Rejection of Carotenoids in Oil Systems by a Nonporous Polymeric Composite Membrane

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ABSTRACT: Earlier studies by the authors on crude vegetable oils showed that color compounds are reduced to the extent of 74–80% during membrane processing. In the present study, attempts were made to understand the rejection mechanism of carotenoids using real and model oil systems. In case of model systems consisting of refined high-oleic sunflower (HOSF) oil, lecithin and β-carotene, the rejection of carotenes was low (11–20%). This could be explained based on the differences between the model and real systems as well as on the solution-diffusion mechanism controlling the material transport across the membrane. This study revealed that β-carotene did not have affinity for the phospholipid reverse micelles present in the oil. Xanthophylls, the major carotenoids present in the crude soybean oil, were rejected to the extent of 60% in the model system (HOSF oil and lutein). High-performance liquid chromatographic analysis of crude oil revealed that there were few other xanthophylls, which are more polar than lutein. These unidentified xanthophylls would be rejected to a much greater extent by the hydrophobic membrane. The extent of color reduction would depend on the actual composition of xanthophylls present in the crude oil and their relative polarity.

Paper no. J9845 in *JAOCS 78*, 803–807 (August 2001).

KEY WORDS: β-Carotene, critical micelle concentration (CMC), lecithin, lutein, nonporous polymeric composite membranes, phospholipid reverse micelle, rejection of carotenoids, solution-diffusion mechanism, triglycerides, xanthophylls.

The removal of color from edible oil is necessary to provide an acceptable finished product to the consumer. The conventional bleaching operation employing a clay product is basically an adsorption process that removes not only color compounds but also other minor impurities. The residual soaps are removed and peroxides are decomposed into aldehydes and ketones due to further oxidation. These decomposition products are also adsorbed to the bleaching agent. Therefore, TOTOX value (the sum of the anisidine value and twice the peroxide value) is used as one of the parameters to evaluate the bleaching operation (1). There are several disadvantages with the current industrial bleaching practice related primarily to the retention of oil, which is in the range of 30–70% of the weight of the activated earth used in the process (2).

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Membrane technology is being looked at as one of the alternate processes for the conventional refining of edible oils. In the last couple of years, degumming and decolorization of crude vegetable oils of soybean, rapeseed, sunflower, and groundnut have been extensively investigated using nonporous hydrophobic polymeric membranes (3–5). These studies revealed that the nonporous membranes were effective in reducing phospholipids, color compounds, and oxidation products while retaining beneficial compounds. However, the permeate flux needs improvement for industrial adoption. Color compounds were rejected to the extent of 80–85% in soybean oil (4). Carotenoids and chlorophyll are the two common color pigments present in most vegetable oils. The chlorophyll content in normal crude soybean oil is 1–1.5 mg/L, and removing these green pigments is considered more difficult than yellow and red pigments (6), mainly carotenoids. However, the nonporous membrane (NTGS-2100) used in our earlier study reduced chlorophyll content to the extent of 78% in crude soybean oil (4). Another membrane from the same series (NTGS-2200) rejected chlorophyll almost completely (over 99%) in model oil system (7). The near-complete removal indicated that the silicon active layer did not have permeability for chlorophyll compounds.

In the conventional process, the color pigments are removed at various steps, and the maximum reduction (85%) occurs during bleaching (4). In the subsequent deodorization step, all the thermally degradable pigments are removed, amounting to total color reduction of 96%. During membrane processing of crude soybean oil, a total color reduction of 74–80% was achieved in a single step by nonporous polymeric composite membranes. In this study, attempts were made to understand the actual rejection mechanism of color compounds during the membrane process using different model oil systems as well as a real system.

