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EXPLORING THE POTENTIAL OF ADDING "EXTRA-DIMENSIONS" TO THE EXTRA VIRGIN OLIVE OIL VOLATILES FINGERPRINTING BY GCxGC-TOF MS AND SOFT IONIZATION



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Aim and Scope

Comprehensive two-dimensional gas chromatography (GCxGC) is the most effective multidimensional separation technique for in-depth investigations of complex samples of volatiles (VOC) in food. It enables highly informative fingerprinting and, if combined with Mass Spectrometry, it has the intrinsic potential to provide a detailed profiling giving access to the higher level of information encrypted in the peculiar distribution of sample's components. Time of Flight Mass Spectrometry (TOF MS) with soft electron ionization adds a further dimension to the analysis thereby enabling more confident identifications but also new challenges for the data processing. The possibility to operate by switching between hard (70 eV) and soft ionization (12, 14 and 16 eV) generates complementary MS signatures (about each component and for each 2D pattern), increasing the dimensionality of the analysis. This approach is particularly effective if soft and hard ionization spectra can be obtained simultaneously affording comprehensive and highly reliable characterization of complex fractions and easing the speciation of isomers.

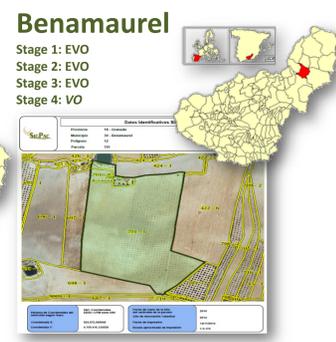
In this study the focus is on the complex volatile fraction from Extra Virgin Olive oils from Spain, variety Picual and obtained from fruits at four different ripening stages. The analytical strategy combines extra-dimensions from volatiles sampling by High Concentration Capacity (HCC) sample preparation (Head Space Solid Phase Microextraction with different polymer(s) combinations) to analytes detection by hard (-70eV) and soft ionization at 12 eV.

The distribution of olives ripening indicators, key-odorants responsible of positive attributes and off-odors for defects assessment is studied; fingerprints from different samples are compared to evaluate critically the beneficial role of adding an extra-dimension to the analysis.



Picual variety harvested in 2014, from Altipiano de Granada (Spain). Baza: conventional production and drip irrigation; Benamaurel: conventional production and drip irrigation. Each sample was available in duplicate and obtained by mixing olives from five different trees in the same plot to have homogeneous and representative samples. Olives were harvested at four different ripening stages: November 10-12, 2014; November 24-28, 2014; December 16-17, 2014; and January 12-15, 2015. Samples were analyzed by an accredited laboratory to define quality parameters: acidity (%), peroxide index (mEq O₂/kg), and UV absorption. Samples also were submitted to sensory evaluation by a recognized/official panel.

Olive Oil Samples



Experimental - GCxGC-TOFMS platform

GCxGC System

Agilent 6890 GC equipped with a standard S/SL injector and fast FID.

Loop-type modulator - Liquid Nitrogen

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

Headspace Solid Phase Microextraction

PDMS: Polydimethylsiloxane 100%
apolar - partition/absorption

PA: Polyacrylate
medium polar - partition/absorption

PDMS/DVB: Polydimethylsiloxane/Divinylbenzene
apolar/medium-polarity - absorption/adsorption

CAR/PDMS/DVB: Carboxen/Polydimethylsiloxane/Divinylbenzene
apolar/medium-polarity - absorption/adsorption

Targeted analytes distribution - fingerprints - have been considered as probes for optimum fiber selection.

Sampling conditions: 100 mg, 40°C, 60 minutes, 20 mL vial

TOF MS

Markes BenchTOF-Select™ with Tandem Ionisation® at 70 eV and 12 eV.

