Cryogenically-modulated comprehensive two-dimensional gas chromatography-high resolution time-of-flight mass spectrometry: fingerprint of the unsaponifiable fraction of vegetable oils

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INTRODUCTION

The present study is focused on the development of a cryogenically-modulated comprehensive two-dimensional gas chromatography-high resolution time-of-flight mass spectrometry (CM GC×GC-HR ToF MS) method for the qualitative profiling of the unsaponifiable fraction of various vegetable oils [extra-virgin olive oil (EVOO), soyabean oil, hazelnut oil, peanut oil]. Particular attention was devoted to the higher molecular weight constituents, such as sterols, triterpenic alcohols, etc. An evaluation of the performance of the HR ToFMS system (operated at a mass resolution of 25,000 fwhm) under the challenging analytical conditions of a CM GC×GC-based experiment was also performed. In general, the MS instrument provided more-than-satisfactory results.

EXPERIMENTAL

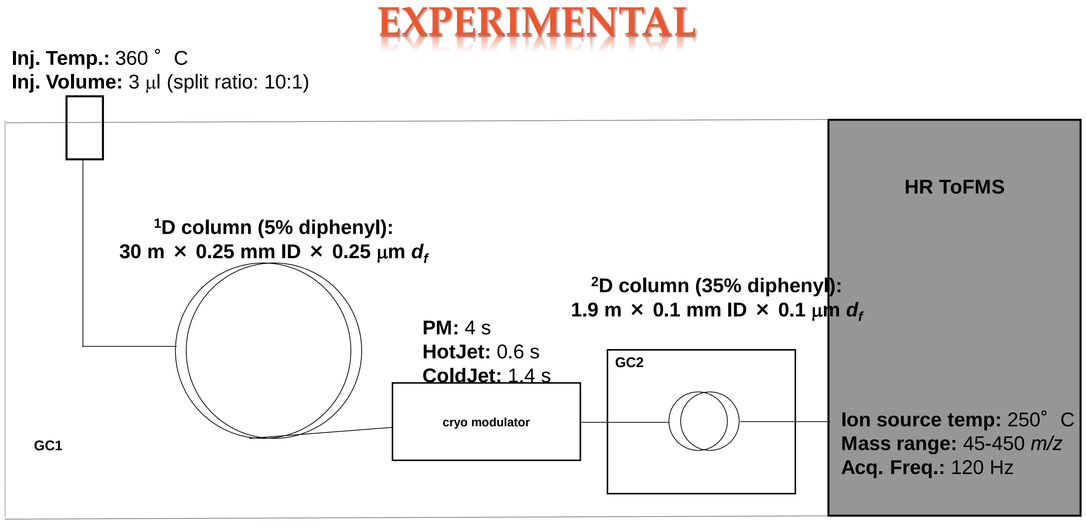

Figure 1. *Experimental conditions*

Sample Preparation:

One gram of vegetable oil was added to 10 mL of a 2 N KOH/EtOH solution, heated at 80 °C, under. After, three extractions were performed with different volumes of diethyl ether and the extracts were washed with 10 mL of distilled water. Finally, the solvent was evaporated under low-pressure conditions at 37°C. The unsaponifiable fraction was dissolved in 500 µL of chloroform, and treated with the derivatization mixture [200 µL of BSTFA (1% TMCS) and 200 µL of pyridine], and then heated at 70 °C for 20 min. The trimethylsilyl (TMS) derivatized sample was then ready for GC injection.

