THE EXTRACTION AND ITS SEPARATION TECHNOLOGY FOR CAROTENOIDS RECOVERY FROM PALM OIL : A REVIEW

TEKNOLOGI ESKTRASI DAN CARA PEMISAHANNYA UNTUK MENDAPATKAN KEMBALI KAROTENOID DARI MINYAK SAWIT : SUATU TINJAUAN

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ABSTRACT

Crude palm oil (CPO)is the richest natural plant source of carotenoids in terms of retinol (pro-vitamin A) equivalent, whereas palm oil mill effluent (POME) is generated from palm oil industry that contains oil and carotenes that used to be treated before discharge. Carotenoids are importance in animals and humans for the purpose of the enhancement of immune response, conversion of vitamin A and scavenging of oxygen radicals. This component has different nutritional functions and benefits to humaan health. The growing interest in the other natural sources of beta-carotene and growing awareness to prevent pollution has stimulated the industrial use of CPO and POME as a raw material for carotenoids extraction. Various technologies of extraction and separation have been developed in order to recover of carotenoids.This article reports on various technologies that have been developed in order to recover of carotenoids from being destroyed in commercial refining of palm oil and effects of some various treatments on the extraction end separation for carotenoid from palm oil and carotenoids concentration. Principally, there are different technologies, and there is one some future which is the use of solvent. Solvent plays important role in the most technologiest, however the problem of solvents which are used is that they posses potentiaal fire health and environmental hazards. Hence selection of the most safe, environmentally friendly and cost effective solvent is important to design of alternative extraction methods.Chemical molecular product design is one of the methods that are becoming more popular nowadays for finding solvent with the desired properties prior to experimental testing.

Keywords *: carotenoids, extraction, separation, technology, crude palm oil, palm oil mill effluent.*

ABSTRAK

Minyak sawit kasar merupakan sumber karotenoid terkaya yang berasal dari tanaman sawit sebagai senyawa yang sama dengan retinol atau pro-vitamin A; sedangkan limbah pengolahan minyak sawit dihasilkan dari industri pengolahan minyak sawit yang berisi minyak dan karotene yang perlu diberi perlakuan terlebih dahulu sebelum dibuang. Karotenoid merupakan bahan penting yang diperlukan pada hewan dan manusia guna memperkuat tanggapan terhadap kekebalan, konversi ke vitamin A dan penangkapan gugus oksigen radikal. Dengan berkembangnya ketertarikan dalam mencari beta-karotene yang bersumber dari alam lain dan meningkatnya kesadaran untuk mencegah adanya pencemaran lingkungan, maka mendorong suatu industri untuk menggunakan CPO dan POME sebagai bahan baku untuk diekstrak karotenoidnya. Berbagai macam teknologi guna mengekstrak dan memisahkan karotenoid telah dikembangkan untuk mendapatkan kembali karotenoidnya. Makalah ini melaporkan dan membahas berbagai jenis teknologi yang telah dikembangkan guna mendapatkan kembali senyawa karotenoid dari kerusakan di dalam proses pemurnian minyak sawit secara komersial dan pengaruh beberapa perlakuan terhadap ekstrasi dan pemisahan karotenoid dari minyak sawit dan konsentrasi karotenoidnya. Pada prinsipnya, berbagai teknologi yang digunakan untuk mengekstrak dan memisahkan karotenoid terdapat perbedaan, dan terdapat salah satu teknologi yang digunakan untuk esktrasi dan pemisahan karotenoid adalah menggunakan bahan pelarut.

Pelarut yang digunakan mempunyai peranan yang penting dalam teknologi ekstrasi; namun pelarut yang digunakan untuk mengekstrak tersebut mempunyai persoalan karena berpotensi mengganggu kesehatan dan membahayakan cemaran lingkungan. Oleh karena itu, pemilihan jenis teknologi yang aman, ramah terhadap lingkungan dan biaya yang efektif untuk penggunaan pelarut merupakan hal penting sebelum dilakukan desain metode/teknologi alternatif untuk esktrasi karotenoid. Pola produk molekuler kimia merupakan salah satu metode yang saat ini menjadi lebih populer untuk mencari pelarut dengan sifat-sifat yang dikehendaki sebelum diujicobakan.

Kata kunci : karotenoid, ekstrasi, pemisahan, teknologi, minyak sawit kasar, limbah industri pengolahan sawit.

INTRODUCTION

Indonesia is the world's largest palm oil producer, contributing around 27,0 million tones (48,78%) of the world production in 2012 (Anonymous, 2011). However, until at this time most of the components of the non-oil have not been used optimally. For example, many palm oil processing by product that can be used as the basis of the oleo-chemical industry, one of which them is beta-carotene that are needed in the food, pharmaceuticals and cosmetic industries *(*Ahmad*et al.,*2009*).*

Crude palm oil (CPO) is a product that resulted from palm oil mills with wet milling process during the extraction palm oil from the fresh fruit bunch; whereas, palm oil mill effluent (POME) refers to the liquid wastes of discharge originating from the mixture of sterilizer condensate, separator sludge and hydrocyclone wastewater (Ahmad *et al.*, 2009). The CPO processing is entirely physical, and mechanical and does not require any chemicals as processing aid (Lim Meng Hon, 2010).

