

Preparation of Trehalose and Sorbitol Fatty Acid Polyesters by Interesterification¹

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Trehalose octaoleate and sorbitol hexaoleate were prepared by a one-stage solvent-free interesterification of methyl oleate with trehalose octaacetate (TOAC) and sorbitol hexaacetate (SOHA), respectively, in the presence of 1–2.5% sodium metal as catalyst. The greatest yield of trehalose octaoleate, 97.5%, and sorbitol hexaoleate, 96.0%, were obtained at a mole ratio of methyl oleate:SOHA of 6:1 and methyl oleate:TOAC of 8:1 at a synthesis time of 2.5 hr, and temperatures of 105–115°C. The structure of both trehalose octaoleate and sorbitol hexaoleate was confirmed by infrared (IR) and nuclear magnetic resonance (C-13 NMR) spectroscopy. Physical properties such as viscosity, HLB, solubility, color, refractive index, specific gravity and density approximated the physical properties of sucrose polyesters (SPE) and vegetable oils. Trehalose octaoleate and sorbitol hexaoleate, as well as sucrose octaoleate, were not susceptible to *in vitro* lipolysis, suggesting potential application as low calorie oils.

The food industry has recently focused attention on the possible use of carbohydrate polyesters, such as sucrose polyesters, as indigestible oils or fats in food products (1,2). There is a current need for a high yielding, inexpensive synthesis of carbohydrate fatty acid polyesters. Several researchers (3–6) have reported the synthesis of carbohydrate fatty acid polyesters, with sucrose polyesters receiving the greatest attention. Akoh and Swanson (7) reported the synthesis of the trisaccharide, raffinose fatty acid polyester. Many interesterification methods (4,8,9) require high temperatures and toxic solvents such as dimethylacetamide, dimethylformamide or dimethylsulfoxide, and the polyesters are not suitable for food applications.

Mattson *et al.* (4) synthesized sorbitol hexaoleate by heating sorbitol and a five-fold excess of ethyl oleate in dimethylacetamide with sodium methoxide as a catalyst. Reaction times of about 5 hr and temperatures near 180°C, were employed (4). Therefore, a potential low temperature, short time, solvent-free process for the synthesis of sorbitol polyesters was explored.

Little research has been published on the chemical synthesis of the novel trehalose fatty acid polyester. Trehalose is a disaccharide nonreducing sugar, the major sugar of insect hemolymph, fungi and yeasts. Trehalose is made of two α -D-glucopyranose components and closely resembles sucrose in physical properties. Reports of solvent-free synthesis of sorbitol or trehalose fatty acid polyesters

by interesterification of SOHA or TOAC with fatty acid methyl ester (FAME) catalyzed by sodium metal are not available. Therefore, the purpose of the present study was to develop a high yielding synthesis of sorbitol hexaoleate and trehalose octaoleate by one-stage solvent-free interesterification, and to compare the physical properties of the synthesized polyesters with the physical properties of sucrose polyesters and vegetable oils.

MATERIALS

Sorbitol hexaacetate and trehalose octaacetate were purchased from Sigma Chemical Co. (St. Louis, MO). Pure methyl oleate was purchased from Aldrich Chemical Co. (Milwaukee, WI). The reaction apparatus for sorbitol and trehalose polyester synthesis is similar to the reaction apparatus described by Akoh and Swanson (7) for one-stage synthesis of raffinose polyesters.

EXPERIMENTAL

Synthesis and purification of trehalose octaoleate and sorbitol hexaoleate followed the one-stage synthesis procedure of Akoh and Swanson (7) for the synthesis of raffinose polyesters, except that trehalose octaacetate (TOAC) and sorbitol hexaacetate (SOHA) were used as reactants instead of raffinose undecaacetate.

A known quantity (Table 1) of methyl oleate and sorbitol hexaacetate or trehalose octaacetate and sodium metal were admixed in a three-necked, round bottom flask equipped with a magnetic stirrer, stopcocks, a vacuum take-off line leading to liquid N₂ cold trap and a vacuum pump. An oil bath was used to maintain a temperature between 100–118°C. The mixture was stirred continuously under dry N₂ atmosphere for a selected reaction period. The pressure was maintained at 0–5 mm Hg with a vacuum pump.

