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EXTRACTION AND ANALYSIS OF BETA-CAROTENE RECOVERY IN CPO AND OIL PALM WASTE BY USING HPLC

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ABSTRACT

Beta-carotene is known to have a high demand in food industry which has made global industry to face challenges especially in fulfilling customers' requirement that are looking for ''environment friendly'' and natural products. This has opened up wide opportunities in utilizing crude palm oil (CPO) and oil palm waste (OPW) as natural sources of beta-carotene. Thus, present study explains recovery of beta-carotene from CPO and OPW of palm pressed fiber (PPF) and empty fruit bunch (EFB). Initially, crude oil from solid OPW samples was extracted by soxhlet extraction. Then, recovered oil from both PPF and EFB along with CPO were used to extract palm carotene. Extraction of beta-carotene from CPO and OPW were performed by using soxhlet adsorption method. High performance liquid chromatography (HPLC) analysis revealed beta-carotene as a major carotene in extracted samples. Results obtained indicated that 3790 ppm of beta-carotene extracted from CPO, 1414 ppm from PPF and 702 ppm from EFB by this soxhlet adsorption method.

Keywords: beta-carotene, HPLC, CPO, EFB, PPF.

INTRODUCTION

Beta-carotene is one of the key products of food industry which has been widely used as nutrients and additives. The global market of carotenoids shows a gradual increase every year, whereby beta-carotene as the most prominent carotenoid. However, recently there are rapid growing concerns about the source of the ingredient along with awareness about potentially harmful synthetic compound from the consumers. These rising consumer awareness and concerns have made the global demand for naturally produced beta-carotene increases gradually. Hence, industries are dynamically searching for new sources as well as introducing different technologies to produce beta-carotene. An interesting alternative to this problem is crude palm oil (CPO) and oil palm wastes (OPW), the natural sources of carotene that is known to contain high concentration of natural carotenoids.

The main carotenoids of palm oil are alpha-carotene and beta-carotene; together they made more than 80 % of the total carotenoids in palm oil (Ooi, Choo *et al.* 1994) with 36.4 % alpha-carotene and 54.4 % beta-carotene (Birtigh, Johannsen *et al.* 1995). It has a greater carotenoids concentration than any other oil or fat (Baharin, Rahman *et al.* 1998) and these carotenes contribute to palm oil stability and nutritional value (Mustapa, Manan *et al.* 2011). The concentration of carotenoids normally ranges between 400 and 3500 ppm (Ahmad, Chan *et al.* 2008) where a total of 11 types of carotenoids in palm oil have been identified (Hudiyono and Septian). However, carotenes in palm oil are destroyed in the present refining process in order to produce light colour oils.

On the other hand, the sustainable conversion of OPW which are widely found in nature can be transformed into high added-value products by extracting phytochemicals that can be useful in cosmetic, pharmaceutical and intermediates for the chemical industry (Ofori-Boateng and Lee 2013). It is known that palm pressed fiber oil is enriched with natural carotene, vitamin E, sterols, squalene, co-enzyme Q10, and phenolic compounds (Lau, Choo *et al.* 2008) and residual fibers from palm oil production contain between 4000 to 6000 ppm of carotenoids which is about six times higher than that found in crushed palm oil (Mustapa, Manan *et al.* 2011).

Carotenoids are lipid-soluble pigments responsible for the colour of a wide variety of foods, where there are about 700 carotenoids in nature (Amorim-Carrilho, Cepeda *et al.* 2014). According to (Pénicaud, Achir *et al.* 2011) carotenoids consist of two classes of molecules known as carotenes and xanthophylls. Commercially carotenes are used in food coloration, vitamin supplements, pharmaceutical and cosmetic products (Ooi, Choo *et al.* 1994). Beta-carotene that belongs to the carotene group is the most widespread in foods with chemical formula of C40H56 and composed of eight isoprene units with specific retinyl rings (Pénicaud, Achir *et al.* 2011). This large aliphatic molecule has a molecular weight of 536.9 g mol-l (Birtigh, Johannsen *et al.* 1995). Figure-1 shows the chemical structure of beta-carotene.

