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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 - (3) *Moringa Oleifera* Seed Oil

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User Benefits

- A fast and direct LC-ESI-QTOF method was established and adopted in identification and composition analysis of TAGs in *moringa oleifera* seed oil. A total of 33 TAGs was identified based on accurate mass MS and MS/MS spectra.
- The result indicates that TAGs of *moringa oleifera* seed oil are mainly consisted of triolein (OOO, 39.7%) and OOX (X=P, S, A, B, Li, G) with ECN at 48~56. It is a high oleic acid containing oil.

■ Introduction

This research is completed in collaboration between Sinarmas Agribusiness & Food (R&D) Marunda Centre and Shimadzu Asia Pacific. *Moringa oleifera* tree is found widely in tropical and subtropical areas worldwide. *Moringa oleifera* seed oil has been used as nutritional supplement, traditional medicines, skin treatment oil and new food resource [1, 2]. Studies on properties of *moringa oleifera* seed oil rely on the analysis of the main components - triacylglycerols (TAGs), their identification and composition. The conventional analytical approach is to determine fatty acids of TAGs. It involves first hydrolysis of TAGs to form fatty acid methyl esters, followed by GC-FID analysis. In recent years, direct analysis of TAGs by LC-MS/MS [2, 3] and SFC-MS/MS [4] have been developed and used for identification of TAGs in various edible oils. In this study, a LC-ESI-Q-TOF method established previously [5,6] was applied in identification of TAGs based on accurate mass spectra and determination of relative composition of the TAGs in *moringa oleifera* seed oil samples.

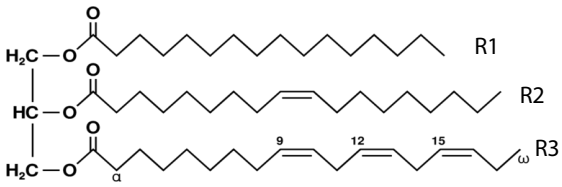

Figure 1 A representative structure of triacylglycerol with saturated fatty acid (SFA, R1), monounsaturated fatty acid (MUFA, R2) and polyunsaturated fatty acid (PUFA, R3).

■ Experimental

Reagents and chemicals

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

Samples and sample preparation

Four *moringa oleifera* seed oil samples, M0, M1, M2 and M3, produced in Indonesia were obtained. Stock solution of 6 mg/mL was prepared by weighing 40 mg of the oil samples and dissolving in chloroform-acetone mixed solvent (v/v=1:1). The stock solution was diluted with mobile phase B to obtain 0.60 mg/mL (or 600 ppm) for analysis on LC-Q-TOF.

Table 1 Analytical conditions on LCMS-9030

LC Conditions	
Column	Shim-pack^{TM} 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)
Gradient program	B%: 0 min, 85% → 8.4~9 min, 95% → 12.1~16 min, 85% → 16 min, stop
Oven Temp.	45°C
Injection Vol.	1 µL
Interface Conditions (LCMS-9030)	
Interface	Heated ESI 4.0 kV
Interface Temp.	150°C
DL Temp.	250°C
Heat Block Temp.	400°C
Nebulizing Gas	3 L/min (N_2)
Heating Gas Flow	10 L/min (Zero Air)
Drying Gas Flow	10 L/min (N_2)
Data acquisition (Q-TOF)	
MS mode (TOF)	Positive, 700-1200, 50 msec
MS/MS (Q-TOF)	Up to 31 precursors, *m/z* 50-1100, CE: -40 V spread (+/-)17 V
Dwell time	20 msec per MS/MS event
Loop time	0.67 sec

LC-Q-TOF conditions

The analytical conditions for TAGs on LCMS-9030 are shown in Table 1. A Shim-pack Velox C18 column was used. Ionization of TAGs is promoted with the addition of ammonium formate in mobile phase A.

■ Results and Discussion

TAGs composition in *moringa oleifera* seed oil

Under the analysis conditions, TAG molecules form ammonium aradduct ions $[TAG+NH_4]^+$, which exhibit high detection sensitivity [5]. Fragmentation of selected precursors was performed in MS/MS mode in a high acquisition speed (20 msec per spectrum). A spread collision energy of -40 (+/-) 17 volts was applied, which led to efficient fragmentation for all TAGs [5, 6].

