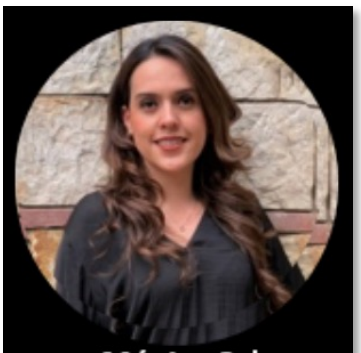
BOOK OF ABSTRACTS

ORGANIZING COMMITEE

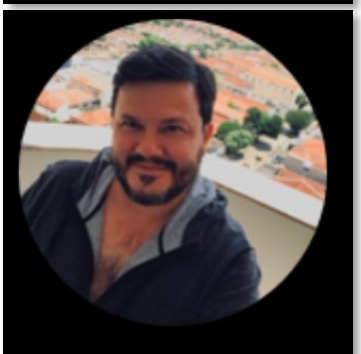


Mónica Cala
Universidad de los Andes
Colombia

Dr. Cala is the Head of the Metabolomics Core Facility – MetCore at Los Andes University in Bogotá, Colombia, which is the first mass spectrometry-based metabolomics facility in Colombia. She is also a founding member of the Latin American Metabolic Profiling Society – LAMPS. At MetCore, she leads research projects applying non-targeted and targeted metabolomics in health, nutrition, and agriculture, providing access to cutting-edge technology in mass spectrometry-based metabolomics, to conduct innovative and multidisciplinary research projects and provide metabolomic services in Colombia and the Latin American region.

María Eugenia Monge
CIBION-CONICET
Argentina

Dr. Monge is an Independent Researcher of the National Scientific and Technical Research Council of Argentina and works at the Centro de Investigaciones en Bionanociencias (CIBION). In 2006, she obtained her Ph.D. in analytical and physical chemistry from the University of Buenos Aires. Since 2014, she has participated in strengthening the Latin American community. Since 2021, she is a founding member of LAMPS. Since 2019, she has been a member of mQACC and the Metabolomics Society. In 2022, she was awarded the Metabolomics Society Medal and was elected as a member of the Metabolomics Society Board of Directors. Her research group develops mass spectrometry-based analytical methods using metabolomics and lipidomics approaches with applications in health and the environment.



Ian Castro-Gamboa
IQ-UNESP
Brazil

Dr. Castro-Gamboa graduated from the University of Costa Rica - UCR (1994) and a Ph.D in Organic Chemistry from the Federal University of São Carlos UFSCar - SP (2000). Currently, he is a professor at the Chemistry Institute of the São Paulo State University - IQ-UNESP. He enjoyed a sabbatical year (2009-2010) at the University of Florida - UF USA, working on metabolomics and dereplication techniques using NMR. Currently, his interests are investigations of bioactive micromolecules, and the development of analytical techniques coupled with NMR and MS for the rational exploration of biodiversity.

PLENARY SPEAKERS

Coral Barbas
Centro de Excelencia en Metabolómica y Bioanálisis (CEMBIO), Universidad San Pablo CEU, Madrid, Spain

Analytical Challenges in Untargeted Metabolomics Workflow

Coral Barbas directs the Center for Metabolomics and Bioanalysis (CEMBIO), which brings together more than 20 specialists, interns and technical staff with intense activity in metabolomics through mass spectrometry coupled with different separation techniques and applied to the search for diagnostic or prognostic markers of a pathology, of evolution before a treatment or a diet, etc. Coral Barbas is one of the 50 most influential women in the world in Analytical Chemistry according to the American magazine 'The Analytical Scientist'. In the field of metabolomics, she also collaborates with top-level international groups. Among the applications, it is worth mentioning the study of cardiovascular diseases, diabetes, pulmonary diseases or leishmania. In addition, she directs the CEU International Doctoral School (CEINDO), a center that coordinates a network of researchers from the three internationally renowned CEU universities. She manages interuniversity doctoral programs and promotes collaboration with prestigious university institutions so that researchers in training can participate in the most relevant European and international research projects.

Karl Burgess
School of Biological Sciences, The University of Edinburgh, Scotland.

RHIMMS and RTmet: Metabolomics Technologies Supporting the Next Generation of Synthetic Biology

Karl Burgess is Senior Lecturer in Biological Mass Spectrometry at the University of Edinburgh. Karl is also the Scientific Director of the EdinOmics core platform, which supports researchers in the UK and abroad in the fields of metabolomics and proteomics. Previously, Karl was a Senior Research Fellow in Metabolomics at the University of Glasgow for 10 years. His topics of interest include the application of metabolomics in the study of bacteria, with special emphasis on industrial biotechnology.

Tim Ebbels
Imperial College, London, UK

Computational Metabolomics: Integration and Interpretation.

Prof Tim Ebbels obtained his PhD in astrophysics from the University of Cambridge and in 1998 moved into bioinformatics via postdoctoral work at Imperial College London. His group focuses on the application of bioinformatic, machine learning and chemometric techniques to post-genomic data, with a particular emphasis on computational metabolomics. He has worked on projects ranging from environmental monitoring, through molecular epidemiology, to toxicogenomics and high-performance computing infrastructures. Much work focuses on modelling of the analytical technologies used to obtain metabolomic data, but his group is also addressing problems of data integration, visualisation, network analysis, time series and metabolite annotation. He is particularly known for the 'BATMAN' software for analysing complex metabolic NMR spectra. Tim is an active member of the metabolomics community, having served as a Director of the international Metabolomics Society from 2012-2018 (Secretary from 2014-16). He has co-organised several international conferences (international scientific committee Metabolomics 2014-17) and is a co-founder of the London Metabolomics Network. He is a member of the OECD Metabolomics Reporting Framework, co-chaired the ECETOC Metabolomics Standards Initiative in Toxicology (MERIT) and is an editorial board member for BMC Bioinformatics and the Journal of Chemometrics. He has a strong commitment to postgraduate education, serving as Director of the MRes in Biomedical Research at Imperial College (>750 students trained), leading its Data Science stream and leading the Data Analysis short course at the Imperial's International Phenome Training Centre. He is a Fellow of the Royal Society of Chemistry and Lifetime Honorary Fellow of the Metabolomics Society.

Facundo Fernández
Georgia Institute of Technology, Atlanta, USA

Next Gen Metabolomics Technologies: Deeper Coverage, Single Cell, Double Bond Pinpointing, Ion Mobility and Imaging

Facundo M. Fernández received his Ph.D. in 1999 from the University of Buenos Aires (Argentina) in the area of analytical chemistry. In 2000-2001 he did a postdoctoral stay at Stanford University (USA) under the supervision of Richard N. Zare and in 2002 he did a second postdoctoral stay at the University of Arizona (USA) under the supervision of Vicki Wysocki. In 2004, he joined the teaching staff of the School of Chemistry and Biochemistry at the Georgia Institute of Technology (Atlanta, USA), where he currently holds the position of Vasser-Woolley Professor in Bioanalytical Chemistry and Deputy Director of the Department. Facundo has received several awards, including the NSF CAREER award, the CETL/BP Teaching award, the Ron A. Hites Award for best publication from the American Society for Mass Spectrometry, and the Beynon Award from Rapid Communications in Mass Spectrometry. He has more than 195 publications in peer-reviewed journals, three patents, and numerous presentations at universities and international conferences. He also serves as Associate Editor of the Journal of the American Society for Mass Spectrometry and Frontiers in Chemistry. His research covers the field of metabolomics by mass spectrometry, including imaging techniques, the development of new ionization methods, and ion mobility instrumentation.

Elaine Holmes
Murdoch University, Perth, Australia

Phenotyping Maternal Child Health

Elaine Holmes is the Principal Investigator at the Australian National Center for Phenomics at Murdoch University in Perth, Australia. Before moving to Perth she led his research at the Department of Surgery at Imperial College London in the UK. She has focused on discovering disease biomarkers in personalized medicine studies or large-scale population studies looking at cardiovascular risks, among others. She has devoted effort to extending population genetic study concepts to the field of metabolomics to study the relationships between diet and metabolic profiles.

Fabien Jourdan
METABOHUB Infrastructure nationale
en métabolomique et fluxomique, Toulouse, France

Making sense of metabolic profiles using network science and knowledge graphs.

Fabien Jourdan holds a PhD in computer science from the University of Montpellier. He is INRAE research director (DR) in the research laboratory TOXALIM, Toulouse (France). He is co-leading a team of 20 scientists (Team MeX "Metabolism and Xenobiotics") working on the metabolic impact of food contaminants on Human health. His main expertise is the development of computational solutions to model metabolism at the cellular or tissue level. Since 2009, he has coordinated the development of the MetExplore web server which is used by more than 800 users worldwide to study omics data in the context of metabolic networks. Since 2021, he is director of French national infrastructure for Metabolomics and Fluxomics MetaboHUB. Former president of French-Speaking Metabolomics and Fluxomics Network (RFMF) he is currently international Metabolomics Society secretary.

Reza Salek
Application Development, Applied Industry and Clinical Division, Bruker BioSpin GmbH, Baden-Württemberg, Germany

How can you make the most out of your data, quality, data sharing and standards.

Dr Reza Salek was awarded his PhD in Molecular Biophysics and Biochemistry from University College London, UK. In the past, he worked at the University of Cambridge, Medical Research Council and EMBL-EBI in Cambridge, UK, as well as The International Agency for Research on Cancer (IARC), Lyon, France. My last position was at Bruker BioSpin GmbH Ettlingen, Germany, as VP, head of application development. I have delivered several data-driven translational solutions and developed and coordinated several open-access data standards leveraging on analytical technology. In addition, I have assembled several infrastructures and knowledge resources while adopting advanced statistics and machine learning solutions for large-scale analysis of metabolomics data.

Ludger Wessjohann
Leibniz-Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Halle, Germany.

Plant metabolic fingerprinting for phylogeny research, bioactives discovery, and resistance breeding support

Professor Wessjohann studied chemistry in Hamburg (Germany), Southampton (UK), and Oslo (Norway, Prof. Skattebøl). He earned his doctorate in 1990 with Prof. Armin de Meijere in Hamburg. After a short period as a lecturer in Brazil, he became a postdoctoral Feodor Lynen fellow of the Alexander von Humboldt Foundation with Prof. Paul Wender at Stanford University (USA), working on the total synthesis of Taxol®. After an assistant professorship in Munich (LMU, 1992–1998), he was appointed to the Chair of Bioorganic Chemistry at the Vrije Universiteit Amsterdam (NL), working on organometallic chemistry and biocatalysis. Since 2000, he has been the director of the Department of Bioorganic Chemistry at the Leibniz Institute of Plant Biochemistry (IPB) in Halle (Germany) and concurrently holds the chair of Natural Product Chemistry at the Martin Luther University of Halle-Wittenberg. From 2010–2017 he served as the Managing Director of the IPB (www.ipb-halle.de (accessed on 19 April 2022).

KEYNOTE SPEAKERS

Paula Burdisso
Instituto de Biología Molecular y Celular de Rosario, Rosario, Argentina

Increasing value-added of export honey by NMR fingerprints

Paula Burdisso has a degree in Biotechnology and a doctorate in Biological Sciences from the National University of Rosario (Argentina) where she trained in the field of Structural Biology. Later, she specialized in the study of metabolic profiles by Nuclear Magnetic Resonance at Imperial College London under the supervision of Drs. Elaine Holmes and Isabel Garcia-Perez. She is currently responsible for metabolomics projects at the Argentine Structural Biology and Metabolomics Platform (PlaBem) of IBR-CONICET, where she works in collaboration with different academic groups in Latin America and with companies in the regional production sector.

Ian Castro-Gamboa
Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais (NuBBE), IQ-UNESP, Araraquara, Brazil.

Natural Products Chemistry in Latin America through the omics optic: challenges and perspectives.

Ian Castro-Gamboa graduated from the University of Costa Rica - UCR (1994) and a Ph.D in Organic Chemistry from the Federal University of São Carlos UFSCar - SP (2000). Currently, he is a professor at the Chemistry Institute of the São Paulo State University - IQ-UNESP. He enjoyed a sabbatical year (2009-2010) at the University of Florida - UF USA, working on metabolomics and dereplication techniques using NMR. Currently, his interests are investigations of bioactive micromolecules, and the development of analytical techniques coupled with NMR and MS for the rational exploration of biodiversity.

Leticia Veras Costa-Lotufo
Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

The invisible beauty of the blue Amazon

Leticia Costa-Lotufo is a full professor of Pharmacology at the Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo. Her lab is focused on the discovery of new anticancer compounds from Brazilian biodiversity and studies on their mechanisms of action. She coordinates a multidisciplinary project to access marine microorganisms' diversity and biotechnological potential along the Brazilian coast and islands. Dr. Costa-Lotufo's activities include the supervision of undergraduate and graduate students and post-doctoral fellows. She has published book chapters, review articles and more than 300 articles in peer-reviewed journals. On 2017, Dr. Costa-Lotufo was nominated as member of the Academy of Science of São Paulo State.

Yamilé López
Universidad Autónoma de Zacatecas, Zacatecas, México

Multi-omics approaches to study acute and post-acute immunometabolic alterations in Mexican COVID-19 patients.

Dr. Yamilé López Hernández is a researcher at the National Council for Science and Technology, attached to the Autonomous University of Zacatecas, Mexico, since 2015. Her undergraduate (Bachelor's Degree in Biochemistry) and postgraduate studies (Master's Degree in Biochemistry and Doctorate in Sciences de la Salud) were held at the University of Havana, Cuba, where she also established herself as a Researcher in the field of Immunology, Vaccinology and Infectious Diseases such as Tuberculosis since 1998. Upon her arrival in Mexico in 2013, she began a new line of research in Metabolomics, applied to the identification of biomarkers of chronic and communicable diseases. She is a member of the National System of Researchers, Level 1 since 2015. She is the author of 34 research articles published in indexed journals and is a co-author of three international patents. She has been the beneficiary of three Grants as Principal Investigator and has contributed extensively to the training of human resources at the Bachelor's, Master's, and Doctorate levels. She has been a member of the Board of Directors of the Mexican Society of Proteomics since 2018. Her contributions have been presented at numerous national and international conferences.

Guillermo Moyna
Universidad de la República (Udelar), Paysandú, Uruguay

Promissory A Short and Biased Account of Metabolomic Analysis in Uruguay: A Few Experiences from the Healthcare and Productive Sectors.

Guillermo Moyna directs the Department of Coastal Chemistry at the University of the Republic (UdelaR) in Paysandú, Uruguay. He completed his undergraduate studies in Chemistry at UdelaR (1992) and obtained his doctorate in Organic Chemistry under the direction of Prof. A. I. Scott at Texas A&M University, USA (1998). He began his academic career as a postdoctoral researcher at the University of the Sciences in Philadelphia, USA, where he later served as Associate Professor and Director of the Nuclear Magnetic Resonance Laboratory until his return to Uruguay in 2011. The research lines of his group cover organic synthesis, organic physical chemistry, and more recently, metabolomics applied to problems ranging from the welfare of farm animals to the diagnosis of liver diseases of autoimmune origin.

Julien Wist
Universidad del Valle, Cali, Colombia; Murdoch University, Perth, Australia.

Covid-19: what have we learnt about it and what have we learnt from it?

A liquid sample can be characterized by NMR within minutes, but it may take hours of interpretation to elucidate a structure or annotate its components, i.e., to convert data into knowledge. This interpretation is mainly achieved by trained spectroscopists and published in scientific journals. However, only a fraction of the data can be published in this way, thus the raw (original) data is often lost and the conclusions are buried into pdf files without proper format, making it very difficult to re-use. While humans can, and prefer to, learn from this summarized information, machine learning or artificial intelligence requires access to the original data. In tandem with Luc Patiny from the Ecole Polytechnique Fédérale de Lausanne (Switzerland) we showcased a database for spectroscopic data https://www.mylims.org in 2008(now offline) that allows users to request services, predict, simulate and view NMR spectra, and manipulate them online so that the data will never leave the lab before being stored into a database. Over the years features were added to promote its use, enabling the creation of interactive teaching material for online courses. The rationale was that students could learn with the same tools that they would later use in the lab. Although valid, this argument was far too simplistic and integrating everything into a single platform was too difficult to maintain. The java applet technology used by then got unsafe and deprecated, and we ported the code to JavaScript. This is a rather unusual choice for building scientific tools and was dictated by the fact that we believed browsers were to become the fastest graphical interfaces, receiving a lot of effort from many giant companies. Moreover, inspired by software practices, the next iteration was built in three pillars that work independently, storage, processing engine and visualization. A working version of it is available at https://www.c6h6.org, and all the necessary code is shared on https://www.github.com/cheminfo under the open source MIT license. The first mentioned database has been up and running at EPFL, at Universidad del Valle and in several industries (thanks to Luc) for 12 years. The smallest one, in Colombia, has > 30,000 entries and 100 active users.

