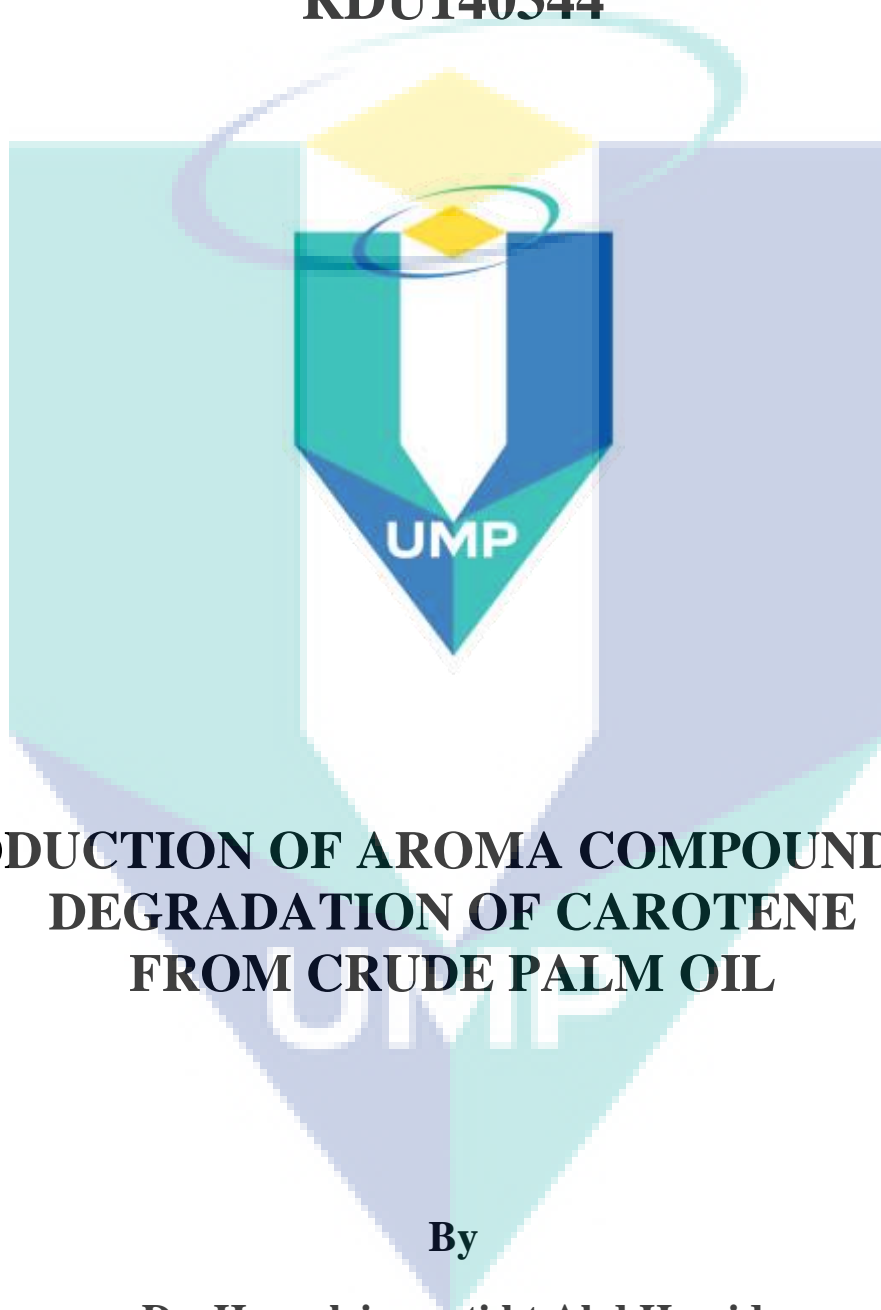


**FINAL REPORT**  
**INTERNAL GRANT UMP**  
**RDU140344**



**PRODUCTION OF AROMA COMPOUNDS BY  
DEGRADATION OF CAROTENE  
FROM CRUDE PALM OIL**

**By**

**Dr. Hazrulrizawati bt Abd Hamid**

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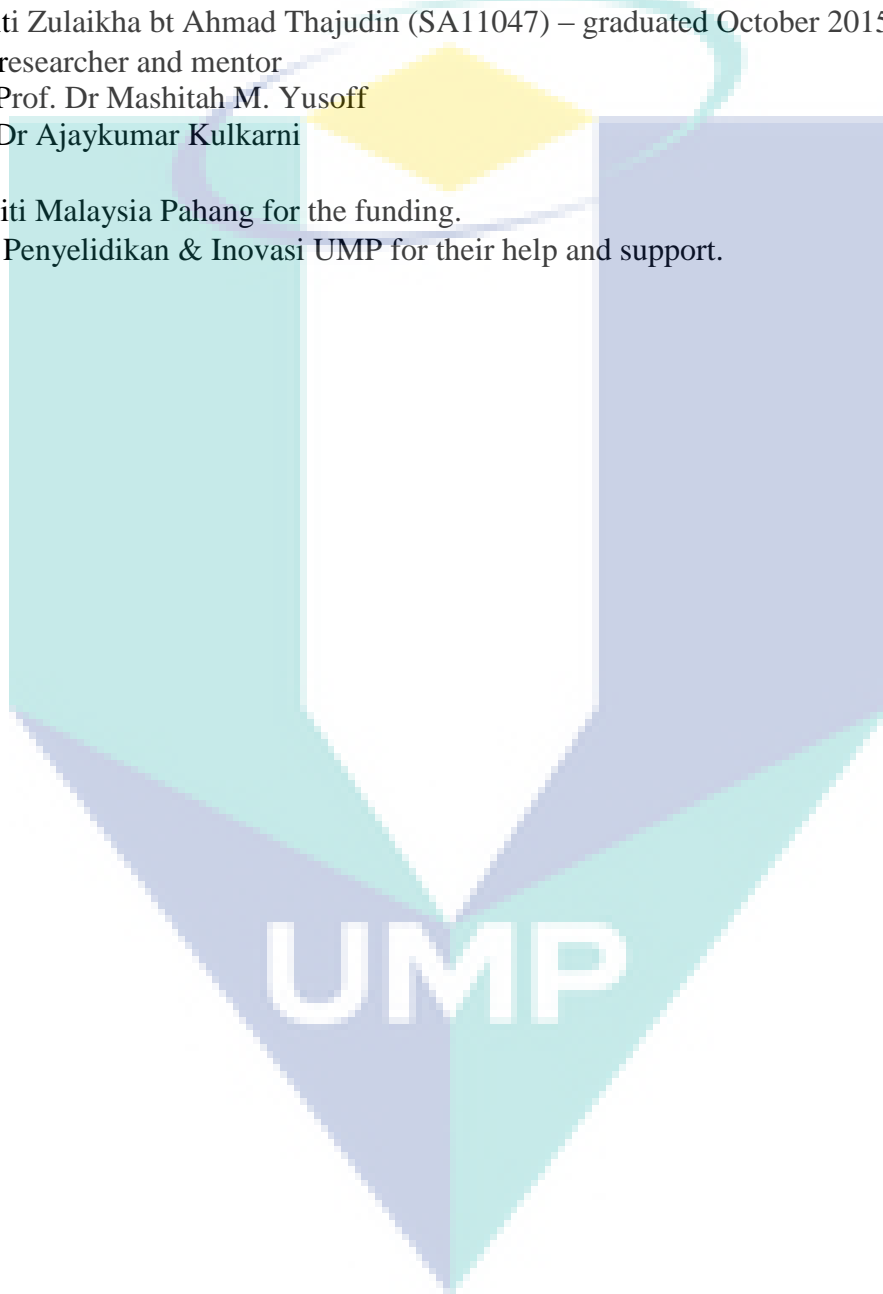
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## ACKNOWLEDGEMENT

I would like to thank the following people and organisations;

- The students working on this project
  - Suria A/P Kupan (MKD14002) – graduated November 2016
  - Siti Zulaikha bt Ahmad Thajudin (SA11047) – graduated October 2015
- The co-researcher and mentor
  - Prof. Dr Mashitah M. Yusoff
  - Dr Ajaykumar Kulkarni
- Universiti Malaysia Pahang for the funding.
- Jabatan Penyelidikan & Inovasi UMP for their help and support.



## REPORT SUMMARY

Production of natural aroma compounds have attained various consideration from industries as well as researchers for the last few decades mainly focusing on cheap and alternative natural sources and also in developing an effective method to reduce cost and time. Hence, this study is believed to address some of the fundamental and practical issues of the area of interest. This research is confined to the formation of aroma compounds by degradation of  $\beta$ -carotene from CPO. Recovery of  $\beta$ -carotene from CPO were performed by using soxhlet adsorption, where the optimum conditions for extraction method were determined by studying different adsorption time and ratio of sample to adsorbent. The concern of this research was focused on the accomplishment of three main goals. Firstly, was to recover  $\beta$ -carotene with high concentration and yield. The recovered palm carotenes were analysed by using HPLC and UV-Vis. Followed by, optimisation of degradation reactions using commercial  $\beta$ -carotene. For thermal degradation reaction, different parameters such as temperature, reaction time and sonication time were studied. Lastly, formation of aroma compounds by degradation of extracted  $\beta$ -carotene under the optimised reaction condition were analysed by using GC-MS and GC-FID. In a nutshell, the focused was given more on qualitative aspects compare to quantitative for the formation of aroma compounds by degradation of  $\beta$ -carotene from CPO. This is because, there is no general reference according to previous research reported on such production and this research is a new attempt in this field which will be the initial stage focusing on formation of aroma compounds by degradation of palm carotene.

Based on results obtained from the research conducted at different IPA extraction time and ratio of CPO: HP-20, highest recovery of  $\beta$ -carotene yield and concentration was being achieved at significantly shorter IPA extraction time which was 1 hr and at ratio of 1: 4 of CPO to HP-20 adsorbent for the soxhlet adsorption extraction method. HPLC and UV-Vis analysis indicated high concentration of  $\beta$ -carotene extracted from CPO. In addition, the optimum parameters on the degradation reaction were determined by using commercial  $\beta$ -carotene. Optimum conditions being predicted based on the results obtained for thermal degradation was 1 hr of sonication time, 5 hr of reaction and at a reaction temperature of 120-130 °C. These developed optimised conditions were used for degradation of extracted  $\beta$ -carotene. Thermal degradation of extracted palm carotene from CPO formed aroma compounds such as  $\beta$ -ionone, DHA, D-limonene,  $\beta$ -ionone epoxide and 3-oxo- $\beta$ -ionone. The

main aroma compound with highest composition formed was DHA (45.91 %) followed by  $\beta$ -ionone analysed by using GC-MS and GC-FID. Hence it was proven that formation of aroma compounds by degradation of  $\beta$ -carotene extracted from CPO is feasible. This research is believed to be vital in contributing fundamental knowledge on the lab scale for the formation of aroma compounds via degradation of  $\beta$ -carotene.

So far, we had total three paper published; one paper published in impact factor journal (International Food Properties, IF , Q2, one paper published in ARPN (Scopus) and another one paper published in IJERT, won 2 awards and have two papers presented in conference. One undergraduate students and one MSc student were graduated from work related to this project. In conclusion, this project has achieved its target for human capital development.

The logo of UMPU (Universitas Muhammadiyah Purwokerto) is a large, stylized shield shape. It is divided into four quadrants by a white vertical line and a white horizontal line that meet at the center. The top-left and bottom-right quadrants are light blue, while the top-right and bottom-left quadrants are light purple. The letters 'UMPU' are written in white, bold, sans-serif font across the center of the shield.

UMPU

## POSTGRADUATE AND UNDERGRADUATE STUDENTS

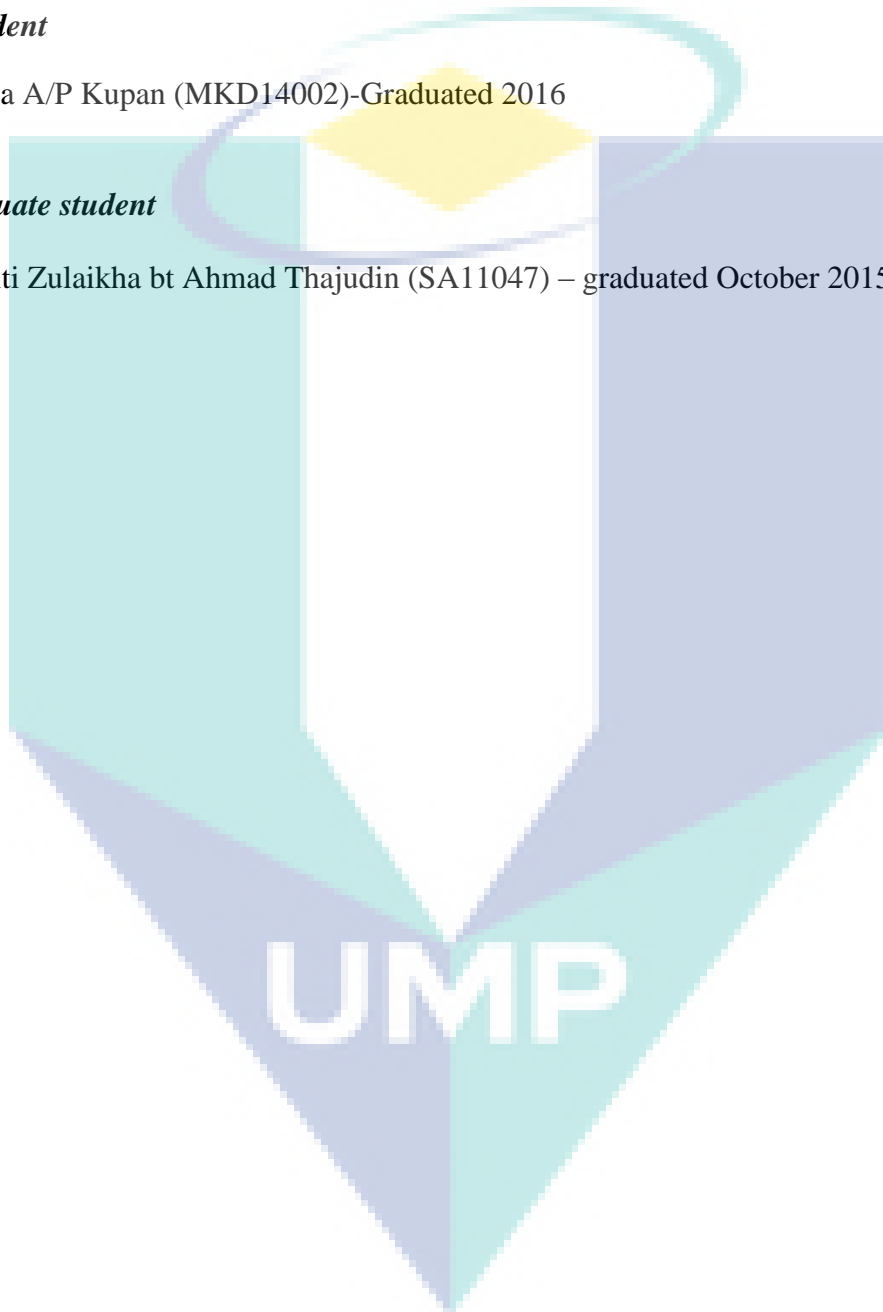
The following student works for this project

### *Master student*

- Suria A/P Kupan (MKD14002)-Graduated 2016

### *Undergraduate student*

- Siti Zulaikha bt Ahmad Thajudin (SA11047) – graduated October 2015



## JOURNALS

This project produced three journal papers

Suria, K., Ajaykumar, D. K., Hazrulrizawati, H. & Mashitah, M. Y. 2015. Extraction of Palm Carotenes and Effect of Oxidative Degradation on  $\beta$ -carotene. *International Journal of Engineering Research & Technology (IJERT)*. 4 (01): 662-667.

Kupan, S., Hamid, H., Kulkarni, A., & Yusoff, M. (2016). Extraction and Analysis of Beta-Carotene Recovery in CPO And Oil Palm Waste By Using HPLC. *ARPN Journal of Engineering and Applied Sciences*. 11 (4):2184-2187

Hamid, Hazrulrizawati, Suria Kupan, and Mashitah M. Yusoff. "Dihydroactinidiolide from thermal degradation of  $\beta$ -carotene." *International Journal of Food Properties* just-accepted (2016).

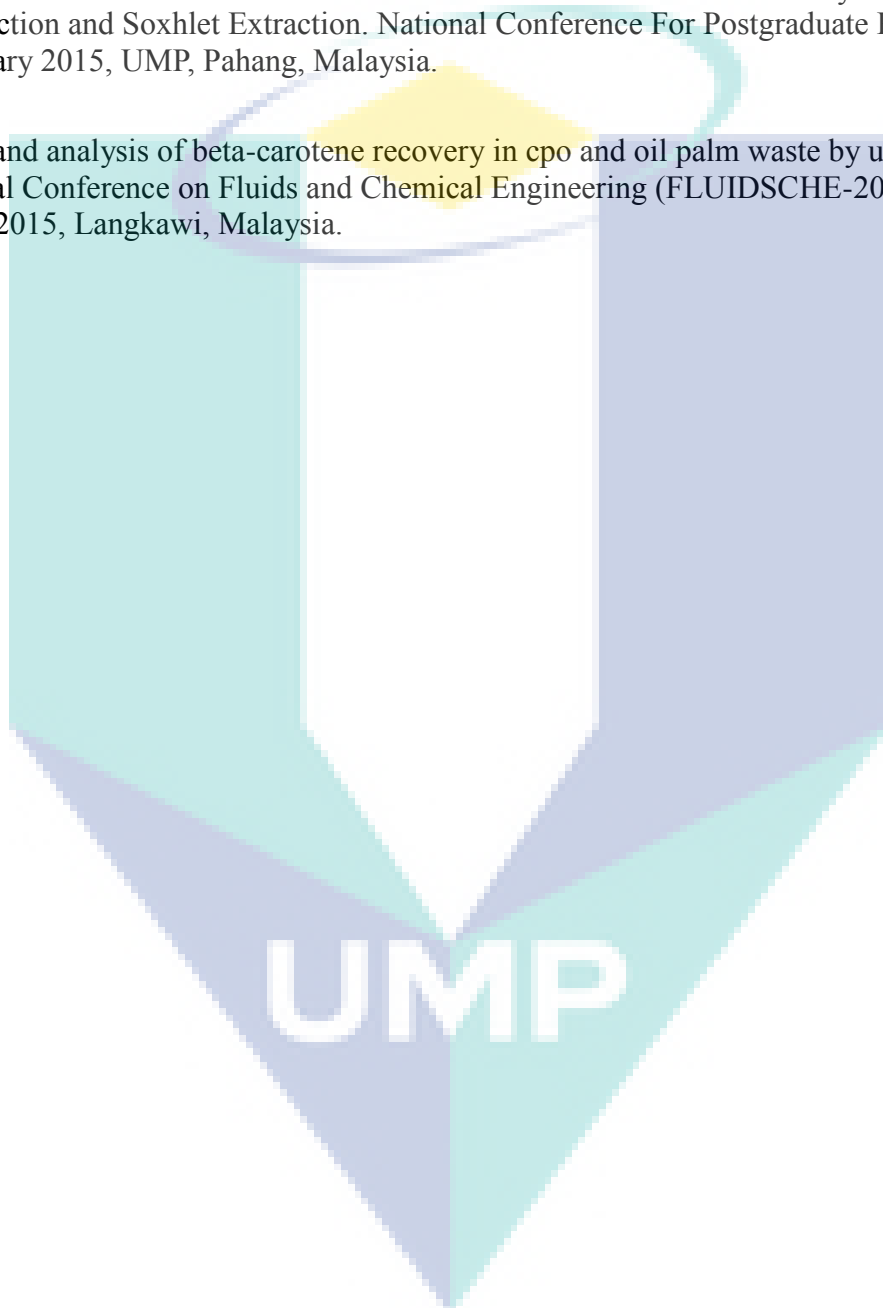


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## PRESENTATION IN CONFERENCE

Comparison of Extraction Method of Palm Carotene from Oil Palm Wastes by Supercritical Fluid Extraction and Soxhlet Extraction. National Conference For Postgraduate Research , 25-25 January 2015, UMP, Pahang, Malaysia.

Extraction and analysis of beta-carotene recovery in cpo and oil palm waste by using HPLC. International Conference on Fluids and Chemical Engineering (FLUIDSCHE-2015), 25-27 November 2015, Langkawi, Malaysia.





## AWARD

Production of aroma compounds by degradation of carotene from crude palm oil and oil palm wastes. Creation, Innovation, Technology & Research Exposition (CITREX), 9-10 March 2015, UMP- **Gold Medal**

Production of aroma compounds by degradation of carotene from crude palm oil and oil palm wastes. 26th International Invention, Innovation & Technology Exhibition ITEX 2015, 21-23 MAY 2015, Kuala Lumpur, Malaysia- ITEX **Bronze Medal**.



