September 19-20, 2024

University of Milan, Italy

Abstracts Book

Polyphenols
APPLICATIONS

17th World Congress on Polyphenols Applications

September 19 – 20, 2024

Milan & Online

Prof. Jan Frederik Stevens

President of Polyphenols Applications World Congress

Oregon State University, USA

Prof. Andreas Schieber

Vice-President of Polyphenols Applications World Congress

University of Bonn, Germany

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Welcome to Polyphenols Applications 2024

Dear Colleagues,

It is a great pleasure to welcome all of you to our 17th World Congress on Polyphenols Applications, which will be held on September 19-20, 2024, at Università degli Studi di Milano Statale, Milan, Italy.

Polyphenols Applications 2024 aims to bring together experts from academia and industry to discuss the latest scientific advancements in fundamental and applied research on polyphenols. This year the conference will feature a balanced mix of invited talks and short talks, based on suggestions from the audience at the previous year's conference.

The conference will cover various topics, including the latest advancements in polyphenol research, focusing on their health benefits, interactions with microbiota, and applications in food processing. Sessions will cover diverse topics, including the potential of anthocyanin-rich extracts in chemotherapy, the antiviral activity of polyphenols, and their impact on obesity and diabetes.

We would like to thank all the speakers and scientific committee members of Polyphenols Applications for their excellent contributions.

We also wish to thank the Local Organizing Committee: Sabrina Dallavalle, Andrea Pinto, Cristian Del Bo', and Daniela Martini from the University of Milan.

Finally, we are grateful for the support of our sponsor HealthTech Bioactives (HTBA).

We hope that you will enjoy the Polyphenols Applications 2024 Congress and that your interactions with colleagues from many countries will stimulate a creative exchange of ideas and challenges.

Prof. Jan Frederik Stevens
President of Polyphenols Applications
Oregon State University, USA

DOI Information

We are pleased to announce that the *Polyphenols Applications 2024 Abstracts Book* will be assigned a **DOI (Digital Object Identifier)**, ensuring a permanent and easily accessible online presence for the entire collection of work.

The DOI will be activated **after the congress**, and it will be communicated to all attendees.

How to cite your paper in the Abstracts Book?

To cite a paper presented at the *Polyphenols Applications 2024 Congress*, include the author's name, the conference date, the paper title (italicized), page number, the conference name, location, and DOI.

Citation example:

Smith, J. (2024, September 19–20). **The impact of polyphenols on cardiovascular health**. *p. 45*, Polyphenols Applications 2024, Milan, Italy. DOI

Practical Information

We would like to take the opportunity to give you some additional information about the meeting arrangements.

The Abstract book contains:
• Speakers' abstracts (the abstracts of the oral presentations follow the order of the program)
• The abstracts of posters on display

Badges

Upon registration you have received your own personal badge. Please wear this badge during the entire meeting including the coffee breaks and lunch.

Instructions for participants

Chairpersons: The Chairpersons will be seated at the president's table.

Speakers: Speakers are invited to give their Power Point presentations for downloading on the computer to the technical team outside and not inside the conference hall. As the schedule is rather tight and to allow sufficient time for discussions, we would be very much obliged if the timing requirements were respected.

Poster Contributors: Please ensure that your poster is displayed at the appropriate location, please respect your poster number. Posters will be divided between day 1 and day 2. Please bring your posters accordingly and fix/remove them according to the day of presentation. The Poster contributors are invited to stand by their poster during the poster sessions.

Speakers Dinner
A dinner is organized on September 19 at Metropolitan Restaurant of Crowne Plaza Milan City Hotel.
If you registered for this dinner, please join the group at 20h00 at the restaurant.

Mobile Phones
As a courtesy to the speakers and other delegates, please turn off your mobile phones or to silent whilst in the conference room. Please do not take pictures of the slides without the consent of the presenting author.

Questions
Please state your name and institution or company before asking your question.

