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Separation of Olein-Stearin from Crude Palm Oil at Different Temperatures and Solvents by Centrifugation

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This work accounts with separation of olein-stearin from crude palm oil by centrifugation at different temperatures (ambient temperature, 0, -25 and -80 ºC) and solvents (acetone, 2-propanol, 1-butanol and diethyl ether). Melting characteristics, triglycerides contents and fatty acid compositions in crude palm oil and stearin fractions at the different conditions were analyzed. Since crude palm oil samples at temperature below 0 ºC were in solid state, separation by centrifugation could not be carried out. It was also difficult to maintain the solutions at the given temperatures throughout an experiment; therefore, effects of solvent polarity were performed only at ambient temperature. Among the experimental conditions, the highest percent yield of stearin (12.03 %) was obtained from crude palm oil dissolved in 2-propanol, while 10.18 % was from crude palm oil sample. Triglyceride is a major constituent (92.5-100 %) in all the separated stearins. Studying on melting characteristics indicated that the stearin obtained from crude palm oil at ambient temperature has the lowest melting point, while the others do not correspond with solvent polarity index. In addition, analysis of fatty acid using GLC with capillary column showed that crude palm oil sample might comprise of some fatty acids which are not commonly found in biological materials and is under determined in this laboratory.

Key Words: Palm oil, Olein-stearin separation, Centrifugation, Triglyceride, Fatty acid.

INTRODUCTION

The palm tree belongs to a family of plants known as palme or palmaceae which include about 3,000-3,700 species grouped among 240-387 genera[1]. The most commonly planted in Thailand are *Eliaes guineensis* and its hybrid species. Mesocarp of the palm fruit is an excellent source of edible oil which could be extruded after cooking at 130 ºC for 1 h under pressure of 45 psi. The semi-solid product is called crude palm oil (CPO) which triglycerides are the major component (more than 90 %). The rests are free fatty acids 3-5 % and minor components such

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as β-carotene (provitamin A) and vitamin E (tocopherols and tocotrienols) *ca.* 1 %. Since trace amount of cholesterol was found in palm oil, it has been worldwide accepted as good quality dietary oil.

Malaysia is the largest palm oil producer in the world, while Indonesia and Thailand are in the second and third ranks, respectively. In 2003, Thailand produced 680,000 metric tons, but most of which are used as local dietary consumption. In comparison, exported values of the products from Malaysia were 3,875 million US dollars, whereas much less of 7.4 million US dollars was exported from Thailand.

Apart from dietary products, palm oil could be used as precursor of other industries such as biodiesel, glycerol and its derivatives, surfactants in cosmetic industries, lubricant, hydraulic, gear box for engines, synthetic polymer *i.e.* polyurethanes, polyamides, polyamidoamides, non-nylon polyamides and modified epoxy resin. It should be noted that most of the Malaysian products are in form of value added, palm olein and oleochemicals while crude palm oil contributes only 10 %.

Physico-chemical properties of triglyceride and its applications depend upon fatty acid constituents in molecule. The differences are due primarily to chain length, degree and position of unsaturation. The shorter chain fatty acids have lower melting point than the longer ones. The higher degree unsaturation exhibits the lower melting point than those of saturated fatty acids of the same chain length. Also, numerous studies have been shown that qualities of edible oils depend largely on fatty acid compositions[2,3].

In general, the two most predominant fatty acids in palm oil are palmitic acid (C16:0; 44 %) and oleic acid (C18:1; 40 %), while linoleic acid (C18:2) and stearic acid (C18:0) are 10 and 5 %, respectively. However, a great variation in the number and concentrations of fatty acid constituents among the palm oils from different origins as well as among different varieties is revealed[1,4].

Food industry requires concentration of the middle range of triglycerides to obtain a product with a desired melting character. Thus separation and fractionation processes are essential part of the oil industry. At present, two conventional processes predominate *i.e.*, vacuum distillation and solvent extraction. However, the isolation of substances from natural products remains problematic, especially with thermally labile materials such as polyunsaturated fatty acids and their glycerides. Vacuum distillation requires relatively high temperature, result in decomposition or degradation of these substances. Some of the disadvantages of solvent extraction are the tendency for the solvent to remain in the product after extraction and the continually shrinking list of safe solvents. For these reasons, much effort has centered on the development of separation and fractionation methods that are clean, efficient and free of thermal degradation of substances.

