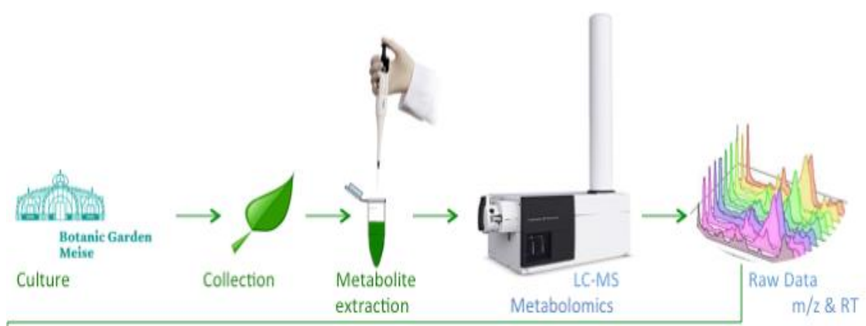
Differentiation of coffee species through untargeted-profiling studies using LC-HRMS in negative ion mode

Florence Souard [a, b], Cédric Delporte [b, c], Piet Stoffelen [d], Andrea Montis [b], Flore Keymeulen [b], Etienne Thévenot [e], Nausicaa Noret [f], Jean-Michel Kauffmann [b], Pierre Van Antwerpen [b, c], Caroline Stévigny [b]

[a] Département de Pharmacochimie Moléculaire, Université Grenoble Alpes, Grenoble, France, [b] RD3-Unit of Pharmacognosy, Bioanalysis and Drug Discovery, Faculty of Pharmacy, Université libre de Bruxelles, Brussels, Belgium; [c] Plateforme analytique de la Faculté de Pharmacie, Université Libre de Bruxelles, Brussels, Belgium; [d] Botanic Garden Meise, Meise, Belgium; [e] CEA, LIST, Laboratory for Data Analysis and Systems' Intelligence, MetaboHUB Gif-sur-Yvette, France; [f] Laboratoire d'Écologie végétale et Biogéochimie, Université Libre de Bruxelles, Brussels, Belgium.

Introduction: Because of their high content of caffeine, chlorogenic acids and other polyphenols, coffee seeds are widely used as beverage but also in cosmetic and food industries. In this study we focused on coffee leaves used to make infusions consumed essentially for medical purposes. We performed metabolomics studies in order to obtain more information about the metabolite biosynthetic pathways and by this fact to improve the composition of future diet supplements.

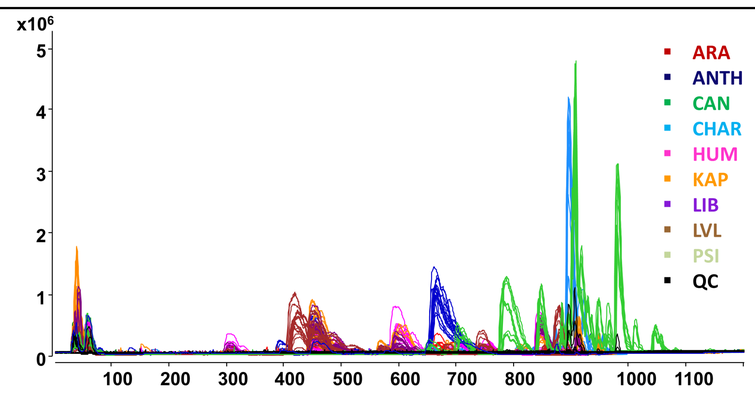
All the studied plants were grown in tropical greenhouses with the same environmental and edaphic conditions. Metabolomics fingerprints of the mature leaves of 9 *Coffea* species (and sub-species) have been undertaken by LC-HRMS analyses in negative ion mode. Then, a comprehensive statistical workflow was designed. It served for univariate hypothesis testing and multivariate modeling by PCA and partial PLS-DA on the Workflow4Metabolomics infrastructure. This strategy permitted to investigate the metabolomics data and their relationship with botanical data.