Crude PalmOil (CPO) contains approximately 1% of valuable minor component including carotenoids and tocopherols. According to previous work, the major components of recovered oil from palm oil mill effluent (POME) were similar to that from crude palm oil, which also contained α- and β-carotene (Ahmad *et al.*, 2009). Carotenes, which impart the distinctive orangered color to palm oil, together with tocopherols contribute to the stability and nutritional value of the oil (Othman *at al.*, 2010). The characteristic orange color of crude palm oil is due to the presence of carotenoids (α- and β-carotenes). These carotenoids are commercial importance as they are utilized as natural coloring agents in edible and pharmaceutical products *(*Mohammad *et al.,* 2013*).* Carotenoids itself are natural pigments synthesized as hydro carbons (carotene viz, lycopene, α-carotene and β-carotene) or their oxygenated derivatives(xanthophylls; lutein, α-cryptoxanthine, and astaxanthine) by plants and micro-organism *(*Das *et al.,* 2007*)*. The majority are hydro carbons of 40 carbon atoms which contain two terminal ring systems, joined by chain of conjugated double bonds or polyene sys*tem (*Fengrova and Beshkova*,* 2009*)*.

The beta (β) carotene is a carotenoid of increasing as precursor of vitamin A, has also been ascribe a central role in cancer prevention and therapy. This is related to the anti-oxidant property of carotenoids with their conjugated polyenestructure, predestined for free radical and singlet oxygen scavenging *(*Siems*et al., 2005)*. Additionally, β-carotene reduces the risk of cardio-vascular diseases, cataract development and macula degeneration *(*Wong *etal., 2011)*. Moreover, the outstanding quencher function of beta-carotene advo-cates dietary supplementation *(*Martano *et al. , 2011).*

Over several decades, various methods have been developed in order to recover carotenoids vitamin E (toco-pherols and tocotrienols) in crude palm oil. These

include saponification(Nesaretnam*et al.*, 2007), selective solventextraction (Nitsche*et al.*, 1999, Heidlas*et al.*, 1998, PORIM., 1989), trans-esterification follow by molecular destillation (Ooiand May, 2000) and further purification by adsorption using synthetic resin (Ooi *et al.*, 1994; Lion Corporation, 1993), silica gel (Tan and Salleh, 1992), reverse phase C_{18} silica (Lion Corporation, 1993) adsorption chromatography (Baharin*et al.,* 2001, Bonnie, 2007) and membrane technology (Baharin*et al.*, 1998).While Kemmerer and Fraps (2007) isolates carotenoids, with a variety of treatments extraction process. The treatment gave the highest carotene are : the sample is dissolved in petroleum ether at room temperature with 82,2% of β-carotene and 16,6% α-carotene, as well as sample saponification in alcoholic KOH for 5 minutes at room temperature with 83,3% β-carotene and 16,3% α-carotene.

Solvent Extraction is a mass transfer operation in which palm oil in extracted with an immiscible or nearly immiscible solvent that exhibits preferential affinity or selectivity towards carotenoids andtocol in palm oil. Solvent is one major commercial importance to the chemical and biochemical industries, as is it often the most efficient method of separation of valuable product from complex feedstock (Othman*et al.*, 2010)

In addition, adsorptive separation studies of β-carotene from methyl ester using MesoporousCarbonCoatedMonolith/MCMM has been studied by Muhamad*at al*. (2013) and optimation carotenoids isolation of the waste crude palm oil using α-amylase, β-amylase, and cellulase has been studied by Hudiyono and Septian (2013). This present study was attempt to review and case study on extraction and separation technology for carotenoids from palm oil.

METHODS/METHODOLOGY

This paper will be using review and case study approach on extraction and separation technology for carotenoids from palm oil, using recent secondary data and information that published data from research and development of bioactive components affairs and in relation on extraction cand separation technology for carotenoids from Crude PalmOil (CPO) and PalmOilMill Effluent (POME) waste. A review on the extraction and separation technology for carotenoids from CPO and POME waste; effect of some variety treatment on the extraction and separation for carotenoids from palm oil, carotene concentration will be illustrated. Selected data from researches and scientific publications by extraction and separation technology journals is used to compare and describes the potential of crude palm oil and palm oil mill affluent as carotenoids, its viability and why it should be promote as source of carotene.

Although the data from researches and scientific publications related to palm oil mill effluent (POME) is used; however, in this to come up for discussion will be focused on crude palm oil issue.

Principally, the extraction and separation technologies for carotenoids from Crude PalmOil(CPO) and PalmOilMillEffluent(POME) waste that have been developed to recover carotenoids can be divided into three main categories,i.e.solvent extraction, trans-esterification and molecular distillation and adsorption (Othman *et al.*, 2010). Subsequently, in order to shift phyto-chemical extraction process to near suistainability as well as offsetting the drawbacks of the con-ventional methods, new methods like ultrasonic-assisted extraction (UAE) are developed and optimized to increase the extraction yields (Wang and Weller, 2006).

