Separation of Palm Carotene from Crude Palm Oil by Adsorption Chromatography with a Synthetic Polymer Adsorbent

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ABSTRACT: Palm carotene was successfully concentrated from crude palm oil by a single-stage chromatographic process on a synthetic porous polymer. Carotene was concentrated to about 10^5 ppm solution, which is about 160 times the original concentration in crude palm oil. Carotene recovery varied from 40 to 65% depending upon chromatographic conditions. The fatty acid composition of the palm oil did not change during the carotene recovery process, and the carotene composition was also almost the same as that in palm oil. Adsorption isotherms of the adsorbent differed from other adsorbents. This new recovery method for palm carotene may be suitable as an edible palm oil pretreatment process due to its efficient mass recovery of a valuable bioresource.

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KEY WORDS: Adsorption chromatography, adsorption isotherm, palm carotene, palm edible oil, synthetic porous resin.

Palm oil has a greater carotenoid concentration than any other oil or fat. Palm oil carotenoids consist of 30% α-carotene (1). Recent studies have shown that carotenoids have anticancer activity, with α-carotene being more effective than β-carotene (2). Various methods of carotenoid recovery from palm oil have been developed; these include saponification (3), adsorption (4), selective solvent extraction (4), and transesterification, followed by both phase separation and distillation of the esters (5,6). Transesterification is the only commercially viable process. This unique method of carotenoid recovery from palm oil has already been developed by Lion Corporation (Tokyo, Japan). It involves the interesterification of triglycerides with methanol to yield methyl esters, followed by phase separation, resulting in a carotene-rich layer and a decolored methyl ester layer. The carotene-rich layer is further concentrated by molecular distillation and chromatographic methods. A similar process, involving transesterification of palm oil, followed by molecular distillation of the esters, to recover the carotenoids has been reported (7), but an edible oil was not obtained. At present, most of the carotenoids in the edible oil are destroyed and discarded in the refining process. Carotenoid extraction by adsorption without a chemical conversion of palm oil has been reported (8–10) but was not commercially viable.

The present study was desirable to develop a separation technique for carotene extraction from crude palm oil (CPO) that maintains an edible-oil quality. A basic patent (11) already describes carotene separation from vegetable oils by using stylene-divinyl benzene copolymers. The chromatographic method described here may be beneficial to edible-oil manufacturers who now discard almost all of the carotenoids in refining palm oil.

EXPERIMENTAL PROCEDURES

Palm oil and chemicals. CPO was obtained from Sime Darby Plantation (Klang, Malaysia). All solvents and chemicals used were of analytical grade. Alumina of chromato-grade, silica gel (Wako gel C-200), and magnesium hydroxide were obtained from Wako Chemicals (Osaka, Japan). Synthetic highly porous resin (Diaion HP-20), a styrene-divinyl benzene copolymer, was obtained from Mitsubishi Chemicals Company (Tokyo, Japan). This resin was selected from both the Diaion HP series (HP 10-50) and SP series (SP 800-875, SP205, SP 207) of synthetic porous resins of the same company (11). This adsorbent has a broad nonpolar and nonionic surface on many small cavities, with a specific surface area of 511 m^2/g.

Column chromatography of CPO. The chromatographic columns were glass tubes (3 cm i.d. by 35-cm length) with an outer jacket for circulating heated water. The adsorbent was slurried in the initial solvent and sonicated for 5 min in a Branson sonicator (Danbury, CT) before packing into the glass tube to about 25-cm high in the column, and the column temperature was kept at 40 to 60°C. CPO, varied from 10 to 60 g, was suspended or almost dissolved in the heated initial solvent, and then loaded onto the column bed. The initial solvents were lower alcohols, about 400 mL of isopropanol (IPA) or ethanol, and the second solvent was *n*-hexane at about 300 mL. Fractions were collected by a fraction collector. The oil content of each fraction was determined gravimet-

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rically after removal of the solvent by a rotary evaporator *in vacuo*. The carotene content was determined by diluting a 1-mL aliquot of each fraction with hexane to the appropriate dilution, and then spectrophotometric measurements were performed with a Hitachi U-2000 recording spectrophotometer at an absorbance of 450 nm.

