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Synthesis and Physical Properties of EOE and EEO, Triacylglycerols Containing Elaidic and Oleic Fatty Acids

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Abstract Symmetrical and non-symmetrical triacylglycerols (TAG) containing oleic (O; 9c-18:1) and elaidic (E; 9t-18:1) acids were required as part of a study relating the physical characteristics and functionality of *trans*-containing TAG with the mouth feel, taste characteristics and related characteristics desired by consumers in frying oils and pastries. To replace the *trans* isomers in frying oils—a significant part of frying oils prepared by partial hydrogenation of vegetable oils—without loss of the sensory properties desired by consumers, required the initiation of a study relating the structure of *trans*-containing TAG with such characteristics as melting range, drop points, and other crystalline properties. Elaidic acid was esterified to trielaidin (EEE), and the EEE partially converted (glycerol/*p*-toluenesulfonic acid) to a mixture containing ca. 40% DAG (the 1,3- and 1,2-isomers). The DAG fraction was separated by silica gel chromatography, the 1,3-dielaidylglycerol (1,3EE-DAG) isomer isolated (structural purity >99%) by crystallization from acetone and esterified with oleic acid (O) to yield EOE. The 1(3)O-MAG was purchased commercially and esterified with E acid to prepare OEE. Both syntheses yielded multi-gram quantities of EOE and EEO, in 80–85% yields, and with structural purities >99%. Thus, by careful selection of the thermodynamically more-stable MAG or DAG precursors, the symmetrical EOE and non-symmetrical EEO isomers could be readily synthesized, and their drop point and melting point values determined.

Keywords Triacylglycerols · ΔH · Melting · Synthesis · Oleic · Elaidic

Abbreviations

TAG	Triacylglycerol
DAG	Diacylglycerol
MAG	Monoacylglycerol
P	Palmitate; 16:0
E	Elaidate; *trans*-9-18:1
O	Oleate; *cis*-9-18:1
L	Linoleate; *cis*-9, *cis*-12-18:2
Ln	Linolenate; *cis*-9, *cis*-12, *cis*-15-18:2
Ag-HPLC	Silver ion high performance liquid chromatography
NMR	Nuclear magnetic resonance
DSC	Differential scanning calorimetry

Introduction

The amounts and types of triacylglycerols (TAGs) in the oil phase of margarines and spreads are considered responsible for such properties as spreadability, resistance to water/oil loss and melting at body temperature [1], with spreadability dependent on the presence of both lower- and higher-melting TAGs. Spreads which contain ''high'' (>50%) levels of TAGs with ''lower'' (<10 °C) melting values tend to be too soft, while those with higher-melting (20–30 °C) TAGs tend to be too hard for good spreadability directly out of the refrigerator.

The 1- and 3- positions on the glycerol backbone of the MAG, DAG and TAG molecules are assumed to be equivalent.

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Hydrogenated vegetable oils, containing lower levels of unsaturated fats and elevated levels of *trans* and saturated fats, have traditionally been used to prepare base feedstocks with the melting point ranges required [2, 3] for structure and spreadability at refrigerator temperatures (i.e. 50 °F, 10 °C). During the catalytic hydrogenation of edible oils, major TAG such as trilinolein and trilinolenin are converted to elaidic acid-containing TAGs such as EEO and EOE, higher-melting TAG which contribute to the desired functional properties (spreadability) of the resultant spreads.

Health, consumer-driven and recent FDA labeling mandates have stimulated research aimed at reducing the levels of *trans* and long-chain saturated fatty acids in shortening and frying oils by such procedures as interesterification, the blending of tropical and liquid vegetable oils, low-temperature fractionation and, more recently, by development of structurally modified oils by transgenic or conventional plant breeding methods [4]. After interesterification, as much as 50% of the symmetrical TAGs of structure ABA are converted to nonsymmetrical TAGs of structure AAB, a change which, when coupled with lower levels of saturated fatty acids (FAs) and the elimination of *trans* isomers, could account for the observed changes in melting point ranges, solid fat content profiles and other parameters contributing to the poor ''mouth feel'' and loss of flavor observed in commercially-available ''low-*trans*'' (<5%) TAG formulations [1]. By careful selection of mono- or diacylglycerol substrates, both the symmetrical (EOE) and nonsymmetrical (EEO) TAG isomers can be chemically synthesized [5, 6], their purities readily determined by silver-ion HPLC (Ag-HPLC), and their drop point [7] and heats of fusion values measured. These values, currently not available, will be utilized as part of a study correlating the sensory properties of TAG formulations with TAG structures (ABA vs. AAB, etc.) and TAG FA (saturated vs. *cis* vs. *trans*) compositions.

