Partial Separation of Polyunsaturated Fatty Acid Esters from FAMEs Mixtures by Adsorption on Silver Nitrate-Impregnated Silica Gel

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ABSTRACT: Mixtures of FAMEs derived from soybean and canola oils were fractionated by contacting their hexane solutions with $AgNO_3/SiO_2$ adsorbents. Methyl linolenate (18:3) adsorbed most strongly, followed by methyl linoleate (18:2), on the $AgNO_3/SiO_2$. Conditions of the extractions ($AgNO_3$ loading, amount of adsorbent, methyl soyate/hexane solution concentration, use of successive extractions, and methods of adsorbent regeneration) were varied. Under optimal conditions, the 7.0% of 18:3 in methyl soyate could be reduced to 0.1%. The described process is a simple method for separating a FAMEs mixture into a fraction that is depleted in polyunsaturated FAMEs and one that is enriched.

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World production of vegetable oils was 201 billion pounds (91.1 million metric tons) in 2000 and has been increasing since (1). In the United States, the amount is 23.5 billion pounds (10.6 million metric tons), of which soybean oil is produced in the largest quantity (16.2 billion pounds). Although these oils are used mostly for food applications, a major emerging use of such oils is as biodiesel fuel, which is generated by the transesterification of the oil with methanol. In the United States, the biodiesel FAMEs mixture is derived primarily from soybean oil, whereas canola/rapeseed is the major source in Europe (2). Biodiesel production worldwide is now approximately 4 billion pounds (1.8 million metric tons) (3). The economical production of these FAMEs mixtures means that such mixtures can serve as feedstocks for the production of other chemicals (4). A major barrier to the use of FAMEs mixtures as a feedstock is that the presence of certain FA makes them unsuitable for a variety of applications. For example, the presence of linolenic acid makes vegetable oils susceptible to oxidation, thereby reducing their usefulness as cooking oils and lubricants. On the other hand, saturated FA and oleic acid are undesirable in oils that might be used as drying oils.

The purpose of the present study is to explore the possibility that the composition of the FAMEs mixtures derived from vegetable oils can be changed by selectively removing certain FA esters thereby leaving a FAMEs mixture that has desirable properties for new applications. For example, removal of linolenic acid methyl ester (18:3) from methyl soyate would leave a FAMEs mixture that, when transesterified with glycerol, would give an oil that is much less sensitive to oxidation than natural soybean oil.

Although $AgNO_3$ adsorbed on silica has long been known (5–7) to serve as a chromatographic support for the separation of various FAMEs mixtures, it has not, to our knowledge, been used to alter the composition of larger amounts of FAMEs mixtures by selective removal of certain FAMEs. In the present paper, we describe the selective removal of 18:3 from FAMEs derived from soybean and canola oils using the solid-phase extractant $AgNO_3/SiO_2$. We also explore more broadly the effects of various extraction conditions on the compositions of FAMEs obtained from $AgNO_3/SiO_2$ extractions. We find that these compositions are substantially different from those of the original oil-derived FAMEs mixtures. A goal of these investigations is to demonstrate that the compositions of relatively large amounts of FAMEs mixtures can be adjusted by extraction with $AgNO_3/SiO_2$. The only other reported method for the partial separation of significant amounts of FAMEs involves solvent extraction with $AgNO_3$ solutions (8), a process that is not as simple as that reported herein.

EXPERIMENTAL PROCEDURES

Materials. AG Environmental L.L.C. (Omaha, NE) supplied the Soy Clear FAMEs mixture (methyl soyate), which was used as received. The canola oil used was purchased from a local store and transesterified with methanol to give methyl canolate using procedures described in the literature (9,10). The mesoporous silica, SBA-15, was prepared using the Pluronic P123 (Aldrich, Milwaukee, WI) surfactant as described in the literature (11). All other chemicals and solvents were reagent grade and were used as received. All manipulations were conducted in air. A gas chromatograph (Hewlett-Packard, 6800 series) equipped with an FID and fused-silica capillary column (SP^{TM} 2330, 30 m × 0.25 mm × 0.25 µm film thickness; Supelco, Bellefonte, PA) was used to determine compositions of the FAMEs mixtures. All weights were measured on an analytical balance and were accurate to ±0.0005 g.

Methods. (i) Conditions of GC analysis. The conditions of GC analysis were as follows: 1.2 mL min^{-1} of He carrier gas; split injection; FID temperature 250°C; injector temperature 250°C and oven temperature 200°C. The response factor for

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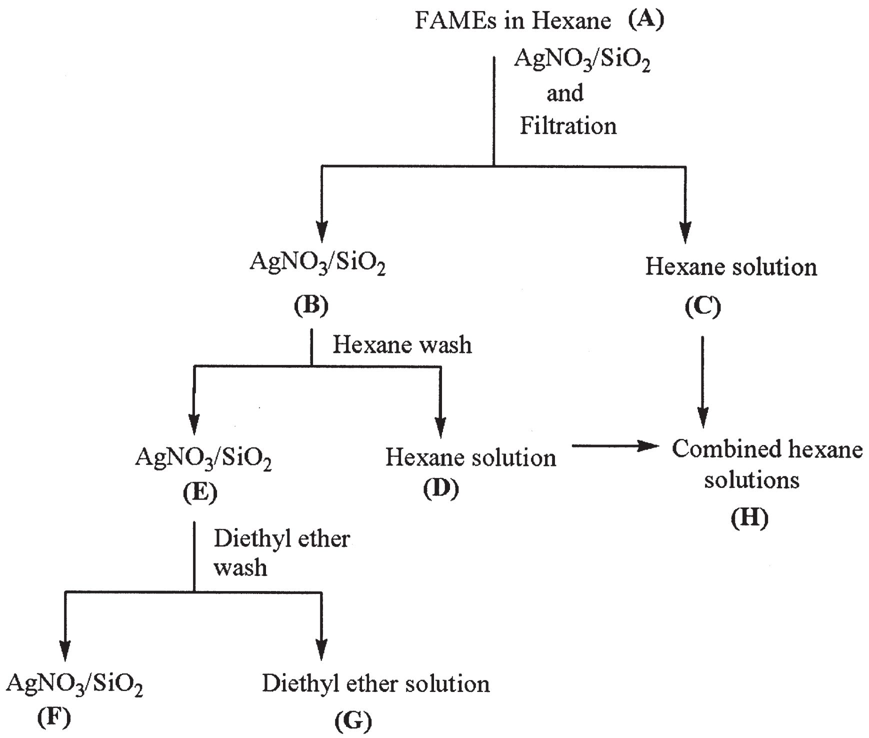

SCHEME 1

each FAME present in the mixture (16:0, 18:0, 18:1, 18:2, and 18:3) relative to the methyl heptadecanoate (17:0) standard was determined (9). As the response factor values were 1.00 ± 0.04, the percentage composition of the FAMEs was calculated without correcting from the relative peak areas in the chromatograms.

