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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 - (2) Palm Oil

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User Benefits

- A fast and direct LC-ESI-Q-TOF method was established for identification of TAGs in palm 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 molecules from accurate mass MS and MS/MS spectra using the LabSolutions Insight Explore s/w.

■ Introduction

The oil palm is a tropical plant originated in the ancient West Africa. In 2018, the world produced 72 million tons of oil palm and 84% of global palm oil production come from Indonesia and Malaysia. Palm oil is used widely as cooking oil, shortenings, margarine etc.. Fatty acid compositions of palm oils have been determined as the key characterization in concerning the health and nutrition [1]. Triacylglycerols (TAGs) are the main form of fatty acids in oils. Analysis of fatty acids in palm oil involves first hydrolysis of TAGs to fatty acid methyl esters, followed by GC-FID analysis. In recent year, direct analysis TAGs by LC-MS/MS [2,3] and SFC-MS/MS [4] have been used for quick identification of TAGs. In this application news, an LC-ESI-Q-TOF method is described for identification and composition analysis of TAGs in palm oil samples.

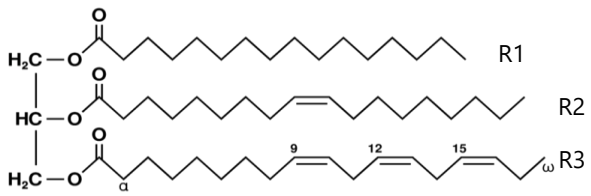

Figure 1 A representative structure of triacylglycerol with saturated fatty acid (SFA, R1), monounsaturated fatty acid (MUFA, R2) and polyunsaturated fatty acid (PUFA, R3).

■ Experimental

Reagents and Chemicals

Acetonitrile (MS grade), 2-propanol (99.9%), chloroform (99.5%) and acetone (99.9%) were obtained from commercial suppliers. Ammonium formate (>99%) of LCMS grade was used as additives in the mobile phase prepared from Milli-Q water.

Samples and sample preparation

Three palm oil samples, P1 (red palm oil), P2 (red palm oil) and P3 (white palm oil), from different brands produced in Malaysia were purchased from local market. Stock solution of 6.0 mg/mL was prepared by weighing 40 mg of palm oil and dissolving in chloroform-acetone mixed solvent (v/v=1:1). The stock solution was further diluted with 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.	1 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 additional of ammonium formate in the mobile phase B was adopted. Under this condition, TAG molecules form ammonium adduct ion $[TAG+NH_4]^+$ with high detection sensitivity. Fragmentation of selected precursor ions was 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 [3].

■ Results and Discussion

TAGs composition in palm oil

Figure 2 shows the TAGs LC-MS profiles of palm oil P1 under the conditions. The TIC peak profile shifts significantly to longer retention time as compared to coconut oil [3], related to the longer hydrocarbon chain fatty acids than that in coconut oil. As shown in Table 2, there is no TAG with only saturated fatty acids (SFAs),

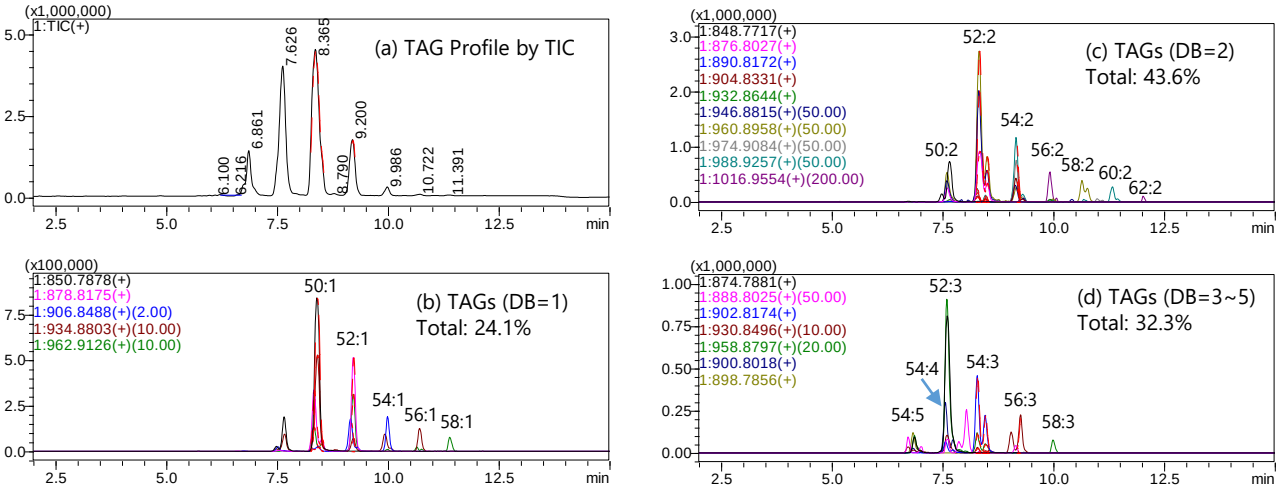

Figure 2 TAG profiles of palm oil by LC-Q-TOF. (a) Total TAGs by TIC; (b) TAGs with one MUFA (DB=1); (c) TAGs with two MUFAs or one PUFA (DB=2) and (d) TAGs with MUFAs and PUFAs (DB=3 and above)

