Development of High Throughput Approaches to Optimise the Nutritional Value of Crops and Crop-Based Foods (FP6 – 036296) DEVELONUTRI

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Project Aims

1. Develop and validate state-of-the-art metabolite profiling and analysis platforms (set of technologies, tools and methodologies) that can be deployed at all stages in the crop improvement, production and processing platforms to ensure optimised nutritional value and safety throughout the food chain.

2. Deploy the validated approaches to fully assess the added value of these technologies in crop and crop-based food analysis using model species which are economically and socially important in Europe. Focus will be on Solanaceous species (tomato, potato) and cereals (wheat).

3. Engage and collaborate with SMEs in the practical assessment of raw and processed materials quality using the validated approaches and to ensure knowledge transfer to policy makers, the commercial sector and the consumer with regard to the value of the research outcomes in the evolution of food quality standards.

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Preliminary experimental approach

- Method development & validation (SOPs)
- Inter-laboratory ring testing
- Database construction to facilitate analytical approach cross comparison
- Demonstration of the approaches
- Potato processing : pasta & bread production: tomato processing & canning

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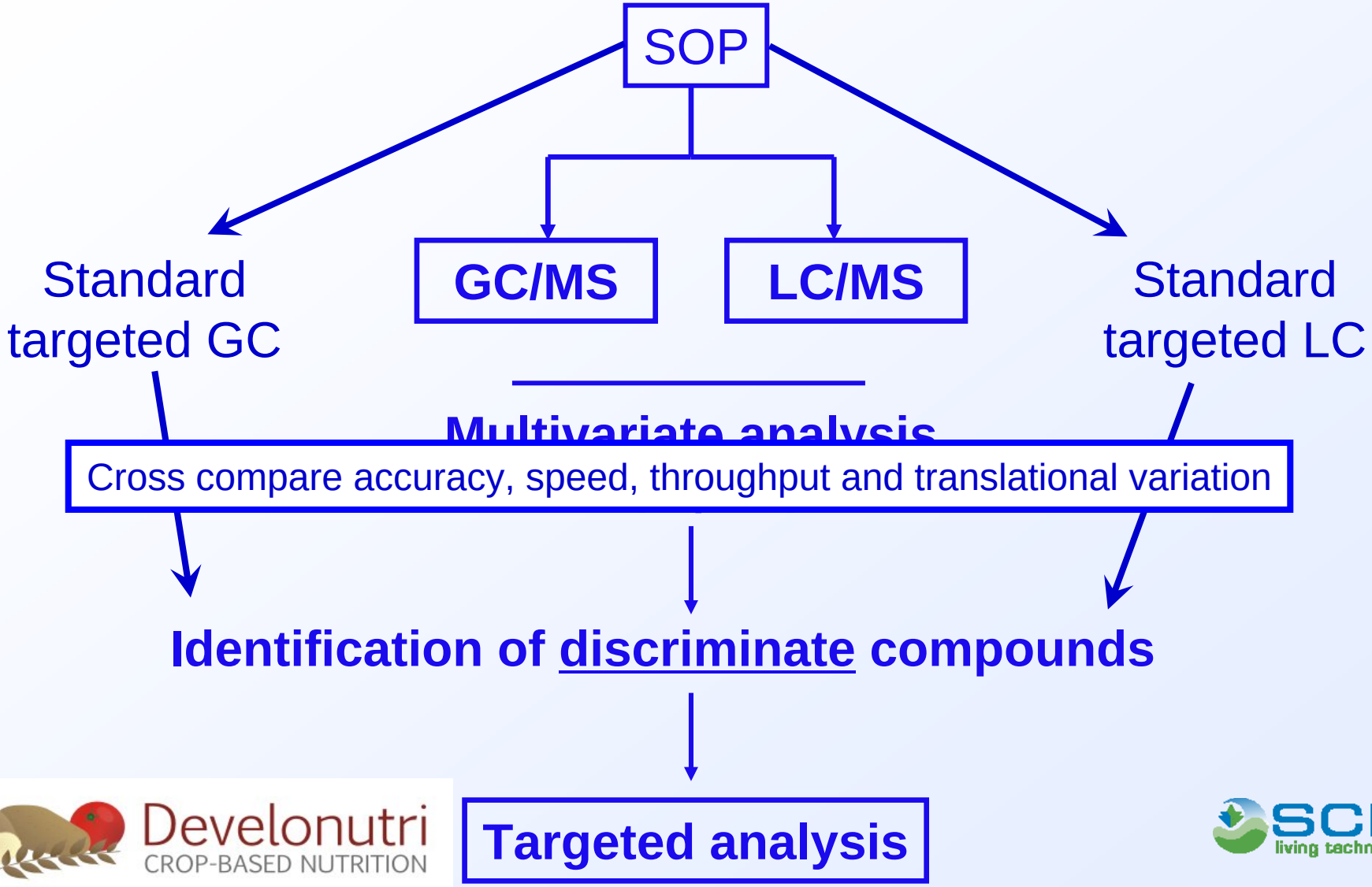
Approach for standard and metabolomic analysis of ring tests and crop samples



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Setting up analytical methods

LC-DAD/MS: Internal and external calibration

Polyphenols:

- Chlorogenic acid
- Sinapic acid
- Ferulic acid
- Gallic acid
- Caffeic acid
- Vanillic acid
- p-Coumaric acid
- o-Coumaric acid
- Myricitrin
- Isoferulic acid
- Isorhoifolin
- Kaempferol-3-O-glucoside
- Naringenin-7-O-glucoside
- Quercetin
- Rutin
- Naringenin
- Kaempferol
- Kaempferol-3-O-rutinoside
- Naringenin chalcone
- Protocatechuic acid
- m-Coumaric acid
- Myricetin

- Delphinidin-3-O-glucoside
- Petunidin-3-O-glucoside
- Malvidin-3-O-glucoside

Glycoalkaloids:

- Tomatine
- Dehydrotomatine

Water-soluble vits:

- Thiamine
- Pyridoxyne
- Ascorbic acid
- Nicotinic acid
- Pantotenic acid
- Folic acid
- Cyanocobalamine
- Riboflavin
- Biotin

Carotenoids and tocols:

- α-Tocopherol
- δ-Tocopherol
- γ-Tocopherol
- Lutein
- Zeaxanthin
- β-Carotene
- Δ-Carotene
- Lycopene
- Phytofluene
- 9-cis-Neoxanthin

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Setting up analytical methods

GC-MS: Internal and external calibration

Phytosterols:

β-Sitosterol
Stigmasterol

Organic acids:

Citric
Succinic
2-Pyrrolidone-5-carboxilic
Malic
Galacturonic
α-Ketoglutaric
Tartaric
Fumaric
Oxalic
Quinnic

