Production of carotenoids from oil palm empty fruit bunches

M T A P Kresnowati, D Lestari, M Anshori, R M Jafar

Food and Biomass Processing Research Group, Industrial Technology Faculty, Institut Teknologi Bandung,
Jalan Let.Jen.Purn.Dr.(HC) Mashudi (Jalan Raya Jatinangor) No.1, Sumedang 45363
kresnowati@che.itb.ac.id

Abstract. Carotenoids are widely used for food colorants to give yellow, orange, or red color in food products. Besides extraction from orange color vegetables/fruits such as carrot, yellow squash, or oil palm, natural carotenoids can also be extracted from microorganisms such as algae or fungi. The processing of oil palm fresh fruit bunches to crude palm oil also produces empty fruit bunches (EFBs) as the biomass waste. This extraction residue still contains traces of oil and thereby also contains carotenoids. The dumped EFBs nearby the palm oil processing plants are also a potential substrate for natural fungal fermentation. This paper explores the potential of EFBs and fungal fermented EFBs as the raw materials for carotenoid extraction. The soxhlet extraction method was applied, using hexane as the solvent. Carotenes were analysed by using spectrophotometer. The obtained results showed that carotene can be extracted from EFBs and fungal fermentation increased the carotene content of EFBs.

1. Introduction
Carotenoids are pigments responsible for red, yellow, and orange colours. They belong to the terpenoids, the group of lipids that has isoprene as the backbone structure. Carotenoids mostly have 40 carbon atoms and cover among others α-carotene, β-carotene, lycopene, astaxanthin, and canthaxanthin. β-carotene, in particular, has been granted permission by Indonesian National Food and Drug Control (BPOM) to be used as colorant in food industries and is widely used as food colorant. β-carotene exhibits antioxidant properties and is the precursor for vitamin A, thereby β-carotene is a molecule of high nutritional value. β-carotene can be produced synthetically via Grignard reaction (the Roche/DSM’s route) or via Wittig reaction (the BASF’s route) [1]. Whereas natural β-carotene is produced via extraction of vegetables or from fermentation of fungal, bacteria, or algae. Natural β-carotene can be consumed at larger quantities.

Oil palm is one of the top Indonesian plantation commodities. Data from Indonesian Statistic Bureau showed that in 2015 the area of oil palm plantations reached 11.3 million hectare and produced around 31.28 million tons of crude palm oil. Besides producing the crude palm oil as the main product, palm oil industry also produces biomass wastes such as empty fruit bunches (EFB), fibers, and shells. EFB is produced at ratio about 0.2 – 0.3 ton/ton fresh fruit bunches (FFB) [2]. EFBs are normally dumped nearby the palm oil plants and are left to be decomposed. Some are used as mulch, but the abundance availability of EFB offers the potential for it to be used as raw materials. Literature study indicated that EFB still contained oil residue as well as other lipid components that dissolved in it. Kupan et al [3] and Manurung et al [4] showed that the carotenoid content of the oil reextracted from EFB was up
to 702 – 915 ppm. The effect of natural fungal growth on carotenoid content of EFB, however, has never been studied. The goal of this research was to explore the potential of oil palm empty fruit bunches and fungal fermented oil palm empty fruit bunches as the raw materials for carotenoid extraction.

2. Materials and methods

2.1 Raw materials

Fresh oil palm empty fruit bunches (EFBs) were collected from PTPN VIII at Cikasungka Plantation, Bogor, West Java, Indonesia. Only the outer part of EFB, i.e., the spikelet, that was used in the experiments. The spikelet of EFB was cut to 5 cm height before further used.

2.2 Extraction

Carotenoids in EFB were extracted by using the Soxhlet extraction method. A 5 g EFB sample was added into the thimble and 300 mL of hexane was used as the solvent for the extraction. Each extraction process was set to proceed for 3 hours.

2.3 Analysis

The carotenoid content in the extract was analysed using a spectrophotometer at 450 nm. The concentration of carotene was obtained by using a calibration curve that was prepared by diluting various concentration of β-carotene standard (Sigma Aldrich).

3. Results and discussion

3.1 Carotenoid content of oil palm empty fruit bunch

Six independent samples of fresh EFB were extracted and consistent carotenoid extracts were obtained (Figure 1). The obtained carotenoid concentration in the fresh EFB extracts ranged from 1.94 to 2.54 ppm, giving an average carotenoid concentration of 2.27 ppm. The variation of carotenoid content in these six samples was also relatively small, less than 10%. Literature study indicated that palm oil was a potential source of β-carotene [1], the obtained data showed that the biomass waste of palm oil industry, EFB, also contained carotene.

