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Profiling of Triacylglycerides present in edible oils consumed in India using LC/MS/MS

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1. Introduction

A triacylglyceride (TAG) is an ester derived from glycerol and three Fatty Acids (FA). TAGs are found in both plant oils and animal fats. Different varieties of oils are used for culinary purposes across the globe. Hence, their characterization is important for nutritional reasons. However, separation and identification of components of the complex mixtures of TAG that constitute fats and oils is a challenging. Characterization of the TAGs present in edible oils requires acquisition of precursor ion *m/z* by full scan MS mode. Full scan MS spectra are generally complicated because of presence of varieties of TAGs. Furthermore, it is necessary to fragment all these precursor ions so as to understand fatty acid composition of TAGs. Selecting these precursor ions manually and creating a method to fragment them is also a tedious task. This task is simplified by Synchronized Survey Scan (SSS) available in LCMS-8040 with LabSolutions software. Here, product ion spectra of all the major precursor ions are obtained as a dependent event of full scan MS in a single analysis, attributed to ultrafast scanning speed of 15000 u/sec of LCMS-8040.

2. Method of Analysis

2-1. Sample Preparation

10 µL of oil sample was diluted in 20 mL of 2-propanol (IPA) and was analyzed by Ultra High Performance Liquid Chromatography (UHPLC) Nexera system coupled with LCMS-8040 triple quadrupole system (Shimadzu Corporation, Japan).

2-2. LC/MS/MS Analytical Conditions

Oil samples were analyzed using LC/MS/MS triple quadrupole system (shown in Fig. 1). The Dual Ion Source (DUIS) consists of an integrated probe for analysis of both ESI and APCI techniques concurrently and continuously without relying on switching between modes. Furthermore, analysis was performed using Shimadzu's Synchronized Survey Scan (SSS) function (shown in Fig. 2). With this automatic MS/MS function, the original MS measurement is used as a trigger, enabling the product ion mass spectra (MS^2 spectra) to be acquired. In this way, by measuring the molecular ion spectra (Q3 scan MS spectra), and then using this as the trigger for measuring the product ion mass spectra (MS^2 spectra), both MS and MS^2 spectra can be acquired simultaneously for a single peak. Neutral loss scan was also used so as to get additional confirmation of fatty acid composition of TAGs. The details of analytical conditions are given in Table 1.

Table 1 LC/MS/MS analytical conditions

Column	: Inertsil C8 (50 mm L × 4.6 mm I.D. × 3 µm)
Mobile phase	: 10 mM ammonium acetate in methanol
Flow rate	: 0.7 mL/min
Oven temperature	: 50°C
Injection volume	: 5 µL
MS interface	: DUIS
Nitrogen gas flow	: Nebulizing gas 1.5 L/min; Drying gas 15 L/min
MS temperature	: Desolvation line 250°C; Heat block 400°C
MS analysis mode	: 1. Q3 scan (*m/z* range 300-1000) 2. SSS (*m/z* range 200-1000) 3. Neutral loss scan

Fig. 1 Nexera with LCMS-8040 triple quadrupole system by Shimadzu

Type	Event#	+/-	Compound Name m/z
Q3 Scan	1	+	300.00:1000.00
|- Product Ion Scan	2	+	100.00 > 200.00:1000.00

Fig. 2 MS method using SSS function

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3. Results

Edible oils from palm, sesame, mahua (*Madhuca longifolia*), sunflower etc. were analyzed using above mentioned method. Workflow is explained here using the data from the analysis of *mahua* oil obtained from *Madhuca* longifolia seeds. This oil is used by some tribes in India for cooking purpose. Other edible oils were analyzed in similar manner. FA composition of *mahua* oil is given in Table 2[1]. Q3 full scan chromatogram of *mahua* oil is shown in Figure 3. Each TAG shows predominantly an $(M+NH_4)^+$ adduct ion (shown in Fig. 4) due to the addition of ammonium acetate in the mobile phase. The true mono isotopic Molecular Weight (M.W.) of a TAG is approximately 0.8 Da greater than the number obtained by adding the integer atomic weights of the elements, owing to the mass defect of hydrogen (actual atomic weight 1.00794) and the large number of hydrogen in the molecule.

