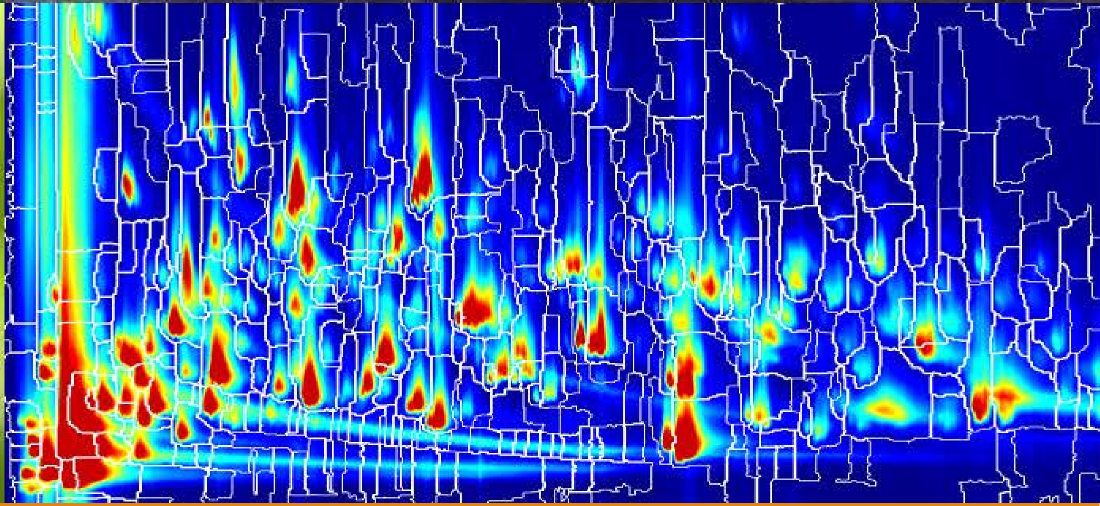


Characterization of odorant patterns by comprehensive two-dimensional gas chromatography: Challenges, strategies and pleasure behind chemistry

Prof. Chiara Emilia Cordero and Prof. Carlo Bicchi

University of Turin - chiara.cordero@unito.it

Foreword

Approaching food compositional complexity to unreveal chemical dimensions and functional properties

Strategies

Multidimensional chromatography and its potential for untargeted-targeted comprehensive investigations

Academic research meets Industrial needs

Example I: hazelnuts and storage quality
Example II: cocoa aroma blueprint

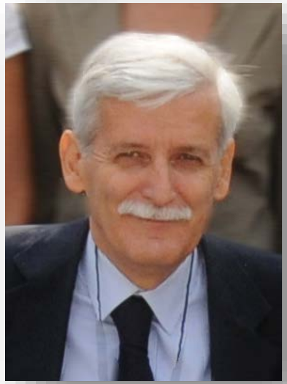
Conclusive remarks

Acknowledgments

UNIVERSITÀ DEGLI STUDI DI TORINO

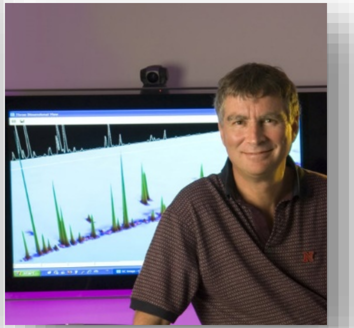
University of Nebraska Lincoln

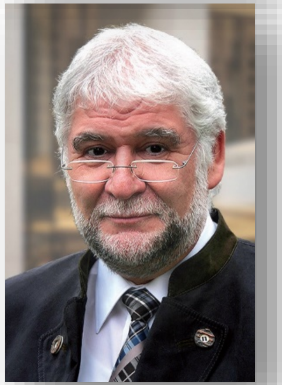
Technical University of Munich



Prof. Carlo Bicchi

Prof. Stephen E. Reichenbach





Prof. Peter Schieberle

Pharmaceutical Biology DSTF-UNITO

Food Chemistry DSTF-UNITO

… the lab Team

JPI a healthy diet for a healthy life

PROGETTO AGER AGROALIMENTARE E RICERCA

FONDAZIONI IN RETE PER LA RICERCA AGROALIMENTARE

FERRERO SOREMARTEC

eit Food

Firmenich

Agilent Trusted Answers

SRA INSTRUMENTS ANALYTICAL SOLUTIONS

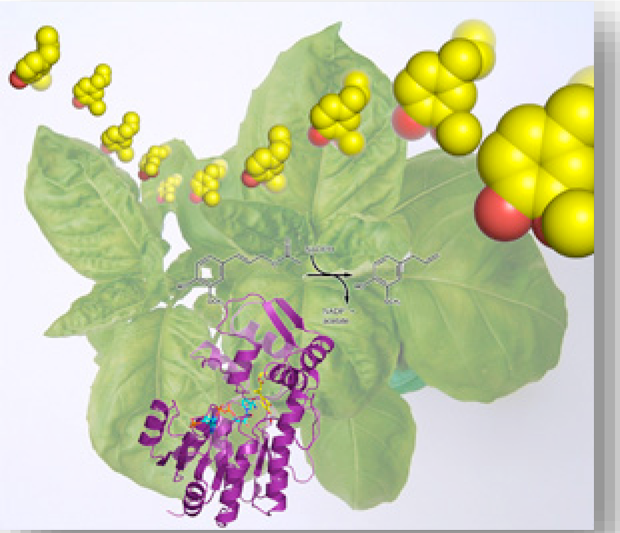
[redacted]

SepSolve Analytical

Foreword

Plant volatilome

Food sensometabolome



Sharing experience and competences

Advanced investigation strategies and **Data interpretation approaches**

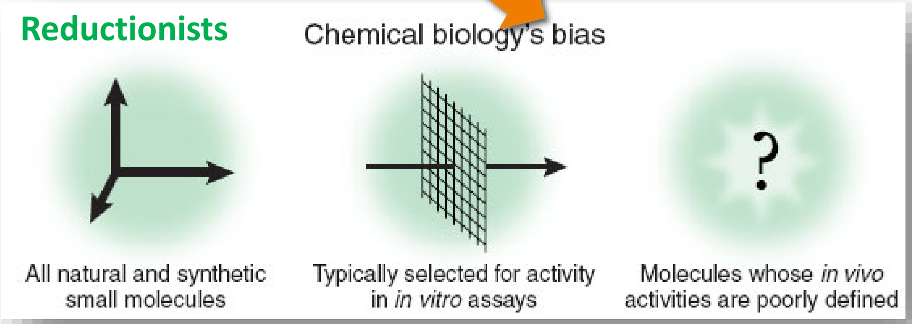
Both disciplines deal with complex biological phenomena

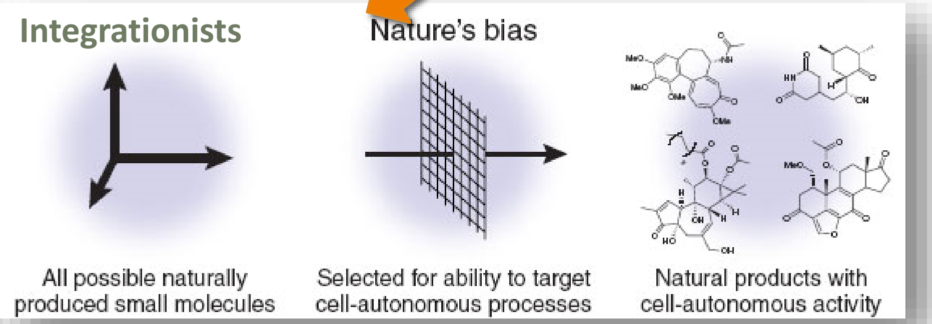
Chemistry is the language

Decoding needs advanced and original analytical approaches

Strategies

Decoding complex phenomena





Adapted from: Randall T. Peterson Commentary on Nature Chemical Biology 4, 635 (2008)

Profiling[1]
detailed analysis of the chemical pattern
sample constituents are studied
by complementary techniques

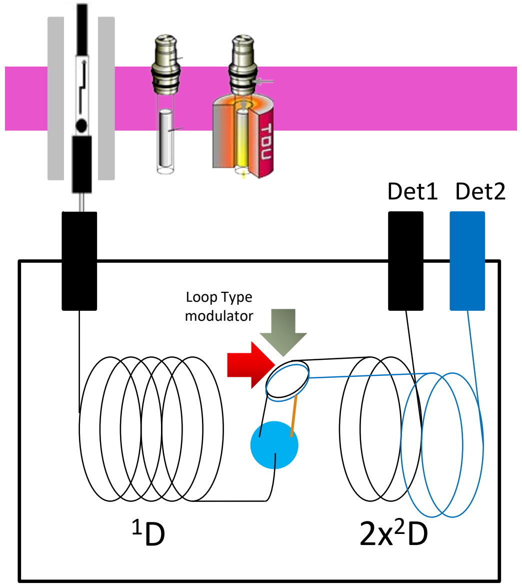
Targeted - Untargeted profiling[2]
multidimensional platforms provide
data on analytes identity (MS signatures)
and amount in the sample

Fingerprinting[1]
general and rapid high-throughput
screening mainly applied
to discriminate and classify samples

Chromatographic fingerprinting[2]
based on pattern recognition principles
extends samples comparison
to all detectable analytes

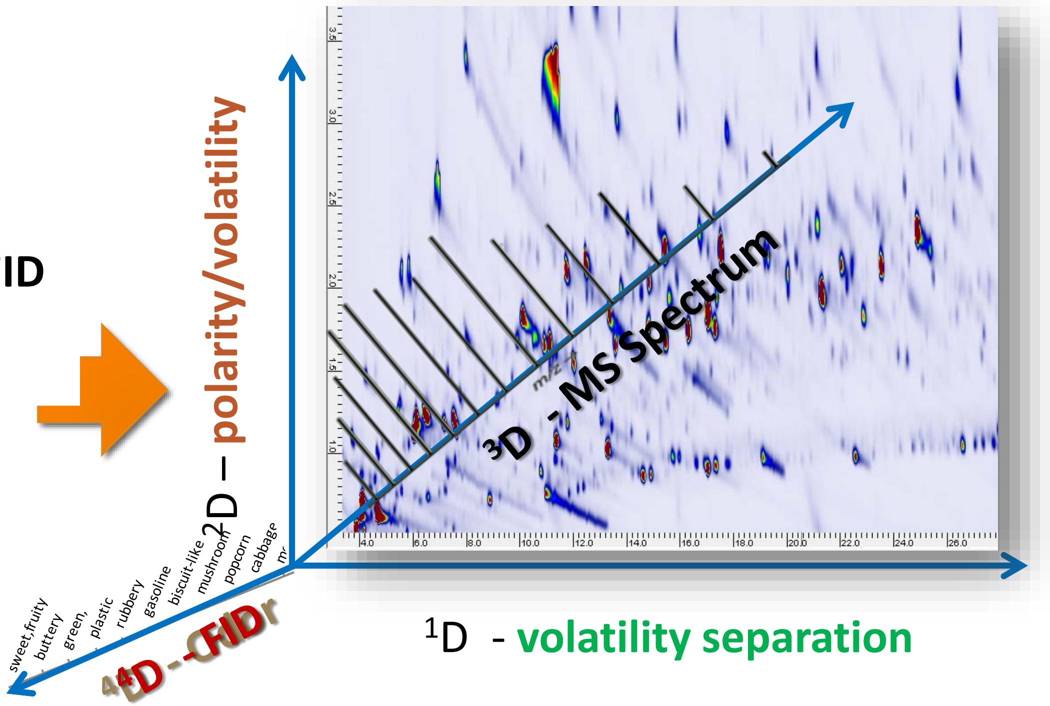
Comprehensive 2D chromatography
unified multi-dimensional platforms