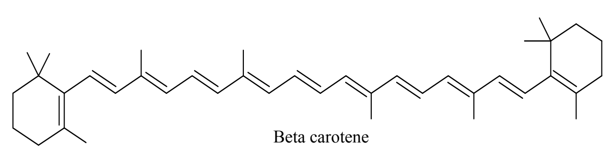

Figure-1. Chemical structure of beta-carotene.

Carotenoids world needs increase every year, especially for food, animal feed and pharmaceuticals. Yet, there is no generally accepted or standard method for

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extraction of carotenoids. Most of the extraction methods follow a common path involving the release of desired components from their matrices by disrupting tissue followed by removal of the unwanted components (Amorim-Carrilho, Cepeda *et al.* 2014). Previously reported methods on carotenoids recovery from palm oil are saponification, adsorption, selective solvent extraction, transesterification followed by distillation, and others [(Ooi, Choo *et al.* 1994); (Latip, Baharin *et al.* 2000); (Latip, Baharin *et al.* 2001), and (Baharin, Latip *et al.* 2001)]. Supercritical fluid extraction using CO2 was applied to enrich crude palm oil from carotenoids up to 200-fold (Davarnejad, Kassim *et al.* 2008). While (Chiu, de Morais Coutinho *et al.* 2009) have studied and applied membrane technology to recover higher concentrations of carotenoids from oil palm biomass.

This study offers an interesting option for recovery of beta-carotene from CPO and OPW of palm pressed fiber (PPF) and empty fruit bunch (EFB). Soxhlet adsorption was employed to extract palm carotene from CPO and OPW. Soxhlet extraction method was initially used to the extract crude oil from the OPW samples which are in solid form. These methods were chosen by considering the risk of beta-carotene degradation and also the physical state of sample being used. As these extraction methods were performed at low temperature, the risk and possibility of degradation of beta-carotene can be lowered. Beta-carotene recovered by this extraction method were further analysed by using HPLC. In a nutshell, this study is believed could support the current demand and can be a good turnover in a process of converting waste to wealth.

MATERIALS AND METHODS

Extraction of Crude Oil from OPW Soxhlet Extraction

About 8 g of dried PPF was weighed and added into the extraction thimble and placed into soxhlet extractor. The soxhlet extractor was assembled by using 200 ml of hexane in a 250 ml round flask. The thimble was inserted in the extractor and heated for 60 min or 10 cycles at 60-65 °C. After the extraction time is complete (hexane in extraction thimble becomes colourless), the solvent is completely removed using rotary evaporator. Soxhlet extraction was repeated few times with PPF and again with same amount of EFB to extract sufficient amount of oil for soxhlet adsorption.

Extraction of Beta-Carotene from CPO and OPW Soxhlet Adsorption

HP-20 (synthetic highly porous resin) of 24 g was transferred into 250 ml conical flask. The adsorption process was started by initially activating the adsorbent using 50 ml of isopropanol (IPA) with continuous stirring for about 30 min. Then, adsorbent was filtered, dried at room temperature and transferred into 250 ml three necks round bottom flask. The round bottom flask was set in a water bath, maintained at a temperature of 40-45 °C and 6 g of CPO that was diluted with 50 ml of IPA was added slowly with continuous stirring for 1 hr. IPA was chosen as first IPA extraction will be carried out and it is important in order to extract and remove maximum fatty acids and polar compounds from sample (Latip, Baharin *et al.* 2001).

Once the adsorption process is completed in, the treated HP-20 was transferred into the soxhlet extraction thimble and polar compounds from sample were extracted from the adsorbent with IPA for 1 hr. Then, followed by carotene extraction using hexane for about 3 hour (adsorbent became colourless). Finally, the solvents were removed from both fractions and concentration of beta-carotene was determined by using HPLC. For this soxhlet adsorption method, different ratio of CPO to HP-20 (1:2, 1:3 and 1:4) and IPA extraction time (0, 1 and 2 hour) were studied to determine the optimum conditions to extract the beta-carotene. The optimised experimental conditions were used to extract palm carotene from CPO and OPW. Figure-2 shows the soxhlet adsorption set up.

Figure-2. Soxhlet adsorption set up.

