ICA 2023

The 1st International Conference on Antioxidants: Sources, Methods, Health Benefits and Industrial Applications

10–12 May 2023 | Barcelona, Spain

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#Antioxidants2023

The First International Conference on Antioxidants: Sources, Methods, Health Benefits, and Industrial Applications

AXA Convention Centre
Barcelona, Spain
10 – 12 May 2023

Conference Chairs

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Prof. Dr. Rosa M. Lamuela Raventós

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The First International Conference on Antioxidants: Sources, Methods, Health Benefits and Industrial Applications

10 – 12 May 2023, Barcelona, Spain

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Conference Program

Wednesday 10 May 2023

14:00 – 15:00 Registration (Check-in)

15:00 – 15:10 Welcome from the Chairs

Opening Session

Session chairs: **Alessandra Napolitano** and **Rosa Lamuela**

15:10 – 15:50 **Plenary Talk : Daniele Del Rio** - Antioxidants and health: evolution of a concept

Flash Poster Presentations

Session chairs: **Alessandra Napolitano** and **Rosa Lamuela**

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15:55 – 16:00 **Daria Trocka -** B4. Can beta-blockers be used as antiglycoxidant drugs? New properties of nebivolol

16:00 – 16:05 **Alba Iglesias Mayor -** A1. BRS Device: A Point-Of-Care Testing System for Total Antioxidant Activity Determination in Biological Samples

16:05 – 16:10 **Bernhard Blank-Landeshammer** – A16. Improved in vitro bioavailability of antioxidant phytochemicals through interaction with plant extracts

16:10 – 16:15 **Abigail García-Villegas -** B12. Revalorization of Avocado By-Products for the Development of Cosmetic Ingredients

16:15 – 16:20 **Paweł Przbylski** - B30. Polyhydroxyphenols with mitochondrial-targeted moiety

16:20 – 16:25 **Marília Goulart** - B27. Extracts, Natural Stilbenes, In Placenta Protection: Which Are They and How Do They Work?

16:25 – 16:30 **Rita Argenziano –** B39. Recovery of low and high molecular weight phenolic compounds endowed with potent antioxidant properties from agri-food byproducts by an ecofriendly and sequential two-steps protocol

16:30 – 16:35 **Raquel Rodríguez Solana -** B43. Using Natural Deep Eutectic Solvents to extract phenolics from in vitro cultures of the Mediterranean plant Thymus lotocephalus produced in response to abiotic factors

16:35 – 16:40 **María Ángeles Martín-Cabrejas** - B24. Phenolic compounds from coffee pulp potentially scavenge reactive oxygen and nitrogen species after in vitro digestion

16:40 – 17:20 **Plenary Talk : Ramón Estruch** - Antiinflammatory Effects of Antioxidant Compounds from Mediterranean Diet

17:20 – 18:00 **Welcome Reception**

Thursday 11 May 2023

Session 1. Part I

Health Outcomes of Antioxidants and Oxidative Stress

Session chairs: **Mario Allegra** and **Anna Tresserra-Rimbau**

09:00 – 09:30 **Raul Zamora-Ros** - Polyphenol exposure and body weight change : epidemiological to clinical evidence

09:30 – 09:45 **Mariano Stornaiuolo** - A wave of pro-oxidant metabolites is released by melanoma cells during chemotherapy and triggers paracrine pro-survival epigenetic changes in the tumor microenvironment

09:45 – 10:00 **Sabrina Donati Zeppa** - High-intensity exercise modifies diet habits and microbiota composition in healthy students

10:00 – 10:15 **José Pérez-Correa** - Overall mass balance analysis of an integrated pressurized liquid extraction and resin purification process for the recovery of phlorotannins from Chilean brown seaweeds

10:15 – 10:30 **Ana Barros** - Fighting antibiotic resistance : giving a second life to natural compounds

10:30 – 11:00 **Coffee Break**

Session 1. Part II

Health Outcomes of Antioxidants and Oxidative Stress

Session chairs: **Daniele del Rio** and **Raul Zamora-Ros**

11:00 – 11:30 **José Viña** - Genistein as a treatment for prodromal Alzheimer's disease. Results of the GENIAL clinical trial (NCT 01982578)

11:30 – 11:45	**Anna Tresserra-Rimbau** - An anti-inflammatory diet, measured by urinary polyphenol excretion, and Metabolic Syndrome in the PREDIMED cohort
11:45 – 12:00	**Victoria Olt** - Identified phenolic compounds of the colonic fraction from biscuits with Tannat grape pomace present potential to improve gut health
12:00 – 12:15	**Raquel Burggraaf-Sánchez de las Matas** - Effects of oral Pterostilbene in patients with diabetic retinopathy, a clinical trial
12:15 – 12:30	**Bence Pál Barta** - Gut-region-specific expression of nuclear factor kappa B p65 in the myenteric ganglia and its microenvironment of streptozotocin-induced diabetic rats
12:30 – 14:30	**Lunch and Poster Session A**

Session 2. Part I
Antioxidant Enzyme Systems

Session chairs: **José Viña** and **Agnieszka Bartoszek**

14:30 – 15:00	**Jiankang Liu** - Nutritionally targeting mitochondria to prevent age-associated diseases: Hydroxytytrosol is a rising star after resveratrol
15:00 – 15:15	**Micol Silic-Benussi** - Disrupting redox homeostasis and the mTOR pathway to kill T-lymphoblastic leukemia (T-ALL) cells
15:15 – 15:30	**Silvia Arribas** - Blood pressure reduction and improved plasma GSH in hypertensive rats supplemented with cocoa shell extract
15:30 – 15:45	**Martina Meinke** - Effects of smoking on skin—application of spectroscopic measurements and biological assays
15:45 – 16:00	**Carmela Spagnuolo** - Aliophen® Formulation Based on Malts and Hops, characterization, biological activities, and applicative outcomes
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Session 2. Part II
Antioxidant Enzyme Systems

Session chairs: **Gian Luigi Russo** and **Martina Meinke**

16:30 – 17:00	**Agnieszka Bartoszek** - Does endogenous antioxidant system indeed require support from exogenous antioxidants to maintain redox homeostasis?
17:00 – 17:15	**Silke Lohan** - Assessment of the redox homeostasis in blood by EPR spectroscopy using UVA irradiation as stressor
17:15 – 17:30	**Laura De Cubas** - Monitoring cytosolic and mitochondrial H_2O_2 and GSH/GSSG levels linked to physiology and pathology
17:30 – 17:45	**George Luo** - A Core Nrf2 Gene Set through Broad Transcriptomic Analysis is Representative of Nrf2 Activity and Reveals Selective Drug Resistance
17:45 – 18:00	**Guillermo López-Lluch** - CoQ10 and endothelial cells activity : relationship with cognitive deficiency

Friday 12 May 2023

Session 3. Part I
Natural and Synthetic Antioxidants: Sources, Analysis and Mechanisms of Action

Session chairs: **José Ricardo Pérez-Correa** and **Grzegorz Litwinienko**

09:00 – 09:30	**Jesús Simal Gandara** - Clinical Metabolomics
09:30 – 09:45	**Adrianna Piasek**- Extract from used coffee grounds as a good source of several antioxidants for skin cells
09:45 – 10:00	**Mario Allegra** - Evaluation of the neuroprotective potential of Indicaxanthin from Opuntia ficus indica fruit against dysmetabolism-related neurodegeneration in high-fat diet-fed mice.
10:00 – 10:15	**Immacolata Castellano** - Low molecular weight thiols with antioxidant activity: natural sources and potential health benefits
10:15 – 10:30	**Iveta Mintale** - Sea buckthorn (Hippophae rhamnoides) seed lipid extract as a supplement to reduce residual risk in patients with coronary heart disease
10:30 – 11:00	**Coffee Break**

Session 3. Part II
Natural and Synthetic Antioxidants: Sources, Analysis and Mechanisms of Action

Session chairs: **Lucia Panzella** and **Iveta Mintale**

11:00 – 11:30	**Jara Pérez-Jiménez** - Exploring natural sources of macromolecular polyphenols
11:30 – 11:45	**Nikola Stamenković** - Can the Universal Acidity Scaling formalism explain scavenging processes in antioxidant theory? A new way to link radical- and ion-driven processes, combining kinetics and thermodynamics
11:45 – 12:00	**Grzegorz Litwinienko** - Polydopamine /melanin nanoparticles as a bionanoantioxidant. Studies in model lipid systems
12:00 – 12:15	**Riccardo Amorati** - Pro-aromatic terpenes from essential oils : a natural strategy to enhance the radical trapping activity of antioxidants and nanomaterials.
12:15 – 12:30	**Layla Simón** - Phlorotannin-containing extracts from Chilean brown algae (Durvillae incurvata) inhibit melanoma cell migration
12:30 – 14:30	**Lunch and Poster Session B**

Session 4. Part I
Natural and Synthetic Antioxidants: Sustainable Methodologies for Recovery and Processing

Session chairs: **Jara Pérez-Jiménez** and **Luca Valgimigli**

14:30 – 15:00	**Lucia Panzella** - Multifaceted application opportunities offered by agri-food waste-derived phenolic compounds
15:00 – 15:15	**Diana Pinto** - Screening of a novel nutraceutical ingredient extracted from chestnut shells by in-vitro and in-vivo assays – An insight into metabolomic approaches
15:15 – 15:30	**Federica Tonolo** - From agri-food waste to functional foods: the molecular mechanism of Nrf2-activating sunflower seed-derived antioxidant bioactive peptides
15:30 – 15:45	**Maria Laura Alfieri** - Hexamethylenediamine-mediated deposition of catechol-based thin films with potent antioxidant activity

15:45 – 16:00	**Salvio Suárez** - A Universal and Versatile Approach to Antimicrobial Coatings via copolymerization of a catechol and a diamine
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Session 4. Part II
Natural and Synthetic Antioxidants: Sustainable Methodologies for Recovery and Processing

Session chairs: **Alessandra Napolitano** and **Rosa Lamuela**

16:30 – 17:00	**Francisco J. Barba** - Antioxidant compounds´ recovery from alternative sources assisted by innovative extraction methodologies
17:00 – 17:15	**Faye Langston** - The impact of broccoli pre-processing on the bioaccessibility and intestinal cell uptake of glucosinolates and carotenoids from broccoli baked snacks
17:15 – 17:30	**Elisabetta Esposito** - Ethosomes and Transethosomes for polyphenol transdermal administration
17:30 – 17:45	**Emily Pilar Laveriano Santos** - Gender Differences between Total Polyphenols in Urine and Cardiovascular Risk Factors in Adolescents from Spain
17:45 – 18:00	**Ancuta Nartea** - The effects of mild baking processing, reproductive cycle and alternative aquafeed in the lipid quality and oxidative stress of fish fillets : the role of carotenoids and tocopherols
18:00 – 18:15	**Awards Ceremony and Closing Remarks**

Welcome from the Chairs

Dear Colleagues,

We are pleased to announce the first International Conference on Antioxidants: Sources, Methods, Health Benefits, and Industrial Applications.

The conference is organized by the MDPI open access journal *Antioxidants* (Impact Factor 7.675) and will be held as a three-day event in Barcelona, Spain, 10-12 May, 2023.

Antioxidants represent a huge area of investigation for researchers from many disciplines, and their importance in a variety of fields from health to food to innovative materials is increasingly appreciated.

Despite the enormous amount of research work related to this issue, our knowledge needs to be improved on the mechanism of action, the bioaccesibility and bioavailability of dietary antioxidants, oxidative stress and antioxidant defence systems, antioxidant-based therapeutic strategies, antioxidant functional food, and the design of biomaterials with antioxidant properties for use in biomedical applications or functional packaging. We have to continue pursuing the search for new natural sources of antioxidants while shifting toward sustainable methodologies for their extraction, and, finally, we need to standardize and validate the methods for their analysis and for evaluating and ranking the antioxidant power.

The main topics and sessions of the conference will cover:

- Health Outcomes of Antioxidants and Oxidative Stress with special emphasis on: cancer, cardiovascular diseases, microbiome and gut health, metabolic disorders, neurodegenerative pathologies, cognitive function and physical exercise and oxidative stress
- Antioxidant Enzyme Systems with special emphasis on: ROS, RNS, and RSS; mitochondria; redox signaling; photooxidative stress and Metalloenzymes
- Natural Antioxidants: Sources, Analysis, and Mechanisms of Action with special emphasis on: occurrence, profiling, analytical methods, bioavailability, and new methodologies for antioxidant power evaluation
- Natural Antioxidants: Sustainable Methodologies for Recovery and Processing with special emphasis on: new eco-friendly methodologies, agrifood wastes and by products, transformation to impart functionality, new materials, nanoparticles and industrial applications

The conference will host a number of internationally renowned speakers and invites submissions for oral presentations and posters. We wish the meeting may offer a unique opportunity for direct communication between scholars, favoring personal contacts between younger and experienced researchers on advanced research topics and technological issues.

All presenters at the conference will be encouraged to submit a full manuscript of their presentation for consideration for publication in a Special Issue of Antioxidants .

We look forward to seeing you at this exciting meeting in Barcelona.

Prof. Dr. Alessandra Napolitano
Conference Chair

University of Naples 'Federico II'
Italy

Prof. Dr. Rosa M. Lamuela Raventos
Conference Chair

University of Barcelona
Spain

General Information

The First International Conference on Antioxidants: Sources, Methods, Health Benefits and Industrial Applications will be held at the AXA Coventiion Centre, Barcelona, Spain, on 10 – 12 May 2023.

This conference seeks to bring together leading researchers from around the world and across all antioxidants-related fields, from health to food to innovative materials, to share the results of their recent studies.

Conference Venue

AXA Coventiion Centre
Avinguda Diagonal, 547, 08029 Barcelona

Registration Desk

The desk for registration, information and distribution of documents will be open from 14:00h on 10 May 2023.

Certificate of Attendance

Participants of the event will be able to downlod an electronic Certificate of Attendance by accessing their dashboards on Sciforum.net once the event is concluded. The certificates will be found under the “My Certificates" category.

Disclaimer

Delegates will receive a name-badge at the Information Desk, upon registration. The badge must be worn prominently in order to gain access to the congress area during the event. Admission will be refused to anyone not in possession of an appropriate badge.

Insurance

The organizers do not accept liability for personal accident, loss, or damage to private property incurred as a result of participation in *ICA 2023 – The First International Conference on Antioxidants: Sources, Methods, Health Benefits and Industrial Applications*. Delegates are advised to arrange appropriate insurance to cover travel, cancellation costs, medical, and theft or damage of belongings.

Barcelona and Catalonia

Barcelona is the capital and largest city of Catalonia and is Spain's second largest city, with a population of over one and half million people.

Located on the northeastern Mediterranean coast of Spain, this city has a rich and diverse history, with its roots dating back to Roman times. The fruitful medieval period established Barcelona's position as the economic and political centre of the Western Mediterranean. The city's Gothic Quarter bears witness to the splendour enjoyed by the city from the 13th to the 15th centuries.

The 20th century ushered in widespread urban renewal throughout Barcelona city, culminating in its landmark Eixample district, which showcases some of Barcelona's most distinctive Catalan art-nouveau, or modernista, buildings. The Catalan Antoni Gaudí, one of the most eminent architects, designed buildings such as La Pedrera, the Casa Batlló and the Sagrada Família church, which have become world-famous landmarks.

In 1992, Barcelona gained international recognition by hosting the Olympic games which brought about a massive upturn in its tourism industry. For visitors, this has translated into the very modern, yet incredibly old city you see now in the 21st century, where new elements work to both preserve and celebrate both the city's heritage and origins.

Barcelona is plenty of outdoor markets, restaurants, shops, museums, and churches. The city is also very walkable, with an extensive and reliable Metro system for more far-flung destinations.

For a complete overview, see wikitravel.org or visit barcelonaturisme.com.

The AXA Convention Centre

Address: Avinguda Diagonal, 547, 08029 Barcelona

The conference will be held at the AXA Convention Centre in Barcelona, Spain. The AXA Convention Centre is located in a vibrant modern zone of the city, next to the main road and with easy access from the airport, as well as the urban and suburban areas.

It is part of "L'Illa Diagonal", a modern complex which include a shopping centre, two 4-stars hotels, several offices, a sports centre, a public park and a parking with 2500 places.

MDPI, the Multidisciplinary Digital Publishing Institute, is a publisher of fully peer-reviewed, Open Access journals with a focus on robust and rapid editorial processes. Our aim is to ensure that high-quality research is verified and made available to the research community as quickly as possible. MDPI stands at the forefront of the Open Access movement, having launched its first online journal Molecules in 1996. Today, MDPI is a leader in Open Access, publishing more than 400 journals across all research disciplines and offering publishing-related initiatives to scholars:

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• JAMS - A complete manuscript submission system that incorporates all steps from initial submission to publication, including peer-review.

• IOAP – An Institutional Open Access Program that supports its members with access to MDPI's submission system, publication data and administrative processes relating to Article Processing Charge (APC) management.

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antioxidants
an Open Access Journal by MDPI

Antioxidants (ISSN ISSN 2076-3921) is an international, peer-reviewed, open access journal, published monthly online by MDPI. It has received an increased Impact Factor of 7.675, and an increased 5-Year Impact Factor of 7.886 in the latest edition of the Journal Citation Reports, published by Clarivate Analytics in June 2022.

Antioxidants provides an advanced forum for studies related to the science and technology of antioxidants, focusing on new insights and ideas on active species and processes of biological relevance, natural products, mechanisms of action, applications and uses.

Journal Webpage: https://www.mdpi.com/journal/antioxidants

Contact persons during the event

Elena González

Email: elena.gonzalez@mdpi.com

Charlotte Gardini

Email: gardini@mdpi.com

Sara Ottolini

Email: sara.ottolini@mdpi.com

Cédric Spinnler

Email: cedric.spinnler@mdpi.com

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Abstracts

Talks

Antioxidants and health: evolution of a concept

Daniele Del Rio

Human Nutrition Unit, Department of Food and Drugs, University of Parma, Italy

The lecture will be based on a historical and personal perspective of the idea of antioxidants, from its application to the preservation of foods from rancidity to the definition of the concepts of antioxidant activity of a molecule and antioxidant capacity (TAC) of a mixture of compounds contained in a specific food, to the wide use of the TAC as a putatively healthy characteristic of a diet and its use as a biomarker of good health. The discussion will then move to the more recently shared awareness of the many transformations dietary compounds that are (chemically) antioxidant undergo after ingestion, with a specific focus on dietary (poly)phenols, highlighting the intrinsic detachment between what is antioxidant in a test tube and what is antioxidant in the human organism, to then conclude showcasing some of the non-antioxidant biological actions of these same compounds as a consequence of their metabolism in humans. A tentative synthesis will be drawn and some future perspectives discussed.

Databases and metabolomics for assessing intake and bioavailability of polyphenols

Claudine Manach

Human Nutrition Unit, INRAE, University Clermont Auvergne, Clermont-Ferrand, France

Polyphenols are important bioactive compounds in our diet. A growing body of evidence supports that some of them may have beneficial effects on human health. However, the understanding of their effects relies on an accurate assessment of their intake. This is challenging due to their huge chemical diversity and wide distribution in plant foods. Current methods based on questionnaires and tables of food composition have inherent limitations, but recent improvements have been made, with a new FFQ focused on polyphenols. The discovery of biomarkers of plant food intake using metabolomics also opened new perspectives to complement questionnaires data.

Beyond the assessment of polyphenol intakes which is crucial for epidemiological studies, the interindividual variation in response to polyphenol consumption is of growing interest. Polyphenols are absorbed and extensively metabolized by host and gut microbiota enzymes, with some important variations between subjects. Certain metabotypes have been observed, characterized by the capacity to produce specific metabolites after intake of isoflavones or ellagitannins. Clinical studies have suggested that the biological response may depend on such metabotypes. Metabolomics is an approch of choice to analyze simultaneously the large range of polyphenol metabolites in biofluids and provide a detailed and comprehensive picture of individuals' true exposure in the body. Despite the enormous potential of untargeted metabolomics many challenges remain, in particular regarding the identification of new metabolites or metabolites with no standard available. PhytoHub is an expert-curated database which has been developed to help in this purpose. New developments that are ongoing in the FoodPhyt project funded by the JPI HDHL will be presented, including an inventory of all known metabolites of dietary phytochemicals, a new approach of in silico prediction of metabolism, and a library of spectral data.

Polyphenol exposure and body weight change: epidemiological to clinical evidence

Raul Zamora-Ros

Unit of Nutrition and Cancer, Epidemiology Research Program, Catalan Institute of Oncology, Bellvitge Biomedical Research Institute (IDIBELL), Hospitalet de Llobregat (Barcelona), Spain

Overweight (BMI 25-29.9kg/m2) and obesity (BMI ≥30kg/m2) has grown to epidemic proportions, where over 1.9 billion adults worldwide are overweight of which 650 million have obesity. Globally, 8% of deaths in 2017 were attributed to obesity – this represents an increase from 4.5% since 1990. Therefore, effective public health strategies to prevent body weight gain are critical to reduce the impact of obesity health-related issues. Polyphenols may have a protective role against body weight gain, with adipose tissue as their potential therapeutic target. In this talk, an overview of the epidemiological evidence, particularly focusing in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, on the associations between polyphenol exposure and body weight will be presented. There will be a comparison of the results obtained from different populations, using different dietary questionnaires or nutritional biomarkers, and different food composition databases. There will be also a discussion of the results of polyphenol supplementation in several clinical trials. Finally, this talk summarizes the current state of the field, provide a critical appraisal of new developments and identify priority areas for future research.

A wave of pro-oxidant metabolites is released by melanoma cells during chemotherapy and triggers paracrine pro-survival epigenetic changes in the tumor microenvironment

Giorgia Musto, Valentina Laurenzi, Mariano Stornaiuolo

Department of Pharmacy University of Napoli Federico II, Napoli Italy Via Domenico Montesano 49 80131 Napoli Italy Phone +39 081 678117

Recent compelling clinical evidence suggests that, in some circumstances, the chemotherapy regimen may play a paradoxical role in tumor treatments and, unwantedly, itself acts as a fosterer of tumor relapse and metastasis. Lung, prostate, bowel, melanoma, and breast cancer are examples of tumors where chemotherapy regimens have indicated the exertion of these unwelcome ''opposite effects'', enhancing the rate of tumor relapse and metastasis even after successful treatment and the eradication of primary tumors.

The rationale behind this unwanted opposite effect of chemotherapy seems to depend on the cytotoxic effect of cancer treatments. By inducing cell damage in the parenchyma of the tumor, chemotherapy promotes the establishment of concerted paracrine signaling in the tumor microenvironment between the surviving cancer cell population and the stroma. This event results in the activation of a "pro-tumoral" stroma that contributes to important steps of the tumorigenic cascade, facilitating relapse, extravasation of cancer cells to secondary metastatic sites, and metastatic growth.

Until now, pro-inflammatory cytokines have been proven to be secreted by chemotherapy-treated cancer cells and act as paracrine factors to activate the stroma. However, the mechanism underpinning the opposite effects of chemotherapy remains largely obscure. Here, we use high-resolution metabolomic profiling to identify glucose and palmitic acid as metabolites secreted by melanoma cells during chemotherapy. We show that glucose acts in a paracrine way and induces oxidative stress in the stroma. Its effect alters stromal metabolism and culminates in epigenetic changes in the stroma, ultimately promoting growth hormone IGF-II secretion and the activation of the WNT/-beta catenin pathway, involved in de-differentiation of the parenchyma and tumor relapse.

Finally, we prove that antioxidants can block this signaling cascade, underlining the possibility and need to design novel therapeutic strategies and effective treatments for metastatic disease, including antioxidants, to avoid ineffective and potentially opposite effects of the chemotherapy regimen for patients.

High-intensity exercise modifies diet habits and microbiota composition in healthy students

Sabrina Donati Zeppa[1], Stefano Amatori[1], Davide Sisti[1], Deborah Agostini[1], Marco Gervasi[1], Valerio Pazienza[2], Alessia Bartolacci[1], Giovanni Piccoli[1], Marco B. L. Rocchi[1], Piero Sestili[1], Vilberto Stocchi[3]

1 Department of Biomolecular Sciences, University of Urbino Carlo Bo, Via Saffi, 2, 61029 Urbino, Italy
2 Fondazione I.R.C.C.S. "Casa Sollievo della Sofferenza" Hospital 71013 San Giovanni Rotondo-FG, Italy
3 Università Telematica San Raffaele, 00166 Rome, Italy

The gut microbiota constitutes a dynamic microbial system constantly challenged by environmental conditions, including physical exercise. This study aimed to investigate the effects of nine weeks of high-intensity interval exercise on gut microbiota composition in seventeen healthy young adults. Before and after the training period, the body composition and maximal oxygen consumption (VO2max) were assessed. Diet was monitored daily from two weeks before the beginning of the training period until the end of the study, and it was performed by call interviews each day after dinner; the variables considered for the analyses were the total energy intake, macronutrient quantities, starch, soluble and insoluble fbres, saturated, monounsaturated, and polyunsaturated fat, Omega-3, Omega-6, iron, vitamins A, C, D, and E, and amino acids (alanine, valine, isoleucine, leucine, tryptophan). The gut microbiota composition was analysed before and after nine weeks of high-intensity interval cycling training by 16S rRNA amplicon sequencing. Physical-exercise-induced changes in microbiota composition, at all taxonomic levels, were analysed. Changes in the relative abundance of eighteen genera were correlated to changes in twenty environmental factors grouped by physical features, sport-related features, and dietary features. Nine weeks of high-intensity exercise induced modifications in gut microbiota composition in healthy male college students, shifting the gut microbial population towards a healthier microbiome with a benefit to human health in general.

Overall mass balance analysis of an integrated pressurized liquid extraction and resin purification process for the recovery of phlorotannins from Chilean brown seaweeds

Camila Vega[1], Nils Huamán-Castilla[2], Luis Omar Mamani[2, 3], María Salomé Mariotti-Celis[4], José Ricardo Pérez-Correa[1]

1 Department of Chemical and Bioprocess Engineering, Faculty of Engineering, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, P.O. Box 306, Santiago 7820436, Chile
2 School of Agroindustrial Engineering, Universidad Nacional de Moquegua, Moquegua 18001, Peru
3 Department of Chemical and Bioprocess Engineering, Faculty of Engineering, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, P.O. Box 306, Santiago 7820436, Chile.
4 Nutrition and Dietetics School, Faculty of Medicine, Universidad Finnis Terrae, Pedro de Valdivia 1509, Providencia, Santiago 7501015, Chile

The integrated pressurized liquid extraction/resin purification (PLE/RP) process can significantly reduce undesirable compounds in polyphenol-rich extracts, increasing their value and applicability. In the extraction of brown seaweeds, the recovery of phlorotannins after RP may be substantially reduced, affecting the economic viability of the integrated process. Identifying the critical stages that determine the overall performance of the integrated process is required to achieve commercial success. A mass balance analysis was applied in a PLE/RP process to define key stages affecting the overall recovery of phlorotannins of Durvillaea incurvata, an endemic Chilean brown seaweed. Seaweeds from two locations in Chile (Concepción and Valdivia) were processed with a PLE/RP method. The total phlorotannins content (Folin–Ciocalteu) and the antioxidant capacity (ORAC) of all the streams of the integrated process (extraction, adsorption, first washing, desorption, and second washing) were determined. PLE extracts from Concepción presented ~%50 higher phlorotannin content (5.30 mg of phloroglucinol equivalent (PE)/g of dry seaweed solid (gds)) and antioxidant capacity (29.67 µmol TE/ gds) than those from Valdivia. In all cases, the adsorption stage retained ~95% of the total phlorotannins, while ~23% of the total phlorotannins were lost in the first washing stage. The desorption stage recovered between 59% and 69% of the phlorotannins retained in the absorption stage, and only ~2% of total phlorotannins were lost in the second washing stage. The antioxidant capacity of the different RP streams showed a similar trend, except for the desorption stage, which recovered ~30% less antioxidant capacity than total phlorotannins. Future research should focus on recovering phlorotannins from the first washing by integrating other RP or membrane stages.

Fighting antibiotic resistance: giving a second life to natural compounds

Ana Barros, Joana Campos, Raquel Fernandes, Mónica Serra, Javier Fidalgo, Hugo Almeida, Ana Casas

Mesosystem, Portugal

Antibiotics prevent millions of deaths every year and remain the primary treatment for potentially fatal bacterial infections. Yet, inappropriate prescription rates and overuse of antibiotics have led to resistance that has created a global health emergency and kills at least 700 000 people a year. If no action is taken, it has been estimated that by 2050, 10 million worldwide deaths could result from antibiotic resistance, making it deadlier than cancer. The antimicrobial resistance mechanisms have spread rapidly in recent decades, namely through the production of enzymes (e.g., extended-spectrum β-lactamases (ESBLs)), conferring resistance to several classes of antimicrobials such as aminoglycosides, quinolones or carbapenems, which may lead to treatment options becoming limited. In this study, we will present not only different methodologies of extraction of these compounds from natural sources, namely a variety of polyphenols and stilbenes that contribute to remarkable biological activities such as anti-inflammatory, antioxidant and antibacterial, among others, as well as their biological activities and applications in the cosmetic and pharmaceutical industries.

Genistein as a treatment for prodromal Alzheimer's disease. Results of the GENIAL clinical trial (NCT 01982578)

Jose Viña

Freshage Research Group Leader, Dpt. Physiology. Fac. Medicine, University of Valencia, Avda. Blasco Ibañez 15- 46010, Valencia, Spain

Polyphenols have many therapeutic applications, especially in degenerative diseases. In relation to Alzheimer's disease, we have observed that genistein, a phytoestrogen present in the leaves and in other foods, is effective in delaying the onset of dementia as well as other pathognomonic signs of the disease both in experimental animals (APP/PS1 double transgenic mice) and in patients with prodromal Alzheimer's disease with mild cognitive impairment. Animal studies have shown that genistein treatment decreases beta-amyloid load, has an anti-inflammatory effect, prevents loss of olfaction, and prevents loss of cognition as measured by routine animal behavior tests in the laboratory.

These results led us to consider a pilot clinical trial in patients with mild cognitive impairment, with a positive charge for beta amyloid (measured by PET). Genistein was administered 120 milligrams daily for 1 year, with assessments at baseline at 6 months and 12 months of treatment. The pilot trial was randomized, bi-centric and placebo controlled.

Results showed that after 12 months of treatment, patients treated with genistein lost significantly less cognition than those treated with placebo. A non statiscally significant improvement was observed in absolutely all the tests used. This trend was statistically significant in 3 of the tests as well as in the consolidated score that includes results from all the tests.

The implications of these results for the therapeutics of prodromal Alzheimer's disease will be discussed.

An anti-inflammatory diet, measured by urinary polyphenol excretion, and Metabolic Syndrome in the PREDIMED cohort

Anna Tresserra-Rimbau[1, 2, 3], Camila Arancibia[1, 2], Inés Domínguez-López[1, 2], Xiaohui Guo[4], Ramón Estruch[3, 5], Miguel Ángel Martínez González[3, 6], Montserrat Fitó[3, 7], Emilio Ros[3, 8], Miguel Ruiz-Canela[3, 6], Rosa M. Lamuela-Raventós[1, 2, 3]

1 Department of Nutrition, Food Science and Gastronomy, School of Pharmacy and Food Sciences XIA, University of Barcelona, 08921 Santa Coloma de Gramenet, Spain
2 Institute of Nutrition and Food Safety (INSA-UB), University of Barcelona, 08921 Santa Coloma de Gramenet, Spain
3 Consorcio CIBER, M.P. Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III (ISCIII), 28029 Madrid, Spain
4 College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China
5 Department of Internal Medicine, Hospital Clinic, Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain
6 University of Navarra, Department of Preventive Medicine and Public Health, IdiSNA, Pamplona, Spain
7 Unit of Cardiovascular Risk and Nutrition, Institut Hospital del Mar de Investigaciones Médicas, Barcelona, Spain
8 Department of Endocrinology, Hospital Clinic, IDIBAPS, Barcelona, Spain

Chronic inflammation is associated with metabolic syndrome (MetS) and other noncommunicable diseases. Diets rich in fruits and vegetables can have strong anti-inflammatory effects, partly due to their polyphenol content. This study aimed to assess the polyphenol intake using a urinary biomarker of an anti-inflammatory diet and associate it with the MetS status. We performed a longitudinal analysis with a subsample of 543 participants (52% women, mean age of 67.5 years) from the PREDIMED cohort with high CVD risk. The total polyphenol excretion (TPE) in urine was determined at baseline and at a five-year follow-up using a validated Folin–Ciocalteu spectrophotometric method. A dietary inflammatory index (DII) was calculated from a validated 137-item food frequency questionnaire. Multivariable linear regression analyses were performed to assess the association between tertiles of changes in TPE with changes in the DII scores and with the MetS status at a five-year follow-up. We found that higher tertiles presented lower anti-inflammatory scores in women. In addition, TPE was inversely associated with changes in the MetS status in both men and women. Therefore, we conclude that urinary polyphenols, at least in women, may represent a potential biomarker of anti-inflammatory diet consumption and are prospectively associated with improvement in MetS.

Identified phenolic compounds of the colonic fraction from biscuits with Tannat grape pomace present potential to improve gut health

Victoria Olt[1], Romina Curbelo[2], Eduardo Boido[2], Eduardo Dellacasa[2], Alejandra Medrano[1], Adriana Maite Fernández[1]

1 Laboratorio de Bioactividad y Nanotecnología de Alimentos, Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República, General Flores 2124, Montevideo 11800
2 Área Analítica Orgánica, Departamento de Química Orgánica, Facultad de Química, Universidad de la República, General Flores 2124, Montevideo 11800, Uruguay

Oxidative stress can cause chronic diseases such as cancer, diabetes, and cardiovascular and neurodegenerative pathologies. The consumption of functional foods rich in bioactive compounds with antioxidant properties may reduce oxidative stress and thus prevent the development of such diseases. Grape pomace, the main winemaking byproduct, contains significant amounts of bioactive compounds with antioxidant capacity. Likewise, the impact of bioactive compounds on disease prevention depends on their bioaccessibility and bioavailability. This study investigated the bioactive (antioxidant, antidiabetic, and antiobesity) properties of the bioaccesible and colonic fractions obtained after digestive simulation of different formulations of biscuits with Tannat (Vitis Vinifera cv Tannat) grape pomace (TGP) and "no added sugar". Additionally, the cell viability and inhibition of intracellular reactive oxygen species (ROS) formation of these two fractions were assessed in Caco-2 cell monolayers. The bioactivities of bioaccesible fractions of TGP biscuits showed no significant differences compared to control biscuits ($p>0.05$). In contrast, colonic fraction bioactivities of TGP biscuits were different from control biscuits ($p<0.05$). Regarding the antioxidant capacity measured by ABTS and ORAC-FL, the colonic fraction of the biscuit with 20% TGP and 4% sucralose showed 397.83 µmol TE/g sample and 161.76 µmol TE/g sample, while the colonic fraction of the control biscuit with 4% sucralose showed 124.2 µmol TE/g sample and 50.86 µmol TE/g sample, respectively. Moreover, the colonic fraction of the biscuit with 20% TGP and 4% sucralose inhibited the formation of ROS under AAPH-induced oxidative stress conditions on Caco-2 intestinal cells. Phenolic compounds in the colonic fraction were identified by HPLC-DAD-MS, mainly showing flavan-3-ols (procyanidins, catechin, and epicatechin), flavonols (quercetin derivatives), and anthocyanins (glucosides and (p-coumaroyl)glucosides of cyanidin, delphinidin, malvidin, peonidin, and petunidin). In conclusion, the results show that the bioactive compounds of TGP remain in the colonic fraction of the biscuits with the potential to reduce oxidative-stress-related chronic diseases.

Effects of oral Pterostilbene in patients with diabetic retinopathy, a clinical trial

Raquel Burggraaf-Sánchez de las Matas[1], Mª Carmen Desco Esteban[2], Ángel Luis Ortega Valero[3], Jorge Mataix Boronat[2]

1 Hospital of Sagunto. Vitreo-Retina and Uveitis Unit
2 FISABIO Oftalmología Médica, Vitreo-retina Unit
3 Faculty of Pharmacy, Department of Physiology, University of Valencia

Introduction: The current management of diabetic retinopathy (DR) by ophthalmologists consists of the observation and control of systemic risk factors until advanced stages of the disease are present. As oxidative stress has been demonstrated to be a crucial factor in the development of DR, oral antioxidants have been postulated as a non-invasive adjuvant treatment during early stages of DR. Pterostilbene (PTER) is an analogue of polyphenol resveratrol, which presents higher bioavailability.

Methods: A prospective, randomized, double-blind placebo-controlled intervention trial was performed. The study included 30 type 2 diabetic patients with mild to moderate nonproliferative diabetic retinopathy. A total of 15 were randomly assigned to the placebo group and the remaining 15 were assigned to the treatment group, taking 250 mg of oral PTER for 12 months. The subjects were followed up for 18 months. Clinical progression of DR, changes in analytic variables, and the effects on systemic risk factors for the progression of DR were analyzed.

Results: The average age was 64.5 years old. A total of 87% of the subjects completed the study; 80.8% were insulin-dependent and 57.7% presented obesity at the beginning of the study. Among the clinical parameters, there was no significant change in visual acuity, nor in the DR stage along the follow-up period in both groups, but a significant reduction in the tortuosity vessel index was observed in the treatment group. The central macular thickness (CMT), which correlates directly with the presence of diabetic macular edema, showed a trend of decreasing in the PTER group (from 269.2 ±35.9 to 261.1 ± 34.7 µm (p=0.086)). Among the systemic risk factors for the progression of DR, the systolic arterial pressure levels dropped significantly from 143.6 ± 17.3 to 133.1 ± 19.3 mmHg (p=0.031) in the Pterostilbene group after 12 months of treatment but increased again after discontinuation. Diastolic arterial pressure showed the same trend (79.6 ±15 to 65.5 ± 21.3 mmHg (p=0.065)) after 12 months of treatment and increased after discontinuation. There was a significant the LDL levels from 95.4 ± 27 to 85.2 ± 25.8 mg/dL (p=0.028) in the treated group in the long term (after 18 months of follow-up). The HbA1c% and blood glucose levels did not change significantly.

Conclusions: The use of oral PTER allowed better control of the systemic risk factors for the progression of DR. No significant clinical changes were observed, but it must be taken into account that DR is a chronic disease in which it is difficult to observe changes at early stages. Given the trend of the CMT to decrease in the PTER group, it would be useful to study larger samples of individuals.

