Characterization of Sucrose Polyesters-Triacylglycerols Mixtures

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High-performance size-exclusion chromatography (HPSEC) and thin-layer chromatography/flame-ionization detection (TLC/FID) have been used for the characterization of mixtures of either monoacid sucrose octaesters with triacylglycerols (TAG) or sucrose polyesters (SPE) prepared from oils with natural oils. In mixtures of monoacid sucrose octaesters/TAG, no significant differences were found between the values obtained by either HPSEC or TLC/FID and the actual component proportions. Additionally, components could be separated by TLC, which was confirmed by fatty acid composition data of each fraction. Analysis of SPE/oil mixtures was attainable by HPSEC, but alternative quantitation by TLC/FID required previous silylation. Likewise, fatty acid composition could be determined only in the total mixture and in the sucrose octaester fraction. A formula derived for calculation of oil fatty acid composition, based on analytical data, showed the validity of the approach used in this study to determine component proportions in functional SPE/oil mixtures.

KEY WORDS: HPSEC, SPE, SPE/oil mixtures, sucrose octaesters, TLC/FID, triacylglycerols.

Previous studies on isolation, characterization and quantitation of sucrose polyesters (SPE) have been carried out recently in our laboratory wherein various chromatographic techniques were successfully applied to quantitate SPE differing in esterification degree (1). Possibility of preparing mixtures of SPE and fats or oils for commercialization (2,3) involves a further analytical challenge for sample characterization. Information on this subject is limited, despite its special interest in terms of the potential contribution of each component in the nutritional properties of the final product. In this regard, the following aspects should be pointed out: (i) Quantitative determination of the relative proportions of both SPE and natural fats is of particular relevance to know the caloric value of the product. Addition of SPE to natural fats or oils does not necessarily change the appearance of the final product, although a decrease of digestibility is expected due to the nonabsorption nature of SPE (4,5). (ii) Fatty acid composition of both SPE and natural fats in mixtures is essential to ascertain the nutritional effects of the product upon absorption. Thus, these data could be of great value when, in practice, addition of SPE to natural fats is directed to gain stability in the final product, e.g., by using SPE enriched in saturated fatty acyls in mixtures with mono- or polyunsaturated oils. In the present study, the use of chromatographic techniques, such as high-performance size-exclusion chromatography (HPSEC) or thin-layer chromatography/flame-ionization detection (TLC/FID), is proposed to approach characterization of mixtures of either pure monoacid sucrose octaesters with triacylglycerols (TAG), or SPE prepared from oils in combination with natural oils.

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EXPERIMENTAL PROCEDURES

Samples. Palm, olive and sunflower oils were obtained from local companies. SPE were prepared according to the solvent-free, sucrate-catalyzed method of Rizzi and Taylor (6), starting from sucrose and fatty acid methyl esters derived from palm or olive oil. Isolation and purification of SPE were carried out by means of silica column chromatography. SPE (50 g) were dissolved in petroleum ether/ethyl ether (95:5, vol/vol) and transferred to a column (4.5 cm i.d. × 42 cm) containing 320 g of silica gel 60 of particle size 0.063–0.200 mm (70–230 mesh ASTM, Merck No. 7734; Merck, Darmstadt, Germany), adjusted to a H_2O content of 5% and suspended in the same mixture of solvents. A first fraction eluted with 1.25 L petroleum ether/ethyl ether (95:5, vol/vol) contained minor nonpolar impurities and was discarded. A second fraction, eluted with 1.25 L petroleum ether/ethyl ether (50:50, vol/vol), contained pure SPE. Sucrose octaoleate (SOO) was prepared, starting from sucrose and excess of oleyl chloride, to form the complete ester (7). Briefly, a molar ratio of one sucrose to twelve fatty acid chloride in pyridine and chloroform was refluxed for 4 h at 40°C under nitrogen stream. Triolein (OOO) and trilinolein (LLL) were obtained by esterification of oleic or linoleic acid, respectively, and glycerol, with *p*-toluene sulfonic acid as catalyst (8). OOO, LLL and SOO were isolated and purified by silica column chromatography. After washing thoroughly, samples were dissolved in petroleum ether/ethyl ether (90:10, vol/vol for OOO and LLL, and 92:8, vol/vol for SOO) and transferred to a column containing silica (20 g/g of sample) resuspended in petroleum ether. Pure samples were obtained by elution with the same solvent mixture than above (150 mL/g of sample)(9).

Separation of SPE according to esterification degree. Octa-, hepta- hexa- and lower sucrose esters were isolated by means of silica column chromatography, and the amount of each component was determined gravimetrically, as described in our earlier work (1).

