Evaporative Light Scattering Mass Detection for High-Performance Liquid Chromatographic Analysis of Sucrose Polyester Blends in Cooking Oils

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A high-performance liquid chromatographic method is described to determine the sucrose polyester (SPE) content in seven blends of cooking oils. Four gel-permeation chromatography (GPC) columns were used in series with an evaporative light scattering mass detector to separate the SPE from the acylglycerols in the final chromatogram. The SPE fraction was collected off the GPC column and injected onto a reverse-phase C-18 column for quantitation with sucrose octaacetate as an internal standard and a gradient of nonaqueous solvents as mobile phase. The chromatograms were interference-free, with only two sharp peaks appearing. The standards were linear from 500 to 5000 μg/mL with a correlation coefficient of r = 0.999. The mean percent recovery (n = 9) and standard deviation were 102 ± 6.7. The detector could detect amounts as low as 5 μg SPE.

KEY WORDS: Cooking oil, chromatography, evaporative light scattering detection, gel-permeation chromatography, HPLC, internal standard, sucrose polyester, reverse-phase chromatography.

One of the rapidly growing areas in the food additive market is that of fat substitutes. Public health concerns and an increased consumer demand for low-fat products has led the food industry to develop fat substitutes and mimetics. One such substitute is olestra or sucrose polyester (SPE). SPE, a nonabsorbable synthetic fat, is a mixture of hexa-, hepta- and octaesters, formed by the reaction of sucrose with long-chain fatty acids (1). SPE has the taste and consistency of conventional fats and oils. However, due to its large molecular weight and resistance to lipase hydrolysis, SPE cannot be metabolized by the body (2,3).

Methods currently used to extract and measure fat content, such as chloroform/methanol extraction, will also measure SPE due to similar solubilities. Thus, erroneous values for total fat will be obtained. It is important to distinguish between digestible and nondigestible fats to meet future nutrition labeling requirements. The new labeling guideline will be revised to include nondigestible fats, such as olestra when the U.S. Food and Drug Administration approves its use in foods.

Birch and Crowe (4) developed a gel-permeation chromatographic (GPC) method to quantitate SPE in feces. Two GPC columns were connected in series to a refractive index detector. The presence of large amounts of triacylglycerols, such as those found in human diets, increased the difficulty of analysis due to similar retention times for the SPE, triacylglycerols and/or triacylglycerol dimers. Because foods contain triacylglycerols as well as added SPE, alternative methods need to be developed.

Recently, Tallmadge and Lin (5) developed a liquid chromatography (LC) method to determine the percent olestra in three olestra–lipid blends, including soybean oil and both heated and unheated cottonseed oil blends with olestra. The samples were diluted in methylene chloride and injected without clean-up onto a reverse-phase nonaqueous LC system with evaporative light scattering mass detection (ELSD). Triacylglycerol molecular species eluted first and were followed by the olestra peak. The olestra peak had a shoulder on it, indicating the potential presence of co-eluting interference peak(s). This was especially evident in the soybean oil/olestra blend.

The objectives of this study were to: (i) analyze SPE in seven cooking oil/sucrose polyester blends; (ii) introduce an internal standard to facilitate quantitation; and (iii) use GPC in conjunction with a reverse-phase LC system to remove the acylglycerol peaks from the final chromatogram.

EXPERIMENTAL PROCEDURES

Materials. Seven commercial cooking oils (canola, olive, corn, soybean, safflower, sunflower and peanut) were purchased at a local supermarket. All solvents were high-performance liquid chromatography (HPLC)-grade and obtained from Fisher Scientific (Norcross, GA).

Internal standard. A 580-mg portion of sucrose octaacetate (Aldrich Chemical Co., Milwaukee, WI) was diluted with methylene chloride to 250 mL to give a concentration of 2320 μg/mL.

SPE standard. The SPE was synthesized from sucrose and the fatty acids from soybean oil by using the method of Akoh and Swanson (6). A 125-mg portion of SPE was diluted to 25 mL with the internal standard solution. Aliquots (1.0, 3.0, 5.0 and 10.0 mL) were diluted to 10.0 mL with the internal standard solution to give concentrations of 500, 1500, 2500 and 5000 μg/mL, respectively.

