ADDING EXTRA-DIMENSIONS TO PRIMARY METABOLOME PROFILING BY GC×GC-TANDEM IONIZATION TOF-MS INSIGHTS ON HAZELNUT (*Corylus avellana* L.) AROMA POTENTIAL

Marta Cialiè Rosso*[1], Carlo Bicchi[1], Erica Liberto[1], Christoph Weinert[2] and Chiara Cordero[1]
[1]Department of Drug Science and Technology, University of Turin, Turin, Italy
[2] Department of Safety and Quality of Fruit and Vegetables, Max Rubner-Institut, Karlsruhe, Germany

Aim and Scope

This study aims at developing and critically evaluating a screening methodology, based on comprehensive two-dimensional Gas Chromatography coupled with Time of Flight Mass Spectrometry (GC×GC -TOF MS), to map the **primary metabolome of hazelnuts.** The approach should focus on characteristic distribution of key aroma precursors and informative chemicals (targeted and untargeted) capable of discriminating the unique metabolite fingerprints of hazelnuts of different origin and quality. This screening methodology includes defatting, hydrophilic extractions of primary metabolites and derivatization through oximation-silylation.
The extra-dimension of analytical information provided by TOF MS featuring Tandem Ionization is considered and evaluated in terms of analytes confident identification and detection.

Experimental

Sample Preparation & Analytical Pratform

Non-volatile fraction

1- Defatting → Hexane extraction

2- Hydrophilic micro-extraction
Extraction in MeOH in order to collect the hydrophilic component, above all aminoacids and sugars

3- Derivatization oximation and silylation
1- **Methoximation** by Methoxamine in Pyridine in order to linearize the sugars, becoming able to react with the sylilant agent
2- **Sylilation** by BSTFA

Reference compounds and solvents were purchased from Sigma-Aldrich (Milan, Italy)

Volatile fraction

In order to evaluate the amount of odorant compound
1. **ROASTING** IN LAB-SCALE:
   - 160°C for 15' for "ideal-roasted" sample
   - 180°C for 15' for "over-roasted" sample

2. HS-SPME
Sampling: 1.50 g of hazelnut powder for Profiling
Temperature: 50°C
Time: 40 min with the pre-loading of the internal standard
Vial volume: 20 mL
Fiber: DVB/CAR/PDMS; 50/30 µm 2 cm
Supelco Bellefonte

3- GC-MS
GC Oven programming: 40°C(1') to 180°C (10') @ 3°/min to 240°C (5')@15°/min
S/SL injector: 250°C Split ratio 1:5
Gas (Hlelium) flow: 1,2 ml/min
MS Transfer line: 270°C Mass Range: 35-350 m/z

GC×GC-TOFMS

GC×GC System

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

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

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

TOF-MS

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

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

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

[1]D - APolar HP5
30 m × 0.25 mm × 0.25µm
He carrier @ 1.3 mL/min

[2]D - Medium polarity OV1701
2,3 m × 0.10 mm × 0.10µm
He carrier @ 1.3 mL/min

GC Oven : 40°C(2') to 240°C (10') @ 3.5°/min
S/SL injector: 250°C Split ratio 1:20
MS Transfer line: 250°C ionization: 70 and 12 eV
MS Aquisition: Mass range 40-350 m/z; frequency 70 Hz
Optimode settings: modulation period **3.5-4s**, hot-jet pulse 250 ms -cold jet stream MFC from 40% to 5% in 70 min

GC-MS

Agilent 6890 GC unit coupled to 5973 MS detector

Samples characteristics

Hazelnuts are widely used as raw material in confectionary industry thanks to the aroma, texture and taste features developed during roasting process. Several chemical reactions, occurring during food heating, are reported in scientific literature and monosaccharides and free amino acids, primary metabolites, are known to be the main precursors of volatile compounds responsible for the aroma.
For the quality assessment, the volatile fraction composition of hazelnuts is well characterized and key-aroma compounds are known, by contrast a correlation between the quali-quantitative distribution of key aroma precursors and volatile composition is not yet established. In particular, the determination of the cultivar and the geographical origin of hazelnut with a greater flavor potential could be a point of great interest for the confectionary industries. For this reason, the study was focused on hazelnuts samples of different cultivars of *Corylus avellana* L., harvested in different geographical areas and treated with different post-harvest condition.