In a typical experiment, substantially anhydrous methyl oleate (52.43 g, 0.1768 mol) and TOAC (15.00 g, 0.0221 mol) were admixed with 2% by weight sodium metal (1.36 g). The reactants were heated to form a homogenous melt at 105–110°C, and interesterification continued for 2.5 hr. The mole ratio of methyl oleate to TOAC was 8:1.

Substantially anhydrous methyl oleate (49.14 g, 0.0276 mol) and SOHA (15.00 g, 0.0345 mol) were admixed with 2% by weight sodium metal (1.22 g). The reactants were heated to form a homogenous melt at 110–115°C, and interesterification continued for 2.5 hr. The mole ratio of methyl oleate to SOHA was 6:1.

At the end of synthesis, the reaction mixture was allowed to cool, neutralized with 1–3 ml glacial acetic acid, dissolved in hexane, bleached with 1 g of activated charcoal, and then filtered and washed with methanol according to the purification method of Akoh and Swanson (7).

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The product was dried over anhydrous sodium sulfate and the solvents removed by rotary evaporation. Sorbitol and trehalose polyesters were further purified by eluting them from a Florisil column with hexane (7,10).

Structure proof and product analysis. Progress of interesterification was followed by TLC with the solvent system (5) chloroform:methanol:water:formic acid 85:15:2:1 (v/v) as the mobile phase. Sugar polyesters were detected on developed TLC plates by spraying them with sulfuric acid-dichromate (50% H₂SO₄ solution) and were visualized as purple-violet spots on a white background when heated at 100–105°C for 5–10 min. Spraying the plates with naphthoresorcinol reagent (7), as in the analysis of raffinose polyesters, did not give purple-violet spots but instead gave light brown-black spots of trehalose and sorbitol polyesters which were detected and were indistinguishable from FAME spots.

The structures of the carbohydrate polyesters were confirmed by both IR and C-13 NMR spectroscopy as described by Akoh and Swanson (7). The physical properties like refractive index, density and specific gravity were determined according to the procedures described previously (7). The viscosities of sorbitol hexaoleate, trehalose octaoleate, sucrose octaoleate and peanut oil were determined with an Ostwald viscosity pipette at 20°C and viscosity expressed in centipoise (cp). Solubility of a 1% solution of the test oils were determined in various solvents (Table 3) at 25°C and 75°C. Hydrophilic-lipophile balance (HLB) was determined according to the method of Gupta *et al.* (11). Ryoto sugar ester standards covering the HLB range 1–15 obtained from Mitsubishi-Kasei Food Corporation (Tokyo, Japan) were used to obtain a calibration curve by plotting water number against HLB. HLB values were reproducible to within ± 0.5 HLB units.

In vitro enzymatic hydrolysis of trehalose octaoleate, sorbitol hexaoleate and sucrose octaoleate by pancreatic, porcine pancreatic lipase, purified lipase and olive oil standard were assayed according to the method of Marchis-Mouren *et al.* (12). After incubation of the mixtures at 37°C for 30 min, the liberated free fatty acids were extracted with hexane and a 10 ml aliquot was titrated according to the method of Pope *et al.* (13). A unit of lipolytic activity is defined as the enzyme activity which liberates one micromole of fatty acid per minute under designated assay conditions. Rates of lipid hydrolysis are reported relative to that of olive oil taken as unity.

RESULTS AND DISCUSSION

Table 1 presents interesterification conditions for the preparation of trehalose octaoleate and sorbitol hexaoleate. The one stage solvent-free synthesis (7) gave excellent yields of trehalose and sorbitol polyesters at temperatures as low as 100–115°C and synthesis times as short as 1.5–2.5 hr. An 8:1 mol ratio of methyl oleate:TOAC and methyl oleate:SOHA (6:1) resulted in the greatest yields of trehalose polyester, 97.5% and sorbitol polyester, 96.0%, respectively. In both cases, acceptable yields were obtained at catalyst concentration of 1.5%. It is not necessary to increase the mole ratio of FAME:TOAC and FAME:SOHA beyond 8:1 and 6:1, respectively. Apparently, carbohydrate acetates and methyl esters react mole to mole for optimum interesterification. Akoh and Swanson (7) and Mieth *et al.* (6) concluded that carbohydrate acetates are good leaving groups and exhibit lower melting points compared to unprotected carbohydrates, thereby eliminating the need for high temperatures during interesterification. The use of carbohydrate acetates as a starting material reduced excessive carbohydrate degradation and caramelization (7).

