EFFECT OF DEGUMMING PROCESS ON CHROMATOGRAPHIC SEPARATION OF CAROTENES FROM CRUDE AND DEGUMMED PALM OIL

L.L.YOU, B.S. BAHARIN[1] and Y.B. CHE MAN

Department of Food Technology
Faculty of Food Science and Biotechnology
University Putra Malaysia
Selangor D.E., Malaysia

AND

S. TAKAGI

Department of Clinical Nutrition
Faculty of Medical Welfare
Kawasaki University of Medical Welfare
Japan

Received for Publication October 24, 2000
Accepted for Publication December 4, 2000

ABSTRACT

Carotene from crude palm oil (CPO) and degummed palm oil (DPO) was separated using synthetic polymer adsorption chromatography. Diaion HP-20 adsorbent was used for reverse-phase chromatography and column temperature was kept at 50C. Both 2-propanol and n-hexane were used as the first and the second eluting solvents, respectively. Phosphoric acid was used to remove the minor components in CPO in the degumming process. The turbidity of DPO was lower than that of CPO and the retention time in the column for DPO was shorter (21.3 min) compared to CPO (29 min) due to the removal of the minor components by phosphoric acid. Degumming process caused a reduction of 1.67 mg of total carotene content and 55.67 ppm of carotene concentration in CPO. This is probably due to the removal of carotenoids during the degumming process. After column chromatography, carotene recovery for CPO was 30.23% and 54.82% for the first and second fractions, respectively. On the other hand, carotene recovery for DPO was 33.91% for the first fraction and 46.25% for the second fraction. This indicated that the separation of carotene in CPO was more efficient than DPO in the column chromatography due to the longer retention time in the column.

[1]Corresponding author: TEL: 603-89486101 ext: 3436; FAX: 603-89423552; E-mail: badli@fsb.upm.edu.my

Journal of Food Lipids **8** (2001) 27-35.

Under the process conditions investigated, carotene compositions were not affected by the degumming and column chromatography process.

INTRODUCTION

Various methods for extracting carotenes from palm oil have been developed, including saponification, adsorption, precipitation, selective solvent extraction and transesterification as well as molecular distillation (Choo 1995). However, only transesterification and distillation have further been developed into commercial-scale processes (Iwasaki and Murakoshi 1992). Unfortunately, in this process, no edible oil is obtained due to the chemical conversion of triacylglycerols to methyl esters. In order to overcome this matter, an adsorption chromatographic process using synthetic polymers has been developed (Baharin *et al.* 1998; Abd. Latip *et al.* 2000a, b). This process is envisaged to be included in the pretreatment stages of CPO refining.

Today, about 90% of Malaysia's palm oil exports are in fully refined or processed forms. The first physical refining plant in Malaysia was erected and operated by Palmex Industries Sendirian Berhad in 1975 (Tang *et al.* 1983). At present, there are more than 20 factories in Malaysia having physical refinery plants. The refining technique consists of water or acid degumming, alkaline refining, bleaching and deodorization (Mohd 1994).

The aim of refining is therefore to convert the crude palm oil to high quality edible oil by removing objectionable impurities to the desired levels in the most efficient manner, if possible, while minimizing losses in the desirable components.

Degumming is an important pretreatment process in palm oil refining. Gum is a general term used to define minor components contained in crude palm oil such as phospholipid, glycolipid, resin-like matter, among others. In this study, phosphoric acid was used to remove the gum from crude palm oil. This degumming process is one of the refining processes which is usually carried out at a temperature of 65-75C. Lower or higher temperatures are not advantageous because at lower temperatures the viscosity of the oil is too high, whereas at 75C and higher, degumming is not completed due to increased solubility of gums. If degumming has no effect on the carotene recovery, crude palm oil can directly be loaded onto the chromatography apparatus. On the other hand, if degumming has a significant effect on the carotene recovery the chromatographic process will be carried out after the degumming treatment.

MATERIALS AND METHODS

Palm Oil and Chemicals

Crude palm oil (CPO) was obtained from Sime Darby Plantation in Klang, Malaysia. The oil sample was stored in a cold room at 4C. All solvents and chemicals used were of analytical grade. Synthetic highly porous resin (Diaion HP-20), a styrene-divinylbenzene copolymer, was obtained from Mitsubishi Chemicals Company (Tokyo, Japan).