Platforms



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

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



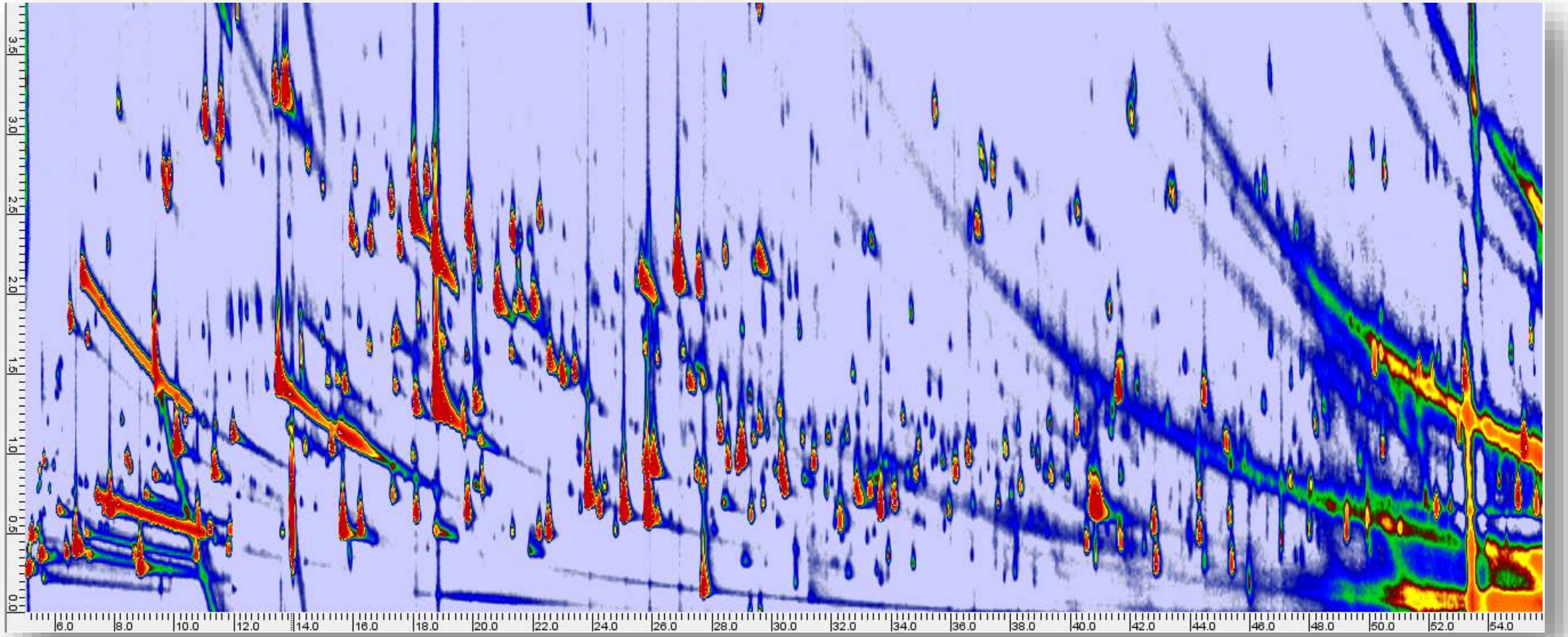
Comprehensive 2D GC
unified multi-dimensional platform

[1] J.C. Giddings, Sample dimensionality: a predictor of order-disorder in component peak distribution in multidimensional separation, J. Chromatogr. A 703(1995) 3–15.

Rational information space

Extra Virgin Olive oil volatiles - Italian origin
HS-SPME (CAR/PDMS/DVB) - 500 mg - 50°C/50 min

Chemical dimensions



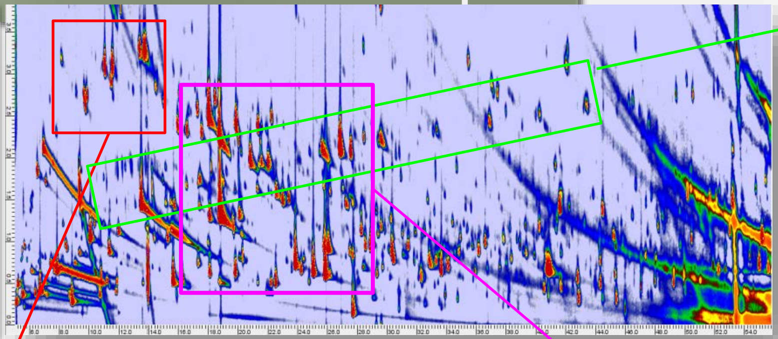
Targeted peaks over more than 800 detectable analytes

255 reliably identified by 70 eV spectrum and I^T coherence

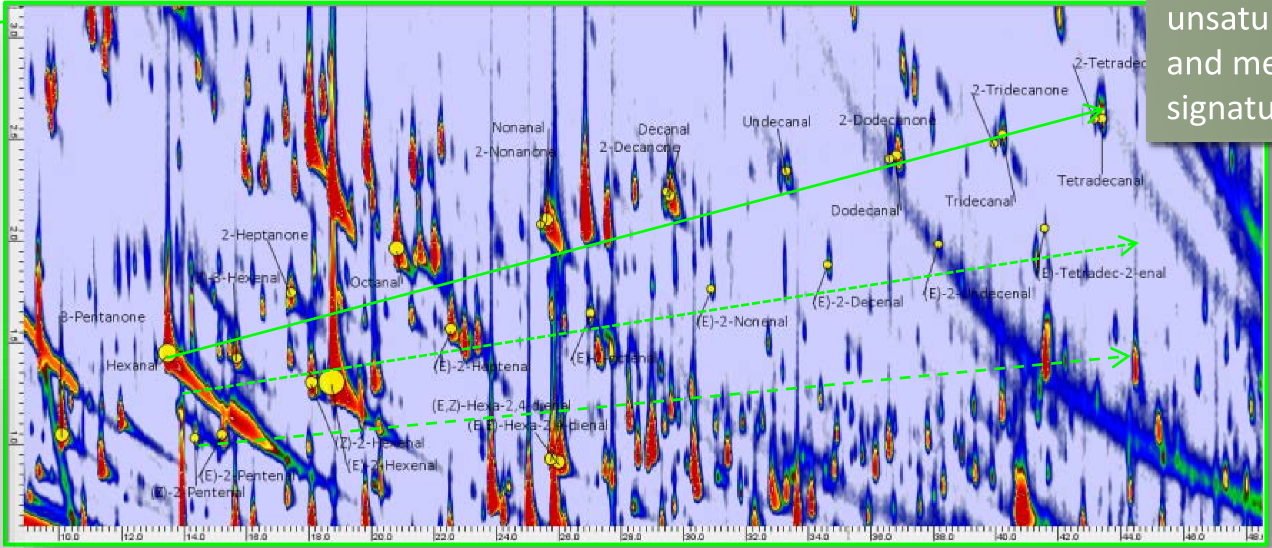
Various chemical classes highly correlated with autoxidation processes , enzymatic peroxidation, aroma compounds and potent odorants

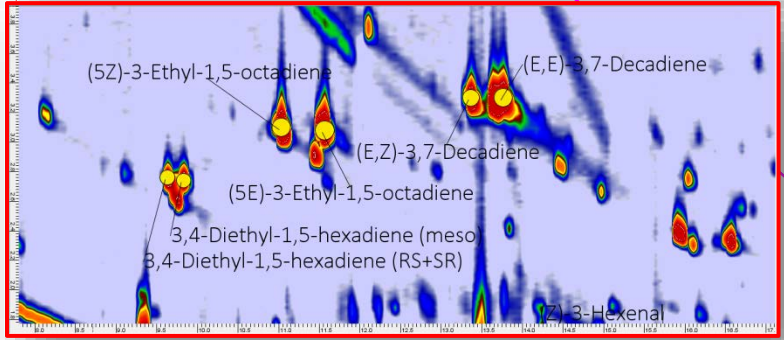
Rational information space

Chemical dimensions

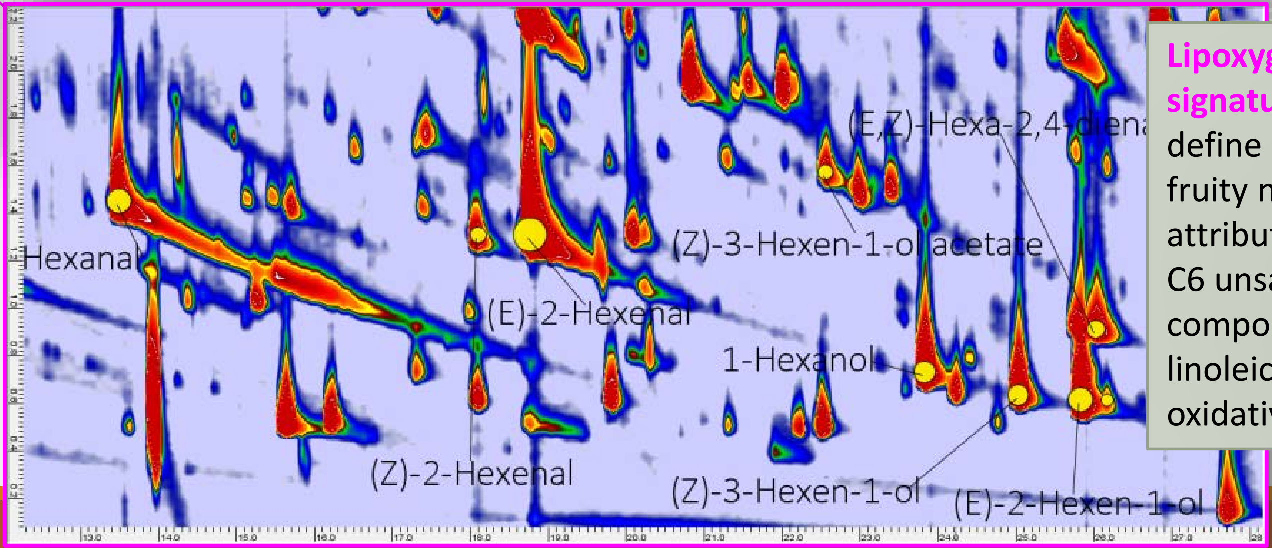


Linear saturated and unsaturated aldehydes and methyl-ketones signatures



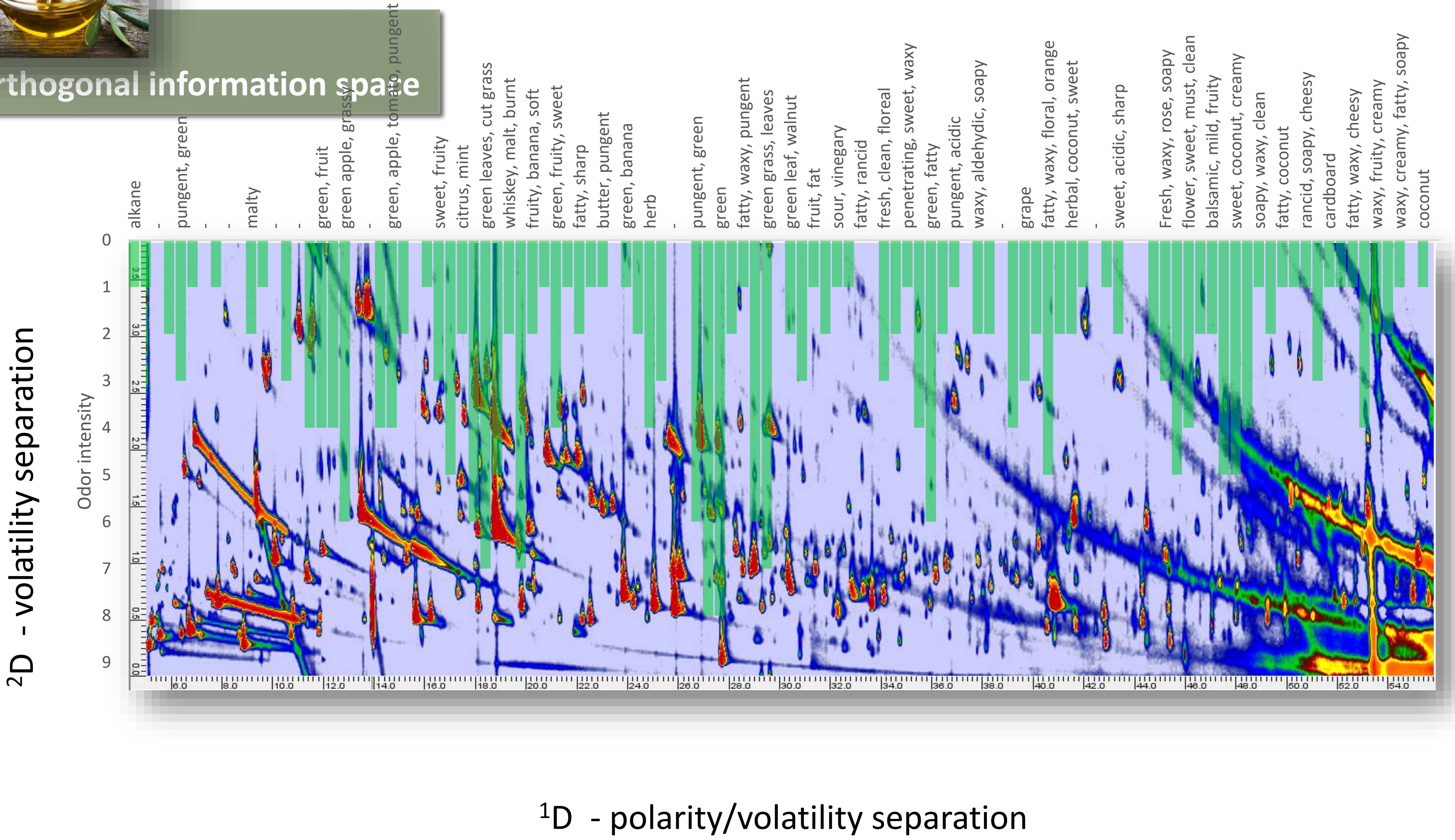


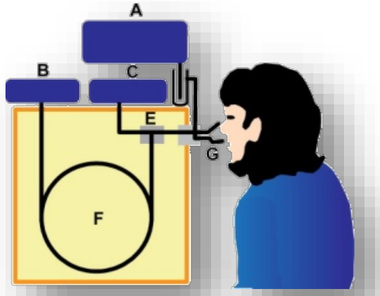
Unsaturated hydrocarbons: distinctive for earlier harvest stages: 3,4-diethyl-1,5-hexadiene (RS þ SR), 3,4-diethyl-1,5-hexadiene (meso), (5Z)-3-Ethyl-1,5-octadiene, (5E)-3-Ethyl-1,5-octadiene, (E,Z)-3,7-decadiene, (E,E)-3,7-decadiene, and (E)-4,8-Dimethyl- 1,3,7-nonatriene.



