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Application News

Triacylglycerols in Tropical Oil / LCMS-9030

A LC-ESI-Q-TOF Method for Identification and Relative Composition Analysis of Triacylglycerols in Tropical Oil - (1) Coconut Oil

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User Benefits

- A fast and direct LC-ESI-Q-TOF method was established for identification of TAGs in coconut oil and determination of the relative composition of various TAGs with different fatty acids.
- A data analysis procedure was set up for unambiguous identification of individual TAG molecule from accurate mass MS and MS/MS spectra using the LabSolutions Insight Explore s/w.

■ Introduction

Tropical oils, namely coconut oil and palm oil etc., are widely used in tropical countries for cooking and other purposes for long history. Triacylglycerols (TAGs, Fig. 1) are the major form of dietary lipid in edible oils including tropical oils. Characterization of TAGs in oils from types to composition is very essential for the evaluation of nutritional values and health impact etc. Traditionally, TAG composition is determined by HPLC-RID method, but identification relies on indirectly GC/MS method via hydrolyzing first to fatty acids, following by derivation and GC/MS analysis. In recent years, direct analysis methods by LC-MS/MS [1-3] and SFC-MS/MS [4] have been used for quick identification of TAGs. In this application note, we introduce a newly-established LC-ESI-Q-TOF method for quick identification of TAGs in coconut oil and determination of their relative composition simultaneously.

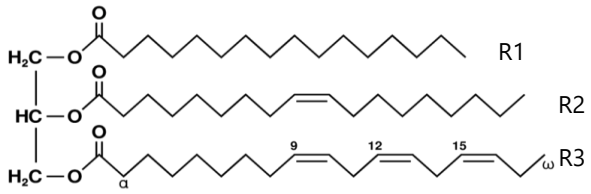

Figure 1 Structure of triacylglycerol with saturated fatty acid (SFA, R1), monounsaturated fatty acid (MUFA, R2) and polyunsaturated fatty acid (PUFA, R3).

■ Experimental

Reagents and Chemicals

Acetonitrile (LC/MS grade), 2-propernol (LC/MS grade), chloroform (LC grade) and acetone (LC grade) were obtained from commercial suppliers. Ammonium formate (>/=99%) of LC/MS grade was used as additives in the mobile phase prepared from Milli-Q water.

Samples and sample preparation

Three coconut oil samples (C1, C2 and C3) from different brands produced in Philippines were purchased from local market. Stock solution of 6.0 mg/mL was prepared by weighing 40 mg of coconut oil and dissolving in chloroform-acetone mixed solvent (v/v=1:1). The stock solution was further diluted with the mobile phase B to obtain 0.60 mg/mL (or 600 ppm) for analysis.

Table 1 Analytical conditions on LCMS-9030

LC Conditions	
Column	Shim-pack Velox™ C18 (2.1 X 100 mm, 2.7 µm), P/N: 227-32009-03
Flow Rate	0.4 mL/min
Mobile Phase	A: 20 mM Ammonium formate in water B: 2-Propanol - ACN = 80:20 (v/v)
Elution mode	Gradient elution, 16 mins
Oven Temp.	45°C
Injection Vol.	5 µL
Interface Conditions (LCMS-9030)	
Interface	HESI 4.0 kV
Interface Temp.	150°C
DL Temp.	250°C
Heat Block Temp.	400°C
Nebulizing Gas	3 L/min
Heating Gas Flow	10 L/min
Drying Gas Flow	10 L/min
Data acquisition (Q-TOF)	
MS mode (TOF)	Positive, 700-1200, 0.05 sec, ID on
MS/MS (Q-TOF)	Up to 31 precursors, 50-1100, CE: -40V spread (+/-)17V
Dwell time	0.02 sec / event
Loop time	0.67 sec / data point

LC-Q-TOF conditions

The analytical conditions on LCMS-9030 are shown in Table 1. Ionization of TAGs by ESI with adding ammonium formate in the mobile phase was adopted [1]. Under this condition, TAG molecules form ammonium adduct ion $[TAG+NH_4]^+$ with high detection sensitivity. Fragmentation of selected precursor ions are performed by Q-TOF and MS/MS spectra were obtained in a high acquisition speed (0.02 sec per spectrum). A spread collision energy of -40 (+/-) 17 was applied, which led to efficient fragmentation for all TAGs.

