Department of Agrifood Chemistry and Biochemistry
University of Agricultural Sciences and Veterinary Medicine
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METABLOMICA VEGETALA IN ERA POSTGENOMICA

PLANT METABOLOMICS BEYOND GENOMICS …

Carmen SOCACIU & col.
Department of Chemistry & Biochemistry,
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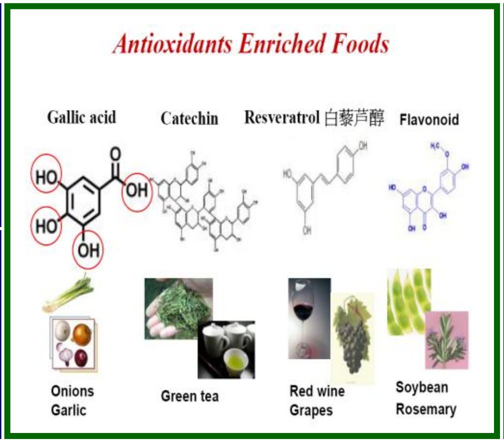
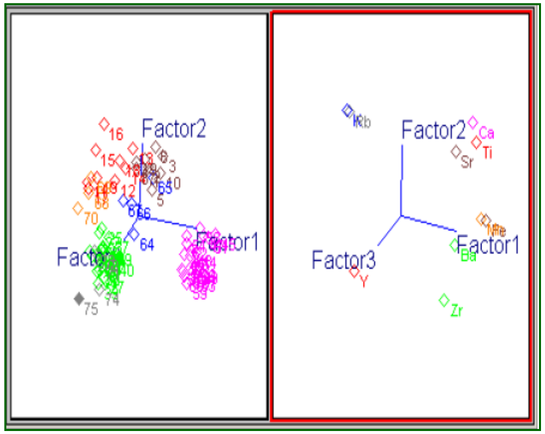




Outline

Systems biology - a multidisciplinary approach

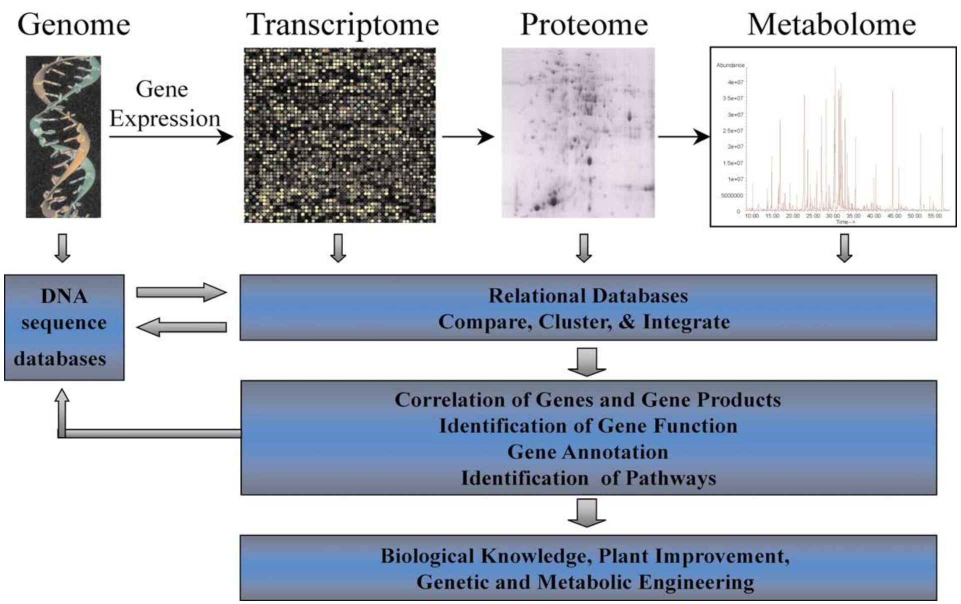
Metabolomics = final result of a cross talk <genome-transcriptome-proteome>

Phytochemicals as markers of plant phenotype - Case studies: taxonomy and biodiversity-biocapacity

Advanced analytics coupled with chemometry

Metabolomics: An INTEGRATED Tool for Studying SYSTEMS BIOLOGY

GENOTYPE + ENVIRONMENT → **PHENOTYPE**



Transcriptomics

Metabolome

METABOLITES

Primary

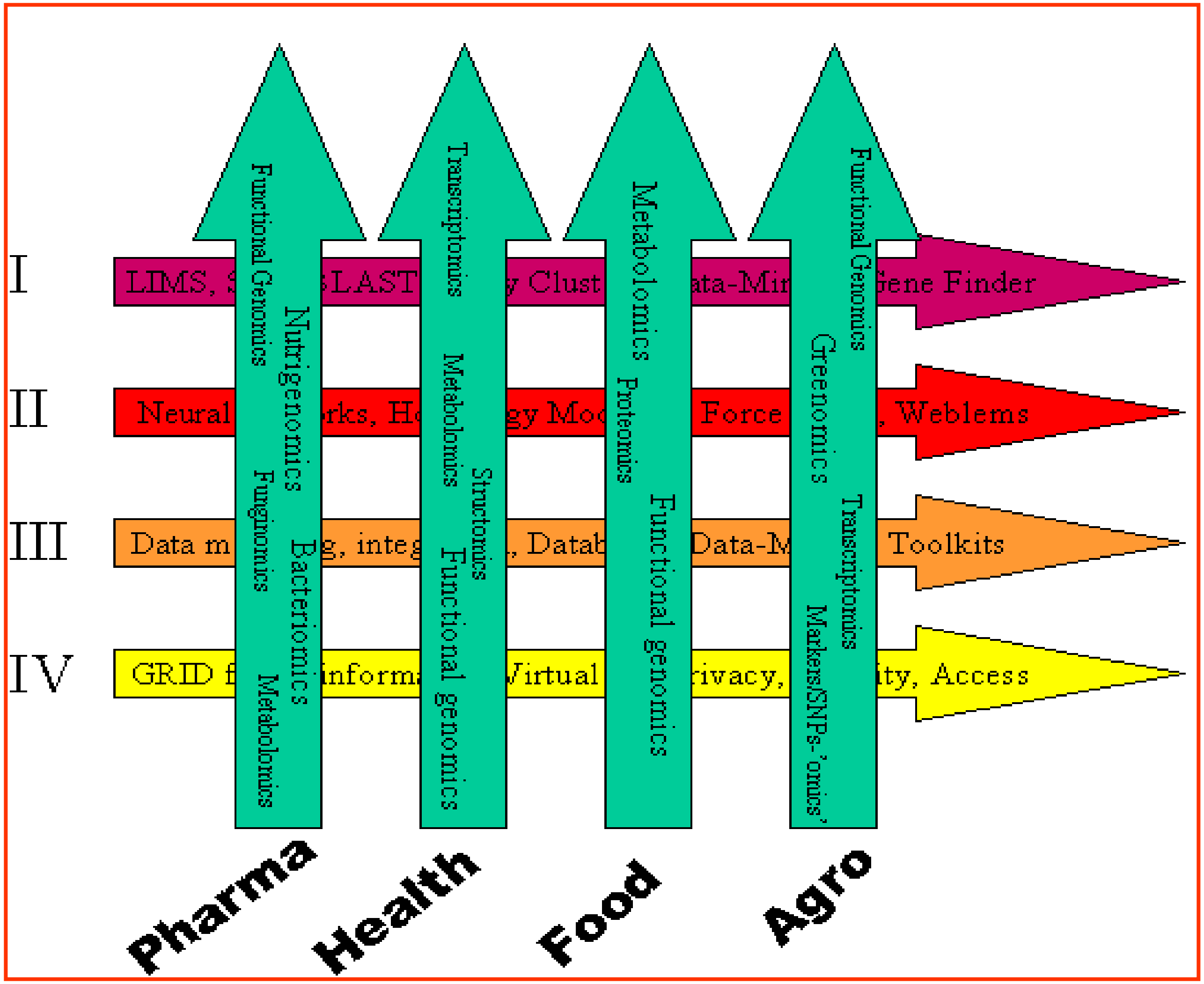
Proteomics

Secondary (small molecules)

Metabolites = end products of gene expression and enzymatic activities

Metabolomics – reflect the activity of a certain BIOSYNTHETIC NETWORK

- complementary method to the large-scale gene transcript analysis (transcriptomics) and proteins fingerprint (proteomics)
- explain and identify the differences between sets of organisms (e.g. differences in genotypes) **CHEMOTAXONOMY**
- elucidate **environmental factors** that influence biochemical events


I
LIMS, BLAST, Clust, ata-Mi, Gene Finder
II
Neural, rks, H, gy Mo, Force, Weblems
III
Data m, g, integ, Datab, Data-M, Toolkits
IV
GRID, informa, Virtual, rivacy, ity, Access
Functional Genomics
Nutrigenomics
Fungiomics
Bacteriomics
Metabolomics
Transcriptomics
Metabolomics
Structomics
Functional genomics
Metabolomics
Proteomics
Functional genomics
Functional Genomics
Greenomics
Transcriptomics
Markers/SNPs-'omics'
Pharma
Health
Food
Agro

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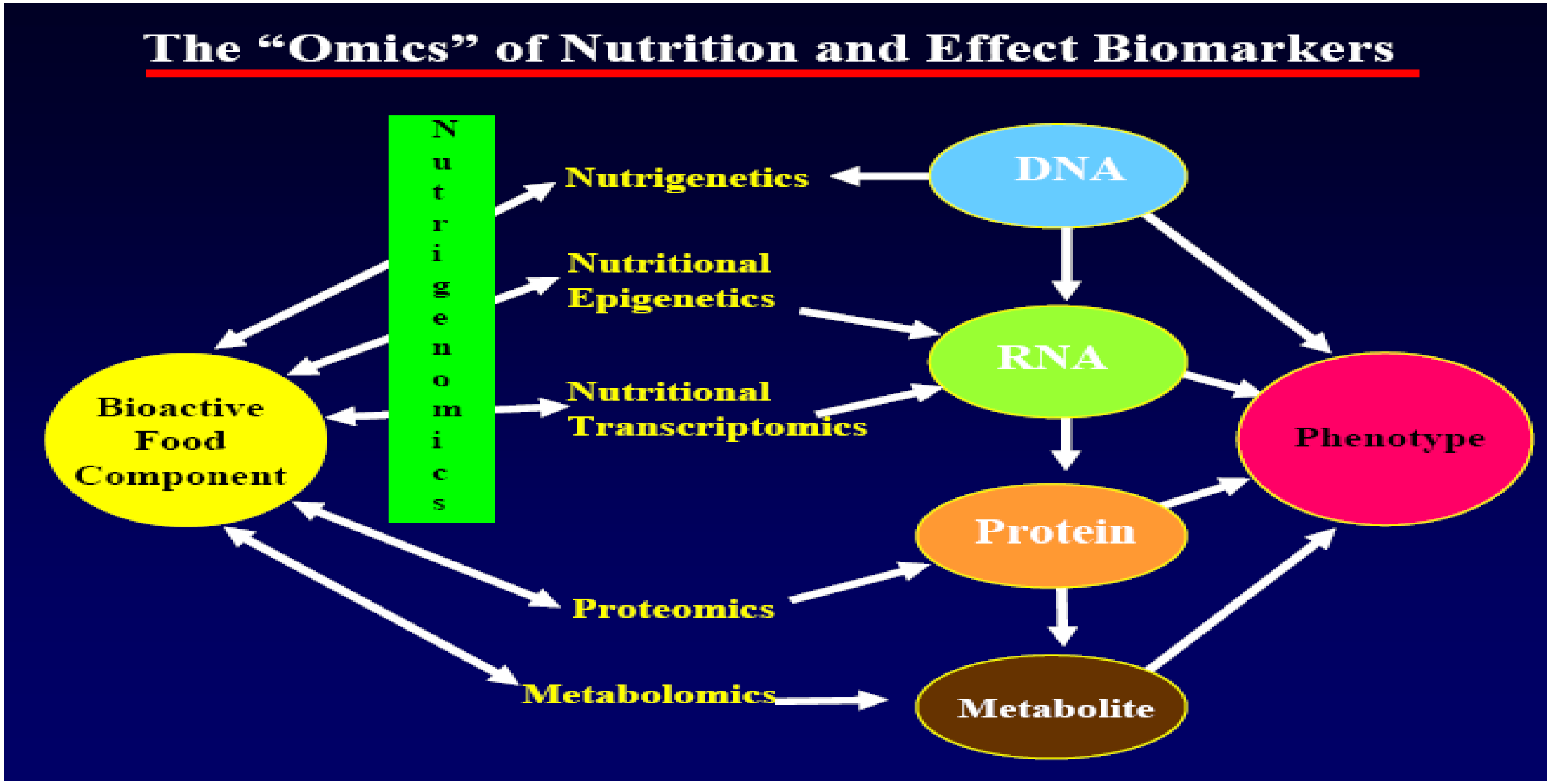
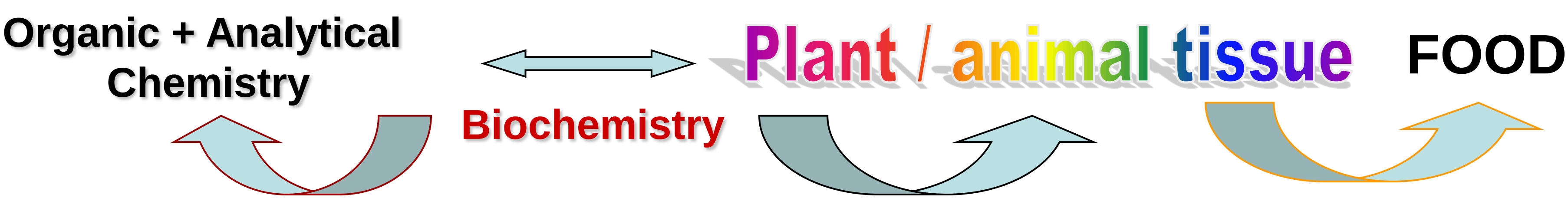


Agrifood metabolomics: characterization of relevant secondary metabolites of plants (phytochemicals) in food/feed products, as authenticity, traceability or quality biomarkers.