![Figure 1. EFB extract and distribution of carotenoid concentration in six independent EFB samples](image)

3.2 Effects of fungal fermentation on carotenoid content of empty fruit bunch

Various types of fungi will naturally grow on the dumped EFB nearby the palm oil plants. Sequential fungal growth is normally observed, which is initiated by yellow orange colour fungi and is continued by white colour fungi until the EFB becomes rotten. The growth may start as early as the second day.
from which the EFB is dumped. Several EFB samples were taken before the fungal growth, during the yellow-orange-colour-fungal growth (during fungal growth), and during the white-colour-fungal growth (after fungal growth). Each sample was extracted and analysed for its carotenoid content (Figure 2).

Significantly different carotenoid content was observed between the groups of EFB samples. The highest carotenoid content was observed in the EFB samples during fungal growth. The average carotenoid contents of the before fungal growth samples, during fungal growth samples, and after fungal growth samples were 2.27, 3.08, and 1.87, correspondingly. This data indicated that the growth of yellow orange fungal in the EFB increased the carotenoid content of the EFB. Thereby it is best to extract the carotene from EFB during this fungal growth.

Fungal production of $\beta$-carotene has been reported. For example, using Blakeslea sp., Phycomonas sp., and Neurospora sp. or yeast species of Rhodotula sp. and Sporobolomyces sp. [1, 5-7]. Commercial $\beta$-carotene production has been performed using Blakeslea trispora, employing submerged fermentation [5]. The obtained data showed that the existence of yellow-orange fungi on EFB increased the carotenoid content of EFB samples. In other words, fungal fermentation using solid EFB as substrate could produce carotene. Production of $\beta$-carotene by solid state fermentation on EFB, by employing endogeneous fungi will significantly offer potential benefit over the submerged fermentation. The feasibility, however, needs to be studied further. This specific EFB endogenous fungal species samples have been isolated and need to be characterised further.

![Figure 2. Effects of fungal growth on EFB on the corresponding carotenoid content](image)

### 3.3 Potential overview of carotenoid production from empty fruit bunch

The potential of carotenoid production from empty fruit bunch is best presented with the yield of carotene from EFB. Table 1 shows that the yield of carotene from EFB ranges from 0.11 – 0.18 mg carotene/g EFB.
Table 1. Carotene yield from EFB

| Type of EFB sample                  | Average Carotenoid concentration in the extract [ppm] | Yield of carotene from EFB [mg/g] |
|------------------------------------|-----------------------------------------------------|----------------------------------|
| Before fungal growth (day 0-2)     | 2.27 ± 0.22                                         | 0.14 ± 0.01                      |
| During fungal growth (day 3-8)      | 3.08 ± 0.55                                         | 0.18 ± 0.03                      |
| After fungal growth (day 9-18)      | 1.87 ± 0.31                                         | 0.11 ± 0.02                      |

The popular sources of carotene are carrots (Daucus carota) and sweet potatoes (Ipomoea batatas) may contain up to 174 and 226 ppm carotene, succeeding [8], whereas the potential oils are palm oil (Elaeis guineensis) and buriti (Mauritia vinifera) that may contain up to 700 and 3380 ppm carotene. The obtained results showed the carotenoid content of EFB were in the range of 0.11 – 0.18 mg/g which corresponded to 110 – 180 ppm. These numbers are comparable to that of carrots. β-carotene can be found in the market as concentrate (in oil) or as solid powder. The final experiment was conducted to evaporate the carotene extract to obtain concentrate, residual oil rich in carotene. 0.065 g carotene-rich-oil was obtained from each g of EFB, containing about 713 ppm of carotene. This number is comparable with the carotenoid content of CPO [8]. Further purification method needs to be applied to obtain higher carotene concentration. Nevertheless, the overall results showed that carotene can be produced from EFB. Considering that EFB is the biomass waste of palm oil industry, the production of natural β-carotene from EFB could be much cheaper. This research indicates the potential of carotene production using EFB as the raw material/substrate.

4. Conclusion
Natural carotene has been successfully extracted from palm oil empty fruit bunch (EFB) during fungal growth using Soxhlet extraction. Fungal growth on EFB had significant effect on carotene yield, where extract from EFB during fungal growth achieved the highest carotene concentration than extract from EFB before fungal growth and after fungal growth. This research indicates the potential of carotene production using EFB as the raw material/substrate.

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