Preparation of Degummed Oil

In the degumming process, 0.4 g of phosphoric acid was added into 200 g of melted crude palm oil and maintained at 60C. This mixture was stirred for 5 min at 60C. After adding Pelite filter aid (Sigma Chemical Co., St. Louis, MO), the mixture was stirred again for another 2-3 min at 60C and subsequently filtered.

Column Chromatography of CPO

The chromatographic column was a glass tube (3 cm i.d. by 35 cm length) with an outer jacket for circulating heated water. Ninety grams of HP-20 were slurried with 2-propanol (IPA) before packing it into the column to a height of 10 cm. Then the column was left to equilibrate overnight. Thirty five grams of melted crude palm oil (60C) were loaded onto the column bed which was kept at 50C. The initial solvent utilized was 550 mL of IPA followed by 250 mL of n-hexane as the eluting solvents. The two fractions were then analyzed. This procedure was repeated by using DPO which had been prepared earlier. Fractions were collected by a fraction collector. The oil content of each fraction was determined gravimetrically after removal of solvent by rotary evaporation at 60C. The turbidity of CPO and DPO before and after column chromatography and quantitative dilution with hexane was possible by recording the secondary the absorbance at 660 nm using a spectrophotometer (Hitachi U-2000, Japan). The absorbances taken were based on apparent turbidity. For determination of total carotene content, 25 mL of n-hexane were added to 1 mL of each fraction to determine the absorbance at 446 nm by spectrophotometer (Hitachi U-2000, Japan). The total carotene content was calculated with E_{446nm} in hexane (2450).

High Performance Liquid Chromatographic (HPLC) Analysis of Carotenes

Carotene components were determined by using HPLC model "LC 10 AS Shidmadzu Liquid Chromatography" with UV detector. Column used was YMC J'Sphere ODS H80 S-4 (Shimadzu Company, Tokyo, Japan), 80A (4.6 mm i.d x 25 cm) while the isocratic mobile phase was acetonitrile/dichloromethane (4:1, v/v). The flow rate was fixed at 1.0 mL/min. Twenty microliters of sample were

injected into the HPLC and the carotene content was determined at 446 nm.

RESULTS AND DISCUSSION

Comparison of Crude and Degummed Palm Oils

Turbidity. The absorbance value obtained was based on apparent turbidity of the sample. From the results obtained, as shown in Table 1, the turbidity for DPO was lower than that of CPO. The lower absorbance value recorded for DPO was expected because some of the pigments in CPO had been removed during the degumming process.

TABLE 1.
CHARACTERISTICS AND MINOR COMPONENTS OF CRUDE PALM OIL (CPO) AND DEGUMMED PALM OIL (DPO)

Sample	OD_{660nm}	Retention Time (min)	Oil Quantity (g)	Total Carotene Content (mg)	Carotene Concentration (ppm)
CPO	0.13±0.01[a]	29.0±1.0[a]	35.03±0.06	23.82±0.01[a]	680.67±0.01[a]
DPO	0.11±0.01[b]	21.3±2.1[b]	35.00±0.01	21.88±0.01[b]	625.00±0.20[b]

Mean ± standard deviation of three experiments. Mean within each column with different superscript are significantly ($p<0.05$) different.

Retention Time. The retention times of both CPO and DPO in HP-20 column by using IPA were determined by the equation below.

Retention time = (volume of adsorbent; cm^3)/(volumetric flow rate; cm^3/s)

The retention time for CPO was longer, 29.0 min, and the flow rate was 6.2 mL/min, due to its high viscosity which was contributed by the presence of minor components in the oil. Most of these minor components had been removed in DPO. DPO of a lower viscosity was able to pass through the column much easier (retention time 21.3 min) and the flow rate was 8.45 mL/min (Table 1).

Total Carotene Content and Carotene Concentration. The total carotene content and carotene concentration were determined by using the equations below.

Total Carotene Content = (Absorbance x Dilution Factor)/ (2450)*
Carotene Concentration = (Total Carotene Content in mg x 10^6) x (Oil Quantity in g)
* $E_{446\,nm}$ in n-hexane.