Preparation of mixtures. SOO was mixed with OOO and LLL at ratios of 20, 40 and 60% SOO. The olive oil SPE/sunflower oil mixtures prepared were 17.1, 27.3 and 47.7% SPE, and those of palm oil SPE/olive oil were 14.8, 27.7 and 46.1% SPE.

Analyses of mixtures. TLC/FID: 1 μL of sample solution (15 mg/mL hexane) was spotted on Chromarods S-III (Iatron Laboratories, Tokyo, Japan). The rods were developed in petroleum ether/ethyl ether/acetic acid (90:10:2, vol/vol/vol) for 35 min and scanned in an Iatroscan MK-5 analyzer (Iatron Laboratories).

HPSEC: Samples were analyzed in a Konik 500 A chromatograph (Konik Instruments, Barcelona, Spain), which included a Rheodyne 7125 injector with a 10-μL sample loop (Hewlett-Packard, Palo Alto, CA). A Hewlett-Packard 1040 A refractive index detector and two 100-Å and 500-Å PLgel columns (particle size: 5 μm), connected in series, were used (Hewlett-Packard). The columns were 30 cm × 0.75 cm i.d., with polystyrene–divinylbenzene highly cross-linked macroporous spherical packing. High-performance

liquid chromatography-grade tetrahydrofuran (THF) served as the mobile phase at a flow rate of 1.0 mL/min, and the sample concentration was 10 mg/mL in THF.

Fatty acid composition of mixture components. Samples were applied to TLC plates (silica gel G) activated at 120°C for 3 h. Plates were developed with 65:35:1 hexane/diethyl ether/acetic acid to its full length. R_f values for sucrose octaesters and TAG were 0.85 and 0.70, respectively. Sucrose octaester and TAG fraction-containing areas of the plate were collected individually and extracted thoroughly with chloroform for the analyses of monoacid sucrose octaesters/TAG mixtures. In SPE/oil mixtures, only the sucrose octaester fraction was collected. Extracts were evaporated under a stream of nitrogen and transesterified with CH_3ONa and HCl/CH_3OH. Methyl esters were analyzed by gas–liquid chromatography in a SP-2380 fused-silica capillary column, 30 m long and 0.25 mm i.d., at a temperature of 180°C.

Statistical analysis. Data are expressed as the mean and standard error of the mean. Differences between the analytical methods used were assessed by Student's *t*-test, and P values < 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Analyses of monoacid sucrose octaesters/TAG mixtures. The first attempts to characterize mixtures were made on simple models, namely the complete oleyl ester of sucrose and monoacid TAG esterified with either oleic or linoleic acid. Table 1 shows the results obtained by HPSEC and TLC/FID of such mixtures. Overall, the results obtained by applying either TLC/FID or HPSEC matched with the actual component proportions in the mixtures. Calibration plots, previously obtained from OOO, LLL and TAG from different oils analyzed by HPSEC, showed no significant differences in response factors under our experimental conditions (10). Likewise, similar response factors were found for sucrose octaesters and TAG in TLC/FID analysis, where the relative responses of LLL and OOO in ratio to SOO were 1.02 and 1.01, respectively. Although no significant differences were found between the values obtained by TLC/FID and HPSEC in SOO/OOO mixtures, there was a slight variation between these data for the mixture of components selected as to be entirely esterified with different fatty acids (SOO/LLL). However, such differences would not be expected in mixtures of SPE with natural oils, where various fatty acids are present.

Fatty acid composition of sucrose and TAG components of SOO/LLL mixtures (Table 2) showed excellent results. Clearly, SOO was isolated with higher purity than was LLL. Still, percentages of oleic acid as low as 1.6 or 1.8 were found in the LLL fraction due to a slight contamination by the upper SOO band, which could have occurred during isolation of TLC fractions. From the data obtained, it appeared that the techniques applied could also be suitable for the analysis of more complex mixtures, such as those formed by SPE and natural oils where a variety of fatty acids are present.

Analyses of SPE/oils mixtures. Table 3 shows proportions of octa-, hepta- and hexa- and lower sucrose esters in SPE, prepared starting from olive oil and palm oil, as analyzed by silica column chromatography, along with the fatty acid composition of each fraction. The last two rows list fatty acid composition of the natural oils used for mixtures. As can be observed, fatty acid composition did not vary among SPE with different esterification degrees.