Experimental

Materials

Oleic (O) and elaidic (E) acids, and 1-monoolein were purchased from Nu-Chek-Prep (Elysian, MN, USA). Sodium methoxide was obtained from Harshaw Chem. Co. (Cleveland, OH, USA), *N*,*N*′-dicyclohexylcarbodiimide, *p*-toluenesulfonic acid and triolein from Sigma-Aldrich (St. Louis, MO, USA), 4-dimethylaminopyridine from Eastman Fine Chemicals (Rochester, NY, USA), Silica gel (60/200) and Florisil© (100/200) from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA). All solvents were either HPLC Grade (acetone, acetonitrile, methanol) or ACS Grade [benzene, carbon tetrachloride, ethyl ether (EE), petroleum ether (PE)], and were used as received.

Methods

1. *Ag-HPLC*: A Spectra-Physics P2000 solvent delivery system (Spectra-Physics Analytical, San Jose, CA, USA/now Thermo-Finnigan), a Rheodyne 7125 injector (Rheodyne, Inc., Cotati, CA, USA) with a 20 μL injection loop, and an ISCO V4 Absorbance Detector (ISCO, Inc., Lincoln, Nebraska, USA) at a wavelength of 206 nm was used. (A representative Ag-HPLC chromatogram of a TAG fraction containing both the EEO and EOE isomers is shown in Fig. 1.) The ChromSpher© Lipids columns (4.6 mm I.D. × 250 mm stainless steel; 5 micron particle size; silver ion impregnated) were purchased from Varian-Chrompack Int., Middelburg, The Netherlands, and used as received.
2. *Thin-Layer Chromatography (TLC)*: Formation of the mono- or dielaidoyl and oleoylglycerol intermediates and the final TAGs were followed by TLC [3]. Samples (5–10 mg) were dissolved in 1.0 mL hexane and applied (ca.10 μL) to 1 in. × 3 in. silica gel TLC [K6] plates (Whatman, Inc., Clifton, NJ, USA). Eluting solvent: 80:20—Hexane: Ethyl Ether (v/v; [8]); visualization by I_2 vapor or by spraying with 10% $CuSO_4$ in 8% H_3PO_4 solution/ heating to120 °C (hot plate).
3. *Gas Chromatography*: Chemical purities of the 1(3)-MAG or 1,3-DAG precursors and of the synthesized TAGs were determined by gas chromatography after conversion (5% HCl in methanol) to FAMEs [9]. A Varian 3400 Gas Chromatograph (GC; Varian Instruments, Palo Alto, CA, USA) equipped with a 100 m × 0.32 mm SP2380 (Supelco, Inc., Bellefonte, PA, USA) capillary column, flame ionization detector (FID) and He as carrier gas (operating conditions: injector, 240 °C; split ratio, 100:1; oven temperature programmed from 155 to 220 °C at 3 °C/min with an initial hold of 15 min; detector, 280 °C) was utilized.
4. *Acylglycerol Structures*: The structural purities of the isolated or purchased MAG and DAG starting materials (after conversion to di- or mono-acetate(s), respectively) and of the synthesized TAGs were determined by Ag-HPLC [10, 11].
5. *Drop Point*: Drop points were determined by AOCS Official Method Cc 18-80. The values listed in Table 1 represent the means of duplicate determinations.