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Staff at the conference registration desk will be happy to deal with any queries you may have. If we receive any messages for you, they will be announced at the break in the session and can be collected from the desk.

Personal Belongings
Please keep your valuables and working materials with you at all times. We would advise you to keep your name on the conference notes, as we may not be able to replace these if lost. Università degli Studi di Milano Statale and Polyphenols Applications can't be held responsible for any loss or damage to your belongings.

17th World Congress on

Polyphenols Applications

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17th World Congress on

Polyphenols Applications

Abstracts for Oral Presentations

Day 1 – September 19, 2024

WHY DEMONSTRATING THE HEALTH BENEFITS OF POLYPHENOLS ISN'T EASY: CONSIDERATIONS ON EXPERIMENTAL DESIGN TO ENHANCE THE IMPACT OF FUTURE POLYPHENOLS RESEARCH

DEL RIO, Daniele

University of Parma, Italy

daniele.delrio@unipr.it

In recent years, epidemiological studies have associated (poly)phenol intake with beneficial health effects on cardiovascular diseases, metabolic diseases, and some types of cancer. However, divergent responses have led to controversial results in many trials, implying that, even after all these years of research, there must be something wrong in the approach applied to explore the potential beneficial effects attributed to dietary phenolic compounds.

This opening lecture will discuss some of the good rules that should be used (but are, indeed, only applied sometimes in polyphenol research) to try and finally unravel the health benefits of these much-praised dietary components.

TOWARDS THE POTENCY OF ANTHOCYANIN-RICH EXTRACTS TO ALLEVIATE SIDE EFFECTS OF CHEMOTHERAPY

MARKO, Doris

Department of Food Chemistry and Toxicology, University of Vienna, Austria

doris.marko@univie.ac.at

Introduction: Colorectal cancer (CRC) represent the third most frequent cancer in western countries. Chemotherapeutic treatment schemes often comprise the topoisomerase poison irinotecan (CPT-11). Topoisomerase poisons stabilize the covalent DNA-topoisomerase intermediate, which is formed during the catalytic cycle of topoisomerases to regulate torsion stress in the DNA and enable crucial processes such as DNA transcription, replication and repair. In proliferating cells, stabilisation of the DNA-topoisomerase intermediates leads to collision of these complexes with the replication fork and DNA damage. As a negative side effect, also non-tumorigenic proliferative tissue such as the intestinal epithelium can be impaired. In vitro studies identified anthocyanins as potential catalytic topoisomerase inhibitors. Catalytic topoisomerase inhibitors prevent the enzyme from the formation of covalent DNA complexes, thus diminishing the toxic impact of therapeutic topoisomerase poisons. Thus, the question arises whether anthocyanin-rich preparations might be used to protect the intestinal tissue without compromising the therapeutic outcome.

Material & Methods: In the present study, anthocyanin-rich extracts of the four berries, blackberry (*Rubus fructicosus*), bilberry (*Vaccinium myrtillus*), black currant *(Ribes nigrum*), and elderberry (*Sambucus nigra*), were produced from natural berry juices and analyzed with respect to their polyphenol profiles by LC-MS/MS. The extracts were applied by oral gavage to Balb/c mice bearing CT-26 murine colon carcinoma tumors in different schedules. Chemotherapeutic treatment was performed by i.p. application of CPT-11. Chemotherapeutic efficacy, the onset of adverse side effects and impact on ADME parameters were determined in different application schedule combinations.

