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Extraction of Palm Carotenes and Effect of Oxidative Degradation on β-carotene

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***Abstract* -The growing demand on beta-carotene has generated huge challenges to global industry to fulfill the customers' requirement that are looking for natural and environment friendly products. This study explains the efficient extraction of carotenoids from Crude Palm Oil by adsorption (column chromatography) where in the suitability of adsorbent is discussed and saponification methods followed by degradation of beta-carotene in an attempt to study the possible norisoprenoids that can be potentially generated using palm carotene in future. The HPLC analysis showed the high concentration of beta-carotene in extracted samples. Gas chromatography/mass spectrometric (GC-MS) analysis showed that, the main degraded compound generated were β-ionone, DHA and ionone epoxide.**

Keywords-Carotenes; β-ionone; β-carotene; HPLC; Degradation

INTRODUCTION

Beta-carotene is one of the main palm oil processing byproducts that can be used in the food [1], pharmaceutical [2], and cosmetics industries [3] apart from oleochemical industries [4]. It is very reactive compound due to its highly unsaturated structure which belongs to the carotenoids group, and also prone to degradation more precisely to isomerisation, especially at high temperature [5]. Basically the range of carotenoid content in crude palm oil (CPO) from Malaysia falls between 500 to 700 ppm [6]. The intense orange color of CPO is due to these carotenoids. Hence, high importance was given to the removal of carotenoids with other impurities during the oil refining process [7]. Even though there has been numerous efforts made to extract carotenoids either by removing chemically converted triglycerides through saponification or transesterification or by using adsorbent materials [6] still more efforts are in progress towards efficient extraction of carotenoids from CPO to fulfill the carotenoids world requirements especially in food, animal feed and pharmaceuticals [4]. Carotenoids are not only responsible for the color, but also important as in nutritional point of view, because some of them have provitamin A activity [8]. Carotenoids are mainly used compared to others dyes in the industry as they are not affected by the existence of ascorbic acid or heating and freezing cycles. Besides, they are particularly known to be strong dyes as they able to impart the desired properties to foods even at ppm levels. As there is high requirements of customer and demanding regulations concerning the usage of artificial dyes, carotenoids are utilized in food technology extensively [9].

On the other hand degradation of carotenoids leads to the formation of aroma compounds that are important in sensorial and food industry [10]. According to [11], carotenoids are extremely prone to degradation due to factors such as heat, low pH, and light exposure that promotes the formation of compounds such as cis-isomers, epoxides, short chain products and also volatile compounds. Through chemical and enzymatic reactions carotenoids able to generate some of aroma compounds, i.e., carotenoids are precursors of norisoprenoids [12]. The C-13 norisoprenoid compounds, such as β-ionone, β-damascone, β-damascenone and others are important aroma compounds associated to carotenoid degradation.

Thus, here we made an effort to an efficient extraction of carotenoids rich in β-carotene from CPO by saponification and column chromatography method followed by oxidative degradation of β-carotene as an attempt to produce aroma compounds. We have used two different adsorbents for column chromatography extraction method which is silica gel and HP-20. The extracted carotenoids were analyzed by HPLC and degraded compounds were analyzed by GC-MS studies.

CHEMICALS, RAW MATERIALS AND METHODS

All the chemicals used were of analytical grade. Silica gel, synthetic highly porous resin (DIAION HP-20) and tartaric acid were bought from TAAT BESTARI SDN BHD. Crude palm oil (CPO) was obtained from Felda Palm Industries Sdn Bhd, Lepar Hilir 3, Pahang. Beta-carotene (type I, brand Sigma) was bought from Permula Chemicals Sdn. Bhd.

