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Isolation of carotenes from palm oil mill effluent and its use as a source of carotenes

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ABSTRACT

Huge amount of palm oil mill effluent (POME) is generated from palm oil industry in Malaysia. POME is highly polluting wastewater which is organic in nature and contains oil and carotenes that need to be treated before discharge. Growing awareness to prevent pollution and increasing importance of carotenes has required POME to be transformed into valuable products. In this study, oil from POME was retrieved by solvent extraction and carotenes were isolated by adsorption chromatography. Synthetic adsorbent with silica-based material was used in the column chromatography. Different types of solvents used in the adsorption chromatography were evaluated on the recovery of palm carotene. Column temperature and oil loading were also studied. Isolation of carotenes from the extracted oil by column chromatography increased the carotene concentration in the oil. Carotene recovery varied from 47 to 92% depending on the chromatographic conditions. Carotene was successfully concentrated to about 70 times of the concentration in the extracted oil by adsorption chromatography process.

Keywords: Palm oil mill effluent; Carotene; Adsorption chromatography; Recovery

1. Introduction

Malaysia is the biggest producer and exporter of palm oil which currently accounts for 51% of world palm oil production and 62% of world exports [1]. Palm oil mills with wet milling process are accounted for major production of palm oil in the country and a significantly large quantity of water is used during the extraction of crude palm oil (CPO) from the fresh fruit bunch (FFB). According to Handbook No. 3, Crude Palm Oil Industry, about half of the water used in the extraction process will result in palm oil mill effluent (POME), and the other half being lost as steam, mainly through sterilizer exhaust, piping leakages, as well as wash waters [2].

POME refers to the liquid wastes of discharge originating from the mixture of a sterilizer condensate, separator sludge and hydrocyclone wastewater. POME is non-toxic but has an unpleasant odour which is predominantly organic in nature and is highly polluting. The organic content of POME, as measured by biochemical oxygen demand (BOD, 3 days, 30°C) averages to about 25,000 mg/L with a chemical oxygen demand of 50,000 mg/L, suspended solids of about 18,000 mg/L and oil content might exceed 6000 mg/L [3].

The impact of POME discharge to a relatively small river can be devastating to its eco-system and beneficial uses. The riverine communities and users of rivers and streams are very vulnerable to the adverse pollution impact of indiscriminate discharges of POME. Therefore,

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the country's Environmental Quality (Prescribed Premises)(Crude Palm Oil) Regulations 1977 requires that all palm oil mills must treat their POME to reduce the oil and grease level to less than 50 mg/L [4]. The residue oil droplets in POME were solvent extractable. Thus, oil can be extracted from POME by solvent extraction.

According to previous work, the major components of recovered oil from POME were similar to that from crude palm oil, which also contained α- and β-carotene [5]. The orange colour of palm oil is due to the presence of these carotenes. Its concentration normally ranges between 400 ppm to 3500 ppm and it contains about 15 times more retinol equivalents (vitamin A) than carrots and 300 times more than tomatoes [6]. Carotenes possess anti-cancer properties for certain types of cancers [7] help to prevent night blindness, eye problems and skin disorders, it also could enhance immunity and protects against toxins, colds, flu and infections. Carotenes do not cause hypervitaminosis A, as conversion of carotenoids to vitamin A is regulated and thus, β-carotene has the advantage in humans that it is nontoxic [8]. On the other hand, vitamin A deficiency is considered to be a public health problem in more than half of all countries and particular β-carotene is the most important vitamin A precursor in human nutrition [9].

In a recent report, the global market value of all commercially-used carotenoids is expected to rise to over $1 billion. In supplements, the natural form can be identified by the phrases, "from an algal source", "from a palm source", or as "natural beta-carotene" on the label. The synthetic form is identified as "beta-carotene." Numerous carotenoids extraction methods have been developed over the years, including saponification, urea processing, adsorption, selective solvent extraction, molecular distillation and transesterification followed by distillation of esters but most of the methods are difficult to perform, inefficient or costly [10]. Most industries have attempted to respond to the stimulated demand by synthetic production of β-carotene or by extracting and subsequently crystallizing β-carotene from natural sources. The consumers in accordance with their present critical attitude towards synthetic products have a clear preference for natural β-carotene.

