Analysis of Sucrose Mono- and Diesters Prepared from Triglycerides Containing C_{12}-C_{18} Fatty Acids

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ABSTRACT

A quantitative gas liquid chromatographic method of analysis for sucrose mono- and diesters, prepared from naturally occurring triglycerides, is described. For esters containing predominantly C_{16}-C_{18} alkyl groups, a common calibration curve was obtained. Calibration standards were prepared by column chromatography using silica gel adsorbent. Methods of analysis for the major impurities present in the sucrose mono- and diesters are also described.

INTRODUCTION

For the purpose of process development in the production of sucrose esters from triglycerides (1) and for quality control, we needed a reliable and reproducible method for the analysis of sucrose esters of fatty acids of a low degree of substitution in reaction mixtures and in various intermediate products in the process of purification of these esters (2).

Methods reported in the literature for analysis of sucrose mono- and di-fatty acid esters included hydrolysis and determination of the sucrose formed (Bertrand's method [3]), the use of high pressure liquid chromatography (HPLC) (4-7), quantitative thin layer chromatography (TLC) (7) and gas liquid chromatography (GLC) (8). For the analysis of mono- and disaccharides, the method recommended by the AOACS (9) is HPLC. However, an initial study of the reversed-phase HPLC of sucrose mono- and diesters of long-chain fatty acids showed that it was difficult to obtain adequate peak separation for quantitative analysis, particularly where glycerides were present as an impurity. A small amount of sucrose and potassium soaps in the samples reduced the column efficiency. We chose to study packed column GLC for three reasons: the mono- and diester components were well separated after derivatization, the glycerol, sucrose and mono- and diglycerides present in small amounts as impurities in the sucrose esters could be determined by the same method of analysis after derivatization, and capillary GLC gave a large number of peaks from each sample. These were difficult to identify and quantitate. It is probable that capillary GLC separated not only sucrose esters of different alkyl chain lengths, but also positional isomers. The 6 and 6′ positions on the sucrose ring are known to be the most reactive (10), but substitution at other positions can occur.

For process control, a relatively rapid method of analysis is required. Either HPLC or GLC fulfils this requirement, although the HPLC method described (7) elutes sucrose esters after 50 min, but quantitative TLC is too time-consuming. The intensity of spots on the TLC plates of sucrose esters visualized by charring with sulfuric acid is difficult to control and the spots themselves are fugitive, making accurate densitometry difficult to achieve (11). Analysis of sucrose and sucrose derivatives by GLC after silylation is an established technique (12,13). In this work a mixture of N, O,-*bis*-(trimethylsilyl)-acetamide (BSA) and trimethyl-chlorosilane (TMCS) was used. A limited amount of work was done using methyl-*bis*(trifluoro)acetamide (MBTFA). The use of these derivatizing agents is well established in the analysis of polyhydric alcohols and their derivatives.

EXPERIMENTAL PROCEDURES

Separation of Mono- and Diesters by Column Chromatography

The apparatus shown in Figure 1 was filled to a depth of ca. 70 cm with silica gel (Mallinckrodt type CC7 or Merck type F60/7737). This was poured dry into the column and an even bed distribution obtained using a tamping rod and a vibrator.

