Determination of Sorbitan Tristearate in Vegetable Oils and Fats

Karen Thyssen* and Karsten Stig Andersen

Aarhus Oliefabrik A/S, Analytical Department, 8100 Aarhus C, Denmark

ABSTRACT: A new method for analysis of Sorbitan Tristearate (STS) in vegetable oils and fats has been developed. The method is based on isolation and hydrolysis of STS compounds in a silica cartridge. The polyalcohols are eluted from the silica cartridge and the final separation and quantitation are done by high-performance liquid chromatography and refractive index detection. Linearity, precision, and recovery satisfy general demands on quantitative methods. The detection limit and the quantitation limit are well below the concentrations normally used to attain functional effects of STS in vegetable oils and fats. *JAOCS 75,* 1855–1860 (1998).

KEY WORDS: Bloom retardation, emulsifier, high-performance liquid chromatography, solid-phase extraction, sorbitan tristearate, STS, vegetable oil.

Fatty acid esters of sorbitol and its anhydrides are approved as food additives in most of the world. They are used as emulsifiers and as fat crystal modifiers to retard bloom in cocoa butter, cocoa butter substitutes, and fat compounds for coating.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) defines Sorbitan Tristearate (STS) as consisting of a mixture of the partial esters of sorbitol and its mono- and dianhydrides with edible stearic acid (1). Even on the assumption that the polyalcohols esterified to the fatty acids in STS only consist of sorbitol, 1,4-sorbitan, 3,6-sorbitan, and isosorbide as mentioned by JECFA, this definition of Sorbitan Tristearate will include more than 200 possible congeners.

Approval of additives calls for analytical methods to determine the content in foods to which they are added. During the last 30 yr there have been more or less successful attempts to meet this need for determination of STS. The analytical methods are usually based on chromatographic techniques: thin-layer chromatography (TLC) (2), gas–liquid chromatography (GLC) (3,4) in combination with column chromatography, and high-performance liquid chromatography (HPLC) (5) in combination with solid-phase extraction. However, none of these methods seemed attractive for the following reasons. The TLC and HPLC methods are only semiquantitative. All methods use lengthy and laborious procedures and hazardous chemicals (e.g., chlorinated solvents, benzene). The GLC methods also use hazardous derivatization reagents [trimethylchlorosilane and *N,N*-bis(trimethylsilyl)trifluoroacetamide].

The work described in this paper has focused on developing a new method which is quantitative, environmentally acceptable, safe in the workplace, and which demands a minimum of manual work for the determination of Sorbitan Tristearate in vegetable oils and fats in the legal concentration range.

EXPERIMENTAL PROCEDURES

Materials. HPLC-grade *tert*-butyl methyl ether and heptane were supplied by Rathburn (Walkerburn, United Kingdom). HPLC-grade acetonitrile and pro analysis (p.a.) sulfuric acid 95–97% were obtained from Merck (Darmstadt, Germany). Water was Milli-Q quality (Millipore, Bedford, MA). Sulfuric acid 10 N and 1 N were prepared by diluting sulfuric acid 95–97% with water. Calcium hydroxide, purum p.a., minimum 96.0%, was purchased from Fluka (Buchs, Switzerland), and D-sorbitol, minimum 98%, from Sigma (St. Louis, MO).

STS was Grindsted STS 30 from Danisco Ingredients (Brabrand, Denmark) or Poem S-65F from Riken Vitamin Co. (Tokyo, Japan). Sorbitan Tristearate solution was prepared by weighing 400 ± 10 mg of Sorbitan Tristearate in a 100-mL volumetric flask and diluting to volume with *tert*-butyl methyl ether.

Equipment. Duran culture tubes of o.d. 16 mm × length 160 mm were supplied by Schott (Mainz, Germany). The tube-heater, regulated to 60 ± 5°C and equipped with N_2 supply, was obtained from Mikrolab (Aarhus, Denmark). Solid-phase extraction columns were Sep-Pak Vac 12 cc (2 g) Silica Cartridges from Waters (Milford, MA). Reservoirs for solid-phase extraction columns were glass tubes with o.d. 15 mm × length 250 mm, enlarged (o.d. ~ 16 mm) at one end to fit the extraction columns. The glycerol bath was regulated to 90 ± 2°C. The liquid chromatographic system was Waters 600 Multisolvent Delivery System equipped with a Waters 410 Differential Refractometer and interfaced to Waters Millenium software. Separations were performed on a 300 mm × 8 mm i.d. Shodex SUGAR SC1011 column supplied by Waters.