Ordu hazelnuts (**O**)

From Ordu region in Turkey, sample composed by hazelnuts from different cultivars (Tombul, Palaz and Çakildak) (harvested in 2015).

Tonda Gentile Romana hazelnuts (**T**)

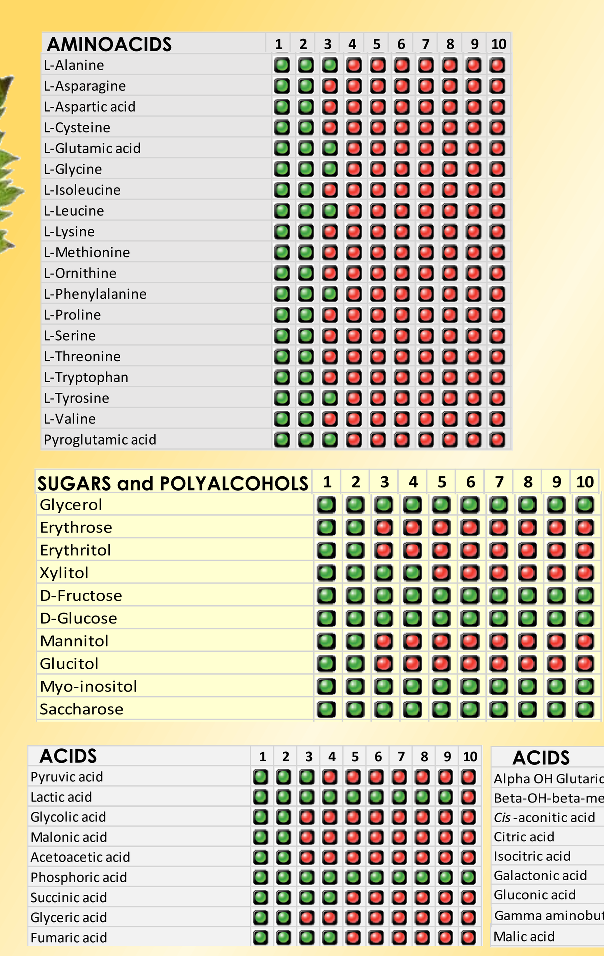
From Lazio region in **Italy (It)**, sample composed by monocultivar hazelnuts - harvested in 2017.

From **Georgia (Ge)**, sample composed by monocultivar hazelnuts (Tonda Gentile Romana)-harvested in 2017.

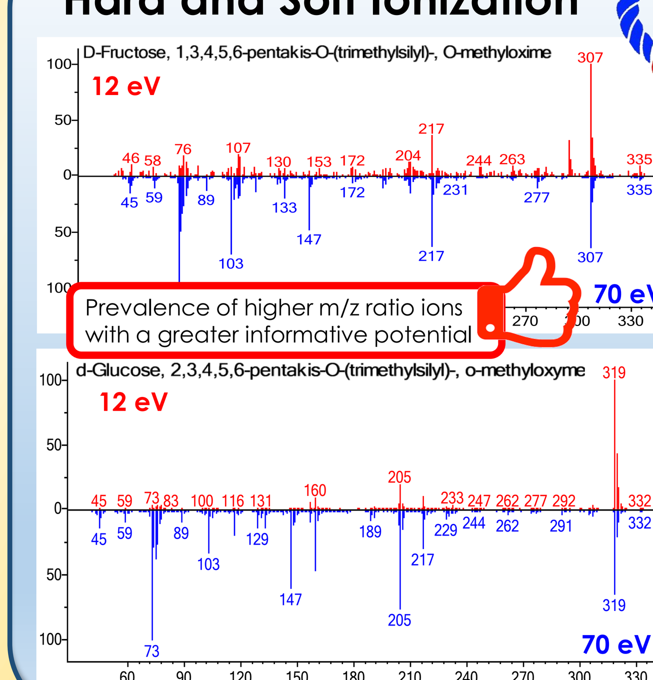
Anakliuri hazelnuts (**An**)

From **Georgia (Ge)**, sample composed by monocultivar hazelnuts (Anakliuri)-harvested in 2017.