EXPERIMENTAL PROCEDURES

Materials. Samples of crude soybean and refined high-oleic sunflower oil (HOSF) oils were obtained by the courtesy of Nippon Lever B.V. (Shimadzu, Japan). Phosphatidylcholine (PC) and lecithin used in the study were of soybean origin. PC was purchased from Nippon Fine Chemical Co. (Takasago, Japan) (PCS; assay min. 95% PC). Refined soybean oil,

β-carotene, lecithin, and chemicals required for analyses were purchased from Wako Pure Chemical Industries (Osaka, Japan). Lutein and β-carotene standards for high-performance liquid chromatography (HPLC) analyses were purchased from Sigma Chemical Co. (St. Louis, MO). The purity of HOSF was very high (>99% triglycerides), and hence it was used as a substitute for triglycerides in the model system study, considering the difficulties in obtaining the latter as pure product.

Oil systems used in the study. The oil systems were: (i) refined HOSF oil along with β-carotene; (ii) refined HOSF/soybean oil along with β-carotene and PC/lecithin; (iii) crude soybean oil; (iv) crude soybean oil along with β-carotene and lecithin; (v) crude soybean and refined HOSF oils along with β-carotene and lecithin; (vi) refined HOSF oil along with lutein and lecithin.

Membrane. Polymeric composite membrane, NTGS-2200 with silicon as active layer and polyimide as support layer, was used in the study (Nitto Denko, Kusatsu, Japan). This is a hydrophobic membrane, denser than reverse osmosis (RO) membrane, which was originally developed for gas-separation applications. The membranes were cut into circular discs (7.5 cm diameter with 32 cm^2 effective area) and fitted in the membrane cell in such a way that the active surface comes into contact with feed material.

Apparatus. Experiments were conducted using a flat membrane test cell (Model C40-B; Nitto Denko) under nitrogen atmosphere, and the required pressure was applied by adjusting the pressure regulator of the nitrogen cylinder. The cell was placed on a magnetic stirrer, and the magnetic spin bar fitted into the cell provided the agitation. The cell and magnetic stirrer were placed in a thermostatically controlled incubator. A schematic diagram of the experimental setup is given in Figure 1. The pressure, temperature, and stirrer spin bar speed were maintained at 4 MPa, 30–40°C, and 400 rpm, respectively. The unit was operated in batch mode by charging the cell with 100 g of crude oil, and the experiment was stopped when permeate collection was *ca.* 10 g in all the experimental runs except in the model studies conducted with lutein. In these experimental runs, the feed was between 20 and 30 g, and the permeate collection was *ca.* 3–4 g.

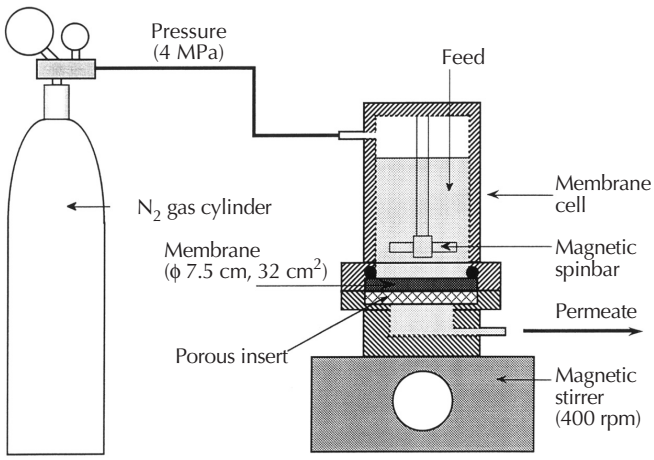

FIG. 1. Membrane apparatus and operating conditions.

Analyses. Association of Official Analytical Chemists (8) Official Method 958.05 was followed for the determination of carotenoids. The absorbance values of samples at 454 nm were also measured for comparison (4). These measurements were carried out using 1- and 2-mm cuvettes, appropriately to be within the sensitive range, with methylene chloride as blank. The spectroscopic data were recorded using a spectrophotometer (V-570; Jasco, Tokyo, Japan). All the values were normalized for 10-mm cuvette using Lambert's law.

