Metabolómica y Alimentómica: Fundamentos y Aplicaciones

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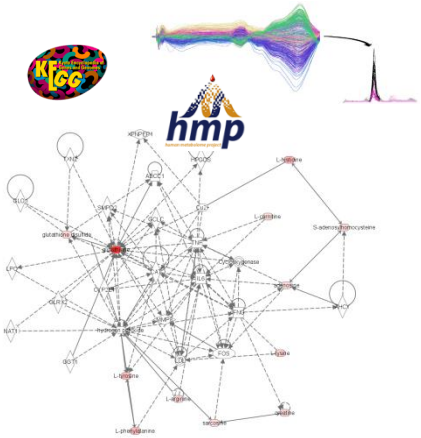


Univ. Zaragoza 2017

La era –ómica

El sufijo “-oma” tiene origen griego y significa “conjunto de”; el empleo de este sufijo define las nuevas aproximaciones masivas hacia las que están derivando distintas disciplinas científicas en el campo de Ciencias de la Vida (p.ej., el estudio del genoma, proteoma, metaboloma…), esto incluye:

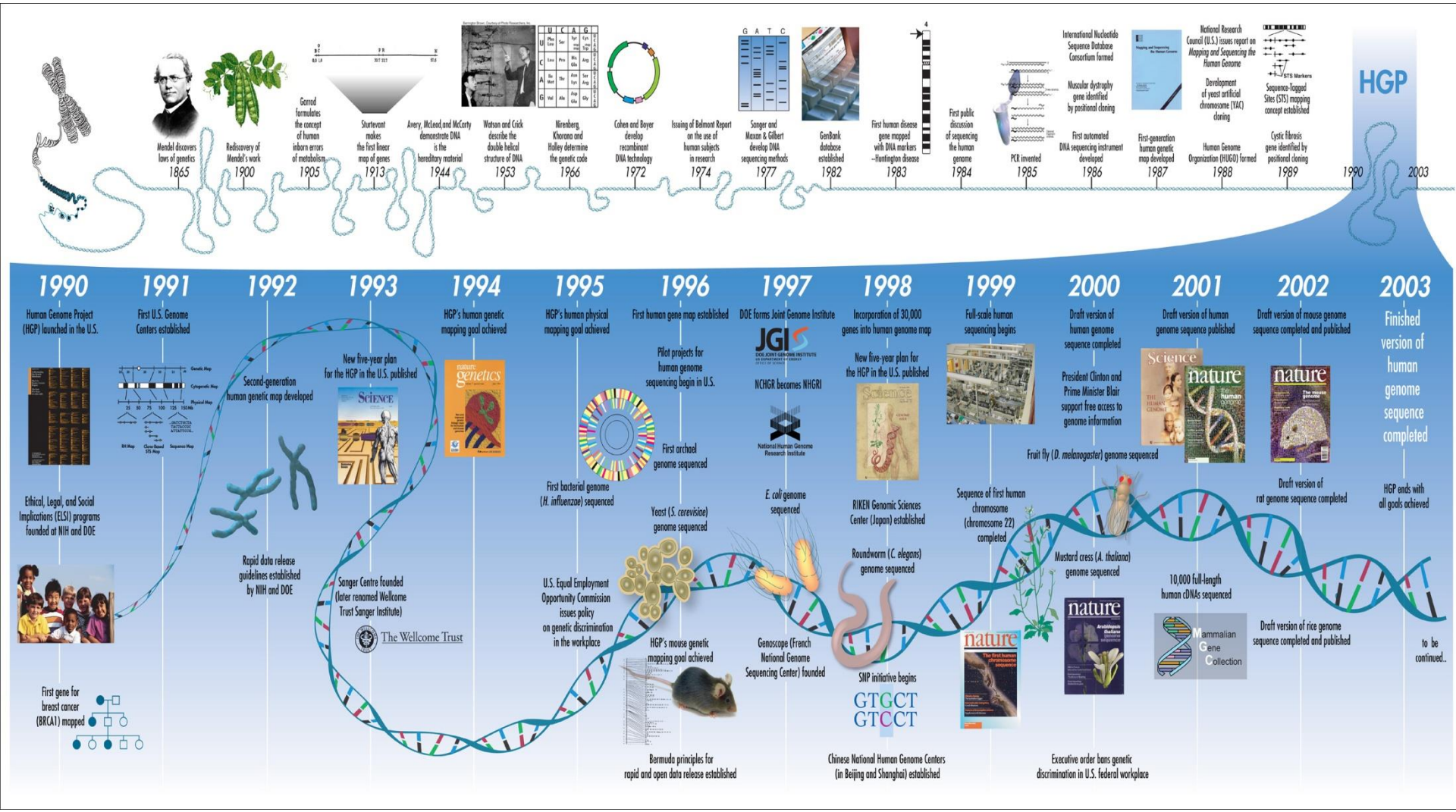
- Perfeccionamiento y desarrollo de nuevas técnicas y metodologías en el campo de la biología, bioquímica, química analítica, bioinformática, etc.

- Nuevas formas de entender y manejar la información disponible.

- Nueva aproximación a las cuestiones que se plantea la humanidad.

OBJETIVO: EL BENEFICIO GLOBAL (OPEN DATABASES)

Proyecto Genoma Humano: El principio

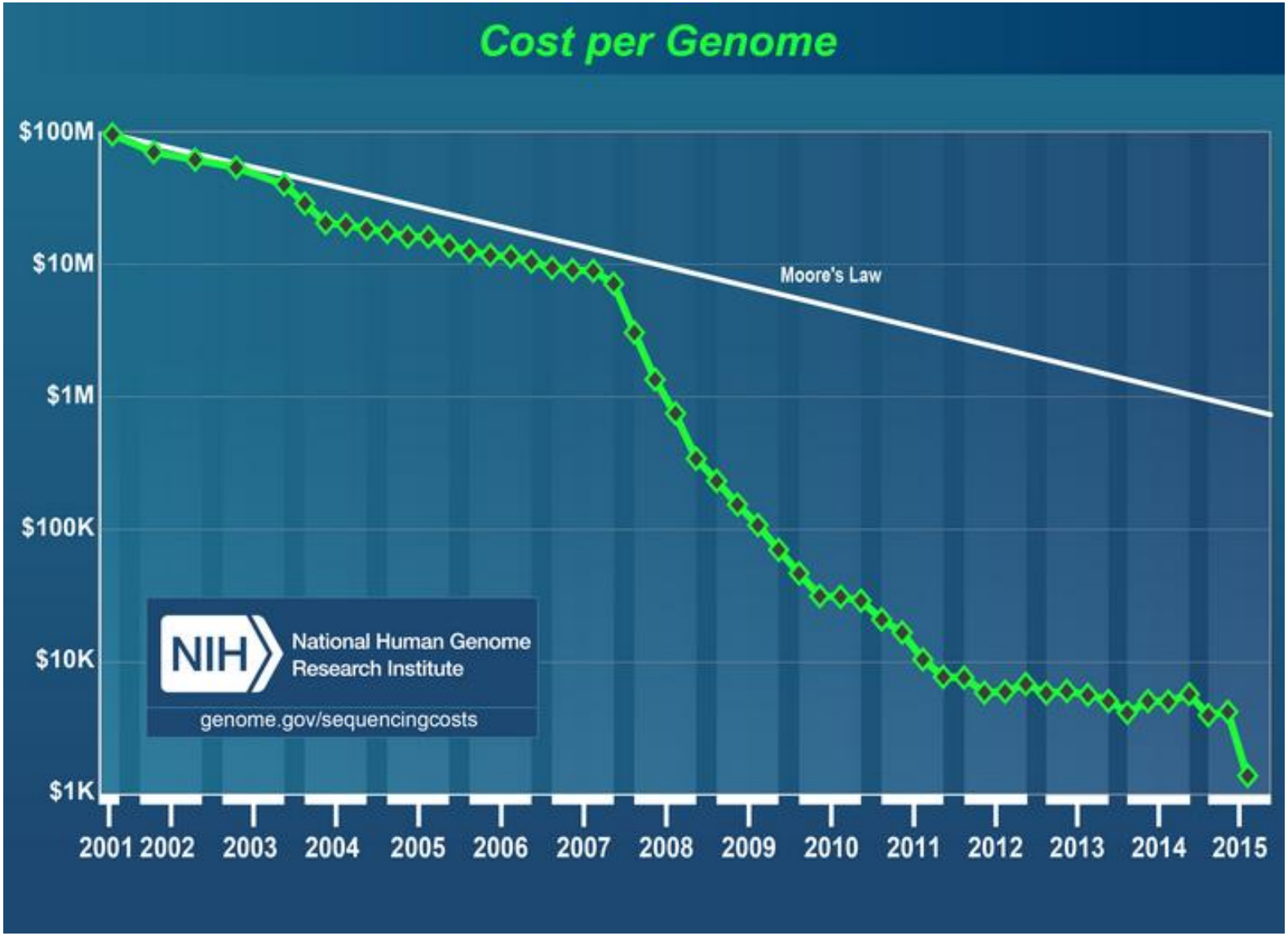


Proyecto Genoma Humano: El objetivo

El objetivo del Proyecto Genoma Humano fue mejorar nuestro conocimiento de la fisiología y salud humanas, ayudando a combatir de forma más eficaz enfermedades infecciosas y no-infecciosas.

El trabajo de interpretación de los genomas secuenciados está aún en desarrollo y se considera que un conocimiento pormenorizado de los mismos proporcionará importantes avances en medicina, farmacología, biotecnología, etc.

La era postgenómica



La (mala) salud en la era postgenómica

-Cuatro enfermedades no transmisibles son hoy las responsables del 80% de las muertes en el mundo: cáncer, enfermedades cardiovasculares, diabetes y enfermedades respiratorias crónicas.

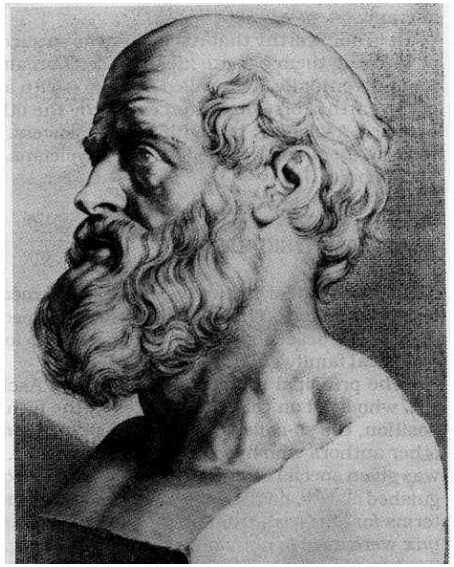
-La población mundial alcanzará los 9.6 billones en 2050, de los cuales un 19% (2 billones) serán mayores de 60 años, mucho más susceptibles a padecer las 4 enfermedades no transmisibles mencionadas (United Nations, 2014).

-Existe una creciente población con sobrepeso (mención especial a la población infantil), con riesgo de padecer obesidad y diabetes tipo 2.

Cómo pueden los alimentos contribuir a estos retos?

Volvamos 2500 años atrás

Los alimentos: Algunas frases de Hipócrates



“Somos lo que comemos”
Hipócrates, siglos IV-V a.c.

“Que el alimento sea tu mejor medicina y tu mejor medicina sea tu alimento”
Hipócrates, siglos IV-V a.c.

“Los jóvenes de hoy no parecen tener respeto alguno por el pasado ni esperanza alguna para el porvenir”
Hipócrates, siglos IV-V a.c.

Otros retos importantes en el Área Agroalimentaria, p.ej.:

1. To produce new functional foods with scientifically proved claims
2. To detect food safety issues at early stage, before they become global!
3. To develop, produce and monitor new transgenic foods
4. To understand the effects of gene-food interaction on human health (Nutrigenomics)
5. To explain the different answers from individuals to food (Nutrigenetics)
6. To establish the global role and functions of gut microbiome
7. To reduce through diet the impact of cardiovascular diseases, obesity and cancer (discovering the molecular mechanisms behind).
8. To get a personalized nutrition: How far we are?

To get a personalized nutrition: How far we are?

Otros retos importantes en el Área Agroalimentaria, p.ej.:

9. To reduce food allergy and food allergens

10. To confirm food quality and traceability

11. Understand the stress adaptation responses of food-borne pathogens

12. To understand the molecular basis of biological processes essential for improving agronomic and farm animal production

13. To understand postharvest phenomena through a global approach (genetics linked to environmental responses: biological networks)

14. To carry out pangenomics of industrial starter cultures and probiotics

15. Bioinformatics (including data processing, clustering, dynamics, or integration of the various 'omics' levels) will have to progress.

Nuevos retos normalmente requieren nuevas respuestas….

Alimentómica

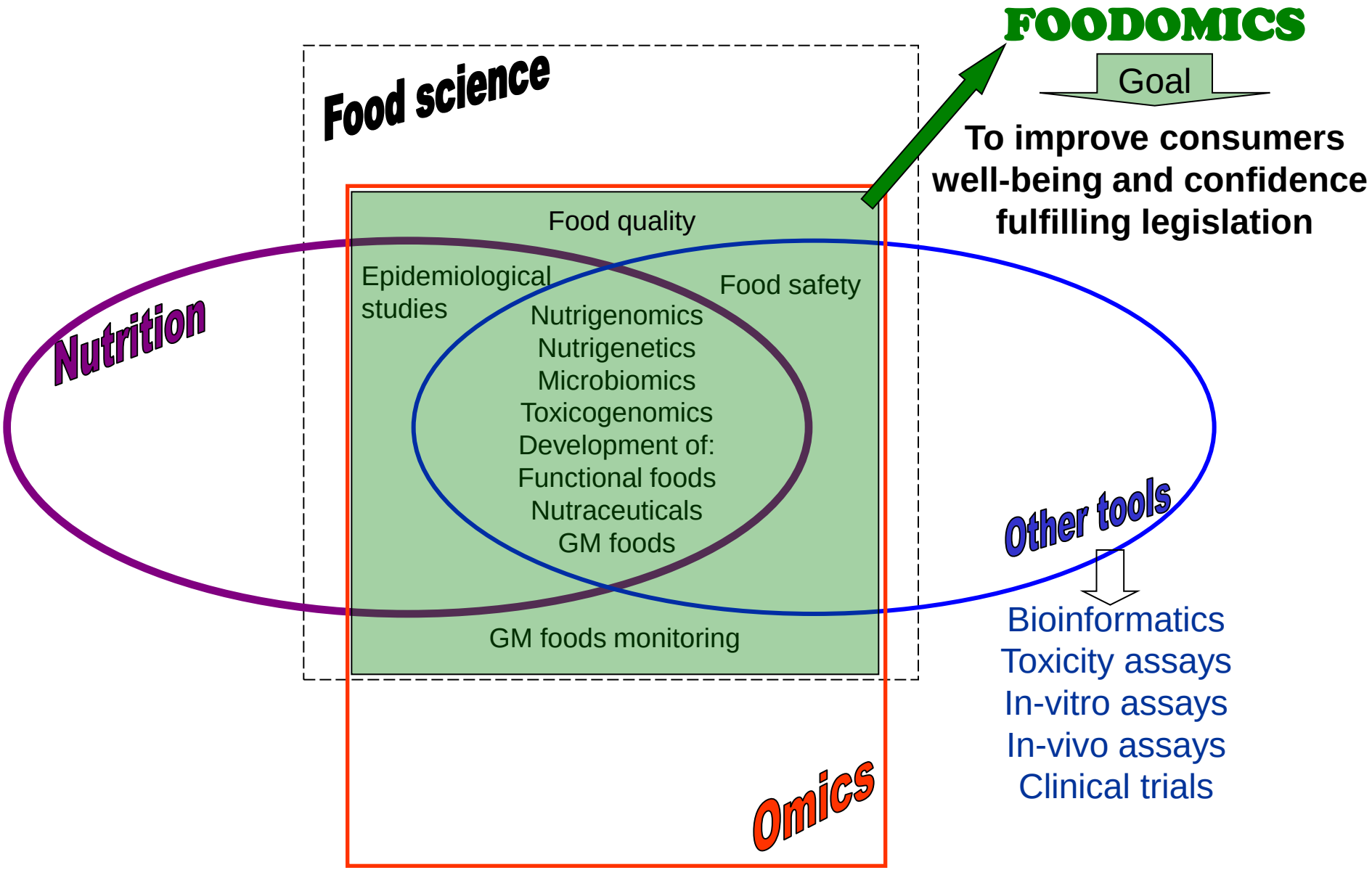
Nuestro grupo ha acuñado el término **Foodomics (Alimentómica)** y lo ha definido por primera vez en una revista SCI como: **una nueva disciplina que estudia los alimentos, incluyendo sus múltiples conexiones con la nutrición y la salud, mediante el empleo de técnicas ómicas con el fin de mejorar la salud y la confianza del consumidor.**

(Cifuentes et al.; *J. Chromatogr. A* 1216 (2009) 7109; *Electrophoresis* 31 (2010) 205; *Mass Spec. Rev. 31 (2012) 49*).

