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Production of Carotenoids from Oil Palm Empty Fruit Bunches: Selection of Extraction Methods

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Highlights:

- Potential of OPEFB as carotene source.
- Hexane is the best solvent for carotene extraction from OPEFB.
- The spikelet has higher carotene content than the stalk.
- A smaller OPEFB cut size gives a higher carotene concentration in the extract.

Abstract. Public awareness of the importance of natural food colorants is increasing. Carotenoids are a coloring agent that is widely used for food applications to give a yellow, orange, or red color to food products. Natural carotenoids can be produced by extraction from various sources, such as carrots and palm oil, or from algae and fungi. Oil palm empty fruit bunches (OPEFB) as the biomass waste of palm oil industry may contain carotenoid residue so that extraction of OPEFB constitutes the crucial step in carotenoid production. The effects of the solvent used for the extraction as well as the size of different parts of OPEFB on the obtained carotenoid were evaluated. The experiments were performed by Soxhlet extraction using n-hexane, ethanol, and isopropyl alcohol as solvents at the boiling point of each solvent. The ratio of OPEFB to solvent was 1:60 (w/v) with variation of OPEFB size. The results showed that the optimum carotene concentration was obtained from extraction using n-hexane on 5-cm OPEFB spikelets.

Keywords: *carotenoid; extraction; empty fruit bunches; solvent; size; spikelet; stalk.*

1 Introduction

Carotenoids are isoprenoid pigments that are synthesized by photosynthetic organisms including plants, some bacteria, yeast, algae, and non-photosynthetic fungi. They are a natural source of yellow, orange, and red colorants. There are about 700 carotenoids in nature [1]. An example of the most widely used

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carotenoid compounds in the food industry is β-carotene, which is used as a coloring agent for oil and fat products such as margarine and cooking oil. This compound has antioxidant properties and is also a natural precursor of vitamin A, therefore it can be added to food products to increase their nutritional value. In the cosmetics and pharmaceutical industries, carotenoids are commonly used to color drug capsules, supplements, and cosmetics [2]. Popular sources of carotene are carrots, sweet potatoes, and palm oil [3]. The greatest economic value of carotene compounds is in nutritional supplements, followed by food, animal feed, and cosmetics [4-6]. β-carotene is the type of carotenoid compound with the highest economic value, i.e., US$ 128 million in 2009.

Palm oil is one of Indonesian top plantation commodities. Based on Indonesian Palm Oil Statistics (2015), oil palm plantations in Indonesia covered 11.30 million hectares, with oil palm production estimated at 31.28 million tons. Carotenoid compounds found in palm oil are α-carotene and β-carotene [7]. Schroeder, *et al.* [8] put forth that carotenoids contained in palm oil are dominated by β-carotene (60%) and α-carotene (34%). The potential of palm oil as vitamin A source is 15 times greater than that of carrots or 300 times that of tomatoes [9].

The processing of palm oil produces a large amount of biomass waste, such as oil palm empty fruit bunch (OPEFB), fibers, and kernels. Oil palm milling annually generates about 1.5 ton of palm oil empty fruit bunches per hectare of palm oil [10]. So far, OPEFB has been used for mulching or as compost. Other uses, e.g., as raw material for biofuel and other biochemicals, are still under development [11-12]. Considering the high concentration of carotene in palm oil, it is expected that OPEFB contains traces of palm oil and OPEFB, which may be a potential source of raw material for carotenoid production. Thus, the utilization of this biomass waste as raw material for carotene production will contribute to its economic value and the circular economy of the palm oil industry. Kupan, *et al.* [13] and Manurung, *et al.* [14] have reported oil re-extraction from palm oil biomass waste, which contained up to 702 to 915 ppm of carotene, while another study indicated that OPEFB contains between 4000 to 6000 ppm of carotenoids [15].

The type of solvent affects the extraction process and the recovery of carotenoids. Carotenoids are compounds of low polarity and therefore soluble in low polarity solvents. Some carotenoid compounds also have polar parts and are thus also soluble in polar solvents. Organic solvents are effective for extracting carotenoids [16]. Extraction is normally performed at high operating temperatures and is affected by the chemical affinity between molecules in the system [17]. The choice of a suitable solvent or solvent combination is one of the most important factors for efficient carotenoid extraction. However, solvent selection is not always easy because other factors also play an important role, such as the functional group (polarity) and the length of the existing carotenoid chain, the sample matrix and its components, and the water content. In addition, in selecting the type of solvent used, several other factors must also be considered, such as sustainability, environmental impact, health, and safety.

This paper describes the development of a method for carotenoid extraction from OPEFB. In particular, the effects of solvent used, size and different parts of OPEFB used in the extraction are discussed.

2 Materials and Methods

2.1 Materials

The OPEFB used in the experiments were collected from PTPN VIII in Cikasungka, Bogor, West Java, Indonesia. The OPEFB parts used in the experiments were the stalk and the spikelet (Figure 1).