Lipoxygenase (LOX) signature: fundamental to define fresh-green and fruity notes (positive attributes) -> biogenesis of C6 unsaturated compounds derived from linoleic and linolenic acids oxidative cleavage.

Orthogonal information space



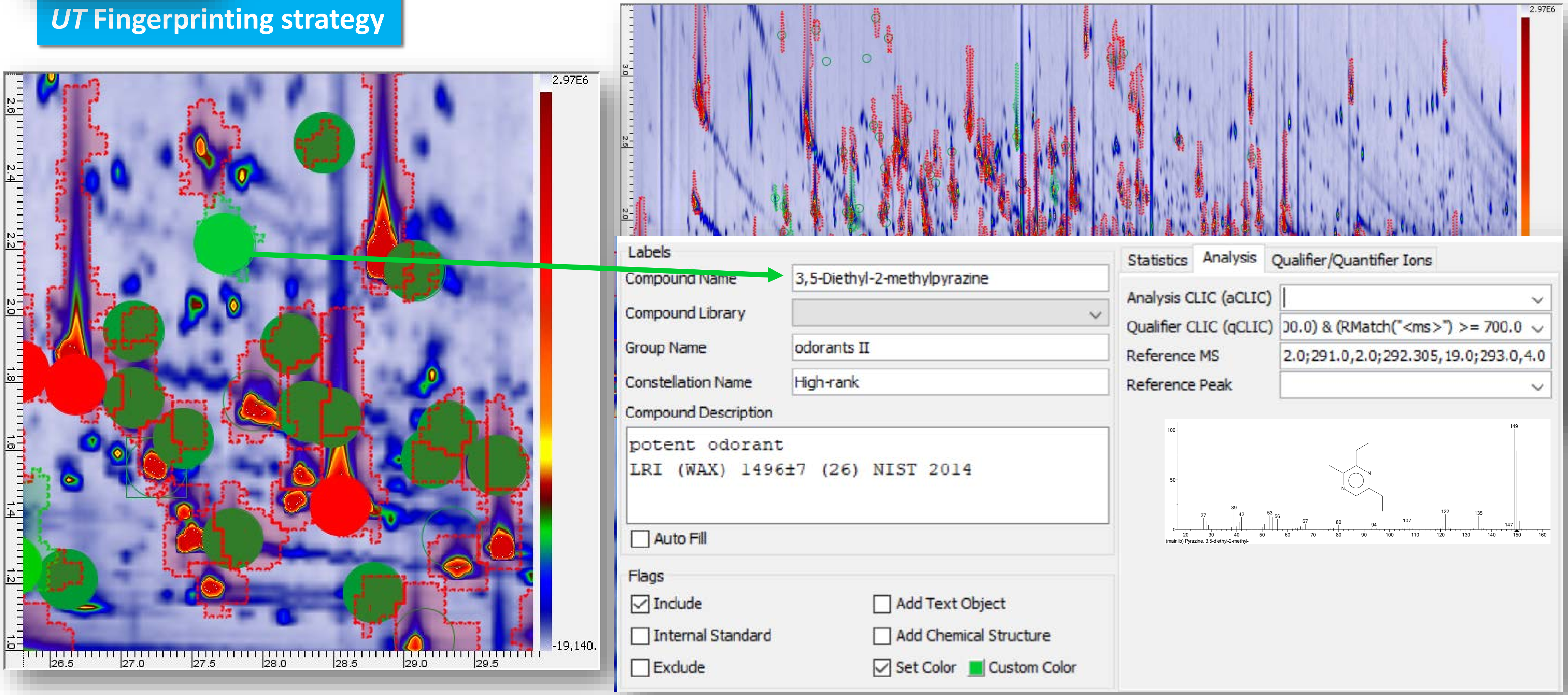


Picture from:
TrAC (2011) 30(11) :1756–1770

STEP 1

Untargeted/Targeted Fingerprinting - comprehensive mapping

UT Fingerprinting strategy



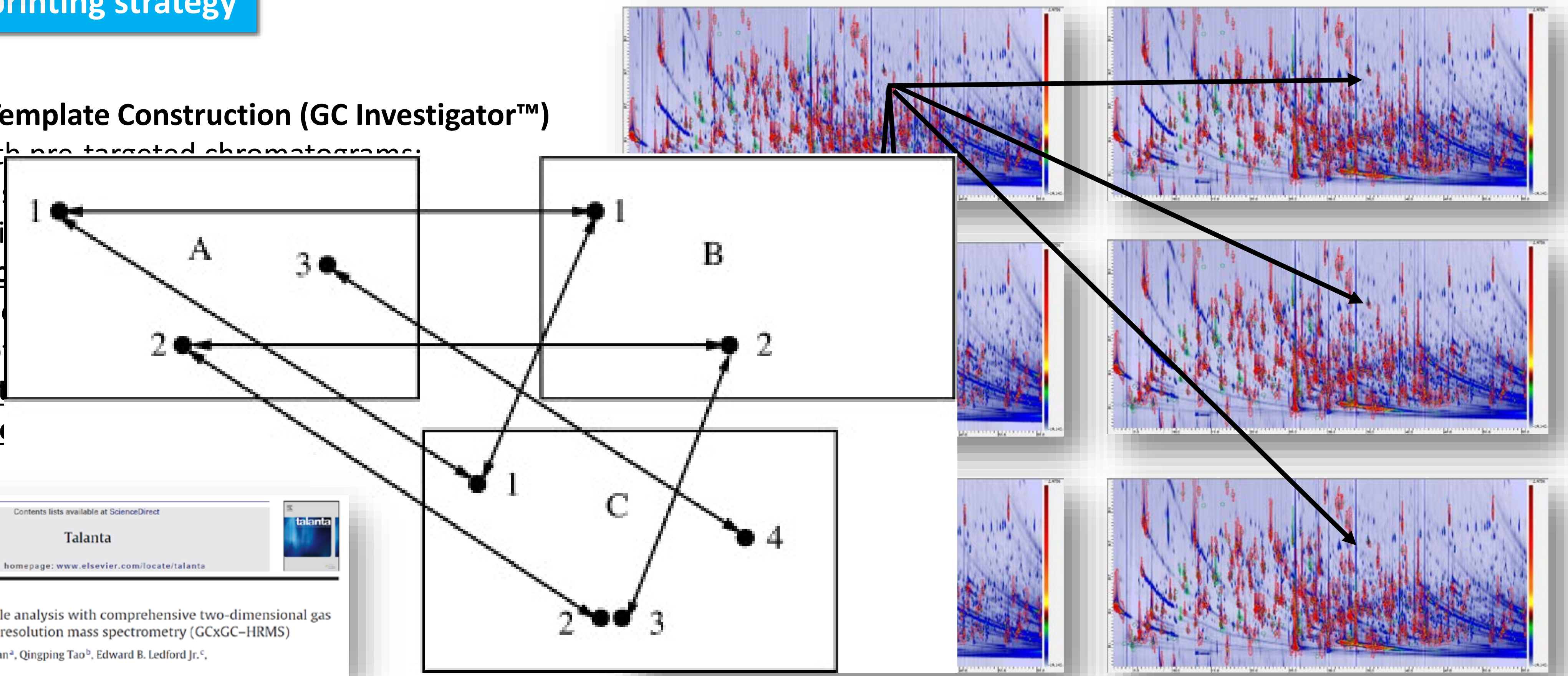
STEP 2

UT Fingerprinting strategy

Untargeted Template Construction (GC Investigator™)

Beginning with pre-targeted chromatograms:

– comprehens
– determinati
– **alignment o**
– generation o
– definition of
– building of **t**
and peak-r



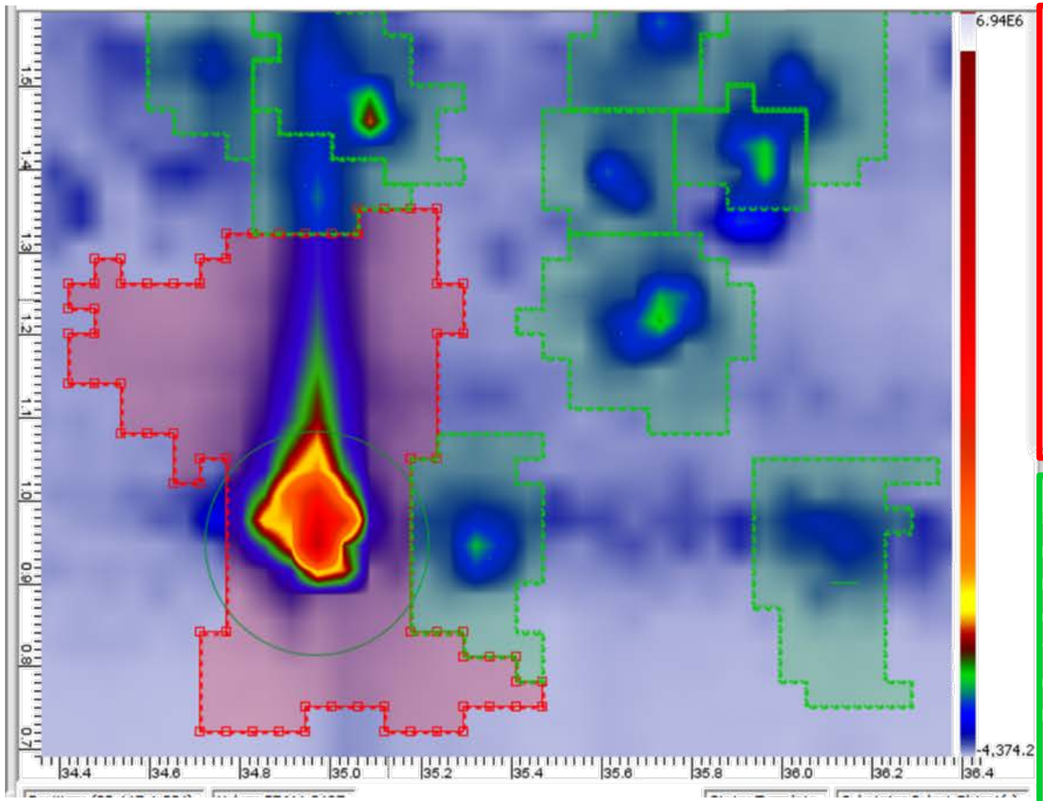
Contents lists available at ScienceDirect
Talanta
journal homepage: www.elsevier.com/locate/talanta
ELSEVIER
talanta

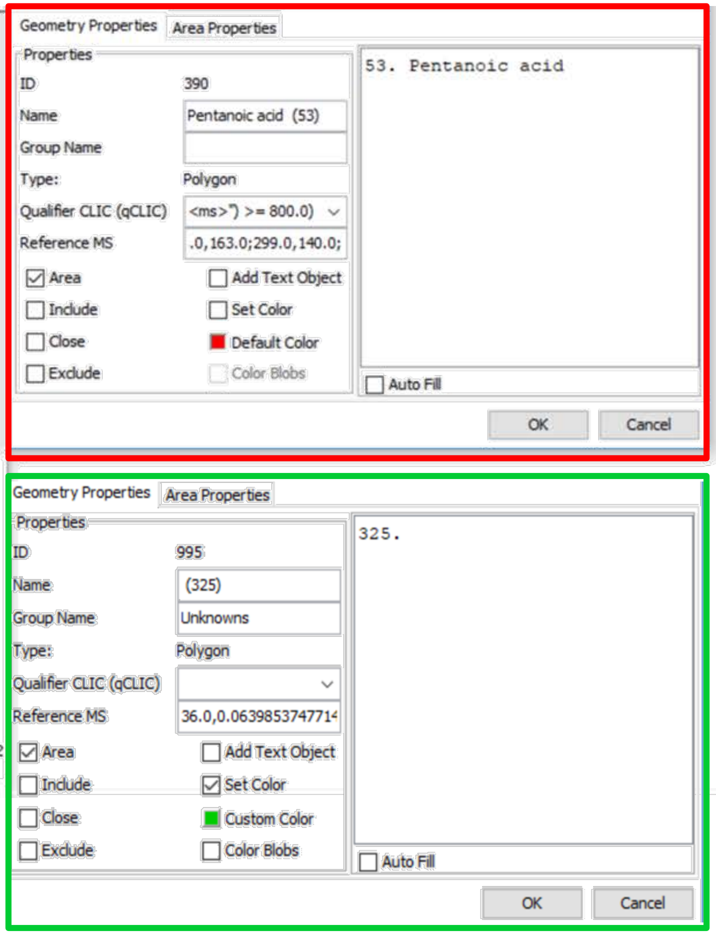
Informatics for cross-sample analysis with comprehensive two-dimensional gas chromatography and high-resolution mass spectrometry (GCxGC–HRMS)

