WALAILAK JOURNAL
http://wjst.wu.ac.th Engineering and Physical Sciences

Separation of Carotenes and Vitamin E from Palm Oil Mill Effluent Using Silica from Agricultural Waste as an Adsorbent

Kanokphorn SANGKHARAK*, Nisa PICHID, Tewan YUNU and Puvanart KINGMAN

Department of Chemistry, Faculty of Science, Thaksin University, Phatthalung 93110, Thailand

(*Corresponding author's e-mail: skanokphorn@yahoo.com)

Received: 23 June 2015, Revised: 4 January 2016, Accepted: 5 February 2016

Abstract

Palm oil mill effluent (POME) was characterized. The effluent had low nitrogen with high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). 6,500 mg/L of oil (78 % of oil recovery) was recovered from 1 L of POME using *n*-hexane. 70 and 71 % of BOD and COD in POME were significantly reduced after oil recovery. The carotenes and vitamin E concentration from recovered oil were 580 and 160 ppm, respectively. α-carotene and β-carotene were presented as a major component in oil. Afterward, carotenes and vitamin E in oil were concentrated by adsorption chromatography using either rice hull silica or rice straw silica as adsorbents. After oil recovery, 4.60 g/L oil was obtained with high concentrations of carotenes (5,590 ppm) and vitamin E (630 ppm). Rice hull silica (RSH) had a higher capability to recover carotenes and vitamin E than rice straw silica (RSS) did. The recovery of carotenes and vitamin E using RSH were 70.77 and 11.88 % recovery, respectively. The carotene and vitamin E recovery method using RHS developed in this work has potential applications in the palm oil industry.

Keywords: Adsorption chromatography, carotenes, palm oil mill effluent, rice hull silica, rice straw silica

Introduction

Red palm oil, a unique nutritious vegetable oil, is derived from the fruits of the palm tree (*Elaeis guineensis*). It is the only oil in nature that provides a bouquet of antioxidants and phytonutrients, such as vitamin E (tocotrienols/tocopherol), mixed-carotenes with high levels of α-carotene (pro-vitamin A), plant squalene, phytosterol complex, and Coenzyme Q10 (CoQ10). No other vegetable oil has such a unique natural combination of phytonutrients. Palm oil possesses 1 % minor components, including carotenes, vitamin E, fatty acids, sterols, pigments, and some other components [1,2]. Carotene concentration in palm oil normally ranges between 400 and 3,500 ppm, and it contains about 15 times more retinol equivalents (vitamin A) than carrots and 300 times more than tomatoes [3,4]. Vitamin E amounts (or tocols), found in terms of tocopherols and tocotrienols, are generally present in common vegetable oils. However, the highest level of tocotrienols is found in virgin red palm oil [5]. Many researchers have evaluated the beneficial health effects of the main phytonutrients in red palm oil, such as vitamin E and pro-vitamin A (mixed-carotenes) [6].

The palm oil industry plays an important role in Thailand's economy. In 2014, there were 25 wet-process crude palm oil factories in Thailand, producing about 0.2 million tons of crude palm oil from 0.9 million tons of fresh fruit bunch (FFB). These factories are traditionally regarded as the major consumers of water and the major sources of organic pollution of surface water bodies. While the oil palm industry has been recognized for its contribution towards economic growth and rapid development, it has also contributed to environmental pollution due to the production of huge quantities of by-products from the oil extraction process [7]. Waste products from oil palm processing consist of oil palm trucks (OPT), oil palm fronds (OPF), empty fruit bunches (EFB), palm pressed fibers (PPF), palm kernel shells (PKS), palm kernel cake (PKC), and liquid discharge POME. POME is generated from various points during processing in an oil mill. These include clarification sludge, sterilization condensates, fruit washing water, hydro cyclone drain-off, and various boiler blowdown, tank, and decanter drainage [8]. For each ton of fresh fiber bunch process, 0.87 tons of POME is discharged [9]. POME is a high volume liquid waste with a high organic content of about 25,000 and 50,000 mg/L, as measured by BOD and COD. POME is highly polluting and needs suitable treatment before discharge. However, many researchers reported that POME contained oil and carotenes in the range of 6,000 mg/L and 0.023 %, respectively [10,11]. Several methods have been developed in order to recover oil and carotenes from POME. The extraction technologies that have been developed to recover carotenes and vitamins E from POME can be divided into 3 main categories; solvent extraction, transesterification, and molecular distillation and adsorption [12-16]. Solvent recovery is the most well known method for recovery. However, the selection of a solvent is a major concern. The selected solvent should be safe, environmentally friendly, economical, and highly efficient [17]. Therefore, the development of a novel and cheap process is an interesting topic.