Workflow4Metabolomics 3.0 - collaborative portal to metabolomics data processing, analysis

LC-(HR)MS data acquisition

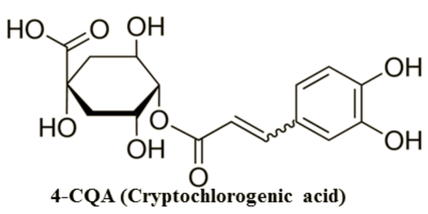
NEGATIVE

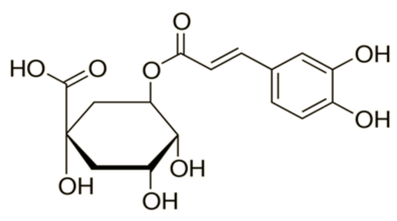


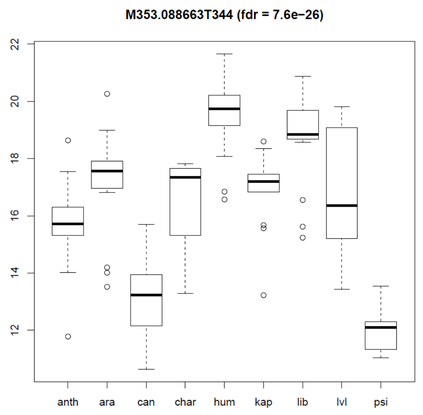
Data processing W4M

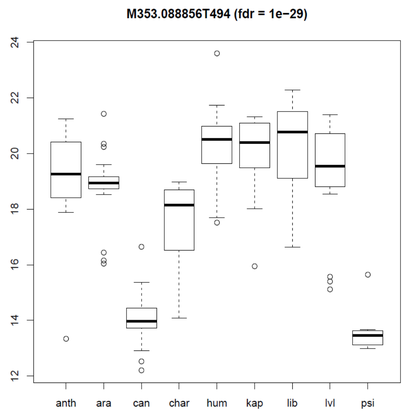
Table1: Set of coffee leaves samples

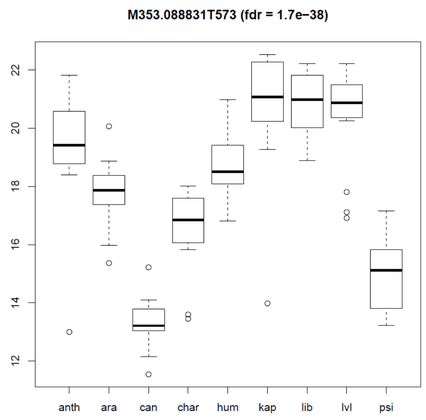
Name	Code	Reference	Origin	Months of collection
C. anthonyi	ANTH	20070347-77	Cameroon	01-03-06-09-11
C. arabica	ARA	19073828	Ethiopia	01-03-06-09-11
C. canephora	CAN	19800409	Central Africa	01-03-06-09-11
C. charrieriana	CHAR	20070349-79	Cameroon	01-03-06-09-11
C. humilis	HUM	20110310-76	Ivory Coast	01-03-06-09-11
C. kapakata	KAP	20110282-48	North-Western Angola	03-06-09-11
C. liberica	LIB	19391724	Central Africa	01-03-06-09-11
C. liberica var liberica	LvL	20110298-64	Ivory Coast	01-03-06-09-11
C. mannii	PSI	20091364-45	Cameroon	01-06-09-11