Stephen E. Reichenbach[a,*], Xue Tian[a], Qingping Tao[b], Edward B. Ledford Jr.[c], Zhanpin Wu[c], Oliver Fiehn[d]

STEP 2

UT Fingerprinting strategy



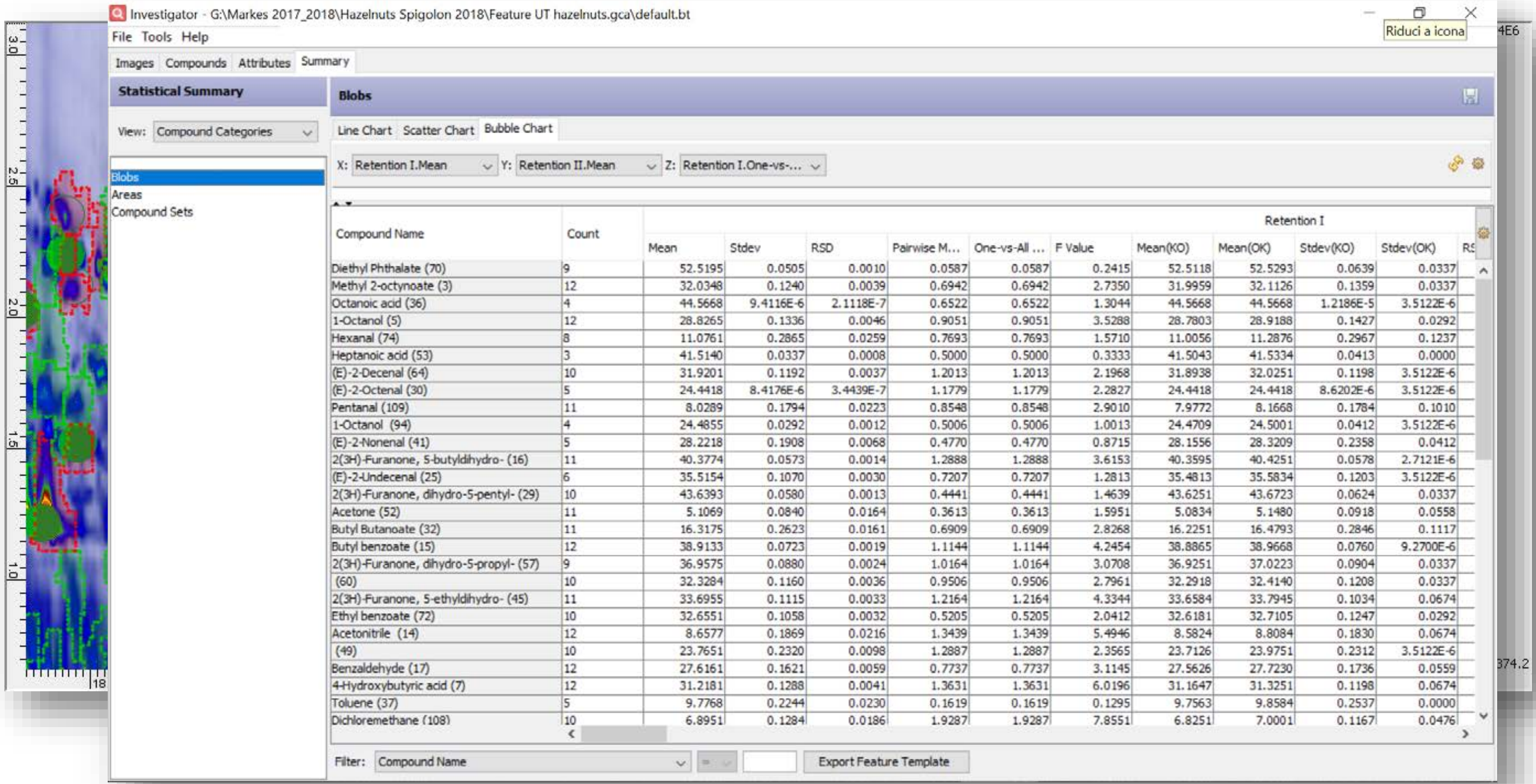


Known - targeted analytes distribution

Unknowns - untargeted analytes distribution

STEP 2

UT **Fingerprinting strategy**



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
Dichloremethane (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 features are cross-aligned between all samples and metadata collected for further processing

Hazelnuts composition

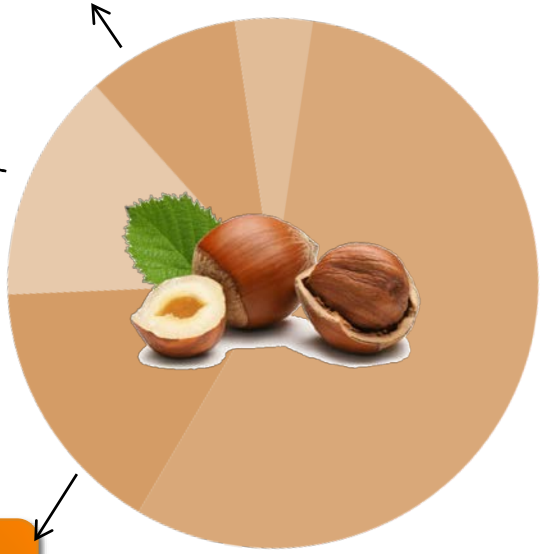
- Dietary fiber → positive effects on blood sugar and cholesterol levels.

WATER (5%)

FIBER (10%)

PROTEINS & AMINO ACIDS (15%)

- Good source of amino acids
- Arginine and Glutamic acids are the most abundant



FATS (60%)

- Organoleptic characteristic, shelf life and nutritional quality
- Rich in MUFA and PUFA
- Poor in SFA

CARBOHYDRATES (17%)

- Soluble sugars: glucose, fructose, *myo*-inositol, sucrose, raffinose and stachyose

Volatiles < 0.1%

Encrypts a lot of information about hazelnut quality

Volatile fraction

Chemical dimensions

- ✓ Hydrocarbons (linear, branched, aromatics)
- ✓ Terpenoids (isoprenoides derivatives C10-C15) hydrocarbons and oxygenated
- ✓ Alcohols (linear and branched)
- ✓ Carbonyl derivatives (aldehydes, ketones)
- ✓ Carboxylic acids (short chain highly volatiles pKa)
- ✓ Esters (FAs and alcohols from aa degradation)
- ✓ Lactones (cyclic esters from hydroxy acids)
- ✓ Heterocycles from Maillard reaction (furanones, pyranones)
- ✓ …

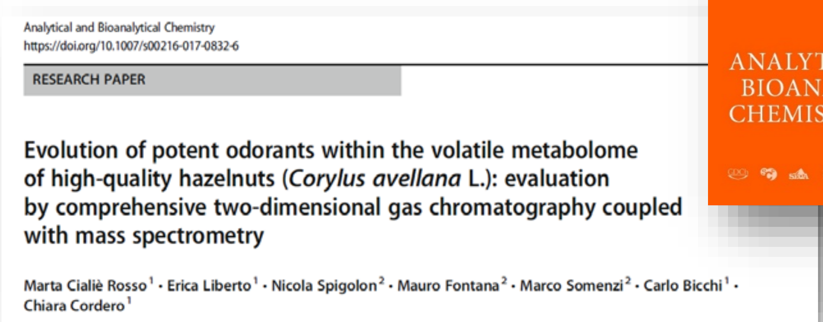
Information dimensions

- ✓ geographical origin and pedoclimatic variations
- ✓ **phenotyping** and chemotyping
- ✓ multitrophic interactions (plants-insects)
- ✓ **presence of bacteria and molds**
- ✓ **scent and odorous compounds**

Roasted hazelnuts

- ✓ **distinctive aroma blueprint**
- ✓ **thermal processes**
- ✓ presence of nutrients and non-nutrients

Example I



Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-017-0832-6

RESEARCH PAPER

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

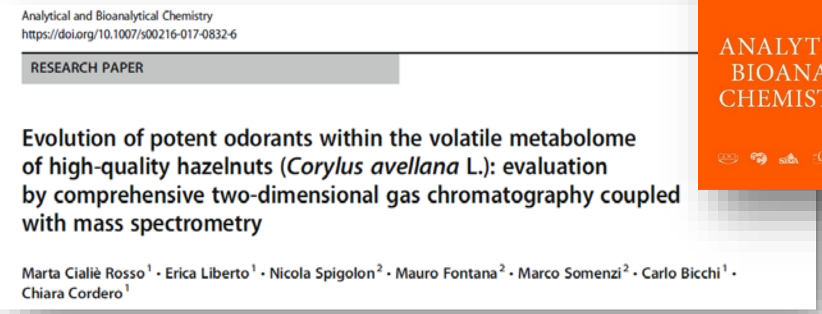
Marta Cialiè Rosso[1] · Erica Liberto[1] · Nicola Spigolon[2] · Mauro Fontana[2] · Marco Somenzi[2] · Carlo Bicchi[1] · Chiara Cordero[1]

Industry trajectory
Post-harvest and storage

- ✓ Origin - botanical / geographical
- ✓ Post-harvest drying (traditional in field, under controlled conditions and at low temperature)
- ✓ Storage 0-12 months at different conditions: normal or modified atmosphere, 5 and 18°C

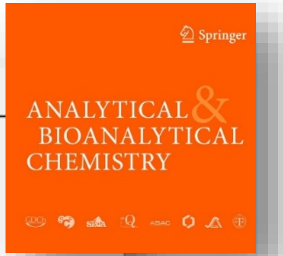
Volatiles formed by lipid oxidation and enzymatic activity (endogenous or exogenous - bacteria, molds)