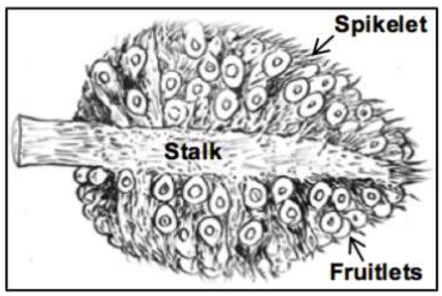

Figure 1 Physical structure of OPEFB. Reference: Yunos, *et al.* [24].

2.2 Methods

Soxhlet extraction was used to extract β-carotene compounds from the OPEFB. The ratio of OPEFB sample to solvent was 1:60 (w/v). Five grams of OPEFB was placed on a thimble and 300 mL solvent was added to a round flask. Each extraction process was carried out for about 3 hours. Three types of solvent were evaluated, i.e., n-hexane, ethanol, and isopropyl alcohol. The extraction process was conducted at the boiling temperature of each solvent.

Initially, three types of OPEFB cutting sizes were tried, 1 cm, 8 cm and 15 cm, using the inner part of the OPEFB, namely the stalk (Figure 1). Later, the outer part of the OPEFB, namely the spikelet, was also tried. One spikelet cut size was used, i.e., 5 cm.

The concentration of carotenoid extract was measured using a visual spectrophotometer at a wavelength of 450 nm. To convert the absorbance into carotene concentration, a calibration curve was set up using pure β-carotene standard (Sigma-Aldrich).

3 Results and Discussions

The experimental results showed that the highest carotenoid concentration was obtained when the OPEFB samples were extracted using n-hexane compared to being extracted using ethanol or isopropyl alcohol (Figure 2). Carotenes are nonpolar so it can be expected that they are more soluble in nonpolar solvents such as n-hexane.

N-hexane is an alkane hydrocarbon compound that has a symmetrical structure, with the chemical formula C_6H_{14}. Because of its symmetrical structure and its C and H only composition, n-hexane is very nonpolar. On the other hand, ethanol (C_2H_5OH) has both polar and the nonpolar groups. The presence of the hydroxyl group in ethanol gives ethanol polar properties, while the alkyl group ($-C_2H_5$) gives ethanol nonpolar properties. Isopropyl alcohol (C_3H_7OH) also belongs to the alcohol group but with a higher molecular weight than ethanol. When compared with ethanol, isopropyl alcohol has more nonpolar properties due to the longer alkyl group than that in ethanol. Indeed, the polarity index of n-hexane, isopropyl alcohol, and ethanol is 0.1, 3.9, and 5.2, respectively [18]. This indicates that n-hexane is very nonpolar compared to isopropyl alcohol or ethanol. N-hexane also has the lowest boiling point among the three evaluated solvents. A lower boiling point minimizes the degradation of carotene during the extraction as well as the further concentration process. Thus, n-hexane is the most suitable solvent to be used for extracting carotene.

N-hexane released into the environment reacts with pollutants to form ozone and photo chemicals during the extraction and recovery process [19]. Several studies have revealed that n-hexane affects the neural system when humans inhale it because of its solubility in neural lipids [20]. In view of the safety, health, and sustainability aspects, it may be better to use ethanol or isopropyl alcohol. However, n-hexane is an effective solvent for this extraction process. Furthermore, n-hexane has been widely used for oil extraction because of its ease of use in the oil recovery process, narrow boiling point (63 to 69 °C), and excellent solubilizing ability [21]. As long as the n-hexane used for extraction meets the standard parameters and is used considering the health and environmental aspects, n-hexane can be used as a solvent in food processes. In addition, it should be noted that in the food processing industry, the n-hexane solvent used is required to be free from aromatic compounds.

N-hexane recovery can be applied to reduce the impact of n-hexane on the environment. The n-hexane recovery process can be carried out by withdrawing vapors overhead from the extractive distillation column and condensing the same and withdrawing the condensate as an n-hexane product of a purity sufficient for use in food processing [22].

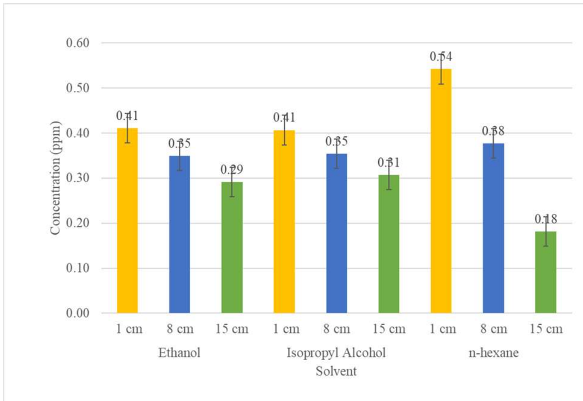

Figure 2 Effect of solvent and OPEFB sample size on carotene extraction from the OPEFB stalk.