*To whom correspondence should be addressed at Aarhus Oliefabrik A/S, Analytical Department, M.P. Bruuns Gade 27, 8100 Aarhus C, Denmark. E-mail: ksa@aarhus.com

Preparation of the sample. The sample was prepared according to IUPAC 2.001 (6) by melting it in an electric oven heated to 60 ± 5°C.

Standard. Sorbitan Tristearate solution (5.0 mL) was transferred to a tube and the solution evaporated to dryness in the tube-heater at 60 ± 5°C by a nitrogen jet. The tube was cooled to room temperature, and 1.00 ± 0.05 g vegetable fat without Sorbitan Tristearate was weighed directly into the tube. The content of the tube was subsequently treated in the same manner as outlined for the melted and weighed sample.

Sample. The melted sample (1.0 g ± 0.1 g) was accurately weighed into a tube to within 0.001 g. The tube was heated in the tube-heater at 60 ± 5°C and 1.0 mL of heptane was added to the sample. The mixture was shaken carefully until it was homogeneous at 60 ± 5°C.

Solid-phase extraction. A silica cartridge was washed with 6 mL of *tert*-butyl methyl ether, dried by a nitrogen flow, and conditioned by a wash with 6 mL of heptane. The solvent level was allowed to drop to the top of the column packing. The sample mixture was transferred to the conditioned silica cartridge. The sample tube was rinsed with 2 × 1.0 mL of 60 ± 5°C warm heptane and the heptane rinsings transferred to the silica cartridge. Solvent was drained off until the level of the sample mixture was about 1 mm above the column packing. The cartridge was then fitted with a reservoir and 20.0 mL of heptane/*tert*-butyl methyl ether (90:10, vol/vol) was applied onto the silica. A triglyceride-containing fraction was eluted by gravitation and discarded. The column packing was dried by a nitrogen flow from the top of the cartridge. Drying was then continued by a nitrogen flow at 0.4 bar passed through the bottom of the silica cartridge for 30 min. If not hydrolyzed immediately, the dried silica cartridge may be stoppered and stored in the refrigerator for up to 14 d before hydrolysis.

Hydrolysis. Hydrolysis was performed with STS deposited on the column packing in the following way. A 4.0-mL aliquot of 1 N sulfuric acid was transferred to the dried silica cartridge and the solvent level allowed to drop to the top of the column packing under mild pressure. Then 1.5 mL of water was applied onto the column packing. The bottom of the cartridge was fitted with a plug, and a plastic lid was loosely placed on top of the cartridge. The silica cartridge was then placed in a glycerol bath at 90 ± 2°C for 24 ± 0.2 h with the top of the column packing about 5 mm below the surface of the glycerol bath.

Elution of sorbitol. The silica cartridge was removed from the glycerol bath and the adhering glycerol rinsed off the cartridge with water. The silica cartridge was allowed to cool to room temperature. Then 4.0 mL of water was applied onto the silica and sorbitol eluted from the column into a 5-mL volumetric flask. If any solvent remained on top of the column packing after the cartridge had reached room temperature, the solvent level was allowed to drop to the top of the column packing before the addition of water. The eluate was eluted with a mild pressure of nitrogen until the volume of the extract was exactly 5.0 mL. If necessary, additional water was added and eluted from the column. The pH of the extract was adjusted to 3.0–7.0 by adding calcium hydroxide. The pH was checked after the addition of 150 ± 2 mg of calcium hydroxide and, if necessary, adjusted by addition of 10-mg portions of calcium hydroxide or 10-µL portions of 10 N sulfuric acid. After that the calcium sulfate was allowed to settle and the solution was filtered through a 0.45-µm filter before analysis.

Chromatographic conditions. The HPLC column was maintained at 50 ± 2°C. The mobile phase consisted of water/acetonitrile (985:15, vol/vol). The flow rate was 0.8 mL/min and the injection volume 50 µL. Refractometer temperature was set to 40°C. The total run time was 30 min.

Expression of results. The STS content in the sample was calculated by the external standard method using the formula: % STS = $A_x/(W \times f)$, where A_x is the peak area of sorbitol in the sample, W is the mass in g of the sample, and f is the response factor.

The response factor was calculated as the average of the response factors that were determined in every analysis series from two standard runs using the formula: $f = A_s \times 200/W_s$, where A_s is the peak area of sorbitol in the standard and W_s is the mass in mg of STS in 100 mL of Sorbitan Tristearate solution.