Formation of a homogenous melt is required for optimum interesterification. Carbohydrate acetates form homogenous melts faster than unprotected carbohydrates such as trehalose and sorbitol, and reduce the time required for synthesis. For example, the two-stage process of Rizzi and Taylor (5), using sucrose as starting material, required temperatures between 130–150°C, reaction times of 8–9 hr and a mole ratio of methyl esters:sucrose of 16:1 that the process is not economical for practical manufacture of carbohydrate polyesters. The solvent-free interesterification process of Feuge *et al.* (3), using molten sucrose, required temperatures between 170–187°C. Temperatures of 105–115°C, pressures of 0–5 mm Hg and reaction times of 2.5 hr employed in the present synthesis resulted in excellent yields of carbohydrate polyesters. Molecular distillation to remove unreacted methyl oleate (14–16) was unnecessary because traces of methyl oleate were easily removed during methanol wash and elution on Florisil column (7). Figure 1 illustrates the reaction pathway for the preparation of trehalose octaoleate.

Product structure and analysis. TLC analyses of trehalose octaoleate, sorbitol hexaoleate and sucrose octaoleate are represented in Figure 2. Trehalose octaacetate, SOHA, methyl oleate and sucrose monostearate

TABLE 1

Interesterification Conditions for Preparation of Trehalose Octaoleate and Sorbitol Hexaoleate^a

Sample	Molar ratio FAME:TOAC or SOHA ^b	Catalyst (% wt of reactants)	Reaction time (hr)	Temperature (°C)	Product yield ^c
Trehalose octaoleate	10:1	1.0	2.5	105–110	91.6
	8:1	1.5	1.5	100–105	94.0
	8:1	2.0	2.5	105–110	97.5
	6:1	2.0	3.0	110–112	89.0
Sorbitol hexaoleate	8:1	1.5	2.0	115–118	92.1
	8:1	2.5	2.5	110–115	94.8
	6:1	2.0	2.5	110–115	96.0

^aReactions carried out between 0–5 mm Hg pressure.

^bFAME:TOAC vs SOHA, Fatty acid methyl ester:trehalose octaacetate or sorbitol hexaacetate.

^cPercent yield by weight of pure trehalose octaoleate and sorbitol hexaoleate based on the theoretical weights.

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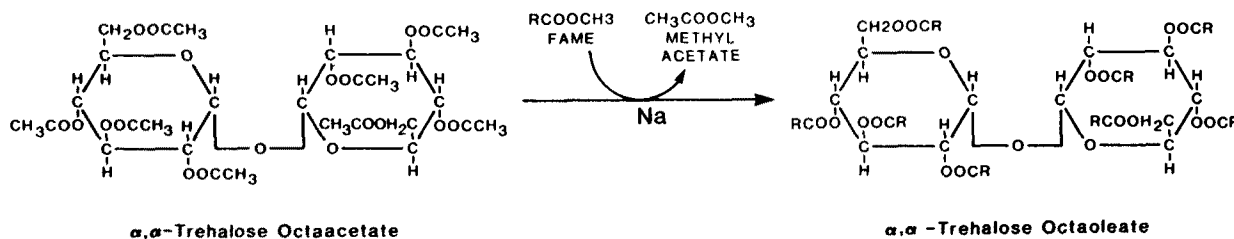


FIG. 1. Reaction pathway for trehalose octaoleate preparation.

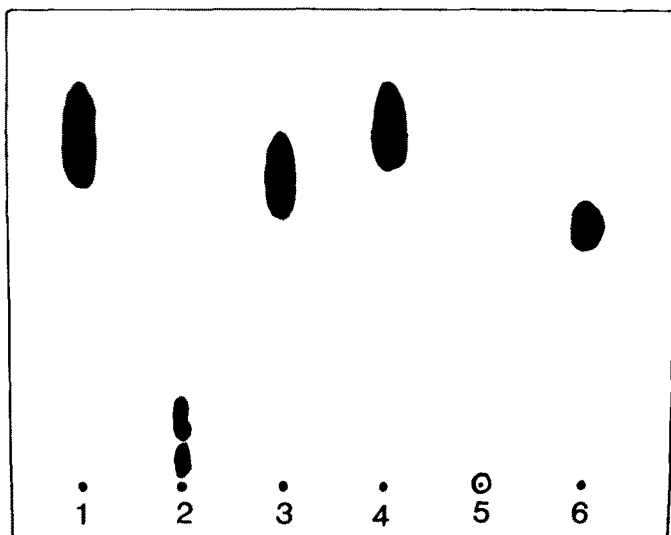


FIG. 2. Thin layer chromatogram of candidate esters. Solvent system = $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}:\text{HCOOH}$ 85:15:2:1 (v/v). Lane 1, purified trehalose octaoleate; Lane 2, sucrose monostearate standard; Lane 3, sorbitol hexaoleate (SOHA); Lane 4, sucrose octaoleate; Lane 5, trehalose octaacetate (TOAC); Lane 6, methyl oleate.