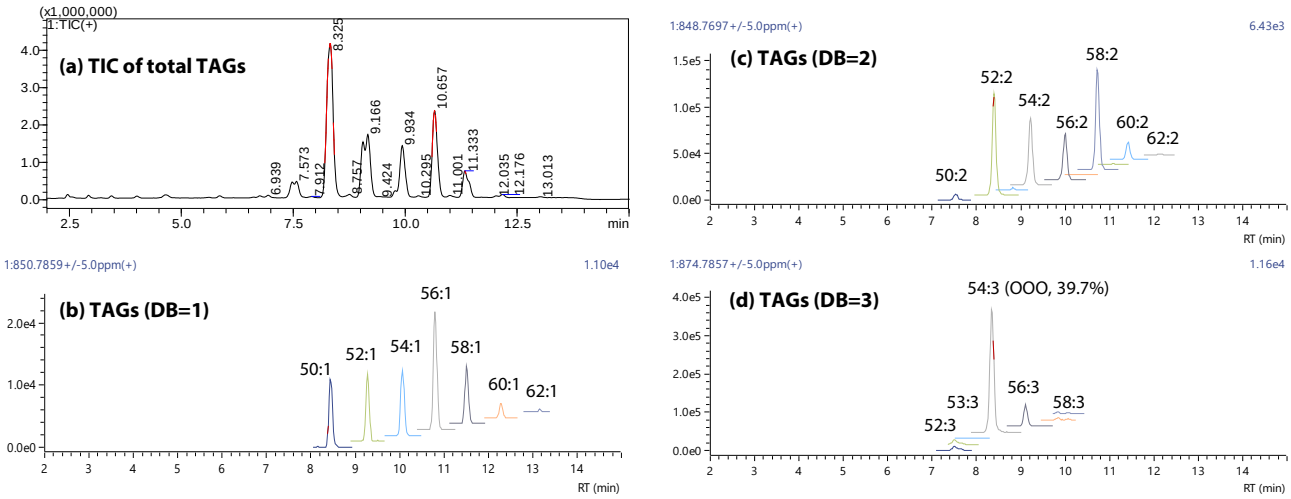

Figure 2 TAG mass chromatograms of *moringa oleifera* seed oil by LC-Q-TOF. (a) Total TAGs by TIC; (b) TAGs with one MUFAs (DB=1); (c) TAGs with two MUFAs or one PUFAs (DB=2); (d) TAGs with MUFAs and PUFAs (DB=3)

Table 2 Results of TAG identification and relative compositions (Area%) in *moringa oleifera* seed oil

P. Code	RT	*m/z*	Formula	CN	ECN	DB	TAG	Area%	Area%
T-850-1	8.36	850.786	C53H100O6	50	48	1	OPP	1.26	
T-878-1	9.23	878.815	C55H104O6	52	50	1	SOP	1.13	
T-906-1	10.00	906.848	C57H108O6	54	52	1	AOP, OSS	1.16	
T-934-1	10.73	934.879	C59H112O6	56	54	1	BOP, OSA	2.03	6.86
T-962-1	11.43	962.910	C61H116O6	58	56	1	POLi, OBS	1.03	
T-990-1	12.18	990.942	C63H120O6	60	58	1	BAO, OSLi	0.23	
T-1018-1	13.02	1018.972	C65H124O6	62	60	1	OBB	0.01	
T-848-2	7.49	848.770	C53H98O6	50	46	2	OPPo, OOM	0.74	
T-876-2	8.36	876.801	C55H102O6	52	48	2	OOP	12.39	
T-890-2	8.76	890.817	C56H104O6	53	49	2	OOH	0.24	
T-904-2	9.16	904.832	C57H106O6	54	50	2	SOO	8.46	
T-932-2	9.98	932.863	C59H110O6	56	52	2	AOO	5.95	42.51
T-946-2	10.29	946.876	C60H112O6	57	53	2	OOHs	0.13	
T-960-2	10.65	960.895	C61H114O6	58	54	2	BOO	12.11	
T-974-2	11.00	974.910	C62H116O6	59	55	2	TOO	0.12	
T-988-2	11.33	988.927	C63H118O6	60	56	2	OOLi	2.26	
T-1016-2	12.04	1016.956	C65H122O6	62	58	2	OGLi, OOHx	0.10	
T-874-3a	7.46	874.786	C55H100O6	52	46	3	OOPo	1.75	
T-874-3b	7.61	874.786	C55H100O6	52	46	3	OLP	0.36	
T-888-3	7.89	888.805	C56H102O6	53	47	3	OOH1	0.08	
T-902-3	8.30	902.817	C57H104O6	54	48	3	OOO	39.71	
T-930-3	9.05	930.848	C59H108O6	56	50	3	GOO	6.26	50.63
T-958-3a	9.77	958.879	C61H112O6	58	52	3	OOCe, GGO	0.57	
T-958-3b	10.01	958.879	C61H112O6	58	52	3	OLB	0.25	
T-900-4	7.58	900.801	C57H102O6	54	46	4	OOL	1.31	
T-898-5	6.93	898.786	C57H100O6	54	44	5	OOLn	0.35	