HPLC Analysis

Water Alliance E2695 HPLC with an automated injector and Photo Diode Array Detector were used to determine beta-carotene in palm carotene extracted. Standard calibration curve was plotted using result from HPLC analysis and concentrations of beta-carotene in extracted samples were calculated. The measurements conditions are at absorbance of 450 nm and at column temperature of 40°C where the C18 reversed phase column was used for analysis. The mobile phase used was acetonitrile/dichloromethane (8:2, vol/vol) at a flow rate of 1 mL/min and analysis time of 45 min.

RESULTS AND DISCUSSION

The calibration curve was plotted based on HPLC results obtained for series of beta-carotene standards with different concentration. Peak area of four different standards was used to plot calibration curve of concentration against peak area. Figure-3 shows the HPLC

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standard calibration curve which was used to determine the concentration of beta-carotene extracted from each samples.

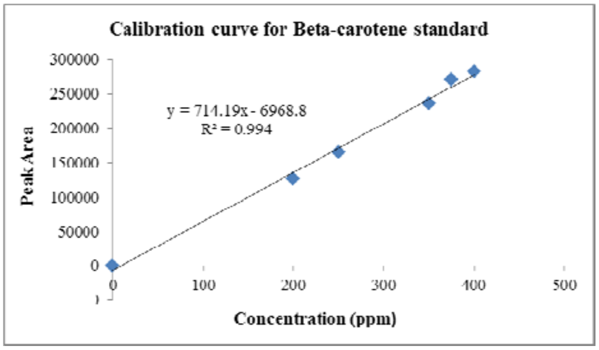

Figure-3. HPLC analyses of standard calibration curve of beta-carotene.

Extraction of palm carotene rich in beta-carotene from CPO and OPW by soxhlet adsorption was performed using HP-20 as adsorbent. Initially CPO was used to optimize the soxhlet adsorption method by studying different ratio of sample to adsorbent and also different IPA extraction time. Then, recovery of beta-carotene under optimised conditions was performed using CPO and OPW. Results for the extraction of palm carotene at different ratio of oil: HP-20 (1:2, 1:3 and 1:4) and different IPA extraction time (0, 1 and 2 hour) were tabulated in Table-1 and Table-2. The concentration of beta-carotene in extracted palm carotene samples was determined by HPLC analysis and calculated using the HPLC standard calibration curve.

Table-1. Concentration of beta-carotene recovery from CPO at different IPA extraction time.

IPA Extraction Time (hr)	Concentration of Beta-carotene Recovery (ppm)
0	706
1	3265
2	2583

For soxhlet adsorption, IPA adsorption is important as it extracts maximum polar and oil compounds while leaving carotene to be extracted using hexane later. Results show that from 6.0 g of CPO used for the extraction, concentration of beta-carotene recovered increases as extraction time increases from 0 to 1 hour then decreases at 2 hr. Based on HPLC analysis results, at 1 hour IPA extraction time the concentration of beta-carotene recovered was 3265 ppm compared to 706 ppm in sample without IPA extraction (0 hr). This is because longer IPA extraction time leads to removal of more oil and polar compounds in the sample. Thus, it increases the concentration of beta-carotene extracted. However, beta-carotene recovery at 2 hr of IPA extraction was lower than 1 hour which was 2583 ppm. This is due to the fact that, longer IPA extraction time also leads to degradation of beta-carotene as extraction using IPA was performed at higher temperature. Overall the results agree with the finding of (Latip, Baharin *et al.* 2001) that beta-carotene recovery increases with shorter IPA extraction time. Therefore it is concluded that 1 hour of IPA extraction is an optimum time for the soxhlet adsorption to recover high concentration of palm carotene.

Table-2. Concentration of beta-carotene recovery from CPO at different ratio of CPO: HP-20.

CPO: HP-20 ratio	Concentration of Beta-carotene Recovery (ppm)
1:2	1143
1:3	2314
1:4	3790

For the case of effect of different ratio of sample to adsorbent which is CPO to HP-20 on the extraction of beta-carotene, extraction by using ratio of 1:2, 1:3 and 1:4 were studied and the results were showed in Table-2 above. Trend of the result shows that as the ratio of CPO: HP-20 increases, the concentration of beta-carotene recovered also increases from 2314 ppm at ratio 1:3 to 3790 ppm at ratio 1:4. This is because as larger amount of adsorbent (HP-20) used, the surface area of this non-polar adsorbent increases. Thus more beta-carotene is being adsorbed on it and being eluted by hexane.