Gut region-specific expression of nuclear factor kappa B p65 in the myenteric ganglia and its microenvironment of streptozotocin-induced diabetic rats

Bence Pál Barta, Benita Onhausz, Afnan AL Doghmi, Abigél Egyed-Kolumbán, Szilvia Kiss, Zita Szalai, János Balázs, Mária Bagyánszki, Nikolett Bódi

Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged, Hungary

Long-lasting hyperglycemia is a serious oxidative stress factor in type 1 diabetes. In our previous studies, we demonstrated that the myenteric neurons located in different intestinal regions display different susceptibilities to diabetic injuries1,2; the expression of tumor necrosis factor alpha3 and its receptors4 also reveals gut-segment-specific alterations in diabetic animals. Nuclear factor kappa B (NF-κB) has important roles in inflammation and stress responses5. Furthermore, tumor necrosis factor alpha can induce the NF-κB signalling pathway, which exerts protective or degenerative effects on neurons5,6.

Therefore, our aim was to investigate the expression of NF-κBp65 in the myenteric ganglia and its microenvironment in different intestinal segments in type 1 diabetes. Ten weeks after the onset of hyperglycemia, tissue samples from the duodenum, ileum, and colon of control, streptozotocin-induced diabetic, and insulin-treated diabetic rats were processed with immunofluorescence, immunogold electron microscopy, and enzyme-linked immunosorbent assay.

In controls, the proportion of NF-κBp65-immunoreactive (IR) myenteric neurons and density of NF-κBp65-labelling gold particles showed an increasing tendency from the proximal to distal part in the ganglia and its microenvironment; however, the NF-κBp65 protein content decreased.

In diabetic rats, the proportion of NF-κBp65-IR neurons significantly increased in the ileum and decreased in the colon, while it remained unchanged in the duodenum relative to controls, which agreed with electron microscopic findings. However, in the intestinal smooth muscles of diabetics, the density of gold labels significantly increased in the duodenum relative to controls. Moreover, the NF-κBp65 concentration of tissue homogenates significantly increased in the diabetic duodenum and ileum compared to controls. Immediate insulin treatment had beneficial effects in the duodenum and colon.

Our results highlight that diabetes-related alterations of NF-κBp65 expression are strictly region-dependent and may contribute to region-specific diabetic enteric neuropathy.

Nutritionally targeting mitochondria to prevent age-associated diseases: Hydroxytytrosol is a rising star after resveratrol

Jiankang Liu

1 University of Health and Rehabilitation Sciences, Qingdao, China
2 Xi'an Jiaotong University, Xi'an, China

Due to the limitation and ineffectiveness of antioxidants in extending life span and preventing age-related diseases, scientists have tried to find new solutions beyond antioxidants for delaying aging and preventing age-related diseases. The French paradox-derived resveratrol has become a star of nutraceutical because of the discovery of its effect on Sirtuins regulation developed by Sinclare's lab at Harvard. Nowadays, resveratrol has been widely studied on retarding aging and preventing/ameliorating numerous disorders in cellular and animal models as well as clinical studies. We have collaborated with DSM scientists to investigate a number of naturally occurring nutraceuticals and found that hydroxytyrosol (HT) has a unique and potent effect on regulating mitochondrial metabolism. HT is abundant in olives and virgin olive oil, which are widely used in Mediterranean diets. Epidemiologic studies have demonstrated that the people of the Mediterranean basin enjoy a healthy lifestyle with decreased incidence of heart disease, prostate and colon cancer, and rheumatoid arthritis. One of the possible reasons is that Mediterranean people have a high intake of HT from olive and olive oil. The beneficial effects of HT have been mainly attributed to its antioxidant effect. However, we have demonstrated that HT is an effective mitochondria-targeting nutraceutical in various cellular and animal models, including age-associated macular degeneration, metabolic disorders, cancer, osteoporosis, and muscle atrophy. Because mitochondrial dysfunction has been implicated in aging and various diseases, the potent effects of HT on regulating mitochondrial metabolism suggest that HT is another rising star of the nutraceutical field after resveratrol and will be widely used for delaying aging and preventing/ameliorating age-associated diseases.

Disrupting redox homeostasis and the mTOR pathway to kill T-lymphoblastic leukemia (T-ALL) cells

Micol Silic-Benussi[1], Evgenyia Sharova[1], Francesco Ciccarese[1], Ilaria Cavallari[1], Vittoria Raimondi[2], Loredana Urso[1, 2], Alberto Corradin[1], Barbara Buldini[3], Samuela Francescato[3], Sonia A. Minuzzo[2], Stefano Indraccolo[1, 2], Donna M. D'Agostino[1, 4], Vincenzo Ciminale[1, 2]

1 Veneto Institute of Oncology IOV – IRCCS, Padova, Italy
2 Department of Surgery, Oncology, and Gastroenterology, University of Padova, Padova, Italy
3 Pediatric Hemato Oncology, Maternal and Child Health Department, University of Padova, Padova, Italy
4 Department of Biomedical Sciences, University of Padova, Padova, Italy

Approximately 20% of patients with pediatric T-cell acute lymphoblastic leukemia (T-ALL) are incurable with current therapy protocols. T-ALL cells frequently present constitutive activation of the mechanistic target of rapamycin (mTOR) pathway and resistance to glucocorticoid (GC)-based chemotherapy. Interrogation of a gene expression dataset from a cohort of therapy-resistant and -responsive T-ALL samples revealed enrichment for transcripts related to mTOR and reactive oxygen species (ROS) in therapy-resistant samples.

We thus employed pharmacological inhibitors and gene silencing to investigate the connection between the mTOR pathway and redox homeostasis. In vitro studies carried out on GC-resistant T-ALL cell lines, primary T-ALL samples, and patient-derived T-ALL xenografts (PDX) showed that the mTORC1 inhibitor everolimus increased the ROS levels, augmented lipid peroxidation, and activated the ROS-sensitive transcription factor NRF2. In addition, everolimus reduced the levels of the pentose phosphate pathway (PPP) enzyme glucose-6-phosphate dehydrogenase (G6PD) and the PPP product NADPH, and it killed leukemic cells while sparing normal T-cells. These effects were blunted by ROS scavengers and were phenocopied by siRNA-mediated silencing of G6PD or the mTORC1 cofactor Raptor. In vivo studies of NOD/SCID mice inoculated with refractory T-ALL PDX demonstrated that everolimus enhanced the antitumor activity of the GC dexamethasone in conditions of high tumor burden that mimicked the clinical setting of acute leukemia.

These findings reveal a link between mTOR pathway activation and ROS homeostasis in T-ALL cells and furnish mechanistic evidence to support the combination of glucocorticoids with mTOR inhibitors to treat GC-resistant T-ALL.

Blood pressure reduction and improved plasma GSH in hypertensive rats supplemented with cocoa shell extract

Santiago Ruvira-Hernando[1], Pilar Rodríguez-Rodríguez[1], David Ramiro-Cortijo[1], Metee Iampanichakul[2], Silvia Arribas[1]

1 Department of Physiology, Faculty of Medicine. Universidad Autónoma de Madrid. C/ Arzobispo Morcillo 2, 28019-Madrid, Spain.
2 Department of Physiology, Faculty of Medicine, Khon Kaen University, Thailand

Cocoa manufacturing produces large amounts of waste, but its by-products may be recycled and transformed into ingredients with health-promoting properties. We previously studied the vascular actions of cocoa shell, a byproduct rich in phenolic compounds, caffeine, and theobromine. An aqueous extract (CSE) and its bioactive compounds induce relaxation in rat arteries, also being able to effectively scavenge superoxide anion (Rodriguez-Rodríguez et al., Antioxidants (Basel). 2022;11(2):429). Given the vasodilatory and antioxidant properties of CSE in vitro, we **aimed** to investigate its possible cardiovascular effects in vivo in a rat model of hypertension. **Methods.** We used 12-month-old male and female rats exposed to undernutrition during the fetal period (MUN), who developed hypertension together with oxidative stress in adult life (only in males). The rats were supplemented with a gelatin cube containing 250 mg/kg CSE, for 2 weeks (5 days/week). We assessed the systolic blood pressure (SBP) by tail-cuff plethysmography and obtained a blood sample through the tail before (basal) and at the end of CSE supplementation. In plasma, we evaluated the SOD and catalase activity and GSH by spectrophotometry and assessed the presence of bioactive compounds (UAM-CIAL Metabolomics Service). **Results.** Bioactive compounds (caffeine and theobromine) were absent in plasma in basal conditions, and they were present after supplementation. Male MUN males had higher SBP compared to females (males 182.2±3.6, n=5; females=138.7±4.9 mmHg; n=5, p-value 0.001). In males, CSE supplementation significantly lowered the SBP (males+CSE=159.9±1.8 mmHg; p-value=0.02), while in females, CSE did not modify it (females+CSE=143.0±6.3 mmHg). In male rats, the plasma GSH was significantly elevated by supplementation (p0.006), but it was not modified in females (p-value= 0.46). Plasma catalase and SOD activities were not modified by CSE supplementation. **Conclusion.** Cocoa shell is a byproduct with potential applications in the context of hypertension.

Effects of smoking on skin—application of spectroscopic measurements and biological assays

Martina C. Meinke[1], Phuong Thao Tran[1, 2], Kerstin Wolk[3, 4], Katinka Jung[5], Silke B. Lohan[1]

1 Charité – Universitätsmedizin Berlin orporate member of Freie Universität Berlin, Humboldt-Universität, Department of Dermatology, Venereology and Allergology, Center of Experimental and Applied Cutaneous Physiology, Charitéplatz 1, 10117 Berlin, Germany
2 Institute of Pharmacy, Department of Pharmacology, Freie Universität Berlin, Königin-Luise-Str. 2+4, 14195, Berlin, Germany
3 Charité – Universitätsmedizin Berlin orporate member of Freie Universität Berlin, Humboldt-Universität, Department of Dermatology, Venereology and Allergology, Psoriasis Research and Treatment Center, and Institute of Medical Immunology, Charitéplatz 1, 1
4 BIH Center for Regenerative Therapies, Charité – Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany; kerstin.wolk@charite.de
5 Gematria Test Lab GmbH, 13187 Berlin, Germany

Smoking is still very common, but each cigarette leads to a boost in free radicals, which must be controlled by antioxidants (AO) in the body. In the present study, the status of the cutaneous AO system compared to the blood was investigated in healthy smokers using electron paramagnetic resonance spectroscopy and biological assays. The basic status compared to non-smokers and the short-term impact of controlled cigarette consumption in smokers were analyzed regarding endogenous and exogenous AO. In smokers, the basic level of carotenoids as representative of exogenous AOs was decreased in skin in vivo and in blood plasma. However, the levels of glutathione (GSH), the prototypical endogenous AO, analyzed in tapes stripped from the skin and blood plasma, were increased in smokers in vivo and in blood plasma. This increase was reflected by elevated overall radical scavenging activity in the skin. To resist permanent stress situations, metabolism produces more endogenous AOs, thus strengthening the skin's AO status. This may indicate long-term adaptation to chronic oxidative stress. This effect was not clearly visible in blood; this compartment may be a suitable immediate indicator of AO consumption. Moreover, decreased levels of AKR1C2, which we show for the first time to be expressed in immune cells, may be a candidate marker for long-term smoking.
Furthermore, direct skin exposure to smoke leads to radical production within the skin. This oxidative stress can be sensitively detected by the fluorescence background using Raman spectroscopy. So, a smoker's skin might be affected by the side stream of the smoke and by its inhalation, explaining the more pronounced effects on skin compared to blood plasma.

References

S.B. Lohan, K. Bühring, A.-C. Lauer, A. Friedrich, J. Lademann, A. Buss, R. Sabat, K. Wolk, M. C. Meinke, Analysis of the Status of the Cutaneous Endogenous and Exogenous Antioxidative System of Smokers and the Short-Term Effect of Defined Smoking thereon, Antioxidants 2020, 9 (537); doi:10.3390/antiox 9060537

Tran PT, Beidoun B, Lohan SB, Talbi R, Kleuser B, Seifert M, Jung K, Sandig G, Meinke MC. Establishment of a method to expose and measure pollution in excised porcine skin with electron paramagnetic resonance spectroscopy. Ecotoxicol Environ Saf. 2022 Nov 4;247:114258. doi: 10.1016/j.ecoenv.2022.114258. Online ahead of print. PMID: 36343452

Aliophen® Formulation Based on Malts and Hops, characterization, biological activities, and applicative outcomes.

Carmela Spagnuolo, Idolo Tedesco, Gianluca Picariello, Eva Adabbo, Iole Meola, Francesco Siano, Gian Luigi Russo

National Research Council, Institute of Food Sciences, 83100 Avellino, Italy

Malts and hops are the main components of beer. They represent rich sources of bioactive compounds in part responsible for the beneficial effects of moderate consumption of this beverage. A new formulation based on barley malts and hops named Aliophen® was recently obtained using a patented production process (PCT/IB2018/056283). Its potential beneficial effect in disease prevention has been investigated in different preclinical models. Aliophen® possesses a high total polyphenolic content, shows antioxidant activity against low-density lipoprotein (LDL) oxidation, and can protect erythrocytes from hemolysis. Moreover, the chemopreventive and antioxidant effects of Aliophen® formulation against colon cancer have been demonstrated in a mouse model of azoxymethane-induced carcinogenesis. To better define the chemical and biological characteristics of Aliophen®, the carbohydrate and protein components were eliminated through a specific protocol; subsequently, the formulation was separated by preparative HPLC in fractions of different polarity. Those rich in pro-anthocyanins, pro-anthocyanidins, and tannins resulted in the most effective in reducing cell viability in different malignant cell lines by mechanisms interfering with redox modulation. Data will be discussed and interpreted in light of the potential applicative outcomes of Aliophen® and its new derivatives as nutraceuticals or supplements in disease prevention or therapy.

Does endogenous antioxidant system indeed require support from exogenous antioxidants to maintain redox homeostasis?

Patrycja Jakubek, Monika Kuczyńska, Klaudia Suliborska, Barbara Kusznierewicz, Jacek Namieśnik, Wojciech Chrzanowski, Agnieszka Bartoszek

Faculty of Chemistry, Gdansk University of Technology, Gdansk, Poland

The food-derived antioxidants are still commonly perceived as additional line of support for endogenous antioxidant barrier in a situation of oxidative stress. However, the clearcut electrochemical parameters of oxidative stress have not been delineated so far and currently the emphasis has shifted towards redox homeostasis maintenance. In the latter case, endogenous systems may be supported by exogenous reducing compounds in their ROS neutralization, but also if overdone, the organism may be pushed into reductive stress. In our recent research, we compared the antioxidant efficacy of exogenous and endogenous reducing compounds. Based on our results, it will be argued that the exogenous reducing agents, if present, seem to take over the antioxidant barrier role to maintain cellular redox homeostasis and by this save endogenous antioxidants, so as they can play their other physiological roles.

Assessment of the redox homeostasis in blood by EPR spectroscopy using UVA irradiation as stressor

Silke B. Lohan[1], Pauline Nibbe[1], Johannes Schleusener[1], Silas Siebert[1], Doreen Brandt[1], Ronja Westphalen[1], Nadine Schüler[2], Bastian Berger[2], Eva M.J. Peters[3, 4], Martina C. Meinke[1]

1 Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Charitéplatz1, Department of Dermatology, Venereology and Allergology, Center of Experimental and Applied Cutaneous Physiology, 10117 Be
2 Freiberg Instruments GmbH, Freiberg, Germany
3 Justus-Liebig-Universität Giessen, Department of Psychosomatic Medicine and Psychotherapy, Psychoneuroimmunology Laboratory, Friedrichstraße 33, 35390 Gießen, Germany
4 Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Charité Center 12 (CC12) for Internal Medicine and Dermatology, Charitéplatz 1,10117 Berlin, Germany

Supplementation with antioxidants has increased worldwide in order to promote health, counteract diseases, and treat health and cosmetic issues, e.g. skin aging. Antioxidants have a positive effect on the redox homeostasis when applied at physiological doses, but at concentrations above physiological acceptability, they can have pro-oxidative effects. Electron paramagnetic resonance (EPR) spectroscopy allows the direct detection of radicals in different systems. An in-situ irradiation source (UVA-LED) and EPR spectroscopy were used as a stressor and readout system, respectively, to develop a method for the determination of the redox status in blood plasma. The method was first established using protein solutions and various plasma samples from a transfusion medicine biobank and were then validated in a double-blind placebo-controlled pilot study in vivo, in which blood plasma samples were analyzed using EPR spectroscopy before and after a defined oral intake of different vitamin C doses.
The approach was validated by reference measurements of the exogenous antioxidants β-carotene and vitamin C as well as glutathione as an endogenous representative. Malondialdehyde was studied for oxidative stress.
Protein samples with antioxidants and plasma samples from volunteers displayed two slopes (m1, m2) for radical production, whereby m1 represented the quantities of antioxidants and proteins and m2 represented the protein content only. These two slopes in combination with the intervening transition area (T) were used to calculate the oxidative stress coping capacity (OSC) value, which correlated positively with the vitamin C concentration in blood plasma. Furthermore, a selective radical quenching mechanism for vitamin C was observed: the proportion of reactive oxygen species (ROS) in the plasma samples was degraded depending on the vitamin C concentration ingested; the proportion of lipid oxygen species (LOS) remained stable. OSC may represent a sensitive method to detect stress and treatment effects on the redox status in vivo to be used in future validation and stress reduction studies.

Monitoring cytosolic and mitochondrial H_2O_2 and GSH/GSSG levels linked to physiology and pathology

Laura De Cubas, Elena Hidalgo

Oxidative Stress and Cell Cycle group, Universitat Pompeu Fabra, Barcelona

Genetically-encoded probes are used to measure H_2O_2 in living cells. Using the two main families of reporters, roGFP-based proteins and HyPer-derivatives, we compared the most sensitive ones to be expressed in fission yeast: roGFP2-Tpx1.C169S and HyPer7. These reporters can detect low micromolar concentrations of extracellular peroxides in Schizosaccharomyces pombe. roGFP2-Tpx1.C169S is around five times more sensitive than HyPer7, and is able to detect cytosolic fluctuations of peroxides upon ETC inhibition. Contrary to roGFP2-Tpx1.C169S, HyPer7 tagged to the mitochondrial matrix is fully functional and capable of measuring steady-state levels and ETC-dependent H_2O_2 fluctuations. MTS-HyPer7, but not cytosolic HyPer7, is partially oxidized under basal conditions up to 40%, and this basal oxidation was abolished by co-expressing different H_2O_2 scavengers in the matrix. The matrix-to-cytosol gradient of peroxides, with high concentrations in the matrix and low nanomolar levels in the cytosol, was eliminated through the depletion of the main peroxiredoxin of S. pombe Tpx1. We conclude that the matrix-to-cytosol gradient is due to the activity of this cytosolic peroxiredoxin, since Δtpx1 has the same degree of oxidation in the matrix and in the cytosol. We will show our recent work combining different reporters expressed at different subcellular localizations, alone or in combination with H_2O_2 scavengers, to improve our understanding on peroxide fluxes, with the aim of unraveling the activation of physiological signaling cascades controlled by peroxide fluctuations.

A Core Nrf2 Gene Set through Broad Transcriptomic Analysis is Representative of Nrf2 Activity and Reveals Selective Drug Resistance

George Luo[1], Harshita Kumar[1], Ethan Jiang[2], Kristin Alridge[1], John Letterio[1, 3, 4]

1 Case Western Reserve University, USA
2 University of Chicago, USA
3 University Hospitals of Cleveland, USA
4 Rainbow Babies And Children's Hospital, USA

Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of antioxidant and detoxifying enzymes in response to cellular stress. Kelch-like ECH associated protein 1 (KEAP1) is an adaptor protein that acts as a negative regulator of Nrf2 by targeting it for ubiquitination and degradation. Normally, Nrf2 is repressed through proteasomal degradation mediated by KEAP1. However, in response to oxidative stress, Nrf2 dissociates from KEAP1 and enters the nucleus to activate the expression of its target genes. The Nrf2-KEAP1 pathway plays an important role in the cellular response to oxidative stress and prevention of chronic diseases such as cancer, neurodegeneration, and inflammation. In our study, we investigate activation of the Nrf2 in human cancers and fibroblast cells through KEAP1 inhibitor, CDDO-2P-Im, and KEAP1/Nrf2 mutations. We define a list of 13 core and 12 nearly universally upregulated Nrf2 target genes from the seven RNA-Seq databases that we generated and analyzed, which can be used to examine Nrf2 activation in a multitude of human tissues and context. Additionally, several novel Nrf2 target genes and context-dependent gene regulation discrepancies were identified. We validate this Nrf2 target gene list using data from other cancers and other published datasets of Nrf2 activation. Using public data, our Nrf2 activity score correlates with sensitivity to PX-12 and necrosulfonamide. However, sensitivity to other drugs such as paclitaxel and bardoxolone methyl shows no significant correlation with Nrf2 activity. We validate this finding in our cell lines, thus suggesting Nrf2 activation causes selective drug resistance. Therefore, our results have implications for cancer therapy and resistance. Overall, we demonstrate our Nrf2 gene set is robust, valid, and useful for measuring Nrf2 activity.

CoQ10 and endothelial cells activity: relationship with cognitive deficiency

Guillermo López-Lluch

1 Department of Physiology, Anatomy and Cell Biology, Andalusian Centre of Developmental Biology, Universidad Pablo de Olavide, Seville.
2 Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, U729). IICS-Madrid, Spain.
3 International Coenzyme Q10 Association.

Coenzyme Q10 (CoQ10) plays many important functions in cells. It is an essential factor for mitochondrial activity and antioxidant protection in tissues and plasma lipoproteins. Due to its antioxidant function, it has been identified as a key component of the protection of cells against ferroptosis.
Deficiency of CoQ10 has been associated with aging and age-related diseases. In recent years, some studies have demonstrated the importance of CoQ10 levels in the maintenance of cognitive capacity and executive function in the elderly. We carried out a cross-sectional study with 64 participants: 17 men and 47 women aged more than 65 years from Active Participation Centers and nursing homes in Seville, Spain. We determined their anthropometric characteristics, physical capacity, and cognitive and functional capacity by using different activity and cognitive tests. The plasma CoQ10 levels showed a strong inverse relationship with frailty risks and sedentarism. Furthermore, high levels of CoQ10 in plasma were associated with the maintenance of higher brain capacity in elderly people.
Interestingly, the activity of CoQ-dependent antioxidant enzymes such as CytB5R3 prevented oxidative damage in endothelial cells, improving the capacity of these cells to maintain the brain–blood barrier capacity. CoQ10 depletion in these cells results in clear deterioration of the endothelium, increasing ferroptosis and cell death.
These results indicate that maintenance of high levels of CoQ10 in plasma in elderly people can prevent endothelial damage, particularly in the brain–blood barrier, preventing neuroinflammation and cognitive decline.

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Clinical Metabolomics

Jesús Simal Gandara

Nutrition and Bromatology Group, Analytical Chemistry and Food Science Department, Faculty of Science, Universidade de Vigo, E32004 Ourense, Spain

Metabolomics is a young field of knowledge that arises linked to other omics such as genomics, transcriptomics, and proteomics. This discipline seeks to understand the performance of metabolites, identifying, quantifying them, and thus understanding its mechanism of action. This new branch of omics science shows high potential, due to its noninvasive character and its close relation with phenotype. Several techniques have been developed to study the metabolome of biological samples, fundamentally nuclear magnetic resonance (NMR), and mass spectrometry (MS), or a combination of several techniques. These techniques are focused to separate, detect, characterize, and quantify metabolites, as well as elucidate their structures and their function on the metabolic pathways they are involved. However, due to the complexity of the metabolome, in most cases it is necessary to apply several of these techniques to understand completely the whole scenery. This contribution is aimed to offer a summary of the current knowledge of these analytical techniques for metabolomics and their application to different fields as food or health sciences. Each technique shows different advantages and drawbacks depending on their technical characteristics and limitations, some factors, such as the aim of the study or the nature of the biological sample will condition the choice. Regarding their applications, NMR has been employed specially to identify new compounds and elucidate structures. The use of MS has gained popularity because of its versatility, easiness to be coupled to separation techniques and its high sensitivity. Metabolomics applications in different science fields are growing each year, due to advances in analytical techniques and combination with other omics that allow to increase the comprehension of metabolic processes. Further development of analytical tools is necessary to continue exploiting all the possibilities of metabolomics [1].

Reference

[1] Fraga-Corral, M.; Carpena, M.; Garcia-Oliveira, P.; Pereira, A.G.; Prieto, M.A.; Simal-Gandara, J. Analytical metabolomics and applications in health, environmental and food science. Critical Reviews in Analytical Chemistry, 2020. https://doi.org/10.1080/10408347.2020.1823811

Extract from used coffee grounds as a good source of several antioxidants for skin cells

Adrianna Maria Piasek[1, 2], Łukasz Wysocki[1, 2], Tomasz Kobiela[1], Anna Sobiepanek[1]

1 Warsaw University of Technology, Faculty of Chemistry, Chair of Drug and Cosmetic Biotechnology, Poland
2 Ecobean sp z o. o., Poland

Global consumption registers over 400 billion cups of coffee annually. To supply this market, 10 million hectares of plantations are needed (mainly in South America and Africa). As a result, humanity generates huge amounts of waste (spent coffee grounds, SCG), and in Europe, this amounts to more than 9 thousand tons daily. Even after the process of roasting the beans and brewing the coffee, SCG contains many useful compounds such as antioxidants. This work focuses on the extraction and characterization of SCG ethanolic extract containing antioxidants. The antioxidant properties were evaluated using common chemical tests (such as DPPH, FRAP, ABTS, and CUPRAC). Chromatographic methods such as TLC and HPLC were used to determine the composition of SCG extracts.

It is rich in compounds classified as polyphenols and flavonoids, but also one of the most abundant components is caffeine. The effect of the obtained extract on skin cells was also tested using the MTT cytotoxicity assay. The protective and repairing properties against UV radiation were tested by quantifying the reactive oxygen species produced by the cells with the DCF-DA compound. All the results obtained suggest that spent coffee grounds are a remarkable source of compounds with antioxidant properties that can be applied in many industries, including the cosmetic, food, and pharmaceutical industries.

Evaluation of the neuroprotective potential of Indicaxanthin from *Opuntia ficus indica* fruit against dysmetabolism-related neurodegeneration in high-fat diet-fed mice

Mario Allegra, Simona Terzo, Ilenia Concetta Giardina, Antonella Amato, Alessandro Massaro, Pasquale Calvi, Alessandro Attanzio, Antonella D'Anneo, Ignazio Restivo, Flavia Mulè, Luisa Tesoriere

University of Palermo, Italy

Overnutrition and modern diets containing high proportions of saturated fat are amongst the major factors responsible for the development of low-grade, systemic, chronic inflammation, dyslipidaemia and hyperglycaemia. Interestingly enough, recent studies have started to recognize oxidative stress-related glucose dysmetabolism as a risk factor for neurodegenerative conditions such as Alzheimer's disease (AD) and other cognitive disorders as well. Coherently, over 80% of AD patients have T2DM or abnormal serum glucose levels. These evidences suggest that the pathogenic mechanisms of T2D and AD might well overlap in a self-feeding, vicious cycle between chronic neuro-inflammation, mitochondrial dysfunction and oxidative stress. In these scenarios, the molecular interconnections between neuro-inflammation and glucose dysmetabolism could represent a potential therapeutic target to prevent or ameliorate neurodegeneration and cognitive impairment.

Indicaxanthin (Ind) is a bioavailable, betalain pigment from Opuntia ficus indica fruit, able to cross the brain–blood barrier. Relevantly, the phytochemical has been demonstrated to interfere with redox-dependent signalling pathways, exerting significant anti-oxidative and anti-inflammatory effects both in vivo and in vitro. Recent evidences have also demonstrated that a nutritionally relevant dose of Ind ameliorates glucose dysmetabolism and counteracts insulin resistance in a murine model of metabolic syndrome. Along these lines and taking into account the strict interconnections between neurodegeneration, oxidative stress and glucose dysmetabolism, this work has explored whether and how Ind, extracted from Opuntia ficus indica fruit, exerts protective effects in an in vivo model of dysmetabolism-related neurodegeneration.

To this end, Ind was purified as detailed in the Italian Patent Application No. 102021000015167 filed on 10.06.2021. C57BL/6J mice (n=24) were grouped as follows: 1. a negative control group was fed with a standard diet for 14 weeks; 2. a positive control group was fed with a high-fat diet (HFD) for 14 weeks; 3. an Ind group was fed with HFD for 10 weeks and subsequently received Ind per os at a nutritionally relevant dose of 0.86 mg/kg/day for 4 weeks within a HFD regimen. At the end of the treatment, the mice were sacrificed, the brains rapidly dissected for hippocampus and cortex, and neuro-apoptosis, neuro-inflammation and oxidative stress were evaluated.

Our results clearly show that Ind treatment counteracted the HFD-induced brain weight reduction and apoptotic nuclei formation (evaluated by TUNEL assay). Moreover, the phytochemical administration also inhibited neuro apoptosis by reducing Fas-L, Bim and P27 mRNA levels and increasing the Bcl-2 and BDNF ones (assessed by qRT-PCR). From a mechanistic perspective, Ind-mediated effects on HFD-induced neurodegeneration were associated with a reduction in brain oxidative stress and neuro-inflammation. Indeed, Ind treatment effectively counteracted the HFD-induced production of reactive oxygen and nitrogen species, malondialdehyde and NO, evaluated using spectrofluorimetric and colorimetric assays. Coherently, an increase in the expression levels of Nrf-2 and its downstream activation products, HO-1 and SOD-2, was also observed in the Ind-group by Western blotting analysis. Along these evidences, Ind administration also ameliorated neuro-inflammation, assessed as expression levels of NF-kB and its downstream activation products, i-NOS and COX-2, using Western blotting.

As a whole, our present results indicate that Ind treatment is able to counteract neurodegeneration in an in vivo model of metabolic syndrome at a nutritionally relevant dose. Modulation of the expression of crucial genes and proteins involved in the oxidative stress-dependent inflammatory reaction underlying the obesity-related neurodegeneration emerges as a possible mechanism for the observed neuroprotective effects. In perspective, our data suggest a potential employment of Ind, alone or in combination therapy, to prevent and/or treat neurodegenerative conditions associated with glucose dysmetabolism.

Low-molecular-weight thiols with antioxidant activity: natural sources and potential health benefits

Immacolata Castellano[1, 2], Annalisa Zuccarotto[3], Serena Leone[3], Monia Teresa Russo[4], Gian Luigi Russo[5]

1 Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, 80131 Naples, Italy
2 Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy
3 Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy.
4 Department of Research Infrastructures for Marine biological resources, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy.
5 National Research Council, Institute of Food Sciences, 83100, Avellino, Italy

Marine derived sulfur-containing amino acids have recently attracted the research community's interest for their therapeutic potential (Castellano and Seebeck 2018). These molecules include 5-thiohistidine and its p-methylated forms, ovothiols, are mainly present in marine invertebrates, bacteria and microalgae. Thanks to the peculiar position of the thiol group on the imidazole ring of histidine, ovothiols are probably the most acidic natural thiols, endowed with unique antioxidant properties. They have been proposed to play a key role in controlling the cellular redox balance considering their ability to carry out redox exchange with glutathione. Ovothiol was first isolated from sea urchin eggs, which still represent the major natural source used to purify and test its biological activities. We have demonstrated that ovothiol inhibits the activity of g-glutamyl-transpeptidase, an enzyme involved in glutathione metabolism and in the control of redox homeostasis, and induces the arrest of cell proliferation through an autophagic mechanism in hepatocellular carcinoma and leukemic cell lines (Brancaccio et al. 2019). More importantly, we observed that ovothiol exhibits no toxicity towards non-proliferating cells, but exerts several beneficial properties in different in vitro and in vivo experiments. For example, it exerts anti-oxidant and anti-inflammatory activity in an in vitro model of endothelial dysfunction and in human keratinocytes (Brancaccio et al. 2022). In addition, it ameliorates damage, when administered to mice affected by liver fibrosis, and in ex vivo inflamed skin tissues. However, sea urchins cannot be considered an eco-sustainable source of these natural compounds for extensive testing and transfer to clinical trials. In this context, microalgae, may represent an optimal alternative natural source for ovothiol biosynthesis (Russo et al. 2023). On the other hand, the biomimetic chemical synthesis of ovothiols, has led to the production of iso-ovothiols, whose biological activities are currently unknown and need to be tested for further potential drug development or dietary supplementation.

Sea buckthorn (*Hippophae rhamnoides)* seed lipid extract as a supplement to reduce residual risk in patients with coronary heart disease

Iveta Mintale[1, 2, 3], Kristaps Erglis[2, 3], Sanda Jegere[1, 2, 3], Ralfs Zuzans[1, 2], Aija Romanovska[3], Eriks Jakobsons[2, 3], Martins Erglis[1, 2, 3], Iveta Bajare[2, 3], Vilnis Dzerve[1, 2, 3], Vadims Bartkevics[2, 4], Andrejs Erglis[1, 2, 3]

1 Latvian Centre of Cardiology, Pauls Stradins Clinical University Hospital, Riga, Latvia
2 University of Latvia, Riga, Latvia
3 JSC "SISTEMU INOVACIJAS", Jaunmarupe, Latvia
4 Institute of Food safety, Animal Health and Environment "BIOR", Riga, Latvia

Background. Despite effective preventive and treatment strategies, patients with coronary heart disease (CHD) remain at high residual risk. Supplementation with plant-derived extracts could play a role in risk reduction.
Aim. To determine whether sea buckthorn seed lipid extract (SBS-LE) containing polyunsaturated and monounsaturated fatty acid Omega-3,6,9 combination, fat-soluble vitamins, and bioactive compounds synergistically reduce the residual risk for CHD patients.
Methods. A total of 35 of 100 patients with CHD and obesity, hypertension, dyslipidaemia, elevated c-reactive protein (CRP) or uric acid level on optimal treatment, including statins ar ezetimibe, were analysed in this pilot trial at the Latvian Centre of Cardiology. The patients received two SBS-LE soft gelatine capsules (2000mg) per day for three months. SBS-LE was produced by supercritical fluid extraction with carbon dioxide. At baseline (V_0) and the three-month (V_3) follow-up, the blood pressure and heart rate were measured, and blood samples were taken for the lipid profile, glucose, CRP, and uric acid.
Results. Of the first 35 patients, 17 (49%) were men, with a mean age of 69.28±10.48 years. A total of 33 patients (94.29%) were adherent to the SBS-LE with good tolerability and no side effects. One patient (2.86%) was hospitalised with worsening heart failure. In two patients (5.71%), the antihypertensive medication was changed. In four patients (11.43%), the dose of statins was increased. At 3 months, the systolic blood pressure was reduced by 3.15±7.86 mmHg (140 [IQR=125;140] mmHg V_0 to 135 [IQR=125;135] mm Hg V_3 (p=0.048)). There was also a trend to a lower blood glucose level (5.95 [IQR = 5.47; 6.55] mmol/l V_0 vs. 5.63 [IQR=5.11;6.35] mmol/l V_3 (p=0.106)). There were no significant differences in other parameters.
Conclusion. The preliminary results of our study show a reduction in systolic blood pressure and lower glucose level. Continuation of the study with more participants could strengthen the hypothesis of the efficacy of the SBS-LE for high-risk patients.

Exploring natural sources of macromolecular polyphenols

Jara Pérez-Jiménez[1, 2], Marisol Villalva[1, 3], Esther García-Díez[1], Sonia Ramos[1, 2], María Ángeles Martín[1, 2]

1 Dpt. Metabolism and Nutrition, Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Madrid
2 CIBER of Diabetes and Associated Metabolic Disease (CIBERDEM), ISCIII, Madrid, Spain
3 Dpt. Chemistry and Physics Applied, Faculty of Sciences, Madrid Autonomous University, Madrid, Spain

Dietary polyphenols have been widely studied in analytical, observational, in vitro, preclinical and clinical experiments, generating cumulative evidence on their health-related properties. However, there is a fraction of dietary polyphenols still understudied, the so-called macromolecular or non-extractable polyphenols. They include either macromolecular structures (high molecular weight proanthocyanidins, hydrolysable tannins or phlorotannins) or small phenolic compounds linked to macromolecules such as dietary fiber by different linkages. Research on these compounds has shown that they are at least partially bioavailable after microbiota action and they contribute to the biological activities of the more available extractable polyphenols. For this reason, there is an interest in the identification of macromolecular polyphenol sources. This talk will summarize recent research on new sources of macromolecular polyphenols, including: a) traditional foods (fruit, vegetables, legumes); b) agrifood byproducts (fruit peels, cereal brans, materials from banana tree); c) underused vegetal materials (seaweeds); d) a combination of several matrixes to generate new food ingredients (cocoa and carob, grape and pomegranate pomaces). Overall, these sources of macromolecular polyphenols have a high potential to be used in several food formulations.

Can the Universal Acidity Scaling formalism explain scavenging processes in antioxidant theory? A new way to link radical- and ion-driven processes, combining kinetics and thermodynamics

Nikola Stamenković[1, 2], Nataša Poklar Ulrih[1], Janez Cerkovnik[2]

1 Biotechnical Faculty, University of Ljubljana
2 Faculty of Chemistry and Chemical Technology, University of Ljubljana

The introduction of modern (hybrid gradient-based) DFT methods in the 1990s[1,2] allowed the scientific community to study quantum processes and reactivities in antioxidant theory in depth.[3,4] The modern counterpart to these theoretical methods can be scale-based theories that predict either the nucleophilic or the electrophilic potential in chemical systems.5-8 The introduction of a new reactivity descriptor, within the framework of Universal Acidity Scale (UAS) formalism, is envisaged to be applied and presented. Moreover, UAS is capable of consequent consideration of both experimentally and theoretically calculated results developed to solve intracidic differentiation phenomenon, and which are thus able to connect reactivities of acids and bases with electrophiles and nucleophiles. Therefore, the aim of this study is to investigate the topologies and reactivities of different scavengers, treating them theoretically and computationally, and eventually compare these to their experimental results of scavenging process. The results are expected to provide the link between thermodynamics and kinetics in general antioxidant theory and provide a simple and rapid way to theoretically evaluate novel synthetic or natural scavengers by relating their acidities with their designated single electron reactive site(s). Henceforth, the results shall include bond dissociation enthalpies and adiabatic ionization potentials, using inversion theory leveling for the computational part of the study, as well as the concrete connection to experimental pKa of selected scavengers.