Sugars:

Glucose
Fructose
Ribose
β-Gentiobioside
Arabinose
Melezitose
Raffinose
Xylose
Mannitol
Maltose
Rhamnose
Sucrose
Sorbitol

Aminoacids:

Glutamic acid
Leucine
β-Alanine
Asparagine
Cysteine
Glutamine
Glycine
Histidine
Methionine
Aspartic acid
Arginine
Lysine
Proline
Tyrosine
Alanine
Phenylalanine
Isoleucine
Threonine
Serine
Tryptophan
Valine

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Ribitol used as an I.S. in all cases

Validation

- Response linearity vs concentration for standards.
- Response linearity vs amount of plant material extracted for both aqueous and non-polar fractions.
- vs volume of aqueous extract derivatised.
- Injection volume.
- Extraction and analysis reproducibility.
- Extraction and analysis reproducibility (multiple injection and/or sample).
- Validation of extract stability whilst on the autosampler tray
- Estimation of sampling errors, via repeat injection of samples or repeat analyses.

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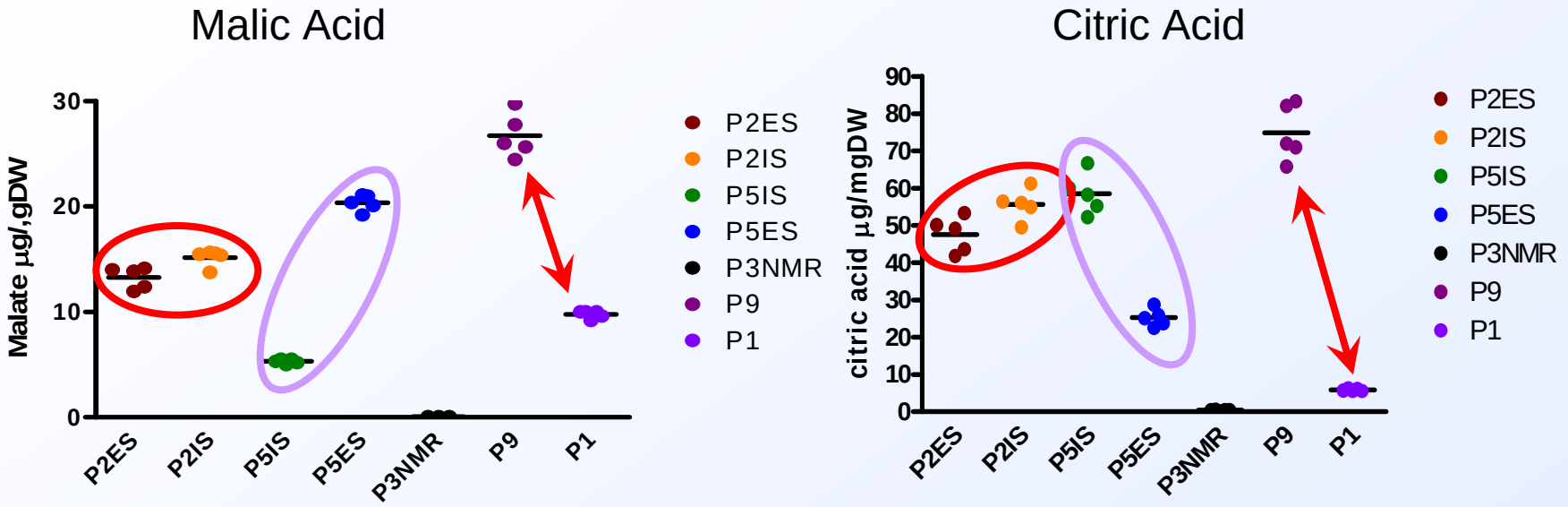
Levels of malic and citric acid determined from select partner GC-MS profiles



Variable factors

- Run conditions
- GC-MS manufacturer
- Standard – internal/external
- Comparison with other technologies, e.g. NMR

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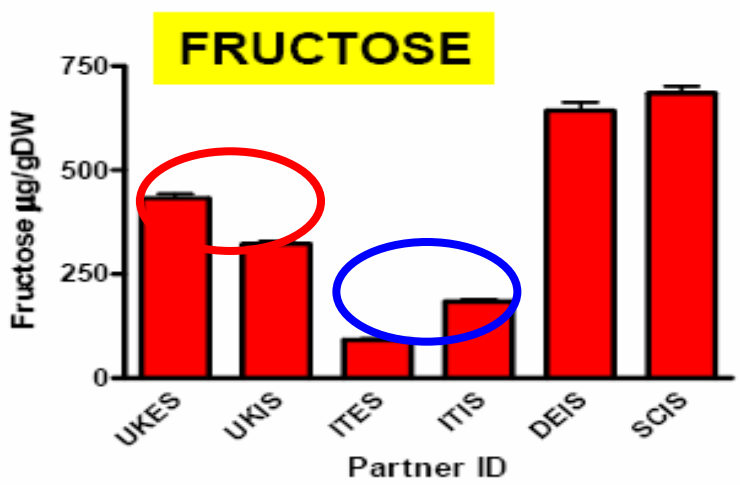
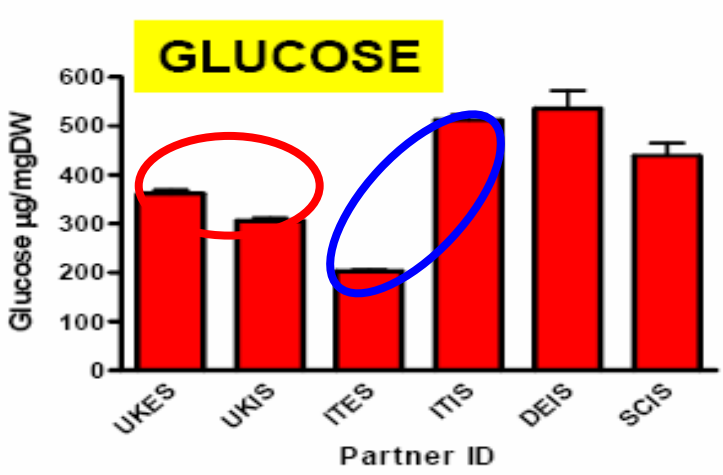
Levels of fructose and glucose determined from select partner GC-MS profiles





