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Artificial Intelligence smelling:
Can multidimensional chromatography play a (key) role?

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Foreword

Is comprehensive two-dimensional gas chromatography worthy to be adopted in flavour characterization? Is it simply a more complicated approach to GC separations? Does it open new opportunities to flavor analysis?

Opinions...

Gestalt: *something that is made of many parts and yet is somehow more than or different from the combination of its parts*

Key-concepts

- ✓ *Artificial Intelligence smelling* based on sensomics (SEBES*)
- ✓ Analytical dimensions of a GC×GC platform
- ✓ Investigation strategies: a change of perspective from 1D -> 2D

Shelling nuts: an *omics* approach to unravel hazelnut quality and flavour by advanced chromatographic fingerprinting techniques based on GC×GC patterns

Conclusive remarks

GC: The State of the Art

Chairperson: Pat Sandra
Participants: Steven Lehotay
Hans-Gerd Janssen
Chiara Cordero
Frank David
John Hinshaw

GC: The State of the Art

November 01, 2017

By **Chiara Cordero**, **Pat Sandra**, **John Hinshaw**, **Hans-Gerd Janssen**, **Frank David**, **Steven Lehotay**

Foreword

Pat Sandra: Comprehensive GC×GC has gained prominence at international meetings and in the literature in recent years. Do you expect a breakthrough in the coming years for routine analyses? Will modulation by temperature or by flow be mostly applied? Is the data handling sufficiently developed in terms of accuracy and speed for routine applications?

Steven Lehotay: GC×GC provides greater selectivity in separations, but as it is commonly used now, it adds too much time to the analysis. Another major problem is that a microbore second-dimension column is easily overwhelmed by high concentration matrix components, which is nearly always the case in real-world samples. GC×GC is overkill in common applications and fails in many difficult ones, thus, it needs to be used in a different way to provide faster separations with more sample capacity. I think a breakthrough in GC×GC would have been possible many years ago if the drivers of the technology had decided to overcome its practical limitations, including excessive liquid nitrogen usage for cryogenic modulation, rather than demonstrate niche applications.

Frank David: GC×GC will definitely find its way to routine application, mainly in petrochemical analysis. All types of modulators can be used, but easier, user-friendly, intuitive software and data handling are needed. Moreover, the application potential of GC×GC should not be overestimated. One-dimensional GC and GC–MS are able to cover most GC-amenable applications.

Hans-Gerd Janssen: GC×GC is already routinely used in the mineral oil area and in the flavour and fragrance industry, simply because one-dimensional GC cannot do the job. For many other applications we are forced, by government policies or for company-internal reasons, to stick with one dimensional GC. I do not expect a dramatic breakthrough for GC×GC, but it could evolve to 10–15% of the GC market.

GESTALT

noun \ gə-'stält

understanding the whole, not merely the sum of its parts.

…something that is made of many parts and yet is somehow more than or different from the combination of its parts[1]…

Flavour

Aroma

Comprehensive 2D GC

[1] Merriam-Webster.com Dictionary, Merriam-Webster, https://www.merriam-webster.com/dictionary/gestalt. Accessed 5 Apr. 2022

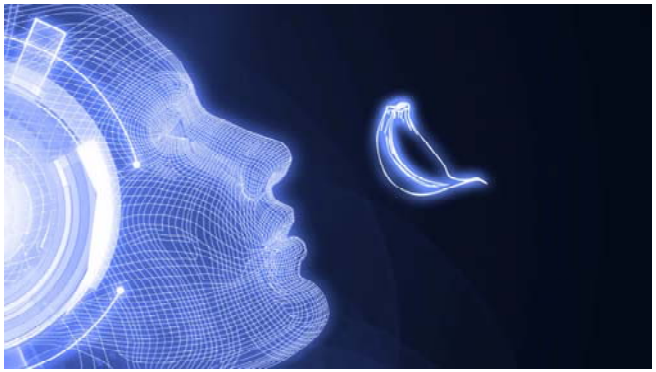
Aroma

AI Smelling

nature machine intelligence ARTICLES
https://doi.org/10.1038/s42256-020-0159-4
Check for updates

Rapid online learning and robust recall in a neuromorphic olfactory circuit

Nabil Imam[1] and Thomas A. Cleland[2]



<u>Our noses are busy beasts</u>.
At any given moment, multiple smells are competing for our attention, and somehow the brain can tell when it's smelling an orange even against a backdrop of other scents, say perfume or soap.

<u>The brain's olfactory bulb has hundreds of receptors tracking odors all the time</u>, and yet somehow keeps everything straight.
<u>Scientists at Cornell University working with researchers at Intel</u>
have just <u>created an AI algorithm trained to recognize 10 scents by mimicking the mammalian olfactory bulb (MOB).</u>
Give the algorithm a computer chip to run on and it can learn to identify new odors.[1]

[1] https://bigthink.com/ Accessed 5 Apr. 2022

Sensomics[1]

AI Smelling

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

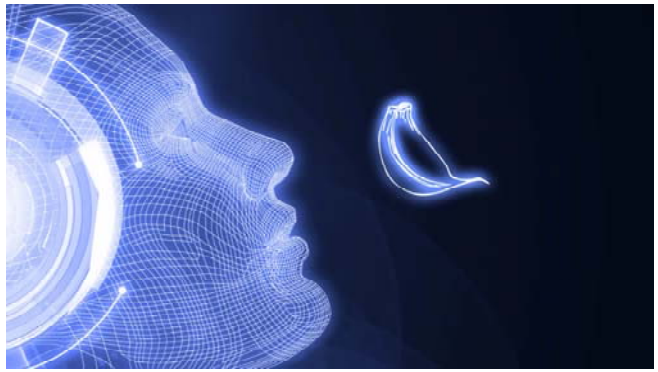
Article

Cite This: J. Agric. Food Chem. 2019, 67, 4011−4022

pubs.acs.org/JAFC

Characterization of Key Aroma Compounds in a Commercial Rum and an Australian Red Wine by Means of a New Sensomics-Based Expert System (SEBES)—An Approach To Use Artificial Intelligence in Determining Food Odor Codes

Luca Nicolotti,[#] Veronika Mall,[#] and Peter Schieberle[*,§]



Context: Sensomics[1]

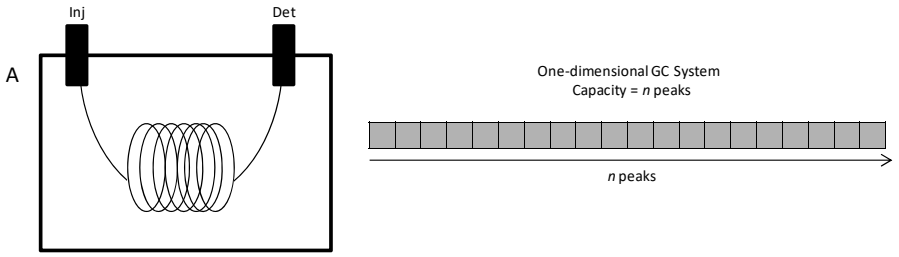
Principle: key-odorants and odorants patterns evoke specific smells/aroma qualities while contributing to define the overall flavor perception of a food - identity

Methods: extract, isolate, quantify potent odorants by reliable and robust methodologies

Outcome: ***Sensomics-Based Expert System[2] (SEBES)*** *that predicts key-aroma signatures of food without using human olfaction.*

[1] Dunkel, A.; Steinhaus, M.; Kotthoff, M.; Nowak, B.; Krautwurst, D.; Schieberle, P.; Hofmann, T. Angew. Chemie - Int. Ed. 53 (28) (2014) 7124–7143.
[2] Nicolotti, L.; Mall, V.; Schieberle, P. J. Agric. Food Chem., 67 (2019) 4011–4022

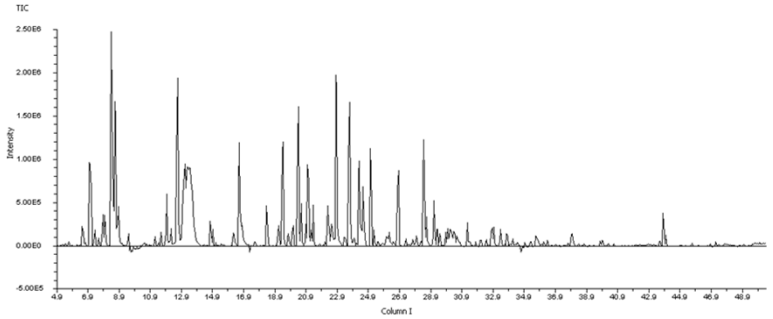
Comprehensive 2D GC



- ✓ Separation power (peak capacity) is given by the product of the two chromatographic dimensions (GC×GC)[1]
- ✓ Independent (almost) displacement in both dimensions produces rational retention patterns for homologue series[1]
- ✓ Band compression (in space - for thermal modulators) produces signal-to-noise ratio enhancement - sensitivity
- ✓ Bi-dimensional peak patterns exploits a 3D space where fingerprinting could be more accurate that in a 2D space (as for 1D-GC profiles)

[1] Giddings, J.C. (1987), Concepts and comparisons in multidimensional separation. J. High Resol. Chromatogr., 10: 319-323.

Conventional 1D GC



Profiling[1]
detailed analysis of the chemical pattern

Target(ed) analysis[2]
GC-MS metadata (retention and spectra)
analytes identity and amount

Chromatographic fingerprinting[1,3]
general and rapid high-throughput screening -> discriminate/classify samples

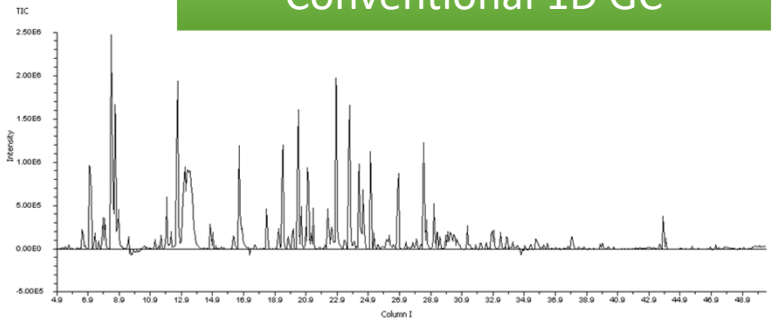
Limits
high chemical dimensionality[4]
complexity of food samples

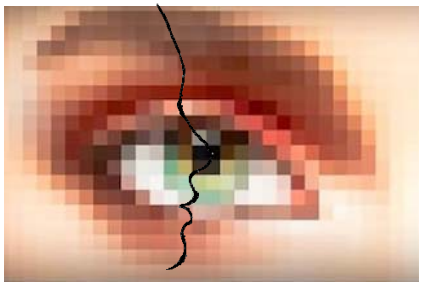
isomers/isobars might co-elute and analytes discrimination becomes challenging

Need of multiple dimensions (separation / detection) to explore compositional complexity[4]

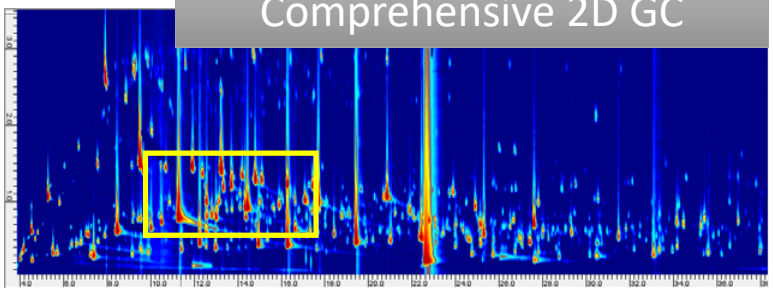
[1] Harrigan G., Goodacre R. (2003) Metabolic profiling: its role in biomarker discovery and gene function analysis. Kluwer Academic Publishers: Boston
[2] S.E. Reichenbach et al. J. Chromatogr. A 1226 (2012) 140– 148
[3] Stilo, F., Bicchi, C., Jimenez-Carvelo, A.M., Cuadros-Rodriguez, L., Reichenbach, S.E., Cordero, C. TrAC Trends Anal. Chem. 134 (2021) 116133
[4] Giddings, J. C. (1995) J. Chromatogr. A. 703, 3–15.

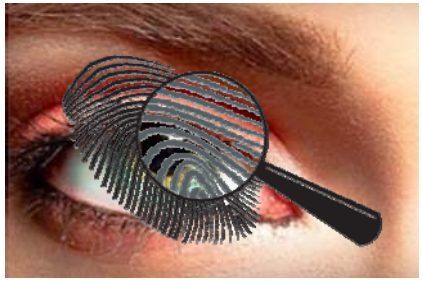
Conventional 1D GC

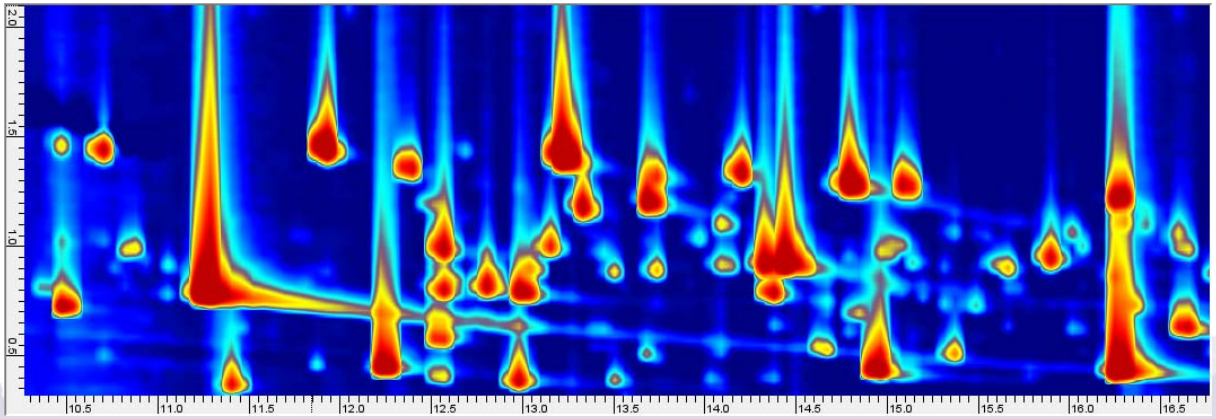




Comprehensive 2D GC







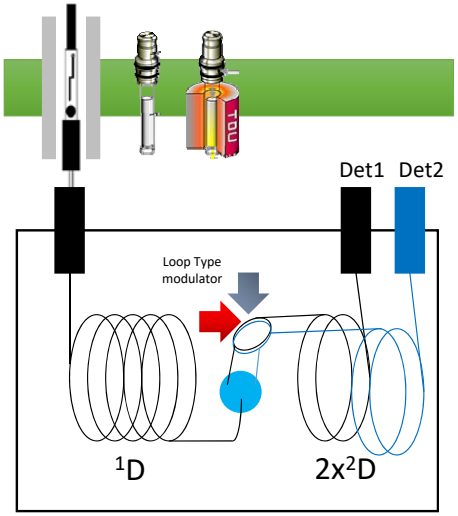
G=STALT

"High resolution" profiling
GC×GC separation power
accurate quantitative profiling

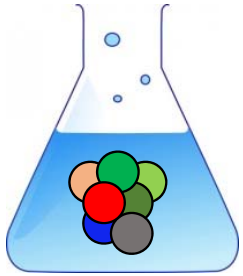
2D/3D Chromatographic fingerprinting[1]
pattern recognition (forensics)
comprehensive sample comparison

Group-Type Analysis
Rational retention logic
Ordered elution patterns

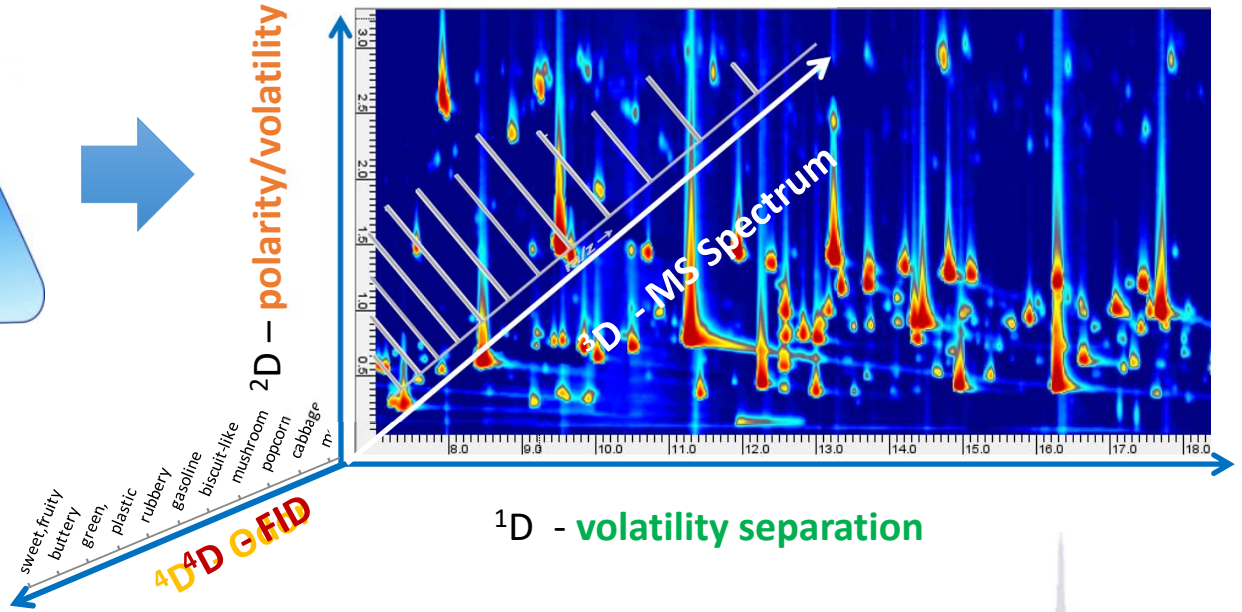
[1] Stilo, F., Bicchi, C., Jimenez-Carvelo, A. M., Cuadros-Rodriguez, L., Reichenbach, S. E., & Cordero, C. (2021). TrAC Trends in Analytical Chemistry, 134, 116133.