Results: Several berry species such as bilberry, blackberry, elderberry and black currant are known as rich sources for anthocyanins, but differ substantially in their respective anthocyanin profile. Within the huge class of polyphenols, anthocyanins possess unique structural elements, requiring a low pH to ensure respective stability. To overcome this problem, anthocyanin-rich preparations are usually acidified prior to application in vivo. One possibility is the acidification with citric acid, an often used strategy in studies comprising anthocyanins. Indeed, in BALB/C mice, the systemic bioavailability of anthocyanins was clearly enhanced by oral application of anthocyanin-rich extracts in citric acid compared to sodium chloride solution. However, in the present study we clearly demonstrated that the application of citric acid to tumor-bearing (CT26 murine colon carcinoma) BALB/C mice stabilized co-applied anthocyanins but citric acid per se strongly suppressed tumor growth. Of note, the tumor suppressing activity of citric acid was partly reverted by co-application of a blackberry extract. Moreover, the application of the blackberry extract was not well tolerated by the animals and substantial toxicity was observed irrespective of application of the extract in citric acid or sodium chloride solution. These results question a potential health benefit of blackberry-based preparations as food supplements. In contrast, oral application of elderberry extract was well tolerated by the animals. However, in combination with chemotherapy (CPT-11, ip) substantial impact on the therapeutic efficacy of the drug was observed.

Conclusion: Taken together, the study underlines the potential of polyphenol-rich food supplements to interfere with chemotherapeutic cancer treatment and the necessity to eventually control or even prevent respective consumption in patients under acute treatment.

Supported by the Austrian Science Fund (FWF, project number P 31805).

ANTIVIRAL ACTIVITY OF POLYPHENOLS: LATEST DEVELOPMENTS

VENTURELLI, Sascha

University of Hohenheim, Germany

sascha.venturelli@uni-hohenheim.de

The events surrounding the COVID-19 pandemic have made it clear how quickly viruses can spread globally, placing a significant burden on the healthcare system. Thus, antiviral agents represent an important complement to vaccination. The administration of therapeutic antivirals is particularly intended for patients who are already infected or do not have adequate vaccination protection. Natural active ingredients that either strengthen the body's own immune system as well as active ingredients that have direct antiviral activity are of particular medical interest. The very large and heterogeneous chemical group of polyphenols contains some promising compounds with these desired biochemical properties. From a medical and nutritional point of view, the subgroup of prenylated hop polyphenols is particularly attractive, because it includes bioactive food ingredients generally well tolerated by the human body.

In this context, various antiviral molecular properties have been described for the chalcone xanthohumol, which is a prenylated plant polyphenol of hops that can be converted into the prenylated derivatives isoxanthohumol, 6- and 8-prenylnaringenin, among others, during the brewing process. For instance, xanthohumol and some of its derivatives showed significant antiviral activity against the positive-sense RNA viruses hepatitis C virus (HCV) or the severe acute respiratory syndrome coronavirus (SARS-CoV-2), which are classified as highly pathogenic to humans. With regard to the replication cycle of HCV, a molecular inhibition of the assembly of HCV virions has been described for xanthohumol, which ultimately leads to a reduction in virus production. In regards to SARS-CoV-2, inhibition of a viral peptidase, which is essential for the processing of the viral polyprotein and thus the formation of infectious virus particles, could be demonstrated for xanthohumol and, in part, its derivatives. This interaction with the viral enzyme ultimately leads to a reduction in viral replication. Such examples show how natural polyphenols could be used directly or as chemical backbones against therapeutically challenging viral human diseases such as HCV or SARS-CoV-2 infections.

POLYPHENOLS AND THE ENTEROENDOCRINE SYSTEM: IMPLICATIONS FOR OBESITY

ARDEVOL, Anna, PINENT, Montserrat, TERRA, Ximena, BLAY, Teresa, BELTRAN-DEBÓN, Raúl and RODRIGUEZ-GALLEGO, Esther

MoBioFood Research Group, Departament Bioquímica i Biotecnologia, Universitat Rovira i Virgili, Tarragona, Spain

anna.ardevol@urv.cat

Obesity remains a persistent and growing health challenge worldwide. While polyphenol consumption has demonstrated efficacy in both preventing and alleviating obesity, the underlying mechanisms of action are not fully understood [1]. Polyphenols are widely recognized for their health-promoting effects, despite their low bioavailability. It is hypothesized that some effects arise from the absorbed forms of polyphenols, while others may result from interactions between non-absorbed compounds and structures within the gastrointestinal tract. Our research supports the hypothesis that polyphenols interact with the enteroendocrine system, which plays a critical role in regulating food intake and metabolic health.