Quantitation of mixtures by HPSEC is presented in Table 4. Efficacy of the separation by HPSEC for olive oil SPE/sunflower oil mixtures is illustrated in Figure 1. The validity of the method relied on the elution of SPE as a single peak and the excellent separation achieved between SPE and TAG peaks, while those compounds present in minor amounts were not detected. However, separation by TLC, either with plates or TLC/FID, was more complicated than that for pure sucrose octaesters/TAG, given that heptaesters, of lower polarity than octaesters, overlapped here with TAG. Hence, for alternative quantitation of SPE/oil mixtures by TLC/FID, samples were analyzed after silylation, thus obtaining only two peaks corresponding to SPE and TAG (data not shown). Still, HPSEC was advantageous because a previous derivatization step was not required.

As mentioned above, TAG overlapped with heptaesters and could not be isolated by TLC. Accordingly, fatty acid composition could be determined in the total mixture and exclusively in the sucrose octaester fraction, which

TABLE 1

Quantitation of Monoacid Sucrose Octaesters/Triacylglycerols Mixtures (wt% of sucrose octaesters on total sample)

Sample[a]	Actual	Found[b] HPSEC	Found[b] TLC/FID
SOO/OOO	20.2	20.4 ± 0.09[c]	19.9 ± 0.39[c]
	39.9	39.7 ± 0.35[c]	40.1 ± 0.37[c]
	59.7	59.4 ± 0.22[c]	59.2 ± 0.34[c]
SOO/LLL	19.5	18.8 ± 0.19[c]	20.4 ± 0.36[d]
	39.2	38.4 ± 0.27[c]	40.8 ± 0.29[d]
	60.3	59.2 ± 0.33[c]	61.0 ± 0.40[d]

[a]SOO: sucrose octaoleate, OOO: triolein, LLL: trilinolein; HPSEC, high-performance size-exclusion chromatography; TLC/FID, thin-layer chromatography/flame-ionization detection.
[b]Means ± SEM of three determinations. Values in rows without common superscript letters are significantly different, $P < 0.05$.

TABLE 2

Fatty Acid Composition of SOO and LLL Fractions in Mixtures (wt% on fraction)

SOO/LLL mixtures	Fraction	Fatty acid composition[a] $C_{18:1}$	Fatty acid composition[a] $C_{18:2}$
19.5% SOO	SOO	100	n.d.[b]
	LLL	n.d.	100
39.2% SOO	SOO	100	n.d.
	LLL	1.6 ± 0.17	98.4 ± 0.17
60.3% SOO	SOO	100	n.d.
	LLL	1.8 ± 0.23	98.1 ± 0.23

[a]Means ± SEM of three determinations. See Table 1 for abbreviations.
[b]n.d., Not detected.

TABLE 3

Characterization of Initial Sucrose Polyesters (SPE) and Oils

Sample	Sucrose esters	Weight percentage	Fatty acid composition (wt%)				
			$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	Others
Olive oil SPE	Octa-	70.8	11.3	4.3	76.5	5.9	2.0
	Hepta-	12.3	10.8	4.2	77.4	5.5	2.1
	Hexa-	10.1	11.0	4.2	77.2	5.5	2.5
	Total	100	10.7	4.6	77.2	5.3	2.2
Palm oil SPE	Octa-	71.5	44.5	5.6	38.8	8.4	2.7
	Hepta-	12.4	44.6	5.7	39.9	8.5	1.3
	Hexa-	6.5	44.7	5.7	39.5	8.3	1.9
	Total	100	44.2	6.2	39.2	8.3	2.1
Olive oil			10.1	3.9	75.2	8.7	2.1
Sunflower oil			7.3	5.3	29.1	56.4	1.9

TABLE 4

Quantitation of SPE/Oils Mixtures by HPSEC (wt% of SPE on total sample)

Sample	Actual	Determined by HPSEC[a]
Palm oil SPE/olive oil	14.8	15.1 ± 0.32
	27.7	28.3 ± 0.49
	46.1	46.9 ± 0.38
Olive oil SPE/sunflower oil	17.1	17.1 ± 0.46
	27.3	26.7 ± 0.29
	47.7	46.7 ± 0.39

[a]Means ± SEM of three determinations. See Tables 1 and 3 for abbreviations.

