

# Bleaching Rapeseed and Soybean Oils with Synthetic Adsorbents and Attapulgites

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**Efficiencies of synthetic adsorbents and attapulgites in bleaching alkali-refined rapeseed and soybean oils ranged from 13–53% and 93–97%, respectively. The Freundlich equation was more applicable than the Langmuir equation to the experimental adsorption isotherms of  $\beta$ -carotene on attapulgites. Bleaching with attapulgites reduced tocopherols by 12.5–29.5% in rapeseed oil and by 18.9–44.8% in soybean oil. Cosmetic-grade attapulgite was superior to the others in bleaching efficiency, equilibrium amount adsorbed and removal of free fatty acids.**

**KEY WORDS:** Adsorbents, adsorption isotherm, attapulgite, bleaching,  $\beta$ -carotene, free fatty acids, tocopherols.

The usual processes of degumming, alkali-refining, bleaching and deodorizing typically remove 97% of the free fatty acids and 32% of the tocopherols from crude soybean oil (1). The optimum bleaching medium reduces the contents of  $\beta$ -carotene and free fatty acids to the greatest extent possible, while retaining tocopherols in bleached vegetable oils (2). The Langmuir and Freundlich equations have been applied to the adsorption isotherms of  $\beta$ -carotene on montmorillonite, sepiolite and standard activated clay from alkali-refined vegetable oils (3). The naturally occurring minerals attapulgite and sepiolite are similar in both fiber shape and structure, but they are appreciably different in chemical composition. Attapulgite contains 10.2%  $\text{Al}_2\text{O}_3$  and 9.4%  $\text{MgO}$ , and sepiolite contains 1.0–1.5%  $\text{Al}_2\text{O}_3$  and 18.3–23.7%  $\text{MgO}$  (4).

The present study was conducted to compare the bleaching efficiencies of attapulgites with those of synthetic adsorbents, standard activated clay and sepiolite (2), and to determine the applicability of the Langmuir and Freundlich equations to the adsorption isotherms of  $\beta$ -carotene on attapulgites.

## MATERIALS AND METHODS

**Materials.** Alkali-refined rapeseed (Honen Oil Co., Ltd., Shimizu, Japan) and soybean (Ajinomoto Co., Ltd., Tokyo, Japan) oils were used because of their significant  $\beta$ -carotene contents (2). The oils were stored in the dark at 4°C until used. The synthetic adsorbents  $\text{Al}_2\text{O}_3 \cdot \text{mH}_2\text{O}$  (SPA1),  $\text{Al}_2\text{O}_3 \cdot \text{nH}_2\text{O}$  (SPA2),  $\text{Al}_2\text{O}_3 \cdot \text{Na}_2\text{O} \cdot 2\text{CO}_3 \cdot \text{xH}_2\text{O}$  (SPA3),  $\text{Mg}_6\text{Al}_2(\text{OH})_{16}\text{CO}_3 \cdot \text{xH}_2\text{O}$  (SPA4),  $2\text{MgO} \cdot 6\text{SiO}_2 \cdot \text{mH}_2\text{O}$  (SPA5),  $2\text{MgO} \cdot 6\text{SiO}_2 \cdot \text{nH}_2\text{O}$  (SPA6) and  $\text{Al}_2\text{O}_3 \cdot 9\text{SiO}_2 \cdot \text{xH}_2\text{O}$  (SPA7) were tested by bleaching media. All the synthetic adsorbents (SPA1–SPA7) were donated by Kyowa Chemical Industry Co., Ltd. (Takamatsu, Japan) and were passed through a 200-mesh screen before evaluation. The attapulgites (Tsuchiya Kaolin Ind., Ltd., Tokyo, Japan) tested were of the following grades: low-temperature dried (A1), high-temperature dried (A2), regular volatile matter sorptive (A3), pharmaceutical (A4), cosmetic (A5) and low-volatile matter sorptive (A6). A1 and A3 were passed through a 200-mesh screen, and A2, A4 and A5 were passed through a 100-

mesh screen. A6 was passed through screens ranging in size from 30 to 60 mesh. Standard activated clay for comparison, certified by the Japan Oil Chemists' Society, was from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). All bleaching media were dried at 110°C for 15 h before use. All other chemicals were reagent-grade.

**Bleaching procedure.** Bleaching was carried out by a previously described method (2). The filtered oil (approximately 2.0 mL) was added to 8.0 mL of cyclohexane in a measuring flask to give a total volume of exactly 10 mL. Residual  $\beta$ -carotene content was measured spectrophotometrically at 453 nm. The relative amount of pigment adsorbed ( $X$ ) and the residual relative amount at equilibrium ( $X_e$ ) were obtained from Equations 1 and 2

$$X = (A_o - A_x)/A_o \quad [1]$$

$$X_e = A_x/A_o \quad [2]$$

where  $A_o$  was the absorbance of unbleached oil and  $A_x$  was the absorbance of bleached oil. The relative amount adsorbed per gram of adsorbent ( $m$ ) was used. Adsorption isotherms were plotted as  $X/m$  vs.  $X_e$  for each amount of adsorbent used.