## FINANCIAL REPORT

The total amount of budget approved for this project is RM 74500.00. At the end of this project RM 73977.12 was spent, representing 99.3% of funding utilisation as shown in Table 1.

Table 1: Budget and spending of the project

<b>Project Leader</b>	<b>Approved</b>	<b>Spending</b>	<b>% spending</b>
<b>DR HAZRULRIZAWATI BT ABD HAMID</b>	<b>39,894.00</b>	<b>39,232.05</b>	<b>98.34</b>



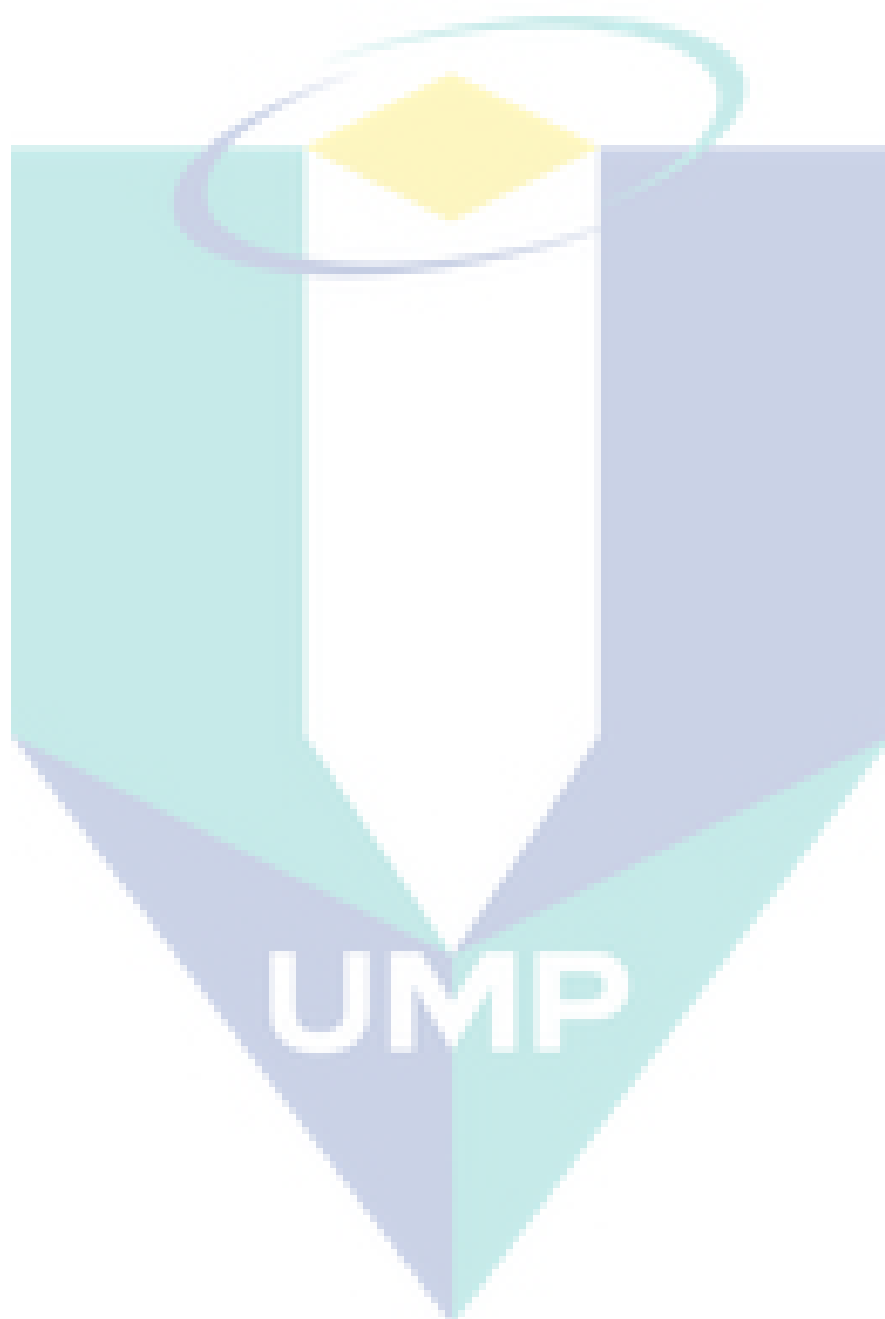
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## ABSTRACT

Carotenoids derived aroma compounds such as  $\alpha$ -ionone,  $\beta$ -ionone,  $\beta$ -damascenone and other few important compounds can be produced via degradation. These aroma compounds have a heavy demand in food products, cosmetics and pharmaceutical industry. Currently, there are growing concerns from consumers particularly about the source of ingredients and awareness about potentially harmful synthetic compound. These have made the global demand for naturally produced aroma compounds increases gradually and gained renewed attention recently. However, extraction of natural carotenoid-derived aroma compounds directly from natural sources is expensive and not economical because isolation of single pure natural aroma compounds directly from plant sources is difficult and involves tedious work as it is present in a very low yield. Therefore, this research which is aims to produce aroma compounds from degradation of carotenoids from CPO and oil palm wastes (OPW) was conducted. The palm carotene extraction process was performed using soxhlet adsorption, adsorption column chromatography, saponification, neutralisation and transesterification for CPO and SFE and soxhlet extraction for OPW of PPF and EFB. The extraction process is mainly aimed in extracting palm carotene rich in  $\beta$ -carotene concentration. In comparison with all the extraction techniques, soxhlet adsorption using HP-20 as adsorbent resulted high concentration of  $\beta$ -carotene extracted which was 3856 ppm of 77.12 % at 1:4 ratio of CPO: HP-20 for 1 hr IPA extraction time. The concentration of  $\beta$ -carotene extracted was calculated by using HPLC standard calibration curve. As for the production of aroma compounds, optimization of degradation reaction was carried out using commercial  $\beta$ -carotene in order to achieve optimum degradation conditions. For the optimization of thermal degradation reaction, different sonication time, reaction time and reaction temperature were studied. While for oxidative degradation, effect of sonication, light and amount of catalyst were studied. Based on the result obtained, the optimum condition for thermal degradation was 1 hr sonication, 5 hr reaction and 120-130 °C whereas for oxidative degradation vial must be completely covered, sealed and kept at room temperature. Then, the extracted palm carotene was degraded using thermal and oxidative degradation under optimized reaction conditions. The oxidative degradation using extracted carotene failed to produce any aroma compounds. However, thermal degradation of extracted carotene from both CPO and OPW produced aroma compounds such as  $\beta$ -ionone, DHA, 2,4-decadienal, D-limonene,  $\beta$ -epoxide, and 3-oxo- $\beta$ -ionone. The major aroma compound with highest composition produced was DHA (37.26 %) followed by  $\beta$ -ionone (9.23 %) analysed by using GC-MS and GC-FID. The results obtained proved that production of aroma compounds by degradation of  $\beta$ -carotene from CPO and OPW is viable. As there is no study was carried out on production of aroma compounds by degradation of carotenoids from these sources, this study is believed could support the current demand of natural aroma compounds and found to be energy and a cost saving method also quality and environment friendly. Further works are still required to completely isolate  $\beta$ -carotene and understands the mechanism involved in degradation reaction to make this effort applicable in industry scale.

## ABSTRAK

Beberapa kompaun aroma penting seperti  $\alpha$ -ionone,  $\beta$ -ionone,  $\beta$ -damascenone dan lain-lain boleh dihasilkan melalui degradasi karotenoid. Sebatian-sebatian aroma ini mempunyai permintaan yang berat dalam industri produk makanan, kosmetik dan farmaseutikal. Kini, kebimbangan daripada pengguna terutamanya tentang sumber bahan-bahan dan kesedaran tentang sebatian sintetik yang mungkin berbahaya semakin meningkat. Ini telah membuatkan permintaan global bagi sebatian aroma semulajadi meningkat secara beransur-ansur dan diberi perhatian baru-baru ini. Walau bagaimanapun, pengekstrakan sebatian aroma semula jadi yang diperolehi daripada karotenoid secara langsung daripada sumber semula jadi adalah mahal dan tidak ekonomik kerana pengasingan tunggal sebatian aroma asli terus daripada sumber tumbuh-tumbuhan adalah sukar dan melibatkan kerja yang susah kerana ia wujud dalam kuantiti yang sangat rendah. Oleh itu, kajian ini yang bertujuan untuk menghasilkan sebatian aroma daripada degradasi karotenoid daripada minyak sawit mentah (CPO) dan buangan minyak sawit (OPW) telah dijalankan. Proses pengekstrakan karotena sawit telah dilakukan dengan menggunakan penjerapan soxhlet, kromatografi, saponifikasi, peneutralan dan transesterifikasi bagi CPO dan SFE dan pengekstrakan soxhlet untuk OPW daripada PPF dan EFB. Proses pengekstrakan adalah bertujuan untuk mengekstrak karotena kaya dengan kepekatan  $\beta$ -karotena yang tinggi. Berbanding dengan semua teknik pengekstrakan, penjerapan soxhlet menggunakan HP-20 sebagai adsorben memberikan ekstrak  $\beta$ -karotena dengan kepekatan paling tinggi iaitu 3.856 ppm (77.12 %) pada nisbah 1: 4 CPO: HP-20 untuk masa pengekstrakan IPA selama 1 jam. Kepekatan  $\beta$ -karotena yang diekstrak telah dikira dengan menggunakan keluk penentuan standard HPLC. Bagi pengeluaran sebatian aroma, pengoptimuman reaksi degradasi telah dijalankan dengan menggunakan  $\beta$ -karotena komersial untuk mencapai keadaan reaksi yang optimum. Untuk mengoptimumkan tindak balas degradasi haba, masa sonikasi yang berbeza, masa reaksi dan suhu tindak balas telah dikaji. Manakala bagi degradasi oksidatif, kesan sonikasi, cahaya dan amaun pemangkin telah dikaji. Berdasarkan keputusan yang diperolehi, keadaan optimum untuk degradasi haba adalah 1 jam sonikasi, 5 jam reaksi dan 120-130 °C manakala bagi degradasi oksidatif botol mesti ditutup sepenuhnya, dimeteraikan dan disimpan pada suhu bilik. Kemudian, karotena sawit yang telah diekstrak telah didegradasi menggunakan degradasi haba dan oksidatif dalam keadaan tindak balas yang telah dioptimumkan. Degradasi oksidatif menggunakan ekstrak karotena gagal memberikan apa-apa sebatian aroma. Walau bagaimanapun, degradasi haba dengan ekstrak karotena daripada kedua-dua CPO dan OPW menghasilkan sebatian aroma seperti  $\beta$ -ionone, DHA, 2,4-decadienal, D-limonene,  $\beta$ -epoksida, dan 3-oxo- $\beta$ -ionone. Sebatian aroma utama dengan komposisi tertinggi dihasilkan adalah DHA (37.26 %) diikuti dengan  $\beta$ -ionone (9.23 %) dianalisis dengan menggunakan GC-MS dan GC-FID. Keputusan yang diperolehi membuktikan bahawa pengeluaran sebatian aroma melalui degradasi  $\beta$ -karotena daripada CPO dan OPW boleh dilaksanakan. Oleh kerana, tiada kajian telah dijalankan ke atas pengeluaran sebatian aroma melalui degradasi karotenoid daripada sumber-sumber ini, kajian ini dipercayai boleh menyokong permintaan semasa sebatian aroma semula jadi dan kaedah ini didapati dapat menjimatkan tenaga dan penjimatan kos juga mesra alam. Kerja lanjut masih diperlukan untuk mengasingkan  $\beta$ -karotena sepenuhnya dan memahami mekanisme yang terlibat dalam tindak balas degradasi untuk membuatkan usaha ini boleh digunakan dalam skala industri.



## CHAPTER 2

### RESEARCH METHODOLOGY

#### 2.1 INTRODUCTION

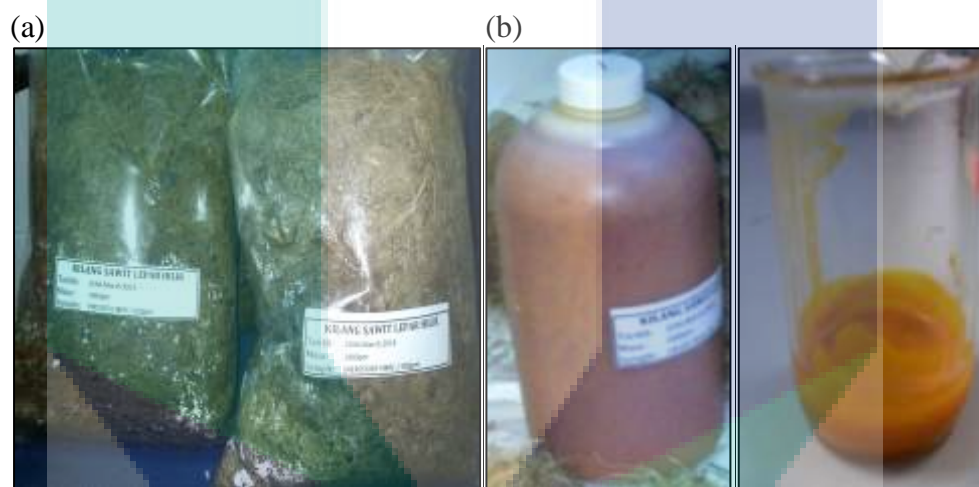
This chapter discusses about the overall methodology of the research whereby  $\beta$ -carotene from both CPO and OPW were recovered by soxhlet adsorption method. Highest concentration of  $\beta$ -carotene was obtained by optimising the extraction method using different ratio of sample to adsorbent also different IPA extraction time. Then, for the thermal degradation using commercial  $\beta$ -carotene different parameters were set up such as temperature, time of reaction, and sonication time to optimize the yield of aroma compounds produced. Also, parameters such as light, sonication and amount of sample and catalyst used were varied to optimize the oxidative degradation of the commercial  $\beta$ -carotene. The extracted palm carotenes were used to form aroma compounds in an optimised degradation reaction conditions. The degraded products were further analysed by using GC-MS and GC-FID.

#### 2.2 MATERIALS AND CHEMICALS

Crude palm oil (CPO) and oil palm wastes (OPW), which are empty fruit bunch (EFB) and palm pressed fiber (PPF) were collected from Felda Palm Industries Sdn. Bhd., Lepar Hilir 2, Pahang, Malaysia. Standard  $\beta$ -carotene and  $\beta$ -ionone were purchased from Merck (Germany). Synthetic highly porous resin (DIAION HP-20) and standard alkane (C<sub>8</sub>-C<sub>20</sub>) for GC-FID analysis were bought from Sigma Aldrich (United States). All the other chemicals and reagents used in this study were of analytical and industrial grade.

## 2.2 SAMPLE PREPARATION

CPO of 1 kg stored in a dark closed container was stored in a room temperature and protected from exposure of direct light. The solid oil palm wastes consisted of a palm pressed fiber (PPF) and empty fruit bunch (EFB) of 1 kg each of these OPW were dried at room temperature for 24 hr. These milled OPW were also protected from exposure to direct light and stored in a room temperature until used for soxhlet extraction. Figure 2.1 below shows the raw materials (a) oil palm wastes (PPF and EFB) and (b) crude palm oil that were collected for this research.



**Figure 2.1:** (a) Oil palm waste and (b) crude palm oil

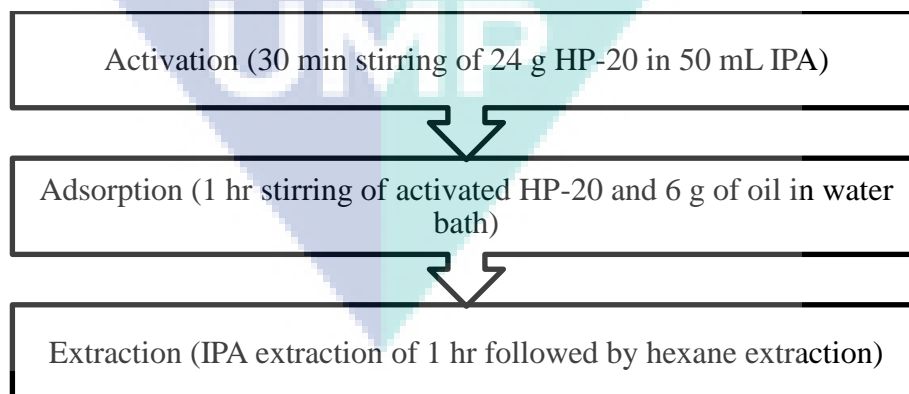
## 2.4 RECOVERY OF PALM CAROTENE

For the recovery of  $\beta$ -carotene from crude palm oil and oil palm wastes, soxhlet extraction method was employed. Palm carotene was extracted by soxhlet adsorption method and concentration of  $\beta$ -carotene was determined by using HPLC and UV-Vis. In order to standardize all the physical properties of the samples, the oil from solid waste of EFB and PPF was initially extracted by soxhlet extraction method.

### 2.4.1 Soxhlet Adsorption

Initially, the extraction of crude oil from solid OPW was conducted by soxhlet extraction. Oil from 8 g of PPF was extracted by using soxhlet extractor. The soxhlet extractor was assembled using 200 mL of hexane at 60-65 °C and heated for 60 min or 10 cycles. After the extraction time was completed, the solvent was removed using rotary evaporator at  $60 \pm 5$  °C. The extracted oil was transferred into a vial and kept in freezer before used for soxhlet adsorption. Soxhlet extraction was repeated few times with PPF and again with same amount of EFB to extract sufficient amount of oil for soxhlet adsorption.

Synthetic highly porous resin (DIAION HP-20) was used for soxhlet adsorption method to extract  $\beta$ -carotene from CPO and OPW. This is an accepted method that is used as reference for the recovery of palm carotene (Baharin et al., 2001, Latip et al., 2000 and Latip et al., 2001). Different ratio of oil to HP-20 (1:2, 1:2 and 1:4) and different IPA extraction time (0, 1 and 2 hr) were studied to determine the best conditions to extract the  $\beta$ -carotene. The optimised experimental conditions were repeated to extract more amount of  $\beta$ -carotene from CPO and these optimised conditions also were then used for recovery of  $\beta$ -carotene from PPF and EFB. Figure 2.2 shows flow chart explaining recovery of carotene by soxhlet adsorption.



**Figure 2.2:** Flow chart of soxhlet adsorption



HP-20 of 24 g was activated by using 50 mL of IPA in a conical flask with continuous stirring for 20 min. The treated adsorbent then 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 and maintained at a temperature of 40-45 °C with continuous stirring for 1 hr. Then, 6 g of CPO diluted with 50 mL of IPA was added slowly into the flask. The mixture of CPO and HP-20 was transferred into the soxhlet extraction thimble. IPA of 200 mL was used first to extract oily and polar compounds from CPO for 1 hr at 75-80 °C. Followed by  $\beta$ -carotene extraction using hexane for about 2 hr at 60-65 °C. Finally, the solvents were removed from both fractions and concentration of  $\beta$ -carotene was determined by using HPLC and UV-Vis. Experiment was repeated with oil extracted from the OPW. Figure 2.2 shows the soxhlet adsorption set up for extraction of palm carotene.



