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Separation of Nonpolar Lipid from Soybean Oil Deodorizer Distillate by Stirred Batch-Wise Silica Gel Adsorption-Desorption

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Abstract: In this study, the stirred batch-wise adsorption-desorption was employed to separate soybean oil deodorizer distillate (SODD) into a nonpolar lipid fraction (NPLF) and a polar lipid fraction (PLF). Starting with SODD that contains 4.35% fatty acid steryl esters (FASEs), 1.92% squalene, 13.19% tocopherols, and 9.22% free phytosterols, it was possible to obtain NPLF enriched with FASEs (19.5%, recovery 96.93%) and squalene (9.15%, recovery 100%). The contents of FFAs, TAGs, tocopherols, and free phytosterols remained in the NPLF were 11.59%, 1.36%, 4.83%, and 1.45%, respectively. The batch extraction employed in this study yielded about the same degree of separation as compared to that of modified soxhlet extraction. However, the advantage of the method of this study is that it can be scaled-up easily.

Keywords: Adsorption, desorption, modified soxhlet extraction, soybean oil deodorizer distillate, stirred batch-wise

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INTRODUCTION

During the deodorization of soybean oil, substances that usually give a bad taste and/or foul odor (aldehydes and ketones) are mostly removed via steam-stripping distillation. Also removed in this operation are tocopherols, phytosterols, free fatty acids (FFAs), triacylglycerols (TAGs), diacylglycerols (DAGs), monoacylglycerols (MAGs), fatty acid steryl esters (FASEs), and hydrocarbons (1–4). The distillate obtained is termed as soybean oil deodorizer distillate (SODD). SODD can be a good raw material for sources of tocopherols, free phytosterols, fatty acids, FASEs, and squalene (5).

Since bioactive compounds such as tocopherols, free phytosterols, squalene, and FASEs are minor components in SODD, enrichment of them in SODD is vital before they can be effectively fractionated and separated into an individual compound. Numerous methods have been proposed for treating SODD to isolate one or two compounds (6–13). In general, the selective separation of compounds in SODD is based on differences in their chemical and physical properties such as molecular weight, charge, solubility, polarity, or differences in volatility.

Molecular distillation or short-path distillation is by far the preferred method for isolating both thermosensitive and high molecular weight compounds. However, the disadvantages of this method are that the equipment is expensive and the operation cost is high. Other separation processes of compounds in SODD based on differences in their polarity were proposed. For example, a modified soxhlet extraction was used to separate crude rice bran oil (14) and soybean oil deodorizer distillate (12,13) into two fractions based on differences in polarity of the constituent compounds. The polarity of compounds in SODD on silica gel thin-layer chromatography (TLC) plate analysis increases in the following order: hydrocarbons < FASEs < aldeydes < ketones < TAGs < tocopherols < DAGs < FFAs < free phytosterols < MAGs (12). The TLC plates were developed in a mixture of solvent (hexane/ethyl acetate/acetic acid = 95/5/1 v/v).

Gunawan et al. (12,13) reported that separation results of modified soxhlet extraction are comparable to those obtained from molecular distillation. The advantages of modified soxhlet extraction over molecular distillation are that it operates under atmospheric pressure and lower temperature, and requires simpler equipment. Although modified soxhlet extraction is capable of eliminating tocopherols, free phytosterols, FFAs, and acylglycerols from SODD in laboratory scale, it is difficult to apply this method in large-scale operations. The problem of uneven packing becomes increasingly severe as the diameter of the column is increased, leading to an increase in height equivalent to a theoretical plate (HETP).

This has proved to be a major obstacle to the scale-up of chromatographic processes (15).

Chu et al. (16) separated vitamin E from palm fatty acid distillate using silica in a stirred batch adsorption. However, information on the separation performance, such as tocopherols recoveries, is not available. In general, a stirred batch process is poorer in its efficiency than a packed-bed column operation.

The objective of this study is to develop a new approach for the separation of non polar lipid fraction (NPLF) from SODD by using a stirred batch-wise hexane desorption to achieve the same degree of separation as that obtained by modified soxhlet extraction that we reported in previously (12,13). The effects of operation parameters, such as silica gel to SODD mass ratio, solvent volume to SODD mass ratio, and adsorption-desorption temperature on separation, were systematically investigated.