The data from the results of researches and scientific publications finding was conducted analyze through descriptive analysis that developed by Hair *et al.* (1987).

DISCUSSION

The extraction and separation technology for carotenoids from CPO and POME waste

Usually solvent extraction was operated at normal condition using hexane as solvent to extract carotenoids and vitamin E from crude palm oil (Latip*et al.*, 2000; Chiu*et al.*, 2009). However hexane process potential fire, health and environmental hazards (Choo*et al.*, 1996).Short chain alcohols, especially ethanol and isopropanol have been proposed as alternative extraction solvent due to their greater safety and reduce probability of regulation (Ping and Gwendoline, 2006). Alcohols tend to extract more non-glyceride materials than hexane due to their greater polarity (Othman *et al.*, 2010).

It was known that extraction is a process to separate a mixture into fractions or its constituents by suitable solvent. Organic solvents are partly toxic, inflamable and may cause explosive. These disadvantages cause to a lot of or attempt to do in solvent recovery and plant safety, Therefore,extraction of palm oil from its mesocorp using SupercriticalFluidExtraction (SFE) technology has been considered a promising way to recover carotenes and prevent their high losses during palm oil milling (Mustapa*et al.*, 2011). This due to its unique advantage including low temperature used, seletive extraction, simpler and cleaner (solvent – free) product recovery.

In addition, SFE is also environmentally benign technology since the process typically general no waste. Because of that application of supercritical fluid extraction (SFE) technology for crude palm oil extraction, and for the recovery of the carotenes from palm mesocarp is expected to have agreat future outlook (Bharath, 2003), and it was and is seen to have agreat potential for replacing the conventional screw press extraction, clarification and vacuum drying processes (Lau *et al.*, 2006). Based on that reason, the extraction technology is set to become more popular in the next 10 years *(*Mustapa *et al., 2011)*.

Recently the use of sub-critical R 134 a (1,1,1,2-tetra fluoroethane) as an alternative to CO_2 solvent for the recovery of palm oil was studied by Mustapa *et al., (2009)*. R 134 a (1,1,1,2-tetra fluoro-ethane) itself is classified as a substance non-toxic, non-reactive, non-inflamable and non-ozone depleting. It also has a high volatility and boiling point at atmosphere pressure of 26.6^{o} C; which means it leaves negligible solvent resi-dues in the products *(*Mustapa *et al., 2011)*.

They performed the extraction of palm oil from mesocarp using R 134 a at sub-critical conditions. The extraction of palm oil was found to increase with temperature and peaked at a maximum of 100 bar and 80^{o} C *(*Mustapa *et al.,2009)* and also reported that the extraction of palm oil using R 134 a has a great potential for application in the palm oil industry. Unfortunately, there are a few disadvantages of SFE technology since it requires operating pressures that are typically much higher than ambient conditions, compression of solvent that requires elaborate recycling measure to reduce energy costs and also high capital investment for equipment *(*SFE Research Group*, 2010)*.

Trans-esterification is the only commercially viable process. This unique method of carotenoid recovery from palm oil has already been developed by Lion Corporation in Tokyo, Japan (Baharin*et al.*, 2001). Trans-esterification process will convert the molecules of triglycerides into smaller molecule of fatty acids methyl ester (FAME), making it much*e*asier to be separated *(*Puah*et al., 2008)*. Mean- while the solubility of compound in solvent depends on its molecular weight, polarity and solvent strength *(*Puah *et al., 2007)*, and trigycerides in CPO itself, also have large molecular weights of 807-885. As a result, triglycerides has lower solubility in the solvent and it was known that the carotenoids are transported together with

triglycerides. Therefore, the fatty acids methyl ester (FAME) is more soluble than triglyrides(Othman*et al.*, 2010).

Trans-esterification is a process involving neutralization and inter-esterification of triglycerides with methanol to yield methyl esters; followed by phase separation, resulting in carotene-rich layer and decolored methyl ester layer. The carotene rich layer is further concentrated by molecular distillation and chromatographic methods (Baharin*et al.*, 2001).However, trans-esterification process convert the CPO irreversibly to methyl ester which is not edible and furthermore, also changes the quality of oil (Puah*et al.*, 2005).

Molecular destillation process represents a special type of vaporization at low pressures and low temperatures. This methods is possible to separate and purity molecules which have high molecular weight and thermally sensitive such as vitamins (Othman *et al.*, 2110). However, trans-esterification process converts the CPO irreversibly to methyl ester which is not edible, and furthermore, also changes the quality of oil (Puah *et al.*, 2005). Further purification will be needed to produce crystalline carotenoids and tocols safe for consumption.

Methods to recover cerotenoid from palm oil using adsorption has been developed and ported by Tanaka*et al.*, (1986) and Memuro*et al.*, (1986), but is was not commercially viable. Subsequently, a process to separate carotene from Crude Palm Oil (CPO) that did not require convertingCPO to methyl ester was also sucsessfully developed based on adsorption with synthetic polymer adsorbent by Baharin*et al.*,(1998), Latip*et al.*, (2000;2001). According to Othman*et al.* (2010), reported that there were two types of solvent are commonly used in adsorption methods. Alcohol such as ethanol and isopropanol (IPA) is used as initial solvent to build an initial layer on the surface area of the adsorbent in order to allow solute contact with liquid phase. The second solvent is n-hexane, which is preferable for eluting the carotene adsorbed on the hydrophobic surface of the adsorbent.