As can be seen from Table 1, there was a reduction of 1.67 mg of total carotene content and 55.67 ppm for carotene concentration in DPO. This was probably due to the removal of the carotenoids during the degumming process.

Adsorption Chromatographic Behavior of CPO and DPO. The porous polymer, Diaion HP-20 is a nonionic styrene-divinylbenzene copolymer and is hydrophobic. The HP-20 resin was used in this experiment due to its high adsorption capacity for carotenoids. Aniza (1995) reported that HP-20 was the best adsorbent compared to silica gel, activated alumina and cyclodextrin. The HP-20 resin was found to be efficient in adsorbing carotenoids probably due to the hydrophobic interaction with carotenoids which are also hydrophobic in nature. Organic solvent, IPA was used to elute the oil from the column because lower alcohols are known as good solvents for CPO whereas n-hexane was used to elute the carotene. This chromatographic adsorption method was a reverse phase system where the stationary phase was nonpolar and a higher polarity solvent such as IPA was used followed by low polarity solvent such as n-hexane. Moreover, compared to components that are nonpolar, polar components were eluted first from the column.

In Table 2, the first fraction in which CPO was eluted with IPA, showed that almost 87.80% of the oil had been eluted whereas only a small amount, 2.37% of the oil was obtained in the second fraction which was eluted with n-hexane. By comparing both values of carotene concentrations before chromatography (23.82 mg from Table 1) and after chromatography (20.26 mg), a reduction of 14.94% in carotene concentration was noticed. This meant that there were carotenes still adsorbed on the resin. The first fraction that was rich in oil (or triacylglycerol) had a total carotene content of 7.20 mg and the second fraction, which was rich in carotene (13.06 mg). Thus, about 30.23% and 54.82% of carotene recoveries can be obtained in the first and second fractions, respectively.

TABLE 2.
OIL QUANTITY, TOTAL CAROTENE CONTENT, CAROTENE CONCENTRATION AND CAROTENE RECOVERY OF CPO AND DPO AFTER HP-20 TREAMENT USING ISOPROPANOL-HEXANE SYSTEM

Sample	Fraction	Oil Quantity (g)	Oil Recovery (%)	Total Carotene Content (mg)	Carotene Concentration (ppm)	Carotene Recovery (%)
CPO	First	30.73	87.80	7.20	234.30	30.23
	Second	0.83	2.37	13.06	15734	54.82
	Total	31.56	90.17	20.26	641.95	85.05
DPO	First	29.08	83.09	7.42	255.16	33.91
	Second	0.90	2.57	10.12	11244	46.25
	Total	29.98	85.66	17.54	585.06	80.16