Metabolomics = systematic study of chemical **fingerprint to realize a metabolite profiling (small molecules)** in a specific matrix (plant, food) NON-TARGETED

***M*etabonomics = quantitative measurements to identify a specific metabolic response** (by key-molecules, e.g. pigments) TARGETED

METABOLOME = complement of all metabolites expressed in a cell, tissue or organism



WHAT WE NEED

Plant/animal metabolome

Processing

Chemistry

Isolation
Purification
Structure
Elucidation

Individuals

IT

Advanced Statistics

High performance

Equipments

Applied BIO-Chemistry

- Direct measurement of a physical property (e.g. color)
- Selective solubilization from a complex matrix
- Group identification and quantification (spectrometry)
- Fingerprint of a certain group (comparisons)
- Individual characterization (MS, NMR)

Physiologia Plantarum 132: 199–208. 2008

REVIEW

Metabolomics for plant stress response

Vladimir Shulaev[a,*], Diego Cortes[a], Gad Miller[b] and Ron Mittler[b,c]

[a]Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
[b]Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV 89557, USA
[c]Department of Plant Science, Hebrew University of Jerusalem, Givat Ram, Jerusalem 91904, Israel

Physiologia Plantarum 132: 190–198. 2008

REVIEW

Metabolomics integrated with transcriptomics: assessing systems response to sulfur-deficiency stress

Rainer Hoefgen[a] and Victoria J. Nikiforova[a,b,*]

[a]Abteilung 1 Molekulare Physiologie, Max-Planck-Institut für Molekulare Pflanzenphysiologie, Am Mühlenberg 1, 14476 Potsdam-Golm, Germany
[b]Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Str 35, Moscow 127276, Russia

Physiologia Plantarum 132: 176–189. 2008

REVIEW

Integration of metabolomics and proteomics in molecular plant physiology – coping with the complexity by data-dimensionality reduction

Wolfram Weckwerth[a,b,*]

[a]Department of Metabolic Networks, Max Planck Institut für Molekulare Pflanzenphysiologie, Am Mühlenberg 1, 14476 Potsdam, Germany
[b]GoFORSYS, Department of Biochemistry and Biology, University of Potsdam, 14469 Potsdam, Germany

Physiologia Plantarum 132: 162–175. 2008

REVIEW

Plant metabolomics and its potential application for human nutrition

Robert D. Hall[a,b,*], Inge D. Brouwer[c] and Melissa A. Fitzgerald[d]

[a]Plant Research International, Business Unit Bioscience, PO Box 16, 6700 AA Wageningen, The Netherlands
[b]Centre for Biosystems Genomics, PO Box 98, 6700 AB Wageningen, The Netherlands
[c]Division of Human Nutrition, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands
[d]Grain Quality, Nutrition and Postharvest Centre, International Rice Research Institute (IRRI), and International Network for Quality Rice,

Metabolomics Unravel Contrasting Effects of Biodiversity on the Performance of Individual Plant Species

Christian Scherling[1], Christiane Roscher[2,3], Patrick Giavalisco[4], Ernst-Detlef Schulze[2], Wolfram Weckwerth[5*]

Physiologia Plantarum 132: 150–161. 2008

REVIEW

Metabolite profile analysis: from raw data to regression and classification

Matthias Steinfath[a,*], Detlef Groth[a], Jan Lisec[b] and Joachim Selbig[a]

[a]Department of Molecular Biology, Institute for Biology and Biochemistry, University of Potsdam, Karl-Liebknecht-Straße 24-25, D-14476 Potsdam-Golm, Germany
[b]Department of Molecular Biology, Max Planck Institute for Molecular Plant Physiology, Am Mühlenberg 1, D-14476 Potsdam-Golm, Germany

Physiologia Plantarum 132: 117–135. 2008

REVIEW

Metabolomic technologies and their application to the study of plants and plant–host interactions

J. William Allwood*, David I. Ellis and Royston Goodacre

School of Chemistry, Manchester Interdisciplinary Biocentre, The University of Manchester, 131 Princess Street, Manchester M1 7DN, UK

The Plant Cell, Vol. 14, 1437, July 2002, www.plantcell.org © 2002 American Society of Plant Biologists

MEETING REPORT

Plant Metabolomics: The Missing Link in Functional Genomics Strategies

SCIENCE DIRECT®

ELSEVIER

Integrating genomics and metabolomics for engineering plant metabolic pathways

Kirsi-Marja Oksman-Caldentey[1] and Kazuki Saito[2,3,4]

PHYTOCHEMICALS as metabolomic markers

- Omega 3,6,7-fatty acids
- Phytosterols
- Carotenoids & chlorophylls
- Vitamins : A, C, E
- Flavours: terpenoids

- Polyphenols: flavonoids, phenolic acids, antocyans, tannins
- Saponins, lignins and indoles
- S-derivatives: thiols and glycosinolates

Plant secondary metabolites (more than 20000 molecules known yet…)

✓Attraction/defence molecules
✓Antioxidant/antibiotic action
✓Beneficial for plant animal & human health.

Functionality = Bioactivity dependent on solubility, stability, bioavailability & redox potential

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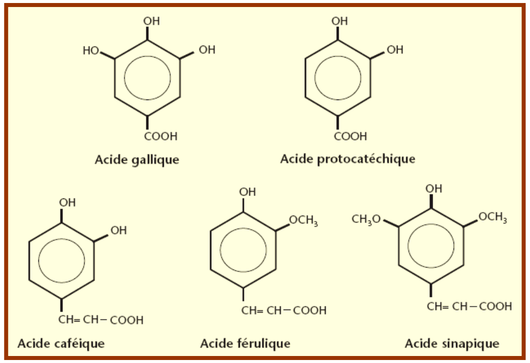
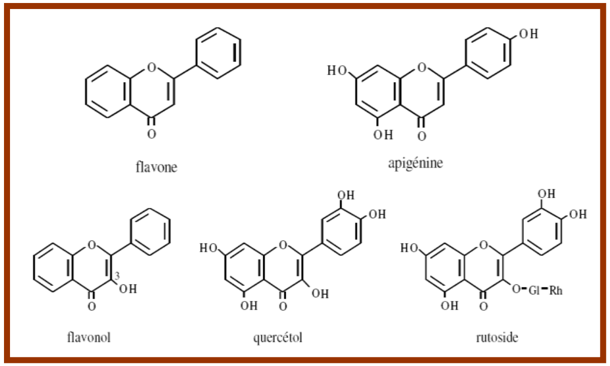
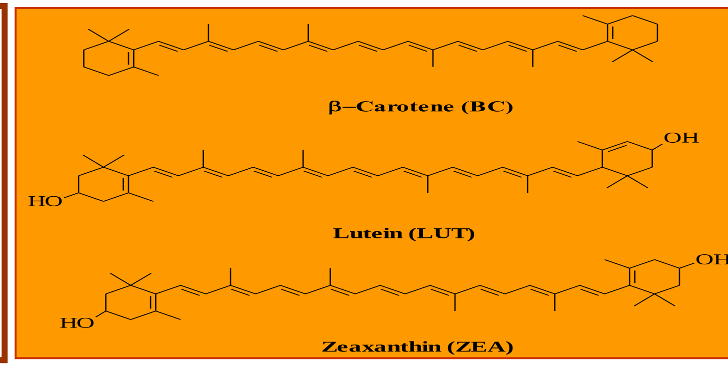
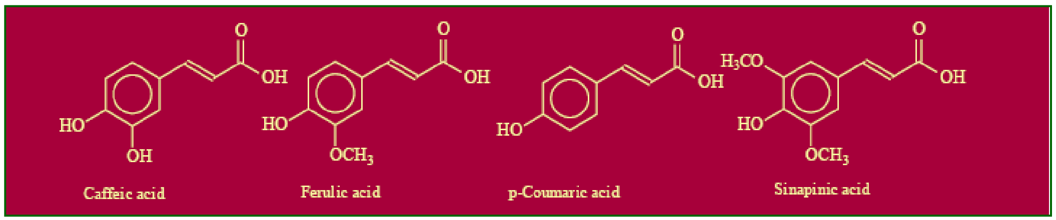
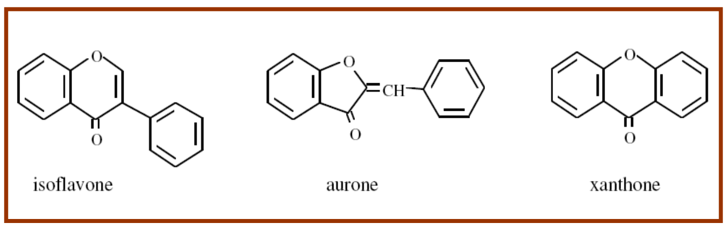
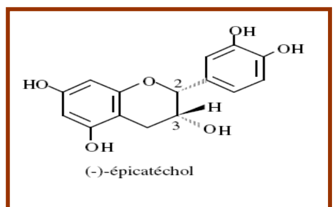