Growing awareness to prevent pollution and increasing importance of carotenes has required POME to be transformed into valuable products. The objective of this study is to extract residue oil from POME and to isolate the carotenes from the extracted oil. Single batch of solvent extraction was used to extract the oil from POME and adsorption chromatography with different settings was employed to recover the palm carotene.

2. Materials and methods

2.1. Materials

POME samples were collected from M.P. Mathew Palm Oil Mill, Penang at a temperature of 80°C. Silica gel was obtained from Sigma Aldrich (M) Sdn Bhd, Malaysia. This silica gel had a particle size of 63–200 μm and pore size of 90 Å. All solvents used were of analytical grade.

POME and *n*-hexane were mixed at a ratio of 1:0.6 (POME:*n*-hexane) in a flocculator for 10 min at 350 rpm. The contents were then transferred to the separating funnels and left to separate into two layers. The extract was filled into a conical flask and solvent was distilled off using rotary evaporator. The drying process was conducted repetitively in an oven at ~100°C and cooled in a desiccator until the weight was constant. The product was the extracted oil from POME and was used in the next experiment. Fig. 1 shows the flow chart of the process.

2.2. Adsorption column chromatography

10 g of silica gel was packed in the column with 10 mm internal diameter with an outer jacket for circulating heated water. The column was then equilibrated with *n*-hexane. About 2.5 g of the extracted oil from POME was loaded in the column chromatography to contact with adsorbent. The initial solvent was a non-polar solvent; about 100 mL of n-hexane was brought into the column. The second solvent was ethanol at about 100 mL was later added to the column chromatography. Fractions of 25 mL were collected regularly in the receiving flask and solvent was removed by using a rotary evaporator. The oil content of each fraction was then determined gravimetrically after drying process was conducted in an oven at ~100°C and cooled in a desiccator. The carotenes content was determined according to PORIM test method [11] by dissolving 0.1 g of the sample in 25 mL of *n*-hexane and measured by using spectrophotometer Genesys 20 at 445 nm. Fig. 2 displays the schematic diagram and flow chart of the adsorption column chromatography process.

The experiments were done at 30, 40 and 50°C. A column with 30 mm internal diameter was used for ex-

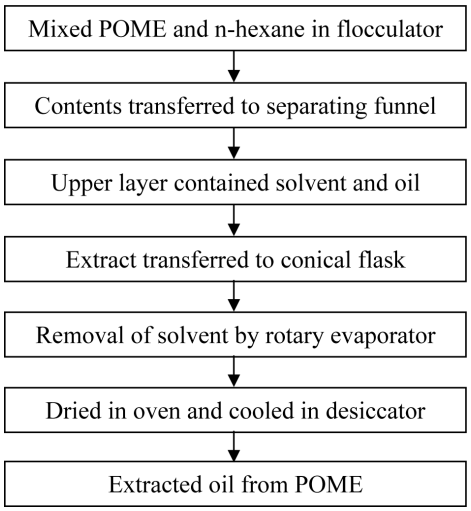

Fig. 1. Flow chart for extraction of oil from POME.