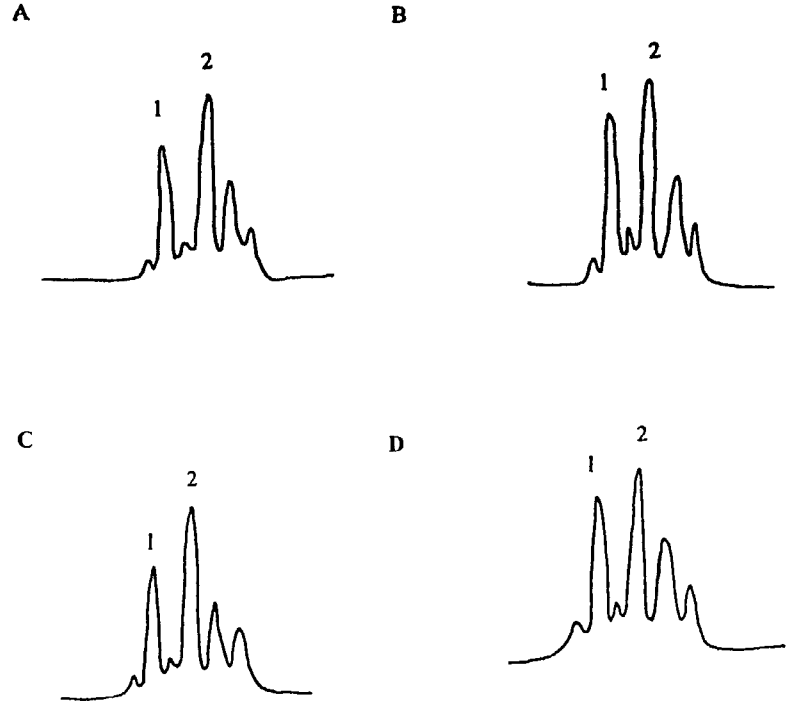

FIG. 1. CHROMATOGRAM FOR CPO AND DPO BEFORE AND AFTER CHROMATOGRAPHY
A: CPO ; B: DPO (Before Chromatography)
C: CPO; D: DPO (After Chromatography)

Peak 1:α-carotene
Peak 2: β-carotene

As can be seen in Table 2, 83.09% of oil quantity was recovered in the first fraction and 2.57% in the second fraction. Carotene concentrations of DPO before and after treatment were 21.88 mg (from Table 1) and 17.54 mg, respectively. Therefore, there was about 20% reduction in carotene concentration in treated DPO. The first fraction that was rich in oil showed 33.91% carotene recovery while for the carotene rich fraction, the total carotene recovery was 46.25%.

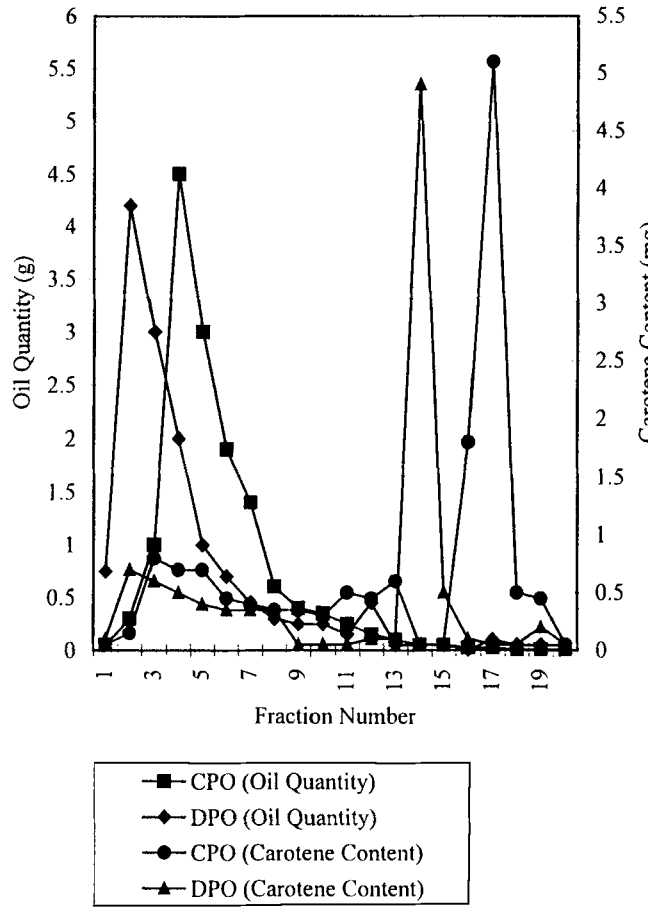

FIG. 2. ELUTION PROFILE FOR CPO AND DPO

In these results, the second fraction obtained show very high carotene concentrations of 15734 ppm and 11244 ppm for CPO and DPO, respectively. These results indicate that the carotene was much concentrated in the second fraction that was eluted with n-hexane. Furthermore, the carotene recovery in the second fraction for CPO was higher than that of DPO. This indicated that separation of carotene from palm oil was more efficient in CPO, perhaps due to the longer retention time of CPO in the column allowing more of the carotene to be adsorbed onto the resin.

Analysis of Carotene Components. Tan *et al.* (1986) reported in detail the separation and identification of carotene in several palm oil fractionation processes

in Malaysia. Seven hydrocarbons which have not been reported previously were identified, namely, phytoene, phytofluene, zeta-carotene, α-zeacarotene, β-zeacarotene, neurosporene and δ-carotene. Furthermore, α-carotene, β-carotene, and lycopene were also identified. Because of their sensitivity to oxidation, carotenoids should be chromatographed immediately after extraction. Figure 1 shows the chromatograms for CPO and DPO before chromatography and after chromatography. Peaks obtained were identified as α-carotene and β-carotene. Based on the number of peaks obtained in the chromatogram it was found that similar peaks were obtained for both CPO and DPO. This clearly indicated that the degumming process did not degrade any of the carotene components in the CPO.