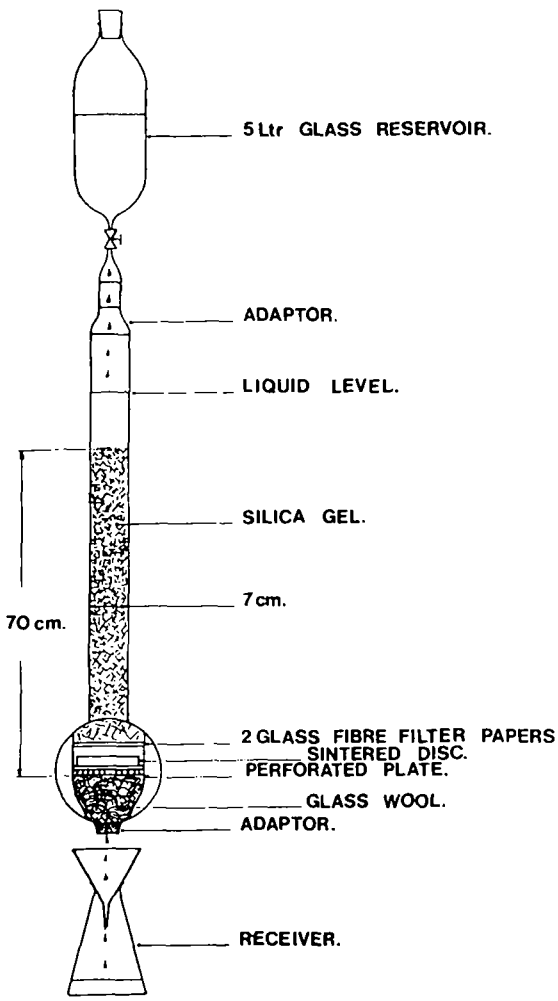

FIG. 1. Large-scale chromatography column.

The silica gel was deactivated by passing ca. 5 L of dry diethyl ether down the column. This ether was discarded. The crude sucrose ester sample was dissolved in 'Analar' toluene (150 g mixed esters in 250 mL toluene) at 60 C, allowed to cool to ca. 30-35 C, and poured onto the column immediately after the final portion of diethyl ether. When the toluene solution of sucrose ester had run onto the column, ca. 100 mL of 'Analar' toluene was added to the top of the column to force the sucrose esters below the surface layer of silica gel.

The residual glycerides and fatty acids were then eluted

off the column by running 5 L of diethyl ether through it. It was then ready for ester separation. Sucrose and residual soaps were retained on the column.

The diesters were eluted using 7-8 L of dry *n*-butanone, and 300-mL fractions collected. An initial fraction (600 mL) of colored product containing higher esters plus some fatty acids was obtained. This was followed by the pure sucrose diester.

The monoester was then eluted using *n*-butanone saturated with water, and ca. 8-10 L were required and again 300-mL fractions were collected. The first portion of water-saturated solvent was added slowly to the top of the column, since water adsorption by the silica gel is an exothermic process and overheating caused the *n*-butanone to boil and disturbed the column bed of silica gel.

The progress of the separation was monitored by TLC using *n*-butanone saturated with water as the eluting solvent (Fig. 2), and, if more dry *n*-butanone was required to remove the diesters, then this was run down the column prior to changing over the wet *n*-butanone.

Small portions (20 mL) of each fraction were evaporated down and redissolved in 2 mL of chloroform for spotting onto a TLC plate. The composition of each fraction was monitored in this way, and the fractions containing pure mono- and diesters were identified. The fractions of interest, containing pure mono- or diesters, were evaporated down using a rotary evaporator under vacuum and a final purification carried out by redissolving them in IPA, filtering if turbidity caused by small particles of silica gel was apparent, and the esters recovered using a rotary evaporator to remove solvent. The final products were dried in a vacuum oven at 60 C. The column was cleaned for reuse by pouring several liters of dry methanol down it.

The mono- and diesters containing unsaturated fatty acid groups were kept in sealed containers in the dark, since they have a tendency to oxidize when exposed to UV light in the presence of air.

The overall recovery of sucrose esters was ca. 40% of the total amount placed on the column. Washing the column with methanol removed most of the remaining sucrose esters, but did not separate them, and methanol could not be used as an eluting solvent.

Analysis of Sucrose Esters by Thin Layer Chromatography

A 20 cm × 20 cm TLC plate (Merck type 2357 without binder) was spotted with the ester samples dissolved in chloroform. The plate was developed by the normal upward elution technique in a tank containing *n*-butanone saturated with water. The plate was developed by spraying either with a sulfuric acid/methanol mixture, or with a carbohydrate-visualizing reagent (14) and heated to 120 C for a few minutes.

Analysis of Sucrose Esters by Gas Liquid Chromatography

The column and conditions used were: column—1 min at 70 C, programmed at 16 C/min to 380 C; final temperature —3 min at 380 C; packing—3% Dexsil 300 GC (registered trade mark of the Olin Matheson Corp.) on Chromosorb WHP; detector—FID; GLC—Varian Model 3700.