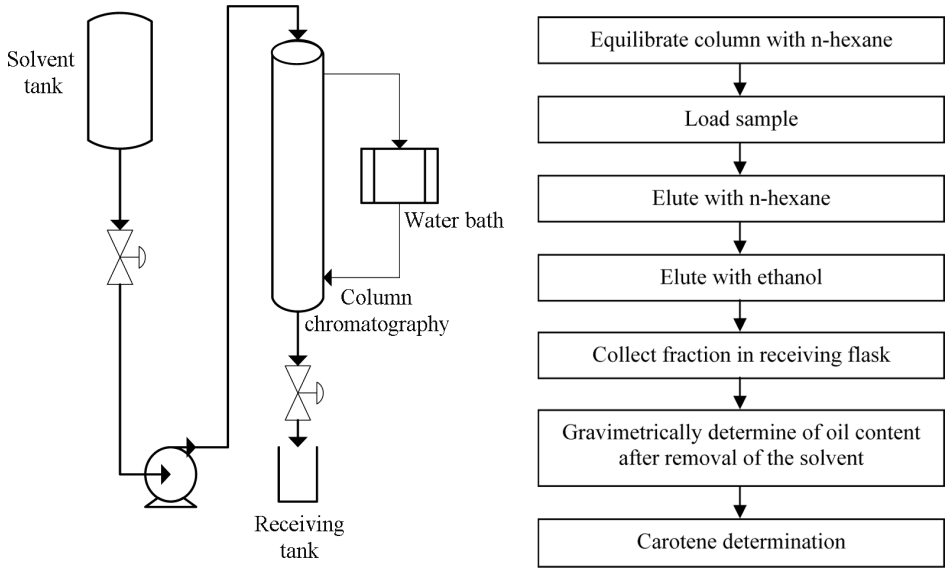

Fig. 2. Schematic diagram and flow chart of the adsorption column chromatography.

periments with different initial loading. The weights of extracted oil:silica gel (w/w) used for ratios of 1:4, 1:5 and 1:6 were 10 g:40 g, 8 g:40 g and 6.67 g:40 g. Beside that, petroleum ether which is another type of initial solvent was used to replace *n*-hexane in the above experiments.

3. Results and discussion

3.1. Solvent extraction

By using single batch solvent extraction, the oil and grease content that can be recovered from POME in this study was about 5000 mg/L. The carotene concentration in the extracted oil from POME was about 453 ppm. High performance liquid chromatography (HPLC) analyses showed that the carotene in the extracted oil from POME was almost similar trend as in CPO. The major components in the extracted oil from POME are α-carotene and β-carotene. Although the carotene concentration in POME is low, extraction of oil and carotenes from POME is worth as the whole study serves a double purpose. First, the wastewater is converted to value added products (carotenes) and second, recovering of organic matter (oil and carotenes) can reduce the BOD load that faces by the palm oil mill in the wastewater treatment system.

3.2. Effect of extracted oil loading on column chromatography

The effect of extracted oil:silica gel ratio on the carotene recovery is displayed in Table 1. Higher ratio of the

Table 1
Effect of extracted oil:silica gel ratio on the recovery of palm carotene

Extracted oil:silica gel ratio	Fraction	Oil recovery (%)	Carotene	
			Recovery (%)	Average concentration (ppm)
1:4	Hexane	42.52	76.45	816
	Ethanol	47.15	18.96	182
	Petroleum ether	82.59	52.33	287
	Ethanol	14.95	4.51	137
1:5	Hexane	18.30	57.93	1436
	Ethanol	74.16	37.18	227
	Petroleum ether	82.27	60.06	331
	Ethanol	17.02	4.85	129
1:6	Hexane	12.84	47.15	1665
	Ethanol	85.65	52.44	278
	Petroleum ether	81.59	92.75	516
	Ethanol	17.81	3.43	87

extracted oil:silica gel represented lower initial loading of the extracted oil into the column. The recovery of palm carotene in hexane fractions decreased when the extracted oil:silica gel ratio increased from 1:4 to 1:6 whereas the palm carotene in ethanol fractions in hexane–ethanol (H–E) system increased when the ratio increased. This happened because the remaining carotene in the column which did not elute by the initial solvent, hexane was eluted by the second solvent, ethanol. On the other hand, the recovery of palm carotene in petroleum ether fractions increased as the extracted oil:silica gel ratio increased and the palm carotene in ethanol fractions in petroleum ether–ethanol (P–E) system remain almost the same.

The oil recovery in hexane fractions also showed a similar trend with the carotene recovery where smaller amount of oil was recovered at higher extracted oil:silica gel ratio. However, the values for oil recovery in petroleum ether just about unchanged. This means that the extracted oil in the column chromatography was more easily eluted by petroleum ether compared to elution by hexane. The oil attached stronger to petroleum ether than silica gel in the P–E system.

The average carotene concentrations in hexane fractions were higher than carotene concentrations in petroleum ether and ethanol fractions. This indicates that palm carotene is more soluble in hexane compared to petroleum ether and least soluble in ethanol in this study. Hexane has a good solvency for oil whereas ethanol has poor solvency for oil [12]. The average carotene concentrations were higher at lower initial loading of extracted oil for both hexane and petroleum ether fractions. In this study, the carotene was concentrated to 26 times (11,933 ppm) of the concentration in the extracted oil by adsorption chromatography in one of the hexane fraction and 70 times (32,052 ppm) of the concentration in extracted oil in one of the petroleum ether fraction.

