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Short communication

Analysis of triacylglycerol positional isomers in food products as brominated derivatives by high-performance liquid chromatography coupled with a flame ionization detection

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Abstract

Reversed-phase HPLC resolution and HPLC–flame ionization detection quantitation of model triacylglycerol positional isomer pairs (important in the study of food formulation lipids) after facile conversion to brominated derivatives is reported. The positional isomers in the triacylglycerol pairs were at least 98% resolved from each other during reversed-phase HPLC. Triacylglycerol quantitation obtained by HPLC–flame ionization detector was checked against standard positional isomer pairs known by mass. The flame ionization detection area percent gave absolute error range of 0.3–1.6% per triacylglycerol.

Keywords: Positional isomers; Derivatization, LC; Triacylglycerols; Fatty acids

1. Introduction

Knowledge of triacylglycerol (TAG) structure or location of particular fatty acids (FAs) on the glycerol primary and secondary carbons is becoming increasingly important in lipid chemistry for metabolic, biosynthetic chemical reactions and food formulation products such as cocoa butter, stereospecific knowledge of particular FA location on specific glycerol *sn*-1, -2, and -3 carbons is required. However, for autoxidation and photoxidation mechanisms of TAGs in regard to oxidative stability only the regiospecific knowledge of particular FA location on glycerol primary (*sn*-1 and -3) and secondary (*sn*-2) carbons is required. There is no need to distinguish between what FA are located on *sn*-1 compared to *sn*-3 carbons.

Oil oxidative stability has been correlated in part with TAG structure. TAGs with different FAs in specified glycerol carbon locations have been synthesized and subjected to oxidative stability experiments [1–3]. These oxidation experiments have shown that the FA location affects TAG oxidative stability. Also, oxidative stability of natural TAG mixtures obtained from various vegetable oils [4–6] as well as TAG mixtures from synthetic oil products [7] have been studied. Statistically significant differences in oxidative stabilities were correlated with distribution of particular FA on TAG glycerol primary and secondary carbons.

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Analyses of TAG structure have involved enzymatic methods, nuclear magnetic resonance spectrometry, mass spectrometry and resolution of triacylglycerol derivatives on chromatographic columns [8–10]. In general, these analytical techniques for regiospecific analysis of TAG structures are labor intensive and time consuming.

Recently, more expedient techniques, which involved direct resolution of TAG positional isomers by HPLC have been described. Jeffery resolved underivatized TAG positional isomers in cocoa butter, a cocoa butter substitute, palm oil and randomized palm oil via argentation HPLC coupled with flame ionization detection (FID) [11]. Adlof resolved a series of model TAG isomers by argentation HPLC coupled with ultraviolet detection [12]. Other workers prepared simple derivatives of TAG for resolution by reversed-phase and normal-phase HPLC. Hammond resolved epoxidized model TAGs, palm oil, beef and lard tallows via polar-phase HPLC on a silica column coupled with FID [13]. Halogenated monounsaturated disaturated TAG positional isomers in highly saturated fats were resolved by reversed-phase HPLC [14,15]. Epoxidized monounsaturated disaturated TAG pairs in vegetable oils were resolved by reversed-phase HPLC coupled with light-scattering detection [16].

New work is presented here for RP-HPLC resolution and FID quantitation of model TAG positional isomers after facile conversion to brominated derivatives followed by reversed-phase HPLC coupled with FID. All the positional isomers were at least 98% to resolved. TAG quantitation was checked against standard positional isomer mixtures known by mass. A qualitative and quantitative HPLC method for regiospecific analysis of selected TAG positional isomers was thus developed.

2. Experimental[1]

2.1. Materials

The following TAGs were obtained from Sigma (St. Louis, MO. USA): 1,2-dipalmitoyl-3-oleoyl (PPO); 1,3-dipalmitoyl-2-oleoyl (POP); 1,2-distearoyl-3-oleoyl (SSO); 1,3-distearoyl-2-oleoyl (SOS); 1,3-dipalmitoyl-2-linoleoyl (PLP) and 1,2-dilinoleoyl-3-oleoyl (LLO) glycerols. The following TAGs were prepared by the procedure of Awl et al. [17] from dihydroxacetone and benzylidene precursors and acylation by the appropriate fatty acid: 1,2-dipalmitoyl-3-linoleoyl (PPL); 1,3-dilinoleoyl-2-oleoyl (LOL); 1,3-dilinoleoyl-2-palmitoyl (LPL); 1,2-dilinoleoyl-3-palmitioyl (PLL); 1,3-distearoyl-2-linoleoyl (SLS); 1,2-distearoyl-3-linoleoyl (SSL); 1(3)-linoleoyl-2-stearoyl-3(1)-palmitioyl (LSP); 1,(3)-stearoyl-2-linoleoyl-1(3)-palmitioyl (SLP); 1,3-linolenoyl-2-linoleoyl (LnLLn) and 1,2-linolenoyl-3-linoleoy l(LnLnL) glycerols. Acetonitrile (ACN) was purchased from EM Science (Gibbstown, NJ, USA) and methylene chloride (DCM) was purchased from Fisher Chemicals (Fair Lawn, NJ, USA). Bromine was purchased from Aldrich (Milwaukee, WI, USA).

2.2. Preparation of the TAG derivative

The brominated derivatives of the TAG pairs were prepared by addition of bromine to the TAG FA double bonds as follows. Briefly, a TAG pair prepared by mass to equal 100 mg was exposed to 100 μl of bromine in a reaction vial sealed with an aluminum-lined cap. A 4-ml volume of DCM was added and the contents allowed to stand a room temperature for 30 min. Then the solution was ready for injection into the HPLC system. *Caution:* avoid exposure to bromine, which is a toxic substance.

