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Adsorption Chromatography of Carotenes from Extracted Oil of Palm Oil Mill Effluent

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Abstract: Carotenes is one of the most important vitamin A precursor in human nutrition which has numerous advantages. Palm Oil Mill Effluent (POME) is wastewater which consists of carotenes in the oil and grease content. Therefore, adsorption chromatography is used to separate the carotenes from the oil and grease in POME. Several types of adsorbents, temperatures and mass loading were studied in the experiments. The 40°C and oil:adsorbent ratio of 1:5 was recommended to be the most suitable temperature and mass loading for separation of carotenes by adsorption chromatography. Silica gel also shows better quality of adsorbent in separation of carotenes in hexane fractions.

Key words: Adsorption chromatography, oil, carotenes

INTRODUCTION

Crude Palm Oil (CPO) is the world's richest natural plant source of carotenes in terms of retinol. Palm oil contains the highest known concentration of agriculturally derived carotenoids of the vegetable oils that are widely consumed. CPO possesses 1% minor components which amongst them are the carotenoids, tocopherols and tocotrienols and sterols (Choo *et al*., 1997). The orange colour of palm oil is due to the presence of these carotenes. Its concentration normally ranges between 400 to 3500 ppm and it contains about 15 times more retinol equivalents (vitamin A) than carrots and 300 times more than tomatoes (Sundram *et al*., 2003). The major carotenes in palm oil are α- and β-carotene, which account for 90% of the total carotenes (Ooi, 1999; Sundram *et al*., 2003; Ng and Tan, 1988). Carotenes possess anti-cancer properties for certain types of cancers (Choo *et al*., 1997), helps to prevent night blindness, eye problems and skin disorders, it also could enhance immunity and protects against toxins, colds, flu and infections (Ravanello *et al*., 2003). Beside from medicinal uses, the importance of carotenoids has also increased due to the more extensive use of natural compounds in the food, cosmetic, pharmaceutical and feed industries for its colorant and antioxidant properties (Bhosale and Bernstein, 2004; Sabio *et al*., 2003).

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 CPO from the Fresh Fruit Bunch (FFB). About half of the water used in extraction process will result in Palm Oil Mill Effluent (POME). About 2.5 tonnes of POME are produced for every tonne of oil extracted in an oil mill (Ho *et al*., 1984; Songip *et al*., 1996). Thus, 17.73 million tonnes of palm oil production in year 2008 resulted in about 44.33 million tonnes of POME. If the POME is discharged untreated, the amount of BOD produced in year 2008 was 1.108 million tonnes. 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 (Ahmad *et al*., 2008).

Two different procedures were used to separate carotenes by adsorption. One method comprised addition of adsorbent to a solution of the carotene mixture until an amount of carotene equal to the most strongly adsorbed carotene had been adsorbed. Another method, the solutions of the carotene mixture were passed over columns composed of the finely divided adsorbent. Normal phase chromatography also known as adsorption chromatography separates molecules based on their polar properties. In the adsorption process, solute and solvent molecules compete for sites on the adsorbent; to be adsorbed, the solute molecule must first displace a solvent molecule (Sewell and Clarke, 1987).

Due to the readily available source of POME and functions of carotenes, the objective of this study is to separate the carotenes from the extracted oil through adsorption chromatographic process.

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MATERIALS AND METHODS

Materials: POME samples were collected from M.P. Mathew Palm Oil Mill Sdn. Bhd., Penang at temperature of 80°C. The oil and grease from POME was extracted by using solvent extraction method as described in previous work (Ahmad *et al*., 2008). Three type of adsorbents were used in the studies which are silica gel, florisil and alumina. Silica gel was bought from Sigma Aldrich Sdn Bhd whereas florisil and alumina were bought from Merck. The silica gel and aluminium oxide had a particle size of 63-200 μm. Florisil had a particle size of 150-250 μm. All solvents used were of analytical grade.