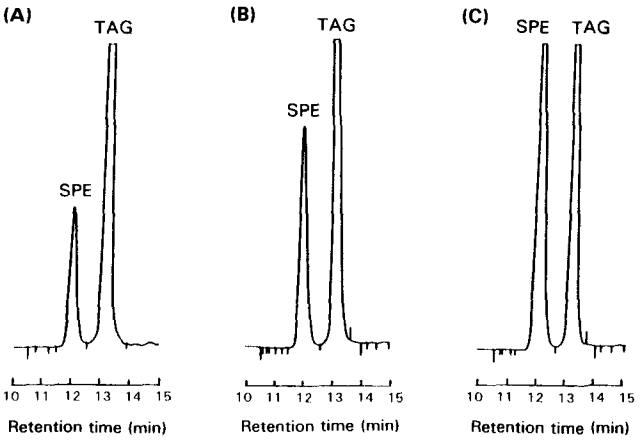

FIG 1. High-performance size-exclusion chromatograms of olive oil sucrose polyesters/sunflower oil mixtures. (A) 17.1% SPE, (B) 26.7% SPE and (C) 46.7% SPE. Abbreviations: SPE, sucrose polyesters; TAG, triacylglycerols.

separated well from the other components. Taking into account that fatty acid composition is similar in all sucrose ester fractions, regardless of the esterification degree (Table 3), the values obtained for sucrose octaesters

TABLE 5

Fatty Acid Composition of Palm Oil SPE and Olive Oil (OO) Fractions in Mixtures (wt% on fraction)

SPE/oils mixtures[a]	Fraction	Fatty acid composition				
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	Others
15.1% SPE	Total	16.2	4.3	69.5	8.4	1.6
	SPE[b]	46.1	5.3	38.7	7.9	2.0
	OO[c]	10.9	4.1	75.0	8.5	1.5
28.3% SPE	Total	19.5	4.6	66.9	7.9	1.1
	SPE	45.0	5.7	38.8	8.1	2.4
	OOO	9.4	4.2	78.0	7.8	0.6
46.9% SPE	Total	28.3	4.8	57.3	8.0	1.6
	SPE	46.5	5.5	38.6	7.7	1.7
	OO	12.2	4.2	73.8	8.3	1.5

[a]Determined by HPSEC. See Tables 1 and 3 for other abbreviations.
[b]Values corresponding to sucrose octaesters.
[c]Values calculated from the equation cited in the text.

were assumed for those corresponding to the total SPE fraction. Fatty acid composition of the oil was calculated from the following equation, applied to the major fatty acids:

$$100 \times \text{wt\% of } C_x \text{ in the mixture} = a \times \text{wt\% of } C_x\% \text{ in oil} + (100 - a)\text{wt\% of } C_x \text{ in sucrose octaesters} \quad [1]$$

where a = wt% of oil in the mixture, as determined by HPSEC and C_x = fatty acid.

Tables 5 and 6 show the results obtained for fatty acid compositions of components in palm oil SPE/olive oil and olive oil SPE/sunflower oil mixtures, respectively. The first column includes SPE percentages as analyzed by HPSEC. The validity of the analytical approach was substantiated by the fact that fatty acid composition of SPE and oil fractions, shown in Tables 5 and 6, conformed well with the values calculated from the fatty acid composition of the mixtures and the actual component proportions. Additionally, there was an excellent agreement between the

TABLE 6

Fatty Acid Composition of Olive Oil SPE and Sunflower Oil (SO) Fractions in Mixtures (wt% on fraction)

SPE/oils mixtures[a]	Fraction	Fatty acid composition				
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	Others
17.1% SPE	Total	7.8	5.2	36.5	48.2	2.3
	SPE[b]	12.0	4.9	73.8	6.4	2.9
	SO[c]	6.9	5.3	28.8	56.8	2.2
26.7% SPE	Total	8.1	4.7	41.5	43.7	2.0
	SPE	11.5	4.5	76.7	5.7	1.6
	SO	6.9	4.8	28.7	57.5	2.1
46.7% SPE	Total	9.5	5.0	52.0	31.9	1.6
	SPE	11.7	4.4	75.4	6.0	2.5
	SO	7.6	5.5	31.5	54.6	0.8

[a]Determined by HPSEC. See Tables 1 and 3 for other abbreviations.
[b]Values corresponding to sucrose octaesters.
[c]Values calculated from the equation cited in the text.

data obtained after isolation of sucrose octaesters and oil TAG calculations, and the values found for the mixture constituents, SPE and oil, individually (Table 3).

Overall, the results presented here for quantitative analyses of the sucrose esters/TAG mixtures tested did not differ significantly from the actual proportions, and differences found in fatty acid composition of mixture constituents were within the ranges expected. In practice, the analytical approaches used in this work can serve to gain insight into the properties of functional mixtures of SPE with natural oils or fats from variable sources.

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