Due to the available POME and the growing value of carotenes and vitamin E, the objective of this study was to separate valuable products, including oil, carotenes, and vitamin E, from POME, using column chromatography. Silica-based resin was extracted from rice hull ash (RHA) and rice straw ash (RSA) to reduce the cost of the recovery process. The economic feasibility and efficiency of silica from agricultural waste was also compared with commercial adsorbents. In addition, the possibility of wastewater treatment during the oil recovery process was also determined by measuring several parameters such as pH, BOD, COD, and the total solids of POME. The development of an inexpensive method for carotene and vitamin E separation by column chromatography using silica from agricultural waste has not previously been reported.

Materials and Methods

Materials

RHA and RSA were obtained from Producers Rice Mill (Phatthalung, Thailand). POME was obtained from Palm Oil Public Co., Ltd. (Krabi, Thailand) at a temperature of 75 - 85 °C. Carotenes, tocopherols and tocotrienols were purchased from Merck (Germany). Commercial silica was obtained from Sigma Aldrich (USA). All solvents and chemicals used were of analytical grade.

The preparation of silica from rice hull and rice straw ash

Silica was extracted from RHA and RSA using the modified method of Kamath and Proctor [18] and Kalapathy *et al*. [19]. The silica from RHA and RSA, namely RHS and RSS, were chemically characterized by an Attenuated Total Reflectance-Fourier Transformed Infrared Spectroscopy (ATR-FTIR) in the frequency range of 4000 - 400 cm^{-1} (Perkin Elmer). In addition, the silica were also determined for moisture content and soluble minerals content, including Na, K, Ca and Mg, by Atomic Adsorption Spectroscopy (AAS) [20].

The application of RHS and RSS for separation of oil, carotenes and vitamin E from POME

POME was first characterized and measured for pH, BOD, COD, total solids, suspended solids, total nitrogen, and oil concentration, followed by the APHA method [20]. Afterwards, oil was extracted from POME by *n*-hexane with an optimal ratio at 1:0.6 (POME:*n*-hexane), following Ahmad *et al*. [11]. The residual was measured as the oil value [4]. Afterwards, recovered oil was utilized for carotene and vitamin E separation.

A measure of 10 g of RHS and RSS was packed in a chromatography column with a 10-mm internal diameter. Adsorbent was packed in the column, and the study was done at room temperature. The column was equilibrated with *n*-hexane. The oil was firstly pretreated by heat at 40 °C and loaded onto the column, following Ahmad *et al*. [11]. About 2.4 g of recovered oil was loaded in the column to be in contact with the adsorbent. Thereafter, 100 mL of *n*-hexane was added, followed by 100 mL of ethanol.

The remaining oil in the column was then recovered by 50 mL of *n*-hexane. Fractions of 12.5 mL effluent were collected regularly in a receiving flask. Twenty fractions were collected in this study, in which fraction numbers 1 - 8 were eluted by 1st *n*-hexane, fraction numbers 9 - 16 were eluted by ethanol, and fraction numbers 17 - 20 were eluted by 2nd *n*-hexane. Each fraction was then determined for the concentration of their carotenes and vitamin E [19,21].