Our studies have specifically shown that grape-seed derived procyanidins (GSPE) reduce food intake through modulation of the enteroendocrine system. In experiments with female Wistar rats, acute administration of GSPE resulted in a 30% reduction in food intake when the rats were offered a palatable diet. This reduction was associated with increased secretion of GLP-1, insulin, and ghrelin. The administration of Exendin, a GLP-1R antagonist, partially prevented this effect, highlighting the role of GLP-1 in mediating the GSPE response [2]. Subchronic GSPE treatment also reduced active ghrelin levels in the plasma and downregulated ghrelin gene expression in the stomach[3]. Additionally, in a model of diet-induced obesity using cafeteria-induced obese female rats, a 15-day GSPE treatment reduced food intake, increased ghrelin and CCK accumulation in the stomach and duodenum, decreased PYY gene expression in the ileum, and modulated GLP-1 and PYY expression and secretion in the colon [4].

We further hypothesize that the effects of GSPE on the enteroendocrine system may be mediated by the interaction of polyphenols with nutrient-sensing proteins in the intestinal lumen. Some flavanols, for example, have been identified as ligands for bitter taste receptors [5]. Our research demonstrated that epicatechin, an agonist of TAS2R5, increased portal GLP-1 levels and reduced food intake in rats, similarly to the effects of Phenantholine, a known TAS2R5 agonist [6]. Moreover, epicatechin gallate modulated ghrelin secretion in MGN3-1 ghrelinoma cells, an effect attenuated by TAS2R antagonists [3]. Additionally, epicatechin (a TAS2R5 agonist) and vanillic acid (a TAS2R14 agonist) differentially induced GLP-1 and PYY secretion in human Hutu-80 cells.

In conclusion, our findings suggest that specific polyphenolic compounds can modulate enterohormone secretion by interacting with bitter taste receptors in the intestinal wall, with effects varying across different intestinal segments. These signaling pathways likely contribute to the health-promoting effects of polyphenols in preventing metabolic disruptions associated with diet-induced obesity. Further elucidation of these mechanisms may guide the development of new treatments for obesity and related metabolic disorders.

Supported by Universitat Rovira I Virgili, Generalitat de Catalunya, Ministerio de Ciencia SPAIN.

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EFFECTS OF POLYPHENOLS ON DIABETES: A MECHANISTIC INSIGHT

RAMOS, Sonia [1, 2]

[1] Department of Metabolism and Nutrition, Institute of Food Science and Technology and Nutrition, Spain

[2] Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas, Spain

s.ramos@ictan.csic.es

Introduction: Diabetic cardiomyopathy (DCM) is a serious complication that accounts for more than half of diabetes-related morbidity and mortality cases, but an appropriate treatment is still lacking. Metformin (MET) is the primary treatment for type 2 diabetes, and flavonoids have been proposed as safe agents to alleviate DCM. This study investigates the effects of (-)-epicatechin (EC), the flavonoid-derived colonic metabolite 2,3-dihydroxybenzoic acid (DHBA), MET, and their combination (MIX) under glucolipotoxic conditions.

Material & Methods: H9c2 cardiomyocytes were exposed to EC, DHBA, MET, or a mixture of all previous compounds (MIX) under a simulated diabetic millieu. ROS generation, GSH and caspase-3 activity were assessed by fluorescence. JNK, Beclin-1, p62, LC3, Bax and Bcl-2 levels were analysed by Western blot.

Results: Under glucolipotoxic conditions, EC, DHBA, MET, and MIX improved redox status and autophagy, and reduced apoptosis. All treatments reinforced antioxidant defenses, inhibited reactive oxygen species generation, and decreased JNK phosphorylation, thereby alleviating oxidative stress. They also reduce apoptosis and activate autophagy by modulating Bax, caspase-3, p62, Bcl-2, beclin-1, and LC3-II/LC3-I.

