High-informative chromatographic fingerprinting of raw hazelnuts volatiles by comprehensive two-dimensional gas chromatography coupled with Time of flight mass spectrometry: challenges in defining odorant patterns related to sensory defects

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Aim and scope

The capture of volatiles patterns encrypting the Chemical Odor Code [1], i.e. the chemical code of odor perception of a food, poses severe challenges for mono-dimensional gas chromatographic platform. Separation power, improved sensitivity and resolution enhancement are key-features that make comprehensive two-dimensional gas chromatography (GC×GC) a platform of choice to achieve accurate and reliable results within *sensomic* workflows [2]. This study explores the potentials of high-informative fingerprinting on raw hazelnuts volatiles by combining head-space solid phase microextraction to GC×GC and Time-of-flight Mass Spectrometry. Hazelnuts of different geographical origin and cultivar, selected by flash-profile descriptive analysis for the presence/or not of sensory defects, are profiled and their 2D patterns processed by combined Untargeted and Targeted (*UT*) fingerprinting based on template matching principles. Visual features fingerprinting is also applied to better highlight pattern differences and peculiarities. Unsupervised and supervised chemometrics are adopted on 2D peaks quantitative descriptors to find reliable and informative peak-patterns for effective discrimination and classification of samples on the basis of their sensory quality.

GC×GC-TOFMS platform

GC×GC System

Agilent 7890 GC equipped with a standard S/SL injector and fast FID

Loop-type thermal modulator

Zoex KT 2004 loop-type thermal modulator
Optimode v2.0 - Cryogenic LN_2

Capillary columns, unions and non-purged tees were from SGE or MEGA (Milan, Italy)

TOF MS

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

2D data were processed by GC Image® GC×GC Edition Software Release 2.7 (GC Image Lincoln NE, USA)

Headspace Solid Phase Microextraction

HS-SPME sampling conditions

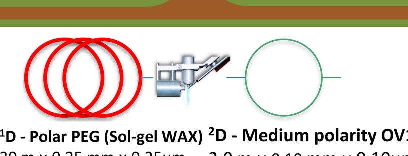
- Sampling: 1.5 g of Hazelnut
- Temperature: 50°C
- Time: 40 min with the pre-loading of the internal standards (α/β-thujone and methyl-2-octynoate)

Vial volume: 20 mL
Fiber: DVB/CAR/PDMS; 50/30 µm; 1 cm Supelco Bellefonte.

¹D - Polar PEG (Sol-gel WAX)
30 m × 0.25 mm × 0.25µm
He carrier @ **1.7 mL/min**

²D - Medium polarity OV1701
2.0 m × 0.10 mm × 0.10µm
He carrier @ **1.7 mL/min**

GC Oven programming: 40°C(2') to 240°C (10') @ 3.5°/min
S/SL injector: 250°C, Split ratio 1:10
MS Transfer line: 250°C tandem ionization: 70 and 12eV
MS Aquisition: Mass range 40-350 m/z; frequency 50 Hz
Optimode settings: modulation period **3.5 s**, hot-jet pulse 250 ms -cold jet stream MFC from 40% to 5% in 70 min



Samples

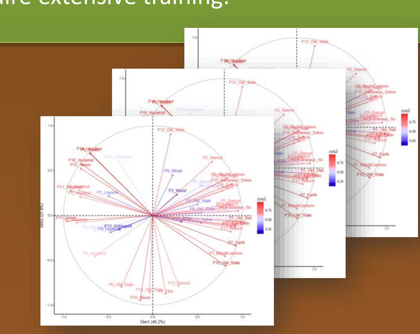
7 OK: Tasty huzelnuts, free from any off-flavor note

7 Uncoded KO: Huzelnuts showing unspecific defects

19 Coded KO:
- 4 Mould-rancid-solvent
- 3 Mould
- 6 Rancid-stale
- 3 Rancid
- 3 Rancid- solvent

Flash profile

Flash profile (FP) is an alternative sensory analysis technique to understand the sensory positioning of products. Untrained subjects select their own terms to describe and evaluate a set of products simultaneously, and then rank the products for each attribute that they individually create. Flash Profile does not require training session because the subjects have to rank products on an ordinal scale for each term. The advantages of this technique are that it is less time consuming and is more cost effective when compared to other available descriptive analysis methods that require extensive training.