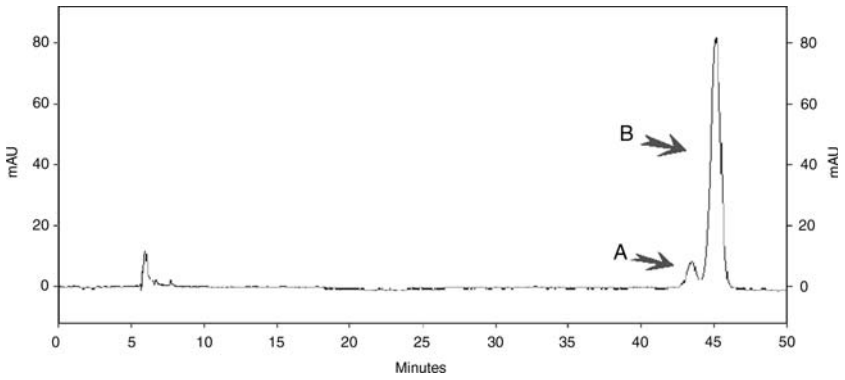

Fig. 1 Analysis of EOE and EEO triacylglycerol mixture by dual-column Ag-HPLC. Sample size: 10 μg. Flow rate: 1.0 mL/min 0.5% ACN in hexane. UV detection at 206 nm. Peaks: **A** = EOE, **B** = EEO

Table 1 Physical and chemical properties of MAG, DAG and TAG

Precursor/product	Chem. (%)	Struct. (%)	Onset of melt (°C)	Melting points (°C)		Heats of fusion (ΔH)	
				Mettler	DSC	cal/g	J/g
EEE	99.9	99.9	39.4	43.5	43.4	36.1	151.1
1,3EE-DAG	99.9	>99	–	–	–	–	
1(3)E-MAG	99.9	98	–	12.5	–	–	
EOE	99.7	>98	11.0	15.9	14.9	22.5	94.2
EEO	99.9	99.6	4.7	14.4	12.2	26.8	112.2
OOO	99.9	99.9	2.4	6.1	6.1	25.6	107.2

6. *Differential Scanning Calorimetry (DSC)*: Heats of fusion (ΔH) and melting points were determined according to AOCS Official Method Cj 1-94.

Syntheses

See Table 1 for drop point and DSC values, as well as chemical and geometrical purities of the MAG and DAG precursors and of the TAG isomers.

1. *Preparation of EEE*: Elaidic acid (66.8 g, 0.25 mol) was combined with glycerol (7.3 g, 0.08 mol) and 0.7 g *p*-toluenesulfonic acid in a 250 mL, 3-necked round bottom (r.b.) flask fitted with an argon inlet and thermometer. The contents were stirred magnetically and heated by oil bath to 115 °C. Water which condensed on the flask walls during the reaction was removed by use of a heat gun. Reaction progress was measured by TLC. After 5 h the reaction mixture was cooled, the solid residue dissolved in 150 mL petroleum ether and eluted through a glass column packed with 500 g Florisil. The EEE was isolated by elution with 1,500 mL 90/10 PE/EE, and the solvents removed to yield 59.2 g (85% yield) of product.
2. *Preparation of 1,3-Dielaidin*: EEE (10.0 g, 0.01 mol) and glycerol (1.0 g, 0.01 mol) were combined in a 25 mL r.b. flask equipped with reflux condenser and argon inlet. Sodium methoxide (0.1 g) was added and the mixture was heated to 115 °C with stirred magnetically. DAG formation was followed by TLC, and 0.1 g portions of glycerol and sodium methoxide added after 7 h. After 24 h, the mixture was cooled to room temperature, dissolved in 20 mL benzene and eluted through a 2.54-cm glass column packed with 50 g silica gel. The TAGs (4.8 g) were eluted with 400 mL benzene, the DAGs (5.4 g; ca. 50% yield) with 90:10 benzene: EE, and the MAGs and FAs (1.0 g) with 100% EE. (Toluene may be substituted for benzene.) After solvent removal by rotary evaporation, the DAG fraction was dissolved in 500 mL acetone, and the solution was slowly cooled (crystal formation noted) to/stored overnight at 5 °C. The crystals (4.8 g) were removed by filtration through a cold Buchner funnel, and dried in a vacuum oven at 40 °C. The DAG structural purity, determined by conversion to the mono-acetate/Ag-HPLC [Method (4), above], was found to be >99% 1,3-dielaidylglycerol.
3. *Preparation of EOE*: EOE was prepared according to the method of Kodali et al. [12]. 1,3-dielaidylglycerol (2.2 g, 3.5×10^{-3} mol) in 80 mL carbon tetrachloride was transferred to a heat-dried, 3-necked, 500 mL r.b. flask equipped with a mechanical stirrer, thermometer and argon inlet. Oleic acid (1.1 g, 3.5×10^{-3} mol, 10%

