Artificial Intelligence (AI) smelling based on GC×GC: a key-tool to make a step forward in food quality measurements

ARTIFICIAL INTELLIGENCE (AI) SMELLING BASED ON GC×GC: A KEY-TOOL TO MAKE A STEP FORWARD IN FOOD QUALITY MEASUREMENTS

Simone Squara, Andrea Caratti, Erica Liberto, Carlo Bicchi, Stephen E. Reichenbach, Chiara Cor

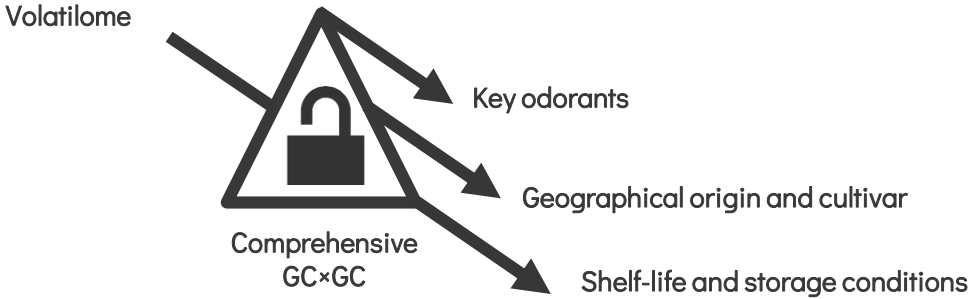
(AI) SMELLING APPROACH

Context: *Sensomics*

Principle: key-odorants and odorants patterns evoke specific smell/aroma of a food

Methods: identification and quantification of potent odorants by robust analytical methods

Results: *Sensomics* based expert system (SEBES) capable to predict key-aroma signatures without the human smell



L. Nicolotti, V. Mall, P. Schieberle, J. Agric. Food Chem. 67 (2019) 4011–4022. doi:10.1021/acs.jafc.9b00708

HAZELNUTS

- “Premium” Confectionery industry raw material
- Constant production growth
- Production areas (largest to smallest):
  Turkey (67%)
  Italy (12%)
  Azerbaijan (5%)
  USA (4%)

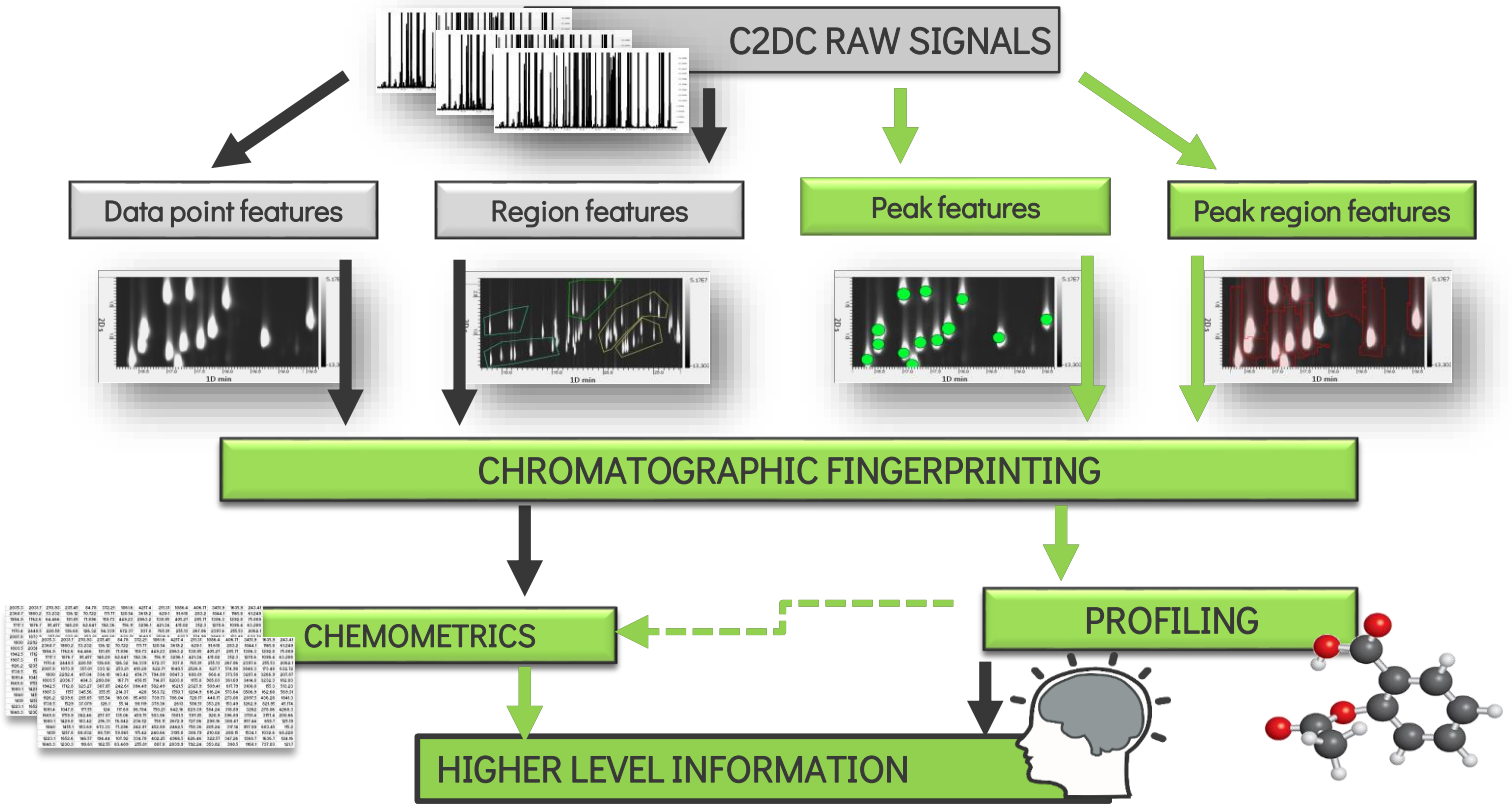
Lipids (60%)

Carbohydrates (12%)

Proteins (18%)

Moisture (5%)
Ashes (3%)
Volatiles (<0.01%)

Data processing work flow



F. Stilo, C. Bicchi, A.M. Jimenez-Carvelo, L. Cuadros-Rodriguez, S.E. Reichenbach, C. Cordero, Chromatographic fingerprinting by comprehensive two-dimensional chromatography: Fundamentals and tools, TrAC Trends Anal. Chem. 134 (2021) 116133. doi:10.1016/j.trac.2020.116133.