Columns were conditioned for 2 hr at 180 C and then for ca. 5 hr at 250 C before use and had nitrogen flowing through them at all times. A short guard column of Chromosorp WHP was used to remove nonvolatile material and increased column life. Following the column conditioning process, new columns were tested by analyzing a sample of sucrose esters of known composition. Samples were dissolved in dry pyridine and silylated with a mixture of BSA and TMCS (12). A limited amount of work using MBTFA (12) showed that ester peaks were eluted at lower temperatures when derivatized with this reagent. For quantitative estimation of the sucrose ester content of an unknown sample, a calibration curve was required. Samples of sucrose mono- and diester separated by column chromatography on silica gel were first analyzed for impurities (glycerol, free fatty acids mono- and diglycerides) as described below, and the percentage purity of the calibration standards established.

For calibration, accurately weighed amounts of mono- or diester were dissolved in 'Analar' grade pyridine in 10-mL volumetric flasks. Three calibration standards were made up for each ester sample, the monoester at concentrations of 0.03, 0.05 and 0.08 g/mL and the diester at concentrations of 0.01, 0.02 and 0.03 g/mL. In samples of sucrose esters produced by the process described (2), the ratio of mono- to diester was ca. 2.4:1 and the calibration ranges covered were adjusted accordingly. A standard solution of *n*-tetracosane in 'Analar' chloroform (0.01 g/mL) was made up as the internal standard. Solutions for analysis were made up in 5 mL reactivials (registered trade mark of Pierce Chemical Co.) and contained 1 mL each of sucrose ester solution, *n*-tetracosane solution and the mixed silylating agents (BSS and TMCS). The reactivials were heated at 60 C for 20 min prior to injecting into the chromatograph, which was operated under the conditions listed above. Duplicate injections were made for each solution.

From the integrated ester peak areas and the known weights of sample and internal standard, two ratios were calculated. These were:

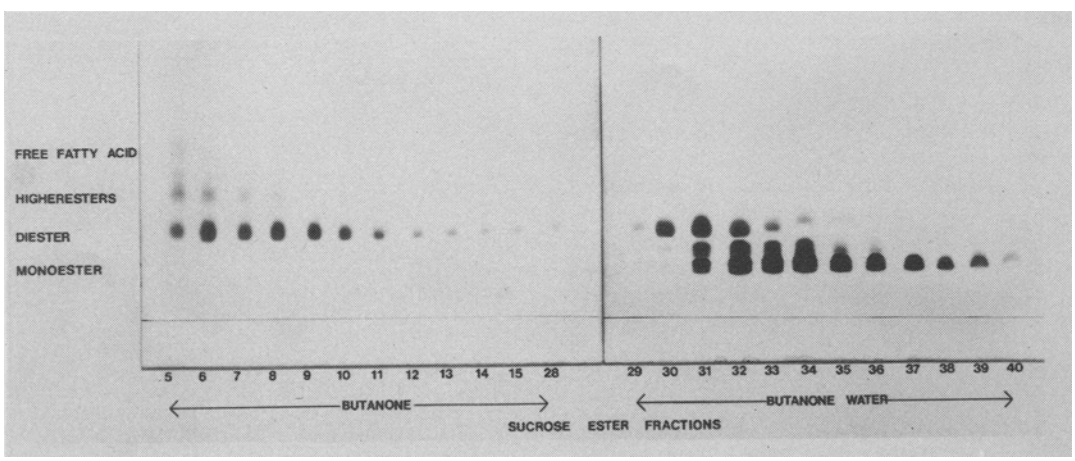

FIG. 2. TLC plate—fractions eluted from chromatography column.

$$\frac{\text{total peak area for all significant ester peaks}}{\text{peak area of } n\text{-tetracosane}}$$

and

$$\frac{\text{corrected weight of sucrose ester injected (actual wt} \times \text{\% purity/100)}}{\text{weight of } n\text{-tetracosane injected}}$$

A graph of total peak area ratio against sample weight ratio was plotted. This was found to be a straight line passing through the origin. From this calibration graph, the composition of sucrose ester sample of unknown composition was calculated.

Analysis of Impurities in Sucrose Esters

Glycerol

Quantitative analysis for glycerol was carried out by silylation with BSA and TMCS, followed by GLC (12). A similar experimental procedure to that described above for the sucrose mono- and diesters was employed. Calibration was carried out using 'Analar' glycerol supplied by B.D.H. Chemicals Limited, Poole, England.