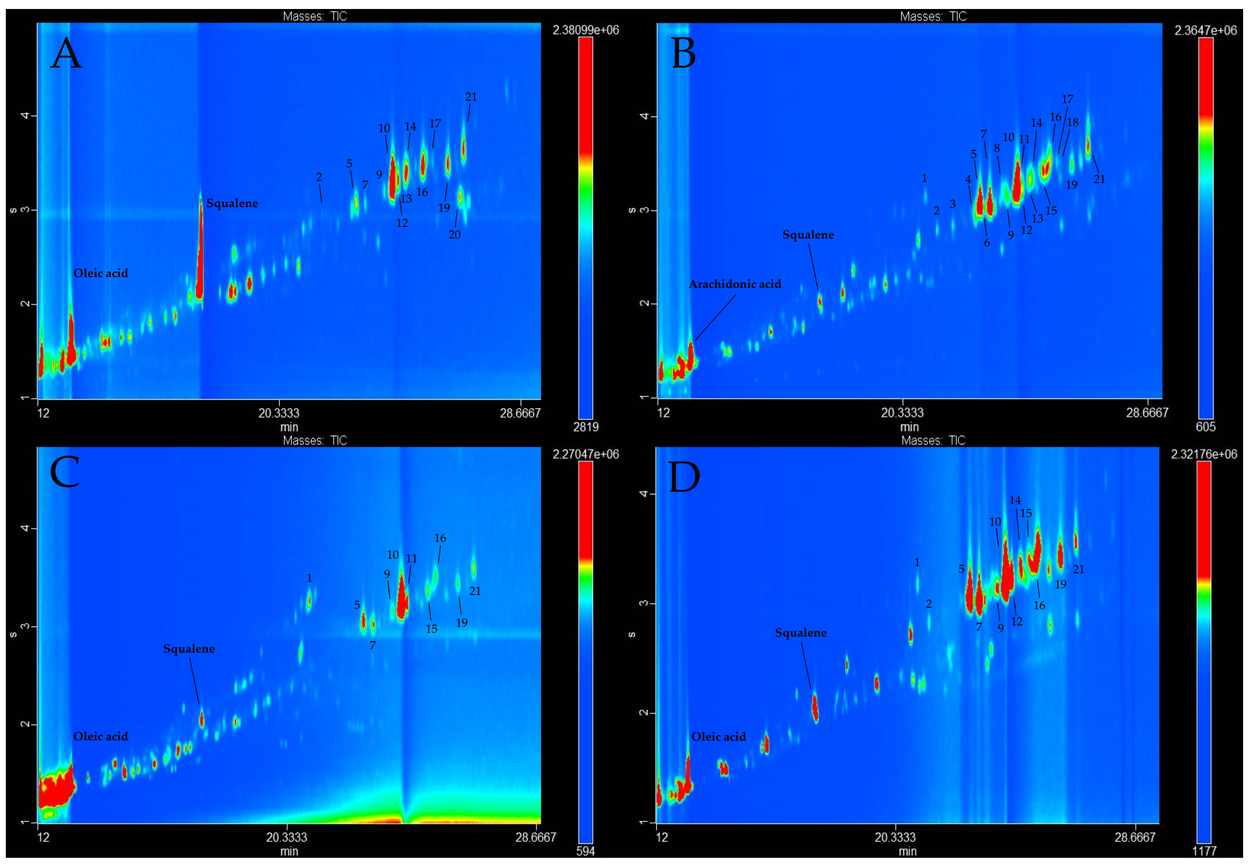

Figure 2. *TIC GC×GC-HR ToFMS bidimensional plots of the unsaponifiable fractions of extra-virgin olive oil (A), soyabean oil (B), hazelnut oil (C) and peanut oil (D)*

Table 1. *Peak assignment, MW information, molecular ion error, for the vegetable oil samples. Abbreviations: DesMeSt = desmethylsterol; TriTerpOH = triterpenic alcohol; DiMeSt = dimethylsterol; TriTerp2OH = triterpenic dialcohol; MeSt = methylsterol, TMS = trimethylsilyl ether.*