Although the numerous studies of fatty acid compositions in palm oil elsewhere have been reported, information from Thailand products has limited. Accordingly, the present study attempts to gain knowledge base of crude palm oil product from Thailand which is an essential background in health food and nutraceutical applications.

The efficiency of using centrifugation techniques and in combination with using some solvents for separation of olein and stearin was also evaluated. In addition, some properties, *i.e.* melting characteristics, triglyceride contents as well as fatty acids compositions of the stearin samples separated by the different conditions were included.

EXPERIMENTAL

Sample collection and stocking: Crude palm oil sample was obtained from Satun Industries Co., Ltd., Satoon, Thailand. To make well comparable data, the sample used throughout these studies was collected from the same batch and was separately stocked under nitrogen in 500 mL tightly sealed glass bottles at 4 ºC. To each experiment, appropriate amounts were taken and were slightly warmed with stirring until homogeneous sample was obtained.

Effects of temperature on the yield of olien-stearin separation: The effects of temperature on yield of olein-stearin separation were planned to evaluate at four conditions, *i.e.* ambient temperature, 0, -25 and -80 ºC. The homogeneous sample was transferred into centrifuge tube and weight. They were left at the desired temperature until equilibrium was reached. Unfortunately, the samples left at 0 ºC and lower were in solid state, therefore, centrifugation could not be performed. Olein-stearin appeared for the samples left at ambient temperature after standing over night. They were fractionated by centrifugation at 7,741g for 20 min (Beckman, J-30i). Stearin fraction was filtered with known weight Whatman filter paper No. 1 under vacuum and washed twice with acetone in cold room at 0-4 ºC. The filter paper with stearin was put in desiccator and was further dried under high performance vacuum (0.05 millibar; Franklin electric, U.S.A.), then was weighed with analytical balance (Mettler, AE 240).

Effects of solvent on the yield of olien-stearin separation: Preliminary study in this laboratory indicated that stearin and olein were not formed by dissolving crude palm oil in low polarity solvents such as hexane. In contrast, phase separation appeared when the sample was dissolved in the higher solvent polarity. In addition, toxicity to human health was also taken into consideration. Accordingly, four solvents were selected to investigate the effects of solvent polarity on separation of stearin and olein, namely, acetone, 2-propanol, 1-butanol and diethyl ether which were in order from the higher to the lesser polarity index. Experiment was carried out by mixing 10 g of the homogeneous crude palm oil sample in centrifuge tube with 10 mL of the selected solvents. To prevent a solvent evaporation, the tubes were sealed with aluminium foil and paraffin and were stood over night at ambient temperature. They were centrifuged at room temperature on top bench centrifuge at 756 g for 20 min.

Analysis of triglyceride: Triglyceride was determined using calorimetric techniques as the procedure described by Van Handel and Zilversmith[5]. Various concentrations (125-2000 µg/mL) of tri-stearin (Sigma Chemicals) were prepared by dissolving in chloroform and were used as standard triglyceride. Standard curve was performed by taking aliquot of 100 μL (12.5-200 μg) of the solutions and drying at 70 ºC. Saponification was achieved by adding 0.5 mL of 1 % KOH in alcohol, heating at 70 ºC for 20 min. The solutions were neutralized with 0.5 mL 0.2 N H_2SO_4 at 70 ºC for 10 min. After cooling, 0.1 mL of 0.5 % sodium metaperiodate was added, followed by 0.1 mL of 5 % sodium bisulfite. The colour was developed by adding 3 mL of 0.25 % chromotropic acid reagent and heating in boiling water for 0.5 h. Then, an absorbance of the colour solutions was determined with spectrophotometer (Shimazu U160A) at wavelength of 570 nm. Similar procedure was performed for unknown samples and their triglyceride contents were determined from the standard curve.