EXTRACTION OF CAROTENOIDS FROM CPO

Column Chromatography

About 50 g of HP-20 (synthetic polymer resin) adsorbent was weighed and transferred into 250 ml beaker for activation. The adsorbent was activated using 100 ml isopropanol (IPA) with continuous stirring for about 30 min. Then, the activated adsorbent was filtered and dried at room temperature. The column was then packed with this dried HP-20 and eluted

with little IPA. Then, 10 g of CPO was weighed and dissolved with a little IPA and then loaded into the column to contact with the HP-20. The column was first eluted with IPA and fractions of IPA that is light in yellow color were collected. Then, the carotenoids were eluted with second solvent which is hexane once the IPA fraction color changes from light yellow to almost colorless. The presences of beta-carotene in all the hexane fractions were determined by using thin layer chromatography (TLC) plates. The solvent from each fraction were removed by a rotary evaporator and the carotene content was determined by using HPLC. Column chromatography experiment was repeated by using 50 g of silica gel without any activation, whereby the column is directly packed with silica gel that is in slurry form. In this case first column was run by IPA and then carotenoids were extracted with hexane.

Saponification

About 5 g of CPO was added to ethanolic potassium hydroxide (3 ml of 60% KOH in water + 5 ml of ethanol). The mixture was kept in freezer for 24 hr (to eliminate lipids and to precipitate polyphenols in the alcoholic phase). The saponified mixture was then placed in a separating funnel with 5 ml of ethyl ether and this phase was washed with water, dried over anhydrous sodium sulphate, and evaporated to dryness. The experiment was carried out at room temperature with minimum light exposure. The final residue was dissolved in chloroform and filtered before HPLC injection.

High Performance Liquid Chromatography Assay of β-carotene (HPLC)

The β-carotene content in carotenoids extracted by column chromatography and saponification method was measured by using high performance liquid chromatography (HPLC). Commercial beta-carotene has been analyzed by using HPLC and standard calibration curve has been plotted to determine the concentration of carotenes in extracted samples. The measurement conditions are at an absorbance of 450 nm and at column temperature of 40 ◦C. The mobile phase used was acetonitrile/dichloromethane (8:2, vol/vol) at a flow rate of 1 mL/min and analysis time of 30 min.

OXIDATIVE DEGRADATION OF B-CAROTENE

This method was adapted and modified based on [13]. About 20 mg of β-carotene was introduced in a 20 ml of hydroalcoholic solution prepared by using 120 ml ethanol, 5 g tartaric acid and pH 3.4 adjusted with 1 M NaOH containing 2.5 mg/liter iron oxide. The solution was left at room temperature for five days. Three different solutions (A, B, and C) were prepared. Sample A was prepared by using 20 mg of β-carotene and covered/sealed completely without any exposure to light while sample B was prepared using the 20 mg of β-carotene and kept without any cover from light. As for sample C, the solution was prepared using 20 mg of β-carotene as well, fully covered and another 2.5 mg of iron oxide was added to study effect of excess in catalyst. After five days, organic compounds were extracted using hexane by liquid–liquid extraction method. Identification of degraded compounds was achieved by GC/MS.

GC/MS Analysis

The J&W DB-5 (95% dimethyl, 5% diphenyl polysiloxane) column was used for GC/MS analysis. The column temperature was programmed at 60 °C (1 min) from 310 °C at a rate of 4 °C min–1 (20 min). The injector temperature was 250 °C; in splitless mode. The ionization energy is 70 eV with transfer-line temperature at 250 °C. Mass spectra were scanned in the m/z = 58-650 range. Identification was achieved by mass fragmentometry, a library search (NIST) and comparison with literature data.

RESULTS AND DISCUSSION

Carotenoids of CPO that are rich in β-carotene were extracted by two different methods which were column chromatography and saponification. Both methods were studied to determine the efficiency by comparing the concentration of carotene being extracted. For column chromatography silica gel and HP-20 were used. The carotene content was determined by using HPLC. As for oxidative degradation of β-carotene, the degraded products were analyzed by using GC/MS.

Carotenoids Analysis

The concentration of beta-carotene in carotenoids being extracted by using column chromatography is presented in Table 1 and Figure 1. Standard beta-carotene has been used as a reference to identify the extracted carotene by comparing the retention time and concentration of carotenes were calculated based on HPLC calibration curve plotted using series of beta-carotene with different concentrations.