Lutein contents in oil samples of the model study were determined in the reversed-phase HPLC generally as per the method followed by Monma *et al.* (9) with slight modification using acetonitrile/ethanol (65:35) as mobile phase. Xanthophylls and β-carotene in crude oils were also analyzed using the same procedure. HPLC details: column—TSKgel ODS-80TM 5 μm, 4.6 × 15 cm (Tosoh Co. Ltd., Tokyo, Japan); Jasco CO-1560 Intelligent Column Thermostat, Jasco PU-1580 Intelligent HPLC Pump, Jasco AS-950 Intelligent Sampler, Jasco UV-1575 Intelligent UV/VIS Detector (Japan Spectroscopic Co. Ltd., Tokyo, Japan). Measurement conditions: absorbance 450 nm, column temperature 25°C, flow 0.5 mL/min and analysis time 35 min. Sample preparation: 1 g oil sample was added to 10 g of mobile phase and mixed periodically using a vortex mixer. After 1 h, the supernatant was collected and used for the analysis.

Performance parameters. The performance of the membrane process was expressed in terms of percent observed rejection (R_o) and percentage reduction (PR) of color compounds in the oil.

R_o was determined, assuming that it was constant during each batch of the experiment, by using Equation 1 (2):

$$R_o = \frac{100\ \ln\left(C_{R,f}/C_{R,i}\right)}{\ln\left(W_i/W_f\right)} \qquad [1]$$

where $C_{R,i}$ and $C_{R,f}$ are the initial and final contents of color compounds in the retentates (kg/kg-oil), and W_i and W_f are the initial and final weights of retentate (kg-oil), respectively.

PR is the overall reduction of color compounds in the feed material compared to the processed oil. PR was calculated using Equation 2:

$$\mathrm{PR} = \frac{100\ (C_F - C_P)}{C_F} \qquad [2]$$

where C_F and C_P are the contents of color compounds in the feed and the processed oils (kg/kg-oil).

RESULTS AND DISCUSSION

Refined HOSF oil along with β-carotene model system. The reduction of carotenes was only 17% in the absence of any addition of surfactants (Table 1). The performance of nonporous denser membranes for oil systems has been explained qualitatively by considering together RO theory as well as denser membrane theory, as the contribution of solution-diffusion to the transport is more than that is usually observed in RO membranes (7). In the present study, permeability of

carotenes was only slightly lower than that of the triglycerides during the membrane process (Table 1). In the absence of any surfactant, the permeation of triglycerides and carotenes is primarily controlled by the solution-diffusion effect. Solution-diffusion mechanism describes the transport of a gas, vapor, or liquid through a nonporous denser membrane, which is given by Equation 3:

$$P_A = S_A \cdot D_A \quad [3]$$

where P_A is the permeability of component A, and S_A and D_A are the solubility and diffusivity of component A in the membrane, respectively (10). Solubility values of β-carotene and triglycerides were predicted based on modified group-contribution lattice-fluid equation of state (GCLF-EOS) model proposed by Lee and Danner (11). This model provides excellent predictions of solvent activity coefficients in polymers both at infinite dilution and at finite concentrations with only input of molecular structures in terms of their functional groups. Polydimethyl siloxane (PDMS), which is identical to silicon rubber, was chosen as the polymer for solubility predictions from the database of the above model. Predicted solubility values suggested that solubility of β-carotene is much higher than triglycerides in PDMS (about 10- to 11-fold) at any given value of activity. Higher solubility of one of the components leads to preferential sorption. In the experimental model, β-carotene concentration was very low (258 mg/kg) and so also its molar concentration. Therefore, the activity of carotene would be much lower than unity, and that of triglycerides would be close to unity at the feed compositions used in the experiments.