SHORT COMMUNICATION SPEAKERS

Andrés Charris, *et al.*
CIBION-CONICET, Centro de Investigaciones en Bionanociencias, NMR Group, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina

Aligning peaks by matching multiplet profiles in NMR metabolomics

Statistical correlation analysis (STOCSY) has been an effective tool used to extract traces with peaks correlated to chosen driver peaks of interest, which allows the generation of peak lists that can be queried in a database. Nevertheless, STOCSY presents limitations where its performance is diminished, mainly in cases of peak misalignment, weak peaks and peak overlap (these also affect multivariate data analysis, MVDA). We recently proposed COCOA-POD and CSMDB to address the issues of peak overlap or weak peaks. These two methodologies were successfully presented for spectral sets with aligned peaks. However, a peak alignment component was missing in our whole workflow. Widely used, spectral binning "avoids" peak alignment, but comes with the cost of drastically reducing data resolution and suffering from peak overlap. Alternatively, peak alignment algorithms can be used, but most fail when peak overlap "distorts" multiplet lineshapes, and also when chemical shifts exchange the frequency order of multiplets among the spectra, aligning incorrect multiplets together. With these limitations in mind, we proposed a new methodology that consists in aligning peaks by matching multiplet profiles of f1 traces on J-resolved spectra. Pairwise comparison of a reference spectrum to all spectra in the set is performed, matching f1 traces within a given tolerance, thereby building a peak correspondence table. STOCSY can be applied directly on the correspondence matching data to identify multiplets correlated to a selected driver peak, and the output can be directly coupled to "CSMDB with ConQuer ABC" for the integrated evaluation of compound identification with biological correlation assessment, using multiplet queries, and also prepared for 1D ^{1}H queries. Furthermore, MVDA can be performed directly on the correspondence matching data matrix.

Keywords: *Alignment; STOCSY; Overlap; COCOA-POD; CSMDB*

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Miguel Fernández-Niño, *et al.*
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From cocoa beans to chocolate: unraveling the biochemistry of cocoa post-harvesting through metabolomic analysis

The alarming loss of antibiotics' effectiveness in treating bacterial infections has shown the urgency to develop new antimicrobial agents. In a world that is running out of new antibiotics, essential oils (EOs) from medicinal plants promise to be an alternative and sustainable source of new drugs. However, the mechanism responsible for the biological activity of several EOs is not yet clear and this has limited the use of these natural products as therapeutic alternatives. This study aims to investigate the metabolic changes in *Escherichia coli* during the antibacterial action of *Lippia Origanoides*'s essential oil. Methods: *E. coli* ATCC11775 was selected for the study and cultured in Lysogeny broth at 37°C. Minimum inhibitory concentration (MIC) was determined for the EO by using the microbroth dilution method. Metabolic profiles of the samples treated with EO, and control bacteria were obtained by using liquid chromatography hyphenated with mass spectrometry. Data processing, metabolite identification, and statistical analysis are currently in progress aided by bioinformatic tools and databases such as Thermo Xcalibur™, XCMS version 3.7.1, MetaboAnalyst version 5.0, and CEU mass-mediator(5). Results and perspectives: The MIC50 for *L. origanoides* EO was 0,24mg/mL. A bacterial inoculum of approximately 1x108 UFC/mL was treated with this concentration of EO. The bacterial cultures were incubated in 2 mL conical tubes for 8 hours at 37°C. Liquid-liquid extraction was performed to obtain cellular extracts and the chemical profiles were analyzed in an UHPLC-ESI-Q-Orbitrap-HRMS equipment. Since this work corresponds to ongoing research, the results concerning metabolic profiling are currently being analyzed. We expect that this strategy will allow us to identify the discriminant metabolites that can be associated with the antimicrobial mode of action of the EO from *L. origanoides* on *E. coli.*

Keywords: *cocoa, chocolate, post-harvesting, metabolomics*

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Fabio Herrera-Rocha, *et al.*
Grupo de Diseño de Productos y Procesos (GDPP), Departamento de Ingeniería Química y de Alimentos, Universidad de los Andes. Bogotá, Colombia.

Lipidomic profiling of bioactive lipids during spontaneous fermentations of fine-flavor cocoa

Lipids are essential components in chocolate and represent more than 50% of the dry weight of cocoa beans. The impact of cocoa lipid content on chocolate quality (e.g., texture, melting, and crystallization temperature) has been extensively described. Nevertheless, few studies have been recently performed to elucidate the cocoa lipid composition, focusing only on triglycerides and fatty acids. Up to date, most of the potential biological activity of cocoa lipids remains unexplored. The present study aims to analyze the lipidome of fine-flavor cocoa fermentations using LC-MS-QTOF and develop a Machine Learning model to assess potential biological activity. We assembled three wooden fermentation boxes, each with 400 Kg of cocoa. Then we collected 1 sample from each one, consisting of 10 cocoa seeds. These samples were analyzed by LC-MS-QTOF in positive and negative ionization modes. Our results revealed that the cocoa lipidome comprises fatty acyls, glycerophospholipids, and other small lipid groups such as glycerolipids, sterol lipids, sphingolipids, and prenol lipids. No significant variations were observed in these groups of lipids during fine-flavor cocoa fermentations. Several Machine Learning algorithms were initially trained using a 492 bioactive and non-bioactive lipids dataset to explore potential biological activity among the identified lipids. After optimizing and testing all the algorithms, we found that K-Nearest Neighbors (KNN) had the best performance. Then, we used this model to classify the identified lipids as bioactive or non-bioactive compounds. Thus, based on their molecular descriptors, 28 molecules were nominated as potential bioactive lipids. None of these compounds have been previously reported as bioactive. Our work is the first untargeted lipidomic study and systematic effort to investigate potential bioactivity in lipids of fine-flavor cocoa fermentations using Machine Learning algorithms. However, further research is still required to dissect the specific biological activity linked to the lipids nominated in this work as potentially bioactive.

Keywords: *Lipidomic, Machine Learning, Cocoa, Fine Flavor, Bioactivity*

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OSMAC approach for assessing the metabolism of *Penicillium javanicum* isolated from *Galianthe thalictroides* (Rubiaceae), a Brazilian Pantanal medicinal plant

Penicillium is perhaps the most well-understood genus of fungi and produces a plethora of medicinal substances, including the famously World-changing penicillin. These fungi can also produce an array of important secondary metabolites, including terpenes, alkaloids, and a variety of peptides. Investigating a given fungus metabolism is challenging, as the isolated cultivation environment is not conducive to the full biosynthetic expression. However, strategies to assist microorganisms in secreting a larger number and variety of metabolites have been developed and proved to be valuable approaches in studying them. OSMAC (One Strain Many Compounds), for instance, introduces different stimuli in the cultures, such as changes in substrates, light regimen, pH levels, presence of epigenetic modulators, among others. In this context, the objective of this work was to assess the metabolic potential of a *P. javanicum* strain isolated from *Galianthe thalictroides* from the Brazilian Pantanal. In an antibiosis co-culture assay, *P. javanicum* inhibited the growth of *Sclerotinia sclerotiorum*, an important phytopathogen, by 72.65%. The OSMAC experiment design used as parameters nutrient availability (Potato Dextrose or Czapek-Dox culture media), the cultivation method (static or dynamic), and cultivation environment (presence or absence of co-culture with *S. sclerotiorum*), resulting in eight experiments. After a 30-day incubation period, the resulting metabolites were filtered from the mycelial biomass and subjected to extraction with ethyl acetate. The chemical profile was obtained by liquid chromatography coupled to high-resolution mass spectrometry and subjected to the GNPS (Global Natural Products Social Molecular Network) platform for spectral library searches and data visualization through molecular networks. The results showed that some metabolites were observed exclusively under certain conditions and allowed the annotation of beauvericin, a non-ribosomal depsipeptide, and both the mevastatin, a polyketide, and its isomer mevinic acid. This is the first report of this endophyte being isolated from *G. thalictroides.*

***Keywords**: Penicillium javaricum; Metabolomics; Sclerotinia scletorotiorum; Molecular Networking; OSMAC*

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Daniel Andrés Pardo Rodríguez, *et al.*
Grupo de Investigación en Fitoquímica; Metabolomics Core Facility—MetCore, Universidad de los Andes (UNIANDES), Bogotá, Colombia

Evaluation of metabolic alterations induced in *Trypanosoma cruzi* (Chagas disease) by *Clethra fimbriata* extracts.

Chagas disease (ChD), caused by *Trypanosoma cruzi*, is endemic in 21 Latin American countries and it is estimated worldwide around 8 million people are chronically infected. Currently, only two drugs are available for therapeutic use against *T. cruzi*: Benznidazole and Nifurtimox. The use of these treatments is controversial because they have presented several issues associated with side effects and low compliance to treatment. Therefore, the search for new tripanocidal agents is necessary. Natural products have been considered as a potential innovative source of effective and selective agents for drug development to treat *T. cruz*i infection. Recently, our research group showed that extracts from Clethra fimbriata have activity against all stages of *T. cruzi*. To evaluate the metabolic alterations induced in *T. cruzi* after the treatment with *C. fimbriata* hexanic extract (CFHEX), a multiplatform metabolomics analysis were performed using RP/HILIC-LC-QTOF-MS and GC-QTOF-MS. A total of 154 altered compounds were found significantly altered in the treated parasites corresponding to amino acids (glycine, valine, proline, isoleucine, alanine, leucine, glutamic acid, and serine), fatty acids (stearic acid), glycerophospholipids (phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine), sulfur compounds (tripanothion) and carboxylic acids (pyruvate and phosphoenolpyruvate). The metabolic pathways that were most affected were mainly related to three biological processes: lipid metabolism, which was found to be preferentially increased, and energy and amino acid metabolism, which were decreased. Further, exogenous compounds of the triterpene type (betulinic, ursolic, and pomolic acid) were found in the treated parasites. Our findings suggest that triterpene-type compounds could contribute to the activity evidenced by CFHEX by inhibiting essential processes in the parasite. Many of which would be related to the functioning of the mitochondria.

Keywords: *Chagas disease, Clethra fimbriata, Energy metabolism, Untargeted metabolomics, Triterpenes, Trypanosoma cruzi.*

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Mónica Quintero, *et al.*
Research and Development Center—Colcafé S.A.S, Bogotá, Colombia

An untargeted metabolomics approach was applied to understand the sensory acceptance of coffee beverages.

Coffee is one of the most consumed beverages globally (third in the global ranking), preceded only by water and tea. New categories have emerged during the last years, with more than 10% claiming unique sensory profiles associated with the extraction process of the product. Concentrated liquid coffees (CLCs) are one of these new product categories, employed as ingredients in the production of canned coffee, cold brews, and other beverages based on coffee. However, the stability of these new categories represents a challenge for coffee. Understanding the chemical markers associated with sensory quality in coffee requires implementing advanced analytical tools such as metabolomics.
This study aimed to apply an untargeted LC/QTOF-MS analysis to identify compounds that positively and negatively affect the acceptance of coffee beverages from liquid coffee concentrates (CLCs) before and after storage. The metabolomic results were integrated with sensory parameters, such as color, pH, titratable acidity, and oxygen contents, by a bootstrapped version of partial least squares discriminant analysis (PLS-DA) to select and classify the most relevant variables regarding the rejection or acceptance of CLC beverages. The OPLS-DA models for metabolite selection discriminated between the percent sensory acceptance (the Accepted group) and rejection (the Rejected group). Eighty-two molecular features were considered statistically significant. Our data suggest that coffee sample rejection is associated with chlorogenic acid hydrolysis to produce ferulic and quinic acids, consequently generating methoxybenzaldehydes that impact the perceived acidity and aroma. Furthermore, acceptance was correlated with higher global scores and sweetness, as with lactones such as feruloyl-quinolactone, caffeoyl quinolactone, and 4-caffeoyl-1,5-quinolactone, and significant oxygen levels in the headspace. Finally, untargeted metabolomics studies might be correlated with sensory analysis to understand the acceptability of coffee beverages.

Keywords: *Untargeted metabolomics, concentrated liquid coffee, sensory analysis, high-performance liquid chromatography, mass spectrometry*

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Maximilian Rey, *et al.*
Centro de Investigaciones en Bionanociencias (CIBION), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

Contributing to preclinical *in vitro* and *in vivo* studies of an anti-Chikungunya drug candidate by means of LC-MS-based metabolomics strategies

Chikungunya fever is a mosquito-borne disease caused by chikungunya virus, which is a RNA virus of the togaviridae family, mostly prevalent in tropical countries. No specific treatment or vaccine is available up to date. Using computer-aided design, a candidate for an anti-chikungunya drug (LB-16)(1) was identified and is currently undergoing preclinical studies. Biotransformation studies are necessary to evaluate drug stability and elimination, and to characterize potentially toxic or active metabolites. Hepatic metabolism is the primary elimination mechanism for xenobiotics, in which the drug undergoes a series of enzymatic reactions to increase hydrophilicity, enhancing urinary excretion. The first step (phase-I) involves reactions such as oxidation, reduction, and hydrolysis. Objectives: To assess phase-I metabolism, we investigated the in-vitro metabolic stability of LB-16 using liver murine microsomes. At different times, samples were analyzed by means of reverse phase HPLC-ESI-SQ-MS and UPLC-ESI-HRMS methods using untargeted and semi-targeted metabolomics approaches to detect LB16 and its metabolites. Results: The assay revealed that LB-16 underwent rapid modification with approximately 90% of the drug modified in 45 min (metabolic half-life (t1/2) of 12.4 min). Results from UPLC-ESI-HRMS provided the tentative identification of an hydroxylated derivative of LB-16 by means of accurate mass, isotopic pattern and MS/MS experiments. Conclusions: The untargeted metabolomics approach was successfully implemented to identify a hydroxylated LB-16 metabolite with MSI confidence level 2. Future perspectives: LB16-OH is being synthesized for structural confirmation by matching retention time and mass spectral descriptors. Moreover, current studies involve the preliminary evaluation of in vivo pharmacokinetic parameters of LB-16 in mice, and serum samples will be analyzed by UPLC-ESI-HRMS to target LB-16 and its metabolites in systemic circulation.

Keywords: *pharmacometabolomics; drug discovery; chikungunya; microsomal stability*

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Alejandra Rojo Sánchez, *et al.*
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Urinary metabolomic profile of systemic lupus erythematosus and lupus nephritis based on liquid and gas chromatography/mass spectrometry (LC-QTOF-MS AND GC-QTOF-MS)

Systemic lupus erythematosus (SLE or lupus) is a chronic autoimmune disease, and kidney involvement with SLE, lupus nephritis (LN), is a frequent and severe complication of SLE that increases patient morbidity and mortalityis. The current gold standard for classifying LN progression is a renal biopsy, an invasive procedure with potential risks. Undergoing a series of biopsies for monitoring disease progression and treatments is unlikely suitable for patients with LN. Thus, there is an urgent need for non-invasive alternative biomarkers that can facilitate LN class diagnosis. Such biomarkers will be very useful in guiding intervention strategies to mitigate or treat patients with LN. The current study aims to explore new biomarker candidates for non-invasive diagnosis of LN and explore the pathogenic mechanisms that contribute to renal injury. Materials and Methods: A metabolomics approach using LC-QTOF-MS in both positive and negative electrospray ionization (ESI) modes and GC-QTOF-MS was developed for comparison of urine metabolic profile of 23 LN patients, 16 SLE patients, and 10 healthy controls (HCs). Differential metabolites were evaluated with univariate (UVA) and multivariate (MVA) analysis using a nonparametric t test, principal component analysis (PCA) and orthogonal partial least squares regression (OPLS-DA). Results: Both UVA and MVA showed a clear discrimination in the urinary metabolome between LN, SLE and HCs. The significant altered metabolites between LN and SLE correspond mainly to fatty acyls, amino acids, bile acids in particular methylglutamic acid, monopalmitin methyl-L-proline, 3-oxo-4-pentenoic acid, glutaric acid, 3-hydroxyglutaric acid, citraconic acid, glutamine, glycocholic acid and ureidoisobutyric acid. Analysis of metabolic pathways shows disturbances in biosynthesis of alanine, aspartate and glutamate metabolism, citrate cycle (TCA cycle) and glutamine and glutamate metabolism. Conclusions: The urinary metabolome of SLE and LN patients made it possible to determine metabolic alterations and discriminate LN patients from SLE patients. If confirmed in larger studies, these urine metabolites may serve as biomarkers to help discriminate between SLE with and without renal involvement.