**Figure 2.2:** Soxhlet Adsorption

## **2.5 OPTIMISATION OF DEGRADATION REACTIONS**

### **2.5.1 Oxidative Degradation of Commercial $\beta$ -carotene**

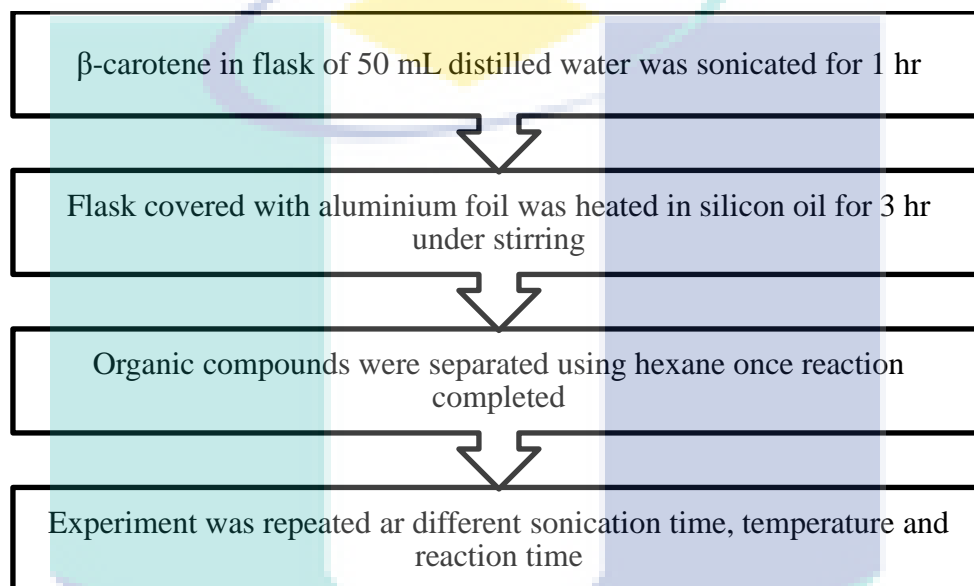
This degradation method was based on those developed by Nonier et al., 2004. Hydroalcoholic solution comprises of ethanol (120 mL), tartaric acid (5 g), 2.5 mg/L iron

oxide, and NaOH of 1 M was prepared. The pH of solution is maintained at 2.4 by adding NaOH and adjusting final volume of the hydroalcoholic solution to 1 L with distilled water. Then, 10 mg of  $\beta$ -carotene was introduced into 10 mL of a hydroalcoholic solution in a vial. Vial that was fully covered and sealed was left in room temperature for five days. After five days, organic compounds were extracted by liquid-liquid extraction using hexane. Firstly, the solution was added with little amount of sodium hydrogencarbonate and filtered. The organic phase was dried over anhydrous sodium sulphate. The final phase was concentrated in a rotary evaporator before GC-MS analysis. In order to optimise the degradation reaction conditions, parameters such as sonication, exposure of light, amount of sample and presence of catalyst was studied. Thus, five different samples; A had 10 mg of  $\beta$ -carotene, B with 20 mg of  $\beta$ -carotene, C with 10 mg of  $\beta$ -carotene and left over without any cover, D with 10 mg of  $\beta$ -carotene with 20 min of sonication prior to degradation and E with 10 mg of  $\beta$ -carotene with catalyst (5.0 mg/L of iron oxide) were prepared for the optimisation. Sample A, B, D and E were covered with aluminium foil to avoid exposure of light. After five days at room temperature, all five samples were extracted using hexane and analysed using GC-MS to identify the degraded compounds. Based on results obtained, optimised conditions were determined from the replicate results.

### 2.5.2 Thermal Degradation of Commercial $\beta$ -carotene

Thermal degradation method was adapted and modified based on research conducted by Kanasawud and Crouzet, (1990). For this method using commercial  $\beta$ -carotene, 10 mg of commercially purchased  $\beta$ -carotene was dispersed in 50 mL distilled water in a 250 mL round-bottom flask that was covered with aluminium foil and sonicated for 1 hr. During the sonication, it is important to minimize the exposure of light to the sample to avoid degradation due to exposure of light factor. After sonication, the flask was connected to condenser and heated in silicon oil bath at  $100 \pm 5$  °C for 2 hr under stirring. Once reaction completed, the organic compounds were separated with hexane for at least three times to completely remove the degraded compounds from the distilled water. Then, anhydrous sodium sulphate was added to remove the remaining water. Analysis was performed by using GC-MS. The experiment was repeated with different reaction time (4, 5

and 6 hr), temperature (110-120, 120-120 and 120-140 °C) and sonication time (0, 1, 2 and 2 hr) to determine an optimised degradation reaction condition that is able to produce higher yield of aroma compounds. Based on results obtained from the replicate samples, optimised conditions for thermal degradation was determined. Figure 2.4 shows the flow chart of thermal degradation.



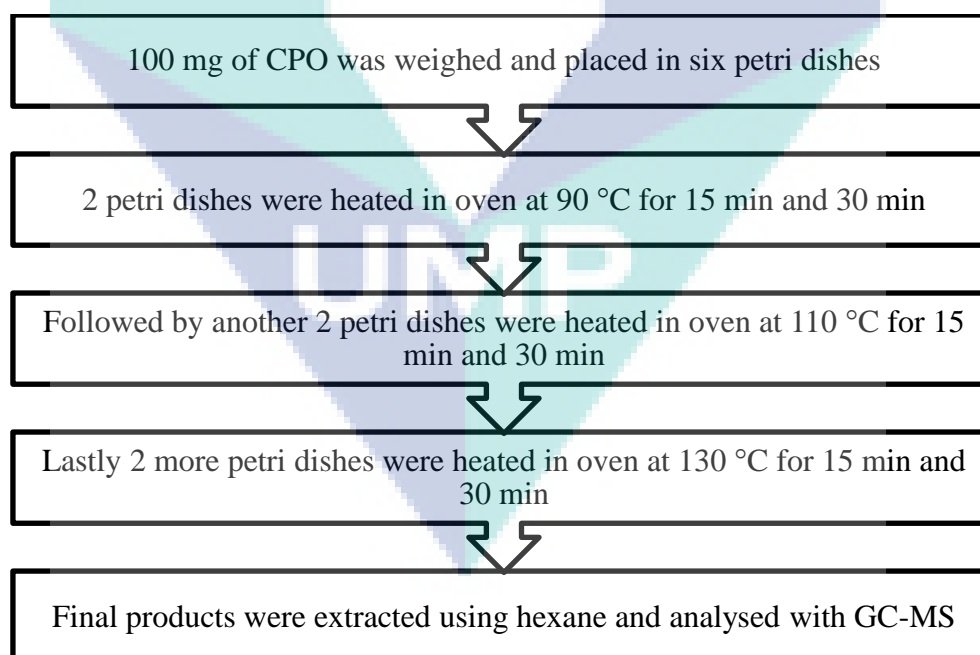
**Figure 2.4:** Flow chart of thermal degradation of commercial  $\beta$ -carotene

## 2.6 FORMATION OF AROMA COMPOUNDS

Aroma compounds (mainly  $\alpha/\beta$ -ionone), can be derived from degradation of carotenoids or via organic synthesis. In this research, aroma compounds are aimed to form from palm carotenes. Thus, initial degradation reactions using commercially purchased  $\beta$ -carotene were conducted in different parameters to determine an optimised reaction conditions that give higher yield of aroma compounds. Then, recovered  $\beta$ -carotene from CPO, PPF and EFB with highest concentration of  $\beta$ -carotene among all the samples were subjected to the optimised degradation reactions conditions. The degraded samples were then subjected to GC-MS and GC-FID analysis.

### 2.6.1 Accelerated Heating, Oxidative and Thermal Degradation of CPO

The degradation of carotene from CPO directly without any extraction was also studied by conducting few experiments, which were by accelerated heating, thermal and oxidative degradation of CPO. For accelerated heating, 100 mg of CPO sample was weighed and placed in six different petri plates. Two samples of each were heated in an oven for 15 and 20 min respectively at 90 °C and followed by heating at 110 and then 120°C for the remaining samples. While for thermal degradation 100 mg of CPO was dissolved in 50 mL distilled water and sonicated for 1 hr in a covered round-bottom flask. Then, flask connected with condenser was heated in silicon oil bath at  $100 \pm 5$  °C for 2 hr under stirring and organic compounds were separated by extraction after reaction completed. The oxidative degradation was carried out by using 100 mg CPO that was added to hydroalcoholic solution in a fully covered and sealed vial and left for five days at room temperature. All the final products were extracted and analysed by using GC-MS. Figure 2.5 below represents the flow chart for the accelerated heating of CPO.



**Figure 2.5:** Flow chart of the accelerated heating of CPO

### 2.6.2 Oxidative Degradation of Recovered Palm Carotene

Extracted  $\beta$ -carotene of 100 mg from CPO was added to hydroalcoholic solution in a fully covered and sealed vial. After five days at room temperature, sample solution was first added with little amount of sodium hydrogencarbonate and then extracted by using hexane. The organic phase was then dried over anhydrous sodium sulphate and final phase was concentrated for analysis. Degradation was also repeated with  $\beta$ -carotene extracted from PPF and EFB.

### 2.6.2 Thermal Degradation of Recovered Palm Carotene

Extracted  $\beta$ -carotene of 100 mg from CPO was dispersed in 50 mL distilled water in a covered round-bottom flask and sonicated for 1 hr. Then, the flask connected to condenser was heated in silicon oil bath at 120-120 °C for 5 hr under stirring. After reaction was completed, organic compounds were separated using hexane and little anhydrous sodium sulphate was added to remove any water excess. Then, extracted  $\beta$ -carotene from PPF and EFB were degraded under the same condition. These reaction conditions were selected based on results obtained from optimisation experiments using commercial  $\beta$ -carotene.

## 2.7 METHODS OF ANALYSIS

The analyses of all extracted  $\beta$ -carotene were performed by UV-Vis and HPLC to determine the concentration of  $\beta$ -carotene recovered. While GC-MS and GC-FID methods were used for analysis of oxidative and thermal degradation products.

### 2.7.1 Analysis of $\beta$ -carotene

(i) *UV-Visible Spectrophotometer (UV-Vis)*

Recovered carotene content in the samples was calculated in terms of  $\beta$ -carotene, using a standard absorbance curve calibrated with standard  $\beta$ -carotene. A 100 mL stock solution was prepared with 0.1 g of  $\beta$ -carotene in chloroform. Aliquots were taken from this solution and diluted to different concentrations (200-1000 ppm). Some previous studies had determined the absorbance to be measured at 446 nm (Ahmad et al., 2009; Baharin et al., 2001, Latip et al., 2000; and Lau et al., 2008).  $\beta$ -carotene content in samples were analyzed using a UV-Vis spectrophotometer Genesys. Prior to analysis, recovered sample were melted and homogenised thoroughly. About 50 mg of extracts were diluted with 10 mL of chloroform. Absorbance readings of samples and standards used for calculation were based on mean values obtained from at least three readings.

(ii) ***High Performance Liquid Chromatography (HPLC)***

Water Alliance E2695 HPLC with an automated injector and Photo Diode Array Detector were used to determine  $\beta$ -carotene extracted, qualitatively and quantitatively. For qualitative analysis, retention time of  $\beta$ -carotene standard peak was matched with extracted sample. While, for quantitative analysis, standard calibration curve was plotted using result from HPLC analysis and concentration of  $\beta$ -carotene in extracted samples were calculated. Firstly, stock solution was prepared by diluting 10 mg of commercial  $\beta$ -carotene standard with 10 mL chloroform. Then, series of standard solutions were prepared in a working range of 200 to 400 ppm. About 50 mg of the all extracted  $\beta$ -carotene samples were re-dissolved in 10 mL of chloroform and 1 mL aliquot of all these samples were used for HPLC analysis. 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 (9.5:0.5, vol/vol) at a flow rate of 1 mL/min and analysis time of 45 min. This method was developed based on study performed in previous studies (Ahmad et al., 2008, Baharin et al., 1998; Baharin et al., 2001).

## 2.7.2 Identification of Degradation Products

### (i) *Gas Chromatography-Mass Spectrometry (GC-MS)*

The analysis was performed by using GC-MS with a silica capillary column DB 5 (J&W) (25 mm X 0.256 mm). The injector temperature was 250 °C with an injection of 2 µl in a splitless mode. Temperature of interface was 250 °C and temperature of ion source was 200 °C. The oven heating was as follows; 60 °C (2 min), temperature rise to 220 °C at 4 °C/min and kept constant at 220 °C for 20 min. The ionization energy was 70 eV with a helium gas flow at 1 mL/min in constant flow mode and scanning of mass spectra at m/z of 58-650.

### (ii) *Gas Chromatography-Flame Ionization Detector (GC-FID)*

The chromatographic analyses were performed on a gas chromatograph (Agilent, 7890 model; Palo Alto, USA) equipped with a split/splitless injector, flame ionization detector (FID), and capillary column DB 5. The conditions used were: injector temperature was at 250 °C with an injection of 2 µL in a splitless mode. Temperature of interface was at 250 °C and temperature of ion source was at 200 °C. The oven heating was as follows; 60 °C (2 min), temperature rise to 220 °C at 4 °C/min and kept constant at 220 °C for 20 min. The ionization energy was 70 eV with a helium gas flow at 1 mL/min in constant flow mode. Co-injection with a hydrocarbon (C<sub>8</sub>-C<sub>20</sub>) standard was used to confirm the compounds identity. Kovats Index demonstrated in mathematic equation is as shown below.

$$I = 100 \times [n + (N-n) \log (t'_{r(\text{unknown})}) - \log (t'_{r(n)}) / \log (t'_{r(N)}) - \log (t'_{r(n)})]$$

Where,

I = Kovats retention index,

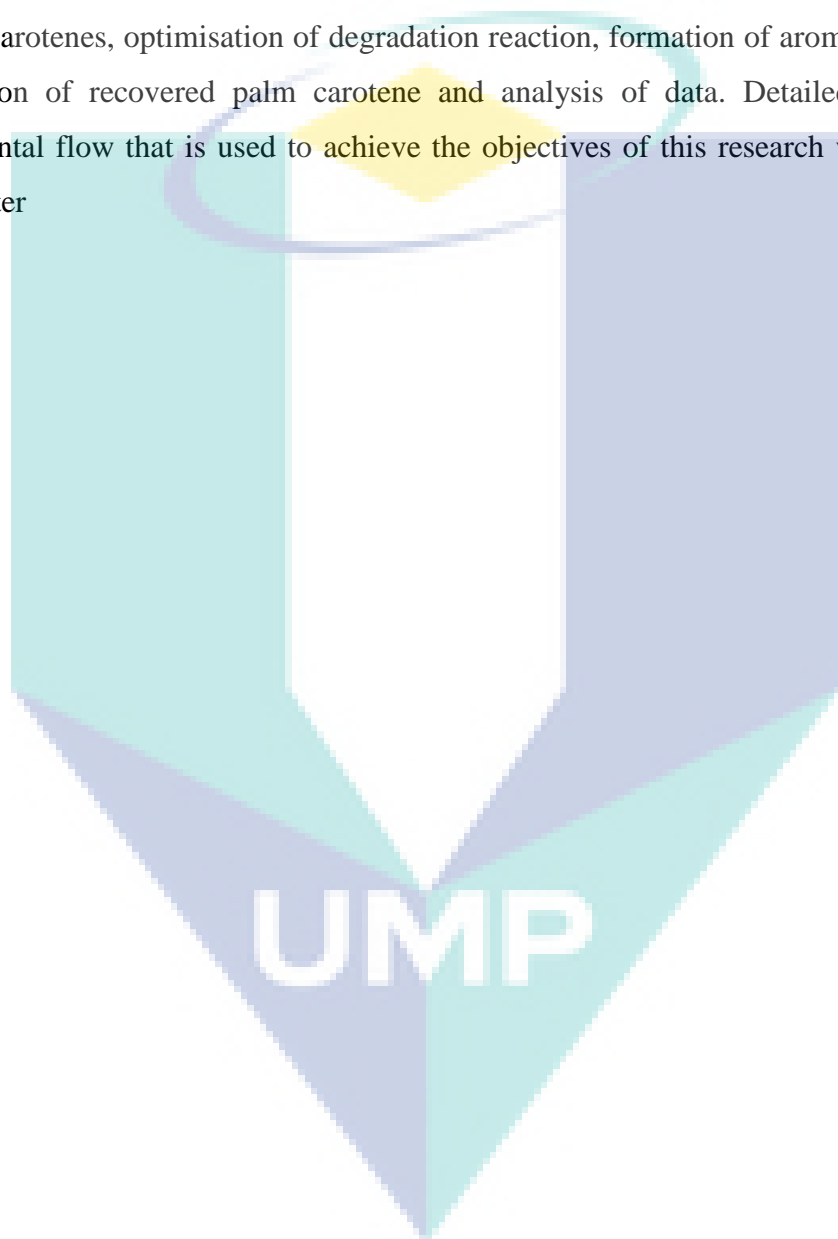
n = the number of carbon atoms in the smaller alkane,

N = the number of carbon atoms in the larger alkane,

t'<sub>r</sub> = the adjusted retention time.

## 2.7 CONCLUSIONS

This chapter summarise the overall methodology involved in this research. It covered some major parts of materials and chemicals also methods especially on recovery of palm carotenes, optimisation of degradation reaction, formation of aroma compounds by degradation of recovered palm carotene and analysis of data. Detailed picture of the experimental flow that is used to achieve the objectives of this research was explained in this chapter





## CHAPTER 3

### RESULTS AND DISCUSSION

#### 3.1 INTRODUCTION

This chapter covers results illustrated in a form of graphs, figures and tables which were obtained using by the method discussed in Chapter 3 followed by discussions. Results of recovery of  $\beta$ -carotene from CPO and OPW (EFB and PPF) were discussed. Besides, degradation results of commercial  $\beta$ -carotene which was used to determine an optimised reaction conditions were conversed and compared with degradation results of recovered  $\beta$ -carotene. Also findings from previous research were refered and quoted to compare with current outcomes.

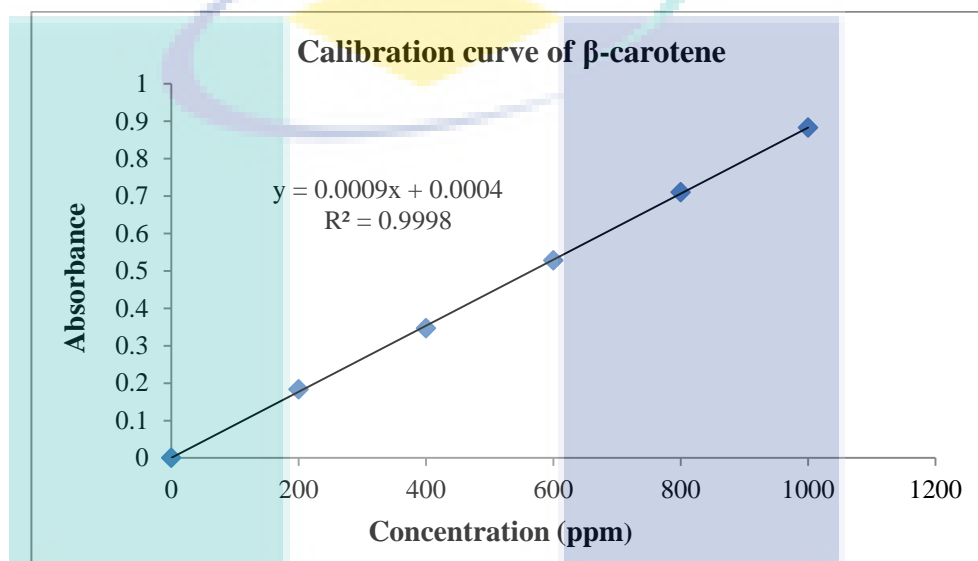
#### 3.2 RECOVERY OF $\beta$ -CAROTENE

Two different sources of palm carotene used were CPO and OPW (PPF and EFB), whereby soxhlet adsorption method was employed to recover  $\beta$ -carotene from the samples. Different IPA extraction time and ratio of sample to adsorbent (HP-20) were studied to optimise the extraction method and recovered  $\beta$ -carotene were analysed using UV-Vis and HPLC to determine the concentration.

##### 3.2.1 Calibration Curve

For analysis using UV-Vis, concentration of the  $\beta$ -carotene standards with five different concentrations were plotted against the absorbance to obtain a straight line.

Absorbance of samples and standards were measured at 336 nm, which corresponded to the maximum absorption of carotenoids (Ahmad et al., 2009; Baharin et al., 2001; Latip et al., 2000 and Lau et al., 2008). The absorbance results used for calculation were based on mean values obtained from three readings. Regression analysis revealed a good relation (correlation coefficient  $R^2 = 0.9998$ ) as showed in Figure 3.1.



**Figure 3.1:** Calibration graph of  $\beta$ -carotene standard by UV-Vis

Table 3.1 shows the analysis result of UV-Vis for the standard  $\beta$ -carotene prepared in a working range of 200 to 1000 ppm (APPENDIX A1). The mean absorbance of 3 readings for each standard (1-5) and standard deviation were calculated.

**Table 3.1:** Results of the analysis of  $\beta$ -carotene standard by UV-Vis

Analysis	Absorbance of $\beta$ -carotene Standard				
	1	2	3	3	5
1	0.183	0.336	0.529	0.709	0.883
2	0.182	0.336	0.528	0.710	0.882
3	0.183	0.337	0.529	0.710	0.883
Mean	0.183	0.336	0.528	0.710	0.883

HPLC also was used to determine the concentration of  $\beta$ -carotene in recovered.  $\beta$ -carotene standard peak was matched with extracted sample to confirm the presence of  $\beta$ -carotene qualitatively. As for quantitative analysis, standard calibration curve was plotted using results from HPLC analysis (APPENDIX B1). Calibration graph for HPLC was based on peak area of five different concentrations of  $\beta$ -carotene standards to determine the linearity in a working range from 200 to 300 ppm with correlation coefficient,  $R^2 = 0.993$ . The method was developed based on study performed in previous studies (Ahmad et al., 2008; Baharin et al., 1998 and Baharin et al., 2001) as described in the methodology section. Figure 3.2 shows the HPLC calibration curve of  $\beta$ -carotene standard.