High-performance liquid chromatography (HPLC) analysis of carotene. HPLC analysis was performed with a Shimadzu LC-10 apparatus, equipped with a Chromato-pak C-R7A column and a YMC J'Sphere ODS H80 S-4, 80A (4.6 mm i.d. × 25 cm); the isocratic mobile phase was acetonitrile/dichloromethane (8:2, vol/vol) (12); the flow rate was 1.0 mL/min; and carotene was determined by absorbance at 450 nm.

Fatty acid analysis. Fatty acid compositions of both CPO and HP-20 column-chromatographic fractions were analyzed by the ordinary gas–liquid chromatography (GLC) method (13). Fatty acids prepared from CPO or the fractions were esterified with 5% HCl-methanol at 85°C for 4 h. Margaric acid was used as an internal standard for absolute quantitative analysis. GLC analysis was accomplished with a Shimadzu 6A apparatus, equipped with a column (2.6 m/m $\phi \times 3$ m) packed with 15% diethyleneglycolsuccinate on shimalite, at 200°C column temperature. The carrier gas was N_2.

Adsorption isotherms of carotene. CPO solution (5 mL) with different concentrations of initial solvent was mixed with 1 g of adsorbent in stoppered test tubes. These tubes were shaken in a water-bath shaker for 20 min at 28°C at a speed of 200 strokes/min. After settling for 10 min, the supernatant was subjected to both oil and carotene determination by the method above-mentioned. A 1-mL aliquot of the supernatant was diluted with hexane to 10 mL, followed by a spectrophotometrical determination of carotene at 450 nm, and the oil quantity was determined after removal of the solvent. The amount of carotene adsorbed was the difference between the original carotene concentration and the supernatant carotene concentration.

RESULTS AND DISCUSSION

Chromatographic separation of palm carotene: solvent system. Three column supports (Diaion HP-20, alumina, and silica gel) were used in adsorption chromatography to separate the carotenes from CPO. Both alumina and silica gel were used for normal-phase chromatography, and Diaion HP-20 for reverse-phase chromatography. Preliminary experiments on alumina and silica gel with hexane or another nonpolar solvent as the initial eluting solvent did not result in a good separation of the oil and carotene in the elution fractions. HP-20 showed a higher adsorption capacity and a superior ability to separate the palm carotene relative to other supports. The ability of this resin was thought to be due to the superior surface area and greater hydrophobicity. Thus, HP-20 resin was adopted as the adsorbent for palm carotene.

The solvent systems were initially examined for HP-20 column chromatography. Lower alcohols, such as methanol, ethanol and IPA, were used as the initial eluting solvents because of reverse-phase chromatography. The second solvent was *n*-hexane, which was preferable for eluting the carotene adsorbed on the hydrophobic surface of the HP-20 resin. As described later, both column temperature and CPO loading quantities largely affected chromatographic behavior. Throughout the experiments with the solvent systems, the column temperature and CPO loading were set at 50°C and 30 g, respectively. Reproducible results were obtained at 50°C by using IPA and ethanol as the initial eluting solvent (Figs. 1 and 2). Methanol could not be used because of the slow elution of oil due to poor solvency in methanol and the high viscosity of oil, even at higher temperature. IPA had good solvency for CPO, and the solution was almost homogeneous at 50°C. The adsorption ability for carotene in IPA was, however, lower than that in ethanol. On the other hand, ethanol had poor solvency for CPO but more carotene was adsorbed on the column (Fig. 2). More ethanol volume was required for eluting the oil, because the elution speed of the oil was lower in chromatography.