Sample prep - GC×2GC-MS/FID
Sample prep - GC(O)×GC-MS



Information dimensions
spectral signature (identity)
volatility/polarity
sensory descriptor (bio-assay)



[1] J.C. Giddings J. Chromatogr. A 703(1995) 3–15.

Rational information space

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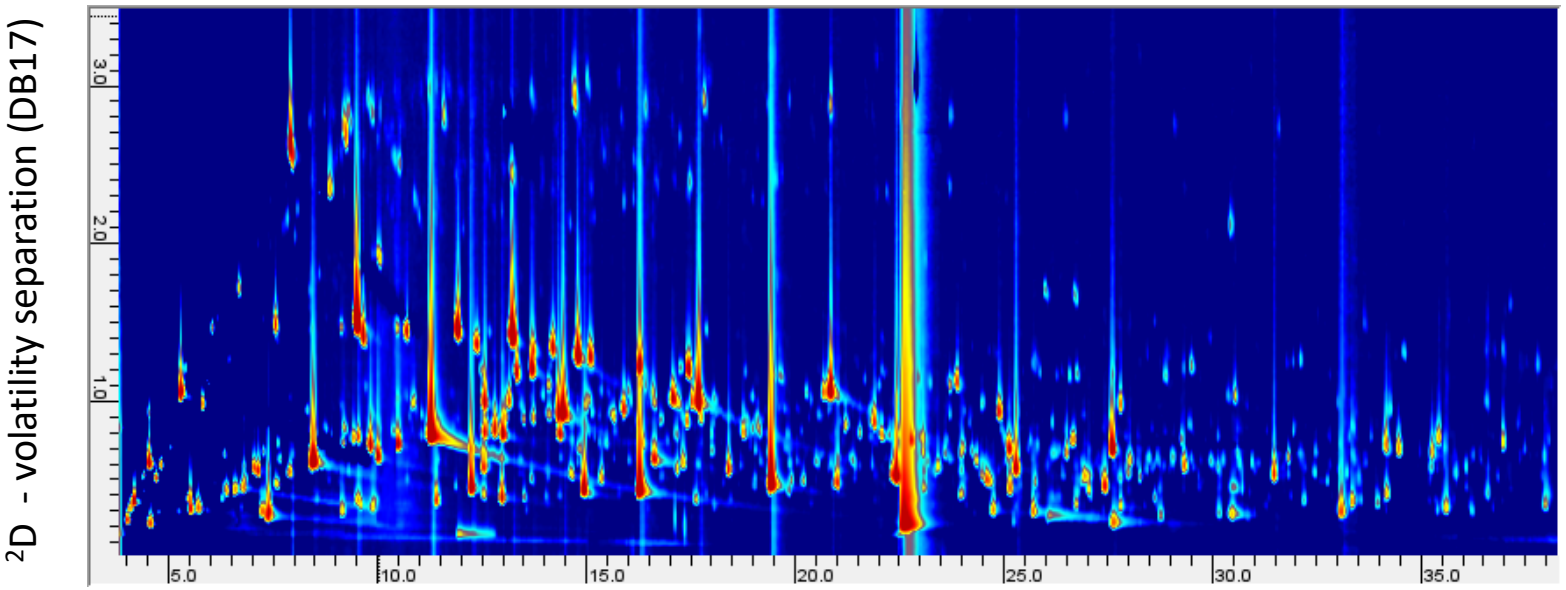
Raw hazelnut volatiles - Rancid sample Origin Turkey
HS-SPME (CAR/PDMS/DVB) - 125 mg - 50°C/50 min

Chemical dimensions



About 700 detectable features (2D peaks) over 20 S/N
Of them 250 reliably identified by 70 eV spectrum and I^T coherence
Various chemical classes highly correlated with **autoxidation** processes , enzymatic **peroxidation**, **aroma compounds** and **potent odorants**

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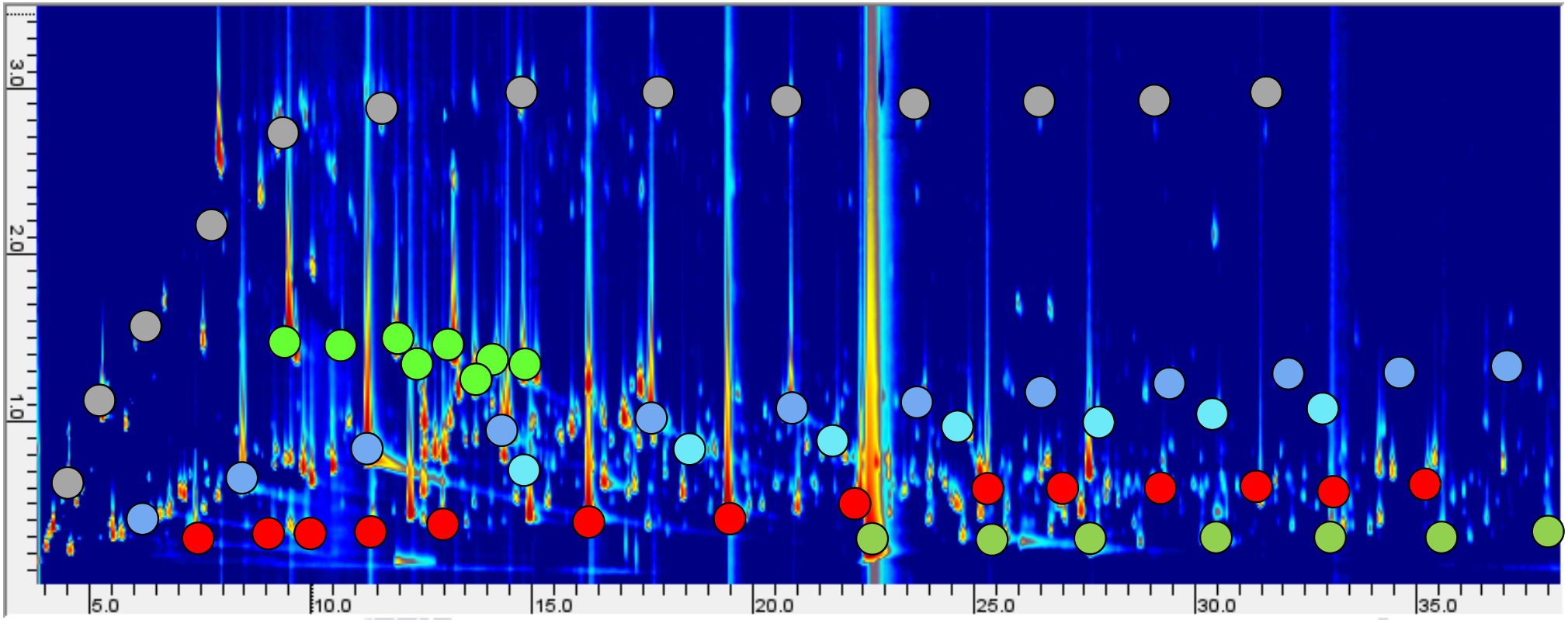
Rational information space

Raw hazelnut volatiles - Rancid sample Origin Turkey HS-SPME (CAR/PDMS/DVB) - 125 mg - 50°C/50 min

Information dimensions



n-alkanes primary alcohols fatty acids saturated aldehydes unsaturated aldehydes monoterpenes

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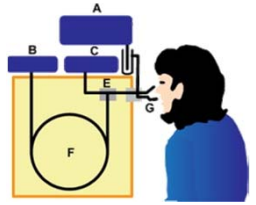
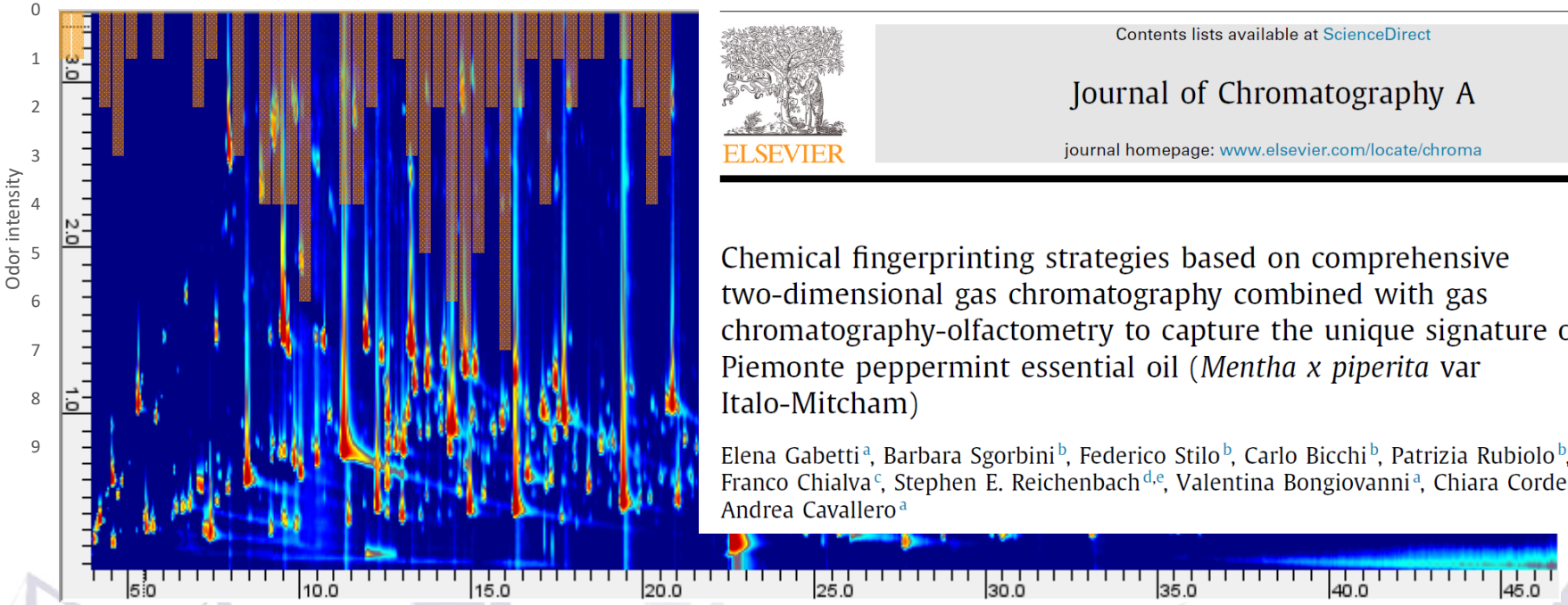
Ortogonal information space

Information dimensions



Picture from:
TrAC (2011) 30(11) :1756–1770

^{2}D - volatility separation



Contents lists available at ScienceDirect

Journal of Chromatography A

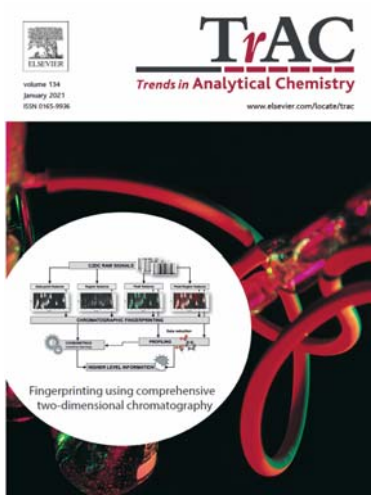
journal homepage: www.elsevier.com/locate/chroma

Chemical fingerprinting strategies based on comprehensive two-dimensional gas chromatography combined with gas chromatography-olfactometry to capture the unique signature of Piemonte peppermint essential oil (*Mentha x piperita* var Italo-Mitcham)

Elena Gabetti[a], Barbara Sgorbini[b], Federico Stilo[b], Carlo Bicchi[b], Patrizia Rubiolo[b], Franco Chialva[c], Stephen E. Reichenbach[d,e], Valentina Bongiovanni[a], Chiara Cordero[b,*], Andrea Cavallero[a]

^{1}D - polarity/volatility separation

Data processing



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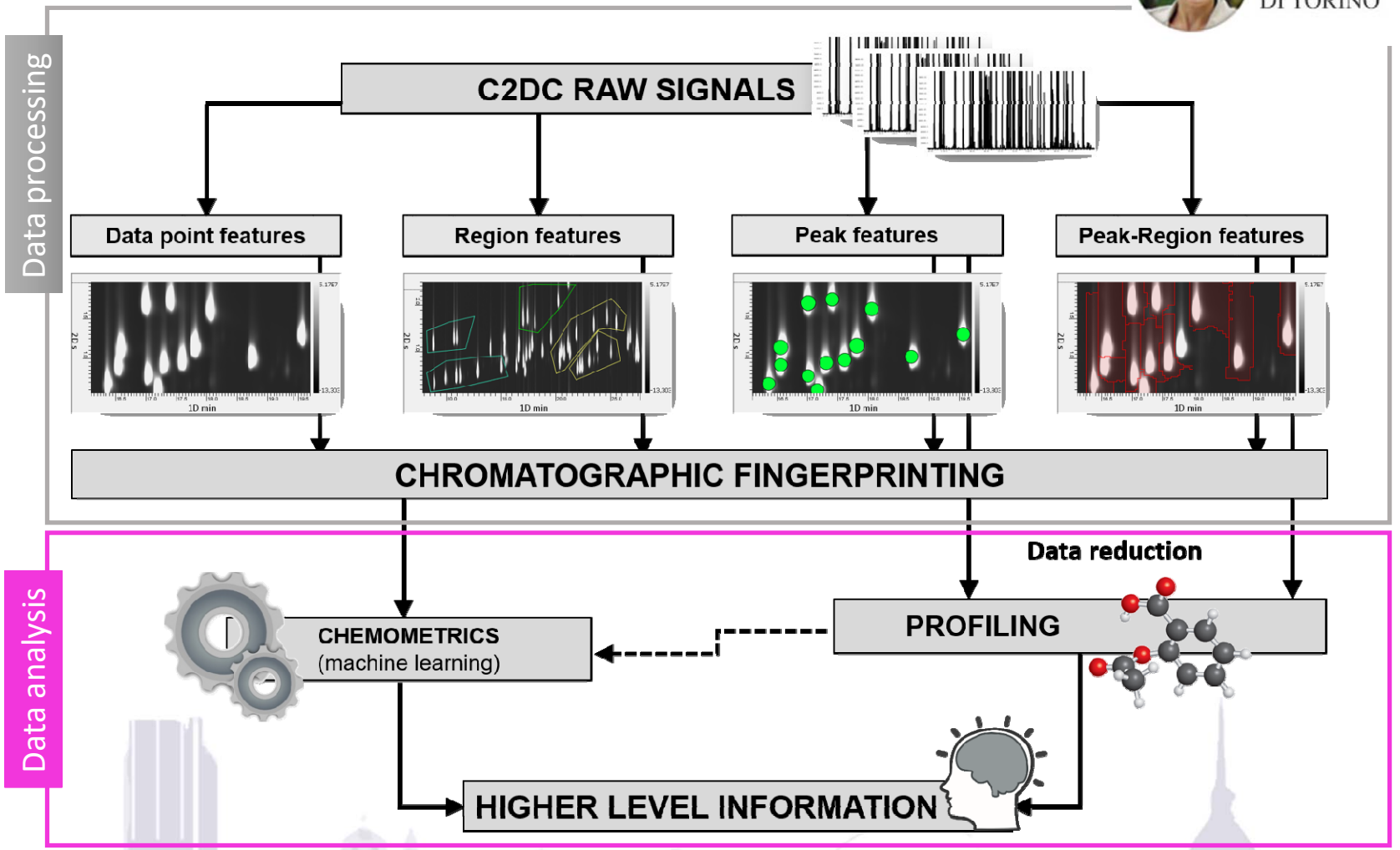