(ii) Preparation of $AgNO_3/SiO_2$. Four different compositions (10, 20, 30, 40%, w/w) of $AgNO_3/SiO_2$ were prepared by adding the necessary amounts of $AgNO_3$ and SiO_2. A typical procedure for the synthesis of 20% $AgNO_3/SiO_2$ was as follows: To a solution of $AgNO_3$ (1.0 g) in deionized water (30 mL) was added 4.0 g of SiO_2 (200–400 mesh) (Aldrich). The mixture was stirred at room temperature for 15 min and then dried in an oven at 120°C for 24 h to furnish a free-flowing white solid. The same procedure was followed for the synthesis of the 10, 30, and 40% $AgNO_3$ loadings.

Similarly, the 20% $AgNO_3$/SBA-15 adsorbent was prepared by mixing a solution of $AgNO_3$ (1.0 g) in deionized water (30 mL) and 4.0 g of SBA-15 with stirring. The mixture was then dried in an oven at 105°C for 24 h. The adsorbent had a 394 m^2/g BET surface area and a 57.5 Å pore size. These parameters were measured at 77 K using the BET/BJH (Brunnauer-Emmett-Teller/Barrett-Joyner-Halenda) method with N_2 gas on a Micromeritics ASAP 2000 (Norcross, GA) instrument.

(iii) General procedure for FAMEs extractions (Scheme 1). A 5.00 g sample of $AgNO_3/SiO_2$ was loaded into a glass column (20 × 3 cm; $AgNO_3/SiO_2$ bed height is ~3 cm) containing a medium-porosity frit. A solution (**A** in Scheme 1) of 1.0 mL of the FAMEs mixture in 100 mL of hexane was added on top of the $AgNO_3/SiO_2$; the mixture was allowed to stand for 30 min during which time the FAMEs partially adsorbed to the $AgNO_3/SiO_2$. Then the solution was gravity filtered (~45 min) through the frit to give hexane solution **C**. The remaining $AgNO_3/SiO_2$ adsorbent (**B**) was then washed with 2 × 50 mL of hexane; this hexane solution (**D**) was added to the gravity-filtered hexane solution (**C**). Then the $AgNO_3/SiO_2$ (**E**) was washed with 3 × 50 mL diethyl ether to desorb the polyunsaturated FAMEs in diethyl ether solution (**G**). The hexane (**D**) and diethyl ether (**G**) extraction solutions were vacuum filtered through the frit. After filtration, the solvents were removed by rotary evaporation under reduced pressure. Then the compositions of the hexane (**H**) and diethyl ether (**G**) fractions were analyzed using GC. All of the extractions were conducted at room temperature.

(iv) Optimization of the extraction process. Preliminary studies (data not shown here) were conducted to determine the optimal conditions (used in the above procedure) with respect to effect of stirring, reaction time, adsorbent type, and solvents for the extraction process. The effect of stirring was first determined with a mechanical stirrer in a 250-mL round-bottomed flask. The flask was charged with a 5.0-g sample of 20% $AgNO_3/SiO_2$ and 1.0 mL of FAMEs in 100 mL of hexane, and the mixture was stirred vigorously for 1.0 h. Then, the solution was gravity filtered through a Whatman filter paper (#5, 110 mm diameter). The remaining $AgNO_3/SiO_2$ adsorbent was then washed with 2 × 50 mL of hexane followed by 3 × 50 mL of diethyl ether. The FAMEs compositions of the hexane and diethyl ether fractions were determined using GC. After stirring, the impeller shaft of the mechanical stirrer was covered with metallic silver, indicating that some of the $AgNO_3$ had leached from the SiO_2 during the stirring process. Hence, use of this stirring mode was avoided.

In a second stirring trial, a magnetic stir bar was used to stir the $AgNO_3/SiO_2$ and FAMEs mixture. The results showed that this method gave the same composition of FAMEs as that found with the mechanical stirring, but there was no evidence of $AgNO_3$ leaching. In the third procedure, the extraction process was conducted without stirring. This method also gave the same composition of FAMEs in the hexane and diethyl ether fractions as those obtained with stirring. Thus, stirring did not appear to improve the process significantly in any way. Therefore, all extractions described in this report were conducted without stirring.

To determine the time required for the extractions to reach equilibrium, we analyzed aliquots of the solution in the $AgNO_3/SiO_2$ and FAMEs/hexane mixtures every 15 min for the first hour and every half hour thereafter. Analyses of these aliquots showed that no change in composition occurred after 15 min of contact of the FAMEs/hexane solution with the $AgNO_3/SiO_2$ extractant at room temperature. Thus, longer times were not required in this extraction process. To ensure that equilibrium was achieved, all extractions were performed for 30 min.