Solute diffusion can be explained by the Wilke-Chang equation (Eq. 4) (12):

$$D_{AB} = 2.946 \times 10^{-11} \times \frac{(\chi M_B)^{1/2} T}{\mu V_A^{0.6}} \quad [4]$$

where D_{AB} is the diffusivity of solute in solvent (membrane material) (m^2/s), χ the association parameter for the solvent (–), M_B the molecular weight of solvent (kg/mol), T the temperature (K), μ the viscosity of solution (Pa · s) and V_A the molar volume of the solute at the boiling point (m^3/mol). According to this relation, the lower the molar volume of the solute, the higher is the diffusivity. The molecular weight of β-carotene is 537 and that of triglyceride is ~885. Hence by virtue of lower molecular weight, the diffusivity of β-carotene would be greater than that of triglycerides in membrane material. Higher solubility and higher diffusivity of carotenes should have resulted in preferential permeation or, in other words, negative rejection. However, the actual condition changed the scenario, resulting in lower positive rejection. In a liquid system, solubility and diffusivity are strongly dependent on the feed composition unlike gas systems where they are constant (13). Further, in a ternary system (a binary mixture and a polymer), the interaction between the components of the binary mixture could alter the solubility and diffusivity of the individual components in the membrane material. Solubility plays a major role in deciding the selectivity since the difference in diffusivity of components is generally smaller in liquid systems (10). Therefore, it can be construed that actual feed composition and thermodynamic interactions between the components in the ternary system resulted in lower positive rejection instead of preferential permeation of carotenes.

TABLE 1
Percentage Reduction and Observed Rejection of Carotenoids in Membrane Processed Refined HOSF/Soybean Model Oil Systems[a]

			Carotenoid content			
Type of oil	Surfactant	Addition (%)	Feed (mg/kg)	Permeate (mg/kg)	PR (%)	R_o (%)
HOSF	Control	—	258	213	17	18
HOSF	PC	0.4	223	186	17	17
HOSF	Lecithin	2.2	244	205	16	17
HOSF	Lecithin	5.4	216	175	19	20
HOSF	Lecithin	5.2	50	45	10	11
Soybean	Lecithin	4.7	32	28	13	13

[a]PR, percentage reduction; R_o, observed rejection; HOSF, refined high-oleic sunflower oil; PC, phosphatidylcholine. The soybean oil was refined.

Refined HOSF/soybean oil along with β-carotene and PC/lecithin model system. Experiments conducted with addition of either lecithin or PC to the model HOSF oil system did not improve the rejection of carotenes (Table 1). Lecithin is an important co-product of edible oil processing obtained during degumming of crude oils. The quality and quantity of PC in the lecithin primarily decide its applications. Among the various phospholipids present in the vegetable oils, PC has the lowest critical micelle concentration (CMC) (14) and hence exhibits the highest surfactant activity. Therefore, the HOSF oil model solution containing the principal phospholipid (PC) could be considered close to the real system. During the membrane process, the rejection of carotenes in the HOSF–PC system was not different from the system without any surfactant (Table 1). The reverse micelles formed with the addition of PC in the oil system did not seem to have affinity for β-carotenes.

When PC was replaced with lecithin, the model system was much closer to the real system. However, there was only a marginal improvement in the rejection of carotenes at higher level of lecithin addition (Table 1). But once again, considering the amount of lecithin addition, the mixed phospholipid reverse micelles formed in the system did not seem to have affinity for the β-carotenes. Further increasing the ratio of lecithin to β-carotene in the feed solution by decreasing the β-carotene addition did not improve the rejection by the membrane. The system of refined soybean oil, β-carotene, and lecithin also behaved in a similar way.

Crude soybean oil system. Our earlier studies showed that nonporous membranes rejected color compounds to the extent of 80–85% in crude soybean oil (4). In the present study, the rejection of carotenoids by NTGS-2200 membrane was 79% (Table 2). Earlier studies on membrane decolorization showed that absorbance at 454 nm could be used as a simple and good index to examine the color changes in the crude and membrane-processed oil samples (4). The normalized absorbance values at 454 nm for crude oil and permeate were 8.22 and 2.32. The corresponding reduction in absorbance during membrane processing was 72%.