■ Results and Discussion

TAGs composition in coconut oil

It is well known that coconut oil has a high content of TAGs with saturated fatty acids (SFAs) and high content of so-called medium chain fatty acids (MCFAs). The current LC-ESI-Q-TOF method allows to differentiate directly the TAGs with different types of fatty acids, i.e.,

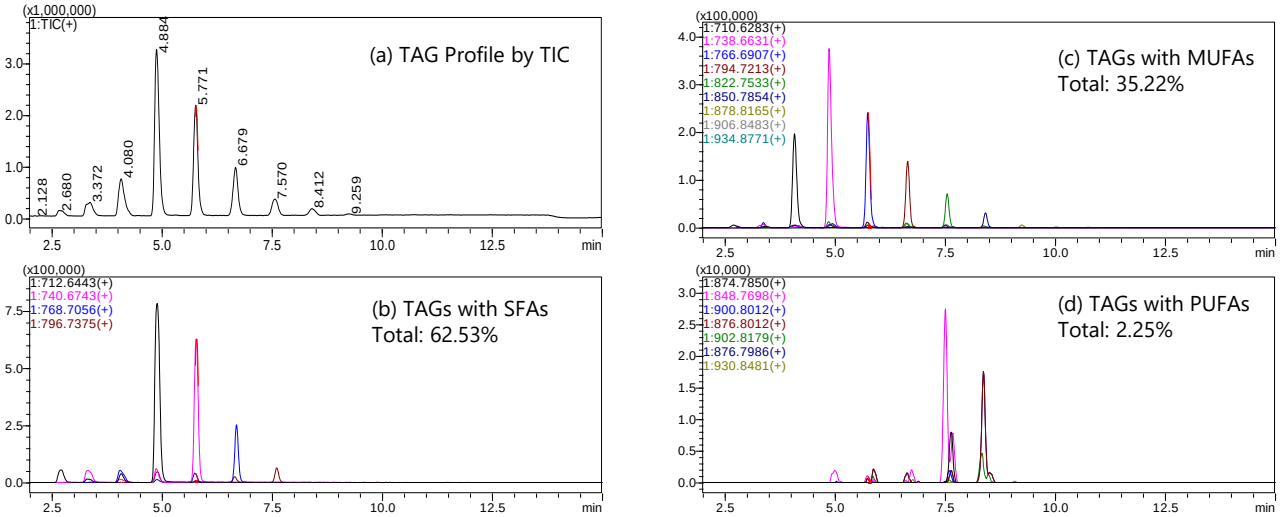

Figure 2 TAG profiles of coconut oil (C1) by LC-Q-TOF. (a) Total TAGs by TIC; (b) TAGs with saturated fatty acids (SFAs); (c) TAGs with monounsaturated fatty acids (MUFAs) and (d) TAGs with polyunsaturated fatty acids (PUFAs)

Table 2 Results of TAG identification and relative compositions (Peak Area%)