Various wash solvents were tried for the removal of the adsorbed FAMEs from the $AgNO_3/SiO_2$ (**E**) adsorbent. The solvents tried were acetone, acetonitrile, methylene chloride, ethanol, methanol, 2-propanol, *n*-butanol, chloroform, ethyl acetate, THF, diethyl ether, and benzene. After the extraction process, the $AgNO_3/SiO_2$ adsorbent was washed with 2 × 50 mL of hexane and 3 × 50 mL of a solvent to remove the unsaturated FAMEs. The solutions were then vacuum filtered through the frit, and the solvents were removed by rotary evaporation under reduced pressure; the FAMEs composition in each solvent was analyzed using GC. When acetone, acetonitrile, methylene chloride, ethanol, methanol, 2-propanol, *n*-butanol, chloroform, ethyl acetate, THF, and benzene solvents were used, some leaching of $AgNO_3$ from the $AgNO_3/SiO_2$ adsorbent was observed. That is, after the solvents were removed by rotary evaporation, the flask was covered with white solid and metallic silver together with the desired solution indicating that some $AgNO_3$ had leached from the SiO_2 solid. However, when diethyl ether was used, excellent desorption of the FAMEs was achieved without leaching of $AgNO_3$. Hence, diethyl ether was chosen for all extractions. Also, no leaching was observed with hexane.

Two other adsorbents ($AgNO_3/Al_2O_3$ and $AgNO_3$/Florisil^{TM}) were also prepared by mixing 1.0 g of $AgNO_3$ in 30 mL of deionized water with 4.0 g of Al_2O_3 (A950–500, neutral, 60–325 mesh; Fisher, Fairlawn, NJ) or Florisil (F100–500, 60–100 mesh; Fisher). The mixture was stirred at room temperature for 15 min and then dried in an oven at 120°C for 24 h. The resulting 20% $AgNO_3/Al_2O_3$ and 20% $AgNO_3$/Florisil adsorbents were then used in the extraction of methyl soyate using procedures similar to those described above and in Scheme 1. However, the extraction results (data not shown here) obtained using these adsorbents indicated that these adsorbents were not as effective for removing 18:3 as $AgNO_3/SiO_2$ (200–400 mesh). For example, the percentage of 18:3 in the hexane (**H**) fraction was 1.1% when 20% $AgNO_3/Al_2O_3$ and 20% $AgNO_3$/Florisil were used as the extractants; this compared with 0.1% when 20% $AgNO_3/SiO_2$ (200–400 mesh) was used.

$AgNO_3/SiO_2$ adsorbents prepared from silica gels (70–270 mesh and 130–270 mesh; Aldrich) were also tested in the extraction process. These silica gels have the same textural properties as that of silica gel (200–400 mesh), i.e., BET surface area (500 m^2/g), pore size (60 Å), and pore volume (0.75 cm^3/g). These silica gels were impregnated with $AgNO_3$ using procedures similar to those described in the second part of the Methods portion of this Experimental Procedures section. However, the extraction results (data not shown here) obtained using these $AgNO_3/SiO_2$ adsorbents indicated that these adsorbents were not as effective for the removal of 18:3 as $AgNO_3/SiO_2$ (200–400 mesh). For example, the percentage of 18:3 in the hexane (**H**) fraction was 0.7% when 20% $AgNO_3/SiO_2$ (70–270 mesh) and 20% $AgNO_3/SiO_2$ (130–270) were used as the extractants; this compares with 0.1% when 20% $AgNO_3/SiO_2$ (200–400 mesh) was used. Hence, data reported in this work are based on extractions using $AgNO_3/SiO_2$-containing silica gel (200–400 mesh).

(v) Analysis of the composition of FAMEs. An aliquot (70 μL) from each of the hexane (**H**) and diethyl ether (**G**) solutions (after evaporation; see preceding paragraphs) was added to 5.0 mL of hexane. Then, 50 μL of each of these solutions was mixed with 50 μL of the internal standard methyl heptadecanoate (17:0). Next, 1 μL of each solution (in triplicate) was injected into the HP 6800 series gas chromatograph fitted with an FID. The concentrations of the individual methyl esters were recorded with the HP 3396 series III integrator. The percentage of each FAME was calculated by comparing the peak area to that of the internal standard. The volumes of FAMEs in the hexane (**H**) and diethyl ether (**G**) fractions were measured using a 1-mL disposable syringe and were accurate to ±0.005 mL.

RESULTS AND DISCUSSION

The extraction process. The overall extraction process for the partial separation of FAMEs is outlined in Scheme 1. It involves simply allowing a hexane solution (**A**) of a FAMEs mixture (e.g., methyl soyate or methyl canolate) to sit with the solid $AgNO_3/SiO_2$ adsorbent for 30 min. Then the solid $AgNO_3/SiO_2$ (**B**) is filtered from the hexane solution (**C**). The $AgNO_3/SiO_2$ (**B**) is washed with hexane to give an additional hexane solution (**D**), which is combined with **C** to give the entire hexane solution (**H**). The $AgNO_3/SiO_2$ adsorbent (**E**) is then washed with diethyl ether, which removes all of the adsorbed FAMEs as the diethyl ether solution (**G**). In general, the saturated (16:0, 18:0) and monounsaturated (18:1) FAMEs tend to remain in the hexane solution, whereas the polyunsaturated (18:2, 18:3) FAMEs tend to adsorb on the $AgNO_3/SiO_2$ and are major components of the diethyl ether solution (**G**).

Effect of increasing the amount of SiO_2 on extractions of methyl soyate by $AgNO_3/SiO_2$. In this series of experiments, extractions were performed with $AgNO_3/SiO_2$ containing the

TABLE 1
Effect of Increasing the Amount of SiO_2 on the FAMEs Composition (wt%) of the Hexane (H) and Diethyl Ether (G) Fractions from Extractions of Methyl Soyate with $AgNO_3/SiO_2$

		% $AgNO_3/SiO_2$ used in extractions[a]			
FAMEs	Feed	10	20	30	40
16:0	10.3	0 (10.3)[b]	27.7 (0.0)[b]	16.0 (0.0)[b]	14.9 (0.0)[b]
18:0	4.5	0 (4.5)	12.4 (0.0)	7.1 (0.0)	6.7 (0.0)
18:1	24.6	0 (24.6)	39.4 (14.2)	32.9 (6.8)	31.3 (6.6)
18:2	53.6	0 (53.6)	20.4 (76.4)	43.2 (74.4)	46.2 (74.2)
18:3	7.0	0 (7.0)	0.1 (9.4)	0.8 (18.8)	0.9 (19.2)
18:2/18:1 ratio	2.2	0 (2.2)	0.52 (5.38)	1.3 (10.9)	1.5 (11.2)
FAME recovered (mL)	1.0	0.0 (1.0)	0.32 (0.65)	0.60 (0.35)	0.70 (0.26)