Data processing

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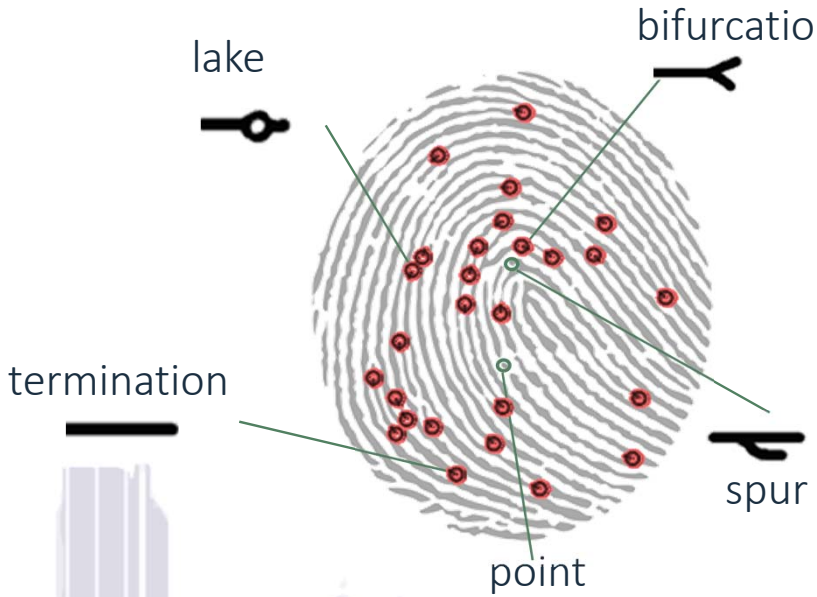
A **fingerprint** is the pattern of ridges and valleys on the surface of a fingertip
-> Everyone has unique fingerprints

Crime scene fingerprint



Database fingerprints

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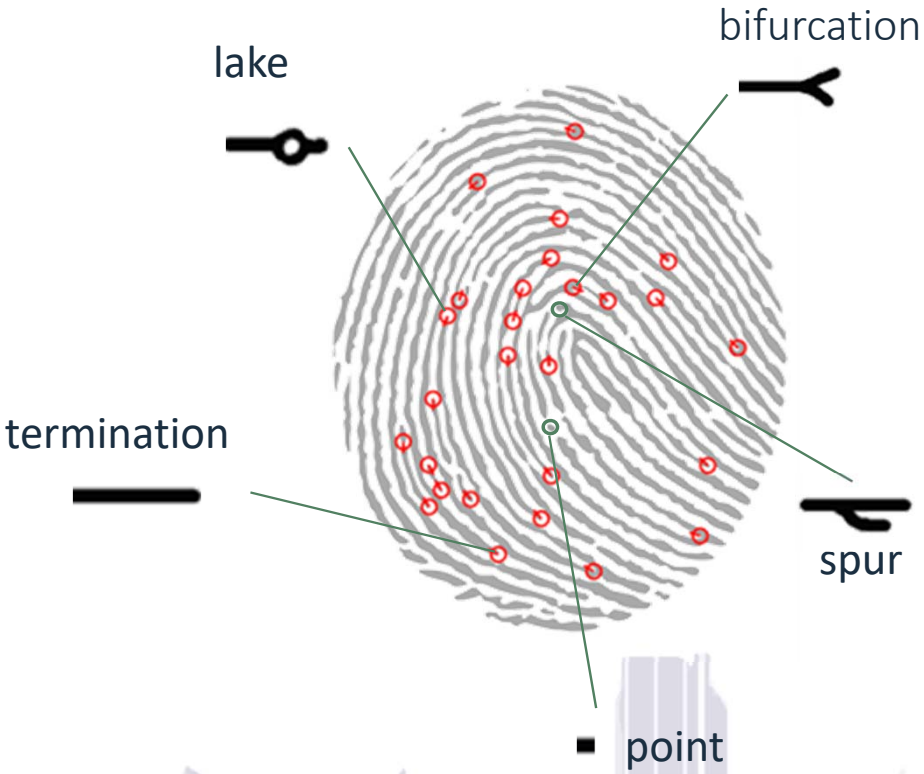
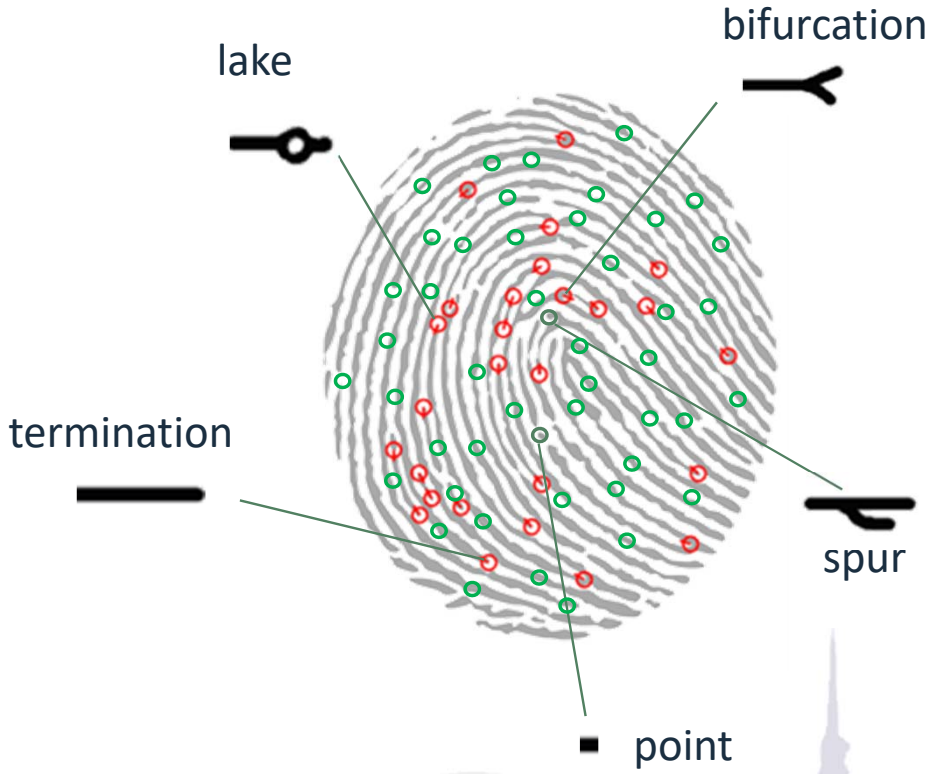
Targeted - minutiae



Untargeted - Targeted

UT - extended investigation



STEP 1

Data processing

Untargeted/*Targeted* Fingerprinting[1-4] - comprehensive mapping

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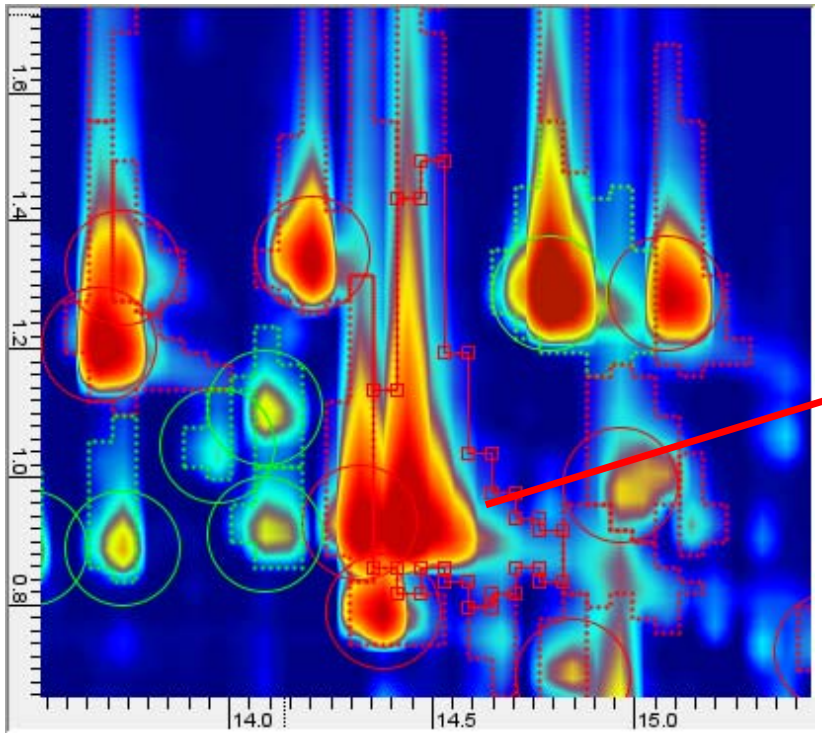
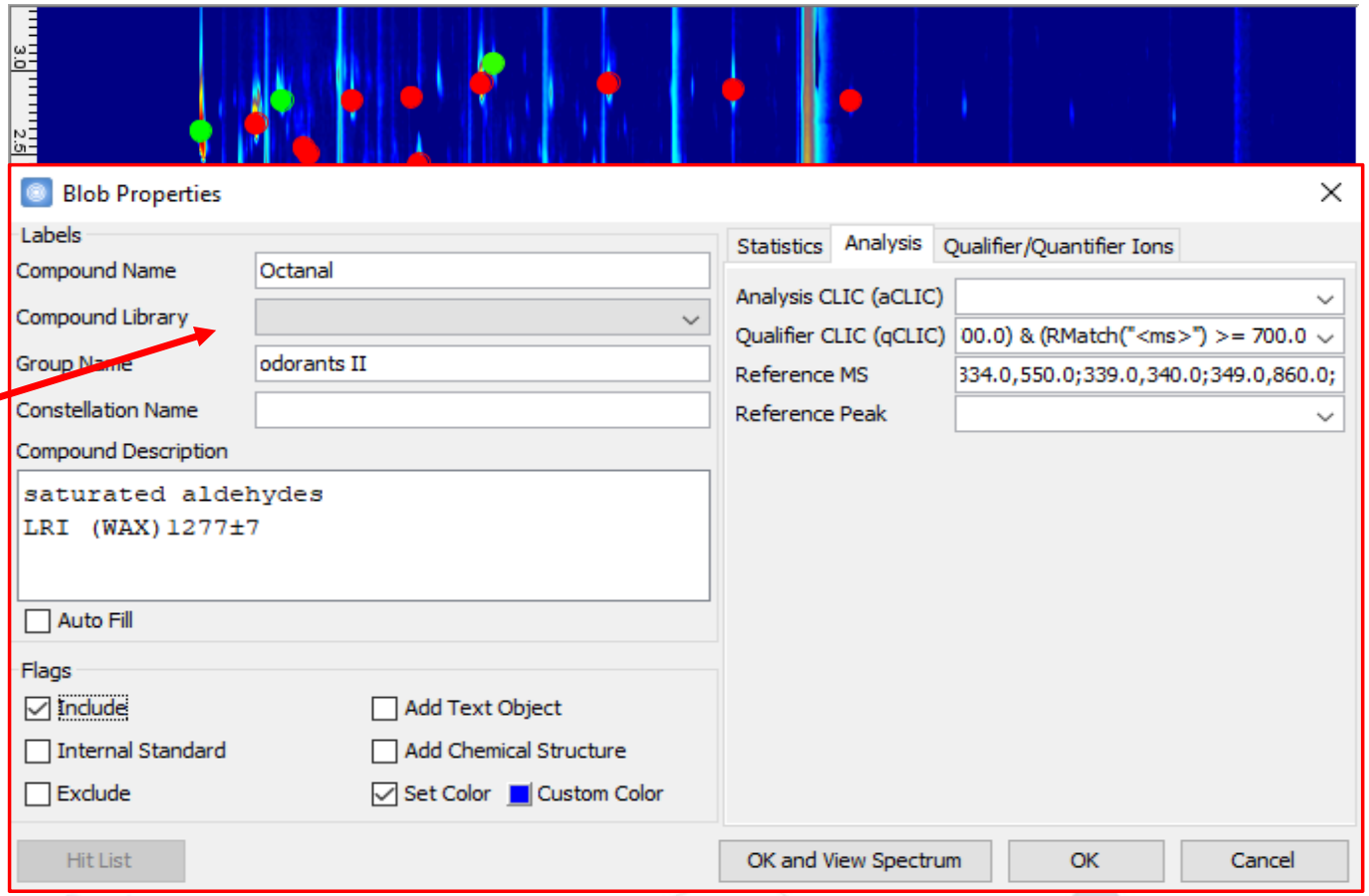


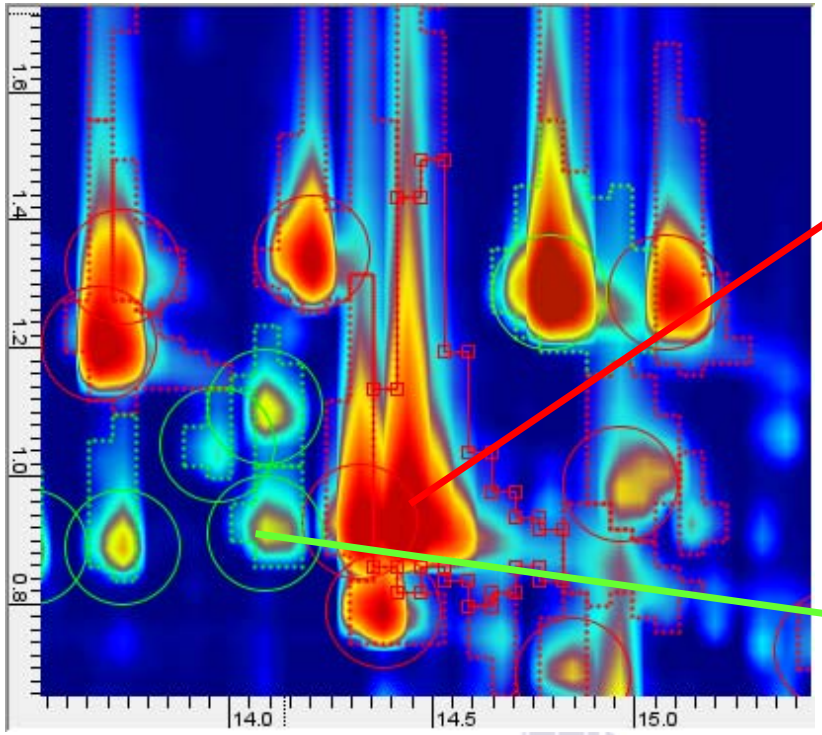


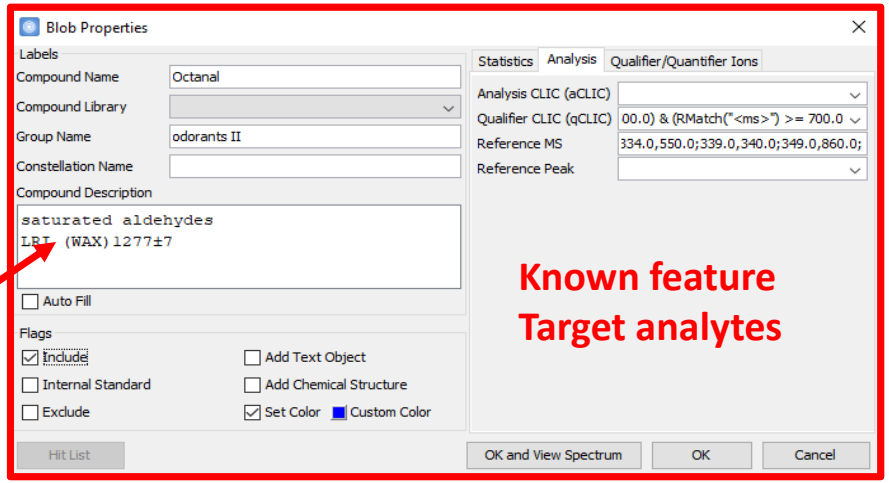
[1] Magagna, F., Valverde-Som, L., Ruíz-Samblás, C., Cuadros-Rodríguez, L., Reichenbach, S. E., Bicchi, C., & Cordero, C. (2016). Analytica Chimica Acta, 936, 245–258.
[2] Reichenbach, S. E., Tian, X., Tao, Q., Ledford, E. B., Wu, Z., & Fiehn, O. (2011). Talanta, 83(4), 1279–1288
[3] Reichenbach, S. E., Zini, C. A., Nicolli, K. P., Welke, J. E., Cordero, C., & Tao, Q. (2019). Journal of Chromatography A, 1595, 158–167
[4] Cordero, C., Guglielmetti, A., Bicchi, C., Liberto, E., Baroux, L., Merle, P., … Reichenbach, S. E. (2019). Journal of Chromatography A, 1597, 132–141