TABLE 2
Percentage Reduction and Observed Rejection of Carotenoids in Membrane-Processed Crude Soybean Oil and Its Model Systems

System	Feed preparation		Carotenoid content			
	Lecithin (%)	Carotene[b] (mg/kg)	Feed (mg/kg)	Permeate (mg/kg)	PR (%)	R_o (%)
Crude oil	0.0	0	47	10	79	79
Model oil[a]	0.0	49	96	51	47	49
Model oil[a]	0.0	25	73	28	62	63
Model oil[a]	5.0	26	71	28	61	62

[a]Model oil = crude oil plus additions indicated under feed preparation.
[b]Added β-carotene. For abbreviations see Table 1.

These polymeric composite membranes rejected phospholipids, a natural surfactant present in crude oils, almost completely (3,5). The rejection of phospholipids could be due either to size exclusion or to solution-diffusion effect. However, a recent study on characterization of phospholipid reverse micelles (15) showed that the solution-diffusion effect is the predominant mechanism for the observed high rejection of phospholipids by nonporous membranes when the phospholipid content is below and above its CMC in the crude oil. Furthermore, in the latter case, size exclusion may provide a synergistic effect. The phospholipid content in the crude oil used in this study was 1.1%, which is above its CMC.

Hydrocarbon (e.g., β-carotene) and oxygenated (e.g., xanthophylls) carotenoids are the two groups of carotenoids present in oil seeds. Xanthophylls (predominantly lutein) are detected in soybean, whereas no hydrocarbon carotenoids including β-carotene are detected except for a trace amount in some varieties of soybean seeds (9). In crude canola oil, carotenoids are reported to be at a level of 95 mg/kg, comprising 90% xanthophylls and 10% carotenes (16). β-Carotene, a photoprotective antioxidant in photosynthetic tissue, decreased more rapidly than other pigments during the process of seed maturation (9). Therefore, xanthophylls would be the major carotenoids present in the oils extracted from soybean and rapeseed.

During membrane processing, the greater rejection of carotenoids in vegetable oils is considered to be mainly due to their association with the reverse micelles of mixed phospholipids formed in the crude oil system. But the greater reduction of color values (65–78%) observed in screw-pressed groundnut and sunflower oils (5) revealed that the solution-diffusion effect is the predominant mechanism in the rejection of color pigments. It seems that the silicon layer of the membrane did not have higher permeability for xanthophylls resulting in greater rejection in crude oils.

Crude soybean oil along with β-carotene and lecithin model system. Lecithin and β-carotene additions and membrane performance are presented in Table 2. When the carotenoids level was nearly doubled in the feed by making the addition of β-carotene equal to the amount present in the crude oil, the absolute reduction remained similar (45 mg/kg) to that of the control experiment (37 mg/kg).

In another experimental run, when the addition of β-carotene was reduced by about 50%, the percentage reduction and rejection of carotenoids increased. However, the net reduction remained at 45 mg/kg. Addition of 5% lecithin to the above system did not appreciably change the net reduction of carotenoids. The reduction in color was 61%, which was still less than the reduction obtained in the control run. The results implied that the membrane rejected mostly xanthophylls present in the crude oil and not the added β-carotene.

Crude soybean and refined HOSF oils along with β-carotene and lecithin model system. In these experiments, crude soybean and refined HOSF oils were used in equal proportion in the feed in the absence and presence of lecithin. Lecithin and β-carotene additions and membrane performance are presented in Table 3. In this system, there was no improvement in the net reduction of color pigments. In all these experimental runs, crude oil content in the feed appeared to be the only predominant factor affecting the amount of rejection of color compounds by the membrane. The added β-carotene largely permeated through the membrane. From these experimental runs, it was once again confirmed that xanthophylls were largely rejected by the membrane, whereas a majority of the β-carotenes were permeating through the membrane.

Refined HOSF oil along with lutein and lecithin model system. The nonporous membrane rejected lutein to the extent of 60% in the absence of any addition of surfactants (Table 4), whereas the β-carotene rejection was only 18% under otherwise similar conditions. Lutein is an oxygenated carotenoid and contains two hydroxyl groups in its molecular structure. These hydroxyl groups make lutein more polar as compared to β-carotene. This may explain the higher rejection of lutein in the model system, since the membrane used in the study is hydrophobic in nature. Experiments conducted with addition of lecithin to the model HOSF oil system showed slight improvement in the rejection of lutein (Table 4). It appears that lutein has some affinity to phospholipid reverse micelles present in the oil system.