Analytical method

The carotenes and vitamin E content were quantitatively determined by UV-spectrophotometer, with absorbance at 446 and 295 nm, respectively [21,22]. Identification and analysis were carried out by comparing carotene and vitamin E standards. In addition, carotenes and vitamin E were qualitatively determined by using a high performance liquid chromatography (HPLC). The HPLC system was equipped with a system controller SCL-10Avp, an autoinjector SIL-10 ADvp, a pump FCV-10ALvp, a solvent delivery module LC-10ATvp, a column oven CTO-10Avp, and a diode array detector SPD-M10Avp. Chromatographic analysis was performed using an analytical scale (250 × 4.6 mm i.d.) C_{30} reversed phase column with a particle size of 5 µm. Eluent A consisted of methanol, trrt-butyl methyl ether (MTBE), and water (81:15:4, v:v). Eluent B was prepared by mixing MTBE, methanol, and water (90:6:4, v:v). Separation was performed at a column temperature of 15 °C by a linear gradient from 100 % A to 56 % B within 50 min at a flow rate of 1 mL/min. Identification of carotenes and tocopherols was based on retention time, co-injection with standards, and UV-VIS spectra. Quantification was carried out by external standards for all-*trans*-β-carotene, all-*trans*-α-carotene, (±)-α-tocopherol, and tocotrienol, monitored at their spectral maximum: all-*trans*-β-carotene (453 nm), all-*trans*-α-carotene (445 nm), α-tocopherol (292 nm), and tocotrienol (320 nm). Calculation of concentrations was based on linear calibration graphs [22,23].

Oil value was determined gravimetrically after removal of the solvent by a rotary evaporator [4]. Na, K, Ca, and Mg were determined by AAS using an air/acetylene flame. Stock standard solutions containing 1000 mg/L, in 2 % HNO_3, of each metal was used for the preparation of calibration standards and in the spiking experiments. All the analyses were carried out using AAS at the wavelengths specific for each metal (**Table 1**). For the preparation of the calibration curve, a series of 4 working standards were prepared for each metal. The working standards were prepared from the intermediate standards that were earlier prepared from the stock solutions (1000 mg/L) [24]. The moisture content of silica gels was determined using an air oven method [25]. All values are the means of triplicate analysis.

Table 1 Instrumental parameters for Na, K, Ca, and Mg determination in biodiesel samples by AAS.

Elements	Wavelength (nm)	Current (mA)	Spectral resolution (nm)	Gas
Na	589.0	3	0.8	C_2H_2/air
K	766.5	4	0.8	C_2H_2/air
Ca	427.7	3	1.2	C_2H_2/N_2O
Mg	285.2	2	1.2	C_2H_2/air

Results and discussion

Oil extraction from POME

POME is the waste generated from palm oil production processes. POME is a thick brownish liquid that contains high solids and organic compounds. POME from Krabi (Thailand) was first characterized. The BOD and COD of POME were estimated to be around 20,000 and 45,000 ppm, respectively. High amounts of oil (8,330 mg/L), total solids (40,000 mg/L), and suspended solids (19,020 mg/L) in POME were detected, and it contributed to high BOD and COD in the effluent [26]. pH and total nitrogen were also determined. The characteristic of POME (this study) are shown in **Table 2**. The characteristics of