PRIMARY METABOLITES Exhaustiveness of extraction

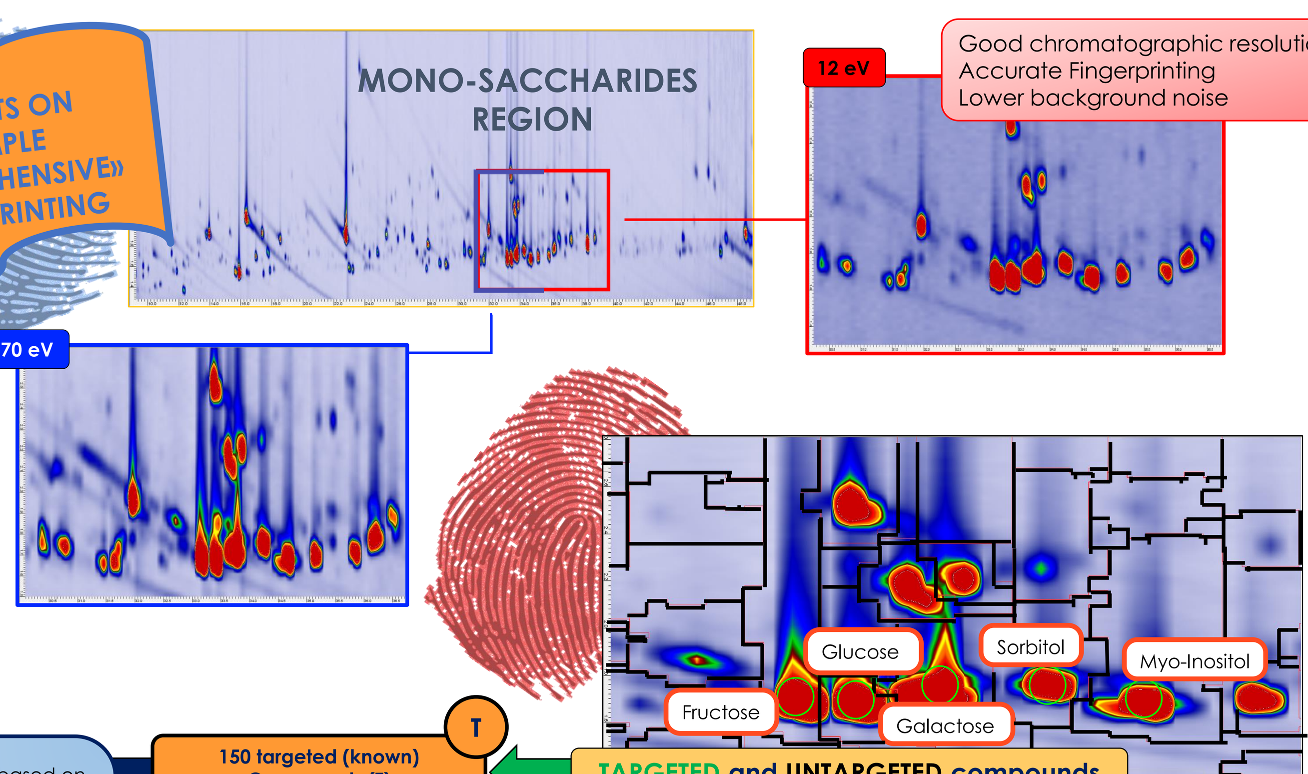


Hard and Soft Ionization



Results and Discussion

INSIGHTS ON SAMPLE «COMPREHENSIVE» FINGERPRINTING

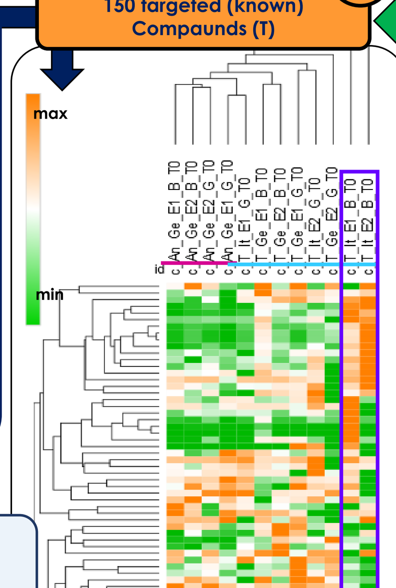


TARGETED and **UNTARGETED** compounds

150 targeted (known) Compounds (T)