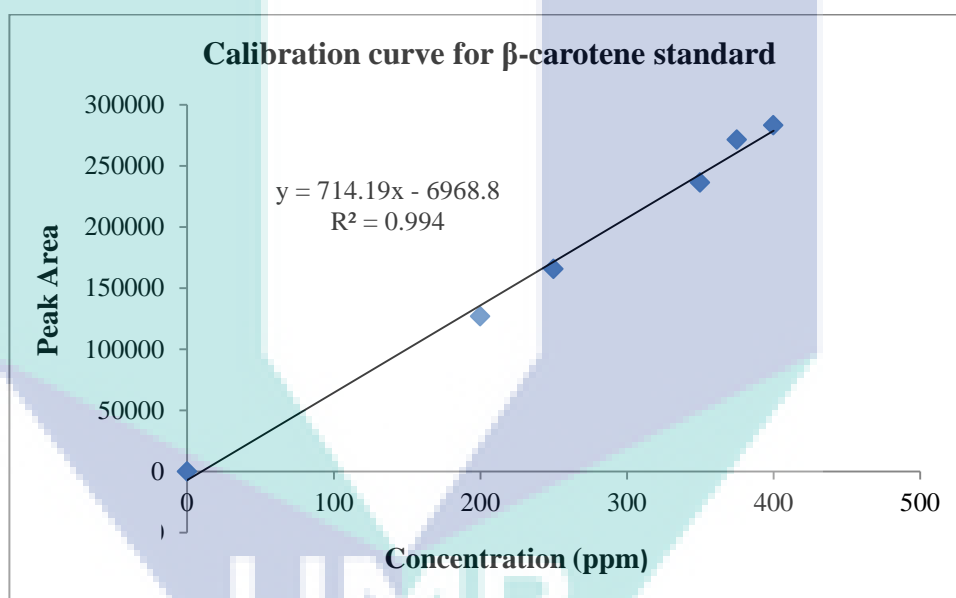


Figure 3.2: Calibration graph of  $\beta$ -carotene standard by HPLC

### 3.2.2 $\beta$ -carotene Recovered from CPO and OPW

Palm oil is one of the richest sources of  $\alpha$ -carotene and  $\beta$ -carotene (300-3500 mg/kg) which constitute more than 80 % of the total carotene (Chiu et al., 2009). However, according to Amorim-Carrilho et al. (2013), the high variability in chemical structure and poor stability greatly contribute to the difficulty of carotene analysis. Carotene content may be different between different parts of the same sample. As a consequence, there is no

generally accepted method or standard method for carotenoids extraction in laboratories. However, most extraction methods involve the release of desired components from their matrices by disrupting tissue followed by removal of the unwanted components. Also, the susceptibility of carotene to oxidation and degradation need to be considered when developing a method for recovery as it very sensitive to light, heat, acid or oxygen exposure (Amorim-Carrilho et al., 2013).

Soxhlet extraction at temperatures between 50 to 70 °C has been reported to give higher phytochemical yields (Ofori-Boateng and Lee, 2013). Therefore, in this study soxhlet adsorption method was employed to recover  $\beta$ -carotene from both CPO and OPW by using HP-20 which is non-polar adsorbents. The extraction method was optimised by varying the IPA extraction time and ratio of sample to adsorbent. These two parameters were studied because, IPA extraction time and amount of adsorbent used were important in improving the concentration as well as yield of  $\beta$ -carotene recovered. Results for the recovery of  $\beta$ -carotene at different IPA extraction time were tabulated in Table 3.2 and for different ratio of CPO: HP-20 was showed in Table 3.3 (APPENDIX A2 and B2).

The effect of different IPA extraction time on recovery of  $\beta$ -carotene from CPO was studied by conducting soxhlet adsorption of IPA at 0 hr which means no extraction with IPA, 1 and 2 hr. IPA adsorption is important as it extracts maximum CPO and polar compounds. Polar compounds in CPO are more soluble in IPA, while non-polar compound such as  $\beta$ -carotene is soluble in hexane. Hence, IPA extraction is required to remove the unwanted compounds and to increase the concentration of  $\beta$ -carotene recovered. Hexane was selected as one of the solvent because carotene is more likely to be eluted by hexane. This was explained by Ahmad et al. (2008) that as carotene was eluted by hexane,  $\beta$ -carotene extracted in hexane fraction is higher in concentration compared to those extracted by other solvent. This indicated that the remaining triglyceride in the CPO was eluted by the polar solvent as it has a slow elution of oil due to poor solvency for oil.

**Table 3.2:**  $\beta$ -carotene Recovery at Different IPA Extraction Time

Extraction Time (hr)	Sample recovered (%)		Concentration of $\beta$ -carotene recovered (ppm)	
	Hexane	IPA	HPLC	UV-Vis
0	93.22	-	706	750
1	13.98	71.81	3265	3231
2	9.33	75.90	2583	2530

Results show that from 6.0 g of CPO used for the soxhlet adsorption, 71.81 % of sample recovered in IPA fraction of 1 hr IPA extraction, while 75.90 % was recovered in 2 hr of IPA extraction. This shows that increase in IPA extraction time leads to increase in sample recovered in IPA fraction which means directly lower the amount of  $\beta$ -carotene recovered in hexane fraction from 13.98 % to 9.33 % respectively at 1 and 2 hr of IPA extraction. This is due to the fact that, longer IPA extraction time leads to degradation of  $\beta$ -carotene as extraction using IPA was performed at higher temperature compared to hexane thus leaving less amount of  $\beta$ -carotene to be recovered in hexane fraction.

However, HPLC and UV-Vis analysis revealed that concentration of  $\beta$ -carotene recovered in hexane fraction increases as extraction time increases from 0 to 1 hr then decreases at 2 hr. Based on UV-Vis analysis results at 1 hr IPA extraction time the concentration of  $\beta$ -carotene recovered was 3231 ppm compared to 750 ppm in sample without IPA extraction (0 hr). Also the  $\beta$ -carotene recovery at 2 hr of IPA extraction was lower than 1 hr which was 2530 ppm. This is because longer IPA extraction time leads to removal of more oil and polar compounds in the sample which was supported by statement from Ahmad et al. (2008) that triglyceride in the CPO is eluted by polar solvent (IPA). Thus, the unwanted compounds from the sample were discarded maximumly which contributes to increase in the concentration of  $\beta$ -carotene recovered. Yet, when the IPA extraction time is increased from 1 to 2 hr, there is a decrease in the concentration of  $\beta$ -carotene recovered. This is because, at the same time this longer time causes decrease in carotene recovery (%) in hexane fraction and increases the amount of sample lost. Overall the results agree with the finding of Latip et al. (2001) that percentage of carotene extracted

increases with shorter IPA extraction time. So, 1 hr IPA extraction is sufficient because even at shorter adsorption time the adsorption capacity of the adsorbent reached its maximum where in other words, it can be concluded that the adsorbent and CPO has reached equilibrium (Latip et al., 2001).

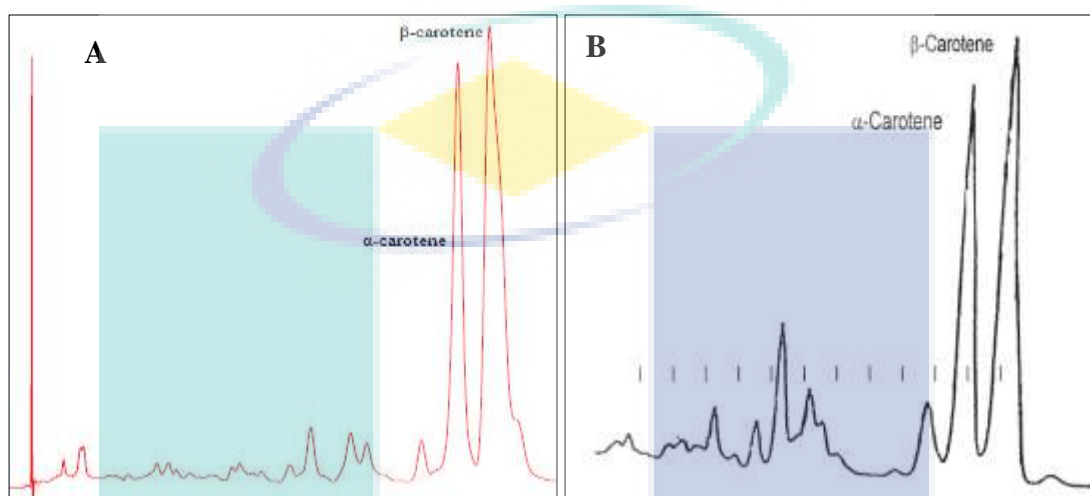
It would be reasonable to believe that IPA adsorption is important mainly in this method, as sufficient time is required for maximum removal of oil and polar compounds. However, longer time of IPA extraction might leads to degradation of  $\beta$ -carotene. Conversely, results obtained prove that percentage of carotene extracted in hexane fraction increases only with shorter IPA extraction time. Besides, the overall sample lost for both 1 and 2 hr of IPA extraction was almost the same with slight increase which was 13.21 % to 13.76 %. This result shows that, the longer extraction time of IPA, the lower concentration of  $\beta$ -carotene recovered and the higher percentage of sample lost. Therefore it is concluded that, 1 hr of IPA extraction is an optimum time for the soxhlet adsorption considering the concentration, yield of recovery and also the amount of sample lost.

**Table 3.3:** Result for soxhlet adsorption at different ratio of CPO: HP-20

CPO: HP-20 ratio	Sample recovered (%)		Sample loss (%)	Concentration of $\beta$ -carotene recovered(ppm)	
	Hexane	IPA		HPLC	UV-Vis
1:2	3.39	86.57	9.03	1133	1250
1:3	11.13	78.52	10.35	2313	2301
1:3	15.30	70.09	13.51	3790	3770

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 1:3 were studied and the results were showed in Table 3.3. Trend of the result shows that as the ratio of CPO: HP-20 increases the percentage of sample loss also increases from 9.03 % to 13.51 %. For the amount of carotene recovered in hexane fraction, it also shows an increase from 3.39 % to 11.13 % at ratio 1:2 to 1:3 and to 15.30 % at ratio of 1:3. This is because as the amount of adsorbent (HP-20) used increases, the surface area of this non-polar adsorbent

also increases. Thus more  $\beta$ -carotene is being adsorbed on it and being eluted by hexane. According to Baharin et al. (1998), the ability of this resin is due to the superior surface area and greater hydrophobicity.



**Figure 3.3:** Comparison of HPLC chromatogram with literature; A: extracted carotene; B: carotene in hexane fraction by Baharin et al. (2001)

Based on the HPLC analysis results, the highest concentration of  $\beta$ -carotene extracted was achieved at ratio of 1:3 which is 3790 ppm. As the ratio of CPO:HP-20 decreases the concentration of  $\beta$ -carotene recovery also decreases from 2313 ppm at ratio 1:3 to 1133 ppm at ratio 1:2. Hence, ratio of 1:3 of CPO to HP-20 adsorbent is efficient in extracting palm carotene using soxhlet adsorption. The 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 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. Besides as stated by Baharin et al. (1998), this adsorbent has high adsorption capacity and ability to separate the palm carotene due to the superior surface area of the HP-20. Availability of binding site for more adsorbing capability increases as the amount of adsorbent increases (Latip et al., 2000).

The advantage of using this adsorbent is the ability to extract high concentration of  $\beta$ -carotene and also the compounds adsorbed onto the resin can be eluted easily using solvent. Besides, this adsorbent can be reused several times by regenerating it using non polar solvents such as hexane (Latip et al., 2001). In this study, hexane was used to regenerate the HP-20 and reused several times to increase the amount of palm carotene extracted for degradation. In short for soxhlet adsorption, 1 hr of IPA extraction time and 1:3 ratio of CPO to HP-20 are the optimum conditions to recover highest concentration of  $\beta$ -carotene from CPO. Therefore, it was finalized that this optimised conditions were used to recover  $\beta$ -carotene from OPW of PPF and EFB.

Waste materials such as OPW are found in abundance and polluting the environment. However, these can be tapped through the process of waste management in order to recover or extract vital nutrients or phytochemicals such as carotene which can be utilized in production of aroma compounds via degradation. As, soxhlet extraction is faster and also cheaper and it does not require sophisticated set up this can be used to recover the  $\beta$ -carotene. Hence, recovery of  $\beta$ -carotene from oil extracted from OPW of PPF and EFB was conducted by using optimised soxhlet absorption conditions.

Based on the results obtained from the analysis, it is proven that  $\beta$ -carotene recovered from PPF was in higher concentration compared to EFB. Results were showed in Table 3.3. The sample lost for both PPF and EFB sample were 13.97 and 13.35 % respectively. While from 6.0 g of oil used, 13.72 % of carotene recovered in PPF and 13.53 % was recovered from EFB of hexane fraction. Concentration of  $\beta$ -carotene recovered in PPF was twice than EFB. By HPLC analysis, the concentration of  $\beta$ -carotene recovered in PPF and EFB were 1313 and 702 respectively, while UV-Vis analysis reveals concentration of 1397 and 687 ppm in PPF and EFB respectively.

Overall, among three different samples used; CPO, PPF and EFB, CPO was proven to contain high concentration of  $\beta$ -carotene. Optimised conditions of soxhlet adsorption method with 1 hr of IPA extraction with ratio of 1:3 of crude oil to HP-20 lead to extraction of highest concentration of  $\beta$ -carotene from CPO which was 3790 ppm by HPLC and 3770



ppm by UV-Vis, followed by PPF, with concentration of 1313 and 1397 ppm by HPLC and UV-Vis analysis respectively. Meanwhile, EFB yielded lowest concentration of  $\beta$ -carotene recovery which was 702 ppm by HPLC analysis.

**Table 3.3:**  $\beta$ -carotene recovery in OPW by soxhlet adsorption

Sample	Sample recovered (%)		Concentration of $\beta$ -carotene recovered (ppm)	
	Hexane	IPA	HPLC	UV-Vis
PPF	13.72	71.31	1313	1397
EFB	13.53	72.01	702	687

### 3.3 OPTIMISATION OF OXIDATIVE AND THERMAL DEGRADATION

Optimisation of the oxidative degradation and thermal degradation was carried out based on one-factor-at-a-time (OFAT) experiments. OFAT experiment is an experiment technique which varies one variable or factor at one time while keeping others fixed to identify the effect of each factor. For both thermal and oxidative degradation, different factors or parameters were tested to optimise the reaction conditions which lead to formation of aroma compounds

#### 3.3.1 Oxidative Degradation

Results of oxidative degradation of five samples with different parameters as mentioned in the methodology were presented in the Table 3.5 (APPENDIX C5). Effect of light on degradation of this commercial  $\beta$ -carotene was studied in sample A and C, as it was reported as important factor in degradation of carotenoid. According to the literature (Penicaud et al., 2011) light is reported among the three factors that influence on the carotenoid degradation. Along with this, amount of sample and catalyst also effect of sonication were studied in order to obtain an optimise oxidative degradation reaction conditions.

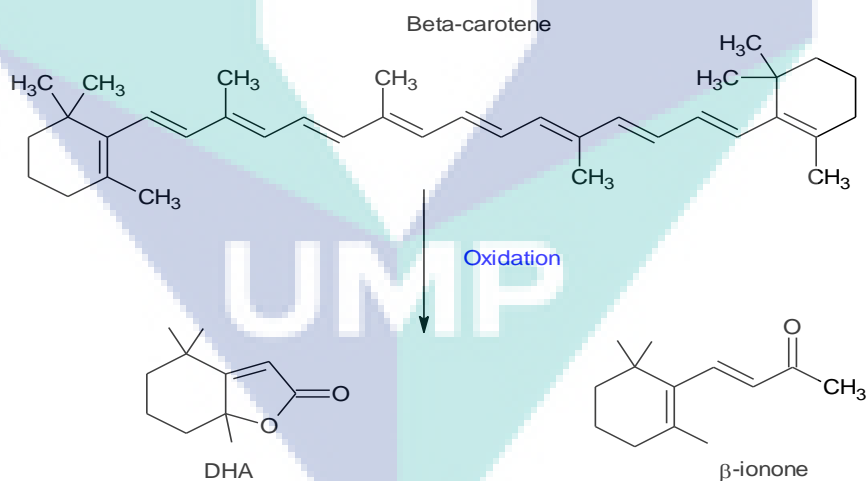
**Table 3.5:** Oxidative degradation products of commercial  $\beta$ -carotene

Compound	Composition (% peak area)				
	A	B	C	D	E
Cyclohexanol, 2,6-dimethyl	1.39	-	1.33	3.97	-
3-Heptanone, 3-methyl	-	5.00	-	-	-
$\beta$ -cyclocitral	-	2.05	1.91	-	-
Butyl isobutyl phthalate	-	-	-	2.65	-
Monolauryl malaete	-	-	-	6.13	-
t-butylhydroquinone	-	2.51	-	-	-
$\beta$ -ionone	1.10	6.11	-	-	3.72
3-oxo- $\beta$ -ionone	1.05	2.28	1.06	1.96	-
Toluene	69.07	-	69.21	-	60.03
3,5-Octanediol, 2,7-dimethyl	1.60	-	-	-	-
Dihydroactinidiolide (DHA)	17.53	38.66	19.50	53.75	29.07
1-Cyclohexene-1-carboxaldehyde, 2, 6,6-trimethyl	-	2.05	-	-	-
(+)-Dihydro- $\alpha$ -ionone	-	-	-	2.88	-
Cyclohexene, 1,5,5-trimethyl-6- acetylmethyl	-	1.33	-	-	-
$\beta$ -ionone epoxide	5.95	15.90	3.76	16.35	6.91
Cyclopropyl ketone	-	6.29	2.25	-	-

<sup>a</sup> Components < 1 % are not included

Based on the results obtained, sample A, B and E produced  $\beta$ -ionone which is the main C-13 norisoprenoid. The formation of  $\beta$ -ionone is further confirmed by its GC-MS mass spectrum (APPENDIX D5). The composition (% peak area) of  $\beta$ -ionone in sample A was 1.10 %, whereas in sample B was 6.11 % and sample E was 3.72 %. This is due to variation of mass of  $\beta$ -carotene used in both samples for degradation which is 10 mg in sample A while 20 mg in sample B. Besides, the other composition of additional aroma compounds such as DHA,  $\beta$ -ionone epoxide and 3-oxo- $\beta$ -ionone were also higher in sample B compared to sample A. This shows that increase in the amount of  $\beta$ -carotene lead to higher production of aroma compounds because more carotene is being cleaved to generate different aroma compounds. Nonier et al. (2003) has proposed that aroma compounds that possess distinct aromatic characteristics are formed during the oxidative degradation of carotenoids. The results obtained also supports those findings.

$\beta$ -carotene is a very reactive compound due to its unsaturated bonds, and thus it is prone to oxidative degradation. The mechanism involved in the degradation is isomerization of  $\beta$ -carotene, followed by formation of radical species and the apparition of cleavage products (Penicaud et al., 2011). Oxygen may attack the  $\beta$ -carotene either on the  $\beta$ -ring or on the chain. Thus, much diverse products in terms of molecular weight, chemical functions and others were produced. DHA was the most common aroma compound identified in all five samples with high composition (A: 17.53 %, B: 38.66 %, C: 19.50 %, D: 53.75 % and E: 29.07 %). It is a volatile compound formed from degradation of  $\beta$ -carotene. Complete oxidative degradation of DHA in all five samples may lead to high formation of  $\beta$ -ionone. Besides DHA, some other aroma compounds formed from this oxidative degradation were 3-oxo- $\beta$ -ionone and  $\beta$ -ionone epoxide. 3-oxo- $\beta$ -ionone was identified in four samples except sample E but showed in very low composition (% peak area). The lower yield of this compound could be due to insufficient or incomplete cleavage of  $\beta$ -carotene. The degradation pattern of  $\beta$ -carotene showing important products is presented in the Figure 3.3.