EXPERIMENTAL METHODS

Materials

Silica gel (70–230 mesh) was obtained from Silicycle (Quebec, Canada). Characteristics of the gel according to the manufacturer were as follows: particle size, 60–200 μm; pore size, 60 Å; pH, 7; water content, 6%; and specific surface area, 500 m^2/g. SODD was donated by TTET Union Corporation (Tainan, Taiwan). TLC aluminum plates (20 cm × 20 cm × 250 μm) were purchased from Machery-Nagel (Schweiz, Germany). Standard nonacosane; farnesene; cholesta-3,5-diene; squalene; fatty acids; α-, δ-, and γ-tocopherol; monooleylglycerol; diolein; triolein; and tripalmitin were obtained from Sigma Chemicals Company (St. Louis, MO). Standard β-sitosterol (practical grade) was obtained from MP Biomedicals, LLC (Aurora, OH). All solvents and reagents were of either high-performance liquid chromatography (HPLC) grade or analytical reagent grade and were obtained from commercial sources.

Adsorption Experiment

The adsorption kinetic of SODD onto silica gel was studied by using a batch reactor. Silica gel was heated in an oven at 150% for one hour to remove water. SODD (5, 10, or 15 g) was dissolved in hexane (50 mL) and the solution was put into a 250 mL glass bottle at 25°C. Silica gel (30 g) was added to this solution. The glass bottle with its content was either operated at room temperature (25°C) or at $-10°C$. The mixture was stirred at 1000 rpm for a certain time (5, 10, 30, 60, or 120 minutes). Afterwards, the mixture was filtered by using a replaceable filter and vacuum pump. Hexane was removed from the resulting filtrate to yield a non polar lipid fraction (NPLF). Soxhlet extraction was applied to extract lipids adsorbed on silica gel with ethyl acetate as the solvent. The extract was designated as polar lipid fraction (PLF).

Separation of Nonpolar Lipids from SODD

Foreign material was removed from SODD by using a 7-µm Advantec filter paper (Toyo Roshi Kaisha Ltd., Tokyo, Japan). The silica gel was kept in a furnace at 150°C for 1 h to remove its water content. A schematic drawing of the experimental set up is shown in Fig. 1. The chamber (2 L) was equipped with a jacket with a capacity of 1.7 L to control the temperature and a mechanical stirrer to give good mixing. A replaceable filter disk with pore size of 40–100 µm was installed at the bottom of the chamber for separating the silica gel and the solution. A water vacuum pump was connected to the bottom section of the chamber. Refrigerated circulating bath with ethanol as the refrigerant supplies coolant for the jacket. SODD (20 g) and a certain amount of hexane were put into the chamber at 25°C or $-6°C$. Silica gel, the amount depending on the mass ratios of silica gel to SODD, was added to this solution. A thermocouple was inserted into the chamber. The mixture was magnetically stirred at 1000 rpm for 10 min. Afterwards, the mixture was filtered by using a replaceable filter and vacuum pump. The process was repeated

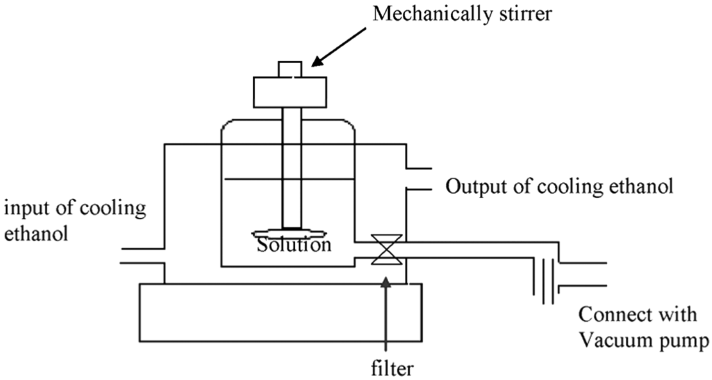

Figure 1. A schematic drawing of the experimental set up.

with the addition of the same volume of hexane. During each cycle, a portion of nonpolar compounds dissolved in the hexane. After many cycles, the desired compounds were concentrated in the filtrate fraction. Desorption was stopped after more than 95% FASEs were recovered. Hexane was removed from the pooled filtrates to yield the NPLF. Lipids adsorbed on the silica gel were then extracted by soxhlet extraction with ethyl acetate for 3 h at 71 ± 1°C, and the extract was designated as the PLF.

Analysis by TLC and High Temperature Gas Chromatography (HT-GC)

Individual components in each fraction were identified by using authentic standards for TLC and HT-GC as described by Gunawan et al. (12). TLC plates were developed in pure hexane for squalene and FASEs analysis and developed in a mixture of solvent (hexane/ethyl acetate/acetic acid = 95:5:1 v/v) for aldehydes, ketones, TAGs, tocopherols, free phytosterols, and FFAs analysis. After the plates had been dried in air, spots on each plate were visualized by exposing the chromatogram to iodine vapor. Spots for FASEs and free phytosterols were detected by spraying with a fresh solution of 50 mg of ferric chloride in a mixture of 90 mL of water, 5 mL of acetic acid, and 5 mL of sulfuric acid. After heating at 100°C for 3–5 min, it was indicated by a red-violet color (17). Spots for aldehydes and ketones were detected by spraying with a fresh solution of 400 mg 2,4-dinitrophenylhydrazine in a mixture of 100 mL methanol and 15 mL sulfuric acid. After heating the plates at 100°C for 5–10 min, the spots were indicated by a wine-red color (17).