The STS content was calculated as the average of two determinations.

RESULTS AND DISCUSSION

Chromatographic separation. Sorbitol was heated to 230°C for 24 h under a nitrogen cover and dissolved in water before HPLC analysis. The chromatogram with five peaks (A, B, C, D, E) is shown in Figure 1. The negative refractive index peak is caused by acetonitrile, which is not in the same concentration in the sample as in the mobile phase. Peak E with reten-

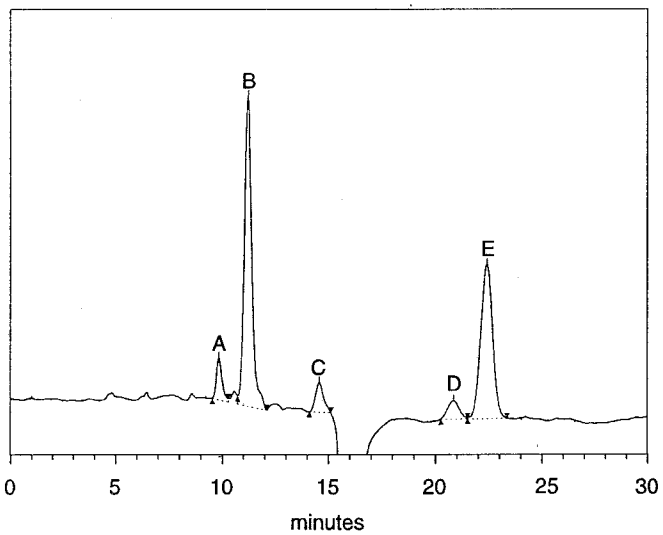

FIG. 1. High-performance liquid chromatogram of polyalcohols formed by heating sorbitol to 230°C for 24 h under a nitrogen cover. For chromatographic conditions, see the Experimental Procedures section. A and D, unknown polyalcohols; B, sorbitan(s); C, isosorbide; E, sorbitol.

tion time (t_r) 22.4 min is sorbitol, peak B (t_r = 11.1 min) represents sorbitan compound(s), and peak C (t_r = 14.5 min) is isosorbide (1,4-3,6-dianhydrosorbitol). The identities of the two other peaks (A and D) are not known, but they could be polymers of C6- polyalcohols. The identities of peak B and peak C are based on the syntheses of 1,4 sorbitan (7) and 1,4-3,6-dianhydrosorbitol (8).

Chromatograms of a blank vegetable oil and of the vegetable oil spiked with 2% STS are presented in Figures 2 and 3, respectively. Four of the five peaks (A, B, D, E) from the heat-treated sorbitol in Figure 1 can be found in the oil spiked with STS and not in the blank oil. The presence of these four peaks in the chromatogram was used as an identification of sorbitan esters in the oil.

Specificity. Compounds in the injection solution can interfere with the early eluting polyalcohols in the chromatogram. The peak with t_r = 8.6 min (Figs. 2 and 3) is caused by salts. The retention time of this peak can fluctuate by several minutes, depending on the condition of the column. If the position of the peak is disturbing the polyalcohols, the retention time of the peak can be changed by purging the column with 50 mL of aqueous 0.1 M $Ca(NO_3)_2$. The column lasts longer if water is used as mobile phase instead of water/acetonitrile. The compounds eluting at 13.6 and 14.5 min are largely due to *tert*-butyl methyl ether having been in contact with the silica cartridge, and are probably reaction products of *tert*-butyl methyl ether. Glycerol also elutes in this area. Isosorbide is hidden in the peak with t_r = 14.5 min and cannot be quantitated as long as *tert*-butyl methyl ether is used in the analytical procedure.

Sorbitol (E) was used to determine the content of STS in the sample because of possible interferences early in the chromatogram. Alternatively, peak D with t_r = 20.9 min, or the sum of the two later eluting peaks (D and E) can be used to measure the content of STS. Sorbitan (B) is usually the highest polyalcohol peak in the chromatogram and can also be used for the STS quantitation if a blank run has shown the absence of interfering peaks. The resolution of peaks D and E can be improved by increasing the acetonitrile concentration in the mobile phase. However, an increased amount of acetonitrile in the mobile phase leads to a more disturbed baseline. A higher temperature also produces a more noisy baseline. A lower flow rate improves resolution, but increases the analysis time.