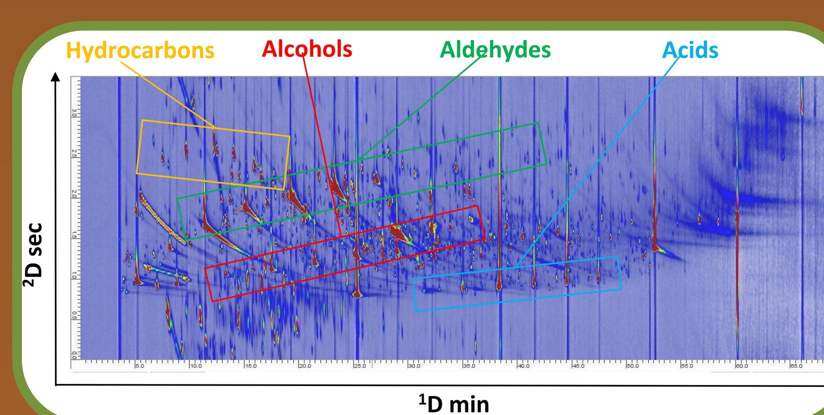


Volatile 2D pattern

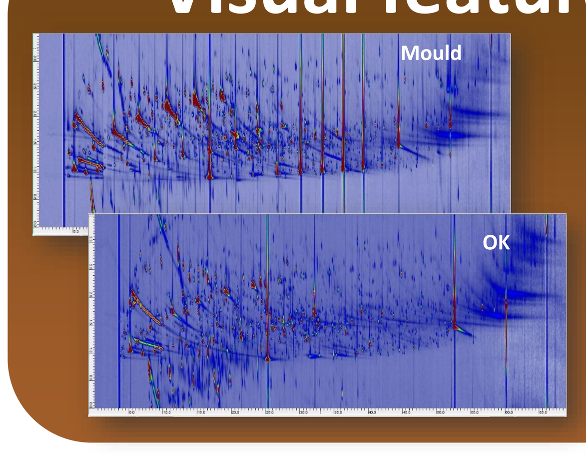
Two basic requirements for a truly multidimensional analysis:

- Orthogonal separation
- Separation achieved in the first dimension must be kept in the second dimension

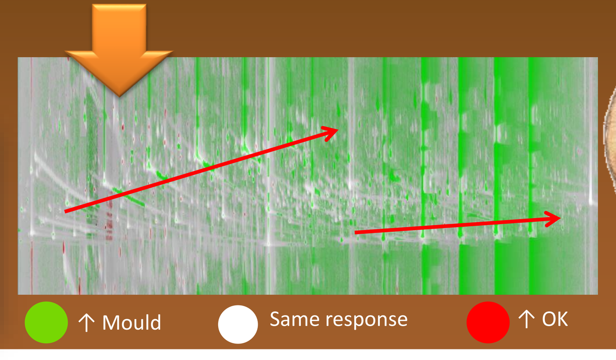
The picture shows a bidimensional chromatographic analysis: different classes of chemical compounds follow a distinctive distribution.



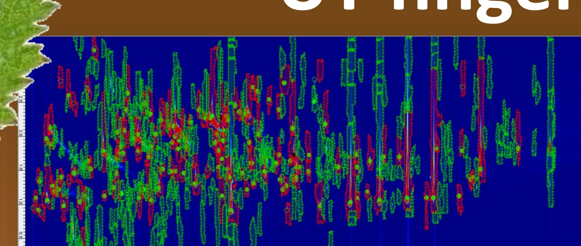
Visual features fingerprinting



Comparative visualization - pair-wise
Pixel based comparison - each pixed is a detector data package



UT fingerprinting

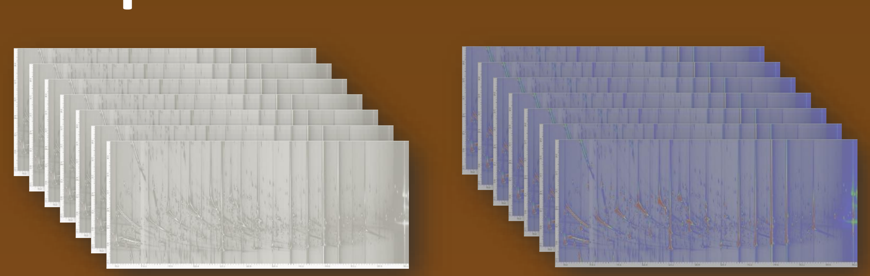


✓Comprehensive mapping of all detactable 2D peaks or 2D peak-regions
✓Realignment of 2D peaks across all chromatograms
✓Pattern recognition and data mining

When a template is comprehensively built on 2D peak patterns from a data set by including targeted and untargeted (UT) analytes reliably matched across samples, a truly comprehensive mapping of sample chemical complexity is possible.
Template features selection rules can be extended to *UT templates* for 2D pattern comparison between batches acquired over large time frames.