Because a process separating carotene from crude palm oil (CPO) by adsorption using absorbent is still not commercially proven and may slow down in the refining process (Othman*et al.*, 2010), so adsorption of beta-carotene onto mesoporous carbon coated monolith (MCCM) in isopropyl alcohol and n-hexane solution has been studied by Muhamad*et al.*, (2010) and thedesorp-tion of beta-carotene from MCCM including isotherm kinetic and rege-neration studies has been also studied by Muhamad*et al.*, (2011). It was reported that the utility of carbonaceous (powder and granular) material in the form of fixed bed for separation is associated with high pressure drops, potential channeling and many other demerits. Compared to cerbonaceous material; mesoporus carbon coated monolith (MCMM) has large external surface area and a very less pressure drop across fixed bed MCCM column. Highmechanical stability and thermal expansion coefficient are some other properties of the MCCM. The MCCM column can also placed in vertical or horizontal position and in mobile system without deforming shape and is easier to be scaled up due to its simple design and uniform flow distribution *(*Muhammad *et al., 2013)*.

In the current technology of extraction for carotenoid, the appropriate extraction method must be employed to conserve resources. Soxhlet extraction and maceration used in solvent extraction are resource-use (energy and materials) intensive and mostly result in degradation of plant vitamins and lipids (Pingret*et al.*, 2012), especially carotenoids as long extraction time required. In order to shift phytochemical extraction process to near sustain ability as well as effecting the drawbacks of the conventional extraction method, new methods like ultrasonic–assisted extraction (UAE), microwave assisted extraction, pressurized fluid extraction, etc. are developed and

optimized to increase the extraction yield (Wang and Weller, 2006 ; Boateng and Lee, 2013).

UAE technology is outstanding in that it is simple and fast technique, witch consume less energy, time and materials, thus producing more pure product at higher yield (Boateng and Lee, 2013). This method has been applied to extract carotenoids from corn (Ye *et al.*, 2011), tomato (Lianfu and Zelong, 2008) etc. with comparatively high yields. Optimization of UAE of carotenoids from plat materials would therefore help improve the overall process for recovery of bio-active substance. Also, other parameters like ultrasonic frequency and power can be optimized in order to improve the mass transfer rate for efficient extraction of carotenoids (Boateng and Lee, 2013).

In order to obtain the solvent suitable to extract high concentration of carotenoids from palm fronds, three food grade solvent (ethanol, n-hexane and acetone) were used for preliminary extraction. Ethanol, n-hexane and acetone are cheap and reusable food, compatible bio-solvents, which have ability to stabilize against oxidation. Thus widely used in recovery of carotenoids from plant tissue (Boateng and Lee, 2013). Briefly, the dry palm fronds (10g) were mixed with 0,1 g L-ascorbic acid already dissolved in the solvent (100 ml), placed in the shaker bath and shaken continuously for 3 hours at 40^0C. The supernatant were collected after filtering and residues were subjected to additional three rounds of extraction when the filtrate was colorless. The three solvent extract were analyzed by HPLC-FLD immediately after extraction.

Effect of Some Various Treatments on The Extraction and Separation for Carotenoids from Palm Oil

Effect of some various treatments on the extraction and separation for carotenoids from palmoil has been conducted by some researches , i. e. : Effect of column temperature on HP 20 column chromatography and effect of CPO loading on the recovery of palm carotene by Baharin*et al.*, (1998), Ahmad *et al.*, (2009); subsequently,Effect of of carotene extraction system on carotene composition and carotene stability during storage (Baharin*et al.*, 2001); Effect of CPO/ silica gel ratio on percentage of carotenes recovered in hexane fraction (Ping, 2007); Effect of extracting solvent on total carotenoid content of crude carotenoids extracts recovered from palm oil meal (Panpipat and Chaijan, 2011); and Effect of temperature, contact time and adsorption mechanism using mesoporous carbon coated monolith *(*Muhammad *et al., 2013)*.

Table 1 : Effect ofcolumn temperature on the recovery of palm carotene with the I-H and E-H solvent system [(a)] (*)

Temperature (oC)	Fractions	Oil recovery (%)	Carotene	
			Recovery (%)	Concentation (ppm)
I – H system				
40	IPA	94.7	42.2	310
	Hexane	5.3	57.8	7.630
50	IPA	96.0	61.1	397
	Hexane	4.0	38.9	6.306
60	IPA	96.6	72.7	434
	Hexane	3.4	27.8	4.559
E – H system				
50	Ethanol	95.8	50.8	357
	Hexane	4.2	49.2	7.953
60	Ethanol	99.8	59.7	404
	Hexane	a;z	40.3	11.647
70	Ethanol	97.3	64.2	250
	Hexane	2.7	35.8	4.964

(*) source :Baharin *et al.*, (1998).(a) CPO loading amount 30 g.