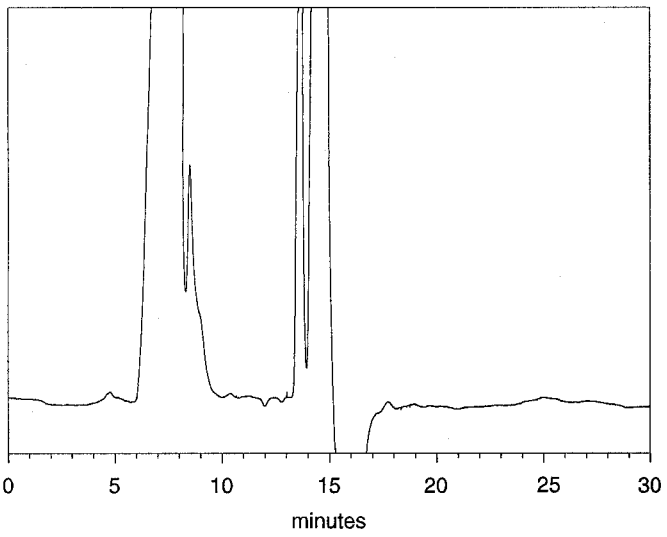

FIG. 2. High-performance liquid chromatogram of a blank vegetable oil. For chromatographic conditions, see the Experimental Procedures section.

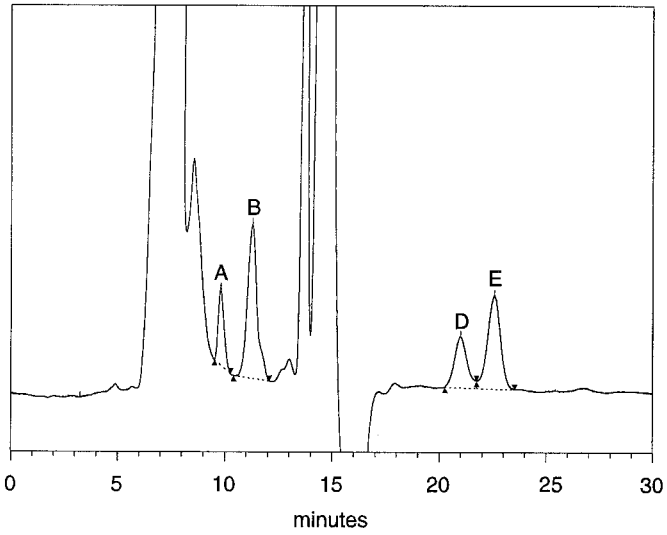

FIG. 3. High-performance liquid chromatogram of a vegetable oil spiked with Sorbitan Tristearate (STS). For chromatographic conditions, see the Experimental Procedures section. A and D, unknown polyalcohols; B, sorbitan(s); E, sorbitol. For abbreviation see Figure 1.

Solid-phase extraction. Polyalcohol compounds are lost together with the triglycerides eluted from the silica cartridge. This was established by eluting the polyalcohol esters with *tert*-butyl methyl ether/methanol/sulfuric acid 1 N (40:60:1, vol/vol/vol) from the silica cartridge after elution of triglycerides. The molecular size distribution of the polyalcohol ester fraction was examined using IUPAC Standard Method 2.508 (9) modified with two extra columns (pore sizes: 100 Å and 500 Å). The size distribution of the polyalcohol ester fraction (Fig. 4) was compared with the size distribution of the STS product that had not been fractionated on the silica cartridge (Fig. 5). A blank, fractionated triglyceride sample was also analyzed with the modified IUPAC method to determine the contribution of nonpolyalcohol compounds to the polyalcohol ester fraction (Fig. 6).

It can be seen from the chromatograms (Figs. 4–6) that the majority of the STS components that coeluted with the triglycerides from the silica cartridge had a molecular size higher than the molecular size of the different isomers of sorbitan tristearate (mol. wt. 962). The amount of polyalcohol esters lost was about 40% (area percent). If we assume that the polyalcohol esters with a molecular size higher than sorbitan tristearate have four fatty acids esterified to the polyalcohols, then the 40% polyalcohol esters corresponds to a loss of about 34% of the C_6-polyalcohols.