Monma *et al.* (9) reported that there are few xanthophylls other than lutein present in the crude oil. HPLC analysis of crude oil used in the study also revealed that there were few other xanthophylls, which are more polar than lutein. The membrane may reject these unidentified xanthophylls to a much greater extent. This could be a probable reason for the greater reduction in color observed in the membrane processing of crude soybean oil.

TABLE 3
Percentage Reduction and Observed Rejection of Carotenoids in Membrane-Processed Crude Soybean and Refined HOSF Model Oil Systems[a]

Proportion soybean and HOSF	Feed preparation		Carotenoid content			
	Lecithin (%)	Carotene[b] (mg/kg)	Feed (mg/kg)	Permeate (mg/kg)	PR (%)	R_o (%)
1:1	0.0	0	24	3	88	88
1:1	0.0	41	65	35	46	48
1:1	5.0	38	60	35	42	43

[a]For abbreviations see Tables 1 and 2.
[b]Added β-carotene.

TABLE 4
Percentage Reduction and Observed Rejection of Lutein in Membrane-Processed Refined HOSF Model Oil System[a]

			Lutein content			
Type of oil	Surfactant	Addition (%)	Feed (mg/kg)	Permeate (mg/kg)	PR (%)	R_o (%)
HOSF	Control	—	8.0	3.4	57.8	60.0
HOSF	Lecithin	8.1	9.6	3.2	66.6	68.6

[a]For abbreviations see Table 1.

From the present and earlier (4) studies, it can be seen that rejection of color pigments (mainly xanthophylls) by the nonporous membranes is significant in crude soybean oil. Hitherto it was contemplated that phospholipid reverse micelles have the affinity for carotenoids, and the nonporous membranes rejected carotenoids associated with the micelles. But in the crude oils, carotenoids were rejected irrespective of the phospholipid content (either above or below the CMC). This implies that xanthophylls, the major carotenoids present in the crude oils, need not necessarily have an affinity for the phospholipid reverse micelles present in the oil system for their rejection. And the rejection seems to be predominantly due to solution-diffusion effect in which the silicon active layer of the membrane does not have higher permeability for xanthophylls. However, some of the xanthophylls, being more polar in nature, may have an affinity for reverse micelles (hydrophilic polar heads are inward in the reverse micelles), which may further increase their rejection. The extent of rejection depends on the actual composition of xanthophylls present in the crude oil. The presence of phospholipid reverse micelles in the oil system could enhance the rejection of xanthophylls.

The rejection of color compounds reported by other researchers in hexane/oil solution in an ultrafiltration process (17) is mainly due to the affinity of these compounds for the reverse micelles formed in the system. Further, from this study it can be inferred that the varying rejection performances observed by these researchers with different crude vegetable oils actually depended on the composition of color compounds and their relative polarity.

β-Carotene content is very low or insignificant in crude oils such as soybean and rapeseed. From the model studies, it was observed that the rejection of β-carotenes by the membrane is very low, and also it can be inferred that β-carotenes do not have an affinity to the phospholipid reverse micelles. The nonporous membrane would not reject β-carotenes so much while rejecting oxygenated carotenoids (xanthophylls) significantly, which are more polar in nature. This would be a desirable feature of the membrane process since β-carotenes are precursors of vitamin A and also exhibit greater pro-antioxidant activity compared to oxygenated carotenoids. Retention of β-carotenes would be beneficial in processing, especially vegetable oils containing higher amounts of β-carotenes such as palm oil.