Table 2 Fatty acid composition of mahua oil[1]

Fatty Acid Name	Lipid Number	Abbreviation Used	Molecular Weight	Neutral Loss monitored (FA M.W. + NH_3)	% fatty acid composition of mahua oil
Oleic	C18:1	O	282.00	299.00	36.7 ± 0.27
Palmitic	C16:0	P	256.00	273.00	21.3 ± 1.01
Stearic	C18:0	S	284.00	301.00	24.3 ± 0.30
Linoleic	C18:2	L	280.00	297.00	15.2 ± 0.64
Arachidic	C20:0	A	312.50	329.50	1.3 ± 0.15
Myristic	C14:0	M	228.00	245.00	-
Euric	C22:1	E	338.00	355.00	-
Behenic	C22:0	B	340.50	357.50	-
Lignoceric	C24:0	Ln	368.60	385.60	-

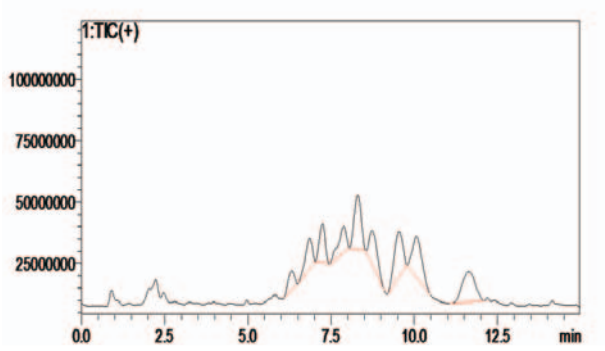

Fig. 3 Q3 full scan MS chromatogram of *mahua* oil

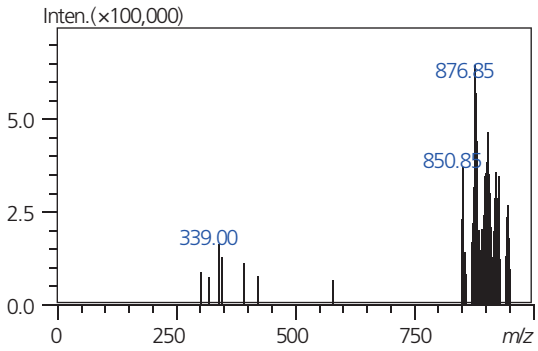

Fig. 4 Q3 full scan MS spectrum of mahua oil

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Additional information can be obtained from extracted ion chromatograms. Overlay of extracted chromatograms for *m/z* 880.80, 878.90, 876.80, 874.70 and 872.80 is shown in Fig. 5. These *m/z* values correspond to triglycerides made up of fatty acids with 0, 1, 2, 3 and 4 double bonds respectively. Such comparison indicates the degree of unsaturation of edible oil. The dominant ion is *m/z* 876.80 and oleic acid is the major fatty acid component in mahua oil. Hence, it is possible that TAG having *m/z* of 876.80 contains two oleic acid which is confirmed by obtaining product ion spectrum of *m/z* 876.80 (Refer to Table 3). Product ion spectra of all major precursor ions are obtained using Synchronized Survey Scan. Representative product ion spectrum of *m/z* 872.9 is shown in Fig. 6. Product ion m/z 855.90 is formed due to the loss of ammonium adduct where as *m/z* 575.65 and 599.30 are formed due to combined loss of one of the fatty acids and ammonia from the TAG species.