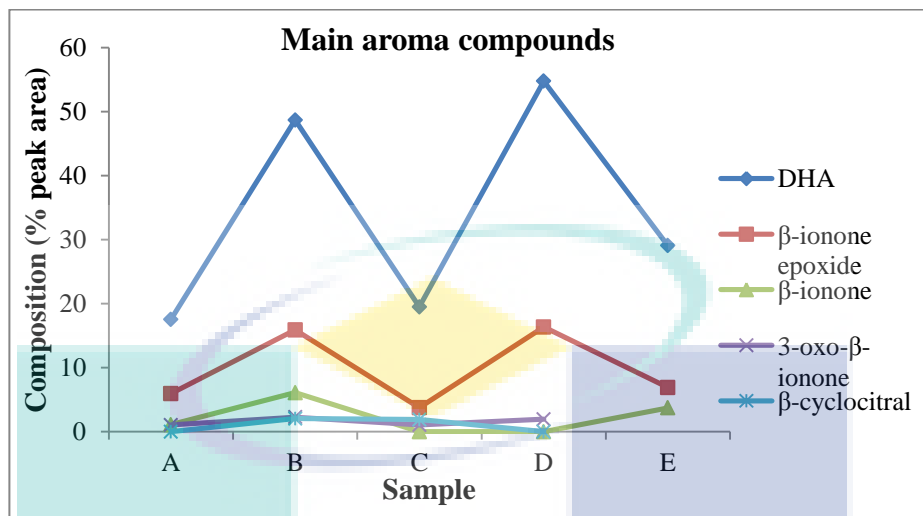
**Figure 3.3:** Main aroma compounds formed by oxidative degradation

$\beta$ -ionone epoxide was identified in all five samples. However, sample B and D contains high composition of this compound which was 15.90 % and 16.35 %. Interestingly, the formation of (+)-dihydro- $\alpha$ -ionone aroma compound took place only in

sample D. This is maybe due to sonication effect in the initial stage prior to degradation, as sonication helps to homogenised the  $\beta$ -carotene in the sample evenly. Apart from all these compounds, cyclohexanol, 2, 6-dimethyl was also obtained in three of the samples with a composition of A (1.39 %), C (1.33 %) and D (3.97 %). The difference in the value of this compound could be due to difference in the mass of initial  $\beta$ -carotene used and the presence of the sonication effect in sample D. Some of the compounds formed have been previously reported by Wache et al. (2003), where  $\beta$ -carotene when cleaved, gives rise to various interesting compounds such as  $\beta$ -ionone and DHA that are often associated with fruity, floral and woody notes to varying degrees.

$\beta$ -cyclocitral was also identified in two samples, B and C with very low yield with a composition of 2.05 % and 1.91 % respectively. Thus, it can be concluded that in order to optimise the yield of aroma compounds produced by oxidative degradation, sample need to be sealed and covered from exposure of light and high amount of  $\beta$ -carotene used. Due to the unsaturated bonds of  $\beta$ -carotene, it is very prone to oxidative degradation.  $\beta$ -carotene undergoes isomerization, followed by formation of radical species that leads to formation different volatile and nonvolatile compounds.  $\beta$ -ionone,  $\beta$ -ionone epoxide, DHA and 3-oxo- $\beta$ -ionone were some of the major compounds determined. Toluene also has been identified in sample A, C and E. According to Onyewu et al. (1982), few past research have reported formation of volatile compounds such as toluene, xylene, ionene, and 2,6-dimethylnaphthalene as thermal degradation products of carotene

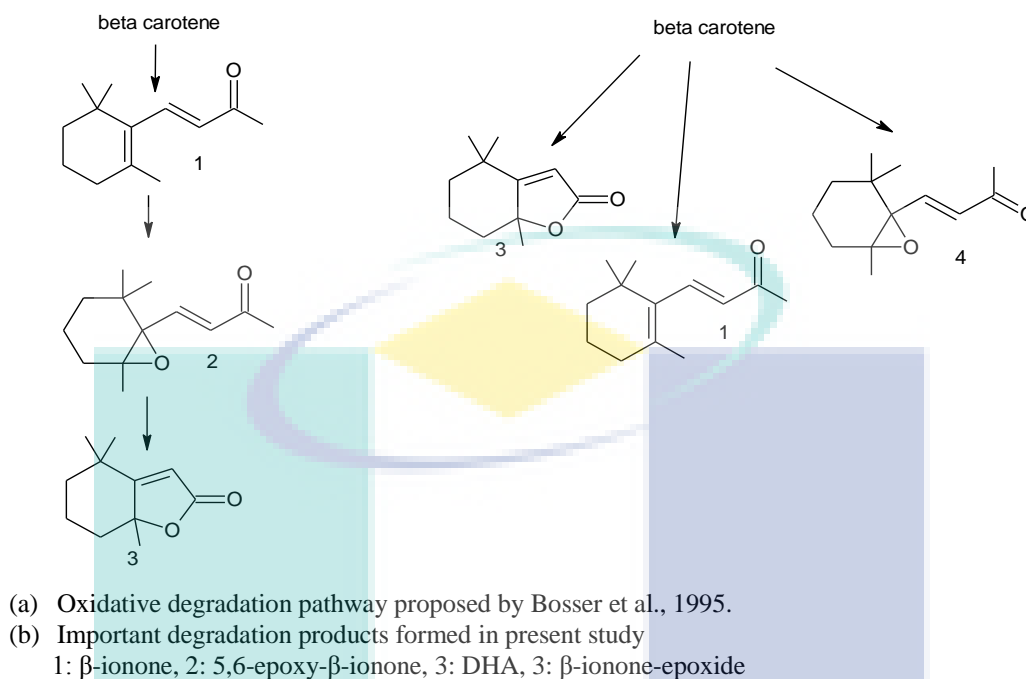
Toluene was one of the volatile compounds formed by degradation of  $\beta$ -carotene in present study and as proposed by Zepka et al. (2013) along with toluene, p-xylene, m-xylene and 2, 6-dimethylnaphthalene formed due to cyclization of polyene carotenoid chain. Rios et al. (2008) also has reported that nor-carotenoid and the aromatic hydrocarbon (m-xylene and toluene) were produced by carotenoids cyclisation reaction. Along with catalyst, carotenoids are also degraded by light, heat and acid. Hence,  $\beta$ -carotene may be oxidized to form new compounds usually that give rise to formation of aroma compounds affected by any of the above mentioned three factors (Mortensen and Skibsted, 2000). Composition of common aroma compounds formed in all samples is shown in Figure 3.5.



**Figure 3.5:** Formation of aroma compounds from oxidative degradation

Yamauci et al. (1993) has stated that  $\beta$ -carotene is an excellent substance for free-radical attack because of the presence of long and conjugated double bonds thus resulting in products that are very complex. In a nutshell, oxidative degradation is best to be conducted without light exposure in a sealed and fully covered container as performed in sample A. Addition of excess amount of catalyst and sonication does not lead to much changes in the amount of aroma compound produced especially  $\beta$ -ionone and DHA. Comparatively, increase in amount of  $\beta$ -carotene used, increases the amount of aroma compounds produced especially  $\beta$ -ionone. This is because as more  $\beta$ -carotene is being degraded, more C-13 norisoprenoid is being generated (Mendes-pinto, 2009).

Figure 3.6 shows the oxidative degradation pathway proposed by (a) Bosser et al. (1995) and (b) shows some important aroma compounds generated in this study. According to Bosser et al. (1995), this degradation pathway (a), it can be concluded that further oxidative degradation of  $\beta$ -ionone (1) leads to the formation of DHA (3). As in the present study, DHA was the highest yield aroma compound formed in all the samples; it shows that most of the  $\beta$ -ionone formed has been converted to DHA. Hence, production of  $\beta$ -ionone can be increased only if the further degradation of  $\beta$ -ionone to DHA is prevented in an earlier stage of oxidative degradation.



**Figure 3.6:** Oxidative degradation scheme

(i) ***Optimised Oxidative Degradation***

Based on the results obtained at different parameters, optimised conditions for oxidative degradation are without light exposure in a sealed and fully covered container. Result of replicate oxidative degradation of commercial  $\beta$ -carotene was summarized in Table 3.6 (APPENDIX C6). Two samples with 10 mg of commercial  $\beta$ -carotene were used for oxidative degradation at the optimised reaction conditions left at room temperature for five days. DHA was found to be the most common aroma compound known that identified highest composition (58.31 %). Remarkably, C-13 norisoprenoid,  $\beta$ -ionone was observed with high composition (% peak area). The retention time of  $\beta$ -ionone formed in the degraded sample based on GC-MS result matches the retention time of standard  $\beta$ -ionone.  $\beta$ -ionone is commercially an important aroma compound that is found naturally in a very low concentration. Besides, some other aroma compounds such as  $\beta$ -ionone epoxide and 3-oxo- $\beta$ -ionone are also produced at this optimised oxidative degradation. Taking into account the results obtained, it is proven that oxidative degradation in an optimised reaction

conditions that are able to increase the yield of aroma compounds produced mainly  $\beta$ -ionone and DHA, and can be used for production of aroma compounds by degradation of extracted palm carotene rich in  $\beta$ -carotene from CPO and OPW.

**Table 3.6:** Oxidative degradation product at optimised condition of commercial  $\beta$ - carotene

Compound	Kovats Index	Composition (% peak area)	Identification
		OO	
Monolauryl maleate	-	7.16 $\pm$ 1.70	MS
t-butylhydroquinone	-	2.81 $\pm$ 0.22	MS
$\beta$ -ionone epoxide	1335	12.33 $\pm$ 8.16	MS,FID
$\beta$ -ionone	1386	3.73 $\pm$ 0.32	MS,FID
Dihydroactinidiolide (DHA)	1357	58.31 $\pm$ 7.56	MS,FID
3-oxo- $\beta$ -ionone	1639	3.99 $\pm$ 1.97	MS,FID

<sup>a</sup> Components < 1 % are not included

<sup>b</sup> Mean value ( $\pm$ SD) of the compounds constituents of two different determination using GC-MS

### 3.3.2 Thermal Degradation

For the thermal degradation of commercial  $\beta$ -carotene, the initial reaction conditions (100  $\pm$  5  $^{\circ}$ C for 3 hr after 1 hr of sonication) was chosen based on previous study by Kanasawud and Crouzet (1990). Analysis of thermal degradation products at this condition were presented in Table 3.7.

**Table 3.7:** Thermal degradation products of commercial  $\beta$ -carotene

Compound	Composition (% peak area)
Cyclohexanol, 2,6-dimethyl	1.33
Cyclopropyl ketone	2.25
$\beta$ -ionone epoxide	3.76
Dihydroactinidiolide (DHA)	19.50
Toluene	69.21
3-oxo- $\beta$ -ionone	1.06

Components < 1 % are not included

The major aroma compound formed was dihydroactinidiolide (DHA) with a composition (% peak area) of 19.50 %, together with some other compounds which were  $\beta$ -ionone epoxide and 3-oxo- $\beta$ -ionone with a composition of 3.76 % and 1.06 % respectively. It is known that carotenoids degradation lead to formation of C-13 norisoprenoids mainly  $\alpha$ -ionone or  $\beta$ -ionone. When intense heat is applied, the ring structures of  $\beta$ -carotene are cleaved and molecular reactions occur involving the double bonds. Bonnie and Choo (1999) have reported that two types of thermal degradation products are formed: volatile fraction with low molecular weight molecules and a non-volatile fraction from the larger fragments of the carotene molecules. However degradation of commercial  $\beta$ -carotene at above mentioned reaction conditions failed to form the C-13 norisoprenoids.

Thus, experimental approach for the thermal degradation using commercial  $\beta$ -carotene under different parameters such as sonication time, reaction time and reaction temperature was used to find the best parameters. The optimum conditions that formed highest yield of aroma compounds were then employed for thermal degradation of recovered palm carotene with high concentration of  $\beta$ -carotene to form high yield of aroma compounds mainly norisoprenoids which are in demand in the global market that play an important role as flavour and fragrance substances. Striking examples are the unusually potent norisoprenoids fragrances  $\beta$ -ionone, compound possess a megastigmane carbon skeleton (Winterhalter and Rouseff, 2001).

(i) ***Different Sonication Time***

The effect of sonication time on thermal degradation of  $\beta$ -carotene was studied by varying the sonication time. Throughout this experimental work, reaction temperature and time maintained constant at  $100 \pm 5$  °C for 3 hr. Four different samples of 10 mg of  $\beta$ -carotene were prepared for thermal degradation with 0 hr of sonication which means without any sonication, 1, 2 and 3 hr of sonication. Table 3.8 shows the result obtained for the effect of different sonication time on thermal degradation using commercial  $\beta$ -carotene (APPENDIX C1).



**Table 3.8:** Thermal degradation products of  $\beta$ -carotene at different sonication time

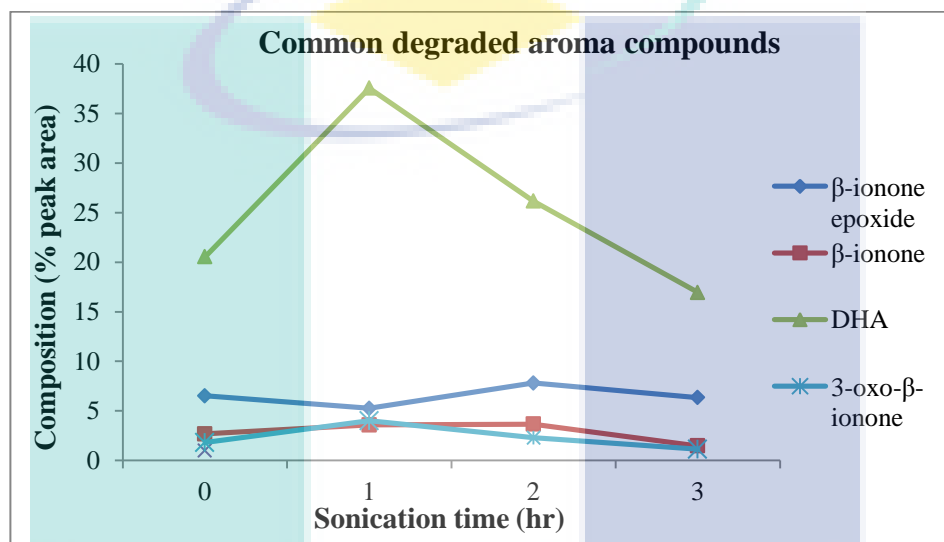
Compound	Composition (% peak area)			
	Sonication time (hr)			
	0	1	2	3
Cyclopropyl ketone	1.82	3.38	-	2.12
t-butylhydroquinone	-	1.52	1.15	-
Cyclohexanol, 2,6-dimethyl	-	2.66	-	-
$\beta$ -cyclocitral	-	1.55	-	-
$\beta$ -ionone epoxide	6.52	5.26	7.82	6.33
$\beta$ -ionone	2.68	3.59	3.67	1.37
Dihydroactinidiolide (DHA)	20.53	37.55	26.16	16.93
$\alpha$ -ionone	-	-	1.56	-
Toluene	61.37	30.68	36.90	67.80
3-oxo- $\beta$ -ionone	1.81	3.00	2.31	1.13
3-hydroxy- $\beta$ -ionone	-	1.33	-	-

Components < 1 % are not included

The main aroma compounds formed were  $\beta$ -ionone,  $\beta$ -cyclocitral, DHA,  $\alpha$ -ionone, 3-oxo- $\beta$ -ionone and 3-hydroxy- $\beta$ -ionone where DHA as the major compound among all the four samples. At 0 hr of sonication, few important compounds were produced such as  $\beta$ -ionone (2.68 %), DHA (20.53 %), 3-oxo- $\beta$ -ionone (1.81 %) and  $\beta$ -ionone epoxide (6.52 %). Among these compounds,  $\beta$ -ionone is the C-13 norisoprenoids of carotenoids degradation. This is due to the fact that, thermal degradation of  $\beta$ -carotene in aqueous medium which involves initially epoxidation and furanoid rearrangement leads to the formation of compounds such as  $\beta$ -ionone,  $\beta$ -cyclocitral, DHA and other reaction products (Mendes-Pinto, 2009).

In addition at 1 hr of sonication, new compounds known as t-butylhydroquinone (3.38 %), cyclohexanol, 2, 6-dimethyl (2.66 %),  $\beta$ -cyclocitral (1.55 %), 3-oxo- $\beta$ -ionone (3.00 %) and 3-hydroxy- $\beta$ -ionone (1.33 %) were formed. Cyclohexanol, 2, 6-dimethyl,  $\beta$ -cyclocitral and 3-hydroxy- $\beta$ -ionone were the compounds determined only in degraded sample of 1 hr of sonication. Meanwhile at 2 hr of sonication time of thermal degradation,  $\alpha$ -ionone (1.56 %) was formed. This compound was only determined at this particular

sonication time and at 3 hr of sonication, the total number of compounds formed decreased compared to the other three samples. Whereas, 3-oxo- $\beta$ -ionone was determined in all four samples. Based on the results obtained, few trends were observed on the four common degradation products from all the four samples by varying the sonication time. These trends are clearly depicted in Figure 3.7.



**Figure 3.7:** Common degraded aroma compounds

The results show that, there is a decrease in production of  $\beta$ -ionone epoxide from 0 to 1 hr of sonication followed by an increase from 1 hr to 2 hr of sonication time. Meanwhile, for  $\beta$ -ionone, DHA and 3-oxo- $\beta$ -ionone, there is an increase followed by decrease in production as sonication time increases. As for DHA, highest production was achieved in 1 hr sonication time which was 37.55 % while for  $\beta$ -ionone, the production was almost the same during 1 and 2 hr of sonication which was at 3.59 and 3.67 % respectively. System of conjugated double bonds makes  $\beta$ -carotene vulnerable to heat. Along with this, there is also formation of toluene in all four samples. Findings on formation of toluene has been previously reported by Rios et al. (2008) where it has been proposed that toluene and m-xylene are most common volatile derived from thermal degradation of carotenoids. According to Mader (1963), toluene, m-xylene and p-xylene are some of the compounds

probably formed by cyclization of this polyene carotenoid chain, whereas the  $\beta$ -ionone ring is involved in the formation of ionone. Cyclization initiated with an eight-electron systems followed by rearrangement which leads to the formation of a four-ring intermediate. This mechanism explains the formation of toluene (Onyewu et al., 1982).

Generally, sonication is the process where energy is supplied to mix well particles in a particular sample. As the result, this leads to complete dispersion of small particles. Therefore, sonication is very important prior to thermal degradation. As  $\beta$ -carotene is not soluble in water, it needs to be mixed well in the distilled water. At the same time an optimum sonication time is crucial to increase the yield of aroma compounds formed. As shown in Figure 3.7, the trend observed clearly attributes that sonication of 1 hr is the optimum time for formation of high yield of aroma compounds. This is because sonication of 1 hr formed most of the aroma compounds in high yield compared to other sonication time. Thus, 1 hr of sonication is sufficient to disperse the  $\beta$ -carotene completely in the medium (distilled water) before degradation begin.

**(ii) Different Reaction Time**

The second parameter being tested in thermal degradation using commercial  $\beta$ -carotene is different reaction time. Mendes-Pinto (2009) found that the extent of  $\beta$ -carotene degradation is strongly dependent on temperature and time of reaction. Three different samples with 10 mg of commercial  $\beta$ -carotene each were thermally degraded for 3, 5 and 6 hr respectively at  $100 \pm 5$  °C with 1 hr of sonication. The degraded samples were then analysed using GS-MS and results are shown in Table 3.9 (APPENDIX C2). Cyclopropyl ketone (1.63 %) was formed only at 3 hr of reaction time, while 3-oxo- $\beta$ -ionone was produced in both 3 and 5 hr of reaction time with a composition of 1.53 % and 1.35 % respectively. Surprisingly isocyclocitral and chiloscyphone were produced at 6 hr of reaction time with a composition of 1.12 % and 1.08 % respectively and  $\alpha$ -ionone was determined only at reaction time of 5 hr. A total of six  $\beta$ -carotene degradation compounds were formed at 6 hr of reaction.

The impact of thermal degradation at different reaction time can be clearly seen in Figure 3.8 where the composition (% peak area) of three common aroma compounds formed in all the three samples was compared. As the reaction time increases, more breakdowns of aroma compounds into other compound occurred (Zepka and Mercadante, 2009). Current findings agree with the theory or concept mentioned earlier, where at maximum reaction time which is 6 hr the amount of DHA,  $\beta$ -ionone,  $\beta$ -ionone epoxide decreases and new compounds such as isocyclocitral and chiloscyphone were formed. According to Zepka et al. (2013), intramolecular cyclization activated by the thermal process followed by an elimination reaction in the chain or a heterolytic fragmentation are the main reaction mechanism involved in the formation of the above mentioned volatiles.