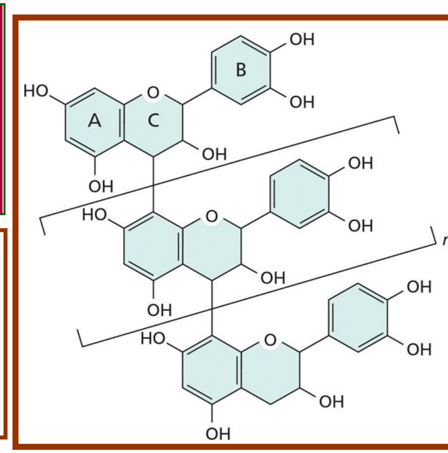




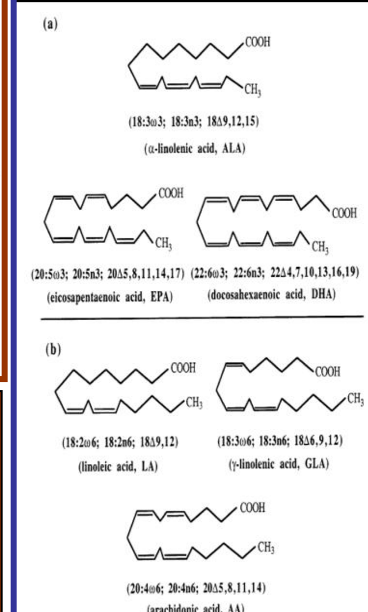


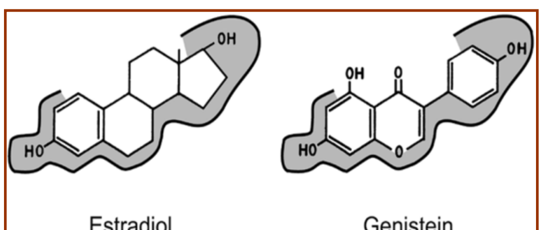


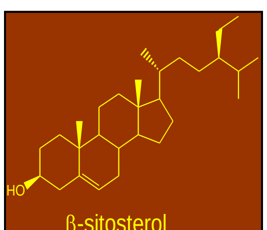


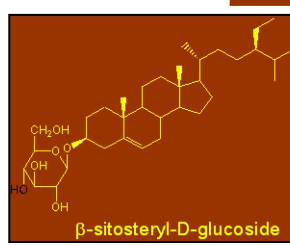


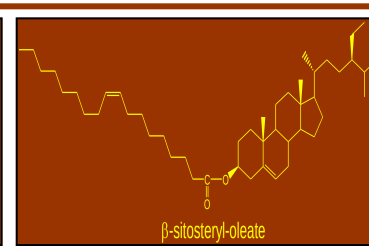
Energy-rich (labile) molecules



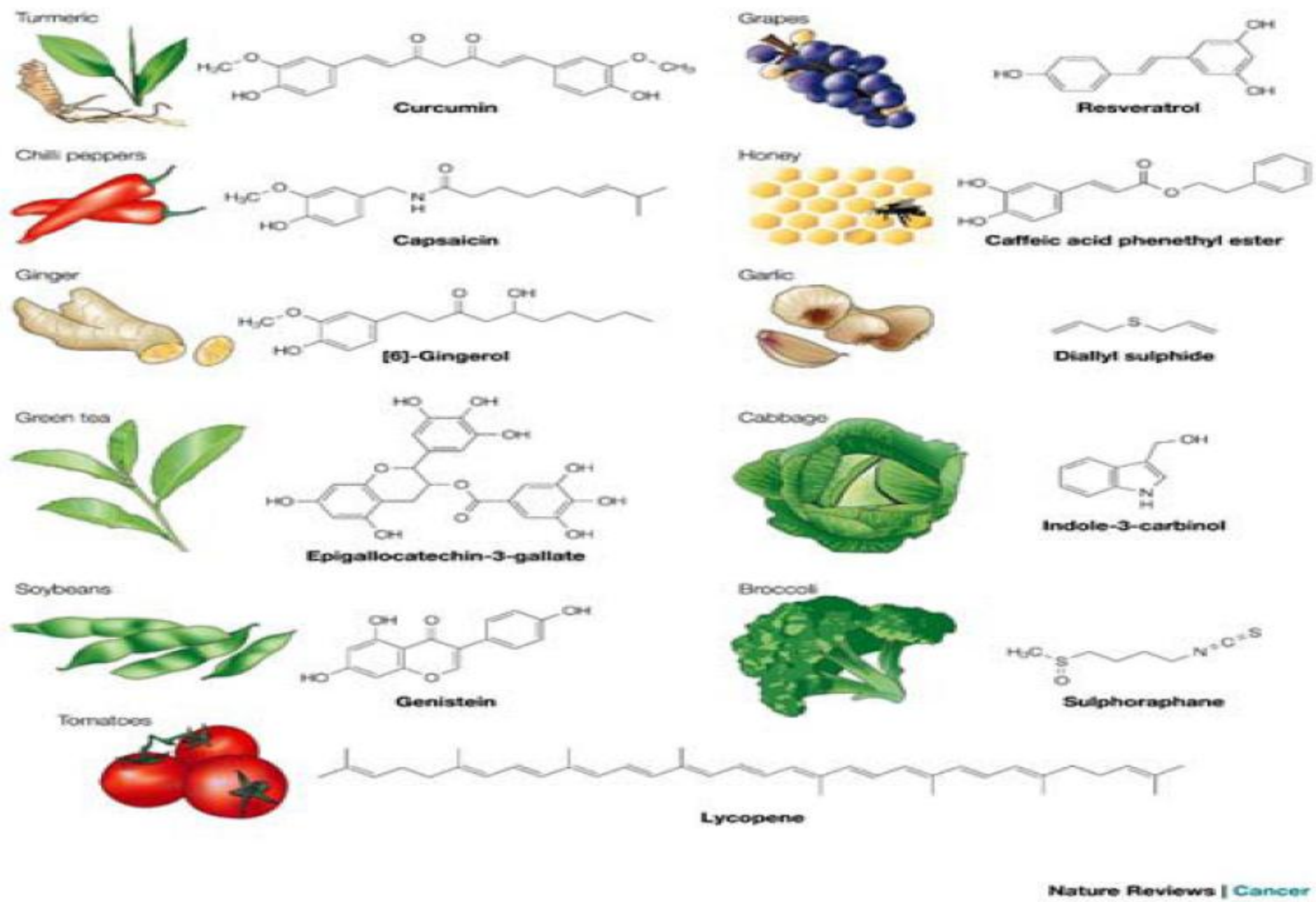








Phytochemical synthesis related to genetic and environmental factors



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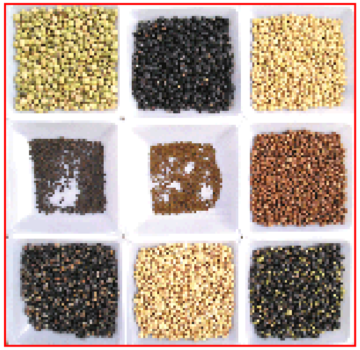
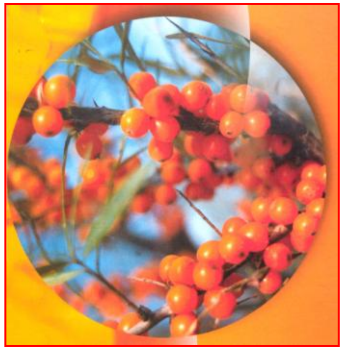
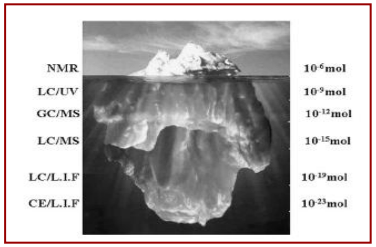
OUR INVESTIGATIONS : OBJECTIVES

- **Fingerprint of phenolics /carotenoids/sterols/PUFAs & quantitation of (HPLC, FTIR-ATR, UV-Vis) in different plants cultivated and originating from Romania**

- **Screening of the specific PUFAs & phytosterols & phenolics fingerprint of functional food (oils and juices) or nutraceuticals**

- **Metabolomic approach- Comparison of HPLC, LC-MS, GC-FID, GC-MS and FTIR data, by Chemometry: Quality & authenticity**



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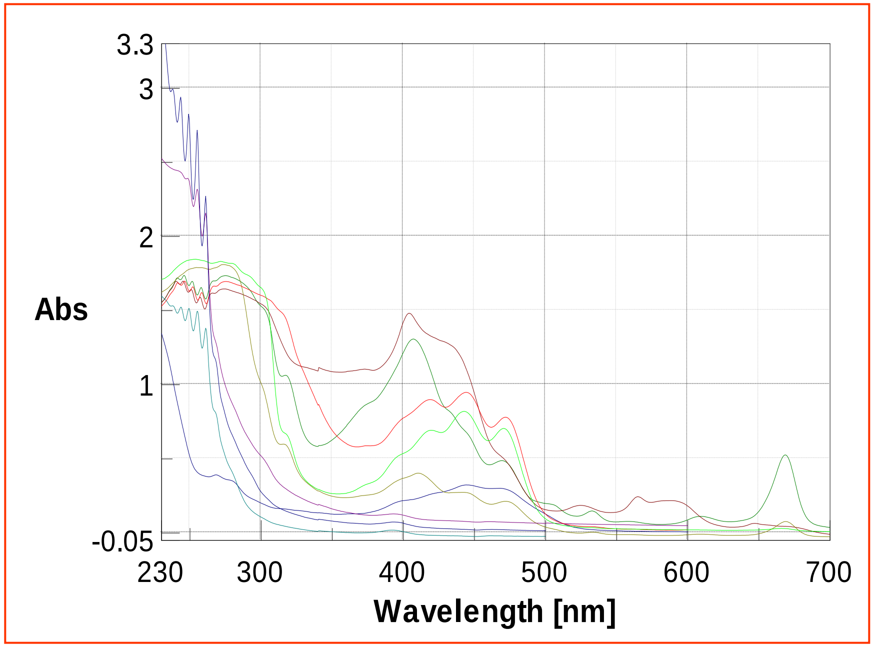
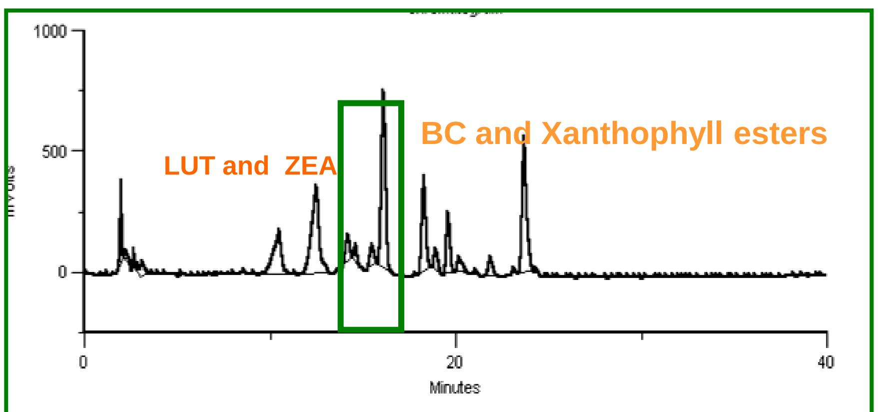
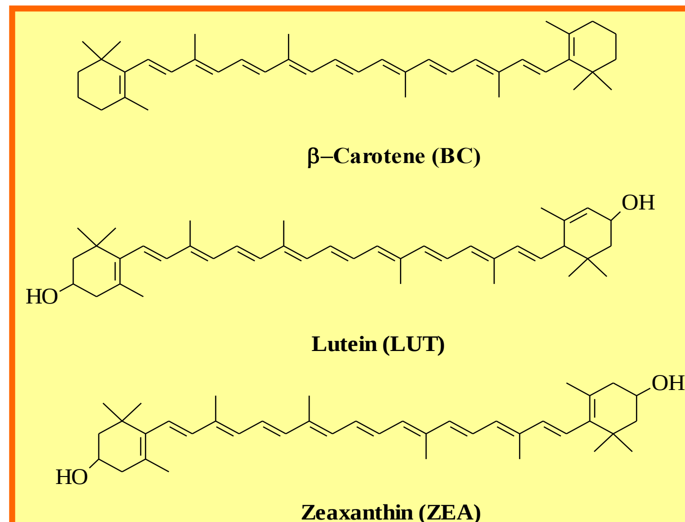


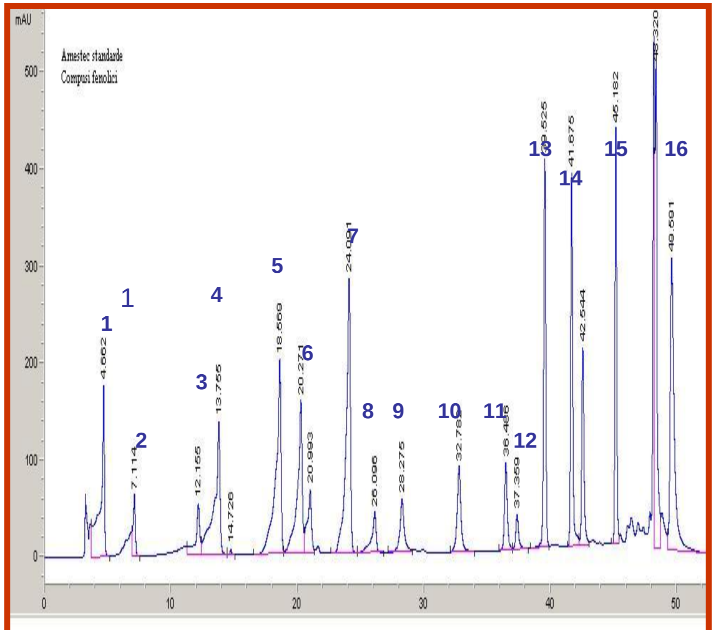
OILS
Seabuckthorn
Hempseed
Pumpkin
Sunflower
(1:1000, PE)
Olives
Wallnut
Rapeseed
Soybean
Sesame

PLANT PIGMENTS

Lipophilic- carotenoids
Hydrophilic- vitamins and phenolics



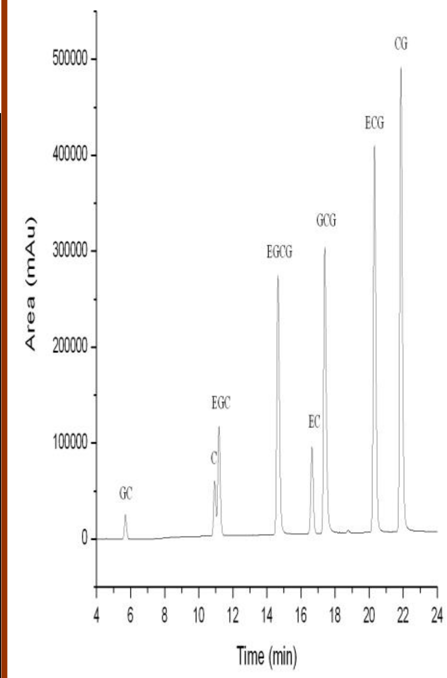




HPLC separation of phenolics (Socaciu et all, 2006)

HPLC – best choice for phenolics

Peak	Name	Pea k	Name
1.	Gallic acid	9.	Rutin
2.	Protocatecuic acid	10	Myricetin
3.	Chlorogenic acid	11	Quercetin
4.	Caffeic acid	12	Kaempferol
5.	p-Coumaric acid	13	Izorhamnetin
6.	Ferulic acid	14	Pinocembrin
7.	Sinapic acid	15	Chrysin
8.	o-Coumaric acid	16	Galangin



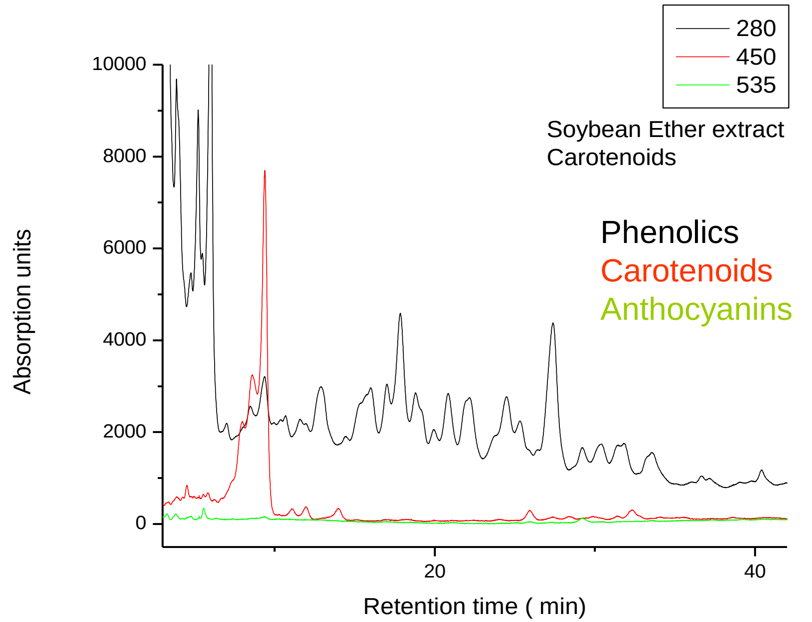
Optimized procedures to separate different <u>phenolics</u> (phenolic acid derivatives and flavonoids) and catechins (C, GC, EC, ECG, EGC, EGCG, CG, GCG)

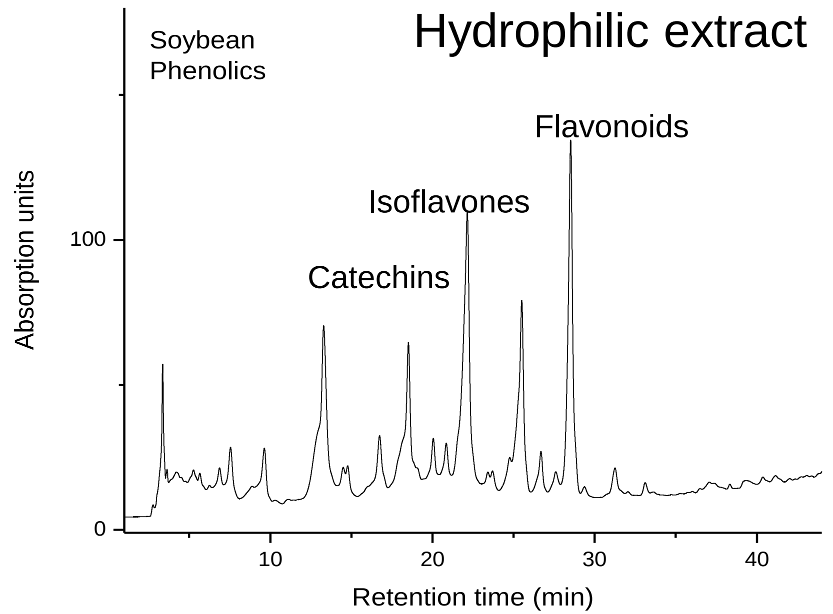
Chromatography (TLC, HPLC, LC-MS, GC, GC-MS)