Esters of polymers of C_6-polyalcohols. Not all the polyalcohol ester components with molecular sizes higher than sor-

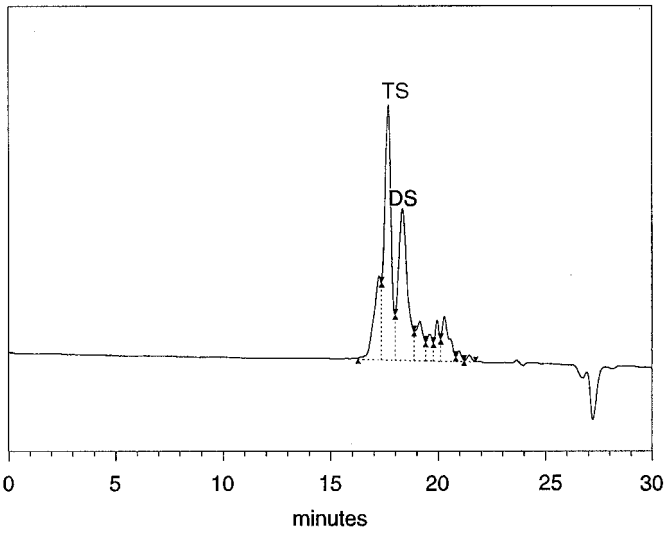

FIG. 4. High-performance liquid chromatogram showing the molecular size distribution of a polyalcohol ester fraction isolated after extraction of triglycerides. For chromatographic conditions, see text. STS, sorbitan tristearate. DS, sorbitan distearate.

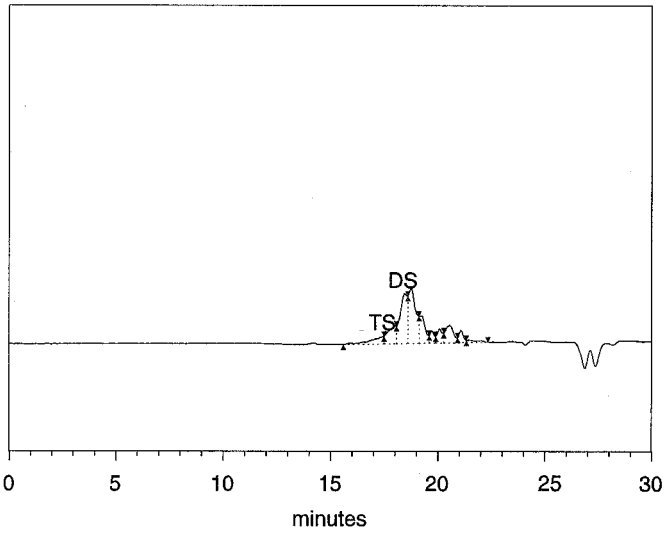

FIG. 6. High-performance liquid chromatogram showing the molecular size distribution of a blank triglyceride fraction. For chromatographic conditions, see text. For abbreviations see Figures 3 and 4.

bitan tristearate coeluted with the triglycerides (Fig. 4). This can be explained by the presence of esters of polymers of C_6-polyalcohols. To investigate molecular size in combination with polarity of the polyalcohol esters, we carried out preparative TLC on 0.25-mm layers of silica gel. The plate was developed in pentane/diethyl ether/acetic acid (90:10:1, vol/vol/vol) and spots of polyalcohol esters were detected by spraying the plate with bromothymol blue dissolved in 0.1 N sodium hydroxide and with ethanolic phosphomolybdic acid. Five fractions of different polarities were isolated from the TLC plate, and size exclusion chromatograms showing the molecular size distribution of each fraction were run. All fractions, regardless of polarity, contained compounds with molecular sizes higher than sorbitan tristearate. An STS component with a polarity and a molecular size higher than sorbitan tristearate must contain esters of at least some dimers of C_6-polyalcohols, and therefore we concluded that the commercial STS product examined contains esters of polymers of C_6-polyalcohols.

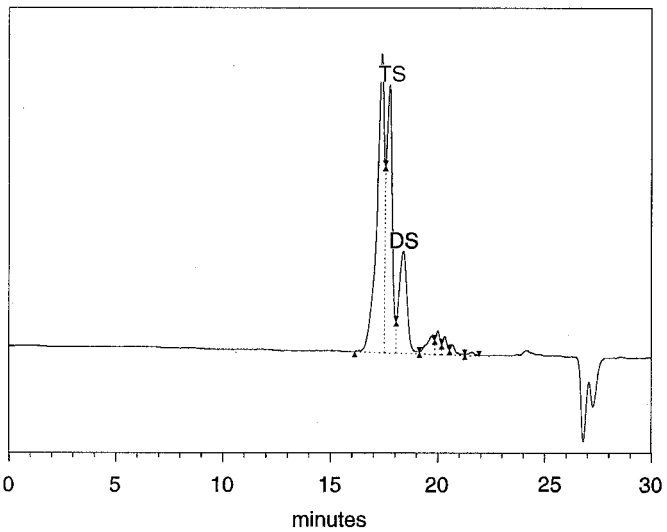

FIG 5. High-performance liquid chromatogram showing the molecular size distribution of STS. For chromatographic conditions, see text. For abbreviations see Figures 3 and 4.