Long-Chain Fatty Acids

An indirect method of analysis for long-chain fatty acids was adopted. The total (soaps plus fatty acids) content was determined (12) by GLC after silylation with BSA and TMCS. The experimental procedure was similar to that followed for the GLC analysis of sucrose mono- and diesters.

The metal soaps content (potassium or calcium) was determined from the potassium or calcium content, which was determined by plasma emission spectrophotometry (15). Since the fatty acid profile of the triglyceride used in the production of a given sucrose ester sample was known, the appropriate metal soap content could be calculated.

By difference, the long-chain fatty acid content was determined. Calibration was carried out using pure stearic and palmitic acids and their metal derivatives. Direct GLC determination of the long-chain fatty acids without silylation proved unsuccessful, since other components present 'tailed' badly off the column.

Sucrose

Analysis of the free sucrose content was made by silylation and GLC (12). The experimental procedure was similar to that described above for sucrose esters. Calibration was carried out using 'Analar' sucrose supplied by B.D.H. Chemicals Limited.

Mixed Glycerides

Analysis of the mono-, di- and triglyceride content was made by silylation followed by GLC (13). The experimental procedure followed was similar to that described for sucrose esters given above. Pure standards of mono- and diglycerides for calibration were prepared by column chromatography using the column shown in Figure 1. The experimental procedure was similar to that used in the separation of sucrose mono- and diesters, but the sequence of elution solvents was 60:80 petroleum ether, then a mixture of 50:50 petroleum ether and diethyl ether, and finally diethyl ether. This eluted the glycerides in the order tri-, di- and then monoglyceride. Composition of the fractions eluted off the column was checked by TLC and the pure fractions were recovered by evaporation under vacuum from the column eluate.

Moisture

Moisture content was determined by the Karl Fischer technique (16). The sucrose esters were soluble in the Karl Fischer reagent and determinations were carried out using an automatic Karl Fischer titrator.

RESULTS AND DISCUSSION

Purification of Sucrose Esters by Column Chromatography

For regular calibration in the GLC method of analysis of sucrose esters, purified mono- and diesters were required. Solvent extraction or partition processes (2) were not selective enough to yield pure mono- or diesters from a mixed ester product. Preliminary investigations showed that Mallinckrodt silica gel Type CC7 gave a good separation of mono- and diesters and a large-scale column chromatographic method was developed. However, Merck Type F60/7737 silica gel was cheaper and proved adequate in most cases. The sucrose ester recovery from the large-scale silica gel columns was poor and only ca. 40% of the total sucrose esters placed on the column were eluted in the fractions collected. However, TLC analysis (Fig. 2) showed that an excellent separation was achieved and the purity of the mono- and diester samples was normally in the range of 90-95% (17). With some triglycerides (e.g., hydrogenated rapeseed oil), complete separation of diesters from higher esters was not achieved (Fig. 3) but TLC analysis indicated that only a small amount of higher esters was present. Typical GLC chromatograms of sucrose mono- and diesters are shown in Figure 4 and for esters containing C_{16}-C_{18} alkyl groups complete separation of the two groups of peaks was achieved. With C_{12} alkyl groups present (e.g., esters prepared from coconut oil), some overlap of the two groups of peaks occurred (17).

Analysis of Sucrose Esters by Thin Layer Chromatography

The TLC plate shown in Figure 3 indicates the mono/diester separation achieved from sucrose esters derived from the following six triglycerides: tallow (IV = 45), soybean oil (IV = 130), hydrogenated soybean oil (IV = 66), hydrogenated rapeseed oil (IV = 64), palm oil (IV = 51) and palm kernel oil (IV = 19).

The diesters from hydrogenated rapeseed oil showed the presence of a significant amount of higher esters, rendering the diester sample unsuitable as a GLC calibration standard.