Almost all of the triglyceride in CPO was eluted by either initial solvent, IPA or ethanol in a larger quantity, and about 40 and 60% of the carotene was adsorbed. The carotene adsorbed on the column was then eluted by hexane, the second solvent, to obtain a sharp peak on the chromatogram (Figs. 1 and 2). In this step, carotene was concentrated to about 39,000 ppm and 6,400 ppm by the IPA–hexane (I–H) and ethanol–hexane (E–H) solvent systems, respectively (Table 1). With the E–H system, the carotene concentration was lower because of the

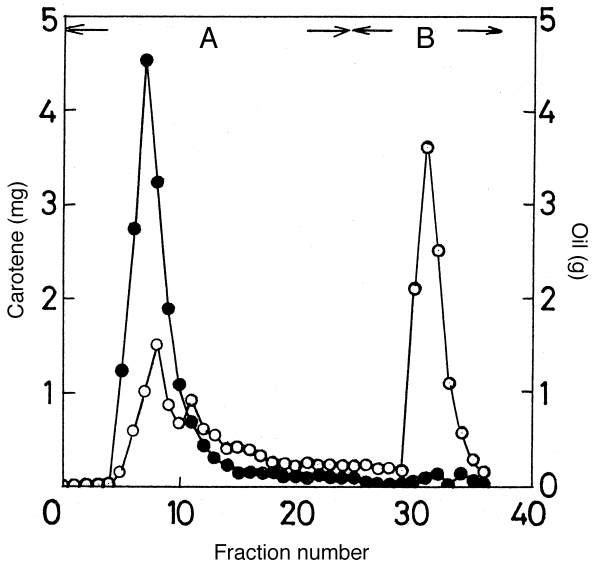

FIG. 1. Diaion HP-20 column chromatography for separation of palm carotene. Initial solvent (A), isopropanol 550 mL; second solvent (B), *n*-hexane 250 mL. One fraction, 23 mL. Column temperature, 50°C. Flow rate, 1.75 mL/min. Crude palm oil (CPO) loading, 30 g. ○—○, Carotene; ●—●, oil.

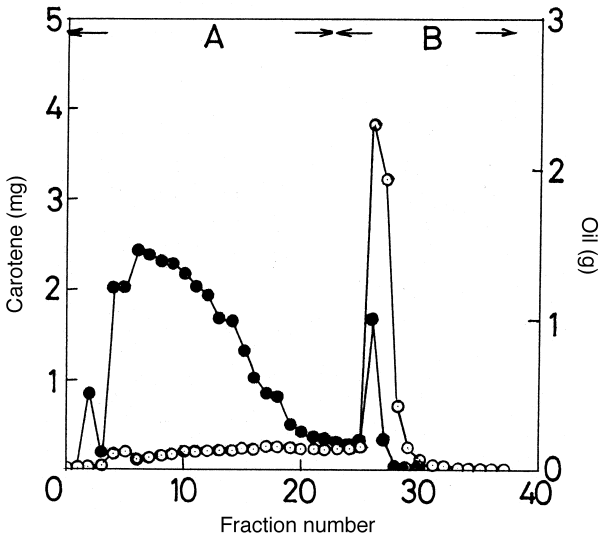

FIG. 2. Diaion HP-20 column chromatography for separation of palm carotene. Initial solvent (A), ethanol 550 mL; second solvent (B), *n*-hexane 250 mL. One fraction, 23 mL. Column temperature, 50°C. Flow rate, 1.75 mL/min. CPO loading, 30 g. ○—○, Carotene; ●—●, oil. See Figure 1 for abbreviation.

larger amount of oil present. Carotene recovery from CPO was 39 and 53% for the I–H and E–H systems, respectively (Table 1). These results clearly show that the I–H system produced a higher carotene concentration and lower carotene recovery, whereas the E–H system produced a lower carotene concentration and higher carotene recovery. When the hexane fraction from the I–H system was subjected to rechromatography with the same column, the carotene concentration increased to about 110,000 ppm with a lower carotene recovery (22.3%). This carotene concentration is 30% more than the 80,000 ppm obtained by transesterification and molecular distillation (7). In the E–H system, a carotene concentration of 22,000 ppm and a 34% recovery were obtained (Table 1).