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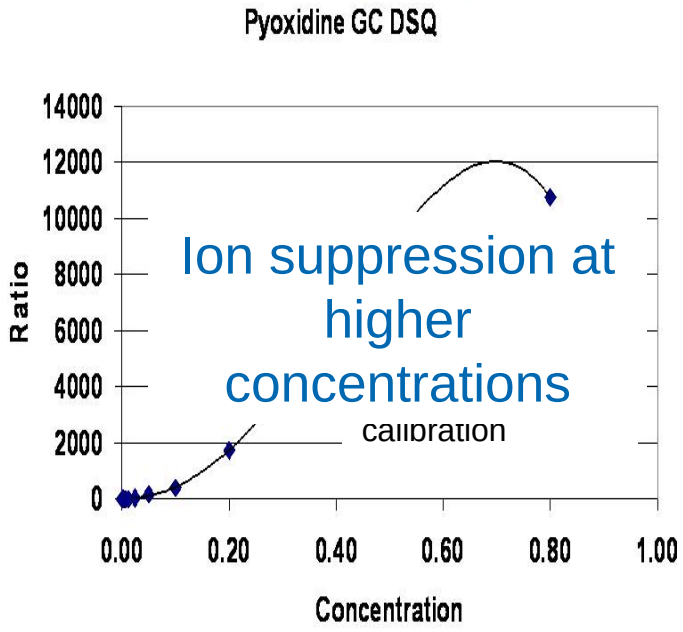
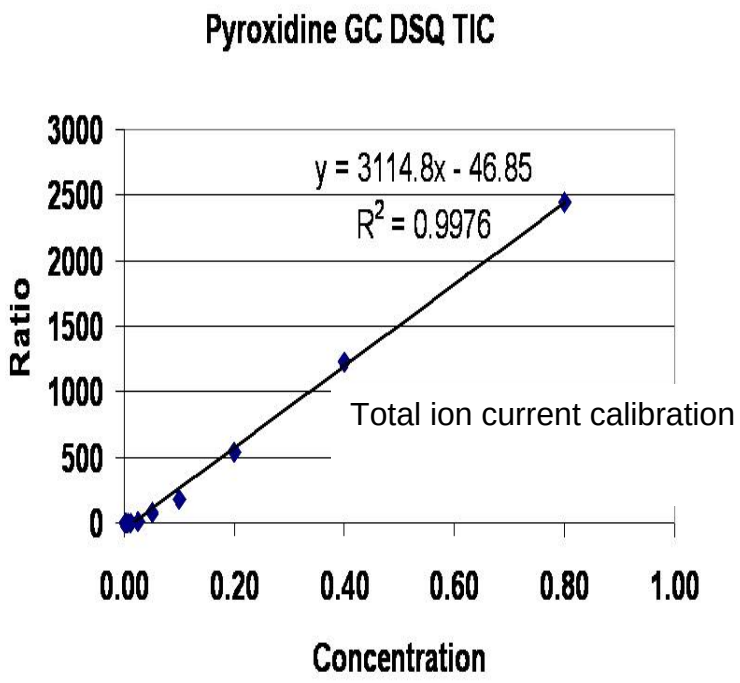
GC-MS calibration curves for pyroxidine (Vit B6) derived from different MS data post acquisition strategies

Pyroxidine – selected ion



Pyroxidine – selected TIC



A clear example of selectivity (selected ion) versus utility (TIC)

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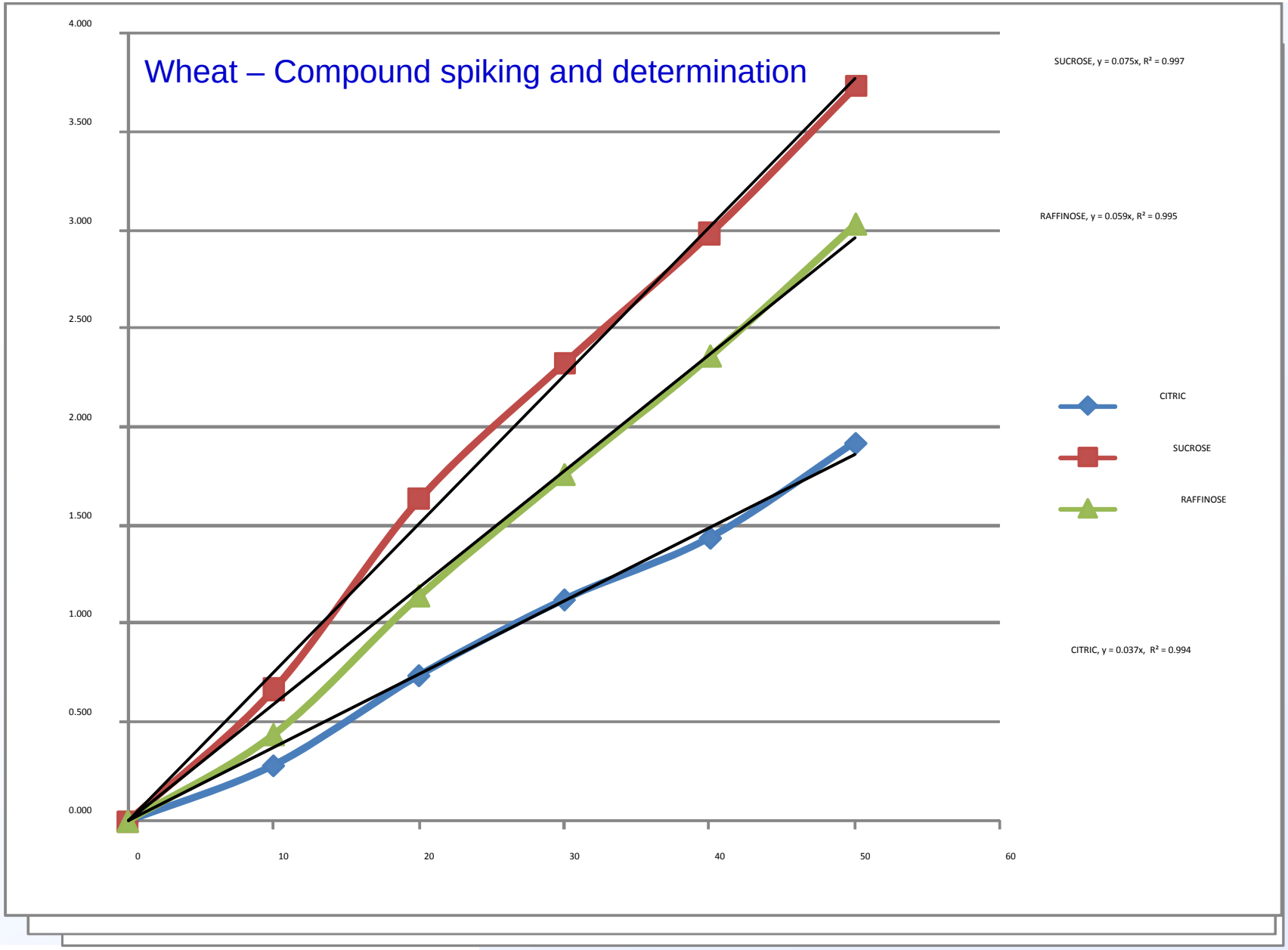