P. Code	Ret. Time	m/z	Formula	CN	ECN	DB	TAGs	Area%	Area%
T-712-0	4.89	712.644	C43 H82 O6	40	40	0	PLaLa, MMLa, SLaC(w)	31.90	
T-740-0	5.78	740.674	C45 H86 O6	42	42	0	SLaLa, PMLa	20.81	62.53
T-768-0	6.70	768.706	C47 H90 O6	44	44	0	SMLa, PPLa	7.79	
T-796-0	7.61	796.738	C49 H94 O6	46	46	0	SPLa, SSC, PPM	2.03	
T-710-1	4.08	710.628	C43 H80 O6	40	38	1	OLaC, OMCy(w)	7.04	
T-738-1	4.87	738.663	C45 H84 O6	42	40	1	OLaLa	12.57	
T-766-1	5.75	766.691	C47 H88 O6	44	42	1	OMLa, OPC(w)	7.73	
T-794-1	6.65	794.721	C49 H92 O6	46	44	1	OPLa, OMM	4.47	
T-822-1	7.55	822.753	C51 H96 O6	48	46	1	OPM, OSLa(w)	2.23	35.22
T-850-1	8.42	850.785	C53 H100 O6	50	48	1	OPP, OSM(w)	0.94	
T-878-1	9.25	878.817	C55 H104 O6	52	50	1	SOP	0.17	
T-906-1	10.03	906.848	C57 H108 O6	54	52	1	OSS, OAP	0.05	
T-934-1	10.76	934.877	C59 H112 O6	56	54	1	OBP, OAS	0.02	
T-874-3	7.64	874.785	C55 H100 O6	52	46	3	OLP	0.26	
T-848-2	7.51	848.770	C53 H98 O6	50	46	2	SLP	0.86	
T-848-2	7.68	848.770	C53 H98 O6	50	46	2	LMS, LPP	0.24	
T-900-4	7.60	900.801	C57 H102 O6	54	46	4	OOL	0.06	
T-876-2	8.38	876.801	C55 H102 O6	52	48	2	OOP	0.58	
T-902-3	8.34	902.818	C57 H104 O6	54	48	3	OOO	0.16	2.25
T-876-2	8.51	876.799	C55 H102 O6	52	48	2	SLP	0.02	
T-930-3	9.10	930.848	C59 H108 O6	56	50	3	GOO	0.004	
T-932-2	9.96	932.863	C59 H110 O6	56	52	2	AOO	0.03	
T-960-2	10.69	960.895	C61 H114 O6	58	54	2	BOO	0.04	
T-988-2	11.38	988.926	C63 H118 O6	60	56	2	OOLi	0.01	

Note: CN, carbon number; ECN, equivalent carbon number; DB, double bond; w, weak peak intensity (low amount).
FA: Cy, Caprylic acid (8:0); C, Capric acid (10:0); La, Lauric acid (12:0); M, Myristic acid (14:0); P, Palmitic acid (16:0); S, Stearic acid (18:0), O, Oleic acid (18:1), L, Linoleic acid (18:2); Ln, Linolenic acid (18:3); A, Arachidic acid (20:0); G, Gondoic acid (20:1); B, Behenic acid (22:0); Li, Lignoceric acid (24:0).

with SFAs, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The TIC and respective XICs are shown in Fig 2. The results of peak identification and relative composition of TAGs are summarized in Table 2. It can be seen in Table 2, four peaks are identified to be TAGs of SFAs (DB=0) with ECN from 40 to 46. The relative composition of the TAG with SFAs is 62.5% (based on peak area). Nine TAG structures were identified from the respective MS/MS spectra. In addition, nine peaks (*m/z* 710.628 etc) were identified to be TAGs with MUFAs, which total relative composition is 35.2%. Furthermore, ten peaks (*m/z* 874.785 etc.) were identified to be TAGs with PUFAs. The total relative content of TAGs with PUFAs is 2.3% (peak area). The level of triolein (OOO) is only 0.16%. If counting ECN 40~46 as MCFAs, the total composition is 94.3%.

Identification of individual TAGs

Identification of TAG is based on HRAM data analysis using LabSolutions Insight Explore (Figure 3). The accurate mass obtained from MS spectrum is used to predict the formula and the number of double bonds in the molecule. The fragments shown in the MS/MS spectrum of the precursor were used to determine the types of fatty acids according to neutral loss principle.

Figure 3 shows the spectra of peak T-712-0 at RT 4.89. The data analysis generates a formula (most-likely) of C43H82O6 with 3 double bonds for *m/z* 712.6450 ion peak. This result matches perfectly with TAG molecule with ECN of 40 and zero double bond on the fatty acid hydrocarbon chains R1, R2 and R3 (Figure 1), i.e., SFAs. The types of fatty acids in the molecule can be derived from MS/MS spectrum. As shown in Table 3, the five fragments observed correspond to neutral losses of five different fatty acids. However, the peak intensities for stearic acid (18:0) and capric acid (10:0) are much weaker than palmitic acid (16:0), myristic acid (14:0), lauric acid (12:0). As such, the main TAG structures are likely PLaLa and MMLa, while the SLaC is in lower amount.