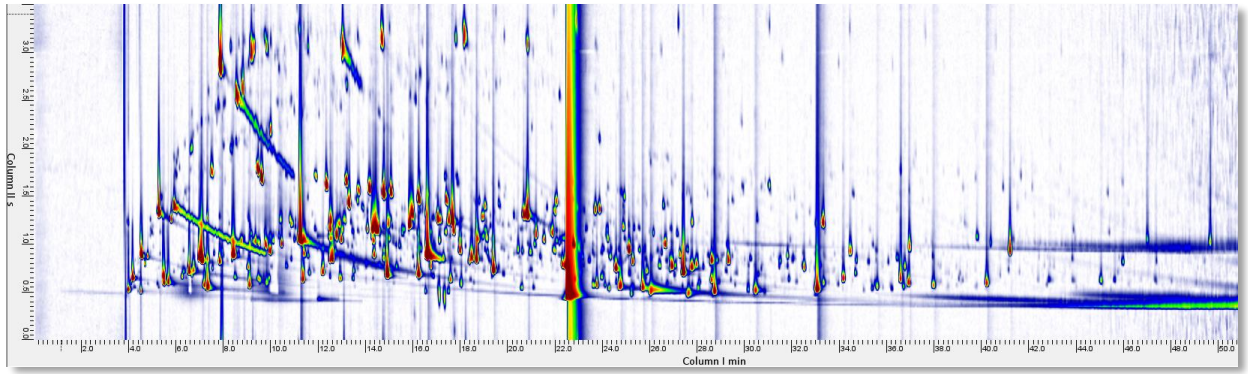
FROM THERMAL TO DIFFERENTIAL-FLOW MODULATION

460 features:

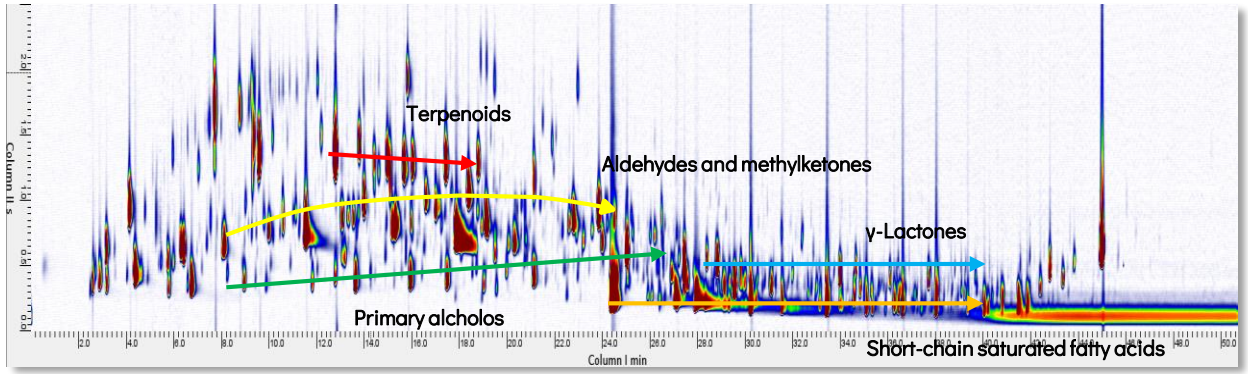
Chemical patterns

Aldehydes
Ketones
γ-Lactones
Short-chain saturated fatty acids
Alcohols
Terpenoids
Aromatics
…