2.3. HPLC of TAG derivatives

The RP-HPLC–FID conditions are: the pump used was a Thermo Separation Products SP 8800 system. A step gradient solvent program was used as follows: initial conditions, acetonitrile–dichloromethane (72:28, v/v) isocratic to 40.0 min then to ACN–DCM (65:35) by 45 min then isocratic to 55.0 min then ACN–DCM (60:40) by 60 min then isocratic to 70 min; then ACN–DCM (55:45) by 80 min then isocratic to 95 min with return to initial conditions ACM–DCM (72:28) by 99 min. The flow-rate was 0.9 ml/min. The columns used were two Inertsil

[1]Names are necessary to report factually on available data; however, the US Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

ODS-80A (GL Sciences, Keystone Scientific, Bellefonte Park, PA, USA), 25 cm×4.6 mm, 5 μm in series. 10 μl of 25 μg/μl sample in dichloromethane were injected. The HPLC detector was a HPLC–FID system (Finnigan, Austin, TX, USA), operated with block temperature 130°C; detector gas, 140 ml/min. hydrogen; cleaning flame, 275 ml/min hydrogen; 175 ml/min oxygen; and compressed air, 0.4 $ft.^3$/min (1 ft.=30.48 cm). The FID response was processed by a Star Chromatography Workstation with version 4.0 software, (Varian, Walnut Creek, CA, USA). Identification and quantitation of the HPLC–FID response was based on known gravimetric mixtures of the positional isomer TAG pairs.

3. Results and discussion

The triacylglycerol pairs of positional isomers prepared above were resolved as brominated derivatives by RP-HPLC and their retention and resolution data are listed in Table 1. The retention data showed that retention times for each of the positional TAG isomers had the AAB (see Table 1) isomer eluting earlier than the ABA isomer, where A was a saturated fatty acid and B an unsaturated fatty acid. Apparently in this study, for the brominated derivatives, the AAB isomer was more polar than the ABA isomer and eluted earlier on the reversed-phase HPLC column. The resolution of each TAG pair of AAB and ABA isomers, while not baseline was a least 98%.

The good quality resolution, along with the excellent quantitation without response factor ability of the HPLC flame ionization detector, gave excellent quantitation for each isomer of the positional TAG pairs. These quantitative data are given in Table 1. The good quantitation is shown by the absolute error for each TAG isomer known by mass percent vs. experimental area percent obtained by FID. The absolute errors ranged from 0.3 to 1.6%.

We found that due to the di-isotopic nature of bromine, brominated derivatives are not suitable for TAG composition analysis by RP-HPLC–mass spectrometry. However, the epoxide derivatives would be expected to be suitable for RP-HPLC coupled to mass spectrometry with an atmospheric pressure chemical ionization source (APCI-MS). Derivatization of TAG positional isomers is usually necessary for their identification and quantitation by mass spectrometry.

The TAG brominated derivatives proved useful for HPLC–FID resolution of the model TAG pairs: LLnLn/LnLLn, LLO/LOL, PLL/LPL, PPL/PLP, SPL/SLP, PPO/POP, SSL/SLS and SSO/SOS. Resolution of TAG position isomers like Sat-Sat-Unsat

Table 1
Quantitative analysis of dereivitized triacylglycerol (TAG) pairs by reversed-phase HPLC–FID[a]

Positional TAG pair	TAG pair[b] identification AAB/ABA	Retention[c] time (Min) AAB/ABA	Mass percent AAB/ABA	Area[d] percent AAB/ABA	Absolute[e] error percent AAB/ABA	R_s[f]
Dipalmitoyloleoyl	PPO/POP	67.3/68.7	58.0/42.0	57.4/42.6	0.6/0.6	1.20
Distearoyloleoyl	SSO/SOS	82.4/83.6	30.0/70.0	31.0/69.0	1.0/1.0	1.00
Dipalmitoyllinoleoyl	PPL/PLP	59.3/60.8	42.4/57.6	41.2/58.8	1.2/1.2	1.00
Dilinoleoyloleoyl	LLO/LOL	49.0/47.7	53.9/46.1	52.3/47.7	1.6/1.6	1.00
Dilinolenoyllinoleoyl	LnLnL/LnLLn	13.5/13.1	55.0/45.0	54.0/46.0	1.0/1.0	1.13
Dilinoleoylpalmitoyl	LLP/LPL	52.0/51.5	54.0/46.0	55.5/44.5	1.5/1.5	1.00
Stearoylpalmitoyllinoleoyl	SPL/SLP	63.3/64.0	54.0/46.0	54.7/45.3	0.7/0.7	1.20
Distearoyllinoleoyl	SSL/SLS	71.3/72.7	66.6/33.3	66.3/33.7	0.3/0.4	1.18

[a] See Experimental section for derivative preparation and reversed-phase HPLC–FID procedure.
[b] A=fatty acid one/B=other fatty acid. L, O, Ln, S, P=linoleic, oleic, linolenic, stearic, palmitic fatty acids, respectively.
[c] Absolute retention time precision for triplicate analyses=±0.50 min.
[d] Area percent (%) precision for triplocate analyses=±0.2%.
[e] Absolute error=difference between known %(w/w) and experimental area% per TAG.
[f] R_s=resolution between individual TAG of the TAG pair on the HPLC chromatogram. R_s=1.18×difference between TAG retention times/sum of peak area width of each TAG at 1/2 peak height. R_s=1.0=98% resolution between the TAGs of the TAG pair. R_s>1.5=100% or baseline resolution between the TAG of the TAG pair. Ref. [18].

from Sat-Unsat-Sat is important. For example, this resolution is important for margarine base stock research, since some of the desired melting property is related to the amount of the desirable Sat-Unsat-Sat TAG present.

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