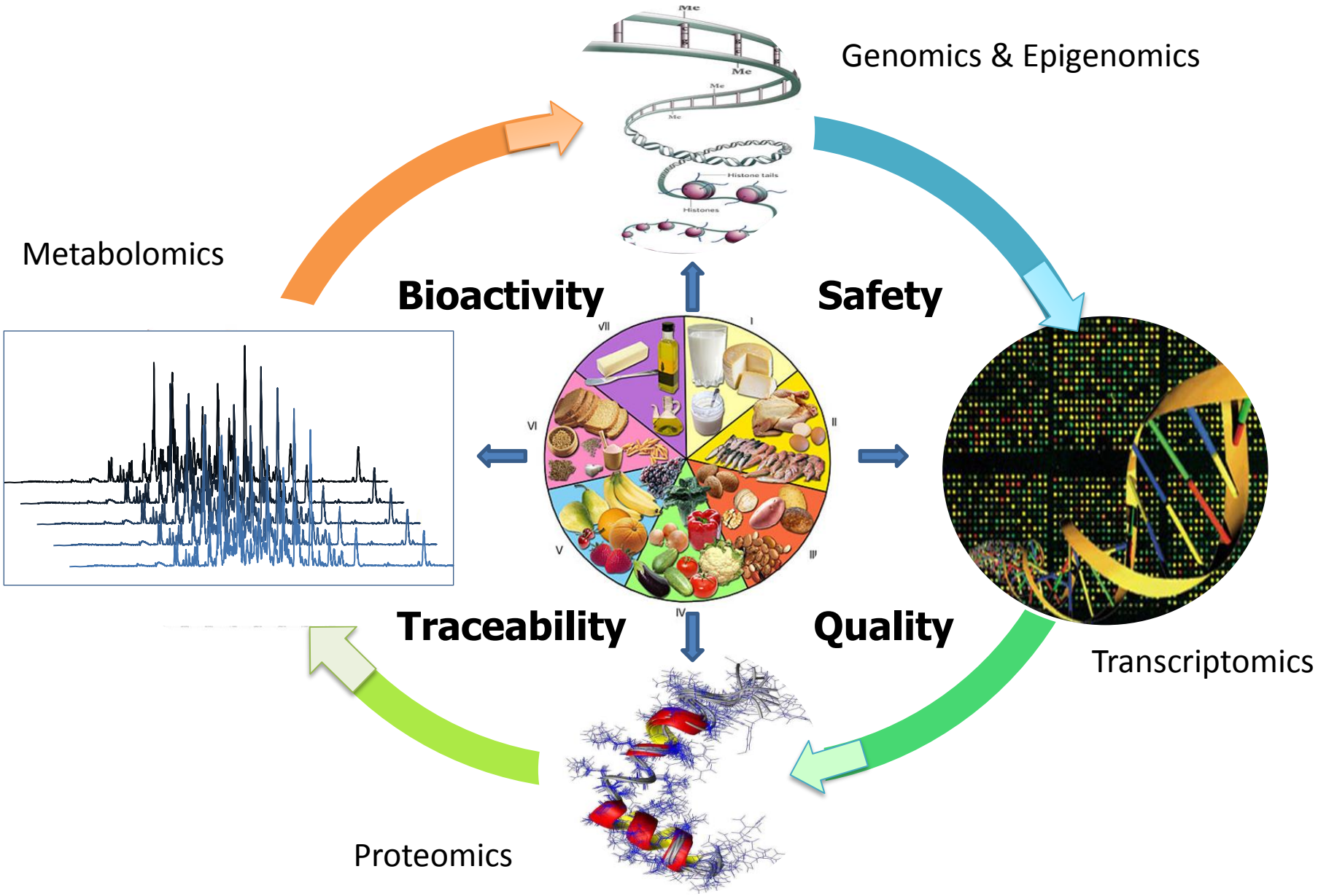
El interés en Foodomics coincide con una clara tendencia en medicina y biociencias hacia la prevención de enfermedades futuras.

MEJORAR EL BIENESTAR
Y LA CONFIANZA
DE LOS CONSUMIDORES Y
ASEGURAR EL CUMPLIMIENTO
DE LA LEGISLACIÓN

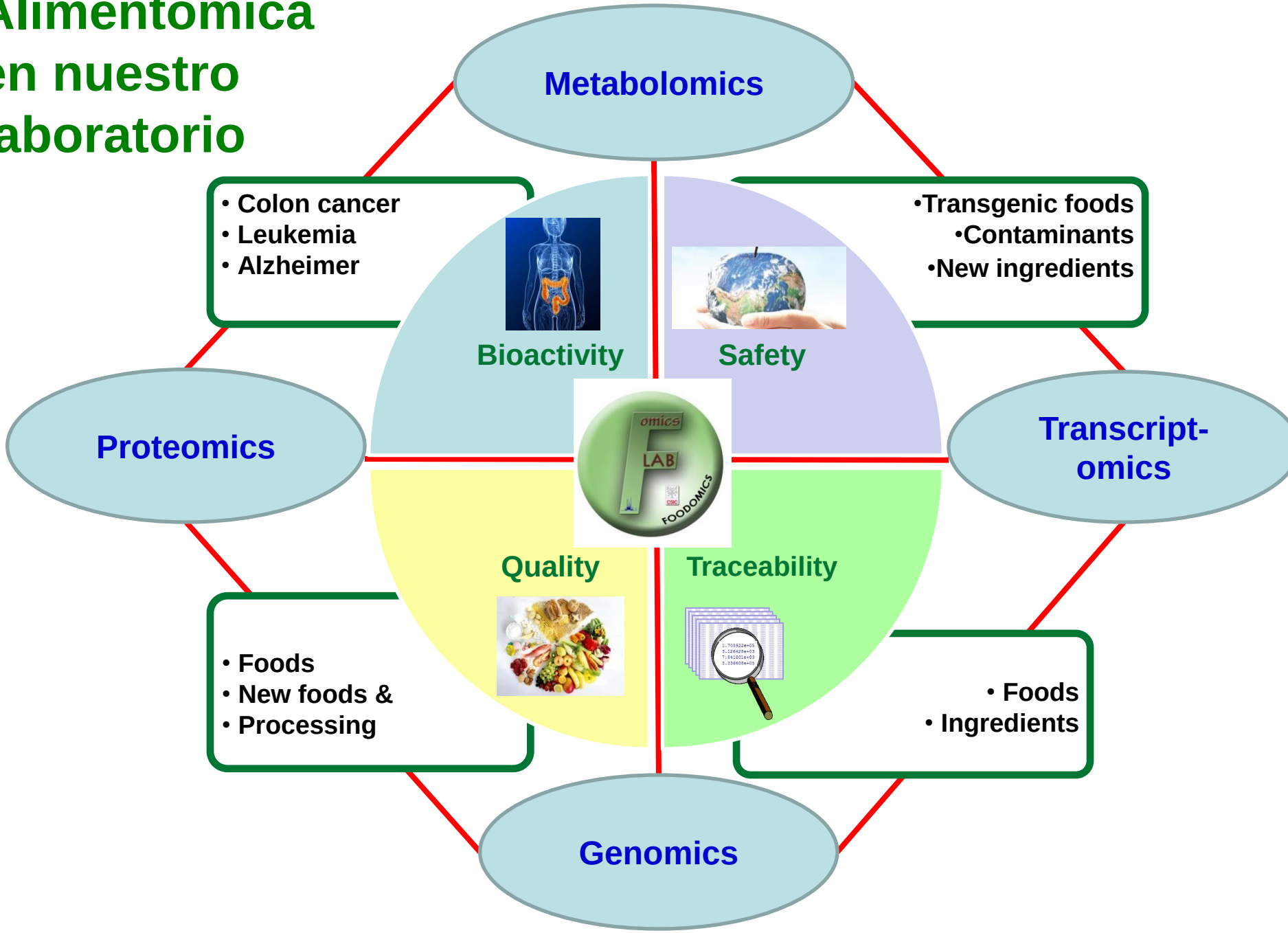
Foodomics: A new omics for a new food era



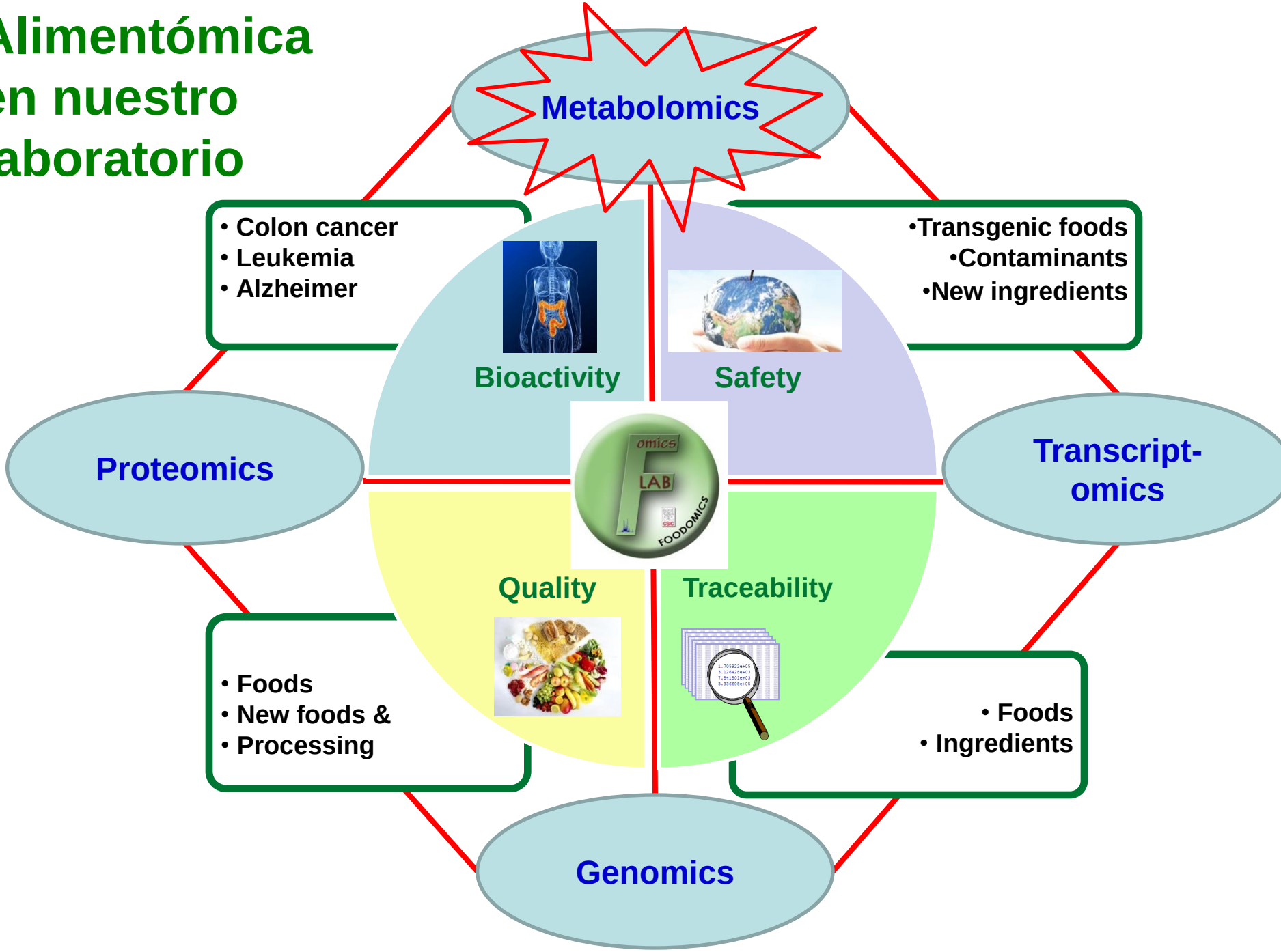
Foodomics tools and applications



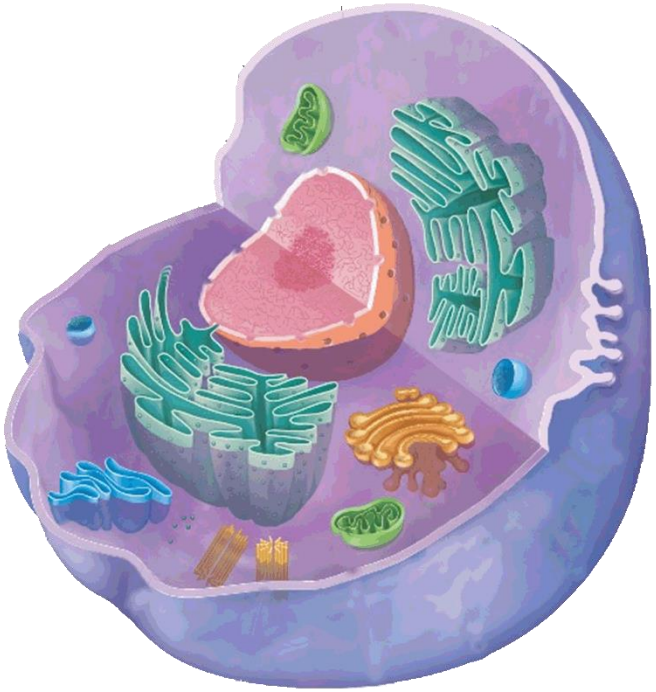
Alimentómica en nuestro laboratorio



Alimentómica en nuestro laboratorio



METABOLÓMICA



METABOLISMO

μεταβολή

METABOLITOS

son los intermediarios y productos del metabolismo

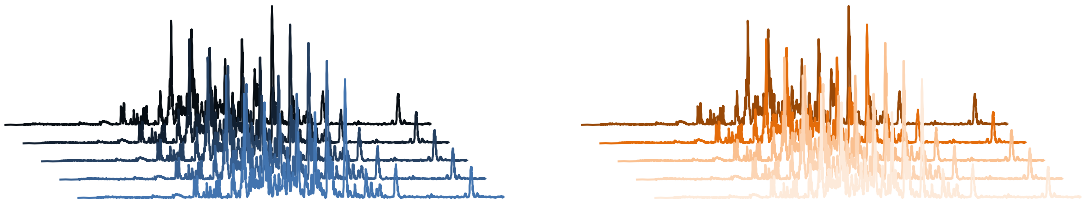
METABOLOMA

es el conjunto de pequeñas moléculas en una muestra determinada: suero, orina, células, tejidos, órganos, organismo

SAMPLE PREPARATION/METABOLITES EXTRACTION

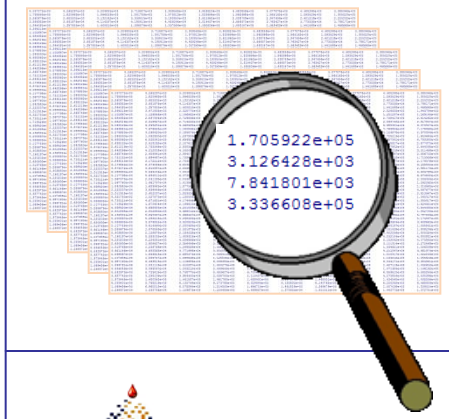
time, reproducibility, selectivity

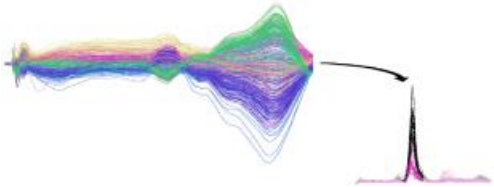
METABOLOMICS/FINGERPRINTING



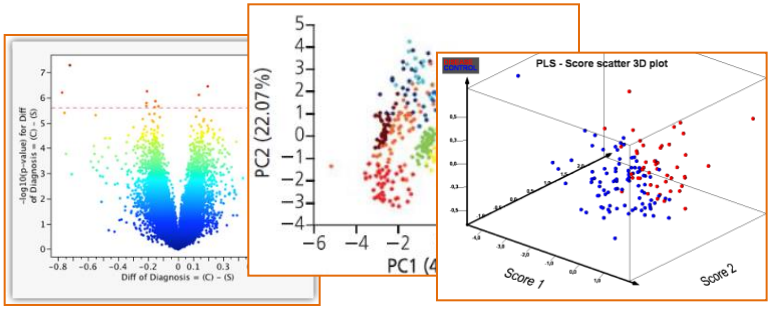
MS
LC-MS
GC-MS
CE-MS
NMR...