Qualitative= fingerprint

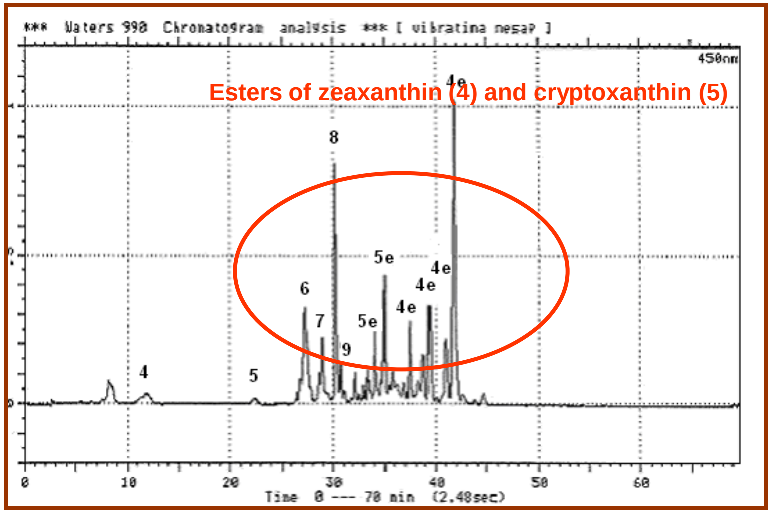
Large variability of composition depending on solvents and separation protocols

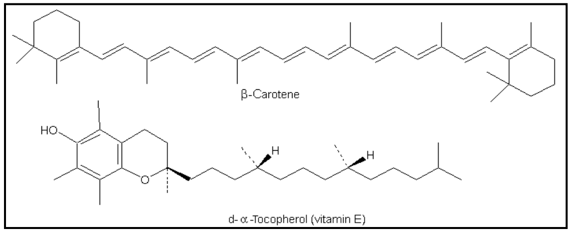
Soybean (*Glycine max*)

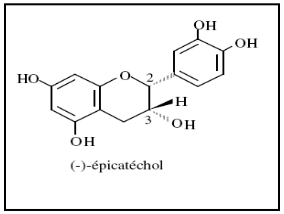


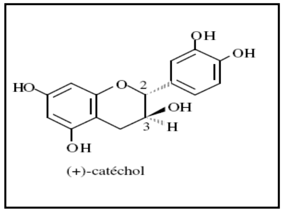


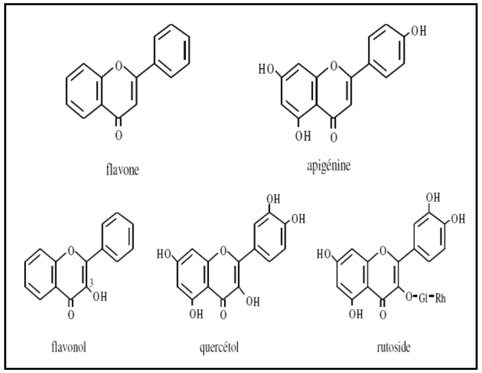
HPLC multilevel investigations for each phytochemical class, subclasses and individuals



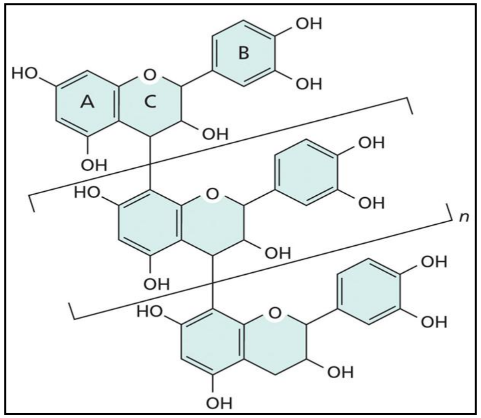


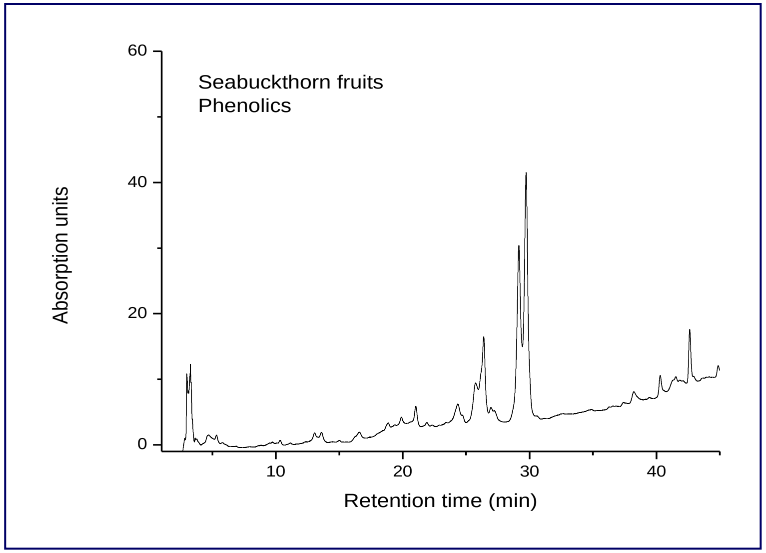


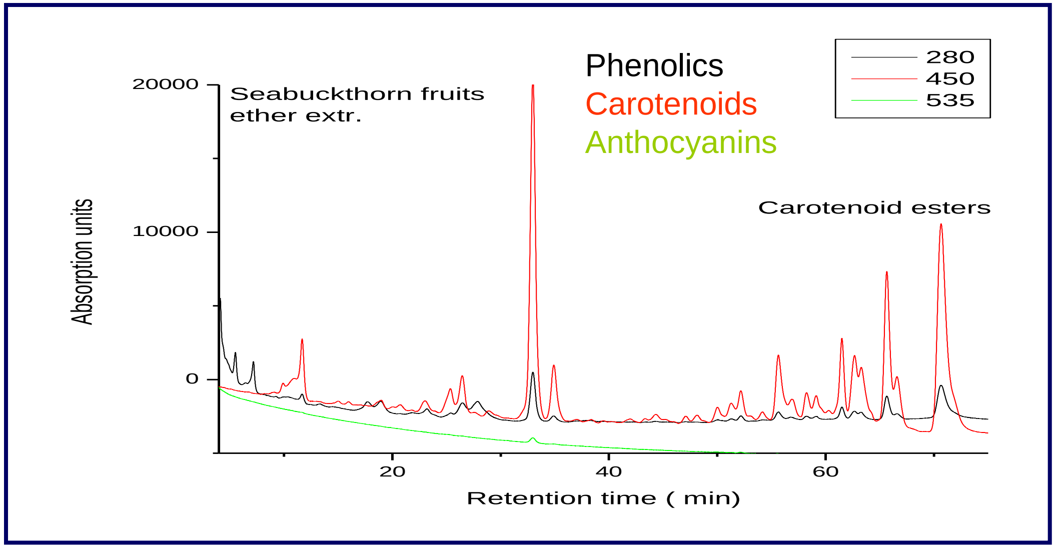


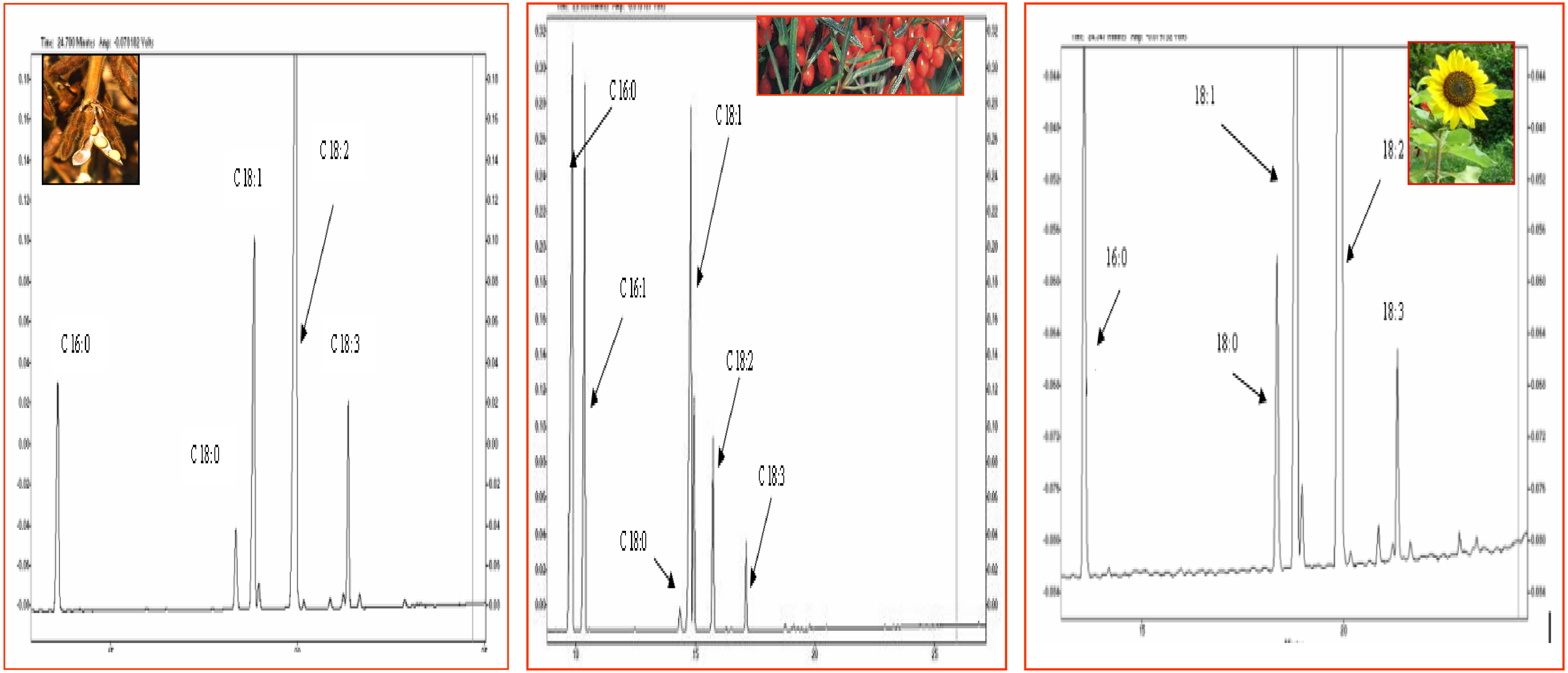


Seabuckthorn fruits









A. B. C.

GC-FID fingerprint for <u>fatty acids</u>, useful to discriminate between soybean oil (SO) (A), seabuckthorn oil (SBO) (B) comparing with sunflower oil (C).