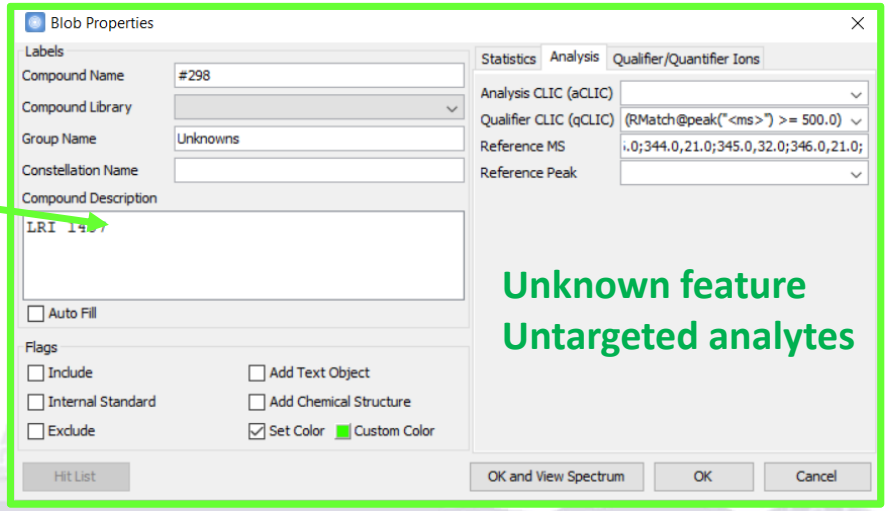
Data processing

STEP 2

Untargeted/Targeted Fingerprinting - comprehensive mapping







STEP 3

Data processing

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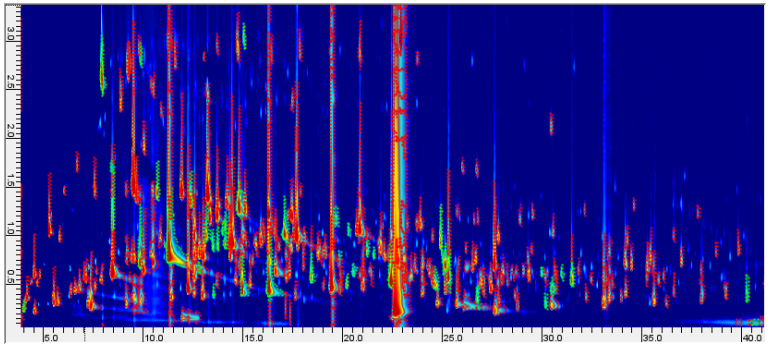

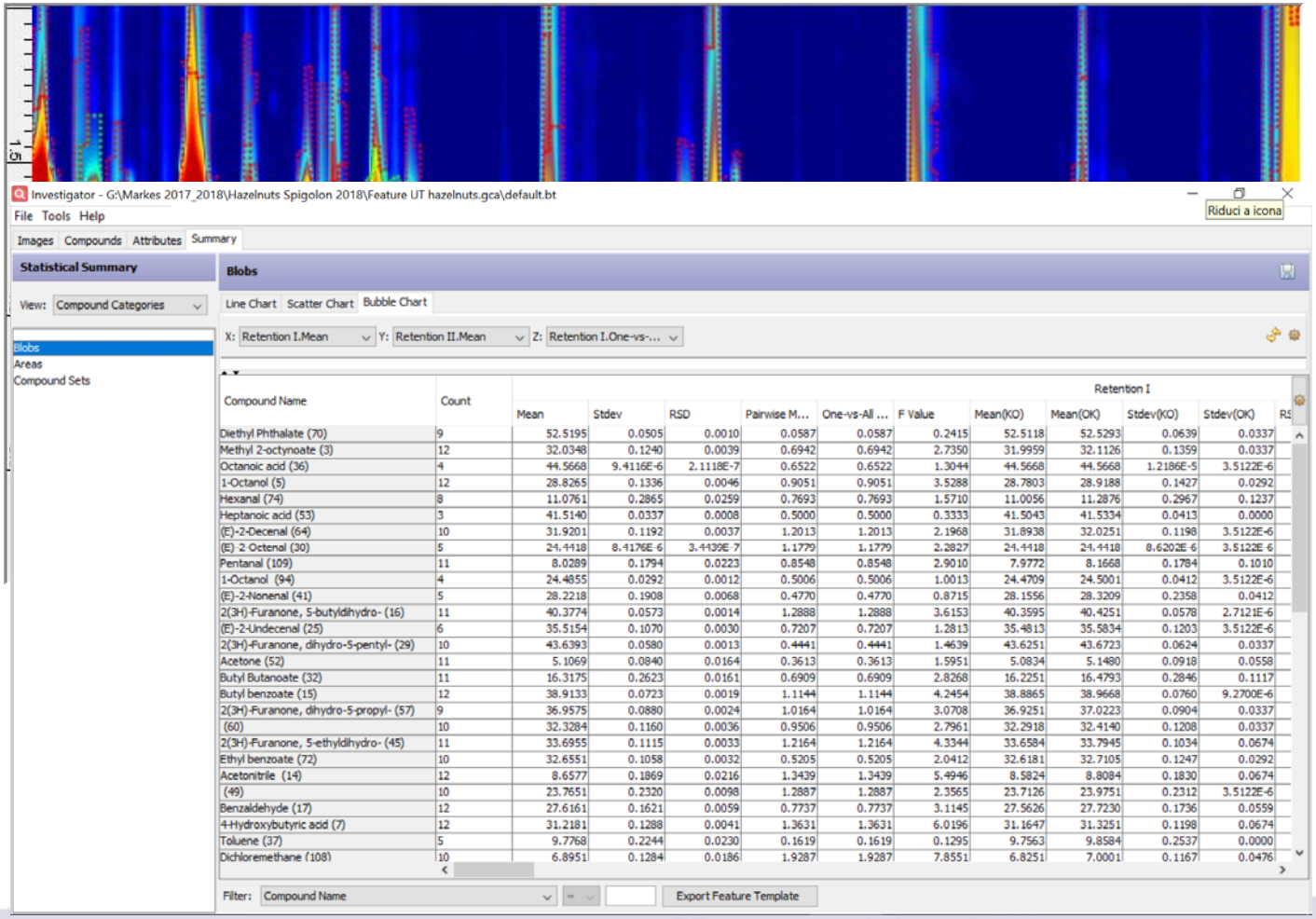

Compound Name	Count	Retention I Mean	Stdev	RSD	Pairwise M...	One-vs-All ...	F Value	Mean(KO)	Mean(OK)	Stdev(KO)	Stdev(OK)
Diethyl Phthalate (70)	9	52.5195	0.0505	0.0010	0.0587	0.0587	0.2415	52.5118	52.5293	0.0639	0.0337
Methyl 2-octynoate (3)	12	32.0348	0.1240	0.0039	0.6942	0.6942	2.7350	31.9959	32.1126	0.1359	0.0337
Octanoic acid (36)	4	44.5668	9.4116E-6	2.1118E-7	0.6522	0.6522	1.3044	44.5668	44.5668	1.2186E-5	3.5122E-6
1-Octanol (5)	12	28.8265	0.1336	0.0046	0.9051	0.9051	3.5288	28.7803	28.9188	0.1427	0.0292
Hexanal (74)	8	11.0761	0.2865	0.0259	0.7693	0.7693	1.5710	11.0056	11.2876	0.2967	0.1237
Heptanoic acid (53)	3	41.5140	0.0337	0.0008	0.5000	0.5000	0.3333	41.5043	41.5334	0.0413	0.0000
(E)-2-Decenal (64)	10	31.9201	0.1192	0.0037	1.2013	1.2013	2.1968	31.8938	32.0251	0.1198	3.5122E-6
(E)-2-Octenal (30)	5	24.4418	8.4176E-6	3.4439E-7	1.1779	1.1779	2.2827	24.4418	24.4418	8.6202E-6	3.5122E-6
Pentanal (109)	11	8.0289	0.1794	0.0223	0.8548	0.8548	2.9010	7.9772	8.1668	0.1784	0.1010
1-Octanol (94)	4	24.4855	0.0292	0.0012	0.5006	0.5006	1.0013	24.4709	24.5001	0.0412	3.5122E-6
(E)-2-Nonenal (41)	5	28.2218	0.1908	0.0068	0.4770	0.4770	0.8715	28.1556	28.3209	0.2358	0.0412
2(3H)-Furanone, 5-butyldihydro- (16)	11	40.3774	0.0573	0.0014	1.2888	1.2888	3.6153	40.3595	40.4251	0.0578	2.7121E-6
(E)-2-Undecenal (25)	6	35.5154	0.1070	0.0030	0.7207	0.7207	1.2813	35.4813	35.5834	0.1203	3.5122E-6
2(3H)-Furanone, dihydro-5-pentyl- (29)	10	43.6393	0.0580	0.0013	0.4441	0.4441	1.4639	43.6251	43.6723	0.0624	0.0337
Acetone (52)	11	5.1069	0.0840	0.0164	0.3613	0.3613	1.5951	5.0834	5.1480	0.0918	0.0558
Butyl Butanoate (32)	11	16.3175	0.2623	0.0161	0.6909	0.6909	2.8268	16.2251	16.4793	0.2846	0.1117
Butyl benzoate (15)	12	38.9133	0.0723	0.0019	1.1144	1.1144	4.2454	38.8865	38.9668	0.0760	9.2700E-6
2(3H)-Furanone, dihydro-5-propyl- (57)	9	36.9575	0.0880	0.0024	1.0164	1.0164	3.0708	36.9251	37.0223	0.0904	0.0337
(60)	10	32.3284	0.1160	0.0036	0.9506	0.9506	2.7961	32.2918	32.4140	0.1208	0.0337
2(3H)-Furanone, 5-ethyldihydro- (45)	11	33.6955	0.1115	0.0033	1.2164	1.2164	4.3344	33.6584	33.7945	0.1034	0.0674
Ethyl benzoate (72)	10	32.6551	0.1058	0.0032	0.5205	0.5205	2.0412	32.6181	32.7105	0.1247	0.0292
Acetonitrile (14)	12	8.6577	0.1869	0.0216	1.3439	1.3439	5.4946	8.5824	8.8084	0.1830	0.0674
(49)	10	23.7651	0.2320	0.0098	1.2887	1.2887	2.3565	23.7126	23.9751	0.2312	3.5122E-6
Benzaldehyde (17)	12	27.6161	0.1621	0.0059	0.7737	0.7737	3.1145	27.5626	27.7230	0.1736	0.0559
4-Hydroxybutyric acid (7)	12	31.2181	0.1288	0.0041	1.3631	1.3631	6.0196	31.1647	31.3251	0.1198	0.0674
Toluene (37)	5	9.7768	0.2244	0.0230	0.1619	0.1619	0.1295	9.7563	9.8584	0.2537	0.0000
Dichloromethane (108)	10	6.8951	0.1284	0.0186	1.9287	1.9287	7.8551	6.8251	7.0001	0.1167	0.0476

Targeted and **untargeted** peak(-region) features are cross-aligned between all samples and metadata collected for further processing.

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Combined untargeted and targeted fingerprinting with comprehensive two-dimensional chromatography for volatiles and ripening indicators in olive oil

Federico Magagna [a, 1], Lucia Valverde-Som [b, 1], Cristina Ruíz-Samblás [b], Luis Cuadros-Rodríguez [b], Stephen E. Reichenbach [c], Carlo Bicchi [a], Chiara Cordero [a, *]

Shelling nuts

Artificial Intelligence smelling: Can multidimensional chromatography play a (key) role?

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Raw ingredient for **confectionery** products **Turkey is the leading** producer (about 75% of world production) **Italy follows** as second in the ranking

- ✓ **Industrial partner** world leader in the production of confectionery products based on hazelnuts
- ✓ Need for **objective evaluation** of ***quality***

Quality assessment at **industrial level** focuses on morphological aspects, presence of damaged kernels, perceivable sensory defects (mould, rancid, *cimiciato*, stale etc..)

***Corylus avellana* L.**

Step-ahead in quality assessment *molecular resolution* probes:

- ✓ qualification (oxidation status, shelf-life storage effectiveness, bacterial and mold grow)
- ✓ *identitation*[1] (cultivar, origin, harvest area)
- ✓ definition of *aroma potential*[2]

***AI* decision makers**

1. *Computer Vision* in defected hazelnuts VOCs patterns
2. *Smelling machine* - aroma blueprint
3. Aroma precursors pattern

1. Cuadros-Rodríguez, L.; Ruiz-Samblás, C.; Valverde-Som, L.; Pérez-Castaño, E.; González-Casado, A. *Anal. Chim. Acta* **2016**, *909*, 9–23.
2. Cialiè Rosso, M.; Mazzucotelli, M.; Bicchi, C.; Charron, M.; Manini, F.; Menta, R.; Fontana, M.; Reichenbach, S. E.; Cordero, C. J. Chromatogr. A **2020**, *1614* (460739)

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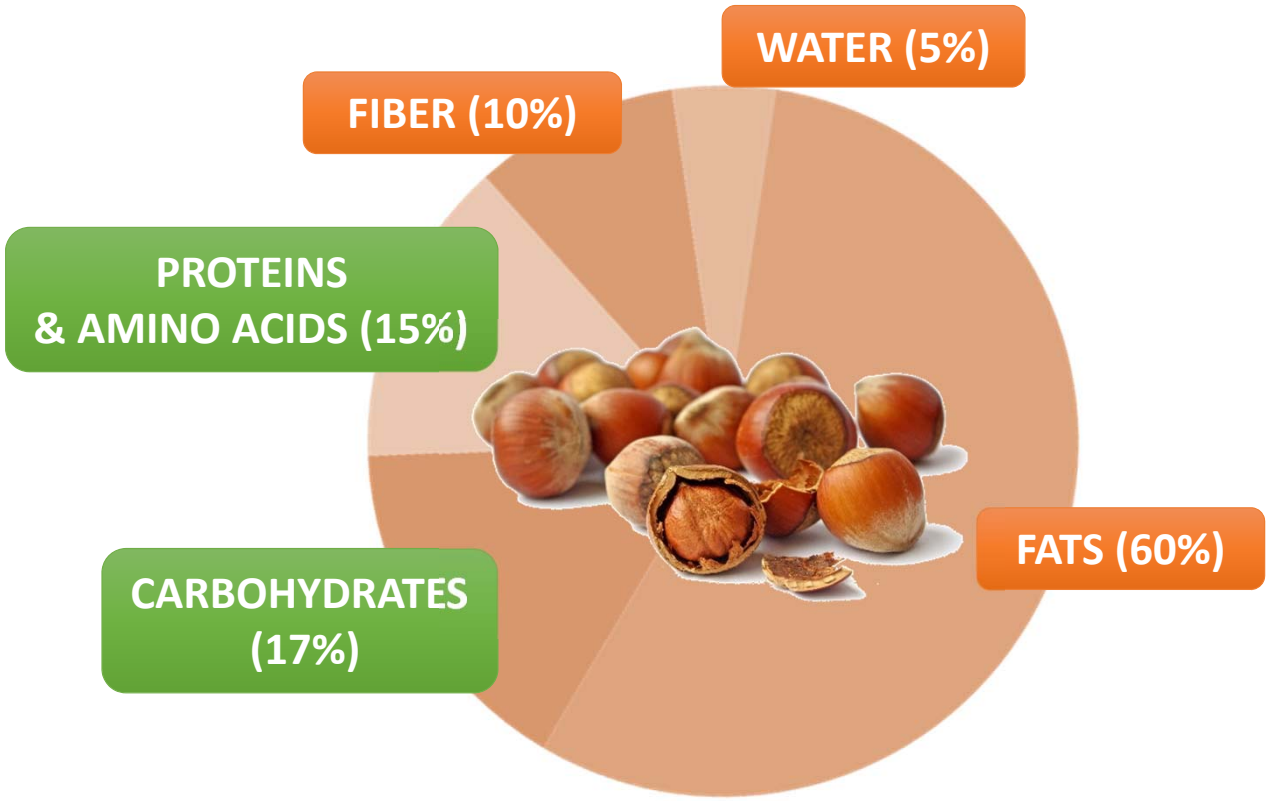