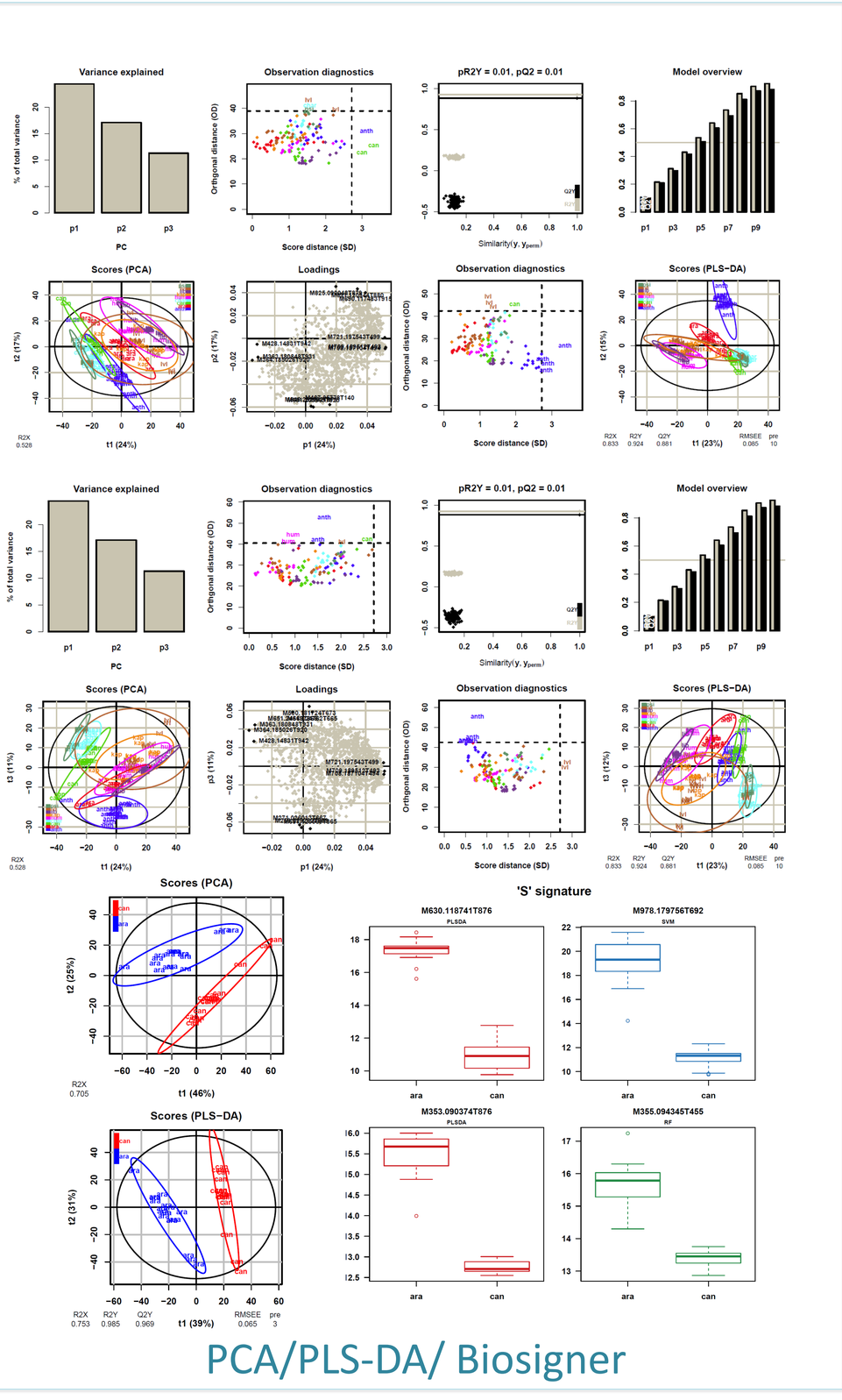




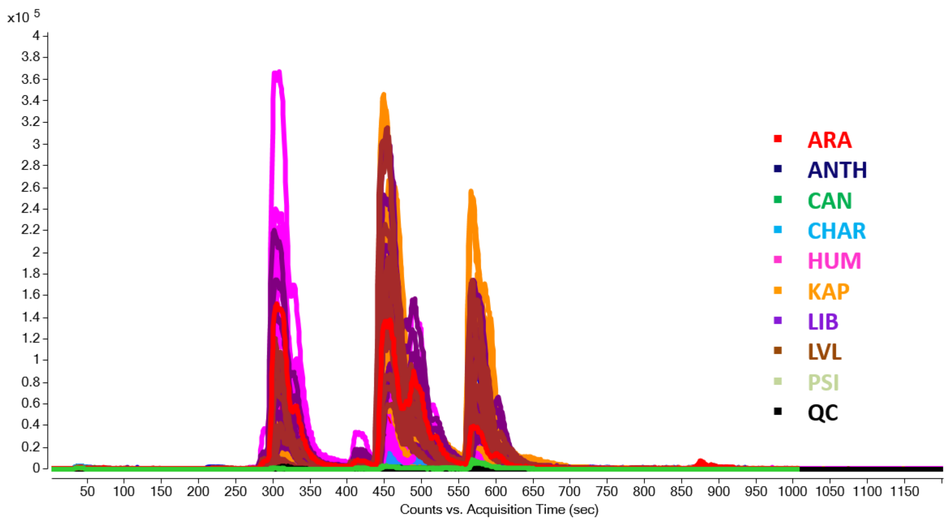








Results: All nine clusters of each species studied were observed on both PCA and PLS-DA score plots. The results presented show a good discrimination between clusters. For each studied species levels of the three main chlorogenic acids were monitored by univariate analysis.



Chlorogenic acid repartition

PCA/PLS-DA/ Biosigner

Conclusion: In the present study, the identification of the main metabolites in negative mode permitted us to point out that it was possible to discriminate *Coffea* species and subspecies by analysing their metabolomics profile. Among the identified metabolites, several chlorogenic acids or (epi)catechin derivates were found discriminant.