POME were similar with the properties of POME as previously reported [27,28]. Untreated POME has high oil and organic contents, which are highly polluting. In addition, the effect of oil interferes with the transmission of light into water bodies, thereby retarding photosynthesis, and is a possible disruption of the food chain in the ecosystem [6,19]. Therefore, POME is a most harmful waste for the environment, and needs to be treated before discharge [7]. In this study, oil was extracted from POME following Ahmad *et al*. [4]. The oil was recovered from POME using *n*-hexane with a mixture ratio of 1:0.6. 6,500 mg/L of oil (with 78 % recovery) was retrieved. However, RHS could not adsorb oil fully, probably because of the carbon residue on the silica surface caused by incomplete carbonization at 500 °C. The similar adsorption efficiency of RSS was also observed. This is probably due to fewer adsorption sites and a smaller pore size [29]. The carotene concentration and total carotenes of oil were 580 ppm and 0.03 %, respectively. However, small amounts of vitamin E (160 ppm) with a total vitamin E at 0.01 % were observed (**Table 2**). Oil was further determined for the concentration of carotenes and vitamin E by HPLC. The HPLC spectrum indicated that oil contained α-carotene and β-carotene as major components at the retention times of 1.86 and 2.10 min, respectively. The percentage of α-carotene: β-carotene was 25:75 %. In addition, vitamin E, in terms of tocotrienols and tocopherols isomer, was also observed. Tocotrienols and tocopherols were eluted at the retention times of 4.56 and 7.50 min, respectively. The percentage of tocotrienols and tocopherols from the sample was 30:70 %. The composition of carotenes and vitamin E from oil was similar to palm oil. This was mainly because the samples originated from sterilizer condensate and sludge separator discharge in the palm oil mill [4]. BOD and COD content were significantly reduced, with 70 - 71 % reduction after the oil recovery process. However, total solid and suspended solid content was 5 % of reduction. The characteristics of POME after oil removal are also presented in **Table 2**.

Table 2 Characteristics of POME from palm oil mills (Krabi, Thailand).

Compositions	**Unit**	**POME**		**Oil residue from POME**
		Before oil removal	**After oil removal**	
Color		Brown	Brown	Light brown
pH		5.0	5.9	-[a]
BOD	ppm	20,000	6,000	-
COD	ppm	45,000	13,000	-
Total nitrogen	ppm	460	450	-
Total solid	mg/L	40,000	38,000	-
Suspended solid	mg/L	19,020	18,069	-
Oil content	mg/L	8,330	1,830	6,500
Carotenes	ppm	-	-	580
Vitamin E	ppm	-	-	160

-[a] : Data not determined

The separation of carotenes and vitamin E from oil

Due to the readily available source of POME and the growing value of carotenes and vitamin E, the oil from POME was selected and utilized as a source for carotenes and vitamin E. The study of the separation of carotenes from POME was already successfully done by Ahmad *et al.* [4] using adsorption chromatography. However, carotenes and vitamin E recovery though adsorption chromatography using RHS and RSS has never been done.

RHS and RSS were first prepared by following Kamath and Proctor [18] and Kalapathy *et al.* [19]. Silica content of RHS and RSS were estimated by EDX data, based on the assumption that all of the silicon was in the form of silica [19]. The silica content of RHS and RSS samples were 95 and 90 %, respectively. RHS and RSS had lower moisture contents in the range between 3.30 - 3.60 %. The impurities of silica gel were also determined using AAS (**Table 3**). The impurity contents were also compared with commercial silica. The results revealed that the Na, K, and Ca contents were similar. The major chemical groups present in isolated silica were identified by the ATR-FTIR spectra. The broad band between 1066 cm^{-1} was due to siloxane bonds (Si-O-Si). The peaks between 972 and 661 cm^{-1} were attributed to the vibration modes of the gel network [18,19].

RHA and RSA, an agricultural residue, are abundant in rice producing countries. RHA and RSA are rich in silica (about 92 - 95 %), highly porous, and lightweight, with a very high external surface area, and can be an economically viable raw material for the production of silica gels and powders [18,19]. The surface area and pore volume are highly dependent on the pyrolysis temperature. The surface area and pore volume decrease with increasing pyrolysis temperature. However, the optimum temperature which yields the highest surface area was 500 °C. RHS has a BET surface area of 345 m^2/g and a pore volume of 0.85 cm^3/g [29]. Its adsorbent and insulating properties are useful to many industrial applications. Silica from RHA has been evaluated as an adsorbent of minor vegetable oil components [27,29]. Although various uses for RHS and RSS have been suggested in the literature, their utilization remains a major concern [19]. In this study, RHS and RSS were applied as adsorbents in adsorption chromatography for carotene and vitamin E separation. The effectiveness and economic feasibility of RHS and RSS were also compared with the commercial silica.