Fig. 3 demonstrates the influence of the ratio of extracted oil:silica gel on the total recovery of palm carotene and oil. The total oil recovery in both H–E and P–E systems increased as a higher ratio of extracted oil:silica gel was used. This indicates that when more extracted oil was loaded onto the column, there was more oil trapped in the column. Therefore, the total oil recovery was the highest when the extracted oil loading was the lowest.

The total carotene recovery in H–E system was almost the same in all ratios studied whereas the total carotene recovery in P–E system increased as the extracted oil:silica gel ratio increased. This may be due to the higher amount of oil recovered at the lower extracted oil:silica gel ratio for P–E system which needed more time for solvent evaporation and caused the deterioration of the palm carotene content. Based on the experiments in this study, the most appropriate ratio of extracted oil:silica gel for the system is 1:6.

3.3. *Effect of column temperature on column chromatography*

Table 2 shows the effect of column temperature on the recovery of palm carotene and oil recovery in H–E system and P–E system. The carotene recovery in hexane fractions by using H–E system did not vary much depending on the column temperature. On the other hand, the carotene recovery in petroleum ether fractions was more than 91% at 30 and 40°C but decreased to 60% at 50°C. This suggests that 50°C is not a suitable temperature to recover palm carotene from the extracted oil by using P–E system.

According to Table 2, the total oil recovery in P–E system increased as the column temperature increased. This may due to the fact that the oil viscosity decreases as the temperature increases and the oil was easier eluted from the column by the solvent system. The oil recovery in hexane fraction slightly fluctuated at different temperatures but the total oil recovery in H–E system was more than 90% with the highest recovery at 40°C.

The influence of temperature on carotene recovery and carotene concentration with H–E and P–E systems is demonstrated in Fig. 4. The total carotene recovery for both H–E and P–E systems achieved more than 90% except for P–E system at a temperature of 50°C which only recovered about 63% carotene. The probable explanation for this is that exposing carotene at a higher temperature will degrade the carotene and thus lower the recovery percentage.

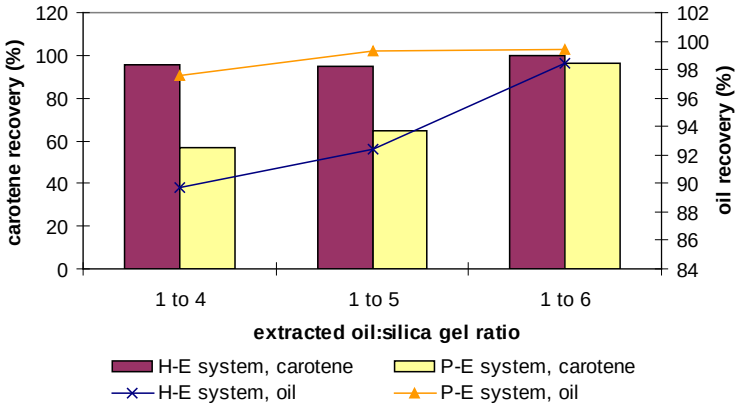

Fig. 3. Effect of extracted oil:silica gel on total recovery of palm carotene and oil.

Table 2
Effect of column temperature on the recovery of palm carotene

Column temperature (°C)	Fraction	Oil recovery	Carotene	
			Recovery (%)	Average concentration (ppm)
30	Hexane	53.64	77.33	652.79
	Ethanol	36.43	7.37	91.67
	Petroleum ether	81.41	91.39	519.22
	Ethanol	15.72	2.34	67.63
40	Hexane	29.27	74.63	1154.55
	Ethanol	67.15	10.83	73.00
	Petroleum ether	90.09	94.03	520.23
	Ethanol	8.41	3.72	220.36
50	Hexane	42.27	74.65	799.59
	Ethanol	50.97	8.75	77.73
	Petroleum ether	93.40	60.13	291.94
	Ethanol	5.84	3.02	234.68

