and HPLC.

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Conflicts of Interest

The authors declare no conflict of interest.

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