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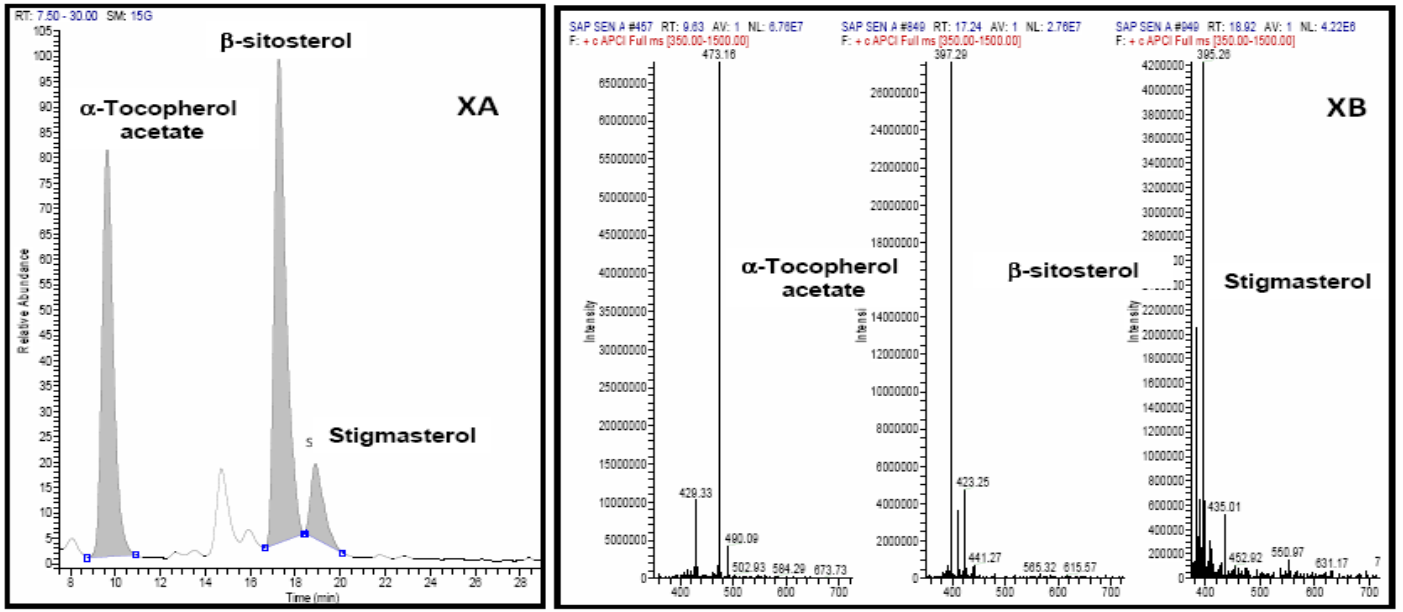
Sterol analysis

P10 - GC-MS: High accuracy but limited chemical diversity apparent

Sitosterol	**Potato**	**Tomato**	**Range**	**Linearity (R^2)**	**LOD**	**LOQ**
Expt 1	16.9 ± 1.3	219.7 ± 3.5	0.8 µg/ml – 0.8 mg/ml	0.999	0.2 µg/ sample	0.4 µg/ sample
Expt 2 (50%)	9.1 ± 0.8	115.3 ± 0.4				

P2 - LC-MS: Acceptable accuracy but excellent chemical diversity apparent



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Inter-laboratory Ring Testing

- Formation of a unified standard extraction protocol
- Material
- Potato: Tuber material freeze-dried and milled
- Wheat (bread): Grain freeze-dried and milled
- Tomato: ripe fruit freeze-dried and milled

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Inter-laboratory Ring Testing

- LC and GC-MS (metabolomics) approaches that, where all other things are equal, the differences that do occur are mainly associated with the type of hardware used. In the case of the GC-MS profiling the composition over 60 metabolites was similar, however quantitative variation was found between partners: same trend different levels
- Exactly the same result was found using the traditional (non-metabolomics based) approaches.
- This was not confined to organics as the nutrient analysis experienced the same problems

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Micronutrient analysis
More problematic than organics?

Standardization of method

From different methods across different labs (Ist ring test)

Defined and unique protocol (IInd ring test)

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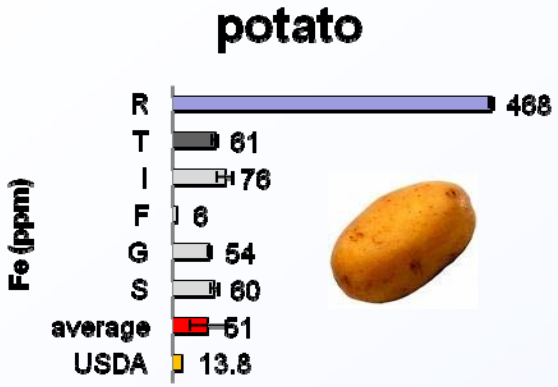
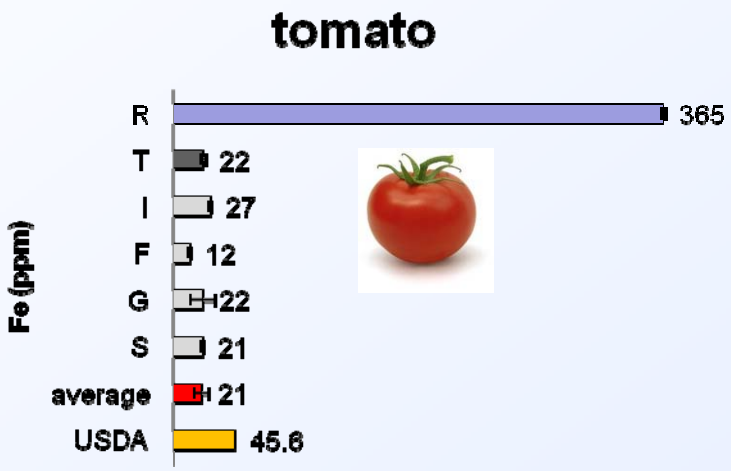
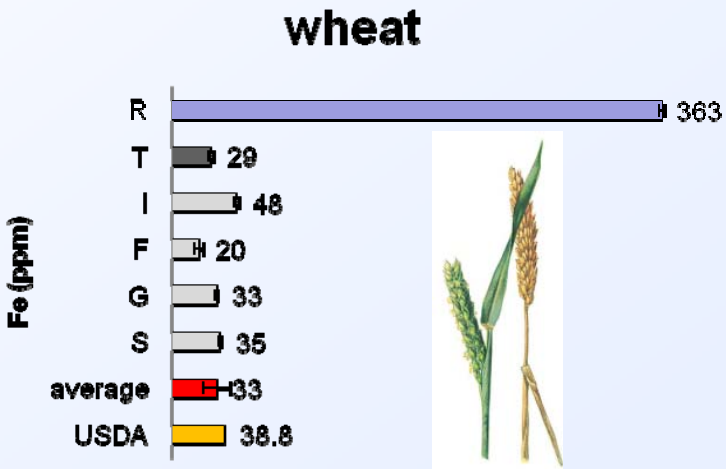
Fe measurements