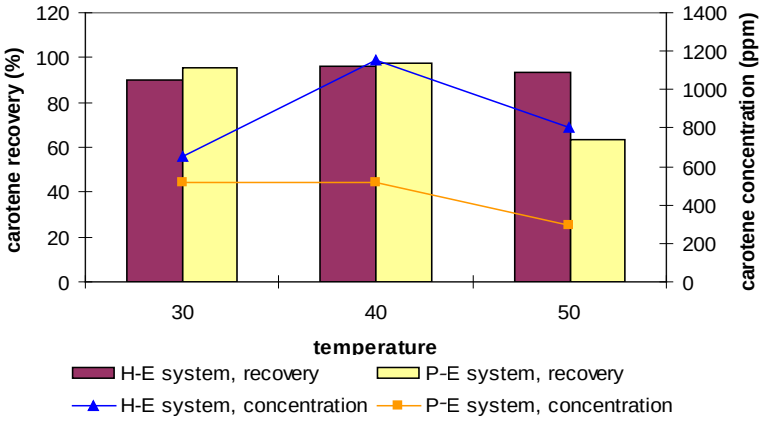

Fig. 4 Effect of temperature on palm carotene recovery and concentration with the hexane:ethanol system and petroleum ether:ethanol system.

The highest average carotene concentration for both H–E and P–E systems fell on 40°C and the lowest average carotene concentrations were at 30 and 50°C for H–E and P–E systems, respectively. From these results, the suggested temperature for adsorption chromatography by using either H–E or P–E system is 40°C.

4. Conclusions

The oil and grease content in POME by solvent extraction was about 5000 mg/L with the carotene concentration of 453 ppm. The carotene was concentrated to 26 times and 70 times of the concentration in the extracted oil by adsorption chromatography in one of the hexane fraction and petroleum ether fraction, respectively. According to the experiment results, the most appropriate extracted oil:silica gel ratio for the system is 1:6 and the recommended temperature for adsorption chromatography by using either H–E or P–E system is 40°C. Overall, H–E system at 40°C and extracted oil:silica gel ratio of 1:6 are the most suitable conditions to isolate the palm carotene from the extracted oil by using adsorption chromatography.

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References

[1] Malaysian Palm Oil Council, http://www.mpoc.org.my/main_ind_01.asp, cited February 2008.
[2] M.I.R.H. Thani, W.W.R. Ibrahim and M.S. Sulaiman, Industrial Processes and the Environment (Handbook No. 3), Crude palm oil industry. Department of Environment, Malaysia, 1999, p. 92.
[3] A.-N. Ma, Environmental management for the palm oil industry. Palm Oil Develop., 30 (2000) 1–10.
[4] Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations 1977 In Vol. Environmental Quality Act, 1974.

[5] A.L. Ahmad, C.Y. Chan, S.R. Abd Shukor and M.D. Mashitah, Recovery of oil and carotenes from palm oil mill effluent (POME). Chem. Eng. J., 141(1–3) (2008) 383–386.
[6] K. Sundram, R. Sambanthamurthi and Y.A. Tan, Palm fruit chemistry and nutrition. Asia Pacific J. Clinical Nutrition, 12(3) (2003) 355–362.
[7] Y.M. Choo, A.-N. Ma and S.C. Yap, Carotenes, vitamin E and sterols in oils from *Elaeis guineensis*, *Elaeis oleifera* and their hybrids. Palm Oil Develop., 27 (1997) 1–8.
[8] N. Chandrasekharan, Red palm oil for the prevention of vitamin A deficiency. Palm Oil Develop., 27 (1997) 20–24.
[9] R. Sambanthamurthi, K. Sundram and Y.-A. Tan, Chemistry and biochemistry of palm oil. Progress Lipid Res., 39(6) (2000) 507–558.
[10] C.K. Ooi, Y.M. Choo, S.C. Yap, Y. Basiron and A.S.H. Ong, Recovery of carotenoids from palm oil. J. AOCS, 71(4) (1994) 423–426.
[11] PORIM Test Methods. Palm Oil Research Institute of Malaysia, Kuala Lumpur, Vol. p2.6, 1995.
[12] B.S. Baharin, K. Abdul Rahman, M.I. Abdul Karim, T. Oyaizu, K. Tanaka, Y. Tanaka and S. Takagi, Separation of palm carotene from crude palm oil by adsorption chromatography with a synthetic polymer adsorbent. J. AOCS, 75(3) (1998) 399–404.