standard were co-plated. Sucrose monostearate, SOHA and TOAC did not migrate far on TLC plates. Methyl oleate appeared as yellow-brown spot on TLC plates. Further evidence of successful synthesis was provided by obtaining the fatty acid composition of trehalose octaoleate and sorbitol hexaoleate as methyl esters according to the transesterification method of Akoh and Swanson (17). The fatty acid composition of trehalose and sorbitol polyesters as determined by GLC did not change when compared to the starting methyl ester, indicating that methyl oleate was incorporated into trehalose and sorbitol under the interesterification conditions.

Figure 3 is the C-13 NMR spectrum signals for trehalose octaacetate. Signals observed include the methyl carbons of the acetate group (20.6 ppm), 1° carbon of glucose moieties (61.8 ppm), carbons of trehalose skeleton (68.2–69.9 ppm), anomeric carbon of glucose (92.1 ppm) and carbonyl carbons of acetyl group (169.5–170.4 ppm). Figure 4 illustrates the C-13 NMR spectra of sorbitol hexaacetate. Signals of the methyl carbons of acetyl group (20.6 ppm), 1° carbons of sorbitol (61.3–61.8 ppm), CDCl_3 (75.2–78.8 ppm), other carbon skeletons of sorbitol (68.3–69.2 ppm) and ester carbonyl carbons of acetyl group (169.6–170.2 ppm) were observed. The C-13 NMR

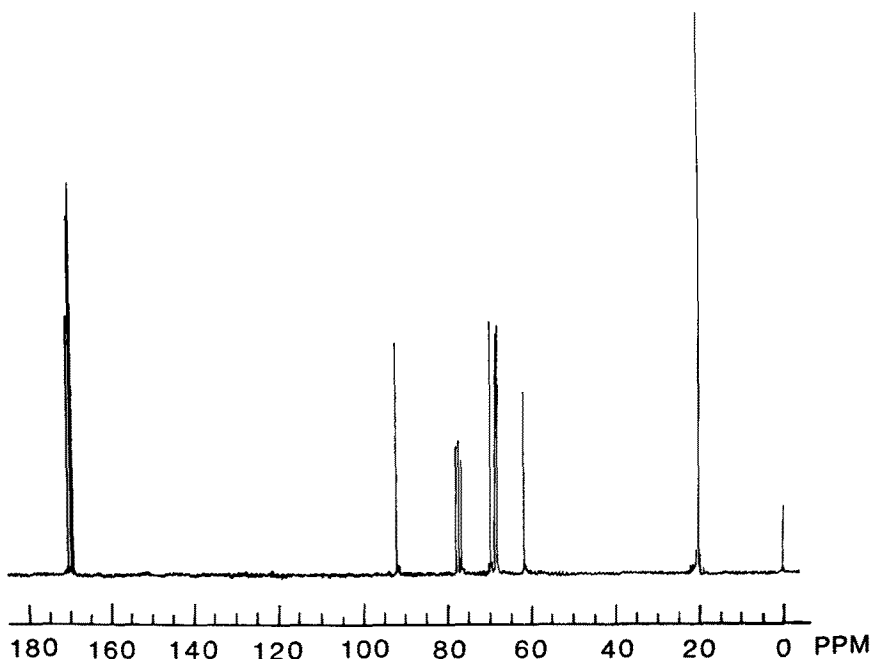


FIG. 3. C-13 NMR spectra of trehalose octaacetate (TOAC).