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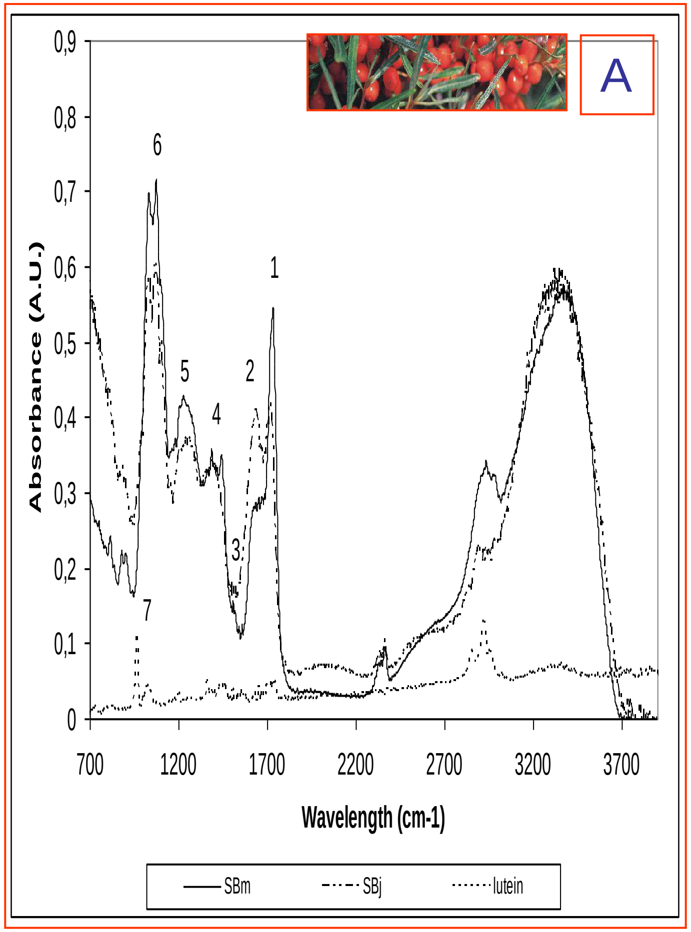
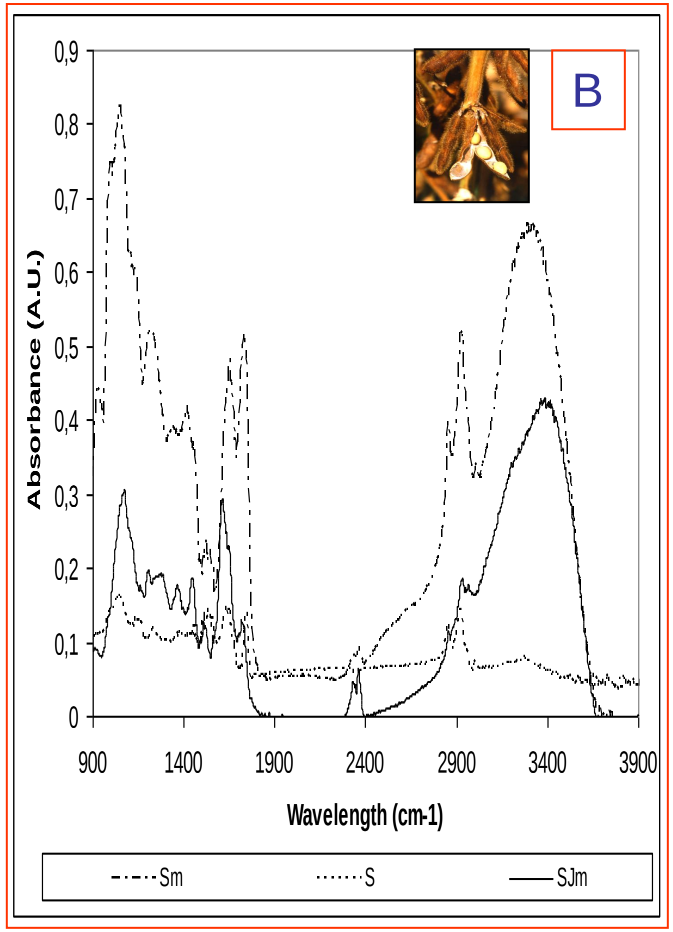
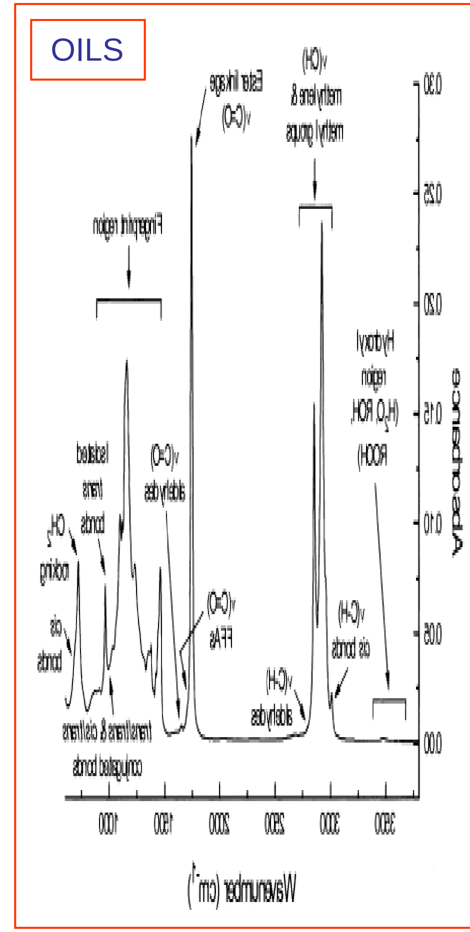
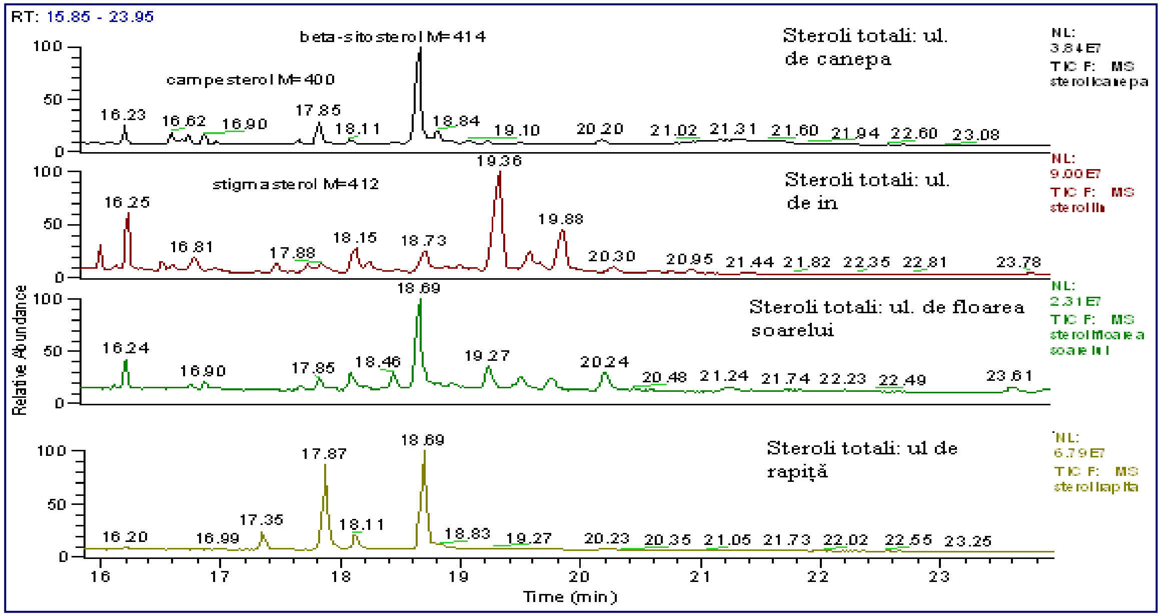


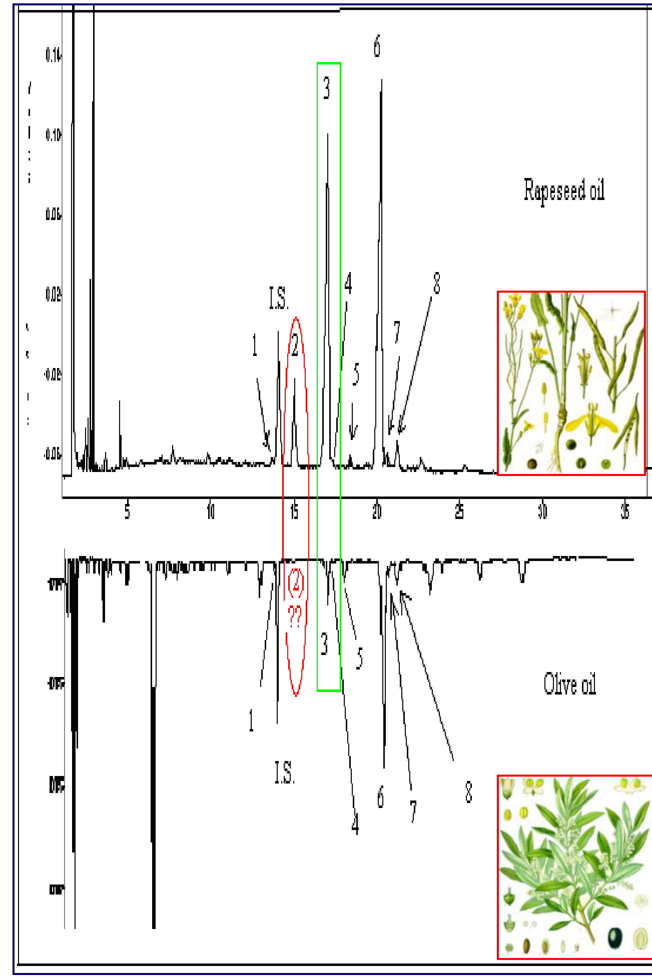


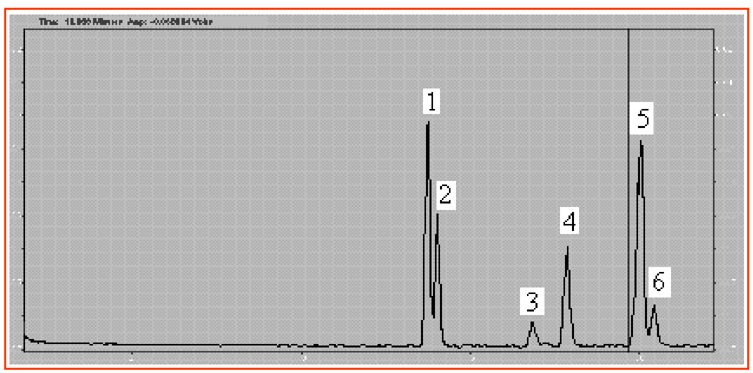


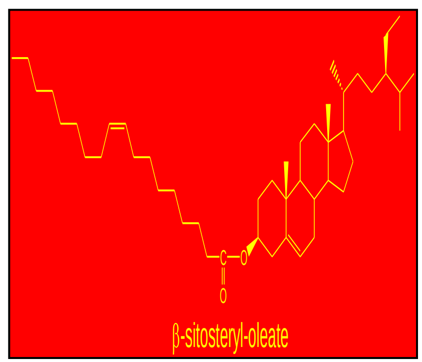
FT(ATR)MIR fingerprint of oils

Edible plant oils authenticity – by GC fingerprints

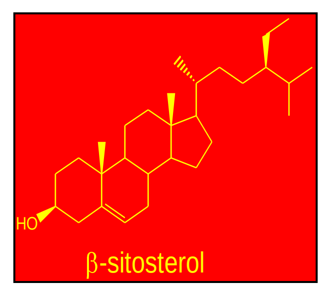


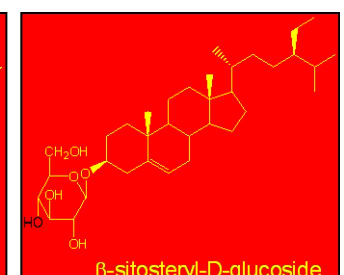






GC-FID for main phytosterols, used as authenticity markers of edible oils: (2)-5α-Colestan-3β-ol, (3)-Campesterol, (4)-Stigmasterol, (5)- β-Sitosterol, (6)- β-Sitostanol. Internal standard: Colesterol (1)

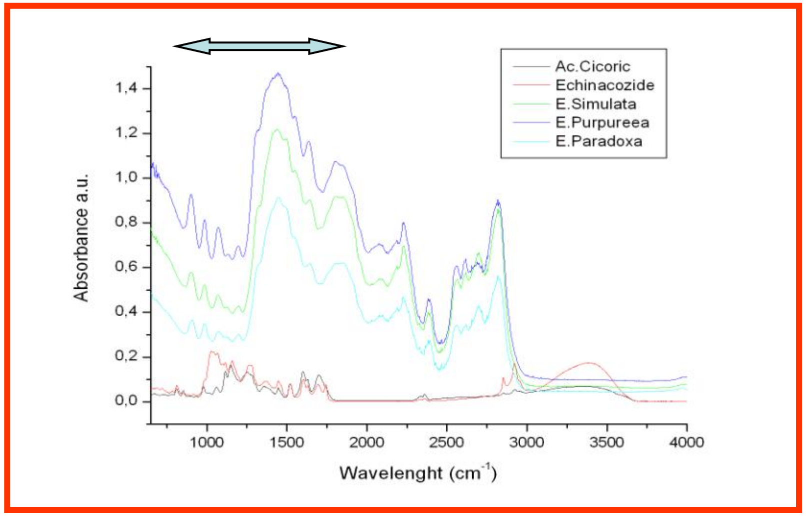


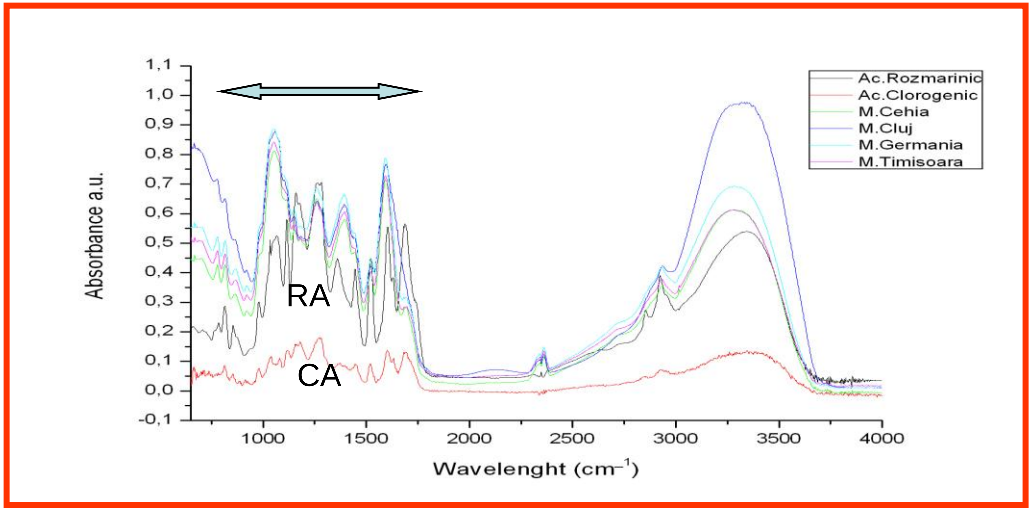


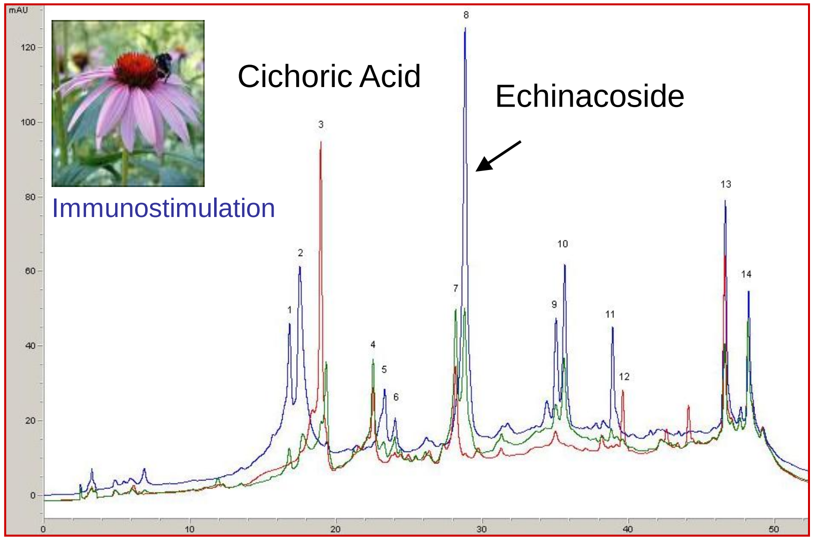
Complementary techniques FTIR and HPLC-PDA

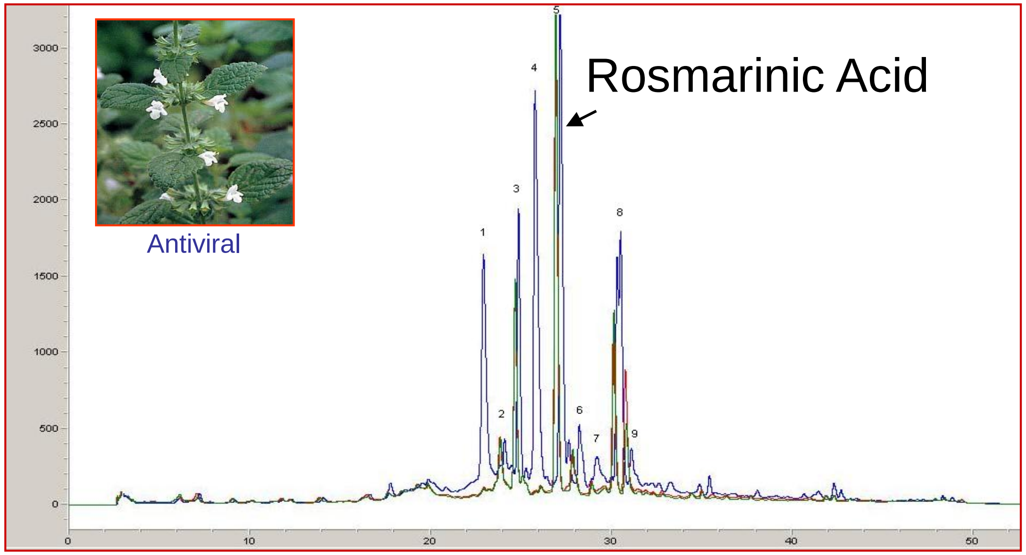
Echinaceea sp.