References:

1. Becke, A.D. J. Chem. Phys. 1993, 98, 5648-5652.
2. Adamo, C. et al. J. Chem. Phys. 1999, 110, 6158-6169.
3. Wang, L. et al. Food Chem. 2019, 275, 339-345.
4. Spiegel, M. J. Chem. Inf. Model. 2022, 62, 2639-2658.
5. Mayer, R.J. et al. J. Org. Chem. 2019, 84, 8837–8858.
6. Justel, P.M. et al. J. Org. Chem. 2021, 86, 5965-5972.
7. Mayer, R.J. et al. J. Am. Chem. Soc. 2020, 142, 5221-5233.
8. Montecinos, R. et al. New J. Chem. 2021, 45, 11495-11505.

Polydopamine/melanin nanoparticles as a bionanoantioxidant. Studies in model lipid systems

Katarzyna Jodko-Piórecka[1], Monika Kluzek[1, 2], Grzegorz Litwinienko[1]

1 University of Warsaw, Faculty of Chemistry
2 Weizmann Institute of Science, Department of Materials and Interfaces

Melanin (including eumelanin and neuromelanin) is a group of natural macromolecular pigments acting as intracellular antioxidants via quenching electronically excited states, sequestration of redox active metal ions and scavenging free radicals. It is believed that intracellular inhibition of free-radical reactions by neuromelanin greatly contributes to prevention against primary or secondary mechanisms underlying various diseases such as atherosclerosis, asthma, and some neurodegenerative disorders such as Parkinson's and Alzheimer's disease. Recently, we described the antioxidant properties of polydopamine (PDA, synthetic analogue of melanin) in nonpolar systems as due to activation by hydroperoxyl radicals, whereas the process mediated by alkylperoxyl radicals was not inhibited by PDA (Guo et al., Angew. Chem. Int. Ed. 2021, 60, 15220). This finding prompted us to examine the interactions of PDA nanoparticles with model lipid bilayers composed of zwitterionic (1,2-dimyristoyl-sn-glycero-3-phosphocholine; DMPC) and anionic (1,2-dimyristoyl-sn-glycero-3-phosphoglycerol, DMPG) lipids. Using the Isothermal Titration Calorimetry (ITC) and Differential Scanning Calorimetry (DSC), we determined thermodynamic parameters of melanin binding to phospholipid membranes, and we evaluated the contribution of the lipid charge to that process. Moreover, we confront the thermodynamic data with the antioxidant activity of PDA nanoparticles, expressed as the ability to scavenge peroxyl radicals, experimentally investigated in model lipid bilayers composed of DMPC and DMPG, doped with methyl linoleate and subjected to initiated peroxidation. PDA nanoparticles displayed the highest antioxidant activity in negatively charged liposomes (DMPG). Surprisingly, we observed a strong synergy between PDA and a model antioxidant 2,2,5,6,7-pentamethyl-6-hydroxychromane (PMHC, the analogue of α-tocopherol) in neutral DMPC liposomes, while no synergy was found in DMPG liposomes. This different behaviour can be interpreted as an effect of strong Coulombic interation of PDA nanoparticles with the negatively charged surface of DMPG membrane, decreacing its mobility.

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Pro-aromatic terpenes from essential oils: a natural strategy to enhance the radical trapping activity of antioxidants and nanomaterials

Riccardo Amorati[1], S. S. R. Kumar Challa[2]

1 Department of Chemistry "G. Ciamician", University of Bologna, Via San Giacomo 11, 40126, Bologna, Italy
2 Xheme Inc, UMass Innovation Institute. Mt. Ida Campus of UMass Amherst, School of Applied Science, 100 Carlson Ave, Newton, MA. 02459

Lipid peroxidation represents a major hurdle in disparate fields ranging from food storage to biomedical applications. Many antioxidant strategies have been put forward to address this problem, firstly based on the use of synthetic additives, including phenols and aromatic amines, and more recently on the "rediscovery" of natural inhibitors with a food-grade or GRAS status, especially for applications linked to the food and pharmaceutical industry. Great interest has been recently devoted to essential oils (EOs), which are complex mixtures of volatile components obtained from aromatic plants which are, in many cases, already present in human diet [1]. The study of the components of EOs has demonstrated antioxidant activity through direct alkylperoxyl radical (ROO•) trapping, or through the regeneration of other antioxidants such as alpha-tocopherol (a-TOH) [2,3]. Gamma-terpinene (g-T) is a terpenic EOs component present in oregano and citrus species, that displays a unique pro-aromatic 1,4-cyclohexadiene ring. When g-T reacts with radicals under O_2, it forms an unstable alkylperoxyl radical that decays to hydroperoxyl radical (HOO•) and to the aromatic terpene para-cymene. Interestingly, HOO• is a reducing radical that can quickly react with various oxidized compounds such as the tocopheroxyl radical, thus prolonging the inhibition of a-TOH [2,3]. Here, we show that g-T at low concentrations has a synergic antioxidant activity with an engineered nanoporous material based on CeO_2, and with other metal oxides nanoparticles such as TiO_2, as a result of the ability of HOO• to reduce the oxidized moieties present on the surface. These materials, which are completely inactive in the absence of g-T, behave in a catalytic fashion. Once they are reduced by HOO• they can quench ROO• with a cyclic mechanism. These findings are important to understand the mechanism of the antioxidant activity of nanomaterials, and for future practical exploitation of the potential antioxidant activity of essential oils.

References:
1) Ruberto, G.; Baratta, M. T. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem. 2000, 69, 167−174.
2) F. Mollica, I. Gelabert, R. Amorati. Synergic antioxidant effects of the essential oil component γ-terpinene on high-temperature oil oxidation. ACS Food Sci. Technol. 2022, 2, 180−186.
3) Guo, Y.; Baschieri, A.; Amorati, R.; Valgimigli, L. Synergic antioxidant activity of γ-terpinene with phenols and polyphenols enabled by hydroperoxyl radicals. Food Chem. 2021, 345, 128468.

Phlorotannin-containing extracts from Chilean brown algae (Durvillae incurvata) inhibit melanoma cell migration

Layla Simón[1, 2, 3], Migdalia Arazo[1, 4], Gonzalo Rivera Andrades[1, 5], Angye Caro[1, 4], Kevin González-Morelo[1, 4], Andrew F.G. Quest[2, 3], Ricardo Pérez-Correa[4], María Salomé Mariotti[1]

1 Nutrition and Dietetic School, Universidad Finis Terrae, Santiago 7501015, Chile
2 Cellular Communication Laboratory, Center for Studies on Exercise, Metabolism and Cancer (CEMC), Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile, Santiago 8380492, Chile
3 Advanced Center for Chronic Diseases (ACCDiS), Faculty of Medicine, University of Chile, Santiago 8380492, Chile
4 Department of Chemical and Bioprocess Engineering, Faculty of Engineering, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, P.O. Box 306, Santiago 7820436, Chile
5 Institute of Nutrition and Food Technology (INTA), Universidad de Chile, Santiago 7830490, Chile

The number of deaths associated with cancer is increasing worldwide and metastasis is the leading cause. Metabolic switching is a hallmark of cancer cells that facilitates metastasis. Caveolin-1 is a tumor promoter that increases glycolysis and thereby the metastatic potential of cancer cells. Brown algae are consumed in Asia and the south of Chile due to their nutritional, antioxidant and antihyperglycemic properties. Phlorotannins are a type of polyphenol present in brown seaweeds. Preliminary results indicate that commercially available phloroglucinol, a monomer of a phlorotannin, can prevent Caveolin-1-induced migration. Our general aim was to study the effect of phlorotannins obtained from Chilean brown seaweeds (Durvillae incurvata) through ultrasound-assisted extraction (USAE) on glycolysis and the metastatic potential of Caveolin-1-expressing melanoma cells. For that reason, 20 g of brown algae were extracted via USAE (20°C, 40% potency, for 30 min) using 200 mL of ethanol solution (32,5%), rotavaporated and lyophilized at -80°C for 72 h. The total polyphenol content and antioxidant capacity of the extract were determined using Folin–Ciocalteu and DPPH assays, respectively. Phlorotannin-rich extracts were used to treat metastatic melanoma cells (B16F10) expressing Caveolin-1. In this way, we evaluated the effect of phlorotannin-rich extracts on glycolysis, as evidenced by measuring the lactate concentration in the cell culture media and migration using Boyden chambers. Extracts with proven phlorotannin enrichment and antioxidant activity were used at a non-cytotoxic concentration (100 µM), and shown to reduce glycolysis and the migration of metastatic melanoma cells. With these experiments, we determined that extracts obtained from an unexploited Chilean raw material using sustainable technologies might have pharmaceutic potential to be used to prevent metastasis and thereby reduce cancer-related deaths.

Multifaceted application opportunities offered by agri-food waste-derived phenolic compounds

Lucia Panzella

Department of Chemical Sciences, University of Naples "Federico II", Italy

Phenolic compounds occur widely in nature and are well-known for their beneficial effects on human health, which have been ascribed in part to their ability to act as potent antioxidants. Recently, the antioxidant properties have prompted the use of phenolic compounds not only as food supplements, but also as additives for the development of functional materials. Within this context, this talk will be focused on relevant examples of how natural phenols can be exploited as multifunctional compounds in the food, cosmetic, and biomedical sectors. Particular attention will be paid to phenolic compounds from agri-food wastes such as spent coffee grounds, exhausted woods from tannin industrial production, and nut shells, which are rich in phenolic polymers including lignin and tannins, endowed with very potent antioxidant properties. The possibility to further improve these antioxidant properties through chemical manipulation will also be presented, together with efficient and green strategies for the recovery of phenolic compounds based on the use of ball milling and/or deep eutectic solvents (DES).

Screening of a novel nutraceutical ingredient extracted from chestnut shells by in vitro and in vivo assays—An insight into metabolomic approaches

Diana Pinto[1], Andreia Almeida[1], Anallely López-Yerena[2], Soraia Pinto[3], Bruno Sarmento[4], Rosa M. Lamuela-Raventós[5], Anna Vallverdú-Queralt[6], Cristina Delerue-Matos[7], Francisca Rodrigues[7]

1 REQUIMTE/LAQV - Instituto Superior de Engenharia do Porto
2 Nutrition, Food Science and Gastronomy Department, School of Pharmacy and Food Science, University of Barcelona, 08028 Barcelona, Spain
3 i3S, Institute for Research and Innovation in Health, University of Porto, 4200-135 Porto, Portugal; INEB, Institute of Biomedical Engineering, University of Porto, 4200-135 Porto, Portugal
4 i3S – Institute for Research and Innovation in Health, University of Porto, Rua Alfredo Allen, 4200-135 Porto, Portugal, INEB – Institute of Biomedical Engineering, University of Porto, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal CESPU – Institute
5 Nutrition, Food Science and Gastronomy Department, School of Pharmacy and Food Science, University of Barcelona, 08028 Barcelona, Spain; Consorcio CIBER, M.P. Fisiopatología de la Obesidad y la Nutrición (CIBERObn), Instituto de Salud Carlos III (ISCIII)
6 Nutrition, Food Science and Gastronomy Department, School of Pharmacy and Food Science, University of Barcelona, 08028 Barcelona, Spain; Consorcio CIBER, M.P. Fisiopatología de la Obesidad y la Nutrición (CIBERObn), Instituto de Salud Carlos III (ISCIII
7 REQUIMTE/LAQV, Polytechnic of Porto, School of Engineering, 4249-015 Porto, Portugal

The nutraceuticals intake plays a leading role in the daily diet of society for health-promoting purposes even though the European legislation regarding the validation of nutraceuticals remains vague and a comprehensive assessment of the in vitro and in vivo bioactivity should be guaranteed before its use as functional ingredient. Chestnut shells (CS) are an exceptional source of bioactive compounds with promising pro-healthy effects.

This study attempts, for the first time, to validate an eco-friendly CS extract prepared through subcritical water extraction (SWE) using in vitro and in vivo assays stepping forward into a nutraceutical use. After an optimization study, the extract was characterized regarding phenolic composition, bioactivity, and intestinal cells viability. In vivo studies were carried out in rats orally treated with CS extract (50 and 100 mg/kg body weight (bw)) to evaluate antioxidant response, blood glucose and lipids levels, metabolic profiling targeted on polyphenols by LC-ESI-LTQ-Orbitrap-MS, and histopathological analysis.

The results corroborated the in vitro antioxidant, hypoglycemic, hypolipidemic, and neuroprotective effects of CS extract ascribed to its richness in phenolic acids and hydrolysable tannins. Strong in vivo antioxidant responses were reported through the upmodulating antioxidant enzymes' activities and downregulating lipid peroxidation. The safety of CS extract was ensured using in vitro assays up to 100 µg/mL in HT29-MTX and Caco-2 intestinal cells and in vivo histopathological studies. Through metabolomic approaches, phenolic metabolites arising from phase I (hydrolysis and oxidation) and phase II (glucuronidation, methylation, and sulfation) reactions were identified in rat

liver, kidney, and blood serum. The outcomes predicted an outstanding contribution of the phenolic metabolites to the in vivo antioxidant efficacy of CS extract, proposing its use as a prominent source of anti-aging molecules for nutraceuticals employed in the prevention and co-therapy of lifestyle-related diseases triggered by oxidative stress. The next steps will be centered on designing a functional food incorporating the CS extract.

From agri-food waste to functional foods: the molecular mechanism of Nrf2-activating sunflower seed-derived antioxidant bioactive peptides

Federica Tonolo[1], Alessandra Folda[1], Stefania Ferro[1], Alessandro Grinzato[2], Oriano Marin[1], Maria Pia Rigobello[1]

1 Dept.of Biomedical Sciences, University of Padova, Padova, Italy
2 European Synchrotron Radiation Facility, Division of Experiments, Grenoble, France

Different compounds present in agri-food waste could be reused to make better foods. Among these bioactive compounds, bioactive peptides are potential ingredients of functional foods that exert antioxidant and anti-inflammatory properties. The extraction of new food-derived bioactive peptides from sunflower seeds' processing by-products and evaluation of their molecular mechanism in cellular model were performed. First, the simulation of physiological gastro-intestinal digestion of sunflower seed protein isolate was performed. Antioxidant peptide-enriched fractions were extracted and purified, and new peptides were identified through LC-MS/MS analysis. To assess the molecular mechanism of the latter, the possible interaction of these molecules with Keap1, involved in redox signalling, was evaluated using a predictive molecular docking approach. The peptides showing a high score of interaction were synthesized and analysed in Caco-2 cells. Some of them exerted a protective effect against oxidative stress induced by TbOOH, observed both as rescue of viability and inhibition of ROS production. Treatment for 24h of Caco-2 cells with some peptides determined Nrf2 translocation from the cytosol to the nucleus. These observations suggested that our peptides modulate Keap1/Nrf2 interaction leading to the transcription of the Nrf2-ARE-regulated antioxidant and phase II enzymes. Therefore, the gene expression of NQO1, SOD1 and HO-1 was analysed with RT-PCR in cells treated for 24h with the pre-selected peptides showing their overexpression. A crosstalk between Nrf2 and NF-κB, through the action of HO-1, is noteworthy. Thus, anti-inflammatory effects of these peptides, besides their antioxidant activity, were evaluate. In Caco-2 cells pre-treated with the most active peptide, followed by TNF-α-induced inflammation, the levels of NF-κB in the nucleus, decreased due to the increased levels of IκB in the cytosol. As inflammation and redox imbalance are related to many diseases, the use of food waste-derived molecules for their regulation is noteworthy.

Hexamethylenediamine-mediated deposition of catechol-based thin films with potent antioxidant activity

Maria Laura Alfieri[1], Sara Ferraris[2], Andrea Cochis[3], Lucia Panzella[1], Silvia Spriano[4], Alessandra Napolitano[1]

1 Department of Chemical Sciences, University of Naples Federico II, via Cintia 4, I-80126 Naples, Italy
2 Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129, Torino, Italy
3 Department of Health Sciences, Center for Translational Research on Autoimmune and Allergic Diseases CAAD, University of Piemonte Orientale, Corso Trieste 15/A, 28100, Novara, NO, Italy
4 Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129, Torino, Italy.

The design and synthesis of bioinspired polyphenol-based systems have been very active research points over the past decade since they might offer advantages with respect to pure polyphenols in terms of stability, antioxidant ability, adhesion properties and controlled release in the target site.
Interesting opportunities for the design and development of functional biomaterials and systems come from the oxidatively-induced cross-linking reaction of catechols with long aliphatic chain diamines such as hexamethylenediamine (HMDA).[1]
Based on these premises, we herein report a simple and efficient titanium surface modification strategy based on the deposition of uniform and thin caffeic acid (CA)-based films by exploiting the HMDA cross-linking ability. The coatings of CA/HMDA on both polished and chemically pre-treated Ti6Al4V alloys were characterized from the chemical and physical standpoints. Interestingly, both coatings exhibited marked antioxidant properties as evaluated with two commonly used chemical assays (2,2-diphenyl-1-pycrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays) that persisted overtime and were not lost even after prolonged storage of the material. Moreover, the coating surfaces showed good cytocompatibility and a high reactive oxygen species scavenging ability, preventing their intracellular accumulation under pro-inflammatory conditions.
HMDA also markedly enhances film deposition from the polymerization of dopamine and a variety of catechol substrates, including the key eumelanin precursors 5,6-dihydroxyindole (DHI) and its carboxylic acid (DHICA), leading to films with attractive properties in terms of morphology and functionalities. Here, we disclose an example of mussel-inspired antioxidant film-forming system hinging on a non-covalent small-molecule partnership between HMDA and 5,6-dihydroxyindole-2-carboxylic acid methyl ester.[2] The films displayed potent antioxidant properties in three different assays and exerted significant protective effects from oxidative stress on HaCat cells stimulated with UV radiation. [2]

References:
1. Iacomino et al. Langmuir 2017, 33, 2096-2102.
2. Argenziano et al. J Colloid Interface Sci 2022, 624, 400-410.

A Universal and Versatile Approach to Antimicrobial Coatings via copolymerization of a catechol and a diamine

Daniel Ruiz-Molina[1], Salvio Suarez[1], Jose Bolaños[2], Victor Yuste[2]

1 Institut Catala de Nanociencia i Nanotecnologia (ICN2-CSIC), Spain
2 Universitat Autònoma de Barcelona (UAB), Spain

Antibacterial materials have gained significant attention in recent years due to growing concerns over antibiotic resistance and the spread of infectious diseases. The most common types of antibacterial materials are those that contain silver or copper ions, which are effective against a wide range of bacteria but may involve environmental and health concerns. Additionally, other materials such as polymers, ceramics, and nanoparticles have also been shown to possess antibacterial properties, though they are still in the early stages of development, so more research is needed to fully understand their potential uses and limitations.

In this scenario, catechol-based materials inhibit the growth of a wide range of bacteria, including both Gram-positive and Gram-negative strains, thanks to their redox activity and ability to disrupt bacterial cell membranes. Additionally, catechol-based molecules are usually non-toxic to human cells, making them potentially useful compounds for the development of antibacterial materials. The presence of catechol groups and other polyphenol-based materials can locally change microenvironments near the bacteria at the bio–nano interface, causing oxidative stress or increasing the solubility of the nanoparticles, which can induce bacterial damage. In any case, in order to inactivate bacteria and reduce their viability and proliferation, the interface must be modified until it can physically disrupt the membrane (membrane depolarisation) or the production of reactive oxygen species (ROS) at the bio–nano interface or inside the bacteria.

We have developed a methodology to produce functional bioinspired coatings based on the combination of different catechol derivatives with amino-based ligands. The synthesis of these catechol-based polymers is a straightforward and eco-friendly procedure, avoiding the use of harmful solvents. Therefore, this universal and bioinspired catechol-based platform could be an environmentally friendly alternative with promising antibacterial applications.

Antioxidant compounds´ recovery from alternative sources assisted by innovative extraction methodologies

Mara Calleja, Juan Manuel Castagnini, Noelia Pallarés, Houda Berrada, Emilia Ferrer, Francisco J. Barba

Preventive Medicine and Public Health, Food Science, Toxicology and Forensic Medicine Department, Faculty of Pharmacy, Universitat de València, Avda. Vicent Andrés Estellés, s/n, 46100 Burjassot, València, Spain

The tragedy of Ukraine, as well as the recent pandemic, have exposed the risks and dangers of dependence on global supply chains of essential products, not only because of their lack of security but also because they can seriously compromise the geopolitical decision-making process at the national and regional levels and therefore directly impact the formulation of policies necessary to counter threats to world peace. From the point of view of food, there is a need to obtain oil and proteins from sustainable and economically profitable sources through the use of innovative, efficient and ecological processes, not only because of geopolitical risks, but also because of the existing commitment worldwide in terms of sustainability. Some of sustainable sources include microalgae, insects and by-products of the agri-food industry. These, in themselves, have a series of drawbacks, mainly derived from the acceptance by the consumer and the potential problems that may arise from the microbiological point of view, thus being necessary the use of processes to be able to convert them into derived products and above all allow them to have an acceptable food safety. In this sense, the use of supercritical fluids (SC-CO2) is a highly recommended and interesting strategy for the recovery of oils and fat-soluble compounds, with this being the approach traditionally used. However, the cake that is produced after the SC-CO2 process of obtaining it has been much less studied. Thus, this study will detail some of the advances made by the ALISOST group in the context of the EXTRAOLIPRO project, within the AGROALNEXT program, evaluating the use of SC-CO2 as a strategy to recover fat- and water-soluble antioxidant compounds from oils and cakes obtained from microalgae, insects and by-products of the agri-food industry.

The impact of broccoli pre-processing on the bioaccessibility and intestinal cell uptake of glucosinolates and carotenoids from broccoli baked snacks

Faye Langston[1], Mario Ferruzzi[2], Alvaro Cruz-Carrión[2], Geoffrey Nash[1], John Bows[3]

1 University of Exeter
2 Arkansas Children's Research Institute
3 PepsiCo International Ltd

Food industries use a range of pre-processed vegetable materials due to their ease of manufacturing, storage, and cost effectiveness. However, it is important to determine how pre-processing impacts the bioavailability of vegetable phytochemicals in finished products to understand their ability to promote health. Vegetable snacks were produced through hybrid baking, microwave technology in tandem with conventional hot-air drying, using fresh, individually quick frozen (IQF), freeze-dried, or air-dried broccoli in a potato flake matrix.

In vitro models that simulate oral, gastric, and small intestinal digestion were used to assess the bioaccessibility of broccoli glucosinolates, carotenoids, phenolic acids and chlorophylls. Pre-processed vegetables and snack products were incubated under controlled temperature and pH conditions to mimic upper gastrointestinal (GI) tract conditions. The incorporation of the ingredients into snack products generally increased the bioaccessibility of the phytochemicals regardless of the pre-processing method. Total phenolic acids, carotenoids and chlorophylls had, on average, a higher bioaccessibility of 6.71%, 17.55%, and 7.32%, respectively, in the snack products than in the ingredients. Using fresh or air-dried broccoli was found to be most beneficial for promoting glucosinolate bioaccessibility in comparison to IQF and freeze-dried for phenolic acid, carotenoid, and chlorophyll bioaccessibility.

Following the assessment of in vitro bioaccessibility as a predictor of bioavailability, subsequent measurement of intestinal uptake was conducted using a CaCo-2 human intestinal cell model. The study was further supported by anaerobic fecal fermentation to provide an insight into the influence of pre-processed vegetables and snack products on gut microbiota and the formation of microbial metabolites of target bioactives. This research demonstrates the significance of the pre-processing technique on phytochemical bioavailability and how food manufacturers can optimize the retention and delivery of select phytochemicals through the consideration of vegetable pre-processing.

Ethosomes and Transethosomes for polyphenol transdermal administration

Elisabetta Esposito[1], Maddalena Sguizzato[1], Francesca Ferrara[2], Markus Drechsler[3], Rita Cortesi[1], Anna Baldisserotto[4], Agnese Bondi[1], Mascia Benedusi[5], Walter Pula[1], Stefano Manfredini[6], Giuseppe Valacchi[7]

1 Department of Chemical and Pharmaceutical Sciences, University of Ferrara, I-44121-Ferrara, Italy
2 Department of Neurosciences and Rehabilitation, University of Ferrara, Ferrara, Italy; I-44121-Ferrara, Italy
3 Bavarian Polymerinstitute "Electron and Optical Microscopy" University of Bayreuth, Germany
4 Department of Life Sciences and Biotechnology, University of Ferrara, 44124, Italy
5 Department of Neuroscience and Rehabilitation, Section of Physiology, University of Ferrara, 44121, Ferrara (Italy)
6 Department of Life and Biotechnology, University of Ferrara, 44121, Italy
7 Department of Neurosciences and Rehabilitation, University of Ferrara, Ferrara, Italy; I-44121-Ferrara, Italy NC State University, Plants for Human Health Institute, Animal Science Dept. NC Research Campus, Kannapolis, NC 28081, USA Kyung Hee University

Nature offers a tremendous potential of phytocompounds suitable to treat many pathologies and disorders. Recent studies have demonstrated that the nanoencapsulation of phytocompounds allows their preservation from degradation and ensures a prolonged action. Notably, nanocarriers based on natural lipids have gained particular attention due to their biocompatibility, capability to encapsulate lipophilic molecules, and easy way of production, avoiding the use of organic solvents and thus ensuring a low toxicity profile. Recently, we investigated the potential of ethosomes and transethosomes in the treatment of inflammatory disorders affecting the skin, mainly induced by oxidative stressors such as pollution, UV exposure, and smoke. Different plant-derived polyphenols have been investigated, such as mangiferin and quercetin. Drug release and permeation was investigated in vitro, using Franz cells associated with different membranes. Particularly, nylon and polytetrafluoroethylene membranes have been employed for in vitro release tests, while in vitro permeation tests have been conducted with STRAT-M® and human stratum corneum–epidermis membranes. In vitro permeation studies revealed an enhancement of polyphenol permeation in the case of ethosomes and transethosomes with respect to conventional formulations. Notably, scratch wound healing and migration assays suggested the potential of quercetin-loaded transethosomes as an adjuvant strategy for melanoma and possibly for other skin iperproliferative conditions, including psoriasis.

References:
[1] Sajadimajd, S.; Bahramsoltani, R.; Iranpanah, A.; Kumar Patra, J.; Das, G.; Gouda, S.; Rahimi, R.; Rezaeiamiri, E.; Cao, H.; Giampieri, F.; et al. Advances on Natural Polyphenols as Anticancer Agents for Skin Cancer. Pharmacol. Res. 2020, 151, 104584.
[2] Ferrara, F.; Benedusi, M.; Sguizzato, M.; Cortesi, R.; Baldisserotto, A.; Buzzi, R.; Valacchi, G.; Esposito, E. Ethosomes and Transethosomes as Cutaneous Delivery Systems for Quercetin: A Preliminary Study on Melanoma Cells. Pharmaceutics 2022, 14, 1038.

Gender Differences between Total Polyphenols in Urine and Cardiovascular Risk Factors in Adolescents from Spain

Emily Pilar Laveriano Santos[1, 2], Isabella Parilli-Moser[3], Camila Arancibia[3], Sonia L. Ramírez-Garza[3], Anna Tresserra-Rimbau[3, 4], Inés Domínguez-López[3, 4], Ana María Ruiz-León[4, 5, 6], Ramón Estruch[4, 5], Patricia Bodega[7, 8], Mercedes de Miguel[7, 8], Amaya de Cos-Gandoy[7, 8], Vanesa Carral[7, 8], Gloria Santos-Beneit[7, 9], Juan M Fernández-Alvira[8], Rodrigo Fernández-Jiménez[8, 10, 11], Rosa M. Lamuela-Raventós[3, 4]

1 Department of Nutrition, Food Science and Gastronomy, School of Pharmacy and Food Sciences XaRTA, Institute of Nutrition and Food Safety (INSA-UB), University of Barcelona, 08921 Santa Coloma de Gramenet
2 Consorcio CIBER, M.P. Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III (ISCIII), 28029 Madrid, Spain.
3 Department of Nutrition, Food Science and Gastronomy, School of Pharmacy and Food Sciences XaRTA, Institute of Nutrition and Food Safety (INSA-UB), University of Barcelona, 08921 Santa Coloma de Gramenet, Spain
4 Consorcio CIBER, M.P. Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III (ISCIII), 28029 Madrid, Spain
5 Department of Internal Medicine, Hospital Clínic, Institutd'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), University of Barcelona, 08036 Barcelona, Spain
6 Mediterranean Diet Foundation, 08021 Barcelona, Spain
7 Foundation for Science, Health and Education (SHE), 08008 Barcelona, Spain
8 Centro Nacional de Investigaciones Cardiovasculares Carlos III (F.S.P.), 28029 Madrid, Spain
9 The Zena and Michael A. Wiener Cardiovascular Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
10 CIBER de Enfermedades Cardiovasculares (CIBERCV), 28029 Madrid, Spain
11 Hospital Universitario Clínico San Carlos, 28040 Madrid, Spain

(1) Background: Observational studies have shown an inverse association between polyphenol intake and cardiovascular risk factors (CVRFs) in adults, but few provide information on adolescents. This study aims to evaluate the relationship between urinary total polyphenol excretion (TPE) and CVRFs in adolescents aged 12.04 ± 0.46 years. (2) Methods: A cross-sectional study was performed on 1194 Spanish adolescents from the baseline SI! (*Salud Integral*) Program with available urine samples. The TPE in urine was determined by the Folin–Ciocalteu method after solid-phase extraction. The TPE by the Folin–Ciocalteu method was positively correlated with the total polyphenols quantified by the HPLC-HRMS method (R=0.22, P 0.001). Additionally, structural equation models (SEM) were used to evaluate the association between TPE and CVRFs. The main dependent variables were triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), and glucose as observed variables; and body composition (BC) (including waist circumference z-score, the body mass index z-score, and body fat percentage) and blood pressure (BP) (including systolic blood pressure z-score and the diastolic blood pressure z-score) as latent variables that were not analyzed directly. TPE was considered an independent variable. (3) Results: Out of 1194

participants, 566 were considered in the SEM analysis after the adjustment of age, gender, physical activity, fasting, Tanner scale, high parental education, place of birth, household income, and intake of energy, fat, fiber, and monounsaturated and polyunsaturated fatty acids. The results revealed that TPE was directly associated with BC and glucose and indirectly associated with BP, TG, LDL-C, and HDL-C only in boys. Although there was no association between TPE and CVRFs in girls, direct associations were found between body composition and TG with BP. (4) Conclusions: Higher concentrations of TPE were associated with a better profile of cardiovascular health, especially in boys.

The effects of mild baking processing, reproductive cycle and alternative aquafeed in the lipid quality and oxidative stress of fish fillets: the role of carotenoids and tocopherols

Ancuta Nartea[1], Sabrina Colella[2], Matteo Zarantoniello[3], Paola Riolo[4], Ike Olivotto[3], Patrick Orlando[3], Luca Tiano[5], Deborah Pacetti[1]

1 Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche
2 Institute for Biological Resources and Marine Biotechnologies, National Research Council (IRBIM CNR), Largo Fiera della Pesca 2, 60125 Ancona, Italy
3 Department of Life and Environmental Sciences, Polytechnic University of Marche, 60131 Ancona, Italy
4 Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche, 60131 Ancona, Italy
5 Department of Life and Environmental Sciences, Polytechnic University of Marche, Via Brecce Bianche, AN, 60131, Italy

Antioxidants play a double role: functional and technological. They offer several beneficial effects on human and animal health, and they counteract the oxidation in food. Hypothetically, fillet quality may depend on the processing, reproductive cycle, and diet of fish, and it could be improved with carotenoids and tocopherols. Thus, the study assessed the following: the impact of mild baking treatments on antioxidant compounds levels and oxidative status of Atlantic salmon (Salmo salar) fillets; the impact of the reproductive cycle of European Sardine (Sardina pilchardus) on lipid quality, oxidative and antioxidant stability combined with mild baking methods; and the impact of Hermetia illucens prepupae, enriched in carotenoids and tocopherols, as alternative aquafeed on health status and fillet quality in rainbow trout (Oncorhynchus mykiss).
Salmon was baked in a conventional oven, CO, steam, SO and, sous-vide, SV. Sardines at different phases of reproductive phases were baked in CO, SO, and SV. Rainbow trout juveniles were fed with a sustainable meal, containing 3 (HM3) or 20% (HM20) of full-fat black soldier fly prepupae. Antioxidant presence in the insects was modulated through an innovative substrate for rearing.
In salmon, all treatments preserved ω3 PUFA and tocopherols and increased Coenzyme Q10 availability, while solely SO enhanced the availability of astaxanthin. Oxidized Coenzyme Q10 (index of oxidative degree) was inversely correlated with astaxanthin and α-tocopherol. Biological parameters grouped raw sardines in the pre-spawning, spawning, and post-spawning phases of reproduction. α-tocopherol and, EPA/DHA increased from post-spawning, pre-spawning to spawning. Baking affected the oxidative degree differently: CO > SO $\geqslant$ SV mitigated by α-T to CO $\geqslant$ SO > SV. HM20 trout used most of the dietary carotenoids to avoid inflammation in response to alternative aquafeeds, rather than accumulating them in the flesh.
The role of antioxidants in physiological responses, reproductive phases, marketable traits, and the processing of fish was demonstrated.

Abstracts

Poster Exhibition

A1. BRS Device: A Point-Of-Care Testing System for Total Antioxidant Activity Determination in Biological Samples

María Díaz-González, Alba M. Casielles, Alba Iglesias-Mayor, Sandra Tamargo-Díaz, Sara Menéndez-Cotarelo, Henar Muñoz-Cimadevilla

BIOQUOCHEM SL (Spain)

The total antioxidant capacity (TAC) has been revealed as an early biomarker of oxidative stress related to different diseases.
The TAC is a global measure of the non-enzymatic antioxidant efficiency that integrates the individual effect of all antioxidants in a sample, and their additive, synergistic, or antagonistic interactions.
The TAC is therefore considered an important parameter to establish the antioxidant status of biological samples.
Classical spectrophotometric methods for TAC analysis present some limitations including sample pretreatment, long assay procedures, native pH alteration, low stability of some reagents, low sensitivity, and the interference of turbid/colour samples.
The BRS device is a new point-of-care electrochemical system for the direct and fast determination of TAC in a wide variety of biological samples, e.g., blood, serum, plasma, erythrocytes, saliva, or urine. Similar to a glucometer, the BRS device comprises a handheld meter and disposable test strips. The meter is controlled by a smart mobile device and has been designed for use by non-laboratory personnel.

A2. Dicaffeoylquinic acids from *Artemisia ludoviciana Nutt*. herb and mitochondria: implications in cancer and hypoxia/reoxygenation injury

Arvydas Strazdauskas[1, 2], Sonata Trumbeckaitė[1, 3], Justina Dambrauskienė[3, 4], Sandra Šilobrit[5], Emilija Paškevičiūtė[5], Rasa Banienė[1, 2]

1 Laboratory of Biochemistry, Neuroscience Institute, Lithuanian University of Health Sciences, Sukileliu Av. 13, LT-50162 Kaunas, Lithuania
2 Department of Biochemistry, Medical Academy, Lithuanian University of Health Sciences, Eiveniu Str. 4, LT-50161 Kaunas, Lithuania
3 Department of Pharmacognosy, Medical Academy, Lithuanian University of Health Sciences, Sukileliu Av. 13, LT-50162 Kaunas, Lithuania
4 Laboratory of Biopharmaceutical Research, Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Sukileliu Av. 13, LT-50162 Kaunas, Lithuania
5 Medical Faculty, Lithuanian University of Health Sciences, Sukileliu Av. 13, LT-50162 Kaunas, Lithuania

Dicaffeoylquinic acids (DCAs) possess various biological properties, such as anticancer, antioxidant, anti-inflammatory, and antiviral activities. Great amounts of DCAs are found in silver wormwood (*Artemisia ludoviciana Nutt*.) herb acetone extract methanolic fraction - 3,4-dicaffeoylquinic, 3,5-dicaffeoylquinic and 4,5-dicaffeoylquinic acids (relatively 26.87 %, 57.78 % and 15.35 %, respectively). We used healthy (RPTEC) and cancerous (Caki-2) renal cells to see whether DCAs fraction from silver wormwood herb would show protective effects against cancer or hypoxia/reoxygenation damage. Both cell lines were incubated with DCAs fraction with final concentrations at 15–350 μg/ml for 24 hours. There was no effect on cell viability, however 250 and 350 μg/ml suppressed Caki-2 proliferation by 75 and 96 %, respectively. We chose average concentrations 63 and 125 μg/ml to see whether they would inhibit Caki-2 metabolism through mitochondria and found that mitochondrial respiration (with glutamate+malate+succinate and ADP) was inhibited by 38 %. DCAs fraction also increased the permeability of the inner membrane and decreased the respiratory control index by 70 %. In another set of experiments, we used healthy RPTEC cells to assess whether DCAs fraction would protect mitochondria during hypoxia/reoxygenation. The 24-hour hypoxia did not alter respiration of mitochondria in leak state (glutamate+malate), however it significantly decreased ADP-dependent respiration rate (also with succinate) and respiration rate during uncoupled state. Even though after 24-hour hypoxia and 24-hour reoxygenation mitochondrial respiration was partially restored, DCAs fraction increased respiration in leak state and decreased at phosphorylation state comparing to hypoxic levels. Although such concentrations of DCAs fraction did not protect mitochondria during hypoxia/reoxygenation, it impaired cancer metabolism through oxidative phosphorylation. In conclusion, our data show that dicaffeoylquinic acids, obtained from silver wormwood, suppresses mitochondrial oxidative phosphorylation of cancer Caki-2 renal cells and had no effect on healthy cells, and might be promising compounds in cancer therapy.