For column chromatography, the ratio of CPO to adsorbent is 1:5 with 10g of CPO and 50 g of HP-20 and silica gel adsorbents. From the result obtained, it is evident that, the concentration of carotene extracted for both adsorbents silica gel (1228 ppm) and HP-20 (1291 ppm) is almost close and suggests HP-20 to be more effective in extracting carotenoids from CPO. This is because, according to the percentage of recovery, HP-20 is able to recover high percentage of carotene which is 73.1 % compared to silica gel which is only 53.5 %. This ability of HP-20 to adsorb carotene from CPO can be ascribed to the similarity of the molecular structures of carotene and the adsorbents and also to hydrophobic interaction between the adsorbents and carotene [14]. The chromatograms are presented in Figure 2. Only hexane fractions were analyzed by HPLC although β-carotene was observed in IPA fractions. This is because, TLC tests done during experiment for both HP-20 and silica gel showed some other polar compound present in IPA fractions.

The concentration of carotene extracted from CPO by saponification method is only 631 ppm which is half the carotene extracted by column chromatography. Also HPLC analysis of saponified extract in Figure 3 gives extra peak beside α- and β- carotene. Thus based on the HPLC analysis, it is proven that column chromatography method is more efficient compare to saponification method. However in terms of adsorbent being used in column chromatography, HP-20 shows high efficiency in extraction of carotenes than silica gel.

Table 1. Conc. of beta-carotene in extracted sample by using different adsorbents

Adsorbent	Hexane Fraction collected (g)	Carotene	
		Recovery (%)	Concentration (ppm)
HP-20	7.3058	73.1	1291
Silica gel	5.3487	53.5	1228

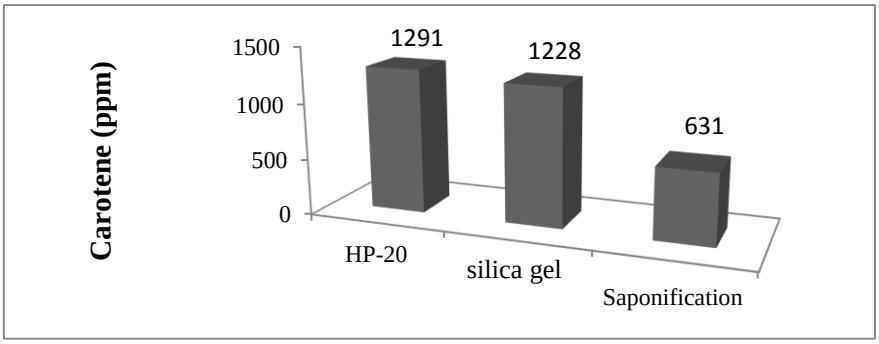


Figure 1. Comparison of conc. of beta-carotene in extracted sample by different methods