Table 2 : Effect Of Column Temperature On The Recovery Of Palm Carotene (*)

Coloumn 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
	Petrol – 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
	Petrol – ether	90.09	94.03	520.23
	Ethanol	8.14	3.72	220.36
50	Hexane	42.27	74.65	799.59
	Ethanol	50.97	8.75	77.73
	Petrol – ether	93.40	60.13	291.94
	Ethanol	5.84	3.02	234.68

(*) Source :Ahmad*et al.*, (2009).

Effect of Column Temperature on Column Chromatography

According to Baharin*et al.*, (1998) study was reported that the effect of column temperature varied with the solvent system. In the iso-proponal-hexane system,carotene recovery reached 58% at 40° C, and at high temperature, carotene recovery become lower. The carotene concentration was 7.600ppm at 40° C, and decreased with increasing temperature in the ethanol – hexane system, the carotene recovery was also higher (50% at 50°C)and gradual by decreased at higher temperature. However, Ahmad *et al.*, (2009) was reported that the carotene recovery in hexane fractions by using hexane – ethanol system didnot very much depending on the column temperature. On other hand, the carotenerecovery in petroleum ether fractions was more than 91% at 30°C and 40°C, but decreased to 60% at 50°C. They suggested that 50°C is not a suitable temperature to recover palm carotene from the extracted oil by using petroleum ether – ethanol system.

Table 1 and 2 shows the effect of column temperature on the recovery of palm carotene and oil recovery in the system that were reported by Baharin*et al.*, (1998) and by Ahmad *et al.*, (2009).The temperature examined were 40, 50, and 60°C in the I-H systems; and 50, 60, and 70°C in the E-H system. The effects of column temperature varied with the solvent system.

Based on Table 1, it can be seen thatin the I-H system, carotene recovery reached 58% at 40°C, and at higher temperature, carotene recovery became lower. The carotene concentration was 7,600 ppm at 40°C, and decreased with increasing temperature.

The oil recoveries were almost the same at the examined temperature (95 to 97%). In the E-H system, the carotene recovery was also higher (50% at 50°C) and gradually decreased at higher temperatures.The oil recovery was not affected much by column temperature. From these results, the recommended column temperature is 40°C in the I-H system, and 50°C in the E-H system (Baharin *et al.*, 1998).

According to Table 2, the total recovery in P-E system increased as the column temperature increased. This may due to the fact that oil viscosity decreases as the temperature increases and the oil was easiereluted from the column by solventsystem (Ahmad *et al.*, 2009). The oil recovery in hexane fraction slightly fluctuated at different temperatures but the total oil recovery in H – E system was more than 80 % with the higher recovery at 40° C.

Effect of crude palm oil (CPO) loading

Baharin *et al.*, (1998) explained and reported that CPO loading on HP – 20 column is important condition, because this largely effects carotene recovery. Effect of CPO loading under the I – H system at 40° C column temperature was shown in Table 3.

Table 3 : Effect of CPO Loading on the Recovery of Palm Carotene with The I-H Solvent System (*)

CPO loading (g)	Fraction	Oil Recovery (%)	Carotene	
			Recovery (%)	Concentration (ppm)
10	IPA	88.9	14.8	144
	Hexane	11,1	85.2	6.645
20	IPA	96,3	35.4	249
	Hexane	3,7	64.6	11,983
30	IPA	94,7	42.2	310
	Hexane	5.3	57.8	7,630
40	IPA	96.3	65.2	401
	Hexane	3.7	34.8	5,529
50	IPA	92.3	73.5	447
	Hexane	7.7	26.5	1,919
60	IPA	93.9	78.2	503
	Hexane	6.1	21.8	2,154

(*) Source :Baharin*et al.*, (1998). IPA :Iso-propyl alcohol (350 ml); hexane (500ml).

According to the Table 3 above, it can be seen clearly that recovery decreased with increasing CPO loading. The higher carotene recovery (85%) obtained at 10 g CPO loading dropped rapidly with a 60-g load to 22%. On the other hand, the high oil recovery (89–96%) did not depend upon CPO loading. The carotene amount eluted by isopropanol reached 78 % at 60 g CPO loading, in spite of 15 % level at 10 g CPO.

These results suggest that carotene recovery depends mainly on two factors : (i) competitive adsorption between the oil and carotene on the HP-20 resin surface, and (ii) the adsorption capacity of the resin for carotene in the presence of the solventisopropanol (Baharin *et al.*, 1998).

Effect of Carotene Extraction System on Carotene Composition and Carotene Stability During Storage

Based on results of Baharin and his coworkers studied in 2001 was reported and summarized that the quality of CPO after the extraction process was slightly deteriorated in terms of free fatty acid, moisture content, impurities, peroxide value, anisidine value, discriminant function and deterioration of bleachability index. However, the CPO still can be refined to product refined, bleached, deodorized palm oil that meets the PalmOilRefiners Association of Malaysian specifications. It was concluded by Baharin*et al.*, (2001) as follows: *firstly* , triglyceride carbon number and fatty acid composition of CPO after going through the carotene extraction process were almost the some as CPO data; *Second ly*, the major component of the carotene fraction similar to CPO, which contain mainly alpha- and beta- carotene; and *finally* the carotene could be store for at least 3 months.