Melissa off.



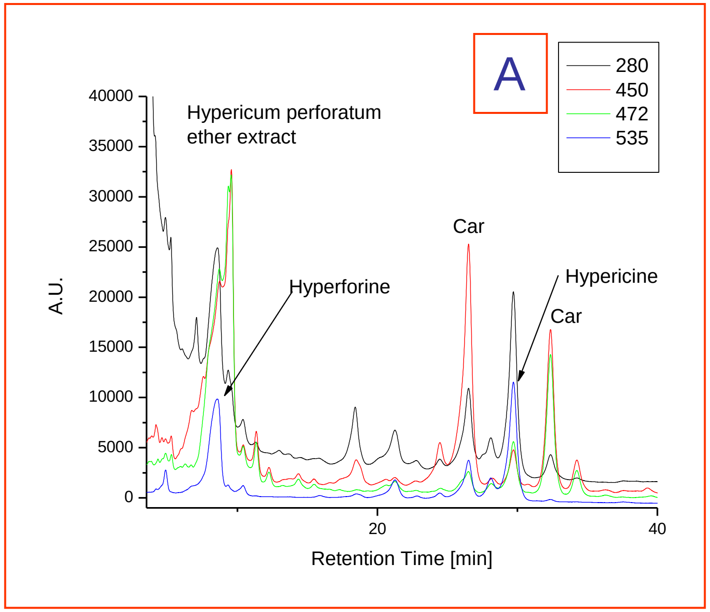


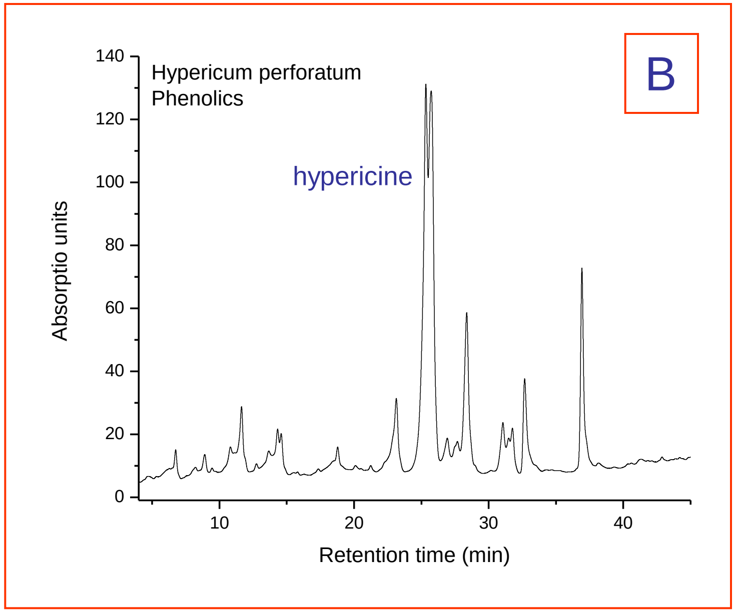


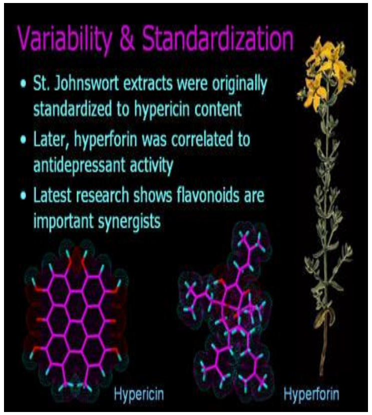


Metabolic/taxonomic markers of *Echinaceea sp.* si *Melissa off.*

Department of Agrifood Chemistry and Biochemistry
University of Agricultural Sciences and Veterinary Medicine
Cluj-Napoca, Romania





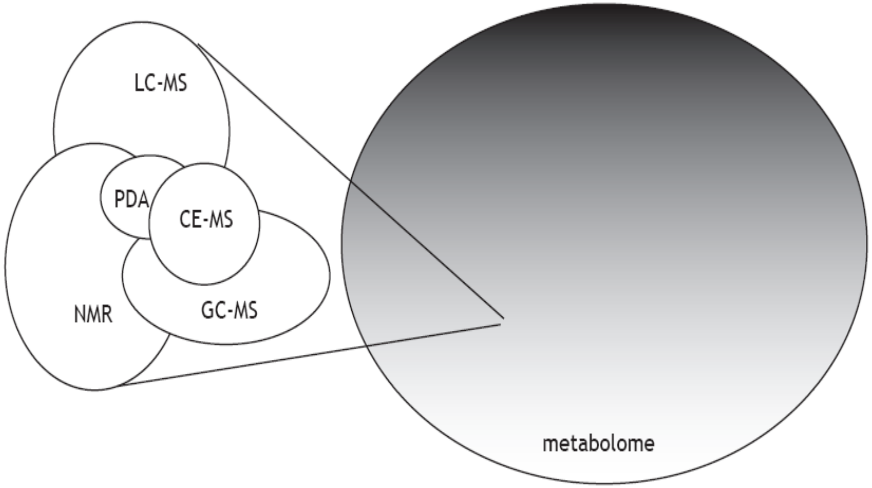


HPLC fingerprint of medicinal plants: carotenoids and hypericine/hyperforine (A).

Identification p-coumaric acid (t_R=18.5 min), lutein (t_R=9.5 min), zeaxanthin and β-carotene (t_R=23.5 min), hyperforine and hypericine.

Metabolomics Technologies

- UPLC, HPLC
- CE/microfluidics
- LC-MS
- FT-MS
- QqQ-MS
- NMR spectroscopy
- X-ray crystallography
- GC-MS
- LIF detection

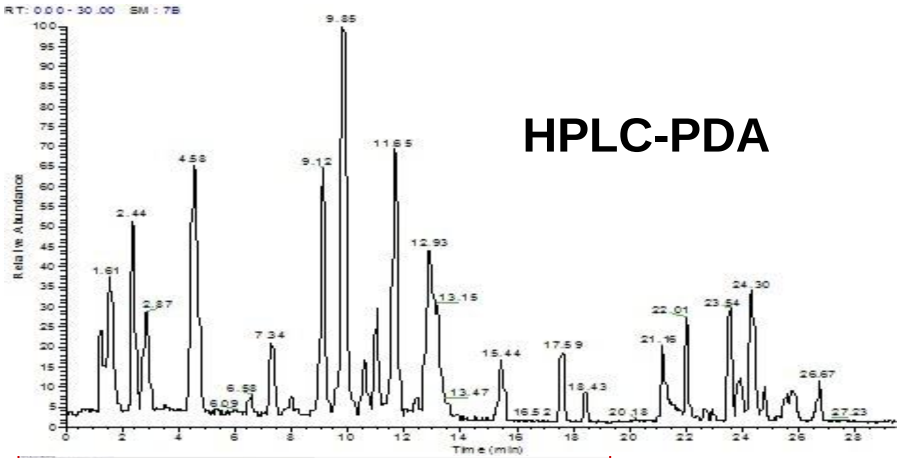


Widely used methods for plant metabolite analysis:

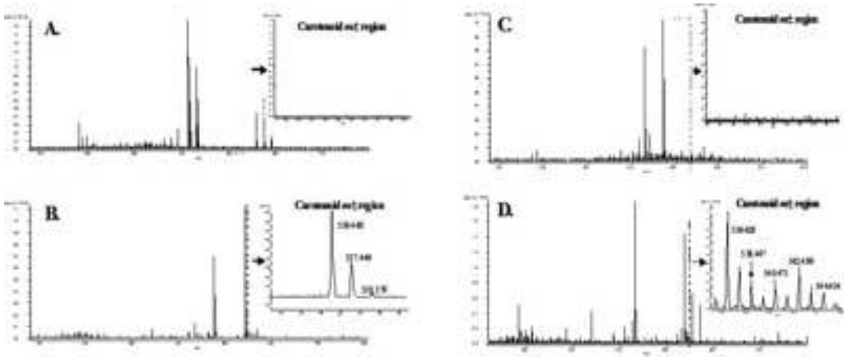
❑GC /MS and LC/MS *(Sumner, Mendes & Dixon (2003) and Dunn & Ellis (2005).*
❑LC/PDA/MS (*Huhman & Sumner, 2002),*
❑Capillary electrophoresis/mass spectrometry (CE/MS (Soga *et al.*, 2003; Sato *et al.*, 2004)
❑Fourier Transformed IR Spectroscopy (*Socaciu, 2009*)
❑Fourier-transform ion-cyclotron mass spectrometry (FT/MS) *(Tohge et al., 2005)*
❑Nuclear magnetic resonance (NMR) (*Ward et al., 2003; Wiklund et al., 2005)*

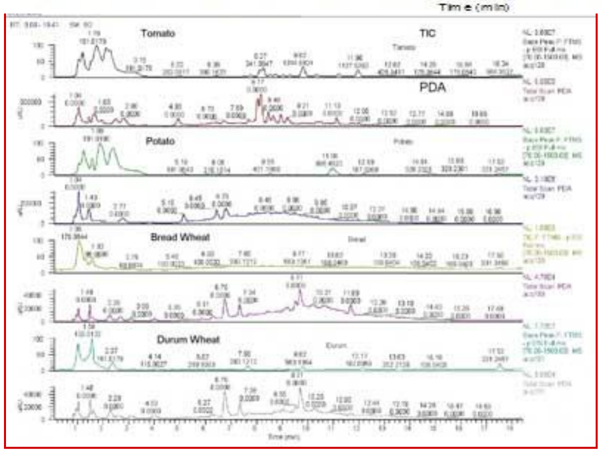
Validation by advanced methods HPLC-PDA or LC-MS