HPLC. A Hewlett-Packard (Avondale, PA) 1090 Win LC, equipped with a diode array detector (DAD) and a Vectra 486 computer, was used to analyze the samples. A Sedex 45 ELSD (Richard Scientific, Novato, CA) was connected to the LC in series after the DAD. The ELSD was set at 40°C, with a nebulizer gas pressure of 2.1 and a gain of 3 for the future GPC columns and a gain of 5 for the nonaqueous reverse-phase system. A Hewlett-Packard 35900 digital A/D analog interface connected the mass detector electronically to the Vectra 486 computer.

Clean-up HPLC. The SPE was separated and isolated from the acylglycerols in each sample by GPC with a mobile phase of methylene chloride, an injection volume of 200 μL, and a flow rate of 1.0 mL/min. Four GPC columns connected in series consisted of a Beckman (San Ramon, CA) Ultrasphere 1000 Å (7.7 mm × 30 cm), a Beckman Ultrasphere 500 Å (7.7 mm × 30 cm), a Waters (Milford, MA) μStyragel 500 Å (7.8 mm × 30 cm) and a Waters μStyragel 100 Å (7.8 mm × 30 cm).

Reverse-phase HPLC. A Beckman 5 μm ODS column (4.6 × 25 cm) was used with a gradient (Table 1) of methylene chloride, acetonitrile and isopropanol, an injection

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TABLE 1

Reverse-Phase High-Performance Liquid Chromatography Gradient[a]

Time (min)	A	B	C	Flow (mL/min)
0	45.5	52.5	2.0	1.0
5	45.5	52.5	2.0	1.0
5.5	98.0	0	2.0	1.5
11.5	98.0	0	2.0	1.5
12.0	45.5	52.5	2.0	1.5
15.0	45.5	52.5	2.0	1.5
17.0	45.5	52.5	2.0	1.0

[a]A, methylene chloride; B, acetonitrile; and C, isopropanol.

volume of 10 μL, a flow rate of 1.0 to 1.5 mL/min and a column temperature of 40°C.

Analysis. Approximately 0.5 g of each cooking oil and SPE were combined and mixed for 2 min with a vortex mixer. The blend was sonicated for 1 min.

Approximately 200 mg of the blend was diluted to 10.0 mL with methylene chloride. Upon mixing, 200 μL was injected into the LC system with the four GPC columns installed in series. The SPE fraction was collected at a predetermined retention time window *via* a three-way valve installed after the DAD and before the ELSD. The collected fraction was evaporated to dryness under nitrogen and reconstituted to 1.0 mL with the internal standard solution. All reactions were carried out in duplicate unless otherwise indicated.

The SPE fractions collected off the GPC from each cooking oil/SPE blend were loaded into the autoinjector for separation on the C-18 column. The SPE peak was identified by predetermining the retention time. The amount of SPE in each oil blend was then quantitated by an on-line computer with sucrose octaacetate as internal standard.

Calculation of SPE amount. The SPE was calculated as follows:

$$\text{mg SPE} = \frac{R_{f_{standard}} \times \text{peak response of sample} \times \text{DF}}{\text{peak response of internal standard} \times 1000} \quad [1]$$

$$\text{the Rf} = \text{response factor of the standard} = \frac{C_{sample} \times \text{peak response of internal standard}}{\text{peak response of sample} \times C_{internal\ standard}} \quad [2]$$

where C is the concentration in μg/mL, DF is the dilution factor and 1000 is used to convert from μg to mg.