**Specific surface area, acid strength and acidity.** Specific surface area, acid strength and acidity of attapulgite were measured by previously described methods (5).

**Determination of tocopherols and free fatty acids.** Residual contents of tocopherols and free fatty acids in unbleached and bleached alkali-refined oils were determined by previously described methods (2,6).

## RESULTS AND DISCUSSION

**Bleaching efficiency.** Bleaching efficiencies for  $\beta$ -carotene are shown in Table 1. Bleaching efficiencies were ranked in the order: A1–A5 (93–97%) > SPA 1 (51–52%) > A6 (45–49%) > SPA2–SPA7 (13–48%). The bleaching efficiency of standard activated clay, used for comparison, was 96% or more.

Table 2 shows the specific surface areas, acid strengths and acidities of the attapulgites. Although surface areas and bleaching efficiencies of bentonite, montmorillonite and sepiolite were significantly correlated [ $r^2 = 0.929$  for rapeseed and  $r^2 = 0.916$  for soybean in our previous paper (2)], surface areas of attapulgites were not significantly correlated with bleaching efficiency (7). The acidities at  $\text{pK}_a = 4.0, 3.3$  or  $1.5$ , and the bleaching efficiencies of A1–A6 were not significantly correlated (7). Acid activation of rice hull ash significantly promoted the adsorption of lutein (3,3'-dihydroxy  $\beta$ -carotene) from soybean oil (8). Although the surface areas (73.4–194.9  $\text{m}^2/\text{g}$ ) of A1–A6 were generally smaller than those of sepiolites (83.0–281.1  $\text{m}^2/\text{g}$ ) (2), and acid strengths ( $\text{H}_o \leq +1.5$ ) of the attapulgites were weaker than those of the sepiolites ( $\text{H}_o \leq -3.0$ ), the attapulgites were superior in bleaching efficiency to the sepiolites (35–85%) (2). Higher bleaching efficiencies of the attapulgites were attributed to less competitive adsorption of triglycerides (9) and the pore structure accessible to  $\beta$ -carotene. Further experimentation is

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TABLE 1

Effect of Bleaching Media on  $\beta$ -Carotene Contents of Alkali-Refined Oils

Medium <sup>a</sup>	Fractional degree of bleaching (%) <sup>b</sup>	
	Rapeseed	Soybean
SPA1	51	52
SPA2	27	21
SPA3	14	14
SPA4	48	39
SPA5	13	18
SPA6	20	13
SPA7	40	37
A1	94	94
A2	96	96
A3	93	95
A4	97	96
A5	97	96
A6	49	45
Standard activated clay	99	96

<sup>a</sup>Media: SPA1,  $\text{Al}_2\text{O}_3\text{mH}_2\text{O}$ ; SPA2,  $\text{Al}_2\text{O}_3\text{nH}_2\text{O}$ ; SPA3,  $\text{Al}_2\text{O}_3\text{Na}_2\text{O} \cdot 2\text{CO}_3\text{xH}_2\text{O}$ ; SPA4,  $\text{Mg}_6\text{Al}_2(\text{OH})_{16}\text{CO}_3\cdot 4\text{H}_2\text{O}$ ; SPA5,  $2\text{MgO} \cdot 6\text{SiO}_2\text{mH}_2\text{O}$ ; SPA6,  $2\text{MgO} \cdot 6\text{SiO}_2\text{nH}_2\text{O}$ ; SPA7,  $\text{Al}_2\text{O}_3\cdot 9\text{SiO}_2\text{xH}_2\text{O}$ ; A1, low-temperature dried; A2, high-temperature dried; A3, regular volatile matter sorptive; A4, pharmaceutical; A5, cosmetic; A6, low-volatile matter sorptive grade.

<sup>b</sup>Fractional degree of bleaching (%) =  $(A_{\text{unbleached}} - A_{\text{bleached}}) / A_{\text{unbleached}} \times 100$ .  $A_{\text{unbleached}}$  and  $A_{\text{bleached}}$  are absorbances at 453 nm of unbleached and bleached oil, respectively. Mean data from three replicates. Coefficients of variation were 0.05–0.11%.

necessary to fully explain the superior bleaching efficiencies of attapulgites.