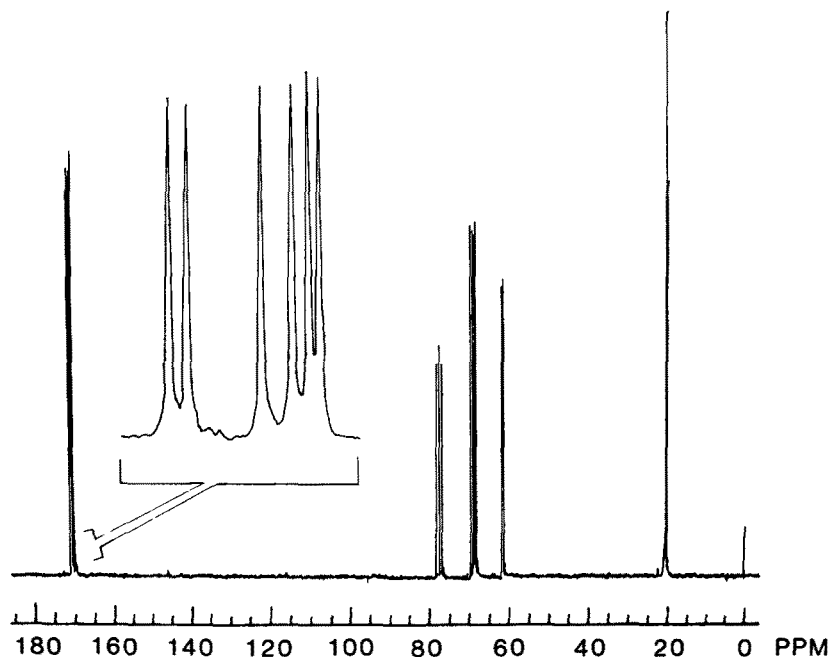


FIG. 4. C-13 NMR spectra of sorbitol hexaacetate (SOHA).

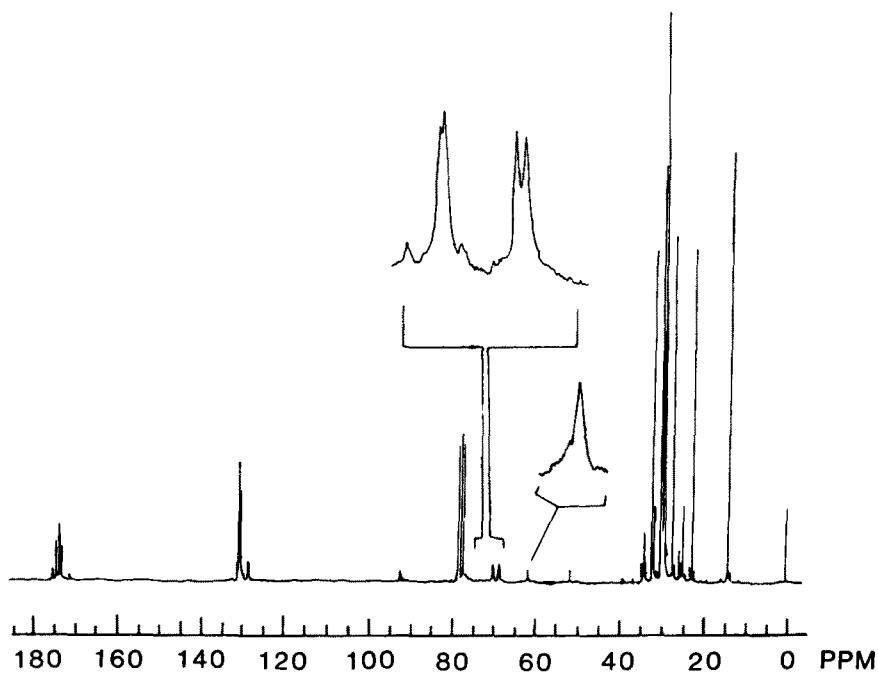


FIG. 5. C-13 NMR spectra of trehalose octaoleate.

spectra established the structure of TOAC and SOHA, respectively, and served as reference for the interpretation of the trehalose octaoleate and sorbitol hexaoleate spectra.