Elution Profile of Carotene Recovery by Adsorption Column Chromatography. Oil quantity and carotene content were calculated for each fraction which were collected by a fraction collector. For IPA fraction in Fig. 2, maximum oil quantity was obtained in fraction number 4 and number 2 for CPO and DPO, respectively. This meant that DPO can easily be eluted from the column compared to CPO. For n-hexane fraction in Fig. 2, maximum carotene content was obtained in fraction 17 and 14 for CPO and DPO, respectively. The results obtained showed that large amounts of carotene were eluted from the column by n-hexane.

CONCLUSION

Based on the results obtained, it may be concluded that degumming is not necessary before the carotene extraction process in column chromatographic process although it is still an important pretreatment step in the refining of CPO.

ACKNOWLEDGMENT

This study was carried out with financial support from IRPA (Intensification of Research in Priority Area) Grant No. 03-02-04-0036 of the National Council for Research and Science Development in Malaysia. The supply of CPO from Sime Darby Plantation in Klang, Selangor, Malaysia is gratefully acknowledged.

REFERENCES

ABD. LATIP, R., BAHARIN, B.S., CHE MAN, Y.B. and ABDUL RAHMAN, R. 2000a. Evaluation of different adsorbents for carotene extraction from crude palm oil by adsorption chromatography. J. Amer. Oil Chem. Soc. *77*, 1277-1281.

ABD. LATIP, R., BAHARIN, B.S., CHE MAN, Y.B. and ABDUL RAHMAN, R. 2000b. The effect of adsorption and solvent extraction process on the percentage of carotene extracted from crude palm oil. J. Am. Oil. Chem. Soc. *78*, 83-87.

ANIZA, A.1995. Extraction of α-carotene components from crude palm oil using resin chromatography method. BSc.(Biotechnology) Thesis, University Pertanian Malaysia, Serdang, Selangor, Malaysia.

BAHARIN, B.S., ABDUL RAHMAN, K., ABDUL KARIM, M.I., OYAIZU, T., TANAKA, K. and TANAKA, S. 1998. Separation of palm carotene from crude palm oil by adsorption chromatography with synthetic polymer adsorbent. J. Am. Oil Chem. Soc. *75*, 399-404.

CHONG, C.L. 1984. Chemical and physical properties of palm oil and palm kernel oil. In *Selected Reading on Palm Oil and Its Uses.* Palm Oil Familiarization Programme POFR. PORIM. (1994). Revised Ed. pp. 60-88, Compiled and edited by the technical committee of 1994 Palm Oil Familiarization Programme.

CHOO, Y.M. 1995. Carotenoids from palm oil. Palm Oil Development, *22*, 1-6.

IWASAKI, R. and MURAKOSHI, M. 1992. Palm oil yields carotene for world markets. Inform *3*, 210-217.

MOHD, S.A.Y.1994. Refining and downstreaming processing of palm and palm kernel oils. In *Selected Reading on Palm Oil and Its Uses.* Palm Oil Familiarization Programme POFR. PORIM. Revised Ed. Compiled and edited by the technical committee of 1994 Palm Oil Familiarization Programme.

TAN, B., GRADY, C.M. and GAWIENOWSKI, E. 1986. Hydrocarbon carotenoid profiles of palm oil processed fraction. J. Am. Oil. Chem. Soc. *63*, 1175-1179.

TANG, T.S., TEOH, K.T. and LEE, Y.Y. 1983. Importance of crude oil quality and pretreatment in physical refining of palm oil products. In *Palm Oil Product Technology in the Eighties*, (E. Pushparajah and M. Rajadura, eds.) pp. 195, A report of the proceeding of the international conference on palm oil product technology in the eighties held in Kuala Lumpur June 22-24, 1981. Sponsored by PORIM and the Incorporated Society of Planters.