[a] 1.0 g of $AgNO_3$ in each adsorbent with different amounts of SiO_2.
[b] The first number is the amount in the hexane fraction (**H**); the number in parentheses is the amount in the diethyl ether fraction (**G**).

same amount of $AgNO_3$ but decreasing amounts of SiO_2, which had the effect of increasing the weight percentage of $AgNO_3$ from 10 to 40%. The $AgNO_3/SiO_2$ samples were prepared by adding 1.0 g of $AgNO_3$ to 9.0 g of SiO_2 (for 10% $AgNO_3$ loading) and to 1.5 g of SiO_2 (for 40% loading). Although each of the loadings contained the same amount of $AgNO_3$, the $AgNO_3$ was distributed over a much larger surface area in the 10% sample than in the 40% sample. Results of the extractions of 1.0 mL of methyl soyate in 100 mL of hexane are shown in Table 1. All (1.0 mL) of the methyl soyate adsorbed on the 10% $AgNO_3/SiO_2$ and was recovered in the diethyl ether fraction (**G**); the same behavior was observed when only SiO_2 (10 g) was the extractant. Also, when 5.0 g of pure SiO_2 was used, nearly all (0.95 mL) of the 1.0 mL of methyl soyate was adsorbed and was recovered in **G**. On the other hand, with a small amount of SiO_2 (2.0 g), most (0.90 mL) of the 1.0 mL of methyl soyate was recovered in the hexane fraction (**H**). The composition of the FAMEs in both the **H** and **G** fractions was essentially the same as that in the original methyl soyate. Thus, the silica itself did not effect a significant fractionation of methyl soyate.

In using 20% $AgNO_3/SiO_2$, in which the $AgNO_3$ units are more closely spaced, the 16:0, 18:0, and 18:1 percentages in **H** are much higher than those in methyl soyate, and the percentages of 18:2 and 18:3 are much lower. Of course, trends in the diethyl ether fraction (**G**) are just the opposite. Thus, 20% $AgNO_3/SiO_2$ is selective for the adsorption of the polyunsaturated FAMEs (18:2 and 18:3). Particularly noteworthy is the reduction in the concentration of 18:3 from 7.0% in methyl soyate to 0.1% in the hexane fraction (**H**). However, it is also important to note the higher selectivity for 18:2 as compared with 18:1; thus, the 18:2/18:1 ratio (0.52) in the hexane fraction (**H**) is much smaller than that (2.2) in methyl soyate. The final row in Table 1 shows that only 0.32 mL of the original 1.0 mL of methyl soyate is found in the hexane fraction (**H**). More (0.65 mL) of the methyl soyate is found in the diethyl ether fraction (**G**). As the $AgNO_3$ loading is increased to 30 and 40% by decreasing the amount of SiO_2, the selectivity for 18:3 and 18:2 decreases somewhat as indicated by the higher (0.9%) percentage of 18:3 and the higher 18:2/18:1 ratio (1.5) in **H**.

In an effort to understand the trends in the compositions of the hexane (**H**) and diethyl ether (**G**) fractions, one needs to consider that 10% $AgNO_3/SiO_2$ has a very high SiO_2 surface area and that the $AgNO_3$ units are widely separated. For a 500 m^2/g surface area of SiO_2, each $AgNO_3$ unit occupies 142 Å^2 on average. The large surface area (mostly SiO_2) is sufficient to adsorb all of the FAMEs. In the 20% $AgNO_3/SiO_2$ extractions, much of the methyl soyate adsorbs (0.65 mL) because of the still relatively high surface area, but each $AgNO_3$ unit now occupies only 71 Å^2, and these units are only 8.4 Å apart on average. This suggests that two (or three) Ag^+ ions may be sufficiently close to coordinate to two (or three) double bonds in 18:2 (or 18:3). Presumably, 18:2 and 18:3 adsorb more strongly than 18:1 because their olefinic groups are coordinated to more Ag^+ ions, which accounts for the substantial removal of 18:3 and the low 18:2/18:1 ratio (0.52). Previous equilibrium studies (12) of the binding of one $[(\eta^5\text{-}C_5H_5)(PR_3)Pd]^+$ complex to one olefin in 18:1, 18:2, and 18:3 showed that binding is very nearly the same for all of these FAMEs.

As the amount of SiO_2 is reduced in the 30 and 40% samples, the amount of adsorbed FAMEs decreases to 0.26 mL, and some of the selectivity for 18:2 and 18:3 is lost. It is not entirely clear why the selectivity decreases; however, we speculate that the SiO_2 surface area per $AgNO_3$ unit (36 Å^2) in the 40% adsorbent means that the SiO_2 surface is completely covered and the SiO_2 pores may be plugged by more than one layer of $AgNO_3$.

The results in Table 1 show that 20% $AgNO_3/SiO_2$ is the most selective for 18:3 removal, but 65% of the original methyl soyate must be removed to achieve that selectivity. With this loading, the saturated FAMEs (16:0, 18:0) are completely separated from the unsaturated FAMEs. Thus, the hexane fraction (**H**) is enriched in saturated (40.1%) and monounsaturated (39.4%) FAMEs, whereas the diethyl ether fraction (**G**) is enriched in polyunsaturated (85.8%) FAMEs and contains none of the saturated FAMEs. On the other hand, 40% $AgNO_3/SiO_2$ extracts only 26% of the methyl soyate, but it is less selective for the polyunsaturated FAMEs. Depending on the application, one can choose a $AgNO_3$ loading that gives the most desirable FAMEs composition in the hexane (**H**) and/or diethyl ether (**G**) fraction.