Figure 5 is the C-13 NMR for trehalose octaoleate. In the spectrum, signals of the end methyl carbons of the oleoyl group (14.1 ppm), CH₂ group of the oleoyl group (22.7–31.9 ppm), 1° carbon of glucose moiety (61.4 ppm),

carbon skeletons of trehalose (68.1–69.8 ppm), CDCl₃ (75.2–78.8 ppm), anomeric carbon of glucose (92.1 ppm), double bonded carbons of 18:1 (129.5–130.1 ppm) and carbonyl carbons of oleoyl acyl group of trehalose octaoleate (169.2–170.8 ppm) were observed. Figure 6 is the C-13 NMR of sorbitol hexaoleate. Signals observed include the end methyl carbons of oleoyl group (14.1 ppm), CH₂ group of oleoyl chain (22.7–31.9 ppm), 1° carbons of

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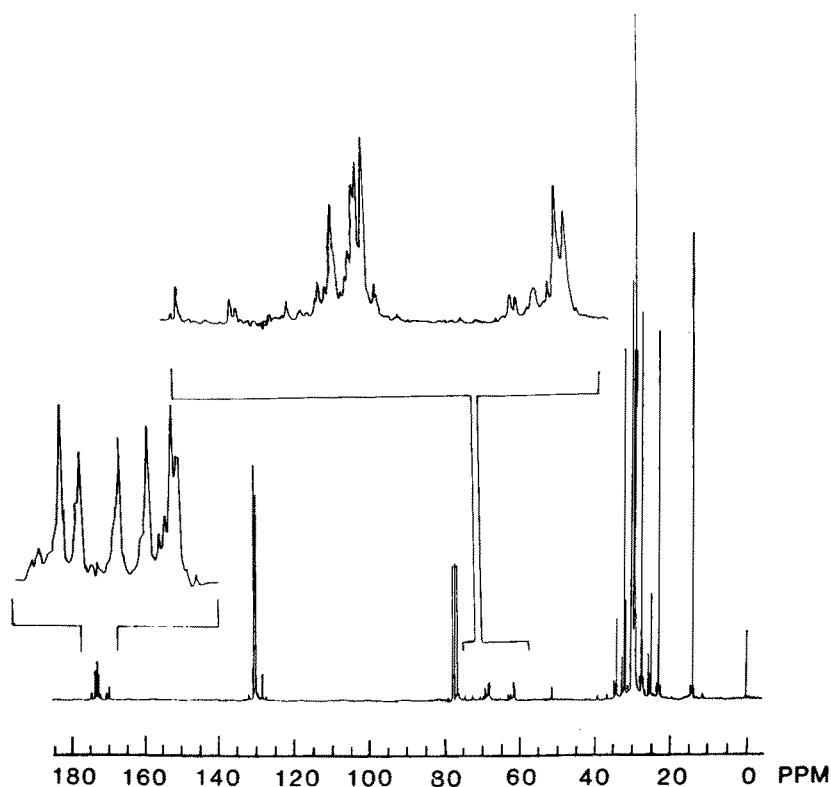


FIG. 6. C-13 NMR spectra of sorbitol hexaoleate.

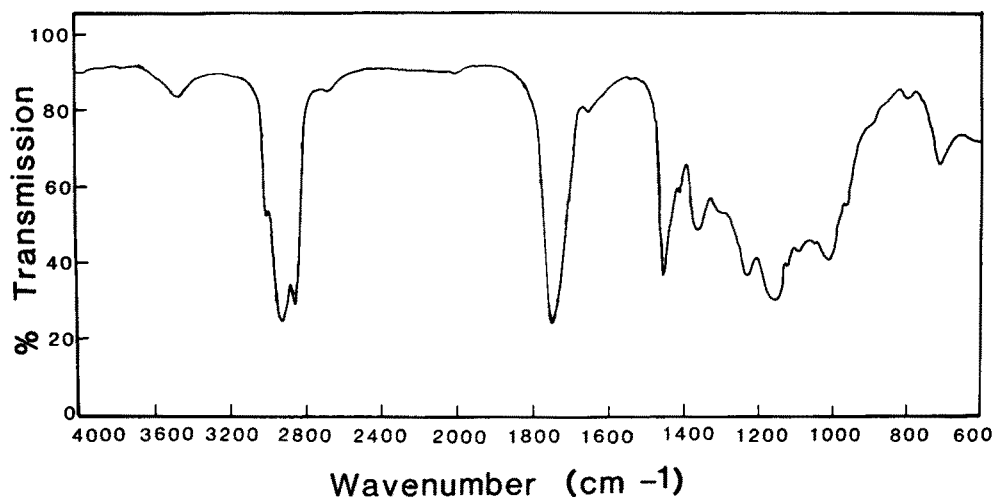


FIG. 7. Infrared spectrum of trehalose octaoleate.

sorbitol (61.3–61.6 ppm), CDCl_3 (75.2–78.8 ppm), other carbon skeletons of sorbitol (68.1–69.1 ppm), double bonded carbons 18:1 (127.8–129.9 ppm) and carbonyl carbons of oleoyl acyl group of sorbitol hexaoleate (172.1–173.0 ppm).