The R_f values of the mono- and diesters were similar for all of the sucrose esters examined. It was found that spraying the TLC plates with a carbohydrate-visualizing reagent (14) gave poor results and charring with sulfuric acid/meth-

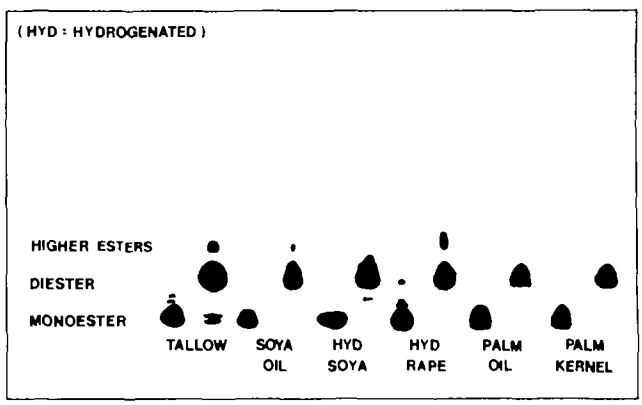

FIG. 3. TLC plate—sucrose esters prepared from six triglycerides.

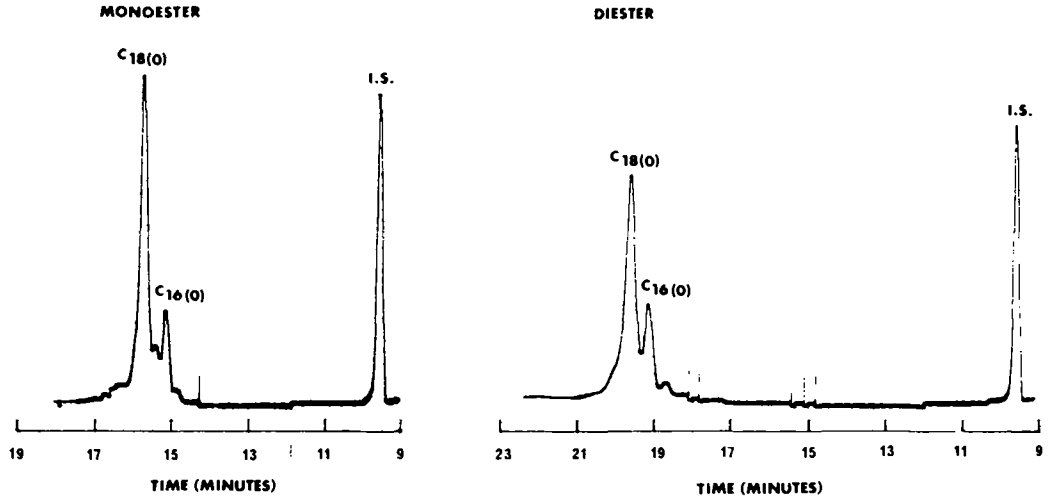

FIG. 4. GLC traces of sucrose mono- and diesters from tallow (IV = 45).

anol was preferred. The degree of charring is difficult to reproduce and the spots are fugitive and accurate densitometry (11) was not possible. However, TLC is a very useful method by which the fractions eluted from large-scale chromatography columns can be monitored.

Analysis of Sucrose Esters by GLC

Typical GLC chromatograms of sucrose mono- and diesters are shown in Figure 4. A typical set of calibration data (for sucrose esters from palm oil) is shown in Table I. Calibration data on a number of sucrose esters prepared from other C_{16}-C_{18} triglycerides are plotted on the calibration graph given in Figure 5. It is clear that all pure mono- and diesters of sucrose give one calibration line if the fatty acid profile of the triglycerides used contained predominantly C_{16}-C_{18} fatty acids. The esters prepared from palm kernel oil (with a high C_{12}-C_{14} fatty acid content) gave separate calibration lines for mono- and diesters. Since the carbon and hydrogen contents of sucrose esters from triglycerides containing mixtures of C_{16} or C_{18} fatty acid groups are similar, then one calibration line should be obtained using purified monoesters with similar fatty acid profiles. The sucrose diesters contain a slightly higher percentage of carbon and hydrogen than the monoester (ca. 65%C, 10.0%H vs 59%C, 9.0%H), but since they are eluted at a higher column temperature with associated peak broadening, both mono- and diesters are found to give one calibration line within experimental error. Palm kernel oil and coconut oil, the two C_{12} triglycerides used, both possess complex fatty acid profiles, giving rise to numerous mono- and diester peaks in the GLC chromatograms. Peak overlap occurred, reducing the quantitative accuracy of the GLC analysis (17). This master calibration graph (Fig. 5) was used for the analysis of sucrose esters containing both mono- and diesters, even if pure standards of mono- and diesters of the particular C_{16}-C_{18} fatty acid profile were not available. It also enabled an accurate analysis to be carried out in cases where pure diesters were difficult to isolate from higher esters since the monoester calibration could be utilized for an ester sample containing predominantly C_{16}-C_{18} fatty acids. As a measure of the reproducibility attainable, ten samples of one batch of sucrose esters prepared from tallow (IV = 45) by the process described (2) were analyzed and the mean and standard deviation calculated for both mono- and diester content. The results showed that for a sample containing 65% monoester and 26% diester, the standard deviations for mono- and diesters were ±3% and ±2%, respectively.