DATA ANALYSIS AND MULTIVARIATE STATISTICS





Peak finding, peak integration, time alignment, adduct removal, normalization, etc...



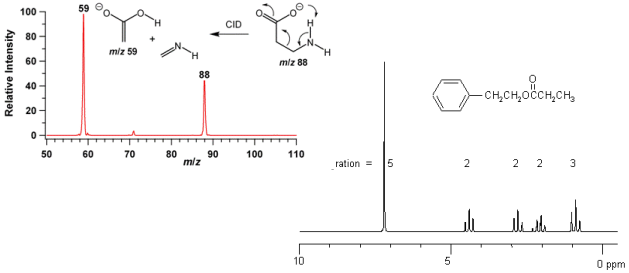
BIOMARKER IDENTIFICATION

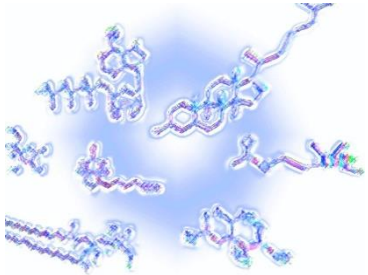
hmp human metabolome project

PubChem

KEGG Kyoto Encyclopedia of Genes and Genomes

Scripps Center For Metabolomics
METLIN: Metabolite and Tandem MS Database





PATHWAY ANALYSIS AND CONFIRMATION

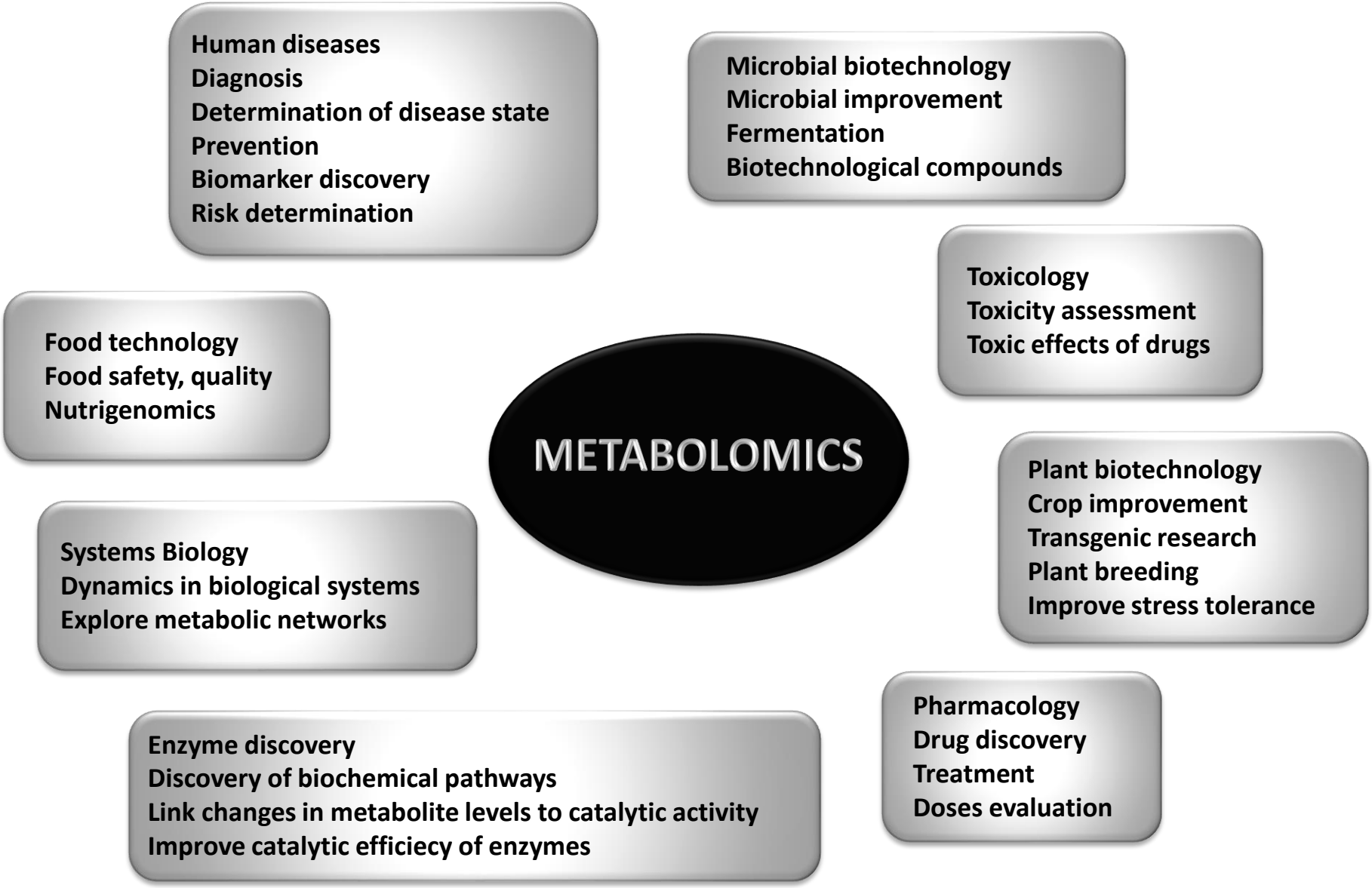


Figure modified from: BioMed Research Internatio Volume 2013, Article ID 792527,

METABOLOMICS and AGROFOOD SCIENCE

Metabolomics: applications to food science and nutrition research

David S. Wishart[a,b,c,*]

Trends in Food Science & Technology 19 (2008) 482–493

International Journal of *Molecular Sciences*
Int. J. Mol. Sci. **2016**, 17, 1871

Review

Metabolomics, a Powerful Tool for Agricultural Research

He Tian, Sin Man Lam and Guanghou Shui *

Metabolomics in Food Science

Juan Manuel Cevallos-Cevallos[*,1], José Ignacio Reyes-De-Corcuera[†]

Advances in Food and Nutrition Research, Volume 67

Traceability

Substantial equivalence

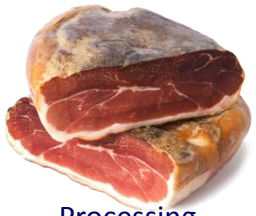
SAFETY

Legislation

QUALITY

Fuctional food
Nutraceuticals

Contamination
Toxicity



Processing

PROCESSING

Allergenicity
Intolerance

Health

Adulteration

BIOACTIVITY

Illuminating a plant's tissue-specific metabolic diversity using computational metabolomics and information theory

Dapeng Li[a], Sven Heiling[a], Ian T. Baldwin[a], and Emmanuel Gaquerel[a,b,1]

[a]Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, 07745 Jena, Germany; and [b]Centre for Organismal Studies, University of Heidelberg, 69120 Heidelberg, Germany

Edited by Jerrold Meinwald, Cornell University, Ithaca, NY, and approved October 11, 2016 (received for review June 23, 2016)

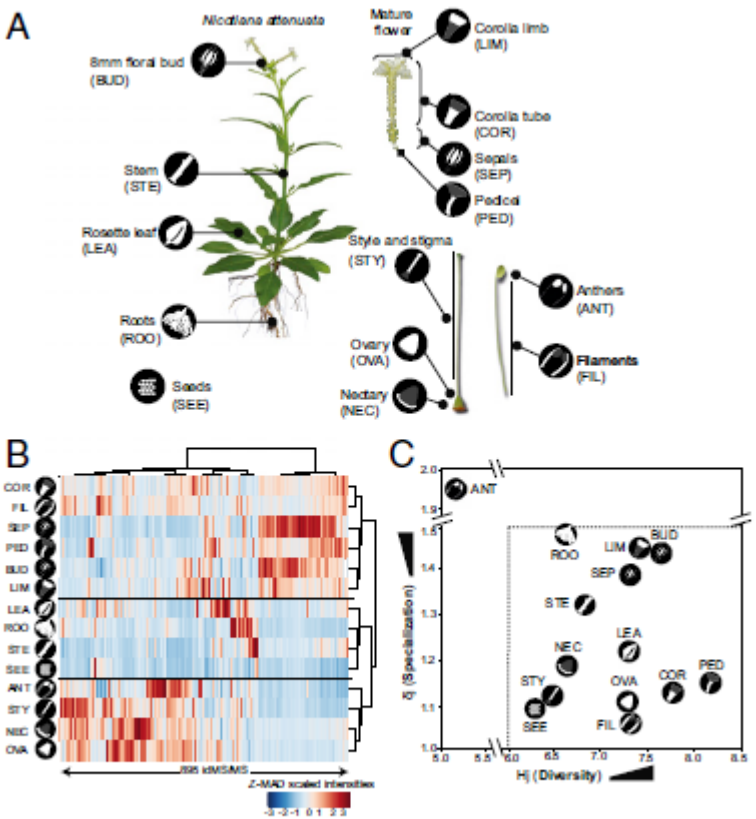


Fig. 1. Integration of MS-based metabolomics and information theory analysis highlights tissue-specific metabolome specialization. (*A*) Tissues were collected

Metabolomics was done using UHPLC-ESI/qTOF-MS.
The metabolic differences among tissues within a plant provide another source of variance that can be harnessed in the quest to understand gene function. The workflow defines a framework for future evolutionary studies on plant tissue metabolic specialization.

PNAS, published online Nov 7th, 2016
www.pnas.org/cgi/doi/10.1073/pnas.1610218113

Contents lists available at ScienceDirect

Biochimie

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

Mini-review

Using lipidomics for expanding the knowledge on lipid metabolism in plants

Hezi Tenenboim [a, 1], Asdrubal Burgos [a, 1], Lothar Willmitzer [a], Yariv Brotman [b, *]

[a] Max-Planck-Institut für Molekulare Pflanzenphysiologie, Potsdam, Germany
[b] Department of Life Sciences, Ben Gurion University of the Negev, Beersheva, Israel

Recent advances in metabolomic and lipidomic technologies and analysis have increased our knowledge of the plant lipidome, its biosynthesis, regulation, adaptation, remodeling, functions, roles, and interactions. This paper discusses specific issues pertaining to lipidomic research in plants, and how lipidomics has helped elucidate key issues in plant cell biology, immunity, response to stress, evolution, crop enhancementd - to name but a few.

Biochimie 130 (2016) 91-96

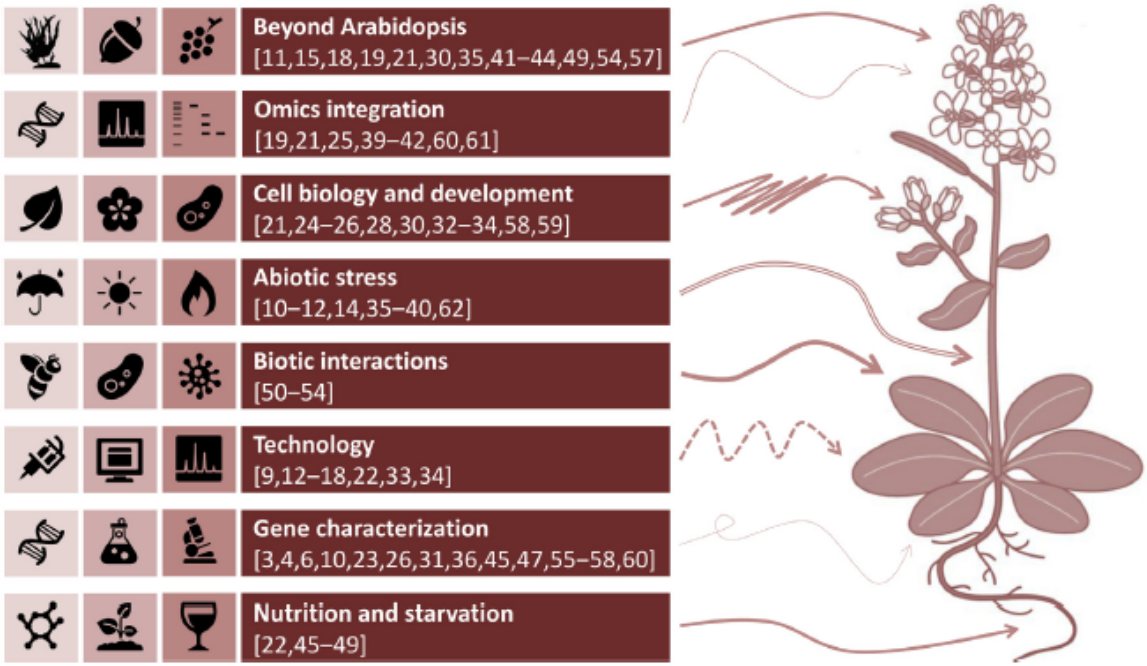


Fig. 1. A graphic summary of some of the topics discussed in this review, along with the relevant references. All icons taken from www.icons8.com.

Trends in Plant Science

CellPress

Special Issue: Unravelling the Secrets of the Rhizosphere

Review

Metabolomics in the Rhizosphere: Tapping into Belowground Chemical Communication

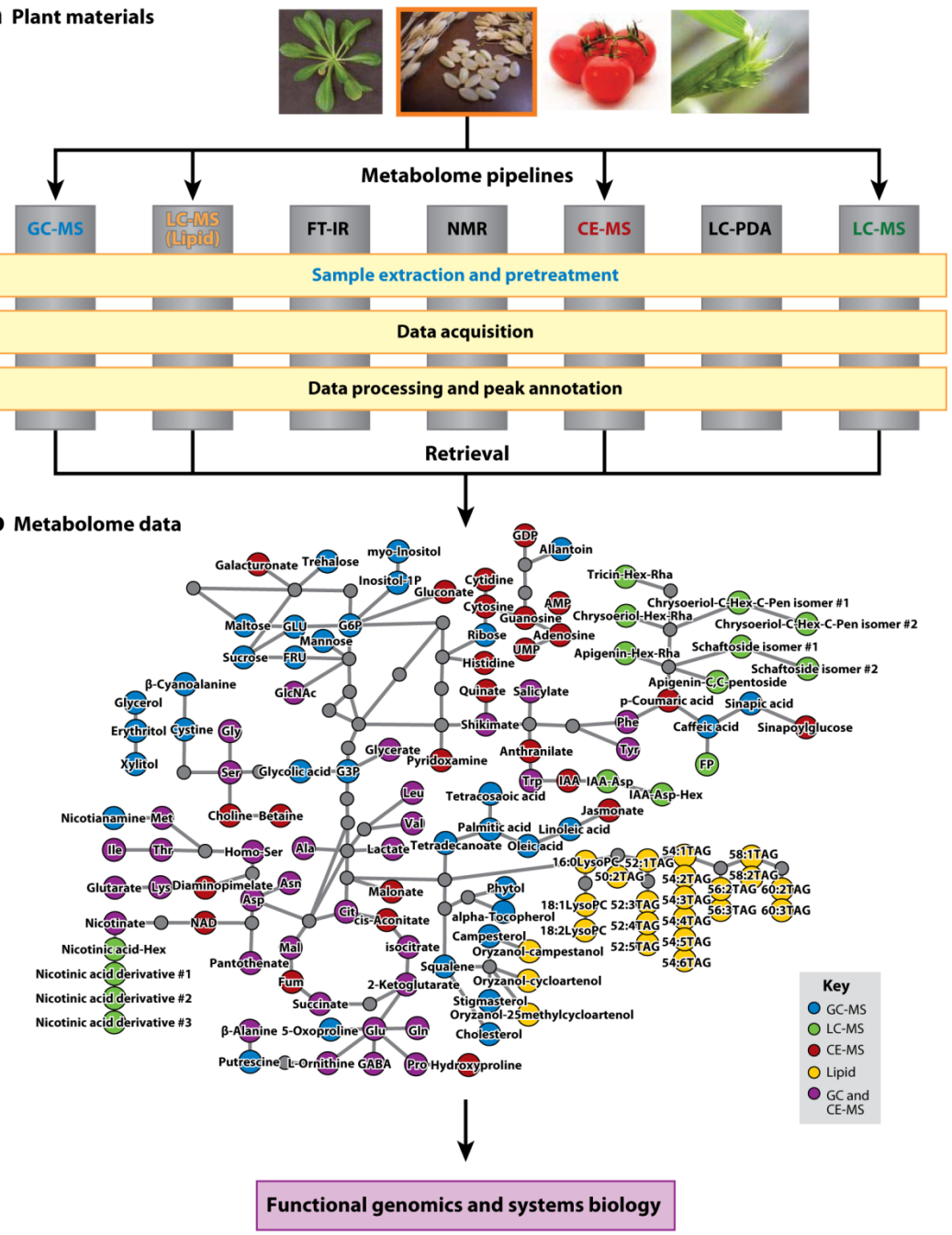
Nicole M. van Dam[1,2,3,*] and Harro J. Bouwmeester[4,*]

Interactions between roots and rhizosphere community members are mostly achieved via chemical communication. Root exudates contain an array of primary and secondary plant metabolites that can attract, deter, or kill belowground insect herbivores, nematodes, and microbes, and inhibit competing plants. Metabolomics of root exudates can potentially help us to better understand this chemical dialogue.