The observed C-13 NMR chemical shifts were consistent with the structures of trehalose octaoleate and sorbitol hexaoleate, respectively. The absence of the acetate

signal at 20.6 ppm in the C-13 NMR spectra of both polyesters indicated complete substitution of acetates with the oleoyl group. The degree of substitution (D.S.) (7) were 8.0 and 6.0 for trehalose and sorbitol polyesters, respectively. Methods for determining D.S. by hydroxyl values have been reported previously (5,14,16).

Further evidence of structure were obtained from IR spectral analyses. Figure 7 is the IR spectra of liquid

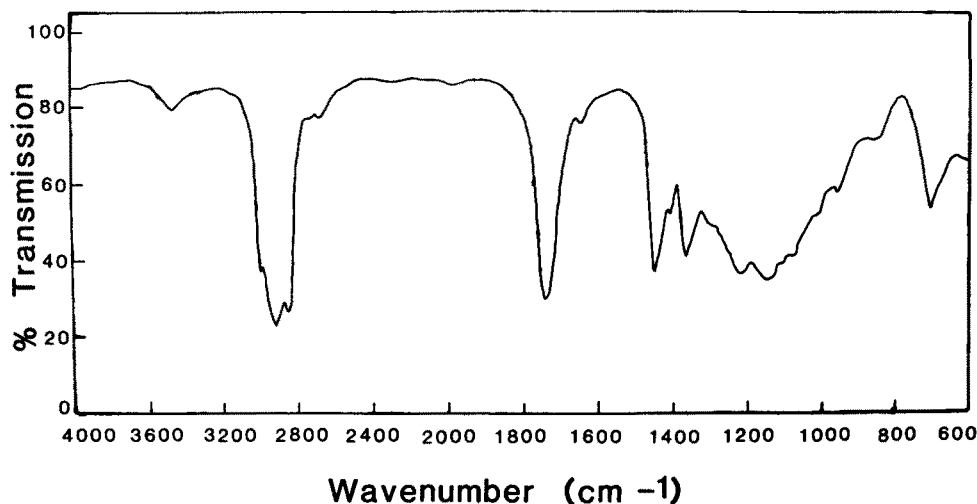


FIG. 8. Infrared spectrum of sorbitol hexaoleate.

trehalose octaoleate. Absorption bands were observed at 3450 cm^{-1} (overtone stretching vibrations), $3000\text{--}2850\text{ cm}^{-1}$ (C-H stretch in CH_3 and/or CH_2), 1750 cm^{-1} (ester carbonyl), 1455 cm^{-1} (C-H deformation in CH_3 and/or CH_2), 1365 cm^{-1} (CH_3 bending vibrations), $1230\text{--}1020\text{ cm}^{-1}$ (C-O-C vibrations), 900 cm^{-1} (pyranose ring vibrations), 800 cm^{-1} (alpha glycosidic bond, "tentative") and 720 cm^{-1} [$(\text{CH}_2)_4$ + in oleic]. In the IR spectrum of sorbitol hexaoleate (Fig. 8), absorption bands were observed at 3460 cm^{-1} (overtone stretching vibrations), $3000\text{--}2840\text{ cm}^{-1}$ (C-H stretch in CH_3 and/or CH_2), 1740 cm^{-1} (ester carbonyl), 1450 cm^{-1} (C-H deformation in CH_3 and/or CH_2), 1365 cm^{-1} (CH_3 bending vibrations), $1220\text{--}1000\text{ cm}^{-1}$ (C-O-C vibrations) and 710 cm^{-1} [$(\text{CH}_2)_4$ + in oleic]. The absence of the pyranose ring vibration (920 cm^{-1}) in sorbitol hexaoleate indicates that sorbitol was present as open chains and that cyclic sorbitol was unlikely.

The TLC, IR and C-13 NMR data suggest that the main products of interesterification reactions were trehalose octaoleate and sorbitol hexaoleate containing long chain fatty acids of oleic acid.