A3. Effects of a supplementation with tart cherry (*Prunus cerasus L.*) juice and its kernel extract on antioxidant status and muscle health

Daniela Beghelli[1], Silvia Damiano[2], Cristina Andreani[3], Caterina Bartolacci[3], Cristina Marchini[1], Augusto Amici[1], Laura Giusti[2], Giulio Lupidi[2]

1 School of Biosciences and Veterinary Medicine, University of Camerino, IT
2 School of Pharmacy, University of Camerino, IT
3 Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH 45219, USA

Tart cherry (fruit of *Prunus cerasus L.*) is nowadays consumed as a nutritional supplement due to its beneficial health effects. Indeed, numerous scientific studies have attested, by in vitro and in vivo assays, its high anti-oxidative and anti-inflammatory properties. However, some investigations have also evaluated its potential use in lowering muscle pain, and accelerating muscle repair. This is especially important for aging individuals who experience loss of muscle mass and strength (sarcopenia). In the present study, we specifically evaluated the effect of tart cherry juice and/or kernel extract supplementation on both regenerative capacity of skeletal muscles and antioxidant status of old male BALB/c mice. After three weeks of dietary supplementation, or not (CTRL), with tart cherry juice (1 mg anthocyanins/mouse/day) and/or modified diet (kernel extract 30mg/kg/day), blood and tissue (liver and kidney) samples were collected to evaluate the antioxidant status. In plasma, the AntiOxidant Power, reactive oxygen metabolites and serum lactate dehydrogenase, creatine kinase and aspartate aminotransferase activities were evaluated, whereas, in the tissue samples, the enzyme activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase were measured. To evaluate the regenerative capacity of skeletal muscles (gastrocnemius, soleus, and extensor digitorum lateralis muscles), the number of satellite cells (stem cells) were determined by Flow cytometry. Data obtained from our in vivo study showed a significant increase in the number of muscle stem cells upon treatment, especially in old male BALB/c mice supplemented with tart cherry seed extract ($p=0.0015$). On the contrary, nonsignificant differences have been stated for the antioxidant status. Our results suggest that consumption of innovative nutraceutical formulations based on tart cherries could be useful to prevent many diseases including sarcopenia and degenerative muscle wasting disorder.

A4. Fisetin Mitigates Diabetes-Induced Skeletal Muscle Atrophy

Elizabeth Sánchez-Duarte[1], Luis Alberto Sánchez-Briones[1], Claudia Ileana Popoca-Guzmán[1], Ma. del Rosario Abraham-Juárez[2], Rocío Montoya-Pérez[3], Karla Susana Vera-Delgado[1], Sergio Márquez-Gamiño[1]

1 Departamento de Ciencias Aplicadas al Trabajo, Universidad de Guanajuato, Campus León.
2 Departamento de Alimentos, Universidad de Guanajuato, Campus Irapuato–Salamanca.
3 Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo.

The presence of skeletal muscle atrophy has substantial adverse effects on the preservation of the body's integrity and function in patients with diabetes mellitus. Fisetin (3,3′,4′,7-tetrahydroxyflavone) is a flavonoid polyphenol with antioxidant and anti-inflammatory activities. It has a broad range of health benefits, but its impact on muscle atrophy is unknown. Therefore, the present work aimed to evaluate the effect of fisetin on the expression of atrogenes in slow- and fast-twitch diabetic rat muscles. Male Wistar rats were rendered diabetic by intraperitoneally applying a single dose of streptozotocin (100 mg/kg). Fisetin treatment (2.5 mg/kg/day) was administered for 3 weeks. At the beginning and end of the intervention, the fasting blood glucose (FBG) and body weight gain were evaluated. Both slow (soleus) and fast (tibialis anterior, TA) skeletal muscles were used to examine muscle weights and for the quantitative analysis of the expression of the atrogenes of interest (MuRF1 and atrogin-1) by using RT-qPCR. The oral administration of fisetin increased body weight gain and the mass of the TA and soleus muscle, and the mRNA levels of atrogin-1 and MuRF1 were significantly downregulated in both types of muscles. Based on the results of this study, fisetin exerts myoprotective effects by attenuating diabetes-induced muscle atrophy.

A5. Sea buckthorn (*Hippophae rhamnoides)* seed lipid extract as a supplement to reduce residual risk in patients with coronary heart disease

Iveta Mintale[1, 2, 3], Kristaps Erglis[2, 3], Sanda Jegere[1, 2, 3], Ralfs Zuzans[1, 2], Aija Romanovska[3], Eriks Jakobsons[2, 3], Martins Erglis[1, 2, 3], Iveta Bajare[2, 3], Vilnis Dzerve[1, 2, 3], Vadims Bartkevics[2, 4], Andrejs Erglis[1, 2, 3]

1 Latvian Centre of Cardiology, Pauls Stradins Clinical University Hospital, Riga, Latvia
2 University of Latvia, Riga, Latvia
3 JSC "SISTEMU INOVACIJAS", Jaunmarupe, Latvia
4 Institute of Food safety, Animal Health and Environment "BIOR", Riga, Latviaç

Background. Despite effective preventive and treatment strategies, patients with coronary heart disease (CHD) remain at high residual risk. Supplementation with plant-derived extracts could play a role in risk reduction.
Aim. To determine whether sea buckthorn seed lipid extract (SBS-LE) containing polyunsaturated and monounsaturated fatty acid Omega-3,6,9 combination, fat-soluble vitamins, and bioactive compounds synergistically reduce the residual risk for CHD patients.
Methods. A total of 35 of 100 patients with CHD and obesity, hypertension, dyslipidaemia, elevated c-reactive protein (CRP) or uric acid level on optimal treatment, including statins ar ezetimibe, were analysed in this pilot trial at the Latvian Centre of Cardiology. The patients received two SBS-LE soft gelatine capsules (2000mg) per day for three months. SBS-LE was produced by supercritical fluid extraction with carbon dioxide. At baseline (V_0) and the three-month (V_3) follow-up, the blood pressure and heart rate were measured, and blood samples were taken for the lipid profile, glucose, CRP, and uric acid.
Results. Of the first 35 patients, 17 (49%) were men, with a mean age of 69.28±10.48 years. A total of 33 patients (94.29%) were adherent to the SBS-LE with good tolerability and no side effects. One patient (2.86%) was hospitalised with worsening heart failure. In two patients (5.71%), the antihypertensive medication was changed. In four patients (11.43%), the dose of statins was increased. At 3 months, the systolic blood pressure was reduced by 3.15±7.86 mmHg (140 [IQR=125;140] mmHg V_0 to 135 [IQR=125;135] mm Hg V_3 (p=0.048)). There was also a trend to a lower blood glucose level (5.95 [IQR = 5.47; 6.55] mmol/l V_0 vs. 5.63 [IQR=5.11;6.35] mmol/l V_3 (p=0.106)). There were no significant differences in other parameters.
Conclusion. The preliminary results of our study show a reduction in systolic blood pressure and lower glucose level. Continuation of the study with more participants could strengthen the hypothesis of the efficacy of the SBS-LE for high-risk patients.

A6. Sea buckthorn (*Hippophae rhamnoides)* seed lipid extract bioavailability assessment

Kristaps Erglis[1, 2], Sanda Jegere[1, 2, 3], Iveta Mintale[1, 2, 3], Ralfs Zuzans[1, 3], Aija Romanovska[2], Eriks Jakobsons[1, 2], Martins Erglis[1, 2, 3], Iveta Bajare[1, 2], Vilnis Dzerve[1, 2, 3], Vadims Bartkevics[1, 4], Indrikis Muiznieks[1], Andrejs Erglis[1, 2, 3]

1 University of Latvia, Riga, Latvia
2 JSC "SISTEMU INOVACIJAS", Jaunmarupe, Latvia
3 Latvian Centre of Cardiology, Pauls Stradins Clinical University Hospital, Riga, Latvia
4 Institute of Food safety, Animal Health and Environment "BIOR", Riga, Latvia

Background A healthy lifestyle and balanced nutrition are key factors for good long-term general health and disease prevention. One of the strategies to prevent disease and decrease risk factors is to supplement daily nutrition with plant-derived nutraceuticals. An important factor affecting nutraceutical efficiency is their bioavailability.
Aim The aim of the study was to determine the bioavailability of sea buckthorn seed lipid extract (SBS-LE) containing 90% polyunsaturated, monounsaturated fatty acid, fat-soluble vitamins and other bioactive compounds.
Methods Five healthy subjects were recruited for this pilot study at the University of Latvia. A single dose of SBS-LE 4000mg (produced by supercritical fluid extraction with carbon dioxide) was given in the form of four standardized soft gelatine capsules in total containing 1480mg of linoleic acid (LA), 1200mg of alpha-linolenic acid (ALA), 680mg of oleic acid and 28mg of beta-sitosterol. Five blood samples were taken before administration of SBS-LE, after 1 hour, 6 hours, 24 hours and 31 hours. The blood samples were analysed with a gas chromatography–flame ionization detector (GC-FID). The subjects were instructed to avoid consuming any fat-containing products.
Results Of the 5 subjects, 3 (60%) were men with the mean age 39.00 ± 12.63 years. The relative increase in unsaturated fatty acid levels between baseline and 6 to 24 hours after a single dose of SBS-LE administration was 10.50±9.68% (p=0.067); this increase was mostly by LA 5.20±4.85% (p=0.077). Conversely, there was a decrease in saturated fatty acid levels by 4.09±6.74% (p=0.122), mostly due to a reduction in palmitic acid C16:0 by 5.97±6.80% (p=0.110). No changes in trans-fatty acids were observed after SBS-LE intake.
Conclusion The results show remarkable trends for increased unsaturated fatty acid and decreased saturated fatty acid concentration after a single dose of 4000mg SBS-LE administration. SBS-LE impacts blood fatty acid profile and has the potential to be used as a nutraceutical in terms of bioavailability.

A7. Antioxidant activity of *Alium cepa* flowers in *Caenorhabditis elegans*

Cristina Moliner[1], Guillermo Casedas1,[2], Francisco Les[1,2], Sonia Nuñez[1], Víctor López[1,2], Carlota Gómez-Rincón[1,2]

1 Departamento de Farmacia, Facultad de Ciencias de la Salud, Universidad San Jorge, Villanueva de Gállego (Zaragoza), Spain
2 Instituto Agroalimentario de Aragón, IA2, Universidad de Zaragoza-CITA, Zaragoza, Spain

Allium cepa L. (*Amaryllidaceae*), commonly known as onion, is one of the most widespread crops in the world. The antioxidant power of the bulb is well studied; however, there are no studies on its flowers. Onion flowers are also edible, and there is increased interest in using the flowers as a culinary ingredient. This study explored the antioxidant activity of *A. cepa* flowers using different approaches, including in vitro methods, as well as *Caenorhabditis elegans*, a nematode model.

The extract was obtained by percolation with soxhlet using ethanol as the solvent. The antioxidant activity was evaluated in vitro through the $DPPH^{\cdot}$ and $O^{2\cdot-}$ scavenging activity as well as FRAP and ORAC assays. For the in vivo evaluation, the model organism *C. elegans* was used. Acute toxicity and the effect of the extract on the resistance against oxidative stress were performed in *C. elegans* N2. Moreover, the activity of antioxidant enzymes (catalase and superoxide dismutase) of worms was assessed through commercial kits after exposure to sublethal oxidative stress and without exposure.

The in vitro assays revealed the scavenging capacity of onion flowers. Supplementation with the extract significantly increased the survival rate of *C. elegans* under lethal oxidative stress induced by juglone 150 µM. Furthermore, exposure to the extract enhanced catalase activity in the nematode. Hence, *A. cepa* flower extract showed promising antioxidant properties in the different models used.

A8. IWCKDDQNPH: a novel bioaccesible bioactive peptide obtained from a hydrolysate of α-lactalbumin with alcalase

Jessica Báez[1], Adriana Maite Fernández-Fernández[1], Valeria Tironi[2], Karen Perelmuter[3], Victoria Olt[1], Rosario Durán[4], Mariela Bollati[3], María Cristina Añón[2], Alejandra Medrano-Fernández[1]

1 Laboratorio de Bioactividad y Nanotecnología de Alimentos, Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República, Montevideo 11800, Uruguay.
2 Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA)—(CCT La Plata-CONICET, CICPBA), Facultad de Ciencias Exactas (UNLP), La Plata, Argentina.
3 Unidad de Biología Celular, Instituto Pasteur de Montevideo. Montevideo 11400, Uruguay.
4 Unidad de Bioquímica y Proteómica Analíticas. Instituto de Investigaciones Biológicas Clemente Estable, Instituto Pasteur de Montevideo. Montevideo 11400, Uruguay.

Bioactive peptides (BPs), amino acid sequences encrypted in proteins of different origin, are capable of exerting biological activities after being released, depending on their physicochemical and structural characteristics. BPs present potential use in the development of functional foods for the prevention and treatment of many chronic diseases. α-lactalbumin (α-La), a protein from the cheese industry by-product (whey) is an interesting source of BPs, and its use allows the application of the circular economy concept for sustainable development. This work studies the in vitro antioxidant activity (cellular and non-cellular), the immunomodulatory effect and the anti-inflammatory capacity of the bioaccessible peptide IWCKDDQNPH obtained from an α-La hydrolysate with alcalase enzyme (HA).
HA underwent a digestive simulation process followed by purification and fractionation by RP-HPLC. From the 55 fractions obtained, the bioaccessible peptides belonging to α-lactalbumin were identified by mass spectrometry. Three peptides were obtained: IWCKDDQNPH, DKFLDDDLTDDIM, and KFLDDDLTDDIM, which were synthesized and analyzed. IWCKDDQNPH showed greater in vitro bioactivity carrying out more analyses. The antioxidant capacity of the peptide was measured using the ABTS, ORAC, and HORAC methods, obtaining IC50 values of 0.45±0.02, 0.015±0.002, and 1.30±0.05 mg/mL, respectively. The inhibition capacity of intracellular ROS formation in Caco-2 intestinal cells was studied under oxidation-induced conditions (AAPH 1mM), presenting no activity.
The immunomodulatory effect of IWCKDDQNPH on the NFkB reporter cell line HT-29-NFkB-GFP was analyzed, showing activity at concentrations 0.75 and 1.5 mg/mL. Finally, the anti-inflammatory capacity of the peptide was studied by measuring nitric oxide production on LPS-induced RAW 264.7 macrophages, presenting significant differences with the control (DMEM with LPS) at a concentration of 1 mg/mL.
In conclusion, the peptide IWCKDDQNPH from α-lactalbumin hydrolysate has antioxidant and anti-inflammatory potential, as well as immunomodulatory effect.

A9. A wave of pro-oxidant metabolites is released by melanoma cells during chemotherapy and triggers paracrine pro-survival epigenetic changes in the tumor microenvironment

Giorgia Musto, Valentina Laurenzi, Mariano Stornaiuolo

Department of Pharmacy University of Napoli Federico II, Napoli Italy Via Domenico Montesano 49 80131 Napoli Italy Phone +39 081 678117

Recent compelling clinical evidence suggests that, in some circumstances, the chemotherapy regimen may play a paradoxical role in tumor treatments and, unwantedly, itself acts as a fosterer of tumor relapse and metastasis. Lung, prostate, bowel, melanoma, and breast cancer are examples of tumors where chemotherapy regimens have indicated the exertion of these unwelcome ''opposite effects'', enhancing the rate of tumor relapse and metastasis even after successful treatment and the eradication of primary tumors.

The rationale behind this unwanted opposite effect of chemotherapy seems to depend on the cytotoxic effect of cancer treatments. By inducing cell damage in the parenchyma of the tumor, chemotherapy promotes the establishment of concerted paracrine signaling in the tumor microenvironment between the surviving cancer cell population and the stroma. This event results in the activation of a "pro-tumoral" stroma that contributes to important steps of the tumorigenic cascade, facilitating relapse, extravasation of cancer cells to secondary metastatic sites, and metastatic growth.

Until now, pro-inflammatory cytokines have been proven to be secreted by chemotherapy-treated cancer cells and act as paracrine factors to activate the stroma. However, the mechanism underpinning the opposite effects of chemotherapy remains largely obscure. Here, we use high-resolution metabolomic profiling to identify glucose and palmitic acid as metabolites secreted by melanoma cells during chemotherapy. We show that glucose acts in a paracrine way and induces oxidative stress in the stroma. Its effect alters stromal metabolism and culminates in epigenetic changes in the stroma, ultimately promoting growth hormone IGF-II secretion and the activation of the WNT/-beta catenin pathway, involved in de-differentiation of the parenchyma and tumor relapse.

Finally, we prove that antioxidants can block this signaling cascade, underlining the possibility and need to design novel therapeutic strategies and effective treatments for metastatic disease, including antioxidants, to avoid ineffective and potentially opposite effects of the chemotherapy regimen for patients.

A10. Anti-inflammatory and antioxidant effects of supercritical CO_2 carrot and pumpkin extracts

Miriana Durante[1], Luca Frattaruolo[2], Maria Stella Cappello[3], Giovanni Mita[3], Anna Rita Cappello[2], Marcello Salvatote Lenucci[4]

1 CNR-ISPA Lecce, Italy
2 Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Via Pietro Bucci, 87036 Arcavacata di Rende, Italy
3 Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Lecce-Monteroni, 73100, Italy
4 Department of Biological and Environmental Sciences and Technologies, University of Salento, Via Lecce-Monteroni, 73100, Italy

Supercritical CO_2 (SFE) represents a sustainable technology, alternative to conventional solvent (CSE), for the extraction of food-grade bioactives from plants. Here, extracts were obtained by SFE and CSE from carrot and pumpkin matrices, as well as from an inert control matrix impregnated with organic hempseed or flaxseed oil as co-solvents. This study aimed to investigate the anti-inflammatory effects of the extracts and their ability to counteract the oxidative phenomena which can disturb the cellular redox homeostasis, promoting the onset of several pathologies.

The extracts presented different quali-quantitative composition of bioactives, such as tocochromanols and carotenoids, as well as healthy lipid indices. The anti-inflammatory activity of the different extracts was analysed by assessing their ability to modulate nitrite oxide production (NP_{lps}) in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. A dose-dependent anti-inflammatory potential of carrot and pumpkin extracts (CP_{ex}) was observed, as well as their ability to decrease the expression levels of several pro-inflammatory cytokines. Interestingly, all extracts interfered with the pro-inflammatory effects of MDA-MB-231 breast cancer cell-conditioned media. Furthermore, CP_{ex} inhibited reactive oxygen species (ROS) accumulation in LPS-stimulated macrophages. The nuclear factor-erythroid-2-related factor 2, a transcription factor, proved to be involved in the mechanisms underlying such effects; its activation by carrot extracts was corroborated by the increase in mRNA levels of several target genes, such as catalase and glutathione-peroxidase. Regardless of matrices, co-solvents and extraction method, although α-tocopherol, lutein, carotenes, and total carotenoids negatively correlated with NP_{lps}, and most carotenoids and their total also negatively correlated with the intracellular ROS levels, a positive correlation was found between total tocopherols/total carotenoids ratio and both NP_{lps} and ROS production. These results suggest an idiosyncratic effect of the pool of bioactives in the CP_{ex} on inflammation-induced oxidative stress, so emphasizing the importance of the interaction among plant extract constituents in antioxidant and anti-inflammatory cellular response.

A11. Vitamin K2: A Promising Antioxidative Regimen for Oxidative Stress Induced Organelle Damage in Neurodegenerative Diseases

Shruti Shandilya, Janne Ruokolainen

Aalto University, Finland

To date, only five drugs have been approved by the FDA, USA, for the treatment of Alzheimer's disease. Current studies are focused on the screening of bioactive compounds for their effect on preventing Aβ formation and oxidative stress reduction, and targeting organelle dysfunction to prevent AD in early stages. Vitamin K2, known for its antioxidative and anti-inflammatory properties, can act as a potent neuroprotective molecule. Despite its action against mitochondrial dysfunction, the mechanism underlying the links between the protective effects of vitamin K2 and endoplasmic reticulum (ER) stress along with basal levels of total tau protein and amyloid-beta 42 (Aβ42) has not been elucidated yet. To understand the neuroprotective effect of vitamin K2 during metabolic complications, human neuroblastoma SH-SY5Y cells were treated with streptozotocin and menadione in a dose-dependent manner, followed by the post-treatment of vitamin K2. The modulating effects of vitamin K2 on cell viability, lactate dehydrogenase release, reactive oxygen species (ROS), mitochondrial membrane potential, ER stress marker (CHOP), an indicator of unfolded protein response (UPR) p-IRE1α, GSK3α/β, total tau and Aβ 42 were studied. Vitamin K2 significantly reduced neuronal cell death by inhibiting cytotoxicity and ROS levels, and retained mitochondrial membrane potential. It significantly decreased the expression of CHOP protein along with the levels and the nuclear localization of p-IRE1 α. In addition, it downregulated the expression of GSK3 α/β and decreased the total tau protein, with a diminutive effect on secreted Aβ 42. Vitamin K2 alleviated mitochondrial damage and ER stress, and tauopathy mediated neuronal cell death, highlighting its role as a novel antioxidative therapeutics targeting related cellular processes.

A12. Antioxidant properties of flavan-3-ols of industrial white, green and black tea (*Camellia sinensis* L.) extracts evaluated *in vivo* and characterized by liquid chromatography techniques

Antonio Manuel Inarejos Garcia[1], Julia Heil[2], Sonia Guilera Bermell[1], Patricia Martorell Guerola1[3], Beatriz Alvarez Pérez[3], Silvia Llopis Pla[3], Gertrud E. Morlock[2]

1 Department of Functional Extracts, ADM® Valencia, 46740 Carcaixent, Spain.
2 Justus Liebig University Giessen, Institute of Nutritional Science, Chair of Food Science, and TransMIT Center for Effect-Directed Analysis, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany.
3 Archer Daniels Midland, Nutrition, Health&Wellness, Biopolis S.L. Parc Científic Universitat de València, 46980 Paterna, Spain

Compared to black tea, green and white tea leaves have significant higher and natural contents of antioxidant flavan-3-ols such as (+)-catechin (C), (–)-epicatechin (EC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC), (–)-gallocatechin gallate (GCG), (–)-epigallocatechin gallate (EGCG), and (+)-gallocatechin (GC). In black tea, due to the enzymatic polymerization of monomeric flavanols during tea processing, also thearubigins and theaflavins are naturally present.
There are several tea extracts in the market, named as nutraceuticals, standardized to different bioactive components, chiefly EGCG, total catechins, etc. The oxidative stability of these antioxidants is different depending on the chemical structure. EGCG is the less stable catechin and several commercial tea extracts are highly concentrated in EGCG. The overall oxidative stability of highly purified tea extracts is limited due to the absence of other natural antioxidants, which are characteristic from *Camellia sinensis L*. and significantly affect oxidative stability of commercial extracts. Therefore, the antioxidant profile and/or bioactive composition analyzed by different chromatographic techniques together with in vivo models are fundamental to evaluate oxidative stability, safety, and therefore healthy properties throughout the entire commercial shelf life of the product.
The main objective of the present study was to characterize the bioactive profile by high-performance thin-layer chromatography and high-performance liquid chromatography of different commercial white, green and black tea extracts, together with referenced tea leaves to assess the antioxidant properties together with the oxidative stress by different *Caenorhabditis elegans* in vivo models.

A13. Screening of enzyme inhibition and antioxidant properties of *Phlomis lychnitis* L. extract

Francisco Les[1], Guillermo Cásedas [1,2], Cristina Moliner[1], Carlota Gómez-Rincón[1], Eduardo Aranda[1], Víctor López[2]

1 Facultad de Ciencias de la Salud, Universidad San Jorge, Villanueva de Gállego, Zaragoza, Spain
2 Instituto Agroalimentario de Aragón, IA2, Universidad de Zaragoza-CITA, Zaragoza, Spain

Introduction: *Phlomis lychnitis* L. (Lamiaceae) is a yellow-flowered shrub found in the Iberian Peninsula and other Mediterranean countries. *P. lychnitis* has been used in traditional medicine to treat illnesses such as colds and gastrointestinal problems. Due to the abundance of polyphenols in its composition, this plant is a source of bioactive compounds for the development of new possible treatments[i], [ii], [iii].
Objective: Determine the bioactive properties of a methanolic extract of *P. lychnitis* L. and quantify the total polyphenols of the extract, its antioxidant capacity, and its ability to inhibit digestive enzymes.
Material and methods: To determine the total polyphenol content, the Follin–Ciocalteu test was carried out. The antioxidant activity was evaluated through the DPPH and xanthine/xanthine oxidase assays. In addition, the ability to inhibit enzymes such as xanthine oxidase and the digestive enzymes α-glucosidase and lipase was evaluated.
Results: The results of these tests showed that the extract has a great phenolic composition. It also had the ability to eliminate free radicals, showing antioxidant properties. Furthermore, it was able to inhibit the enzyme α-glucosidase, lipase, and xanthine oxidase. These two properties proved to be concentration dependent.
Conclusion: The extract has bioactive compounds as polyphenols with antioxidant and enzyme inhibitory activity. This plant could have a potential use in the therapeutic treatment of different pathologies such as diabetes, obesity, and ageing, but more studies must be performed.

References:

[i] Catalán J. Plantas medicinales de Monlora y las Cinco Villas Orientales. Zaragoza: Centro de estudios de las Cinco Villas.

[ii] López V, Jäger AK, Akerreta S, Cavero RY, Calvo MI. Antioxidant activity and phenylpropanoids of *Phlomis lychnitis* L.: a traditional herbal tea. Plant Foods Hum Nutr. 2010;65(2):179-85.

[iii] Rivera D, Verde A, Fajardo J, Obon C, Consuegra V, Garcia-Botia J, et al. Ethnopharmacology in the Upper Guadiana River area (Castile-La Mancha, Spain). Journal of Ethnopharmacology. 2019; 241:70.

A14. Bioeconomy of Guacamole Industry Residues for the Development of New Pharmacological and/or Tecnoactive Products

Alejandro Rojas-García, María de la Luz Cádiz-Gurrea, Álvaro Fernández-Ochoa, Felipe Huertas-Camarasa, David Árraez-Román, Antonio Segura-Carretero

University of Granada, Department of Analytical Chemistry, Spain

Over the last decade, the guacamole industry has grown significantly, making avocado processing increasingly attractive to the business sector. In 2019, total avocado production was 6.4 million tons worldwide. In Spain, 20,000 hectares are dedicated to avocado cultivation, 90% of which are located in Malaga, Granada, Cadiz and Huelva.

However, the presence of the peel and seed, 30% of the total weight of the fruit, as well as the residue of the pulp, is estimated to waste 39% of the food corresponding to 1.92 million tons per year of waste, which entails a high environmental and economic impact. This highlights the need to find alternatives to revalue this waste since both peel and seed contain significant quantities of bioactive compounds with antioxidant power and beneficial health effects. Moreover, these phytochemical-rich by-products can be considered natural alternatives to synthetic compounds, thus contributing to a more sustainable production.

Therefore, the aim is to make a comprehensive and sustainable use of guacamole waste by using economically viable revalorization strategies to produce high added-value products. To this end, methods of waste stabilization, extraction and characterization of bioactive compounds will be optimized. In addition, the bioactive potential of these compounds will be evaluated, and revalorization strategies will be developed for their incorporation in different industries such as pharmaceuticals, animal feed or biodegradable packaging.

The results obtained will lead to the implementation of an integral revalorization model transferable to industries of different types and with different plant matrices, thus reducing pollution and allowing the development of new high added-value products.

Bibliography:

- Pérdidas y Desperdicio de Alimentos. 2019.
- Rojas-García et al. (2022). “Biological Evaluation of Avocado Residues as a Potential ...”.
- Salazar-López et al. (2020). “Avocado fruit and by-products as potential ...”.
- Ezgi-Bezirhan et al. (2015). “Investigation of Bioplastics”.
- Morales-Ramos et al. (2020). “Self-selection of agricultural by-products and food ingredients”.

A15. In Vitro Studies on Potential Utilization of Rosehip Leaves in Poultry Nutrition as Source of Bioactive Compounds with Antioxidant Activity

Petru Alexandru Vlaicu[1], Mihaela Saracila[1], Arabela Elena Untea[1], Alexandra Gabriela Oancea[1], Corina Nicoleta Predescu[2]

1 Department of Food and Feed Quality, National Research and Development Institute for Animal Nutrition and Biology
2 Faculty of Veterinary Medicine, University of Animal Science and Veterinary Medicine of Bucharest, Romania

Rosehip (*Rosa canina L.*) is a common wild plant in some European countries that produces fruits rich in bioactive compounds, which have been explored and utilized in various ways. However, in vitro studies revealed that its leaves present higher concentrations of bioactive compounds with antioxidant activity than the fruits. For this study, we selected rosehip leaves to assess their potential utilization as a feed ingredient in poultry nutrition. Reverse-phase high-performance liquid chromatography (RP-HPLC) was used to investigate phytochemical constituents such as the polyphenol profile, carotenoids (lutein, zeaxanthin and beta-carotene), and vitamin E (tocopherols isomers). The fatty acid profile was determined by gas chromatography while the antioxidant capacity (DPPH, total antioxidant capacity (TAC), ABTS, iron chelating activity), bioactive compounds such as the total polyphenol content (TPC), total flavonoids (TF), and in vitro peroxidation inhibition capacity were determined spectrophotometrically. The results showed that from the polyphenols profile, the flavan-3-ols group (catechin 11259.36 and epicatechin 845.45 mg/kg) was the most abundant, followed by methoxycinnamic (601.18 mg/kg). The dominant polyphenols from acids were tannic and caffeic (555.43 and 586.16 mg/kg), from the flavonols was rutin (310.79 mg/kg), and from stilbenes group was resveratrol (11.22 mg/kg). The carotenoids profile showed that the leaves have 325.05 mg/kg lutein, 1929.19 mg/kg zeaxanthin, and 4761.46 mg/kg beta-carotene. Alpha, delta, and gamma tocopherol accounted for 681.28 mg/kg of vitamin, of which alpha isomer represented 95%. The DPPH concentration determined in rosehip leaves was 1636.17 mM Trolox and ABTS was 773.07 mM Trolox, followed by TAC (643.66 mM equiv. ac. ascorbic) and TPC (143.96 mg/g GAE). From the total n-3 fatty acids (38.65 g/100g), α-linolenic was the most abundant (37.04 g/100g). The rosehip leaves being an important source of n-3 fatty acids, the counteracting potential of the oxidation process was also evaluated. The results showed 38.06% inhibition by using an in vitro-induced lipid peroxidation method. The promising results obtained in this study about rosehip leaves' bioactive compounds support their potential utilization as a feed ingredient in poultry nutrition.

A16. Improved in vitro bioavailability of antioxidant phytochemicals through interaction with plant extracts

Bernhard Blank-Landeshammer, Lisa Mitter, Melanie Wallner, Gerald Klanert, Julian Weghuber

University of Applied Sciences Upper Austria

Lipophilic phytochemicals suffer from poor intestinal absorption and thus generally exhibit low bioavailability. Through the modulation of both physicochemical and biochemical parameters, plant extracts have the potential to modulate the absorption process and positively influence the bioavailability of certain target substances. However, these interactions are often limited to specific conditions and the mechanisms and potential synergisms are poorly understood. In this work, we used a human intestinal cell line to investigate the uptake characteristics and delivery of dietary antioxidants lutein, zeaxanthin, and coenzyme Q10, as well as the transport rates of soy isoflavones. Different formulations were subjected to simulated gastrointestinal digestion and their cellular bioavailability was determined using HPLC-DAD and LC-MS. Further, extracts from *C. longa* (curcuma), *Z. officinale* (ginger), *P. nigrum* (black pepper), and *P. ginseng* (ginseng) were investigated for their potential interaction with the target substances in vitro. We found increased isoflavone transport rates with curcuma and ginger extract as well as increased uptake of lutein and Q10 with curcuma and black pepper, respectively. Through the investigation of mixed micelle incorporation efficiency, we could dismiss changes in bioaccessibility as a potential enhancing mechanism in our experimental setup. We further conducted a rhodamine 123 efflux assay and discovered inhibition of P-glycoproteins by the ginger and black pepper extracts, highlighting a plausible route of action leading to increased isoflavone bioavailability.

A17. Antioxidant compounds formed in Maillard reaction of glucose and glycine

Sara Bolchini, Ksenia Morozova, Matteo Mario Scampicchio

Free University of Bozen-Bolzano, Italy

Maillard reaction is a complex series of chemical reactions that occur between reducing sugars and amino acids residues in food when heated. Its products (MRPs) are responsible for aroma production and food browning and in literature some of them are reported as antioxidants. However, the molecules responsible for antioxidant activity and their mechanism of production remain still unclear. Therefore, this study, based on the kinetic model of the reaction developed by van Boekel's group (Martins and Van Boekel 2005), aimed at identifying the antioxidant compounds evolved during the course of the Maillard reaction and determining their kinetic of production. The spectrophotometric assay Oxygen Radical Antioxidant Capacity (ORAC) and HPLC coupled with three different detectors—Diode Array Detector, CoulArray Detector, and High-Resolution Mass Spectrometer—were used to follow the production of antioxidant MRPs and allowed their identification. 1H and 13C NMR analyses have also been applied to follow the consumption of glucose and glycine during the reaction and the production of some MRPs, such as acetic and formic acid. According to the results obtained, it was possible to conclude that antioxidant molecules are produced during the reaction and their chemical structures have been putatively identified as 2-acetylpyrrole and dehydrate 1- and 3- deoxyglucosones.

References:
Martins, Sara I.F.S., and Martinus A.J.S. Van Boekel. 2005. "A Kinetic Model for the Glucose/Glycine Maillard Reaction Pathways." Food Chemistry 90(1–2): 257–69.

A18. Phenolics from lingonberry raw materials—natural versatile antioxidants with promising therapeutic applications

Gabriele Vilkickyte, Lina Raudone

1 Laboratory of Biopharmaceutical Research, Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Sukileliu av. 13, LT-50162 Kaunas, Lithuania
2 Department of Pharmacognosy, Lithuanian University of Health Sciences, Sukileliu av. 13, LT-50162 Kaunas, Lithuania

Lingonberry (*Vaccinium vitis-idaea* L.) is a perennial evergreen shrub, the raw materials of which are extremely rich in phenolic compounds with high potential in biomedical applications due to their versatile antioxidant activity. Nevertheless, there are not enough scientific data to confirm the predominant agents, responsible for the antioxidant activity and traditional therapeutic use of lingonberries. Therefore, crude dry extracts of lingonberry leaves and fruits (L1 and F1) were fractionated by column chromatography using Sephadex LH-20, eluting successively with different polarity solvents to remove non-phenolic lingonberry constituents as sugars (waste) and to obtain anthocyanins and phenolic acids (F2), arbutin (L2), flavonols and flavan-3-ols (L3 and F3) and proanthocyanidins-rich (L4 and F4) fractions. For each fraction, the phytochemical composition was determined by the validated HPLC-PDA method, while reactive oxygen species and free radical scavenging, reducing, and chelating activities were investigated using spectrophotometrical H_2O_2 scavenging, ABTS, FRAP, and FIC assays, accordingly. The radical scavenging activity assessed by ABTS assay ranged between 1751.4 and 7832.7 μM TE/g DW, with the lowest and highest values determined in F1 and L4, respectively. In the FRAP assay, L3 and F3 fractions surpassed all other fractions by the greatest reducing activity, followed by L4 and F4 fractions, whereas the lowest reducing activity was found in F1. Similarly in the FIC assay, the highest chelating activity was expressed in L4 and F4 and the lowest one was in F1. Furthermore, phenolic fractions were found to be active against H_2O_2 in a concentration-dependent manner (31.25–625 μg/mL). Average H_2O_2 scavenging activity values decreased in following order: L4 > F4 > ascorbic acid > L1 > L3 > F3 > F2 > F1 > L2. Present results displayed higher bioactivities of particular fractions than that of crude extracts and elucidated lingonberry proanthocyanidins as antioxidant activity markers, which could be promising candidates in phytopharmaceuticals.

A19. *Sorbus* L. fruits, leaves and inflorescences as an underutilized valuable resource of phytochemical diversity and excellent antioxidant capacity

Lina Raudonė[1, 2], Kristina Zymone[2, 3]

1 Department of Pharmacognosy, Lithuanian University of Health Sciences, Lithuania
2 Laboratory of Biopharmaceutical Research, Lithuanian University of Health Sciences, Lithuania
3 Department of Analytical and Toxicological Chemistry, Lithuanian University of Health Sciences, Lithuania

The research of new, natural, safe plant extracts and phytochemicals that might be employed in a variety of industries to produce cosmetics, nutraceuticals, and pharmaceuticals is becoming more challenging as consumers' awareness of the health benefits and safety of foods, and demand for products with clean and clear labelling grows [1]. Plants that can withstand poor soils and harsh environments are of particular interest [2]. *Sorbus* L. species and cultivars are widely distributed and environment-resilient [3]. All parts of the plant, namely fruits, leaves, barks, and inflorescences, have a unique composition of biologically active compounds. The aim of our study was to determine the qualitative and quantitative comparative phytochemical profiles of fruits, leaves and inflorescences of various *Sorbus* genotypes and to characterize their importance for possible use as the source of valuable specialized metabolites and antioxidants. Significant quantitative and qualitative variation of phytochemicals was highly dependent upon the plant matrix. The prevalence of neochlorogenic and chlorogenic acids in the phenolic profile was characteristic to all *Sorbus* genotypes tested and could serve as fast-acting antioxidant markers for the genus. The leaves showed significant superiority in terms of phenolic compounds compared to fruits and inflorescences. The genotype had a remarkable impact on the profile of specialized metabolites. *S. commixta*, *S. aucuparia* var. *xanthocarpa* and cultivars of group *Rossica* distinguished themselves with an extraordinary composition and antioxidant potential, and could be used as promising genotypes with the defined markers for standardization.

References:
1. Helal NA, Eassa HA, Amer AM, Eltokhy MA, Edafiogho I, Nounou MI. Nutraceuticals' Novel Formulations: The Good, the Bad, the Unknown and Patents Involved. Recent Pat. Drug Deliv. Formul. 2019; 13:105-156.
2. Mikulic-Petkovsek M, Krska B, Kiprovski B, Veberic R. Bioactive Components and Antioxidant Capacity of Fruits from Nine Sorbus Genotypes. Food Science. 2017:82;647-658.
3. Aldasoro JJ, Aedo C, Navarro C, Garmendia FM. The Genus Sorbus (Maloideae, Rosaceae) in Europe and in North Africa: Morphological Analysis and Systematics. Syst. Bot. 1998:23;189–212.