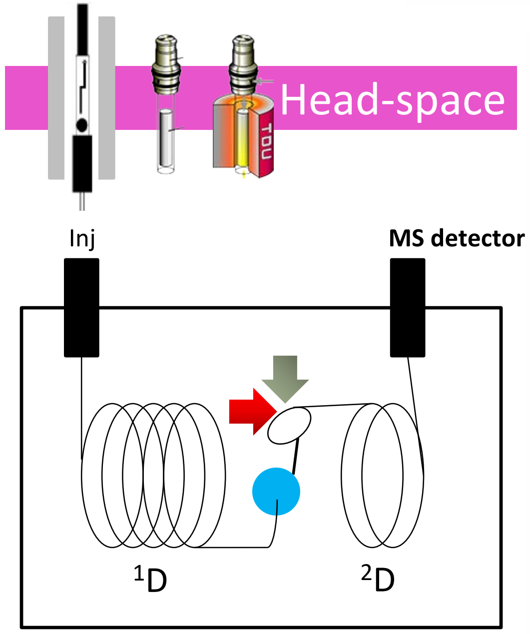
Example I


Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-017-0832-6
RESEARCH PAPER

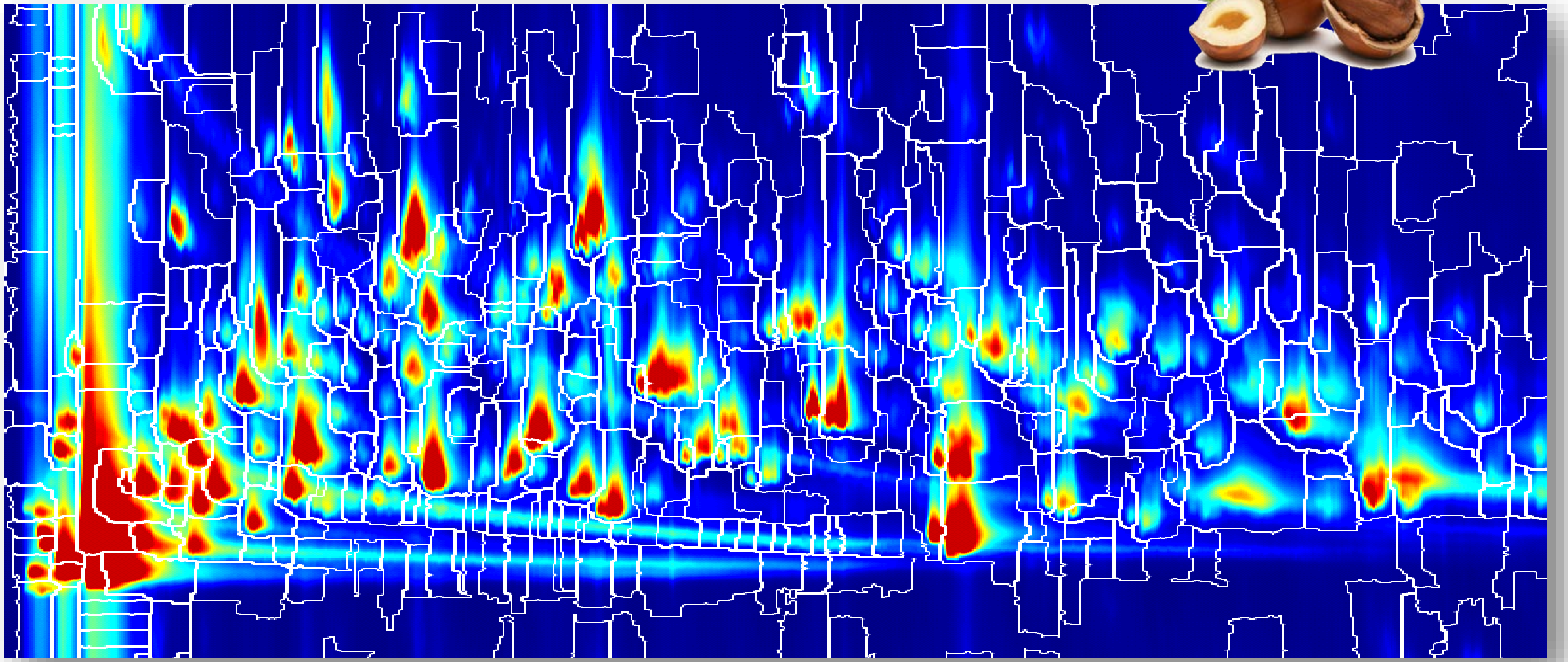
Evolution of potent odorants within the volatile metabolome of high-quality hazelnuts (*Corylus avellana* L.): evaluation by comprehensive two-dimensional gas chromatography coupled with mass spectrometry

Marta Cialiè Rosso [1] · Erica Liberto [1] · Nicola Spigolon [2] · Mauro Fontana [2] · Marco Somenzi [2] · Carlo Bicchi [1] · Chiara Cordero [1]



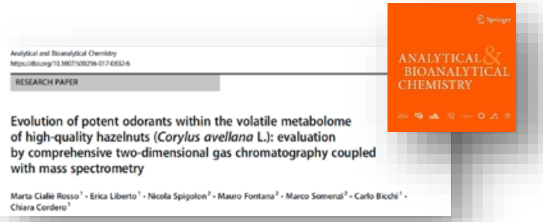


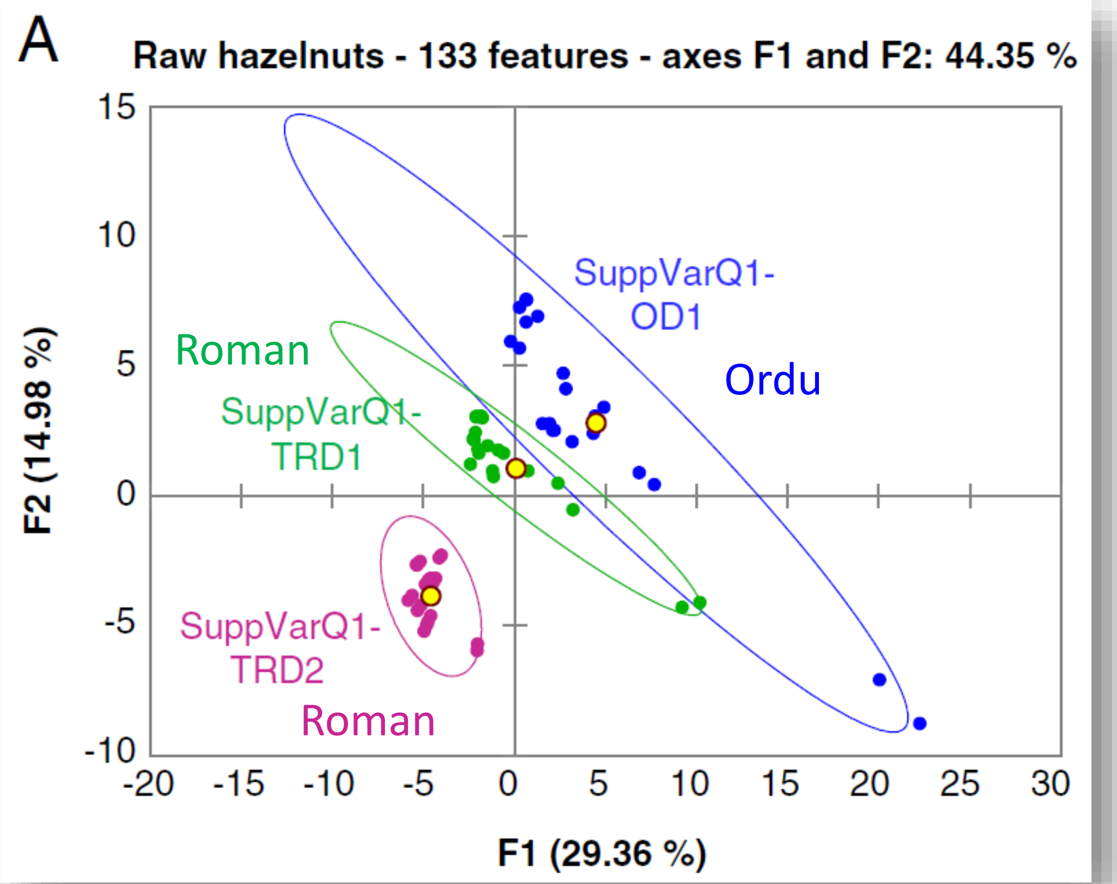
GC×GC-MS



Example I

Ordu D1- traditional drying
Roman D1 - traditional drying
Roman D2 - low temperature drying





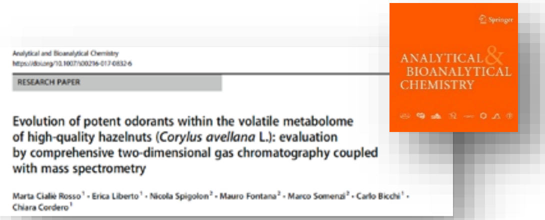
133 volatiles - targeted fingerprinting

Enable a clear clusterization of samples according to origin and post-harvest treatment

Fisher ratio (F), driven **by post-harvest drying** (D1 vs. D2).
Fisher critical value at the 95% of confidence level (α = 0.05 Fcrit) for the data matrix dimensions was 2.16.

Most informing variables (F value 202 -22): series of linear and branched alcohols (2-heptanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-ethyl-1-hexanol, benzyl alcohol), esters (ethyl acetate, butyl butanoate, 2-methyl-butyl propanoate) and acetic acid.

Example I



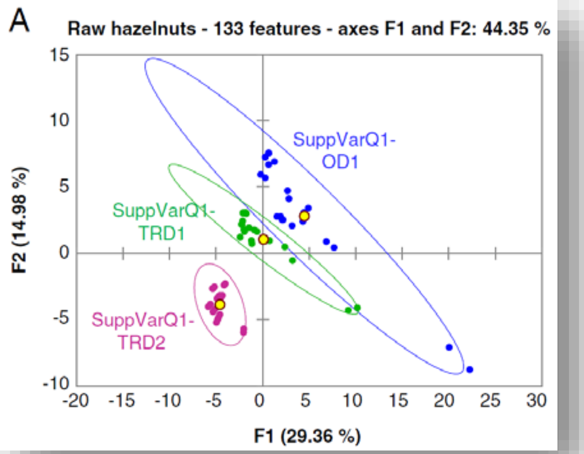
Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-017-0832-6

RESEARCH PAPER

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

Marta Cialiè Rosso[1] · Erica Liberto[1] · Nicola Spigolon[2] · Mauro Fontana[2] · Marco Somenzi[2] · Carlo Bicchi[1] · Chiara Cordero[1]

Exploring the information dimensions

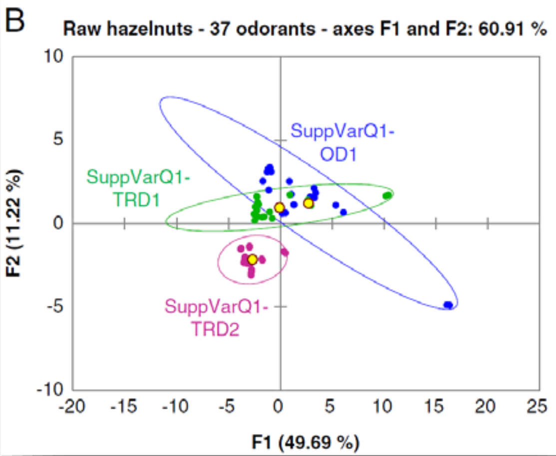


Ordu D1- traditional drying
Roman D1 - traditional drying
Roman D2 - low temperature drying

Fruit viability
Enzimatic inactivation

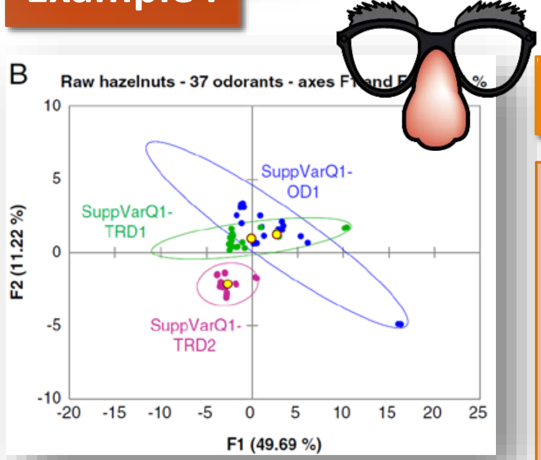
Most of the informative compounds have been correlated with nut **ripening** and/or **fermentation processes** occurring in vegetables

3-Methyl-1-butanol (i.e., isoamyl alcohol) is a fermentation product formed from L-leucine
2-Methyl-1-propanol - L-valine as precursor
2-Heptanol is formed from β-ketoacids hydrolysis and subsequent decarboxylation
2-Ethyl-1-hexanol found in fermented soybean foods

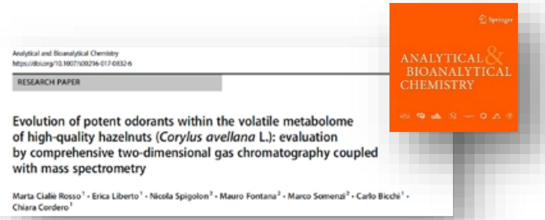


The **most potent odorants** (OT values up to 2500 µg/L) correlated closely (> 0.800) with storage time: 1-heptanol (*green*, *chemical*), 2-octanol (*metal*, *burnt*), 1-octen-3-ol (*mushroom*), (E)-2-heptenal (*fatty*, *almond*), hexanal (*leaf-like, green*), heptanal (*fatty*), octanal (*fatty*) and nonanal (*tallowy*, *fruity*)

Example I



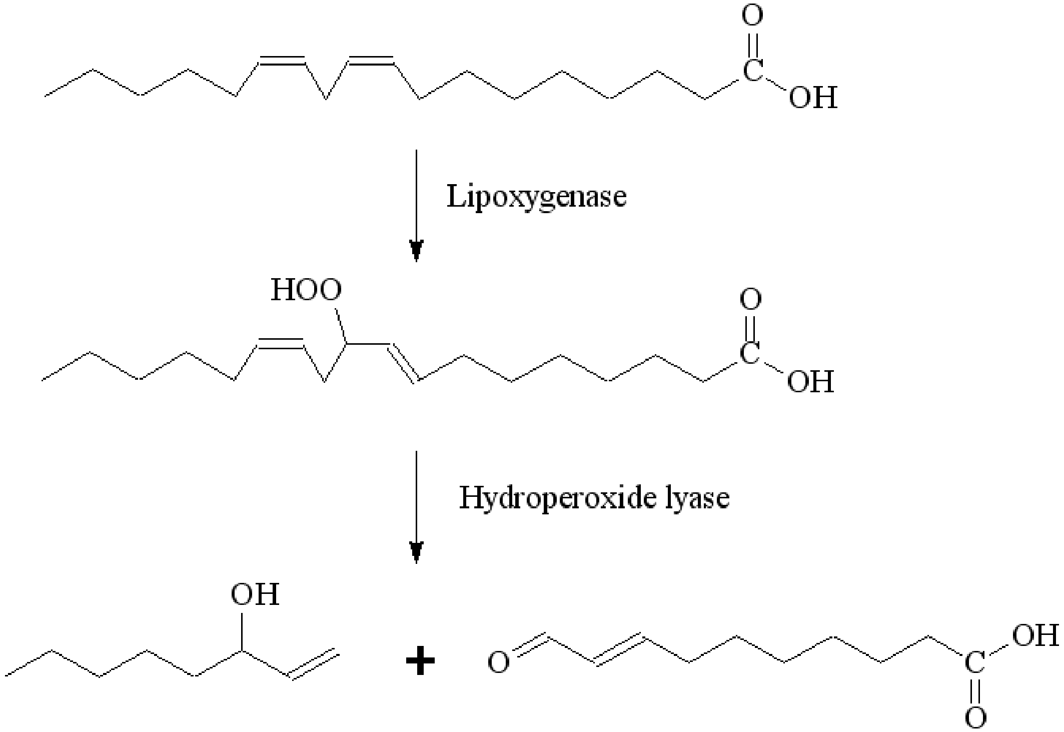
Ordu D1- traditional drying
Roman D1 - traditional drying
Roman D2 - low temperature drying