TABLE I

GLC Calibration Data – Sucrose Mono- and Diesters from Palm Oil

Solution concentration (g/mL)[a]	Weight ratio		Peak area ratio	
	SME[b]	SDE[c]	SME[b]	SDE[c]
0.0102	–	0.95	–	0.62
0.0195	–	1.81	–	1.38
0.0325	–	3.02	–	2.44
0.0390	3.98	–	2.78	–
0.0504	5.04	–	4.06	–
0.0646	6.59	–	5.29	–

[a]Corrected for impurities present in the sucrose esters.
[b]SME = Sucrose monoesters.
[c]SDE = Sucrose diesters.

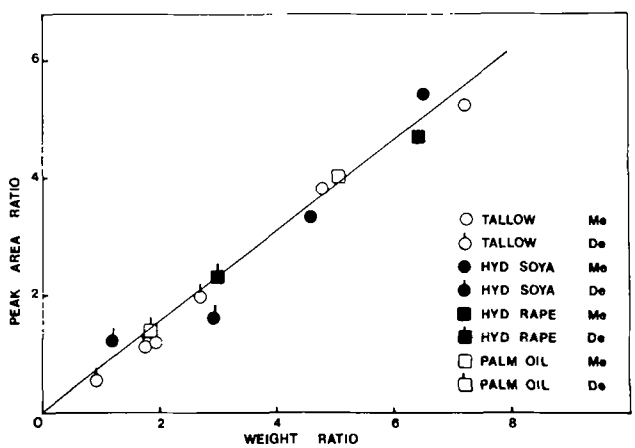

FIG. 5. Master calibration graph—sucrose mono- and diesters from C_{16}-C_{18} triglycerides (GLC analysis).

Capillary GLC, because of the large number of peaks obtained from esters with different alkyl chains and from positional isomers, could not be used for quantitative analysis.

Factors Affecting the GLC Analysis

The GLC method of analysis, using packed columns, has a number of inherent errors which are difficult to quantify. Whereas monoester calibration standards of better than 95% purity can be obtained, diester standards often contain some higher esters, making it difficult to assess their purity. To obtain symmetrical sucrose diester and triglyceride

peaks without excessive 'tailing', a short (50-cm) column and a rate of temperature rise of 16 C/min was used. For accurate quantitative analysis, a column 1 m long and a rate of temperature rise of not more than 8 C/min are recommended (19), in order to attain equilibrium conditions on the column. However, we found that operating a 1-m Dexsil 300 column under these conditions did not give symmetrical sucrose diester peaks. Even operating at a rate of temperature rise of 16 C/min gave rise to some 'tailing' of the diester peaks, with a loss of reproducibility in the computation of the peak areas and a shorter (50-cm) column was preferred. The pyridine solutions of the sucrose ester fractions are not stable for long periods, and it has been observed that degradation of the sucrose esters does occur after silylation. This is shown by an increase in the levels of sucrose present and often occurs if the derivatized esters are left standing at room temperature for 24 hr.

Analysis of Impurities Present in Sucrose Esters

A typical analysis of an impure sample of sucrose esters is given in Table II.

Glycerol was determined with good reproducibility. A good peak separation of glycerol was obtained, after silylation, from the other components present.