TAGs profile and distribution

Three coconut oil samples were analyzed under the same condition to compare the TAGs profile and distribution of different types of TAGs. The results of sample C1, C2 and C3 are shown in Figure 4.

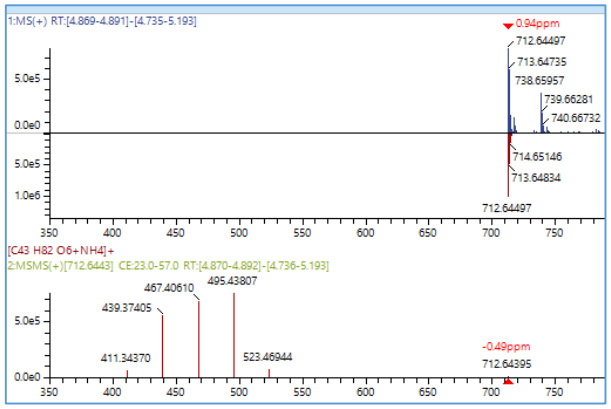

Figure 3 Displaying and processing of HRAM spectra (MS and MS/MS) of peak T-712-0 on LabSolutions Insight Explore.

Table 3 MS/MS fragment, neutral loss and TAG structure

MS/MS (of *m/z* 712.645)			TAG
Fragment	NL	FA	
411.344-*w*	284.278	S [18:0]	PLaLa, MMLa, SLaC(*w*)
439.374	256.247	P [16:0]	
467.406	228.215	M [14:0]	
495.438	200.183	La [12:0]	
523.469-*w*	172.152	C [10:0]	

Note: "-w" indicates weak peak intensity and low amount

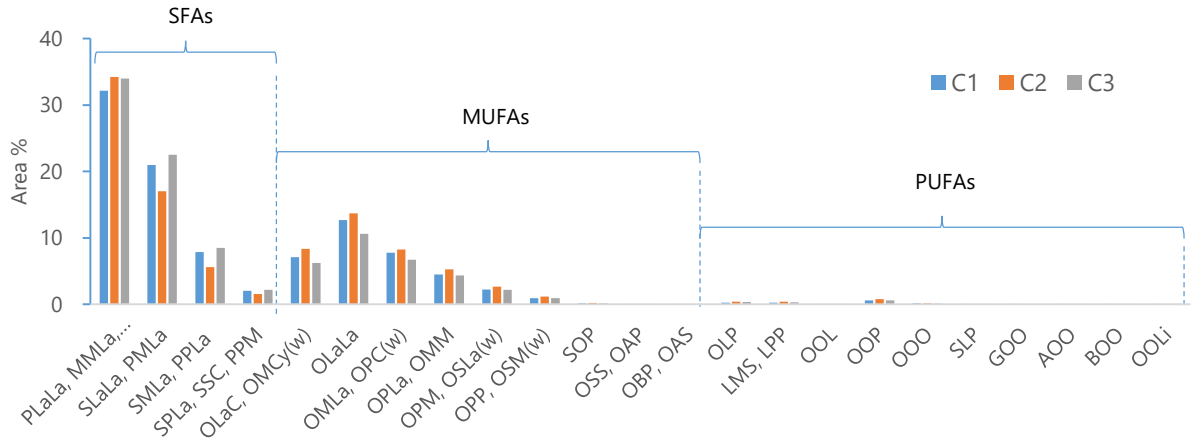

Figure 4 Comparison of TAG profile and distribution in coconut oils (C1, C2 and C3) from different brands

■ Conclusion

A fast and direct LC-ESI-Q-TOF method was established and used for the identification and composition analysis of TAGs in coconut oils. A total of 38 TAGs were identified based on accurate mass MS data and MS/MS spectra. The composition analysis results (relative) shows a consistent profile and distribution of TAGs in the three different samples, i.e., 62.5% of TAGs with SFAs, 35.2% of TAGs with MUFAs and 2.3% of TAGs with PUFAs.

■ References

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