A20. Optimization of the extraction targeting for phenolic content and antioxidant capacities of *Agrimonia eupatoria* L

Gerda Šaltytė[1], Modestas Žilius[2], Lina Raudonė[1,3]

1 Department of Pharmacognosy of Lithuanian University of Health Sciences, Sukileliu av. 13, LT-50162 Kaunas, Lithuania
2 Department of Clinical Pharmacy, Lithuanian University of Health Sciences, Sukileliu av. 13, LT-50162 Kaunas, Lithuania
3 Laboratory of Biopharmaceutical Research, Lithuanian University of Health Sciences

An essential initial stage in the creation of products rich in phytochemicals is the extraction process of phytochemicals from plant materials. In recent years, the demand for natural-origin antioxidants in the food and pharmaceutical industries has been increasing. Phenolic compounds have been distinguished as a widespread group in the plant world with extraordinary antioxidant activity and epidemiological evidence in managing various chronic degenerative diseases. *Agrimonia eupatoria* L. has been used in traditional medicine in the treatment of diarrhoea, liver and urinary system diseases, and inflammation of oral mucosa. Phytochemical studies related *A. eupatoria* have led to the isolation and identification of rich profiles of tannins and flavonoids. The scientific uncertainties lie within the solvent selection, the ratio of material and solvent, and the extraction time. The aim of the study was to modulate the extraction parameters using the Response Surface Methodology. The experimental plant consisted of 15 runs. The experimental estimates were the total phenolic content (evaluated using the Folic-Ciocalteu method) and the radical scavenging (ABTS assay). The total phenolic content ranged from 13.01 mg/g up to 120.60 mg/g of gallic acid equivalents. The radical scavenging activity also significantly varied from 94.21 TE µmol/g up to 1984.33 TE µmol/g. The amounts of phenolic compounds and antioxidant activity highly depend on the extraction parameters. The modulation of extraction techniques could provide a standardized procedure for the manufacturing of antioxidant trich smart ingredients with reliable final compositions.

A21. Accumulation of antioxidant enzymes and antioxidant activity in different *P. Sylvestris* half-sib families after pre-sowing seed treatment with low-temperature plasma

Ieva Cesniene[1], Diana Miskelyte[2], Vaida Sirgedaite-Seziene[1]

1 Institute of Forestry, Lithuanian Research Centre for Agriculture and Forestry, Liepų Str. 1, Kaunas District, LT-53101 Girionys, Lithuania
2 Faculty of Natural Sciences, Vytautas Magnus University, Vileikos Str. 8, LT-44404 Kaunas, Lithuania

Woody plants, especially tree residues, contain a large amount of antioxidants, which are useful for food industry. *Pinaceae* species are considered a natural source of antioxidant compounds and are characterized by their pharmaceutical and nutraceutical properties. Pre-sowing seed treatment with the new alternative method of low-temperature plasma not only induces plant growth and increases yield, but also supposedly increases the amount of biologically active compounds, especially antioxidants. In our study, we selected nine different half-sib families (different genetic properties) of Scots pine. These seeds were treated with low-temperature plasma for 1 min and 2 min. Our research showed that genetic properties and treatment duration have a significant impact on the accumulation of antioxidants in Scots pine needles. One half-sib family (from all nine families) has an increased amount of antioxidant enzyme (APX and GR), after a longer time of seed treatment (2 min), compared to control seeds. Meanwhile, seed treatment with low-temperature plasma for 2 min has decreased the amount of enzymes of CAT compared to the control in the same half-sib family. Antioxidant activity (which was evaluated using the ABTS and DPPH methods) showed that seed treatment time and half-sib family have a significant impact on these compounds' accumulation in Scots pine needles. Our study determined that eco-friendly technologies could increase pharmaceuticals properties in Scots pine needles, and that these are dependent on genetic properties and treatment time. Furthermore, antioxidants, which are extracted from the treatment of Scots pine needles, can be used in the food and pharmaceutical industries.

A22. Fluorescent probes containing catechol moiety as markers of peroxyl radicals in lipid membranes

Jaroslaw Kusio, Adrian Konopko, Grzegorz Litwinienko

University of Warsaw, Faculty of Chemistry, Poland

Due to their optical and chemical properties, boron dipyrromethanes (BODIPY) are valuable building blocks for new fluorescent sensors for chemical and biological research or medical diagnostics [1,2]. Herein, we describe a methodology for obtaining the fluorescent probe (FP) of Reactive Oxygen Species (ROS). The presented FP is an OFF/ON type sensor, where the BODIPY part of the molecule acts as a "reporter" of the signal (in this case, fluorescence intensity), and a phenolic group performs the role of the "receptor" which is a moiety sensitive to ROS. The change in the molecule's fluorescence is triggered by the Photoinduced electron Transfer (PeT) that occurs after the oxidation of the phenolic part of the probe [1].
BODIPY-based OFF/ON FPs have several advantages: chemical inertness of the BODIPY part, high fluorescence intensity at low concentrations (μM), and enhancement in emission proportional to the reaction progress. There are some known examples of FPs containing tocopherol or ubiquinol moieties as receptors [3], however, we decided to incorporate catechol as the receptor to obtain the reactivity reflecting the antiradical activity of catecholamine neurotransmitters [4]. Additionally, non-polar, carbon-side chains are added to the BODIPY-based molecule to tune the compound's polarity and tailor it to be localised inside the lipid bilayers, where peroxyl radicals are the primary mediators of peroxidation.

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References:
[1] Kusio J., et al., Lipid Oxidation in Food and Biological Systems, editor: C. Bravo-Díaz, *Springer Nature Switzerland AG*, **2022**, 49
[2] Kusio J., et al., *Antioxidants*, **2020**, 9(1), 88
[3] Oleynik P., et al., *J. Am. Chem. Soc.,* **2007**, 129, 1842
[4] Jodko-Piorecka K., et al., *J.Org. Chem.*, **2022**, 87, 1791.

A23. Association between the APOE gene polymorphism and lipid profile following long-term orange juice intake

Layanne Nascimento Fraga[1], Dragan Milenkovic[2], Camille Perella Coutinho[1], Adriana Campos Rozenbaum[1], Franco Maria Lajolo[1], Neuza Mariko Aymoto Hassimotto[1]

1 Food Research Center (FoRC) and School of Pharmaceutical Sciences, University of São Paulo, 05508-000, São Paulo, Brazil
2 Department of Nutrition, University of California Davis, Davis, CA, USA

Introduction: Orange juice intake has been associated with a reduced risk of cardiovascular disease. However, large inter-individual variability in response to orange juice for lipid profile has been observed. This heterogeneity in responsiveness could be associated with single nucleotide polymorphism genes (SNP) of lipid metabolism, which has not been previously addressed. **Objective:** Thus, this study aimed to investigate the influence of SNP in the APOE gene in the heterogeneity in biological response after long-term orange juice intake. **Methodology:** Forty-six volunteers ingested 500mL daily for sixty days and plasma lipid profile, triglycerides (TG), total cholesterol (TC) and, HDL, LDL were measured before orange juice intake (T0) and after sixty days (T60). Additionally, SNP in the APOE gene was genotyped in the same volunteers. **Results:** Five genetic models (codominant, dominant, recessive, over dominant, and log-additive) were applied to analyze the associations between APOE polymorphism and orange juice intake on a lipid profile. Following association analysis, the genotype of the APOE gene variant rs429358 alters the effect of orange juice intake on total TG and HDL levels ($p<0.05$). The CC genotype was associated with an increase in TG and a decrease in HDL levels, in both the co-dominant, as well as in the recessive models; additionally, the TT genotype was associated with a decrease in TG and HDL. **Conclusion:** These results identified genetic polymorphism in the APOE gene associated with the heterogeneity in lipid profile changes after chronic orange juice intake, results that may provide bases for future precise nutritional recommendations regarding this flavanone-rich food to lower the risk for cardiovascular disease.

A24. The Catechol/Quinone/Superoxide system as a non-conventional catalytic antioxidant mechanism that explains the antioxidant potential of natural polyphenols

Wenaki Pan, Alice Cariola, Federico Simoncioni, Yafang Guo, Riccardo Amorati, Luca Valgimigli

University of Bologna, Italy

The hydroperoxyl radical HOO·, the neutral form of superoxide anion, is ubiquitous in living systems and is considered among the main mediators of oxidative damage. It is also implied in the triggering of Ferroptosis, a recently recognized form of non-apoptotic cell death mediated by iron. On the other hands, quinones, the oxidized products of antioxidant catechols, are commonly regarded as toxic electrophiles, responsible for biomolecules' alteration and for amplifying oxidative-stress-related damage.
Interestingly, we have recently found that, in the presence of HOO·/O_2·⁻, quinones (Q) are effectively reduced stepwise to the semiquinone radicals (QH·), then to the corresponding catechols (QH_2). This counterintuitive behavior renders *o*-quinones extremely effective chain-breaking antioxidants in the presence of a source of HOO· radicals or superoxide radical anion, able to protect oxidizable substrates, such as dietary lipids more effectively than catechols. The overall picture sees o-quinones behave as catalysts in the cross-dismutation of alkylperoxyl radicals (ROO•) and HOO• radicals, either formed during the autoxidation of hydrocarbons, or from the mitochondrial respiratory chain in living organisms, and this chemistry was found to be key to the antioxidant activity of melanins, of which *o*-quinones represent the structural motive. Our recent results obtained inhibited autoxidation studies in homogenous solution, in micelles and in biomimetic liposomal models suggest that the same chemistry has the potential to protect cells from oxidation thereby counteracting ferroptosis. Since the catechol function is most represented in natural phenolic antioxidants, from simple phenolic acids to more complex flavonoids and tannins, this chemistry also explains their often incompletely understood antioxidant behavior in living organisms, and allows the rational design on novel unconventional antioxidant systems based on the synergic interplay among natural antioxidants like terpenoids and flavonoids.

A25. Supercritical antisolvent precipitation of marjoram extracts to enhance their cellular antioxidant activity

María de las Nieves Siles-Sánchez, Irene Fernandez-Jalao, Susana Santoyo, Laura Jaime de Pablo

Institute of Food Science and Research (CIAL), Universidad Autónoma de Madrid (CEI UAM+ CSIC), 28049 Madrid, Spain

Marjoram (*Origanum majorana* L.) is an aromatic plant widely known for its antioxidant properties which are mainly attributed to its phenolic composition and terpenes, among others.

The aim of the present study was to achieve a fractionation of the antioxidant compounds of a marjoram ethanolic extract using supercritical antisolvent precipitation (SAS). This technology allowed the fractionation of the extract according to their solubility in supercritical CO_2, obtaining a precipitate of microparticles (non-soluble compounds) and an oleoresin (soluble compounds found in the subsequent separator). Thus, 40 and 50 ºC and a pressure ranging from 100 to 250 bar were used as SAS process conditions. Subsequently, the chemical characterization of phenolic compounds was carried out by HPLC-PAD and the cellular antioxidant activity (CAA) of all the samples was evaluated. For this latter purpose, human intestinal cells (Caco-2) were employed.

The results obtained showed how the SAS fractionation of the extract compounds affected their CAA. Regarding the fractions recovered in the precipitation vessel, the one obtained at 150 bar and 40 ºC showed the best CAA. Furthermore, the highlights of this precipitate fraction consisted of powder microparticles with uniform size distribution and a size of 5.29 µm. Regarding the fractions collected in the separator, 200 bar and 50 ºC were the condition that exerted the best CAA. In order to determine the CAA found in these fractions, their composition was simulated through a mixture of pure standards and their CAA evaluated. The results showed that the fraction obtained in the precipitation vessel CAA is related to its composition of phenolic compounds, although the CAA of the separator was only partially due to the presence of these phenolic compounds.

Therefore, this work has demonstrated how the use of a sustainable and novel technology such as SAS can allow the obtainment of fractions enriched in antioxidant compounds from marjoram leaves.

A26. Cellular antioxidant and anti-inflammatory activity assessed in edible mushroom extracts

Vanessa Grifoll[1], Rebeca Lavega[1], Paula Bravo[1], María de las Nieves Siles-Sánchez[2], Susana Santoyo[2], Margarita Pérez-Clavijo[1]

1 Centro Tecnológico de Investigación del Champiñón de La Rioja (CTICH), Autol, Spain
2 Department of Production and Characterization of Novel Foods, Institute of Food Science Research-CIAL (UAM + CSIC), Campus de Cantoblanco, Universidad Autónoma de Madrid, 28049 Madrid, Spain

Mushrooms possess appreciative chemical composition with high amounts of functional proteins, low total fat level, polysaccharides, terpenoids, phenolic compounds, and low molecular weight compounds. Some of them have been reported to be responsible for the antioxidant and anti-inflammatory effects detected in mushrooms.

The aim of this study was to evaluate the antioxidant and anti-inflammatory potential of cultivated basidiomes of *Agaricus bisporus* (AB), *Agaricus brunnecens* (ABp), *Agaricus subrufescens* (ABz), *Lentinula edodes* (LE), *Agrocybe aegerita* (AA), *Pleurotus citrinopileatus* (PC), *Hericium erinaceus* (HE), *Hericium coralloides* (HC), *Pleurotus ostreatus* (PO), *Pleurotus citrinopileatus* (PC), *Pleurotus ferulae* (PF), *Pleurotus eryngii* (PE), *Pholiota nameko* (PN), *Auricularia auricula-judae* (AAj), *Grifola frondosa* (GF), *Hypsizygus tessulatus* var.grey (HTg), Flammulina velutipes var.white (FVb), and *Flammulina velutipes* var.yellow.

Mushrooms were harvested, frozen, and freeze-dried for further analysis. Extraction with ethanol was performed.

In order to study the antioxidant capacity of the extracts, the analyses carried out were as follows. The cellular antioxidant activity assay was quantified as the ROS inhibition in a Caco-2 cell line. Additionally, antioxidant enzyme activity of SOD, CAT, and GPx were determined. Lastly, the expression of antioxidant metabolic genes (NRF-2, GSTA-1, and PRX-2) was evaluated.

The anti-inflammatory activity of the ethanolic extracts was evaluated on THP-1 cells. The release of TNF-α, IL-1β, IL-6, and IL-8 was measured in the supernatants of THP-1/M cells using ELISA kits. Finally, the expression of inflammatory genes (iNOS, COX-2 and NLRC-3) has been studied.

From all the results it can be concluded that the three species of *Agaricus* (ABz, ABp and AB), HE and LE have the highest antioxidant capacity. Whereas all the species assessed showed anti-inflammatory capacity.

Our observations suggest that including industrially cultivated mushrooms in the diet may help to prevent diseases associated with oxidative damage and inflammation.

A27. Phytochemical analysis and in vitro antioxidant potential of black chokeberry pomace (*Aronia melanocarpa* L.) as by-product

Mihaela Saracila[1], Petru Alexandru Vlaicu[1], Alexandra Gabriela Oancea[1], Arabela Elena Untea[1], Corina Nicoleta Predescu[2]

1 Feed and Food Quality Department, National Research and Development Institute for Biology and Animal Nutrition, Calea Bucuresti, No.1, 077015 Balotesti, Ilfov, Romania
2 Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine, 105 Splaiul Independentei, 050097 Bucharest, Romania

Black chokeberry fruits (*Aronia melanocarpa L*.) are recognized for their high content of bioactive constituents with a strong antioxidant capacity. However, due to their undesirable astringency, chokeberry fruits are processed into various products, such as juice, jam, etc. Chokeberry pomace (CP) is discarded as waste after extracting the juice. This study aimed to evaluate the potential of CP as a source of valuable bioactive constituents for human or animal nutrition by identifying and quantifying the phytochemical compounds and investigating the antioxidant potential. The total polyphenols, flavonoids, carotenoids (beta-carotene, lutein, zeaxanthin, canthaxanthin), and tocopherols were evaluated. The screening and quantification of polyphenols were performed using RP-HPLC. Antioxidant activity of CP was determined using four different methods (ABTS·+, DPPH, Fe^{2+}-chelating power and the phosphomolybdenum method). In vitro antioxidant potential of the CP ethanolic extract was evaluated by induced lipid peroxidation on organic tissue (treated with an oxidised mixture of 100 µM $FeCl_2$+/500 µM AA). Chokeberry pomace contained high levels of hydrophilic (22.94±1.52 mg/g GAE total polyphenols, 1.89±0.08 mg/g QE flavonoids) and lipophilic antioxidants (4516.76±45.35 mg/kg ß-carotene, 52.35±3.21 mg/kg lutein, 286.87±2.45 mg/kg zeaxanthin, 11.99±1.48 mg/kg canthaxanthin, 114.37±0.57 mg/kg α-tocopherol, 21.19±0.31 mg/kg γ-tocopherol, and 21.63±0.24 mg/kg δ-tocopherol). Twelve polyphenolic constituents were identified in CP by RP-HPLC, with flavan-3-ol, epicatechin, rutin and catechin being dominant phenolic compounds. Results showed that CP has potent antioxidant activity: 126.94±12.94 mM eq Trolox (ABTS assay), 2.37±0.14 mg/g eq EDTA (Fe^{2+}-chelating power), 201.99±21.99 mM eq ascorbic acid (phosphomolybdenum method), and 388.24±20.07 mM eq Trolox (DPPH assay). The application of a CP ethanolic extract on the peroxidised tissue sample inhibited peroxidation with an efficiency of 32.06%. Even though this is a waste, CP is a valuable source of health-promoting phytochemicals with a potent antioxidant activity that might be used for various applications, particularly functional ingredients or as a supplement in animal feed.

A28. Antioxidant Activity of hydrosol extracts from *Veronica* species

Marija Nazlić[1], Karla Akrap[1], Dario Kremer[2], Valerija Dunkić[1]

1 Department of Biology, Faculty of Science, University of Split, Ruđera Boškovića 33, 21000 Split, Croatia
2 Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia

Free volatile compounds (FVCs) were isolated in water fractions (hydrosols) of five Croatian *Veronica* species obtained by hydrodistillation (HD) and microwave-assisted extraction (MAE). These extraction methods yield two layers, the lipophilic layer (LL), or essential oil, and the aqueous layer (hydrosols). Hydrosols are aromatized waters produced by condensation of water vapor in distillation processes that contain volatiles, water-soluble components, and are, therefore, safer for human use compared to components from lipophilic phase. In general, research on the aroma profiles of hydrosols and their biological efficacy is still limited. Composition of volatile compounds and antioxidant activity of hydrosols for five species is presented in this research: *V. cymbalaria, V. hederifolia, V. officinalis, V. persica,* and *V. polita*. The compounds *β*-ionone, benzene acetaldehyde, *(E)*-caryophyllene, and caryophyllene oxide were detected in all five *Veronica* hydrosols studied. *(E)-β*-Damascenone is significantly present in all isolates except MAE isolates of *V. cymbalaria*. Hydrosols from both extraction techniques were tested by two methods: ORAC and DPPH. *V. officinalis* had the highest activity in ORAC for both extraction methods (217.54 ± 14.98 µmol TE/mL for HD extraction and 359.9 ± 44.40 µmol TE/mL for MAE). Considering that both extraction methods isolated the same major constituents, our results suggest that microwave extraction is a good alternative for the extraction of FVCs. This conclusion is partly supported with the results for antioxidant activity, according to which hydrosols for four out of five investigated species resulted in better activity for MAE extracts for ORAC method. Comparing the tested antioxidant activity of all hydrosols, it can be concluded that besides *V. officinalis* (which is generally known for showing good results for in vitro biological tests of its extracts), *V. cymbalaria* also showed higher antioxidant activity than other tested species. Further research should be based on the study of other biological activities of these extracts for possible pharmaceutical use.

A29. Evaluation of antioxidant activity from two varieties of *Phoenix dactylifera* L. for future functional food applications

Clara Muñoz-Bas, Nuria Muñoz-Tébar, Laura Candela-Salvador, Juana Fernández-López, José Ángel Pérez-Álvarez, Manuel Viuda-Martos

IPOA Research Group, Agro-Food Technology Department, Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Miguel Hernández University, 03312, Orihuela, Spain

Phoenix dactylifera L., also known as date palm, is a crop native to South Africa and Africa regions with tropical and subtropical climates whose fruit is mainly oblong or ellipsoid containing a lone seed enclosed by a thin endocarp. Its nutritional profile in addition to its composition in bioactive compounds, such as phenolic acids, flavonoids, carotenoids, procyanidins, tocopherol, tocotrienols, and sterols, has attracted the interest of researchers and food industries for its potential application as a novel ingredient in the development of functional foods. In the present work, two date varieties (Confitera and Medjoul) at the Tamar ripening stage were used to obtain the pulp by peeling and removing the pit from the date fruit. The antioxidant activity of the edible parts of date fruit was analyzed using the ferric reducing power (FRAP) and ferrous ion chelating (FIC) methods along with the DPPH and ABTS radicals scavenging activities. The variety that showed the highest antioxidant activity was Confitera with mean FRAP values of 0.78 vs. 0.64 mg Trolox/g pulp, DPPH values of 32.33 vs. 26.46 µg Trolox/g pulp, FIC values of 4.63 vs. 3.75 µg EDTA/g pulp. However, in the ABTS radical scavenging activity, it was observed that Medjoul had slightly higher antioxidant capacity than Confitera (1.14 vs. 0.91 mg Trolox/g pulp). The results indicate that the date pulp from both varieties represents a valuable source of antioxidant compounds that can be potentially used as a novel raw ingredient for the development of food enriched with bioactive compounds, although this is a preliminary study and further studies on the incorporation of date pulp in several food matrices should be carried out.

A30. Antioxidant properties of date seeds (*Phoenix dactylifera*) co-products. A potential ingredient in the development of functional foods

Laura Candela-Salvador, Nuria Muñoz-Tebar, Clara Muñoz-Bas, José Ángel Pérez-Álvarez, Juana Fernández-López, Manuel Viuda-Martos

IPOA Research Group, Agro-Food Technology Departament, Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Miguel Hernández University, 03312, Orihuela, Spain

The growing attention by food industry and consumers by "clean label" food products is serving to improve the management and handling of agri-industrial co-products due to these co-products possibly being a rich source of bioactive compounds. A very important co-product, due to the large volume it generates and the bioactive compounds that it is possible to find in their composition, is the date seeds obtained from the date fruit industrialization. Thus, the aim of this study was to assess the antioxidant properties of flours with different granulometries obtained from date seeds *var.* medjool. To obtain the flours, the date seeds were dried for 48 h at 55 ºC. Subsequently, they were ground and passed through sieves to obtain two date seed flours with different particle sizes: 0.712 > Ø > 0.412 mm (HDSF) and 0.412 > Ø > 0.210 mm (LDSF). The antioxidant activity was determined using three different assays, such as DPPH, ABTS, and FRAP. As regard to the antioxidant activity analyzed with DPPH assay the results showed values (p<0.05) of 1.28 and 1.96 mg Trolox equivalent/g of sample for HDSF and LSDF, respectively, whilst in ABTS assay, the HDSF and LSDF had values (p<0.05) of 11.75 and 13.39 mg Trolox equivalent/g of sample, respectively. Finally, in FRAP assay, again HDSF showed lower (p<0.05) antioxidant values than LHDSF, (3.22 and 4.80 mg Trolox equivalent/g of sample, respectively). This trend may be explained due to for larger particle sizes, the matrix is less altered, producing less release of bioactive compounds with antioxidant properties while reducing the size of the matrix by grinding could cause a greater release of bioactive compounds with antioxidant properties. The results obtained showed that date seed flour could be use as ingredient in the development functional food due to its high antioxidant properties.

A31. Assessment of the antioxidant properties of white mushroom (*Agaricus bisporus*) and oyster mushroom (*Pleurotus ostreatus*) co-products: A potential functional food ingredient

Patricia Bermúdez[1], Margarita Pérez[1], Manuel Viuda[2], Juana Fernández[2], Pablo Martínez[1], Vanessa Grifoll[1]

1 Centro Tecnológico de Investigación del Champiñón de la Rioja (CTICH), Spain
2 Universidad Miguel Hernández de Elche, Spain

In Spain, the white mushroom and oyster mushroom production is estimated to be 70,740 and 6.738 tons/year, respectively. In the production and industrialization processes of these products, a large volume of co-products, around 11,700 tons/year, are generated, the majority of which correspond to stem. The co-products generated can be used as potential ingredients in the development of functional foods because it is possible to find bioactive compounds with antioxidant properties in their composition. Thus, this work aimed to assess the antioxidant properties of white mushroom (WMC) and oyster mushroom (OMC) co-products. Both mushroom co-products were dried at 35 ºC for 24 h and subsequently ground until obtaining flour. Then, the antioxidant activity was carried out using four different methods (DPPH, ABTS, FRAP, and FIC assays).

In all antioxidant assays used, the highest values ($p < 0.05$) of antioxidant activity were obtained in the WMC, except in the case of the FIC assay, where no differences were found ($p > 0.05$) between WMC and OMC with values of 0.69 and 0.63 mg EDTA equivalents/g sample, respectively. In DPPH assay, the values obtained were 410.53 and 90.80 µg Trolox equivalents (TE)/g sample, for WMC and OMC, respectively. In the same way, the values obtained in the ABTS method were 1852.23 and 550.62 µg TE/g sample for WMC and OMC, respectively. Finally, in the FRAP assay, WMC had a value of 9.90 mg TE/g sample, while OMC showed a value of 9.90 mg TE/g. The results from this work suggest that white mushroom and oyster mushroom co-products constitute an appreciated alternative for the food industry due to it being possible to find a high content of valuable bioactive compounds including vitamins, minerals as well as, polyphenolic compounds with a demonstrated antioxidant activity in their composition. These co-products could be used as potential ingredients in the development of functional foods.

A32. Madagascar's endemic plants: an amazing source of natural antioxidant compounds

Rianatiana Hasina Ranaivoarisoa[1, 2], Njakarinala Ranarivelo[2, 3], Henintsoa Rakotoarivelo[1, 2], Angelo Randriatsalama[1, 2], Vahinalahaja Razafintsalama[2]

1 Doctoral School Valorization of Renewable Natural Resources, University of Antananarivo, Madagascar
2 National Center for Applied Pharmaceutical Research (CNARP), Rue RP Rahajarizafy Ambodivoanjo – Ambohijatovo BP 702, Antananarivo, Madagascar
3 CIRM – Laboratory of Pharmacognosy, University of Liège, Avenue Hyppocrate 15, B36, 4000 Liège, Belgium

The use of antioxidants in the treatment of many human diseases related to oxidative stress is helpful. The Madagascan flora is characterized by high endemism. Over 80% of the 10,900 accepted vascular species are endemic. However, this resource is little explored. In this study, the screening of antioxidant activity of methanol extracts from twenty endemic species to Madagascar, belonging to six families, was conducted to evaluate and classify their potential antioxidant property. The purpose is to estimate their potential biological utility and find new sources of natural antioxidant compounds. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH°) free radical and the Antioxidant Activity Index (AAI) is calculated to compare the antioxidant strength of different plant extracts. The IC_{50} values of the extracts ranged between 9.36 and 110.17 µg/L, with AAI ranged of 4.58 and 0.39. Five species including *Hyperacanthus poivrei*, *Cryptocaria acuminata*, *Hyperacanthus thouvenotii*, *Hyperacanthus mangoroensis* and *Dilobea thouarsii*, have very strong activity with AAI's above two according to Scherer and Godoy AAI scale. Phenolic compounds and flavonoids that may support antioxidant activity are identified by HPLC-MS in these plants extracts. Considering the growing interest in assessing the antioxidant capacity of natural products, these plants with strong antioxidant activity are interesting to explore. In the long term, further characterization of active compounds from these species with appreciable antioxidant activity will be valuable in the treatment of diseases where free radical-induced tissue damage plays a role.

A33. Different harvest periods modify the polyphenol composition of the freeze-dried peel of jaboticaba-sabará (*Myrciaria jaboticaba* (Vell.) O. Berg), a native Brazilian fruit

Roberto de Paula do Nascimento[1], Renata Galhardo Borguini[2], Manuela Cristina Pessanha de Araújo Santiago[2], Mario Roberto Marostica Junior[1]

1 Laboratory of Nutrition and Metabolism (LANUM), Department of Food Science and Nutrition (DECAN), School of Food Engineering (FEA), University of Campinas (UNICAMP), Campinas, SP, Brazil
2 Brazilian Agricultural Research Corporation (EMBRAPA), Embrapa Food Technology, Rio de Janeiro, RJ, Brazil

Jaboticaba-sabará is a berry native to Brazil's Atlantic Forest. Recently, pre-clinical studies have shown that jaboticaba-derived products can act against oxidative stress and inflammation. Jaboticaba peel's rich composition in bioactive compounds, mostly anthocyanins, is probably behind its health benefits. It is poorly known, however, if different harvest periods can change the bioactive capacity of jaboticaba. Therefore, the objective of this work was to compare the composition and antioxidant capacity of the peel of jaboticaba harvested in two distinct moments. Ripe fruit was collected during May and the peak season of August–October, from Casa Branca, SP, Brazil. The peel was freeze-dried and the extracts were produced according to the literature, using ethanol or methanol as the main solvents. Total phenolic compounds (TPC), total flavonoids, monomeric anthocyanins, ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) were analyzed using spectrophotometry. Specific anthocyanins, other flavonoids, phenolic acids, and carotenoids were quantified via high-performance liquid chromatography (HPLC). The results were quantified in the dry weight of powder. In general, the August–October sample showed higher bioactivity potential. The levels of total flavonoids (12.62 mg/g), monomeric anthocyanins (7.22 mg/g), and FRAP (1131 µmol/g) were 92%, 28%, and 23% higher for the August–October sample. Such differences were corroborated by HPLC. Cyanidin-3-*O*-glucoside (14.52 mg/g), delphinidin-3-*O*-glucoside (1.23 mg/g), and ellagic acid (2.98 mg/g) were found in greater amounts (48%, 105%, and 26% higher, respectively) in the August–October sample. Interestingly, the May sample had 20% more gallic acid (3.93 mg/g). The levels of other polyphenols and carotenoids were low (1 mg/g) in both samples. The TPC and ORAC results did not differ greatly between the samples. Jaboticaba peel's darker purple-to-black color during the peak season probably explains its higher bioactive potential. However, independent of the harvest period, ripe jaboticaba maintains a rich composition that can be promising for disease management.

A34. The advantages of using biopolymers as encapsulation systems for essential oils: development of microcapsules and thin films, with applications in industry

Roxana Gheorghita, Ancuta Veronica Lupaescu, Monica Iavorschi

Faculty of Medicine and Biological Sciences, Stefan cel Mare University of Suceava, Romania

The aim of this study was to test the effect of encapsulating different essential oils (EO) and to observe the differences between the methods of incorporating active substances, comparing the results obtained after the development of films and microcapsules. Thus, lemon, grapefruit, orange, cinnamon, cloves, ginger, garlic and pepper essential oils were microencapsulated in biopolymeric materials based on agar and sodium alginate. Similarly, biopolymer films, made of agar, sodium alginate, glycerol and water, with the addition of 10 and 15% (w/v) essential oil, respectively, were developed using the casting method. In order not to negatively influence the antioxidant activity of the oils, they were added after the film-forming solution was obtained and the temperature was reduced by approximately 55°C (from 90°C to 35°C). The control samples were obtained only from biopolymers, plasticizer and water, without the addition of EO. After development, the microcapsules and films were tested in order to identify the microstructure, physical and optical properties (mass, thickness, density, hydrophobicity, tensile strength, elasticity, water activity index, color, opacity and transmittance), antioxidant and antibacterial properties. Both the microcapsules and the microcapsules and the films presented a homogeneous microstructure, without pores or fissures, with regular edges, and were smooth and pleasant to the touch, with a smell and taste specific to the oil added to the composition. The thickness of the films was greatly influenced by the volume of EO added, increasing considerably with the increase in its concentration. For example, the film thickness with lemon oil increased from 84.66 µm (10% EO added) to 101 µm (15% EO added). Increasing the volume of grapefruit oil facilitated the increase in film thickness, from 73.66 µm to 109.66 µm. The increase in density and transmittance was directly proportional to the addition of EO in the composition, and the water activity index was reduced by approximately 30%. The result is an expected one, if we take into account the strong hydrophobic nature of essential oils. The antioxidant and antimicrobial activity were strongly influenced by the addition of essential oils. The results indicated the possibility of using antioxidant substances in microencapsulated form, which can be added to basic food products, such as dairy products or sweets, making it more accessible to consume substances with a beneficial effect on the human body. After testing the films with the addition of essential oils in the structure, the results confirmed the possibility of developing materials that can be used for

packaging food supplements and can potentiate the effect of the substances they contain (natural powders, vitamins, protein shakes, etc.). Moreover, they can be used as active substances and drug delivery systems, with the technology for obtaining and handling microcapsules and membranes being easy to apply, and can be successfully scaled at an industrial level.

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A35. Impact of Pepsin and Alkali Hydrolysis on Antioxidant Activity of Pollen Proteins

Ancuta Veronica Lupaescu[1], Monica Iavorschi[1], Roxana Gheorghita[1], Mircea Oroian[2]

1 Faculty of Medicine and Biological Sciences, Stefan cel Mare University of Suceava, Romania
2 Faculty of Food Engineering, Stefan cel Mare University of Suceava, Romania

Bee pollen is a natural product from honeybees known since ancient times for its healthy properties and nutritional value. Given its diverse composition, bee pollen was reported to have strong health benefits such as antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic effects, yielding compounds for the pharmaceutical and cosmetic industries. Although the protein content of pollen varies between plant species (from 10 to 40% of dry mass), its antioxidant response can be improved through enzymatic hydrolysis, fermentation, pH changes, heat or chemical treatments. Therefore, this study aims to evaluate the impact of hydrolysis treatment on the antioxidant activity of pollen proteins. In this regard, the pollen proteins previously extracted using an ultrasonic method were subjected to fragmentation under denaturing conditions. The antioxidant potential of hydrolysates was determined through DPPH and ABTS radicals. For qualitative information, sodium dodecyl sulfate polyacrylamide gel electrophoresis and FTIR analysis were used. The results obtained highlighted an improvement of the antioxidant activity as a result of the hydrolytic treatment, which allows a superior utilization of the pollen protein matrix.

A36. Characterization of the Biological Activity of the Ethanolic Extract from the Roots of Cannabis sativa L. Grown in Aeroponics

Fabio Ferrini[1], Sabrina Donati Zeppa[2], Vittoria Carrabs[1], Alessia Bartolacci[1], Piero Sestili[1]

1 Department of Biomolecular Sciences, University of Urbino Carlo Bo, Via Saffi, 2, 61029 Urbino, Italy.
2 Department of Biomolecular Sciences, University of Urbino Carlo Bo, Via Saffi, 2, 61029 Urbino, Italy

Cannabis sativa var. Kompolti, a variety routinely used for food production purposes, is characterized by a low concentration of psychoactive molecules, although containing many other biologically attractive metabolites in all parts of the plant, including the roots. In the present work, we evaluate the specific biological activities of the roots' extract from plants cultivated through aeroponics, an affordable and reliable method facilitating the isolation and processing of roots, with the advantage of being suitable for industrial scale-up. Furthermore, aeroponics results in an increased net accumulation of the most biologically attractive constituents (β-sitosterol, friedelin, and epi-friedelanol) found in the roots. The ethanolic extract of the aeroponic roots of C. sativa (APEX) and its separate components are studied to evaluate their anti-inflammatory (modulation of the expression level of specific markers upon LPS stimulation in U937 cells, such as IL-6, IL-8, TNF-α, IkB-α, iNOS, IRAK-1, and miR-146a) and antioxidant (in either acellular or cellular settings) activities. The APEX anti-inflammatory and antioxidant capacities are also functionally benchmarked using the wound-healing assay. On the whole, the data obtained show that APEX and its main components showed significant anti-inflammatory and antioxidant activities, which may render the exploitation of roots as a source of natural antioxidants and anti-inflammatory agents highly attractive, with the additional technical and economic advantages of aeroponics compared to soil cultivation.

A37. Antioxidant Activity and Capacity of Fruits and Vegetables Juices by a Kinetic-Based ORAC Assay

Umme Asma, Matteo Scampicchio

Faculty of Science and Technology, Free University of Bozen-Bolzano, Piazza Universitá 1, 39100 Bolzano, Italy

The kinetics of the reaction between antioxidants and radicals is crucial to understand the activity and functionality of many bioactives. However, most of the methods available that study the antioxidant-radical interactions in water-based samples, such as the classical ORAC assay, are often limited by: (a) the expression of results in terms of arbitrary units (i.e., area under the curve), and (b) the lack of information on how fast antioxidants react with free radicals. Accordingly, in this work, we proposed a kinetic-based ORAC assay that provided not only the ORAC index, but also the absolute rate constants of the reaction between antioxidants and free radicals (antioxidant activity), as well as their stoichiometry (i.e., the number of radicals scavenged by the antioxidant, which corresponds to the antioxidant capacity). Through the simulation and fitting of the experimental data using a kinetic model based on a competitive mechanism, we were able to express the radical scavenging activity of 13 individual antioxidants and 17 fruits and vegetable juices. Overall, this work will improve the information that can be derived from the classical ORAC assay and could be of interest to many food and supplement manufacturers for enhancing their screening capacity towards antioxidants.

A38. Repurposing Chestnut Shells as Antioxidant-Rich Ingredient

Ana Sofia Ferreira[1, 2], Ana Margarida Silva[1, 2], Diana Pinto[1, 2], Ricardo Ferraz[3], Manuela M. Moreira[1], Jaroslava Švarc-Gajić[4], Paulo Costa[5, 6], Cristina Delerue-Matos[1], Francisca Rodrigues[1]

1 REQUIMTE/LAQV, ISEP, Polytechnique Institute of Porto, Porto, Portugal
2 Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
3 Ciências Químicas e das Biomoléculas (CQB) e Centro de Investigação em Saúde e Ambiente (CISA), Escola Superior de Saúde do Instituto Politécnico do Porto, Porto, Portugal
4 Faculty of Technology, University of Novi Sad, Novi Sad, Serbia
5 UCIBIO – Applied Molecular Biosciences Unit, MedTech-Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
6 Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

Subcritical water extraction (SWE) is a highly promising green technology for recovering bioactive compounds from food by-products due to its low cost, minimal solvent usage, quick processing, and high yields[1]. Chestnut (*Castanea sativa*) fruits are ecologically and economically important and their industrial processing generates significant amounts of by-products, mainly shells[2]. This study aimed to develop a bioactive extract with high antioxidant, antimicrobial, and antiradical scavenging properties, as well as low toxicity effects on human buccal epithelial cell lines (HSC3 and TR146), by optimizing the extraction temperature (110 °C-180 °C) of chestnut shells using SWE.