Define the Industrial quality

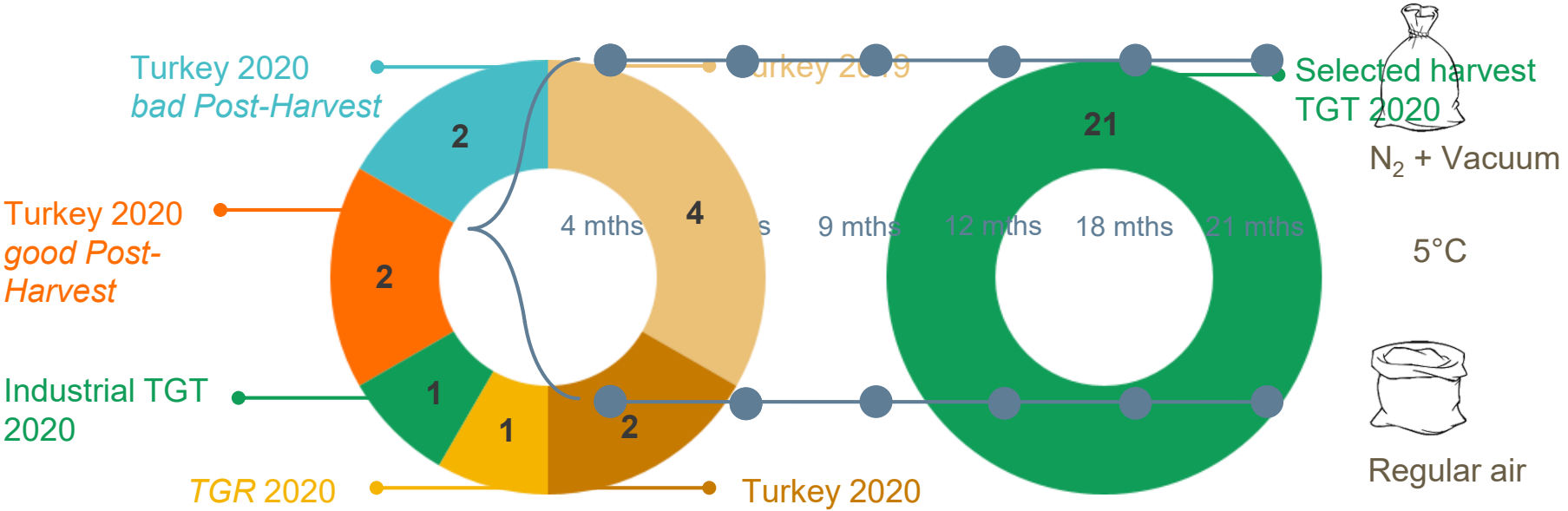


Thermal modulator

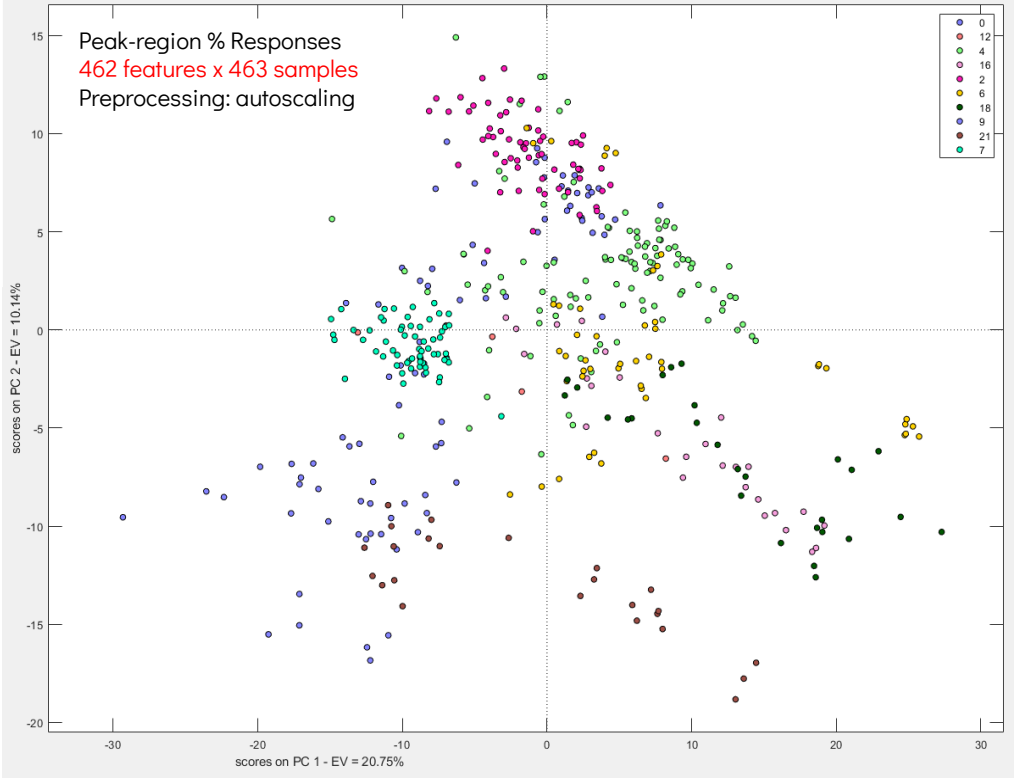


Differential flow modulator

SAMPLING DESIGN



Confounding variables

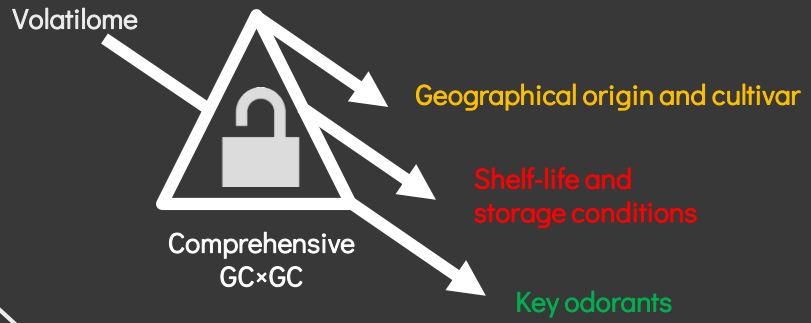


Colours according to storage months

Many simultaneous phenomena:

- 2 harvest years (2019 and 2020)
- Shelf-life (0-21 months)
- 4 varieties (2 monocultivars – *Tonda gentile Trilobata*, *Tonda gentile Romana*, 2 blends – Akçakoca, Giresun)
- 2 post-harvest conditions simulating extreme climate
- events (drought vs. heavy rain)
- 2 storage conditions (under vacuum vs. standard atmosphere)

Industrial quality

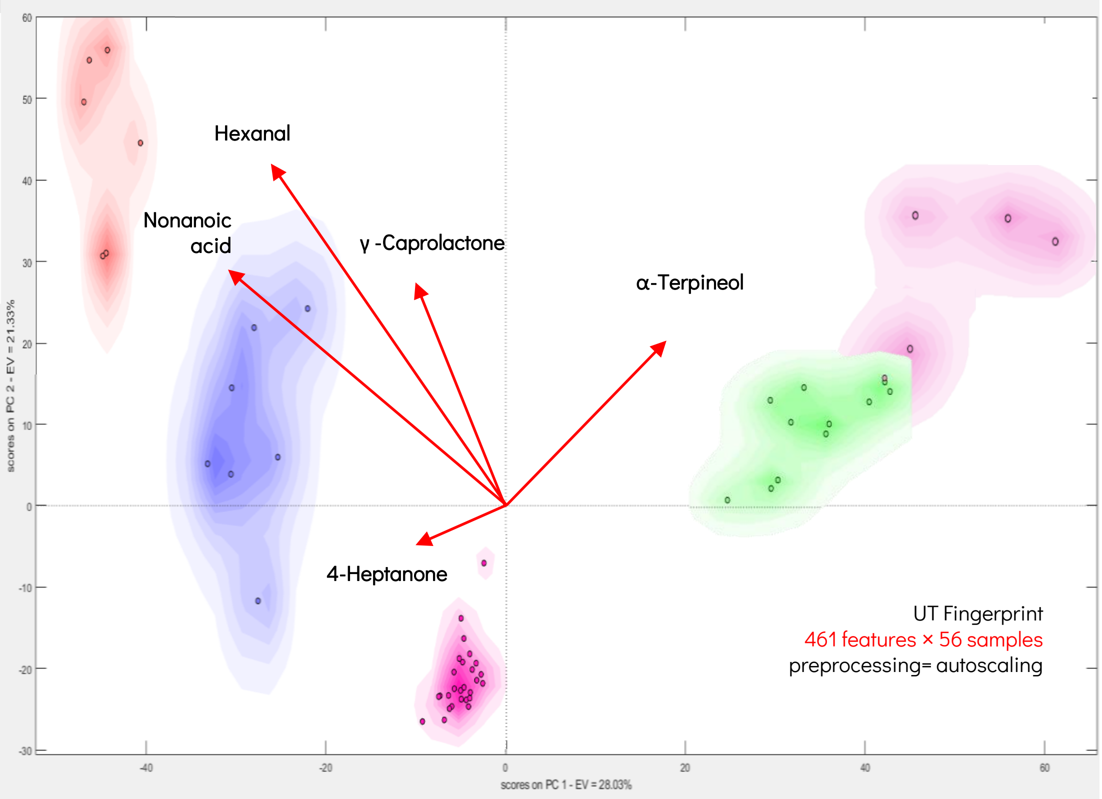


Quality from an industrial point of view:

- Discrimination of the gold standard

- Rancidity
- Aroma blueprint and spoilage (oxidative and enzymatic pathways)