R = Romania
T = Turkey
I = Italy
F = Finland
G = Greece
S = Switzerland

= ICP-AES
= ICP-MS
= ICP-OES

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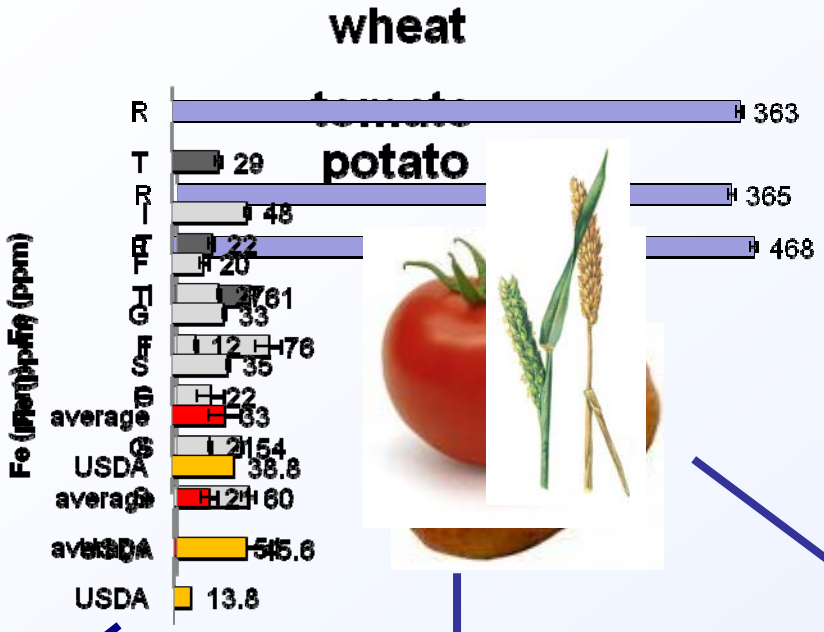
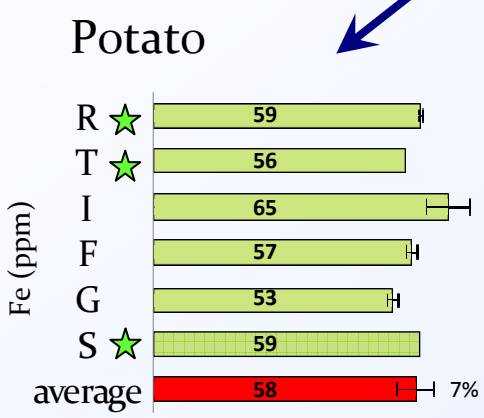
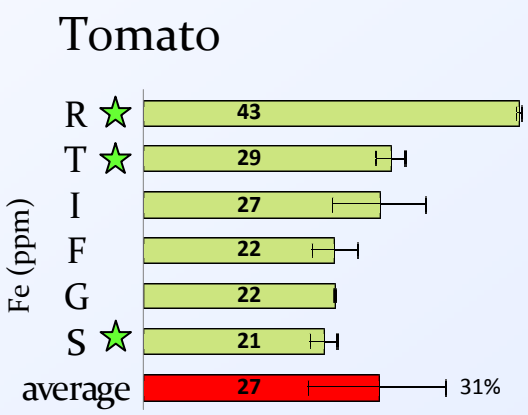
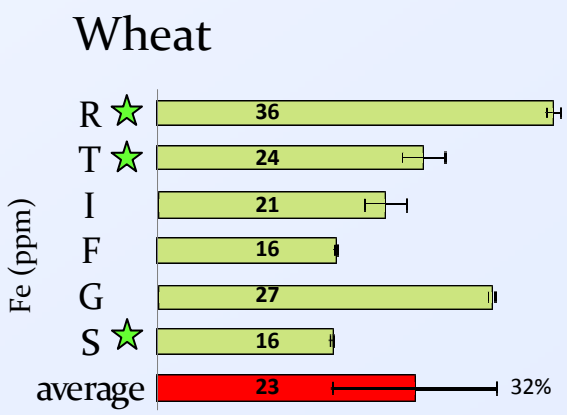
Fe measurements