The extract obtained at 110 °C showed the highest phenolic and flavonoid contents (239.53 mg of gallic acid equivalents (GAE)/g dry weight (dw) and IC_{50}=148.68 µg/ml, respectively) as well as the highest antioxidant activity (4240.38 µmol of ferrous sulphate (FeS)/g). Additionally, the extract obtained at 110 °C demonstrated the best antiradical activity (IC_{50}=426.88 µg/ml for DPPH assay) and a good ability to scavenge the reactive oxygen species HOCl and $ROO^{\cdot}$ (IC_{50}=4.47 µg/ml and 0.73 µmol of Trolox equivalents/mg dw, respectively). All extracts demonstrated antimicrobial activity against several pathogens, including *Staphylococcus aureus*, *Enterococcus faecalis,* and *Escherichia coli*, species that may be present in the oral cavity during the oral mucositis condition. The cell viability results indicated that the extract obtained at 110 °C achieved the lowest IC_{50} values in cell assays (IC_{50}= 1325.03 and 468.15 µg/ml, respectively, for HSC3 and TR14). Therefore, the extract obtained at 110 °C was considered the optimal one, according to its bioactive properties, for incorporation into a fast-dissolving oral film with a polymer matrix for application in oral mucositis treatment. Studies are currently being performed to validate the physicochemical properties of the oral film as well as the encapsulation of the extract into microparticles, aiming to develop a drug delivery system with sustained release.

References:

[1] Ferreira, A.S.; Macedo, C.; Silva, A.M.; Delerue-Matos, C.; Costa, P.; Rodrigues, F. Natural Products for the Prevention and Treatment of Oral Mucositis-A Review. Int. J. Mol. Sci. 2022, 23, 4385. https://doi.org/10.3390/ijms23084385.

[2] Barreira J.C.M. et al. (2020) Bioactive Compounds of Chestnut (Castanea sativa Mill.). In: Murthy H., Bapat V. (eds) Bioactive Compounds in Underutilized Fruits and Nuts. Reference Series in Phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-030-30182-8_18

A39. Pressurized Liquid Extraction from Olive Pomace: Optimization of Extraction Parameters and Application

Cecilia Dauber[1], Melissa Romero[1], Elena Ibáñez[2], Adriana Gámbaro[1], Ignacio Vieitez[1]

1 Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República, Uruguay
2 Laboratory of Foodomics, Institute of Food Science Research, CSIC, Spain

Olive pomace has been reported to be a rich source of phenolic compounds, which may be used as natural food additives with health-promoting properties. Considering the growing interest in adding value to agroindustrial waste by means of environmentally friendly technologies, the aim of this work was to study pressurized liquid extraction (PLE) technology as a way to obtain natural extracts with antioxidant properties from olive pomace with potential use as food additives. Samples of cv. Arbequina were used for the experiments. The extraction temperature (from 100 ºC to 140 ºC) and composition of solvent (from 50% to 100 % ethanol/water mixtures) were studied as independent variables of the process using a face-centered central composite design. A total of 15 runs were carried out in a laboratory-scale extractor, including three central point repetitions (120 ºC, 75:25 ethanol/water). Each run had a 10-minute static time followed by a 30-minute dynamic extraction time (flow of 2 mL/min). The process pressure remained constant for all runs at 10 MPa.The extraction yield (% w/w) was calculated after solvent evaporation, ranging from 18.85 to 28.25%. Over the dried extracts, the total phenol content (TPC) and Trolox equivalent antioxidant capacity (TEAC) were determined using Folin–Ciocalteau and $ABTS^{+\cdot}$ assays, respectively. TPC of the extracts ranged from 41.2 to 72.4 mg GAE/g, while TEAC ranged from 623.2 mmol/g to 1134.5 mmol/g. According to the quadratic model fitted to data (p0,05), higher values of TPC and TEAC are obtained at 120 ºC using between 60 and 80% ethanol. The extract obtained at 120 ºC with 75% ethanol was able to protect purified sunflower oil subjected to an accelerated oxidative stability test (Rancimat) when added at 1000 mg/kg, increasing its induction period 2.4-fold.

A40. Pomegranate peel extracts: characterization and antioxidant properties

María Noel González, Cecilia Dauber, Adriana Fernández, Alejandra Medrano, Ignacio Vieitez

Facultad de Química, Departamento de Ciencia y Tecnología de Alimentos, Universidad de la República

Production and consumption of pomegranates are increasing both worldwide and in Uruguay. However, large amounts of by-products are generated in which peels take up about 40-50% of the total weight of the fruit. It has been reported that pomegranate peels contain many bioactive compounds such as polyphenols, dietary fiber, vitamins, minerals, etc., but little information is available about these properties in Uruguayan fruits.

The objective of this study was to compare two different extraction techniques: pressurized liquid extraction (PLE) and maceration (MAC) from the pomegranate peel in order to obtain natural extracts as a source of polyphenols with high antioxidant activity.

The peels were dried for 5 hours at 105 °C and then ground. After that, the moisture, protein, fiber, ashes, and fat of the dried peels were determined.

In PLE extractions, the temperature ranged from 100 ºC to 120 ºC and the composition of the solvent from 75% to 100% ethanol/water mixtures. Each run had 10 minutes of static time followed by 30 minutes of dynamic extraction time (2 mL/min). Process pressure remained constant for all runs at 100 bar. In MAC, approximately 2.0 g of the peels were extracted with 30 mL of 75% ethanol/water mixture in 4 hours. The extraction yield (% w/w) was calculated after solvent evaporation and over the dried extracts; total phenol content (TPC) was determined using the Folin-Ciocalteu method; and antioxidant activity (TEAC) was determined using and ABTS+ and ORAC-FL assays.

The highest yield was obtained with the MAC extract (66.9±0.8%); however, the highest TPC (200.6±30.6 mg GAE/g) and TEAC were obtained with the PLE extract achieved at 120 °C and with 100% of ethanol as the solvent.

The results reinforce the beneficial use of green extraction technologies to obtain antioxidants compounds from natural sources and especially from by-products.

A41. Process design and optimization for the extraction of bioactives from pomegranate marc with super- and subcritical fluids: A fascinating biorefinery opportunity

Riccardo Tornese[1], Miriana Durante[2], Anna Montefusco[1], Marcello Salvatore Lenucci[1]

1 Department of Biological and Environmental Sciences and Technologies, University of Salento, Via Lecce-Monteroni, 73100, Italy
2 Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Lecce-Monteroni, 73100, Italy

The industrial technology for pomegranate (*Punica granatum* L.) juice extraction through the hydraulic pressing of fruits generates large amounts of pomegranate marc (PM), a by-product constituted of both rinds and kernels extraordinarily rich in lipophilic and hydrophilic health-promoting compounds. Dehydrated PM contains up to 3.5% oil, mainly from the kernels, comprising polyunsaturated fatty acids of the family of conjugated α-linolenic acids, as well as large amounts of phenolics, soluble and insoluble dietary fibers from both kernels and rinds. These compounds can be efficiently recovered through biorefining strategies to obtain ingredients of excellence for pharmaceutical, cosmeceutical and/or nutraceutical use, contributing to the exploitation of this agri-food by-product and to the sustainability of the pomegranate production/processing chain in a context of circular economy. Supercritical carbon dioxide extraction (SFE) and subcritical water extraction (SWE) are green and environmentally friendly technologies complementing each other for the effective extraction of high-value lipo- and hydro-soluble natural molecules, and these are gaining a foothold in industrial production of solvent-free vegetable phytocomplexes. Nevertheless, the industrial application of SFE and SWE requires a careful optimization of the operative parameters to make the process efficient from both an economic and a productive point of view. In this context, a response surface methodology approach, based on the Box–Behnken design, was used to model and maximize SFE and SWE sequential extraction of PM oil and phenols, respectively, while optimizing the main operative parameters (temperature, pressure, solvent flow and extraction time).
Overall, our results prove that the novel developed biorefinery process is appropriate for the valorisation of PM by-products and that, under the optimized operating conditions, it is efficient in the extraction of added-value fractions with potential antioxidant and bioactive activities, as well as an exhausted residue rich in insoluble fibers (mostly cellulose) useful to the food/feed industry or as soil conditioner.

A42. Lignin from chestnut shells as a potent reducing agent for preparation of silver nanoparticles and implementation of highly antioxidant and antibacterial poly(lactic acid) electrospun fibers

Sara Viggiano[1], Rita Argenziano[1], Andrea Cochis[2], Antonietta Pepe[3], Lucia Panzella[1], Alessandra Napolitano[1]

1 Department of Chemical Sciences, University of Naples "Federico II", Naples, Italy
2 Department of Health Sciences, University of Piemonte Orientale, Novara, Italy
3 Department of Science, University of Basilicata, Potenza, Italy

Natural phenolic polymers from plant sources are attracting considerable scientific interest as robust and multifunctional compounds. In this scenario, a prominent role is played by lignin, which is endowed with potent antioxidant properties that have prompted its applications in a variety of fields, such as food packaging, wound healing, drug delivery, and tissue regeneration.[1,2] On this basis and in a green chemistry perspective, several studies have been directed to the exploitation of sustainable and easily available sources of this polymer, such as by-products and wastes from the agri-food industry. In this context, we recently reported a combined mechanochemical/deep eutectic solvent (DES)-based protocol for the recovery of lignin from the shells of edible nuts.[3] Among the recovered samples, very efficient antioxidant properties in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays were determined, in particular, for chestnut shell lignin.
Herein, we report the use of chestnut shell lignin as a reducing agent for a solvent-free mechanochemical synthesis of silver nanoparticles (AgNPs), involving ball milling of lignin/$AgNO_3$ in a 85:15 w/w ratio for 180 min at 50 osc/s. Subsequently, poly(D,L-lactic acid) (PDLLA)-based electrospun fibers containing different amounts of AgNPs/lignin were prepared. The antioxidant properties of the materials were evaluated by the DPPH and FRAP assays, showing good results particularly for the fiber containing PDLLA (96.9% w/w), AgNPs/lignin (2.1% w/w), and chitosan (1% w/w). Finally, the presence of AgNPs conferred antibacterial properties to the fibers where the surface colonization from the pathogens *Escherichia coli* and *Staphylococcus epidermidis* was reduced of about 1.5 logarithms in comparison to the bulk PDLLA.

References:
[1] Panzella, L.; Napolitano, A. Natural phenol polymers: Recent advances in food and health applications. *Antioxidants*, **2017**, 6, 30.
[2] Lu, X.; Gu, X.; Shi, Y. A review on lignin antioxidants: Their sources, isolations, antioxidant activities and various applications. *Int. J. Biol. Macromol*. **2022**, 210, 716–741.
[3] Argenziano R., Moccia F., Esposito R., D'Errico G., Panzella L., Napolitano A. Recovery of Lignins with Potent Antioxidant Properties from Shells of Edible Nuts by a Green Ball Milling/Deep Eutectic Solvent (DES)-Based Protocol. *Antioxidants,* **2022**, 11, 1860.

A43. The role of hydrogen sulfide in the effects and expression of delta opioid receptors in mice with osteoarthritis pain

Gerard Batallé, Xue Bai, Ignacio Martínez-Martel, Olga Pol

1 Grup de Neurofarmacologia Molecular, Institut d'Investigació Biomèdica Sant Pau (IIB SANT PAU), Sant Quintí 77-79, 08041 Barcelona, Spain
2 Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

Osteoarthritis is a chronic multifactorial and degenerative disorder of unknown etiology and one of the most common forms of arthritis characterized by a progressive loss of articular cartilage, intense pain, and functional deficits. Oxidative stress is a key factor in the initiation and progression of osteoarthritis. Current therapies are limited, with significant undesirable effects. In this study, we evaluated the combination of hydrogen sulfide (H2S) with delta opioid receptor (DOR) agonists as a possible treatment to inhibit osteoarthritis pain. Therefore, using female C57BL/6J mice with osteoarthritis provoked by the knee injection of monosodium acetate (MIA), we assessed the effects of the co-administration of diallyl disulfide (DADS) or morpholin-4-ium 4-methoxyphenyl(morpholino) phosphinodithioate dichloro-methane complex (GYY4137), two slow-releasing H2S donors, with UFP-512 (a DOR agonist) on the mechanical allodynia and grip strength deficits caused by osteoarthritis. The effects of both H2S donors on oxidative stress and the expression of DOR and BDNF in the dorsal root ganglia (DRG) of MIA-injected mice were further assessed. Our results showed, for the first time, that the systemic and local administration of UFP-512 inhibited the allodynia and grip strength deficits in MIA-injected animals. The administration of low doses of DADS or GYY4137 potentiated the antiallodynic actions and the recovery of grip strength produced by the intraperitoneal and intra-articular injection of UFP-512. Both H2S donors enhanced the expression of DOR and the antioxidant enzymes, superoxide dismutase 1 and glutathione S-transferase Mu1, in the DRG of animals with osteoarthritis pain. In addition, the upregulation of BDNF provoked by osteoarthritis was normalized in DADS- and GYY4137-treated animals. This study proposes that the co-treatment of H2S donors with DOR agonists, systemically or locally applied, may be a therapeutic approach for osteoarthritis pain.

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A44. Hydrogen sulfide potencies the antinociceptive effects of cannabinoid 2 receptors during neuropathic pain in mice

Xue Bai, Gerard Batallé, Ignacio Martínez-Martel, Olga Pol

1 Grup de Neurofarmacologia Molecular, Institut d'Investigació Biomèdica Sant Pau (IIB SANT PAU), Sant Quintí 77-79, 08041 Barcelona, Spain
2 Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

Treatment with slow-releasing hydrogen sulfide (H2S) donors increases the antinociceptive effects of opioids in animals with inflammatory and neuropathic pain, but the impact of this gaseous neurotransmitter on the analgesic actions of cannabinoid 2 receptors (CB2R) during neuropathic pain has not been evaluated. Using male C57BL/6 mice with nerve-injury-provoked neuropathic pain, we assessed if the treatment with two H2S donors, diallyl disulfide (DADS) and morpholin‐4‐ium 4‐methoxyphenyl (morpholino) phosphinodithioate dichloromethane complex (GYY4137), could enhance the antinociceptive effects of JWH-133 (a selective CB2R agonist). The effects of DADS and GYY4137 on the expression of the nuclear factor-κB (NF-κB), Nrf2 transcription factor, heme oxygenase 1 and CB2R in the prefrontal cortex (PFC) were also analyzed. Our results showed that the systemic injection of JWH-133 inhibited the nociceptive responses generated by nerve injury in a –dose–response manner and that both H2S donors improved its antiallodynic and anti-hyperalgesic effects. In addition, both DADS and GYY4137 normalized the over-expression of phosphorylated-NF-kappa-B inhibitor alpha (p-IKBα) caused by nerve damage, activated the antioxidant system (Nrf2 and HO-1) and induced the up-regulation of CB2R in the PFC. This suggests that the anti-inflammatory and antioxidant properties of H2S in this brain area might participate in the activation of CB2R, a key factor in increasing the effects of JWH-133 during neuropathic pain. These data reveal that slow H2S releasers potentiated the antinociceptive effectiveness of CB2R agonists, and using this co-treatment could be a suitable approach to relieving neuropathic pain in mice.

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A45. A positive interaction between molecular hydrogen and heme oxygenase 1 in the inhibition of the allodynia and affective deficits associated with chemotherapy in mice

Ignacio Martínez-Martel, Xue Bai, Gerard Batallé, Olga Pol

1 Grup de Neurofarmacologia Molecular, Institut d'Investigació Biomèdica Sant Pau (IIB SANT PAU), Sant Quintí 77-79, 08041 Barcelona, Spain
2 Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain

Chemotherapy-provoked peripheral neuropathy and its associated affective disorders are significant adverse effects in cancer patients, and their treatment is not completely resolved. A recent study revealed a positive interaction between molecular hydrogen (H_2) and a heme oxygenase (HO-1) enzyme inducer, cobalt protoporphyrin IX (CoPP), in the inhibition of neuropathic pain provoked by nerve injury. Nonetheless, the impact of CoPP co-administered with hydrogen-rich water (HRW) on the allodynia and the emotional disorders associated with paclitaxel (PTX)-induced neuropathic pain has not yet been analyzed. In this study, using male C57BL/6 mice injected with PTX, we examined the effects produced by the co-administration of CoPP and HRW in the mechanical allodynia, thermal allodynia, and anxiety- and depressive-like behaviors provoked by PTX. Moreover, the effects of this combined treatment on the oxidative stress caused by PTX in the dorsal root ganglia (DRG) were also studied. Our results revealed that the antiallodynic actions of the co-administration of CoPP plus HRW are higher than those produced by each of them individually administered and also reduced the treatment time. This combination further inhibited the anxiodepressive-like behaviors related to PTX administration, normalized the up-regulation of 4-hydroxynonenal, and activated the expression of the antioxidant enzymes HO-1, superoxide dismutase 1, and glutathione S-transferase mu 1 in the DRG. This suggests the involvement of the antioxidant system in the enhanced effects induced by CoPP plus HRW in PTX-injected mice. This study proposes the co-administration of CoPP plus HRW as a potential treatment for chemotherapy-provoked peripheral neuropathy and its accompanying emotive deficits.

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B1. Bee pollen antioxidants present potential to prevent/treat metabolic disorders

Adriana Maite Fernández-Fernández, Victoria Olt, Jessica Báez, Marcelo Miraballes, Adriana Gámbaro, Alejandra Medrano-Fernandez

Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República, General Flores 2124, Montevideo 11800, Uruguay

Metabolic disorders such as diabetes are associated with oxidative stress and excessive glucose and lipid intake. Thus, the use of natural sources of antioxidants to counteract reactive oxygen species (ROS) and inhibit enzymes related to the hydrolysis of carbohydrates and fat for enabling their absorption may prevent/treat metabolic disorders. In this sense, bee pollen commercialization has increased over the years because of its health-promoting properties, which could be used for this purpose. The aim of this study was to evaluate the in vitro biological activity (antioxidant and anti-inflammatory capacities and inhibition of carbohydrates and fat enzymes) of four commercial bee pollens from a Uruguayan local market for use as a functional ingredient: MT, EN, and CA from China and MA from Spain. MA showed the highest antioxidant capacity by ABTS and ORAC-FL (61.03–91.81 and 80.28–137.67 µmol TE/g pollen, respectively). MA and CA showed the inhibition of intracellular ROS formation in Caco-2 intestinal cells under AAPH (1mM)-induced oxidation conditions at a range of concentrations (700–1000 µg/mL) when compared to the control (DMEM with AAPH, $p<0.05$). Regarding the inhibition of enzymes involved in carbohydrates and fat absorption, the samples showed the inhibition of α-glucosidase with no significant differences between them (IC50=6.95–7.51 mg/mL, $p>0.05$). In contrast, MA and EN showed the highest pancreatic lipase inhibition (IC50 values of 25.05 and 22.29 mg/mL, respectively) with no significant differences between these two samples ($p>0.05$). CA showed anti-inflammatory potential at concentrations of 300–800 µg/mL when evaluating nitric oxide production by RAW264.7 macrophages under LPS-induced conditions compared to the control (DMEM with LPS, $p<0.05$). In conclusion, commercial bee pollen samples present the potential to promote human health by reducing oxidative stress and biochemical events related to metabolic disorders such as diabetes when used as a nutraceutical and/or as a functional ingredient.

B2. The impact of prepartal liver glutathione on milk performance parameters and welfare of peripartal dairy cows

Ana Flavia Souza Lima[1], Johan Estevez Osorio[1], Gustavo Goncalves Begalli[1], Maria Helena Oliveria[1], Rodrigo Chaves Barcellos Grazziotin[1], Jessica Halfen[1], Erminio Trevisi[2]

1 School of Animal Science, Virginia Tech, Blacksburg, VA, USA
2 Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Sciences, Universita Cattolica del Sacro Cuore, Piacenza, Italy

The transition period in dairy cows is commonly defined as the last 3 weeks before and 3 weeks after parturition. This challenging period is particularly characterized by a negative energy balance (NEB) condition, which occurs when energy requirements to sustain lactation are greater than energy intake from feed consumption. This NEB condition leads to excessive lipid mobilization, hepatic fatty acid oxidation, and, consequently, oxidative stress. Oxidative stress is caused by the imbalance between increased oxidative factors (e.g., reactive oxygen metabolites (ROM)) and depleted antioxidants in the body. Oxidative stress is an underlying factor for the dysfunctional immune system in dairy cows during the transition period, undermining animal welfare, lactation performance, and sustainability. Glutathione (GSH) is a potent antioxidant that dairy cows can store in the liver and use later during high oxidative stress periods, such as the transition period. Nutritional strategies to enhance liver GSH concentration before parturition could be a new tool for dairy farmers to decrease oxidative stress after calving while increasing milk production in dairy cows. Our objective was to define the mechanisms that control liver GSH synthesis and storage in transition dairy cows and assess prepartal liver GSH concentration effects on milk performance parameters and welfare postpartum. We conducted a retrospective analysis based on prepartal liver GSH, at -10 days before parturition, on lactation performance parameters and welfare in transition dairy cows. Blood and liver samples were collected at various time points between -21 and +21 days relative to parturition to evaluate biomarkers related to oxidative stress, metabolism, inflammation, and liver function. Further analyses of this study are on their way to investigating the impact of the cellular mechanisms that control GSH synthesis and storage in the bovine liver before parturition.

B3. Elderflower (*Sambucus nigra* L) hydrophilic extracts possess antioxidant and cell viability protective effects against oxidative stress

Daiva Majiene[1, 2], Aurelijus Laurutis[1], Julius Liobikas[1, 3]

1 Laboratory of Biochemistry, Neuroscience Institute, Lithuanian University of Health Sciences, LT-50161 Kaunas, Lithuania
2 Department of Drug Technology and Social Pharmacy, Lithuanian University of Health Sciences, Sukileliu Str. 13, LT-50162 Kaunas, Lithuania
3 Department of Biochemistry, Medical Academy, Lithuanian University of Health Sciences, Eiveniu Str. 4, LT-50103 Kaunas, Lithuania

Elderflowers have been used as a first-line treatment for colds and flu since ancient times because they have antiviral, antibacterial, anti-inflammatory effects. There are data demonstrating that aqueous elderflower extracts have antioxidant activity. However, the analyses were mostly performed using only chemical methods, for example, DPPH, ABTS, and other assays.
Therefore, it seems relevant to produce hydrophilic extracts from elderflowers using water (WES) and a mixture of water + 20% polyethylene glycol (Pg-WES), to compare their chemical composition, and to evaluate the antioxidant activity in cells under normal conditions, and to analyze their effect on cell viability under oxidative stress conditions.
The elder flowers were obtained from a pharmacy (a manufacturer "Emili"). The extracts were prepared in an ultrasonic bath at room temperature for 20 minutes. The phenolic compounds were determined by the Folin–Ciocalteu method. The extracellular and intracellular ROS concentrations in C6 glial cells were assessed fluorometrically. The viability of cells was performed after staining of nuclei. Data analysis was performed by using SigmaPlot.
The results showed that the total amount of phenolic compounds (PC) detected in WES was about 4-fold less than in ethanolic extracts, but about 10 times higher if compared to the results of other scientists who produced the tea infusions. The addition of PEG solvent to water increased the total phenolic content by more than twice.
Both extracts reduced the level of extracellular and intracellular ROS in a concentration-dependent manner. Notably, the Pg-WES was found to be effective at much lower concentrations.
Under high oxidative stress, when exposure to 100 μM H_2O_2 for 24 h resulted in the death of all cells, the Pg-WES had the high protective effect, since it reduced the population of dying cells by half when applied at 40 μg/mL PC, whereas the WES at the same concentration was half as effective. At milder oxidative stress conditions (75 μM H_2O_2 for 6 h and only 9% of cell death), both hydrophilic extracts (starting at 20 μg/mL) restored the cell viability to the control levels.

B4. Can beta-blockers be used as antiglycoxidant drugs? New properties of nebivolol

Daria Trocka[1], Miłosz Nesterowicz[1], Karolina Dańkowska[1], Kamil Lauko[1], Mateusz Maciejczyk[2]

1 Students' Scientific Club "Biochemistry of Civilization Diseases" at the Department of Hygiene, Epidemiology and Ergonomics, Medical University of Bialystok, Białystok, Poland
2 Department of Hygiene, Epidemiology and Ergonomics, Medical University of Bialystok, Białystok, Poland

Introduction: Cardiovascular diseases (CVD) remain one of the leading causes of death worldwide. The risk factors for CVD include age, improper diet, low physical activity, increased blood pressure, diabetes, overweight and obesity. This explains why more people are affected by CVD than any other disease in civilization. According to recent reports, protein glycation and oxidative stress are involved in the development of CVD. To prevent such processes, it is preferable to use drugs that are already well-known in cardiology. Beta-blockers—the most common class of cardiological drugs—may have antiglycation and antioxidant properties. However, to date, little is known about the antiglycoxidant properties of nebivolol.

Materials and methods: This study was conducted to assess the antiglycooxidant activity of the beta-blocker nebivolol in an in vitro model of oxidized bovine serum albumin (BSA). The experiment was performed in three series, each time in duplicate. Measures of 1 mM nebivolol and 0.09 mM BSA were incubated for six days with 0.5 M glucose as a glycation agent. Aminoguanidine was used as a glycation inhibitor. The DPPH radical scavenging capacity; total thiols – a product of protein oxidation; as well as tryptophan and β-amyloid – products of protein glycation, were assessed colorimetrically/fluorometrically.

Results: The beta-amyloid level was significantly lower, while the tryptophan and total thiol level were significantly higher in samples with nebivolol than in samples without this drug. The in vitro DPPH radical scavenging capacity of nebivolol shows intense antioxidant activity. This is proof that nebivolol has antiglycation and antioxidant properties in an in vitro model. Nebivolol may be more successfully used in the treatment of CVD, especially in patients with coexisting diabetes mellitus. However, such studies in animal and human models still need to be conducted.

B5. Polyphenolic fraction from *Cannabis sativa* as an endogenous antioxidant system modulator on SH-SY5Y cell line

Guillermo Cásedas[1, 2], Henar Rojas-Márquez[3], Cristina Moliner[1], Carlota Gómez-Rincón[1, 2], Francisco Les[1, 2], Filippo Maggi[4], Víctor López[1, 2]

1 Universidad San Jorge, Spain
2 Instituto Agroalimentario de Aragón-IA2, Spain
3 Universidad del País Vasco/Euskal Herriko Unibertsitatea, Spain
4 University of Camerino, Italy

The rise of supplements based in CBD (cannabidiol) and cannabis derivatives requires an exhaustive research to determine why the Cannabis sativa plant has these potential benefits in human health [1]. But not only CBD is the bioactive compound that provides these beneficial properties, recent studies have shown that the C. sativa plant harbors a high content of polyphenols [2]. These compounds have been used and commercialized for decades due to their main antioxidant activity on cells and the ability to reduce the reactive oxygen species (ROS) that are generated [3–5]. After having carried out a first screening on the neuroprotective activity of this extract in the N2a mouse cell line (neurons) and demonstrating the antioxidant and cytoprotective profile [6], it is convenient to continue researching the molecular mechanisms affected by this extract on other human cell lines. This extract was tested on SH-SY5Y cells which are a human neuroblastoma cell line. MTT assay was carried out in order to double-check cytoprotective effect on this neurons. After that, the activity of endogenous antioxidant enzymes, such as catalase and superoxide dismutase, were measured. Finally, to establish a molecular approach to this project, the expression of antioxidant proteins PRDX1 and PRDX3 were quantified, as well as Nuclear factor-erythroid 2 related factor 2 (Nrf2), which is the master regulator of oxidative stress, ensuring the balance between reduction and oxidation reactions in cells.
The polyphenolic extract of Cannabis sativa was not cytotoxic and enhanced the activity of endogenous antioxidant enzymes as well as decreased lipid peroxidation and modulating gene expression and protein translation related to oxidative stress on SH-SY5Y cell line.

References:
1. Andre, C.M.; Hausman, J.F.; Guerriero, G. Cannabis sativa: The plant of the thousand and one molecules. Front. Plant Sci. 2016, 7, 19.
2. Mazzara, E.; Carletti, R.; Petrelli, R.; Mustafa, A.M.; Caprioli, G.; Fiorini, D.; Scortichini, S.; Dall'Acqua, S.; Sut, S.; Nuñez, S.; et al. Green extraction of hemp (Cannabis sativa L.) using microwave method for recovery of three valuable fractions (essential oil, phenolic compounds and cannabinoids): a central composite design optimization study. J. Sci. Food Agric. 2022.
3. Cásedas, G.; Bennett, A.C.; Burgos, E.G.; Serranillos, M.P.G.; López, V.; Smith, C.; Smith, C. Polyphenol - associated oxidative stress and inflammation in a model of LPS - induced inflammation in glial cells: do we know enough for responsible compounding ? Inflammopharmacology 2018.

4. Cásedas, G.; Les, F.; González-Burgos, E.; Gómez-Serranillos, M.P.; Smith, C.; López, V. Cyanidin-3-O-glucoside inhibits different enzymes involved in central nervous system pathologies and type-2 diabetes. South African J. Bot. 2018, 120, 241–246.
5. Les, F.; Arbonés-Mainar, J.M.; Valero, M.S.; López, V. Pomegranate polyphenols and urolithin A inhibit α-glucosidase, dipeptidyl peptidase-4, lipase, triglyceride accumulation and adipogenesis related genes in 3T3-L1 adipocyte-like cells. J. Ethnopharmacol. 2018, 220, 67–74.
6. Cásedas, G.; Moliner, C.; Maggi, F.; Mazzara, E.; López, V. Evaluation of two different Cannabis sativa L. extracts as antioxidant and neuroprotective agents. Front. Pharmacol. 2022, 13, 1–11.
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B6. Metallofullerenol $Sc_3N@C_{80}(OH)_{18}$ as a new generation of radioprotectors—studies of radical processes occurring in human erythrocytes under irradiation

Jacek Grebowski[1], Szymon Porebski[1], Maciej Studzian[1], Anna Konarska[2], Marian Wolszczak[3], Łukasz Pulaski[4], Grzegorz Litwinienko[5]

1 Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland
2 Institute of Applied Radiation Chemistry, Technical University of Lodz, Wroblewskiego 15, 93-590 Lodz, Poland
3 Institute of Applied Radiation Chemistry, Technical University of Lodz, Wroblewskiego 15, 93-590 Lodz, Poland.
4 Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-236 Lodz, Poland.
5 Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland.

Metallofullerenols (MFs) are functionalized endohedral fullerenes that combine at least three levels of matter organization: atomic, molecular and supramolecular, which results in their unique activity at the nanoscale. The biomedical applications of MFs began with gadolinium contrast agents, but now their potential medical applications extend far beyond diagnostics, such as resonance imaging. In many cases, preclinical studies have shown a high therapeutic value of MFs, therefore we have examined the interaction of obtained metallofullerenol with high-energy radiation and reactive oxygen species generated during irradiation as a basis for potential applications in modern cancer therapy. We tested the radioprotective potential of metallofullerenol $Sc_3N@C_{80}(OH)_{18}$ on human erythrocytes irradiated with gamma (γ) radiation. We determined the reaction rate constant of $Sc_3N@C_{80}(OH)_{18}$ by pulse radiolysis with radicals $CCl3OO^{\bullet}$ (1.29×10^7 $dm^3mol^{-1}s^{-1}$). The results show that $Sc_3N@C_{80}(OH)_{18}$ at a concentration of 25 μM protects human erythrocytes against radiation-induced hemolysis compared to unprotected cells. The protective effect of $Sc_3N@C_{80}(OH)_{18}$ was confirmed by the reduced release of potassium cations compared to cells irradiated without $Sc_3N@C_{80}(OH)_{18}$. Our results indicate that $Sc_3N@C_{80}(OH)_{18}$ protected erythrocyte membrane proteins exposed to 2000 Gy γ radiation, as demonstrated by SDS-PAGE, and protected the band 3 protein from radiation-induced degradation. We suppose that scavenging of ROS by $Sc_3N@C_{80}(OH)_{18}$ and adsorption of metallofullerenol to membrane proteins as well as protection of -SH groups against oxidation are responsible for the observed effects.

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B7. Antioxidant And Cytotoxic Activities of *Cornus Mas* Fruit Extract in Gentamicin-Induced Nephrotoxicity on Mice Renal Cells

Mara Aurori[1], Daniela Hanganu[2], Emoke Pall[1], Mihai Cenariu[1], Mihaela Niculae[1], Sanda Andrei[1]

1 Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, 400374 Cluj-Napoca, Romania
2 Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Haţieganu", 400372 Cluj-Napoca, Romania

The fruits of *Cornus mas* tree, also known as cornelian cherry, are a good source of antioxidants as they contain polyphenolic compounds, mono- and triterpenoids, carotenoids, and vitamins. The antibiotic Gentamicin, an aminoglycoside, is used to treat bacterial infections, but it also causes undesirable side effects, including ototoxicity and nephrotoxicity.
The study aim was to investigate the antioxidant and cytotoxic effects of cornelian cherry extracts against Gentamicin-induced stress on mice renal epithelial cells. Using the Folin-Ciocâlteu method, the total polyphenolic content of the ethanolic extract obtained from *Cornus mas* fruits was determined. The antioxidant activity of the ethanolic extract was assessed using DPPH and FRAP assays. The results showed a good antioxidant activity, with DHPP = 0.466 mg/mL and FRAP = 23.09 µmol Trolox/mL extract. Primary renal epithelial cells were obtained from mouse fetuses' renal tissue, which was further treated with Gentamicin (100 µg/µL) and *Cornus* extract in three different concentrations (130, 195, and 260 µg/µL). Cytotoxicity study was performed using the MTT assay and Annexin V-FITC cell apoptosis detection kit using flow-cytometry. These tests revealed that the extract-treated groups had higher cell viability than antibiotic-treated groups. Additionally, increased viability was observed in the groups treated with extract and antibiotic, which was comparable to the extract-treated cells. At the highest concentration, however, a decrease in viability was observed, most likely due to the combined cytotoxic effect of the extract and gentamicin.

B8. The Effects of Antioxidant Supplementation on Inflammatory and Oxidative Stress Parameters in Rat Models of Diet-Induced Obesity

Mihaela Cotul, Daria Antonia Dumitras, Andrei Paul Ungur, Alexandra Dreanca, Sanda Andrei

Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Obesity is a major public health concern that is associated with a variety of chronic diseases, such as diabetes, cardiovascular pathologies, or cancer. The use of dietary supplements, including antioxidants, has become increasingly popular as a means of preventing or treating obesity.

The purpose of the present study was to investigate the efficacy of *Calendula officinalis* (rich in carotenoids) and *Ribes nigrum* (rich in polyphenols) powders on oxidative stress markers in obesity induced in rats. The Sprague–Dawley rats were fed either a normal diet (ND) or a high fat diet (HFD) diet with and without antioxidant powder in flaxseed oil (200 mg/kg CO and 200 mg/kg RN), delivered by daily gavage for 8 weeks.

Multiple blood markers of inflammation (interleukin IL-6 and TNF-a) and oxidative stress (total antioxidant capacity-TAC, catalase activity-CAT, lipids peroxides-LPO, and 8-hydroxy-2′-deoxyguanosine-8-OH-dG) were measured and compared between the supplemented and non-supplemented groups. The HFD induce a significant increase in the body weight (BMI of 0.84–0.92) and in the adipose tissue (adiposity index of 2.8–3.5%), accompanied by significantly higher levels of oxidative stress and inflammation. The antioxidants significantly reduced the body weight increase (14%) and BMI (0.3 g/cm2). Moreover, the antioxidant powder ameliorated the inflammatory status, and showed a significant antioxidant effect by modulating de CAT activity, and reduction of LPO and 8-OH-dG.

B9. *Pelargonium sidoides* DC. active substances therapy protected periodontal tissue decreased inflammation

Rasa Baniene[1], Evelina Alkimaviciene[2], Nomeda Baseviciene[2]

1 Biochemistry Department, Lithuanian University of Health Sciences
2 Clinic of dental and oral pathology, LSMU Hospital, Kaunas Clinics, Medical academy, Lithuanian University of Health Sciences, Eivenių g. 2, LT-50161 Kaunas, Lithuania

Periodontitis is a chronic inflammatory disease affecting the supporting structures of the teeth. This disease affects more than 10% of the population and is the most common cause of tooth loss worldwide. Periodontitis is caused by bacteria that adhere to and grow on the tooth's surfaces, along with an over-increased immune response against these microorganisms and induced activation of the molecular cascade of inflammation. Biologically active compounds of *Pelargonium sidoides* root extract (PSRE) demonstrated the possibility to modulate bacterial virulence, stimulate host immune responses, and reduce inflammation. Thus, they are coming under considerable scrutiny as potentially new non-aggressive therapeutic agents for managing periodontal infections. **The purpose of this study** is to evaluate changes in molecular markers MMP-3 and TIMP-1 in association with clinical signs of periodontitis and assess the protective effect of *Pelargonium sidoides* root extract (PSRE). **Methods**: Saliva from 20 healthy persons (control group), 20 periodontitis patients, and 20 periodontitis patients treated with PSRE were collected using the SalivetteÒ saliva sample collection kit. Samples were centrifuged at 3650 rpm, aliquoted, and stored at -80ºC until analysis. The amount of MMP-3 and TIMP-1 in the saliva of periodontitis patients and healthy persons was evaluated using commercial ELISA kits. **Results:** The MMP-3 concentration was significantly higher in the saliva of the periodontitis patient group as compared to healthy persons. After treatment with PSRE, the MMP-3 level in the patient group saliva decreased and returned to the control group level. The level of TIMP-1 in the saliva of periodontitis patients was significantly lower as compared to healthy persons. After treatment with PSRE, the TIMP-1 concentration in the saliva of the patient group increased significantly as compared with the TIMP-1 concentration in the same group before treatment. **Conclusion:** Biologically active compounds of *Pelargonium sidoides* root extract decreased inflammation and protected periodontal tissue.

B10. Air pollution and the skin—a new method to verify the efficacy of anti-pollution products

Phuong Thao Tran[1, 2], Parichat Tawornchat[1], Burkhard Kleuser[3], Batoul Beidoun[1], Silke Lohan[1], Grit Sandig[4], Martina Meinke[1]

1 Center of Experimental and Applied Cutaneous Physiology, Department of Dermatology, Venereology and Allergology, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany, corporate member of Freie Universität Berlin, Humboldt-Universitä
2 Institute of Pharmacy, Department of Pharmacology, Freie Universität Berlin, Königin-Luise-Str. 2+4, 14195 Berlin
3 Institute of Pharmacy, Department of Pharmacology, Freie Universität Berlin, Königin-Luise-Str. 2+4, 14195 Berlin, Germany
4 Gematria Test Lab GmbH

Rising levels of air pollution cause serious health problems worldwide. Air pollution affects skin health and has been linked to a rise in skin disorders. The skin, the largest organ of the human body, is continually exposed to stress from the environment. Because of this, oxidative stress is increased, which can lead to early skin aging, harm to the skin's protective layer, and cellular damage caused by free radicals. There are several ways to evaluate the amount of pollution on the skin or the effectiveness of the materials employed as preventative agents. Electron paramagnetic resonance spectroscopy (EPR) has been used to develop a new method for assessing the potential harmfulness of air pollution to the skin and confirming the effectiveness of skin protection measures. Using cigarette smoke as a model pollutant, spin-labeled PCA (3-(carboxy)-2,2,5,5-tetramethyl-1-pyrrolidinyloxy) was utilized to assess the generation of free radicals in excised skin. Additionally, the combined effects of UVA radiation and smoke exposure were studied. The aim of this study was to validate the efficacy of anti-pollution products against cigarette smoke. For both ex vivo and in vivo research, a smoke chamber was constructed to repeatedly expose skin to cigarette smoke. Initial studies have shown that smoking promotes free radical production in the skin and that there is a direct relationship between nicotine levels and free radical production. This method was used to investigate different ingredients and determine the protective effect against cigarette smoke. Several antioxidants were incorporated in a placebo formulation and were tested against a branded product. It has been shown that these products show protection against radicals compared to unprotected skin.