Trends in Plant Science

Figure 1. Belowground Chemical Communication of Plants with other Organisms. (A) Plants exude phenolic

Trends in Plant Science 21 (2016) 256-265
http://dx.doi.org/10.1016/j.tplants.2016.01.008



Saito K, Matsuda F. 2010.
Annu. Rev. Plant. Biol. 61:463–89

Metabolomics for Functional Genomics, Systems Biology, and Biotechnology

Kazuki Saito[1,2] and Fumio Matsuda[1]

[1]RIKEN Plant Science Center, Tsurumi-ku, Yokohama 230-0045, Japan

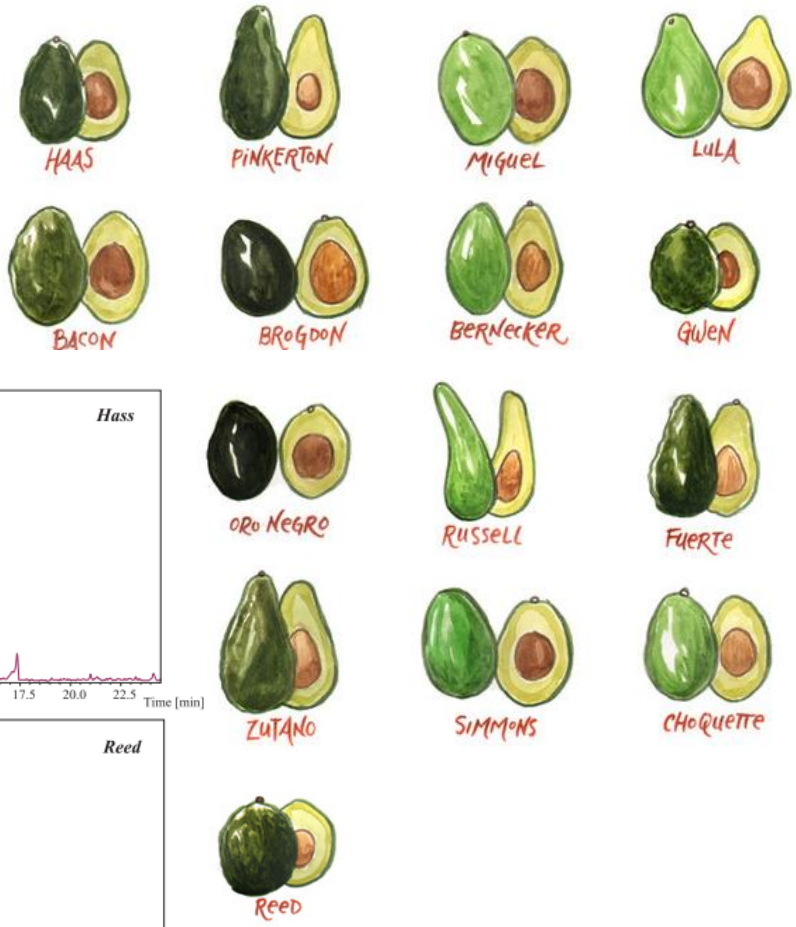
Annu. Rev. Plant Biol. 2010. 61:463–89

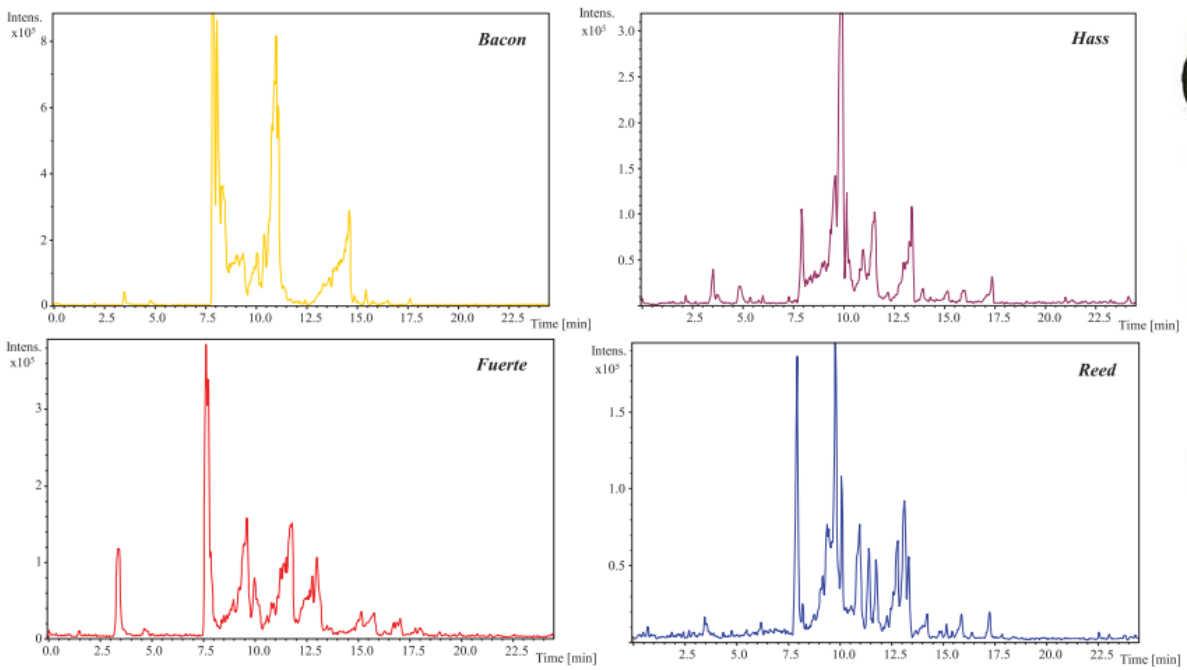
Metabolomics plays a significant role in fundamental plant biology and applied biotechnology. Plants collectively produce a huge array of chemicals, far more than are produced by most other organisms. Metabolomics applications include quantitative loci analysis, prediction of food quality, and evaluation of genetically modified crops. Systems biology driven by metabolome data will aid in deciphering the secrets of plant cell systems and their application to biotechnology.

Determination of changes in the metabolic profile of avocado fruits (*Persea americana*) by two CE-MS approaches (targeted and non-targeted)

Paulina K. Contreras-Gutiérrez[1]
Elena Hurtado-Fernández[1]
María Gómez-Romero[2]
José Ignacio Hormaza[3]
Alegría Carrasco-Pancorbo[1]
Alberto Fernández-Gutiérrez[1]

Electrophoresis 2013, *34*, 2928–2942

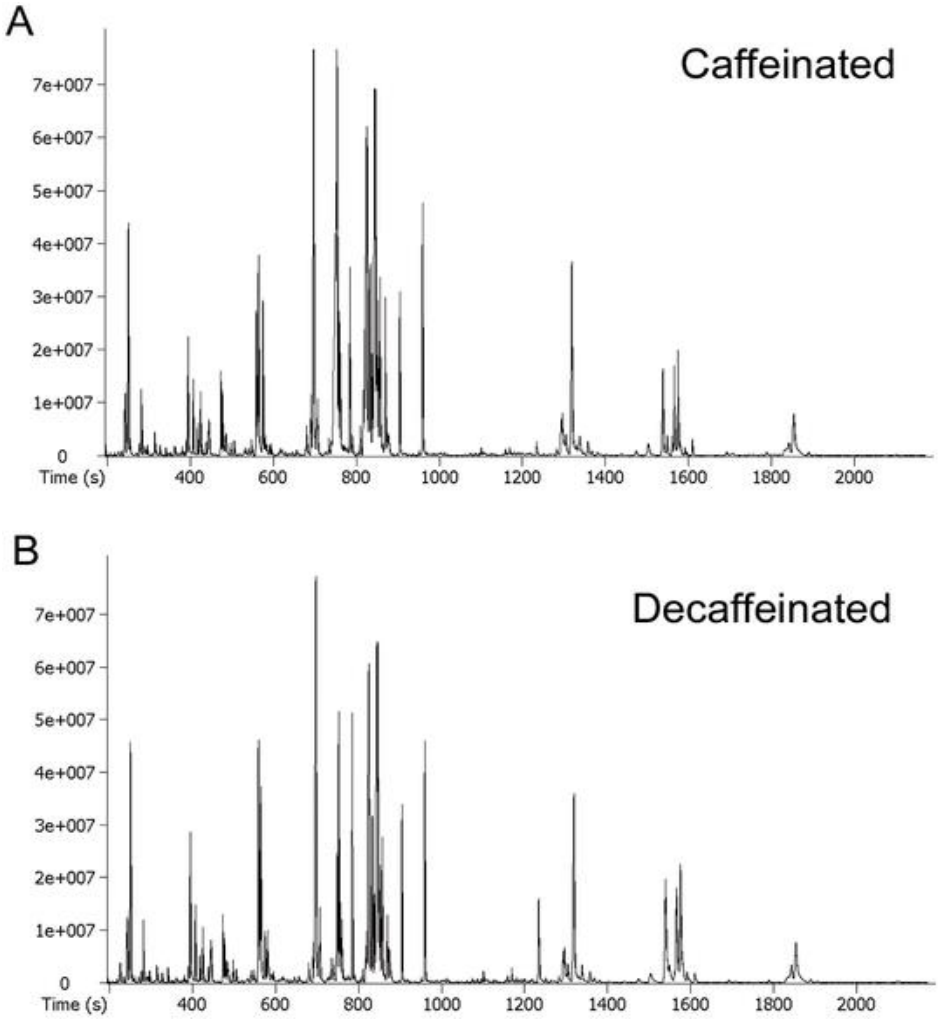




Bare fused-silica capillaries with 50 μm id and a total length of 85 cm from Beckman Coulter Inc. were used. The running buffer was 40 mM ammonium acetate at pH 9.5, voltage was set at 30 kV.

Perseitol, quinic, chlorogenic, *trans*-cinnamic, pantothenic and abscisic acids, as well as epicatechin and catechin decreased during the ripening process, whereas ferulic and *p*-coumaric acids showed the opposite trend.

Figure 1. Metabolomic profiling of coffee samples using GC-TOF-MS.

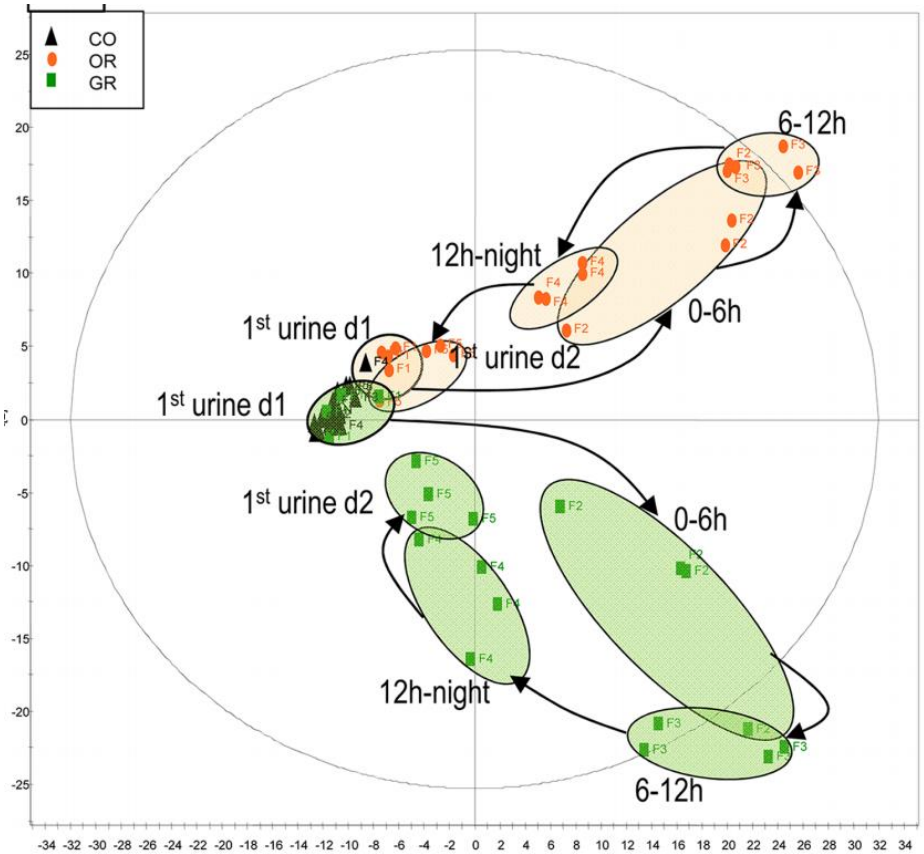


Chang KL, Ho PC (2014) Gas Chromatography Time-Of-Flight Mass Spectrometry (GC-TOF-MS)-Based Metabolomics for Comparison of Caffeinated and Decaffeinated Coffee and Its Implications for Alzheimer's Disease. PLOS ONE 9(8): e104621. doi:10.1371/journal.pone.0104621
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0104621

Mass Spectrometry-based Metabolomics for the Discovery of Biomarkers of Fruit and Vegetable Intake: Citrus Fruit as a Case Study

Estelle Pujos-Guillot,[†,‡] Jane Hubert,[†,‡] Jean-François Martin,[†,‡] Bernard Lyan,[†,‡] Mercedes Quintana,[†,‡] Sylvain Claude,[†,‡] Bruno Chabanas,[†,‡] Joseph A. Rothwell,[†,‡] Catherine Bennetau-Pelissero,[§,∥] Augustin Scalbert,[⊥] Blandine Comte,[†,‡] Serge Hercberg,[#] Christine Morand,[†,‡] Pilar Galan,[#] and Claudine Manach*[,†,‡]

J. Proteome Res. 2013, 12, 1645−1659



Control

Orange juice

Grapefruit

Principal Component Analysis (PCA) of these significant ions shows the dynamic evolution of the urine metabolome after ingestion of orange and grapefruit juice

Foodomics projects in our lab on:

Safety, quality and traceability of

Transgenic foods
Other foods & ingr

GM corn, GM soya, GM yeasts…

DNA, proteins and metabolites

In collaboration with **GSF** (Munich, Germany)

Bioactivity of food ingredients against:

Alzheimer

Population study

Biological sample: Cebrospinal fluid (CSF)

In collaboration with **Karolinska Institute** (Stockholm, Sweden)

Colon cancer

Human cell lines
Animal models

Biological samples: DNA, RNA, proteins and metabolites

Leukemia

Human cell lines

Biological samples: DNA, RNA, proteins and metabolites

In collaboration with
Univ. Miguel Hernandez, Elche, Spain
University of Granda, Granada, Spain

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In collaboration with
Univ. Miguel Hernandez, Elche, Spain
University of Granda, Granada, Spain

Transgenic maize (Bt corn)

A new CryIA(b) gene (encodes for a *Bacillus thuringiensis* protoxin) is inserted by recombinant DNA techniques into the maize genome.

The new protoxin acts as insecticide against lepidopters.

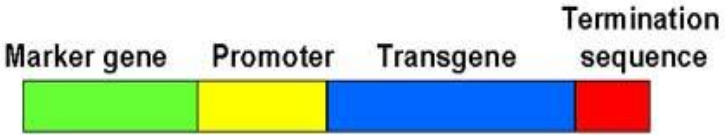
Transgenic soybean (RR soybean)

A new CP4 EPSPS gene from Agrobacterium (that encodes for a CP4 5-enolpyruvylshikimate-3-phosphate sintase, CP4-EPSPS) is inserted by recombinant DNA techniques into the soy genome.

The new CP4-EPSPS enzyme allows to the GM plant to resist the effect of the herbicide glyphosate.