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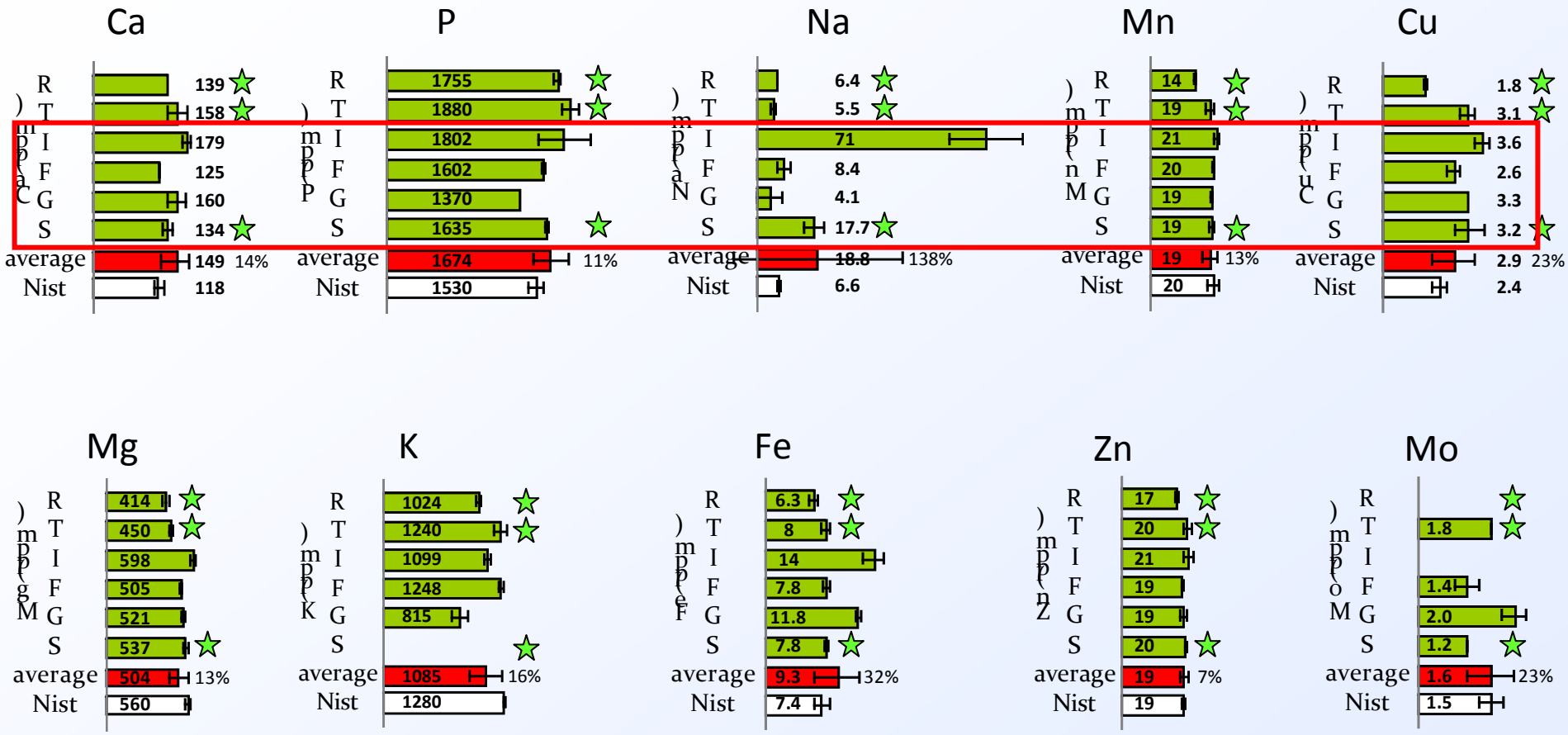
NIST 2nd Ring test



R = Romania: ICP-AES
T = Turkey: OCP-MS
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G = Greece: ICP-OES
S = Switzerland: ICP-OES

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Parripak Gretna (SME) Potato Processing

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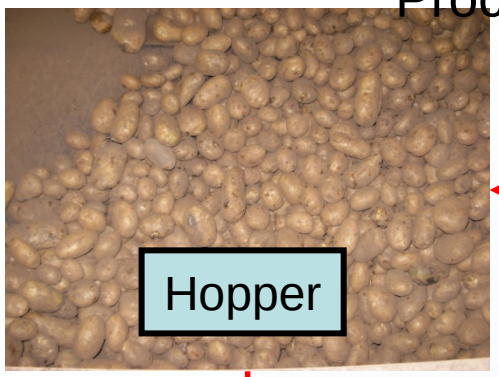
Storage

Amb Feb-March (can be heated if temp falls below 10°C)