RESULTS AND DISCUSSION

The chromatograms from the analytical C-18 column were interference-free, with only two sharp peaks appearing (Fig. 1). The internal standard eluted at 2.7 min, and the SPE eluted at 8.9 min. The standards were linear from 500 to 5000 μg/mL with a correlation coefficient of r = 0.999. Because 10 μL was injected on the system, the amount of SPE on the column ranged from 5.0 to 50 μg. The lower end of the range at 5.0 μg is 25% less than

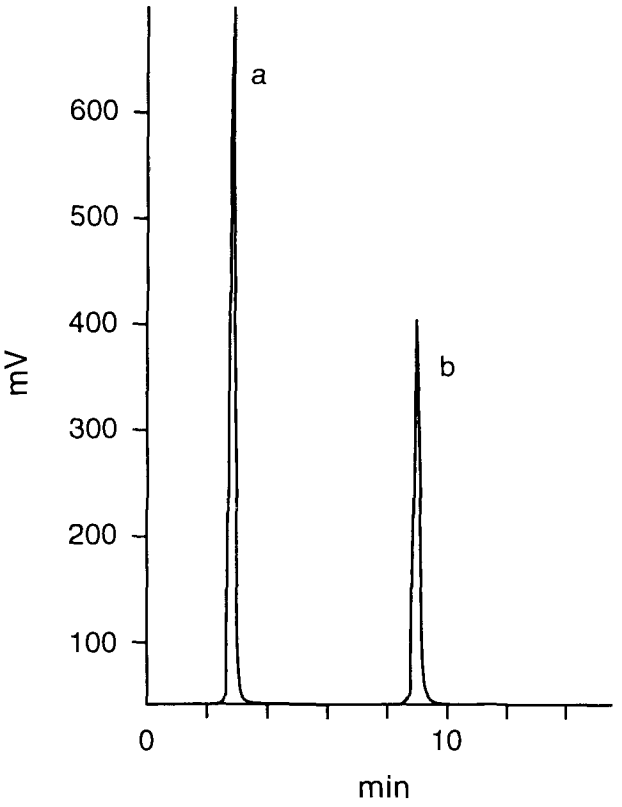

FIG. 1. High-performance liquid chromatography chromatogram (C-18 column) of sucrose polyesters (SPE) and the internal standard; a is the internal standard and b is the SPE peak. An injection volume of 10 μL with a gradient program and evaporative light scattering mass detection was used.

the lower end of the range obtained by Tallmadge and Lin (5).

The gradient in this study (Table 1) starts with 45.5% methylene chloride. It was observed that a lower amount of methylene chloride, such as that used by Tallmadge and Lin (5), gives a broad SPE peak. In fact, less than 60% methylene chloride caused broadening of the SPE peak. We overcame the SPE peak broadening effect by using 2% isopropanol in the gradient. As a result, the peaks were much sharper than those in the chromatograms presented by Tallmadge and Lin (5). This observation is a definite improvement over the gradient system reported by Tallmadge and Lin (5).

The use of an internal standard eliminates the need of subjecting data to linear regression analysis for quantitation. When choosing an internal standard, heptadecanoic acid methyl ester was initially evaluated. However, for the internal standard to have a peak response similar to that of 500 μg/mL of SPE standard, a concentration of 10,000 μg/mL was required. Due to the large amounts required for this response, another internal standard was sought. According to the ELSD manufacturer (Sedex; Richard Scientific), the signal obtained from the substance analyzed is dependent on the formula $A = am^b$, where A is the diffused light, m is the mass of the substance being analyzed and ^{b} is the slope of the line from a plot of log A vs. log m. An internal standard such as sucrose oc-

taacetate with a molecular structure and weight similar to that of the SPE was used for this reason.

Listed in Table 2 are the quantities of SPE added to the blend, the amount detected and the percent recovered for each blend of cooking oils studied. The mean percent recovery (n = 9) and standard deviation were 102 ± 6.7. Each blend contained approximately 50% SPE. To study the recoveries at various levels, additional soybean oil was blended with SPE at levels of 25, 50 and 75%. The respective recoveries were 98.3, 109 and 99.6%. The system reproducibility was evaluated by completing five replicate analyses of canola oil/SPE blends. The mean value in mg SPE ± standard deviation and coefficient of variation (CV) was 103 ± 9.9 (CV, 9.6%).