Methods: Ten grams of adsorbents was packed in the column with 10 mm internal diameter and 13.5 mm length. 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 extracted oil was heated at about 40°C to melt it before loaded in the column. A non-polar solvent, 100 mL of n-hexane was then brought into the column. One hundred milliliter of ethanol, a polar solvent was later added to the column chromatography. Fractions of 25 mL were collected regularly in the receiving flask. The oil content of each fraction was then determined gravimetrically after removal of the solvent by using a rotary evaporator. The carotenes content was determined according to PORIM test method (1995) by dissolving 0.1 g of the sample in 25 mL of n-hexane and measured by using spectrophotometer Spectroquant Pharo 300 at 446 nm.

The experiments were done at 30, 40 and 50°C. Different initial mass loading of extracted oil to the column adsorbent were also being studied. Column with 30 mm internal diameter was used for experiments with different initial loading and fractions of 50 mL were collected. The ratio of extracted oil:adsorbent (w/w) used in the experiments were 1:4, 1:5 and 1:6. The experiments using 30 mm internal diameter column was done at room temperature. The non-polar and polar solvents used were 300 mL, respectively.

RESULTS AND DISCUSSION

Effect of temperature: Experiments were conducted with the 1 cm diameter column at 10°C intervals between 30 and 50°C. The adsorption chromatography done in this study is a normal phase chromatography where non polar solvent, hexane was eluted first and followed by polar solvent, ethanol. Therefore, the non polar compounds such as carotenes will be eluted first in hexane fractions before the polar compounds.

Table 1: Effect of temperature on adsorption chromatography

		Carotenes	
Fraction	Oil recovery (%)	Recovery (%)	Average concentration (ppm)
30°C			
Silica gel			
Hexane	53.64	77.33	652.79
Ethanol	36.43	7.37	91.67
Florisil			
Hexane	81.69	74.39	596.59
Ethanol	18.23	24.69	887.28
Aluminium oxide			
Hexane	46.16	73.25	805.27
Ethanol	40.92	19.71	244.40
40°C			
Silica gel			
Hexane	29.27	74.63	1154.55
Ethanol	67.15	10.83	73.00
Florisil			
Hexane	62.41	54.68	570.81
Ethanol	36.89	19.48	344.04
Aluminium oxide			
Hexane	42.96	85.87	1045.39
Ethanol	41.01	13.83	176.34
50°C			
Silica gel			
Hexane	42.27	74.65	799.59
Ethanol	50.97	8.75	77.73
Florisil			
Hexane	93.52	77.11	536.28
Ethanol	6.41	4.62	468.76
Aluminium oxide			
Hexane	43.29	86.10	681.83
Ethanol	37.89	6.71	60.69

Table 1 shows the effect of temperature on the adsorption chromatography on oil and carotenes recovery using different type of adsorbents. Oil recovery for hexane fractions varied from 29.27 to 93.52% with mean value of 55.02% whereas oil recovery for ethanol fractions varied from 6.41 to 67.15% with mean value of 37.32%. In most of the situations, oil recovery in hexane fractions are higher that in ethanol fractions. This implies that the oil is more soluble in hexane rather than ethanol.

The mean value carotenes recoveries are 75.33 and 12.89% in hexane and ethanol fractions, respectively. This also indicates that carotenes compound is easily soluble in hexane and less soluble in ethanol. The carotene recovery in hexane fractions for florisil at 40°C was much more lower than the mean value which maybe due to uneven loading of the sample that cause the elution of carotene being affected.

The highest average carotene concentration, 1154.55 ppm was achieved by using 40°C in hexane fractions with silica gel. Table 1 shows that the average carotenes concentration at 40°C were higher than that the average carotenes concentration at 30 and 50°C. The average carotenes concentrations were mainly higher in hexane fractions compare to ethanol fractions despite of different temperatures and adsorbents.