Profiling and quantitation



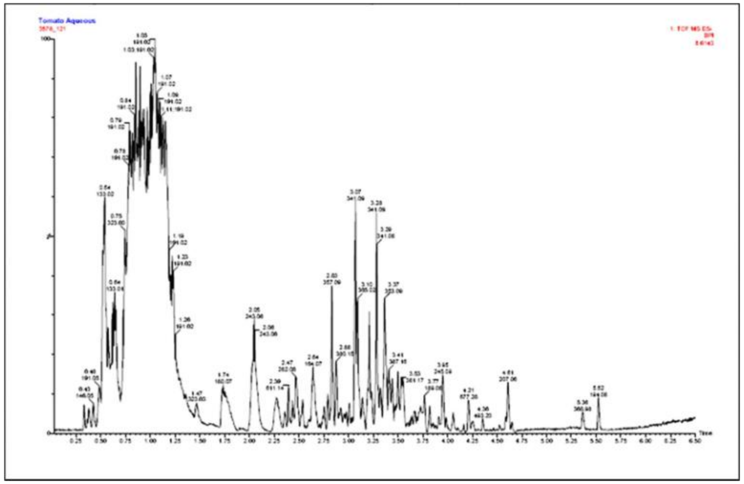
HPLC-PDA





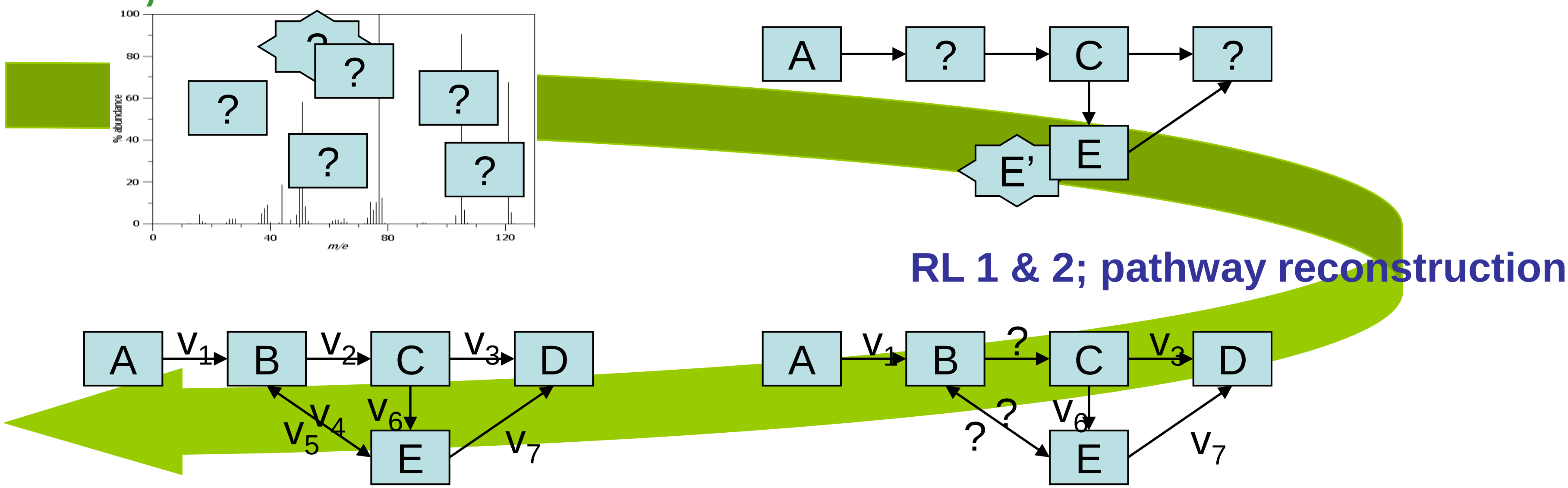
MALDI-ToF-MS profiles

LC-QToF-MS



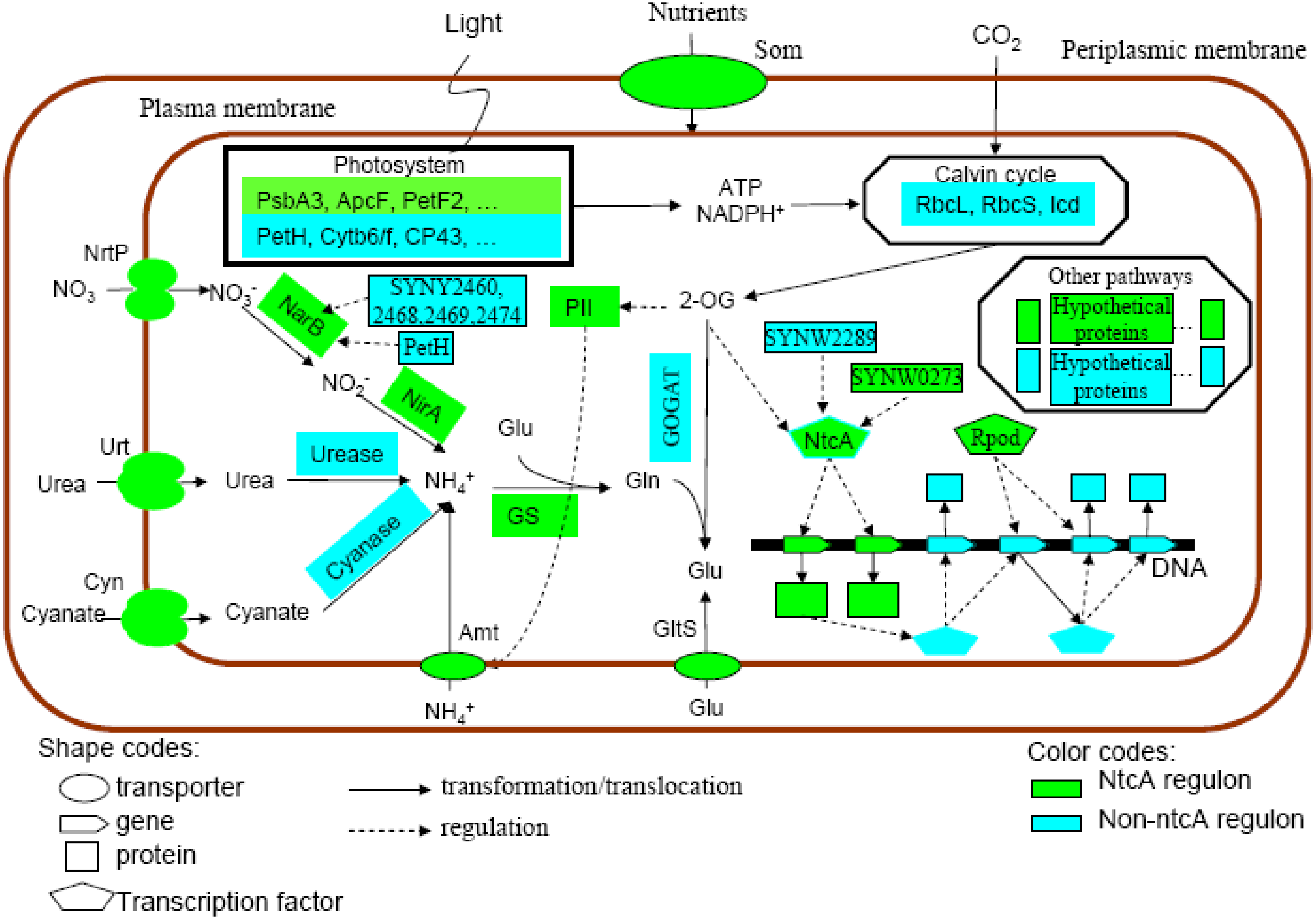
Metabolomics & Fluxomics

RL 1; identified metabolite



RL 2; metabolic flux analysis (Fluxomics)

Cross-talk Network between Nitrogen Assimilation and Photosynthesis



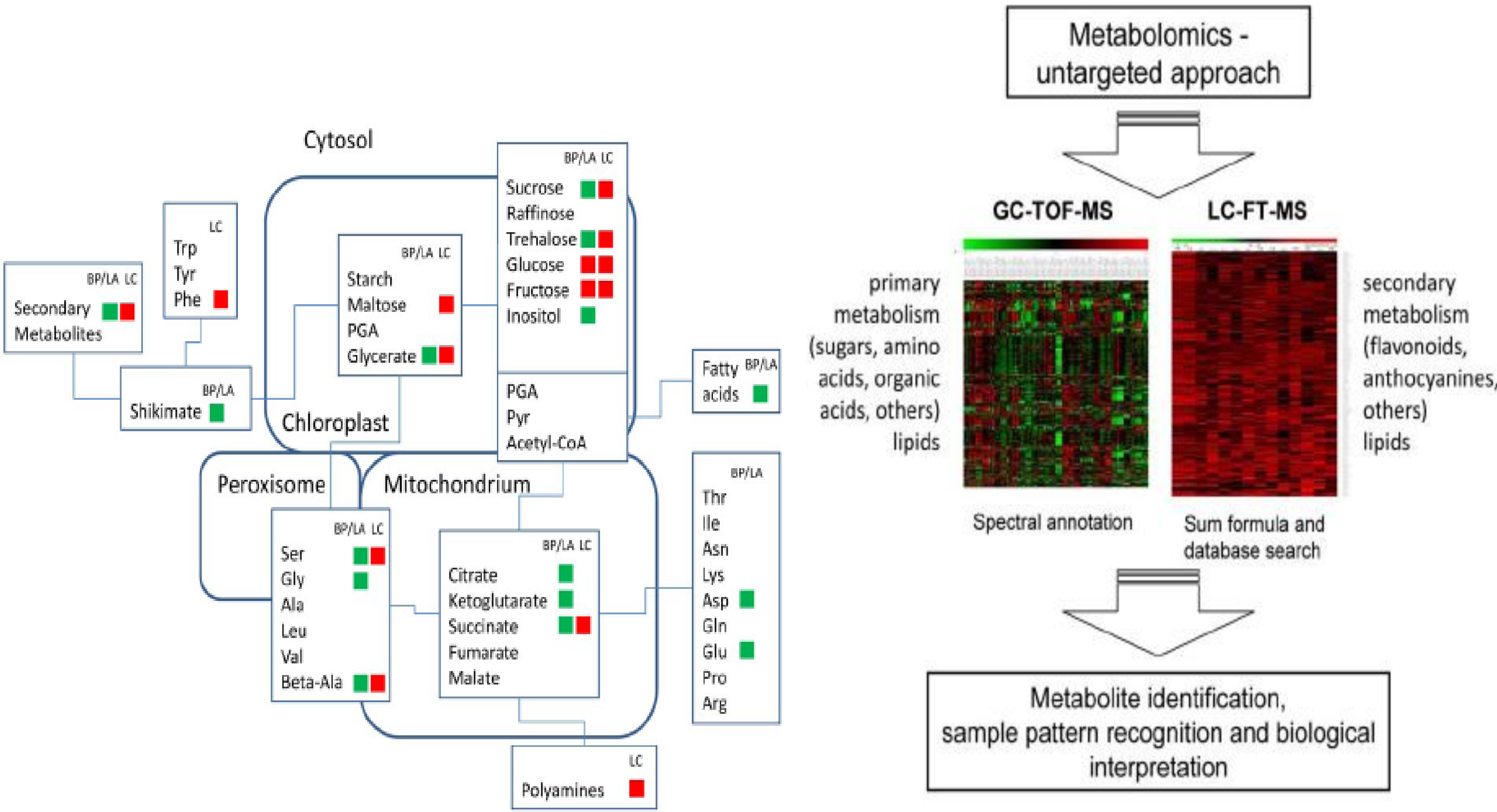


Figure 4. Metabolic signatures of the primary metabolism of small-statured plant species in response to plant diversity. Significant changes were symbolized via heat map (red = relative increase in response to plant diversity; green = relative decrease in response to plant diversity). BE/LA corresponds to relative metabolite concentration changes of *Bellis perennis* and *Leontodon autumnalis* in response to species richness. LC corresponds to relative metabolite concentration changes of *Lotus corniculatus* in response to increasing proportions of legumes.
doi:10.1371/journal.pone.0012569.g004

METABOLOMICS _ ADDED VALUE

•DNA microarrays are expensive and often unavailable, protein analysis is time-consuming and hindered by the complexity of the molecules

•Many gene and proteins' functions remain unknown, but metabolites can be assigned to particular tissues and diseases

•Metabolic methods - highly effective.

•Databases of metabolomes and metabolic biomarkers are yet available / increase exponentially.

Limitations, opposite of those posed by genomics:

If the genetic source of a disease is too far "upstream" of the pathology to identify, metabolic changes might be too far "downstream," and “diluted” by protein activities, environment, and other intermediate biochemical events.

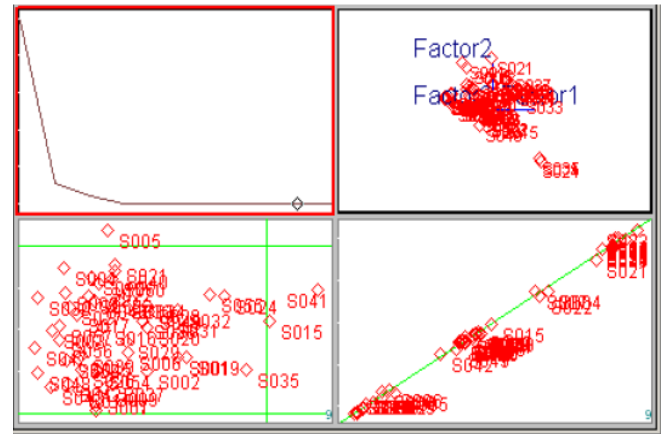
Metabolomic profiles are subject to random fluctuations, and can be highy influenced by environmental factors (climate, stress)

Some conclusions

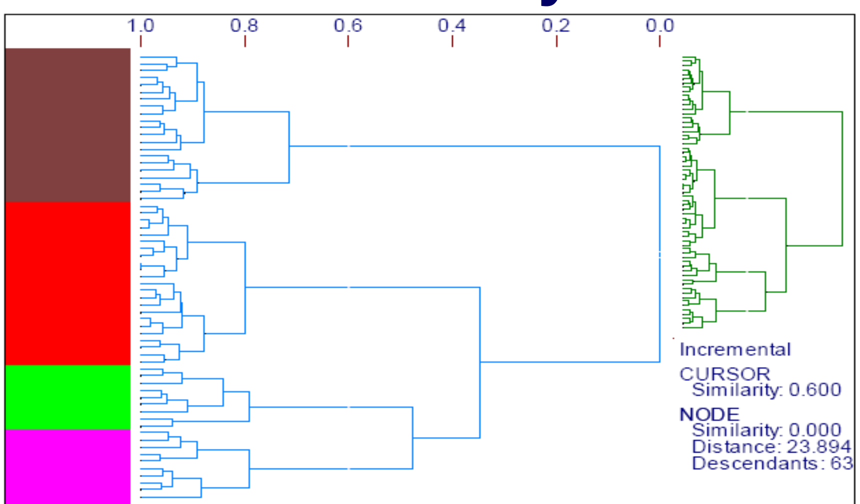
1. Metabolome analysis technologies are still in early developments. Especially in plants and food **metabolome analysis has to deal with a highly diverse range of biomolecules, at minor concentrations , but with high impact in biodiversity, nutrition and health**
2. The technical progress in development of **new instruments and methods** (LC-MS, GC-MS, TOF-MS/MS, NMR, FTIR, etc) with powerfull capacity induced exponential development of metabolomics
3. **Combinations of different analytical platforms** are required for comprehensive metabolomic studies.
4. **Chemometry** is a *sine-qua non*-condition for appropiate interpretation for many analytical data, difficult to correlate
5. **Cohorts of case studies and databases are required for further developments of Metabolomics**

Chemometrics

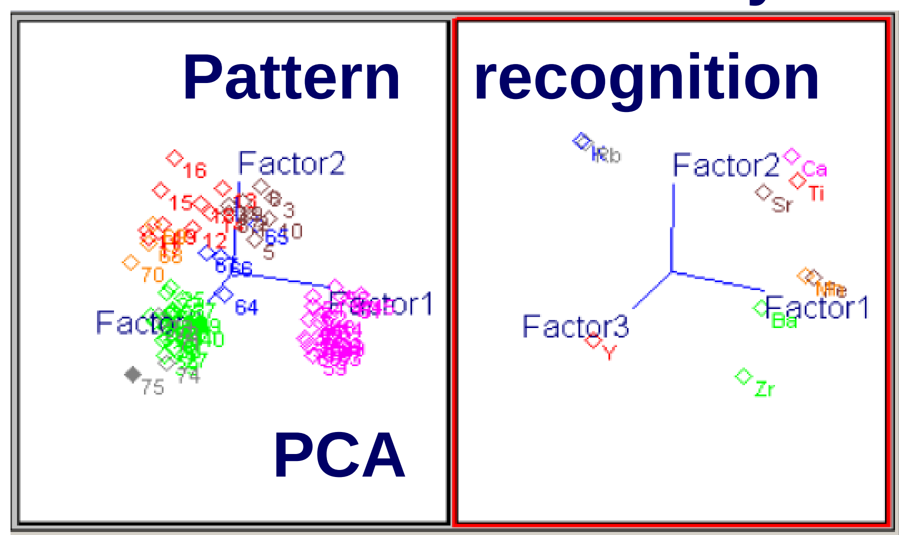
Preprocessing by Metalign
Genemaths
SIMCA
Mathlab
Infometrix:
Pirouette 4.0