Exploring the information dimensions

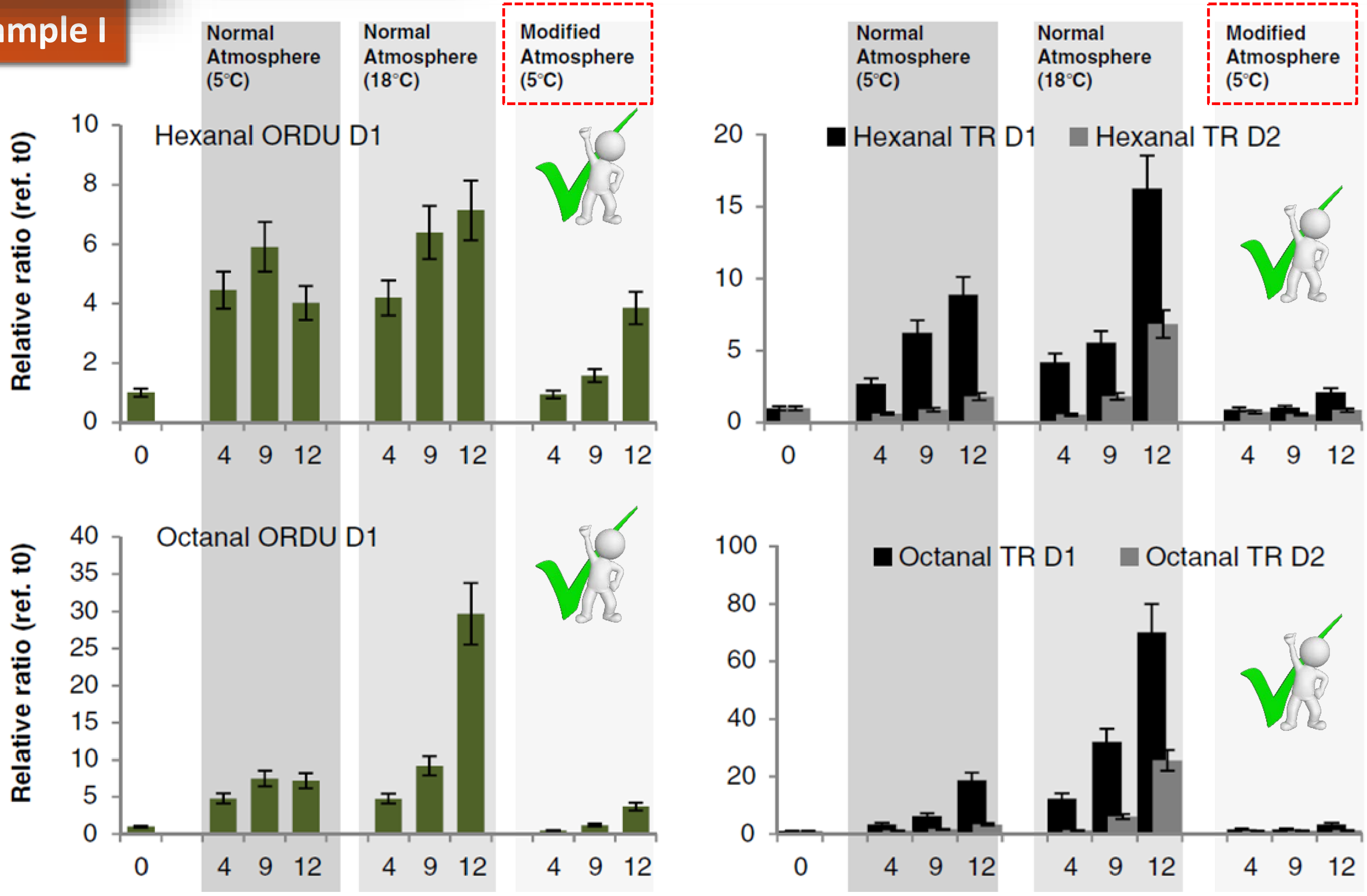
2-octanol and 1-octen-3-ol known products of linoleic acid cleavage, which are generally promoted by fungal lipoxygenase/hydroperoxide liase enzymes

The increasing trend of these alcohols might be correlated to the occurrence of **off-odors** related to *metallic* and *mushroom-like* notes