Table 4 : Effect of CPO/silica gel ratio on percentage of carotenes recovered in hexane fractions*

Ratio/Silica gel	Carotene				Carotene recovery in hexane fractions (%)	Oil recovery (%)
	Content (mg)		% Carotene			
	F_1	F_2	F_1	F_2		
1 : 5 (a)	1,9	-	0,15	-	93	52
1 : 5 (b)	1,3	0,39	0,56	0,54	82	49
1 : 5 (c)	1,41	0,29	0,97	0,35	85	50
1 : 8 (a)	0,52	0,49	1,7	2,0	87	97
1 : 8 (b)	0,34	0,47	1,1	3,1	66	98
1 : 10 (a)	0,16	0,41	2,1	4,4	73	99
1 : 10 (b)	1,12	-	11,2	-	92	99
1 : 10 (c)	1,18	0,007	12,9	0,6	90	99
1 : 10 (d)	0,10	0,14	8,3	4,7	32	-
1 : 10 (e)	0,15	0,15	14,8	5,5	21	99
1 : 10 (f)	0,16	0,21	14,7	7,1	26,6	99,5
1 : 10 (g)	0,06	0,22	0,04	6,0	54	99
1 : 15	0,05	0,14	1,7	9,8	57	99
1 : 20	0,07	0,02	1,2	0,03	13	-

(*)Source : Ping (2007)

Note :

- Letters in parenthese represent the number of extraction for the ratio used.
- Ratio 1 : 10 (g) represent extractions using silica gel with pore size of 100 A°.
- All silica gel used was activated except for ratio 1:10 (d) to (f).

Effect of CPO/Silica gel ratio on percentage of carotenes recovered in hexane fractions

The effect of the above treatments was studied and reported the by Ping (2007) in his paper with the topic " Palm Carotene Concentrates from Crude Palm Oil using Vacuum Liquid Chromatography on silica gel ". The weights of CPO : silica gel (w/w) used for ratios of 1:5, 1:8, 1:10, 1:15, and 1:20 were 3g : 15g ; 1,9g : 15,2g ; 2g : 20g ; 1g : 15g and 1g : 20g, respectively. The silica gel was activated in an oven at 120° C for 12 hours and cooled in silica gel dessicator prior to its use in the column. For the ratio 1:10, two treatments, activated [1:10 (a) to (d)] and non-activated [1:10 (d) to (f)] silica gel, were evaluated. Using activated silica gel with a pore size of 100 A° [1: 10 (g)]. Result of the studied to the effect of CPO/silica gel ratio on percentage of carotenes recovered in the hexane fractions is presented in Table 4.

Based on Table 4 above, it can be explained that further increase in the ratio of CPO : silica gel to 1:20, reduced the carotene eluted in the hexane to 1,2 %. The reduction could be because of build up pressure from the high amount of adsorbent and eluting solvent which showed down the oil movement, thus affected the arotene concentrations. Ping (2007) reported and summarized that the highest carotene concentrations (> 10%) was achieved using a ratio of 1 : 10 (w/w) crude palm oil : silica gel. For this ratio, activated silica gel improved the carotene recovery to 72-79% compared to 21 – 32% by non activated silica gel. High recovery (99% - 99.5%) of the bulk oil recovered from the silica gel was achieved by elution with a polar solvent, e.g. ethanol or isopropanol followed by a non-polar solvent, e.g. hexane.

Effect of extracting solvent on total carotenoid content of crude carotenoid extracts re-covered from palm oil meal.

The effect of the above treatment for extraction of carotenoids from palm oil

meal was studied and reported by Panpipat and Chaijan (2011). Carotenoid extraction from palm oil meal was carriedout using different solvent mixtures including 10% alcoholic KOH; ethanol : hexane (4 : 3, v/v), acetone : hexane (2:3, v/v), and acetone : ethanol : hexane(1:1:2 v/v) with a palm oil meal / extracting solvent ratio of 1:2,5 (w/v). The result of the treatment was showed that the highest recovery did not represent the highest carotene carotenoid content as indicated in Figure 1.