"GOLD" STANDARD DISCRIMINATION



Loadings "Honorable mentions":

- Tonda Gentile Tril obata 2020 samples
- Akçakoca 2020 samples
- Akçakoca 2019 samples
- Rabold samples
- Mouldy samples

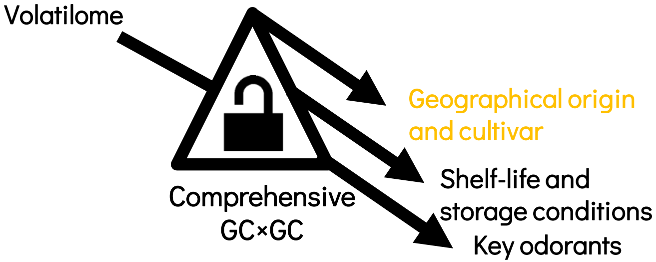
4-Heptanone: Ketones class has *nutty* and *fruity* smell

Nonanoic acid: Short chain fatty acids derive from lipid degradation, *cheesy* and *waxy* smell

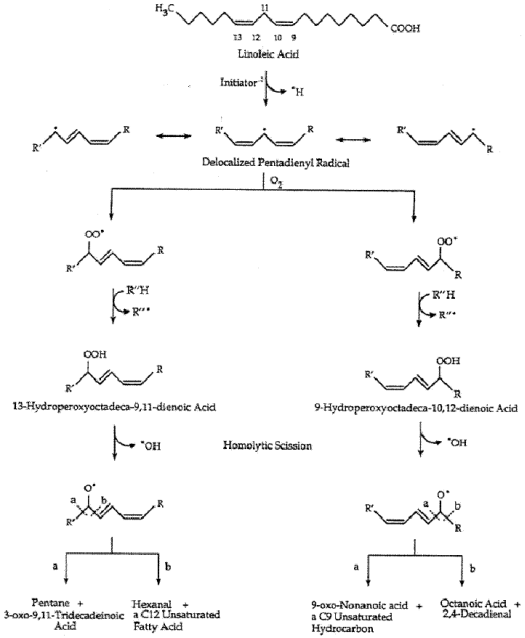
γ –Caprolactone: formed within the mold metabolic path, *sweet* notes

α-Terpineol: Terpenoids have defensive roles in plants, *floral* smell

Hexanal: Aldehydes are secondary products of lipid oxidation

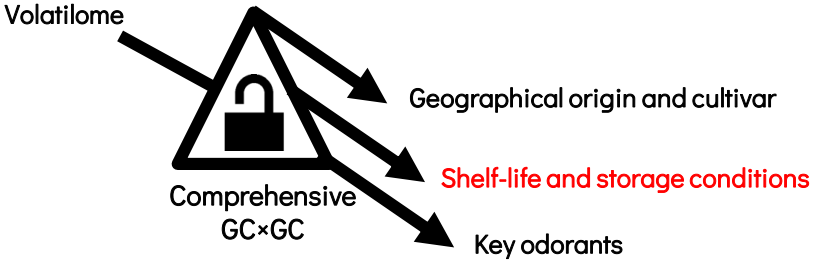


RANCIDITY ASSESSMENT



Aldehydes, ketones and alcohols are the secondary product of oxidative processes on lipids

Short-chain fatty acids as markers of further oxidation processes on the corresponding aldehydes



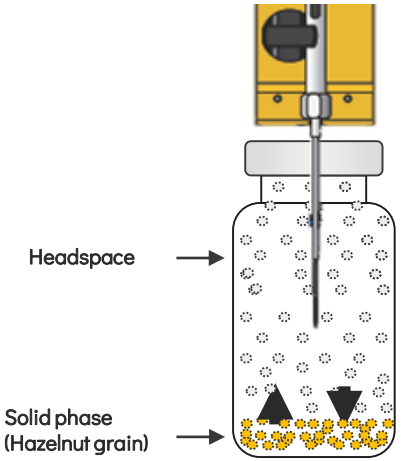
Shahidi F. Assessment of lipid oxidation and off-flavour development in meat and meat products. *Flavor of Meat and Meat Products*. Springer, Boston, MA, 1994.

M. Cialiè Rosso *et al.*, "Evolution of potent odorants within the volatile metabolome of high-quality hazelnuts (Corylus avellana L.): evaluation by comprehensive two-dimensional gas chromatography coupled with mass spectrometry," *Anal. Bioanal. Chem.*, vol. 410, no. 15, pp. 3491–3506, 2018.

ANALYTICAL PLATFORM

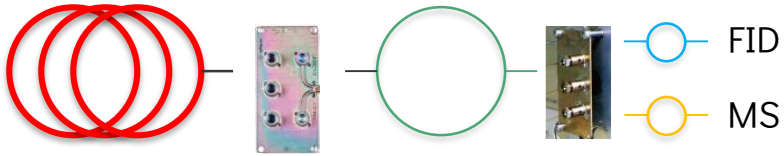
Solid phase microextraction (SPME)

- DVB/CAR/PDMS d_f50/30 µm – 2cm
- 50 °C
- 50 minutes
- 100mg ± 0.2 mg of raw grain



GC×GC system setup

- GC Oven ramp: 40°C(2') to 130°C @ 4°/min, to 260°C (10') @ 8°/min
- S/SL injector: 250°C, pulsed split, split ratio 1:5, injection pulse 250 kPa Until 2.5 minP
- Reverse fill/flush differential flow modulator: P_M 2.5s, Inj 250ms
- Passive splitter to parallel detector MS/FID
- MS Transfer line: 270°C Agilent 5977B High-efficiency source single quadrupole. Mass range 40-250 m/z



^{1}D - Polar PEG (Sol-gel WAX)
20 m × 0.18 mm × 0.18µm
He carrier @ **0.4 mL/min**

^{2}D – Medium polarity OV17
1.8 m × 0.18 mm × 0.18µm
He carrier @ **10 mL/min**

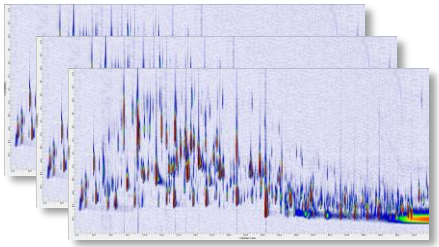
Passive splitter
0.5 m × 0.1 mm to MS
1.1 m × 0.18 mm to FID
FID:MS ratio = **70:30**

