HIGH-THROUGHPUT CHROMATOGRAPHIC FINGERPRINTING OF EXTRA VIRGIN OLIVE OIL VOLATILES BY GC×GC-MS/FID AND DIFFERENTIAL FLOW MODULATION

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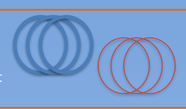
Aim and scope

Comprehensive two-dimensional gas chromatography combines the information power of detailed profiling and effective fingerprinting by producing samples unique 2D patterns helpful for discrimination and classification purposes. The possibility to routinely perform highly informative fingerprinting/profiling on large sample set is attractive, but requires analytical platforms with low operational costs, simpler laboratory management and robust and repeatable performances over time. This study explores the feasibility of transferring a fingerprinting method for Extra Virgin Olive oils (EVOOs) volatiles from a loop-type thermal modulated (TM) GC×GC-MS to a reverse-inject differential flow modulated (FM) platform. The principles of method translation are adopted to effectively transfer the application from TM to FM by preserving analytes elution order, ^{1}D resolution and 2D pattern coherence.

EXPERIMENTAL – GCxGC-MS/FID platform

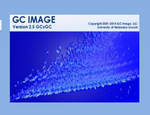
Agilent 7890B GC equipped with 7650A autosampler and **5977B HES MSD** operating in EI mode at 70 eV - FID detector
Scan speed 12,500 amu/*s HES Tune* option

Capillary columns, unions and non-purged tees were from SGE and Agilent



Raw data were acquired by Enhanced MassHunter (Agilent Technologies)

2D data were processed by GC Image® GC×GC Edition Software Release 2.6 (GC Image Lincoln NE, USA)



Headspace linearity

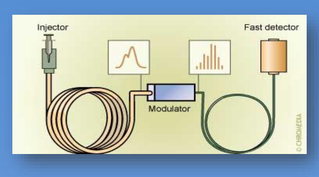
The adoption of HS Solid Phase Microextraction with multi-component fiber was optimized for time and temperature to match for **headspace linearity conditions and increase fingerprinting sensitivity. Sample amounts below 100 mg avoided saturation effects.**

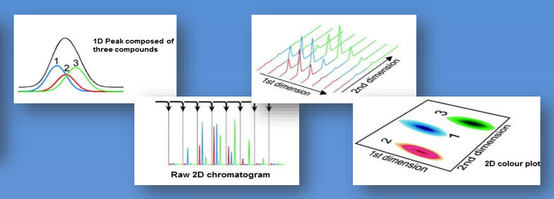
HS-SPME sampling conditions

- Sampling: 100 mg of EVOO
- Temperature: 40°C
- Time: 60 min with the pre-loading of the internal standards (α/β-thujone and methyl-2-octynoate)
- Vial volume: 20 mL
- Fiber: DVB/CAR/PDMS; 50/30 µm; 1 cm Supelco Bellefonte.

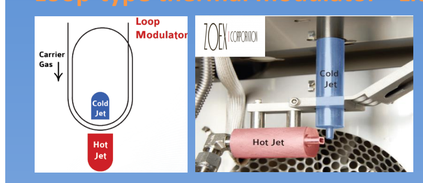
GCxGC principles and modulators

GC×GC separation multiplies the **separation power** of two chromatographic dimensions. The resulting 2D separation is a **pattern** of 2D peaks spread over a **bi-dimensional space** where the **relative position** is a function of the **differential retention** by each dimension.





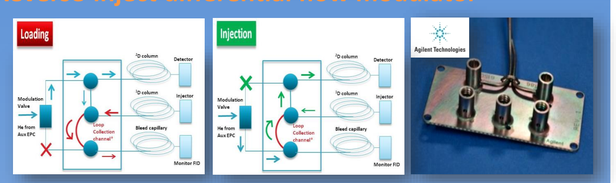
Loop-type thermal modulator - Liquid Nitrogen



"Single jet – two stage modulator" deactivated silica capillary is rolled up in a loop held by a metallic glide.

Zoex KT 2004 loop-type thermal modulator
Optimode v2.0 - Cryogenic liquid nitrogen. www.zoex.com.

Reverse-inject differential flow modulator



Schematic representation of the differential flow modulation with "reverse fill/flush" dynamic in the loading and injection state. www.agilent.com

The "loop-type" thermal modulator operates by focusing sample components in a two-stage dynamics. A continuous stream of refrigerated nitrogen (cold jet) traps analytes eluting from the ^{1}D dimension column. Every few seconds (modulation period - typically 2-6s) an hot jet fires for a fixed time interval (typically 200-400 ms) diverting the cold stream and enabling the re-volatilization (release) of condensed analytes towards the ^{2}D column. These operations are periodically repeated across all the chromatographic run. The modulator geometry and the capillary loop dimensions enable a very effective process by trapping and releasing analyte at least two-times before enter into the 2D.