Effect of column temperature on HP-20 column chromatography. The effects of column temperature on adsorption of both carotene and triglyceride were examined in both I–H and E–H systems (Table 2). These experiments were carried out with 30 g CPO and were conducted twice. The temperatures examined were 40, 50, and 60°C in the I–H system, and 50, 60, and 70°C in the E–H system. The effects of column temperature varied with the solvent system. In the I–H system, carotene recovery reached 58% at 40°C, and at higher temperatures, 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 temperatures (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.

Effect of CPO loading. CPO loading on the HP-20 column is an important condition, because this largely affects carotene recovery. CPO loading was examined from 10 to 60 g under the I–H system at 40°C column temperature (Table 3). Figure 3 shows the relationship of CPO loading to carotene and oil recovery. Clearly, carotene 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 the 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 solvent isopropanol. The adsorption capacity for oily materials (CPO and its fatty acid esters) on the HP-20 resin has already been determined, and a 0.2- to 0.5-g loading of oily materials per gram resin has been recommended (11). In our research, the capacity of HP-20 resin at 10 g CPO loading was only 0.05 g per gram of resin, and it was 0.3 g at 60 g CPO. These differences depend

TABLE 1
Diaion HP-20 Column Chromatography for Separation of Palm Carotene with the IPA–Hexane (I–H) System and Ethanol–Hexane (E–H) System

			Carotene		
Step	Fraction[a]	Oil quantity (g)[b]	Content (mg)	Recovery (%)	Conc. (ppm)[c]
First	IPA	30.0	12.03	60.6	410
chromatography	Hexane	0.2	7.83	39.4	39,150
	Ethanol	28.2	9.19	47.3	326
	Hexane	1.6	10.24	52.7	6,400
Rechromatography[d]	IPA	0.2	3.24	16.3	18,224
	Hexane	0.03	4.36	22.0	108,929
	Ethanol	1.2	3.60	18.5	3,000
	Hexane	0.3	6.60	34.0	22,000

[a]IPA, isopropanol; eluate by each solvent.
[b]Weight after evaporation of solvent.
[c]Original concentration in crude palm oil (CPO), about 600 ppm.
[d]Hexane fraction in each solvent system was subjected to rechromatography; see text.

TABLE 2
Effect of Column Temperature on the Recovery of Palm Carotene with the I–H and E–H Solvent Systems[a]

			Carotene	
Temperature (°C)	Fraction	Oil recovery (%)	Recovery (%)	Concentration (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	27.3	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	0.2	40.3	11,647
70	Ethanol	97.3	64.2	250
	Hexane	2.7	35.8	4,964

[a]CPO loading amount,30 g. See Table 1 for abbreviations.

on the kind of oily material, its loading quantity, the solvent system, and column temperature. Carotene concentrations in the hexane fractions were affected by the amount of oil eluted at the same time, but carotene was concentrated to about 10 times when the CPO loading was below 30 g. Thus, under our chromatographic conditions, a 20-g loading of CPO was suitable for good recovery with a high concentration of carotene.

HPLC analyses of carotene in the hexane fraction (I–H solvent system) clearly showed almost the same pattern as that in CPO (Fig. 4). The major components of the carotene fraction were similar to CPO, which contains α- and β-carotene. Carotenoids in CPO were almost equally concentrated in the hexane fraction.

Fatty acid composition of IPA fraction. Fatty acid analyses were performed for the CPO and IPA fractions to check whether the fatty acid compositions were the same. The fatty acid compositions of both IPA and hexane fractions were almost the same as CPO data (Table 4). These results indicate that the fatty acids of CPO do not change chemically during the carotene recovery processes.

Adsorption isotherms of palm carotene. The adsorption activity of Diaion HP-20 was determined in both IPA and hexane solvent (Fig. 5). HP-20 resin tended to adsorb much more carotene in isopropanol than in hexane. The adsorption ratios of carotene by HP-20 resin were obtained as a percentage of carotene adsorbed-to-total carotene loaded, which were 93.7 and 43.7% in IPA and hexane, respectively. The carotene that corresponds to the difference of these ratios can be recovered.