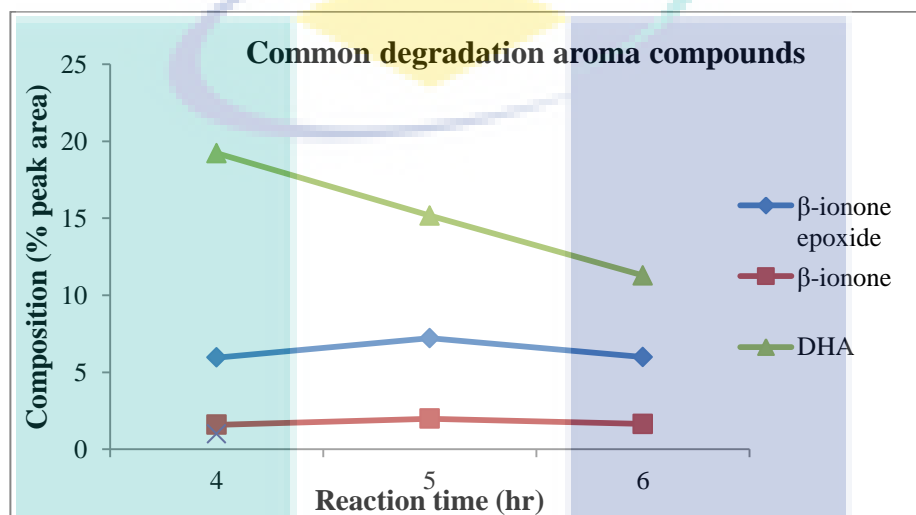
**Table 3.9:** Thermal degradation products of  $\beta$ -carotene at different reaction time

Compound	Composition (% peak area)		
	Reaction time (hr)		
	3	5	6
Cyclopropyl ketone	1.63	-	-
$\beta$ -ionone epoxide	5.95	7.21	5.98
$\beta$ -ionone	1.59	1.98	1.63
Dihydroactinidiolide (DHA)	19.22	15.17	11.28
3-oxo- $\beta$ -ionone	1.53	1.35	-
Chiloscyphone	-	-	1.08
Toluene	62.89	65.31	73.37
Isocyclocitral	-	-	1.12
$\alpha$ -ionone	-	1.00	-

Components < 1 % are not included

Highest yield of  $\beta$ -ionone formed at the 5 hr of reaction time with a composition of 1.98 % along with  $\beta$ -ionone epoxide with 7.21 %. According to Silva Ferreira et al. (2008)  $\beta$ -ionone is 'violet' and can be formed from degradation of  $\beta$ -carotene. This C-13 norisoprenoid could come from the direct degradation of carotenoid molecules such as  $\beta$ -carotene. Meanwhile, DHA production was highest at 3 hr of reaction time which was 19.22 %, whereas toluene detection was remarkable in all three samples with the highest composition at 6 hr of reaction time. DHA is the most frequently cited among heat induced

volatiles from carotene (Kanasawud and Crouzet, 1990). As the reaction time increases the amount of DHA decreases, so it can be assumed that DHA as the first volatile compound formed during heating is probably degraded to other compound in longer reaction time. Taking into account of all the possibilities and yield of aroma compounds mainly  $\beta$ -ionone, 5 hr of reaction time is believed as an optimum reaction time for degradation of  $\beta$ -carotene.



**Figure 3.8:** Common degradation aroma compounds at different reaction time

### (iii) *Different Reaction Temperature*

Temperature changes also were studied to optimise the yield of thermal degradation reaction products. Temperatures ranging from 110 to 130 °C were studied and results are shown in Table 3.10 (APPENDIX C3). Temperature is one of the factors that influence the degradation of carotenoids (Penicaud et al., 2011). A total of five degradation products were determined from the GC-MS analysis of reaction at 110-120 °C with DHA at highest composition (% peak area), 31.08 % along with  $\beta$ -ionone epoxide (5.88 %) and 3-oxo- $\beta$ -ionone (3.93 %) as important aroma compounds. Thermal degradation of  $\beta$ -carotene at 120-130 °C resulted several main aroma compounds. Again, DHA an intermediate compound is the most common aroma compound identified with a composition of 28.66 %. Despite all of the facts discussed earlier,  $\beta$ -carotene an unstable compound consisting of a polyene

chain with 11 conjugated double bonds and a  $\beta$ -ring at each end of the chain, thus can be easily degraded to a variety of products at elevated temperatures (Marais, 1992).

Besides DHA, other aroma compounds formed such as cyclohexanol, 2, 6-dimethyl (1.76 %), 3-oxo- $\beta$ -ionone (2.95 %),  $\beta$ -cyclocitral (1.20 %) and  $\beta$ -ionone epoxide (3.80 %). Toluene was determined in all three samples under different temperature. This was supported by findings from Zepka et al. (2013), who proposed that even under mild thermal conditions, studies concerning the generation of volatile compounds by  $\beta$ -carotene degradation usually reported the formation of toluene and few other compounds such as m-xylene and 2, 6-dimethylnaphthalene. The main aroma compound which is C-13 norisoprenoids was successfully produced, which is  $\beta$ -ionone with a composition of 2.33 %. Also, at 130-130 °C, five degradation products were observed. DHA and 3-oxo- $\beta$ -ionone were determined with a yield of 17.31 % and 3.00 % and 3-hydroxy- $\beta$ -ionone (1.53 %) was compound formed only at this particular reaction temperature. Based on the overall result obtained, it would be reasonable to believe that temperature between 120-130 °C is found to be an optimum thermal degradation temperature as most of the important carotenoid derived aroma compounds were formed at this temperature with considerably high yield.

**Table 3.10:** Thermal degradation products of  $\beta$ -carotene at different reaction temperature

Compound	Composition (% peak area)		
	Temperature (°C)		
	110-120	120-130	130-130
Monolauryl maleate	3.03	2.89	1.18
Cyclohexanol, 2,6-dimethyl	-	1.76	-
Toluene	36.91	50.35	76.96
$\beta$ -cyclocitral	-	1.20	-
$\beta$ -ionone epoxide	5.88	3.80	-
$\beta$ -ionone	-	2.33	-
Dihydroactinidiolide (DHA)	31.08	28.66	17.31
3-oxo- $\beta$ -ionone	3.93	2.95	3.00
3-hydroxy- $\beta$ -ionone	-	-	1.53

Components < 1 % are not included

Result of present study shows that degradation of water suspension of  $\beta$ -carotene at different temperature follows the same kinetics as degradation in homogeneous organic solution which is zero order reaction kinetics (Kanasawud and Crouzet, 1990). Based on the results given in Table 3.10, maximum numbers of the components have been previously identified in the volatile fraction produced by thermal degradation of  $\beta$ -carotene in the solid state or in solution.  $\beta$ -carotene is a very reactive compound due to its unsaturated bonds, thus this compound is prone to thermal degradation. As the temperature rises, production of volatiles greatly accelerates, especially toluene. DHA remains as one of the degradation aroma product found in larger quantities than the other compounds studied. Nonier et al. (2003) stated that  $\beta$ -carotene deteriorates very slowly but the degradation speeds rises as the temperature increases. However, an optimum temperature is required as very high/extreme temperature tend to further convert the desired aroma compounds into some other compounds.

(iv) *Optimised Thermal Degradation*

Two samples 10 mg of commercial  $\beta$ -carotene were used for thermal degradation at the optimised reaction conditions which is at 120-130 °C, for 5 hr with 1 hr sonication. The thermal degradation products of these replicated samples were analysed using GC-MS and results were showed in Table 3.11 (APPENDIX C3).

**Table 3.11:** Thermal degradation products of commercial  $\beta$ -carotene at optimised condition

Compound	Kovats Index	Composition (% peak area)	Identification
		OT	
Monolauryl maleate	-	5.33 ± 0.57	MS
$\beta$ -cyclocitral	-	3.21 ± 0.08	MS
$\beta$ -ionone	1386	9.60 ± 0.03	MS,FID
Dihydroactinidiolide (DHA)	1357	61.23 ± 12.25	MS,FID
3-oxo- $\beta$ -ionone	1639	3.57 ± 0.05	MS,FID

<sup>a</sup> Components < 1 % are not included

<sup>b</sup> Mean value ( $\pm$ SD) of the compounds constituents of two different determination using GC-MS

DHA is the most common aroma compound known to be one of the first volatile compounds that formed by thermal degradation of  $\beta$ -carotene (Kanasawud and Crouzet, 1990) and also in this study, DHA is identified as highest yield with a composition of 61.23 %. More interestingly C-13 norisoprenoid,  $\beta$ -ionone was observed with high yield. The yield of  $\beta$ -ionone formed in the degraded was 9.60 %.  $\beta$ -ionone is one of the commercially important aroma compound which is observed in plant tissues in a very low concentrations. Tedious and laborious extraction processes increases the production cost of  $\beta$ -ionone (Wache et al., 2006). Thus, this present method is a direct convenient production of  $\beta$ -ionone which serves the need of food and aroma industry. Also, high yield of DHA was observed which can conveniently be converted into  $\beta$ -ionone by further photo-oxygenation reaction (Mendes-pinto, 2009).

Based on the result obtained, it is proven that thermal degradation in an optimised reaction conditions increases the yield of aroma compounds produced, mainly  $\beta$ -ionone and DHA and can be further employed for degradation of extracted carotene from CPO and OPW to produce aroma compounds. Besides the major formation of  $\beta$ -ionone and DHA, some other useful aroma compounds formed from this thermal degradation were 3-oxo- $\beta$ -ionone and  $\beta$ -cyclocitral. Although  $\beta$ -cyclocitral was identified in both samples, its yield was low (3.21 %). The low yield of  $\beta$ -cyclocitral compound could be due to insufficient degradation of  $\beta$ -carotene. Citral is one of the compounds being used to produce  $\beta$ -ionone via organic synthesis. As a whole these findings agree with Silva Ferreira et al. (2008) who has reported that norisoprenoids could originate from direct degradation of carotenoids molecules such as  $\beta$ -carotene.

### **3.3 FORMATION OF AROMA COMPOUNDS BY DEGRADATION**

Carotenoids are extremely susceptible to degradation by factors such as heat, low pH, light and others which causes rearrangement or formation of degradation compounds such as epoxides, short chain products and also volatile compounds (Zepka and Mercadante, 2009). According to Penicaud et al. (2011),  $\beta$ -carotene is a major carotene in carotenoids group which is a very reactive compound. Subsequently, it is also prone to



degradation particularly at high temperature due to its highly conjugated double bond which renders it electronically rich by delocalization of  $\pi$ -electrons. Hence,  $\beta$ -carotene in CPO and OPW are believed could generate various volatile compounds such as  $\beta$ -ionone, DHA and others by degradation. Thus, degradation of carotene in CPO directly without any extraction was initially studied by conducting few experiments which were by accelerated heating, oxidative and thermal degradation. Also, extracted  $\beta$ -carotene from CPO, PPF and EFB with highest concentration among all the extracted samples was subjected to the optimised degradation reactions conditions.

### 3.3.1 Oxidative and Thermal Degradation of CPO

The effect of degradation on CPO prior to extraction of  $\beta$ -carotene was studied. Firstly, accelerated heating was performed on CPO and GC-MS results on changes in composition of CPO after heating in oven over a period of time were tabulated in Table 3.12 (APPENDIX D1). Based on the results obtained, palmitic acid and oleic acid remained as major degradation compounds in all ranges of temperature being tested for both 15 and 30 min. Oleic acid composition decreased upon increase in the accelerating heating time from 15 to 30 min. Citric acid was only found in samples heated at 90 and 130 °C and surprisingly compounds such as metaraminol, cyanoacetylurea, 15-hydroxypentadecanoic acid and ethyl palmitate were determined at 110 °C.

Overall it can be concluded that, accelerated heating directly on CPO does not contribute to any formation of aroma compounds or C-13 norisoprenoids, although there is presence of  $\beta$ -carotene in it. Hence, the suggested pathway is the recovery of palm carotene which will yield carotene with high concentration of  $\beta$ -carotene from CPO followed by degradation to obtain desired aroma compounds. This method can be applied to OPW in order to recover high concentration of  $\beta$ -carotene by discarding the fatty acids and other polar compounds in it. Since CPO is a cheap source and PPF and EFB are wastes from palm oil industry, the production of aroma compounds by degradation of  $\beta$ -carotene from these starting materials will be economically viable.

**Table 3.12:** Product of accelerated heating of CPO

Compound	Composition (% peak area)					
	Heating Temperature (°C)					
	90		110		130	
	Heating Time (min)					
	15	30	15	30	15	30
Palmitic acid	55.79	53.99	50.21	53.17	52.12	39.88
Linoleic acid	2.77	2.88	-	-	3.13	-
cis-Vaccenic acid	1.35	30.00	-	-	1.53	1.93
Methyl palmitate	-	-	1.33	-	-	-
Stearic acid	2.75	1.90	-	-	1.60	3.17
Metaraminol	-	-	3.33	1.68	-	-
Cyanoacetylurea	-	-	1.36	2.52	-	-
15-Hydroxy pentadecanoic acid	-	-	3.83	9.31	-	-
Oleic Acid	27.82	7.22	33.27	28.26	31.67	32.55
2-Chlorohistidine	-	-	-	-	3.20	-
Ethyl Palmitate	-	-	3.73	2.16	-	-
Palmitelaidic acid ethyl ester	-	-	-	-	-	1.71
Propanamide	-	-	-	-	-	1.36
Palmitic anhydride	5.88	-	-	-	-	-

Components < 1 % are not included

Besides, thermal degradation and oxidative degradation CPO also was performed to study the difference in result obtained by degradation of  $\beta$ -carotene in CPO prior and after extraction. The basic degradation reaction conditions were employed. However results obtained found to be unimpressive as shown in Table 3.13 which proved that there is no conversion of  $\beta$ -carotene to aroma compounds. Two samples of 100 mg each were degraded thermally and oxidatively. For thermal degradation of CPO, three common compounds exist which was palmitic acid, oleic acid and cis-vaccenic acid. Palmitic and oleic acid made the major portion of CPO. Also few other compounds such as stearic acid, methyl palmitate and others are determined in a small composition by using GC-MS analysis.

**Table 3.13:** Oxidative and Thermal Degradation of CPO

Compound	Composition (% peak area)	
	T-CPO	O-CPO
Palmitic acid	50.73 ± 7.37	-
Methyl palmitate	-	27.66 ± 5.06
Ethyl palmitate	-	22.01 ± 0.67
Oleic acid	32.79 ± 9.99	-
cis-vaccenic acid	3.33 ± 0.88	-
Methyl elaidate	-	25.73 ± 3.53

<sup>a</sup> Components < 1 % are not included

<sup>b</sup> Mean value (±SD) of the compounds constituents of two different determination using GC-MS

<sup>c</sup> All the fatty acids related compounds matches more than 90 % of library search by GC-MS

These explained the high content of saturated fatty acids in palm oil and the need for extraction prior to degradation. As extraction is able to remove fatty acids content, hence the concentration of  $\beta$ -carotene can be increased. On the other hand, oxidative degradation of CPO results shows that there is no formation of any aroma compounds when CPO is directly used for oxidative degradation. Methyl palmitate, ethyl palmitate and methyl elaidate were the common compounds determined with high composition (% peak area). It is proven that though the concentration of  $\beta$ -carotene in CPO is considerably high, it is necessary to separate the  $\beta$ -carotene from the fatty acids other polar compounds in the oil. This is because for degradation to occur, the conjugated double bonds in the  $\beta$ -carotene must be broken down. This will be possible only if extraction or separation process carried out prior to degradation, as this step increases the concentration of  $\beta$ -carotene as well as discard the unwanted fatty acids and other compounds from the palm carotene.

### 3.3.2 Oxidative Degradation of Recovered $\beta$ -Carotene

Recovered  $\beta$ -carotene from CPO and OPW were studied for oxidative degradation under optimum reaction conditions. Due to their extended conjugated system, carotenoids are susceptible to attack by a variety of reactive chemical agents (Mortensen and Skibsted, 2000). Thus, they are prone to degradation (Zeb and Murkovic, 2011) and oxidized. GC-

MS analysis identified the compounds formed and results obtained were tabulated in Table 3.13. Results were found to be unimpressive in the case of formation of aroma compounds by oxidative degradation of extracted  $\beta$ -carotene from CPO also PPF and EFB. This might be due to the fact that recovery of palm carotene by soxhlet adsorption increases the concentration of  $\beta$ -carotene, however presence of fatty acids in the palm oil is still noted.

**Table 3.13:** Oxidative degradation products of  $\beta$ -carotene from CPO and OPW (PPF & EFB)

Compound	Composition (% peak area)		
	CPO1	PPF1	EFB1
methyl palmitate	3.79 $\pm$ 3.12	20.18 $\pm$ 0.33	20.76 $\pm$ 19.38
ethyl palmitate	12.67 $\pm$ 3.18	27.39 $\pm$ 0.06	-
palmitic acid	36.73 $\pm$ 1.61	-	-
methyl elaidate	3.65 $\pm$ 2.23	13.53 $\pm$ 0.18	21.33 $\pm$ 15.56
ethyl oleate	7.53 $\pm$ 3.12	23.03 $\pm$ 0.19	-
cis-vaccenic acid	13.31 $\pm$ 18.27	-	33.09 $\pm$ 1.36
ethyl tetradecanoate	-	6.63 $\pm$ 0.21	-
ethyl stearate	-	3.98 $\pm$ 0.32	-

<sup>a</sup> Components < 1 % are not included

<sup>b</sup> Mean value ( $\pm$ SD) of the compounds constituents of two different determination using GC-MS

<sup>c</sup> All the fatty acids related compounds matches more than 90 % of library search by GC-MS

Increasing temperature increases the oxidation and degradation rate (Zeb and Murkovic, 2013). However, unlike thermal degradation, oxidative degradation could not offer high temperature as the reaction was carried out in room temperature. Though oxidative degradation of commercial  $\beta$ -carotene produced aroma compounds, it would be reasonable deduce that there are difficulties to purify the isomers of carotenes as they are not much difference in polarity also structure. For instance the only difference between  $\alpha$ - and  $\beta$ -carotene is the position of the double bond in one of the cyclohexane rings. So, the recovered  $\beta$ -carotene of CPO and OPW is not 100 % pure and contains impurities. Thus, oxidative degradation of these extracted palm carotenes does not produce any aroma compounds but only few compounds associated with the fatty acids of palm oil as shown in table above.

### 3.3.3 Thermal Degradation of Recovered $\beta$ -carotene

Recovered  $\beta$ -carotene from CPO and OPW with highest concentration was studied by conducting thermal degradation with 100 mg of extracted sample under optimum reaction conditions. Results obtained proves that  $\beta$ -carotene extracted from CPO and OPW was successfully converted to aroma compounds mainly  $\beta$ -ionone, DHA, 3-oxo- $\beta$ -ionone and D-limonene. The breakdown products of carotenoids are carbonyl compounds with C13 that are the most abundant norisoprenoids in nature with the megastigmane structure including the family of ionones and C11 such as  $\beta$ -cyclocitral and DHA (Mendes-Pinto, 2009). Analysis results for two degraded sample of each extracted sample from CPO, PPF and EFB were tabulated in Table 3.15 (APPENDIX D2).

**Table 3.15:** Thermal degradation products of  $\beta$ -carotene from CPO and OPW (PPF & EFB)

Compound	Kovats Index	Composition (% peak area)			Identification
		CPO2	PPF2	EFB2	
$\beta$ -ionone	1386	3.38 $\pm$ 3.83	1.53 $\pm$ 0.25	-	MS,FID
3-oxo- $\beta$ -ionone	1639	8.13 $\pm$ 5.11	-	-	MS,FID
D-Limonene	1026	-	7.36 $\pm$ 7.85	-	MS,FID
$\beta$ -ionone epoxide	1335	-	-	3.69 $\pm$ 2.96	MS,FID
DHA	1357	35.91 $\pm$ 33.05	-	23.32 $\pm$ 19.72	MS,FID
Methyl elaidate		-	5.98 $\pm$ 6.15	-	MS
Ethyl Oleate		-	5.18 $\pm$ 5.28	-	MS
oleic acid		-	-	33.59 $\pm$ 18.92	MS

<sup>a</sup> Components < 1 % are not included

<sup>b</sup> Mean value ( $\pm$ SD) of the compounds constituents of two different determination using GC-MS

<sup>c</sup> All the fatty acids related compounds matches more than 90 % of library search by GC-MS

The main aroma compounds formed in CPO were  $\beta$ -ionone (3.38 %) and 3-oxo- $\beta$ -ionone (8.13 %) and DHA (35.91 %). While in sample PPF, the composition (% peak area) of  $\beta$ -ionone produced was 1.53 % and D-limonene was formed with a composition of 7.36 %. DHA and  $\beta$ -ionone epoxide were formed successfully by degradation of  $\beta$ -carotene extracted from EFB with composition of 23.32 and 3.69 % respectively. Formation of this

aroma compounds was supported by findings of Silva Ferreira (2008), that norisoprenoids can be produced by the direct degradation of carotenoid mainly from  $\beta$ -carotene.  $\beta$ -ionone identification was further confirmed by comparing the retention time with reference of standard  $\beta$ -ionone. The GC-MS chromatogram for standard  $\beta$ -ionone is attached in APPENDIX D3. GC-FID analysis was done to further confirm the aroma compounds produced by comparing the Kovats index (RI).