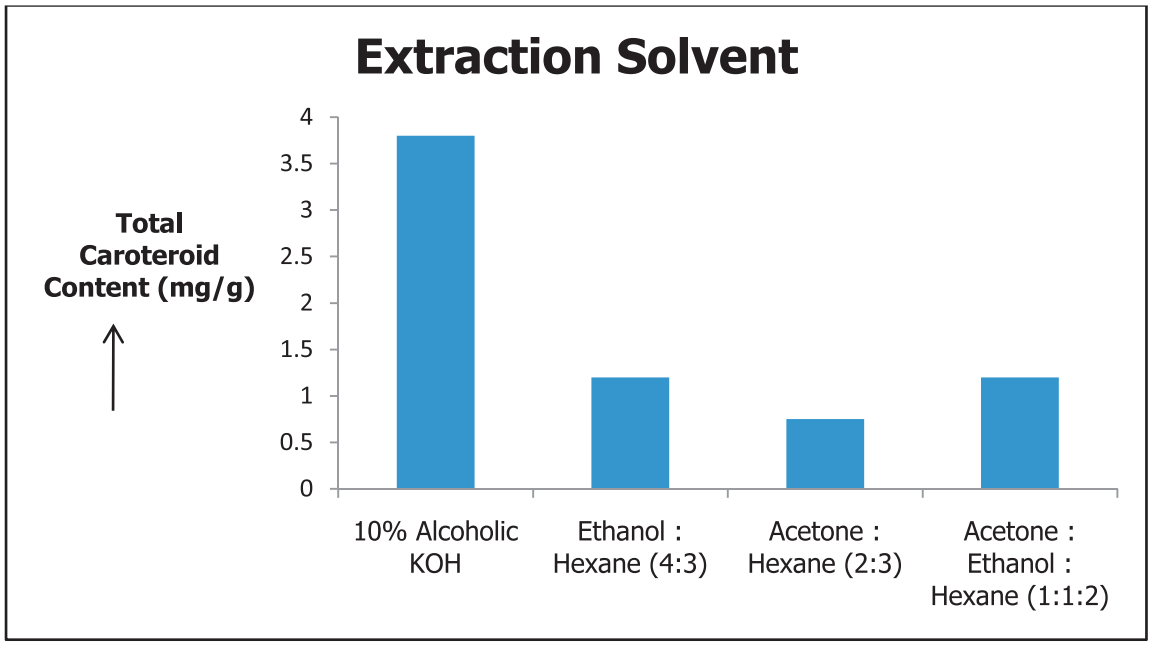

Figure 1. Effect of extracting solvent on total carotenoid content of crude extracts recovered from palm oil meal (Source : Panpipat and Chaijan, 2011).

Based on Figure 1, it can be reported that the 10% alcoholic KOH exhibited the highest carotene content ($p<8.05$). The lowest recovery of crude extract with highest carotene was found in the extract 10% KOH indicating the lowest impurities From the results, it was noted the highest purity of crude carotenoid extract might be obtained when the extraction was done with 10% alcoholic KOH. Therefore, the optimum solvent used for carotenoid extraction from palm oil meal was 10% alcoholic KOH.

According to Panpipat and Chaijan (2011), based on the total carotenoid content determination using spectrophotometer at 450 nm, it was known that the major carotenoid found in the extract of 10% alcoholic KOH from palm oil meal was alpha-carotene and beta-carotene. This result was in the same agreement with Kiokias and Gordon (2004), who reported that beta-carotene and alpha-carotene were the major found in palm oil witch contributed about 80% - 90% of total carotenoids.

Effect of Temperature and Contact Time

According to Muhamad*et al.*, (2013) it was reported that beta-carotene adsorption increases with temperature and suggesting that the intraparticle diffusion rate of the adsorbate molecules into the pores increased with increase in temperature since diffusion is an endothermic process. Physical adsorption is normally considered to be the dominant adsorption mechanism for temperature lower than 100^0C (Ma and Lin, 2004). The pigment is adsorbed only on outer surface of the adsorbent at lower temperature, and both of the outer surface and pore surface a higher. However, at higher temperature

destruction of beta-carotene mayoccure (Ahmad *et al.*, 2009). There-fore, the adsorption experiments were carried out up to 50^0 C.

Meanwhile, an increase in reaction temperatures causes a decrease in solution viscosity leading to an increase in beta-carotene molecules rate of diffusion across the external boundary layer and into the internal pores of the adsorbent. In addition, an increase in temperature increases MCCM (Meso-porous Carbon Coated Monolith) equili-brium capacity for beta-carotene. This may be result of increase in beta-carotene movement with temperature. An increasing number of molecules may also acquire sufficient energy to undergo an interaction with active sites. As presented in Table 5 the beta-carotene adsorption capacity onto MCCM increased from 8.128 to 10.775 mg/g with an increase in reaction temperature from 30^0 C to 50^0 C, indicating that the process is endothermic (Bulut and Aydin, 2006). Beta-carotene adsorption on MCCM for various adsor-bate concentrations was fast initially,thereafter, the adsorption rate decreased slowly as the available adsorption sites decreased gradually.

Muhamad*et al.*, (2013) was also reported that the mechanism for beta-carotene removal by adsorption may assumed to involve three successive transport steps : (i) film diffusion, (ii) intraparticle or pore diffusion and (iii) sorption onto interior sites. The last step is considered negligible as it is assumed to be rapid. Beta-carotene uptake on MCCM active sites can mainly be governed by either liquid phase mass transfer or intraparticle mass transfer rate.

Based on the reports and results of experiments and scientific publications; so, according to the author opinion is that extraction temperature should be used in the range of 30, 40, and 50°C. Because in the range extraction temperature for carotenoids extractions used have a significant effects to carotenoids concen-trations. Thus, the temperature range of 30 – 50°C was considered optimal for preliminary experiments.