Keywords: *systemic lupus erythematosus, lupus nephritis, urine biomarkers, metabolomics*

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Metabolic Signatures Associated with Chronic Hypoxia in well-trained adolescents

Training in hypoxic conditions is used by athletes who aim to increase physical performance at sea level and to improve hypoxic resistance during competitions in altitude environments. Hypoxic training interventions might also contribute to preventing and treating cardiovascular diseases. Comparisons between lowland and highland populations provide an exceptional opportunity to understand the mechanisms of hypoxia resistance. Its molecular basis has not been extensively studied in trained adolescents, and the metabolomic adaptation remains poorly understood. The aims of this study were to compare the blood metabolic profiles between moderate altitude trained and low altitude trained athletes to identify the potential metabolic pathways underlying these differences. Metabolic profiling of serum samples from 80 adolescent male athletes (15 ± 1 years, VO2max 60.6 ± 6.0 ml·min-1·kg-1) specialized in aerobic endurance sports were analyzed and were classified as altitude natives (N=40) or lowlanders (N=40) attending to the altitude of their place of birth (~2600m above sea level and Cali ~1000m, respectively). Samples were analyzed by LC–MS-QTOF. Differences between profiles from altitude natives and lowlanders groups, with univariate (UVA) and multivariate (MVA) analysis using a nonparametric t test, principal component analysis (PCA) and orthogonal partial least squares regression (OPLS-DA). A total of 94 compounds were identified with LC–MS-QTOF. Partial least-squares discriminant analysis showed a clear separation between groups. Significant variations in metabolites highlighted group differences in diverse metabolic pathways, including lipids, amino acids, bile acids, purines, pyrimidines, among in under basal condition. The metabolite with highest increase was C17 Sphinganine (fold change 17.2, p = 0.01). The data show that altitude natives can present altered metabolic state related to complex lipids and fatty acids, notably glycerophospholipids and sphingolipids among other key metabolic pathways related to human to cardiorespiratory fitness. Our findings propose relevant plasma metabolites that could contribute to a better understanding of the metabolic alterations associated with hypoxia resistance and cardiorespiratory fitness in trained adolescents.

Keywords: *altitude, endurance performance, mass spectrometry, biomarkers, sportomics*

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Metabolomics and machine learning for bioprocess optimisation: A *Bacillus* case study

Metabolomics and machine learning complement each other to address challenges in biotechnology. To demonstrate the advantage of this approach, we have developed a novel pipeline combining machine learning and metabolomics applied to optimise the yield of a bioprocess in a sample-efficient way. We selected Bacillus subtilis and surfactin as our model study system: surfactin is a valuable lipopeptide with antibiotic activity and can be used for several biotechnology applications. To improve surfactin yield, we performed the optimisation of carbon and nitrogen concentrations in a defined basal minimal medium for *Bacillus subtilis* DSM 3256, via an iterative machine-learning-based testing loop. Methods were fine-tuned to achieve high-quality growth curve data for different carbon and nitrogen combinations (coefficient of variation = 12%). In addition, a flow-injection method for fast measurement of surfactin using multiple reaction monitoring (MRM) was implemented. Thus, considering both experimental data and computational results, samples from the optimisation loop were selected and further analysed by metabolomics to uncover metabolic shifts towards the optimal surfactin yield. This also allowed determination of the presence/amount of specific metabolites, such as other lipopeptides, and correlate them with increased/reduced surfactin yield, as well as understanding their coupling with growth rates. Results revealed intricate relationships between secreted lipopeptides' yields across the tested carbon/nitrogen space, including multiple surfactin variants and iturin A. This work provides an overall framework for bacterial strain improvement using high-throughput metabolomics and machine learning algorithms, opening the scenario for future applications in bacterial production. The platform can analyse 100 samples in ~2 hours, allowing loop experiments to be performed in a few days, providing a seamless approach that will be further automated in the future.

Keywords: *Metabolomics, Machine Learning, Loop, Flow-injection, Optimisation*

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In search of potential biomarker panels for ccRCC detection: Integrative metabolomics approaches

Kidney cancer is accepted to be a metabolic disease. Renal cell carcinoma (RCC) is the main type of kidney cancer with more than 50% of cases incidentally diagnosed. Clear cell RCC (ccRCC) is the most common histological subtype and is characterized for being a glycolytic and lipogenic tumor, exhibiting abnormal cholesterol metabolism and fat storage. Previous findings from our research using an ultraperformance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS)-based lipidomics-machine learning strategy provided a 16-lipid panel, which allowed discriminating ccRCC patients from controls with 77.1% accuracy in an independent test set. Objectives: Serum samples from the same cohort comprised of ccRCC patients and controls were interrogated with a different reverse phase chromatographic method aimed at detecting metabolites of medium polarity and providing additional tools to investigate ccRCC biology and disease progression. In an attempt to increase analysis throughput, we have additionally evaluated the feasibility of detecting the 16-lipid panel using Direct Analysis in Real Time (DART)-HRMS. Results: The UPLC-HRMS-based metabolomics method coupled to a L1-regularized logistic regression model provided a 10-feature panel, currently under identification, that allowed discriminating ccRCC patients from controls with 80.6% accuracy in an independent test set. Regarding the DART-HRMS-based method, a subgroup of the 16-lipid panel comprised of LPC(16:0/0:0) and PC(18:2/18:2) in addition to cholesterol, allowed differentiating ccRCC patients from controls in a pilot sub-cohort (n=18) with 83.3 % accuracy. Conclusions: High classification model performance was accomplished with an independent test set for the metabolomics assay, and with a training set under cross-validation for DART-MS experiments. For the latter, cholesterol, which is only detected by means of chemical ionization mechanisms operating in DART and is known to be altered in ccRCC, improved the classification performance. Future perspectives: Results are promissory for translating these fingerprints to the clinical setting after developing targeted assays in larger cohorts.

Keywords: *metabolomics, lipidomics, clear cell renal cell carcinoma, ultraperformance liquid chromatography, ambient mass spectrometry*

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POSTER PRESENTATIONS

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Promissory photoprotective extracts from Colombian Caribbean brown algae.

Introduction: The increasing number of dermatological illnesses, the higher radiation levels on earth's surface, and the increasing evidence of the impacts of some synthetic filters on the environment and human health, reflect the necessity of finding new effective skin photoprotection products. In this scenario, Colombian phycodiversity has an attractive potential, considering the reports related to the photoprotective activity, stability, and biocompatibility of some previously algae-isolated compounds. However, only recently have we started a systematic evaluation of the chemical composition of these resources in our country. Due to the application of the algal compounds with photoprotective activity sought here, we proposed a metabolic profiling strategy based on HPLC-DAD profiles and sun protection factors. To our knowledge this is the first report of a metabolic profiling-based selection approach in the photoprotection research area. Objective: Develop a methodology for the selection of brown macroalgae extracts with photoprotective activity, correlating the results of the in vitro evaluation of sun protection factors, antioxidant activity, and HPLC-DAD metabolic profile. Results: Interesting SPF concentration-dependent values between 0.403 and 2.915 in 50 ppm extract solutions, UVAr between 0.167 and 3.623, λ between 335 and 393 nm, and antioxidant activity in six organic and four butanolic extracts. Heatmaps with this information grouped extracts according to their polarity, displaying the highest values in the organic extracts conglomerate. Metabolic HPLC-DAD profile analysis through the MCR-ALS algorithm differentiated 268 compounds with different UV-Vis absorptions. Conclusion: Selection of the organic extracts from *Dictyota* sp. (DP12301), *Stypopodium zonale* (EP10501-10901-11101), and *Turbinaria tricostata* (UP10201) with the most promising photoprotective activity.Future Perspectives: Chemical characterization of the selected extracts to identify compounds with the observed activity. *In vivo* evaluation of the sun protection factors and sunscreen pre-formulation assays. Testing of this photoprotection analytical-metabolic profiling-based screening approach in other organisms.

Keywords: *brown algae, photoprotection; sun protection factors; antioxidant activity; metabolic profile.*

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Untargeted metabolomics analysis for unrevealing the mode of antibacterial action of essential oils from Colombian medicinal plants

The alarming loss of antibiotics' effectiveness in treating bacterial infections has shown the urgency to develop new antimicrobial agents. In a world that is running out of new antibiotics, essential oils (EOs) from medicinal plants promise to be an alternative and sustainable source of new drugs. However, the mechanism responsible for the biological activity of several EOs is not yet clear and this has limited the use of these natural products as therapeutic alternatives. This study aims to investigate the metabolic changes in *Escherichia coli* during the antibacterial action of *Lippia Origanoides*'s essential oil. Methods: *E. coli* ATCC11775 was selected for the study and cultured in Lysogeny broth at 37°C. Minimum inhibitory concentration (MIC) was determined for the EO by using the microbroth dilution method. Metabolic profiles of the samples treated with EO, and control bacteria were obtained by using liquid chromatography hyphenated with mass spectrometry. Data processing, metabolite identification, and statistical analysis are currently in progress aided by bioinformatic tools and databases such as Thermo Xcalibur™, XCMS version 3.7.1, MetaboAnalyst version 5.0, and CEU mass-mediator(5). Results and perspectives: The MIC50 for *L. origanoides* EO was 0,24mg/mL. A bacterial inoculum of approximately 1x108 UFC/mL was treated with this concentration of EO. The bacterial cultures were incubated in 2 mL conical tubes for 8 hours at 37°C. Liquid-liquid extraction was performed to obtain cellular extracts and the chemical profiles were analyzed in an UHPLC-ESI-Q-Orbitrap-HRMS equipment. Since this work corresponds to ongoing research, the results concerning metabolic profiling are currently being analyzed. We expect that this strategy will allow us to identify the discriminant metabolites that can be associated with the antimicrobial mode of action of the EO from *L. origanoides* on *E. coli.*

Keywords: *Drug development; natural products; pathogenic bacteria; mass spectrometry, metabolomics*

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Chemometric study of Colombian cocoa crops: flavonoid and methylxanthine content in unfermented raw cocoa beans.

Introduction: *Theobroma cacao* L. has been recognized as an important source of polyphenols and alkaloids, and a variety of pharmacological properties have been attributed to these compounds, that is why cocoa should be considered for its application in the development of functional ingredients for food, cosmetic and pharmaceutical formulations. Objectives: The aim of this study was to evaluate the levels of chemical markers in unfermented raw cocoa beans in introduced and regional clones cultivated in Colombia, using multivariate statistical methods for the analyze of flavanol monomers (epicatechin and catechin), flavanol oligomers (procyanidins) and methylxanthine alkaloids (caffeine and theobromine) of cocoa samples. Results: The results showed that geographic origin, the harvest conditions of each region and the year of harvest may contribute to major discrepancies between cocoa beans, although significant differences were not observed between universal and regional clones. Turbo cocoa samples were notable for their higher flavanol monomer content, Chigorodó cocoa samples for the presence of both types of polyphenol (monomer and procyanidin contents) and the Northeast cocoa samples for the higher methylxanthine content. The univariate analysis allowed us to establish that EET-96 had the highest contents of both flavanol monomers (13.12 ± 2.30 mg/g) and procyanidins (7.56 ± 4.59 mg/g). Conclusions: The establishment of characteristics other than agronomic ones or production yields, such as the contents of compounds with beneficial properties for health, is an important milestone for decision making based on the projections of cocoa cultivation, in this context, EET-96 clone, due to its low variability and higher contents of polyphenols and methylxanthines, is the most promising one for commercial purposes. Future perspectives: The development of a functional ingredient enriched in these metabolites to be incorporated into a final consumer product. It would contribute to added value in the cocoa production chain and its sustainability.

Keywords: *catechins, methylxanthines, procyanidins, clones, cocoa*

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Estudio volatilómico de las especies *Stenospermation weberbaueri* Engl. y *Anthurium caramantae* Engl. (Araceae) con espacio de cabeza dinámico y cromatografía de gases acoplada a espectrometría de masas.

Los Compuestos Orgánicos Volátiles (COV) son producto del metabolismo secundario de las plantas que aportan información acerca de procesos bioquímicos que se dan al interior de las células. En el caso de la familia Araceae, los COV median la atracción de polinizadores durante la antesis. Sin embargo, los estudios de este tipo en esta familia se han concentrado en pocos géneros. En Colombia, los estudios sobre análisis de fragancias y su rol biológico van en crecimiento, sin embargo, son nulos en lo referente al análisis de perfiles volátiles de la familia como tal. Se estudiaron los COVs de *S. weberbaueri* y *A. caramantae* por medio de la técnica de muestreo DHS, el análisis del perfil volátil se hizo por medio de CGEM. La identificación de los metabolitos se hizo por comparación con librerías espectrales y el cálculo de índices de retención. Se llevó a cabo el PLSDA y VIP score para encontrar los metabolitos que aportan diferencias significativas en las fases de antesis; finalmente la interpretación biológica se hizo con Biocyc, Chebi y KEGG para el análisis ontológico de los compuestos y las posibles rutas biosintéticas de los metabolitos. Durante las fases de antesis se obtuvieron 28 metabolitos de *A. caramantae* y 54 metabolitos de *S. weberbaueri*. El PLSDA para A. caramantae mostró que 8 compuestos son quienes tienen un factor discriminante entre fases, de los cuales se pudieron identificar 4; en el caso de *S. weberabueri* se obtuvieron 20, de los cuales se pudieron identificar 9. En conclusión el uso de DHS junto con la CGEM permitió conocer el perfil volátil de las dos especies durante las fases de antesis, demostrando la eficiencia y versatilidad al permitir recolectar in situ los volátiles, al no causar daños mecánicos en las plantas que podrían alterar la composición química. El PLSDA junto con el gráfico VIP permitieron determinar una clara separación entre fase a partir de los COV, en donde estos metabolitos resultaron tener alguna relación en procesos de atracción de polinizadores en otras especies de Araceae y otras familias. Los COV involucrados resultan ser producto de la ruta de los ácidos grasos y los terpenos.

Keywords: *Dynamic Headspace; Cromatografía de gases acoplado a espectrometria de masas; Compuestos orgánicos volátiles; Araceae*

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Alteraciones metabólicas ocasionadas por intoxicaciones letales con cianuro y monóxido de carbono: Aplicaciones en forense

En Colombia en el año 2019, el INML y CF realizó 24.404 necropsias por muertes violentas. El 12% fueron accidentales y el 11% suicidios, donde están involucrados tóxicos como el monóxido de carbono (combustiones incompletas, gases) y el cianuro (ingesta en conductas suicidas). El alto número de casos que ingresan al sistema medicolegal generan represamientos en los análisis, reprocesos y dificultades en la administración de justicia. Objetivo: Estudiar las alteraciones metabólicas en muertes ocasionadas por intoxicaciones con monóxido de carbono y cianuro, debido a la poca estabilidad de estos analitos en la matriz, el incremento casuístico y al interés en extender el conocimiento en los mecanismos de toxicidad. Resultados: El análisis multivariado evidenció discriminación entre CO (monóxido) contra MV (muertes violentas) y CN (cianuro) contra MV, generando modelos con valores aceptables de varianza predicha (R2) y capacidad predictiva (Q2). Se obtuvieron 30 características moleculares significativas para la plataforma UHPLC/QTOF-MS y 31 para GC/QTOF-MS. Dentro de los tipos de compuestos alterados están las carnitinas, glicerofosfolípidos, glicerofosfocolinas, carbohidratos, aminoácidos y ácidos. En la intoxicación con CO se observan alteraciones en las rutas metabólicas de la D-Glutamina y D-glutamato, la piridina y la biosíntesis de la arginina. También hubo un incremento en las carnitinas involucradas en el transporte mitocondrial de los ácidos grasos y alteraciones en el metabolismo de la glicina, la serina la metionina y la betaína. En las intoxicaciones con CN se observan cambios en el metabolismo de la galactosa. Para ambos tóxicos se evidenciaron alteraciones en el metabolismo del nicotinato y la nicotinamida. Donde los cofactores derivados del nicotín adenin dinucleótido (NAD) son fundamentales para funciones esenciales como la respiración mitocondrial, el ciclo de Krebs y la producción de ATP. Esta inhibición es consistente en ambos casos con la hipoxia como signo de envenenamiento. Conclusiones: Los resultados confirman que un enfoque metabolómico multiplataforma no dirigido permite una amplia cobertura de metabolitos en sangre total. Éste es el primer estudio documentado que compara la composición química del metaboloma de muertes por CO y CN. Las alteraciones en los perfiles metabólicos pueden ser potenciales diferenciadores y/o clasificadores en intoxicaciones con los tóxicos volátiles de estudio en aplicaciones de interés forense.