TOF-DS

Raw data were acquired by TOF-DS software (Markes International, Llantrisant, UK)

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

GC Oven programming: 40°C(1') to 260°C(10') @ 3.5°/min

S/SL injector: 250°C Split ratio 1/20

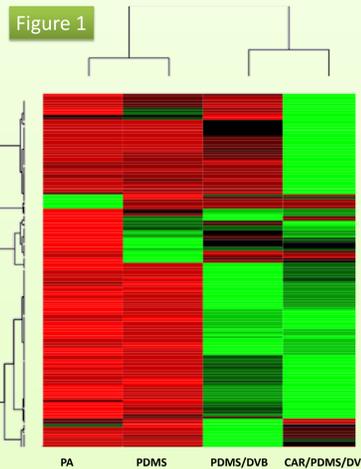
MS Transfer line: 250°C tandem ionization: -70 and -12eV

MS Acquisition: Mass range 40-350 m/z; frequency 50 Hz

Optimode settings: modulation period 4s, hot-jet pulse time 250 ms - cold jet stream MFC from 40% to 5% in 80 min

Results and Discussion

VOCs Fingerprint information potential



The selection of the best component/polymer combination for headspace extraction of VOCs was driven by targeted fingerprinting.

A group of **155 targeted analytes** reliably identified at 70eV by matching with those from commercial and in-house databases (NIST Identity Search algorithm Match quality threshold ≥ 900 and I_5 coherence) was mapped across 2D patterns from four different commercial fibers. Results are visualized as heat-map (Figure 1) based on Quantifier responses (mean and centering normalization) from Baza plot sample at the second stage of harvest.

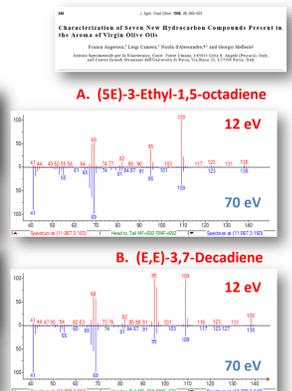
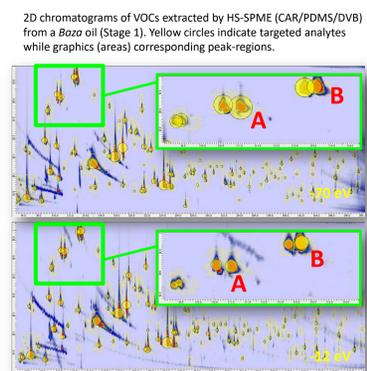
To make data comparable, the sample amount was kept constant and within headspace linearity (**100 mg**) at **40°C - 60 minutes**. A design of experiments defined optimal sampling conditions (data not shown).

The combination of different polarities and extraction mechanisms (absorption/adsorption) have a clear impact on the fingerprint information potential. The best combination included Carboxen-Polidimethylsiloxane-Divinylbenzene (1 cm - Stableflex) with, on average, higher analyte concentration factors and wider polarity range.

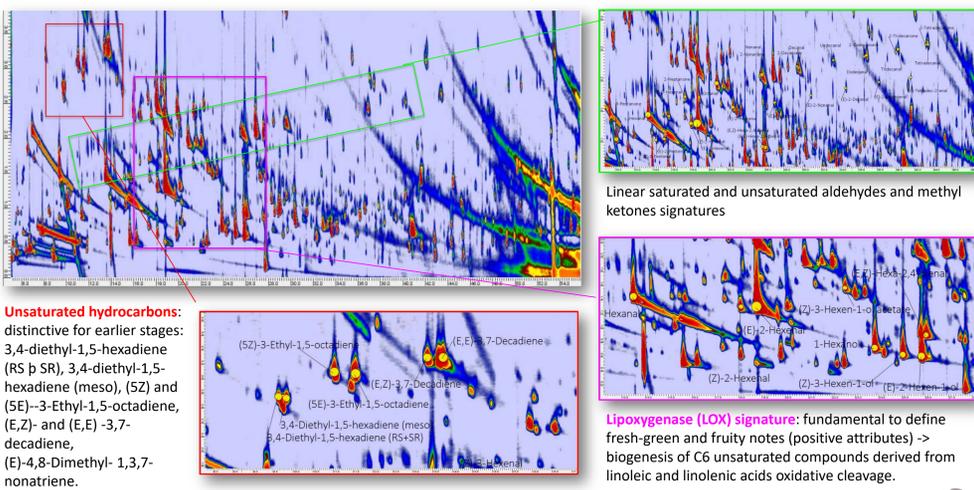
Exploring the potentials of 12 eV ionization

Tandem Ionisation® at 70eV and 12eV offers the possibility to cross-validate in fingerprinting investigations.