Conclusion: EC and DHBA provide similar benefits to MET in reducing cardiomyocyte damage by improving antioxidant capacity, autophagy, and apoptosis, but no additional effects were observed when combined with MET.

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INTERPLAY BETWEEN PHENOLIC COMPOUNDS AND BIOLOGICAL RHYTHMS

MUGUERZA, Begoña

Universitat Rovira i Virgili, Spain

begona.muguerza@urv.cat

The bioavailability and bioactivity of (poly)phenols depend on external and host factors. In addition, our results also show that the time at which (poly)phenols are consumed must be considered when investigating their effects. Hence, the time factor arises as a determinant factor of the effect of diet on health, reinforcing the importance of biological rhythms and chrononutrition, a relatively new discipline that has gained great importance in recent years. Moreover, the interaction between phenolic compounds and biological rhythms seems to be bidirectional, as some of them have demonstrated their activity as modulators of circadian and seasonal rhythms.

In this regard, our group has demonstrated that proanthocyanidins can modulate central and peripheral biological rhythms in both healthy and obese rats, and the interaction of these phenolic compounds with the clock system could be a potential mechanism for their beneficial effects. Therefore, there is a bidirectional interaction between dietary phenolic compounds and biological rhythms, and the time of the day and season of the year in which they are consumed influence their health effects, which, in turn, could be mediated by the interaction of these compounds with the clock system, acting as synchronizers.

FLAVONOL AND HYDROXYBENZOIC ACID INTAKE IS PROSPECTIVELY ASSOCIATED WITH DEPRESSIVE SYMPTOMS IN YOUNG AUSTRALIAN ADULTS

GAMAGE, Elizabeth [1]; LANE, Melissa M [1]; MOHEBBI, Mohammadreza [2]; LOTFALIANY, Mojtaba [3]; ASHTREE, Deborah N [1]; JACKA, Felice N [1]; ORR, Rebecca [1]; O'NEIL, Adrienne [1]; DAWSON, Samantha [1]; MARX, Wolfgang [1]

[1] Institute for Mental and Physical Health and Clinical Translation, Food & Mood Centre, School of Medicine, Deakin University, Barwon Health, Geelong, Victoria, Australia, 3220;
[2] Biostatistics Unit, Faculty of Health, Deakin University, Melbourne, Australia;
[3] Institute for Mental and Physical Health and Clinical Translation, School of Medicine, Deakin University, Geelong, Australia

egamage@deakin.edu.au

Introduction: Research suggests an association between polyphenols and depression; however, this association is relatively unexplored in younger populations. As such, the aim of this study was to assess the prospective association between polyphenol intake and depressive symptoms in young adults.

Methods: Data from the Raine Study's Generation 2 20-, 22-, and 27-year follow-ups were used (N = 1,484). Polyphenol intake was estimated using Phenol-Explorer. Energy-adjusted polyphenol intake was categorised into quartiles. Depressive symptoms were assessed via the 21-item Depression, Anxiety, and Stress Scale. Linear mixed-effects models were used and sociodemographic characteristics and lifestyle- and health-related behaviours were adjusted for.

Results: Participants in the highest quartiles for flavonol and hydroxybenzoic acid intake had lower depressive symptoms than participants in the lowest quartiles [flavonols (Q4 v Q1 mean difference: -1.41, 95%CI: -2.51, -0.31); hydroxybenzoic acids (Q4 v Q1: -1.42, CI: -2.54, -0.29)]. We found no evidence of an association for total polyphenols, and other polyphenol classes and subclasses.

Conclusion: These results suggest that the intake of some polyphenol subclasses may be useful targets for novel prevention strategies for depression. However, further mechanistic studies in human populations, and prospective studies in young adults and across the lifespan are required.