***Keywords**: Forense, monóxido, cianuro, toxicología, medicina legal*

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NMR spectroscopy applied to the metabolic analysis of homemade products based on *Cannabis sativa* for medicinal purposes

Cannabis sativa is a versatile plant often used to produce fiber, oil and medicine. It is currently gaining popularity due to the high nutritional properties of the seed oil and the pharmacological activity of the cannabinoid fraction in the inflorescence. In recent years, new laws and regulations allowed the use of homemade medicinal products around the world. In Argentine, the use and production of homemade cannabis-based medical products were legalized in 2017. Although its popularity has increased since then, home production still faces uncertainties about the cannabinoids content in medical preparations. Key factors such as the use of stable genotypes or reproducible extraction methods have a significant influence on the final composition of medicinal products. In this context, facile and rapid quantitative methods to determine the cannabinoid content are crucial. The current standard methods (e.g. liquid chromatography combined with tandem mass spectrometry), require a time-consuming multistep sample preparation. Results: In this study, a quantitative nuclear magnetic resonance spectroscopy (qNMR) method for screening cannabinoids in medicinal products was developed. Using this qNMR method, we analyze the chemical composition of a population of 250 cannabis-based medicinal products made by users and producers from Buenos Aires, Argentina. We found that more than 63% of the analyzed preparations have a total concentration below 10 mg/mL, with a median of 7.54 mg/mL. Δ9-tetrahydrocannabinolic acid (Δ9-THCA) and Δ9-tetrahydrocannabinol (Δ9-THC) were the most concentrated cannabinoids found in 90% of the samples. In contrast, the concentrations of cannabidiol (CBD) and cannabidiolic acid (CBDA) were significantly lower, with a median of 0.07 and 0.08 mg/mL, respectively. Conclusion: Our results indicate that, despite the different variables considered in the manufacture of artisanal preparations, there are significant differences in concentration levels compared to the commercial products available for medicinal use in Argentina. Expanding this research to a broader population and geographic areas will be crucial in gaining valuable quantitative data of homemade cannabis products and thus providing users and doctors with better tools for their application in specific therapies.

Keywords: *NMR, Metabolomics, Cannabis, Medicinal, Homemade*

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Metabolomic comparison between wild and cultivated *Porophyllum ruderale* Jacq. Cass (Asteraceae) using UPLC-ESI-QTof-MS technique.

Porophyllum ruderale Jacq. Cass (Asteraceae) is a non-convention edible plant found in south of Brazil with interesting and complex chemical profile. Objectives - Thus, the present study phytochemically explore *P. ruderale* and compare individuals of wild and cultivated plant samples. Results: UPLC-ESI-QToF-MS technic afforded putatively identification of 36 substances including mainly phenolic acids, fatty acids and chlorophyll derivatives. Of that, 21 substances were tentatively identified as diterpene aminoglycoside wedeloside derivatives, described for the first time in *P. ruderale*. Designed for that, PCA (principal component analysis) and cluster analysis were used to distinguish different metabolites among wild and cultivated *P. ruderale* samples, that were separated into well-defined groups. The principal components (PCs) of all detected signals in positive mode (n=3846) where computed in order to reduce dimensionality and eliminate redundancy. The algorithm centered the data and used the singular value decomposition (SVD) to compute the PCs. Working with the firsts 23 PCs, that explains more than 99% of the dataset variance, was built an hierarchical binary cluster tree. A k-means clustering algorithm was also used to check the clustering partitioning coherence. The optimum number of clusters for the k-means method was determined as eight clusters. Conclusions - Finally, PC3 cumulative variance could explain more than 81% of the total data variance and the ions m/z 918, 920 and 962 influenced the PC3 cumulative percent on 99%, 99%, 97% and 97%, respectively. These ions correspond to new skeletons of diterpene aminoglycosides, supported by tandem mass (MS2) fragmentation profile. Future perspectives: Unequivocally elucidate the identified diterpene aminoglycoside wedeloside derivatives through chromatographic isolation, and fully characterize those using spectrometric technics, especially NMR.

Keywords: *Porophyllum ruderale, diterpene aminoglycosides, UPLC-ESI-QToF-MS, Principal Component Analysis, K-means analysis.*

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Effect of potassium phosphite application on the metabolite biosynthesis during the interaction carnation (*Dianthus caryophyllus* l.) - *Fusarium* oxysporum f. sp. diathi (fod)

Colombia is recognized worldwide for its huge floricultural diversity, with more than 50 flower species and more than 1500 varieties produced annually. In particular, carnation represents 14% of flower exports. The country is positioned as the largest producer of carnations globally, with more than 100 varieties planted under hydroponic cultivation. However, its production is greatly affected by the fungus *Fusarium oxysporum* f. sp. dianthi (Fod), the vascular wilting-causing phytopathogen. The resistance induction by using inducers of biotic or abiotic origin can represent alternative for efficiently controlling the disease with a low environmental impact. In order to understand such induction in terms of the involved biochemical response, the present study investigated the interaction between carnation and Fod, determining those metabolites synthesized in plant by the effect of commercial potassium phosphite application. The resulting information rationalizes the development of a novel alternative for managing and efficiently controlling plant disease. The untargeted metabolomics-based analysis allowed us to identify 64 metabolites for the plant group treated with potassium phosphite and, subsequently, challenged with Fod. Compared to the control group, the results revealed increased levels of proline, a marker of stress. In addition, the accumulation of glutamine, citrulline, urea, and glutamate suggested stimulating the arginine biosynthesis pathway, resulting in the urea and citrate cycle. It has also been shown that the accumulation of nitric oxide from arginine is an important signaling component of the plant defense response against pathogen infection. From this outcome, potassium phosphite can be considered an effective resistance inducer, activating the plant defense mechanisms against Fod, involving an earlier and more robust response. However, futuretranscriptional and field studies are necessary to determine the role of this resistance inducer against vascular wilting.

Keywords: *Untargeted metabolomics; Resistance Induction; Fusarium oxysporum, carnation; vascular wilting*

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Estudio de variaciones en el metaboloma de pacientes con Endometriosis con y sin tratamiento mediante ^{1}H RMN

La endometriosis es una enfermedad crónica de etiología desconocida definida por la implantación y crecimiento benigno del tejido endometrial fuera del útero. El "patrón de oro" para su diagnóstico es la visualización directa de lesiones mediante laparoscopía, procedimiento invasivo e inapropiado para el seguimiento de pacientes.
Se plantea el desarrollo de nuevos métodos no invasivos, rápidos, y de costo moderado capaces de diagnosticar la endometriosis desde el comienzo de los síntomas. Los métodos quimiométricos basados en espectroscopia de ^{1}H RMN de muestras biológicas de fácil acceso, sangre y orina, son una alternativa con enorme potencial, y desarrollarlos es un objetivo de este proyecto. Debido a la variación en los tratamientos recibidos, y para evaluar el alcance y robustez de la metodología, comparamos muestras de pacientes tratadas con el fármaco dienogest (n=24), pacientes sin tratamiento actual (n=15), pacientes que núnca recibieron tratamiento (n=16), y voluntarias sanas (n=24). Se obtuvieron los perfiles de ^{1}H RMN de muestras de suero mediante experimentos CMPG con presaturación de agua y análisis estadísticos multivariados, incluyendo análisis de componentes principales (PCA) y regresiones de mínimos cuadrados parciales (OPLS-DA).
La comparación de datos de enfermos con individuos sanos indica una clara diferenciación, independiente de los tratamientos farmacológicos. Un estudio de los valores de carga del OPLS-DA permitió correlacionar al colesterol, VLDL, LDL y lactato con los individuos sanos, y NAc-colina, NAc-glucosamina y acetoacetato, con los individuos enfermos. El análisis no permite discriminar pacientes bajo distintos tratamientos, lo que nos habla de la robustez e independencia del método para calificar a las pacientes enfermas. Se obtuvo un modelo preliminar que permitiría diagnosticar a las pacientes con endometriosis, independientemente del tratamiento recibido. A futuro se pretende elaborar un modelo paramétrico que será evaluado en muestras de pacientes con sospecha de la enfermedad que están a la espera de una laparoscopía.

***Keywords**: molecules of high added value; cupuaçu; chemistry of natural products; amazon biodiversity*

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Estudio volatilómico en aguacate Hass (*Persea americana* Mill. cv.) para la identificación de biomarcadores de toxicidad.

En el mundo actual las ciencias ómicas tiene diversas aplicaciones, cada una de ellas comprende el desarrollo de nuevas tecnologías que permitan una mejor comprensión y desarrollo de estas ciencias. Para el caso de la metabolómica, dentro de esta ciencia se encuentran los estudios volatilómicos, los cuales se caracterizan por estudiar el perfil volátil (volatiloma) que genere una determinada matriz, es decir, analizar la presencia de los compuestos orgánicos volátiles (COVs) en una matriz. Hoy en día, los estudios volatilómicos tienen diversas aplicaciones en la detección temprana de enfermedades, evaluación del origen alimenticio, valoración de la calidad alimentaria, identificación de biomarcadores de maduración y toxicidad, entre otros. Por ello, es importante realizar una evaluación de la calidad alimentaria en el aguacate Hass (*Persea americana* Mill.) cv. puesto que, para evitar pérdidas en los cultivos y en el producto al momento de su poscosecha, es habitual que el campesino realice un rociado con diversos tipos de plaguicidas para evitar el desarrollo y proliferación de estos fitogatógenos. En el mundo de la química analítica e instrumental, se han reportado metodologías como QuEChERS y la microextracción en fase sólida (SPME), para el análisis residual de plaguicidas. Estos métodos apoyan los estudios volatilómicos permitiendo generar un gran impacto en los procesos de inocuidad alimentaria, evaluando la calidad de los alimentos y la presencia de agentes tóxicos en estos. Por ello, se pretende evaluar los procesos de inocuidad alimentaria del aguacate Hass, a través de la identificación y cuantificación de los residuos de plaguicidas presentes en cáscara, pulpa y semilla de este fruto; posteriormente por medio de un estudio volatilómico se evaluará la presencia de los metabolitos respuesta generados en *Persea americana* Mill. cv. por la presencia de plaguicidas en dicha matriz, a través de cromatografía de gases con espectrometría de masas (GC – MS).

Keywords: *Volatilómica, aguacate Hass, biomarcadores, SPME, QuEChERS*

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MALDI-Imaging analysis of haemantidine and 6-epihaemantidine in leaves of *Crinum scabrum* Herb. (AMARYLLIDACEAE)

Crinum scabrum is a plant of the Amaryllidaceae family. This family is known as a producer of unique isoquinolinic alkaloids that have many biological activities described in the literature. It is no data about the distribution of these alkaloids in these plants. Many of these alkaloids is useful against pathogens and the distributed along the tissues of the plants. To demonstrate the dispersion of the main compound found in *Crinun scabrum*, leaf cuts were performed in a cryostat embedded with tissue tek® (Sakura), the cut thickness was 70µm and the temperature used was -20 °C. Then, the HCCA matrix at a concentration of 7 mg mL-1 diluted in 1:1 acetonitrile:water containing 0.1% TFA was applied on the sections using imageprep (Bruker®). Subsequently, the plate coated with HCCA matrix was inserted into the MALDI-TOF/TOF Ultraflextreme equipment (Bruker Daltonics) and the parameters used to acquire the spectra that resulted in the formation of the image of interest were: reflector mode of analysis, 500 laser shots per spectrum, PIE (Pulsed ion extraction) 130 ns, positive analysis mode, laser frequency 1000 Hz. For external calibration of the equipment, quercetin standard was used. Spectra were acquired in the 0-1000 Da mass range. After the analysis, was demonstrated that the main compound is distributed for all analyzed tissue. That fact could be related to the protection against some pathogens like caterpillar that very common as predator in Amaryllidaceae species.

Keywords: *Amaryllidaceae, Maldi-Imaging, protection, alkaloids*

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Untargeted phyto-metabolomics using LC-MS-QTOF reveals the influence of altitudinal variation in chemical composition of Bamboo species.

Bamboo species have traditionally been used as a building material and as a potential source of bioactive substances. These species produce a wide variety of phenolic compounds considered biologically active, especially flavonoids and hydroxycinnamic acid derivatives. However, the effect of altitude is still insufficiently known on the metabolome of these species. Aim of the study was to investigate the changes induced by altitudinal variation in the chemical composition of phenolic compounds from 12 different bamboo species classified into two groups: low-altitude (~0 - 1500 m) and high-altitude (~1500 - 3000 m) using untargeted phyto-metabolomics approach based on LC-MS-QTOF. Differences in metabolic profile between groups were evaluated by univariate and multivariate analysis. According to the results obtained, the chemical classes of metabolites increased with low-altitude were hydroxycinnamic acid derivatives highlighting quinic acid (QA) (fold change: 5.04, p-value: 0.001). QA is a precursor compound in the biosynthesis of caffeoylquinic acids and other bioactive compounds with important role in the defense against biotic or abiotic stress in plants. On the other hand, flavonoids were the group of metabolites that increased with high-altitude, being an opportunity to explore the biological potential of these compounds. As far as we know, this is the first report of changes induced by altitudinal variation in the chemical profile of different bamboo species. Our results could provide new strategies to enhance the production of phenolic compounds in bamboo crops in our country.

Keywords: *Bamboo, phyto-metabolomics, natural products, altitudinal variation, and flavonoids*

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Metabolic profiling of ethanolic extracts from Colombian species of *Guadua Kunth* (Poaceae)

The *Guadua Kunth* (Poaceae) genus is recognized worldwide for its ecological, social, economic, cultural, and commercial applications. Within the reported studies of chemical composition for this genus, only preliminary phytochemical analyzes are presented, determining the presence of groups of secondary metabolites corresponding mainly to flavonoids and derivatives of phenolic acids. Related to pharmacological aspects, its applications as an antioxidant, antimicrobial and antiseptic stand out. However, to this date, there are no reports that contribute to establishing its metabolic and phytochemical profile aimed at the search for compounds with biological potential. In this research, the metabolic profile of 29 ethanolic extracts belonging to 10 different species of the genus *Guadua Kunth* was studied to perform their chemical characterization based on chromatographic methodologies. The methodology included the collection and preparation of the extracts, followed by the study of the metabolic profile using different chromatographic techniques such as HPTLC, UPLC-DAD, and LC-MS-QTOF. The study of the metabolic profile allowed us to determine the presence of different groups of secondary metabolites such as flavonoids in their aglycone and glycosidic form, derived mainly from phenolic acids and terpenes. In the case of glycosidic flavonoids, a significant presence was determined in some species such as *Guadua angustifolia* Kunth and *Guadua amplexifolia* Presl. It is important to highlight those previous reports have identified this type of metabolites by means of preliminary phytochemical analysis. This research contributes to the chemical characterization of these species using different modern instrumental techniques, providing the basis to initiate in-depth chemical and biological studies to find compounds with biological potential. Correlation studies are currently being carried out using a metabolomic approach with these species, based on biological activity and chemical information to identify compounds with biological potential.

Keywords: *metabolic profiling, natural products, Guadua, flavonoids and biological potential*

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Serum metabolomics analysis of patients with Sjögren's syndrome

Sjögren's syndrome (SS) is a chronic autoimmune systemic disease, characterized by the compromise of exocrine glands secondary to lymphocytic infiltration, especially the lacrimal and salivary ones. SS is a progressive and complex disease with a genetic component that also involves the activation of immune cells and the production of autoantibodies. Although recently metabolomics studies have been carried out to find biomarkers in tears, plasma, and urine; the pathophysiological process of the disease has not yet been clarified, so it is necessary to continue investigating this pathology. Aims and methods: This study sought to identify metabolites in serum from SS patients and healthy controls that could serve to characterize the disease. Serum samples from 43 SS patients and 101 healthy controls were obtained. The metabolite profiles of serum were analyzed by LC-MS in both positive and negative electrospray ionization modes, followed by data alignment and filtration. The selection of statistically significant molecular characteristics was performed by multivariate (MVA) and univariate (UVA) statistical analysis. The MVA was performed using the SIMCA-P + 16.0 software (Umetrics) and the UVA was performed using Matlab. Regarding the UVA analysis, the p value was determined by parametric tests (Mann-Whitney U test) and the p value was calculated with a post hoc False Discovery Rate (FDR) correction. Results: The main metabolites in samples from SS patients were related to the metabolism of phospholipids, fatty acids, and amino acids such as alanine and histidine. Conclusions: In conclusion, our findings showed separate metabolic profiles for SS patients compared to healthy controls that could be useful to better understand the pathophysiological processes of this disease.