Volatiles < 0.01%

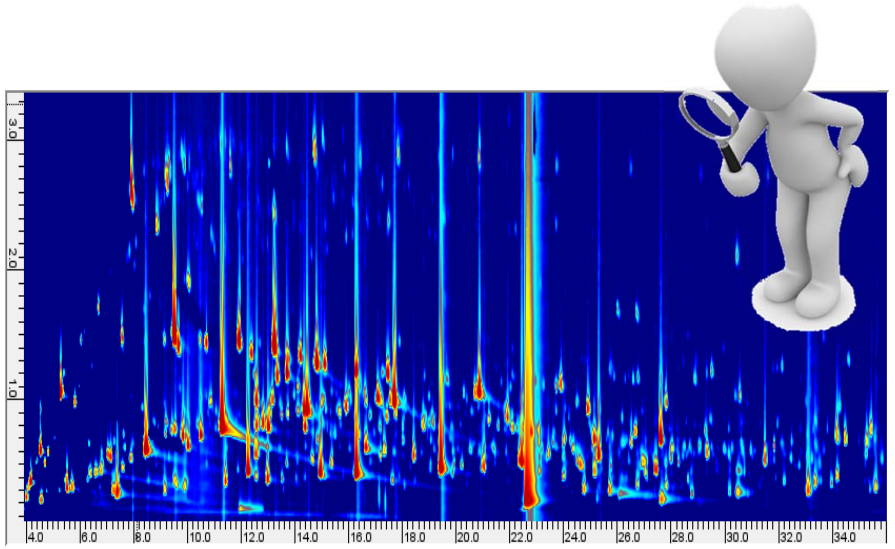
- Hydrocarbons
- Terpenoids
- Alcohols (linear and branched)
- Carbonyl derivatives
- Carboxylic acids
- Esters
- Lactones

Encrypts a lot of information

- geographical origin
- **phenotyping** and chemotyping
- multitrophic interactions (plants-insects)
- **presence of bacteria and moulds**
- **scent and odorous compounds**

- **distinctive aroma blueprint**

Shelling nuts



Quality assessment at **industrial level** focuses on **morphological aspects, presence of damaged kernels**, perceivable sensory defects (mould, rancid, *cimiciato*, stale etc..)

AI **decision makers**
Computer Vision **in defected hazelnuts volatilome patterns**

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Computer Vision strategy
Classification trees

Volatiles patterns diagnostic of spoilage

Computer Vision tools
Prompt identification of non-conform samples and confident rejection

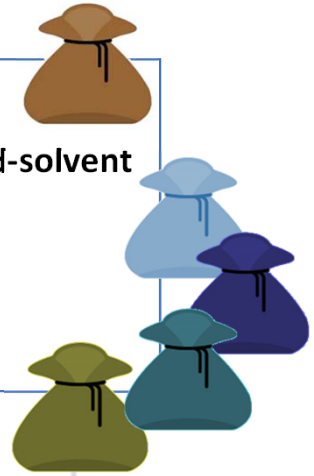
Good (OK) Rancid (KO) Spoiled (KO)

Collection of defected hazelnuts

- ✓ harvest years
- ✓ origin
- ✓ shelf-life stage

Flash profiling

Mould
Mould-rancid-solvent
Rancid
Rancid-stale
Solvent
Uncoded KO



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Platform

p_i p_{Aux} p_{Tee}

Tee-union

FID (70%)

MS (30%)

^{1}D - Polar Heavy Wax (J&W)
20 m × 0.18 mm × 0.18 µm
He carrier @ **0.4 mL/min**

^{2}D - Medium polarity OV17
1.8 m × 0.18 mm × 0.18 µm
He carrier @ **8 mL/min**

Tools

Headspace SPME

ISTDs Pre-loading

α and β-Thujone
100 mg/L (5.0 µL)

Methyl-2-octynoate
100 mg/L (5.0 µL)

5 min
50°C

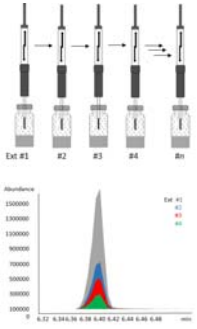
Hazelnuts
powder
0.100 g

50 min
50°C

Multiple Headspace SPME - quantitation

MHS-SPME enables **accurate quantitation** of **several markers (ESTD and response factors)**

secondary products of lipid oxidation (hexanal, heptanal, octanal, nonanal, (E)-2-octenal, (E)-2-nonenal);
key-aroma compunds (3-methylbutanal, ethyl 2-methylbutanoate, (E)-β-damascenone, 2-nonanone, heptanoic acid etc);
markers of defected hz (nonanoic acid, butyric acid, 4-heptanol, 1-pentanol, propanoic acid, 2-heptanol, pentanoic acid etc)



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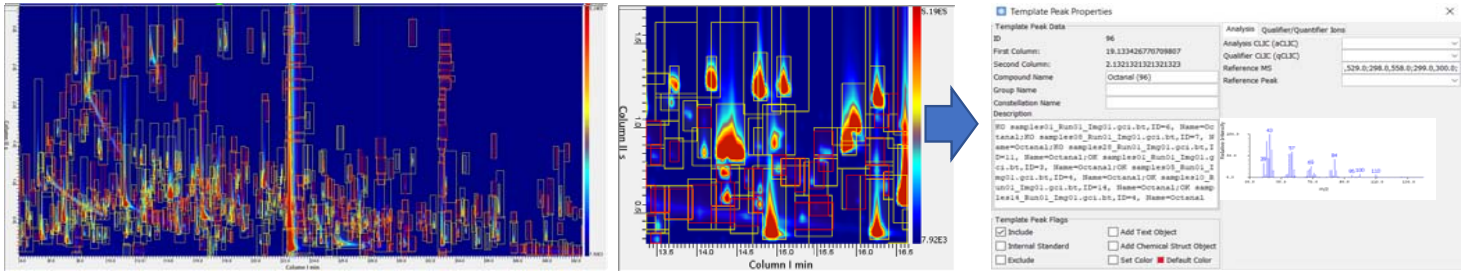
Untargeted/Targeted (UT) fingerprinting on single chromatograms

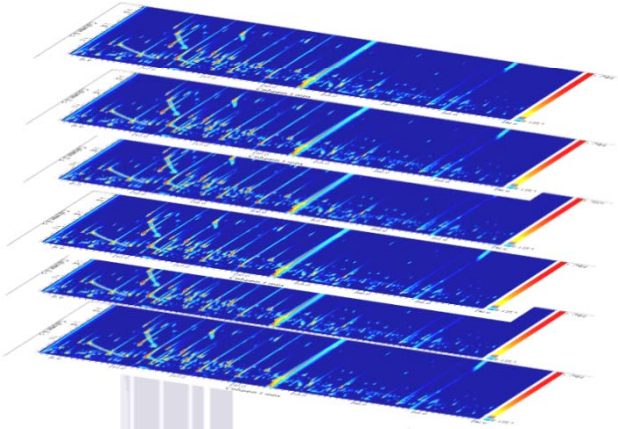
Food Chemistry

An effective chromatographic fingerprinting workflow based on comprehensive two-dimensional gas chromatography – Mass spectrometry to establish volatiles patterns discriminative of spoiled hazelnuts (*Corylus avellana* L.)

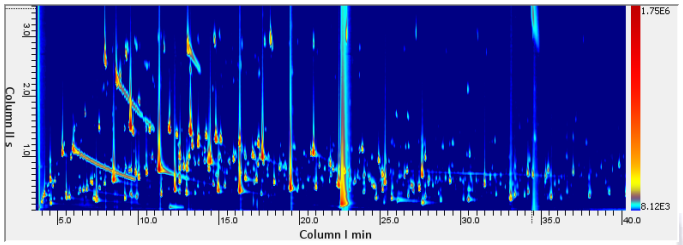


Generation of composite class-images from samples groups - one for each sensory defect

✓ patterns re-alignment by reliable 2D peaks and raw data summation (composite image)



Cumulative class-image "Mould"



The effect of dominant variables (origin, harvest year, cultivar, shelf-life etc..) is minimized while the "signature" of *mold* sensory defect emphasized - easier detection

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Composite class-images from samples groups - one for each sensory defect

Food Chemistry

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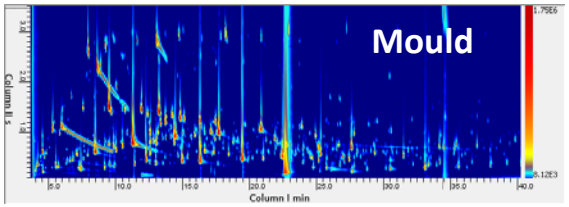
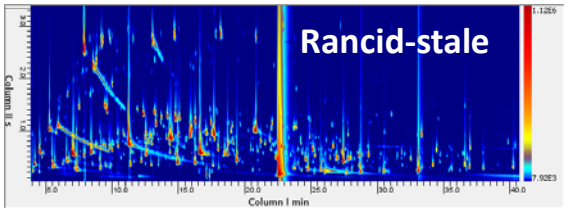
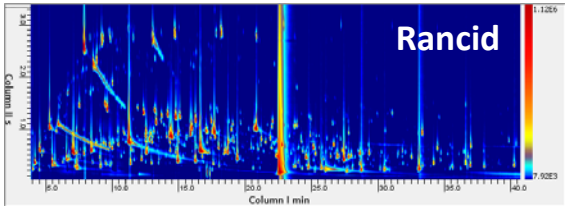
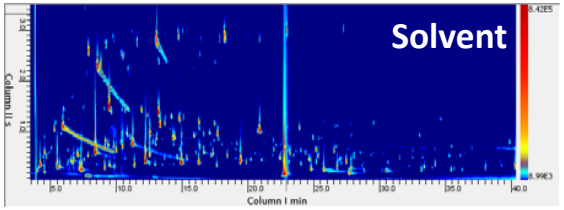
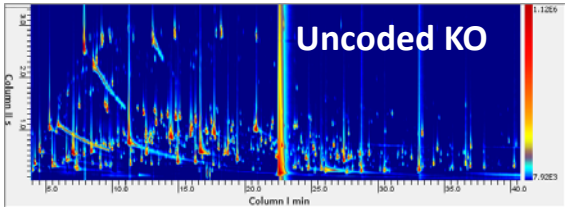
Federico Stilo[1], Erica Liberto[1], Nicola Spigolon[1], Giuseppe Genova[1], Ginevra Rosso[1], Mauro Fontana[1], Stephen E. Reichenbach[1,2], Carlo Bicchi[1], Chiara Cordero[1,*]

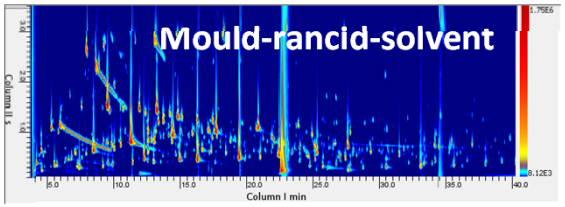


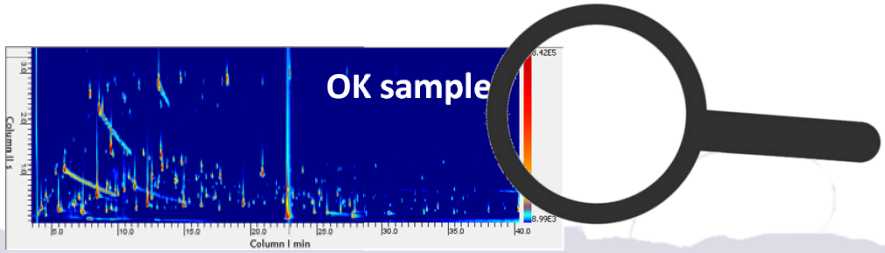












Mould
Mould-rancid-solvent
Rancid
Rancid-stale
Solvent
Uncoded KO

Reference OK samples

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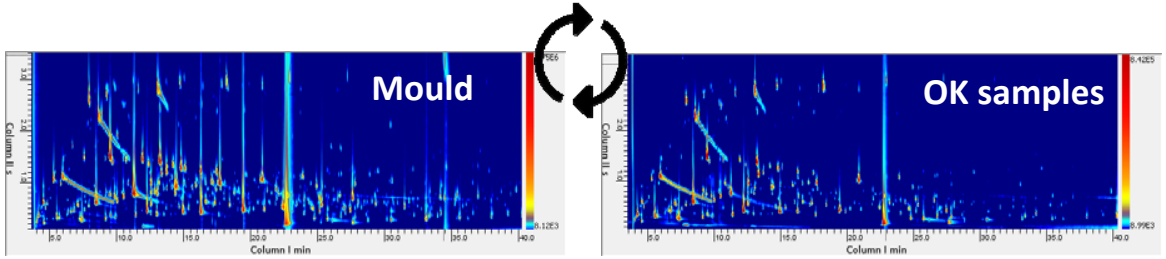

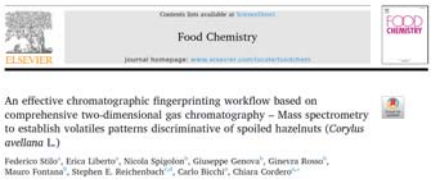

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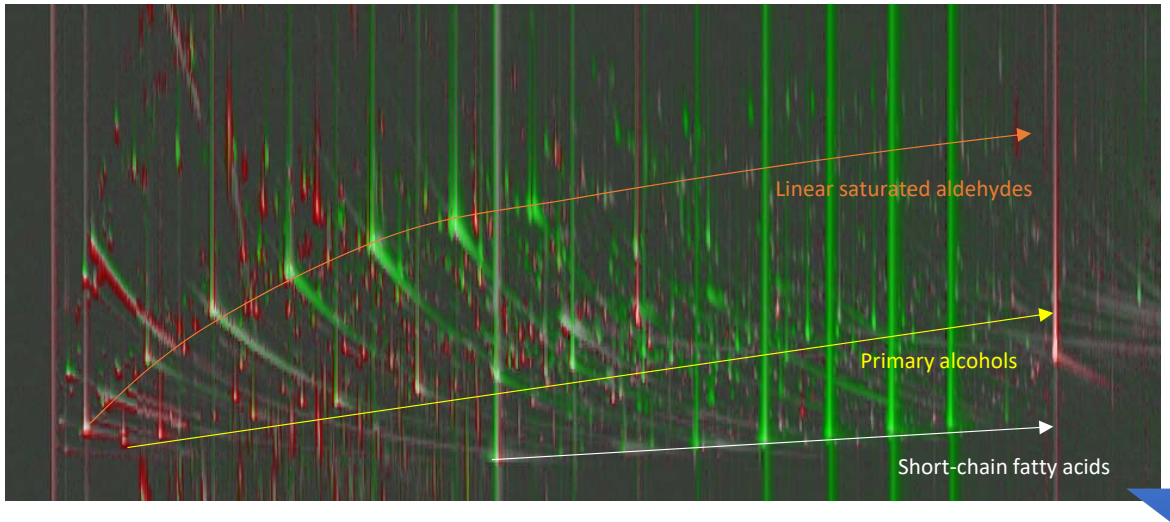