excess) in 10 mL of carbon tetrachloride was added via syringe. 4-Dimethylaminopyridine (0.5 g in 5 mL CCl_4) was added in one batch and 1.34 g *N*,*N*′-dicyclohexylcarbodiimide dropwise over a 30-min period. The reaction was stirred at 28 °C for 2 h (precipitate noted). The precipitate was removed by vacuum filtration and the solvents by rotary evaporator. The EOE residue (4.2 g) was purified by elution (5% EE in PE) through a 2.54 cm diameter glass column packed with 30 g silica gel to yield 2.7 g (86% yield) of product. Analysis of the EOE by Ag-HPLC and, after conversion to FAME, by GC yielded structural and chemical purities of 98 and >98%, respectively. (see ''Materials'' and ''Methods'' above and Table 1).

4. *Preparation of OEE*: Commercially-available 1(3)-monoolein (3.0 g, 8.4×10^{-3} mol) was esterified with elaidic acid (5.4 g, 1.9×10^{-2} mol) to prepare OEE (6.5 g; 87% yield) as described in ''Preparation of EOE'', above.

Results and Discussion

Multi-gram (4–6 g) quantities of highly-pure symmetrical or non-symmetrical TAG could be prepared by esterification of the appropriate MAG or DAG precursors (also commercially available), as described above. Silica gel chromatography was utilized to isolate the DAG and MAG fractions, with the thermodynamically more stable 1-MAGs and 1,3-DAGs predominating (thermodynamic ratios of 80/20 1(3)- vs. 2-MAGs and 1,3- vs. 1,2-DAGs), and could be readily isolated by low-temperature crystallization/filtration from acetone with structural purities exceeding 98%. (The percentage of MAG or DAG present in the final mixture can be adjusted by the amount of ethylene glycol added, and the thermodynamically more-stable MAG or DAG isomers increased by addition of 1% of an acid such as HCl [8].) The thermodynamically less-stable 1,2-DAGs, present at only 20–25% in the original DAG fraction, could also be utilized for preparation of non-symmetrical TAG isomers, but only mg quantities of highly-pure (>99%) samples could be isolated by Ag-HPLC [13]. The commercially-available (and less-expensive) 1O-MAG was therefore esterified with elaidic acid to yield the non-symmetrical OEE isomer. This flexible synthetic approach [5–7, 10, 12] offers a viable alternative to enzymatic synthesis [14–20] for preparation of TAGs present in chemically-interesterified commercial spreads and formulations.

Ag-HPLC [9–11, 21] is a rapid and reproducible method to analyze the isolated symmetrical and non-symmetrical TAGs, with a detection limit of <0.5%. A single column was sufficient to achieve the desired separation(s), but two ChromSpher© Lipids columns connected in series resulted in improved sample capacity and improved peak-to-peak resolution. Ag-HPLC is easy to use and yields reproducible results within 25–50 min. UV detection at 206 nm was used to determine structural purities of the EEO and EOE isomers and, as noted previously [9], the results were comparable to those achieved by lipolysis/gas chromatography.

The chemical and physical properties of the structured TAGs and their precursors are shown in Table 1. Included are Mettler dropping points (MDP) and melting points/point ranges obtained by differential scanning calorimetry. Heats of fusion, also determined by DSC, are also included. The difference (by DSC) between the onset of melt and the melting points indicates that EEE, EOE, and EEO TAG are sharply-melting materials, since their melting points are about 4–7 °C above the onset of melt. As would be expected, EEE shows a higher heat of fusion than EOE or EEO. On the other hand, EEO and EOE have heats of fusion similar to OOO. By comparison, completely saturated TAGs such as tristearin and tripalmitin show heats of fusion of 45.0 and 39.6 cal/g, respectively [22]. Thus, EEE and SSS have similar heats of fusion, and the introduction of oleic acid into the molecule lowers ΔH by approximately a factor of 2 (i.e. from 36.1 cal/g for EEE to 22.5 and 26.8 cal/g for EOE and EEO, respectively).

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