Figure 2 shows the chromatogram obtained with the GPC columns. Excellent separation was obtained between the SPE peak and the triacylglycerol peak. A DAD detector was used to take ultraviolet (UV) scans at various points on the SPE peak. However, because methylene chloride exhibits strong absorbance at the same wavelengths as SPE, the baseline from the methylene chloride was zeroed out so that any absorbance due to SPE or triacylglycerol could be obtained. The UV scans taken at various points on the SPE peak did not overlap, thus indicating a possible impurity or a co-eluting interference on the GPC system. Accurate quantitation could not be done with the GPC columns. Therefore, the SPE fraction was collected and assayed on a reverse-phase C-18 column for quantitation. UV spectra by DAD from various points on the SPE peak from the C-18 column (Fig. 1) could not be obtained due to the "gradient effect." The methylene chloride on the C-18 column was not run isocratically as it was on the GPC columns. In fact, the methylene chloride increased by 52.5% in a 0.5-min period. Therefore, due to the sharp increase in the methylene chloride content and a UV cutoff for methylene chloride of 230 nm, the only spectrum observed was that of the methylene chloride absorbance. The only apparent means to identify the peaks on the C-18 system was the use of retention times. In an attempt to further confirm that the SPE peak was pure on the C-18 column, the solution of oil blend was injected directly onto the C-18 column without prior GPC clean-up. Figure 3 illustrates the triacylglycerol molecular species peaks that eluted before the SPE peak. Quantitation of this SPE peak on the C-18 column without GPC clean-up was comparable to the results of the SPE peaks on the C-18 column from the collected fraction of the GPC columns. The SPE peak was much sharper than that reported by Tallmadge and Lin (5). The introduction of 2% isopropanol in our gradient system greatly sharpened the peaks. This also indicates that we have a cleaner SPE product (octaester) than the hexa-, hepta- and octaester (olestra) product reported (5).

This observation raises the question as to why is the GPC system necessary? The use of SPE in cooking oils is only one application of this fat substitute. More methods for the analysis of SPE in different food matrices need to be developed. Food processors will expand SPE use to other foods with more complex matrices, including frying. Some of the SPE manufactured by different companies will differ in degree of fatty acid substitution (e.g., olestra with hexa-, hepta- and octaesters). A simple "dilute and shoot" method with a C-18 column will quickly become obsolete and may shorten the life of the C-18 column. Tallmadge and Lin (5) noted that their reverse-phase method is not applicable to: (i) blends of unheated olestra with previously heated triacylglycerol because of the high levels of triacylglycerol polymers that may co-elute with the olestra; and (ii) the analysis of olestra in olestra/triacylglycerol blends that have been co-heated under frying conditions due to the formation of olestra/triacylglycerol polymers. Combining GPC and C-18 columns as illustrated in this study circumvents this problem and gives a powerful tool to quantitate SPE in various food matrices. This method can easily be extended to the analysis of SPE with various degrees of substitution when present in food matrices.

Initially in this study the ELSD was pushed to its lower detection limits. A standard series of 5 to 27 μg/mL was used on the C-18 column with an injection volume of 100 μL at a more sensitive gain setting of 7. It was virtually impossible to obtain linearity. Despite the manufacturers' claims that an ELSD can be utilized with a gradient, we found that ELSD is sensitive to gradients. Upon injecting a methylene chloride blank, a peak was observed at the same retention time as the SPE. The peak was a result of an increase in methylene chloride concentration in the gradient. The work of Tallmadge and Lin (5) was reproduced at a sensitive gain setting of 7, and the methylene chloride peak was observed to be 15% of the total SPE peak. Once the sensitivity is lowered to a gain of 5, the methylene chloride peak effect becomes neglible.

This method provides a rapid technique to quantitate SPE in cooking oil blends. The use of an internal standard greatly facilitates the calculation process. The use of the GPC columns provides an excellent means to further adapt the method to other, more complex food matrices by first separating the fat from the SPE and subsequently analyzing the SPE on a C-18 column. This method will find application in future nutrition labeling requirements.