Hydrolysis of STS. As demonstrated by the molecular size distribution of the polyalcohol ester fraction eluted from the silica cartridge, a considerable amount of polyalcohol esters is lost with the elution of triglycerides. However, when we deposited STS dissolved in 4 mL of *tert*-butyl methyl ether on the silica column packing and hydrolyzed the polyalcohol esters, we could not determine any significant difference between the amount of sorbitol formed from this hydrolysis and the amount of sorbitol formed from the hydrolysis of STS after triglyceride elution. This unexpected result occurred because the hydrolysis of the polyalcohol esters was not completed within 24 h and because the eluted STS compounds contained relatively more ester bonds to be hydrolyzed per STS molecule than the compounds staying in the cartridge.

Figure 7 shows the progress of the formation of sorbitol during 48 h of hydrolysis of STS isolated from a representative sample. It can be seen from the graph that the conversion of STS to sorbitol and other hydrolysis products was not completed after 24 h and probably not after 48 h. Increasing the sulfuric acid concentration from 1 to 2 N did not have any significant effect on the hydrolysis rate. The higher acid concentration also required the addition of twice as much base to the sample solution, making the analytical procedure more difficult. Raising the temperature sped up the hydrolysis rate, but a temperature higher than 90°C would require the silica cartridge to be closed more firmly to prevent the water from evaporating and the reaction from taking another direction, resulting in a lower recovery of sorbitol. We have not yet

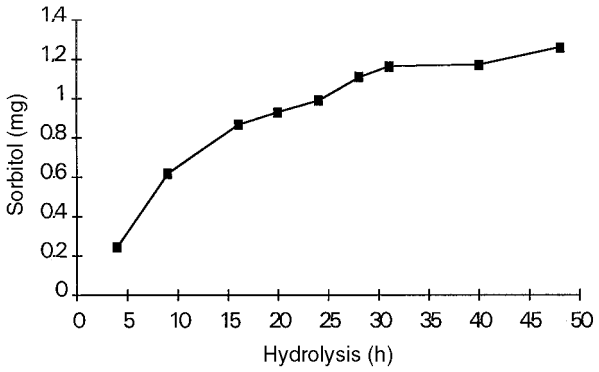

FIG. 7. The amount of sorbitol formed during hydrolysis of 40 mg of STS. For abbreviation see Figure 3.

found a practical way to close the cartridge adequately to be able to raise the temperature above 90°C. The chosen hydrolysis time (24 h) is a compromise between detection limit and time of analysis.

Recovery of polyalcohols. Isosorbide is hidden in the peak with t_r = 14.5 min (Fig. 3) and cannot be quantitated as long as *tert*-butyl methyl ether is used in the analytical procedure, as mentioned earlier. With the purpose of estimating the amount of isosorbide in the polyalcohol extract, we deposited STS on the silica column packing by means of warm heptane and hydrolyzed the polyalcohol esters. A blank extract was also produced without using *tert*-butyl methyl ether. The blank run showed a small peak still eluting at 14.5 min. By subtracting the interfering peak present in the chromatogram of the blank extract from the corresponding peak in the chromatogram of the polyalcohol extract, we estimated the amount of isosorbide in proportion to the other polyalcohols.

The relative amounts of the polyalcohols eluted could have changed by substituting heptane for *tert*-butyl methyl ether, but no significant differences were found in the ratios (area counts) of the polyalcohol peaks A, B, D, and E (Fig. 3), whether *tert*-butyl methyl ether or heptane was used in the analytical procedure.

The total amount of polyalcohols hydrolyzed was much less in the absence of *tert*-butyl methyl ether than when *tert*-butyl methyl ether was used in the analytical procedure. The reason for this might be that the two solvents do not distribute the STS components in the same way along the silica packing, resulting in differences in the ability of the hydrolyzing medium to gain access to the polyalcohol ester bonds.