Fruit viability
Enzimatic inactivation

Example I



leaf-like, green

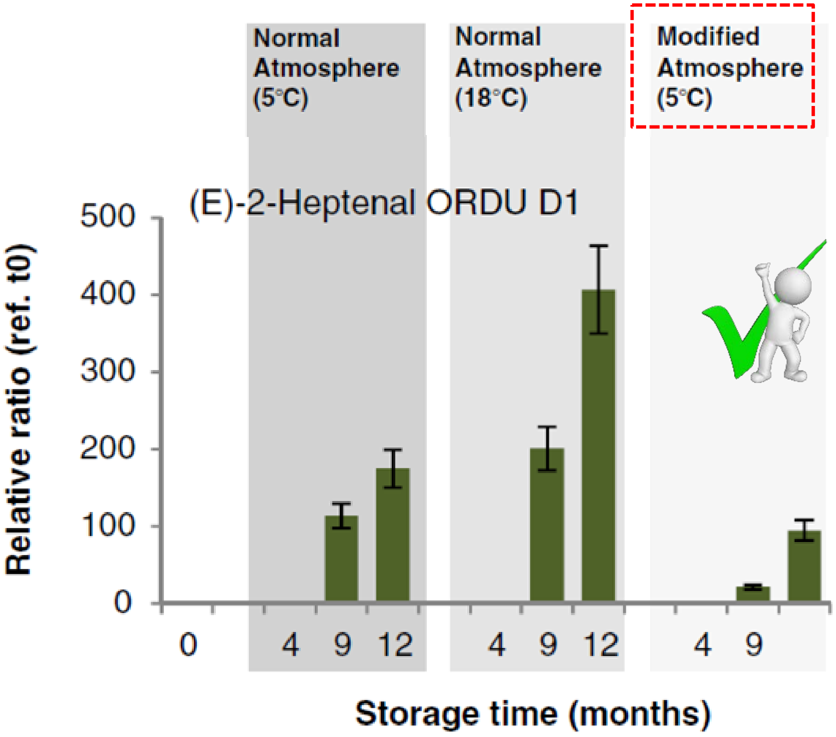
fatty

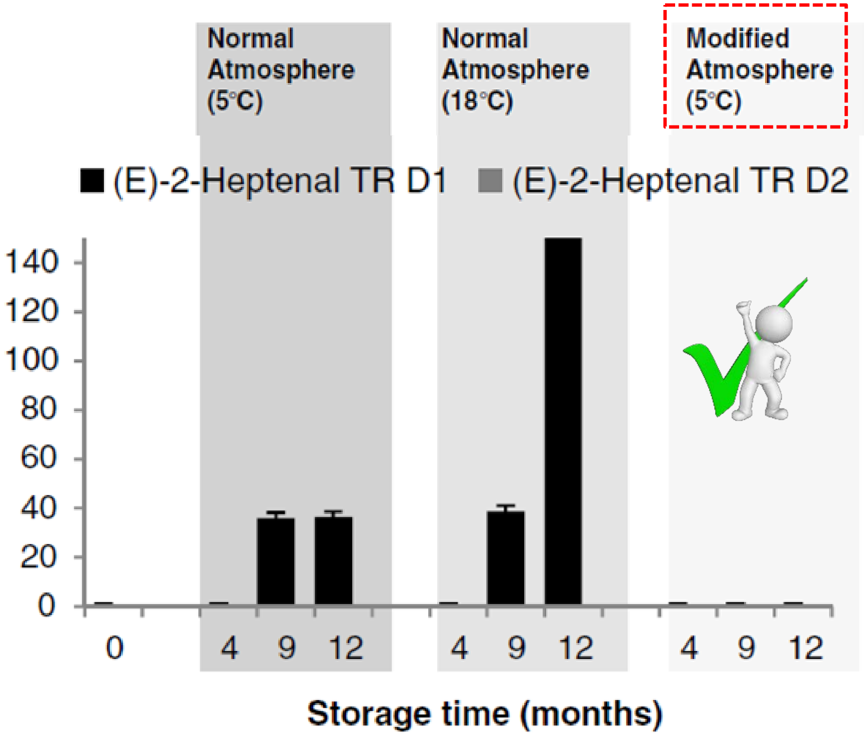

ANALYTICAL & BIOANALYTICAL CHEMISTRY

RESEARCH PAPER

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

Example I





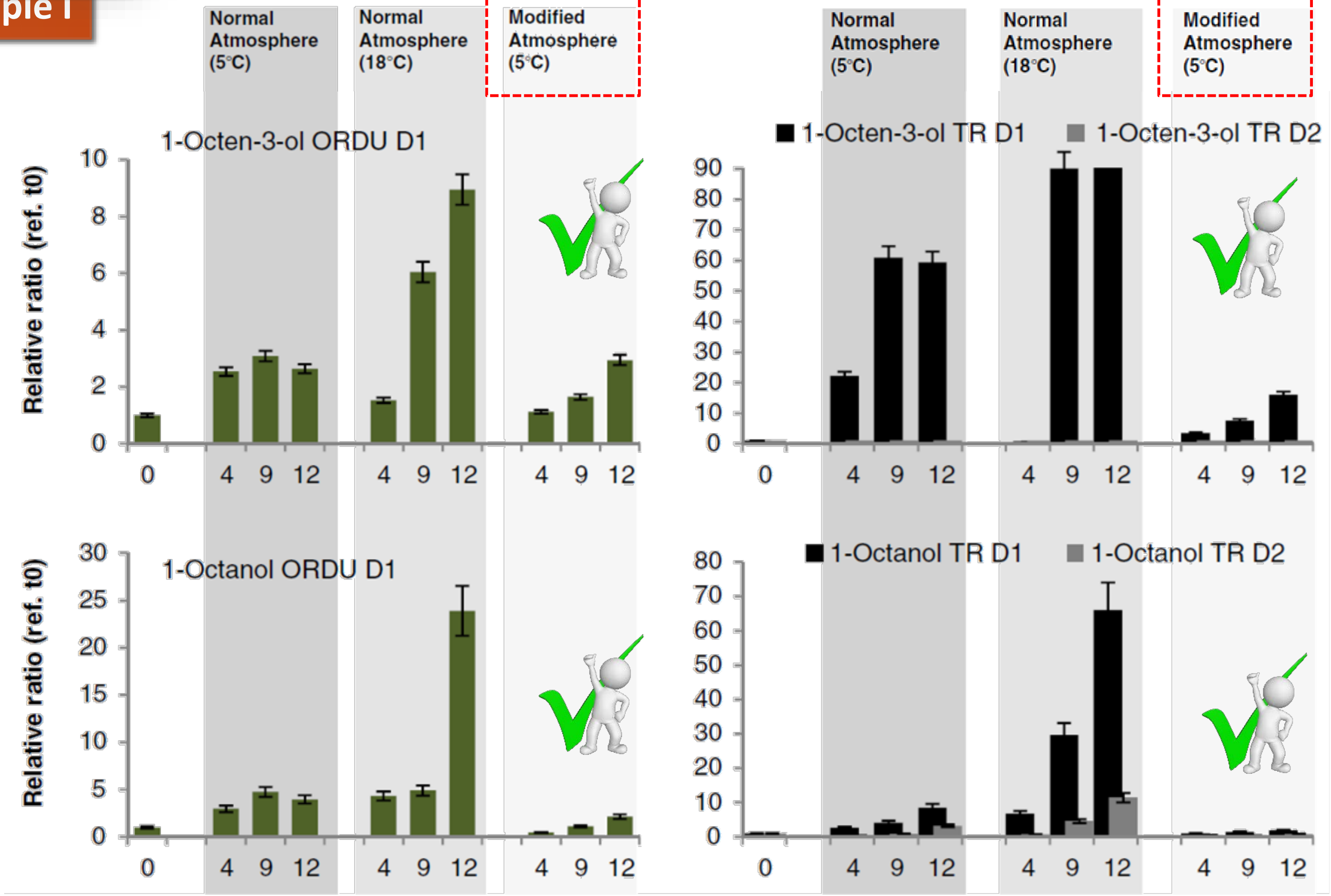
fatty, almond


ANALYTICAL & BIOANALYTICAL CHEMISTRY

RESEARCH PAPER

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

Example I



mushroom

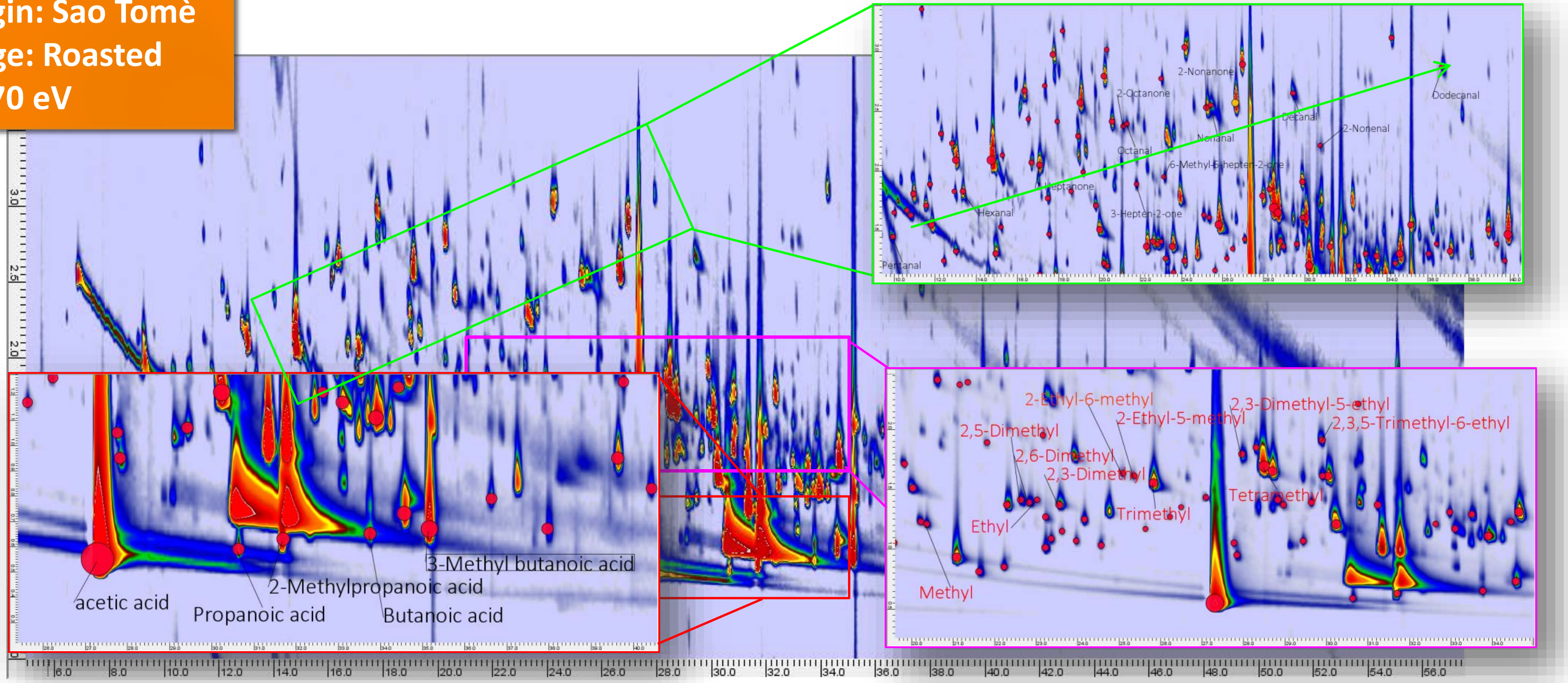
metal, mushroom

Example II

Industry trajectory How capture cocoa aroma blueprint from high-quality origins?

Origin: Sao Tomè
Stage: Roasted
EI: 70 eV

Linear saturated aldehydes and ketones signature



Short chain fatty acids signature: linear and branched chain FAs derived from beans fermentation during post-harvest

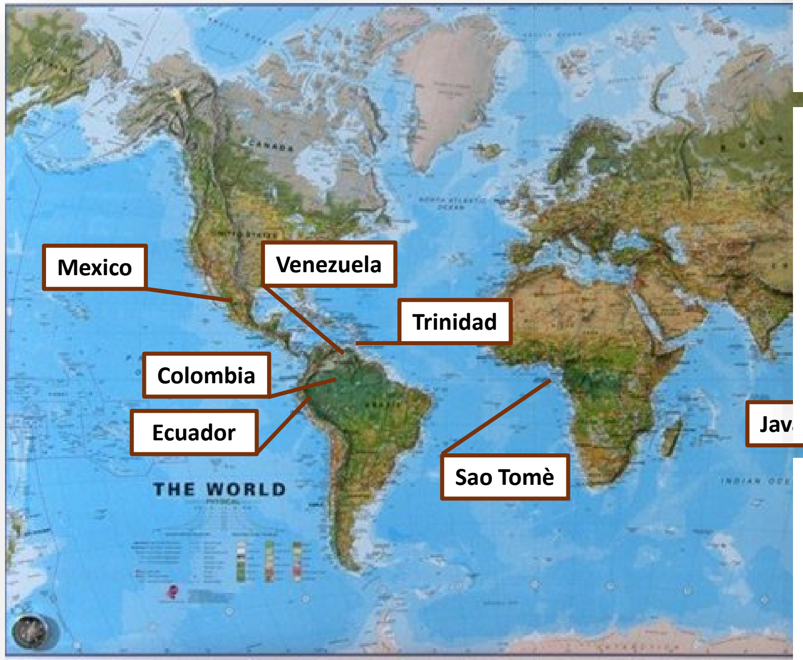
Pyrazines signature: this chemical group of volatiles, formed through Maillard reaction of di-carbonyls and aminoacids, is informative about geographical origing of cocoa. Pyrazines are also key-odorants imparting earthy and roasty notes

Example II

Geographical origins: Central/South America, Africa, Asia

Harvest year 2014
Genetic differences
Commercial Samples

Technological stages: raw, roasted, steamed and nibs



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
Article
pubs.acs.org/JAFC

Comprehensive Chemical Fingerprinting of High-Quality Cocoa at Early Stages of Processing: Effectiveness of Combined Untargeted and Targeted Approaches for Classification and Discrimination

Federico Magagna,[†] Alessandro Guglielmetti,[†] Erica Liberto,[†] Stephen E. Reichenbach,[‡] Elena Allegrucci,[§] Guido Gobino,[§] Carlo Bicchi,[†] and Chiara Cordero[*,†]

Contents lists available at ScienceDirect
ELSEVIER
Journal of Chromatography A
journal homepage: www.elsevier.com/locate/chroma

Advanced fingerprinting of high-quality cocoa: Challenges in transferring methods from thermal to differential-flow modulated comprehensive two dimensional gas chromatography

CrossMark

Federico Magagna[a], Erica Liberto[a], Stephen E. Reichenbach[b], Qingping Tao[c], Andrea Carretta[d], Luigi Cobelli[d], Matthew Giardina[e], Carlo Bicchi[a], Chiara Cordero[a,*]

Contents lists available at ScienceDirect
ELSEVIER
Journal of Chromatography A
journal homepage: www.elsevier.com/locate/chroma

Comprehensive two-dimensional gas chromatography coupled with time of flight mass spectrometry featuring tandem ionization: Challenges and opportunities for accurate fingerprinting studies[☆]

Chiara Cordero[a,*], Alessandro Guglielmetti[a], Carlo Bicchi[a], Erica Liberto[a], Lucie Baroux[b], Philippe Merle[b], Qingping Tao[c], Stephen E. Reichenbach[c,d]

Example II

"Comprehensive" fingerprinting

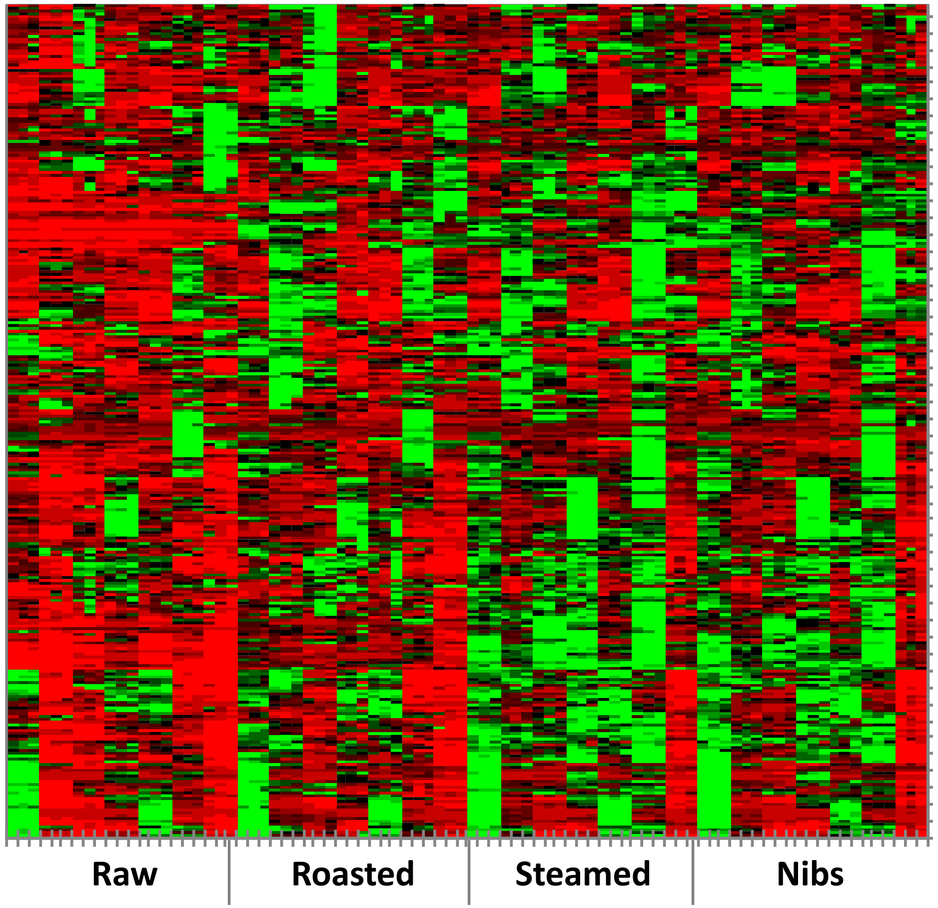
data matrix 595 peak-regions × 168 runs

7 origins × 4 step of processing × 3 batches × 2 replicates

Heat-map (mean and centering normalization) based on Normalized 2D volumes of **595 peak-regions** including **130 targeted analytes** and **17 key-odorants** eliciting characteristic cocoa notes.