As discussed earlier, among these aroma compounds,  $\beta$ -ionone and DHA are norisoprenoids of carotenoids degradation. As stated by Uenojo and Pastore (2010)  $\beta$ -ionone is a compound that formed from  $\beta$ -carotene rupture. This particular aroma compound is widely used in aroma and fragrance industries because of its floral, fruity and sweet aromas. In addition, few other compounds associated with the palm oil's fatty acids determined such as methyl palmitate, oleic acid, ethyl oleate and other silicon group compounds were determined. Identification of silicon group related compounds might be due to mixing of the grease that has been used to set up the glass reflux apparatus for soxhlet adsorption and thermal degradation. A more severe thermal effect on these compounds was found at higher temperature. This shows that although soxhlet extraction was employed, this method only able to concentrate the recovered  $\beta$ -carotene, but does not completely removes the fatty acids. Thus, high temperature is needed to completely degrade the mixture sample and break down the fatty acids so that  $\beta$ -carotene extracted can be converted to aroma compounds. In addition, degradation using bigger amount of sample increases the yield of aroma compounds produced. Therefore, degradation in a bigger scale enables the isolation of single aroma compounds that can be useful in industry.

### 3.5 CONCLUSIONS

This chapter summarise overall results of recovery of  $\beta$ -carotene from CPO and OPW, optimisation of degradation using commercial  $\beta$ -carotene and formation of aroma compounds by optimised degradation reaction conditions using recovered  $\beta$ -carotene. Objectives of this research were achieved, whereby formation of aroma compounds by degradation of recovered  $\beta$ -carotene from CPO and OPW was proven to be viable.

## CHAPTER 4

### CONCLUSIONS AND RECOMMENDATIONS

#### 4.1 INTRODUCTION

In this chapter, summary of this research outcome, limitations faced during present research and recommendations for upcoming research were discussed concisely.

#### 4.2 CONCLUSIONS

Formation of aroma compounds by degradation of  $\beta$ -carotene extracted from CPO and OPW (PPF and EFB) under optimised reaction conditions were investigated. This research can be divided into three major parts which was recovery of  $\beta$ -carotene from CPO and OPW of EFB and PPF. This was performed by using soxhlet adsorption method whereby the extraction conditions were optimised by studying different IPA extraction time and ratio of sample to adsorbent (CPO: HP-20). Next was the optimisation of degradation reactions by using commercial  $\beta$ -carotene which was thermal degradation and oxidative degradation. For optimisation of thermal degradation different sonication time, reaction time and temperature was studied whereas effect of light, sonication, amount of sample and catalyst were investigated. The parameters tested showed significant influence qualitatively and quantitatively especially on the yield of product formed especially aroma compounds. Lastly was formation of aroma compounds by both degradation methods in optimised reaction conditions using recovered  $\beta$ -carotene from CPO and OPW.

Based on results obtained from the research conducted at different IPA extraction time and ratio of CPO: HP-20, highest recovery of  $\beta$ -carotene yield and concentration was being achieved at significantly shorter IPA extraction time which was 1 hr and at ratio of 1: 4 of CPO to HP-20 adsorbent for the soxhlet adsorption extraction method. Comparison of two different analysis methods which were by HPLC and UV-Vis analysis indicated that there were significant differences on concentration of  $\beta$ -carotene extracted from both CPO and OPW. Soxhlet adsorption method tends to extract more palm carotene with high concentration from CPO compared to OPW of EFB and PPF.

In addition, the optimum parameters on the degradation reaction were determined by using commercial  $\beta$ -carotene. Optimum conditions being predicted based on the results obtained for thermal degradation was 1 hr of sonication time, 4 hr of reaction and at a reaction temperature of 120-130 °C, whereas for oxidative degradation vial must be completely covered, sealed and kept at room temperature. These developed optimised conditions were used for degradation of extracted  $\beta$ -carotene. Oxidative degradation using extracted  $\beta$ -carotene failed to form any aroma compounds. Conversely, thermal degradation of extracted palm carotene from both CPO and OPW formed aroma compounds such as  $\beta$ -ionone, DHA, D-limonene,  $\beta$ -ionone epoxide and 3-oxo- $\beta$ -ionone. The main aroma compound with highest composition formed was DHA (44.91 %) followed by  $\beta$ -ionone analysed by using GC-MS and GC-FID.

Hence it was proven that formation of aroma compounds by degradation of  $\beta$ -carotene extracted from CPO and OPW of PPF and EFB is feasible. This research is believed to be vital in contributing fundamental knowledge on the lab scale for the formation of aroma compounds via degradation of  $\beta$ -carotene.

#### **4.3 RECOMMENDATIONS FOR THE FUTURE RESEARCH**

In a nutshell, a thorough understanding about the  $\beta$ -carotene extraction from CPO and OPW and formation of aroma compounds by degradation of recovered  $\beta$ -carotene under optimised reaction conditions was obtained through this research. At the same time,



some improvements were found to enhance the upcoming studies on extraction process and the degradation reaction. Throughout this study, the  $\beta$ -carotene extraction method especially by soxhlet adsorption method was conducted by using smaller soxhlet extraction set up. Therefore, it is recommended to scale up the process by enlarging the extraction set up. Thus, bigger sample amount can be used for one time extraction which will leads to extraction of palm carotene with higher yield and concentration of  $\beta$ -carotene.

Besides, based on the results obtained degradation of extracted  $\beta$ -carotene using larger amount will lead to formation of higher amount of aroma compounds. Hence, it is also recommended to conduct the thermal degradation reaction in optimised reaction conditions using more amount of  $\beta$ -carotene in a larger scale, so that high yield of aroma compounds can be formed and isolated. In addition, the GC-FID and GC-MS also has its drawbacks where some problems were encountered in an attempt to identify the aroma compounds formed. Firstly, failure to match a Kovats index of few compounds from degraded sample exactly with mass spectrum in GC-MS. Besides, inability to match the Kovats index of degraded sample with previous study as there is no previous publications on formation or production of aroma compounds from CPO or OPW. Thus, it is necessary to use few other instrumentation methods such as LC-MS for the analysis of degraded samples along with GC-MS and GC-FID. By implementing these recommendations on the future research it is believed that a better experimental design on this field can be obtained.

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## Dihydroactinidiolide from thermal degradation of $\beta$ -carotene

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### ABSTRACT

The formation of dihydroactinidiolide by thermal degradation of  $\beta$ -carotene was studied. A comparison of yields of dihydroactinidiolide in commercial  $\beta$ -carotene and  $\beta$ -carotene derived from crude palm oil was investigated. Thermal degradation of commercial  $\beta$ -carotene promoted the formation of dihydroactinidiolide with the highest yield, 61.21%. Thermal degradation of recovered  $\beta$ -carotene yielded 29.23% of dihydroactinidiolide. The lower recovery of  $\beta$ -carotene was due to the mixture of compounds in the extract. Further investigation indicated some other useful aroma compounds formed from this thermal degradation were  $\beta$ -ionone, 3-oxo- $\beta$ -ionone, and  $\beta$ -cyclocitral. The outcome provided wide opportunities in utilizing crude palm oil as natural source of  $\beta$ -carotene to produce aroma compound.

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$\beta$ -carotene; Crude palm oil; Dihydroactinidiolide; Thermal degradation; Aroma compounds;  $\beta$ -ionone;  $\beta$ -cyclocitral

## Introduction

Dihydroactinidiolide (dhA) was first isolated as feline's attractants from leaves of *Actinidia polygama*<sup>[1]</sup> and identified as a flavor component in many plants, such as tobacco and tea. dhA is one of three components of the pheromone for queen recognition of the workers of the red imported fire ant (RIFA), *Solenopsis Invicta*,<sup>[2]</sup> and in mammals such as the cat and the red fox.<sup>[3]</sup> Since its discovery in the early 1930s, RIFA has become a major agricultural and urban pest throughout the southeastern United States. In addition, fire ants cause both medical and environmental harm.

The dhA molecule contains a carbonyl group that can react with nucleophilic structures in macromolecules, providing this compound with a high potential reactivity. The dhA is also found to exhibit cytotoxic effects against cancer cell lines.<sup>[4]</sup> In contrast, little is known on the actions of dhA in vascular plants. Nevertheless, dhA has been identified as a major component of ethyl acetate extracts of cyanobacteria or aquatic macrophytes, which inhibit seed germination and seedling growth.<sup>[5]</sup> This compound has also been identified in wheat glumes, which acts as a germination inhibitor.<sup>[6]</sup>

A large number of synthetic approaches either to racemic<sup>[7]</sup> or to enantioselective<sup>[8]</sup> syntheses of dhA has been developed over the last 40 years based on the previously mentioned biological properties. The most classical approach is that described by Mori and Nakazono.<sup>[9]</sup> However, some of the reported enantioselective syntheses suffer from significant drawbacks. The most significant synthetic issues concerns the need of using lengthy multi-step procedures, specially prepared chiral catalysts or enantioenriched starting materials. More efficient methods for the transformation of tetrahydroactinidiolide into dhA, still need to be studied.

Thermal degradation of  $\beta$ -carotene at 180°C in an oxygen-free environment first studied by Mulik and Erdman<sup>[10]</sup> and Day and Erdman,<sup>[11]</sup> which show the formation of toluene, m-xylene, 2,6-dimethylnaphthalene, and ionone. LaRoe and Shipley<sup>[12]</sup> found  $\alpha$ -ionone and  $\beta$ -ionone in small

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amounts along with toluene, xylene, 2,6-dimethylnaphthalene, and ionone when  $\beta$ -carotene was heated at 188°C for 72 h. The mechanism and kinetic study for the formation of volatile compounds conducted by Kanasawud and Crouzet<sup>[13]</sup> shows that dhA is the first compound produced during heat treatment of  $\beta$ -carotene at 97°C in water. Kinetics studies indicate that dhA may also be produced through 5, 6-epoxy- $\beta$ -ionone, which is an important intermediate reaction. This compound acts as a precursor for different volatiles such as  $\beta$ -ionone, 2-hydroxy-2, 6, 6-trimethylcyclohexanone, and 2-hydroxy-2, 6, 6-trimethylcyclohexane-1-carboxaldehyde. However, the amount of volatile compounds formed in the thermal degradation is still limited.

The synthetic method was simplified by analysing the thermal degradation of  $\beta$ -carotene and the amount of dhA formed was reported. The source of  $\beta$ -carotene, from crude palm oil (CPO) is rich in carotenes at approximately 600 ppm, and Malaysia is one of the major consumers and exporters of palm oil. Various methods of carotenoid recovery from palm oil have been reported. These include saponification, soxhlet adsorption, selective solvent extraction, and transesterification followed by distillation, and supercritical fluid extraction using CO<sub>2</sub>.<sup>[14]</sup> To-date, supercritical fluid extraction technology R134a as a solvent was introduced to prevent degradation of carotene during extraction.<sup>[15]</sup> These sources are becoming more important to recover the carotenes in palm oil because most of them are destroyed in the present refinery process to produce light color oils. The thermal degradation of  $\beta$ -carotene has been studied since 1963, but none of the studies reported the amount of dhA formed. Nonetheless, many studies have been done focusing only on the extraction of palm carotenes and no studies have been carried out on the production of aroma compounds by degradation of these carotenoids from CPO.

## Materials and methods

### Materials

CPO was collected from Felda Palm Industries Sdn. Bhd. (Lepar Hilir 3, Pahang, Malaysia). Standard  $\beta$ -carotene and  $\beta$ -ionone were purchased from Merck (Germany). Synthetic macroporous resin (DIAION HP-20), a styrenic polymeric bead type resin design for adsorption with a surface area of 500m<sup>2</sup>/g was bought from Sigma Aldrich (United States). All the other chemicals and reagents used in this study were of analytical and industrial grade.

### Soxhlet adsorption

Twenty-four grams of HP-20 adsorbent was weighed and transferred into a 250 mL conical flask. Next, 50 mL of isopropanol (IPA) was added and maintained with continuous stirring for 30 min to activate the adsorbent. The activated adsorbent was filtered and dried at room temperature and transferred into a 250 mL three neck round-bottom flask. Six grams of CPO diluted with 50 mL of IPA was then added for a period of 1 h under constant stirring and maintained for 1 h at the same temperature. The mixture of CPO and HP-20 was then transferred to the soxhlet extraction thimble. Two hundred milliliters of IPA was added into the 250 mL round-bottom flask of soxhlet extractor and extracted for 1 h at 80 ± 5°C. Next, palm carotene was extracted from the adsorbent at 65 ± 5°C until the adsorbent became colorless (3 h). The experiment was conducted in a dark room.

### $\beta$ -carotene analysis

$\beta$ -carotene content was determined by diluting 50 mg extracted carotene in 10 mL solvent and measuring absorbance in a Shimadzu UV-1601 (Shimadzu Corporation, Kyoto, Japan) at 446 nm. In addition, Water Alliance E2695 HPLC with an automated injector and photo diode array detector were used to determine  $\beta$ -carotene extracted, qualitatively and quantitatively. The isocratic mobile phase was acetonitrile/dichloromethane (9.5:0.5, vol/vol). A low rate of 1.0 mL/min, and carotene

was determined by measuring absorbance at 450 nm. The concentration of extracted palm carotene was determined using standard calibration curve of  $\beta$ -carotene and the results were expressed in ppm. Standard solutions were prepared within a working range of 200 to 400 ppm.

### **Thermal degradation of $\beta$ -carotene**

Ten milligrams of commercially purchased  $\beta$ -carotene was dissolved in 50 mL distilled water in a 250 mL round-bottom flask that was covered with aluminium foil and sonicated for 1 h. The flask was connected to a condenser and heated in silicon oil bath at 110–120°C in an oil bath for 4 h with continuous stirring. This optimized reaction conditions were chosen based on results obtained through two different tested parameters, reaction time (4, 5, and 6 h) and different temperature (110–120, 120–130, and 130–140°C). The organic compounds were separated with hexane when the experiment was completed. Anhydrous sodium sulphate was added to the extract to remove the remaining water. The degraded products were extracted by hexane and an analysis was performed by using gas chromatography-mass spectrometry (GC/MS). The experiment was conducted in a dark room. Then, degradation process was repeated with  $\beta$ -carotene recovered from CPO. All samples were degraded in triplicate.

### **GC spectrometry**

The J&W DB-5 (95% dimethyl, 5% diphenyl polysiloxane; 30 m  $\times$  0.25 mm i.d) 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<sup>-1</sup> (20 min). The injector temperature was 250°C; in splitless mode. The ionization energy was 70 eV with transfer-line temperature of 250°C. Mass spectra was scanned in the  $m/z$  = 58–650 range. Identification was achieved by mass spectral library search combined with retention index comparison by peak area normalization method.

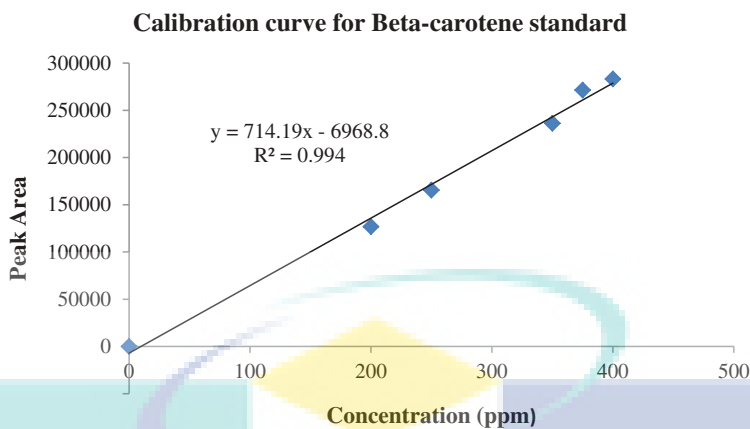
## **Results and discussion**

### **Recovery of $\beta$ -carotene from CPO**

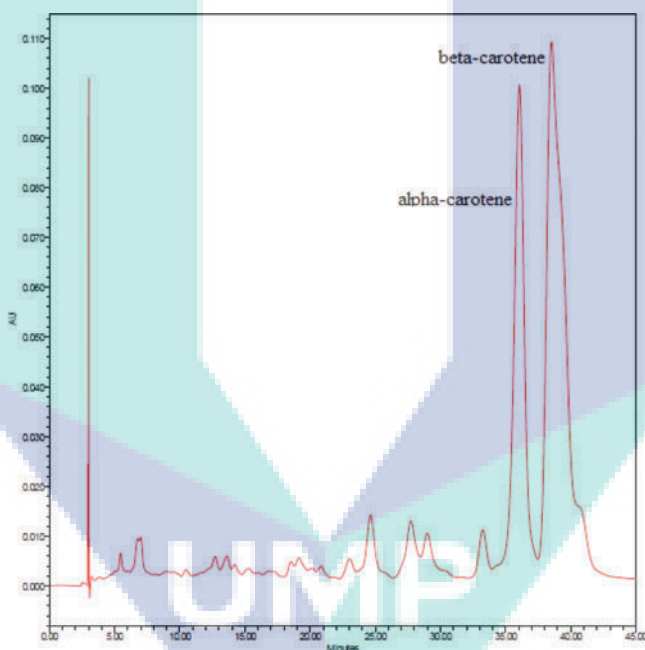
Palm oil is known to contain a high concentration of carotenoid, but according to Amorim-Carrilho et al.,<sup>[16]</sup> a high variability in chemical structure and poor stability greatly makes analysis difficult. Therefore, there is no general or standard method for carotenoids extraction in laboratories, whereby many extraction methods involve the release of desired components from their matrices by disrupting tissue followed by removal of the unwanted components. Besides, when choosing an extraction method, susceptibility of carotene to oxidation and degradation must be considered as it is very sensitive to light, heat, acid, or oxygen exposure.<sup>[16]</sup> Soxhlet extraction at temperatures between 50–70°C have been reported to give higher phytochemical yields.<sup>[17]</sup> Therefore, the Soxhlet adsorption method was employed to extract  $\beta$ -carotene from CPO by considering the possible recovery and risk of  $\beta$ -carotene degradation. Calibration graph for high-performance liquid chromatography (HPLC) was based on peak area of five different concentrations of  $\beta$ -carotene standards to determine the linearity in a working range from 200 to 400 ppm with correlation coefficient,  $R^2 = 0.994$  (Fig. 1). Results show that from 6.0 g of CPO used for the extraction, 71.81% of sample recovered in IPA fraction and concentration of  $\beta$ -carotene from CPO determined by HPLC was 3790 ppm (Fig. 2).

### **GC analysis of thermal degradation of commercial $\beta$ -carotene**

Volatile compounds were identified by mass spectral library searching combined with retention index comparison by peak area normalization method. The thermal degradation products of these triplicate samples were analysed using GC/MS results as shown in Table 1. dhA is the most common



**Figure 1.** HPLC analyses of standard calibration curve of  $\beta$ -carotene.



**Figure 2.** HPLC chromatogram of palm carotene.

aroma compound known to be one of the first volatile compounds that are formed by thermal degradation of  $\beta$ -carotene.<sup>[13]</sup> In this study, dhA is identified with a high yield. More interestingly, C-13 norisprenoid,  $\beta$ -ionone formed in the degraded was 9.60%.  $\beta$ -ionone is one of the commercially important aroma compound which is observed in plant tissues at a very low concentration. Tedious and laborious extraction processes make this  $\beta$ -ionone production costly.<sup>[18]</sup> The production of this compound is expensive and less economical due to low concentration which leads to a tedious and laborious extraction and isolation process of  $\beta$ -ionone. Some other useful aroma compounds formed from this thermal degradation are 3-oxo- $\beta$ -ionone and  $\beta$ -cyclocitral. A high yield of dhA was observed which could be further purified and solve racemic and enantioselective issues in synthetic approaches. Due to its economic significance, the present method is convenient

**Table 1.** Thermal degradation products of commercial  $\beta$ -carotene.

Compound <sup>a</sup>	Kovats Index	Composition <sup>b</sup> (% peak area)
		OT
Monolauryl maleate	2143	5.4 $\pm$ 0.6
$\beta$ -cyclocitral	1218	4.2 $\pm$ 0.1
$\beta$ -ionone	1485	9.6 $\pm$ 0.0
Dihydroactinidiolide (dhA)	1424	61.2 $\pm$ 0.3
3-oxo- $\beta$ -ionone	1665	4.6 $\pm$ 0.1

<sup>a</sup>Components <1% are not included.

<sup>b</sup>Mean value ( $\pm$ SD) of the compounds constituents of triplicate samples determine by using GC-MS.

<sup>c</sup>Thermal degradation was conducted at 110–120°C for 4 h under stirring.