Table 2: Highest carotene concentration by using various temperatures in adsorption chromatography

Temperature (°C)	Silica gel	Florisil	Aluminium oxide
30	3959.08	977.29	3752.12
40	4951.79	731.92	971.80
50	1567.06	655.82	1361.40

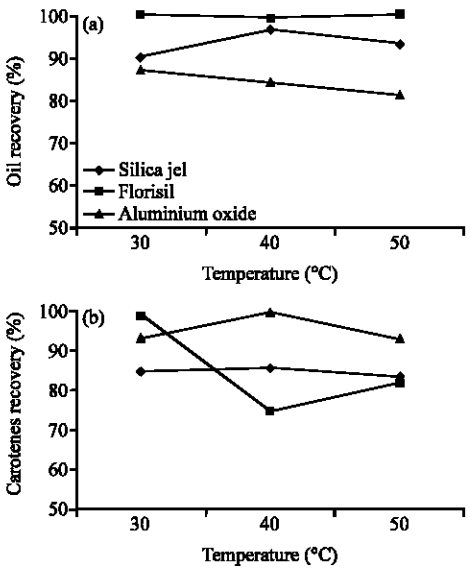

Fig. 1: Effect of temperature on total oil recovery (a) and total carotenes recovery and (b) on adsorption chromatography

Table 2 presents the highest carotene concentration achieved in hexane fraction by using different type of temperatures in adsorption chromatography. By using silica gel as adsorbent, the highest carotene concentration was attained at 40°C whereas by using florisil and aluminium oxide, the highest carotene concentration was attained at 30°C. This indicates that at higher temperature (50°C) the oil viscosity is lower and easier to elute in the column and separation of carotenes into high concentration was ineffective. By using silica gel, the highest carotene concentrations were attained among different type of adsorbents at various temperatures.

Figure 1 displays the effect of temperature on total oil and total carotenes recoveries on adsorption chromatography. Figure 1a demonstrates that florisil had the highest oil recovery followed by silica gel and aluminium oxide. This denotes that most of the oil eluted out from the column when using florisil as adsorbent. Florisil had bigger particle size compare to silica gel and aluminium oxide which enable the oil to pass through the column without obstruction.

Figure 1 b displays that silica gel and aluminium oxide had the highest carotenes recovery at 40°C while florisil had the highest carotenes recovery at 30°C. However, florisil exhibits the lowest carotenes recovery at 40°C which maybe explain by longer time used during evaporation and drying which cause the degradation of carotenes.

Table 3: Effect of mass loading on adsorption chromatography

		Carotenes	
Fraction	Oil recovery (%)	Recovery	Average concentration (ppm)
1 to 4			
Silica gel			
Hexane	42.52	76.45	815.56
Ethanol	47.15	18.96	182.44
Florisil			
Hexane	56.07	59.19	535.67
Ethanol	40.31	21.65	272.56
Aluminium oxide			
Hexane	78.18	90.27	585.92
Ethanol	21.07	8.32	200.39
1 to 5			
Silica gel			
Hexane	18.30	57.93	1435.56
Ethanol	74.16	37.18	227.40
Florisil			
Hexane	46.39	50.73	554.94
Ethanol	50.62	31.93	320.05
Aluminium oxide			
Hexane	64.31	78.45	618.98
Ethanol	27.27	12.09	225.03
1 to 6			
Silica gel			
Hexane	12.84	47.15	1665.46
Ethanol	85.65	52.44	277.70
Florisil			
Hexane	49.19	57.03	570.76
Ethanol	49.21	24.54	253.03
Aluminium oxide			
Hexane	75.17	97.10	655.46
Ethanol	24.72	2.51	51.51

On the whole, the experiments were done to achieve separation of carotenes from extracted oil. Therefore, carotenes recoveries in hexane fractions and carotenes concentration are more important than other responses. Temperature at 40°C is recommended to be most suitable to separate the carotenes compound.

Effect of mass loading: Experiments of different mass loading were conducted with a 30 mm internal diameter column. The weight ratio of oil: adsorbent studied in this research were 1:4, 1:5 and 1:6 as shown in Table 3. The adsorbent weights were kept constant while the extracted oil weights were varied. At ratio 1:4, the oil mass loading are greater than ratio 1:5 followed by ratio 1:6.

The mean value of oil recovery in hexane fractions was 49.22% while the mean value of oil recovery in ethanol fractions was 46.68%. This elucidate that oil is more soluble in hexane rather than ethanol as mentioned in the section above. The oil recovery in hexane fractions increased from using silica gel as adsorbent to florisil and the highest oil recovery by aluminium oxide.