TABLE 2
Effect of Increasing $AgNO_3$ Loading on the FAMEs Composition (wt%) of the Hexane (H) and Diethyl Ether (G) Fractions from Extractions of Methyl Soyate with 5.0 g of $AgNO_3/SiO_2$

		% $AgNO_3/SiO_2$ used in extractions[a]			
FAMEs	Feed	10	20	30	40
16:0	10.3	25.4 (1.5)[b]	27.3 (0.0)[b]	22.6 (0.0)[b]	18.4 (0.0)[b]
18:0	4.5	11.5 (0.6)	12.2 (0.0)	10.0 (0.0)	8.2 (0.0)
18:1	24.6	31.1 (20.0)	39.3 (12.8)	39.3 (8.9)	37.2 (6.1)
18:2	53.6	25.4 (69.3)	21.1 (78.1)	27.9 (81.7)	36.0 (80.8)
18:3	7.0	6.6 (8.5)	0.1 (9.1)	0.2 (9.4)	0.2 (13.1)
18:2/18:1 ratio	2.2	0.82 (3.5)	0.54 (6.1)	0.71 (9.2)	0.97 (13.2)
FAMEs recovered (mL)	1.0	0.50 (0.45)	0.30 (0.65)	0.60 (0.35)	0.65 (0.30)

[a] 5.0 g of $AgNO_3/SiO_2$ with different amounts of $AgNO_3$ and SiO_2.
[b] The first number is the amount in the hexane fraction (**H**); the number in parentheses is the amount in the diethyl ether fraction (**G**).

Effect of increasing $AgNO_3$ loading on extractions of methyl soyate by $AgNO_3/SiO_2$. In this series of experiments, extractions were performed with $AgNO_3/SiO_2$ containing varying amounts of both $AgNO_3$ and SiO_2 but keeping the total mass of the $AgNO_3/SiO_2$ adsorbent constant (5.0 g). Four different $AgNO_3$ loadings were studied: 10, 20, 30, and 40%. The FAMEs compositions in the hexane (**H**) and diethyl ether (**G**) fractions as a function of the percentage of $AgNO_3$ loading are shown in Table 2. When 10% $AgNO_3/SiO_2$ was used, the percentages of 16:0, 18:0 and 18:1 in the hexane (**H**) fraction increased, whereas those of 18:2 and 18:3 decreased, presumably as a result of Ag^+ coordination to the polyunsaturated FAMEs, but also because of the increase in 16:0 and 18:0. The different extraction results for 10% $AgNO_3/SiO_2$ in Tables 1 and 2 were caused by the larger total amount of the extractant used in Table 1 (10 g) as compared with that in Table 2 (5.0 g). With 10 g of 10% $AgNO_3/SiO_2$, all of the methyl soyate adsorbed, whereas 5.0 g has a lower surface area and adsorbed only 45% of the FAMEs in methyl soyate.

When the methyl soyate was extracted with 20% $AgNO_3/SiO_2$ (the same amount as in Table 1), the hexane (**H**) fraction contained primarily 16:0, 18:0, and 18:1 and a very small amount of 18:3 (0.1%). On the other hand, the diethyl ether (**G**) fraction was enriched in the polyunsaturated methyl esters (18:2 and 18:3) and contained none of the saturated methyl esters (16:0, 18:0). Thus, by using the 20% $AgNO_3/SiO_2$ adsorbent, the polyunsaturated methyl esters (18:2, 18:3) were completely separated from the saturated methyl esters (16:0, 18:0). Moreover, there was also a higher selectivity for 18:2 as compared with 18:1 (Table 2). The 18:2/18:1 ratio (0.54) in the hexane (**H**) fraction was much smaller than that (2.2) in methyl soyate. Also, included in Table 2 are data for the amounts of FAMEs in the hexane (**H**) and diethyl ether (**G**) fractions, which were 0.30 and 0.65 mL, respectively. Thus, 0.95 mL of the original 1.0 mL was recovered.

When 30 and 40% $AgNO_3/SiO_2$ were used as the extractants, selectivity for 18:2 and 18:3 was less than that for the 20% mixture but was still higher than reported in Table 1, where the amount of 40% $AgNO_3/SiO_2$ was only 2.5 g. The larger amount (5.0 g) of 40% $AgNO_3/SiO_2$ used to generate the data in Table 2, when compared with the 2.5 g used to develop Table 1, extracted slightly more of the FAMEs (0.30 vs. 0.26 mL), but it reduced the 18:3 level to only 0.2% as compared with 0.9% in Table 1; the 18:2/18:1 ratio (0.97) indicates a higher selectivity of 5.0 g of 40% $AgNO_3/SiO_2$ as compared with 2.5 g of 40% $AgNO_3/SiO_2$ for which this ratio is 1.5.

Effect of concentration of the methyl soyate/hexane solution.

TABLE 3
Effect of Increasing the Amount of Hexane in Methyl Soyate/Hexane Solutions on the FAMEs Composition (wt%) of the Hexane (H) and Diethyl Ether (G) Fractions from Extractions of Methyl Soyate with 5.0 g of 20% $AgNO_3/SiO_2$

		Hexane used in methyl soyate/hexane solutions			
FAMEs	Feed	25 mL	50 mL	100 mL	150 mL
16:0	10.3	29.2 (1.0)[a]	27.4 (0.0)[a]	27.3 (0.0)[a]	27.1 (0.0)[a]
18:0	4.5	13.5 (0.4)	12.5 (0.0)	12.2 (0.0)	11.9 (0.0)
18:1	24.6	40.6 (15.6)	37.1 (12.6)	39.3 (12.8)	40.8 (12.0)
18:2	53.6	16.3 (74.2)	22.8 (78.7)	21.1 (78.1)	20.1 (78.6)
18:3	7.0	0.4 (8.8)	0.2 (8.7)	0.1 (9.1)	0.1 (9.4)
18:2/18:1 ratio	2.2	0.40 (4.8)	0.61 (6.2)	0.54 (6.1)	0.49 (6.6)
FAMEs recovered (mL)	1.0	0.17 (0.80)	0.30 (0.68)	0.30 (0.65)	0.35 (0.60)

[a] The first number is the amount in the hexane fraction (**H**); the number in parentheses is the amount in the diethyl ether fraction (**G**).