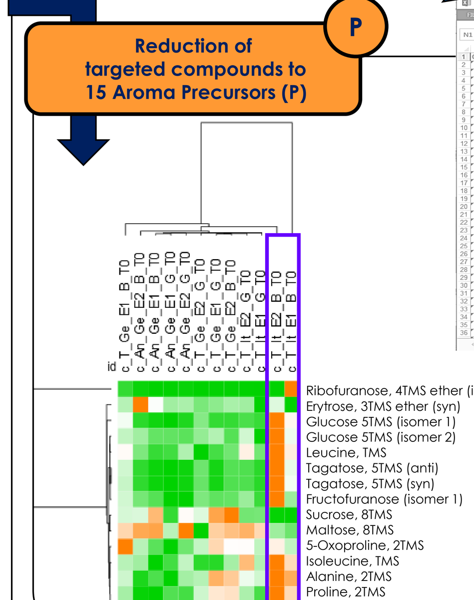
HEAT-MAP based on Percent Response from targeted compounds. Mean and centering normalization. Samples - columns/ targeted blobs- rows.

Hierarchical clustering is in accordance with the cultivar (Anakliuri and Tonda Gentile Romana) with the exception for only one samples that, in any case, presents the same geographical origin (Georgia).



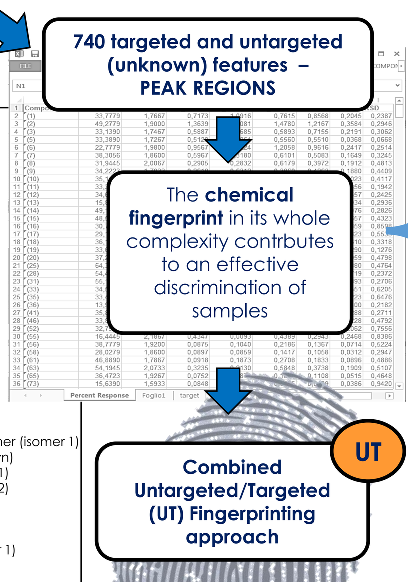
CULTIVARs (**An** and **T**) are clustered coherently

Reduction of targeted compounds to 15 Aroma Precursors (P)



Italian hazelnuts (Tonda Romana) are independently clustered based on both targeted analytes and precursors distribution

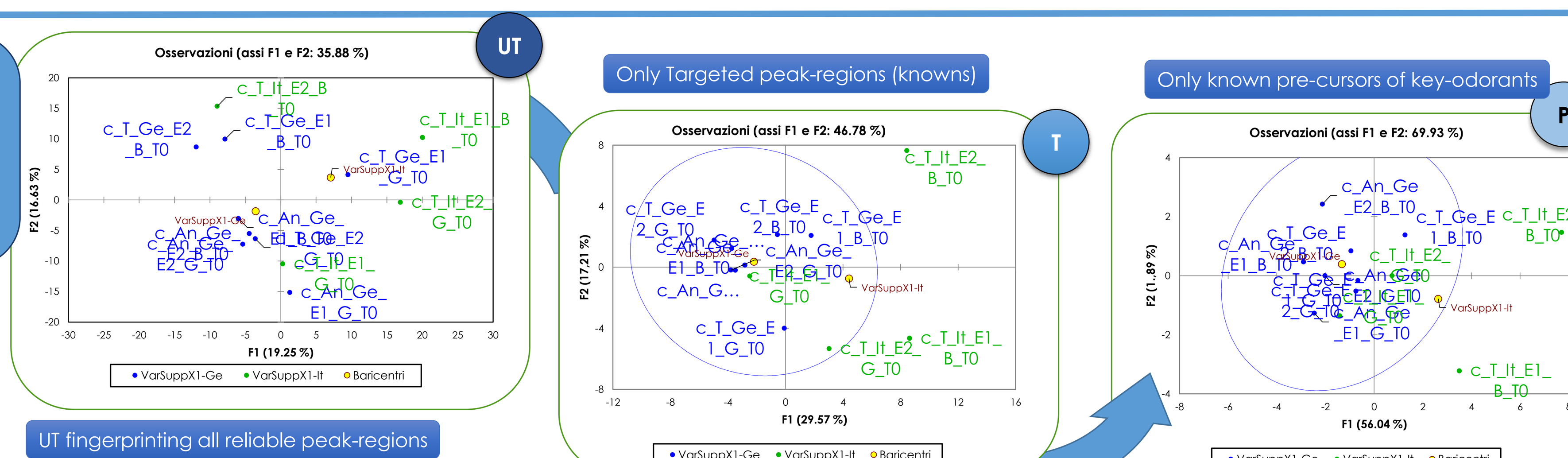
740 targeted and untargeted (unknown) features – PEAK REGIONS