Melting characteristics: Melting characteristics of sample were analyzed with differential scanning calorimeter (Perkin-Elmer, 7 DSC). Accurately weight of 3-5 mg was introduced into aluminium pan and sealed with a pan crimper. Initial temperatures were set at -10 and 25 ºC for crude palm oil and stearin samples, respectively. After holding at the initial temperatures for 1 min, they were heated to 25 and 90 ºC by a raising rate of 10 ºC min^{-1}.

Preparation of fatty acid methyl ester (FAME): Fatty acid methyl ester (FAME) was prepared as the procedure recommended by Morrison and Smith[6]. In brief, 50 mg sample was accurately weighed in ampule. It was mixed with 1 mL of boron trifluoride-methanol (140 g BF_3 per liter of methanol), flushed with nitrogen free oxygen (OFN) and sealed to minimize oxidation. The reaction mixture was left in an oven at 100 ºC for 45 min. After cooling, fatty acid methyl esters were extracted by adding 1 volume of water and 2 volumes of hexane. The fattty acid methyl ester in hexane layer was taken and dried with nitrogen free oxygen stream. The residue was re-dissolved in hexane to obtain an optimum concentration for further analysis with GLC.

Determination of fatty acid methyl esters: Fatty acid methyl esters were analyzed on Hewlett-Packard 5890 series chromatograph equipped with flame ionization detector (FID). One μL of sample was injected at 220 ºC in the splitless mode onto a non-polar bondable PEG capillary column (HP-Innowax 30 m × 0.32 mm i.d., 0.5 μm film thickness, Hewlett Packard) using a Hewlett Packard (HP 7673) automatic sampler. The oven temperature was programmed as following: initiated with 140 ºC for 1 min, increased to 190 ºC with the rate of 25 ºC/min and maintained at this temperature for 25 min, raised to 220 ºC with the rate of 3 ºC/min and further maintained at this temperature for 0.5 h. Helium was used as a carrier gas with a flow rate of 1 mL/min. The detector temperature was 275 ºC. Peak identification of unknown was determined by comparison with the relative retention time (RRT) of 21 standard fatty acids (Sigma, U.S.A.).

RESULTS AND DISCUSSION

Effects of temperature and solvents on fractionation of olein-stearin: Since crude palm oil samples at 0, -25 and -80 ºC, crude palm oil were in solid state, centrifugation at the conditions could not be performed. The results reflected the high melting properties of samples which may due to an effect of the two most predominant constituents in crude palm oil *i.e.* palmitic acid and oleic acid.

Table-1 shows that stearin of 10.18 % could be fractionated from crude palm oil after standing the samples at ambient temperature over night. Slightly higher percent yields were obtained when the crude palm oil was dissolved in acetone and 2-propanol. However, the use of lower polarity solvent (diethyl ether), the lower stearin content could be separated.

TABLE-1
PER CENT YIELD OF STEARIN FRACTIONATED FROM CRUDE PALM OIL (CPO), CPO IN ACETONE, 2-PROPANOL, 1-BUTANOL AND DIETHYLETHER WITH 1:1 RATIO (w/v)*

Sample	Yield (%)
CPO	10.18
CPO:Acetone	10.25
CPO:2-Propanol	12.03
CPO:1-Butanol	10.20
CPO:Diethyl ether	5.125

*Values are the mean of 4 replications

Triglyceride contents in stearin: Results in this study indicated that the CPO sample comprising of 92.5 % triglyceride which is slightly lower than that reported by Ong *et al.*[7]. The variation may be due to the difference of procedure used for determi-nation. The contents observed in stearin fractionated were shown in Table-2. It was found that the stearin sample CPO:1-butanol contained 100 % triglyceride which consisted of high proportion of saturated fatty acid. Therefore, much less saturated fatty acid was left in the olein fraction. Moreover, it would be easier for extracting other minor components such as β-carotenes (provitamin A), vitamin E (tocopherols and tocotrienols), *etc*.