The adsorption capacities of the normal-phase chromato-

TABLE 3
Effect of CPO Loading on the Recovery of Palm Carotene with the I–H Solvent System[a]

			Carotene	
CPO loading (g)	Fraction	Oil recovery (%)	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

[a]Solvent volume, 550 mL IPA and 250 mL hexane. For conditions, see text. Column temperature, 40°C. See Table 1 for abbreviations.

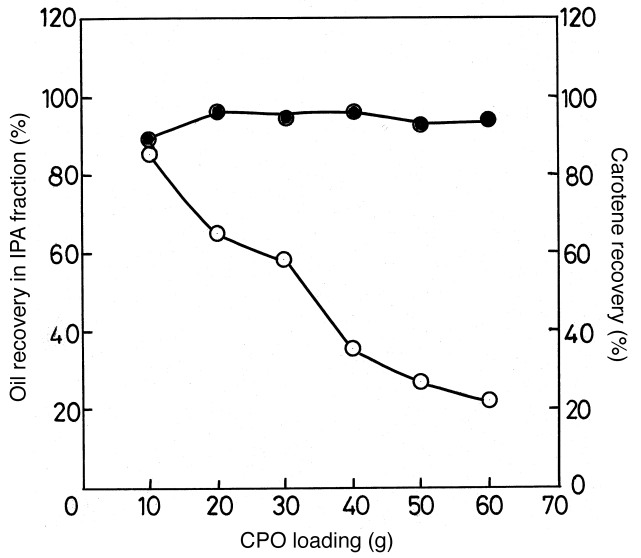

FIG. 3. Effect of CPO loading on the recovery of palm carotene and oil with the isopropanol–hexane (I–H) system. Column temperature, 40°C. ○—○, Carotene; ●—●, oil. For conditions, see text. See Figure 1 for other abbreviation.

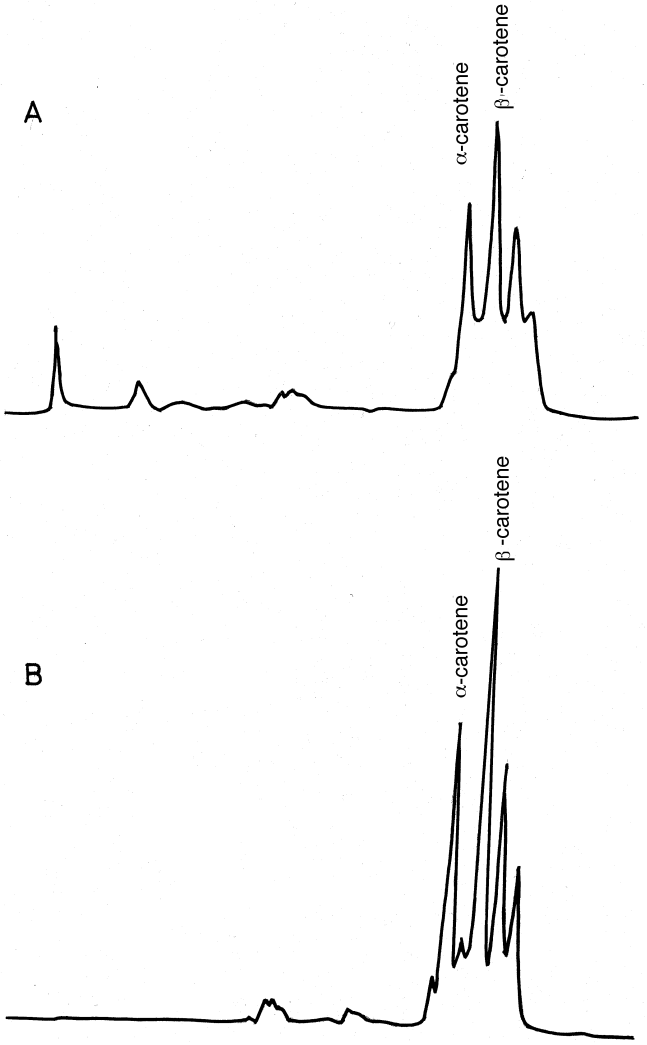

FIG. 4. High-performance liquid chromatography (HPLC) chromatogram of carotenes in the CPO (A) and hexane fraction (B) of Diaion HP-20 column chromatography. See Figure 1 for abbreviation.