TABLE 4
Effect of an Increasing Amount of 20% $AgNO_3/SiO_2$ on the FAMEs Composition (wt%) of the Hexane (H) and Diethyl Ether (G) Fractions from Extractions of Methyl Soyate

		20% $AgNO_3/SiO_2$ used			
FAMEs	Feed	2.0 g	4.0 g	6.0 g	8.0 g
16:0	10.3	13.0 (1.0)[a]	17.5 (0.0)[a]	28.8 (0.0)[a]	51.2 (0.0)[a]
18:0	4.5	5.9 (0.4)	8.0 (0.0)	11.9 (0.0)	21.8 (0.0)
18:1	24.6	28.8 (5.1)	33.4 (9.2)	38.7 (14.3)	19.9 (25.2)
18:2	53.6	50.2 (64.5)	40.4 (75.2)	20.5 (75.6)	7.1 (67.7)
18:3	7.0	2.1 (30.4)	0.7 (15.6)	0.1 (10.1)	0.06 (7.1)
18:2/18:1 ratio	2.2	1.74 (12.6)	1.21 (8.2)	0.53 (5.3)	0.36 (2.7)
FAMEs recovered (mL)	1.0	0.85 (0.12)	0.58 (0.36)	0.30 (0.65)	0.20 (0.75)

[a] The first number is the amount in the hexane fraction (**H**); the number in parentheses is the amount in the diethyl ether fraction (**G**).

It is expected that the amount of FAMEs adsorbed on the $AgNO_3/SiO_2$ will increase as the concentration of the methyl soyate in hexane (**A**) increases. In this series of experiments (Table 3), extractions were performed on solutions containing 1.0 mL of methyl soyate dissolved in 25.0, 50.0, 100.0, and 150.0 mL of hexane using 5.0 g of 20% $AgNO_3/SiO_2$. As expected, the total amount of FAMEs extracted increased as the concentration of methyl soyate increased. For the more dilute solutions containing 50.0, 100.0, and 150.0 mL of hexanes, the percent composition of the FAMEs in the hexanes (**H**) and diethyl ether (**G**) fractions depended relatively little on the concentration of the methyl soyate/hexane solution. However, in the most concentrated solution (25.0 mL hexane), more 18:2 and less 18:3 was removed from the FAMEs as compared with extractions from the more dilute solutions. Also, the selectivity for 18:2 as compared with 18:1 was higher (0.40) in the more concentrated (25.0 mL hexane) solution.

Effect of the amount of 20% $AgNO_3/SiO_2$. In this series of experiments, different amounts (from 2.0 to 8.0 g) of 20% $AgNO_3/SiO_2$ were used to extract a solution of 1.0 mL of methyl soyate in 100 mL of hexane (**A**). As expected, the total volume of FAMEs extracted increased as the amount of 20% $AgNO_3/SiO_2$ increased (Table 4). As the amount of 20% $AgNO_3/SiO_2$ increased, more and more of 18:1, 18:2, and 18:3 were extracted from the methyl soyate leaving the hexanes (**H**) extract with ever higher percentages of the saturated FAMEs. At the same time, the diethyl ether (**G**) fraction contained high concentrations of 18:1, 18:2, and 18:3. Also, the 18:2/18:1 ratio decreased substantially as the amount of adsorbent increased, indicating a higher selectivity for 18:2 as compared with 18:1.

Two-stage extraction with 20% $AgNO_3/SiO_2$. In exploring the possibility that two consecutive extractions would provide greater separations of the FAMEs, 1.0 mL of methyl soyate (**A**) in 100 mL of hexane was treated with 2.0 g of 20% $AgNO_3/SiO_2$. The primary result of the first extraction was the reduction of the percentage of methyl linolenate (18:3) in the hexane (**H**) fraction from 7.0 to 2.1% (Table 5), which was the same as that described in Table 4. The amounts of FAMEs recovered from the hexane (**H**) and diethyl ether (**G**) fractions were 0.85 and 0.12 mL, respectively.

In the second stage, the 0.85 mL of FAMEs obtained from the hexane fraction (**H**) of the first extraction was dissolved in 100 mL of hexane and extracted again using 2.0 g of 20% $AgNO_3/SiO_2$. This gave a further reduction in 18:3 (from 2.1 to 0.2%) in the hexane (**H**) fraction; this fraction consisted of significant amounts of all the FAMEs except 18:3. The diethyl ether (**G**) fraction, however, consisted primarily of 18:2 (83.2%) with smaller amounts of 18:1 (13.0%) and 18:3 (0.2%). The hexane (**H**) fraction contains 0.55 mL of FAMEs, and the diethyl ether (**G**) fraction contains 0.25 mL of FAMEs. Thus, the two-stage extraction using 2.0 g of 20% $AgNO_3/SiO_2$, as compared with a single extraction using 4.0 g 20% $AgNO_3/SiO_2$ (Table 4), produced a FAMEs mixture in **H** that contained a lower concentration of 18:3 (0.2 vs. 0.7%) while extracting essentially the same amount (0.37 vs. 0.36 mL) of FAMEs from methyl soyate. These results suggest that two or more successive extractions will accomplish separations that may be desirable for certain applications.

Extraction of FAMEs derived from canola oil. The FAMEs mixture prepared from canola oil consisted of 4.5% methyl palmitate (16:0), 2.5% methyl stearate (18:0), 66.0% methyl oleate (18:1), 19.4% methyl linoleate (18:2), and 7.6% methyl linolenate (18:3) as determined by GC analysis (Table 6). The selectivity of the $AgNO_3/SiO_2$ adsorbent for 18:3 and 18:2 suggested that

TABLE 5
FAMEs Composition (wt%) of the Hexane (H) and Diethyl Ether (G) Fractions from Successive Extractions of Methyl Soyate with 2.0 g of 20% $AgNO_3/SiO_2$

FAMEs	Feed	1st	2nd[a]
16:0	10.3	13.0 (1.0)[b]	18.9 (0.0)[b]
18:0	4.5	5.9 (0.4)	8.3 (0.0)
18:1	24.6	28.8 (5.1)	34.1 (13.0)
18:2	53.6	50.2 (63.5)	38.5 (83.2)
18:3	7.0	2.1 (30.0)	0.2 (3.8)
18:2/18:1 ratio	2.2	1.74 (12.5)	1.12 (6.4)
FAMEs recovered (mL)	1.0	0.85 (0.12)	0.55 (0.25)

[a] The feed for the second extraction was the **H** fraction from the first extraction.
[b] The first number is the amount in the hexane fraction (**H**); the number in parentheses is the amount in the diethyl ether fraction (**G**).