Table 3 Mineral content of silica prepared from rice hull ash and rice straw ash.

Minerals	**Unit**	**Silica gel sources (This study)**		**Commercial silica**[a]	**Silica**[b]
		Rice hull ash	**Rice straw ash**		
Sodium (Na)	%	0.061	0.020	0.006	0.021
Potassium (K)	%	0.100	0.084	0.020	0.039
Calcium (Ca)	%	0.011	0.015	0.030	0.019
Magnesium (Mg)	%	0.010	0.010	Nd[c]	0.006
Silica content	%	95	90	98	-[d]
Moisture content	%	3.30	3.60	1.20	-

[a] Commercial silica from Merck (Germany)
[b] The information was obtained from [19]
[c] Nd = Not detectable
-[d] = No data

Table 4 Carotenes, vitamin E and oil recovery using rice hull silica (RHS) and rice straw silica (RSS) under *n*-hexane-ethanol system.

Silica	Oil		Carotenes			Vitamin E		
	Quality (g/L)	**Recovery (%)**	**Content (g)**	**Average conc. (ppm)**	**Recovery (%)**	**Content (g)**	**Average conc. (ppm)**	**Recovery (%)**
RHS[a]	4.60	70.77	0.69	1,902	68.32	0.12	289	11.88
RSS	4.32	66.46	0.50	1,555	49.50	0.09	250	8.91
Commercial	4.74	72.92	0.72	2,050	71.28	0.11	301	10.89
Commercial[b]	0.88	36.43	0.81	1,632	74.31	-[c]	-	-

[a]RHS = Rice hull silica; RSS = Rice straw silica
[b]data from Ahmad *et al.* [11] with carotene content obtained from hexane fraction and oil content from ethanol fraction.
-[c] data not available.

RHS and RSS were applied in the adsorption column chromatography. The separation method contained oil:silica at 1:5 under 40 °C using the *n*-hexane-ethanol system [11]. Extraction using solvent has several advantages. Currently, hexane, a solvent obtained from petrochemical sources, is the solvent used for oil extraction. It gives a higher yield and less turbid oil than mechanical extraction, and has a relative low operating cost. The amounts of carotenes, vitamin E, and oil after the recovery process are presented in **Table 4**. Carotene and vitamin E content recovered from RHS was higher than RSS. This may be due to their silica contents, which were related to their adsorption efficiency. The highest oil quality (4.60 g/L, 70.77 % recovery), carotenes (0.69 g, 68.32 % recovery) and vitamin E (0.12 g, 11.88 % recovery) were observed by column using RHS. Twenty fractions were recovered, and each fraction was determined for carotene and vitamin E content. Carotenes were recovered in the fraction number 2 which was eluted by 1st *n*-hexane. The average carotene concentration was in a range of 1,555 - 1,902 ppm. The results indicated that carotenes were soluble in *n*-hexane. However, vitamin E (0.09 - 0.12 g) and oil (4.32 - 4.60 g/L) remained in ethanol fraction (fraction number 9 - 16). Results indicated that carotenes, a non-polar compound, were soluble in non-polar solvent (*n*-hexane). However, the oil and vitamin E were attached to the adsorbent and eluted later by ethanol (a polar solvent) [11]. The carotene recovery was also compared with commercial silica and other works. The content and % of recovery was not significantly different. The separation of carotenes and vitamin E from POME under optimal conditions using RHS and RSS as adsorbents are illustrated in **Figure 1**. The figures show the sharp peaks on the chromatogram for carotene, oil, and vitamin E concentration. The highest carotenes (5,590 ppm) were presented at the fraction number 2 from the optimal condition using RHS. The concentration of carotenes was 10 times higher than the concentration of oil. However, the highest oil and vitamin E were eluted at fraction 12, with 4.60 g/L and 630 ppm, respectively. In addition, the highest % recovery of carotenes (68.32 %) and vitamin E (11.88 %) were also achieved using RHS.