B11. Pycnogenol polyphenols support the resistance of erythrocyte membranes

Tulendy Nurkenov, Gulnara Tashenova, Amantai Kunakbaev, Dina Kulzhanova

Abai KazNPU, Kazahstan

In this work, the influence of biologically active substances on the resistance of erythrocyte membranes and activity of endogenous antioxidant enzymes in vitro was studied.

Since pharmacological drugs often have side effects, extensive research is being conducted to study and use non-drug means to correct the decrease in the resistance capabilities of the body's membrane systems, using natural biologically active substances. Among them, grape seed extract (Pycnogenol) is of particular interest. This is a complex preparation containing more than 40 biologically active components, with powerful antioxidant properties.

Correction of structural and functional membrane disorders with biologically active drugs can be a successful prophylactic strategy to prevent the negative consequences of stress.

Volunteer students from the faculties of physical education, natural science and geography at Abai KazNPU acted as the object of this study. Under in vitro conditions, the effect of the concentration series of Pycnogenol on the osmotic and peroxide resistance of the erythrocyte membranes of young men was tested.

Osmotic resistance slightly increases with the introduction of Pycnogenol into a hypoosmotic NaCl solution (0.35 g/100 ml); a significantly pronounced antioxidant effect is observed at a concentration of grape seed extract of 15 µl. The addition of Pycnogenol at concentrations of 10, 15, 25, and 50 µl to erythrocytes in an incubation medium of 0.4 g/100 ml of NaCl solution led to a significant protective effect—the level of hemolysis decreased by 35.4%, 31.3%, 32%, and 26.4%, respectively. Incubation of erythrocytes in 0.45 g/100 ml of NaCl solution with the introduction of the same concentrations of Pycnogenol into the incubation medium led to an increase in osmotic resistance two or more times compared to the control value.

Thus, the protective effect of the examined drug under in vitro conditions on the state of erythrocyte membranes of young men is clearly traced.

B12. Revalorization of Avocado by-Products for the Development of Cosmetic Ingredients

Abigail García-Villegas, María de la Luz Cádiz-Gurrea, Alvaro Fernández-Ochoa, Maialen Uriz-Martínez, David Arráez-Román, Antonio Segura-Carretero

Department of Analytical Chemistry of the University of Granada, Spain

The guacamole industry has grown exponentially, generating large volumes of waste. The guacamole manufacturing process produces over 39% of by-products, mainly peels and seeds [1]. In this sense, the bioactive potential of avocado peels sand seeds has been reported due to their high content of phenolic compounds, which provides biological properties such as antioxidant and anti-inflammatory capacities [2] related to high protection against UV radiation and oxidative stress, which constitute the main causes of premature ageing and the onset of skin damage and disease [3]. The main objective of the revalorization of these wastes is to obtain powerful ingredients with bioactive properties for the development of high-value-added cosmeceuticals. For this purpose, the compounds present in the avocado peel and seed extracts were annotated with HPLC-ESI-qTOF-MS. In addition to that, these compounds were tested using the Folin–Ciocalteu, FRAP, TEAC and ORAC methods for studying the total phenolic content and their antioxidant activity. The ROS scavenging capacity and the inhibitory effect of enzymes related to skin ageing, such as collagenase, hyaluronidase, tyrosinase, elastase and xanthine oxidase, were also evaluated. Finally, the extracts were incorporated into a cosmetic formulation in gel form and their accelerated and long-term stability were evaluated.

The avocado peel extract, which showed a significantly higher antioxidant and anti-ageing potential than the seed extract, was selected for testing of its efficacy in vivo using a skin platform. This research work is carried out within the national strategic project GUACABIOTAL oriented toward ecological transition with code TED2021-132043B-I00.

Bibliography

1. García-Vargas et al., 2020. ''Avocado-Derived Biomass as a Source of Bioenergy and Bioproducts.''
2. Flores et al., 2019. ''Avocado Oil: Characteristics, Properties, and Applications.'
3. Araújo et al., 2018. ''Avocado By-Products: Nutritional and Functional Properties.''

B13. Untargeted Low-Molecular Metabolomics Approach To Engineered *Sideritis Scardica*-Clinoptilolite Assembly

Adina-Elena Segneanu[1], Gabriela Vlase[2], Crina Andrea Sicoe[2], Ioan Grozescu[3]

1 Institute for Advanced Environmental Research-West University of Timisoara (ICAM-WUT) Oituz nr.4, Timișoara, Romania
2 West University of Timisoara, Blvd.V. Parvan 4, 300223, Timisoara, Romania
3 University Politehnica Timisoara, 2 P-ta Victoriei, 300006, Timisoara, Romania

Sideritis is one of the most well-known healing plants from Eastern Europe, whose name originates from the Greek botanist and pharmacologist Pedanius Dioscorides, the author of De Materia Medica. Their health benefits have been appreciated and exploited in traditional medicine from the Balkan region since ancient times. Several recent studies have investigated the chemical composition and demonstrated the remarkable health benefits of sideritis. The clinoptilolite mineral has multiple modern applications. However, the investigations regarding the therapeutic properties of clinoptilolite are relatively few and refer in particular to its detoxification, antibacterial, immunostimulant, immunomodulating, and potential antiviral activity. This study reports, for the first time, the development of a simple, target phyto-engineered assembly that brings together the beneficial effects of *Scardica sideritis* and clinoptilolite.

The complete metabolites profile of Balkan wild-grown sideritis was carried out based on gas chromatography–mass spectrometry and mass spectrometry. A comprehensive characterization (FT-IR, UV-Vis, SEM, EDS, DLS and XRD) was performed to study the morpho-structural properties of this newly phyto-engineered assembly. Antioxidant activity was evaluated using a combination of in vitro methods (total phenolic assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and total antioxidant capacity (TAC).

The results of the antioxidants assays showed that the phyto-engineered assembly shows higher antioxidant properties than its raw materials: sideritis and clinoptilolite. These collective data confirmed the possibility of increasing the area of new multifunctional antioxidant therapy theoretical knowledge.

B14. Nutritional characterization of oregano leaves as source of natural antioxidants with potential use in ruminants' nutrition

Alexandra Gabriela Oancea, Arabela Elena Untea, Mihaela Saracila, Alexandru Vlaicu, Catalin Dragomir, Gabriel Lucian Radu

The National Research - Development Institute for Animal Biology and Nutrition

The quality of dairy products represents one of the most important subjects for ruminants' industry, animal nutrition being an important strategy to modulate milk composition in order to obtain nutritionally-enriched dairy products which can help to achieve the required nutrients for a healthy diet, by valorising of the various feedstuffs. This study presents an overview of the antioxidant potential of oregano leaves, including the bioactive compounds, such as isomers of vitamin E, xanthophylls, β-carotene, polyphenols, flavonoids and antioxidant capacity, iron chelating capacity, diphenylpicrylhydrazyl (DPPH) assay, and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test. Our results showed that oregano leaves can be considered a valuable source of antioxidant compounds, with a total vitamin E composition of 118.28 mg/kg (α-tocopherol being the most abundant isomer). The xanthophylls profile of the herb was 150.48 mg/kg lutein, 1.62 mg/kg astaxanthin and 0.12 mg/kg canthaxanthin. Additionally, it was observed that oregano can be a rich source of carotenoids, with a composition of 1200 mg/kg β-carotene. The total polyphenols content was 113.47 mg/g gallic acid equivalent, and the content of flavonoids was 6.25 mg/g. The most abundant polyphenols were rutin (12247.83 mg/kg), catechin (5597.93 mg/kg), and caffeic acid (941.43 mg/kg). Moreover, the ABTS test (2869.27 mM Trolox equivalent), DPPH assay (1100.59 mM Trolox equivalent), antioxidant capacity (684.85 mM ascorbic acid equivalent), and iron chelating capacity (28.62 mg/g EDTA equivalent) recorded, indicate the great antioxidant activity of oregano leaves. The in vitro study on efficiency of lipid peroxidation inhibition revealed that oregano leaves have high antioxidant potential, with an inhibition percentage of 47.65%, being more efficient than a synthetic antioxidant (vitamin E) usually used in animal feeds. These results suggest that oregano leaves can be successfully used in ruminants' nutrition with potential beneficial effects on dairy products quality.

B15. Antioxidant profile and inhibitory effect of raw garlic leaves on iron-induced lipid peroxidation

Arabela Elena Untea, Alexandra Gabriela Oancea, Petru Alexandru Vlaicu, Mihaela Saracila

Feed and Food Quality Department, National Research and Development Institute for Biology and Animal Nutrition, Calea Bucuresti, No.1, 077015 Balotesti, Ilfov, Romania

Garlic (*Allium sativum*) has been used in world cuisines and as a health promoter due to its ability to inhibit lipid peroxidation or due to its antioxidant, antithrombotic, antihypertension, anticancer, and antimicrobial effects. Therefore, raw garlic leaves can be used as sources of functional compounds for the development of new structures of food or feed products. The aim of the study was to determine the antioxidant potential, the concentrations of bioactive compounds with antioxidant effects, and the in vitro inhibitory effect on iron-induced lipid peroxidation. The antioxidant potential was evaluated by measuring the ABTS•+, DPPH•, and O_2•− scavenging activity, and the results showed an inhibition percentage as follows: 8.26 % ABTS•+ inhibition, 27.30 % DPPH• inhibition, and 18.37 % O2•− inhibition. The antioxidant capacity was determined using the phosphomolybdenum method (131.04 mM eq ascorbic acid). The hydrophilic bioactive compounds with antioxidant potential were determined in terms of the total polyphenols (11.38 mg/g eq gallic acid) and total flavonoids (5.32 mg/g eq quercetin). The polyphenolic chromatographic profile revealed that methoxycinnamic acid (1.85 mg/g) is the most abundant in the raw garlic matrix, a phenolic acid known for its strong antimicrobial effect. The lipophilic bioactive compounds with antioxidant potential were determined in terms of vitamin E (144.97 mg/kg total content, of which 139.14 mg/kg was alpha tocopherol, 4.16 mg/kg was gamma tocopherol, and 1.67 mg/kg was delta tocopherol) and xanthophyll (lutein and zeaxanthin 3369.12 mg/kg and astaxanthin 17.15 mg/kg). The determined concentration of beta carotene was 24.30 g/kg, which proved that garlic is a rich source of lipophilic antioxidant compounds. The determined valuable antioxidant profile showed that raw garlic leaves can be a potential functional ingredient in the formulation of food and feed nutritional supplements.

B16. The Effect Citrox BCL on *Legionella pneumophila* Mechanisms of Biofilm Formation, Oxidative Stress and Virulence

Eugenia Butucel[1, 2], Igori Balta[2], David McCleery[1], Cosmin Calin Popescu[3], Tiberiu Iancu[4], Ioan Pet[2], Adela Marcu[2], Nicolae Marinel Horablaga[3], Lavinia Stef[2], Nicolae Corcionivoschi[1, 2]

1 Bacteriology Branch, Veterinary Sciences Division, Agri-Food and Biosciences Institute, Belfast BT4 3SD, UK
2 Faculty of Bioengineering of Animal Resources, University of Life Sciences King Mihai I from Timisoara, 300645 Timisoara, Romania
3 Faculty of Agriculture, University of Life Sciences King Mihai I from Timisoara, 300645 Timisoara, Romania
4 Faculty of Management and Rural Development, University of Life Sciences King Mihai I from Timisoara, 300645 Timisoara, Romania

Legionella pneumophila is responsible for causing Legionnaires' disease and Pontiac fever, also known as legionellosis. The aim of this study was to investigate the mechanistic effect of a mixture of natural antimicrobials (Citrox BCL) in preventing *L. pneumophila* biofilm formation and reducing its in vitro virulence. The minimum inhibitory concentrations were detected at 0.06%, and the MBC was established at 0.125%. Based on the growth curve profile, the sub-inhibitory concentration of 0.02% was further used to study the mechanistic implications in the absence of a cytotoxic effect on A549 cells. At 24 h post-infection, Citrox BCL reduced ($p = 0.005$) the intracellular growth of *L. pneumophila* when the A549 cells or the bacteria were pre-treated with 0.02% Citrox BCL. This result was replicated when Citrox BCL was added during the 24 h infection assay leading to a reduction in intracellular growth ($p = 0.003$). Here, we show that at the sub-inhibitory concentration of 0.02%, Citrox CBL lowers the ROS levels in infected A549 cells and causes a 45% reduction in *L. pneumophila* EPS production, a reduction associated with the decline in biofilm formation. Overall, our results corroborate the low c-di-GMP production with the decrease in biofilm formation and low EPS levels. The low EPS levels seemed to be caused by the downregulation of the *tat*B and *tat*C gene expressions. Moreover, inhibition of *pvc*A and *pvc*B gene expressions, leading to lower siderophore levels, suggests that Citrox BCL reduces the ability of *L. pneumophila* to sequester iron and reduce biofilm formation through iron starvation.

B17. The Antioxidant Effect of Natural Antimicrobials in Shrimp Primary Intestinal Cells Infected with *Nematopsis messor*

Igori Balta[1, 2, 3], Lavinia Stef[1], Eugenia Butucel[1, 4], Gratiela Gradisteanu Pircalabioru[5], Adelina Venig[6], Patrick Ward[7], Myriam Deshaies[7], Ioan Pet[1], Ducu Stef[8], Osman Yasir Koyun[9], Todd R Callaway[9], Ozan Gundogdu[10], Nicolae Corcionivoschi[4, 11]

1 Faculty of Bioengineering of Animal Resources, University of Life Sciences King Mihai I from Timisoara, 300645 Timisoara, Romania
2 Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine,400372 Cluj-Napoca, Romania
3 Bacteriology Branch, Veterinary Sciences Division, Agri-Food and Biosciences Institute, Belfast BT4 3SD, UK;
4 Bacteriology Branch, Veterinary Sciences Division, Agri-Food and Biosciences Institute, Belfast BT4 3SD, UK
5 Research Institute of University of Bucharest, 300645 Bucharest, Romania
6 Faculty of Engineering and Management, University of Oradea, 410087 Oradea, Romania
7 Auranta, Nova UCD, Belfield, D04 V2P1 Dublin, Ireland
8 Faculty of Food Processing Technologies, Banat University of Agricultural Sciences and Veterinary Medicine, King Michael I of Romania, 300645 Timisoara, Romania
9 Department of Animal and Dairy Science, University of Georgia, Athens, GA 30602, USA
10 Department of Infection Biology, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK
11 Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine, 400372 Cluj-Napoca, Romania

Nematopsis messor infections severely impact shrimps' health, causing devastating economic consequences for shrimp farming. In a shrimp primary intestinal cells (SGP) model of infection, a sub-inhibitory concentration (0.5%) of natural antimicrobials (Aq) was able to reduce the ability of *N. messor* to infect (*p* 0.0001). To prevent *N. messor* infection of SGP cells, Aq inhibits host actin polymerization and restores tight junction integrity (TEER) and the expression of Zo-1 and occluding. The oxidative burst, caused by *N. messor* infection, is attenuated by Aq through the inhibition of NADPH-produced H_2O_2. Simultaneous to the reduction in H_2O_2 released, the activity of catalase (CAT) and superoxide dismutase (SOD) were also significantly increased (*p* 0.0001). The antimicrobial mixture inactivates the ERK signal transduction pathway via tyrosine dephosphorylation and reduces the expression of DCR2, ALF-A, and ALF-C antimicrobial peptides. The observed in vitro results were also translated in vivo, whereby through the use of a shrimp challenge test, we show that in *N. messor*-infected shrimp, the mortality rate was 68% compared to the Aq-treated group, where the mortality rate was maintained at 14%. The significant increase in CAT and SOD activity in treated and infected shrimp suggested an in vivo antioxidant role for Aq. In conclusion, our study shows that Aq can efficiently reduce *N. messor* colonization of shrimp's intestinal cells in vitro and in vivo and the oxidative-induced cellular damage, repairs epithelial integrity, and enhances gut immunity.

B18. The protective effect of flavonoids-enriched phytoextract blend in an inflammatory bowel disease in vitro model

Elena Lonati[1, 2], Paolo Corbetta[1], Stefania Pagliari[2,3], Laura Botto[1], Emanuela Cazzaniga[1,4], Luca Campone[2,3], Massimo Labra[2, 3], Paola Palestini[1,2], Alessandra Bulbarelli[1, 2]

1 School of Medicine and Surgery, University of Milano-Bicocca, via Cadore 48, 20900 Monza, MI, Italy
2 Bicocca Center of Science and Technology for Food, University of Milano-Bicocca, Piazza della Scienza, 2, 20126 Milano, Italy
3 Department of Biotechnology and Biosciences, ZooPlantLab, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy
4 Bicocca Center of Science and Technology for Food, University of Milano-Bicocca, Piazza della Scienza, 2, 20126 Milano, Italy.

Inflammatory bowel disease (IBD) is characterized by intestinal epithelial barrier disruption, increased inflammatory mediators production and excessive tissue injury. In recent years, the incidence of IBD is increasing, probably due to eating habits changes. In this scenario, there is a growing interest in specific diets development and in functional food formulation with polyphenol-rich phytoextracts. The aim of this study consists in evaluating the protective effects of artichoke, fenugreek and caihua (AFC) phytoextract original blend, firstly subjected to INFOGEST in vitro static digestion protocol. In order to mimic the intestinal barrier subjected to an inflammatory environment, Caco-2 cells were cultured and polarized on a transwell system and then exposed to a pro-inflammatory cytokine cocktail (TNFα e IL-1β). Initially, the AFC digested extract protective effect was evaluated by measuring the transepithelial resistance (TEER) as a marker of barrier integrity. The results showed TEER values improvement in cells pre-treated with AFC blend with respect to inflamed cells, suggesting a regulation in tight junction protein expression and/or localization. Then, the nuclear factor kappa B (NF-κB) pathway was investigated analysing the protein levels of the transcription factor itself and of two targets involved in inflammatory mechanisms: COX-2 and IL-8. As expected, cytokines induced p65NF-κB activation with a 160% increase of COX-2 protein levels. Moreover, a 40-fold increase in IL-8 release was observed. Interestingly, the pre-treatment with AFC blend showed a 50% decrease of activated p65NF-κB as compared to inflamed cells and a consequent reduction of about 50% COX-2 and 30% IL-8. Taken together, these results highlight the anti-inflammatory AFC blend potential, probably due to the presence of polyphenols such as luteolin, apigenin and chrysin. These experimental evidences suggest that AFC blend could be a good ingredient for food functionalization further used in nutritional strategies.

B19. Evaluation of *Epilobium hirsutum* L. optimized extract's anti-inflammatory potential

Ana-Maria Vlase[1], Anca Toiu[2], Dana Muntean[3], Tibor Casian[3], Laurian Vlase[3], Adriana Filip[4], Ioana Bâldea[4], Nicoleta Decea[4], Remus Moldovan[4], Gianina Crisan[1]

1 Department of Pharmaceutical Botany, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, 8 Victor Babeş street, 400012 Cluj-Napoca, Romania
2 Department of Pharmacognosy, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, 8 Victor Babeş street, 400012 Cluj-Napoca, Romania
3 Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, 8 Victor Babeş street, 400012 Cluj-Napoca, Romania
4 Department of Physiology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, 8 Victor Babeş Street, 400347, Cluj-Napoca, Romania

Epilobium hirsutum L. (*Onagraceae* family), commonly known as hairy willowherb, is a perennial herbaceous plant native to Europe and Asia. In Romania, the *Epilobium* genus contains 17 species which are used in folk medicine for various purposes, including to alleviate benign prostate hyperplasia symptoms, and in anti-inflammatory applications; in addition, several studies also support the use of this species for the treatment of gastrointestinal disorders. The present study aimed to evaluate the anti-inflammatory potential of *Epilobium hirsutum* optimized extract in an animal model of acute paw inflammation induced by carrageenan. White male Wistar rats were divided into three groups: negative control, positive control (indomethacin), and group 3, the animals of which received the optimum extract of *E. hirsutum*. After inducing paw acute inflammation, animals were sacrificed at 2h and 24h. From the paw tissue homogenate, oxidative stress was assessed by determining malondialdehyde, reduced glutathione, oxidized glutathione, and their ratio, catalase, and glutathione peroxidase, respectively. In addition, IL-6 and TNF-α cytokine levels were assessed in plantar tissue homogenates. Western Blot analysis was performed for COX-2, NFkB, pNFkB, and GAPDH. The results verified the anti-inflammatory potential of this species as the levels of determined markers and cytokines for vegetal extract were comparable to those obtained for the positive control group treated with indomethacin.

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B20. Assessment of *Epilobium hirsutum* L. optimized extract's antitumor activity in an animal model

Ana-Maria Vlase[1], Anca Toiu[2], Dana Muntean[3], Tibor Casian[3], Laurian Vlase[3], Adriana Filip[4], Ioana Bâldea[4], Nicoleta Decea[4], Remus Moldovan[4], Vlad-Alexandru Toma[5], Piroska Virag[6], Gianina Crișan[1]

1 Department of Pharmaceutical Botany, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, 8 Victor Babeş street, 400012 Cluj-Napoca, Romania
2 Department of Pharmacognosy, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, 8 Victor Babeş street, 400012 Cluj-Napoca, Romania
3 Department of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, 8 Victor Babeş street, 400012 Cluj-Napoca, Romania
4 Department of Physiology, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, 8 Victor Babeş Street, 400347, Cluj-Napoca, Romania
5 Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania
6 Department of Radiobiology and Tumor Biology, Prof. Dr. Ion Chiricuţă Oncology Institute, 34-36 Republicii Street, 400015 Cluj-Napoca, Romania

Epilobium is a genus of flowering plants in the *Onagraceae* family, comprising around 200 species. They are mostly herbaceous perennials found in temperate regions of the world. Species of *Epilobium* have medicinal properties and are used to treat various ailments, including prostate problems and skin conditions. The present study aimed to investigate the anti-tumor potential of *Epilobium hirsutum* optimized extract in an animal model. Ehrlich ascites carcinoma was induced in Swiss albino mice who were divided into four groups: negative control, positive control (cyclophosphamide), group 3 (cyclophosphamide and *E. hirsutum*), and group 4 (*E. hirsutum*). Ascites fluid, liver, and heart samples were collected and analyzed to assess the oxidative stress and inflammation associated with this type of cancer. With this purpose, malondialdehyde, reduced glutathione, oxidized glutathione, and their ratio, the activity of glutathione peroxidase and catalase were determined. In addition, IL-6 and TNF-alfa were assessed on all samples. Ascites fluid was analyzed using Western blot to determine the mechanism of cell death and to evaluate the markers of cancer cell progression and prognosis. In this view, caspase-3 and 9, p53, Bcl-2, Bax and GAPDH were investigated. In addition, the liver biopsies were processed and sectioned at 5 microns; then, the slides were subjected to different staining (Mallory, Van Gieson, and Reticulin). The obtained results show that the optimized extract of *E. hirsutum* reduced the inflammation and oxidative stress associated with this type of tumor, which is comparable with the results of the positive control group, and verified its anti-tumor potential.

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B21. Phenolic and Antioxidant Properties of Italian Pasta from Different Price Categories

Giuliana Vinci[1], Luana Gallotta[2], Sabrina Antonia Prencipe[2], Marco Ruggeri[2], Roberto Ruggieri[2]

1 Department of Management - Sapienza University of Rome, Italy
2 Department of Management, Sapienza University of Rome. Via del Castro Laurenziano 9, 00161, Rome, Italy

Pasta is known and appreciated worldwide due to its nutritional qualities, enabling it to be the basis of the Mediterranean diet, especially in Italy, where 3.6 million tons (1/4 of all the world's) are produced, for a revenue of EUR 5.6 million and an annual *per capita* consumption of 23.5 kg (2021). Pasta has a key role in human nutrition mainly due to its bioactive components, including phenolic compounds and antioxidants, which may have anti-inflammatory properties and improve gastrointestinal health. Therefore, given its nutritional and economic value, the aim of this study is to evaluate the relationship between the antioxidant and polyphenolic potential of Italian pasta from different price categories. In particular, thirty-five samples of Italian pasta, representative of three retail price categories (low, medium, and high), will be analyzed using spectrophotometric assays for the determination of polyphenol, flavonoid, and total phenolic acid content, and for antioxidant activity with ABTS and DPPH assays. The results will thus be evaluated with univariate and multivariate chemometric tools (ANOVA and PCA). From the preliminary data obtained, there is a statistically significant ($p<0.05$) variation in the content of bioactive compounds among price ranges. In particular, the higher-priced pasta showed higher values for both phenolic and antioxidant compounds of about +35% compared to lower-priced pasta samples.

B22. Valorization of Ancient Tomato Production Chain from Campania Region by Their Potential of Phenolic and Antioxidant Compounds

Laura Gobbi, Fabrizio D'Ascenzo, Anna Mallamaci, Sabrina Antonia Prencipe, Giuliana Vinci

Department of Management, Sapienza University of Rome. Via del Castro Laurenziano 9, 00161, Rome, Italy

Tomato (*Solanum lycopersicon*) is a cultivar broadly consumed worldwide, both raw and in processed form, due to its nutritional properties. Indeed, tomato fruit contains several phytochemicals and antioxidants, such as carotenoids (lycopene and β-carotene), ascorbic acid, phenols and flavonoids, thus promoting beneficial effects for human nutrition and health. In Italy, the tomato annual production chain is of about 6 million tons, of which more than 50% comes from Campania, for a per capita consumption of about 2.92 kg per month. During tomato processing, large quantities of by-products are generated, amounting to 3–5% (w/w) of the total raw tomatoes. They consist of peels, seeds and small amount of tomato pomace, which still contain biological compounds with high added value, that are often discharged, creating the main disposal issue for the tomato processing industry. In this regard, the objective of this study is to evaluate the potential of polyphenolic and antioxidant compounds in "*Pera d'Abruzzo*", and "*Antico Pomodoro Riccio*" tomato production chains (raw tomatoes, tomato sauce, and pomace) from the Campania region, through spectrophotometric analysis for the determination of total polyphenols and flavonoids, phenolic acids, carotenoids, antioxidant activity (ABTS and DPPH assays), and lycopene determination by means of HPLC-FD. The results show that the bioactive compounds most present within the tomato chain are carotenoids, including lycopene, which account for about 40% of the total polyphenols determined, in addition, lycopene recovery from tomato processing by-products is shown and was particularly significant (p value < 0.05) compared to raw tomato and tomato sauce.

B23. A Standardized Kinetic-Based Stopped-Flow DPPH Assay to measure the Antioxidant Activity and Capacity of Ascorbic Acid and Fruit Juices

Lucrezia Angeli, Ksenia Morozova, Matteo Mario Scampicchio

Free University of Bolzano, Italy

Food antioxidants play a major role in improving public health because they fight oxidative stress in cells. Thus, monitoring the kinetics of antioxidants during their reaction with radicals is crucial to better understand their functions. However, determining the antioxidant-radical interactions remains challenging for fast antioxidants, such as ascorbic acid, because the reaction is completed in just a few seconds. Accordingly, we proposed a standardized method for the determination of the rate constants of the reaction between fast antioxidants and DPPH• reagent, at room temperature. The method involved a stopped-flow spectrophotometric system, which was used to monitor the absorption decay of DPPH• at 515 nm during its interaction with antioxidants. A kinetic model of pseudo-second order describes the reaction between DPPH• and common antioxidants. Such a reaction can follow a simple model or a more complex model employing a secondary reaction, related to the development of reaction products, which is called a side reaction. The fitting of the DPPH• decay yields the rate constant k_1, which describes the main reaction between antioxidants and DPPH•, and the rate constant k_2, which is attributed to a slower side reaction considering the products generated between the transient radicals (A•) and another molecule of DPPH•. The presence of the side reaction was demonstrated with high resolution mass spectrometry in our previous work (Angeli et al. 2021). Moreover, the stoichiometric factor of the reaction can be easily assessed. Ascorbic acid was the fastest antioxidant and reacted with DPPH• in 2 s. The method was then applied to several antioxidant standards and ascorbic rich fruit juices. Overall, this kinetic-based stopped-flow DPPH• method is recommended to study the radical scavenging activity of individual antioxidants and fruit juices.

References:
Angeli, Lucrezia; Imperiale, Sebastian; Ding, Yubin; Scampicchio, Matteo; Morozova, Ksenia (2021): A Novel Stoichio-Kinetic Model for the DPPH• Assay: The Importance of the Side Reaction and Application to Complex Mixtures. In *Antioxidants (Basel, Switzerland)* 10 (7). DOI: 10.3390/antiox10071019.

B24. Phenolic compounds from coffee pulp potentially scavenge reactive oxygen and nitrogen species after in vitro digestion

Silvia Cañas, Miguel Rebollo-Hernanz, Cheyenne Braojos, Shuai Hu, Maria Martín-Trueba, Alicia Gil, Vanesa Benítez, Yolanda Aguilera, Maria Angeles Martín-Cabrejas

1 Department of Agricultural Chemistry and Food Science, Faculty of Science, C/ Francisco Tomás y Valiente, 7. Universidad Autónoma de Madrid, 28049, Madrid, Spain.
2 Institute of Food Science Research (CIAL, UAM-CSIC). C/ Nicolás Cabrera, 9. Universidad Autónoma de Madrid, 28049, Madrid, Spain.

The coffee pulp (CP) is the main by-product obtained during the wet processing of coffee, and it contains a high content of phenolic compounds and caffeine. In this study, we evaluate the influence of the in vitro gastrointestinal digestion of coffee pulp flour (CPF) and aqueous extract (CPE) on the phenolic composition, and their effects on oxidative stress biomarkers. Our results show that the CPF and CPE contain a high amount of phenolic compounds, especially phenolic acids (chlorogenic and gallic acids), as well as flavonoids (quercetin derivatives). Caffeine exhibits higher concentrations than phenolic compounds in CPF and CPE. The antioxidant capacity increased throughout simulated digestion in both matrices. The $O_2^{\cdot-}$ scavenging capacity of the CPF digested fractions increases, with the phenolic compounds being responsible; in particular, the cryptochlorogenic acid (trans) correlates significatively with $O_2^{\cdot-}$ scavenging capacity (r = 0.956, $p < 0.05$) in CPE. The highest values of H_2O_2 scavenging activity are found in the gastric and intestinal phases that might be due to the highest concentration of hydroxybenzoic acids in CPF, or in case of CPE, by the appearance of new compounds derived from phenolic transformations, such as caffeic acid. In addition, the NO scavenging capacity increases as digestion succeeds in the CPF, correlating with the content of neochlorogenic acid (r=0.998, $p< 0.05$), and the $ONOO^-$ scavenging activity significantly correlates with the neochlorogenic acid content (r=0.999, $p< 0.05$) in CPF, and the hydroxycinnamic acids concentration (r=0.976, $p< 0.05$) in CPE. Then, both matrices may potentially scavenge reactive oxygen and nitrogen species even after in vitro digestion. Thus, CP could be used as a potent food ingredient for preventing oxidative stress thanks to its high content of antioxidant compounds.

B25. Cell antioxidant activity of phenolic compounds from amaranth flour after simulated gastrointestinal digestion

Mariela Rodríguez[1], Ariel Fontana[2], Valeria Tironi[1]

1 Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) (CCT La Plata-CONICET, UNLP). 47 y 116, La Plata, Buenos Aires, Argentina
2 Instituto de Biología Agrícola de Mendoza (IBAM),CONICET-Facultad de Ciencias Agrarias-UNCUYO; Almirante Brown 500,M5528AHB Chacras de Coria, Mendoza, Argentina

Amaranth flour (AF) is a source of different antioxidant components with potential health benefits for consumers, including polyphenols. This work aimed to study the antioxidant properties of polyphenols after simulated gastrointestinal digestion (SGID) of amaranth flour using in vitro acellular and cellular systems (Caco2-TC7 cultures).

The whole *A. mantegazzianus* grain was milled (Udy mill), and a SGID was performed according to an internationally agreed protocol involving oral, gastric and intestinal stages. Polyphenolic compounds (PCs) obtained from 70/30 MeOH/water extraction were analyzed; the total polyphenols content (TPC) was determined using the Folin-Ciocalteu method and PC profiles and concentration with LC-DAD-FLD. Antioxidant activity was evaluated: a) in acellular systems (ABTS, ORAC); b) in cellular systems: colon carcinoma cell cultures (Caco2-TC7) exposed to an oxidative-stress-inducing factor (H_2O_2). Cell viability was determined using lactate dehydrogenase (LDH) assay and intracellular ROS content through 2',7'-dichlorofluorescein diacetate oxidation (DCFH-DA). Using commercial kits, reduced GSH content as well as SOD and GPx activities were measured in cell lysates sequentially exposed to phenolic extract (MeOH/water) evaporated and resuspended in phosphate buffer (35mM, pH 7,8) and then to H_2O_2 as an oxidative stress inducer.

AF digestion (DAF) released/modified some phenols, reduced the diversity of PCs, and increased the total amount extracted given mainly by compounds of the flavanol family and stilbene trans piceatannol. The PTC of DAF was about 20 times greater than that determined for AF. Compounds extracted from DAF showed in vitro antioxidant activity in chemical assays (ORAC and $ABTS^{+\cdot}$ scavenging), as well as the ability to inhibit intracellular ROS, increase SOD activity and GSH contents and raise GPx activity (to basal level) in H_2O_2 -induced cells. These results indicate activity against the cellular oxidative stress of bioaccessible AF-derived PCs, positioning the AF as a potential ingredient for the development of functional foods.

B26. Exploration of grape pomace flour and amaranth flour as functional ingredients in the elaboration of breads: phenolic composition, antioxidant capacity and simulated digestion

Mariela Rodríguez[1], Corrado Rizzi[2], Barbara Simonato[2], Ariel Fontana[3], Valeria Tironi[1]

1 Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) (CCT La Plata-CONICET, UNLP). 47 y 116, La Plata, Buenos Aires, Argentina
2 Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy
3 Instituto de Biología Agrícola de Mendoza (IBAM),CONICET-Facultad de Ciencias Agrarias-UNCUYO; Almirante Brown 500,M5528AHB Chacras de Coria, Mendoza, Argentina

Grape pomace (GP) represents the main solid by-product of the wine industry. Due to it richness in phenolic compounds (PCs), it addition to breads could be a suitable alternative for GP application as a functional ingredient. Otherwise, amaranth flour (AF) presents interesting nutritional and functional properties, including antioxidant capacity (AC). Unfermented GP (*Vitis vinifera* Cabernet Sauvignon) resulting from the production of a sparkling wine was dried (45°C) and pomace flour (GPF) was obtained (Udy mill). White wheat bread (WB) and breads with wheat flour (WF) substitutions were prepared: 5% GPF (5GPFB), 20% AF (A20B), 20% AF and 5% GPF (A20GPF5B). The original flours, breads and digested breads were characterized. A simulated gastrointestinal digestion (SGID) was performed involving oral, gastric, and intestinal stages. The PCs obtained through different extraction protocols (MeOH/HCl 97/3 or MeOH/water 70/30) were analyzed. The total phenolic content (TPC) was determined using the Folin-Ciocalteu method and the PCs profiles and concentration through LC-DAD-FLD. AC was evaluated using ABTS and ORAC.
The TPC of flours extracted using both methods was WF < AF << GPF. MeOH/HCl extracts showed a higher extraction of flavanols such as (-)-epigallocatechin and a reduction in the total anthocyanin content of GPF, with a decrease in the proportion of acylated derivatives possibly hydrolysed by the strong acidic medium. GPF addition to the breads (5GPFB and A20GPF5B) provided an increase in the concentration of epigallocatechin (MeOH/HCl) and others, anthocyanin and non-anthocyanin PCs. The substitution (AF and/or GPF) provided a variety of PCs to the breads, with the highest diversity observed in A20GPF5. After SGID, TPC increased strongly, suggesting a release of compounds. AC correlated with TPC content, increasing after digestion for both extracts by both techniques. In the ABTS assay, a higher AC was recorded for all MeOH/water extracts, while for the ORAC assay, this trend was only found for MeOH/water extracts of digested breads. The results showed that both AF and GPF have potential as antioxidant functional ingredients in bread formulation.

B27. Extracts, Natural Stilbenes, in Placenta Protection: Which Are They and How Do They Work?