TABLE 6
FAMEs Composition (wt%) of the Hexane (H) and Diethyl Ether (G) Fractions from an Extraction of Methyl Canolate with 2.0 g of 20% $AgNO_3/SiO_2$

FAMEs	Feed	20% $AgNO_3/SiO_2$
16:0	4.5	6.8 (0.0)[a]
18:0	2.5	3.5 (0.0)
18:1	66.0	78.2 (35.0)
18:2	19.4	9.8 (43.1)
18:3	7.6	1.7 (21.6)
18:2/18:1 ratio	0.29	0.13 (1.2)
FAMEs recovered (mL)	1.0	0.70 (0.27)

[a] The first number is the amount in the hexane fraction (**H**); the number in parentheses is the amount in the diethyl ether fraction (**G**).

TABLE 7
FAMEs Composition (wt%) of Hexane (H) and Diethyl Ether (G) Fractions from an Extraction of Methyl Soyate with 2.0 g of 20% $AgNO_3$/SBA-15

FAMEs	Feed	20% $AgNO_3$/SBA-15
16:0	10.3	14.0 (1.5)[a]
18:0	4.5	6.2 (0.5)
18:1	24.6	29.4 (12.2)
18:2	53.6	48.9 (64.2)
18:3	7.0	1.5 (21.6)
18:2/18:1 ratio	2.2	1.66 (5.3)
FAMEs recovered (mL)	1.0	0.65 (0.30)

[a] The first number is the amount in the hexane fraction (**H**); the number in parentheses is the amount in the diethyl ether fraction (**G**). SBA-15: mesoporous silica prepared using Pluronic P123 (Aldrich, Milwaukee, WI) (11).

it would be possible to produce a FAMEs mixture that had a high methyl oleate (18:1) content by extracting 18:3 and 18:2 from the methyl canolate. In Table 6 is shown the composition of the FAMEs after extraction of 1.0 mL of methyl canolate in 100 mL of hexane with 2.0 g of 20% $AgNO_3/SiO_2$. The hexane (**H**) fraction (0.70 mL) consisted primarily of 18:1 (78.2%) and lesser amounts of the other FAMEs. The diethyl ether (**G**) fraction (0.27 mL), on the other hand, had significant amounts of 18:1, 18:2, and 18:3. As with methyl soyate, there was a significant preference for the adsorption of 18:3 and 18:2 over 18:1 as indicated by the lower 18:2/18:1 ratio in hexane (**H**) fraction.

Effect of adsorbent type. To explore the possibility that the regular and relatively large pores in a mesoporous silica would give more selective extractions of the various FAMEs mixtures, SBA-15 was prepared (11). SBA-15 is a mesoporous silica gel (SiO_2) prepared using tetraethyl orthosilicate (TEOS) and poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) (average M.W. = 5800 g/mol) as the organic structure directing agent (11). SBA-15 has a well-ordered hexagonal array of one-dimensional parallel channels. After calcination, its BET surface area was 532.8 m^2/g as measured by the BET/BJH method using N_2 gas. After impregnation with 20% $AgNO_3$, the surface area of the resulting solid (20% $AgNO_3$/SBA-15) was reduced to 394.0 m^2/g. Clearly, the introduction of $AgNO_3$ resulted in a significant decrease in the adsorbent surface area. Using 2.0 g of 20% $AgNO_3$/SBA-15 to extract 1.0 mL of methyl soyate gave hexane (**H**) (0.65 mL) and diethyl ether (**G**) (0.30 mL) fractions (Table 7) that had compositions very similar to those obtained in the analogous extraction with 20% $AgNO_3/SiO_2$ (Table 4). Thus, both amorphous and mesoporous silica supports gave approximately the same results.

Regeneration of the $AgNO_3/SiO_2$ adsorbent. Several procedures for regenerating and re-using the $AgNO_3/SiO_2$ adsorbent were investigated. In each of these experiments, 1.0 mL of methyl soyate in 100 mL of hexane was added to 5.0 g of 20% $AgNO_3/SiO_2$ and allowed to sit for 30 min. After the solution was filtered from the adsorbent, the adsorbent was washed with 2 × 50 mL of hexane followed by 3 × 50 mL of diethyl ether. (i) In the first regeneration process, the 20% $AgNO_3/SiO_2$ was dried under vacuum overnight at room temperature. The 20% $AgNO_3/SiO_2$ was then reused in subsequent extractions by vacuum drying the adsorbent between extractions. Table 8 shows the FAMEs compositions of the hexanes (**H**) and diethyl ether (**G**) fractions after each of four successive extractions using the same 20% $AgNO_3/SiO_2$. These data showed that less (0.8 vs. 0.1%) 18:3 was extracted in the second reuse of 20% $AgNO_3/SiO_2$. In the third and fourth uses of the 20% $AgNO_3/SiO_2$, the amount of 18:3 in the hexane (**H**) fraction increased slightly to 1.1%. Also, the 18:2/18:1 ratio increased slightly after the first use. (ii) The second regeneration process differed from the first only by not using the vacuum drying between extractions. Thus, after the first use of the 20% $AgNO_3/SiO_2$, and after the third diethyl ether wash, the second

TABLE 8
FAMEs Composition (wt%) of the Hexane (H) and Diethyl Ether (G) Fractions from Extractions of Methyl Soyate with 5.0 g of Regenerated 20% $AgNO_3/SiO_2$

		20% $AgNO_3/SiO_2$			
FAMEs	Feed	1st	2nd	3rd	4th
16:0	10.3	27.3 (0.0)[a]	23.8 (0.0)[a]	24.6 (0.0)[a]	24.8 (0.0)[a]
18:0	4.5	12.2 (0.0)	11.0 (0.0)	11.3 (0.0)	11.5 (0.0)
18:1	24.6	39.3 (12.8)	38.1 (13.1)	36.7 (14.4)	35.8 (15.2)
18:2	53.6	21.1 (78.1)	26.3 (73.8)	26.5 (74.2)	26.8 (73.8)
18:3	7.0	0.1 (0.9)	0.8 (13.1)	0.9 (11.4)	1.1 (10.8)
18:2/18:1 ratio	2.2	0.54 (6.1)	0.69 (5.6)	0.72 (5.2)	0.74 (4.9)
FAMEs recovered (mL)	1.0	0.65 (0.32)	0.60 (0.35)	0.57 (0.37)	0.55 (0.40)

[a] The first number is the amount in the hexane fraction (**H**); the number in parentheses is the amount in the diethyl ether fraction (**G**).