Keywords: *Sjögren's syndrome; metabolomics; serum; fatty acids; amino acids.*

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Blood serum lipidomics of patients with the severe respiratory syndrome, positive and negative for SARS-CoV-2, by NMR techniques

SARS-CoV-2 is a recent coronavirus that causes a disease characterized by acute respiratory infections. Metabonomics and lipidomics can be used to study how an organism responds to determined stress conditions and allow us to understand the infection process and how it affects the patient. Objectives: To map serum metabolic changes in hospitalized patients with moderate and severe COVID-19 symptoms when compared to individuals that tested negative for SARS-CoV-2, clinically with similar symptoms with COVID-19 patients, and healthy volunteers. Methodology: Serum samples were obtained from patients and volunteers at the University Hospital (UNICAMP, Campinas, Brazil) and stored at -80 °C until analysis. Lipids were analyzed using diffusion-edited ^{1}H-NMR in D_2O-diluted serum samples and after methanol/ chloroform extraction. High-resolution ^{1}H-NMR spectra were obtained on the Bruker AVANCE III 600 MHz instrument at 25 °C. Results: Lipid loads in severe and moderate COVID-19 patients differed in quantity and specie types. Fatty acids, cholesterol, and cholesterol esters CH_3-groups distinguished the analyzed groups of samples, while -CH_2- and -CH=CH- groups distinguished SARS-CoV-2 positive and negative patients in cohorts of patients with severe and moderate symptoms. On the other side, higher levels of phosphorylcholines and sphingolipids were detected in the control group compared to the patients. The two COVID-19 groups of patients showed characteristic very-low-density lipoprotein (VLDL) and low-density lipoprotein levels. Conclusions and future perspectives: Samples from COVID-19 patients were successfully analyzed using ^{1}H-NMR and showed significant differences in lipid levels when compared to healthy volunteers or clinically similar cohorts of patients that tested negative for SARS-CoV-2. The observed differences can be linked to virus replication, and the severity of the symptoms suffered among COVID-19 patients. It is still to be discovered if lipidomics can be applied to predict the gravity of the disease.

Keywords: *COVID-19; respiratory infections; 1H-NMR; Metabonomics; Lipidomics*

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Description of the metabolomic profile of hCMEC/D3 cells (BBB model) under treatment with vasoactive peptides in a cell culture environment.

Metabolomic studies are one of the most informative tools in descriptive studies regarding the comparison of conditions in biological models. In this study, a metabolomic analysis and comparison was performed in hCMEC/D3 cell groups cultured under treatment conditions with Angiotensin II (Ang-II) and Angiotensin 1-7 (Ang 1-7). The results are insightful for further exploration of the pathways associated with the metabolomic response observed. Objectives and Methods: The scope of this study was to describe the metabolomic patterns present in the cells treated with vasoactive peptides to elucidate metabolic pathways associated with responses to their activity. hCMEC/D3 cells were cultured under manufacturer-specified conditions. Treatments with Ang-II, and Ang 1-7 were performed in the log phase at a 1.2 µmolar as stated: (i) Group 1: Ang-2, (ii) Group 2: Ang 1-7, (iii) Control (Normal Saline). Metabolomic analysis was performed with HPLC-MS and GC-MS. Statistical analysis was performed with SIMCA, R and MetaboAnalyst. Results: Differences were found in the metabolomic profile of the treated cells. More than 80 substances were found to be expressed differentially between the groups. High differences were observed in the expression of metabolites associated with aminoacid, fatty acyls and glycerophospholipid pathways. Conclusions: The preliminary results in this study are insightful for recognizing several metabolic pathways associated with stress responses in the treated cells. Further projects are needed to explore the mechanisms of these pathways in the physiological response of the BBB in the presence of vasoactive peptides. Further Remarks and Perspectives:
Studies associated with vasoactive peptides and BBB barrier models are promising in elucidating new action mechanisms of these molecules in different pathologies. Multiplatform experimentation will be needed to explore these findings further.

Keywords: *vasoactive peptides, angiotensins, BBB, hCMEC/D3*

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Lipid profile alterations in a group of Colombian patients with moderate rheumatoid arthritis and systemic lupus erythematosus

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are autoimmune diseases in which genetic, immunological and environmental factors promote the loss of immune tolerance to self-cellular components.Their complexity is remarkable and it is necessary to implement new tools to gain a better understanding of both diseases. Metabolomics and lipidomics aim to measure the complete set of metabolites and lipids in a biological sample. Their levels might be easily correlated with disease phenotypes. Objective: To compare the lipid profile between a group of patients with RA, SLE and healthy controls. Results: We obtained plasma samples from 23 patients with RA, 22 with SLE and 27 healthy controls (HC) and the metabolites and lipids extracted were analyzed using RP LC MS. The significantly altered metabolites were mainly lipids. Most of the phosphatidylcholines (PC), phosphatidylethanolamines (PE) and phosphatidylinositols (PI) were decreased in RA and SLE, but the corresponding lysophospholipids (lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE)) were only significantly increased in RA. A considerable percentage of Sphingomyelins (SM) were decreased, while triglycerides (TG) were increased in both diseases. The lipids PE, PI, LPC, LPE and TG were increased in RA with respect to SLE. Conclusions and perspectives: This study describe the importance of lipid metabolism in the phisiopathology of RA and SLE. The altered levels of phospholipids and sphingomyelins are the result of their constant turnover in key cellular processes like membrane cell composition and cell proliferation. The lower levels of phospholipids in SLE patients respect to RA can be associated with the fact that many SLE patients have detectable levels of phospholipid autoantibodies. The lipid metabolism is essential for immune cell processes. This study shows the importance of describing the lipid profile to differentiate RA and SLE from HC but also to distinguish RA from SLE patients.

Keywords: *phospholipids, sphingomyelins, rheumatoid arthrtitis, systemic lupus erythematosus*

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Metabolic profile of ascidian *Euherdmania* sp. and associated bacteria

Prolific producers of diverse and unique compounds, ascidians are marine invertebrates comprised in the Tunicata phylum. *Euherdmania* sp. is a colonial ascidian found in the northeast cost of Brazil which has yielded potent cytotoxic extracts from both the invertebrate and its associated bacteria. Indeed, previous studies revealed 13 out of 28 bacteria recovered from *Euherdmania* sp. produced extracts that inhibited proliferation of HCT-116 cell line. Methods: Herein, these bacteria were cultivated in solid media, extracted and analyzed for their chemical profiles. Additionally, four samples of *Euherdmania* sp. were collected at Taíba Beach (State of Ceará), extracted, partitioned and chemically probed. All samples were assessed through HPLC-MS/MS. In this sense, chromatograms were processed by MZmine 2.32, while MS/MS data were uploaded to the Global Natural Products Social (GNPS) molecular networking platform. Results and Conclusions: Metabolomic analysis of crude extracts of *Euherdmania* sp. and of their methanolic and ethyl acetate fractions indicated that compounds recovered in the ethyl acetate fraction clustered better with unfractionated extracts, while most peaks shown in the methanolic fraction were not observed therein. Moreover, in crude extracts and fractions, aminoacids, phosphocoline, lipid derivatives groups, and staurosporines (exclusively to one ascidian sample), were identified; the later can be correlated with its microbiota. The analyses of active and non-active bacteria-derived extracts showed that these do not share surfactin and desferrioxamine with ascidian extracts and fractions, indicating that unique metabolites may be linked to the bacteria. More studies are ongoing to better understand the metabolic profile of *Euherdmania* sp. and of its microbiota. Future perspectives: Metagenomics analyses are ongoing to correlate identified compounds with the phylogeny of bacteria.

Keywords: *Metabolic profile, ascidian, Euherdmania, associated bacteria*

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Metabolomic signatures of systemic lupus erythematosus

Autoimmune diseases (AD) occur because of the combination of genetic and environmental factors. These diseases are characterized by a loss of tolerance of the immune system against self-antigens. The pathophysiological mechanisms associated with systemic lupus erythematosus (SLE) are still unknown. Recently, high-throughput metabolomic profiles have been described allowing the description of molecules that could be related to different ADs. Several metabolites have been involved in essential functions for the development of processes associated with inflammation. Unfortunately, the metabolomic alterations in this group of pathologies are still uncertain, so knowing their alterations is essential to understand the development and behavior of ADs. Objectives: A case-control study was conducted 27 patients with SLE, and 30 healthy controls. Patients with poliautoimmunity or multiple autoimmune syndrome were excluded. The serum samples were analyzed under the platform RP-LC/MS-QTOF in electrospray ionization modes. For all comparisons, selection of statistically significant molecular features was performed by multivariate and univariate statistical analysis, *p*-value was determined by Mann–Whitney U test and calculated with post hoc false discovery rate. Results: 160 altered metabolites were found, either upregulated or downregulated, of which most correspond to fatty acids, glycerophospholipids, amino acids, and nucleosides. In addition, metabolites associated with the metabolism of prostaglandins, taurine and hypotaurine were found, as well as metabolites associated with mitochondrial beta-oxidation, androgens estrogens, purines and bile acids. Compared to the healthy control group, the results showed that most metabolites were upregulated in SLE. Conclusions: These metabolic routes are directly involved in the participation of humoral and cellular immune response, which are key processes in the inflammatory response and the loss of immune tolerance.

Keywords: *Metabolomics, Autoimmunity, Inflammation, Systemic Lupus Erythematosus.*

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Metabolic composition of *Myrciaria dubia* (Kunth) McVaugh (camu-camu) subjected to different levels of thermosonication.

Camu-camu belongs to the Myrtaceae family, it is native to the Amazon being present in Colombia, Peru, Brazil, and Venezuela which share borders in this region. Camu-camu has been identified as a rich source of vitamin C, it also presents antioxidant, antiedematogenic, anti-inflammatory, antimicrobial, antihypertensive, neuroprotective, antiparasitic, and analgesic activity.
To increase the concentration of bioactive compounds, thermosonication-assisted extraction was performed. For this purpose, the thermosonication conditions were optimized for camu-camu pulp; and the metabolites extracted from the fruit pulps subjected to the optimal thermosonication conditions were characterized using LC-MS.
Optimization was performed using a Response surface methodology with a Central composite design. Temperature of 30°C to 60°C and time of 10 to 30 min were the parameters selected. Ascorbic acid concentration was used as the response, calculated by differential pulse voltammetry technique. LC-QTOF-MS Untargeted metabolomics approach was performed to metabolic characterization and, Multivariate statistical models was used to identify the metabolites of interest.
Ascorbic acid content was higher at 10 minutes and 30ºC. Metabolic characterization indicated that the content of multiple metabolites increases in the range of 5% to 17% extracting the camu camu fruit to the optimized treatment conditions. PCA and oPLS-DA statistical tests showed a clear separation between the conventional extraction fruit and thermosonication extraction groups. Twenty-five metabolites, some of which no previous report has been found, were annotated.

Keywords: *Untargeted metabolomics, thermosonication, Response surface methodology, camu-camu*

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Metabolomic differences between severe COVID-19 and CAP in patients with acute respiratory failure (ARF) requiring invasive mechanical ventilation (IMV) admitted to the intensive care unit (UCI).

In recent years, lower respiratory tract infections have been the leading cause of mortality and morbidity worldwide, represented mainly by COVID-19 and community-acquired pneumonia (CAP). The main focus has been on the metabolic differences in healthy patients to identify potential prognostic biomarkers and therapeutic targets. Reports about the differentiation of metabolic profiles between patients with COVID-19 and CAP are still scarce, even more regarding the underlying mechanisms of acute respiratory failure (ARF). Thus, we compared the metabolic pathways among severe COVID-19 and CAP patients with ARF. Objectives: To determine the metabolic differences and alterations between COVID-19 and CAP pneumonia patients with ARF. Material and methods: This observational prospective cohort study was conducted in Bogotá, Colombia. Patients diagnosed with ARF secondary to CAP or COVID-19, requiring admission to the intensive care unit (ICU) and invasive mechanical ventilation (IMV), were included. Plasma samples were taken at admission and examined using an untargeted metabolic and lipidomic analysis by reverse phase-liquid chromatography-quadrupole time-of-flight mass spectrometry (RP-LC-QTOF-MS) and hydrophilic interaction liquid chromatography (HILIC-LC-QTOF-MS). Results: A total of 127 patients were included in the study. The metabolomic analysis determined 70 altered metabolites compiling with p-value (≤0.05) and VIP (≥1), suggesting a role for the following altered metabolic pathways: metabolism of arachidonic acid, glycerolipids, glycerophospholipids, linoleic acid, pyruvate, glycolysis, and steroid biosynthesis. Most of these routes involve inflammatory, hypoxic, and pro-thrombotic processes. Conclusions: These metabolic alterations suggest inhibition of innate anti-inflammatory and anti-thrombotic mechanisms in COVID-19 patients, leading to increased viral proliferation, uncontrolled inflammation, and thrombi formation. While in CAP, markers driving hypoxia were found. These results could be used as predictive biomarkers to identify potential therapeutic targets. More studies are needed to corroborate these findings.

Keywords: *COVID-19, community-acquired pneumonia (CAP), Metabolomic, acute respiratory failure (ARF)*

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Plasma metabolomics by Nuclear Magnetic Resonance reveals biomarkers and metabolic pathways associated with the control of HIV-1 infection/progression.

HIV exposure leads to infection, but some individuals show natural resistance to infection, while others, although infected, have a control of the virus replication. Those exposed to the virus who remain seronegative are called HESN. While, within the people who become infected there are two categories: Progressors, who present the typical progression of HIV-1 infection to AIDS, and Controllers, who naturally control HIV-1 replication. Until now, it has not been possible to demonstrate what biological mechanisms determine whether a person is within one group or another, but metabolomics emerges as an alternative.
Objectives: Identify biomarkers and metabolic pathways associated with resistance to infection (HESN) and progression (progressors and controllers) of HIV-1 through NMR analysis of plasma.
Results: We demonstrate using PLS-DA that there are differences in the metabolic profile of progressors, controllers, and HESNs when they are compared to healthy controls. In the discriminant models, 13 metabolites associated with HESN, 14 with progressors, and 12 with controllers were identified, which presented statistically significant mean differences (Wilcoxon test). In progressors, the discriminant metabolites were creatinine, tyrosine, and lipoproteins. On the other hand, in controllers, the discriminant metabolites identified were glutamate, pyruvate, and acetate. The HESN group was characterized by a change in the concentration of lactate and phosphocholine metabolites. In addition, with the help of the KEGG global metabolic network 24 genes closely related to HIV-1 were identified, genes associated with the significant metabolites of each group.
Conclusions and future perspectives: In summary, our results indicate that HIV-1 exposure and infection progression affect the metabolism of individuals and that this phenomenon can be studied by NMR metabolomics. We hope that our research will serve as a basis for future studies that help explain the mechanisms associated with natural resistance to infection in HESN people, and between progressors in controllers of the infection.

***Keywords**: NMR, Metabolomics, HIV-1, Biomarkers, Pathways.*

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Development of an optimized method for processing peripheral blood mononuclear cells for ^{1}H-nuclear magnetic resonance based metabolomic profiling.

Human peripheral blood mononuclear cells (PBMCs) are part of the innate and adaptive immune system and form a critical interface between both systems. Studying the metabolic profile of PBMC could provide valuable information about the response to pathogens, toxins or cancer, the detection of drug toxicity, in drug discovery and cell replacement therapy. Objectives: The primary purpose of this study was to develop an improved processing method for PBMCs metabolomic profiling with nuclear magnetic resonance (NMR) spectroscopy. To this end, an experimental design was applied to develop an alternative method to process PBMCs at low concentrations. Results: The design included the isolation of PBMCs from the whole blood of four different volunteers, of whom 27 cell samples were processed by two different techniques for quenching and extraction of metabolites: a traditional one using organic solvents and an alternative one employing a high-intensity ultrasound probe, the latter with a variation that includes the use of deproteinizing filters. Finally, all the samples were characterized by ^{1}H-NMR and the metabolomic profiles were compared by the method. As a result, two new methods for PBMCs processing, called Ultrasound Method (UM) and Ultrasound and Ultrafiltration Method (UUM), are described and compared to the Folch Method (FM), which is the standard protocol for extracting metabolites from cell samples. Conclusions and future perspectives: We found that UM and UUM were superior to FM in terms of sensitivity, processing time, spectrum quality, amount of identifiable, quantifiable metabolites and reproducibility. In future works, this method can be directly applied to perform high-throughput metabolomics analyses in clinical studies, especially for studying diseases in which the immune system plays an important role. In particular, we intend to apply it to research about the human immunodeficiency virus (HIV), to identify biomarkers and metabolic pathways associated with AIDS development.