The availability of an additional MS signature at lower voltages offers the possibility of confirming the identity of analytes not available as reference compounds. Alkenals signature in olive oils is an example.



Separation patterns and fingerprint information



Ripening indicators - Baza samples

Heat-map based on Quantifier responses for 155 targeted analytes (mean and centering normalization). Samples are ordered from left to right according to olive harvest stage. Coloring from red (low abundance) to green (high abundance) illustrates the evolution of volatiles from Stage 1 (predominance of C6 LOX derivatives and low-molecular weight hydrocarbons -) to Stage 4 where reach their maximum abundance, e.g. 2-nonanone, (E)-2-nonenal, nonanal, octane, 6-methyl-5-hepten-2-one and octanol.

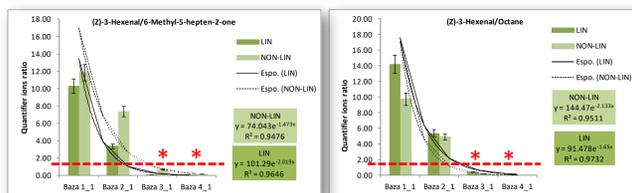
The ratio between informative analytes can be adopted to monitor optimal ripening. Baza oils at third and fourth stage of olive ripening were classified as VO (virgin) and lampante (*).



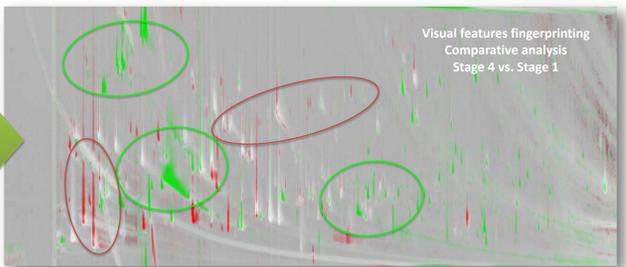
Ripening indicators

Stage-dependent evolution of ripening indicators based on Quantifier ions response ratios between key-analytes was used to define a "cut-off" value for quality classification from EVO to LO and Lampante (*).

TOF sensitivity at 70 eV enabled to work in HS linearity conditions, thereby exploring a wider fingerprint dynamic range and simultaneously cross-validating ripening indicators¹.



Fingerprint evolution from EVO to VO and lampante



The algorithm computes the **difference at each data point between pairs of TICs**; a data point is the output of the detector at a point in time. These differences are mapped into Hue-Intensity-Saturation (HIS) color space to create an image for visualizing the relative differences between image pairs in the retention-times plane. In the regions colored in green, the difference is positive indicating that the relative response is higher in the analyzed image (Stage 4) whereas red pixels (negative difference) indicate a larger relative response in the reference image (Stage 1). The intensity depends on the magnitude of the difference; white saturation indicates pixels at which peaks have nearly equal relative detector responses in the reference and analyzed image.

Oils from Benamaurel plot varied in quality as a function of olives ripening and, as a consequence, their commercial classification. The last harvest stage (beginning of January 2015) produced virgin (VO) oils. This de-qualification was due to the presence of aroma defects (not qualified by the panel) Md>0.00. VOCs pattern at the latest stage resulted rich of saturated and unsaturated aldehydes (autoxidation and LOX products), some saturated hydrocarbons (octane) and, within carbonyl derivatives, 6-methyl-5-hepten-2-one was the most relevant.

Conclusions

GCxGC-TOF MS at 70 eV allowed us to identify about 160 analytes reliably, including potent odorants belonging to furanones and pyranones groups (lactones) present in the oils at ppb level. HS-SPME sampling under headspace linearity conditions is a clear advantage in terms of method dynamic range and quantitation potentials.

Acknowledgments