**Adsorption isotherms of  $\beta$ -carotene on attapulgites.** The adsorption capacity of the bleaching medium can be evaluated by determining the equilibrium amount adsorbed at equal equilibrium concentrations of pigment. Only the adsorption isotherms of  $\beta$ -carotene on attapulgites were measured because of higher adsorption capacities as compared with those of the synthetic adsorbents. The relative amounts of  $\beta$ -carotene adsorbed on A1–A5 at lower relative equilibrium concentrations ( $X_e = 0.1$ ) and higher concentrations ( $X_e = 0.5$ ) were obtained from the adsorption isotherms at 110°C (Table 3). Although there were almost no differences in bleaching efficiencies of A1–A5 (Table 1), the relative amounts adsorbed on A5 were considerably larger than those adsorbed on A1–A4. Therefore A5, with a higher adsorption capacity, was the most effective for bleaching alkali-refined rapeseed and

soybean oils. The relationships between specific surface areas or acidities at  $\text{pK}_a = 4.4, 3.3$  and 1.5 and relative amounts adsorbed at  $X_e = 0.1$  or 0.5 were not significantly correlated. The relative amounts adsorbed [ $X = (A_o - A_x) / A_o = 1.0 - 6.4 (-)$ ] on sepiolites at  $X_e = 0.5$  were approximately 2–5 times larger than at  $X_e = 0.1$  (3). The relative amounts adsorbed [5.2–9.9 (-)] on attapulgites at  $X_e = 0.5$  were approximately 1.3–2.2 times larger than at  $X_e = 0.1$  (Table 3). Thus, the attapulgites had higher adsorption capacities than the sepiolites over the wide range of equilibrium concentrations used. The differences in the amounts of  $\beta$ -carotene adsorbed on the attapulgites were not large between the lower and higher equilibrium concentrations as compared with those of the sepiolites.

The constants in the Langmuir and Freundlich equations are useful in designing adsorption process equipment (10). Figures 1 and 2 show the Langmuir isotherms for the adsorption of  $\beta$ -carotene on A1–A5 from rapeseed and soybean oils, respectively. Table 4 shows the Langmuir isotherm constants. The a and b values are constants that represent the surface area of the adsorbent and the intensity of adsorption, respectively (10). However, the a values (Table 4) and specific surface areas (Table 2) were not significantly correlated. Langmuir isotherm plots had weak points so that the differences in experimental amounts adsorbed at lower equilibrium concentrations could not be represented (Figs. 1 and 2).

Figures 3 and 4 show the Freundlich isotherms for the adsorption of  $\beta$ -carotene on A1–A5 for rapeseed and soybean oils, respectively. The Freundlich isotherm constants are summarized in Table 5. The Freundlich isotherms of pigments from rapeseed and soybean oils on standard activated clay had inflections in the range of  $\log X_e = -1.2$  to  $-1.1$ ; whereas those on sepiolites formed straight lines (3). The fact that inflections were observed for A1–A5 in the ranges of  $\log X_e = -1.6$  to  $-1.1$  for rapeseed and  $-1.3$  to  $-1.0$  for soybean oil indicates that attapulgite is similar in pore size distribution to the standard activated clay rather than to the sepiolites. Therefore,  $\beta$ -carotene is preferentially adsorbed into the smaller pores of the attapulgites at lower concentrations and then into the larger pores at higher concentrations, as explained by Boki *et al.* (3). The constant, k, in the Freundlich equation represents a measure of the surface area of the adsorbent (10); however, the k values (Table 5) and the specific surface areas (Table 2) were not significantly correlated. Because the constants n and k varied at lower and higher equilibrium concentrations, suitable values must be

TABLE 2

Specific Surface Area, Acid Strength and Acidity of Attapulgite

Medium <sup>a</sup>	Specific surface area ( $\text{m}^2/\text{g}$ )	Acid strength	Acidity (mmol/g) in $\text{H}_0$ range <sup>b</sup>		
			$+1.5 \geq \text{H}_0 > +3.3$	$+3.3 \geq \text{H}_0 > +4.0$	$+4.0 \geq \text{H}_0$
A1	194.9	$\text{H}_0 \leq +1.5$	0.068	0.104	0.094
A2	174.7	$\text{H}_0 \leq +1.5$	0.049	0.092	0.096
A3	163.7	$\text{H}_0 \leq +1.5$	0.052	0.103	0.072
A4	98.9	$\text{H}_0 \leq +1.5$	0.029	0.066	0.039
A5	178.0	$\text{H}_0 \leq +1.5$	0.060	0.113	0.111
A6	73.4	ND <sup>c</sup>	ND	ND	ND

<sup>a</sup>Media described in Table 1.

<sup>b</sup>Mean data from three replicates. Coefficients of variation were 0.15–0.65%.

<sup>c</sup>Not detected (observable) because of dark brown color of A6.

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TABLE 3

Relative Amounts of  $\beta$ -Carotene Adsorbed<sup>a</sup> on Attapulgite at 0.1 and 0.5 Relative Equilibrium Concentrations<sup>b</sup>

Medium <sup>c</sup>	Rapeseed		Soybean	
	0.1	0.5	0.1	0.5
A1	2.82	5.47	3.88	5.99
A2	2.97	5.18	4.59	5.91
A3	2.54	5.69	3.91	6.05
A4	3.41	6.39	4.69	6.65
A5	4.83	7.58	7.86	9.85

<sup>a</sup>Coefficients of variation were 0.11–0.35%.