Properties. Table 2 presents physical properties of liquid trehalose octaoleate and sorbitol hexaoleate

compared to sucrose octaoleate and peanut oil. The color and consistency of the sorbitol and trehalose polyesters resemble that of sucrose polyester and peanut oil. With the exception of trehalose octaoleate, which has slightly greater values for refractive index, specific gravity and density, both sorbitol hexaoleate and sucrose octaoleate have R.I., specific gravity and density values approximating that of peanut oil. Sorbitol hexaoleate had a lower HLB value compared to equivalent HLB values for trehalose and sucrose octaoleate (HLB 3.5).

The apparent viscosities of trehalose octaoleate and sucrose octaoleate were similar, while the apparent viscosities of sorbitol hexaoleate was slightly less. Peanut oil had the lowest apparent viscosity. The apparent viscosities of sucrose and trehalose octaoleate were approximately twice the apparent viscosities of peanut oil. The apparent viscosities were apparently dependent on the number of fatty acid chains esterified to glycerol, sorbitol, sucrose or trehalose. Jandacek and Webb (18) reported that the apparent viscosity of homogenous sucrose octaesters increased with fatty acid chain length. The apparent viscosities of sucrose octaoleate and trehalose octaoleate, as well as their refractive indices and densities, were in close agreement with values reported for pure

TABLE 2

Physical Properties of Liquid Trehalose Octaoleate, Sorbitol Hexaoleate, Sucrose Octaoleate and Vegetable Oils

Oil	Mol wt	Refractive index (at 40°C)	Specific gravity (at 20/20°C)	Density, g/cm ³ (at 20/20°C)	Approximate HLB	Viscosity (cp) at 20°C	Color
Trehalose octaoleate	2458.0	1.4671	0.9389	0.9373	3.5	240	Golden yellow
Sorbitol hexaoleate	1769.0	1.4669	0.9186	0.9169	2.0	195	Pale yellow
Sucrose octaoleate ^a	2458.0	1.4669	0.9152	0.9136	3.5	235	Golden yellow
Peanut oil ^b	—	1.4670	0.9159	0.9142	N.D. ^c	140	Pale yellow

^a Sucrose octaoleate synthesized by the same procedure.

^b Peanut oil contains about 54% oleic acid as determined by gas liquid chromatography.

^c N.D., Not determined.

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TABLE 3

Solubility Properties of Trehalose Octaoleate, Sorbitol Hexaoleate and Sucrose Octaoleate

Solvent	Temperature	Trehalose octaoleate	Sorbitol hexaoleate	Sucrose octaoleate
Water	25°C	I	I	I
	75°C	I	I	I
Ethanol	25°C	I	I	I
	75°C	PS	PS	PS
Soybean oil	25°C	S	S	S
	75°C	S	S	S
Glycerin	25°C	PS	PS	PS
	75°C	S	S	S
Chloroform	25°C	S	S	S
	75°C	S	S	S
Hexane	25°C	S	S	S
	75°C	S	S	S

I, insoluble; PS, partially soluble or dispersible; S, soluble.

sucrose octaesters (18). Both trehalose and sorbitol polyesters exhibit physical properties that will allow potential substitution for sucrose polyester in food systems without adversely altering the functional properties of the food.

Table 3 illustrates the solubility properties of trehalose octaoleate, sorbitol hexaoleate and sucrose octaoleate at 25°C and 75°C. The synthesized polyesters were insoluble in water, but totally soluble in hexane, soybean oil and chloroform at 25°C and 75°C, respectively. The polyesters were partially soluble in glycerin and ethanol at 25°C and 75°C. The insolubility of the polyesters in water suggests a nonpolar nature. The solubility of trehalose, sucrose octaoleate and sorbitol hexaoleate in soybean oil suggests that the polyesters can be blended with vegetable oils during formulation of low or reduced calorie food systems without employing organic solvents.

The relative values of *in vitro* lipolysis of trehalose octaoleate, sorbitol hexaoleate and sucrose octaoleate were compared to triolein as control. The highly substituted polyesters (D.S. 6 and 8) for sorbitol hexaoleate, trehalose and sucrose octaoleate, respectively, were not hydrolyzed

by the three enzymes when compared to hydrolysis of triolein (D.S. 3) by the same enzymes. Apparently, both sorbitol and trehalose polyesters can compete with sucrose polyester as potential indigestible oils. Digestibility and absorbability of trehalose polyester *in vivo* remains to be investigated. Mattson *et al.* (19) reported that sorbitol and sucrose polyesters were neither digested nor absorbed in either rats and human subjects. The potential for using trehalose and sorbitol polyesters in food systems is highly promising and requires further investigation.

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