Peak/Compound	MW	Molec. Ion Error (ppm)			
		EVOO	Soyabean	Hazelnut	Peanut
1. Stigmasta-3,5-diene	396.375600		-4.01	0.87	-1.22
2. Cholesterol (DesMeSt-TMS)	458.394393	×	-7.82		-3.22
3. Brassicasterol (DesMeSt-TMS)	470.394393		-2.54		
4. 24 MethyleneCholesterol (DesMeSt-TMS)	-		×		
5. Campesterol (DesMeSt-TMS)	472.410043	-3.55	0.33	3.58	1.12
6. Campestanol (DesMeSt-TMS)	474.425693		1.03		
7. Stigmasterol (DesMeSt-TMS)	484.410043	-1.81	-0.67	0.97	0.03
8. Δ^7-Campesterol (DesMeSt-TMS)	472.410043		3.01		
9. Clerosterol (DesMeSt-TMS)	484.410043	4.47	-1.92	1.05	-2.15
10. β-sitosterol (DesMeSt-TMS)	486.425693	-1.98	-0.22	2.60	1.54
11. Sitostanol (DesMeSt-TMS)	488.441342		2.21	2.37	
12. Δ^5-Avenasterol (DesMeSt-TMS)	484.410043	-4.91	-2.87		-0.27
13. Parkeol (DiMeSt-TMS)	498.425693	0.10	0.82		
14. β-Amyrin (TriTerpOH-TMS)	498.425693	0.56	4.04		3.03
15. Δ^7-Stigmastenol (DesMeSt-TMS)	486.425693		0.32	-2.46	1.58
16. Cycloartenol (DiMeSt-TMS)	498.425693	4.66	-4.64	6.35	8.50
17. α-Amyrin (TriTerpOH-TMS)	-	×	×		
18. Δ^7-Avenasterol (DesMeSt-TMS)	-		×		
19. 24-Methylenecycloartanol (DiMeSt-TMS)	-	×	×	×	×
20. Erythrodiol (TriTerp2OH-TMS)	-	×			
21. Citrostadienol (MeSt-TMS)	498.425693	-7.74	4.66	×	×
	average	3.3	2.6	2.5	2.3

Note: × denotes compounds identifed without molecular ion info

RESULTS AND DISCUSSION

A GC×GC-HR ToFMS method was developed with focus on the ≥ C18 fingerprint: providing a specific detailed view of the heavier MW compounds (**Figure 2**). With regards to sterols, a total amount of 21 compounds were identified. Soyabean oil was found to be the vegetable oil with the higher amount of sterols, while hazelnut oil was the sample with less sterols identified. All the compounds found in the samples are listed in **Table 1**, along with molecular ion error (ppm) information. Noteworthy is the presence of stigmasta-3,5-diene (peak 1) in all of the samples, apart from extra-virgin olive oil. This compound comes from the degradation of β-sitosterol during the refining steps. The high-resolution mass spectrum of stigmasterol TMS is illustrated in **Figure 3**. A molecular ion with high mass accuracy (-0.03 ppm) is evident. Mass accuracy for all compounds was very good: an average value between 2.3 and 3.3 (absolute value) was calculated for all the oils.

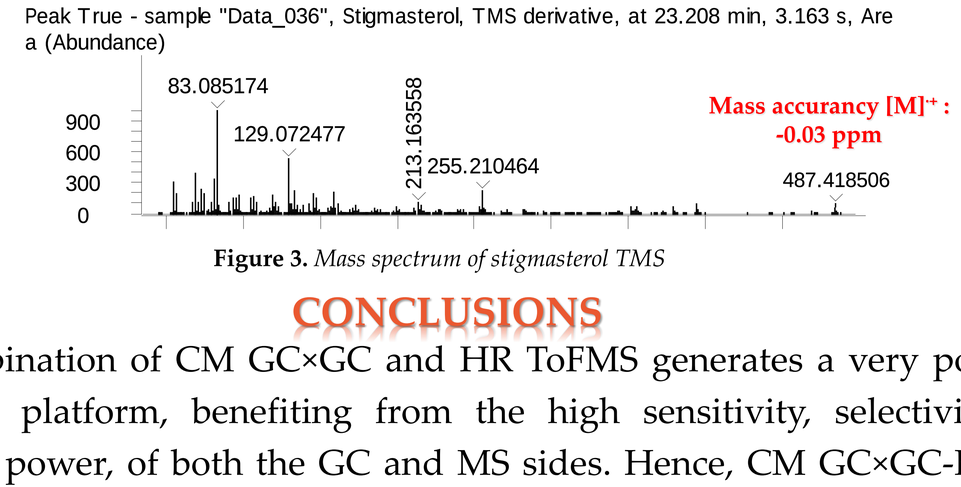

Figure 3. *Mass spectrum of stigmasterol TMS*

CONCLUSIONS

The combination of CM GC×GC and HR ToFMS generates a very powerful analytical platform, benefiting from the high sensitivity, selectivity and resolving power, of both the GC and MS sides. Hence, CM GC×GC-HR ToF MS has the capability to perform in-depth investigations, and generate detailed fingerprints, of complex mixtures of volatile lipids.

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