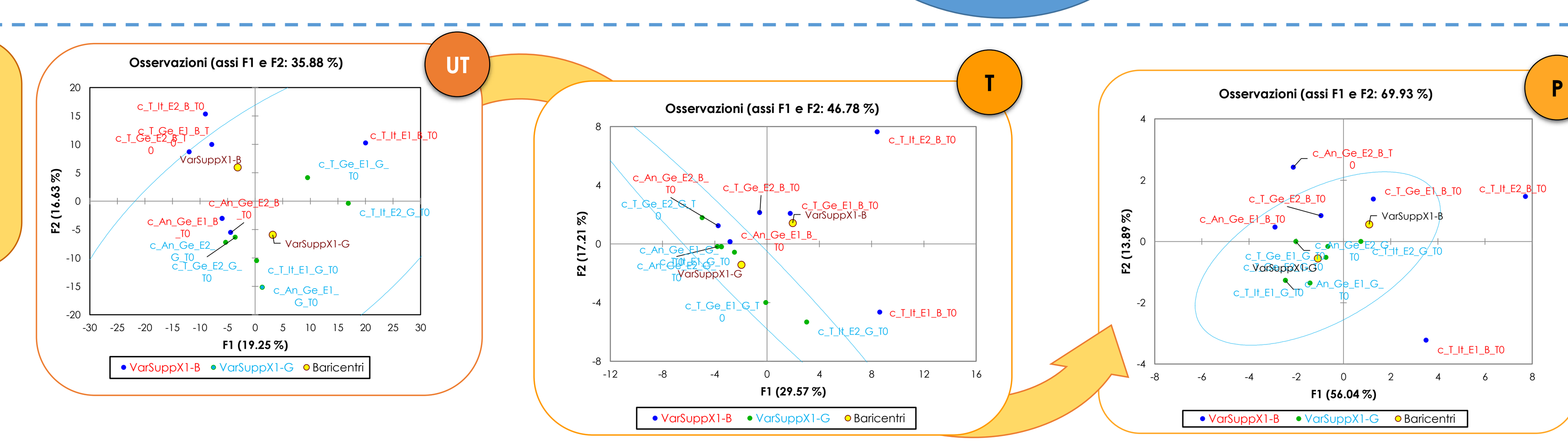
The **chemical fingerprint** in its whole complexity contrbutes to an effective discrimination of samples

Combined Untargeted/Targeted (UT) Fingerprinting approach

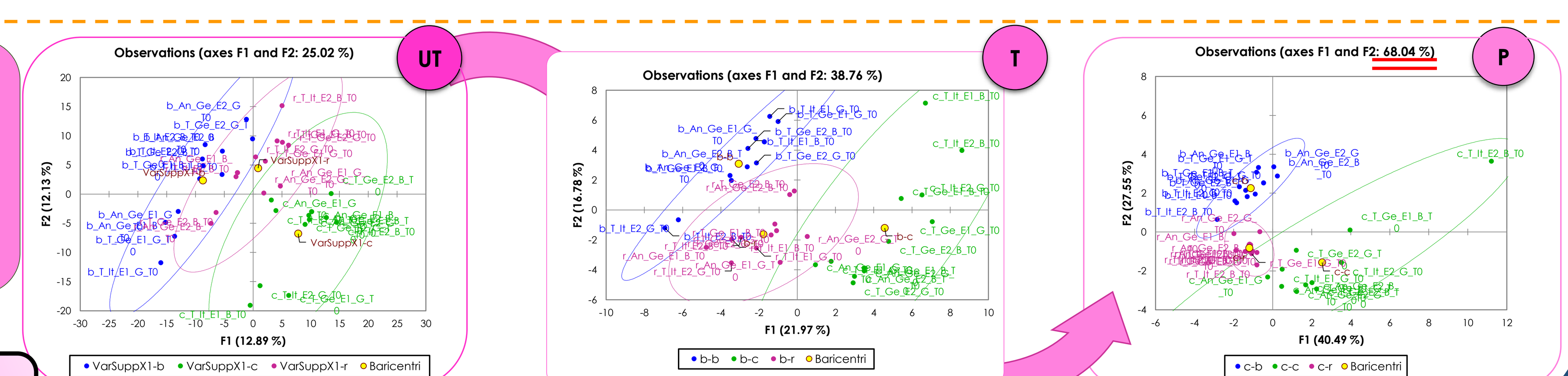
PCA study of the influence of the **GEOGRAPHICAL ORIGIN** on the primary metabolome of **raw hazelnuts**