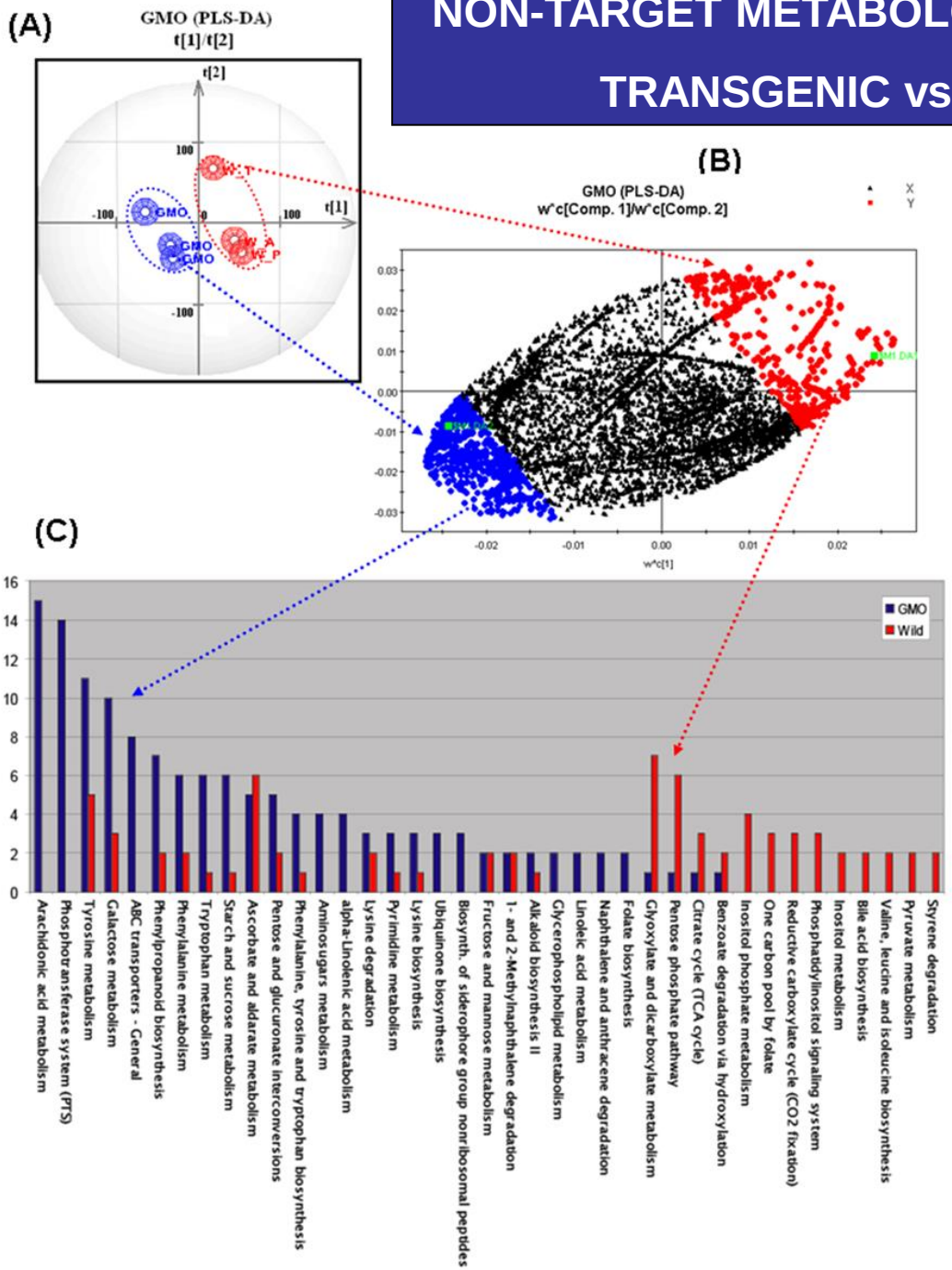
Can the new inserted genes give rise to other unintended effects?

The European Food Safety Agency (EFSA) recommends the development of profiling techniques to study these unexpected effects.



NON-TARGET METABOLOMICS: FT-ICR-MS & CE-TOF-MS

TRANSGENIC vs. CONVENTIONAL MAIZE



The score scatter plot underlines a different pattern for the transgenic (blue color) and wild maize lines (red color). The different properties of the discriminative masses (represented in blue and red in the loading plot) were investigated with MassTRIX.

Statistical analysis of the data from non-target metabolomics (based on FT-ICR-MS and CE-TOF-MS analysis) lead to the tentative identification of possible biomarkers specific of GM vs. wild organisms.

analytical chemistry

Article

pubs.acs.org/ac

Capillary Electrophoresis Time-of-Flight Mass Spectrometry for Comparative Metabolomics of Transgenic versus Conventional Maize

Tuuli Levandi,[†] Carlos Leon,[‡] Mihkel Kaljurand,[†] Virginia Garcia-Cañas,[‡] and Alejandro Cifuentes*,[‡]

Faculty of Science, Tallinn Technical University, Ehitajate tee 5, 19086 Tallinn, Estonia, and Institute of Industrial Fermentations (CSIC), Juan de la Cierva 3, Madrid, Spain

ELSEVIER

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Metabolomics of transgenic maize combining Fourier transform-ion cyclotron resonance-mass spectrometry, capillary electrophoresis-mass spectrometry and pressurized liquid extraction

Carlos Leon[a], Irene Rodriguez-Meizoso[a], Marianna Lucio[b], Virginia Garcia-Cañas[a], Elena Ibañez[a], Philippe Schmitt-Kopplin[b,*], Alejandro Cifuentes[a,**]

[a] *Institute of Industrial Fermentations (CSIC), Juan de la Cierva 3, Madrid, Spain*
[b] *Institut für Ökologische Chemie (Helmholtz Zentrum Muenchen), Ingolstaedter Landstrasse 1, Neuherberg, Germany*

ELECTROPHORESIS

Electrophoresis 2010, *31*, 1175–1183

1175

Carolina Simó[1]
Elena Domínguez-Vega[2]
Maria Luisa Marina[2]
María Concepción García[2]
Giovanni Dinelli[3]
Alejandro Cifuentes[1]

[1]Department of Food Analysis,

Research Article

CE-TOF MS analysis of complex protein hydrolyzates from genetically modified soybeans – A tool for foodomics

Foodomics projects in our lab on:

Safety, quality and traceability of

Transgenic foods

Other foods & ingr

GM corn, GM soya, GM yeasts…

DNA, proteins and metabolites

In collaboration with **GSF** (Munich, Germany)

Bioactivity of food ingredients against:

Alzheimer

Population study

Biological sample: Cebrospinal fluid (CSF)

In collaboration with **Karolinska Institute** (Stockholm, Sweden)

Colon cancer

Human cell lines
Animal models

Biological samples: DNA, RNA, proteins and metabolites

Leukemia

Human cell lines

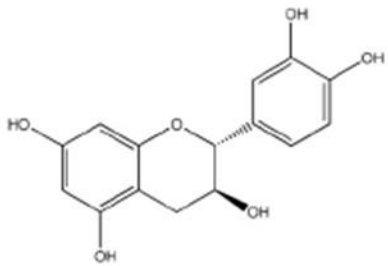
Biological samples: DNA, RNA, proteins and metabolites

In collaboration with
Univ. Miguel Hernandez, Elche, Spain
University of Granda, Granada, Spain

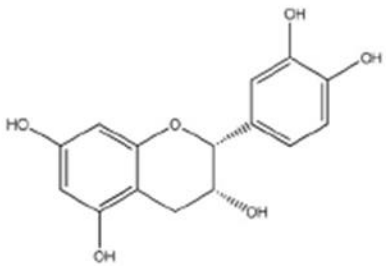
Procyanidins

PROPERTIES

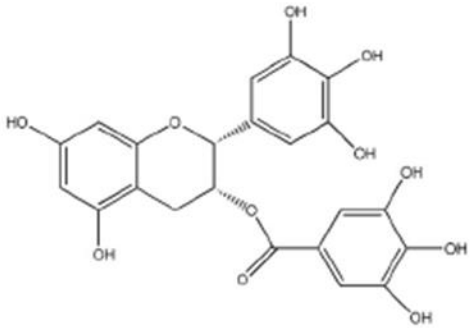
· Type of phenolic compounds abundantly found in vegetables.
· Consumed in high amounts in diet.
· Responsible for sensory properties: astringency, bitterness, aroma, colour.
· Different bioactivities described: antioxidant, anti-proliferative, anti-inflammatory, among others.



(+)-Catechin



(-)-Epicatechin



(-)-Epigallocatechin gallate

Grape Seed Procyanidins

METHOD OPTIMIZATION

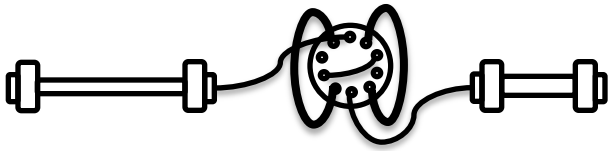
» LC x LC –DAD-MS instrument.

» Operated using a 10-port 2-position switching valve.

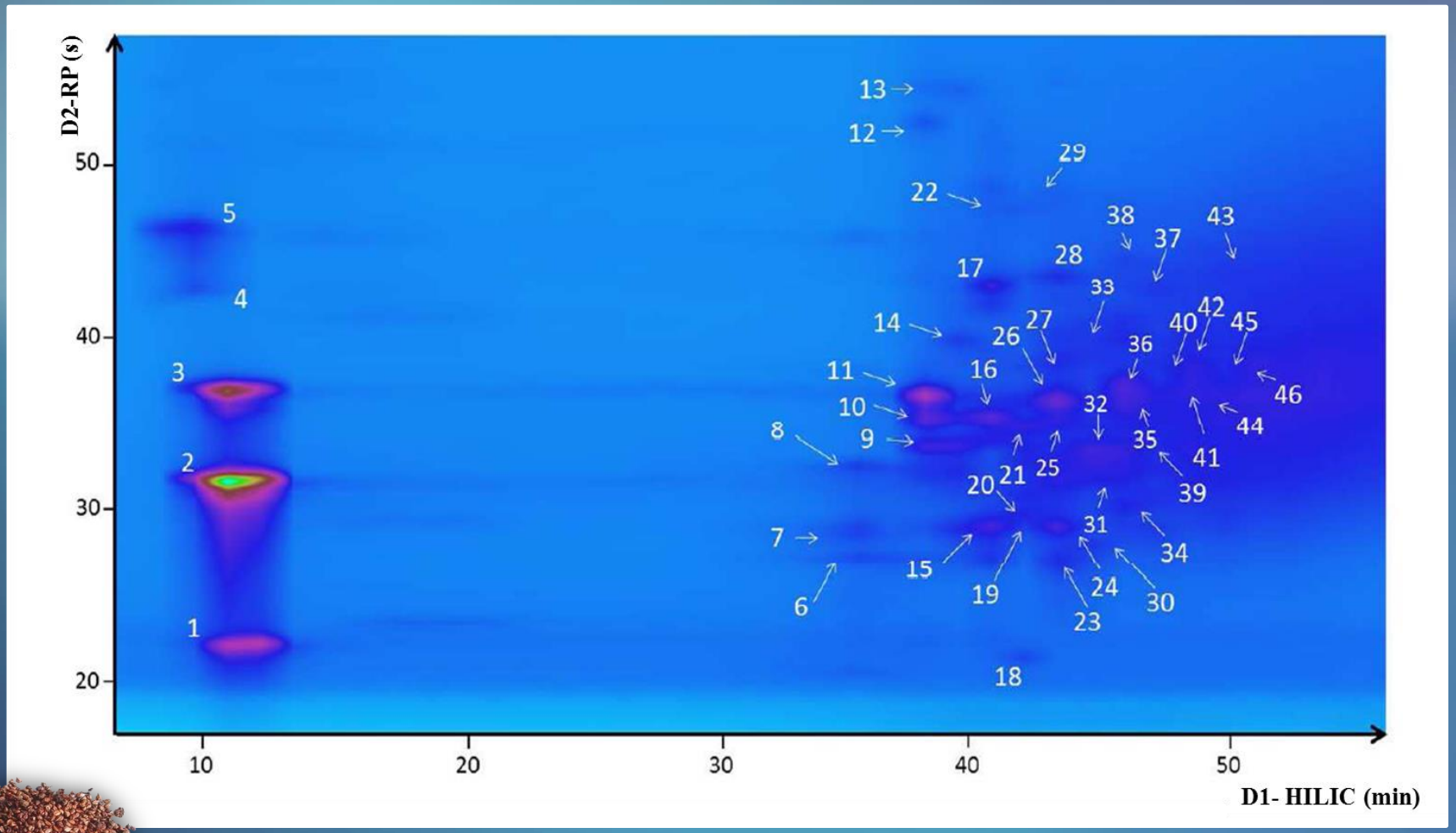
» Interface using two identical injection loops

Method requirements

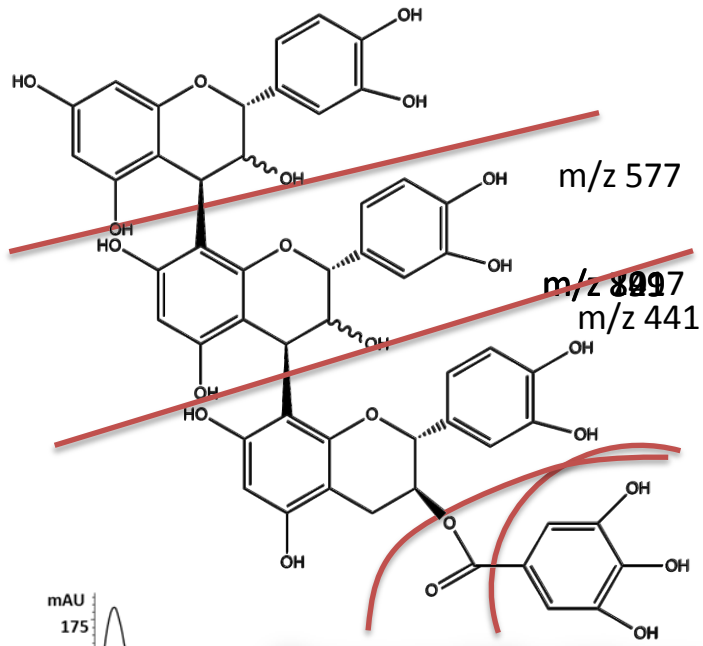
· First dimension: microbore column – very low flow rates
· Second dimension: fast separations
· D2 separation time ≤ time to fill injection loop with D1 eluent.