Gas chromatography is the most applied technique in the analysis of the deodorizer distillates because it allows the analysis of different compounds in the same sample under similar condition (4,13). The contents of squalene, FASEs, free phytosterols, tocopherols, FFAs, and acylglycerols in each fraction were determined by HT-GC as described by Gunawan et al. (13). External standard calibration curves were obtained by using 0.2–20 mg pure standards. Cholesteryl stearate was selected for the determination of the FASE calibration factor and was used for all FASEs. The calibration factors of squalene, stearic acid, stigmasterol, α-tocopherol, monooleylglycerol, diolein, and tristearin were used to quantify squalene, FFAs, free phytosterols, tocopherols, MAGs, DAGs, and TAGs, respectively.

The chromatographic analysis was performed on a TLC plate and a Shimadzu GC-17A (Kyoto, Japan) gas chromatograph equipped with a flame ionization detector. Separations were carried out on a DB-5HT (5%-phenyl)-methylpolysiloxane nonpolar column (15 m × 0.32 mm i.d.; Agilent Technologies, Palo Alto, CA). The temperatures of the injector and the detector were both set at 370°C. The temperature of the column was started at 80°C, increased to 365°C at rate of 15°C/min, and maintained at 365°C for 8 min. The split ratio was 1:50 using nitrogen as carrier gas with a linear velocity of 30 cm/s at 80°C. A twenty-milligram sample was dissolved in 1 mL of ethyl acetate, and a 1-µL sample of this solution was taken and injected into the HT-GC instrument.

The total recovery of each component was calculated by the equation

$$\begin{aligned}\text{Total recovery} = {} & \{[(\text{weight of the product, g}) \\ & \times (\text{content of the compounds in the product, \%})] \\ & /[\text{weight of feed, g}) \\ & \times (\text{content of the compounds in feed(\%)}]\} \times 100\%\end{aligned} \tag{1}$$

Statistical Analysis

The reliability of the results was checked by a statistical analysis. The standard deviation (S) of the measures was calculated considering the difference between the value obtained for an individual experiment, x, and the mean value of three independent experiments, $\bar{x}$, using the formula

$$S = \sqrt{\{[\Sigma(x - \bar{x})^2]/(n - 1)\}} \tag{2}$$

where n represents the total number of experiments. The Fisher method was used to compare all pairs of treatment means (18). The statistical significance of the effects, such as silica gel to SODD mass ratio, hexane volume to SODD mass ratio, number of batches, and temperature upon separation was reported as p-value.

RESULTS AND DISCUSSION

Contact time of solid and liquid is an important parameter in adsorption-desorption. Batch adsorption isotherms of bioactive components in SODD on silica gel were studied firstly. Table 1 shows composition of SODD used in this study. The studies were conducted at an initial SODD concentration of 0.1 g/mL and 30 g of silica gel at 25 or −10°C. The results showed that squalene, FASEs, and tocopherols adsorption achieved equilibrium in less than 5 min. Therefore, an agitation time of 10 min was used through this study which should be sufficient to ensure

Table 1. SODD composition

Compounds	Composition (wt.%)	
	This work[a]	Gunawan et al. (13)
FFAs	36.45 ± 2.21	41.63 ± 0.50
TAGs	8.71 ± 1.77	8.07 ± 0.06
DAGs	1.73 ± 0.39	2.18 ± 0.12
MAGs	0.11 ± 0.03	0.12 ± 0.01
Squalene	1.92 ± 0.03	2.05 ± 0.12
FASEs	4.35 ± 0.76	4.12 ± 0.23
Tocopherols	13.19 ± 0.96	14.89 ± 0.22
Free phytosterols	9.22 ± 0.47	11.25 ± 0.14
Others[b]	24.34 ± 0.86	15.69 ± 0.70

[a] Averages of three independent measurements.

[b] Hydrocarbons, aldehydes, ketones, pesticides, herbicides, and breakdown products of tocopherols and free phytosterols.

that equilibrium has been achieved for all adsorption-desorption experiments. This result agrees with the previous observation (16) that vitamin E (tocopherols and tocotrienols) adsorption rate on silica gel was considered fast (about 5 min).