Keywords: *Metabolomic, design, method, NMR, cells.*

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Evaluation of the metabolomic profile and its relationship with the clinical spectrum of positive subjects for SARS-COV-2 in a Bogota population

One of the main characteristics of COVID-19 pandemic is the low predictability of the COVID-19 lethality. In this study, multiplatform metabolomic and lipidomic approaches by GC-QTOF-MS, and LC-QTOF-MS were performed with the main purpose to explore the predictability of critical complications of COVID-19 using metabolic profiles of SARS-CoV-2 positive patients, also to identify risk factors and prognostic biomarkers of critical disease and death, we used a cohort of one hundred patients from Colsanitas hospital in Bogotá, Colombia, the sample were categorized into 4 experimental groups according its clinical characteristics, as follows: Control(C): 10, Mild(Md): 30, Moderate(Mt): 30 and Severe(S): 30. The differentiating metabolites in plasma samples were involved in Carbohydrates, Amino acids, Fatty Acyls, Organic acids, Organonitrogen compounds, Organooxygen compounds, Peptides, Sphingolipids, Sterol Lipids, Glycerolipids, and Glycerophospholipids metabolism. The metabolite with highest increase in LC-QTOF-MS was the Sphingolipid NeuAcHexCer (42:2;O2) (fold change 1.37, p = 0,008) for SvsMt and in GC-MS was Ribose (fold change 6.87, p < 0.001) for MtvsMd. The largest affected group of metabolites was Carbohydrates and derivatives in SvsMt, all decreased. Glycerophospholipids and Sterol Lipids in MtvsMd and SvsMd were also decreased. The sphingolipids and glycerophospholipids have a structural role, regulate signal transduction and immune activation pathways as well as inflammatory responses, the overall downregulation of serum lipids is linked to liver damage. The increase in amino acids, such as Glutamic acid, regulator of T cell function, (fold change 2.84, p < 0.001) in SvsMd and (fold change 1.87, p < 0.001) in SvsMt, suggests the existence of a metabolic stress condition and correlates with similar studies reported. Final analyzes in this study reveal factors that correlate with disease severity and lays the foundations possible biomarkers and/or therapeutic strategies were identified in the case of eventual outbreaks and/or new future diseases caused by these viruses.

***Keywords**: Sars-CoV-2, Untargeted metabolomics, Biomarkers, Severity, Chromatography.*

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Exploration of the serum metabolic determinants of mammography density, as a risk factor for breast cancer, in women screening in a reference hospital in Bogotá, 2021

Assessing the risk of breast cancer (BC) in a woman is essential when advancing towards personalized detection and reduction of morbidity and mortality rates. Mammographic density (MD) is a well-known risk factor and has the potential to improve the quality of risk prediction models, however discriminatory accuracy remains limited at the individual level. Serum metabolic differences according to the percentage of mammographic density could represent an innovative and useful risk identification tool in clinical practice. Objectives: In this pilot study, the possible association and predictive capacity of the serum metabolic profiles of low and high risk of developing breast cancer was evaluated in a group of Colombian women. Sixty patients were recruited from the mastology unit of the Clínica Colombia in Bogotá. Results: A pilot metabolomics approach was used to obtain a global picture of the metabolic alterations that occur at different levels of BC risk by mammographic density. The samples were analyzed by gas chromatography coupled mass spectrometry (GC-MS). Differences between group profiles were assessed with univariate (UVA) and multivariate (MVA) analysis using a nonparametric t-test, principal component analysis (PCA), and orthogonal partial least squares regression (OPLS-DA). The sample was distributed as follows: Low risk: 28, Moderate risk: 16 and High risk: 16. The significant demographic and clinical variables between the risk groups were: age p(<0.001), sociodemographic stratum p(0.025), visceral fat level p(0.013), and hormonal status p(0.001). In the multivariate models, we found significant differences (CV-ANOVA= p: 1.84323e-05) in the comparison between the low and high risk groups (Q2=0.511, R2=0.545). Conclusions and future perspectives: The most important metabolic pathway in the discrimination of risk groups was the biosynthesis of phenylalanine, tyrosine and tryptophan, demonstrating that there are metabolites with a good capacity to determine the development of a high risk for MD.

Keywords: *Breast Neoplasms, Risk Factors, Mammographic density, metabolomics, metabolites*

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Metabolite identification in methanol extract of a Solanaceae Colombian plant with antiviral activity against dengue, Zika and chikungunya

Diseases caused by viruses (DENV, ZIKV, and CHIKV) are a global public health problem since there are no specific treatments. The major source of molecules of pharmacological interest have been natural products, and despite Colombia's biodiversity, few studies evaluate their antiviral potential. In this study we evaluated the in vitro antiviral potential of eight extracts obtained of native plants from Colombian coffee region belonging to the Solanaceae family against (CHIKV / Col, ZIKV / Col, and DENV-2 / S16803). Based on the MTT assay, the concentration of 62.5 ug/mL was no toxic. At this concentration the methanolic extract of *Solanum ovalifolium* exhibited a high antiviral activity with 100% of inhibition against the three arboviruses. Afterward, we analyzed the phytochemical compounds in this extract by TLC, finding steroidal-like compounds, alkaloids, and polyphenols. Subsequently, we fractionated the extract using a sintered funnel with SiO2 and increasing polarity with different solvents (hexane-Fraction A, ethyl acetate-Fraction B and, methanol-Fraction C) and we tested these fractions against the three arboviruses. The fraction C showed better antiviral activity against DENV, ZIKV, and CHIKV with 54.67%, 61.1% and 92.3% of inhibition respectively. Our findings from the preliminary liquid chromatography/Mass spectrometry (LC-MS) and nuclear magnetic resonance (NRM ^{1}H, ^{13}C) analysis suggested that we have a saponin enrichment mixture of molecules, since we have a positive saponin foam test and typical signals of anomeric carbons (^{13}C 110-100 ppm), methylene carbons linked to hydroxyl groups (60-80 ppm) and aglycone carbons (aliphatic region) as well in ^{1}H NMR spectrum we found similar patrons (anomeric hydrogens). Based on M^{+1} ions, we were able to compare spirostanol/furostanol/spirosolane-type molecules found in the genus *Solanum*. We also have performed LC-MS/MS analysis using the new tool SIRIUS to have the metabolite structure information. *Solanum ovalifolium* is a potential source of compounds with anti-arbovirus activity.

Keywords: *Antiviral, Arbovirus, Solanaceae, metabolite identification.*

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Search for HIF-2 inhibitors from marine seaweed *Laurencia obtusa*

Introduction: Seaweed has an important role in the studies of new treatments for various diseases, such as diabetes, hypertension and bacterial, fungal and viral infections, cancer, and others. Among them, red algae are the main producers of bioactive compounds. The genus *Laurencia*, for example, has secondary metabolites known for their cytotoxic action, such as sesquiterpenes, diterpenes and acetogenins. Cancer-associated mortality worldwide has been growing over the years, and consequently, the need for new treatments as well. Pro-angiogenic activity within the tumor microenvironment is regulated by the HIFs family and therefore control over these factors would contribute to decrease the effects of pathological angiogenesis. Objectives: So, we performed the search of active compounds from *Laurencia obtusa* in SWMD (Seaweed Metabolites Database) database. Afterward, through in silico methods, study was carried out on the possible mechanism of action of the compounds found in the red algae *Laurencia obtusa*, with the analysis of the crystallographic complex 4GHI, chosen among 21 complexes found in the PDB (Protein Data Bank), and docking calculations. Redocking was performed using a known HIF-2α inhibitor, 0X3 ((N-(3-chlorine-5-fluorofenil)-4-nitro-2,1,3-benzoxadiazole-5-amine), and the PLP score function in radius of 8 Å was chosen for docking calculations. Results: 96 compounds obtained from *L. obtusa* reported in the SWMD database were tested in the PAS-B domain of the HIF-2α protein and scanlonenyne acetogenin presented a score of 68.40 indicating affinity of the molecule by the site and interaction with the aminoacids His298, Tyr281, Ser292 and Met252 - fundamental for alllosteric inhibition of the protein - followed by prevezol B (60.80), epoxide (57.46), β-snyderol (56.34) and 13-epipinnatifidenyne (55.16). Conclusions and future perspectives: The results obtained indicate that molecules present in *L. obtusa* have the ability to inhibit the metastasis process during cancer progression and may be potential treatments for various neoplasms.

Keywords: *seaweed, Laurencia, cancer, in silico, metastasis*

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Metabolomics analysis of MRSA isolates with heterogeneous vancomycin-intermediate (hVISA) phenotype in Latin America

Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for a wide range of infections in humans, have disseminated worldwide and, are associated with high morbidity and mortality rates. Vancomycin is the mainstay of treatment for severe MRSA infections, however, low-level glycopeptide resistance in S. aureus has been reported. Vancomycin (VAN) is a first-line therapeutic option in severe infections caused by MRSA in Latin-America (LA). Development of reduced susceptibility to VAN has been associated with multiple changes in genes encoding pathways for cell wall metabolism and envelope stress responses. Nevertheless, a detailed and coherent mechanistic model to explain the phenotype remains elusive. Objective: Characterize the metabolomic profile of hVISA isolate from Latin America (LA). Methods: The untargeted metabolic profiles of intracellular metabolites were analysed in four clinical isolates (two hVISA and two VSSA [VAN susceptible S. aureus]) belonging to Chilean / Cordobes clone-ST5, predominant lineage of hVISA in LA, and reference strains Mu3 and N315. Samples were analyzed by reverse phase liquid chromatography and hydrophilic interaction. Differences between hVISA and VSSA were evaluated with multivariate (MVA) and univariate (UVA) analysis using principal component analysis (PCA), orthogonal partial least squares regression (OPLS-DA) and nonparametric t-test. The affected metabolic pathways were identified with MetaboAnalyst. Results: Among the differences identified in the metabolic profiles of hVISA respect to VSSA, 69 metabolites were relevant. Of these, 47 were fatty acids (including glycerol), 7 amino acids and 6 nucleosides. We found that glycerolipids and glycerol were more abundant in hVISA than in their VSSA. In contrast, monoglycosyldiacylglycerol was 28% more abundant in VSSA. Amino acids as valine, threonine, leucine and tyrosine were more abundant in VSSA than hVISA. Arginine, glycine and betaine were more abundant in hVISA. Additionally, nucleosides and FAD, NADH cofactors, were more abundant in VSSA. The enrichment analysis show that altered metabolic pathways in hVISA strains, were purine and pyrimidine pathway, glycolysis, metabolism of amino and aminoacyl tRNA biosynthesis. Conclusions: Our results show metabolic alterations in TCA, pentose phosphate pathway and purine intermediates in hVISA-ST5 isolates are the main pathways involved in the reduced susceptibility to VAN as reported in VISA isolates, which contributes to the understanding of this complex phenotype.

Keywords: *Metabolomics, Staphylococcus aureus, LC-MS, Antimicrobial resistance.*

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Changes in the volatile compounds of concentrated liquid coffees related with the acceptance of the coffee cup.

The coffee market is one of the commodities in the world and Colombia is the third-producing country with the Arabic variety, characterized by soft, sweet, and fruity notes. Actually, the new generation prefers cold beverages like ready-to-drink coffees, also known as RTD coffees and cold brews, but the consumer's value of the coffee cup's remains the principal attribute is its aroma, which will be mainly responsible for their acceptance, but the knowledge about sensory qualities is beginning. Understanding the chemical reactions related to the sensorial deterioration of liquid coffee concentrates (CLCs) is one of the primary needs for quality assurance in the coffee industry. Objective: This study aimed to identify the different volatile organic compounds related to the acceptance or rejection of CLCs, which consist of coffee extracts passed through an ultra-pasteurized system and packed to get microbiological stability. For this untargeted HS-SPME/GC/MS previous and posterior to storage was used. Univariate and multivariate statistical methods are implemented to select the significant metabolites. A volcano plot was used to identify changes and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) permitted to classify the samples into accepted and rejected. Results: The results showed that the most significant metabolites depicted accurate separation of both groups (i.e., accepted and rejected), and related well to the sensory attributes and physicochemical features evaluated in CLCs. 33 Metabolites were identified as significant compounds, of which 2-Butanone and 2-Methylfurane have been associated with the freshness of the coffee beans in previous literature and depict a significant reduction in rejected samples. Conclusion: Consequently, a significant reduction of such compounds can be indicative of the product's sensory rejection. Future perspectives: So untargeted analysis permitted a broader spectrum of the metabolites related to acceptance in the CLCs coffee cups to find the freshness that might be used in the quality control.

Keywords: *Coffee liquid concentrate, volatile organic compounds, sensory deterioration, SPME/GC/MS, multivariate statistical analysis*

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Detection of pyrazinamide resistance in Mycobacterium tuberculosis by ^{1}H-NMR

At least a third of the world´s population is infected with *Mycobacterium tuberculosis* (Mtb) and approximately 1.3 million deaths from tuberculosis are reported annually. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains challenge treatments. Pyrazinamide (PZA) is a drug, recommended by the WHO, widely used in first and second line regimen. However, at present, there is no efficient and accurate susceptibility assay that can be used as routine analysis in clinic. Statistically 50% of MDR and 90% of XDR clinical strains are resistant to pyrazinamide and some recently studies have shown that PZA use in resistant patients is associated with higher mortality. So the development of a fast and precise method for better prescription is urgent. The molecular mechanism of PZA is not fully understood. It has been demonstrated that PZA crosses the membrane of Mtb by passive diffusion, and it is hydrolyzed to its active form pyrazinoic acid (POA) by a nicotinamidase (PZAse) encoded by the pncA gene in Mtb. Up to 99% of clinical PZA-resistant strains have mutations in the pncA gene, suggesting these mutations to be the most likely mechanism of resistance to PZA. Several mutations scattered along the pncA structural gene or the putative promoter region have been identified. However not all pncA mutations confer PZA resistance, only the mutation that decreased PZAse enzymatic activity or expression, that limited POA production, leads to resistance. Since there is a high correlation between loss of PZase activity and Mtb PZA resistance, the sensitivity or resistance of a strain may simply be addressed by its ability to form or not POA. Her we present a method to accurately quantify pyrazinoic acid, the active form of PZA, in the supernatant of sputum cultures using Nuclear Magnetic Resonance. The ability of clinical sputum cultures to hydrolyze PZA were measured and correlated to different biochemical and molecular PZA-drug susceptibility assays. The results suggest that this method could become the gold standard for PZA-susceptibility determination.

Keywords: *Pyrazinamide, drug-resistant, NMR, Mycobacterium tuberculosis, PZA-susceptibility.*

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Application of NMR-based metabolomics to the evaluation of dry and wet aging protocols for beef loins from grazing Hereford cattle

In addition to factors that include breed, sex, age, feeding, and animal welfare, meat quality correlates strongly with its aging or maturation process. Attributes that are crucial for consumer acceptability, such as tenderness, juiciness, and taste, depend on maturation, and therefore improving our understanding of the process can have a direct effect on meat marketability. In this report we apply metabolomics based on 1H nuclear magnetic resonance (NMR) to evaluate 30- and 60-day dry and wet aging protocols at the molecular level and correlate our findings to results from consumer panels. Data from aqueous extracts from the longgissimus dorsi of two-year-old grazing Hereford steers were employed to interrogate the different maturation conditions using multivariate analysis. Pair-wise comparisons through orthogonal partial least squares discriminant analyses (OPLS-DA) showed that the levels of certain aminoacids, including alanine, isoleucine, and valine, increased for longer maturation times independently of the maturation conditions, while the opposite was observed for β-hydroxybutyrate, lactate, scyllo-inositol, betaine, and creatine. A decrease in the levels of creatine, hydantoin and its microbiological derivative *N*-methylhydantoin, as well as anserine, a dipeptide derived from carnosine, was generally observed in dry aging. When data from 60-day maturations was compared, a positive correlation between the levels of lactate, coline, and adenine with dry aging was observed, while those of aminoacids, syllo-inositol, and simple amines, such as methylamine, increased in wet aging. Many of these metabolites have a detrimental effect on taste, and thus these findings are consistent with results from sensory panels that reveal a preference for meats undergoing shorter dry aging processes. Overall, this study showcases the potential of metabolomics for the judicious selection of maturation parameters in the production of high-quality meat products.