TABLE-2
TRIGLYCERIDE CONTENTS (%) IN STEARIN FRACTIONATED FROM CRUDE PALM OIL (CPO), CPO IN ACETONE, 2-PROPANOL, 1-BUTANOL AND DIETHYL ETHER WITH 1:1 RATIO (w/v)

Sample	Triglyceride content* (%)
CPO	92.5
CPO:Acetone	95.6
CPO:2-Propanol	98.5
CPO:1-Butanol	100.0
CPO:Diethyl ether	97.1

*Values are the mean of 4 replications.

Melting characteristic: From the experiment in this study, the melting point of crude palm oil before a centrifugation was 3.5 ºC. The results of melting thermograms of stearin separated at different conditions were shown in Fig. 1. The melting

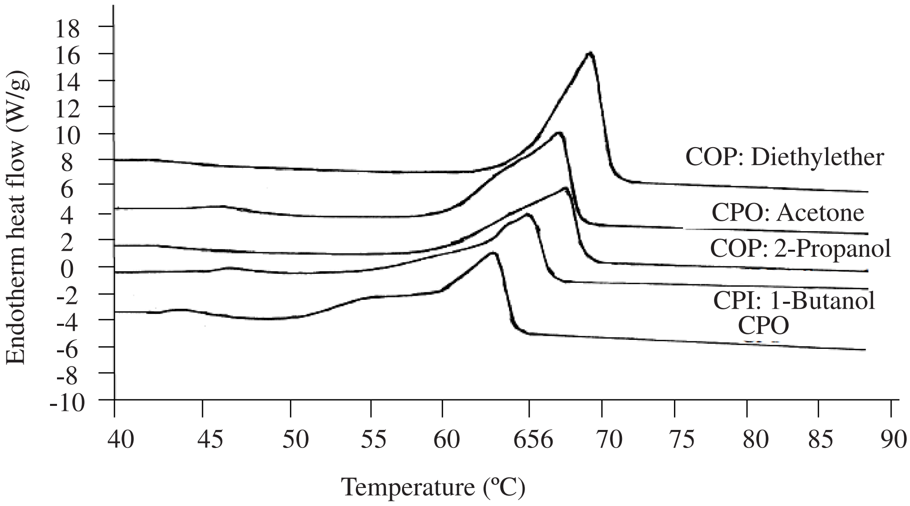

Fig. 1. DSC thermogram of the stearin fractionated from crude palm oil (CPO), CPO in acetone, 2-propanol, 1-butanol and diethyl ether with 1:1 ratio (w/v)

thermogram of the stearin separated from crude palm oil sample has the lower melting point than the samples of crude palm oil in acetone, 2-propanol, 1-butanol and diethyl ether. At least two clear peaks were observed in the heating thermogram of the crude palm oil sample, indicative of more than two melting forms present in the sample. A high melting peak at 63 ºC was obtained. While the thermograms of crude palm oil samples in acetone, 2- propanol, 1-butanol and diethyl ether illustrated a sharp endotherm at 67.5, 68.0, 65.7 and 68.7 ºC, respectively. The high melting components at 65-69 ºC could be due to the high proportion of saturated fatty acid. Therefore, C16:0 and C18:0 would be including in the palm stearin. However, from the experimental data[8] illustrated that 44 % C16:0 contained in crude palm oil while only 4.5 % C18:0 was found, this confirms that C16:0 would be a major component in the palm stearin rather than other saturated fatty acids.

Fatty acid analysis: Fig. 2 shows chromatogram of 21 standard fatty acid methyl esters which are commonly found in biological samples. This suggested that the use of GLC chromatographic conditions as described above provided well resolution for fatty acid analysis. With identical conditions, the satisfied result was also obtained by injection of the low concentration of atty acid methyl esters from crude palm oil (Fig. 3). In consistent with other reports, C16:0 and C18:1ω9 appeared to be the predominant constituents in crude palm oil.