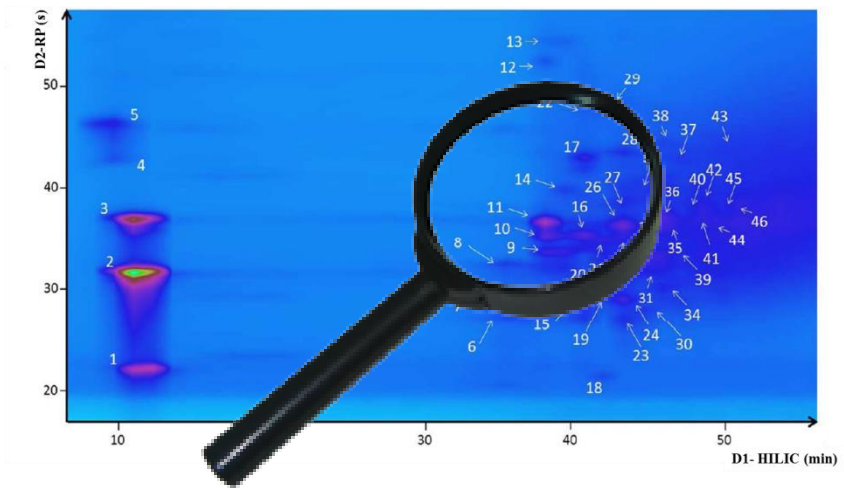


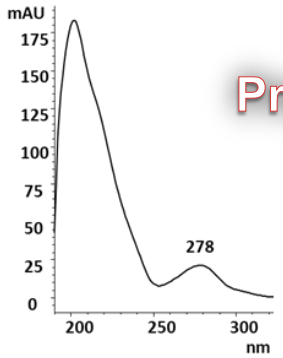
Grape Seed Procyanidins



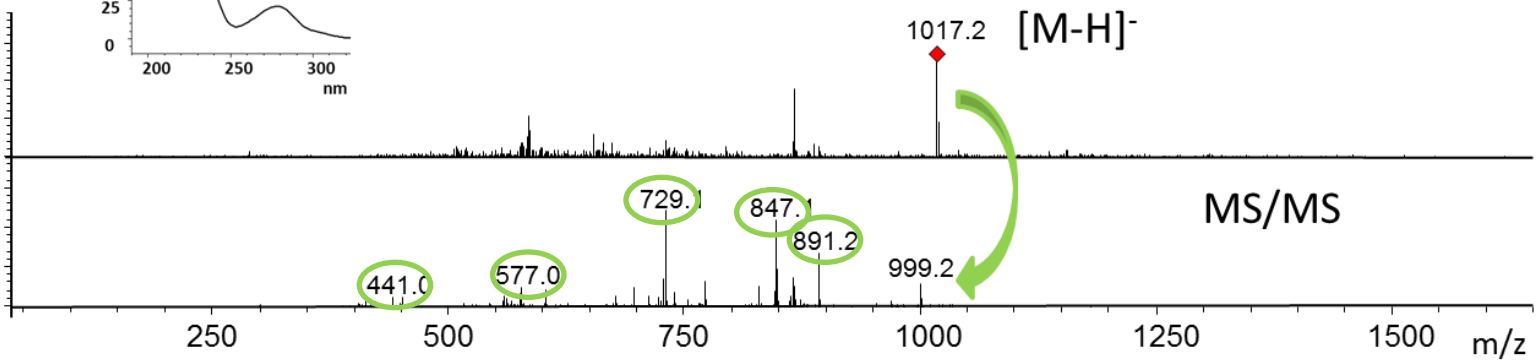
Grape Seed Procyanidins





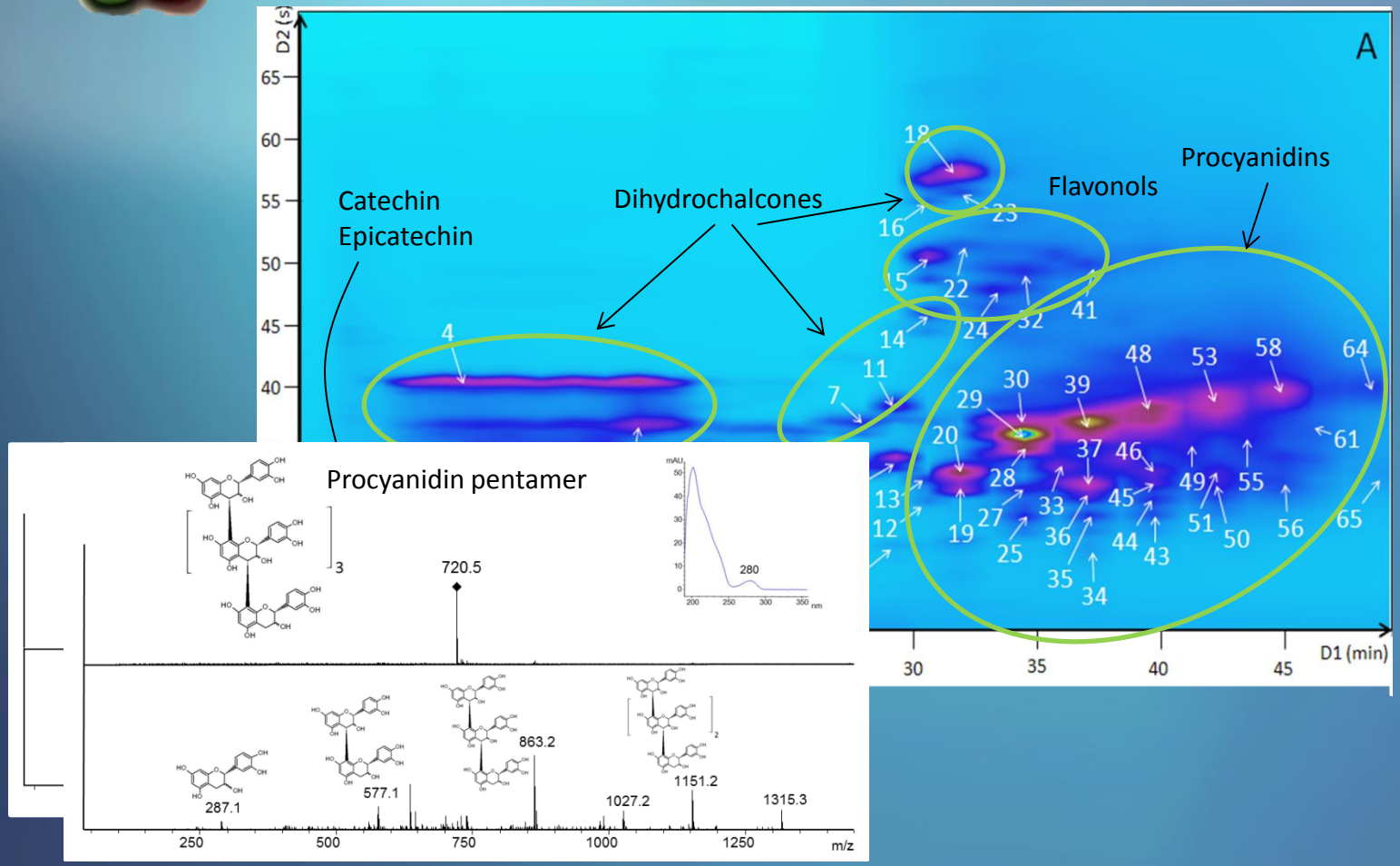


Procyanidin trimer – monogallate PEAK 17



Apple Polyphenols

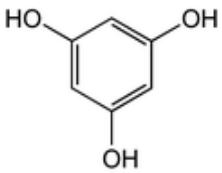
Samples analysis and peak identification



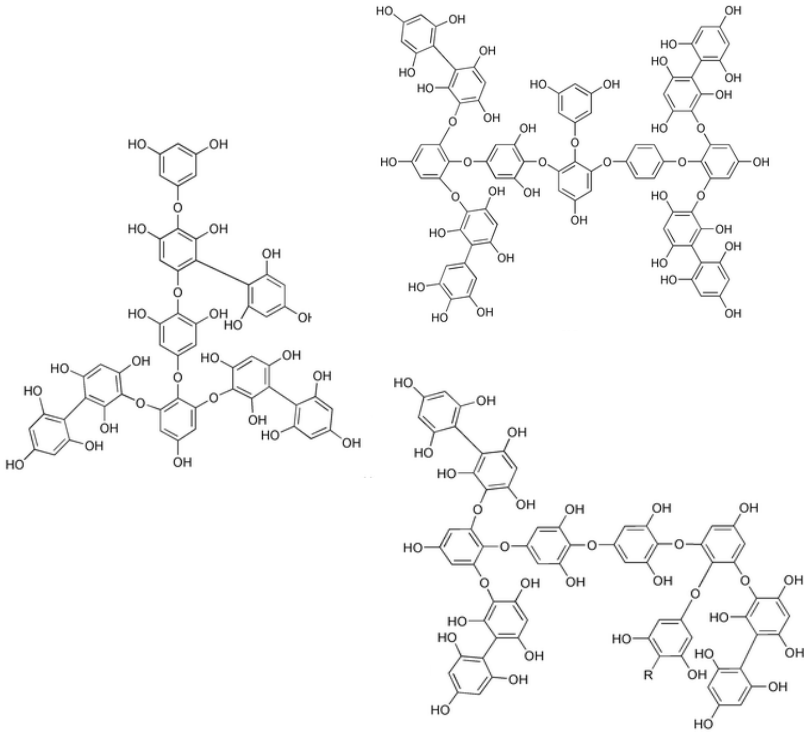
Phlorotannins in Algae

PHLOROTANNINS

- Type of polyphenols only found in algae.
- Polymeric forms.
- Basic unit: phloroglucinol.
- Important bioactivities *in-vitro*:
  - » Antioxidant
  - » Anticancer
  - » Anti-allergenic
  - » Anti-inflammatory
  - » Antimicrobial



Phloroglucinol



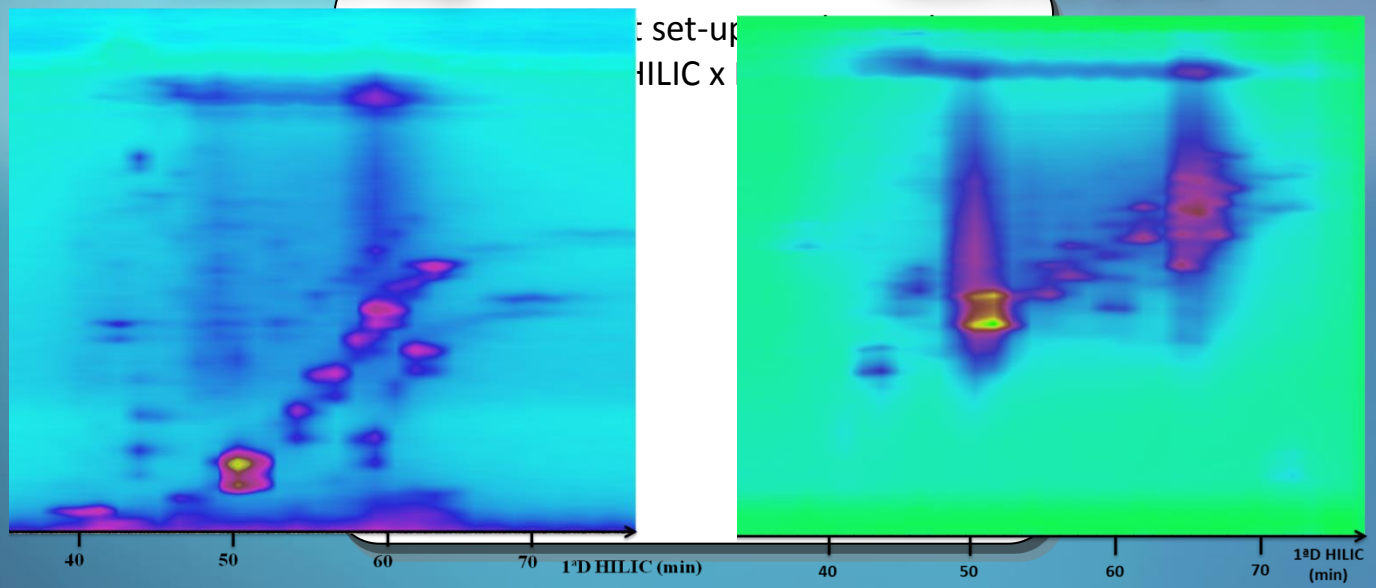
Phlorotannins in Algae

Work under way...

» Development of a LC x LC method to separate and identify the phlorotannins fraction in *Cystoseira abies-marina* brown algae.

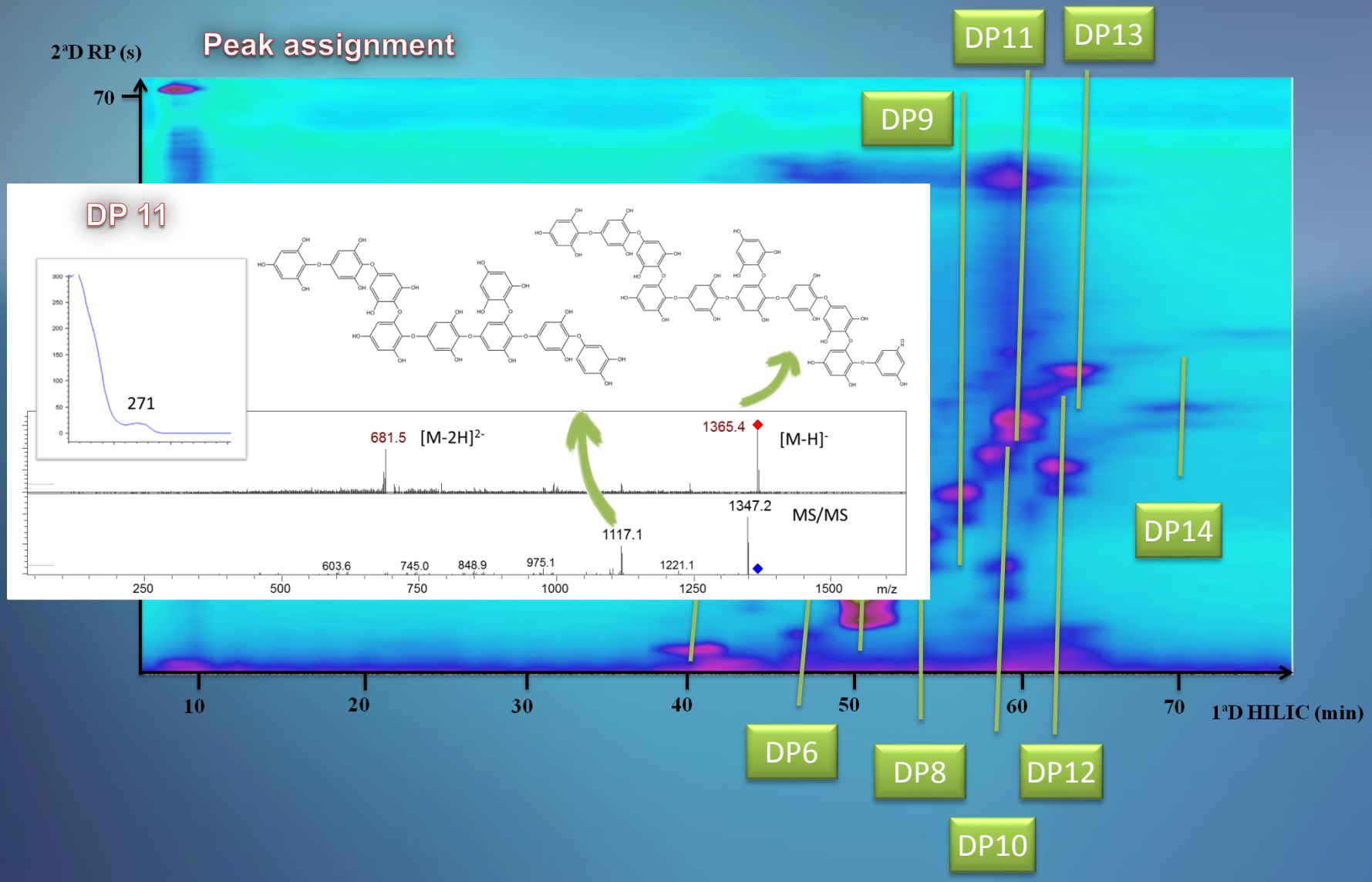
HILIC x RP (C_{18})

HILIC x RP (PFP)



Preliminary results

Phlorotannins in Algae



Journal of Chromatography A, 1313 (2013) 275–283

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

Profiling of phenolic compounds from different apple varieties using comprehensive two-dimensional liquid chromatography

Lidia Montero, Miguel Herrero*, Elena Ibáñez, Alejandro Cifuentes

Laboratory of Foodomics, Institute of Food Science Research (CIAL, CSIC), Nicolás Cabrera 9, Campus Cantoblanco, 28049 Madrid, Spain

Anal Bioanal Chem (2013) 405:4607–4616
DOI 10.1007/s00216-012-6687-y

ORIGINAL PAPER

Optimization of clean extraction methods to isolate carotenoids from the microalga *Neochloris oleoabundans* and subsequent chemical characterization using liquid chromatography tandem mass spectrometry

María Castro-Puyana · Miguel Herrero · Iratxe Urreta · Jose A. Mendiola · Alejandro Cifuentes · Elena Ibáñez · Sonia Suárez-Alvarez

Foodomics projects in our lab on:

Safety, quality and traceability of

Transgenic foods
Other foods & ingr

GM corn, GM soya, GM yeasts…

DNA, proteins and metabolites

In collaboration with **GSF** (Munich, Germany)

Bioactivity of food ingredients against:

Alzheimer

Population study

Biological sample: Cebrospinal fluid (CSF)

In collaboration with **Karolinska Institute** (Stockholm, Sweden)

Colon cancer

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Biological samples: DNA, RNA, proteins and metabolites

Leukemia

Human cell lines

Biological samples: DNA, RNA, proteins and metabolites

In collaboration with
Univ. Miguel Hernandez, Elche, Spain
University of Granda, Granada, Spain

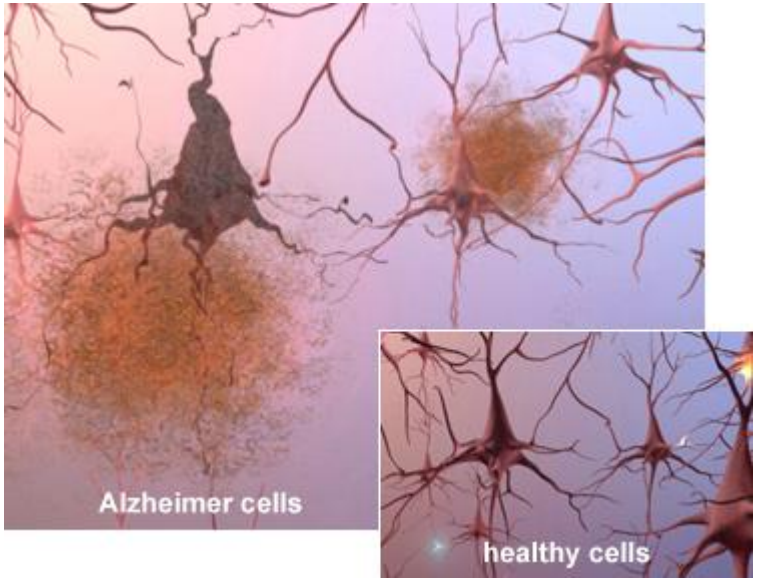
ALZHEIMER'S DISEASE

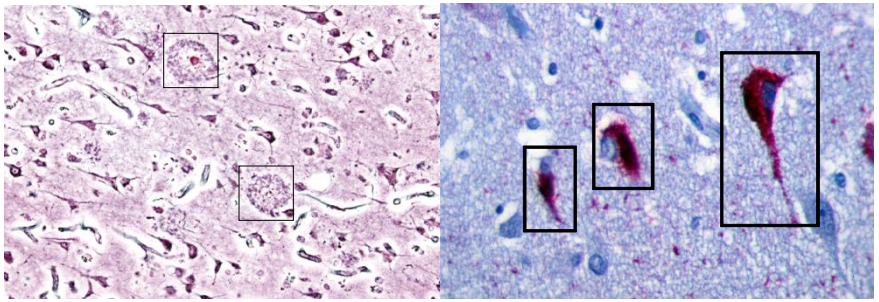
- Most prevalent dementia among aged people. Increasing incidence (WHO):

  >20% older than 64 years old.