Data processing

- GC Image

Data mining

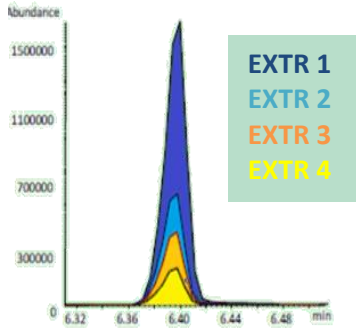
- Matlab



ACCURATE QUANTIFICATION

Quantification approach:

- External calibration
- Multiple headspace extraction (MHE)



$$A_T = \sum_{i=1}^{=\infty} A_i = A_1 \frac{1}{(1 - e^{-q})} = \frac{A_1}{(1 - \beta)}$$

$0.4 < \beta < 0.95$

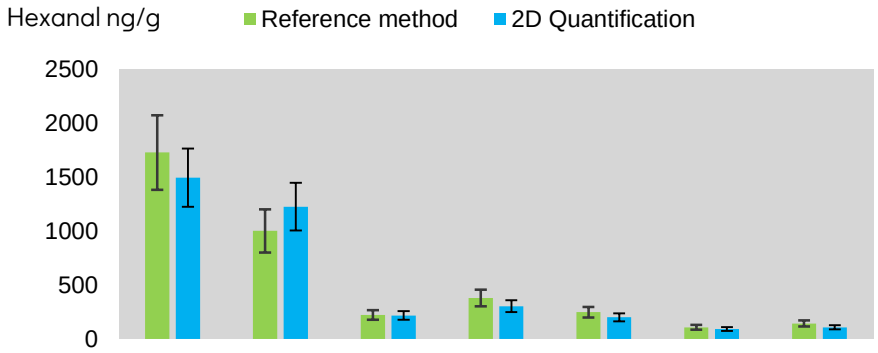
R. Costa, L. Tedone, S. De Grazia, P. Dugo, L. Mondello, Multiple headspace-solid-phase microextraction: An application to quantification of mushroom volatiles, Anal. Chim. Acta. 770 (2013) 1–6. doi:10.1016/j.aca.2013.01.041.

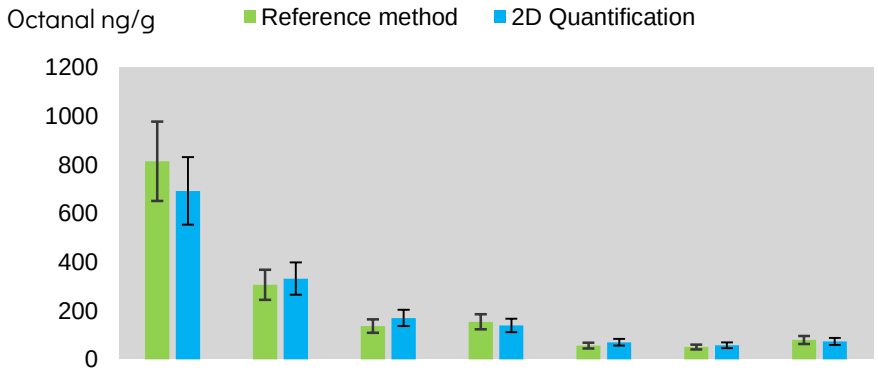
Method validation

Validated 1D-GC-MS method for the quantification of saturated aldehydes used as a reference

Validation on 30 samples at different oxidation stages

Mean % Errors	Old samples	Medium old	Fresh
Hexanal	17.9	13.6	19.6
Octanal	11.6	19.2	10.3





EXTENDED LIST OF QUANTIFIED ANALYTES

A total of 29 rancidity related markers identified (aldehydes, ketones, alcohols, organic acids)

Simplifying the procedure

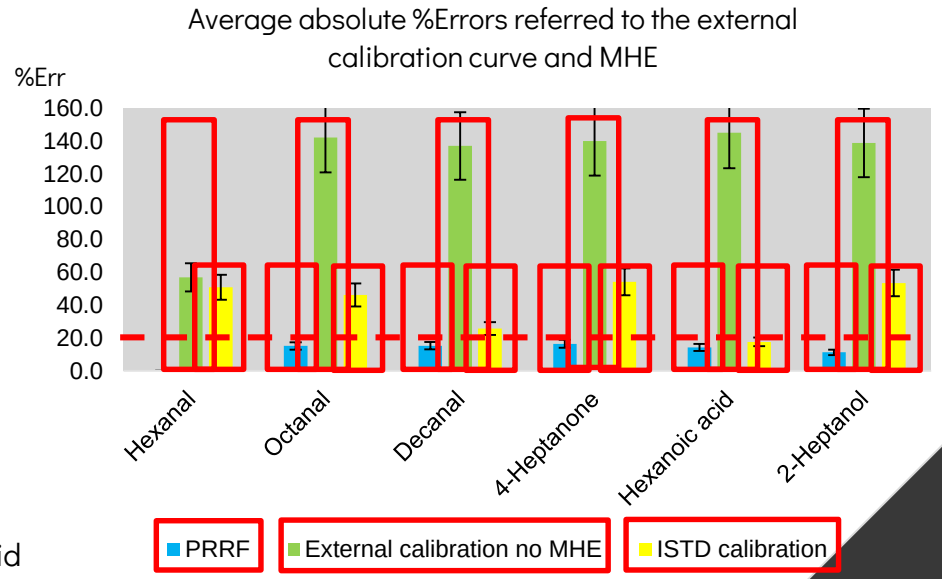
Exploiting the FID wider linearity range

Use of predicted response factors (PRRF) relative to Hexanal

Quantified rancidity markers:

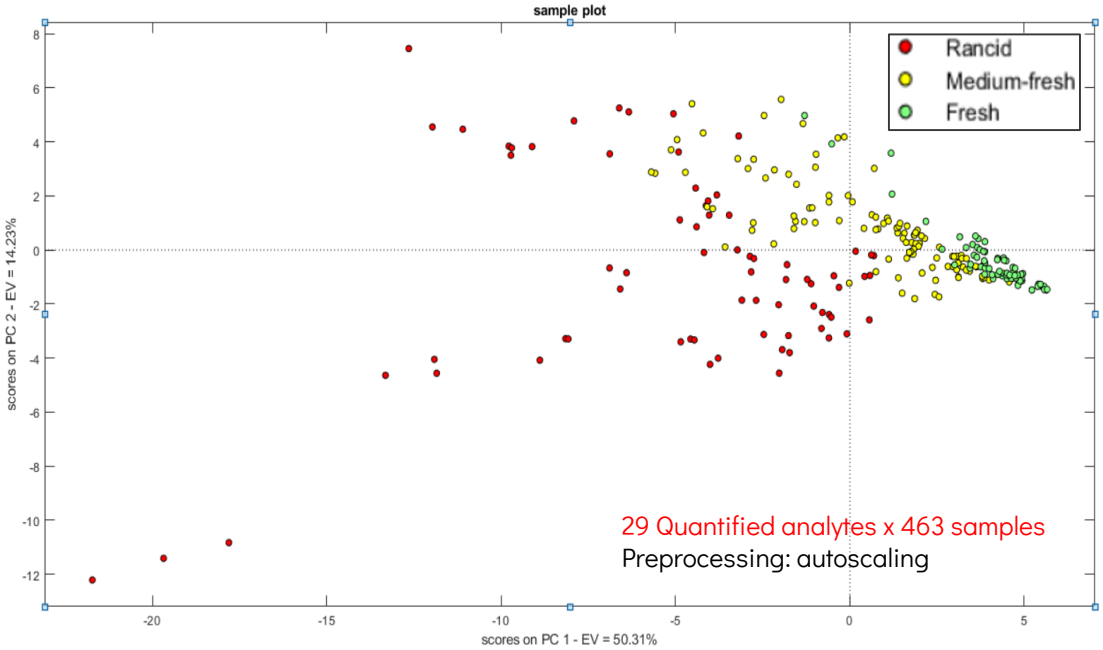
Pentanal	4-Heptanol
Hexanal	2-Heptanone
Heptanal	2-octanone
Octanal	2-Nonanone
Nonanal	2-Decanone
Decanal	Propanoic acid
(E)-2-Hexenal	Butanoic acid
(E)-2-Heptenal	Pentanoic acid
(E)-2-Nonenal	Hexanoic acid
(E)-2-Decenal	Heptanoic acid
1-Pentanol	Octanoic acid
1-Hexanol	Nonanoic acid
1-Octanol	Decanoic acid
2-Hexanol	3-Methylbutanoic acid
2-Heptanol	

$$RRF=10^3 * \left(\frac{MW_i}{MW_{IS}}\right) * (-61.3 + 88.8nC + 18.7nH - 41.3nO + 6.4nN + 64.0nS - 20.2nF - 23.5nCl - 10.2nBr - 1.75nI +$$



J.Y. De Saint Laumer, S. Leocata, E. Tissot, L. Baroux, D.M. Kampf, P. Merle, A. Boschung, M. Seyfried, A. Chaintreau, Prediction of response factors for gas chromatography with flame ionization detection: Algorithm improvement, extension to silylated compounds, and application to the quantification of metabolites J. Sep. Sci., 38 (2015), pp. 3209-3217, 10.1002/jssc.201500106

RANCIDITY HIERARCHY



Classes created with K-mean clustering K=3

Important decision making tool for shelf life strategies

Rancidity assessment with incoming raw materials

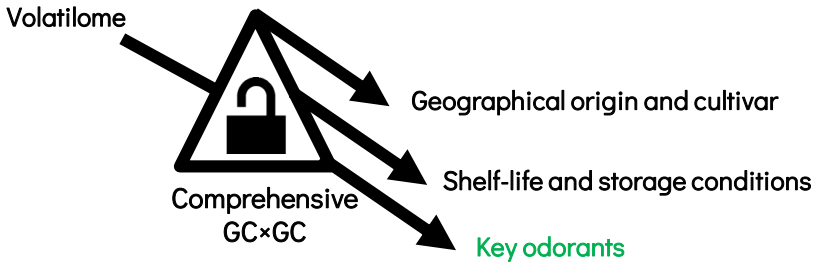
(Al) SMELLING AND SPOILAGE

42 Quantified analytes

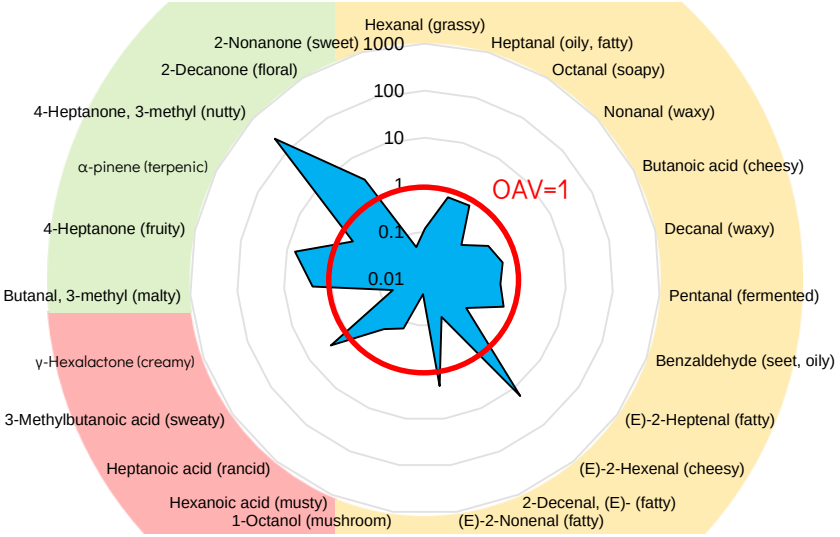
Odour threshold (OT): lowest concentration of a certain odorant that is perceivable by the human sense of smell