Datapoint features fingerprinting combined to peak-regions UT fingerprinting

Computer vision and chemical patterns

First Image

BlobID	Compound ...	Retention I	Retention II	Peak Value
9	Heptanoic acid	41.417	0.661	1027913.00
10		10.908	1.351	2002502.00
11		14.642	1.562	1919573.00
12	Nonanoic acid	47.367	0.721	1150889.00
13		21.000	0.931	1722252.00

Second Image

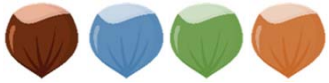
BlobID	Compound ...	Retention I	Retention II	Peak Value
97	Heptanoic acid	41.533	0.631	165949.000
98	1-Decene	10.208	2.883	165851.000
99	3-Penten-2-...	12.658	1.021	163385.000
100	Oxirane, pe...	13.592	1.682	162084.000
101	1-Nonene	7.467	2.132	161864.000

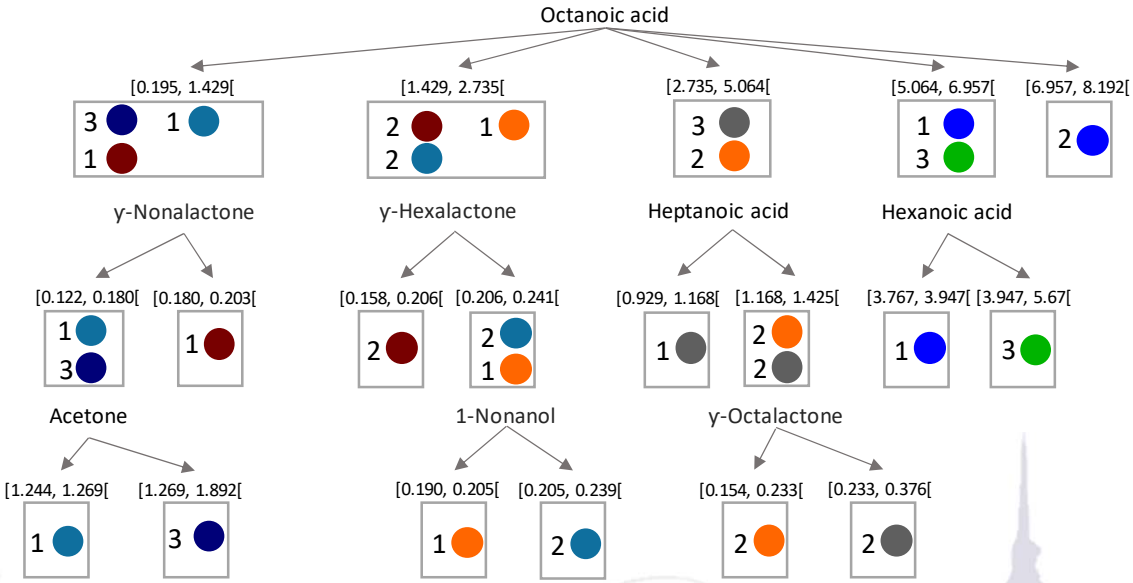
Shelling nuts

OK
Uncoded KO
Rancid
Rancid-solvent
Rancid-stale
Mould
Mould-rancid-solvent

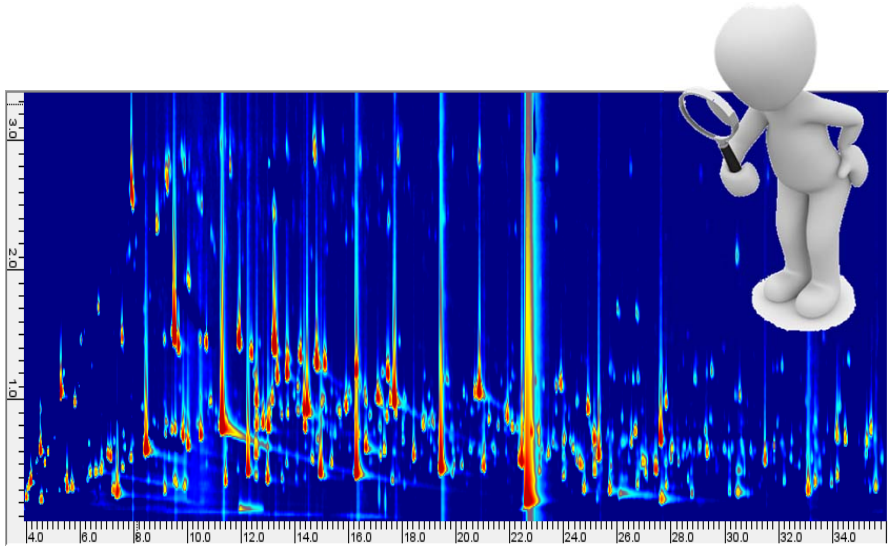
VOCs patterns distinctive of spoiled hazelnuts guide effective classification into seven classes.

- ✓ **Octanoic acid** guides the classification tree being positively correlated to **mould**;
- ✓ **y-nonalactone**, **y-hexalactone**, **acetone**, and **1-nonanol** are decisive to classify OK and rancid samples;
- ✓ **heptanoic** and **hexanoic** acids and **y-octalactone are** present in high relative abundance in rancid-solvent and rancid-stale samples





Shelling nuts



Quality assessment at **industrial level** focuses on morphological aspects, presence of damaged kernels, **perceivable sensory defects** (mould, rancid, *cimiciato*, stale etc..)

AI **decision makers**
AI Smelling machine **- aroma blueprint**

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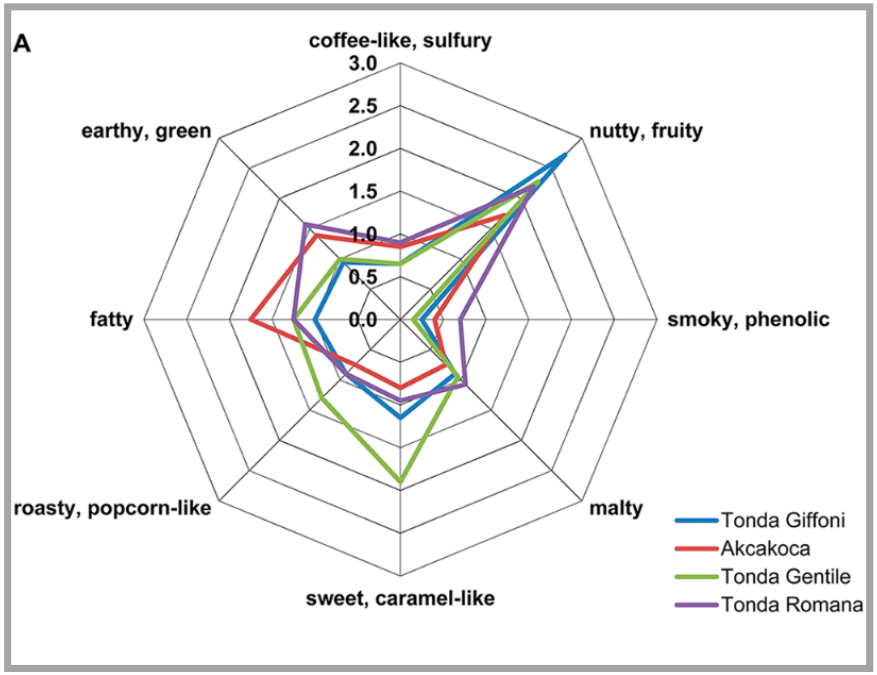
Develop a sensomics-based expert system acting as AI *smelling machine*



Aroma profile of raw hazelnuts from different cultivar/origin[1]

Key-aroma compounds - raw hazelnuts odorants occurring in amounts that exceed the OT (Odor Activity Value > 1).
Their omission in aroma recombinates does not reproduce the flavour blueperint of the original product.

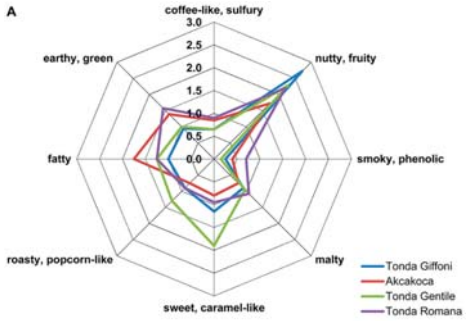
	'Tonda Romana'	'Tonda Gentile'	Akçakoca
hexanal	3	<1	8
3-methyl-4-heptanone	141	126	93
5-methyl-(E)-2-hepten-4-one	2	2	2
2-acetyl-1-pyrroline	24	24	24
dimethyl trisulfide	1	1	1
2-propionyl-1-pyrroline	22	22	22
2-furfuryl mercaptan	8	8	8
3-(methylthio)propionaldehyde	15	15	15
3,5-dimethyl-2-ethylpyrazine	1	1	1
2,3-diethyl-5-methylpyrazine	9	9	9
3,7-dimethylocta-1,6-dien-3-ol	12	12	12
2-acetyl-1,4,5,6-tetrahydropyridine	46	46	46
2-acetyl-3,4,5,6-tetrahydropridine	36	36	36
3-methylbutanoic acid	2	1	1
(E,E)-2,4-nonadienal	6	3	29

1. Kiefl, J.; Schieberle, P. J. Agric. Food Chem. 2013, 61 (22), 5236–5244.

Shelling nuts

Storage quality markers
1-heptanol (*green*, *chemical*), 2-octanol (*metal*, *burnt*), 1-octen-3-ol (*mushroom*), (E)-2-heptenal (*fatty*, *almond*), hexanal (*leaf-like, green*), heptanal (*fatty*), octanal (*fatty*) and nonanal (*tallowy*, *fruity*).

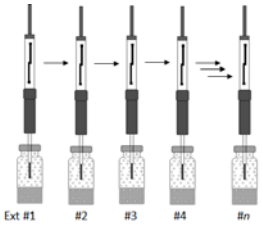
Key-aroma compounds



Spoiled hazelnuts markers
octanoic acid positively correlated to **mould**; γ-nonalactone, γ-hexalactone, acetone, and 1-nonanol are decisive to classify **OK** and **rancid** samples; heptanoic and hexanoic acids and γ-octalactone are present in high abundance **rancid-solvent** and **rancid-stale** samples.

Strategy

Multiple Headspace SPME
Accurate quantification / ESTD and RF



46 analytes
key-aromas
markers

Differential-flow modulator
parallel detection qMS/FID

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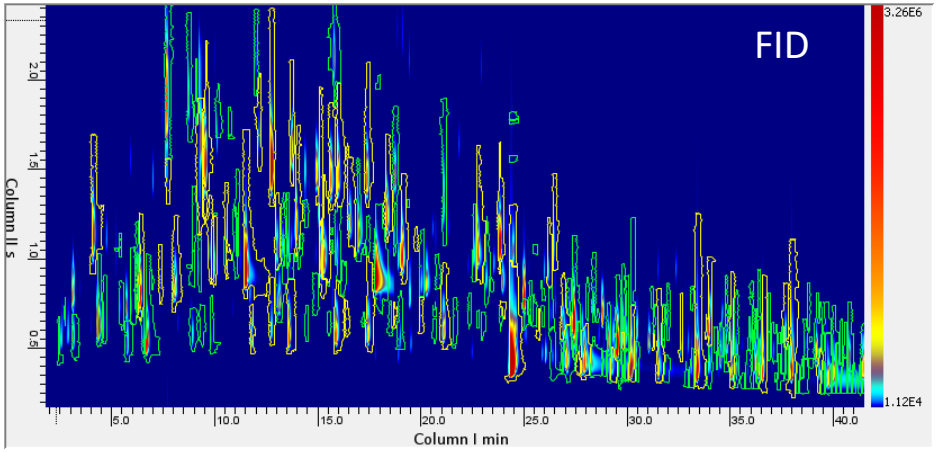

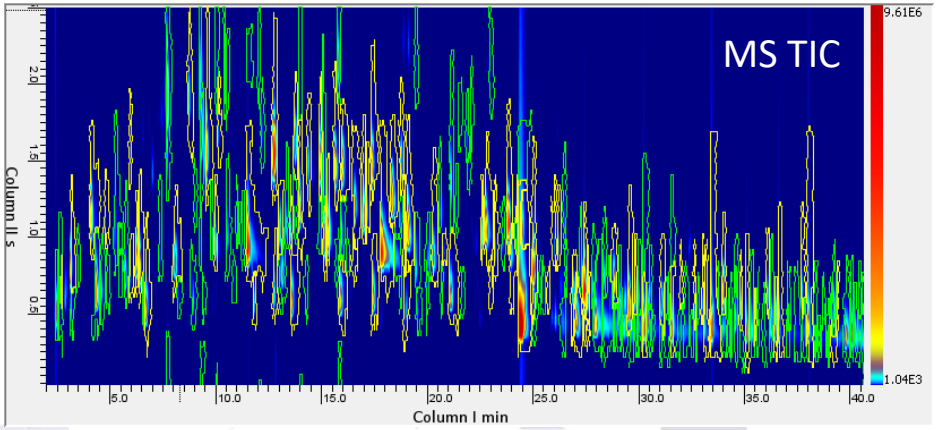

Shelling nuts

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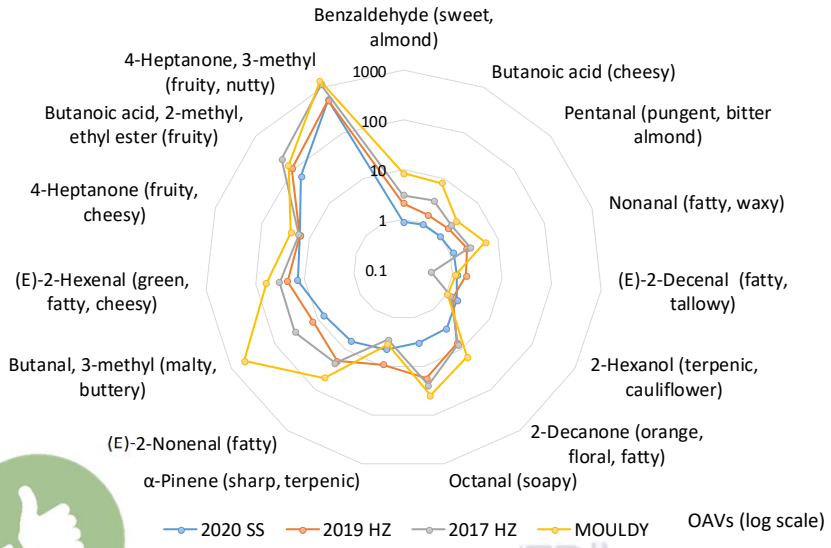
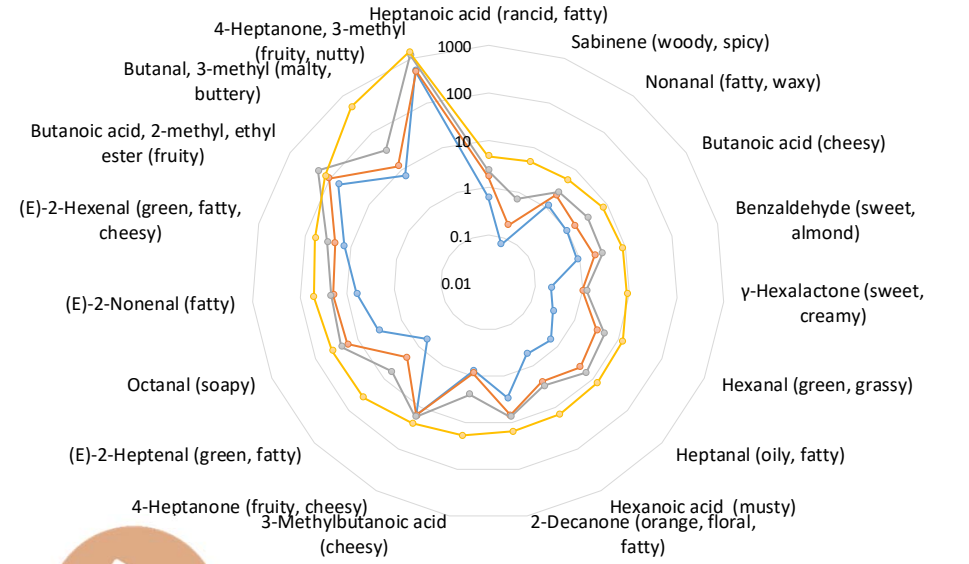
Artificial Intelligence smelling
Aroma blueprint - OAVs >1





Artificial Intelligence smelling
Aroma blueprint of spoiled hazelnuts OAVs >1

Step-ahead in quality assessment
molecular resolution probes:
✓ definition of *aroma potential*

***AI* decision makers**
Aroma precursors pattern

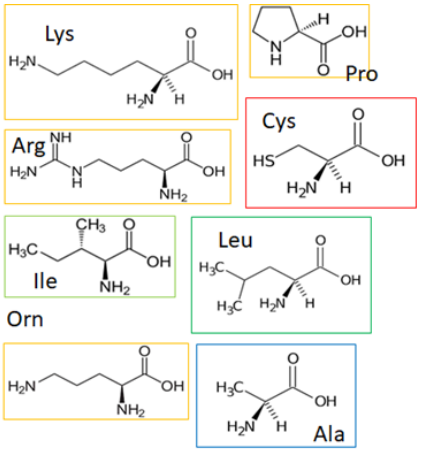
QUESTIONS ON AROMA POTENTIAL

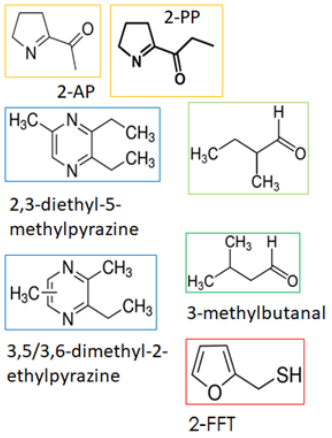
1) Is there a robust correlation between non-volatile precursors and key-aroma compounds in roasted hazelnuts?