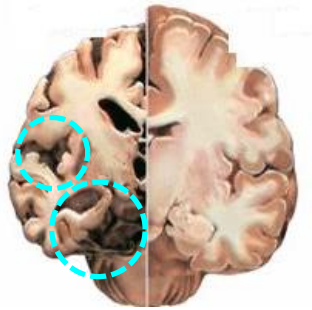
- Alzheimer's disease (AD) description 100 years ago; however origin and causes are unknown.

- Progressive destruction and atrophy of brain cortex: neurofibrillary tangles and amyloid plaques.



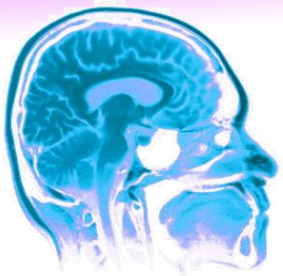


Amyloid plaques Neurofibrillary tangles



AD Healthy

ALZHEIMER'S DISEASE



-Neurophysiological evaluation

- Clinical examination

-Protein analysis including total tau (T-Tau), phospho-tau (P-Tau) and $A\beta_{1-42}$

-Brain imaging: NMR, PET, SPECT.

90% <u>advanced</u> cases of AD *6000-10000 € / patient*

MCI: Mild cognitive impairment cannot be detected

We have developed a metabolomics approach for the early diagnosis of AD based on CSF analysis

ALZHEIMER'S DISEASE

ELECTROPHORESIS

Electrophoresis 2013, 34, 2799–2811

Clara Ibáñez
Carolina Simó
Alejandro Cifuentes

Laboratory of Foodomics, CIAL (CSIC), Madrid, Spain

Received December 21, 2012
Revised February 21, 2013
Accepted February 23, 2013

Review

Metabolomics in Alzheimer's disease research

Alzheimer's disease (AD) is a neurodegenerative mo
still unknown. The majority of AD biochemical mo
developed as an extension of targeted physiological
hypothesis." The potential of metabolomics for the di
cidation of new biochemical pathways modified in
in this review work. A variety of nontargeted metab
tion between healthy subjects and AD patients are
metabolomics to predict progression to AD in indi
is also presented.

Keywords:
Alzheimer's disease / Biomarker / Metabo
Multivariate statistical analysis

1 Introduction to Alzheimer's disease

The incidence of many diseases increases rapidly with aging. Alzheimer's disease (AD) is the most prevalent cause of dementia among older people [1]. It is an incurable, degenerative, and terminal multifactorial disease. Although the initiating events are still unknown [2], AD seems to result from a combination of genetic, environmental, and lifestyle risk factors [3]. One of the hallmarks of AD is the observation of amyloid plaques (or senile plaques) and neurofibrillary tangles. Amyloid plaques are produced by the accumulation of amyloid beta (Aβ) peptides. The "amyloid hypothesis" assigns a critical role to abnormal processing of amyloid precursor protein (APP), which is sequentially cleaved by α-, β-, and γ-secretase-originating neurotoxic-soluble Aβ peptides that aggregate in oligomers to form these plaques. On the other hand, neurofibrillary tangles are intracellular filamentous aggregates of the microtubule-associated protein tau. In its hyperphosphorylated status, tau protein detaches from the microtubules and, consequently, the microtubules fall apart and tau tends to aggregate inducing breaks in the microtubular tracks and neuronal death. It has been estimated that AD

process begins
dementia [4]. AI
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Correspondence: Dr. Carolina Simó, Laboratory of Foodomics, CIAL (CSIC), Nicolás Cabrera 9, 28049 Madrid, Spain
E-mail: c.simo@csic.es
Fax: +34-910017905

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; ApoE, apolipoprotein E; APP, amyloid precursor protein; CSF, cerebrospinal fluid; ECA, electrochemical array; LDA, linear discriminant analysis; MCI, mild cognitive impairment; MDMS-SL, multidimensional MS-based shotgun lipidomics; Mo, monomeric-enriched fraction; PCA, principal component analysis; Po, polymeric-enriched fraction; QqQ, triple quadrupole; T-tau, total tau; UPLC, ultra-performance LC.

analytical chemistry

Toward a Predictive Model of Alzheimer's Dis
Capillary Electrophoresis–Mass Spectrometry

Clara Ibáñez,[†] Carolina Simó,[†] Pedro J. Martín-Álvarez,[†] Miia Kivipe
Angel Cedazo-Mínguez,[‡] and Alejandro Cifuentes*,[†]
[†]Laboratory of Foodomics, CIAL (CSIC), Nicolas Cabrera 9, 28049 Madrid, Spain
[‡]Karolinska Institute, NVS Department, KI-Alzheimer's Disease Research Center, 14

Supporting Information

ABSTRACT: Alzheimer's disease (AD) is the most prevalent form of dementia
an estimated worldwide prevalence of over 30 million people, and its inciden
expected to increase dramatically with an increasing elderly population. Up until
cerebrospinal fluid (CSF) has been the preferred sample to investigate central ne
system (CNS) disorders since its composition is directly related to metal
production in the brain. In this work, a nontargeted metabolomic approach bas
capillary electrophoresis–mass spectrometry (CE–MS) is developed to ex
metabolic differences in CSF samples from subjects with different cognitive
related to AD progression. To do this, CSF samples from 85 subjects were ob
from patients with (i) subjective cognitive impairment (SCI, i.e. control grou
mild cognitive impairment (MCI) which remained stable after a follow-up peri
years, (iii) MCI which progressed to AD within a 2-year time after the initi
diagnostic and, (iv) diagnosed AD. A prediction model for AD progression usi
biomarkers (Aβ42 and tau) but need to be obtained predictive values were hi
MS metabolomics of CSF samples was obtained using 73 CSF samples. Using
100% of the samples in the diagnostic groups. The prediction power was confir
reaching a 83% of diagnostic accuracy. The obtained predictive values were hi
proline, serine, histidine, creatine, carnitine, and suberylglycine were identified
results suggest that CE–MS metabolomics of CSF samples can be a useful t

Alzheimer's disease (AD) is characterized by progressive loss of memory and other cognitive functions leading to dementia. The long duration of AD and its increased incidence with age constitutes a large emotional and financial weight for patients, their families, and society. It is predicted that the worldwide number of AD cases, presently about 36 million, will triple by 2050.[1] Thus, it is of extreme importance to solve the most important AD questions raised, i.e., origin, causes, treatment, prevention, and early and accurate diagnosis. Most AD biomarkers deeply studied so far are protein molecules. Thus, increased total tau (t-tau) and phospho-tau (p-tau) while decreased amyloid beta (Aβ) levels have been observed in AD subjects, in comparison with nondemented subjects.[2] Sensitivity and specificity values of 80−88% are obtained in the diagnosis of advanced cases of AD when these biomarkers are combined.[3,4] On the other hand, advanced medical brain imaging techniques (computed tomography, nuclear magnetic resonance imaging, and single photon or positron emission computed tomography) may help to diagnose the existence of dementia but not specific dementia due to AD.[5] The combination of the analysis of specific protein levels and brain imaging might increase AD diagnosis up to 90%,[4] but the economic cost per patient arises immensely (~6 000−10 000 euros approximately per patient).

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

A new metabolomic workflow for early detection of Alzheimer's disease

Clara Ibáñez [a], Carolina Simó [a,*], Dinesh K. Barupal [b], Oliver Fiehn [b], Miia Kivipelto [c], Angel Cedazo-Mínguez [c], Alejandro Cifuentes [a,**]
[a] Laboratory of Foodomics, CIAL (CSIC), Nicolas Cabrera 9, 28049 Madrid, Spain
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[c] Karolinska Institute, NVS Department, KI-Alzheimer's Disease Research Center, 14186 Stockholm, Sweden

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UHPLC–MS
Multivariate statistical analysis

ABSTRACT
Alzheimer's disease (AD) is the most prevalent cause of dementia among older people. Although AD probably starts 20–30 years before first clinical symptoms become noticeable, nowadays it cannot be diagnosed accurately in its early stages. In this work, we present a new MS-based metabolomic approach based on the use of ultra-high performance liquid chromatography–time-of-flight mass spectrometry (UHPLC–TOF MS) to investigate cerebrospinal fluid (CSF) samples from patients with different AD stages. With the aim to obtain wide metabolome coverage two different chromatographic separation modes, namely reversed phase (RP) and hydrophilic interaction chromatography (HILIC), were used. RP/UHPLC–MS and HILIC/UHPLC–MS methods were optimized and applied to analyze CSF samples from 75 patients related to AD progression. Significant metabolic differences in CSF samples from subjects with different cognitive status related to AD progression were detected using this methodology, obtaining a group of potential biomarkers together with a classification model by means of a multivariate statistical analysis. The proposed model predicted the development of AD with an accuracy of 98.7% and specificity and sensitivity values above of 95%.

1. Introduction

The incidence of many diseases increases rapidly with aging. Alzheimer's disease (AD), the most prevalent cause of dementia among older people, is a multifactorial disease in which age is the main risk factor. Although the initiating events are still unknown [1], AD results from a combination of genetic, environmental and lifestyle factors [2]. Considering the continuous increase in life expectancy [3], it has been estimated that 35.6 million people would suffer dementia in 2010 [4], reaching 115.4 million in 2050. As public awareness of AD increases, the need for methodologies for early diagnosis is becoming imperative in an elderly population. However, early detection of AD is currently a huge challenge since AD probably starts 20–30 years before first clinical symptoms become noticeable. Moreover, early symptoms of AD are shared by various neuropathological disorders, including dementia.

Up to date there is no clinical method to determine which mild cognitive impairment (MCI) individuals will progress to AD except for a long clinical follow-up period. In approximately 80% of cases, MCI progresses to dementia when these subjects are followed up to 6 years [5]. Although there are several drugs that have been proven to slow disease progression and treat symptoms, so far, no treatment can effectively modify AD [6]. Early diagnosis of AD is expected to strongly impulse more research on potential AD risk factors, advances on more efficient drugs and cognitive stimulation programs.

Nowadays, AD can be definitively diagnosed only after death through an examination of brain tissue and pathology in an autopsy. However, it has been reported that increased total tau (t-tau) and phospho-tau (p-tau) while decreased amyloid beta (Aβ) levels have been observed in AD subjects in comparison with non-AD subjects [7]. When these biomarkers are combined, sensitivity and specificity values of 80–88% are obtained in the diagnosis of advanced cases of AD when these biomarkers are combined. On the other hand, advanced medical brain imaging techniques (computed tomography, nuclear magnetic resonance imaging, and single photon or positron emission computed tomography) may help to diagnose the existence of dementia but not specific dementia due to AD [10]. As imaging advances are being presented, parallel work is being carried out to identify reliable and valid markers in biofluids indicative of AD pathology [11]. In this

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Foodomics projects in our lab on:

Safety, quality and traceability of

Transgenic foods
Other foods & ingr

GM corn, GM soya, GM yeasts…

DNA, proteins and metabolites

In collaboration with **GSF** (Munich, Germany)

Bioactivity of food ingredients against:

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Biological sample: Cebrospinal fluid (CSF)

Colon cancer

Human cell lines
Animal models

Biological samples: DNA, RNA, proteins and metabolites

Leukemia

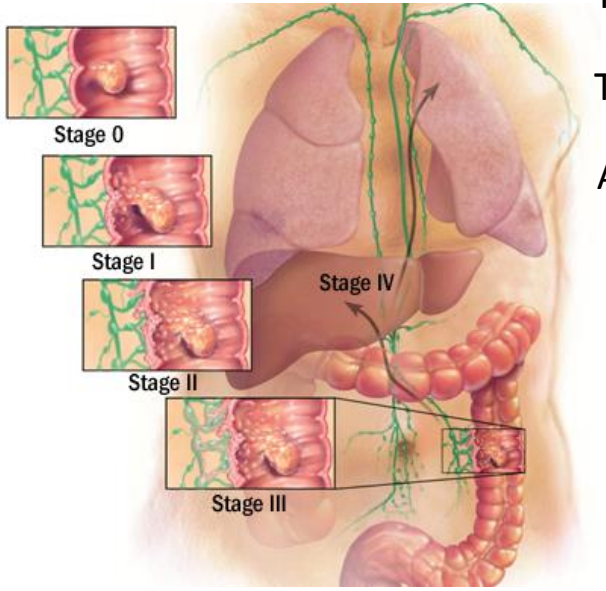
Human cell lines

Biological samples: DNA, RNA, proteins and metabolites

In collaboration with **Karolinska Institute** (Stockholm, Sweden)

In collaboration with **Univ. Miguel Hernandez**, Elche, Spain **University of Granda**, Granada, Spain

Colon cancer and diet



The most diagnosed cancer in Spain: 25000 new cases every year

The 2nd cause of death by cancer in Europe and 4th in the world

According to several studies, **80% of the cases are related to diet**

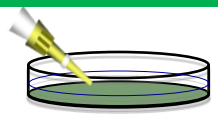
Can we reduce the proliferation speed of colon cancer through diet? This would be a great help since this cancer has a high percentage of recovery if intervention can commence before the period of tumor proliferation preventing the series of events leading to metastasis

Foodomics evaluation of dietary ingredients vs. Human colon cancer cells proliferation

Natural source

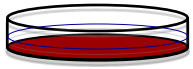
SFE

Polyphenols enriched extracts characterized by LC-UV-MS



Treated

HT29 colon cancer cells



Control

Selection of the polyphenols enriched extract with the highest anti-proliferative activity at 10 µM

RNAs. proteins and metabolites fractions obtained from control and treated HT29 cells (minimum x 3)

Transcriptomics

RNAs analysis by Human Gene 1.0 ST microarrays. Genes expressed differentially confirmed by RT-qPCR

Proteomics

Proteins analysis by 2-D electrophoresis and identification of differential proteins by MALDI-TOF-TOF