It was noted, however, that when the higher concentration of fatty acid methyl esters from crude palm oil was injected, asymmetric peak was observed around C18:0. This suggested the co-elution of unknown fatty acids associated with C18:0 and that was illustrated in Fig. 4 while the more amount of sample was injected. Therefore, it was presumed that crude palm oilmight consist of uncommon fatty

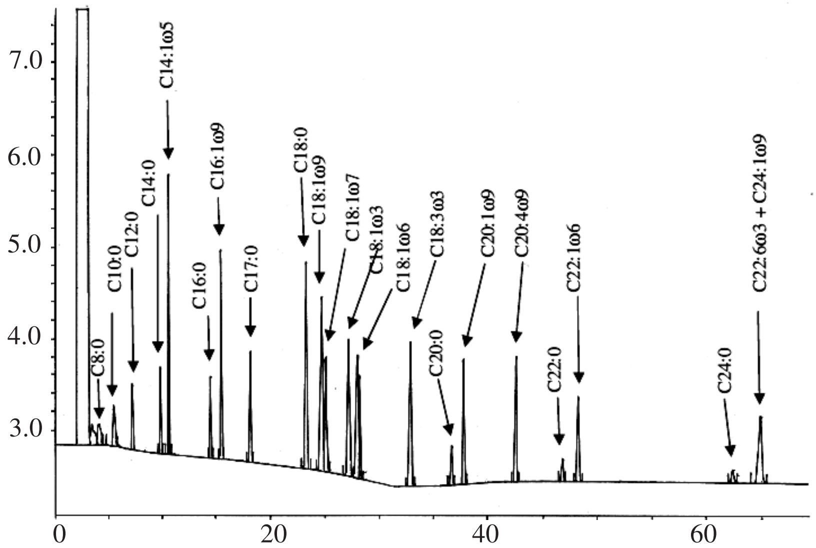

Fig. 2. Chromatogram of 21 standard fatty acid methyl esters

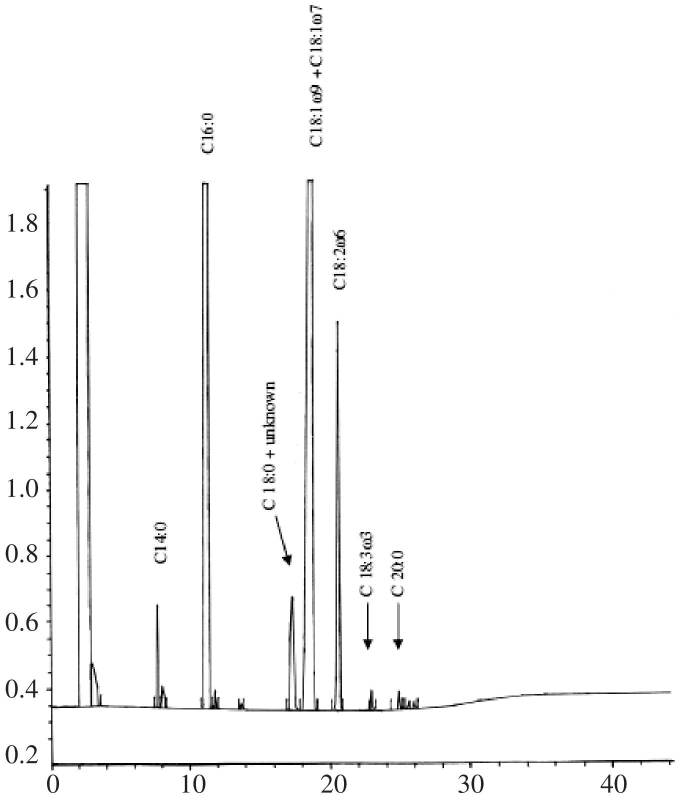

Fig. 3. Fatty acid profile of crude palm oil by injection of the low concentration of fatty acid methyl esters