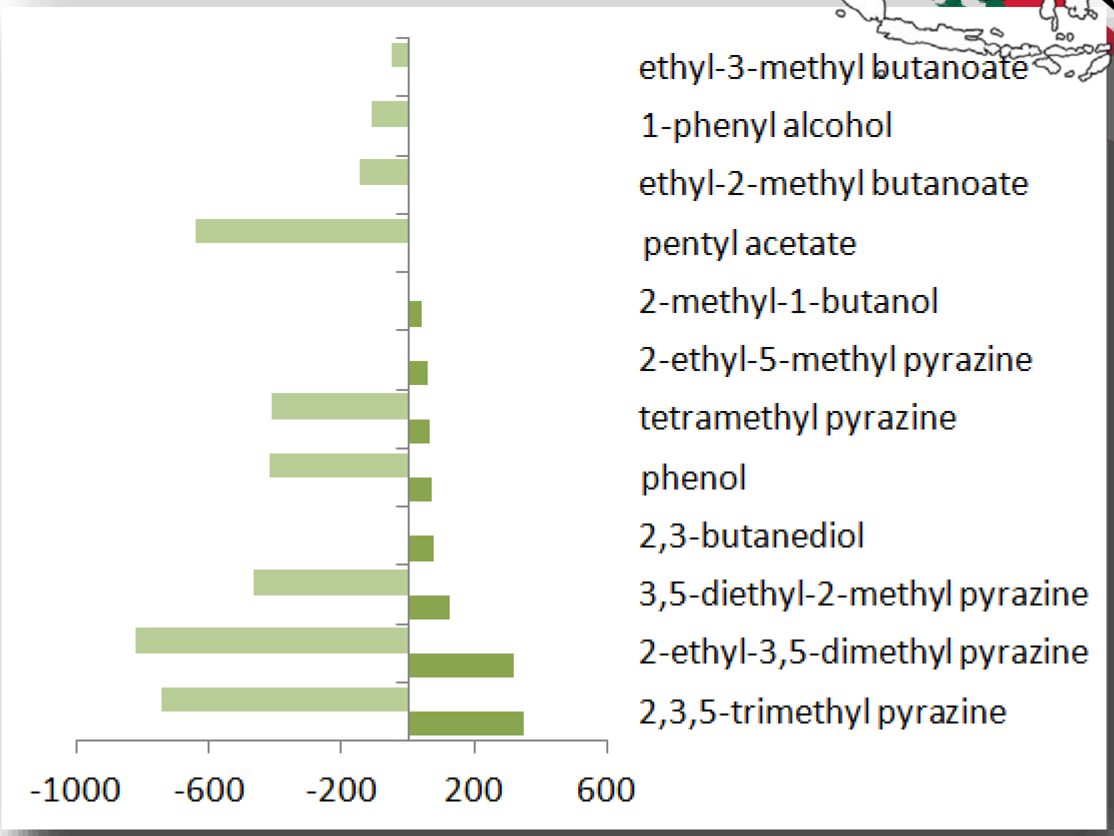
Combined Untargeted and Targeted (*UT*) *fingerprinting*$

Coloring from red (low abundance) to green illustrates the evolution of volatiles from raw beans (predominance of red spots) to nibs where most of the volatiles reach their maximum abundance.



Example II

Supervised approaches inform about those analytes capable of discriminating samples
Which is the impact of roasting and/or steaming?



Fisher ratio values (one *vs.* all)

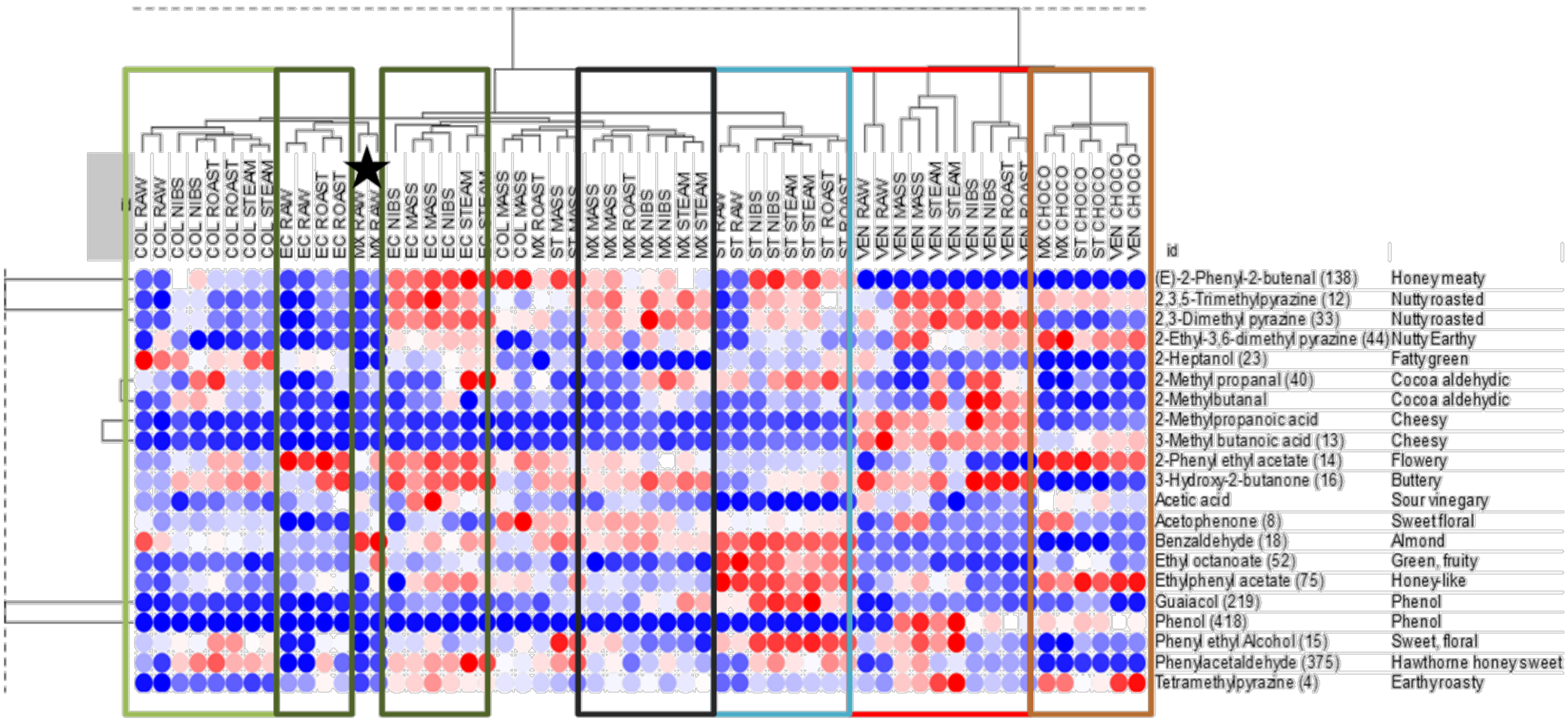
Fisher ratios are used to measure class separation for individual features relative to the variance within classes.

<u>For more than 20 samples</u>
<u>a Fisher ratio of 6.45 exceeds 99% confidence</u>

Example II

Heat-map based on normalized percent responses for detected key-odorants

HC is based on Euclidean distances; data is normalized by Z-score.



Example II

Approach named Multiple Headspace Extraction

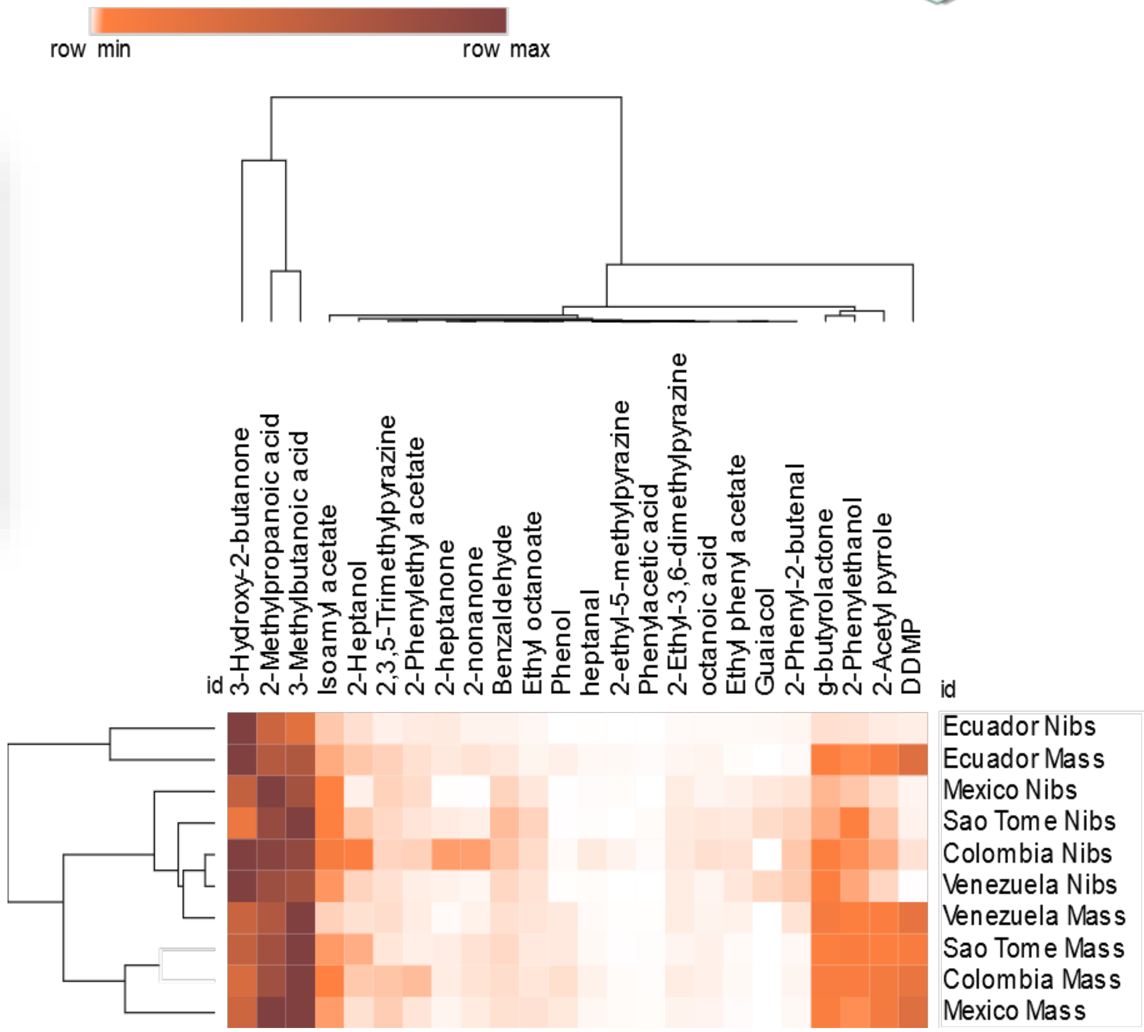
Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Odorants quantitation in high-quality cocoa by multiple headspace solid phase micro-extraction: Adoption of FID-predicted response factors to extend method capabilities and information potential

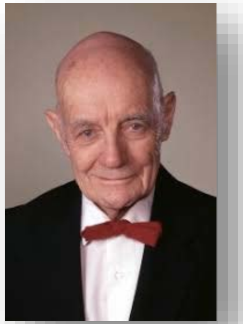
Chiara Cordero [a,*], Alessandro Guglielmetti [a], Barbara Sgorbini [a], Carlo Bicchi [a], Elena Allegrucci [b], Guido Gobino [b], Lucie Baroux [c], Philippe Merle [c]



Key-odorants quantitative distribution in selected samples: Nibs and Mass

Quantiation is by MHE on HS-SPME and by combining MS external calibration and FID predicted relative response factors (RFFs)

Conclusive remarks



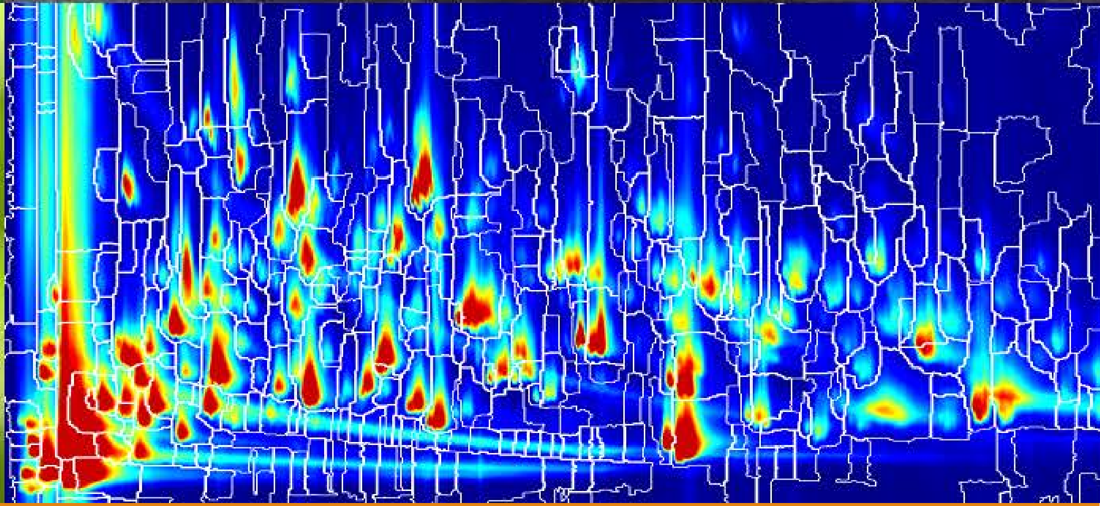
John B. Fenn Nobel Prize for Chemistry in 2002

"You can say that you feel fulfilled as a scientist when, and only when, your scientific achievements help somebody else to solve his problem(s)"

Advancements in analytical chemistry should be exploited to solve practical problems from real-life and to improve our knowledge on complex phenomena (e.g., sensory perception).

OMICs measurement concepts and investigation strategies may accelerate innovation and improve products quality - define new concepts of quality - synergies with industrial research



Thank you for your attention

Prof. Chiara Emilia Cordero

University of Turin - chiara.cordero@unito.it