**Table 2.** Thermal degradation products of recovery  $\beta$ -carotene from CPO.

Compound <sup>a</sup>	Composition <sup>b</sup> (% peak area)
$\beta$ -ionone	1.5 $\pm$ 0.5
Dihydroactinidiolide (dhA)	29.2 $\pm$ 9.5
3-oxo- $\beta$ -ionone	3.9 $\pm$ 0.9
4-hydroxy- $\beta$ -ionone	1.7 $\pm$ 0.8
Methyl palmitate	9.5 $\pm$ 7.9
Palmitic acid	22.0 $\pm$ 13.7
Elaidic acid methyl ester	5.6 $\pm$ 3.7
Oleic acid	20.8 $\pm$ 0.3

<sup>a</sup>Components <1% are not included.

<sup>b</sup>Mean value ( $\pm$ SD) of the compounds constituents of triplicate samples determine by using GC-MS.

<sup>c</sup>Thermal degradation was conducted at 110–120°C for 4 h under stirring.

for the production of dhA and degradation in a bigger scale, and enables the isolation of single aroma compounds that can be useful in the industry.

### GC analysis of thermal degradation of recovery $\beta$ -carotene from CPO

Recovered  $\beta$ -carotene from CPO was studied by conducting thermal degradation under optimum reaction conditions. Results obtained proves that  $\beta$ -carotene extracted from CPO was successfully converted to aroma compounds mainly dhA, along with 3-oxo- $\beta$ -ionone,  $\beta$ -ionone, and few other compounds; the results are shown in Table 2. The breakdown products of carotenoids are carbonyl compounds with C13 that are the most abundant norisoprenoids in nature with the megastigmane structure including the family of ionones, and C11 such as  $\beta$ -cyclocitral and dhA.<sup>[19]</sup> The thermal degradation of commercial  $\beta$ -carotene produced higher yield of dhA compared to recovered palm carotene. This is because although soxhlet adsorption was employed for recovery of palm carotene, this method only helped to concentrate the  $\beta$ -carotene recovered, but did not completely remove the fatty acids and other compounds. There are difficulties to purify the isomers of carotenes as they are not much different in polarity and structure, the only difference between  $\alpha$ - and  $\beta$ -carotene is the position of the double bond in one of the cyclohexane rings. Hence, the recovered  $\beta$ -carotene of CPO is not pure and is a mixture of other compounds. These leads to lower production of dhA in thermal degradation of recovered palm carotene compared to commercial  $\beta$ -carotene.

### Conclusions

In this study, the degradation of  $\beta$ -carotene recovered from CPO was investigated as a model reaction. The formation of dhA was investigated in detail through thermal degradation of two different sources of  $\beta$ -carotene, which are commercial (synthetic) and natural sources, respectively. dhA is a major product for both reaction system of commercial and recovery  $\beta$ -carotene

from CPO. The purity of  $\beta$ -carotene plays an important role in thermal degradation that leads to the formation of different yields of dhA. The formation of dhA from thermal degradation of pure  $\beta$ -carotene is 61.21%, which is higher than the synthetic yield of 45.00%. The recovered  $\beta$ -carotene by Soxhlet adsorption method from CPO was successfully degraded under optimized thermal degradation to produce 29.23% of dhA. The results demonstrated that dhA is a major product which can be increased if the  $\beta$ -carotene recovered from CPO is being purified. The method is simple compared to the synthesis approach and can be applied in bulk without any tedious process. The thermal degradation of  $\beta$ -carotene leads to a formation of some notable aroma compounds especially dhA that can be very useful in the flavor and fragrance industry. However, the extraction of this aroma compound directly from plant sources is expensive and not economical. This has created opportunities in utilizing CPO as natural source of  $\beta$ -carotene to produce aroma compounds.

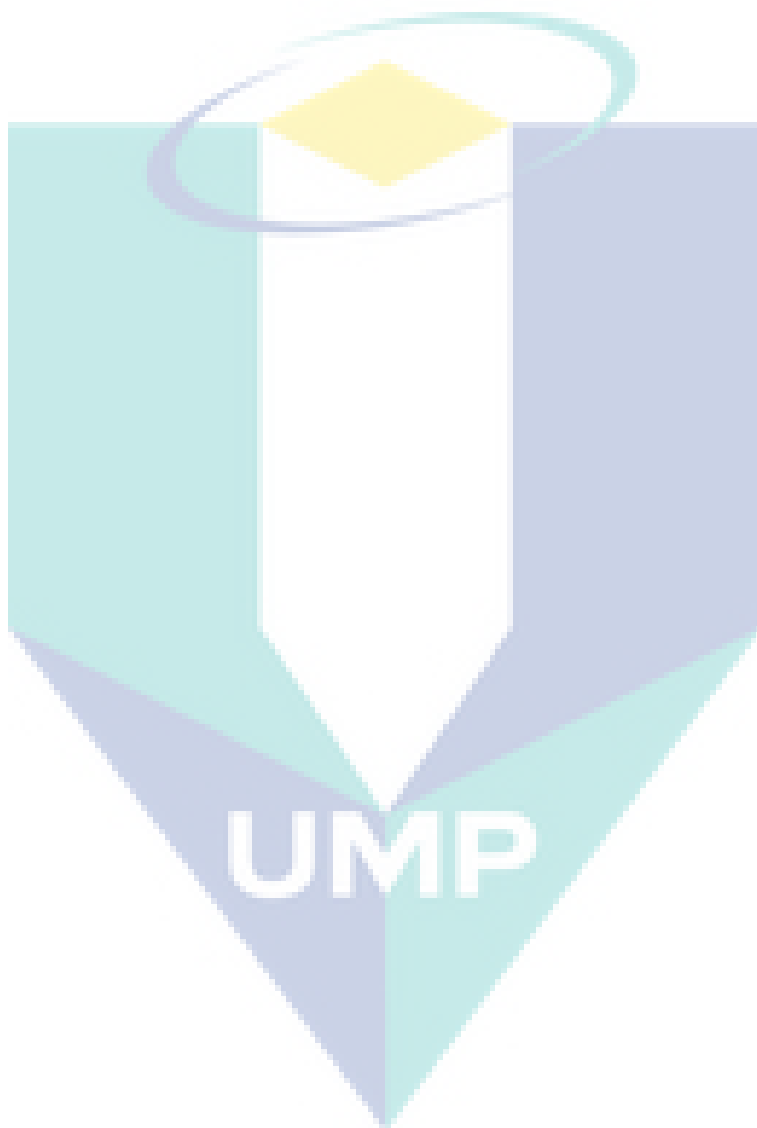
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# Extraction of Palm Carotenes and Effect of Oxidative Degradation on $\beta$ -carotene

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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  $\beta$ -ionone, DHA and ionone epoxide.

**Keywords**-Carotenes;  $\beta$ -ionone;  $\beta$ -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  $\beta$ -ionone,  $\beta$ -damascone,  $\beta$ -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  $\beta$ -carotene from CPO by saponification and column chromatography method followed by oxidative degradation of  $\beta$ -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 $\beta$ -carotene (HPLC)*

The  $\beta$ -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  $\beta$ -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  $\beta$ -carotene and covered/sealed completely without any exposure to light while sample B was prepared using the 20 mg of  $\beta$ -carotene and kept without any cover from light. As for sample C, the solution was prepared using 20 mg of  $\beta$ -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<sup>-1</sup> (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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\alpha$ - and  $\beta$ - 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

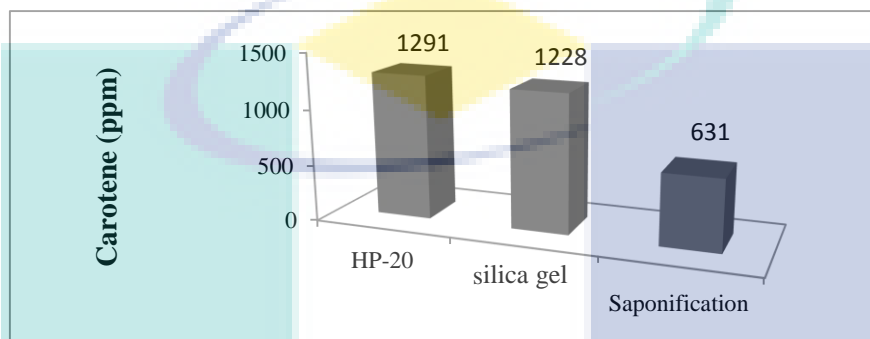


Figure 1. Comparison of conc. of beta-carotene in extracted sample by different methods

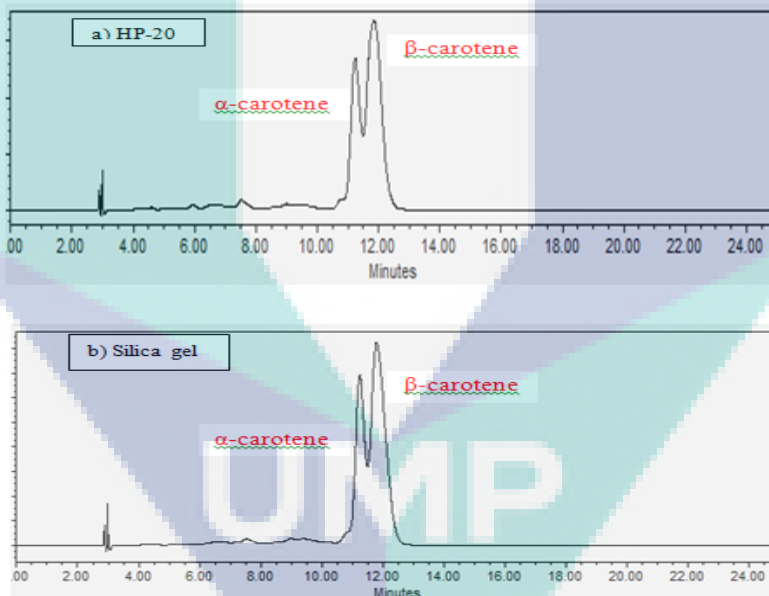


Figure 2. HPLC Chromatograms showing carotenoids of extracted samples

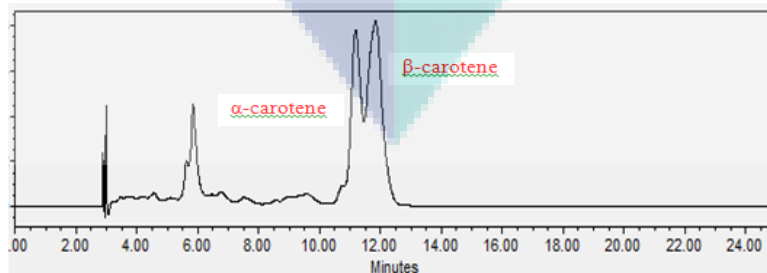


Figure 3. HPLC chromatogram of sample extracted by saponification method

### 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  $\beta$ -carotene. In the present study  $\beta$ -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  $\beta$ -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- $\beta$ -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- $\beta$ -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- $\beta$ -ionone	-	+	-

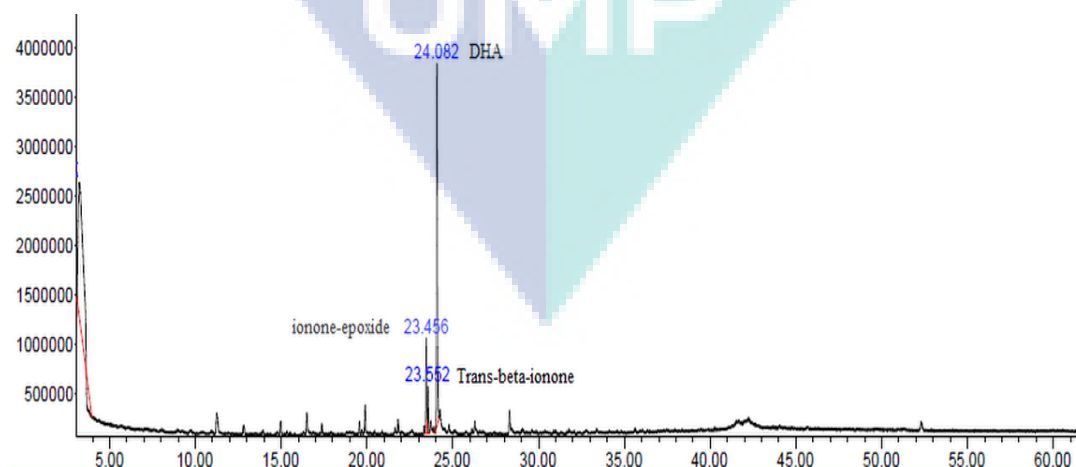


Figure 4. GC/MS Chromatogram of oxidative degradation

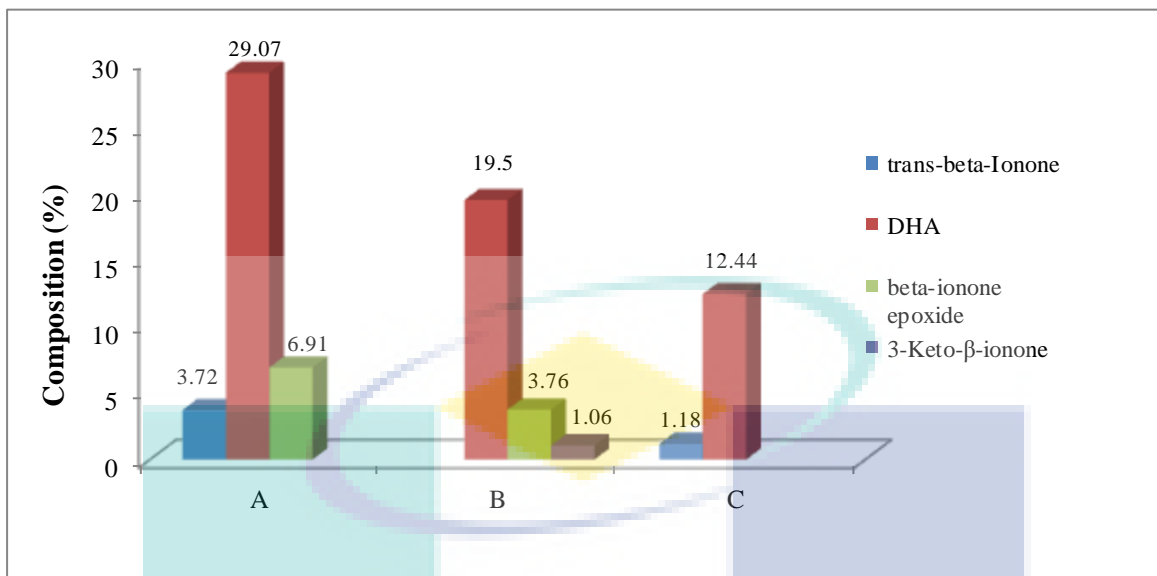
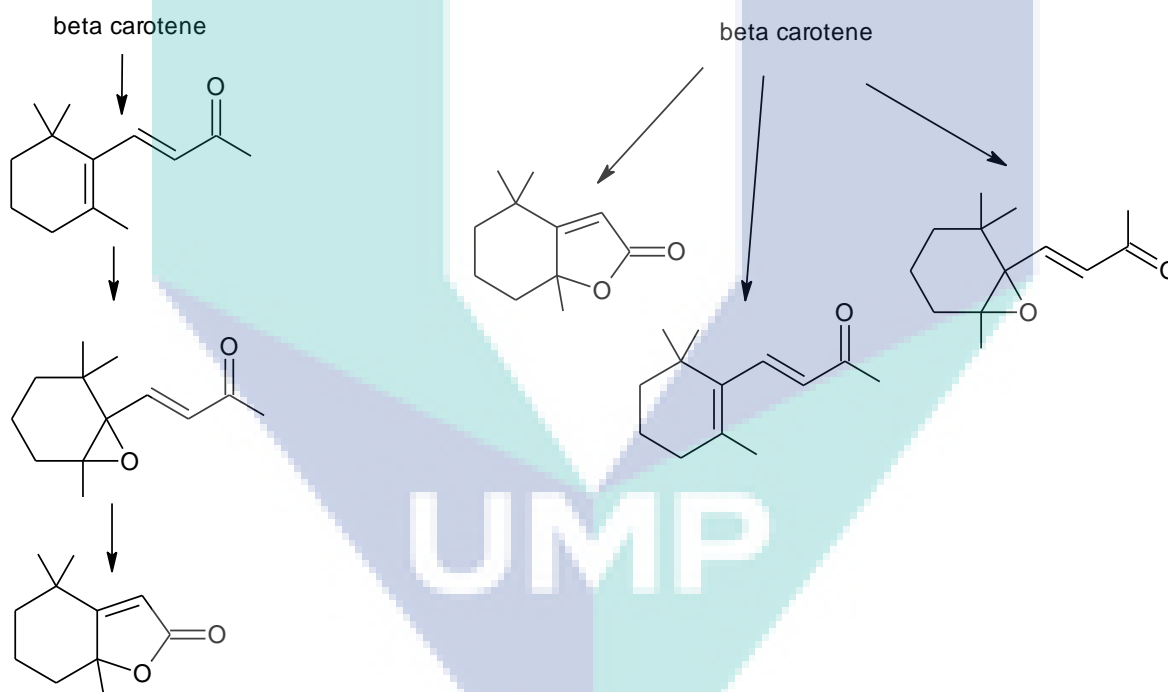


Figure 5. Important aroma compounds generated during oxidative degradation

Scheme: 1



(a) Oxidative degradation pathway proposed by [19].

(b) Important degradation products formed in present study

1:  $\beta$ -ionone, 2: 5,6-epoxy- $\beta$ -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  $\beta$ -ionone, ionone epoxide, DHA, etc were formed by oxidative degradation of  $\beta$ -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.

## ACKNOWLEDGMENT

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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 C<sub>40</sub>H<sub>56</sub> 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<sup>-1</sup> (Birtigh, Johannsen et al. 1995). Figure 1 shows the chemical structure of beta-carotene.

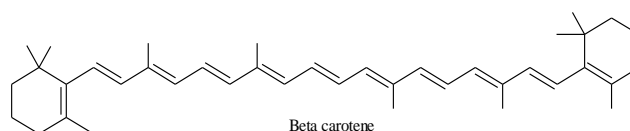


Figure 1: Chemical structure of beta-carotene



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Carotenoids world needs increase every year, especially for food, animal feed and pharmaceuticals. Yet, there is no generally accepted or standard method for 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 CO<sub>2</sub> was applied to enrich crude palm oil from carotenoids up to 200-fold (Davarnejad, Kassim et al. 2008). While (Chiu, de Moraes 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 hr (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 hr) 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 standard calibration curve which was used to determine the concentration of beta-carotene extracted from each samples.

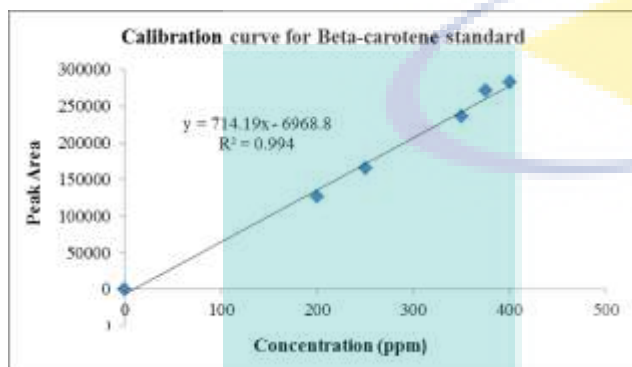


Figure 3: HPLC analyses of standard calibration curve of beta-carotene

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 hr) 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 hr then decreases at 2 hr. Based on HPLC analysis results, at 1 hr 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 hr 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 hr 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.



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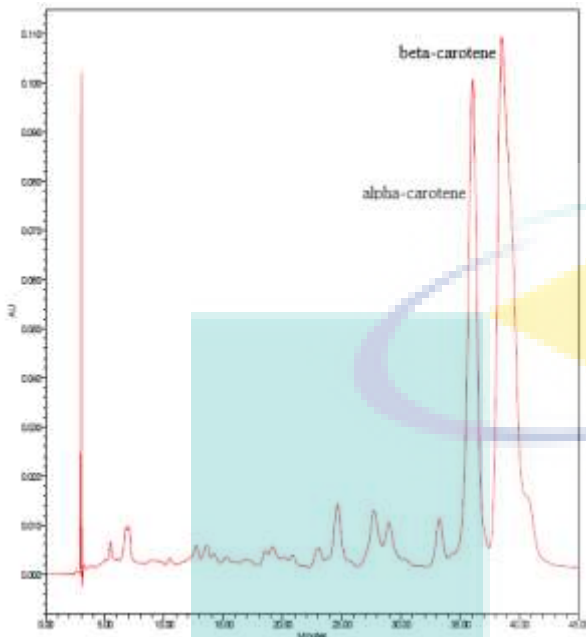


Figure 4: HPLC chromatogram of palm carotene

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 hr 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.

## ACKNOWLEDGMENT

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