Effects of Silica Gel to SODD Mass Ratio

Table 2 shows the effect of silica gel to SODD mass ratio on the composition of the NPLF. The most important criterion for a successful separation is to recover all squalene and most FASEs in the NPLF, while concentrate most more polar compounds, such as tocopherols, free phytosterols, FFAs, MAGs, DAGs, and TAGs in the PLF. A lower silica gel to SODD mass ratio is more desirable in stirred batch adsorption because it requires less adsorbent, and can yield a higher amount of the NPLF. However, lower silica gel to SODD mass ratio is generally poorer in its separation performance than that of higher silica gel to SODD mass ratio. Higher silica gel to SODD mass ratio results in lower recovery of compounds, such as FFAs, TAGs, tocopherols, and free phytosterols in the NPLF. As a result, the amount of the NPLF obtained per unit mass of SODD also decreases because of the increasing adsorption area available per unit mass of SODD. At a silica gel to SODD mass ratio of 3, FASEs, tocopherols, free phytosterols, FFAs, and acylglycerols recoveries were significantly higher ($p < 0.05$) than those obtained while using higher silica gel to SODD mass ratios as can be seen in Table 2.

Table 2. The effects of the silica gel to SODD mass ratio on the composition of the NPLF[a–c]

	Silica gel to SODD mass ratio				
Compounds	3	6	9	12	15
Squalene	7.14 ± 0.12	14.10 ± 0.27	10.89 ± 0.19	8.52 ± 0.24	6.59 ± 0.14
	(100.0 ± 0.00)	(99.80 ± 1.48)	(68.07 ± 1.00)	(52.30 ± 0.30)	(39.80 ± 0.88)
FASEs	11.70 ± 0.39	5.20 ± 0.26	1.22 ± 0.12	1.08 ± 0.06	0.51 ± 0.00
	(68.40 ± 1.39)	(15.30 ± 1.24)	(3.20 ± 0.30)	(2.80 ± 0.23)	(1.30 ± 0.00)
FFAs	25.50 ± 0.07	26.40 ± 0.30	24.45 ± 0.00	21.53 ± 1.82	21.72 ± 1.07
	(36.36 ± 0.37)	(9.40 ± 0.42)	(7.70 ± 0.20)	(6.60 ± 0.45)	(7.00 ± 0.75)
Tocopherols	3.79 ± 0.20	1.60 ± 0.02	1.86 ± 0.15	1.40 ± 0.12	1.12 ± 0.32
	(7.70 ± 0.55)	(1.70 ± 0.10)	(1.70 ± 0.20)	(1.30 ± 0.11)	(1.00 ± 0.27)
Free phytosterols	0.32 ± 0.08	0.30 ± 0.02	0.18 ± 9.00	0.12 ± 0.02	0.12 ± 0.03
	(0.80 ± 0.17)	(0.30 ± 0.01)	(0.20 ± 0.00)	(0.10 ± 0.04)	(0.20 ± 0.06)
MAGs	0.02 ± 0.03	0.00 ± 0.00	0.02 ± 0.00	0.10 ± 0.00	0.01 ± 0.00
	(3.60 ± 0.01)	(2.60 ± 0.10)	(2.30 ± 0.00)	(0.90 ± 0.60)	(1.00 ± 0.60)
DAGs	0.25 ± 0.03	0.10 ± 0.00	0.03 ± 0.01	0.04 ± 0.02	0.05 ± 0.02
	(3.80 ± 0.01)	(0.60 ± 0.02)	(0.20 ± 0.00)	(0.30 ± 0.13)	(0.30 ± 0.14)
TAGs	1.62 ± 0.00	0.30 ± 0.02	0.05 ± 0.00	0.02 ± 0.01	0.03 ± 0.00
	(9.50 ± 0.14)	(0.80 ± 0.04)	(0.10 ± 0.00)	(0.10 ± 0.02)	(0.10 ± 0.03)
Others[d]	38.83 ± 0.32	52.80 ± 0.27	61.30 ± 0.18	67.26 ± 1.83	69.88 ± 1.29
	(38.20 ± 0.81)	(25.40 ± 0.76)	(27.20 ± 1.00)	(29.00 ± 1.44)	(29.60 ± 0.94)
NPLF/SODD, %	0.26 ± 0.003	0.13 ± 0.004	0.12 ± 0.005	0.11 ± 0.003	0.11 ± 0.057

Operation conditions: 20 g SODD, 800 mL hexane, temperature = 25°C, and stirrer speed = 1000 rpm.

[a] Each value represents the mean of three independent experiments.

[b] Values reported as contents (wt%), with recoveries (%) in parenthese.