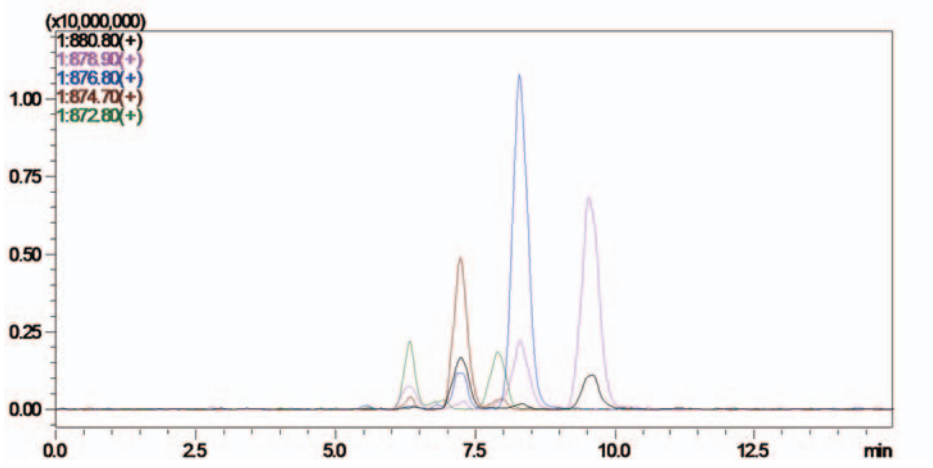

Fig. 5 Overlay of extracted ion chromatograms of *mahua* oil

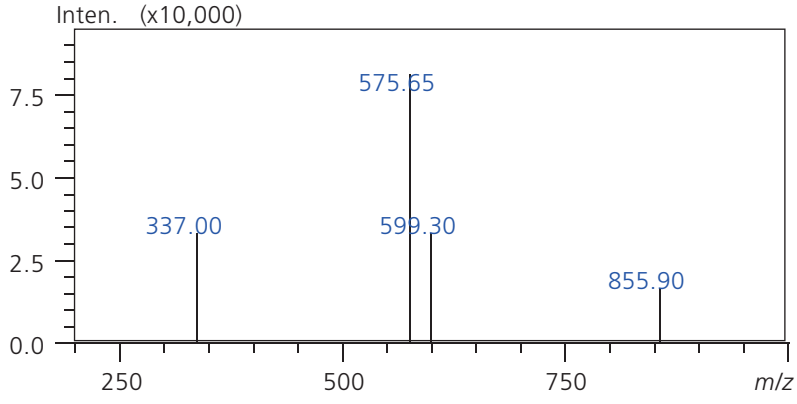

Fig. 6 Representative product ion spectrum of *m/z* 872.90 obtained using SSS

Interpretation of product ion spectra are generally straightforward. First, the identity of the FA lost to give each Diacyl product ion is determined. This is done by subtracting the mass of the Diacyl product ion from the mass of the TAG precursor ion and subtracting further 17 for ammonia to get the M.W. of the FA lost in the fragmentation process. Then relative abundance of Diacyl product ions are compared so as to assign positions of fatty acids on the glycerol. Assignment of the sn (stereospecific number) position of FA is based on the observation that the loss of the sn-1 FA is generally preferred to sn-2 FA[2]. Results obtained from Q3 full scan MS and SSS as well as their interpretation is given in Table 3.