2) Are precursors patterns distinctive of hazelnut origin?

3) Is it possible to assess the "potential" of aroma development through precursors patterns in raw hazelnuts?

4) Have precursors patterns a predictive potential to assess raw hazelnut quality toward its industrial processing trajectory?





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Food metabolomics domain

MRI Max Rubner-Institut

Collaboration with Max Rubner Institut
Department of Safety and Quality
of Fruit and Vegetables Food Quality Area Metabolism
Dr. Sabine Kulling and Dr. Christoph Weinert

Shelling nuts

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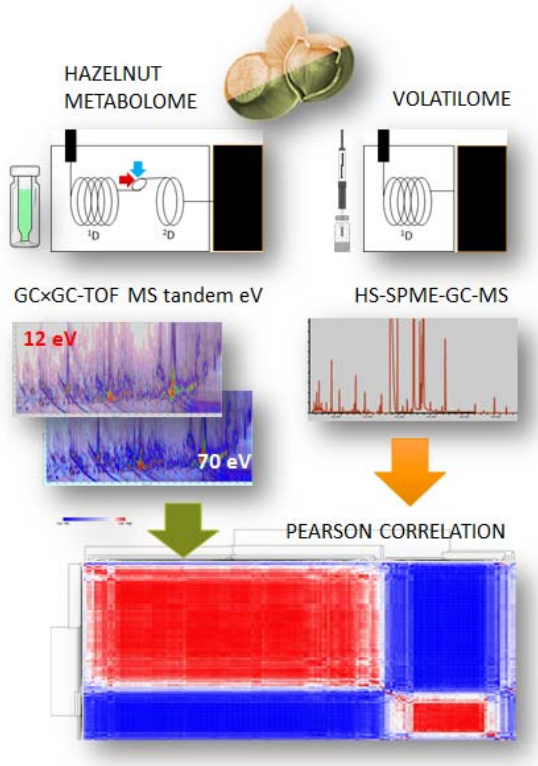
Platform

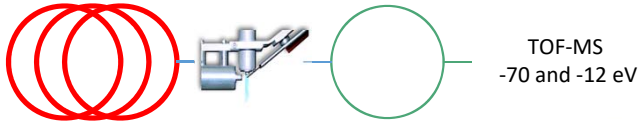
Tools

Sample preparation

Adaptation and validation of a sample preparation procedure from *Nature Protocols* [6, 1483–1499 (2011)]

Extraction with H_2O/CH_3OH 98:2;
Metoximation with MOX solution in pyridine - 60°C for 2 h
Silylation (N,O- bis(trimethylsilyl)trifluoroacetamide) BSTFA - 60°C for 1 h.





TOF-MS
-70 and -12 eV

[1]D - Apolar (HP5 -)
60 m × 0.25 mm × 0.25µm
He carrier @ **1.3 mL/min**

[2]D - Medium polarity OV17
2.0 m × 0.15 mm × 0.10µm
He carrier @ **1.3 mL/min**


Journal of Chromatography A
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Adding extra-dimensions to hazelnuts primary metabolome fingerprinting by comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry featuring tandem ionization: Insights on the aroma potential

Marta Cialiè Rosso[a], Maria Mazzucotelli[a], Carlo Bicchi[a], Melanie Charron[b], Federica Manini[b], Roberto Menta[b], Mauro Fontana[b], Stephen E. Reichenbach[c,d], Chiara Cordero[a,*]


applied sciences
MDPI

Article

Combined Untargeted and Targeted Fingerprinting by Comprehensive Two-Dimensional Gas Chromatography to Track Compositional Changes on Hazelnut Primary Metabolome during Roasting

Marta Cialiè Rosso [1], Federico Stilo [1], Carlo Bicchi [1], Melanie Charron [2], Ginevra Rosso [2], Roberto Menta [2], Stephen E. Reichenbach [3,4], Christoph H. Weinert [5], Carina I. Mack [5], Sabine E. Kulling [5] and Chiara Cordero [1,*]

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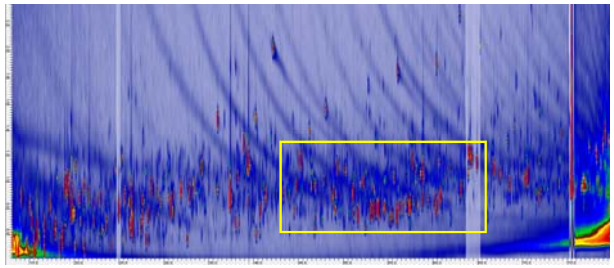
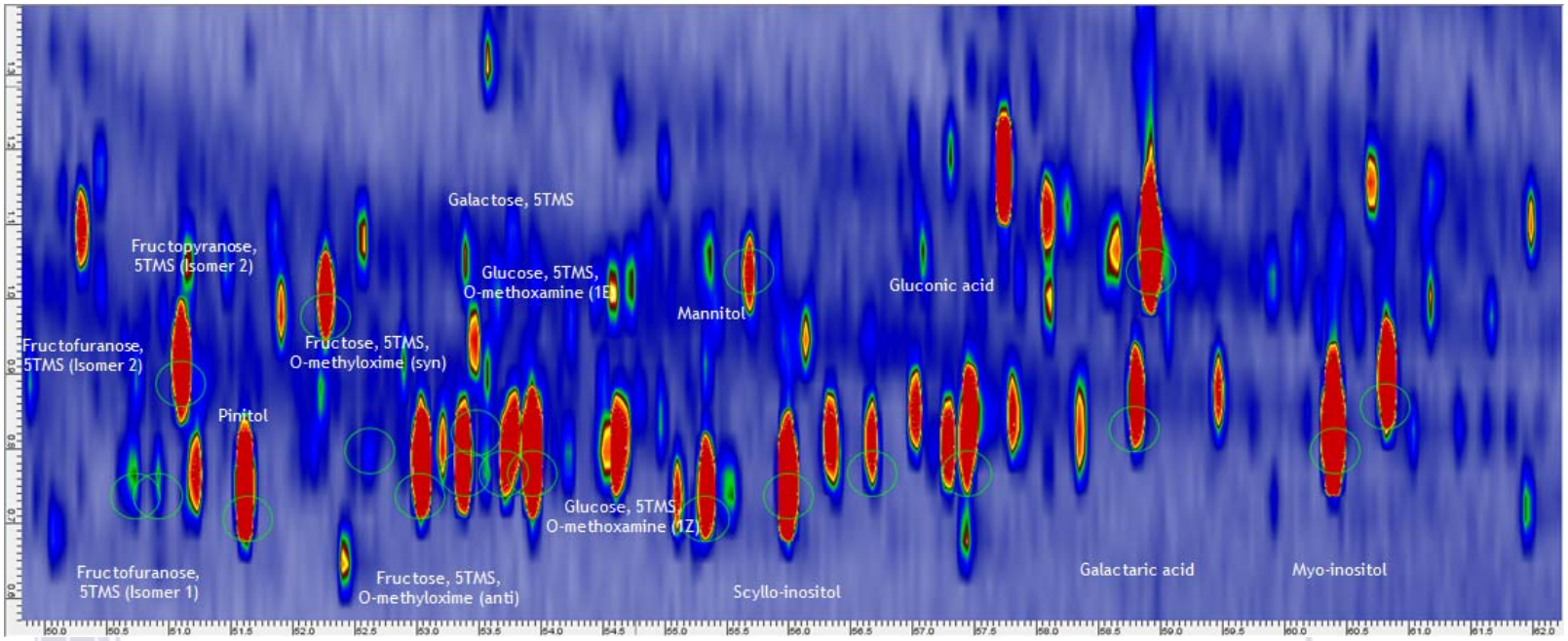
Italian Tonda Gentile Trilobata - Raw Harvest 2017 - mono-saccharides elution region



The primary metabolome of raw hazelnut kernels is complex and accounts for:
1,000 UT features
130 reliably identified metabolites (80 standard confirmation - 50 by LRI and spectra similarity 70 eV and MI confirmation by low energy ionization at 12 eV)

15 amino acids; 25 organic acids; 10 polyols; 16 mono/di saccharides; 5 sugar acids, etc.



GC×GC set-up: ^{1}D: **Rxi-5SilMS** 60 m x 0.25 mm ID x 0.25µm - ^{2}D: **BPX50** 2.2m x 0.15mm ID x 0.15µm - GC Oven: 90°C to 160°C @2°C/min to 290 °C @3.25°C/min to 320 (10') @6°C/min. Run Time: 90' - OPTIC4 injector: 90°C to 280°C, split ratio 1:5, after 1' 1:100, after 20' 1:30 - Modulation settings: modulation time 2,7s, hot-jet pulse jet 250 ms

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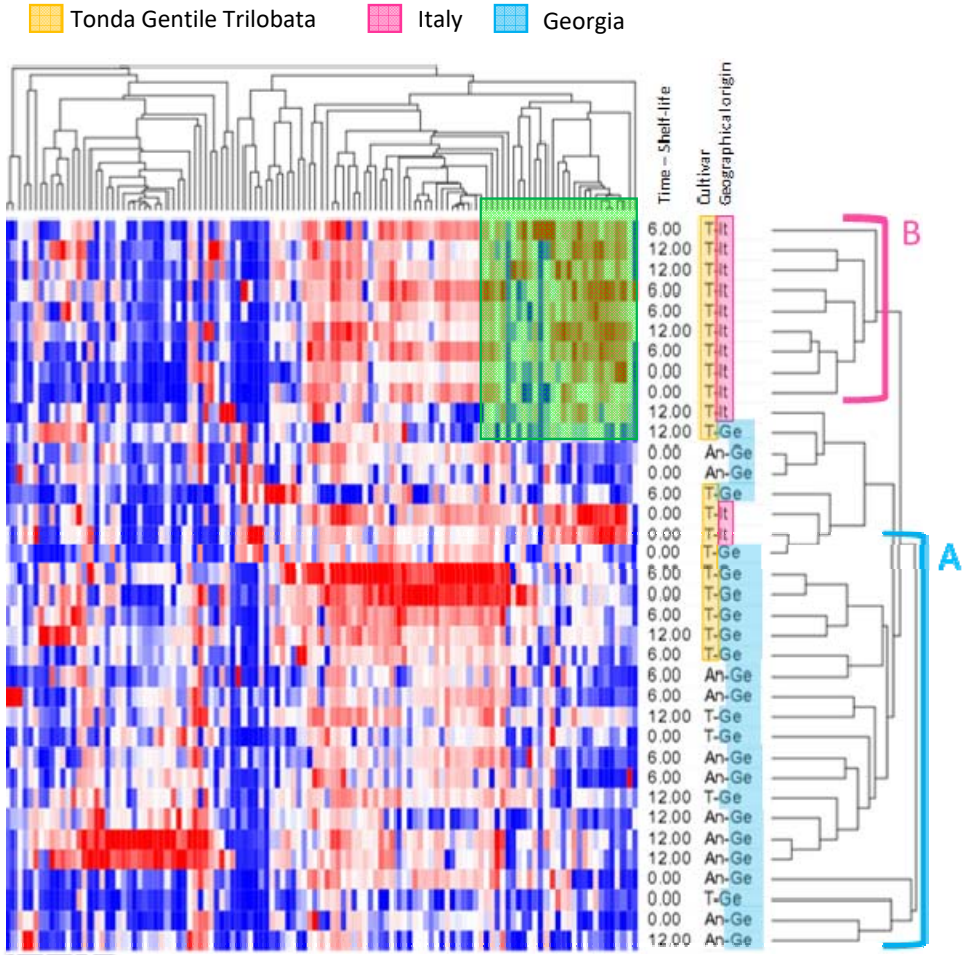
QUESTIONS ON AROMA POTENTIAL

2) Are precursors patterns distinctive of hazelnut origin?

3) Is it possible to assess the "potential" of aroma development through precursors patterns in raw hazelnuts?

4) Have precursors patterns a predictive potential to assess raw hazelnut quality toward its industrial processing trajectory?



Hierarchical clustering based on Euclidean distances of 112 targeted metabolites normalized responses (Pareto scaling and Z-score normalization). Heat-map visualization in blue-red scale.
Hazelnut samples:
T-GE: Tonda Gentile Trilobata from Georgia (2017-2018)
T-IT: Tonda Gentile Trilobata from Piedmont Italy (2017-2018)
AN-GE: Anakliuri Georgia (2017-2018).

Cultivars and pedoclimatic condition of harvest region influence precursors pattern.

- ✓ Tonda Gentile Trilobata hazelnuts harvested in Georgia are grouped together (**light blue** – cluster A)
- ✓ Tonda Gentile Trilobata harvested in Italy (T_IT tag-cluster B) is connoted by a higher relative distribution of several analytes, especially sugar, polyalcohols and amino acids (**light green box**)

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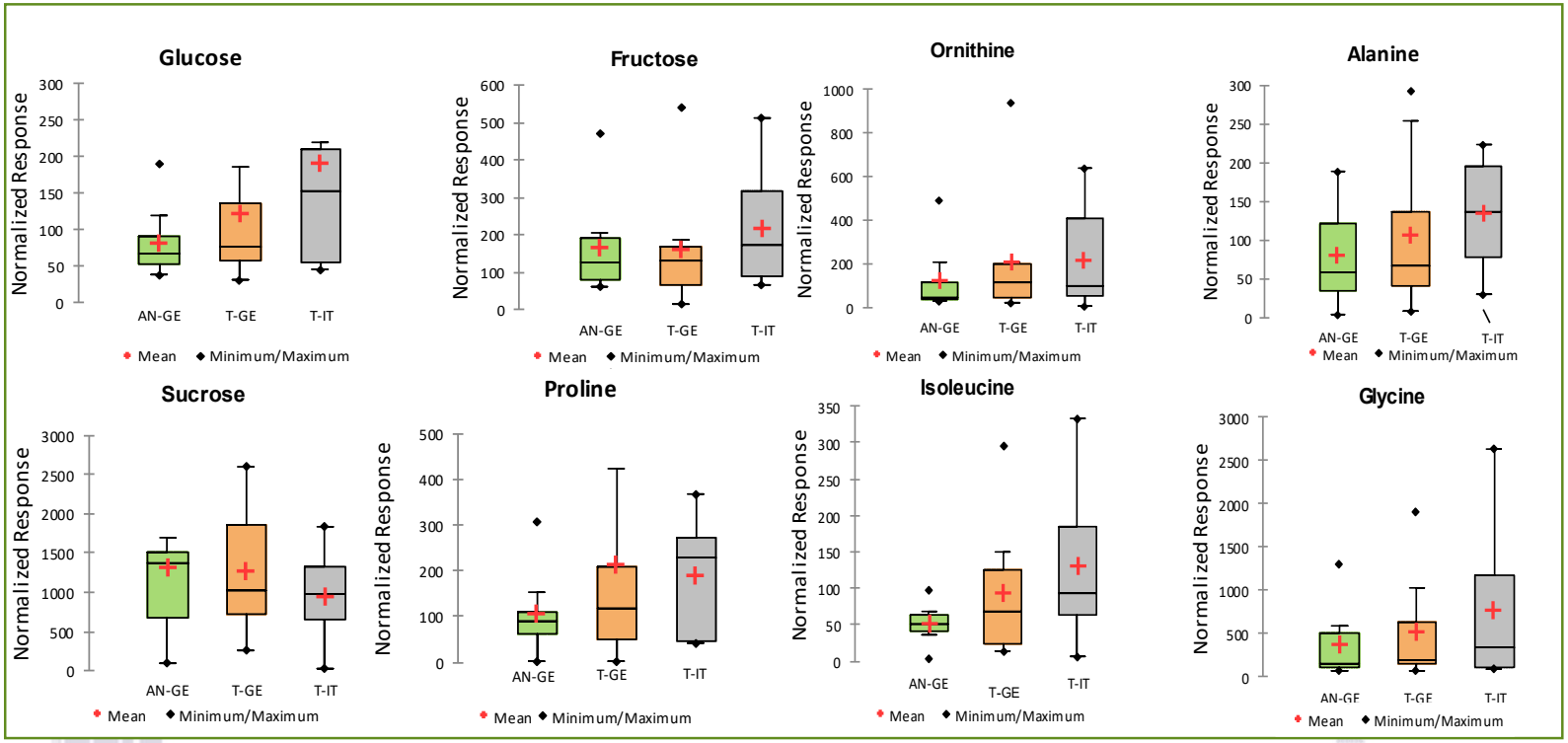
Univariate statistics (i.e., box-plots) illustrating the average normalized response for targeted precursors in the selected hazelnut samples