PCA study of the influence of the **POST-HARVEST TREATMENT** on the primary metabolome of **raw hazelnuts**



PCA study of on the primary metabolome of **raw** and **roasted** hazelnuts to evaluate the influence of the **TECHNOLOGICAL PROCESS**

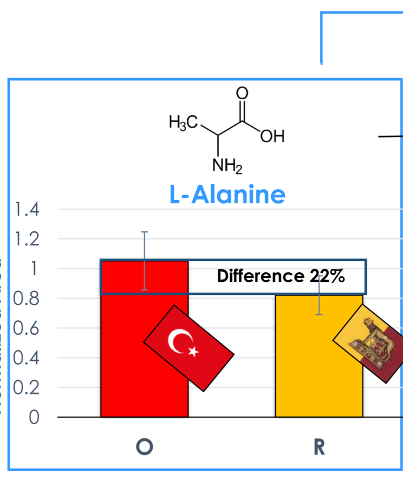


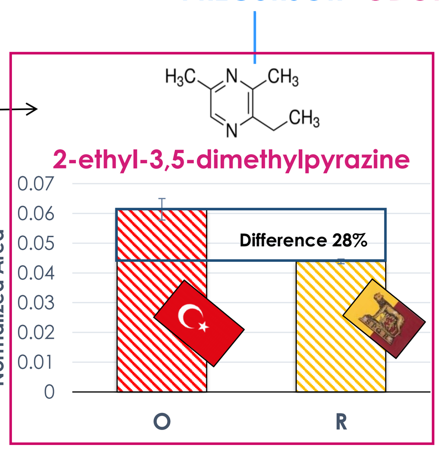
ROASTING GROUPS
c: Raw hazelnuts
r Ideally-roasted hazelnuts
b: Over-roasted hazelnuts

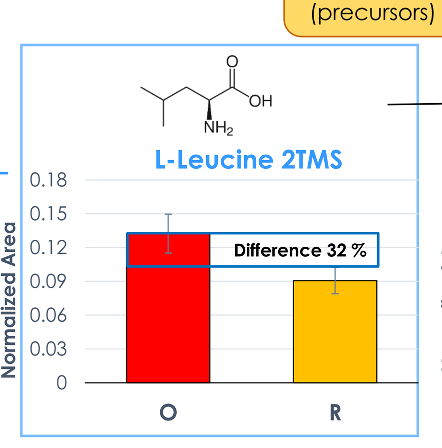
O: Ordu - Tombul, Palaz and Çakildak
R: Lazio - Tonda Gentile Romana, harvested in Italy

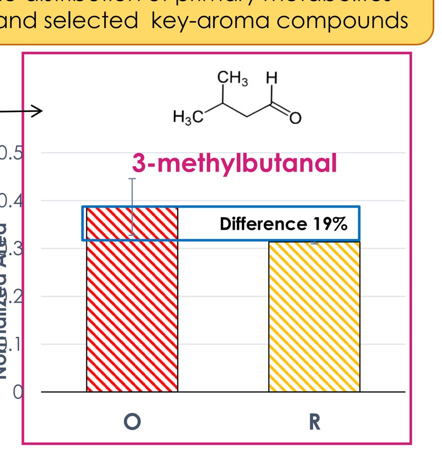
CORRELATION PRECURSOR- ODORANT COMPOUND

Preliminary data suggest good correlation between the distribution of primary metabolites (precursors) and selected key-aroma compounds









Conclusions

SET A GC×GC-TOF MS PROCEDURE FOR A «COMPREHENSIVE» FINGERPRINTING including targeted and untargeted compounds

- Enhancement of S/N ratio at 12 eV
- **Sensitivity maximum at 70 eV**
- Increased separation power

Confident identification of chemical compounds by means of Tandem Ionization at high and low electron energies

Effective fingerprinting of hazelnut cultivars with a focus on the primary metabolome and its correlation with the flavour potential

If you wish to have some extra-information please contact: marta.cialierosso@unito.it