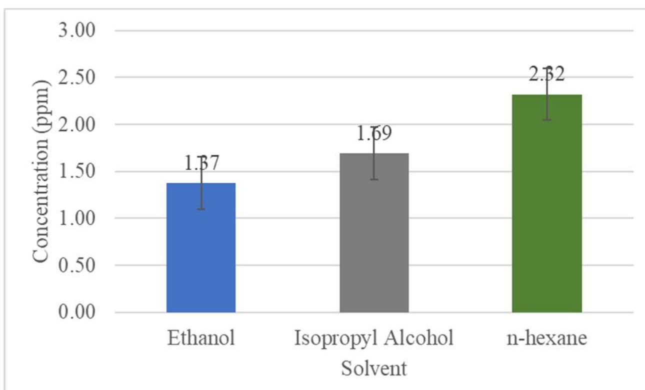

Figure 3 Effect of solvent on carotene extraction from OPEFB spikelets.

The experimental results showed that the highest carotenoid concentration was obtained from extraction of the OPEFB samples with the smallest cut size, i.e., 1 cm (Figure 2). A consistent trend was observed for all types of solvent used. The cut size of the OPEFB samples affects the available contact area between the solvent and the samples. The smaller size of the OPEFB sample provides a larger contact (surface) area between the sample and the solvent. The larger the contact surface area between the solvent and the samples, the more carotenoid can be extracted. A similar trend has been observed in other extraction processes [23-24].

Overall, the results of the present experiment showed that the optimum carotene extraction conditions were obtained using n-hexane solvent and 1 cm OPEFB cut size. However, the ANOVA analysis showed that the solvent's p-value was 0.983, while the p-value of the OPEFB cut size was 0.05. This shows that the effect of sample size on the concentration of the carotene extract was more significant than the effect of type of solvent used in the extraction.

To confirm the effect of type of solvent used on the concentration of the carotene extract, a similar set of experiments was carried out using samples from another OPEFB part, i.e., the spikelet (Figure 1). However, the effect of OPEFB sample size could not be varied as the spikelet is very tough and difficult to break. Instead, 5 cm-length OPEFB-spikelet samples were used for all experiments. The obtained results are presented in Figure 3. Despite the different structures of the stalk and the spikelet, the results presented in Figure 2 and Figure 3 show a consistent trend. Samples extracted using n-hexane gave the highest carotene concentration compared to samples extracted using the other solvents, i.e., ethanol and isopropyl alcohol.

Interestingly, the samples extracted from the spikelet (Figure 3) contained a significantly higher carotene concentrations compared to the samples extracted from the stalk (Figure 2). The data in Table 1 summarize the concentrations of carotene in the samples extracted using n-hexane.

Table 1 Comparison of carotene samples extracted from different parts of OPEFB using n-hexane.

Sample OPEFB	Concentration of carotene (ppm)
8 cm length – OPEFB stalk	0.38 ± 0.06
1 cm length – OPEFB stalk	0.54 ± 0.20
5 cm length – OPEFB spikelet	2.32 ± 0.01

Despite the difference in the OPEFB cut size used in the extraction, the concentrations of carotene in the samples extracted from the spikelet were four-to six-fold higher than from the stalk. This indicates that the part of OPEFB used in the extraction process affects the concentration of carotene in the extract. This is also supported by research on the physical and chemical characteristics of the residual oil in OPEFB, which states that the oil content in the spikelet is higher than in the stalk [25]. However, further study is necessary to precisely quantify the proportion of stalk and spikelet in OPEFB. If the stalk only is only a small portion of OPEFB than it may not be necessary to use the stalk for carotene extraction at all.

Overall, the best result obtained in this study was for the carotene sample extracted using n-hexane from the OPEFB spikelet at 5 cm length. The carotene concentration in the corresponding sample was 2.32 ± 0.01 ppm. The result was comparable with our previous result [26], which reported the concentration of carotene extract from fresh, molded (fungal fermented), and old OPEFB. This concentration is very low and thus further concentration or purification may be necessary before its application. Although some other literature on carotene extraction from oil palm biomass waste is available [13-14,27], a fair comparison between the obtained results is difficult to make as different extraction methods were applied. For example, Kupan, *et al.* [13] obtained 702 ppm of β-carotene extract from the extraction of OPEFB followed by adsorption. Manurung, *et al.* [14] applied maceration extraction as well as a larger OPEFB sample size to obtain 915 ppm of carotene extract and suggested that re-extraction of palm oil from oil palm waste reduces the greenhouse emission of the palm oil industry.

4 Conclusions

The obtained results show that carotenes can be extracted from OPEFB. Extraction of carotene from the spikelet gives a higher concentration compared to the stalk and n-hexane is the optimum solvent for extraction. The cut size of the OPEFB significantly affects the carotene concentration in the extract. However, preparing small cut size OPEFB spikelets for further processing may be difficult. In order to provide final carotene products that can be used in industry or other applications, the extract should be concentrated and/or purified further. Considering the large availability of OPEFB as biomass waste from the palm oil industry, OPEFB has large potential to be used as raw material for carotene production.

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