[c] Recovery = {[(NPLF mass, g) × (content of the compound in the NPLF, %)]/[(SODD mass, g) × (content of the compound in SODD (%)]} × 100%.

[d] Hydrocarbons, aldehydes, ketones, pesticides, herbicides, and breakdown products of tocopherols and free phytosterols.

Contents and recoveries of free phytosterols, and acylglyecrols were not significantly different ($p > 0.05$) among the silica gel to SODD mass ratios (from 6 to 15) studied. However, the squalene recovery at silica gel to SODD mass ratio of 3 was not significantly different ($p > 0.05$) from that obtained while using a silica gel to SODD ratio of 6. If it is desirable to concentrate squalene and FASEs into the NPLF, then a silica gel to SODD mass ratio of 6 is preferred due to the higher recovery of squalene and FASEs and the lower recovery of tocopherols, free phytosterols, and acylglycerols in NPLF. From Table 2, it can be seen that silica gel to SODD mass ratios greater than 6 did not result in successful separation.

Nonpolar compounds such as squalene and FASEs were weakly adsorbed onto silica gel and were easily dissolved by hexane during adsorption. On the other hand, more polar compounds, such as FFAs, and TAGs, as well as tocopherols and free phytosterols, were more strongly adsorbed onto silica gel. A higher silica gel to SODD mass ratio yielded a higher adsorption area available per unit mass of SODD, hence a poorer recovery of FASEs in the NPLF, and less NPLF obtained. Besides, higher silica gel to SODD ratio also resulted in a higher number of extractions in order to achieved 100% recovery of squalene in the NPLF (12,13).

This result agrees with previous observation that the presence of a hydroxyl group on silica gel imparts a degree of polarity to the surface, so that polar molecules such as water, alcohols, phenols, and amines (which can form hydrogen bonds) and unsaturated hydrocarbons (which can form π-complexes) are adsorbed preferentially over nonpolar molecules such as saturated hydrocarbons (15). The amount of FFAs adsorbed per unit area of silica gel was found to increase in proportional to the surface area of the silica gel (19), which is again consistent with the results shown in Table 2.

Effects of Hexane to SODD Ratio

A higher amount of solvent used in each batch is more favorable because it not only reduces the total number of batches needed to acquire a certain degree of separation but also yields a higher amount of NPLF. However, successful separation was not achieved when a hexane to SODD ratio (ml/g) of more than 40 was used due to a poor separation on tocopherols. Table 3 shows the relation between the amount of hexane and the number of batches required to achieve 100% squalene recovery and more than 95% recovery of FASEs in the NPLF. The lowest amount of hexane investigated in this study was 200 ml to avoid sludge-like formation.

Table 3. Effects of amount of hexane on the composition of the NPLF[a–c]

Compounds	Hexane to SODD ratio (mL/g) 10	 20	 40
Squalene	7.81 ± 0.02[b] (100)[c]	7.69 ± 0.02 (100)	6.26 ± 0.14 (100)
FASEs	16.58 ± 0.49 (96.61 ± 0.41)	17.05 ± 0.59 (96.02 ± 0.84)	13.54 ± 0.25 (96.37 ± 0.84)
FFAs	22.39 ± 1.11 (15.60 ± 0.96)	28.67 ± 1.10 (20.02 ± 1.40)	24.55 ± 0.28 (26.22 ± 0.46)
Tocopherols	11.55 ± 0.11 (21.51 ± 0.29)	16.08 ± 0.05 (30.31 ± 0.13)	16.32 ± 0.89 (40.33 ± 1.23)
Free phytosterols	1.77 ± 0.15 (4.84 ± 0.51)	3.91 ± 0.19 (11.13 ± 1.52)	4.46 ± 0.12 (16.17 ± 1.59)
MAGs	0.02 ± 0.01 (3.63 ± 1.21)	0.01 ± 0.00 (3.64 ± 0.45)	0.01 ± 0.00 (2.27 ± 0.92)
DAGs	0.45 ± 0.18 (4.59 ± 2.43)	0.27 ± 0.02 (4.03 ± 0.49)	0.23 ± 0.02 (4.13 ± 0.29)
TAGs	2.37 ± 0.25 (4.91 ± 0.89)	1.16 ± 0.19 (3.41 ± 0.41)	0.90 ± 0.02 (3.17 ± 0.15)
Others[d]	36.91 ± 0.67 (38.12 ± 0.21)	27.33 ± 2.60 (28.02 ± 3.07)	39.61 ± 5.49 (52.03 ± 8.85)
NPLF/SODD, %	25.07 ± 1.16	27.22 ± 0.60	33.52 ± 0.43
Number of batches	75	50	34

Operation conditions: 20 g SODD, silica gel to SODD mass ratio = 6, temperature = 25°C and stirrer speed = 1000 rpm.