Rice hull is a biological source of silicate in the form of tridymite and cristobalite [7]. The amorphous silica in RHA were considered to be the key material for the adsorption of carotenes and vitamin E. Amorphous silica from RHA can be extracted easily using low-temperature alkali extraction. The development of a method for recovering carotenes and vitamin E using RHS seems to be realistic, and makes it a promising method for industrial applications. However, the purification and application of carotenes and vitamin E seems to be an important issue to be considered in the development process.

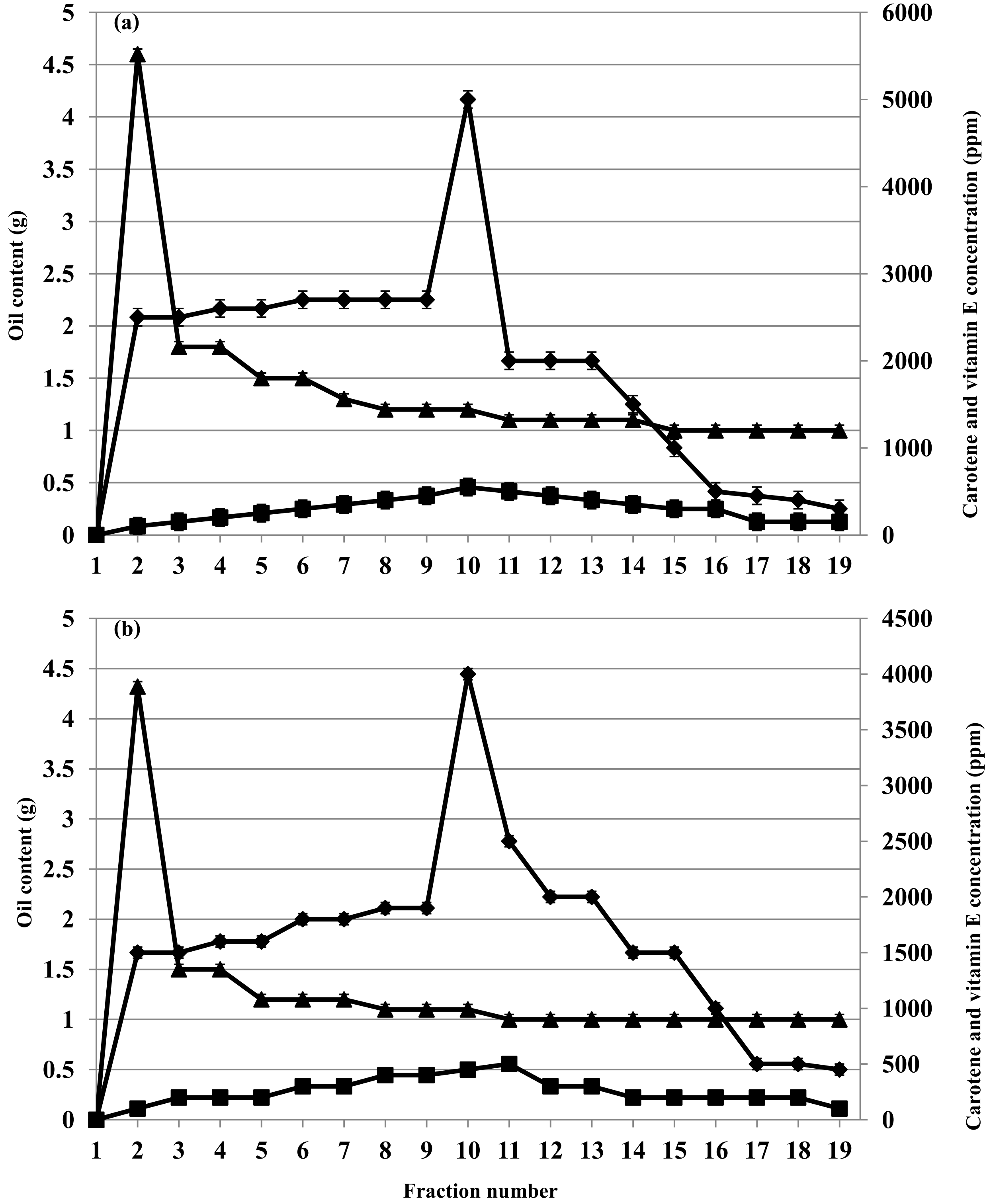

Figure 1 Carotenes (▲), vitamin E (■) and oil (♦) separated from oil from POME by adsorption column chromatography using rice hull silica (a) and rice straw silica (b) as adsorbents.