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

P. Code	Ret. Time	m/z	Formula	CN	ECN	DB	TAGs	Area%	Area%
T-850-1	8.40	850.788	C53 H100 O6	50	48	1	OPP	15.10	
T-878-1	9.22	878.818	C55 H104 O6	52	50	1	SOP	7.42	
T-906-1	9.99	906.849	C57 H108 O6	54	52	1	AOP, OSS	1.24	24.05
T-934-1	10.72	934.880	C59 H112 O6	56	54	1	BOP, ASO	0.18	
T-962-1	11.40	962.913	C61 H116 O6	58	56	1	POLi, OBS(w)	0.10	
T-848-2a	7.48	848.770	C53 H98 O6	50	46	2	OOM, OPPo(w)	1.84	
T-848-2b	7.66	848.770	C53 H98 O6	50	46	2	LPP	12.47	
T-876-2a	8.34	876.803	C55 H102 O6	52	48	2	OOP	17.64	
T-876-2b	8.51	876.803	C55 H102 O6	52	48	2	SLP	4.43	
T-890-2	8.76	890.817	C56 H104 O6	53	49	2	OOH	0.16	
T-904-2	9.16	904.833	C57 H106 O6	54	50	2	SOO	6.25	
T-932-2	9.93	932.864	C59 H110 O6	56	52	2	AOO	0.57	43.63
T-946-2	10.42	946.882	C60 H112 O6	57	53	2	TLP	0.01	
T-960-2a	10.65	960.896	C61 H114 O6	58	54	2	BOO	0.11	
T-960-2b	10.77	960.896	C61 H114 O6	58	54	2	LiLO	0.06	
T-974-2a	10.99	974.908	C62 H116 O6	59	55	2	TOO	0.01	
T-974-2b	11.11	974.908	C62 H116 O6	59	55	2	PxLP	0.00	
T-988-2	11.34	988.926	C63 H118 O6	60	56	2	OOLi	0.08	
T-1016-2	12.03	1016.955	C65 H122 O6	62	58	2	OOHx	0.00	
T-874-3	7.61	874.788	C55 H100 O6	52	46	3	OLP	15.31	
T-888-3a	7.87	888.803	C56 H102 O6	53	47	3	OOH1	0.02	
T-888-3b	8.04	888.803	C56 H102 O6	53	47	3	OLH	0.07	
T-902-3a	8.28	902.817	C57 H104 O6	54	48	3	OOO	6.15	
T-902-3b	8.46	902.817	C57 H104 O6	54	48	3	SOL	3.07	
T-930-3a	9.05	930.850	C59 H108 O6	56	50	3	GOO	0.17	32.31
T-930-3b	9.26	930.850	C59 H108 O6	56	50	3	AOL	0.31	
T-958-3	10.00	958.880	C61 H112 O6	58	52	3	BOL	0.05	
T-900-4a	7.56	900.802	C57 H102 O6	54	46	4	OOL	4.09	
T-900-4b	7.74	900.802	C57 H102 O6	54	46	4	SLL	1.17	
T-898-5	6.83	898.786	C57 H100 O6	54	44	5	OLL	1.88	

CN, carbon number; ECN, equivalent carbon number; DB, double bond; M, Myristic acid (14:0); P, Palmitic acid (16:0); Po, Palmitoleic acid (16:1); H1, cis-10-Heptadecenoic acid (17:1); S, Stearic acid (18:0), O, Oleic acid (18:1), L, Linoleic acid (18:2); A, Arachidic acid (20:0); G, Gondoic acid (20:1); B, Behenic acid (22:0); T, Tricosanoic acid (23:0); Li, Lignoceric acid (24:0); Px, Pentacosanoic acid (25:0); Hx, Hexacosanoic acid (Cerotic acid) (26:0).

but 24.1% TAGs with only one monounsaturated fatty acid (MUFA), 43.6% TAGs with two MUFAs or one polyunsaturated fatty acid (PUFA) (L, 18:2), and 32.3% TAGs with MUFAs and PUFAs (BD=3 and above). The highest composition TAG in each group are OPP (15.1%), OOP (17.6%) and OLP (15.3%). The ECN 50~52 represent the major TAG components, contributing 74.2% of total TAGs.