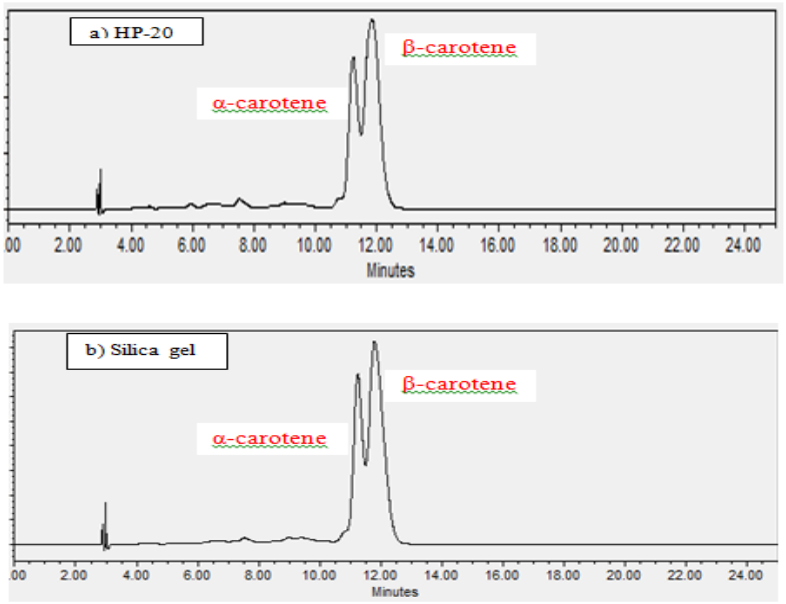


Figure 2. HPLC Chromatograms showing carotenoids of extracted samples

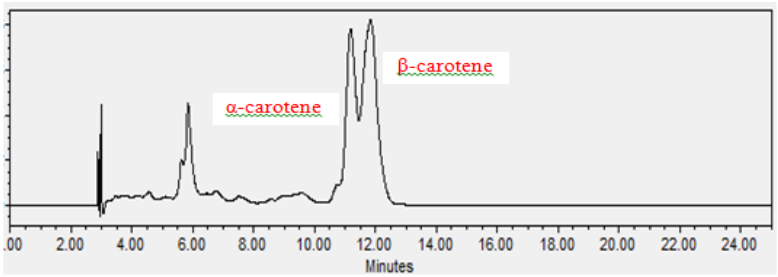


Figure 3. HPLC chromatogram of sample extracted by saponification method

Oxidative Degradation

Though many studies have been reported on biodegradation of beta-carotene [15; 16; 17], very less importance has been given to oxidative degradation of β-carotene. In the present study β-carotene was subjected to oxidative degradation to study the generation of different series of aroma compounds by a very simple method. GC/MS chromatogram is presented in Figure 4. In sample A and B the effect of light were studied against oxidative degradation as light plays an important role in beta-carotene degradation [5], whereas, presence of excess catalyst was monitored in sample C. The degradation products formed by oxidative degradation were subjected to GC/MS and are presented in Table 2.

Due to the unsaturated bonds of β-carotene, it is very prone to oxidative degradation where beta-carotene's undergoes isomerization, followed by formation of radical species and the apparition of cleavage products [5]. Hence, various degradation products are generated and few important aroma compounds in this study were depicted in Figure 5. Trans-beta-ionone, ionone epoxide, DHA and 3-Keto-β-ionone were some of the major compounds determined. Toluene also has been identified in all three samples. Toluene is one of the volatile compounds formed by degradation of beta-carotene along with p-xylene, m-xylene and 2, 6-dimethylnaphthalene due to cyclization of polyene carotenoid chain [18]. Dihydroactinidiolide (DHA) is the most common aroma compound identified in all three samples with high yield (A: 29.07 %, B: 19.5 % and C: 12.44 %).

Samples A and C produced trans-beta-ionone which is a main norisoprenoid. The yield of beta-ionone formed in sample A is 3.72 %, whereas in sample C is 1.18 % while in sample B there is no presence of beta-ionone. This is due to exposure of light to sample B and led to formation of 3-Keto-β-ionone, 2,6-dimethyl cyclohexanol and methanone, dicyclopropyl only in this particular sample. In sample C, the production of trans-beta-ionone is low compare to sample A, though excess of catalyst (iron oxide) has been added. This showed that the increase in amount of catalyst did not contribute to higher production of main norisoprenoid compound and surprisingly a new compound, hexylresorcinol has been determined in sample C.