T-GE: Tonda Gentile Trilobata from Georgia (2017-2018)
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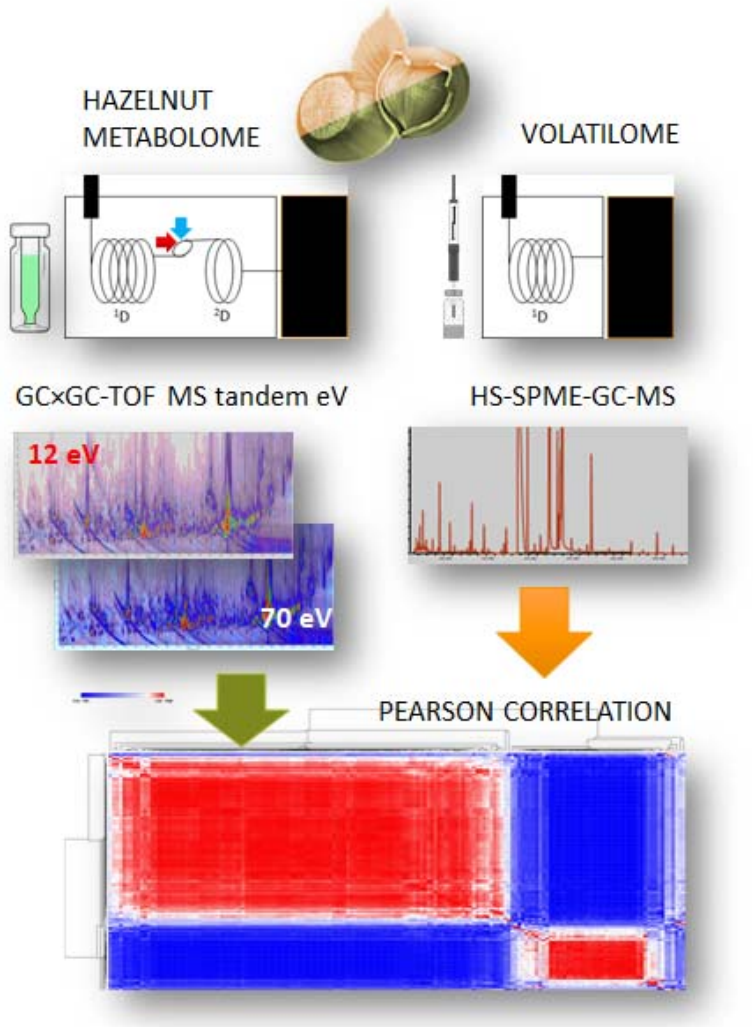

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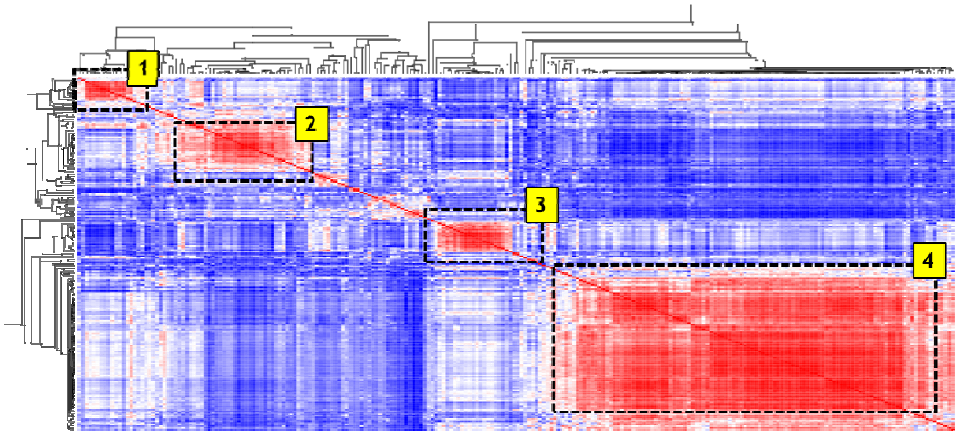


Heat-map based on the Pearson correlation coefficient (r) calculated between primary metabolites % responses (GCxGC-TOF MS) and volatiles % responses after lab-scale roasting. Pareto scaled data - matrix 245 × 45 (features × samples).

R: Tonda Gentile Romana of Italy (2017); O: Ordu blend of Turkey (2015)
P: Tonda Gentile Trilobata from Piedmont Italy (2015); T-GE: Tonda Gentile Trilobata from Georgia; T-IT: Tonda Gentile Trilobata from Piedmont Italy
AN-GE: Anakliuri of Georgia.

1. Native volatiles, dicarbonyls and furans

2. Roasting markers, above all alkyl pyrazines (Maillard reaction signature)

QUESTIONS ON AROMA POTENTIAL

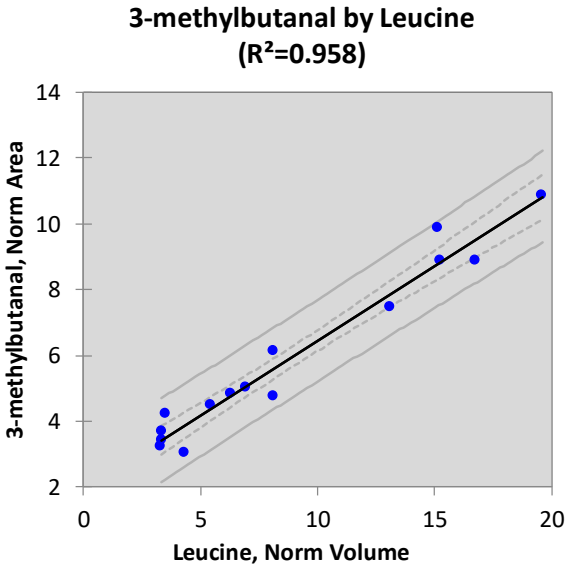
aldehydes)

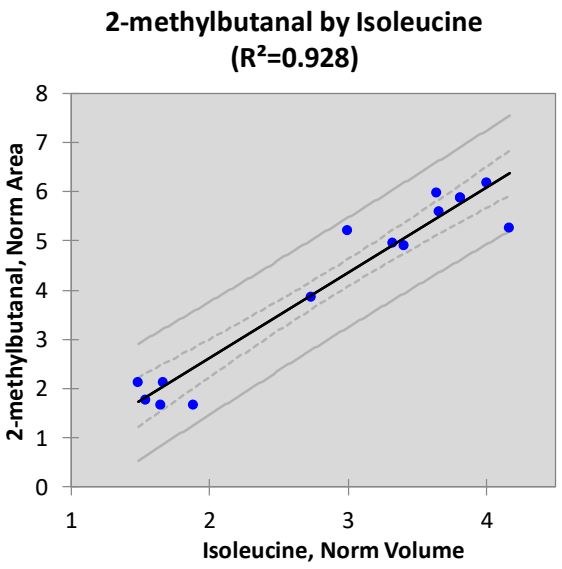
4) Have precursors patterns a predictive potential to assess raw hazelnut quality toward its industrial processing trajectory?

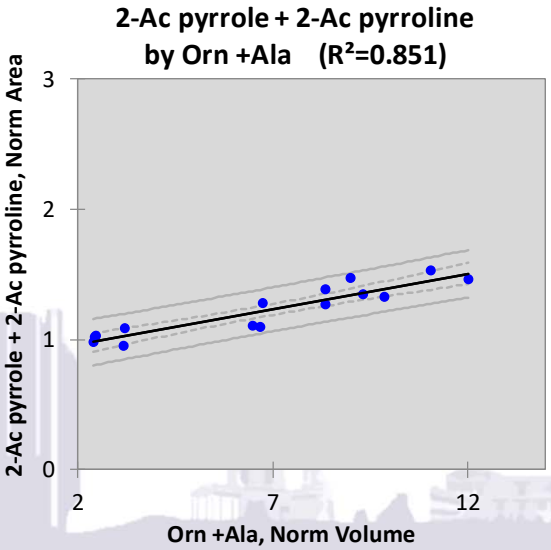
4. Primary metabolites (r≥ 0.89) in accordance to evidences revealed by diagnostic patterns related to cultivars and origin.

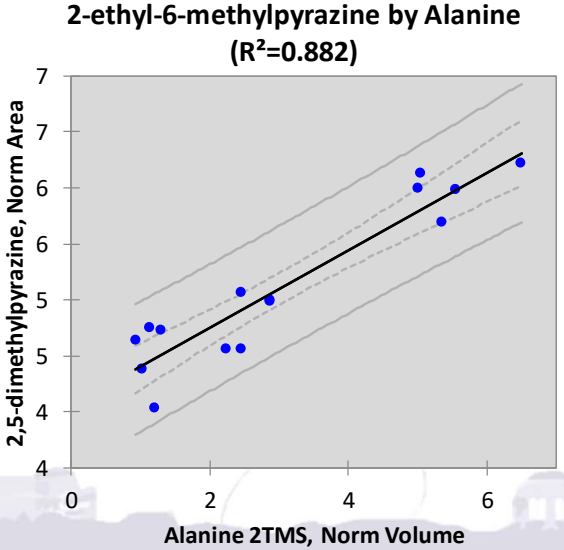
QUESTIONS ON AROMA POTENTIAL

4) Have precursors patterns a predictive potential to assess raw hazelnut quality toward its industrial processing trajectory?









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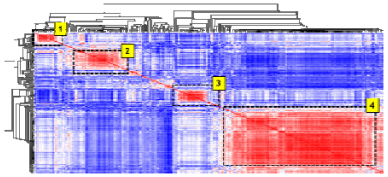

Shelling nuts

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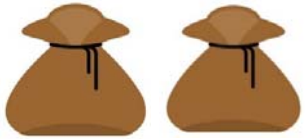
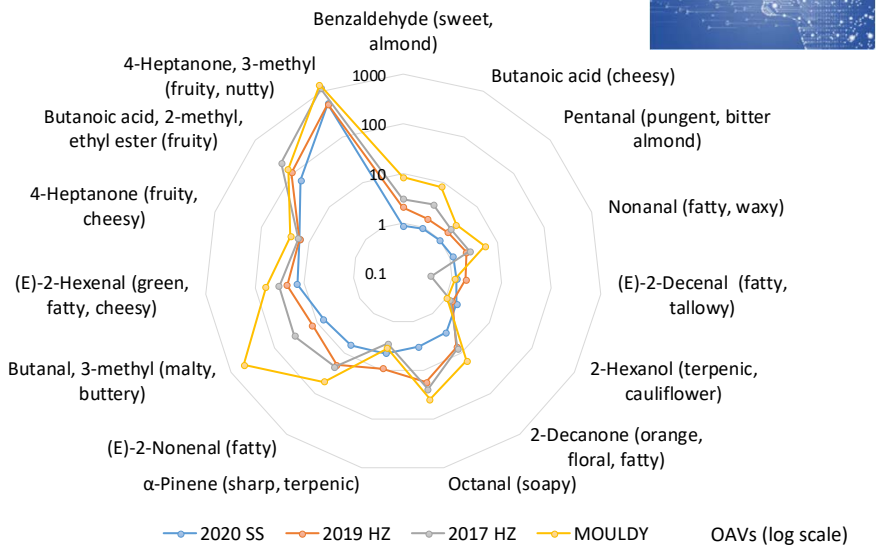
Computer Vision

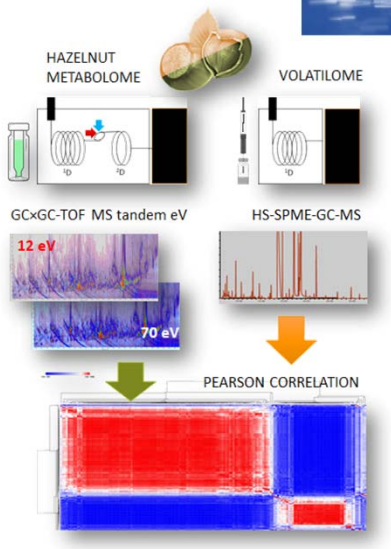


Good (OK) Rancid (KO) Spoiled (KO)

Artificial Intelligence smelling



Aroma potential



GESTALT

noun \ gə-'stält

understanding the whole, not merely the sum of its parts.

Fingerprinting/profiling to unreveal predictive markers

Chromatographic Fingerprinting and Computer vision

Artificial Intelligence smelling machine molecular resolution tool

GC: The State of the Art

Chairperson: Pat Sandra

Participants: Steven Lehotay, Hans-Gerd Janssen, Chiara Cordero, Frank David, John Hinshaw

GC: The State of the Art

November 01, 2017

By **Chiara Cordero**, **Pat Sandra**, **John Hinshaw**, **Hans-Gerd Janssen**, **Frank David**, **Steven Lehotay**

GESTALT

noun \ gə-'stält

understanding the whole, not merely the sum of its parts.

Chiara Cordero: GC-GC cannot be an alternative to GC×GC! They are both capable of expanding the potentials of 1D-GC where a single dimension is not sufficient or selective enough to solve an analytical challenge. However, one is still a multiple 1D-GC approach (that is, GC-GC) and does not require a change of mindset or skills for new users while, as already mentioned, GC×GC requires a "jump" towards new measurement concepts. Once we overcome this gap, we cannot turn back!

Chiara Cordero: I see GC×GC growing in core application areas, including petrochemical, environmental, food and flavours, natural products, and metabolomic studies, and in my research activity I've met new users approaching this technique with curiosity but also with many prejudices and false convictions. My feeling is that we still are in the "induction period".

The possibility of applying dedicated pattern recognition approaches to the analysis of 2D chromatographic data opens new perspectives for fingerprinting studies. This last aspect is a key feature of the technique and it will soon trigger the widespread use of GC×GC in many fields. As experts and passionate chromatographers we have to continue research in the direction of making this technique more intuitive and easy to use with new data analysis tools and approaches to create a "toolbox" for various applications.

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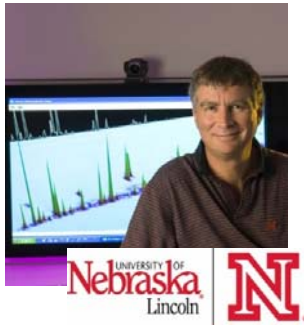
Thank you for your attention

Acknowledgments

Prof. Carlo Bicchi

Prof. Stephen E Reichenbach



Dr. Andrea Caratti

Dr. Simone Squara

Agilent

Applications and Core Technology University Research (ACT-UR) Project #4294

GC IMAGE
Software for Multidimensional Chromatography

Dr. Qingping Tao

Dr. Federico Stilo PhD
Tentamus Group

Dr. Marta Cialiè Rosso PhD
Agilent Technologies

Dr. Nicola Spigolon PhD
Soremartec

SRA INSTRUMENTS
ANALYTICAL SOLUTIONS

SOREMARTEC
Gruppo FERRERO