Identification of individual TAGs

As reported previously [3], identification of individual TAG is based on a data analysis procedure using LabSolutions Insight Explore. First, the accurate mass obtained from MS spectrum is used to identify TAGs via

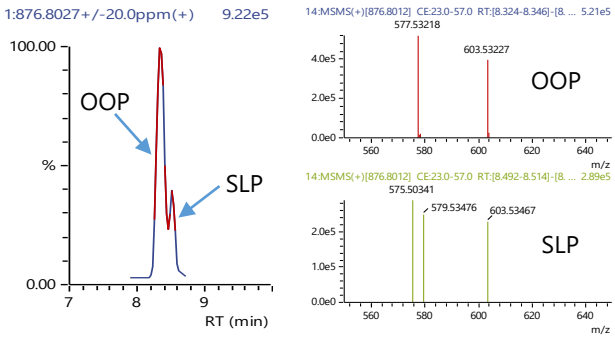

Figure 3 XIC (*m/z* 876.803) and MS/MS spectra. The fragments of left peak is attributed to OOP and the right peak to SLP.

predicting the formula ($CxHyO_6$) and giving 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 XIC and MS/MS spectra of peak T-876-2a and T-876-2b at RT 8.36 and 8.53 min. The obtained formula from accurate mass is C55H98O6 with 4 double bonds in the molecule. This result matches perfectly with TAG molecule with ECN of 52 and one double bond in the fatty acid hydrocarbon chains R1, R2 and R3 (Figure 1), i.e., only one MUFA present. The fragments of the two peaks are different (Figure 3), indicating the different types of fatty acids in the molecules. As shown in Table 3, the TAGs identified for the two peaks are OOP and SLP, respectively. Figure 4 shows another example of TAG identification, which contains 5 double bonds in the molecule. The MS/MS fragments indicate the TAG structure to be OLL (Table 3).

TAGs profile and distribution

Three palm oil samples P1, P2 and P3 were analyzed under the same condition to compare the TAGs profile and distribution of different types of TAGs. The results are shown in Figure 5. Although P1 and P2 are red palm oils and P3 is white palm oil, the TAGs distributions are highly consistent.

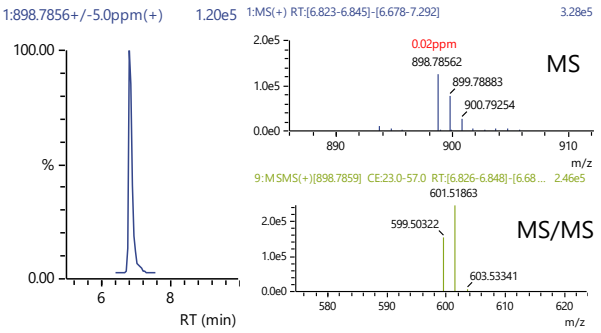

Figure 4 XIC (*m/z* 898.786), MS and MS/MS spectra. The accurate mass generates a formula of C57H100O6 with 5 DB.

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

RT	Presursor	Fragment	NL	FA	TAG
8.36	876.803	577.521	282.255	O [18:1]	OOP
		603.536	256.240	P [16:0]	
8.53	876.803	575.504	284.272	S [18:0]	SLP
		579.535	280.242	L [18:2]	
		603.535	256.242	P [16:0]	
6.93	898.786	599.503	282.256	O [18:1]	OLL
		601.509	280.251	L [18:2]	

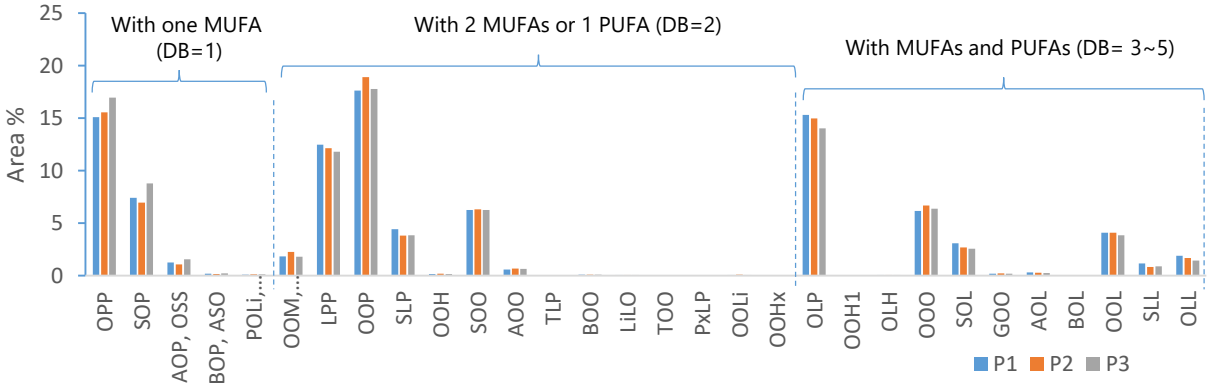

Figure 5 Comparison of TAG profiles and distributions in red palm oils (P1, P2) and white palm oil (P3) from different brands

■ Conclusion

A fast and direct LC-ESI-Q-TOF method was established and used for the identification and composition analysis of TAGs in palm oils. A total of 34 TAGs was identified based on accurate mass MS data and MS/MS spectra. The results show a consistent profile and distribution of TAGs among two red palm oil samples and one white palm oil sample. There is no TAG with only SFAs like coconut oil. The TAGs with one MUFAs is 24.1%, that with two MUFAs or one PUFA is 43.6% and the rest (three MUFAs and/or PUFAs) is 32.3%.

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