[a] Each value represents the mean of three independent experiments.

[b] Values reported as contents (wt%), with recoveries (%) in parenthese.

[c] Recovery = {[(NPLF mass, g) × (content of the compound in the NPLF, %)]/[(SODD mass, g) × (content of the compound in SODD (%)]} × 100%.

[d] Hydrocarbons, aldehydes, ketones, pesticides, herbicides, and breakdown products of tocopherols and free phytosterols.

It can be seen from Table 3 that the amount of the NPLF obtained, in terms of the percentage of SODD, increases ($p < 0.05$) with the amount of hexane. This is because the use of a higher hexane to SODD ratio yielded a larger amount of hexane, hence, more compounds dissolved in hexane. However, the total recoveries of squalene, FASEs, MAGs, DAGs, and TAGs were not significantly different ($p > 0.05$). Moreover, this study shows that as the hexane to SODD ratio decreases from 40 to 10, the total recoveries of tocopherols, free phytosterols, and FFAs reduce from 40.33% to 21.51%, 16.17% to 4.84%, and 26.22% to 15.6%, respectively, whereas the total batch number increases from 34 to 75.

The adsorption process is often conceptualized as a multi-step process involving molecular diffusion, surface diffusion, pore diffusion, and sorption (20). The adsorbate is adsorbed by the adsorbent through molecular diffusion, surface diffusion, and pore diffusion. In desorption, the steps are reversed: pore diffusion, surface diffusion, and molecular diffusion. The most important parameter that affects desorption is the amount of the solvent. If the concentration of the solute is high, desorption is controlled by molecular diffusion. As the concentration of the solute is reduced, the effects of surface diffusion and pore diffusion should be considered.

Table 4. Effects of adsorption temperature on the composition of the NPLF[a–c]

	Adsorption temperature	
Compounds	25°C	−6°C
Squalene	6.26 ± 0.14[b] (100)[c]	9.15 ± 0.12 (100)
FASEs	13.54 ± 0.25 (96.37 ± 0.84)	19.50 ± 0.29 (96.93 ± 0.70)
FFAs	24.55 ± 0.28 (26.22 ± 0.46)	11.59 ± 3.15 (6.63 ± 1.85)
Tocopherols	16.32 ± 0.89 (40.33 ± 1.23)	4.83 ± 0.64 (8.00 ± 1.15)
Free phytosterols	4.46 ± 0.12 (16.17 ± 1.59)	1.45 ± 0.16 (3.40 ± 0.36)
MAGs	0.01 ± 0.00 (2.27 ± 0.92)	0.03 ± 0.00 (1.34 ± 0.45)
DAGs	0.23 ± 0.02 (4.13 ± 0.29)	0.46 ± 0.11 (5.80 ± 0.49)
TAGs	0.90 ± 0.02 (3.17 ± 0.15)	1.36 ± 0.66 (3.30 ± 0.16)
Others[d]	39.61 ± 5.49 (52.03 ± 8.85)	51.52 ± 3.21 (45.07 ± 4.20)
NPLF/SODD,%	33.52 ± 0.43	21.37 ± 0.54
Number of batches	34	70

Operation conditions: 20 g SODD, hexane to SODD ratio = 40 (mL/g), silica gel to SODD mass ratio = 6, and stirrer speed = 1000 rpm.

[a] Each value represents the mean of three independent experiments.

[b] Values reported as contents (wt%), with recoveries (%) in parenthese.

[c] Recovery = {[(NPLF mass, g) × (content of the compound in the NPLF, %)]/[(SODD mass, g) × (content of the compound in SODD (%)]} × 100%.

[d] Hydrocarbons, aldehydes, ketones, pesticides, herbicides, and breakdown products of tocopherols and free phytosterols.

In summary, the results of a stirred batch-wise silica gel adsorption-desorption study show that a lower amount of hexane leads to lower contents and recoveries of FFAs, tocopherols, and free phytosterols in NPLF. However, a higher amount of hexane can reduce the total number of batches required to achieve a specified separation.

Effect of Temperature

Table 4 shows the relation between adsorption-desorption temperature and number of batches required to achieve 100% squalene recovery and over 95% FASEs recoveries in the NPLF. Only two temperatures were investigated in this study. A lower adsorption-desorption temperature is favorable because it minimizes the degradation of bioactive compounds in SODD. The lowest temperature investigated in this study was −6°C due to the capacity of the cooling system. The highest temperature used was 25°C (room temperature). Although higher temperature resulted in higher amount of NPLF and smaller number of total batches required, it yielded poorer separation (12).