Keywords: *NMR, meat, metabolomics*

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Effect of growth under co-culture conditions on the metabolic expression of the biocontrol strain *B. velezensis* IBUN 27755 and identification of bioactive metabolites

The use of biocontrol organisms is an increasingly accepted practice to reduce the use of agrochemicals in crops. In this context, the objective of this work is the chemical study of the *Bacillus velezensis* IBUN 2755 strain, which has been shown to have strong antibacterial and antifungal activity *in vitro* against rice crop pathogens. In this study, not only the chemical characterization of the compounds responsible for the activity was sought, but it was also aimed to establish whether the co-cultivation of the strain with rice pathogens, could be a strategy to induce the production of these bioactive compounds. To identify the IBUN 2755 strain compounds responsible for the antagonistic activity against rice pathogens, the acidic extract of the MOLP medium was fractionated over RP-HPLC; and the activity for each fractions evaluated. The active fractions were evaluated by NMR and LC-MS/MS. Fractions F4 and F5 showed to be the most active against the three pathogens and showed to contain Surfactin C14 as major compound. Surfactin like compounds have been reported as antifungal and antibacterial. To stablish the metabolic profiling of the IBUN 2755 strain under coculture conditions with each of the pathogens, molecular networks were constructed for each of the three extracts from the cocultures, and from the respective monocultures. The results showed that, in effect, when the strain is under stress conditions, such as facing another microorganism, the expression of bioactive secondary metabolites rises drastically and diffuses towards the growth zone of the pathogen

Keywords: *biocontrol, surfactins, coculture, molecular networking.*

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Effect of different biotic and abiotic factors on metabolic expression of biocontrol strain *B. velezensis* IBUN 2755

The study of the metabolome of the *Bacillus velezensis* IBUN 2755 strain is relevant because it has been shown to have strong antibacterial and antifungal activity *in vitro* against three rice pathogens that are the cause of great crop losses. The study of the metabolic profile of this biocontrol strain includes the effect of different biotic and abiotic factors on its metabolic expression. To evaluate the effect of culture conditions on the metabolic expression of the biocontroler, the following were tested: 1. Different culture media: selecting MOLP and MES media. 2 Different extraction methodologies, using: a) liquid-liquid partitioning using ethyl acetate and butanol; and b) acid precipitation. 3. Different culture media, using two different volumes: 25 and 200 ml of medium. The twelve extracts obtained were characterized by LC-MS/MS. These data were used for the construction of molecular networks in GNPS and were dereplicated using the associated GNPS and SIRUS databases. It was possible to establish that the expression of secondary metabolites, by the biocontrol strain IBUN 2755, was higher in the MOLP medium using 25ml of culture. It was also shown that the extracts obtained by acid precipitation and ethyl acetate extraction were the most enriched in cyclic depsipeptide type compounds, which have been reported as antifungal and antibacterial compounds. The butanol extracts were the least complex. Finally, it was possible to establish that although the IBUN 2755 strain can express the same families of compounds in the two culture media; however, differences can be observed not only in the quantity but also in the identity of the compounds produced in both culture media. The expression of molecules of interest such as surfactins, fengycins and Iturins was evidenced.

Keywords: *Biocontrol, molecular networking, culture media.*

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Lipid Isomer Separation Using Travelling Wave Cyclic Ion Mobility Mass Spectrometry

The analysis and structural characterization of lipids remain challenging due to the chemical structural diversity and isomeric nature of lipids. In recent years, liquid chromatography coupled to ion mobility-mass spectrometry (LC-IM-MS) for lipidomics has shown advantages in lipid separation and identification. In particular, collision cross section (CCS) obtained from the IM measurements represents a physical property that can be used to enhance the confidence of lipid identification. In this poster we will discuss the use of a time of flight mass spectrometer combined with cyclic™ ion mobility (cIM) device operated in multi-pass mode to resolve complex lipid isomers. Data were collected on a hybrid quadrupole cIM orthogonal acceleration time-of-flight instrument operated in infusion mode. Lipids were obtained either as authentic standards or via isolation from biological fluids. The cIM device consists of 100cm path length RF ion guide comprising over 600 electrodes around which T-Waves circulate to provide mobility separation. It provides the option to perform either a single pass, or multiple passes until the desired resolution is achieved. MS and CID fragmentation data were obtained on precursor IM separated lipids followed by ToF mass measurement. Using the advanced travelling WAVE technology, a portion of the IMS separation was selected and stored in a Pre-Array trap region. The stored ions were then re-injected to enable ion mobility analysis. Cyclic ion mobility MS allows the separation of isomeric lipid species. These isomeric lipids can have different biological implication and it is therefore important to separate them. The cIMS allows the possibility of increasing the IM resolution and separating the lipid isomers with its multi-pass IMSn capabilities. It provides a mobility resolution of 65 even with a single pass. Multi-pass IM was investigated for the separation and structural characterization of isomers from different lipid classes. Different lipid class standards were obtained from Avanti Polar Lipids with positional isomer (sn1/sn2 vs sn2/sn1), different double bond positions, cis and trans isomers, glucosyl and galactosyl ceramide isomers, phosphatidyl-mono, di, tri-phosphates and ganglioside isomers were investigated. First, individual standards were analyzed to determine their drift time and then an equimolar mixture of the standards were analyzed. Both positive and negative ion mode with different adduct ions were investigated to enhance the mobility separation. The results show that some of the isomers (glucosyl-galactosyl ceramides, and gangliosides) were baseline separated only after 5 passes (approximately IMS resolution 150□/□□) with a drift time of 210.53ms (GalCer) vs 214.49ms GluCer and 41.22ms (GD1b) vs 42.58ms (GD1a). However other lipid isomers remained unresolved after even 50 passes (approximately IMS resolution 450□/□□) mainly due to the rotation of the lipid isomers in the electrostatic field. In each case, to confirm the identity of the standards in the mixture, individual standards of each species were infused into the cIMS and collected at different passes. In summary, cIMS provides novel, scalable ion mobility resolution that is useful to separate isomeric lipid species. Advanced modes of operation with ion activation followed by ion mobility separation offers new insights into lipid structural characterization.

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Determination of metabolomic profiles related to prognosis and recurrence in differentiated thyroid tumors

Differentiated thyroid cancer (DTC) is characterized by its biological heterogeneity and variability in clinical outcomes. This is the most common malignant endocrine neoplasm and constitutes 1-2% of all cancers, with a high variability of recurrence risk according to pathological and clinical characteristics, however, the diagnostic classification tools are still invasive and late. Objective: In this study, we propose to identify metabolites that discriminate between the different prognostic and recurrence classifications of DTC by using metabolomic analysis. Results: In order to explore the metabolic pathways involved in the prognosis and recurrence risk, we applied gas chromatography coupled mass spectrometry (GC-MS) in tumor tissue with different prognoses (low, moderate, high) by risk of recurrence according to the American Thyroid Association Classification (ATA) (low, moderate, high) (n = 29). Evidenced by partial least squares discriminant analysis (PLS-DA), 62 candidate metabolites, of which 23 are significantly altered in the model that compares high risk with moderate risk (FDR < 0.05 Benjamini-Hochberg). Among which are Amino acids, carboxylic acids, Fatty Acids, Hydroxy acids, Keto acids, Organic phosphoric acids, Organooxygen compounds, Purines and Pyridines, metabolites that help to establish differences between the various risks of recurrence and other types of characteristics clinics. Conclusions: Metabolomics represents a promising tool for the identification of patients at high risk of recurrence in patients with DTC who are deficient in post-surgical adjuvant therapy.

Keywords: *Differentiated thyroid cancer, Recurrence risk, metabolomics, prognosis.*

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CitOMICs by drone-SPME: an ascendent platform for monitoring and control of the Gaeumannomyces graminis fungus in rice crops in the Department of Tolima, using drone-SPME and analyzed by GC-MS

Introduction: *Oryza sativa* or rice as it is commonly known, which is a cereal of great importance in many culinary cultures, as well as in Latin America. This project seeks to contribute in the future, to control pests in the agricultural sector of the country, in mitigating the loss of crops and saving expenses. Based on an early, rapid, non-invasive and low-cost warning. With precision agriculture mediated by a DRONE-SPME, volatile organic compounds (VOCs) can be monitored, which are a series of substances released into the environment by living organisms. In this sense, plants, humans, animals and microorganisms predominantly release this type of substance into the environment; Objectives: To identify secondary metabolites (biomarkers) generated in the infection of rice plantations affected by the *Gaeumannomyces graminis* fungus, through the CitOMICs drone-SPME platform, as a strategy for early, rapid and non-invasive detection of the disease, and that allows to build a future support system for decision-making in crop health; Results: VOCs compounds are produced by fungi, bacteria and plants, they are distinguished by having low molecular weight and high vapor pressure and are generated under certain environmental conditions or stress within the body. In this sense, in the different samplings carried out so far, heptadecane, *n*-heptadecanol and dotriacontyl isopropyl ether were identified; Conclusions: So far in preliminary tests, some VOCs have been identified. However, deeper sampling is needed, both in vitro and in-situ; future perspectives: It is intended to identify the biomarkers generated by the pathogenic fungus *in vitro* and then carry out field sampling by means of a drone coupled to a SPME fiber, to generate a comparison of secondary metabolites produced in both environments, mediated by chemometric analysis.

Keywords: *heptane; biomarkers; VOCs; rice; volatile*

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MALDI-ToF mass fingerprinting as a tool to study green coffee shelf-life

The sensorial quality of coffee is impaired by its storage. Aged coffee presents an off-note taste which is called "rested coffee flavor" which is only determined through sensory analysis. This quality reduction is a manifestation of chemical changes and could be monitored using metabolomics techniques. Therefore, the suitability of MALDI-ToF mass fingerprinting was tested to discriminate between unrested ("fresh") and rested Colombian Arabica (Coffea arabica) coffee samples. Optimized mass spectrum profiles of green coffee oil were used to develop a Random Forest model which demonstrated a specificity of 72% to detect rested coffee across different days of sample analysis. The high throughput of MALDI-ToF mass fingerprinting enables the broad screening of green coffee materials for quality monitoring and shelf-life studies.

Keywords: *Coffee, lipids, quality, shelf-life, mass spectrometry*

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Metabolic alterations induced in the leukemic cell line K562 by *Petiveria alliacea* (anamu) extracts

It has been described that energy metabolism, mitochondrial and redox homeostasis in acute leukemia favors chemoresistance to doxorubicin, anthracyclic and Ara-C. Given the importance of "metabolic flexibility" in tumor progression and resistance, natural products have been extensively studied, and it has been shown that plants can directly or indirectly regulate tumor metabolism. Isolated natural products or standardized complex extracts such as those obtained from *Petiveria alliacea* have been shown to have cytotoxic activity on different tumor cell lines and antitumor activity in animal models of breast cancer. Among the mechanisms participate in death, the alteration of proteins involved in metabolism was observed, leading to a decrease in glycolysis and mitochondrial respiration, which leads to lower ATP and lactate production. The purpose of this research was to identify metabolomic alteration in the endometabolome of K562 tumor cells treated with *Petiveria alliacea* (anamu) extracts. The study was performed on K562 leukemic cell lines treated with the mean inhibitory concentration of extracts obtained from *Petiveria alliacea* leaves by supercritical fluid (SC) and infusion (Esp) and negative controls. Samples were analyzed by liquid chromatography coupled to mass spectrometry in positive ionization mode. Differences between profiles were evaluated with univariate and multivariate analysis. Compared with the negative control, a total of 48 metabolites were altered in the SC group and 34 in the Esp group. The chemical classes of metabolites that were found to be altered were Indoles, glycerophospholipids, nucleosides, nucleotides, fatty acyls, carboxylic acids, purine nucleosides and sphingolipids. The metabolome of cell treated with Esp identified metabolic alterations mainly in the metabolism of glycerophospholipids, purine and biotin; for SC, alterations in the metabolism of glutathione, purine and biotin were identified. Esp and SC extracts modulate metabolism in tumor cell lines K562 compared to the negative controls.

Keywords: *metabolism, leukemia, Petiveria alliacea, metabolomics.*

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Serum metabolomic profile of patients with systemic sclerosis: A Pilot Study

Systemic sclerosis (SSc) is an autoimmune disease characterized by immunological alterations that trigger vascular compromise, and fibrosis. Like other autoimmune diseases, SSc is a heterogeneous disease with a variable and unpredictable course. To date, the pathogenic mechanisms involved in SSc have not been fully elucidated. For this reason, it has been proposed to identify biochemical pathways in the pathophysiology of SSc, since the metabolites generated may play a key role in the establishment of personalized treatments, as well as diagnostic and prognostic biomarkers. This study used a multiplatform metabolomics approach to obtain a global picture of metabolic alterations in patients with SSc. The study was performed on serum samples from 15 SSc patients and 101 healthy controls. Samples were analyzed by LC-MS in positive and negative electrospray ionization modes, followed by data alignment and filtering. Statistically significant molecular features were identified by multivariate (MVA) and univariate (UVA) statistical analysis. MVA was performed with SIMCA-P+ 16.0 software (Umetrics) and UVA with Matlab. The metabolites identified in the serum samples were associated with the metabolism of glycerolipids, glycerophospholipids, amino acids, and fatty acids. In addition, this study detected the presence of metabolites such as neamine involved in the inhibition of cell proliferation and angiogenesis, imidapril used to protect the progressive deterioration of autoimmune nephritis, and uric acid involved in interleukin 1 β -mediated inflammation, by activating the inflammasome-associated NOD-like receptor protein 3, a multimolecular complex whose activation appears to be central to many pathological inflammatory conditions. These findings propose relevant serum metabolites that could contribute to a better understanding of the underlying metabolic changes driven by dysregulation in the immune system and endothelial damage seen in SSc.

Keywords: *Scleroderma, Systemic sclerosis, Metabolites, Inflammation, Immune response, Angiogenesis.*

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Metabolomic and lipidomic profiling of patients with acute leukemia: A pilot study

Acute leukemias (AL) are hematopoietic neoplasms, characterized by lack of control and clonal expansion of lymphoid and/or myeloid precursors, which results in bone marrow failure. In Colombia, during the period of January 2021 and June 2022 the number of prevalent cases was 397 to Acute Lymphoid Leukemia (ALL) and 73 to Acute Myeloid Leukemia (AML). Objectives: The purpose of our study was explored metabolic differences between Colombian adult population with AL and healthy individuals applying a non-targeted approach to characterize metabolic and lipidomic profiling by GC–MS and LC-MS techniques in both positive and negative electrospray ionization (ESI) modes to improve metabolome coverage. Results: Plasmas were collected from 20 healthy individuals (HI) and 20 patients with AL comprising: 9 ALL, 9 AML, 1 promyelocytic myeloid leukemia and 1 mixed phenotype AL. Differences between profiles from AL and HI groups were evaluated with univariate (UVA) and multivariate (MVA) analysis. Compared with the HI group, a total of 267 metabolites was altered in the AL groups, 56 by MF in LC-MS, 179 by LF in LC-MS and 32 by MF in GC-MS. The chemical classes of metabolites that upregulated were amino acids, carnitines, glycerolipids, organic acids and organooxygen compounds, on the other hand, the group of metabolites that downregulated were fatty acyls, sphingolipids, steroids, prostaglandins, glycerophospholipids and indoles. The plasmatic metabolome allowed to identify metabolic alterations mainly in the linoleic acid and glycolysis metabolism, several amino acids metabolism and glycerophospholipid metabolism. Conclusions: The metabolic alterations evidenced mainly in lipid metabolism can be a new treatment approach in this type of neoplasia and deserved further validation. This work is the first report of metabolic alterations in patients with AL of Colombian origin, thus contributing to a better understanding of the metabolic pathways involved.