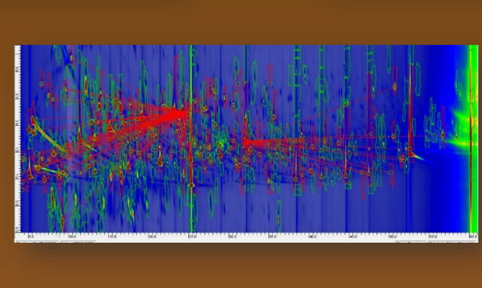
RESULT AND DISCUSSION

Reference patterns - cumulative image creation



Creation of an **average reference image** for each sample class – Images sum and normalization

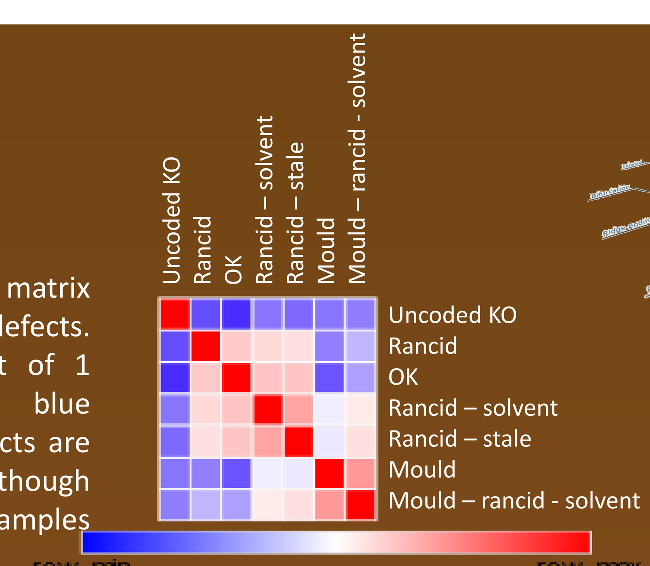
Reference for OK samples
Reference for KO not coded samples
Reference for Mould samples etc….



Reference pattern(s), representative of samples diversity, mapped for all untargeted and targeted 2D peaks and peak-regions are submitted to pattern recognition.

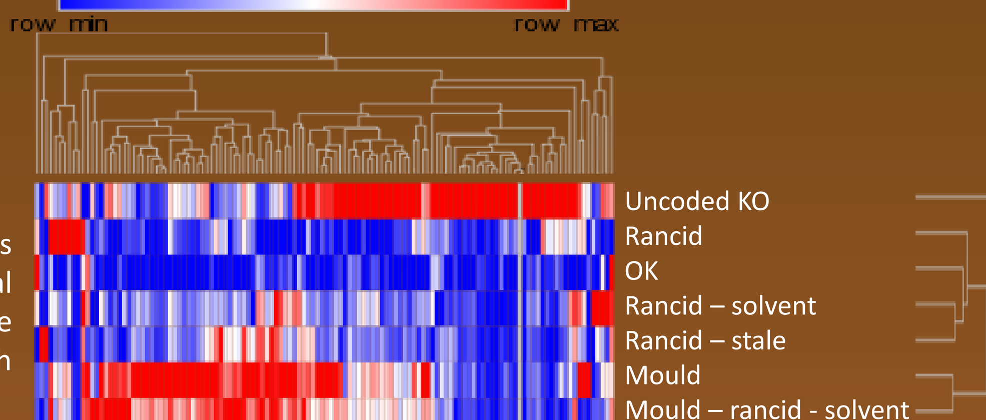
Correlation matrix

Heatmap showing the results of the correlation matrix between sets of variables describing coded defects. Red corresponds to a correlation coefficient of 1 (maximum degree of correlation) while blue corresponds to 0 (no correlation). Some defects are connoted by distinctive patterns of volatiles although some analytes are in common between samples (Rancid and Mould).