Conclusions

POME, liquid waste from palm oil mill, contains low nitrogen content with high BOD (20,000 ppm) and COD (45,000 ppm). However, POME retained several valuable products, such as carotenes and vitamin E, enabling its wide application in the chemical, food and pharmaceutical industries. To recover valuable products, POME was taken and the oil was extracted using *n*-hexane. Oil, carotenes, and vitamin E were recovered at concentrations of 6,500 mg of oil/L, 580 ppm, and 160 ppm, respectively. BOD and COD of POME were reduced significantly after the oil extraction process. α-carotene and β-carotene were presented as major components in oil. Afterward, the carotenes and vitamin E in oil were concentrated by adsorption chromatography using RHS and RSS as adsorbents. After the recovery process, 4.60 g/L of oil with high concentrations of carotenes (5,590 ppm) and vitamin E (630 ppm) was observed. The carotene and vitamin E concentrations were 10 and 4 times higher than the concentration in oil. RHS showed a higher ability to recover than rice straw silica. This suggested that carotenes and vitamin E can be recovered by rice hull silica using the *n*-hexane-ethanol system. The overall carotenes and vitamin E which can be recovered from this experiment were 70.77 and 11.88 % recovery, respectively.

Acknowledgement

The authors would like to thank the Agricultural Research Development Agency (Public Organization), the Thailand Research Fund (RTA5780002), and the Research and Development Institute, Thaksin University, for financial support. Finally, I would like to thank Mr. Christopher Joseph Forti (English adviser and English proof-reader, Thaksin University) for useful comments and suggestions on the language and structure of our manuscript.

References

[1] SH Goh, YM Choo and SH Ong. Minor constituents of palm oil. *J. Am. Oil Chem. Soc.* 1985; **62**, 237-40.

[2] A Embrandiri, MH Ibrahim and RP Singh. Palm oil mill wastes utilization; sustainability in the Malaysian Context. *Int. J. Sci. Res. Publ.* 2013; **3**, 1-6.

[3] K Sundram, R Sambanthamurthi and YA Tan. Palm fruit chemistry and nutrition. *Asia. Pac. J. Clin. Nutr*. 2003; **12**, 355-62.

[4] AL Ahmad, CY Chan, SR Abd Shukor and MD Mashitah. Recovery of oil and carotenes from palm oil mill effluent (POME). *Chem. Eng. J*. 2008; **141**, 383-6.

[5] E Caldwell. Tocotrienol form of natural vitamin E helps against stroke. Ohio State University Medical Center press release. Available at: http://www.medicalcenter.osu.edu/mediaroom/press/article.cfm?ID=2420&i=1, accessed May 2012.

[6] E Dauqan, HA Sani, A Abdullah, H Muhamad and AGM Top. Vitamin E and beta carotene composition in four different vegetable oils. *Am. J. Appl. Sci*. 2011; **8**, 407-12.

[7] PF Rupani, RP Singh, H Ibrahim and N Esa. Review of current palm oil mill effluent (POME) treatment methods: vermicomposting as a sustainable practice. *World Appl. Sci. J.* 2010; **10**, 1190-201.

[8] JC Iqwe and CC Onyegbado. A review of palm oil mill effluent (POME) water treatment. *Global J. Environ. Res.* 2007; **1**, 54-62.

[9] S Prasertsan and P Prasertsan. Biomass residues from palm oil mills in Thailand: An overview on quality and potential usage. *Biomass Bioenerg*. 1996; **11**, 387-95.