Table 2. Oxidative degradation products

Products	Identification (GC/MS)		
	A	B	C
Toluene	+	+	+
Trans-beta-Ionone	+	-	+
Hexylresorcinol	-	-	+
Beta-ionone epoxide	+	+	-
Dihydroactinidiolide (DHA)	+	+	+
2,6-dimethyl Cyclohexanol	-	+	-
Methanone, dicyclopropyl	-	+	-
3-Keto-β-ionone	-	+	-

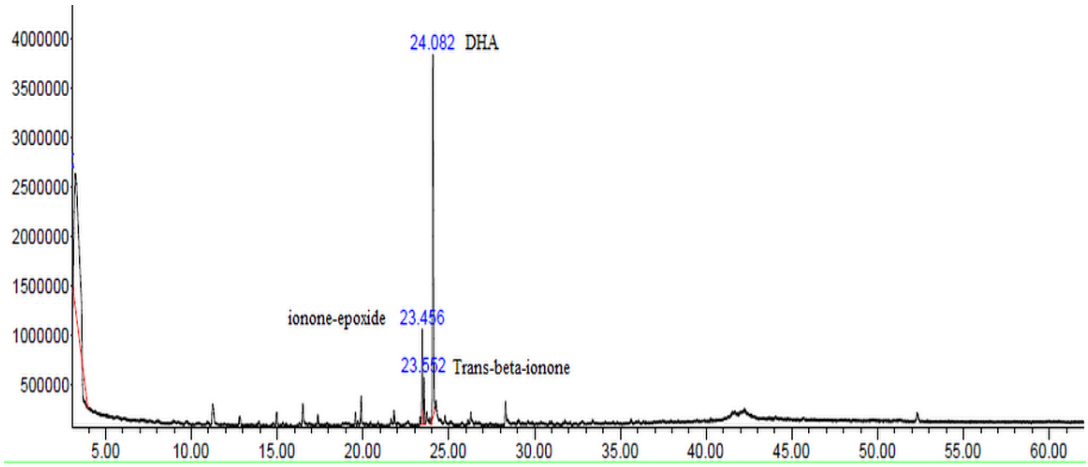


Figure 4. GC/MS Chromatogram of oxidative degradation

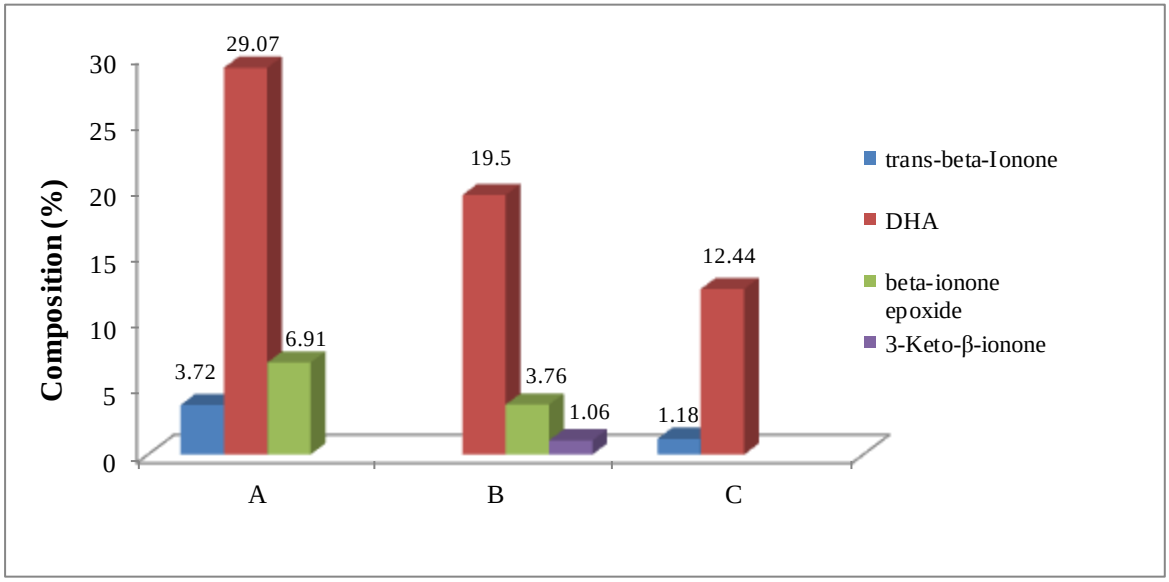
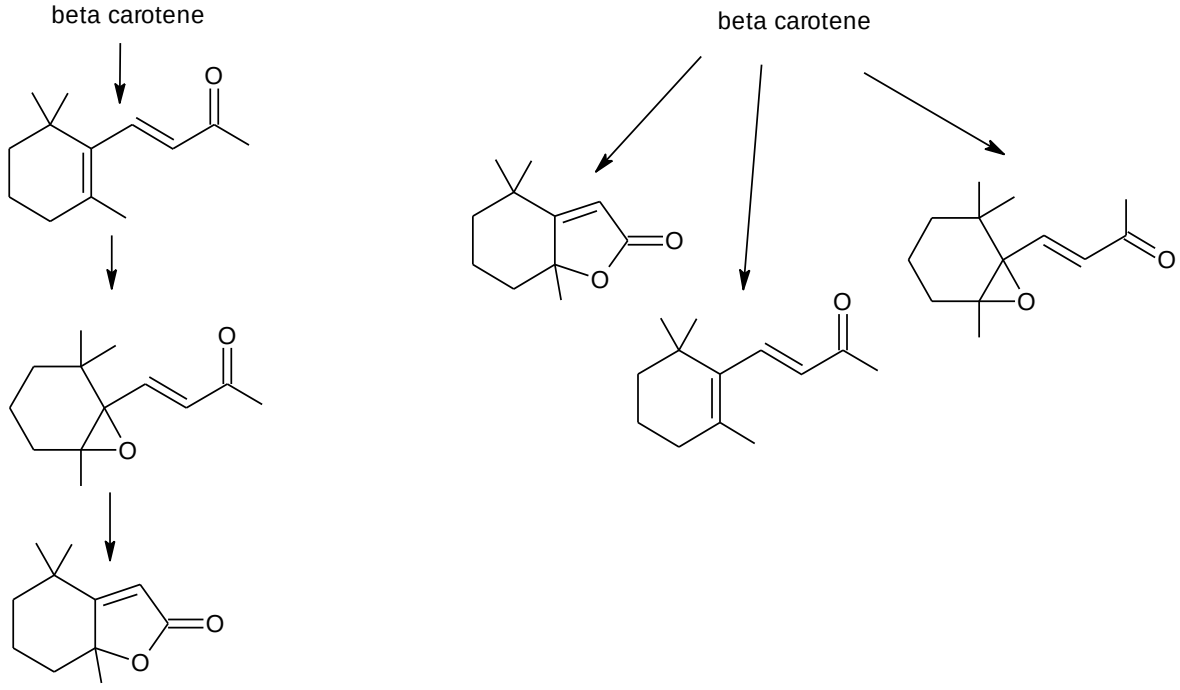


Figure 5. Important aroma compounds generated during oxidative degradation

Scheme: 1



(a) Oxidative degradation pathway proposed by [19].
(b) Important degradation products formed in present study
1: β-ionone, 2: 5,6-epoxy-β-ionone, 3: DHA, 4: ionone-epoxide

Scheme 1 (a) shows the oxidative degradation pathway proposed by [19] and (b) shows some important norisoprenoids generated in this study. According to Scheme 1 (a), it can be concluded that further oxidative degradation of beta-ionone (1) led to formation of DHA (3). As in present study, DHA is the highest yield compound formed in all three samples; it shows that most of the beta-ionone formed has been converted to DHA. Hence, the production of beta-ionone as desired/targeted compound can be increased only if the further degradation of beta-ionone to DHA is prevented in earlier stage of oxidative degradation.

CONCLUSIONS

It can be summarized that column chromatography especially using HP-20 able to extract high concentration of carotenoids from CPO compare to saponification method. Several important aroma compounds viz β-ionone, ionone epoxide, DHA, etc were formed by oxidative degradation of β-carotene. However, addition of extra amount of catalyst did not contribute in increasing formation of beta-ionone and it is proven that during oxidative degradation it is important to cover sample from exposure of light. As, light influence the degradation of beta-carotene and combination of light with oxidation led to formation of some other compounds that are not required.

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