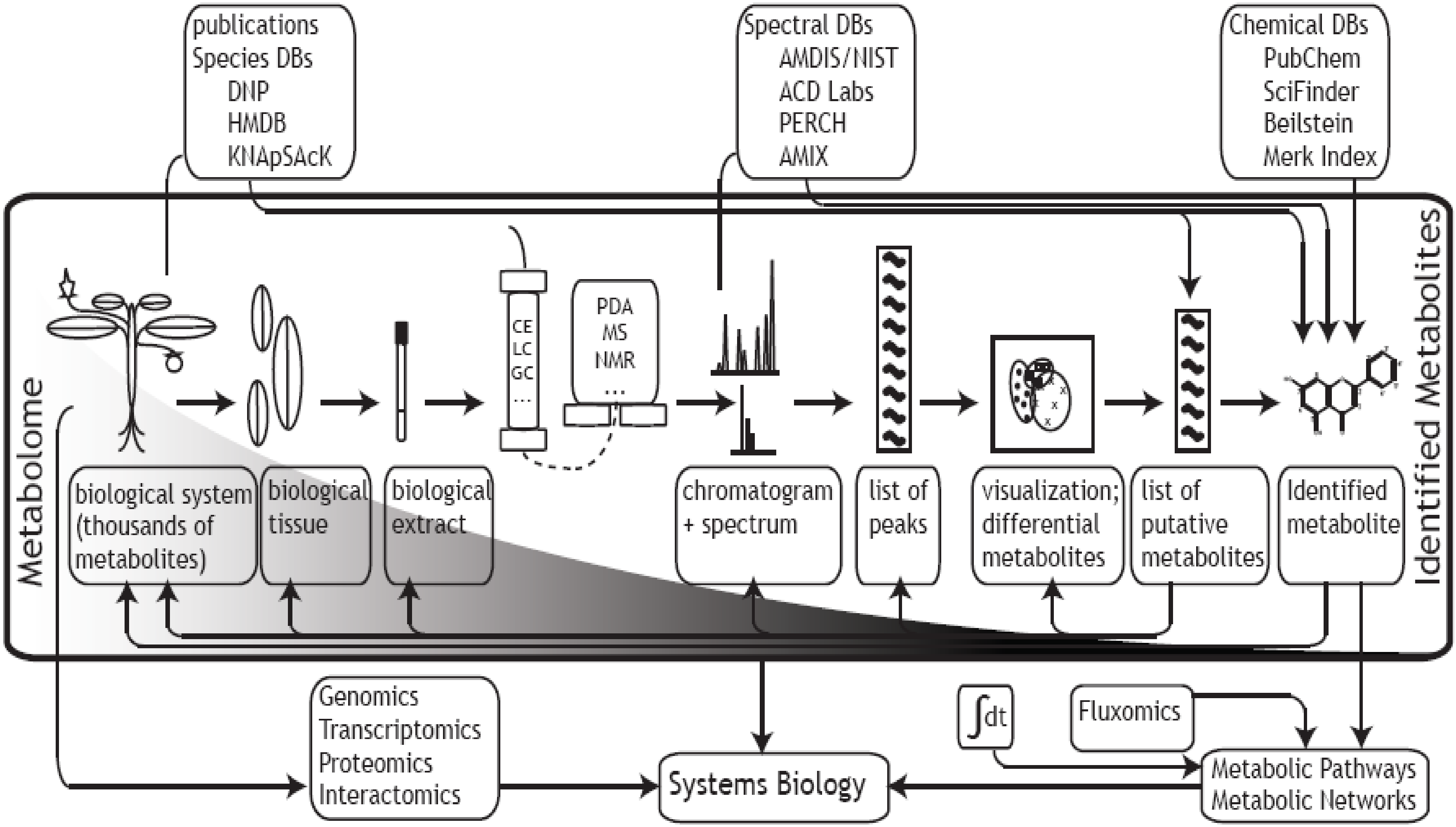
Cluster analysis



PLS Analysis

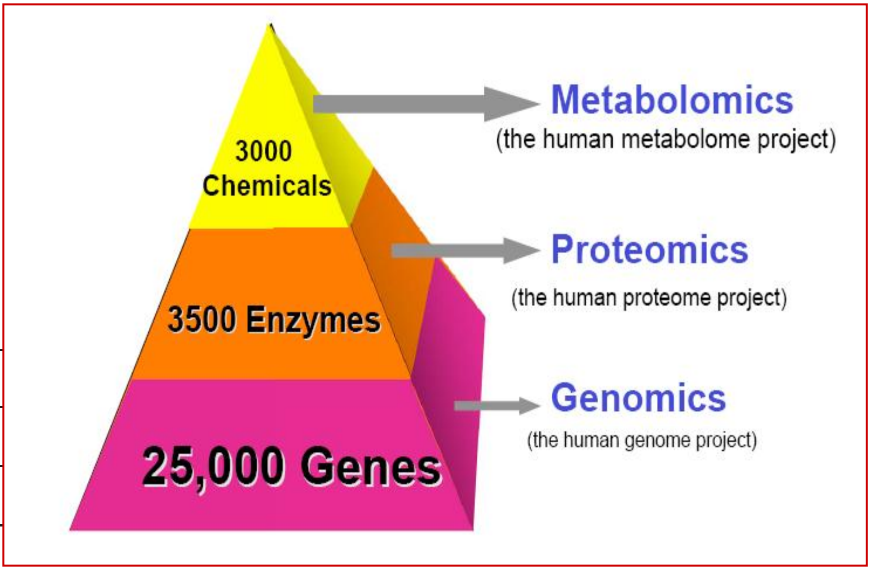


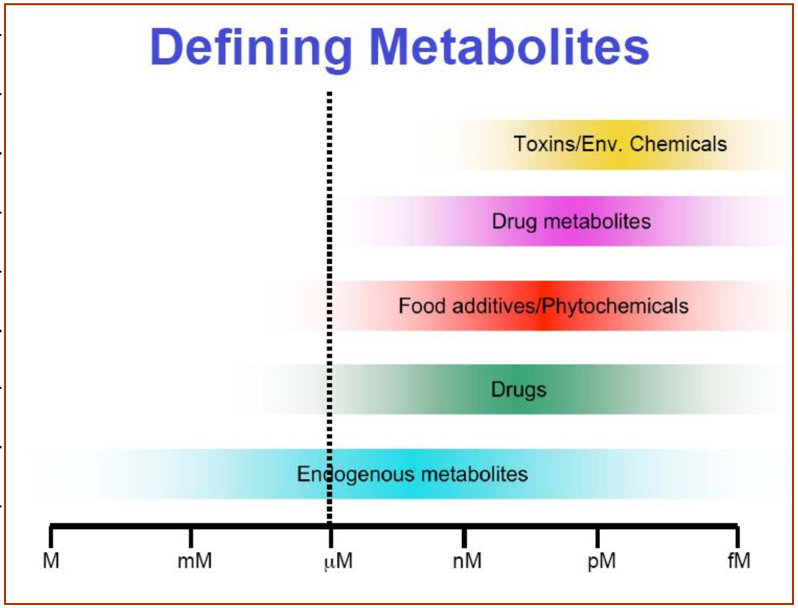
Towards a systems biology approach: steps from metabolome to identified metabolites



METABOLOMIC APPLICATIONS

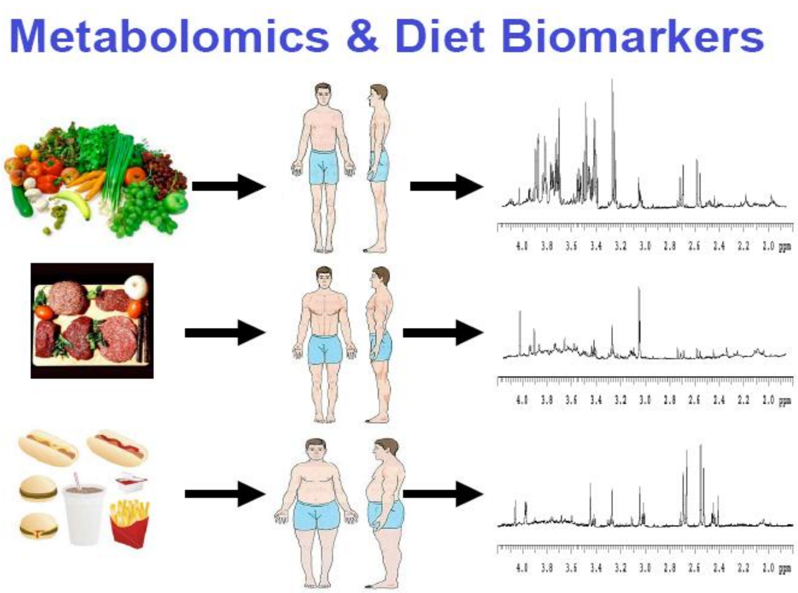
Area of application
Plant breeding and crop quality assessment
Food assessment and safety
Toxicity assessment
Nutrition assessment
Medical diagnosis and assessment of disease status
Pharmaceutical/drug developments
Yield improvement in crops and fermentations
Biomarker discovery
Technological advances in analytical chemistry
Genotyping
Environmental adaptations
Gene function elucidation
Integrated in systems biology

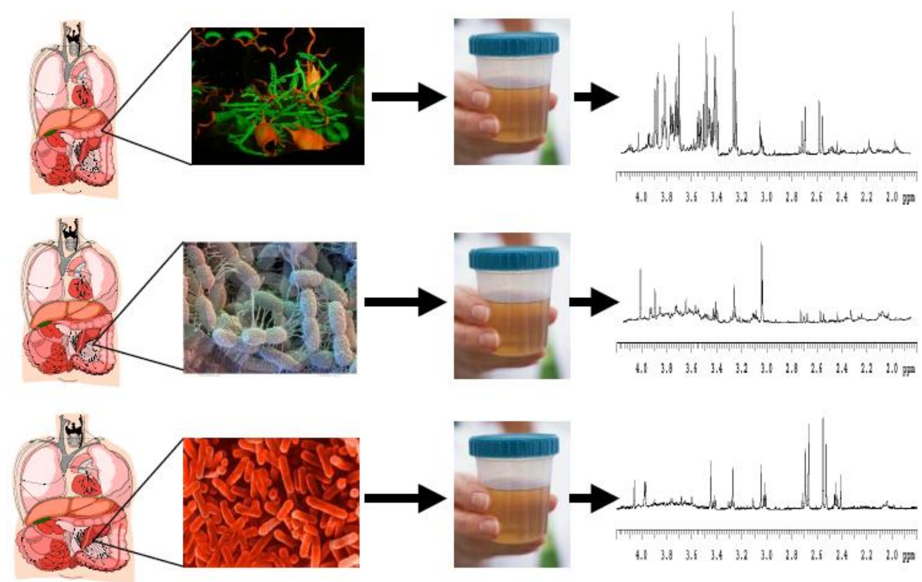




FOOD METABOLOMICS –
IMPACT IN NUTRITION and HEALTH

- A useful tool of food quality and safety evaluation (authenticity /traceability adulteration)
- The key for nutritional sciences development /objective, quantitative interpretation
- Integrated with GENOMICS will become critical for furthering nutrigenomics and the personalized nutrition



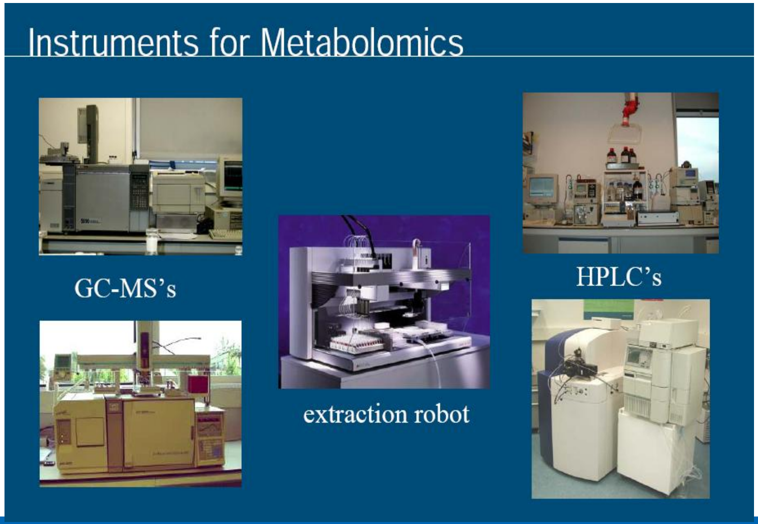


First Metabolomics program in Romania

Carmen SOCACIU coordinator

Floricuta RANGA – UV-Vis, HPLC, and LC-MS (phenolics)
Florinela FETEA – FTIR (plants and food)
Andreea BUNEA- HPLC (carotenoids)
Francisc DULF, PhD : GC-FID and GC-MS (phytosterols)
Constantin BELE and Cristian Matea :GC-FID (fatty acids)
Monica TRIF – FTIR and NMR (functional oils and capsules)
Raluca PARLOG: LC-MS and NIRS (fruits & juices&wine)
Loredana LEOPOLD: FTIR Raman spectroscopy

MeT-RO : A major initiative to establish the Centre for Plant and Food Metabolomic Analysis
Metabolomics Society
Reseaux Metabolomics and Fluxonomics –France
MetaboP- EU Project
META-PHOR



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TRADITION and MODERNITY