It can be seen from Table 4 that the amount of the NPLF obtained, expressed as percentage of SODD, increases with increasing temperature. In addition, the total number of batches required decreases from 70 to 34

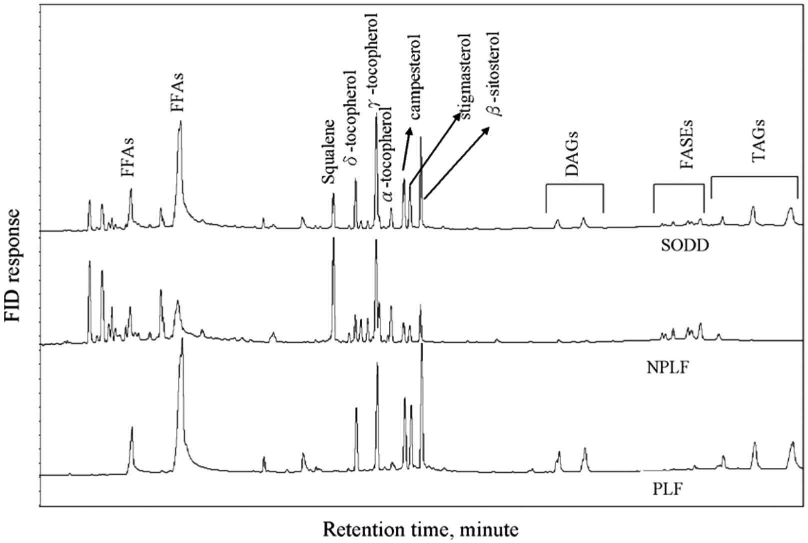

Figure 2. Results of HT-GC analyses. Operation conditions for stirred batch desorption: 20 g SODD, silica gel to SODD mass ratio = 6, hexane to SODD ratio = 40 (mL/g), temperature = −6 °C, and total number of batches = 70.

as temperature increases from −6°C to 25°C. All contents and recoveries of tocopherols, free phytosterols, FFAs, MAGs, DAGs, and TAGs were significantly lower ($p < 0.05$) at −6°C than those obtained at 25°C. At −6°C, most of the tocopherols, free phytosterols, FFAs, and TAGs prefer to exist in silica gel.

It was found that stirred batch-wise adsorption-desorption resulted in a lower number of batches and a higher the NPLF to SODD mass ratio when operated under higher temperature. However, it was less efficient at removing FFAs, TAGs, tocopherols, and free phytosterols from the NPLF. Since adsorption is usually an exothermic process, an increase in temperature will result in a decrease of polar compounds adsorbed onto silica gel. A decrease in adsorption-desorption temperature yielded a better separation of polar and nonpolar components, as can be seen in Table 4. These results agree with previous observations that the ultimate capacity of silica gel is generally higher at lower temperature (15).

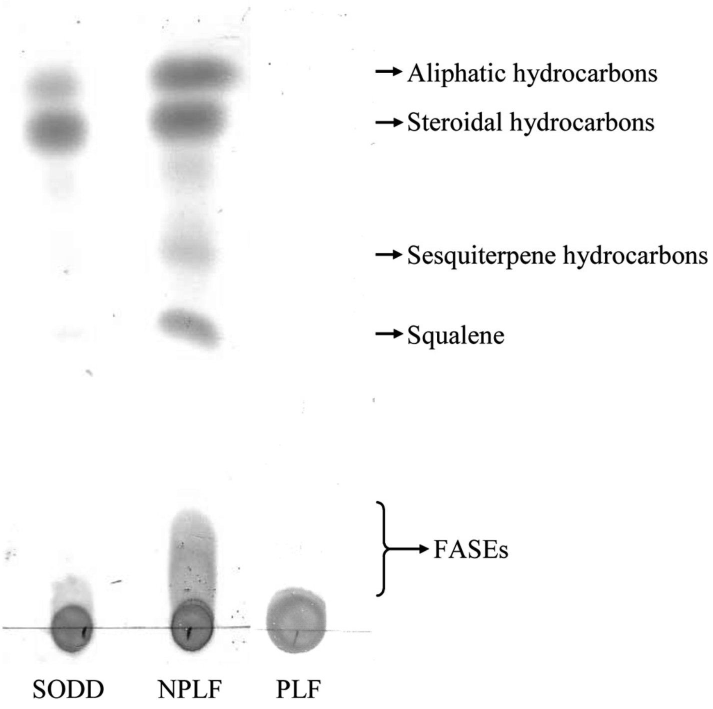

Figure 3. TLC analysis of the SODD, the NPLF and the PLF developed in pure hexane. Operation conditions for stirred batch desorption: 20 g SODD, silica gel to SODD mass ratio = 6, hexane to SODD ratio = 40 (mL/g), temperature = −6 °C, and total number of batches = 70.