A differential-flow modulator exploits the principles of valve-based multidimensional systems. The modulator plate, made by the Capillary Microfluidics Technology™, hosts a collection channel (loop) connected, on one side of the plate, to the ^{1}D column and, on the other side, to a bleeding capillary. During the modulation, the effluent coming from the ^{1}D is collected (loading - stage) into the loop while an auxiliary carrier is diverted by a solenoid valve toward the ^{2}D column. This loading stage typically lasts for few seconds (2-6 s). During the injection stage, the auxiliary gas is diverted towards the Tee connection on the bottom of the plate flushing the loop and transferring analytes toward the ^{2}D.

✓ • Very high separation power (product of two dimensions)
• Improved sensitivity (band compression effect S/N)
• Structured patterns for analytes chemical classes
• Great flexibility in modulation parameters tuning

✗ • Rather high instrumental costs
• Additional costs for cryogenics

✓ •Limited operational and hardware costs
•Relative ease of use and simple maintenance

✗ •Columns configuration (diameter, length)
•High operative flows (2D)

Result and discussion

Translation of chromatographic parameters

Original method - 2D pattern

HS-SPME - GC×GC-MS
Loop-type Thermal Modulation

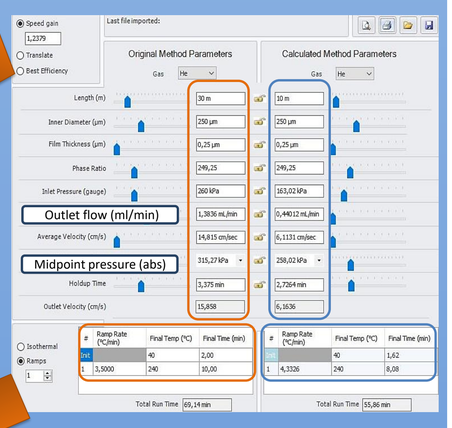
GC Oven: 40°C(2') to 240°C (10') @ 3.5°/min
S/SL injector: 250°C, split ratio 1:20
MS in EI mode, 70 eV Full Scan, 50 Hz, range 40-350 m/z
Modulation settings: P_M **3,5s**, hot-jet pulse 250 ms

135 targeted compounds

...by translating
Preserve the **elution order**
Keep **coherent elution pattern**
Keep original method **Resolution***
Exploit all **information dimensions**
Compensate for reduced loading capacity
Speed-up the analysis.
(***If number of theoretical plates is preserved**)

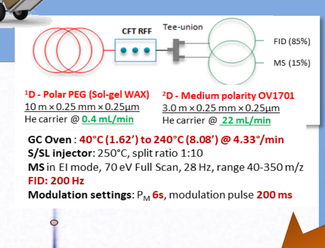
Application transfer

Method translation [1,2]

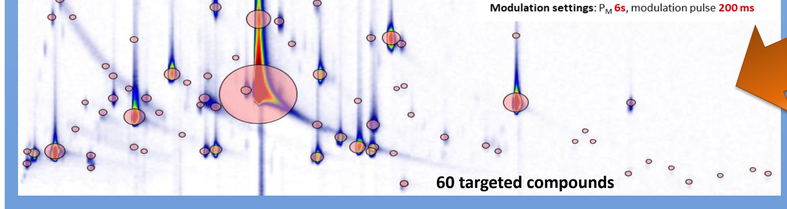


Translated method – 2D pattern

HS-SPME - GC×2GC-MS/FID
Differential Flow Modulation with Reverse Inject



GC Oven : 40°C (1.62') to 240°C (8.08') @ 4.33°/min
S/SL injector: 250°C, split ratio 1:10
MS in EI mode, 70 eV Full Scan, 28 Hz, range 40-350 m/z
FID: 200 Hz
Modulation settings: P_M **6s**, modulation pulse **200 ms**

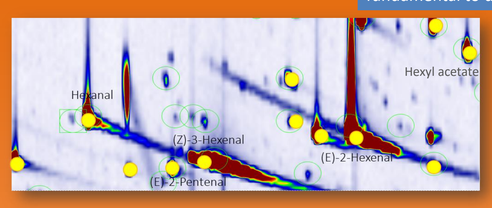


60 targeted compounds

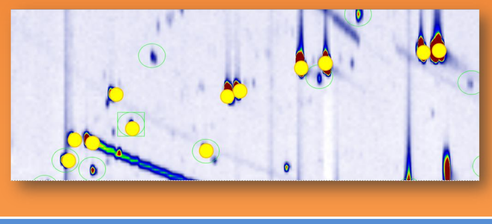
✓**Analytes elution order is preserved**
✓**Analysis time reduced**

Original method

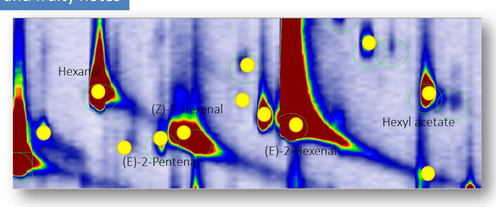
Lipoxygenase (LOX) signature
fundamental to define fresh-green and fruity notes