TABLE 2

Quantities of Sucrose Polyesters (SPE) Recovered from Cooking Oil Blends[a]

Sample	A	B	C	D	E	F	G	H	I
Cooking oil in blend (mg)	202.0	205.0	201.0	199.0	211.0	213.0	200.0	202.0	209.0
SPE in blend (mg)	100.0	104.0	101.0	97.4	103.0	106.0	101.0	151.0	56.7
Amount of SPE found (mg)	91.1	102.0	99.4	97.0	112.0	116.0	110.0	165.0	55.7
% SPE recovered	90.5	97.3	97.8	99.6	107.0	109.0	109.0	109.0	98.3

[a]A, canola oil; B, olive oil; C, corn oil; D, soy oil; E, safflower oil; F, sunflower oil; G, peanut oil; H, soy oil, and I, soy oil.

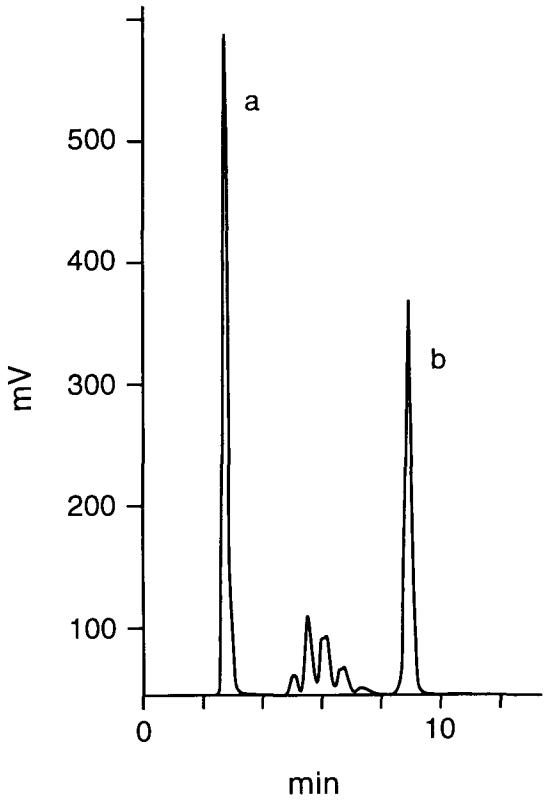

FIG. 2. High-performance liquid chromatography chromatogram of the oil blend on gel-permeation chromatography column (series of four); a is the sucrose polyesters peak and b is the triacylglycerol peak. An injection volume of 200 μL with a flow of 1.0 mL/min and evaporative light scattering mass detection was used.

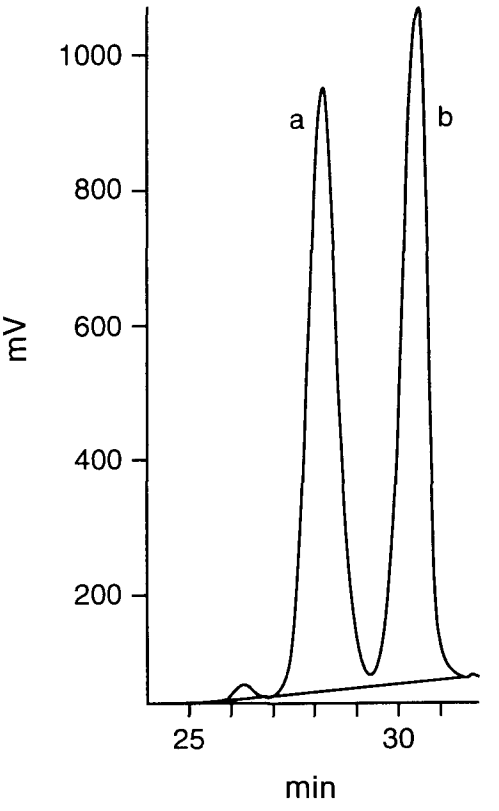

FIG. 3. High-performance liquid chromatography chromatogram of an oil blend without prior gel-permeation chromatography clean-up; a is the internal standard and b is the sucrose polyesters. Between a and b are the triacylglycerol molecular species. An injection volume of 10 μL with a gradient program and evaporative light scattering mass detection was used.

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