Table 5. Kinetics Data for Beta-carotene Adsorption on MCCM (*)

Temp. ^{0}C	Co (mg/L)	Adsorption capacityExperiment (mg/g)	Pseudo –first Order			Pseudo-second-Order		
			Adsorption capacityCalculation (mg/g)	Constant a I (k1) (l/menit)	R^2	Adsorption capacity of experimental(mg/g)	Constanta II(k2) (g/mg-menit)	R^2
50	50	3.099	1.842	0.0221	0.9791	3.262	0.0249	0.9997
50	250	5.969	2.818	0.0235	0.9475	6.203	0.0187	0.9998
50	500	10.775	5.212	0.0237	0.9576	11.186	0.0105	0.9999
30	500	8.218	4.756	0.0196	0.9311	8.772	0.0073	0.9983
40	500	9.615	5.145	0.0216	0.9548	10.152	0.0081	0.9997

(*) Source : Muhamad*et al.*, (2013).

Note : Co = initial concentration at solution phase (mg/L); Q_e = adsorption capacity at experimental (mg/g)

Carotene Concentration

Carotene concentration refer to the purity and yield as the basis of economic measure on its viability in term of process or methods refers as technology. Numerous tests were done (still is) to increase carotenoid-rich palm oils' concentration to elevate carotene concentration such as industrial process called fractionation. Fractionation extends the uses of palm oil, the products obtained are liquid oil (olein, 70-80%) and solid fat (stearin, 20-30%). The carotenoidcontent of various palm oil fractions as shown in

Table 6. One method involves the selective adsorption of carotenoids ob-tained from reverse-phase adsorption material (*Choo et al,* 1992), with the esters of high polarity being first eluted out from the column.

A subsequentimprovement a recovery rate of greater than 90% can be achieved, with carotenoid concentration of 8000-9000 ppm (as shown in Table 7) both of which are higher than those obtained by other methods (*Choo et al.,*1992). A further improvement, an oil with afinal carotenoid concentration of 80,000 ppm been achieved through molecular distillation.

Table 6. Carotenoid content of various palm oil fractions (*)

Palm oil fraction	Carotenoid content (ppm)
-Crude palm oil	630-700
-Crude palm olein	680-760
-Crude palm stearin	380-540
-Residue oil from fibre	4000-6000
-Second press oil	1800-2400

(*) Source : *Choo et al* (1989).

Table 7. Results of various methods of carotenoids recovery and concentration (*)

Methods/ treatment	Carotenoid content (ppm)	Recovery rate (%)
***Through Methyl Esther**		
-Carbon-18 reverse phase Adsorption	8000-9000	>90
-Carbon adsorption	5000-7000	<50
-Vacuum distillation	>20,784	<46
-Molecular distillation	>80,000	>80
***From Crude Palm Oil**		
-Activated carbon adsorption	3700-5600	<80
-Molecular distillation	1290-1990	-

(*) Source : *Choo et al* (1992)

According to Boateng and Lee (2013), it was reported that extraction temperature is one of the important materials. At higher temperature (above 30,14 ^{o}C) with ultrasonic-assisted extraction (UAE) technology, a lower content of carotenoids are obtained as opposed to the conventional maceration, which releases carotenoids at temperatures yet with minimal yield. However, carotenoids concentrations were decrease at elevated temperatures during the preliminary extraction for solvent selection (Pingret *et al., 2012)* have also reported the degradation of lipids at high ultrasonic temperatures.

The concentration of beta-carotene increased with increase in extraction temperature and extraction time 30.14 ^{o}C and 37.11 minutes, respectively, and then decreased significantly (P<0,05) at temperatures between 40-70^{o}C and extraction time from 30-50 minutes. However, there is no significant increase of beta-carotene concentration in the solvent=sample ratio, carotenoids are found to degrade at elevatedtemperatures; thus, this study corresponds to the report by *Gu et al.,* (2008) who also reported an optimum temperature of 30^{0} C for carotenoid extraction. Despite technological improvement, production of carotene-enriched palm oil has been costly in terms of multi stage process. This could be the reason why many plants, have been either currently at testing, demonstration or at pilot plant stage respectively.

According to the author opinion, from the results of the studies and experiments that have been conducted by many researchers; it can be explained that

molecular destillation methods (through methyl ester treatment) is fitting used technology for carotenoids extraction and recovery, because carotenoid concentrations is the highest results.

CONCLUSION

There are many technologies to extract and to separate of carotenoids from palm oil and palm oil mill effluent. Various technologies have been developed in order to recover carotenoids component from being destroyed in commercial refining palm oil.These include saponification, selective solvent extraction, trans-esterification followed by molecular distillation and further purification by adsorption using synthetic resin, silica gel and reverse phase C_{18} silica, adsorption chromatography, mem-brane technology and ultrasonic-assisted extraction technology.

Effect of some various treatment on the extraction and separation for carotenoid from palm oil have been also developed, in order to search the optimum extraction condition for carotenoid and carotene concentration and to determine the performance of the technology. Two novel methods are in producing carotene-rich product such as carotenoid concentrate with concentrations greater then 80,000 ppm through molecular distillation.

In general, the art of extraction and separation technology is improving, with new methods and procedures rapidly being developed. Solvent extraction has been improved by other methods, in order to obtain better yield. The molecular distillation methods through methyl ester treatment is the most interesting to choice for carotenoids extractions and developed with high yield concentration.

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