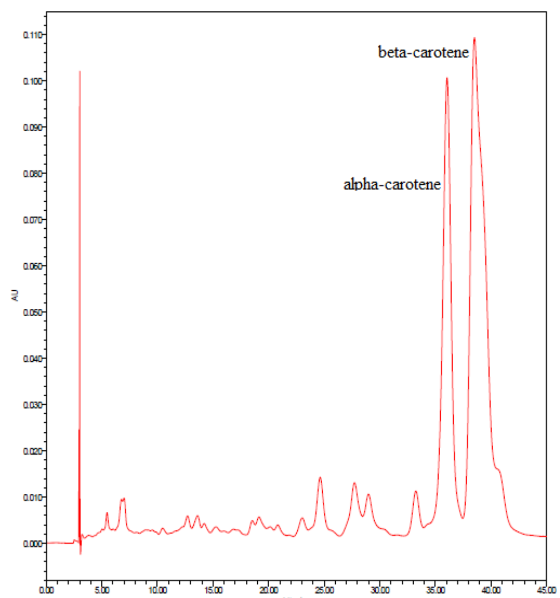

Figure-4. HPLC chromatogram of palm carotene.

Hence, ratio of 1:4 of CPO to HP-20 adsorbent is efficient in extracting palm carotene using soxhlet adsorption. HP-20 adsorbent used in this study is three dimensional cross-linked polymers resin with macropores that is suitable in extracting beta-carotene. This was supported by the findings of (Latip, Baharin *et al.* 2000) who reported that this synthetic adsorbent with large pores is suitable for the adsorption of large molecules and organic substances by means of van der Waals' forces. Beside, according to (Baharin, Rahman *et al.* 1998) the ability of this resin is due to the superior surface area and greater hydrophobicity. Availability of binding site for more adsorbing capability increases as the amount of adsorbent increases (Latip, Baharin *et al.* 2000). HPLC chromatogram of palm carotene extracted by soxhlet adsorption is demonstrated in Figure-4. From the chromatogram, it can be concluded that mixture of carotene mainly alpha- and beta-carotene was extracted. However, the major peak is representing beta-carotene agree with the chromatogram presented by (Baharin, Rahman *et al.* 1998) presenting the palm carotene extracted using HP-20. On the other hand, waste materials such as OPW are found in abundance and polluting the environment. However, this issue or problem can be tapped through the process of waste management. Via this waste management, vital nutrients or phytochemicals such as carotene can be recovered and extracted which also then can be utilized in production of aroma compounds via degradation. Soxhlet adsorption is faster and cheaper and it does not require sophisticated set up, thus it can be used to recover the beta-carotene from these sources. Recovery of beta-carotene from crude oil of PPF and EFB also CPO at optimized soxhlet absorption condition was performed.

Optimised conditions of soxhlet adsorption method were 1 hour of IPA extraction and ratio of 1:4 of crude oil to HP-20. Based on the results obtained from the analysis, it is proven that beta-carotene recovered from CPO is in higher concentration compared to OPW. Results were showed in Table-3 below. Based on HPLC analysis, the concentration of beta-carotene recovered in PPF and EFB were 1414 and 702 respectively, while in CPO was 3790 ppm. Overall, among three different samples used which were CPO, PPF and EFB, CPO was proven to contain high concentration of beta-carotene.

Table-3. Concentration of beta-carotene recovery from CPO and OPW.

Sample	Concentration of Beta-carotene Recovery (ppm)
PPF	1414
EFB	702
CPO	3790

CONCLUSIONS

High recovery of beta-carotene is achieved at significantly shorter IPA extraction time which is 1 hr and at ratio of 1: 4 of CPO to HP-20 adsorbent for the soxhlet adsorption method. Comparison of two different sources of palm carotene indicated that there were significant differences on concentration of beta-carotene extracted from CPO and OPW. Soxhlet adsorption method tends to extract more palm carotene with high concentration in CPO compared to OPW. This study is believed could support the current demand as it can be a good turnover in a process of converting waste to wealth. In conclusion, utilizing CPO and OPW as natural sources of beta-carotene will fulfill customers' requirement that are looking for environment friendly and natural products.

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