<sup>b</sup>Relative amounts of pigments adsorbed per gram of attapulgites were obtained at  $X_e = 0.1$  and  $X_e = 0.5$  relative equilibrium concentrations from adsorption isotherms at 110°C.

<sup>c</sup>Media described in Table 1.

selected based on the concentration of  $\beta$ -carotene in alkali-refined oil to design adsorption processing equipment.

*Residual tocopherols in bleached alkali-refined oil.* Tocopherols are important components of oil because of their antioxidant properties and vitamin E activity. Therefore, it is desirable to preserve the natural tocopherols as much as possible during oil bleaching. Table 6 shows the original contents of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols in unbleached oil, residual contents in bleached alkali-refined oils, and the percentage decrease in total tocopherols. Bleaching with

A1–A5 reduced tocopherols in alkali-refined oils by 12.5–29.5% in rapeseed oil and 18.9–44.8% in soybean oil. During bleaching with A5, which has a higher adsorption capacity, tocopherols decreased 29.5–44.8% and were similar to the values (2.9–43.5%) obtained after bleaching with standard activated clay (2). The decrease in tocopherol contents and surface areas or acidities at  $pK_a = 1.5, 3.3$  and 4.0 were not significantly correlated. Because the relative compositions of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols in unbleached and bleached alkali-refined oils were roughly constant, attapulgites did not selectively adsorb  $\alpha$ -,  $\beta$ -,  $\gamma$ - or  $\delta$ -tocopherols. These results were in agreement with those obtained when bleaching with sepiolites (2).

*Removal of free fatty acids by bleaching.* The contents of free fatty acids in unbleached and bleached alkali-refined oils were quantitatively determined to elucidate whether the amounts of free fatty acids decreased or increased in oil after filtering through the bleaching medium (Table 7). Hau and Nawar (11) reported that stearic or linoleic acids were adsorbed on surface silanol groups of silica gel. Taylor *et al.* (12) reported that the adsorption of heptanoic and oleic acids was governed by the molecular sieving properties of zeolites. These reports (11,12) indicate the differences in adsorption for different free fatty acids. Because the contents of free oleic and linoleic acids were approximately five times as much as those of palmitic, stearic and linolenic acids in the unbleached oils (Table 7), the bleaching medium that preferentially