FAMEs–hexane solution was added to the solid. This procedure gave FAMEs separations during four uses that were very similar to those in procedure (i) (Table 8). (iii) The third regeneration process sought to address the concern that some 18:3 and 18:2 might remain strongly adsorbed on the 20% $AgNO_3/SiO_2$ and not be removed by the diethyl ether wash. It is well known that Ag^+ coordinates to a variety of olefins but especially to ethylene (13–15). To facilitate the removal of 18:3 and 18:2 from the 20% $AgNO_3/SiO_2$, ethylene gas was slowly bubbled through the third diethyl ether wash for 4 h. As in procedure (i), the adsorbent was dried under vacuum before being reused. Results from four consecutive extractions were nearly the same as those from procedure (i); i.e., the second and subsequent reuses were somewhat less efficient at removing 18:3 than the first use. (iv) In this procedure, the 20% $AgNO_3/SiO_2$ was regenerated by heating the adsorbent after the third diethyl ether wash in an oven in air at 120°C overnight. During the drying process the color of the solid changed from white to brown and darkened with each reuse. This method of adsorbent regeneration gave similar distributions of FAMEs in the hexanes (**H**) and diethyl ether (**G**) fractions as for procedure (i).

Of the four procedures, (ii) presents some advantages, because it does not require vacuum drying or treatment with ethylene. By using this procedure, fractions having the composition presented in Table 8 could be obtained repeatedly without special treatment of the $AgNO_3/SiO_2$ adsorbent. Furthermore, no change in the color or textural properties of the $AgNO_3/SiO_2$ adsorbent was observed after four consecutive reuses.

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REFERENCES

1. O'Brien, R.D., *Fats and Oils. Formulating and Processing for Applications*, 2nd edn., CRC Press, New York, 2004.
2. *Vegetable Oils in Food Technology. Composition, Properties and Uses*, edited by F.D. Gunstone, CRC Press, New York, 2002.
3. Pahl, G., *Biodiesel. Growing a New Energy Economy*, Chelsea Green Publishing Co., White River Junction, Vermont, 2005, p. 54.
4. Biermann, U., W. Friedt, S. Lang, W. Lühs, G. Machmüller, J.O. Metzger, M. Rüsch gen Klaas, H.J. Schäfer, and M.P. Schneider, New Syntheses with Oils and Fats as Renewable Raw Materials for the Chemical Industry, *Angew. Chem. Int. Ed. 39*:2206–2224 (2000).
5. Christie, W.W., Solid-Phase Extraction Columns in the Analysis of Lipids, in *Advances in Lipid Methodology–One*, edited by W.W. Christie, The Oily Press, Ayr, Scotland, 1992, pp. 1–17.
6. Flieger, J., and H. Szumilo, Optimizing Chromatographic Conditions for Separation of Fatty Acid Methyl Esters by Argentation Thin-Layer Chromatography, *J. Planar Chromatogr. 13*:426–431 (2000).
7. Keller, G.E., A.E. Marcinkowsky, S.K. Verma, and K.D. Williamson, Olefin Recovery and Purification *via* Silver Complexation, in *Separation and Purification Technology*, edited by N.N. Li, and J.M. Cabo, Marcel Dekker, New York, 1992, pp. 59–84.
8. Teramoto, M., H. Matsuyama, N. Ohnishi, S. Uwagawa, and K. Nakai, Extraction of Ethyl and Methyl Esters of Polyunsaturated Fatty Acids with Aqueous Silver Nitrate Solutions, *Ind. Eng. Chem. Res. 33*:341–345 (1994).
9. Duan, J.-R., I. Billault, F. Mabon, and R. Robins, Natural Deuterium Distribution in Fatty Acids Isolated from Peanut Seed Oil: A Site-Specific Study by Quantitative ^2H NMR Spectroscopy, *ChemBiochem. 3*:752–759 (2002).
10. Dmytryshyn, S.L., A.K. Dalai, S.T. Chaudhari, H.K. Mishra, and M.J. Reaney, Synthesis and Characterization of Vegetable Oil Derived Esters: Evaluation for Their Diesel Additive Properties, *Bioresour. Technol. 92*:55–64 (2004).
11. Zhao, D., Q. Huo, J. Feng, B.F. Chmelka, and G.D. Stucky, Non Ionic Triblock and Star Diblock Copolymer and Oligomeric Surfactant Synthesis of Highly Ordered, Hydrothermally Stable, Mesoporous Silica Structures, *J. Am. Chem. Soc. 120*:6024–6036 (1998).
12. Ghebreyessus, K.Y., and R.J. Angelici, Equilibrium Studies of the Binding of Unsaturated Fatty Acid Methyl Esters to Palladium(II), *Organometallics 24*:1725–1736 (2005).
13. Brandt, P., Addition Compounds of Olefins with Metals, Part 2, *Acta Chem. Scand. 13*:1639–1652 (1959).
14. Hartley, F.R., Thermodynamic Data for Olefin and Acetylene Complexes of Transition Metals, *Chem. Rev. 73*:163–190 (1973).
15. Nymeijer, K., T. Visser, W. Brilman, and M. Wessling, Analysis of the Complexation Reaction Between Ag^+ and Ethylene, *Ind. Eng. Chem. Res. 43*:2627–2635 (2004).

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