CN, carbon number; ECN, equivalent carbon number; DB, double bond; 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); Ln, Linolenic acid (18:3); A, Arachidic acid (20:0); G, Gondoic acid (20:1); Hs, Heneicosanoic acid (21:0); B, Behenic acid (22:0); T, Tricosanoic acid (23:0); Li, Lignoceric acid (24:0); Hx, Hexacosanoic acid (Cerotic acid) (26:0).

Figure 2 shows the mass chromatograms of *moringa oleifera* seed oil (M0). The total ion chromatogram (TIC) peak profile shifts significantly to longer retention time as compared to that of coconut oil and palm oil [5], which is in consistent with their higher ECN distributions. As shown in Table 2, 6.9% of TAGs contain one MUFA, 42.5% TAGs with two MUFAs (mostly OO) and 50.6% TAGs with MUFAs and PUFAs (DB=/>3). The highest-content TAG found in the sample is triolein (OOO, 39.71%). This content is essentially closed to that reported in literatures [1, 2]. In addition to OOO, the contents of OOP (52:2), OOS (54:2), OOA (56:2), OOB (58:2), OOLi (60:2) and OOG (56:3) are also in relatively higher levels. This result indicates that the TAGs of *moringa oleifera* seed oils are mainly consisted of OOO and OOX (X=P, S, A, B, Li, G) with ECN at 48~56. This is in consistent with that reported in literature [1] the content of oleic acid in moringa oil was as high as 70%.

Identification of TAGs

As reported previously [3], identification of individual TAG is based on a data analysis procedure using LabSolutions Insight Explore*TM*. First, the accurate mass obtained from MS spectrum is used to identify TAGs via predicting the formula ($C_xH_yO_6$) 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.

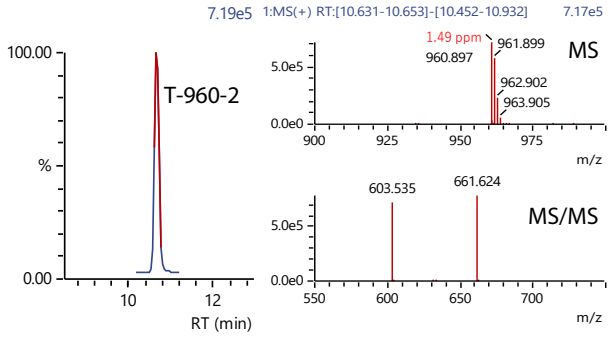

Figure 3 XIC (*m/z* 960.897) and MS, MS/MS spectra. The measured accurate mass of precursor matches a formula of C61H114O6 with 1.49 ppm error.