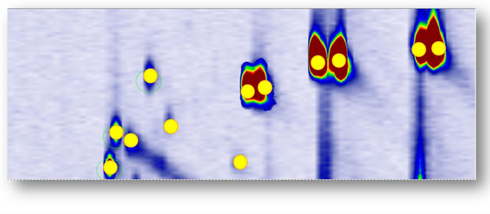


Hydrocarbons
informative about the ripening stage of the olives



Translated method



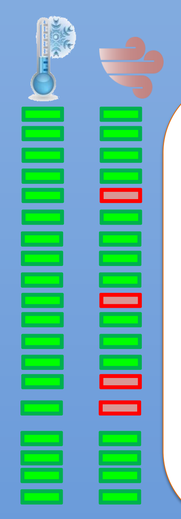


Fingerprint effectiveness

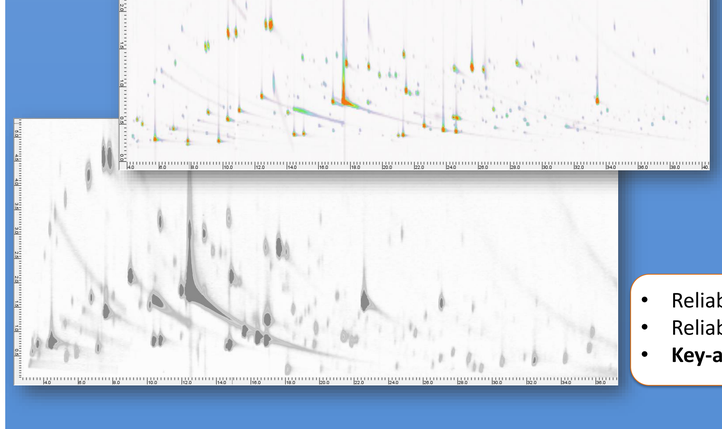
Chemical class distribution for the 135 known analytes detected by the **original method** based on GC×GC-TOF MS and thermal modulation

Potent odorants: Key aroma compounds

Compounds	Descriptor
(E)-2-Hexenal	Green, fresh
(Z)-2-Hexen-1-ol	Green, fresh
Hexanal	Green, fatty
(Z)-3-Hexenal	Green, fatty
(E)-2-Heptenal	Green, fatty
(E)-2-pentenal	Green, pungent
(E)-3-Hexen-1-ol	Green, floral
1-Penten-3-ol	Green, pungent
(E,E)-2,4-Hexadienal	Green, sweet
Pentanal	Fermented, fruity
1-Pentanol	Fermented, pungent
Hexyl acetate	Fruity, green
3-Penten-2-one	Fruity, acetone
1-Octen-3-ol	Earty, green
(E,E)-2,4-Heptadienal	Fatty, green
(E)-2-Nonenal	Fatty, green
(E)-2-Octenal	Fatty, fresh
Nonanal	Aldehydic, waxy
Octanal	Aldehydic, waxy



Distribution of some potent odorants [3] in Extra Virgin Olive Oils (EVOOs) as they are captured by with thermal and flow modulations (data are reported in in logarithmic scale).
The most abundant compounds contributing favourably to the aroma of EVOOs are the C5 and C6 aldehydes and alcohols, which relate to sweetness.
On the other hand the main factors that characterize off-flavors are the C7-C12 aldehydes, alcohols and other volatile compounds with low odour thresholds, with the simultaneous low abundance of the C6 aldehydes, C6 alcohols and esters.



Translation effectiveness

The effective translation of the fingerprinting method from **Thermal (TM)** to **Differential Flow modulation (FM)** does not impact on fingerprinting information potential.
Original TM templates of reliable peaks (257 peaks) from EVOOs volatiles, when applied to 2D pattern obtained in translated conditions, achieve a 43% of positive matches. However, when targeted analytes are considered (i.e. known markers of EVOOs quality) the positive matches are higher (46%), while for potent odorant, both C5 and C6 aldehydes/alcohols and the off-flavor characteristics compounds the coverage is of about 80%.

- Reliable peaks (untargeted) **257** *vs.* **110** → **43 %**
- Reliably identified targets **135** *vs.* **60** → **46 %**
- **Key-aroma compounds** **20** *vs.* **16** → **80 %**

Conclusions

Experimental results on guided method translation, confirm that EVOOs volatiles fingerprinting is feasible by FM GC×GC by preserving 2D patterns information potential and providing mutually coherent sample clustering. The information potential of samples' fingerprints based on known markers is also partially preserved, despite the loss of sensitivity, and 16 over 20 key-aroma compounds are successfully detected.

If you wish to have some extra-information please contact: federico.stilo@unito.it

References

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[2] M.S. Klee, L.M. Blumberg. Theoretical and Practical Aspects of Fast Gas Chromatography and Method Translation, J. Chromatogr. Sci. 40 (2002) 234–247.
[3] C.M.Kalua *et al.* Olive oil volatile compounds, flavour development and quality: A critical review, Food Chemistry 100 (2007) 273–286.
[4] Violin Project - Progetto Ager Fondazioni in rete per la ricerca Agroalimentare http://www.progettoager.it/

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