By adding the area counts of the five peaks representing polyalcohols, we estimated that about 29% of the polyalcohols in the extract is sorbitol. If the STS product is composed of fatty acids and polyalcohols in a ratio of three to one, then 49% (m/m) of the polyalcohols is recovered in the analysis. The obtained recovery percentage of polyalcohols must be taken with some reservation. We do not know if the STS product contains the theoretical percentage of polyalcohols. The amount of isosorbide in proportion to the other polyalcohols is estimated on the basis of a different analytical procedure (no *tert*-butyl methyl ether) and by subtracting an interfering compound. We also assumed that the response factor is the same for all the hydrolysis products.

The low recovery of polyalcohols is due to the elution of polyalcohol esters with the triglyceride fraction and the incomplete hydrolysis of the polyalcohol esters. An approximate mass balance for the polyalcohols is presented in Figure 8. The accuracy of the method is not influenced by the loss of polyalcohols because the method automatically corrects for the incomplete recovery by subjecting the calibration samples to the same treatment as the samples to be analyzed.

Sorbitol/sorbitan ratio. STS products with different lot numbers or from different producers can contain various relative amounts of polyalcohols, depending on the reaction conditions during the syntheses of the polyalcohol esters. Therefore, it is necessary to pay attention to whether the STS used for making the standard is similar to the STS added to the samples to be analyzed. It must be checked that the sorbitol/sorbitan peak ratio of the sample matches the sorbitol/sorbitan peak ratio of the standard to ensure that the correct standard is used. A different sorbitol/sorbitan peak ratio may also imply that sorbitan monostearate has been applied. The identity of the emulsifier can be deduced from a chromatogram showing the molecular size distribution of the polyalcohol esters.

Deodorization. A product containing 2.4% STS was deodorized at four different temperatures, 200, 220, 230, and 240°C, to see if the deodorization temperature had any effect on the content of STS and/or the sorbitol/sorbitan peak ratio. The STS contents determined after the deodorizations were 2.5, 2.1, 1.3, and 0.7%, respectively. At a deodorization temperature of 220°C a decrease in the STS content was already observed. The difference in the STS content at 200 and 220°C could be explained by the uncertainty of the method. However, a difference in the two samples concerning STS was confirmed by the fact that the molecular size distribution of STS was changed by raising the temperature to 220°C. The

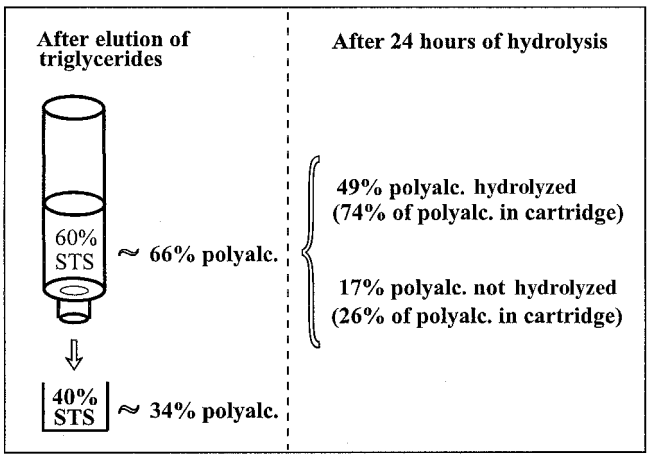

FIG. 8. An approximate mass balance for STS and polyalcohols in the analytical procedure. polyalc., polyalcohols. For other abbreviation see Figure 3.

molecular size distributions of the polyalcohol esters in the deodorized samples were examined by eluting the STS components from the silica cartridges after elution of triglycerides and applying IUPAC Standard Method 2.508 (9), as described earlier. The chromatograms show that higher deodorization temperatures result in a decrease in components with molecular sizes higher than or equal to sorbitan tristearate (mol wt 962) and an increase in components with lower molecular sizes. We could not detect any change in the sorbitol/sorbitan peak ratio due to a higher deodorization temperature. Besides the lower content of STS in the product caused by a higher deodorization temperature, the change in the molecular size distribution might influence the obtained effect of the sorbitan esters in the product.

Accuracy. The accuracy was determined by applying the method to vegetable fats containing known amounts of STS and calculating the recovery of STS. Oils and fats typically used in the confectionery industry and normally used oil additives (lecithin, butylated hydroxytoluene, and tocopherols) were mixed in the laboratory. STS (1, 2, or 4%) was added and the mixtures analyzed with and without STS. Three commercial products with a content declaration of STS were also analyzed. The average percentage recovery of STS added to the fats mixed in the laboratory was 97% (standard deviation SD = 5.7, $n = 7$). The average percentage recovery of STS from the commercial products was 109% (standard deviation SD = 11.2, $n = 3$).