ACKNOWLEDGMENTS

The first author thanks the Department of Science and Technology, New Delhi, India, and Japan Society for Promotion of Science, Tokyo, Japan, for the award of JSPS-Ronpaku Fellowship. This research was partially supported by Bio-oriented Technology Research Advancement Institution (BRAIN), Japan. Takeo Yamaguchi and Bao-Guo Wang at the University of Tokyo provided the predicted solubility data based on modified GCLF-EOS model. Raghavarao S.M.S. Karumanchi and Jaganmohanrao Lingamallu at CFTRI provided valuable advice. Reiko Nagata and Takeo Kamada at NFRI helped in HPLC analysis.

REFERENCES

1. Hodgson, A.S., Refining and Bleaching, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996, Vol. 4, pp. 157–212.
2. Cheryan, M., *Ultrafiltration and Microfiltration Handbook*, Technomic, Lancaster, 1998, pp. 293–298, 406–413.
3. Subramanian, R., and M. Nakajima, Membrane Degumming of Crude Soybean and Rapeseed Oils, *J. Am. Oil Chem. Soc. 74*:971–975 (1997).
4. Subramanian, R., M. Nakajima, and T. Kawakatsu, Processing of Vegetable Oils Using Polymeric Composite Membranes, *J. Food Eng. 38*:41–56 (1998).
5. Subramanian, R., M. Nakajima, T. Kimura, and T. Maekawa, Membrane Process for Premium Quality Expeller-Pressed Vegetable Oils, *Food Res. Int. 31*:587–593 (1998).
6. Sipos, E.F., and B.F. Szuhaj, Soybean Oil, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996, Vol. 2, 497–601.
7. Subramanian, R., K.S.M.S. Raghavarao, H. Nabetani, M. Nakajima, T. Kimura, and T. Maekawa, Differential Permeation of Oil Constituents in Nonporous Polymeric Membranes, *J. Membr. Sci. 187*:57–69 (2001).
8. *Official Methods of Analysis of AOAC International*, 16th edn., 4th revision, AOAC International, Gaithersburg, 1998, Method 958.05.
9. Monma, M., J. Terao, M. Ito, M. Saito, and K. Chikuni, Carotenoid Components in Soybean Seeds Varying with Seed Color and Maturation Stage, *Biosci. Biotech. Biochem. 58*:926–930 (1994).
10. Humphrey, J.L., and G.E. Keller, *Separation Process Technology*, McGraw-Hill, New York, 1997, pp. 225–287.
11. Lee, B.-C., and R.P. Danner, Prediction of Polymer-Solvent Phase Equilibria by a Modified Group-Contribution EOS, *AIChE J. 42*:837–849 (1996).
12. Kagaku Kougaku Binran (*Chemical Engineering Handbook*), 6th edn., The Society of Chemical Engineers, Tokyo, Japan, 1999, pp. 111–112.
13. Mulder, M.H.V., *Basic Principles in Membrane Technology*, Kluwer Academic Publishers, Dordrecht, 1991, pp. 280–415.
14. Kanamoto, R., Y. Wada, G. Miyajima, and M. Kito, Phospholipid-Phospholipid Interaction in Soybean Oil, *J. Am. Oil Chem. Soc. 58*:1050–1053 (1981).
15. Subramanian, R., S. Ichikawa, M. Nakajima, K.S.M.S. Raghavarao, T. Kimura, and T. Maekawa, Characterization of Phospholipid Reverse Micelles in Vegetable Oils, in *Proceedings of the Third International Soybean Processing and Utilization Conference (ISPUC-III)*, Tsukuba, Japan, 2000, pp. 514–515.
16. Eskin, N.A.M., B.E. McDonald, R. Przybylski, L.J. Malcolmson, R. Scarth, T. Mag, K. Ward, and D. Adolph, Canola Oil, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996, Vol. 2, pp. 1–95.
17. Koseoglu, S.S., K.C. Rhee, and E.W. Lusas, Membrane Processing of Crude Vegetable Oils: Laboratory-Scale Membrane Degumming, Refining, and Bleaching, in *Proceedings of World Conference on Edible Fats and Oils Processing: Basic Principles and Modern Practices*, edited by D.R. Erickson, American Oil Chemists' Society, Champaign, 1990, pp. 182–188.

[Received December 18, 2000; accepted May 18, 2001]