Keywords: *Acute myeloid leukemia, acute lymphocytic leukemia, metabolomics, LC-MS, GC-MS*

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Changes in Sphingolipids Composition in Cells and Cell-derived Exosomes for Characterization of Glioblastoma Stem-like Cell Phenotypes

Glioblastoma (GBM) is one of the most malignant central nervous system tumour types. It is characterized by its persistence through self-renewing highly tumorigenic cancer stem-like cells (CSCs). Two main subtypes of CSCs are reported; proneural (PN) and mesenchymal (MES), they are characterized by exhibiting different metabolic, growth, and malignancy properties. Only a few studies have investigated the whole composition of sphingolipids in GBM stem-like cells (GSCs).The aim of this study was the characterization of sphingolipid profile of three Glioblastoma stem-like cells phenotypes, PN and MES (including nodular and semi-nodular dissemination) and suggest a potential role in cancer drug resistance. GSCs lines were isolated from fresh surgical tissue of GBM diagnosed patients and were grown in vitro as neurospheres cultures. EXO were isolated from the GSCs phenotypes through differential and serial ultracentrifugations at 4 °C for further analysis. Sphingolipids and other lipids of medium polarity were extracted using cold methanol. Samples were measured using ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC/QTOF-MS) in an untargeted method. The lipidomic profile was obtained. The multivariate analysis resulted in suitable discrimination between cells phenotypes. After complete statistics, sphingolipids showed a significant presence among the discriminating features. Here, we present the sphingolipid's complete characterization, and we show their pattern among the three GSCs phenotypes. Iterative MS/MS datasets were used for lipid molecular species identification following a strategy that relied on three independent software tools (MS-DIAL 4, Lipid Annotator and Lipid Hunter) followed by manual validation. Complete characterization of the sphingolipids profile, in GSC phenotypes, showed differential distributions among them. Results indicate significant changes for ceramides, sphingomyelins, hexylceramides, sphingosine and sphinganine among GSC phenotypes, being MES-SN phenotype the most different compared with the other two phenotypes for cells, while in cell-derived exosomes this pattern was not followed. Finally, the critical role of lipids and, in particular, sphingolipids for the formation, secretion, and function of cells and exosomes may lead to a radically new understanding of cancer biology and therapy and in the study of GSCs phenotypes.

Keywords: *Metabolic profile, ascidian, Euherdmania, associated bacteria*

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Metabolic profiling by Nuclear Magnetic Resonance (^{1}H-NMR) of brown algae from shallow environments north of the island of San Andrés.

To evaluate the metabolic diversity of brown algae from the shallow ecosystems of the north of the island of San Andrés, the Colombian Caribbean, a metabolic approximation from ^{1}H NMR spectra was presented. Five collections of the most abundant species were made, in different climatic periods, taking into account the variables of the taxonomic identity of each individual, collection depth, and growth substrate. Seven species of abundant algae were found, 6 brown (*Canistrocarpus crispatus*, *Stypopodium zonale*, *Dictyopteris delicatula*, *Sargassum polyceratium, Padina gymnospora*. and *Dictyota* spp.) together with a red algae (*Trichogloeopsis pedicellata*). In addition, differences in the diversity (abundance and composition) of the algal community were observed in each colecting. Organic fractions soluble in CH_2Cl_2 were acquired from these samples and analyzed by ^{1}H-NMR. The multivariate analyzes applied to these fractions showed that *S. zonale* and *C. crispatus* form differentiated groups from the rest of the species, from which characteristic signals of specialized metabolites (meroditerpenes and diterpenes, respectively) were identified. In addition, differences can be observed between the spectra of *S. zonale* correlated to the growth substrate (rocky bottom vs. dead coral), and differences in metabolic production in *P. gymnospora* correlated with the demonstration season (dry season vs. rainy season). The metabolomic experiment allowed us to find in complex mixtures signals of interest characteristic of specialized metabolites, in this case, diterpenes produced by *C. crispatus*.

Keywords: *Brown Algae, Metabolic profiling, Diterpenoids, Nuclear Magnetic Resonance, Spectroscopy*

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Design of an extraction methodology and metabolic profile of a collection of brown and red algae collected in the Colombian Caribbean using NMR^{1}H.

Seaweed are representative organisms of coral reefs, this supplies food, habitat, and protection to many fish and invertebrates. In recent years, their biomass has increased, due to the rapid degradation of marine ecosystems, and the increase in nutrients because of external intervention of the reefs, to the point of becoming an ecological problem for this ecosystem. Less than 1% of the identified species of these organisms are used in the food, pharmaceutical and cosmetic industries even though they could have the potential to be used. This research shows the development of the extraction methodology and the metabolomic study of 27 samples of brown algae and 12 samples of red algae, collected in different locations in the Colombian Caribbean, using ^{1}H-NMR. With the aim of identifying those species that present compounds of interest for the cosmetic, food, and agricultural industries, among others. Extraction methodologies with solvents of different polarities were used, followed by liquid-liquid extraction and selective precipitation. The foregoing to obtain extracts enriched in specific compounds for each sample, each one of them not very complex to facilitate dereplication. The MVDA analysis of the ^{1}H-NMR data for each extract was performed using unsupervised methods (PCA and HCA), seeking to establish the similarity between the extracts obtained by the proposed methodologies, make a comparison between the samples, and characterize the most abundant compounds. present in them by dereplication.In this study, an extraction methodology was developed for the study of brown algae and another for red algae. The metabolic profile of the extracts was established using 1D and 2D NMR (HMBC, HSQC, COSY and J resolved) and dereplication techniques. In this way, abundant seaweed extracts were found in the Colombian Caribbean, enriched in compounds such as phlorotannins, mycosporin-type amino acids (MAAs), diterpenes, etc. which are potential sources of ingredients for the industry. The extracts of each of the HCA clusters are undergoing enzyme inhibition tests of interest to the industry, to establish their potential as raw materials with added value.

Keywords: *Seaweed, Ochrophyta, Rhodophyta NMR, dereplication.*

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Metabolomics as a tool for evaluating the nutritional status of an individual and the nutritional content of food using molecular networking

Molecular networking (MN), applied to LC-MS obtained metabolomics datasets, is a trending tool in the study of the metabolites associated to biological systems. It uses computational strategies to identify similarities among all MS/MS spectra, propagating annotation to unknown related molecules and allowing the interpretation of complex data arising from MS analysis. The approach has emerged as an efficient method for novel specialized metabolites discovery and isolation. Here, we applied total molecular networking to unravel the complex biological chemistry environment of samples from human gut microbiome and a fermented food matrix, aiming to establish the individual nutritional state and the food nutritional potential, respectively. Datasets were obtained through untargeted metabolomics for each sample by duplicates, at METCORE facility (UniAndes), using C18-LC/MS-QTOF. Analyses were performed using GNPS and Cytoscape, grouping samples per treatment, such as physiological state for gut microbiome samples and fermentation time for the food matrix. We identified differential traits in the gut microbiome samples within the cohort of volunteers, such as phospholipids and acylcarnitine being in higher amounts within the breastfeeding women group, vitamin E and antihistamines being in higher proportions in some volunteers that declared their consumption, and steroid hormones being higher in pregnant women. In fermented foods, we found more bioactive compounds such as organic acids, terpenes, and flavones, within 72 and 96 hours of fermentation. This approach allowed us to visualize a snapshot of the chemical species of each sample and their relative abundance. Furthermore, it delivers data to establish a nutritional score for an individual's gut or a specific food, based on MN metabolomics and the proved biological functions most metabolites have in the metabolism. Such a scale of the metabolic state of the individual or food matrices, can act as a tool that aids in translating metabolomics to practical applications in health and nutrition.

Keywords: *Metabolomics, Molecular Networking, nutritional status, nutiritonal potential, GNPS*

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Rational Exploration of Fermented Cupuaçu (*Theobroma grandiflorum*) Aiming to Identify Secondary Metabolites of Commercial Interest Through Dereplication

Introduction: Identifying value-added composts originating from the byproduct of cupuaçu fermentation (*Theobroma grandiflorum*) can contribute to a more rational exploration of this fruit. The exudates adhered to cupuaçu seeds, synthesized in bench-scale fermentation assays, were subjected to analysis by coupling the High-Performance Chromatography System, hyphenated to the Mass Spectrometer (HPLC-MS) in order to trace the related metabolic profiles to microbial activity on this substrate for seven days (168 hours). Exploratory analyzes were executed, building a molecular network, using the GNPS platform (Global Natural Products Social Molecular Networking), and identifying clusters with promising chemical entities. Additionally, data were processed using MS-Dial software and database scans (via MS-Finder) to perform a molecular annotation (and dereplication) process. Objectives: To identify molecules of high added value from exudates generated during the spontaneous fermentation process of cupuaçu seeds (*Theobroma grandiflorum*). Results: Different molecular classes (epicatechin, vitexin, and flavonoids, among others) were recorded during the spontaneous fermentation of cupuaçu seeds. The types of molecular classes annotated depended on the solvent (ethanol or ethyl acetate) used during the collected samples' chemical maceration (sequential). Conclusions: Creation of a database of molecules already cataloged and recognizing signs of classes of organic molecules in cupuaçu fermented. Future perspectives: It is encouraging the expansion of conventional cupuaçu processing practices by making it possible to obtain and commercialize fermented concentrates that contain biomolecules with high added value.

Keywords: *molecules of high added value; cupuaçu; chemistry of natural products; amazon biodiversity*

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Evaluation of fresh, frozen, and lyophilized feces by SPME and derivatization using GC×GC-TOFMS

The field of fecal metabolomics has grown significantly in recent years, however, standard protocols for sample preparation and analysis have yet to be established. Fecal samples provide a direct insight into human health allowing researchers to better understand the complex interactions between the gut microbiome and the host. Despite the increased attention, fecal sample preparation remains a challenge given the heterogeneous matrix and microbial-rich composition. Bacteria and enzymes present within feces remain active and continue to alter the metabolite profile after the sample leaves the body. Therefore, proper controls of pre-analytical conditions including storage and handling are of utmost importance to reduce the metabolic changes and ensure the results are representative of the sample at the time of collection. In this work, the impacts of sample storage and handling conditions on fecal metabolite abundance and profile were evaluated using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS). GC×GC is a powerful technique for analyzing complex samples resulting in enhanced separation and superior sensitivity compared to one-dimensional GC techniques, and along with the TOFMS, enables us to tentatively identify compounds within the samples. Fecal samples were prepared using solid phase microextraction (SPME) and chemical derivatization, two complementary techniques providing an expanded coverage of the fecal metabolome. Fresh, frozen, and lyophilized feces were used to assess metabolite alterations during the pre-analytical handling. Our work provides in-depth understanding of the physicochemical changes taking place within fecal samples during storage and handling with a comprehensive evaluation of the fecal metabolome. The results presented herein contribute towards the standardization of fecal sample preparation methods for metabolomics studies.

Keywords: *Fecal metabolomics; derivatization, SPME, multidimensional separations*

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Exploratory analysis of complex matrices of cupuaçu fermented (*Theobroma grandiflorum* (Willd. ex Spreng.) Schum.): the use of cheminformatics for the bioprospecting of molecules of high added value

Introduction: The rational exploration of the natural fermentation of cupuaçu (*Theobroma grandiflorum*) and the formation of a database that provides subsidies for the promotion of biotechnological initiatives for the Amazon region is an urgent topic. The extraction of metabolites from the fermentation of cupuaçu seeds was carried out by sequential maceration, using ethanol and ethyl acetate. The crude extracts were analyzed by High-Performance Chromatography and hyphenated in a Mass Spectrometer (HPLC-MS). The data were explored in high-performance software, running scans to recognize evidence of biotechnological and industrial interest molecular signals and for pattern recognition and molecular annotations cataloged in the literature (specifically, on established platforms such as Chembl DataBase, Drug Bank, NuBBEDB, and PubChem). Objectives: Demonstrate the relevance of Chemoinformatics, with an emphasis on Deep Learning and Big Data Technologies, in the structural mining of chemical entities of different biological origins, deposited on molecular platforms, aiming at the construction of models for the recognition of biomolecules from the natural fermentation of cupuaçu seeds. Results: After removing the Smiles (Simplified Molecular Input Line Entry Specification), the annotations explored made it possible, using already consolidated algorithms, to calculate different descriptors, which served as input parameters for the classification of artificial neural networks. For example, artificial neural networks could adequately separate more than 90% of the structures of plants, fungi, and bacteria, thus allowing the construction of a mathematical model applicable to cupuaçu molecules. Conclusions: Mathematical mining of chemical entities allowed the derivation of chemical descriptors, which were packaged as chemical fingerprints, efficient in predicting the metabolic variability of exudates from fermented cupuaçu seeds. Future Perspectives: Construction of new models capable of processing data in high volume, speed, and veracity to direct more assertive studies, which promote pharmacological initiatives different from the conventional exploitation of cupuaçu.

Keywords: *microbial synthesis products; cupuaçu; machine learning; amazon biodiversity*

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From cacao ripening to fine flavor cholate. Integrated Metabolomics Studies.

Introduction: Systemic sclerosis (SSc) is an autoimmune disease whose etiology is still unknown. It is characterized by having a variable and unpredictable course, with high morbidity and mortality. Recent studies suggest that there is a link between the loss of immunological tolerance, the mechanisms that regulate cell metabolism, and the appearance of SSc. Therefore, metabolomics might be essential for a better understanding of these pathogenic mechanisms. Objective: This study aims to determine the possible altered metabolic pathways responsible for the mechanisms associated with the appearance of SSc. This would allow to improve the diagnosis, prognosis, and treatment. Methodology: A systematic review of the literature in PubMed, EMBASE, Web of Science, and Scopus was performed from January 2000 to September 2022. Based on predefined inclusion and exclusion criteria, three reviewers independently reviewed the literature. The review included metabolites from different biological samples, such as serum, plasma, and urine, which were analyzed using different metabolomics analytical platforms. Results: The main metabolic families detected that could play an important role in SSc were fatty acids, glycerophospholipids, sphingolipids, and amino acid-derived metabolites. These results indicate the presence of alterations in fatty acid beta-oxidation, lipid metabolism, and amino acid pathways in SSc patients associated with inflammation, vascular endothelial dysfunction, and fibrosis. Future perspectives: Metabolomics offers new insights into the altered metabolic state in patients with SSc, being key to understanding the behavior of the disease, which is still unclear. Likewise, this analysis will help elucidate possible metabolites that can be used as biomarkers for diagnosis, prognosis, and course of the disease.

Keywords: *Systemic sclerosis; Metabolomics; Systematic review.*

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Metabolic fingerprinting of Systemic Sclerosis: A systematic review

The use of biocontrol organisms is an increasingly accepted practice to reduce the use of agrochemicals in crops. In this context, the objective of this work is the chemical study of the *Bacillus velezensis* IBUN 2755 strain, which has been shown to have strong antibacterial and antifungal activity *in vitro* against rice crop pathogens. In this study, not only the chemical characterization of the compounds responsible for the activity was sought, but it was also aimed to establish whether the co-cultivation of the strain with rice pathogens, could be a strategy to induce the production of these bioactive compounds. To identify the IBUN 2755 strain compounds responsible for the antagonistic activity against rice pathogens, the acidic extract of the MOLP medium was fractionated over RP-HPLC; and the activity for each fractions evaluated. The active fractions were evaluated by NMR and LC-MS/MS. Fractions F4 and F5 showed to be the most active against the three pathogens and showed to contain Surfactin C14 as major compound. Surfactin like compounds have been reported as antifungal and antibacterial. To stablish the metabolic profiling of the IBUN 2755 strain under coculture conditions with each of the pathogens, molecular networks were constructed for each of the three extracts from the cocultures, and from the respective monocultures. The results showed that, in effect, when the strain is under stress conditions, such as facing another microorganism, the expression of bioactive secondary metabolites rises drastically and diffuses towards the growth zone of the pathogen

Keywords: *biocontrol, surfactins, coculture, molecular networking.*

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MliR, a Novel MerR-like Regulator of Iron Homeostasis, Impacts Metabolism, Membrane Remodeling and Cell Adhesion in the Marine Bacteroidetes *Bizionia argentinensis*

INTRODUCTION: The central role of iron in bacteria makes it a determinant of pathogenesis, invasiveness and survival. Significant progress has been made towards the regulation of iron metabolism in model organisms. However, mechanisms of iron homeostasis in Bacteroidetes, one of the dominant phyla in animal gut, soil and oceans, remain largely unknown. RESULTS: Here, we identified a novel transcriptional regulator of the MerR superfamily (MliR), phylogenetically unrelated to cognate Fur proteins, involved in iron homeostasis in the marine bacterium Bizionia argentinensis JUB59 and widely conserved in bacteria from a variety of environments. This regulator was named MliR (MerR-like iron responsive Regulator). Deletion of the mliR gene led to decreased cell growth, increased cell adhesion and filamentation. Genome-wide transcriptomic analysis showed that genes associated with iron homeostasis were downregulated in mliR-deletion mutant. Through NMR-based metabolomics, ICP-MS, fluorescence microscopy and biochemical analysis we evaluated metabolic and phenotypic changes associated with mliR deletion. CONCLUSION: The findings presented here bring together transcriptional regulation, energy metabolism, membrane remodeling and cell adhesion, providing insights into the global response to iron availability that may raise interest in microbiology in general.

Keywords: *Iron metabolism, Transcriptional regulation, Bacteirodetes, Filamentation, Iron uptake, Energy metabolism*

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