Chu et al. (16) found that lower temperature led to higher vitamin E uptake at equilibrium, indicating that vitamin E adsorption by silica is an exothermic process. The binding strength between silica and acid is higher than that between silica and ester, which is still higher than that between silica and alcohol (19). In another work, Moreira and Baltanas (21) showed that separation of free phytosterols and tocopherols is preferably performed through fractional crystallization because free phytosterols tend to precipitate at lower temperature.

HT-GC chromatograms in Fig. 2 show that after stirred batch desorption-extraction, most of the FFAs along with tocopherols and free phytosterols were concentrated in the PLF. The FFAs region in the chromatogram of the NPLF shows interferences by minor components, which are hydrocarbons such as aliphatic, sesquiterpene, and tetraterpene (squalene) hydrocarbons as shown in Fig. 3. Steroidal hydrocarbons exist along with tocopherols and free phytosterols. These steroidal hydrocarbons may have arisen from the oxidation of tocopherols and free phytosterols during the refining of soybean oil. HT-GC analysis of

Table 5. A Comparison of the NPLF obtained from SODD by stirred batch adsorption-desorption and modified soxhlet extraction[a]

	Separation method			
	Stirred batch-wise adsorption-desorption[b]		Modified soxhlet extraction[c]	
Compounds	Content, %	Recovery, %	Content, %	Recovery, %
Squalene	9.15	100	8.16	100
FASEs	19.50	96.93	14.05	92.47
FFAs	11.59	6.93	31.71	19.18
TAGs	1.36	3.30	0.55	1.71
Tocopherols	4.83	8.00	3.98	6.67
Free phytosterols	1.45	3.40	0.29	0.64
Others[d]	51.52	45.07	40.88	65.67
Fraction/SODD (%)	21.37		25.16	

SODD compositions are shown in Table 1.

[a] Each value represents the mean of three independent experiments.

[b] Operating conditions: silica gel to SODD mass ratio = 6; Temperature = −6°C; and number of batches = 70.

[c] Reference 13. Operating conditions: silica gel to SODD mass ratio = 3; Temperature = −6°C; and number of extractions = 88 cycles.

[d] Hydrocarbons, aldehydes, ketones, pesticides, herbicides, and breakdown products of tocopherols and free phytosterols.

the PLF showed absence of squalene. Significant amounts of aldehydes and ketones exist both in the NPLF and the PLF.

Stirred batch-wise adsorption-desorption operated on SODD at −6°C could recover 100% squalene and 96.93% FASEs in the NPLF, decrease tocopherols content by 1/3 and free phytosterols content and 1/6 in the NPLF. These results are comparable with those obtained by modified soxhlet extraction as shown in Table 5 which shows that modified soxhlet extraction operated on SODD could recover squalene and FASEs by 100% and 92.47% in the NPLF, respectively. Moreover, tocopherols and free phytosterols contents decrease from 14.89% to 3.98% and from 11.25% to 0.29%, respectively.

CONCLUSION

Separation of SODD into a NPLF and a PLF by batch-wise silica gel adsorption-desorption to achieve the same degree of separation as the modified soxhlet extraction was carried out in this study. From results obtained in this study, it can be seen that stirred batch-wise silica gel adsorption-desorption, which is much easier to scale up than modified soxhlet extraction, can concentrate hydrocarbons and FASEs into the NPLF. Higher mass ratio of silica gel to SODD provides more adsorbent and yields lower production of the NPLF, better separation, and higher total number of batches. A higher production of the NPLF and a lower total number of batches can be achieved while using a higher amount of hexane for each batch; however, it results in poorer separation. Temperature is another important parameter. Lower temperature gives better separation but results in a lower amount of the NPLF produced, and a higher total number of batches. In addition, the NPLF contains squalene and FASEs and can be processed further to obtain pure squalene and FASEs as described in our previous work (12,13).

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NOMENCLATURE

DAGs	diacylglycerols
FASEs	fatty acid steryl esters

FFAs	free fatty acids
HETP	height equivalent to a theoretical plate
HTGC	high-temperature gas chromatography
MAGs	monoacylglycerols
NPLF	nonpolar lipid fraction
PLF	polar lipid fraction
S	standard deviation of the measures
SODD	soybean oil deodorizer distillate
TAGs	triacylglycerols
TLC	thin-layer chromatography
n	total number of experiment
p	probability
x	value of individual experiment
$\bar{x}$	mean value of three independent experiments

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