Marília Oliveira Fonseca Goulart[1, 2, 3], Jadriane X Almeida[4], Monika Oliveira[1, 5], Elaine L. Mendonça[6], Rayane Martins Botelho[1, 7], Camilla Mendes Gonçalves[1, 7], Karen Borbely[1, 8], Alane Menezes de Oliveira[1, 8], Antonio Euzebio Goulart Santana[1, 9], Alexandre URBAN Borbely[1, 7], Orlando P. Araújo[4], Fabiana Andrea Moura[1, 8]

1 UNIVERSIDADE FEDERAL DE ALAGOAS, Brazil
2 Federal University of Alagoas - Institute of Chemistry and Biotechnology, Brazil
3 Tabuleiro dos Martins, Maceio, Alagoas, Brazil, 57072970
4 Federal University of Alagoas - Institute of Chemistry and Biotechnology, Maceio, Alagoas, Brazil, 57072970
5 Instituto de Química e Biotecnologia, Maceio, Alagoas, Brazil, 57072-970
6 Federal University of Alagoas - Institute of Chemistry and Biotechnology, Maceio, Alagoas, Brazil, 57072970 - RENORBIO Program
7 Instituto de Ciências Biológicas e da Saúde, Laboratório de Biologia Celular, CEP 57072-970 Maceió, AL, Brazil
8 Faculdade de Nutrição, UFAL, Maceió, Alagoas, Brazil
9 Centro de Ciências Agrárias, Maceió, Alagoas, Brazil

Essential aspects concerning maternal and child health during pregnancy are mainly related to pre-eclampsia, gestational diabetes, and infection with ZIKA virus (ZIKV). The last infects cells from the developing central nervous system and causes severe anatomical, physiological and cognitive alterations. In this context, preventing placental infection caused by ZIKV and mitigating the symptoms of other diseases in the mother–fetus dyad are urgently required. As such, the aims of the present work address the investigation of natural products, which may inhibit ZIKV infection of trophoblast cells, and with a potential role as an alternative/combined therapy, in animal models, towards the cited diseases. We investigated natural products, mainly residues, in extracts or in a pure form (stilbenes/flavonoids), towards inhibition of ZIKV infection of trophoblast cells. Ethanolic extracts obtained from Schinus terebinthifolius could reduce ZIKV infection and modulate placental responses. Additionally, Passiflora edulis seed extract could also prevent ZIKV infection in Vero and HTR-8/SVneo trophoblast cells. We evaluated the molecular mechanisms, concerning inflammation profile, oxidative stress markers, and modulation of nuclear factors. The extracts, in several concentrations, were shown to be non-toxic, using several models. Both vegetal extracts were able to remarkably decrease the viral load of both MR766 and PE243 strains in cellular supernatants, in addition to other results obtained on the mechanisms of molecular action. In conclusion, the results unveil the role of natural antioxidants (in a mixture or alone) in protecting maternal and child health and providing extra value to products from family farming.

B28. Evaluation of biogenic selenium nanoparticles and curcumin nanospheres on growth, hematology, antioxidant defense and gastrointestinal health in a mice model

Mohammad Moniruzzaman, Taesun Min

Department of Animal Biotechnology, Bio-Resources Computing Research Center, Sustainable Agriculture Research Institute (SARI), Jeju National University, Jeju 63243, Republic of Korea

Selenium is an essential trace metal that is considered an important antioxidant for human and animal health. In the past few decades, most of the studies conducted were related to the utilization of selenium nanoparticles (SeNP) from inorganic sources, which were toxic at high doses and also hazardous to the ambient environment. However, in recent years, it has been reported that green or biogenic synthesis of selenium nanoparticles could be safe to the organisms and environmental friendly. On the other hand, curcumin is a bioactive compound extracted from the plant, Curcuma longa which has antioxidant, antimicrobial and anti-inflammatory properties. However, due to its low solubility in water and rapid elimination from the body, curcumin's bioavailability is very poor in the subject. For this, nanocurcumin with liposomal nanocarriers (hereafter, curcumin nanospheres, CN) could improve the bioavailability in organisms. In this study, SeNP was green synthesized with soybean sprout as capping agent and ascorbic acid as reducing agent. We aimed to orally administer the biogenic SeNP with or without CN in a 3×2 factorial design of experiment for 28 days. Eight groups of mice, each containing three mice/cage, were orally administered with 0, 2 and 4 mg/kg body weight of SeNP and two levels of CN as 0 and 2 mg/kg weight of mice in triplicate. The treatments are: control (saline water), SeNP2 (2 mg/kg weight), SeNP4 (4 mg/kg weight), SeNP2CN2 (2 mg SeNP with 2 mg CN/kg weight), SeNP4CN2 (4 mg SeNP with 2 mg CN/kg weight), and one group contained only curcumin (CUR) at 20 mg/kg and inorganic sodium selenite (NaSe) at 4 mg/kg body weight. At the end of the 28 days of the experiment, we evaluated the growth, hematology, antioxidant defense and gastrointestinal health in male ICR mice by obtaining mice weight, blood, liver, intestine, kidney, etc.

B29. Antioxidant activity of bilirubin in micellar and liposomal systems is pH dependent

Pawel Przybylski, Michał Żebrowski, Grzegorz Litwinienko

University of Warsaw, Faculty of Chemistry

Bilirubin (BR) is a common metabolite of hemoglobin produced from the tetrapyrrolic ring of heme via biliverdin (processes catalyzed by heme oxygenase and biliverdin reductase). There is strong evidence that a high concentration of serum-free BR (hyperbilirubinemia) has significant effects on morbidity and mortality whereas mildly elevated serum BR protects from stress-mediated diseases [1,2]. Although the antioxidant, anti-inflammatory, and immunomodulatory activity of BR is well documented, the mechanism of its antioxidant action during peroxidation in the lipid/water interface still remains an unsolved problem. Since the elementary step of de-activation of a radical by antioxidants depends on the microenvironment, including acid/base equilibria, we tested the role of pH on the anti-radical properties of BR. The results of our studies on the kinetics of peroxidation of polyunsaturated lipid dispersed in the micellar (TritonX-100) and liposomal (DMPC) system initiated by the water-soluble azo-initiator at a pH range of 4.0–9.0 indicate that BR is a retardant of peroxidation at pH 6 while for pH 7 and 8 it exhibits a relatively good radical trapping ability. The rate constant for reaction with alkylperoxyl radicals, $k_{inh} = 3.5 \times 10^4\ M^{-1}s^{-1}$ (pH 7, liposomal system), is comparable or higher than k_{inh} obtained for 2,2,5,7,8-pentamethyl-6-hydroxychromane (analogue of a-tocopherol, most active lipid-soluble natural antioxidant). The significant role of pH might be correlated with the protonation equilibria of bilirubin and the pattern of internal hydrogen bonds that govern the conformation of bilirubin, thus facilitating the reaction with alkylperoxyl radicals at neutral pH.

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References:

[1] Bianco, A., et al. "The extent of intracellular accumulation of bilirubin determines its anti-or pro-oxidant effect." *IJMS* **2020***, 21,* 8101.

[2] Adin, C. A. "Bilirubin as a therapeutic molecule: challenges and opportunities." *Antioxidants* 2021, 10, 1536.

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B30. Polyhydroxyphenols with mitochondrial-targeted moiety

Pawel Przybylski, Martyna Cybularczyk-Cecotka, Konrad Mroziński, Wojciech Witkowski, Grzegorz Litwinienko

University of Warsaw, Faculty of Chemistry

Among cellular organelles, mitochondria are the most exposed to oxidative stress because during generation of energy, the mitochondria facilitate the reduction in molecular oxygen. High redox activity makes them very susceptible to oxidative damage, resulting in the dysregulation and dysfunction of the whole cell. In recent years, a number of antioxidants, such as vitamin E, flavonoids, and polyphenols, have been used due to their beneficial effects against oxidative stress; unfortunately, however, none of them adequately protect mitochondria due to a lack of proper localization of the antioxidant molecule. A covalent linking of a lipophilic cation such as an alkyltriphenylphosphonium (TPP^+) moiety is one of the most effective ways of delivering the small molecular drug to mitochondria; in addition, depending on the cell and transmembrane potential, the final accumulation of mito-compound can be 1000 times higher than the concentration of the nonderived compound. Here, we present results of our current project focused on the regioselective synthesis of a series of quercetin derivatives with structural motifs aimed at modifying the polarity of this antioxidant and targeting mitochondria. The integral part of the project is the study of the structure–activity relationship based on the kinetics of antioxidant action in model micellar and liposomal systems (mimicking the mitochondrial environment) as well as calorimetric measurements for interactions of mito-antioxidants with model lipid bilayers.

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B31. Baicalin and baicalein: mechanism of antiradical action of two flavonoids with a strongly acidic catechol moiety

Pawel Przybylski, Piotr Łetowski, Grzegorz Litwinienko

University of Warsaw, Faculty of Chemistry

Among 30 flavonoids isolated from *Scutellariae radix* (a root of *Scutellariae baicalensis*), two compounds, 5,6,7-trihydroxyflavone (baicalein) and its 7-*O*-β-d-glucuronide (trivial name baicalin), attracted great attention due to many pharmacologic benefits, such as the reduction in the total cholesterol level, decrease in blood pressure, cholagogic, diuretic, and cathartic action, suppression of the production of leukotriene C4 and D4, anti-inflammatory and antibacterial and anticancer activity. Some of those health benefits have been attributed to anti-radical and antioxidant effects. Both compounds have a unique structural feature, as hydroxy groups have a catechol (or pyrogallol) arrangement in ring A, which makes them highly acidic and prone to react with radicals not only via hydrogen atom transfer (HAT) but via electron transfer (ET) from phenolate anion too. Our preliminary measurements indicated kinetic domination of ET over HAT for baicalein reacting with diphenyl-picrylhydrazyl (dpph) radicals in alcohols.[1] Although dpph radicals are isoelectronic to peroxyls, a more complete picture can be obtained for a real peroxidation process; therefore in this presentation, we provide the kinetic data for the peroxidation of polyunsaturated fatty acid carried out in model micellar and liposomal systems in the presence of baicalin and baicalein with extended pH range, which allowed the researchers to obtain an insight into the role of glucuronic moiety for the overall antioxidant effect of baicalin and the stability of pyrogallol-like arrangement of hydroxy groups in the ring A of baicalein.

Participation in this conference was supported within the Excellence Initiative Research University (PSP 501-D112-20-0002210).

References:

[1] Przybylski P.; Konopko A.; Łętowski P.; Jodko-Piorecka K.; Litwinienko G., Concentration-dependent HAT/ET mechanism of the reaction of phenols with 2,2-diphenyl-1-picrylhydrazyl (dpph·) in methanol. *RSC Adv.* **2022**, *12*, 8131–8136.

B32. Sustainable Extraction of Antioxidants from Murtilla Leaves

Natalia Fuentes-Jorquera[1], Jara Pérez-Jiménez[2], María González-Miquel[3], Roberto Canales[1], José Ricardo Pérez-Correa[1]

1 Department of Chemical and Bioprocess Engineering, Faculty of Engineering, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, P.O. Box 306, Santiago 7820436, Chile
2 Department of Metabolism and Nutrition, Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), José Antonio Novais 10, 28040 Madrid, Spain
3 Department of Industrial Chemical Engineering and the Environment, ETSI Industriales, Polytechnic University of Madrid, C/ José Gutiérrez Abascal 2, Madrid, 28006, Spain

Murtilla (*Ugni moline* Turcz), a berry that grows from the center to the south of Chile, has attracted attention due to its high content of antioxidants with potential benefits to human health. These berries also contain significant amounts of vitamins, minerals, and dietary fiber. The Mapuche culture, from the south of Chile, uses murtilla to make a leaf infusion to treat urinary and throat infections. The bioactive composition of the leaves and fruits of murtilla includes a wide variety of phenolic compounds and anthocyanins with antioxidant and antimicrobial activity. The extraction of antioxidants with deep eutectic solvents (DES) is sustainable and safe. These solvents present low vapor pressures, are biocompatible, nonflammable, nontoxic, and can be prepared using renewable materials. This work reports the conventional and microwave extraction of polyphenols from freeze-dried murtilla leaves using DES prepared with choline chloride as a hydrogen bond acceptor (HBA) and 1,2-butanediol, 1,3-butanediol, and 1,2- propanediol as hydrogen bond donors (HBD). The polyphenol composition of the extracts was analyzed using HPLC-DAD. More polyphenols were obtained in less time using microwave extractions with ChCl:1,3-butanediol. The most abundant polyphenols identified in the extracts of murtilla leaves were phenolic acids (gallic acid, p-coumaric acid, and caffeic acid) and flavonoids (catechin and rutin).

B33. Fermentation of Murta Juice (*Ugni Molinae Turcz.*) with a Native *Leuconostoc Mesenteroides* Strain to Obtain a Functional Drink

Natalia Escobar, José Ricardo Pérez-Correa, Wendy Franco

Department of Chemical and Bioprocess Engineering, Faculty of Engineering, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, P.O. Box 306, Santiago 7820436, Chile

This research describes a study on the fermentation of murta juice (*Ugni molinae Turcz*.) using native lactic acid bacteria (LAB) to create a new functional food with high added value. We isolated several bacteria from the *Gluconobacter* and *Leuconostoc* families, ultimately choosing the latter since it was the only BAL identified; additionally, it is commonly used to produce fermented foods. Fermentations were conducted using three different types of murta juice (natural, freeze-dried powder, and commercial) and evaluated the impact of adding glucose at the beginning of fermentation (+G). *L. mesenteroides* showed a fermentative behavior consistent with the literature, improving the juice's antioxidant capacity and inhibitory activity of α-glucosidase. However, no improvements in the inhibitory capacity of α-amylase were observed. The aromatic profile of the juices was expanded by the increase in aromatic compounds produced by fermentation. A sensory panel revealed that fermentation positively impacted the juice's flavor, sweetness, and acidity. The preferred fermented juices were the commercial and natural type +G. Overall, the fermentation of murta juices with *L. mesenteroides* enhances their bioactivity and organoleptic characteristics.

B34. Evaluation of the antioxidant properties and bioaccessibility of phlorothaninsphlorothanins-rich extracts obtained from Chilean brown seaweed by ultrasound-assisted extraction

Gonzalo Rivera[1, 2], Layla Simón[1], Migdalia Arazo[1, 3], Angye Caro[1, 3], Kevin Gonzalez[1], Camila Vega[1, 3], Francisco Pérez-Bravo[2], Ricardo Pérez-Correa[3], María Salomé Mariotti-Celis[1]

1 Nutrition and Dietetics School, Faculty of Medicine, Universidad Finis Terrae. Pedro de Valdivia 1509, Providencia, Santiago 7501015, Chile.
2 Institute of Nutrition and Food Technology (INTA), Universidad de Chile, Santiago 7830490, Chile.
3 Department of Chemical and Bioprocess Engineering, Faculty of Engineering, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, P.O. Box 306, Santiago 7820436, Chile.

Sarcopenia is an age-related condition, with high worldwide incidence, characterized by the progressive decline of the mass, strength, and function of the skeletal muscle. Phlorotannins, a high-value group of polyphenols only found in brown seaweeds, might retard the skeletal muscle loss and function impairment of older adults due to their superior antioxidant properties. However, if phlorotannins are administered orally, they must support the drastic conditions of digestion before reaching their targets. This work evaluated the bioaccessibility and antioxidant potential of phlorotannin-rich extracts obtained via ultrasound-assisted extraction (USAE) of *Durvillea incurvata*, an endemic Chilean brown seaweed. Seaweeds from the cities of Valdivia and Concepción were submitted to USAE (32.5% of ethanol, 40% of potency, 30 minutes). The total polyphenol content (TPC) and antioxidant capacity (AOXC) of freeze-dried extracts were determined using Folin–Ciocalteu and ORAC assays, respectively. Then, extracts were subjected to an in vitro digestion process according to the INFOGEST methodology, adjusting the protocol to the physiological conditions of an older adult. At each stage of the simulated gastrointestinal digestion, TPC and AOXC were determined. *Durvillea incurvate* USAE extracts of both cities presented similar values of TPC (~ 22.46-24.34 mg equivalent phloroglucinol/g of dry solid extract; $p > 0.05$). However, the AOXC of USAE extracts from Concepción (100.06 µmol of TE/ g of dry solid extract) was significantly higher (50%; $p > 0.05$). The bioaccessibility of phlorotannins of both USAE extracts decreased by 30 % after the in vitro gastrointestinal digestion. AOXC followed a similar trend, decreasing by 40 % after the intestinal phase. *Durvillea incurvata* USAE extracts present antioxidant properties; however, their future application in oral-delivery systems which control the sarcopenia of older adults will require the evaluation of barrier technologies that preserve the bioaccessibility of phlorotannins.

B35. Optimization of ultrasound-assisted extraction process to obtain heavy metals-free and phlorotannin-rich extracts from brown seaweed (*Durvillea incurvata*)

Kevin Jose Gonzalez-Morelo[1, 2], Angye Caro-Cabarcas[1, 2], Gonzalo Rivera-Andrades[1, 3], Layla Simón[1], Ricardo Pérez-Correa[2], María Salomé Mariotti Celis[1]

1 Nutrition and Dietetics School, Faculty of Medicine, Universidad Finis Terrae. Pedro de Valdivia 1509, Providencia, Santiago 7501015, Chile.
2 Department of Chemical and Bioprocess Engineering, Faculty of Engineering, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, P.O. Box 306, Santiago 7820436, Chile.
3 Institute of Nutrition and Food Technology (INTA), Universidad de Chile, Santiago 7830490, Chile.

Brown seaweeds are a resource of phlorotannins, phenolic compounds with antioxidant and anti-inflammatory effects in both in vitro and in vivo studies. *Durvillea incurvata* is a brown seaweed widely distributed in central and southern Chile. Recent studies have shown the presence of heavy metals in this Chilean endemic phlorotannin raw material. Therefore, the development of selective extraction techniques is required to obtain safe phlorotannin extracts suitable for human consumption. This research aimed to optimize an ultrasound-assisted extraction process (USAE) to obtain heavy metals-free and phlorotannin-rich extracts from *D. incurvata*. Samples were collected from the coasts of Concepción, Chile, and characterized in terms of protein, ash, moisture, fiber, oil, and carbohydrate contents. Response surface methodology based on a Box–Behnken design was used to optimize the operating parameters (extraction time 10-45 min, power of cavitation: 20%-60% and ethanol concentration of extraction solvent: 15%-50%). The total polyphenol content (Folin–Ciocalteu assay) and antioxidant capacity (ORAC and DPPH assays) of extracts were determined. Likewise, the heavy metals content (As, Hg, Cd, Pb, and Sn) of brown seaweed and USAE extracts were determined. The optimal extract, defined as that which presents the lowest heavy metal content and highest total polyphenol content (24.33 ± 1.47 mg Phloroglucinol/g dry extract) and antioxidant capacity (DPPH: 30 µmol Trolox/g dry extract; ORAC: 200 µmol Trolox/g dry extract), was obtained at 27.5 min extraction time, power 40%, and 32.5 % ethanol concentration. Some experimental treatments were found to have significant cadmium levels (1.2 mg/kg dry weight) compared to international standards; therefore, a purification step is suggested. The incorporation of brown seaweeds extracts obtained using USAE in food matrices for in vitro digestion studies could be a research opportunity to counteract inflammatory conditions prevalent in the elderly.

B36. Characterization and classification of honeydew and blossom honeys based on their antioxidant capacity

Mónica Fernández-Estellé[1], Javier Saurina[2, 3], Oscar Núñez[1, 4, 5], Sonia Sentellas[5, 6, 7]

1 Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, Universitat de Barcelona, Martí i Franquès 1-11, E08028, Barcelona, Spain
2 Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, E08028 Barcelona, Spain
3 Research Institute in Food Nutrition and Food Safety, University of Barcelona, Av. Prat de la Riba 171, Edifici Recerca (Gaudí), E08921 Santa Coloma de Gramenet, Spain
4 Research Institute in Food Nutrition and Food Safety, Universitat de Barcelona, Av. Prat de la Riba 171, Edifici Recerca (Gaudí), E08921 Santa Coloma de Gramanet, Barcelona, Spain
5 Serra Húnter Fellow, Departament de Recerca i Universitats, Generalitat de Catalunya, Via Laietana 2, E08003 Barcelona, Spain.
6 Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, Universitat de Barcelona, Martí i Franquès 1-11, E08028, Barcelona, Spain.
7 Research Institute in Food Nutrition and Food Safety, Universitat de Barcelona, Av. Prat de la Riba 171, Edifici Recerca (Gaudí), E08921 Santa Coloma de Gramanet, Barcelona, Spain.

Honey is a widely appreciated product for its nutritional characteristics and its benefits for human health, comprising antioxidant, anti-inflammatory, antifungal, and antibacterial activities. These attributes depend on the specific composition of each honey variety, with the botanical origin being one of the distinctive features. Indeed, honeydew and blossom honeys show different physicochemical properties, the antioxidant capacity, which mainly relies on the phenolic compound content, being one of the most important.

In this work, aiming at characterizing honeys according to their botanical origin, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity were determined for a total of 73 honeys (50 blossom and 23 honeydew honeys). The Folin–Ciocalteu assay was used for the TPC determination. Regarding flavonoid content, two different methods, both based on the formation of aluminum chelates, were evaluated, observing that the response of compounds belonging to different flavonoid subfamilies depends on the experimental conditions. Lastly, the ferric reducing antioxidant power (FRAP) method was selected for the determination of the antioxidant capacity.

Mean TPC values ranged from 0.17 to 0.7 mg eq. gallic acid g^{-1}, honeydew honeys being the ones with higher values. Regarding TFC, mean values above 1.5 mg eq. quercetin g^{-1} (method applied in the absence of $NaNO_2$) were obtained for honeydew honeys and heather honey. Lower and not discriminatory values (below 0.3 mg eq. epicatechin g^{-1}) were obtained in the presence of $NaNO_2$. The maximum antioxidant capacity was observed for thyme honeys (2.2 mg eq. Trolox g^{-1}) followed by honeydew and heather honeys. PLS-DA models were constructed for honey classification using TPC, TFC, and FRAP data. Honeydew and blossom honey were satisfactorily discriminated (error 5%) with the PLS-DA models constructed. In addition, blossom honeys were perfectly classified according to their botanical origin based on two-class PLS-DA classification models.

B37. Characterization of Nutraceuticals and Functional Food Products Based on Polyphenolic Profiling by Liquid Chromatography-Mass Spectrometry

Oscar Vidal-Casanella[1], Mercè Granados[1], Oscar Núñez[2, 3, 4], Javier Saurina[1, 5], Sonia Sentellas[1, 4, 5]

1 Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, Universitat de Barcelona, Martí i Franquès 1-11, E08028, Barcelona, Spain.
2 Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, Universitat de Barcelona, Martí i Franquès 1-11, E08028, Barcelona, Spain
3 Research Institute in Food Nutrition and Food Safety, Universitat de Barcelona, Av. Prat de la Riba 171, Edifici Recerca (Gaudí), E08921 Santa Coloma de Gramanet, Barcelona, Spain
4 Serra Húnter Fellow, Departament de Recerca i Universitats, Generalitat de Catalunya, Via Laietana 2, E08003 Barcelona, Spain.
5 Research Institute in Food Nutrition and Food Safety, Universitat de Barcelona, Av. Prat de la Riba 171, Edifici Recerca (Gaudí), E08921 Santa Coloma de Gramanet, Barcelona, Spain.

Polyphenols are secondary metabolites ubiquitously present in plants. They belong to a large family of molecules with one or more phenolic rings in their structures. Hundreds of polyphenols are found in plant-based products, and most of them can be classified, depending on their chemical structures, as phenolic acids, flavonoids, stilbenes, lignans, or tannins. Their medicinal properties, mainly associated with antioxidant features, are exploited by the pharmaceutical industry to produce nutraceuticals from different plant parts, including stalks, leaves, and flowers (i.e., grapevine, and artichoke) and fruits (i.e., cranberry, raspberry, and grape).

In this study, several phenolic-rich nutraceuticals and food products were analyzed. Solid samples were treated with MeOH/H2O/HCl (70:29:1, v:v:v) for compound recovery using ultrasound-assisted extraction. The resulting extracts were characterized by liquid chromatography coupled to low-resolution mass spectrometry (LC-LRMS) in MRM mode. The separation relied on a reversed-phase C18 column under an elution gradient using 0.1% (v:v) formic acid aqueous solution and acetonitrile as the components of the mobile phase.

The identification and quantification of the major polyphenols present in these samples were carried out by LC-LRMS using the optimized transitions of 29 polyphenol standards. As an example, some abundant polyphenols were as follows: catechin in cranberry (ca. 9000 $mg \cdot Kg^{-1}$), resveratrol in grape-based products (ca. 9000 $mg \cdot Kg^{-1}$), 4-ethylcatechol in raspberry and tea (ca. 8000 $mg \cdot Kg^{-1}$), and epicatechin in chocolate (ca. 1698 $mg \cdot Kg^{-1}$). According to the overall polyphenolic content, berries-based extracts—including grape, cranberry, and raspberry—and tea resulted in the matrices with the highest polyphenolic levels.

B38. Purification of polyphenols in natural deep eutectic solvent extracts: assessing polymeric resins

Aina Mir-Cerdà[1, 2], Verónica Ramos[3], Mercè Granados[3], Javier Saurina[3, 4], Sonia Sentellas[3, 4, 5]

1 Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, Universitat de Barcelona, Martí i Franquès 1-11, E08028, Barcelona, Spain
2 Research Institute in Food Nutrition and Food Safety, Universitat de Barcelona, Av. Prat de la Riba 171, Edifici Recerca (Gaudí), E08921 Santa Coloma de Gramanet, Barcelona, Spain
3 Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, Universitat de Barcelona, Martí i Franquès 1-11, E08028, Barcelona, Spain.
4 Research Institute in Food Nutrition and Food Safety, Universitat de Barcelona, Av. Prat de la Riba 171, Edifici Recerca (Gaudí), E08921 Santa Coloma de Gramanet, Barcelona, Spain.
5 Serra Húnter Fellow, Departament de Recerca i Universitats, Generalitat de Catalunya, Via Laietana 2, E08003 Barcelona, Spain.

Olive oil production, a main agro-industrial activity in southern European countries, generates large amounts of waste that contains polyphenolic compounds at relevant concentrations. In recent years, interest in the recovery of these compounds to take advantage of their natural antioxidant properties from a circular bioeconomy perspective has grown.
Natural deep eutectic solvents (NADES), which can be considered green solvents, have been shown to efficiently extract phenolic compounds from agri-food waste. The extracts are very complex and must be purified before the potential application of recovered polyphenols in pharmaceutical, food, or cosmetic sectors.
In this study, several polymeric resins (PuroSorb PAD900, PuroSorb PAD950, PuroSorb PAD610, Macronet MN202 and Macronet MN270) were evaluated for the purification of NADES extracts (Choline chloride-Glycerol (1:5); 30% water) from olive leaves. Batch experiments were carried out to assess their adsorption and desorption performances. Adsorption was investigated at the natural conditions of the extract, and ethanol was used in the desorption experiments. High performance liquid chromatography with UV detection was used to quantify the performance of the retention and elution steps.
Overall, resins PAD900 and MN202 provided better results in terms of polyphenols recovery, with values close to 100% for several target polyphenols.

B39. Recovery of low and high molecular weight phenolic compounds endowed with potent antioxidant properties from agri-food byproducts by an ecofriendly and sequential two-steps protocol

Rita Argenziano, Federica Moccia, Iolanda Corrado, Cinzia Pezzella, Lucia Panzella, Alessandra Napolitano

Department of Chemical Sciences, University of Naples "Federico II", Via Cintia 4, I-80126, Naples, Italy

The agri-food industry is responsible for the generation of high volumes of organic wastes, the disposal of which entails a significant cost to the food processor and a negative impact on the environment. On the other hand, these materials represent a sustainable and easily available source of antioxidant phenolic compounds.[1] Remarkable examples of largely produced agri-food byproducts rich in low- and high-molecular-weight phenolic compounds include spent coffee grounds (SCG) and pomegranate peel and seeds (PPS). On this basis, a sequential two-steps conventional/deep eutectic solvent (DES)-based treatment is here reported as an efficient strategy for the recovery of antioxidant low- and high-molecular-weight phenolic compounds from a defatted SCG (dSCG) and PPS. In particular, a first treatment was performed using conventional solvents at lower temperatures. Ellagic acid, punicalagin, chlorogenic acid and caffein were identified as low molecular weight phenolic component of PPS and dSCG extracts, respectively. In the second step, the residual solids obtained after the first treatment were used as starting materials for the subsequent experiments. Based on a procedure previously reported in the literature,[2] different DESs at 120 °C for 24 h were explored, which allowed the researchers to obtain solid samples characterized by high-molecular-weight phenolic compounds, such as lignin, as confirmed via spectroscopic and chromatographic characterization. The recovered high- and low-molecular-weight phenolic compound samples exhibited remarkable antioxidant properties as evaluated using two widely used assay such as DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and FRAP (ferric-reducing antioxidant power) assays and a high phenolic content as determined through TPC (total phenolic content) assay. The proposed eco-friendly approach allows the full exploitation of selected agri-food by-products for the recovery of high-value phenolic compounds at high antioxidant activity properties for applications in the biomedical, food, and/or cosmetic fields.

References:
1. S. Ganesh, A. Sridhar, S. Vishali; *Chemosphere*, 287, **2022**, 132221.
2. R. Argenziano, F. Moccia, R. Esposito, G. D'Errico, L. Panzella, A. Napolitano; *Antioxidants*, 11(10), **2022**, 1860.

B40. Potential human health-enhancing effect of sustainable "high fibre" and antioxidant breads made with the addition of Brewer's spent grain

Jessica Baez, Victoria Olt, Adriana Maite Fernández-Fernández, Alejandra Medrano

Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República, Uruguay

Following the sustainable food and health world trends which encourage the application of the circular economy concept, the use of industry by-products as a source of bioactive compounds for the prevention and/or treatment of chronic diseases is being re-evaluated. The study of by-products such as brewer's spent grain (BSG) as a source of fibre and phenolic compounds presents an alternative to the use of traditional ingredients in the preparation of baked goods. This work studies the potential antioxidant effect of the bioaccessible and colonic fractions of the BSG bread optimized in previous studies.

The in vitro simulation of the digestion was carried out on BSG, the bread with BSG (BBSG) and a control bread without BSG (CB) using the INFOGEST method. The antioxidant capacity was determined through the ABTS and ORAC-FL methods, and the total polyphenol content (TPC) with Folin–Ciocalteu. Inhibition of intracellular reactive oxygen species (ROS) formation of the bioaccessible and colonic fractions was assessed on Caco-2 intestinal cells under oxidation-induced conditions (AAPH, 1mM).

BBSG showed an increase in TPC and antioxidant capacity compared to CB. After digestive simulation, BBSG showed a decrease in TPC but an increase in antioxidant capacity, which could be due to the release during digestion of other compounds (e.g., peptides) with antioxidant activity. BSG bioaccessible and colonic fractions presented inhibition of ROS formation at the tested concentrations (1/200-1/75 and 0.5-10 mg/mL, respectively), showing the lowest ROS formation at 1/75 (13.0±2.2%) and 10 mg/mL (16.2±5.5%). The colonic fractions of BBSG showed inhibition of ROS formation at the highest tested concentration (0.5-15 mg/mL) when compared to the control (DMEM with AAPH, 1mM).

By verifying the bioactive properties at the cellular level and through in vitro determinations after the digestion of BBSG, it is concluded that BSG bioactive compounds present in the bread have a potential antioxidant effect.

B41. Sustainable methodologies of different types of sweet potatoes (*Ipomoea batatas* L.) peel extractions as a source of bioactive compounds

Bruna Moreira, Cristina Caleja, Juan Manuel Castagnini, Francisco J. Barba, Lillian Barros

University of Vigo, Spain

According to the cultivars, *Ipomoea batatas* L. (sweet potatoes) have different quantity of bioactive compounds as β-carotene, anthocyanins, phenolics, dietary fiber, vitamins, minerals, and other bioactive compounds [1]. This tuberous root also received the attention of the food industry, considering that the peel contains stronger antioxidant activity than the flesh part [2]. For this reason, sustainable methodologies may be the best technique to improve extraction efficiency, stability of phenolic compounds, and also be an eco-friendly method [3]. The objective of this work was to evaluate the content of phenolic compounds and flavonoids in purple and orange sweet potato peel using five different types of extractions methodologies (conventional (water and agitation), pulsed electric field (PEF), supercritical carbon dioxide extraction (SC- CO2), conventional combined with SC-CO2 and PEF combined with SC-CO2). The results showed that for both of analyses, total phenolic and flavonoids content, the methodology that had the maximum yield of phenolic substances was conventional combined with SC-CO2, extracting 10.91mg GAE/g DW and 7.72 mg GAE/g DW for the purple and orange peel, respectively. For the total flavonoids content, purple sweet potato peel showed best results, being 5.88 mg CTH/g DW, against 4.44 mg CTH/g DW for the orange peel. All these results confirmed that emerging techniques are best suited for the development of the sustainable production process for the food industry, being the best to maximize the extraction of bioactive compounds, such as phenolics and flavonoids.

B42. Genotoxicity Assessment of Nutraceuticals Containing Natural Antioxidants and Bioactive Molecules Extracted from Thinned Nectarine (*Prunus persica L.*) and Grape Seed (*Vitis Vinifera L.*) Waste Biomasses

Giorgia Musto[1], Elisabetta Schiano[1], Fortuna Iannuzzo[1], Gian Carlo Tenore[1], Ettore Novellino[2], Mariano Stornaiuolo[3]

1 Department of Pharmacy, University of Naples Federico II
2 Department of Medicine and Surgery, Università Cattolica del Sacro Cuore, Rome, Italy
3 Department of Pharmacy University of Napoli Federico II, Napoli Italy Via Domenico Montesano 49 80131 Napoli Italy Phone +39 081 678117

Several pharmaceutical companies are nowadays considering the use of agri-food waste as an alternative raw material for the extraction of bioactive compounds and antioxidants to include in nutraceuticals and food supplements. This recycling activity is encountering the support of authorities, who are alarmed by the air, soil, and water pollution generated by agricultural waste disposal. Waste reuse has several economic advantages: (i) its low cost; (ii) its abundance; (iii) the high content of bioactive molecules; and (iv) the financial support received by governments eager to promote eco-compatible and pollution-reducing practices.

Grapes and nectarines are widely cultivated fruits in the Mediterranean basin, and their agricultural processing is responsible for the production of a large amount of bio-waste. The reuse of this food biomass has become an important source of phytochemicals (polyphenols from grapes and abscisic acid from nectarines), used to produce dietary supplements, functional foods, and food additives for human consumption.

While nutraceuticals produced from biowaste are becoming popular, products that have been risk-assessed in terms of safety are quite rare. This, in virtue of the chemical complexity of waste biomass, could, in many cases, undermine the overall safety of the final product. Developers and producers of nutraceuticals are thus advised to assess the safety of their final nutraceutical products, in compliance with European Food Safety Authority regulations.

Here, we use the Ames test (OECD 471) and the Micronucleus test (OECD 487) to assess the mutagenicity of two nutraceuticals obtained from thinned nectarine (*Prunus persica L.*) and grape seed (*Vitis Vinifera L.*) waste biomasses: the results show that these nutraceuticals are genotoxically safe and, actually, anti-mutagenic, confirming the safety of the products.

B43. Using Natural Deep Eutectic Solvents to extract phenolics from in vitro cultures of the Mediterranean plant *Thymus lotocephalus* produced in response to abiotic factors

Inês Mansinhos[1], Sandra Gonçalves[1], Raquel Rodríguez-Solana[1, 2], Anabela Romano[1]

1 MED – Mediterranean Institute for Agriculture, Environment and Development; CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
2 Department of Agroindustry and Food Quality, Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Avenida Menendez-Pidal, SN, 14004 Córdoba, Spain

Climate change is affecting every region on a global scale, and the Mediterranean is one of the regions with the most potential of being affected. In this context, adverse environmental conditions are a big threat faced by plants. In vitro simulation of abiotic stress conditions is an adequate approach to investigate plants' responses to those factors because it is performed under controlled conditions and is free of pathogens, allowing plant material production on a large scale and without compromising species conservation. The main goal of this study was to evaluate the impact of different abiotic stress conditions on phenolic compounds production and antioxidant activity of green extracts from cultures of *Thymus lotocephalus* López & Morales, an endemic species from the Algarve region (south of Portugal). Cultures were produced in vitro in stressful conditions of drought (2, 5, and 7% polyethylene glycol), temperature, (15 and 30°C), UV-B radiation (4 h/1 day; 4 h/4 days), and nutrients deficiency [nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), and iron (Fe)]. Cultures were used to prepare different extracts via ultrasound-assisted extraction with a natural deep eutectic solvent and the phenolic contents and antioxidant activity were determined. It was observed that temperature and Fe, P, and Mg deficiencies increase the accumulation of phenolics and antioxidant activity evaluated using different chemical assays (DPPH, FRAP, ABTS, and ORAC). In contrast, simulated drought conditions decreased both phenolics production and antioxidant activity. The results of this investigation provide new insights into the involvement of phenolics in response to adverse environmental conditions and the impact of those conditions on the potential application of nontoxic green extracts from *T. lotocephalus*.

B44. Is the future of antioxidants mineral? Nanozymes and other nanotechnology solutions

Victor Puntes

1 Vall d'Hebron Research Instiute (VHIR), 08035 Barcelona, Spain
2 Instiut Català de Nanociència I Nanotecnologia (ICN2), CSIC, The Barcelona Institute of Science and Technology (BIST), Campus UAB, Bellaterra, 08193 Barcelona, Spain
3 Institució Catalana de Recerca I Estudis Avançats (ICREA), 08010 Barcelona, Spain
4 Networking Research Centre for Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Instituto de Salud Carlos III, Madrid, Spain

The role of antioxidants in biology became popular in the second half of the 20th century at times when Linus Pauling (1954 and 1962 Nobel laureate) worked on the so-called orthomolecular medicine, based on nutritional supplementation and high doses of ascorbic acid. It bumped again in the 90s, as consequence of a large human study suggesting that vitamin E supplements could be associated with a reduced risk of heart diseases. During these years, other works, basically pre-clinical and epidemiological, also reported beneficial effects of antioxidant substances in chronic inflammation, neurodegeneration, and cancer. As a consequence of that, antioxidant therapies were evaluated in placebo-controlled trials involving tens of thousands of patients and, despite pathophysiologic, epidemiologic, and mechanistic compelling evidence, these clinical trials have been, to date, mostly negative. This has given rise to a pessimistic view on antioxidant therapies. This has been attributed to the non-drug-likeness of available antioxidant compounds. These compounds have high unspecific uncontrolled reactivity, poor solubility, and hence limited absorption profiles, low bioavailability and low concentrations at the target site. During this time, nanomaterials has been proposed for use in treating human diseases, primarily as drug delivery agents, showing potential benefits in terms of pharmaceutical flexibility, selectivity, dose reduction, and minimization of adverse effects. Thus, efforts have been made towards loading antioxidant molecules such us coenzyme Q10, vitamin E and vitamin A, resveratrol and polyphenols, curcumin, lycopene, silymarin, and superoxide dismutase in nanocarriers such as liposomes, polymeric NPs, lipid NPs, and self-emulsifying systems. More recently, nanotechnology has shown us how rare earth mineral antioxidant NPs, especially cerium oxide NPs, nanoceria, are powerful antioxidant and consequent anti-inflammatory agents that can treat many inflammation-related diseases. This is a new paradigm, where the nanoparticle itself, thanks to its nanometric form and high concentration of oxygen vacancies at its surface, is the active principle, not a vehicle. Interestingly, nanoceria is safe, xenobiotic, and highly traceable material.

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