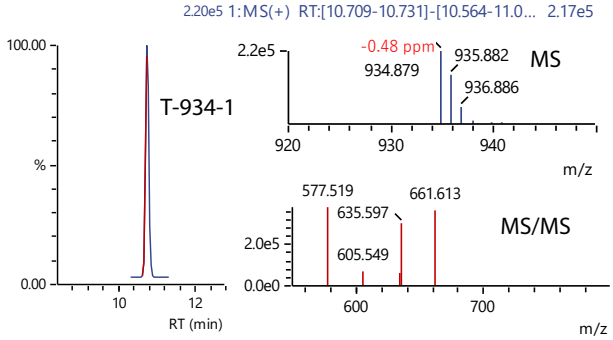

Figure 4 XIC (*m/z* 934.879), MS and MS/MS spectra. The measured accurate mass matches a formula of C59H112O6 with -0.48 ppm error.

Figures 3, 4 and Table 3 shows two examples of TAG identification. The peaks T-960-2 (10.65 min) and T-934-1 (10.73 min) were identified to be BOO and BOP (with small amount of OSA). First, the formula obtained from the accurate mass spectrum are C61H114O6 (DBE=5) and C59H112O6 (DBE=4), which matches perfectly with TAG molecules of 58:2 and 56:1, respectively. As shown in Figure 4, two small fragments (*m/z* 605.549 and 633.581) were detected, which indicates the presence of co-eluted TAG, i.e., OSA.

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

RT	Precursor	Fragment	NL	FA	TAG
10.65	960.897	603.535	340.335	B [22:0]	BOO
		661.624	282.246	O [18:1]	
10.73	934.879	577.519	340.334	B [22:0]	BOP, OSA
		635.597	282.256	O [18:1]	
		661.613	256.240	P [16:0]	
		605.549	312.303	A [20:0	
		633.581	284.272	S [18:0]	

TAGs distribution

Four samples M0, M1, M2 and M3 were analyzed under the same conditions to compare the TAGs distribution in the samples. The results shown in Figure 5 indicates that the TAGs distributions in the four samples are highly consistent.

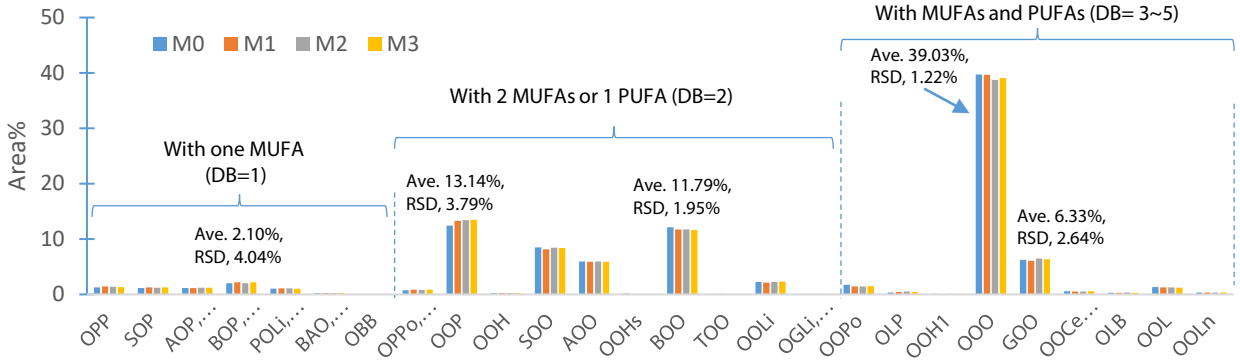

Figure 5 Comparison of TAG distributions in *moringa oleifera* seed oils (M0, M1, M2 and M3)

■ Conclusion

A direct LC-ESI-Q-TOF method was used for the identification and composition analysis of triacylglycerols in *moringa oleifera* seed oil. A total of 33 TAGs was identified based on accurate mass MS and MS/MS spectra. The results show a consistent distribution of TAGs among the four samples studied. The relative content of triolein (OOO) in these samples is 39.7%. In addition to OOO, OOP (52:2), OOS (54:2), OOA (56:2), OOB (58:2), OOLi (60:2) and OOG (56:3) are found as main TAGs. The result indicates that TAGs of *moringa oleifera* seed oil are mainly consisted of OOO and OOX (X=P, S, A, B, Li, G) with ECN at 48~56. It is a high oleic acid containing oil.

■ References

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