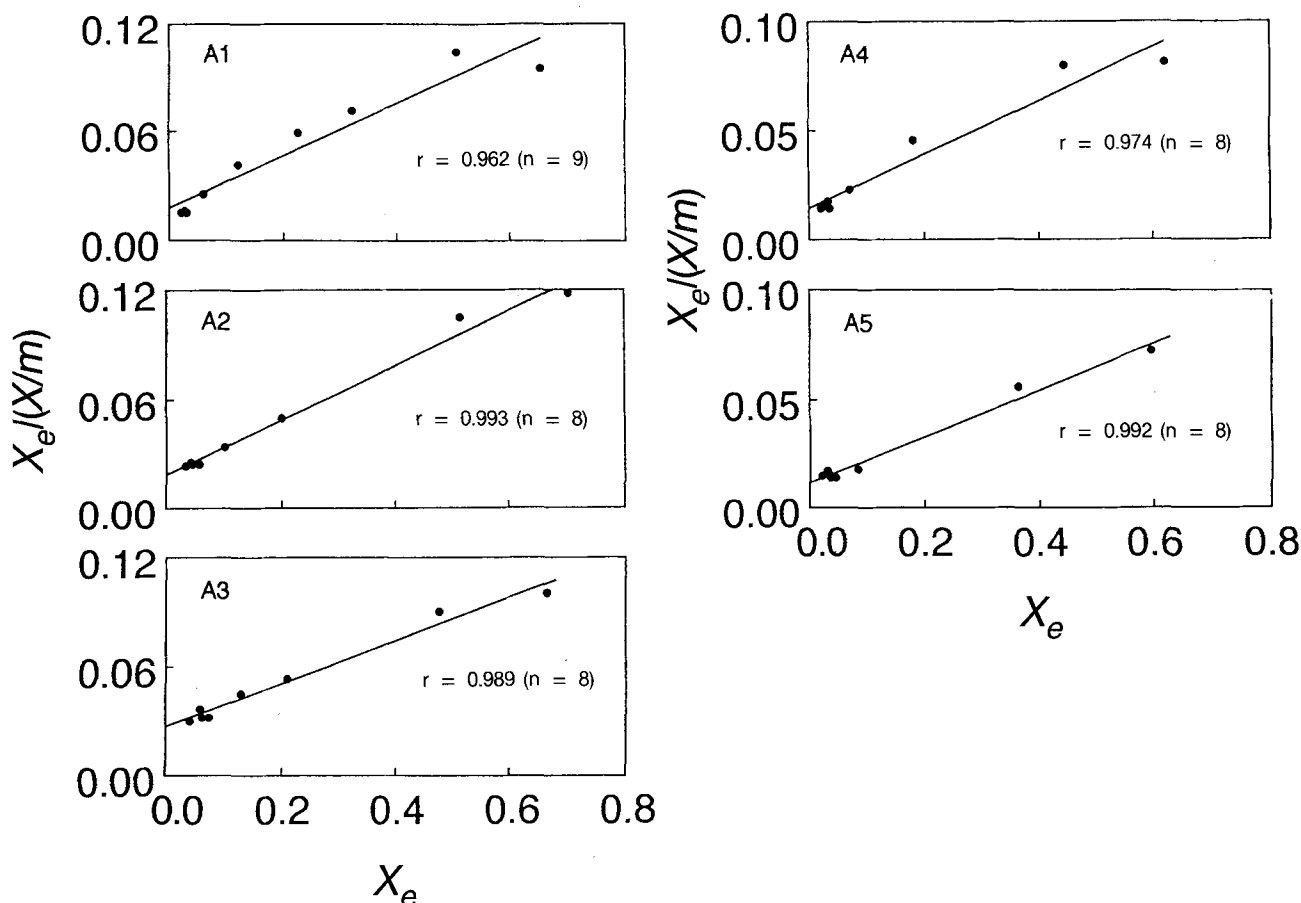


FIG. 1. Langmuir isotherms for adsorption of  $\beta$ -carotene from rapeseed oil on attapulgites  $X_e$ , residual relative amount of  $\beta$ -carotene at equilibrium;  $X/m$ , relative amount of  $\beta$ -carotene adsorbed per gram of adsorbent; A1–A5 as described in Table 1.