An indirect method for the determination of long-chain fatty acid content was chosen since the standard titration methods described in ISO R660 (16) and a direct GLC method, without derivatization (20), gave poor agreement. With impure and colored samples of sucrose esters, the endpoint has proved difficult to determine in the ISO R660 titration. There was also evidence that degradation of sucrose esters to fatty acids occurred in the injection port of the GLC when the technique described by Ottenstein and Supiña (20) was used. The metal soaps of the long-chain fatty acids were determined on the basis of the metal content of the sucrose esters by plasma emission spectroscopy. The latter method was chosen in preference to flame photometry or atomic absorption spectrophotometry since a preliminary investigation showed that it gave more reproducible results. The standard titrimetric method (16) for metal soaps analysis gave poor reproducibility at the low levels of soaps present in the sucrose esters produced by the solvent extraction process (2). For samples containing higher levels of potassium soaps, such as TAL 25 (Table II), it could be used successfully. Several standard methods were available for determination of free sucrose. In the sucrose ester samples which also contained glycerol and mixed glycerides as impurities, the silylation and GLC method (7) was chosen, since these components and sucrose could be determined concurrently. However, with some triglycerides peak overlap of mono- and diglyceride peaks with those of sucrose or long-chain fatty acids occurred and careful choice of the peak used for analysis was required particularly with C_{12}-C_{14} triglycerides.

Moisture contents were normally less than 0.5% by weight. Since sucrose mono- and diesters are hygroscopic, they should be stored in sealed containers to prevent ingress of moisture.

HPLC Analysis

Some work was carried out on the use of reversed-phase HPLC for the analysis of sucrose esters. Since no derivatization is required and analysis is carried out at ca. 30-40 C, it has advantages over the GLC technique. However, from published work (5) in this field it is clear that proposed methods are not ideal. In a paper by Kaufman and Garti (4) the use of reversed-phase HPLC using IPA/methanol or IPA/water mixtures as solvent is discussed. The published chromatograms show poor resolution of the mono- and di-

TABLE II

Analysis of TAL 21T, TAL 25T and Impure Sucrose Esters (GLC)

Component %	TAL 21T[a]	TAL 25T	Impure esters
Glycerol	–	1	1
Calcium tallowate soaps	36	–	–
Potassium tallowate soaps	–	35	2
Mixed glycerides	30	5	7
Sucrose esters	30	32	85
Sucrose	2	25	3
Long-chain fatty acids	2	2	2

[a]It is necessary to add a few drops of acetic acid to the pyridine solvent, prior to silylation, for the GLC analysis of TAL 21T to convert the calcium soaps to the corresponding fatty acids which then silylate readily.

esters, the peaks appearing on the 'tail' of the solvent peak. Incorrect attribution of peaks is given for the samples of TAL 21 and TAL 25 (code numbers of TAL Chemicals Company), since methyl stearate is identified as a component of both products. The typical composition of TAL 21 and TAL 25, as determined by GLC, is given in Table II. Both TAL 21 and TAL 25 were prepared by a process in which triglycerides and sucrose were reacted (1). No methyl esters or methanol were used, and no methyl esters of long-chain fatty acids were present in the products. TAL 25 contains ca. 5% mixed glycerides, and these cannot form the major component identified by Kaufman and Garti (4). At the time when this work was carried out, the TSK columns (21) for carbohydrate analysis were not available and the standard reversed-phase HPLC columns gave poor results.

The presence of residual sucrose plus potassium or calcium soaps has a deleterious effect on the performance of reversed-phase HPLC columns and makes the correct identification of the peaks difficult. Although TLC analysis of sucrose esters on silica gel plates is well established (7), it has proved difficult to obtain quantitative HPLC using silica gel columns, since the sucrose esters tend to be absorbed very strongly on the silica gel. An HPLC technique which separates the sucrose esters by degree of substitution is required, rather than one which gives individual peaks for esters for each fatty acid present.

At the current state of development of analytical techniques, quantitative analysis of mixtures of sucrose mono- and diesters of long-chain fatty acids can best be accomplished by GLC after derivatization. HPLC would probably be a superior and more rapid method if the experimental difficulties could be resolved. Chemical methods of analysis are of limited value, since a mono/diester ratio has to be assumed. The quantitative analysis of the major impurities present in sucrose esters prepared from triglycerides can be carried out accurately, with the exception of the long-chain fatty acid content where further work is required.

The GLC method of analysis of sucrose mono- and diesters reported here has been developed and refined over a period of 5 years and has been used routinely in quality control. Provided that good quality GLC columns were used and frequent calibration checks were carried out, it was found to be a reliable and reproducible technique.

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