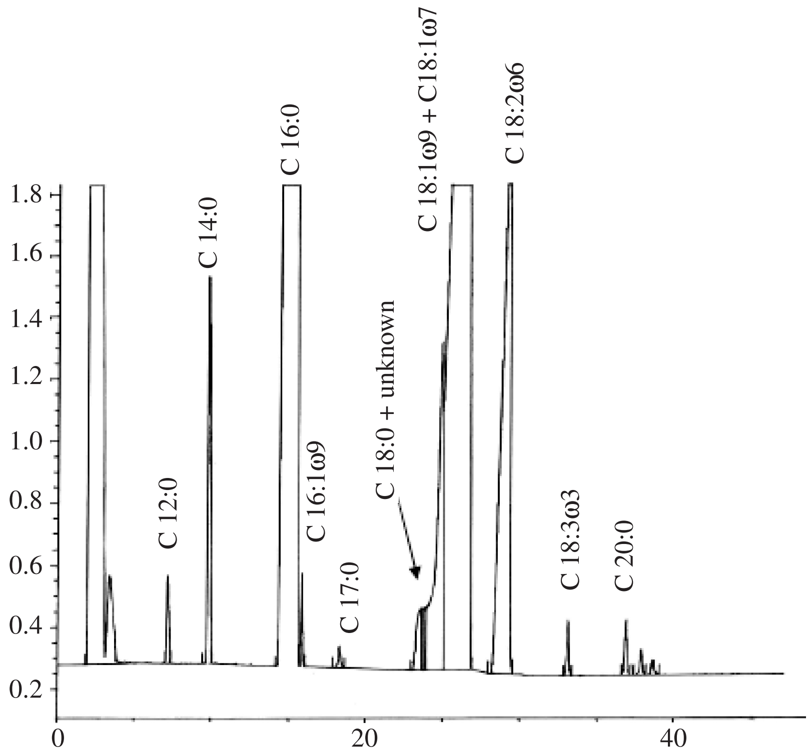

Fig. 4. Fatty acid profile of crude palm oil by injection of the higher concentration of fatty acid methyl esters

acids which have never been found in literature such as branch chain, unusual trans-isomers as well as others fatty acid derivatives. This is under investigating in this laboratory in order to re-evaluate the nutritional value of palm oil.

Conclusion

Separation of stearin from crude palm oil by centrifugation could not be achieved at temperature lower than 0 ºC, but it was easily performed at ambient temperature with the yield of 10.2 %. Relatively similar yield was obtained when crude palm oil was dissolved in acetone, 2-propanol and 1-butanol. It was found that triglyceride is principal constituent in stearin separated from all experimental conditions. Studying on melting characteristics of stearin separated at different conditions indicated that the sample obtained form crude palm oil at ambient temperature has the lowest melting point. Results indicated, however, that melting point of the stearin separated from the different solvents does not correspond with polarity index. Analysis of fatty acid using GLC with capillary column showed that crude palm oil sample might comprise of some fatty acids which are not commonly found in biological materials.

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REFERENCES

1. P.S. Bora, R.V.M. Rocha, N. Narain, A.C. Moreira-Montereiro and R.A. Moreira, *Bioresour. Technol.*, **87**, 1 (2003).
2. A.S. Ong and S.H. Goh, *Food Nutr. Bull. Mar*; **23**, 11 (2002).
3. K. Sundram, *Asia Pacific J. Clin. Nutr.*, **6**, 12 (1997).
4. S. George and C. Arumughan, *J. Sci. Food Agric.*, **56**, 210 (1991).
5. E. Van Handel and D.B. Zilversmith, *J. Lab. Clin. Med.*, **50**, 152 (1957).
6. W.R. Morrison and L.M. Smith, *J. Lipid Res.*, **5**, 600 (1964).
7. S.H. Ong, C.C. Chuah and P.L. Boey, in eds.: D.A. Earp and W. Newall, Preliminary Study on Olein Stearin Separation of Palm Oil, In International Developments in Palm Oil, Rajiv Printers, Kuala Lumpur, pp. 281-290 (1977).
8. A. Noh, N. Rajanaidu, A. Kushairi, Y. Mohdrafii, A. Mohd Din, Z.A. Mohd Isa and G., Saleh, *J. Oil Palm Res.*, **14**, 18 (2002).

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