Cold storage

Box Tipper



Hopper

Dip Tank

Light spray

Water Tank

Peeler

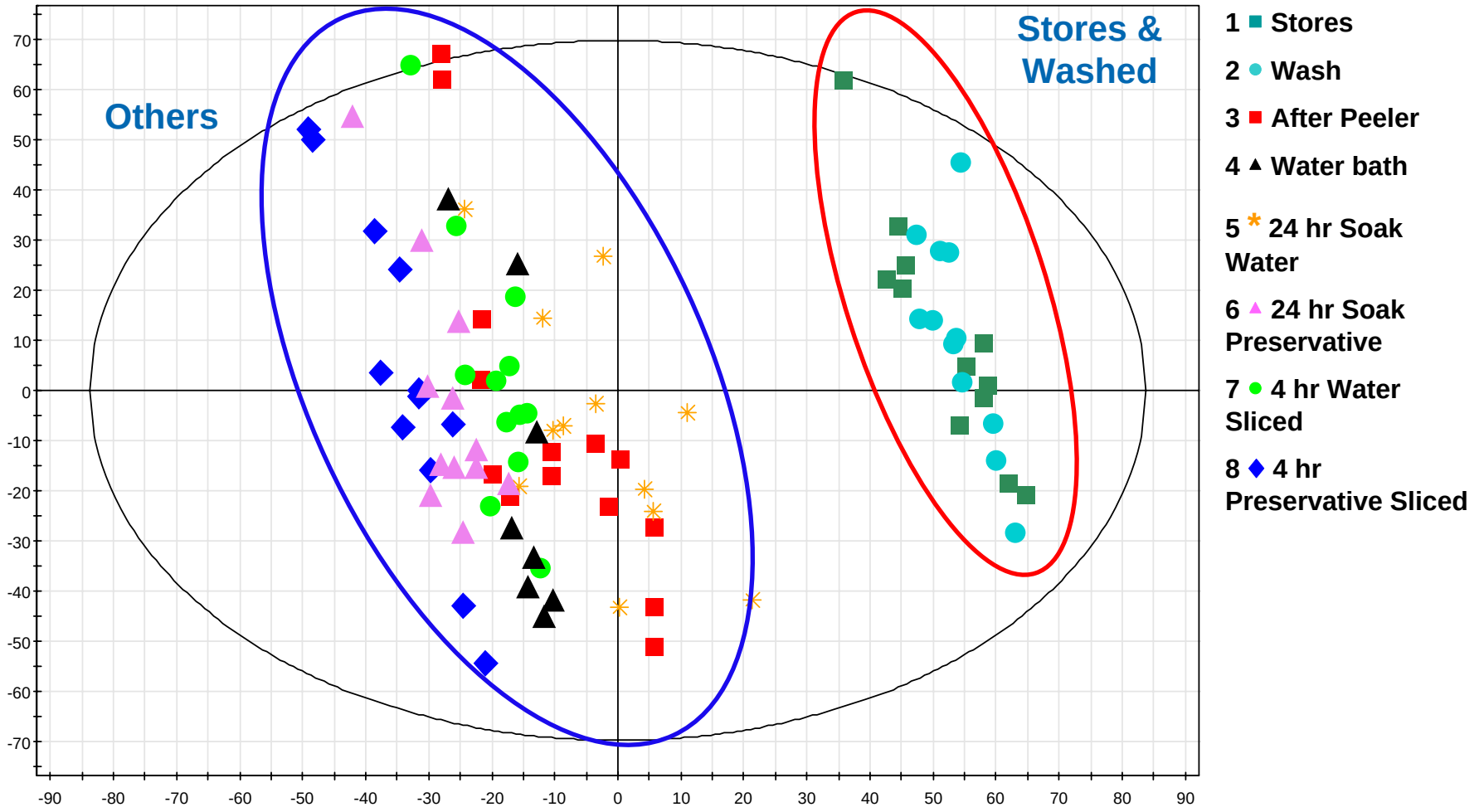
Dice/Slice

Whole/ quartered Water / Sodium Metabisulphate Tank

Dressing Table/Metal Detector

Water / Sodium Metabisulphate Tank

LC-MS Potato Processing - Positive Mode



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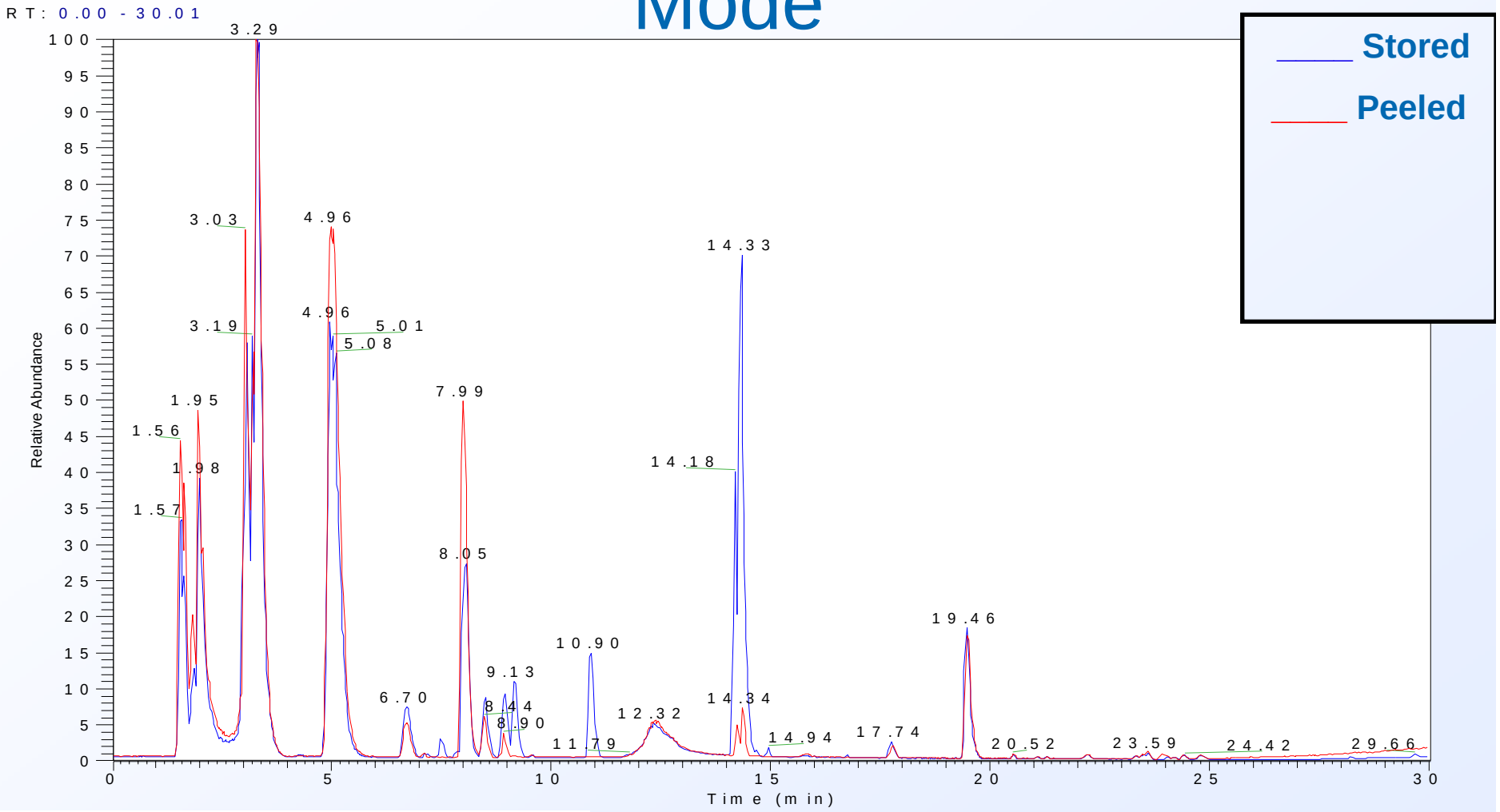
LC-MS Potato Processing - Positive Mode



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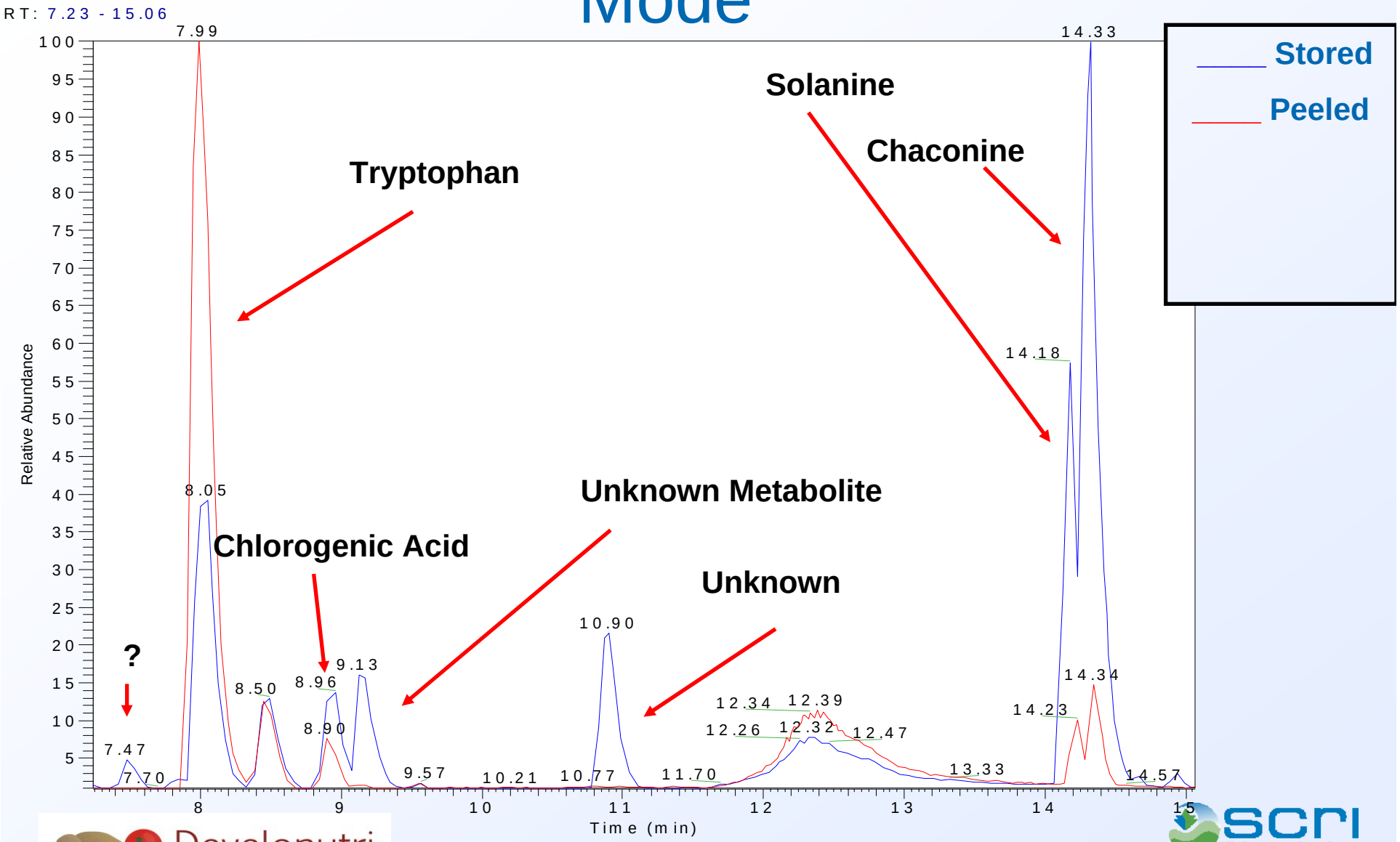
LC-MS Potato Processing - Positive Mode