Table 4: Highest carotenes concentration in hexane fraction by using various mass loading in adsorption chromatography

Oil: adsorbent ratio	Silica gel	Florisil	Aluminium oxide
1 to 4	3424.74	899.47	1877.66
1 to 5	11932.64	957.08	1210.15
1 to 6	10982.53	795.54	1350.89

The carotenes recoveries in hexane fraction varied from 47.15 to 97.10% while the carotenes recoveries in ethanol fraction varied from 2.51 to 52.44%. The mean value of carotenes recovery in hexane fraction was 68.26% while the mean value of carotenes recovery in ethanol fraction was 23.29%. The result for effect of mass loading on carotene recoveries is similar to the effect of temperature on carotene recoveries where the carotenes recoveries are higher in hexane fraction than in ethanol fraction.

The average carotene concentration increased when the ratio of oil:adsorbent increased from 1:4 to 1:6. The best average carotene concentration was achieved by using silica gel where the concentration increased extensively from 815 to 1665 ppm. Whereas the average carotene concentration for florisil and aluminium oxide did not change significantly with the mean of 554 and 620 ppm for florisil and aluminium oxide, respectively.

Table 4 shows the highest carotenes concentration in different types of adsorbent using various oil:adsorbent ratio. The carotenes concentrations were highest by using silica gel in the column chromatography even with a variety of mass loading. The highest carotenes concentration were achieved at ratio 1:5 by using silica gel and florisil as adsorbent whereas ratio 1:4 for aluminium oxide. Among different types of adsorbent, silica gel had the greatest carotenes concentration while florisil had the least carotenes concentration. Florisil which had a larger particle size did not manage to separate and concentrate the carotenes efficiently.

Figure 2 shows the effect of oil:adsorbent ratio on total oil and total carotenes recovery using different types of adsorbent. The total oil recovery increased from ratio 1:4 to 1:6 for three types of adsorbent except for the total oil recovery at ratio 1:5 using aluminium oxide. This indicates that when the mass loading is small, the percentage of oil trapped in the column also little. When initial oil loading is more, the percentage of oil trapped in the column also increased resulting in lesser oil eluted out of the column and oil recovery percentage drop.

According to Fig. 2b, the total carotene recoveries by using silica gel and florisil alter slightly at about 96.7 and 81.7%. However, the total carotene recoveries for aluminium oxide drop at ratio 1:5 which maybe due to the oil recovery for that condition was low and subsequently the carotene content also decreased. The most suitable

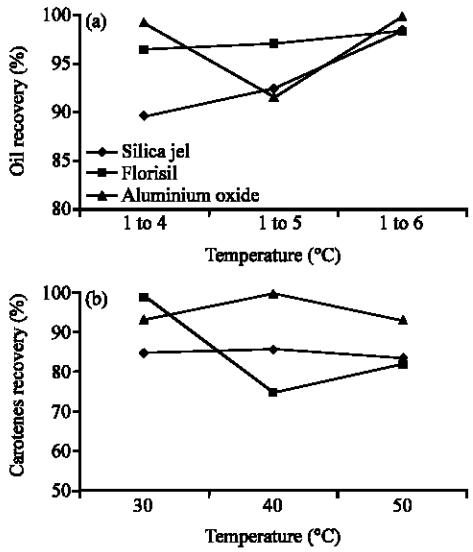

Fig. 2: Effect of mass loading on total oil recovery (a) and total carotene recovery and (b) on adsorption chromatography

oil:adsorbent ratio for separation of carotenes from extracted oil was chosen based on highest carotenes recovery in hexane fraction and high carotene concentration. Therefore, in order to balance these two variables, oil:adsorbent ratio of 1:5 was suggested to be the most effective for separation of carotenes from extracted oil.

CONCLUSION

In conclusion, adsorption chromatography was able to separate carotenes from extracted oil of POME. Most of the carotenes were soluble and eluted by non polar solvent, hexane. Silica gel showed superior quality in separation of carotenes where it highest carotene concentration and high carotenes recovery percentage. From the results, 40°C was the suitable temperature for adsorption chromatography of carotenes from extracted oil of POME. Besides, oil:adsorbent ratio of 1:5 was the appropriate mass loading for adsorption chromatography due to high oil and carotenes recoveries percentage and high average carotenes concentration.

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