$$OAV = \frac{[Analyte\ ng/g]}{OT\ ng/g}$$

Odour activity value (OAV)>1 for analytes that have an impact on the aroma



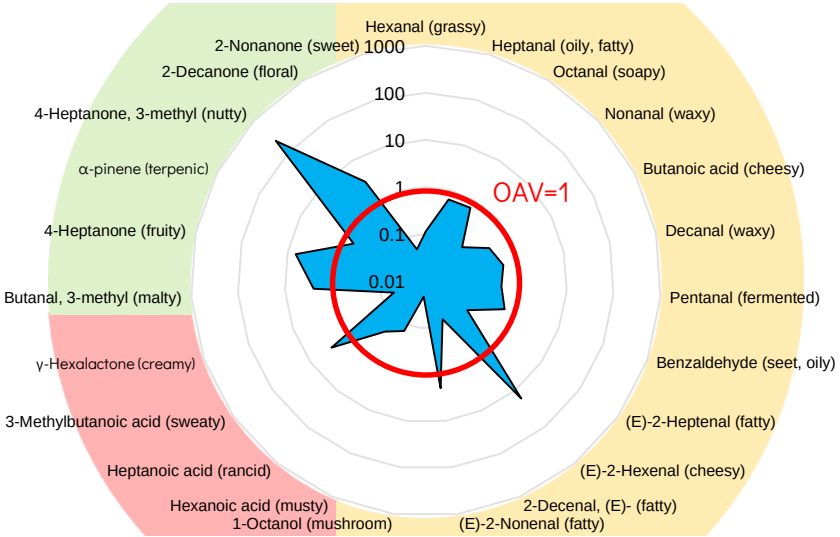
Tonda Gentile Trilobata 2020



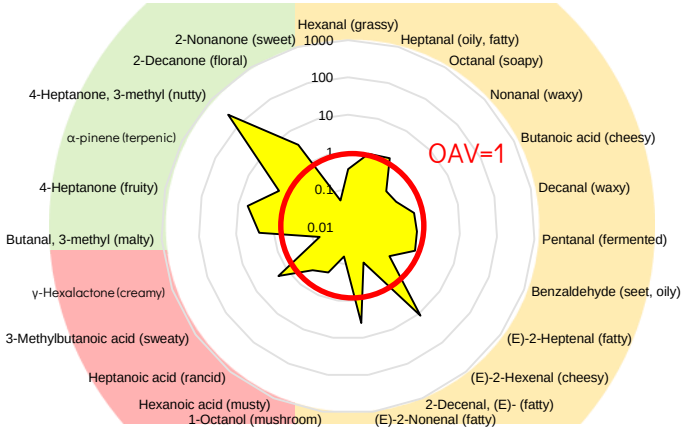
Sensory maps created from OAV values and visualized in log(10) scale

Aroma blueprint and Spoilage

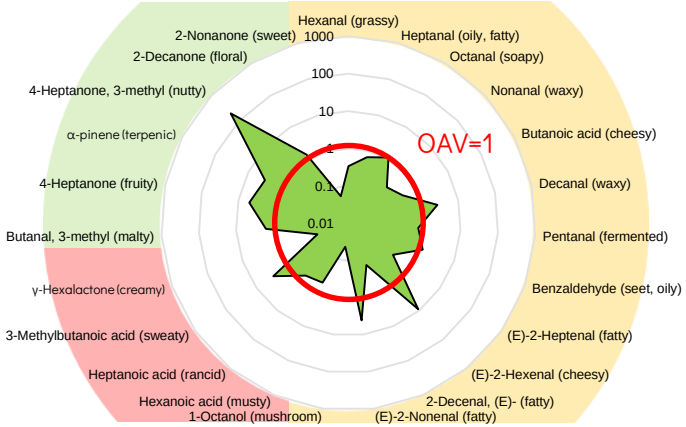
Tonda Gentile Trilobata 0 months



Akçakoca blend 0 months

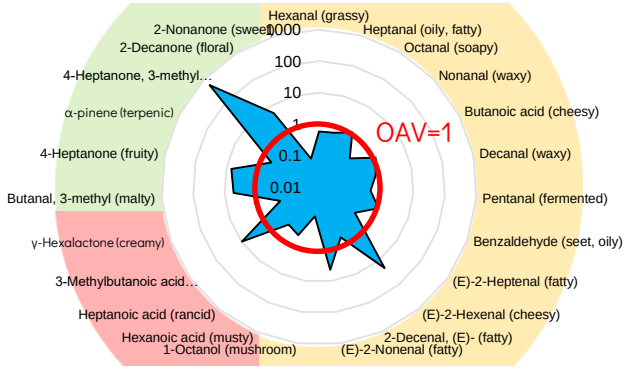


Tonda Gentile Romana 0 months

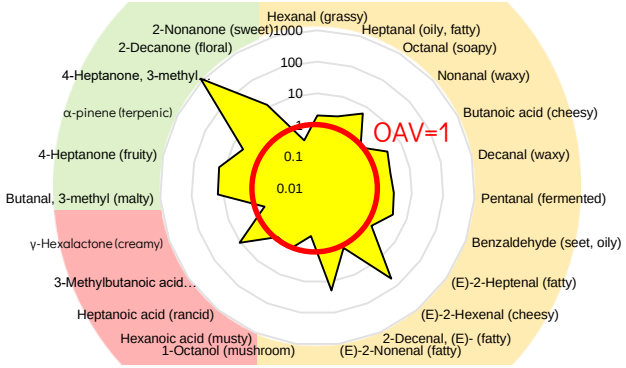


Sensory maps created from OAV values and visualized in log(10) scale

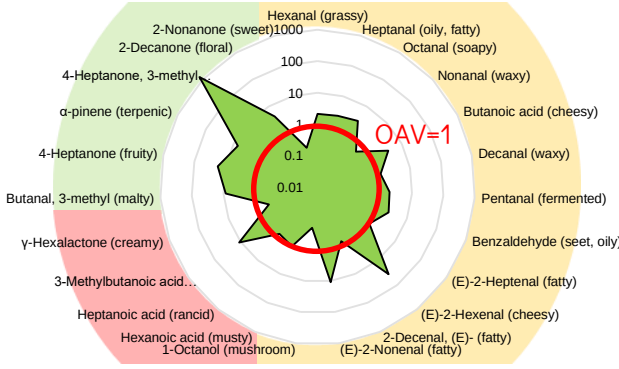
Tonda Gentile Trilobata
9 months - Under vacuum



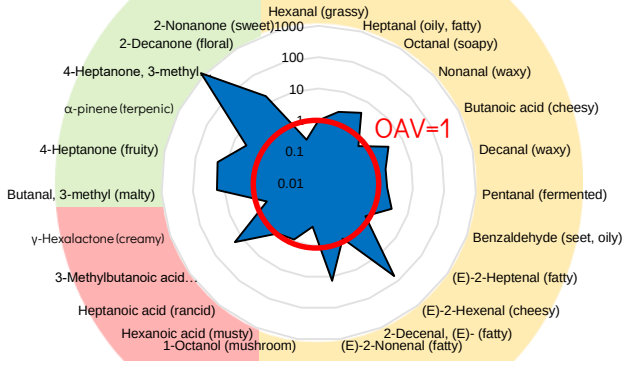
Akçakoca 9 months - Under vacuum



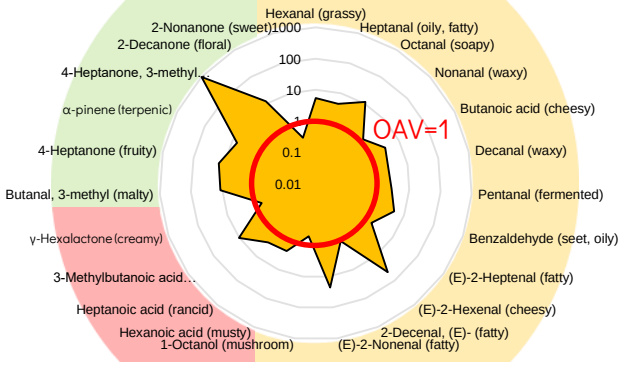
Tonda Gentile Romana
9 months - Under vacuum