[10] MAB Habib, FM Yusoff, SM Phang, KJ Ang and S Mohamned. Nutritional values of chironomid larvae grown in palm oil mill effluent and algal culture. *Aquaculture* 1997; **158**, 95-105.

[11] AL Ahmad, CY Chan, SR Abd Shukor and MD Mashitah. Separation of oil and carotenes from palm oil mill effluent by adsorption chromatography with silica based adsorbent. *Asia-Pac. J. Chem. Eng.* 2009; **4**, 717-22.

[12] M Nitsche, W Johannisbauer and V Jordan. Process of obtaining carotene from palm oil. US Patent 5902890, 1999.

[13] BS Baharin, RA Latip, YB Che-Man and RA Rahman. The effect of carotene extraction system on crude palm oil quality, carotene composition, and carotene stability during storage. *J. Am. Oil Chem. Soc.* 2001; **78**, 851-5.

[14] CK Ooi and YC May. Recovery of Carotenes. US Patent 6072092, 2000.

[15] TYP Bonnie. Palm carotene concentrate from crude palm oil using vacuum liquid chromatography on silica gel. *J. Oil Palm Res*. 2007; **19**, 421-7.

[16] K Nesaretnam, WY Wong and MW Basri. Tocotrienols and cancer; beyond antioxidant activity. *Eur. Lipid Sci. Tech*. 2007; **109**, 445-52.

[17] N Othman, ZA Manan, SR Wanalwi and MR Sarmidi. A review of extraction technology for carotenoids and vitamin E recovery from palm oil. *J. Appl. Sci.* 2010; **10**, 1187-91.

[18] SR Kamath and A Proctor. Silica gel from rice hull ash: Preparation and characterization. *Cereal Chem*. 1998; **75**, 484-7.

[19] U Kalapathy, A Proctor and J Shultz. A simple method for production of pure silica from rice hull ash. *Bioresour. Tech.* 2000; **73**, 257-64.

[20] APHA (American Public Health Association). *In Standard Methods for the Examination of Water and Wastewater*. 20th ed. American Public Health Association, Washington DC, 1998.

[21] Porim Test Methods. *Palm Oil Research Institute of Malaysia*. Kuala Lumpur, 1995, p. 25.

[22] A Schieber, M Marx and R Carle. Simultaneous determination of carotenes and tocopherols in ATBC drinks by high-performance liquid chromatography. *Food Chem*. 2002; **76**, 357-62.

[23] N Saekung, W Chitdsmphandhvej, P Ketkaew, S Angkaew, W Tubgjaroenchai and W Somboon. *In*: Proceeding of the 10th International Seminar on Environment and Architecture and the 1st International Conference on Engineering Environment, Economic Safety and Health, Manodo, Indonesia, 2009, p. D-8.

[24] AOAC. *In Official Methods of Analysis*. 15th ed. Association of Analytical Chemists, Washington DC, 1990.

[25] Z Alias and IKP Tan. Isolation of palm oil-utilising, polyhydroxyalkanoate (PHA)-producing bacteria by an enrichment technique. *Bioresour. Tech.* 2005; **96**, 1229-34.

[26] M Ali Hassan, Y Shirai, N Kusubayashi, MIA Karim, K Nakanishi and K Hashimoto. The production of polyhydroxyalkanoate from anaerobically palm oil effluent by *Rhodobacter sphaeroides*. *J. Ferment. Bioeng*. 1997; **83**, 485-8.

[27] A Proctor. X-ray diffraction and scanning electron microscope studies of processed rice hull silica. *J. Am. Oil Chem. Soc.* 1990; **67**, 576-84.

[28] AN Ma. Environmental management for the oil palm industry. *Palm Oil Develop*. 2000; **30**, 1-10.

[29] M Kim, SH Yoon, E Choi and B Gil. Comparison of the adsorbent performance between rice hull ash and rice hull silica gel according to their structural differences. *LWT-Food Sci. Tech*. 2008; **4**, 701-6.