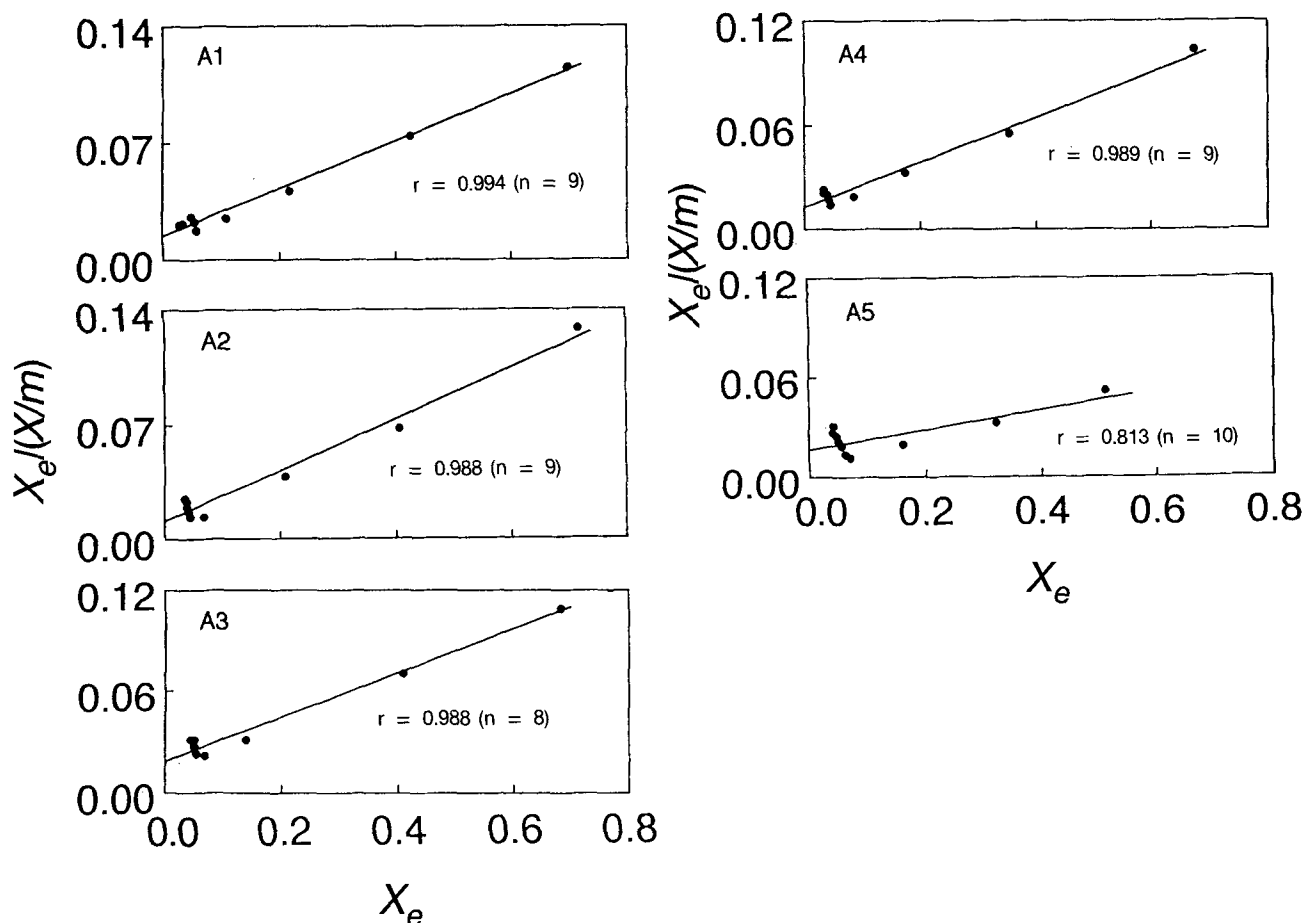


FIG. 2. Langmuir isotherms for adsorption of  $\beta$ -carotene from soybean oil on attapulgites  $X_e$  and  $X/m$  as described in Figure 1; A1–A5 as described in Table 1.

TABLE 4

Langmuir Isotherm Constants<sup>a</sup> for Adsorption of  $\beta$ -Carotene on Attapulgite

Medium <sup>b</sup>	Rapeseed		Soybean	
	a	b	a	b
A1	6.95	8.24	7.06	10.00
A2	6.62	8.17	6.40	14.16
A3	8.45	4.38	7.76	6.88
A4	8.09	8.66	7.71	10.54
A5	9.25	9.17	16.25	3.73

<sup>a</sup>Constants a and b were calculated from the slope and intercept of the Langmuir isotherm plots in Figures 1 and 2.

<sup>b</sup>Media described in Table 1.

adsorbs oleic and linoleic acids will decrease the residual contents of free fatty acids after bleaching. The preferential media were A1–A3. The total contents of free fatty acids in rapeseed oil bleached with A1 and A5, and in soybean oil bleached with A2 and A5 decreased while the contents of linolenic acid increased in comparison with the unbleached oils. Bleaching with A4 increased the total contents of free fatty acids, whereas bleaching with A1–A3 and A5 decreased the total contents of free fatty acids. The results indicate that free fatty acids were

adsorbed on the medium and that triglycerides decomposed into free fatty acids and glycerol during bleaching. Triglycerides composed of linoleic and linolenic acids were easily decomposed by the media A4 and A5. Because residual free fatty acids decrease the oxidative stability of oil, A4 was not suitable for bleaching rapeseed and soybean oils. The decrease in free fatty acid contents and total acidities (sum of acid amounts at  $pK_a = 1.5, 3.3$  and  $4.0$ ) were significantly correlated ( $r^2 = 0.711, n = 10$ ). The higher the total acidity at weaker acid strength, the greater the decrease in free fatty acid content. Acid strength of attapulgite was significantly weaker than that ( $H_o \leq -5.6$ ) of standard activated clay. Neutral or slightly acidic clays often reduce free fatty acid contents by means of preferential adsorption (12). The fact that acid sites with weaker acid strength reduced the contents of free fatty acids in the bleached oils was contrary to observations that decreased amounts of free fatty acids adsorbed on rice hull ash activated with 20% sulfuric acid (13). The decrease in free fatty acid contents and surface area were significantly correlated ( $r^2 = 0.756, n = 10$ ). The decrease in residual content was attributed to the adsorption of free fatty acids on weak acid sites distributed over the surfaces of the attapulgites. A5 was superior to the other attapulgites in bleaching efficiency, equilibrium adsorption capacity and removal of free fatty acids, but it also adsorbed more tocopherols.