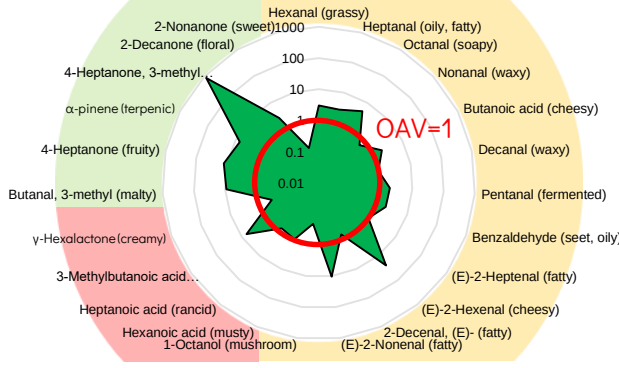
Tonda Gentile Trilobata
9 months - Air



Akçakoca 9 months - Air

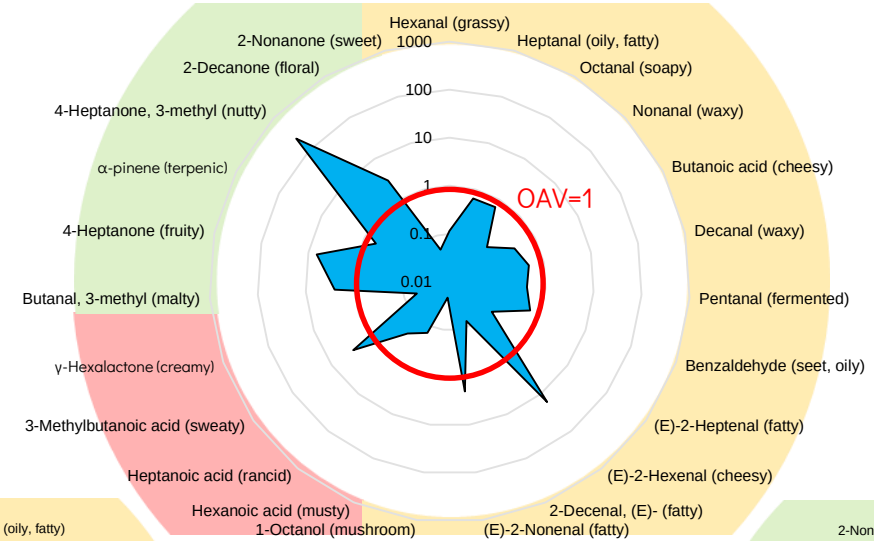


Tonda Gentile Romana
9 months - Air

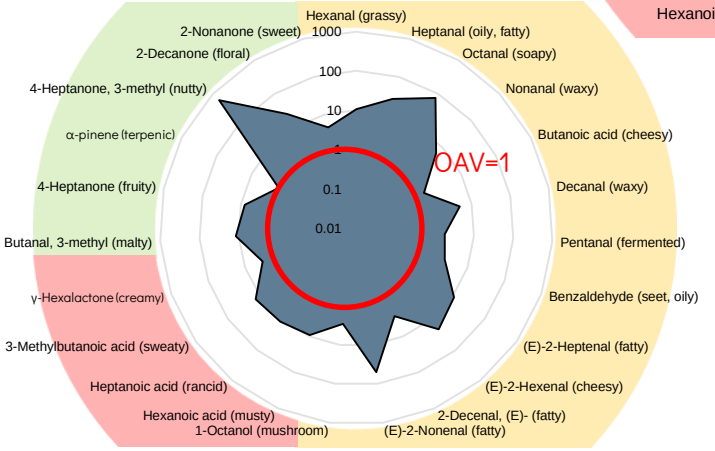


Sensory maps created from OAV values and visualized in log(10) scale

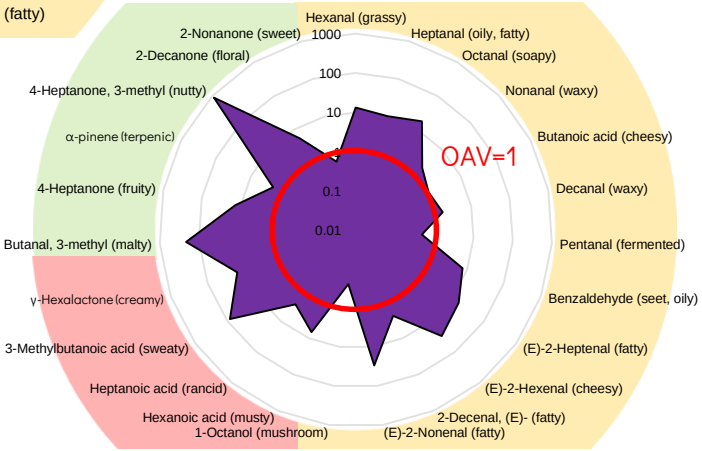
Tonda Gentile Trilobata 0 months



Rancid defect



Mouldy defect

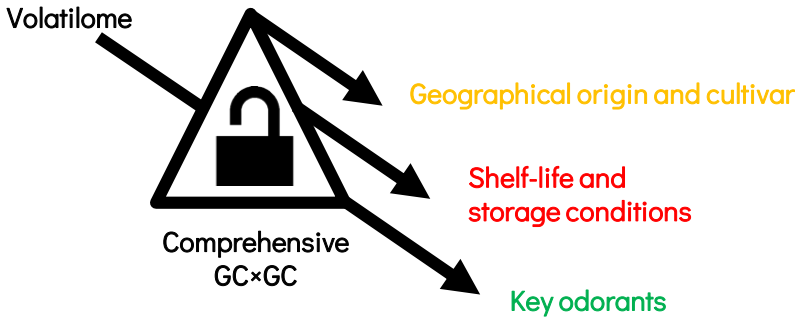


Sensory maps created from OAV values and visualized in log(10) scale

CONCLUSIONS

Routine-ready system for hazelnut fingerprinting

Comprehensive qualification of the hazelnut quality through the *sensomics* AI smelling concept implemented on GC×GC



THANK YOU!

Prof. Chiara E. Cordero

GC IMAGE
Software for Multidimensional Chromatography

Agilent

SRA INSTRUMENTS
ANALYTICAL SOLUTIONS

APPLICATIONS AND CORE TECHNOLOGY UNIVERSITY RESEARCH (ACT-UR) PROJECT #4294

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