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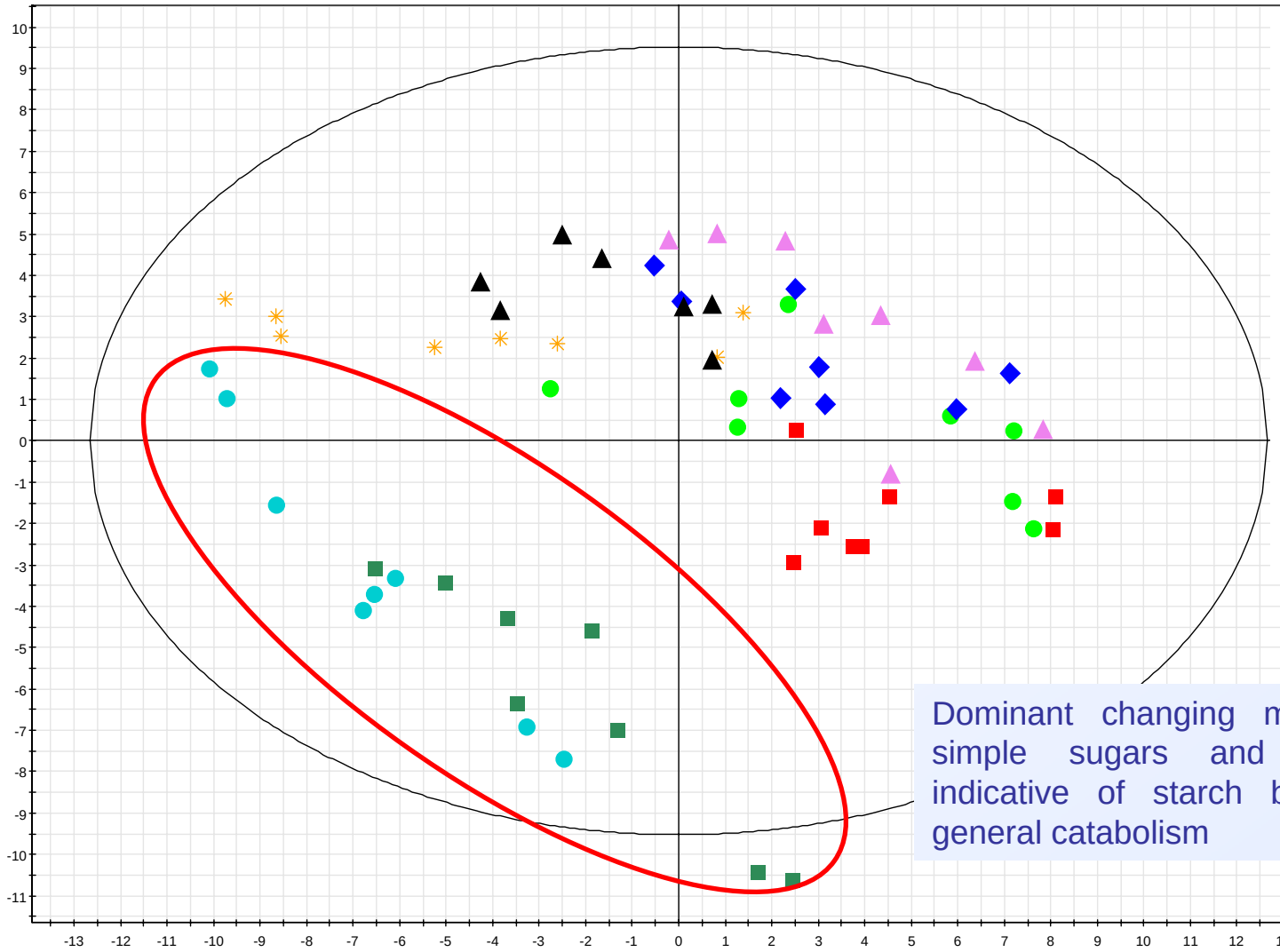
GC-MS Polar Score 1 vs 3



1 ■ Stores
2 ● Wash
3 ■ After Peeler
4 ▲ Water bath
5 * 24 hr Soak Water
6 ▲ 24 hr Soak Preservative
7 ● 4 hr Water Sliced
8 ◆ 4 hr Preservative Sliced

Dominant changing metabolite were simple sugars and amino acids indicative of starch breakdown and general catabolism

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Conclusions

- The metabolomics approaches show promise as selective and quantitative means to report of food composition and safety.
- They offered phenomenal advantages with respect to simultaneous compound coverage and speed (multiple simultaneous reporting).
- Source of variation amongst both new and traditional approaches were numerous: machine operator, preparatory method, rigour of good laboratory practice.
- In “real” scenarios the metabolomic approach can generate quantitative data at a massive level reporting on 10^2 compounds allowing a detailed description of the dynamic processes ongoing in a raw-through-to-processed foodstuff.

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Thanks to the DEVELONUTRI Team

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