Linearity. STS (160–1000 mg) was accurately weighed in a 100-mL volumetric flask and diluted to volume with *tert*-butyl methyl ether to yield Sorbitan Tristearate solutions of 1.6, 2, 3, 4, 5, 6, 8, and 10 g/L. Aliquots (5.0 mL) of each of the STS solutions were transferred to a tube and treated as described for the standard in the experimental procedure section. Each concentration was assayed in duplicate. Visual evaluation of the plot of peak areas as a function of these eight concentration levels showed linearity in the investigated concentration range 8–50 g STS/kg fat. A regression line was calculated by the method of least squares ($y = -1892 + 12863x$, y in arbitrary units). The correlation coefficient of peak area to concentration was $r = 0.9961$.

Precision. The repeatability and the intermediate precision were evaluated by performing two repetitive analyzes of a sample containing 2.9% STS on nine different days. Application of Cochran's test did not yield statistical outliers at the $P = 0.05$ level of significance.

The within-day repeatability standard deviation s_r and the within-day repeatability value *r,* expressed as percentage of STS, were 0.19 and 0.53, respectively.

The within-day repeatability standard deviation s_r is the standard deviation of test results obtained where mutually independent test results are obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time.

The within-day repeatability value r is the value below which the absolute difference between two single test results obtained under within-day repeatability conditions may be expected to lie within a probability of 95%.

The day-to-day repeatability standard deviation s_R and the day-to-day repeatability value *R,* expressed as percentage of STS, were 0.18 and 0.51 respectively.

The day-to-day repeatability standard deviation s_R expresses within-laboratory variations: different days and different analysts.

The day-to-day repeatability value R is the value below which the absolute difference between two single test results obtained under day-to-day repeatability conditions may be expected to lie within a probability of 95%.

Detection and quantitation limits. The detection limit (LOD) and the quantitation limit (LOQ) were calculated according to the following formulas: $LOD = 3.3 \times (SD/S)$ and $LOQ = 10 \times (SD/S)$ where SD is the standard deviation of the response and S is the slope of the calibration curve. The residual standard deviation of the regression line was used as the standard deviation. The detection limit and the quantitation limit were 0.4 and 1.4% of STS, respectively.

REFERENCES

1. Joint FAO/WHO Expert Committee on Food Additives, *Compendium of Food Additive Specifications, Addendum 3*:113 (1995).
2. Dieffenbacher, A., and U. Bracco, Analytical Techniques in Food Emulsifiers, *J. Am. Oil Chem. Soc. 55*:642–646 (1978).
3. Sahasrabudhe, M.R., and R.K. Chadha, Chromatographic Analysis of Sorbitan Fatty Acid Esters, *Ibid. 46*:8–12 (1969).
4. IUPAC, Commission on Oils, Fats, and Derivatives, Determination of the Ester-Emulsifiers Components After Hydrolysis and Silylation by Gas Chromatography, *Standard Methods for the Analysis of Oils and Fats and Derivatives,* 7th edn., Oxford, Blackwell Scientific Publications, 1987, 1992, 6.001:pp. 1–5.
5. Sutton, P.M., and P.T. Slack, Determination of Emulsifiers in Foods. Part III. A Semi-Quantitative Method for Determination of Sorbitan Esters, *Leatherhead Fd. RA Res. Rep. No. 629* (1988).
6. IUPAC, Commission on Oils, Fats, and Derivatives, Preparation of the Sample, *Standard Methods for the Analysis of Oils and Fats and Derivatives,* 7th edn., Oxford, Blackwell Scientific Publications, 1987, 1992, 2.001:pp. 27–28.
7. Soltzberg, S., R. M. Goepp, Jr., and W. Freudenberg, Hexitol Anhydrides. Synthesis and Structure of Arlitan, the 1,4-Monoanhydride of Sorbitol, *J. Am. Chem. Soc. 68*:919–921 (1946).
8. Montgomery, R., and L.F. Wiggins, The Anhydrides of Polyhydric Alcohols. Part IV. The Constitution of Dianhydro Sorbitol, *J. Chem. Soc. 149*:390–393 (1946).
9. IUPAC, Commission on Oils, Fats, and Derivatives, Determination of Polymerized Triglycerides in Oils and Fats by High Performance Liquid Chromatography, *Standard Methods for the Analysis of Oils and Fats and Derivatives,* 7th edn., Blackwell Scientific Publications, Oxford, 1987, 1992, 2.508:1–5.

[Received November 13, 1997; accepted July 25, 1998]