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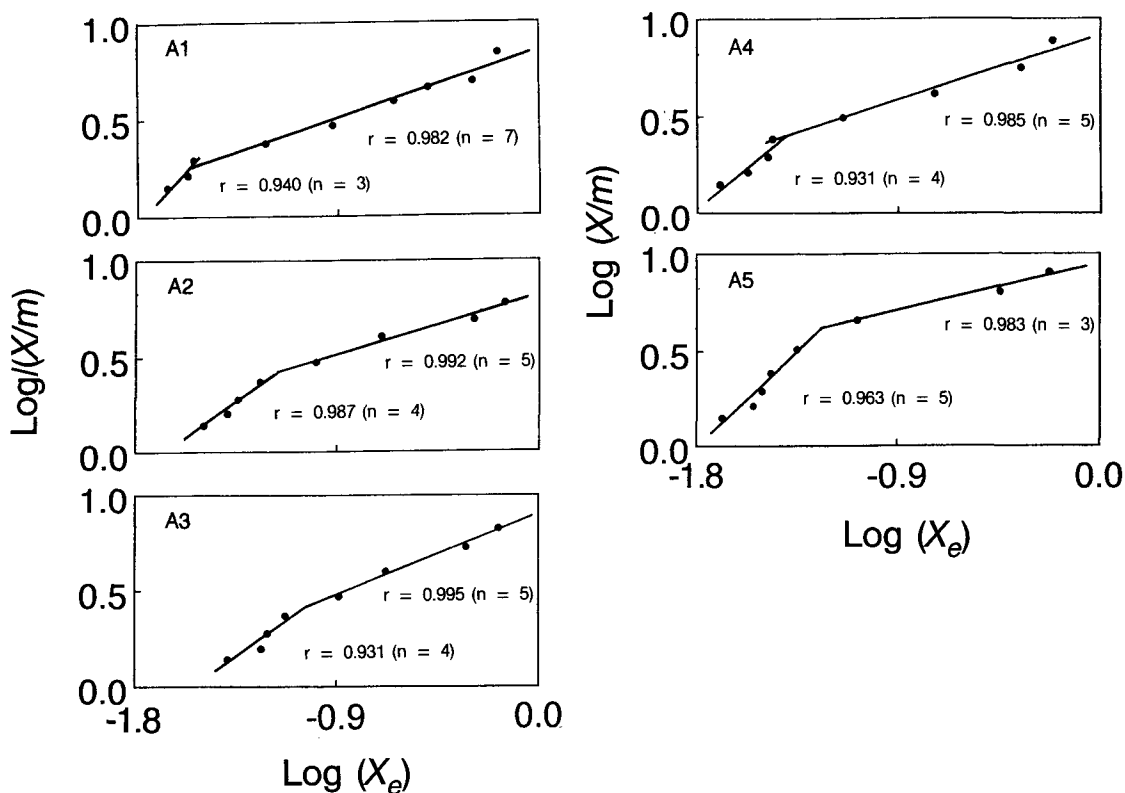


FIG. 3. Freundlich isotherms for adsorption of  $\beta$ -carotene from rapeseed oil on attapulgites.  $X_e$  and  $X/m$  as described in Figure 1; A1-A5 as described in Table 1.

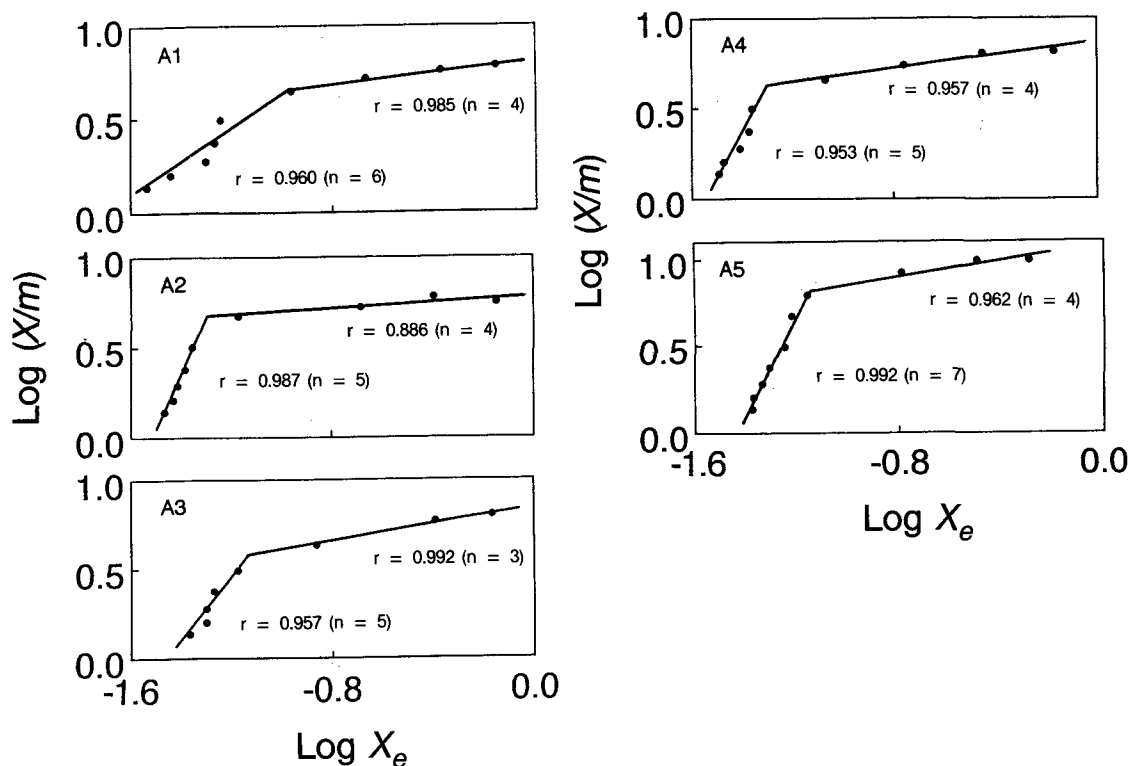


FIG. 4. Freundlich isotherms for adsorption of  $\beta$ -carotene from soybean oil on attapulgites.  $X_e$  and  $X/m$  as described in Figure 1; A1-A5 as described in Table 1.