graphic adsorbents, alumina and silica gel, were compared with the capacity of the HP-20 reversed-phase material. The adsorption isotherms for the normal-phase supports were determined in hexane, which was the initial solvent in normal-phase chromatography (Fig. 6). Adsorption ratios of both alumina and silica gel were much lower (about 50%) than the ratio of HP-20 in IPA (94%). The maximum adsorption capacity of HP-20 was about 10 times that of the other adsorbents. This result indicates that the HP-20 resin has a superior ability to adsorb palm carotene in the initial solvent, IPA, relative to alumina and silica gel, and may be applied in the initial process of manufacturing edible palm oil.

The authors have applied for a Malaysian patent on this process (14).

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TABLE 4
Fatty Acid Composition of CPO and HP-20 Column Chromatography Fractions[a]

	Fatty acid (%)					
Sample oil	C_{12}	C_{14}	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
CPO	0.84	0.96	48.00	3.50	36.63	9.19
IPA fraction	0.62	1.35	47.29	3.64	36.06	10.03
Hexane fraction	0.51	1.79	48.34	3.59	34.57	9.86

[a]See Table 1 for abbreviations.

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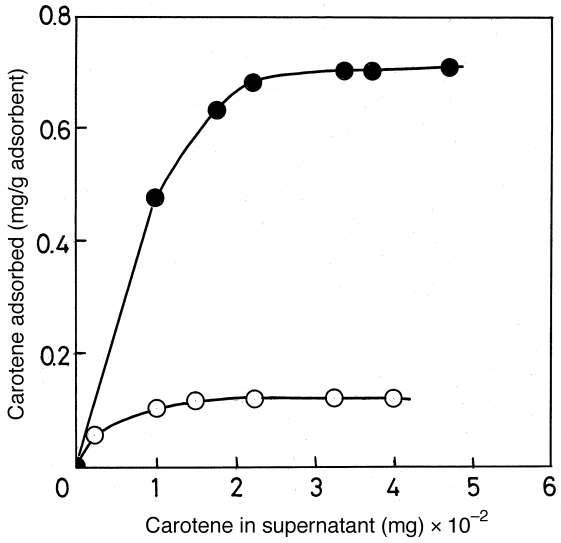

FIG. 5. Apparent adsorption isotherms of palm carotene by Diaion HP-20 with IPA and hexane. ●—●, In IPA; ○—○, in hexane. For conditions, see text. See Figure 1 for abbreviation.

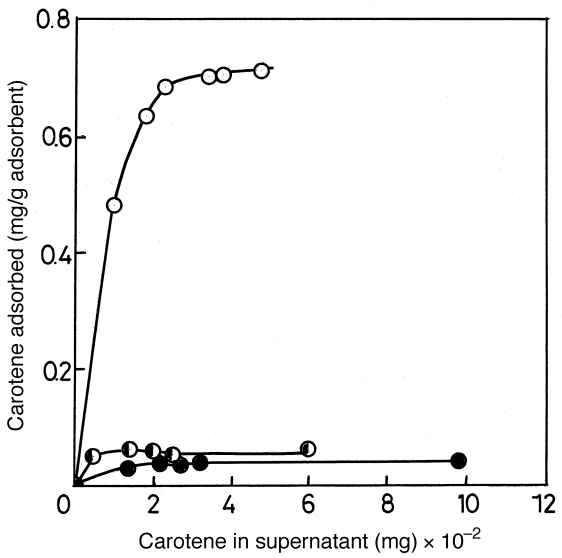

FIG. 6. Apparent adsorption isotherms of palm carotene with various supports in the initial solvent. ○—○, Diaion HP-20; ◐—◐, alumina; ●—●, silica gel. For conditions, see text.

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