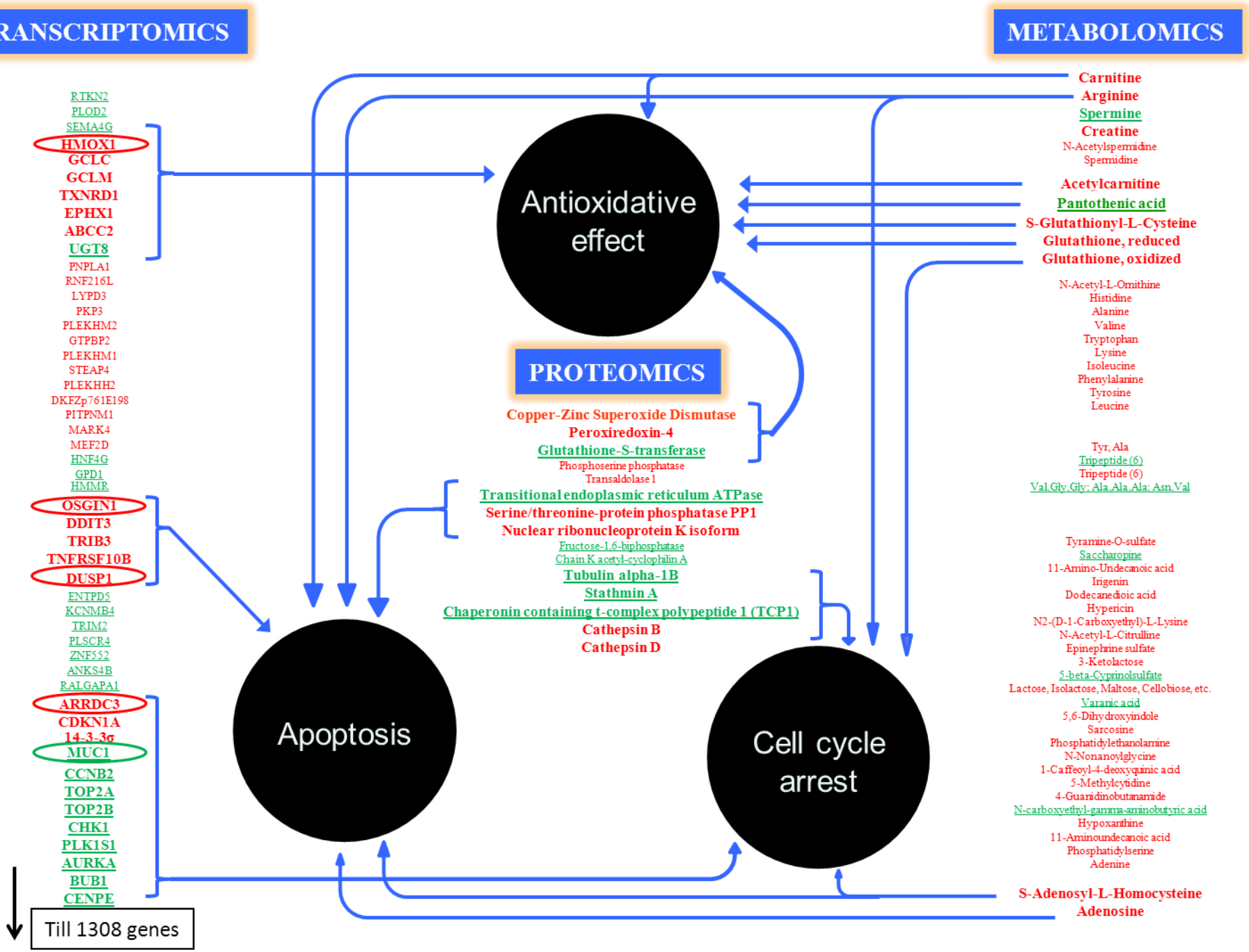
Metabolomics

Metabolites analysis by CE-MS. RP/UPLC-MS and HILIC/UPLC-MS. Identity confirmation using standards

Data processing

Statistical analysis

Biomarkers identification and confirmation. Pathway analysis and biological process understanding


TRANSCRIPTOMICS
METABOLOMICS
RTKN2
PLOD2
SEMA4G
HMOX1
GCLC
GCLM
TXNRD1
EPHX1
ABCC2
UGT8
PNPLA1
RNF216L
LYPD3
PKP3
PLEKHM2
GTPBP2
PLEKHM1
STEAP4
PLEKHH2
DKFZp761E198
PITPNM1
MARK4
MEF2D
HNF4G
GPD1
HMMR
OSGIN1
DDIT3
TRIB3
TNFRSF10B
DUSP1
ENTPD5
KCNMB4
TRIM2
PLSCR4
ZNF552
ANKS4B
RALGAPA1
ARRDC3
CDKN1A
14-3-3σ
MUC1
CCNB2
TOP2A
TOP2B
CHK1
PLK1S1
AURKA
BUB1
CENPE
Till 1308 genes
Antioxidative effect
PROTEOMICS
Copper-Zinc Superoxide Dismutase
Peroxiredoxin-4
Glutathione-S-transferase
Phosphoserine phosphatase
Transaldolase 1
Transitional endoplasmic reticulum ATPase
Serine/threonine-protein phosphatase PP1
Nuclear ribonucleoprotein K isoform
Fructose-1,6-biphosphatase
Chain K acetyl-cyclophilin A
Tubulin alpha-1B
Stathmin A
Chaperonin containing t-complex polypeptide 1 (TCP1)
Cathepsin B
Cathepsin D
Apoptosis
Cell cycle arrest
Carnitine
Arginine
Spermine
Creatine
N-Acetylspermidine
Spermidine
Acetylcarnitine
Pantothenic acid
S-Glutathionyl-L-Cysteine
Glutathione, reduced
Glutathione, oxidized
N-Acetyl-L-Ornithine
Histidine
Alanine
Valine
Tryptophan
Lysine
Isoleucine
Phenylalanine
Tyrosine
Leucine
Tyr, Ala
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Val,Gly,Gly; Ala,Ala,Ala; Asn,Val
Tyramine-O-sulfate
Saccharopine
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Irigenin
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Hypericin
N2-(D-1-Carboxyethyl)-L-Lysine
N-Acetyl-L-Citrulline
Epinephrine sulfate
3-Ketolactose
5-beta-Cyprinolsulfate
Lactose, Isolactose, Maltose, Cellobiose, etc.
Varanic acid
5,6-Dihydroxyindole
Sarcosine
Phosphatidylethanolamine
N-Nonanoylglycine
1-Caffeoyl-4-deoxyquinic acid
5-Methylcytidine
4-Guanidinobutanamide
N-carboxyethyl-gamma-aminobutyric acid
Hypoxanthine
11-Aminoundecanoic acid
Phosphatidylserine
Adenine
S-Adenosyl-L-Homocysteine
Adenosine

Journal of Chromatography A, 1248 (2012) 139–153

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Journal of Chromatography A

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

Global Foodomics strategy to investigate the health benefits of dietary constituents

Clara Ibáñez[a], Alberto Valdés[a], Virginia García-Cañas[a], Carolina Simó[a], Mustafa Celebier[a], Lourdes Rocamora-Reverte[b], Ángeles Gómez-Martínez[b], Miguel Herrero[a], María Castro-Puyana[a], Antonio Segura-Carretero[c], Elena Ibáñez[a], José A. Ferragut[b], Alejandro Cifuentes[a,*]

ELECTROPHORESIS

2328

Electrophoresis 2012, *33*, 2328–2336

Clara Ibáñez[1]
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Research Article

CE/LC-MS multiplatform for broad metabolomic analysis of dietary polyphenols effect on colon cancer cells proliferation

analytical chemistry

Feature

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Present and Future Challenges in Food Analysis: Foodomics

The state-of-the-art of food analysis at the beginning of the 21st century is presented in this work, together with its major applications, current limitations, and present and foreseen challenges.

Virginia García-Cañas,[†] Carolina Simó,[†] Miguel Herrero, Elena Ibáñez, and Alejandro Cifuentes*

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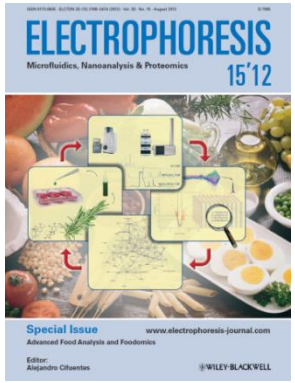
Supporting Information

ELECTROPHORESIS

(impact factor: 3.303)

"Foodomics and Advanced Food Analysis"

August 2012. Editor: A. Cifuentes



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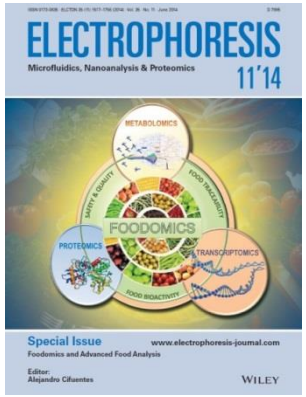
"Foodomics and Advanced Food Analysis"

June 2014. Editor: A. Cifuentes



analytical chemistry

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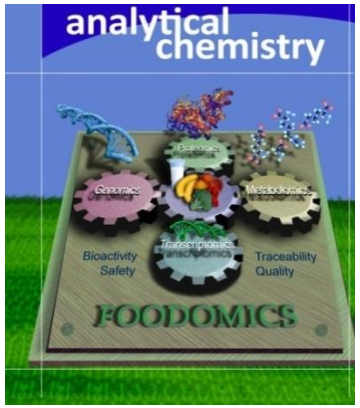
"Foodomics" Cover and Feature Article December 2012



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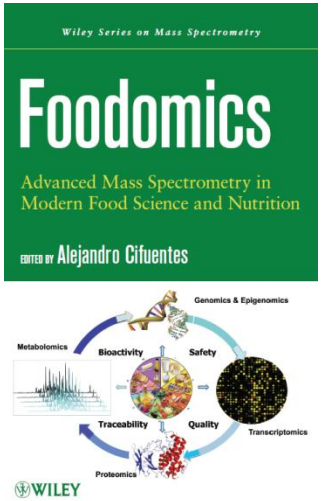

TRENDS IN ANALYTICAL CHEMISTRY

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"Green extraction techniques"

September 2015

Editors:
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TRENDS IN ANALYTICAL CHEMISTRY

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"Modern *Food Analysis and Foodomics*"

December 2013

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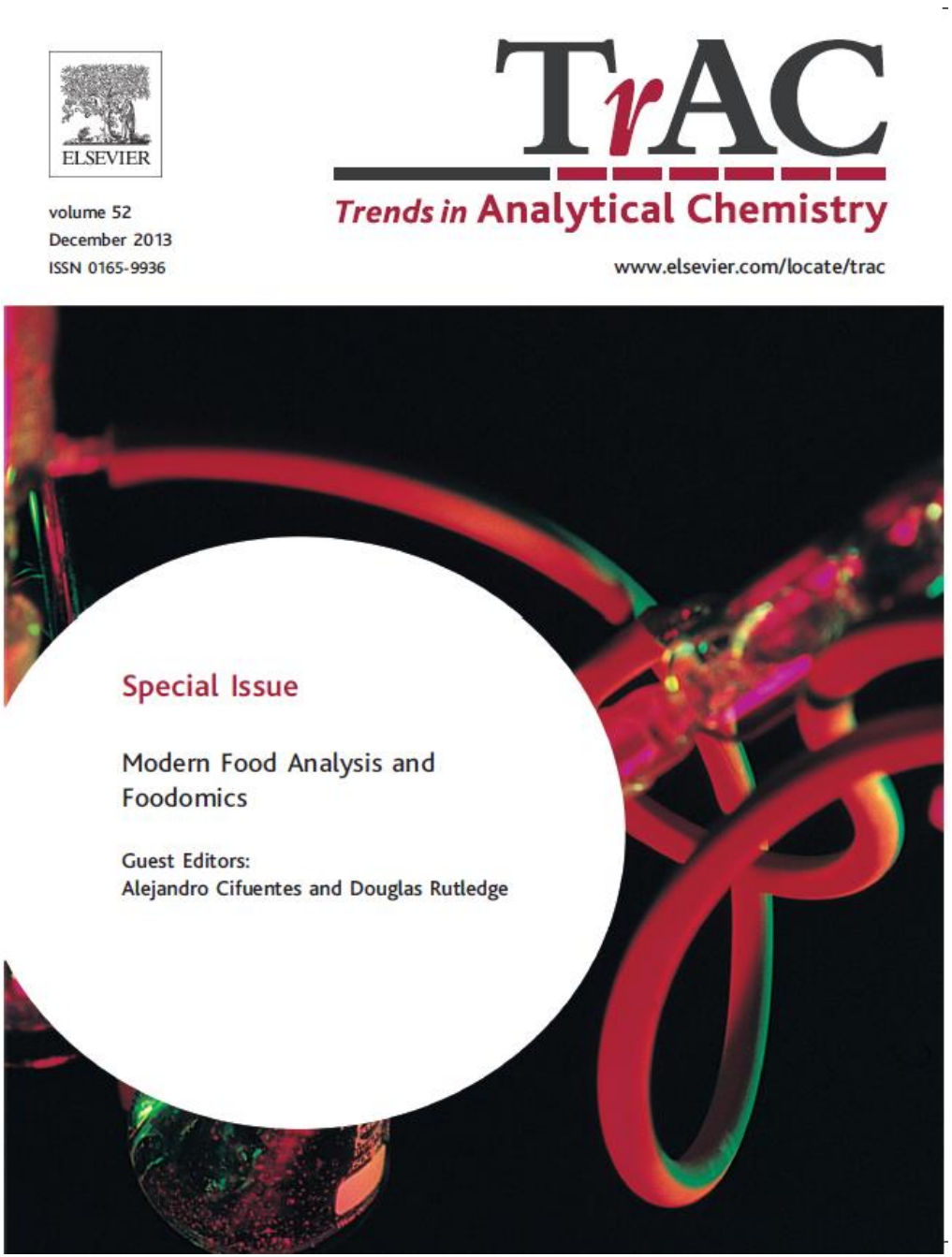
D. Rutledge



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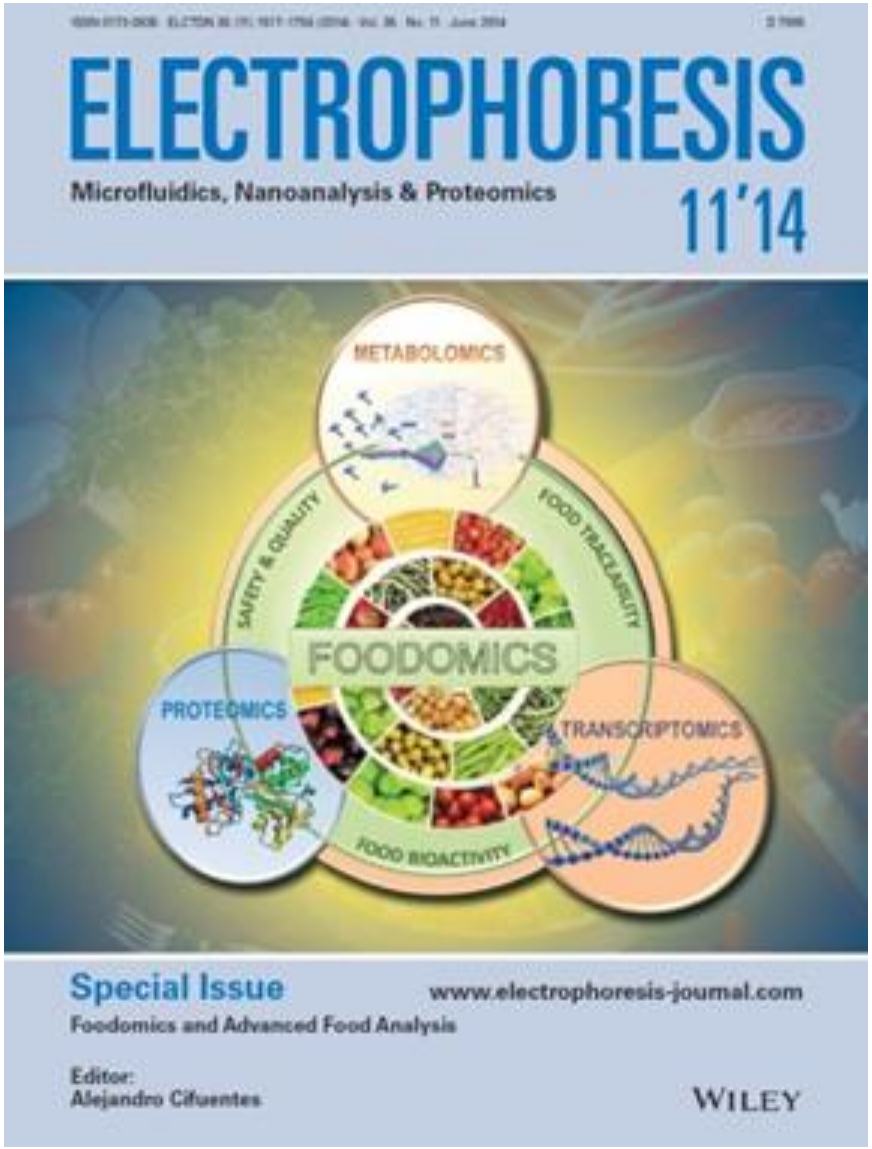
a.cifuentes@csic.es



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