TABLE 5

Freundlich Isotherm Constants for Adsorption of  $\beta$ -Carotene on Attapulgit

Medium <sup>a</sup>	Rapeseed <sup>b</sup>				Soybean <sup>b</sup>			
	n <sub>1</sub>	k <sub>1</sub>	n <sub>2</sub>	k <sub>2</sub>	n <sub>1</sub>	k <sub>1</sub>	n <sub>2</sub>	k <sub>2</sub>
A1	2.64	7.00	0.95	NS <sup>c</sup>	6.06	6.56	0.99	NS
A2	2.87	6.57	1.06	NS	10.97	6.07	0.30	NS
A3	2.14	7.82	1.17	NS	4.17	7.04	0.50	NS
A4	2.69	8.18	1.15	NS	5.56	7.38	0.42	NS
A5	3.62	9.00	0.90	NS	4.28	12.09	0.34	NS

<sup>a</sup>Media described in Table 1.<sup>b</sup>The constants, n<sub>1</sub> and k<sub>1</sub>, were calculated from the slope and intercept (log X<sub>e</sub> = 0.0), respectively, of the Freundlich isotherm plots in the range of approximately log X<sub>e</sub> = -1.3-0.0 in Figures 3 and 4. The constants, n<sub>2</sub> and k<sub>2</sub>, were calculated from the slope and intercept (log X<sub>e</sub> = 0.0) of the Freundlich isotherm plots in the range of approximately log X<sub>e</sub> = -1.6 through -1.3 in Figures 3 and 4.<sup>c</sup>Not significant because the data are not useful in designing adsorption process equipment.

TABLE 6

## Residual Contents of Tocopherols in Unbleached and Bleached Alkali-Refined Oils and Percentage of Decrease in Total Tocopherol Contents During Bleaching

Alkali-refined oil	Medium <sup>a</sup>	Contents of tocopherols ( $\mu\text{g/g}$ ) <sup>b</sup>				Decrease (%)
		$\alpha$	$\beta$	$\gamma$	$\delta$	
Rapeseed	None	181 (17.5) <sup>c</sup>	ND <sup>d</sup>	855 (82.5)	ND <sup>d</sup>	
	A1	151 (18.4)	ND	671 (81.6)	ND	20.7
	A2	173 (19.1)	ND	733 (80.9)	ND	12.5
	A3	135 (15.5)	ND	735 (84.5)	ND	16.0
	A4	128 (14.7)	ND	743 (85.3)	ND	15.9
	A5	78 (10.7)	ND	652 (89.3)	ND	29.5
Soybean	None	74 (3.6)	15 (0.7)	1500 (72.6)	476 (23.1)	
	A1	46 (2.8)	10 (0.6)	1218 (72.7)	400 (23.9)	18.9
	A2	45 (2.8)	9 (0.5)	1181 (72.3)	400 (24.4)	20.8
	A3	49 (3.0)	10 (0.6)	1174 (71.0)	420 (25.4)	20.0
	A4	39 (2.4)	11 (0.7)	1180 (74.5)	355 (22.4)	23.2
	A5	22 (1.9)	0 (0.0)	784 (68.8)	334 (29.3)	44.8

<sup>a</sup>Media described in Table 1.<sup>b</sup>Mean data from three replicates. Coefficients of variation were 0.96-1.36%.<sup>c</sup>The relative compositions (%) are shown in parentheses.<sup>d</sup>ND, not detected.

TABLE 7

## Contents of Free Fatty Acids in Unbleached and Bleached Alkali-Refined Oils and Change in Content of Free Fatty Acids During Bleaching

Alkali-refined oil	Medium <sup>a</sup>	Content of free fatty acid (mg/g) <sup>b</sup>					Decrease or increase (%) <sup>c</sup>
		16:0	18:0	18:1	18:2	18:3	
Rapeseed	None	0.265	ND <sup>d</sup>	1.887	1.145	0.351	
	A1	0.114	ND	0.696	0.931	0.934	-26.7
	A2	0.133	ND	1.449	1.078	0.150	-23.0
	A3	0.104	ND	0.973	0.971	0.118	-42.0
	A4	0.155	ND	2.221	1.440	0.669	+22.9
	A5	0.115	ND	1.310	1.255	0.401	-15.6
Soybean	None	0.362	ND	0.466	2.334	0.172	
	A1	0.334	ND	0.430	2.143	0.124	-9.1
	A2	0.299	ND	0.338	2.124	0.506	-2.0
	A3	0.280	ND	0.488	1.832	0.070	-19.9
	A4	0.670	ND	1.034	5.386	0.671	+132.8
	A5	0.320	ND	0.415	2.318	0.207	-2.2

<sup>a</sup>Media described in Table 1.<sup>b</sup>Mean data from three replicates. Coefficients of variation were 0.72-1.35%. 16:0, Palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.<sup>c</sup>Decrease or increase in contents of free fatty acids are shown as - or +, respectively.<sup>d</sup>ND, not detected.

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