Table 3 Results of Q3 scan and SSS analysis of *mahua* oil

Retention time (min)	TAG adduct ion (*m/z*)	Diacyl product ion (*m/z*)	M.W. of FA lost*	Name of FA lost from TAG species	Abundance of Diacyl product	Probable FA composition of TAG**
6.31	872.90	575.65	280.45	Linoleic	93138	LLP
		599.30	256.40	Palmitic	78383	
6.85	848.80	575.55	256.25	Palmitic	235777	PLP
		551.55	280.25	Linoleic	124438	
7.22	874.70	601.60	256.10	Palmitic	251762	OLP
		575.50	282.20	Oleic	193787	
		577.40	280.30	Linoleic	138732	
7.65	900.90	601.60	282.30	Oleic	99017	OLO
		603.70	280.20	Linoleic	70447	
7.84	850.70	577.65	256.05	Palmitic	1110286	POP
		551.40	282.30	Oleic	303603	
8.31	876.80	577.55	282.25	Oleic	387032	PLS/POO
		603.60	256.20	Palmitic	330750	
		579.55	280.25	Linoleic	110746	
		575.50	284.30	Stearic	107569	
8.77	902.90	603.55	282.35	Oleic	234842	OSL
		605.55	280.35	Linoleic	79227	
		601.55	284.35	Stearic	58450	
9.52	878.90	577.50	284.40	Stearic	215388	SOP
		605.45	256.45	Palmitic	128274	
		579.50	282.40	Oleic	71130	
10.12	904.90	605.55	282.35	Oleic	233175	SLS/SOO
		603.55	284.35	Stearic	185741	
		607.85	280.05	Linoleic	27498	
10.54	930.80	633.60	280.20	Linoleic	49143	OLA ***
11.61	906.90	605.65	284.25	Stearic	114606	SOS/POA
		607.60	282.30	Oleic	80971	
		577.45	312.45	Arachidic	13423	
		633.60	256.30	Palmitic	7022	

* M.W. of FA lost = m/z of TAG - (*m/z* of Diacyl product + NH_3)
**For abbreviations refer to Table 2
*** Interpretation is based on neutral loss scan data (refer to Table 4)

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The $[M+NH_4]^+$ ions of TAGs undergo the characteristic loss of a single neutral species RCOOH + NH_3, for each fatty acyl group present and so neutral loss scan mode can be used for analysis of ammoniated adducts of TAGs[3]. Several neutral loss scans as mentioned in Table 2 were used to determine a complete fatty acyl profile of the TAGs present in edible oils. Results obtained from neutral loss scan and their interpretation is given in Table 4.

Table 4 Results of neutral loss scan analysis of *mahua* oil

Retention time (min)	Fatty acid neutral loss monitored	Observed precursor ion *(m/z)*	Abundance	Probable TAG composition
6.31	Linoleic	872.90	High	LLP
	Palmitic		medium	
6.85	Palmitic	848.80	high	PLP
	Linoleic		medium	
7.22	Oleic	874.70	high	OLP
	Palmitic		medium	
	Linoleic		low	
7.65	Oleic	900.90	high	OLO
	Linoleic		medium	
7.84	Palmitic	850.70	high	POP
	Oleic		medium	
7.90	Palmitic	852.80	high	PSP
	Stearic		medium	
8.31	Palmitic	876.80	high	PLS/POO
	Oleic		medium	
	Stearic		low	
	Linoleic		low	
8.77	Oleic	902.90	high	OSL
	Linoleic		medium	
	Stearic		low	
9.52	Stearic	878.90	high	SOP
	Palmitic		medium	
	Oleic		low	
9.57	Stearic	880.80	high	SPS
	Palmitic		medium	
10.12	Oleic	904.90	high	SLS/SOO
	Stearic		medium	
	Linoleic		low	
10.54	Oleic	930.80	high	OLA
	Arachidic		medium	
	Linoleic		low	
11.61	Stearic	906.80	high	SOS
	Oleic		medium	

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4. Conclusion

- Profiling of TAGs present in *mahua* oil is done for the first time using LC/MS/MS triple quadrupole system.
- Synchronized Survey Scan enables acquisition of MS and MS^2 spectra simultaneously for given peak and hence simplifies the task of profiling of TAGs from edible oils.
- Ultra high scanning speed of 15000 u/sec allows the acquisition of Q3 full scan, SSS and NL in a single run and hence large amount of information can be obtained to determine fatty acid composition of TAGs present in edible oil.

5. References

[1] Rupali Dhara, Dipak K. Bhattacharyya et al., Analysis of sterol and other components present in unsaponifiable matters of *Mahua*, *Sal* and Mango kernel oil, Journal of Oleo Science, Volume 59 (4), (2010), 169-176.
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