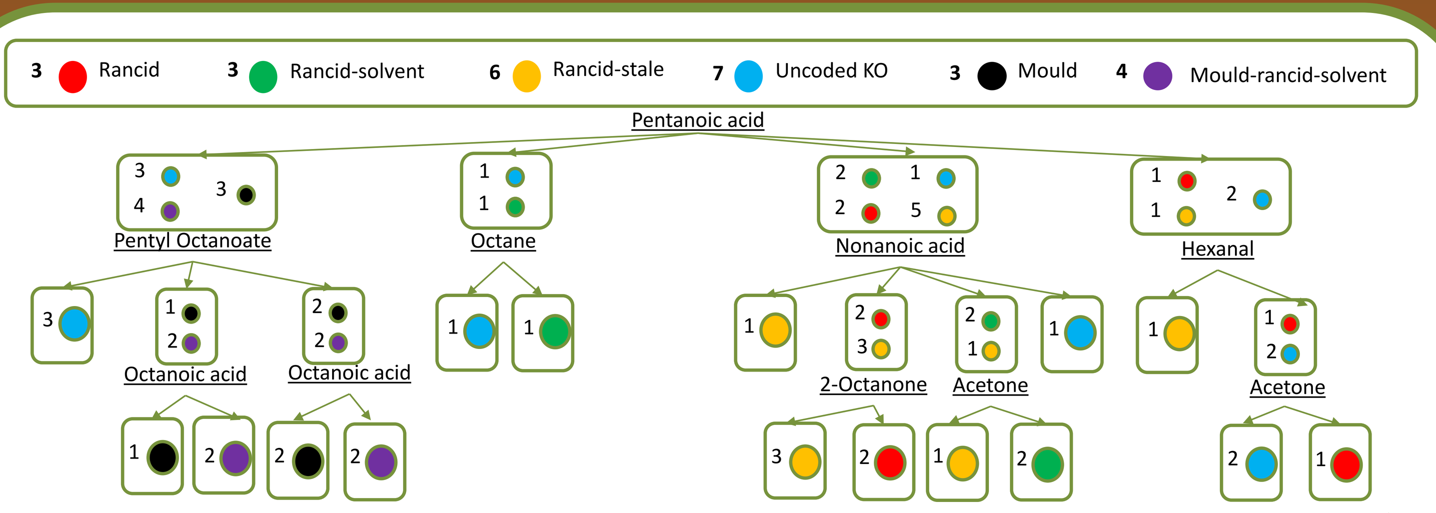


Hierarchical clustering

Heatmap showing the variables distribution across the set of samples together with the hierarchical clustering, based on Euclidean distances - Z-score normalization, indicating group of chemicals with common trends within the set.



Regression tree



By splitting samples with a ratio 4:1 in training set and validation set, the average confusion matrix shows a **96.15%** of correct classification.
This regression tree shows a specific case of 100% of correctness

The 2D patterns of volatiles from good quality and defected hazelnuts show a great complexity; about 350 2D peak-regions are detectable with about 120 reliably targeted analytes. ***UT fingerprinting*** performed on 70 eV EI data, merges targeted and untargeted reliable peak-features delineating patterns of analytes capable of clearly clusterize samples with *mouldy* notes, those with *rancid* and *solvent-like* odors. In addition, samples with *mouldy* notes show diagnostic peak-patterns dominated by several aldehydes (**Nonanal, Heptanal, (E)-2-Decenal, (E)-2- Nonenal, (E)-Undecenal**), short chain fatty acids (**Acetic acid, Pentanoic Hexanoic Heptanoic Octanoic Nonanoic acids**), linear alcohols (**1-Hexanol, 1-Heptanol, 1-Nonanol**) and furanones (**5-Butyldihydro-2-(3H)-Furanone, 5-Ethyldihydro-2-(3H)-Furanone**).
On the other hand, *rancid* samples are not clearly clustered being, this sensory defect, accompanied by additional perceptions like *stale* and *solvent-like* odors.
To create a "model peak-pattern" to be adopted as diagnostic probe for effective fingerprinting of defected hazelnuts, **visual features fingerprinting is applied** [3]. 2D patterns from samples characterized by specific defects are re-aligned and summed to obtain a cumulative image. The capture of unique odorants pattern is therefore more reliable and external sources of variability (cultivar, origin and storage time) better compensated. Through supervised chemometrics (regression trees and PLS-DA) informative analytes are selected and their discrimination role validated.

Conclusions

Experimental results confirm the effectiveness of high-informative fingerprinting by GC×GC-TOF MS and pattern recognition approaches based on template matching for quality assessment of raw hazelnuts based on chemical signatures of sensory defects. The higher level of information explored by "high-resolution" **chromatographic fingerprinting** drives to objective and univocal identification of chemical patterns related to sensory defects thanks to the high sensitivity and specificity of the approach. **Visual features fingerprinting** offers a unique option to minimize the effect of confounding variables and, at the same time, effectively drives to conclusive results.

References

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[3] S.E. Reichenbach, X. Tian, C. Cordero, Q. Tao, Features for non-targeted cross-sample analysis with comprehensive two-dimensional chromatography, J. Chromatogr. A. 1226 (2012) 140–148. doi:10.1016/j.chroma.2011.07.046.

Aknowledgements

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