Gamage E, Orr R, Travica N, Lane MM, Dissanayaka T, Kim JH, et al. Neuroscience & Biobehavioral Reviews. 2023;151:105225

INCREASED RISK FACTORS OF CARDIOMETABOLIC DISEASES ARE ASSOCIATED WITH LOW-POLYPHENOLS INTAKE AND PRO-INFLAMMATORY DIETS

LARA GUZMÁN, Óscar Javier; CORRALES AGUDELO, Vanessa; ESCOBAR RESTREPO, Juan Sebastian; MUÑOZ DURANGO, Katalina; SIERRA RESTREPO, Jelver

Vidarium–Nutrition, Health, and Wellness Research Center, Colombia

ojlara@serviciosnutresa.com

Introduction: Pro-inflammatory diets have been linked to poorer cardiometabolic outcomes and higher mortality rates. Dietary polyphenols, known for their anti-inflammatory properties, have been associated with enhanced metabolic health. We evaluated the association among cardiometabolic risk factors, and gut microbiota composition with diet quality as measured by the energy-adjusted dietary inflammatory index (E-DII), and polyphenol intake.

Material & Methods: Diets were assessed through 24-hour dietary recalls in a cohort of 459 Colombian subjects (51.6% female) aged 18-62 years. Foods and polyphenol intake were analyzed using a custom database integrating data from USDA and Phenol-Explorer v3.6.

Results: Total polyphenols intake averaged 1168 ± 914 mg/day. Men consumed more polyphenols than women (1067.5 vs. 1311.7 mg). Individuals with medium or high polyphenols intake (>1000 mg/day) and a low E-DII (<0.1) exhibited lower BMI (<27 kg/m2), relative fat mass (<33.7%), leptin (<6.5 ng/mL), leptin/adiponectin (<1.3) and high-sensitivity C-reactive protein (<3 mg/L). In contrast, a low polyphenol intake was associated with poorer glucose tolerance, insulin resistance, and a gut microbiota featuring more potentially harmful bacteria.

Conclusion: Dietary patterns in Colombians were assessed using a customized database, enabling for a more accurate estimation of polyphenol intake, inflammatory potential of the diet, and their relationship with health.

Supported by Vidarium/Grupo empresarial Nutresa.

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COEVOLUTIONARY ROLE OF HOST AND GUT MICROBIOTA IN (POLY)PHENOL HEALTH EFFECTS: METABOTYPES AND PRECISION HEALTH

ESPÍN, Juan Carlos; JARRÍN-OROZCO, María Paula; OSUNA-GALISTEO, Leire; ÁVILA-GÁLVEZ, María Ángeles; ROMO-VAQUERO, María; SELMA, María Victoria

Laboratory of Food & Health; Research Group on Quality, Safety and Bioactivity of Plant Foods, CEBAS-CSIC, 30100 Murcia, Spain

jcespin@cebas.csic.es

Dietary (poly)phenols are non-nutritional xenobiotics (yes, we can live without them). Thus, the term "personalized nutrition," aiming to improve health outcomes for non-responders, is i) utopian and ii) unsuitable regarding (poly)phenols and health. Instead, "precision health," encompassing lifestyles, may be more suitable. (Poly)phenols can threaten the host (as antinutrients) and gut microbiota (as antimicrobials). To this end, evolution has reduced the bioavailability and reactivity of (poly)phenols to protect the host and its microbiota (promoting excretion, phase II conjugations, double bond reductions, ring cleavages, removal of hydroxyl groups, etc.). These processes vary among individuals, resulting in high interindividual variability, meaning different health effects depending on each person. Thus, the host and its gut microbiota determine (poly)phenol health effects through this coevolutionary adaptation. (Poly)phenols also exert prebiotic-like effects, primarily through their antimicrobial activities, usually reducing gut microbial diversity and modulating functionality by impacting microbial composition and transcriptomics. The gut microbiota also influences (poly)phenol effects by producing metabolites with different activities than their precursors. Moreover, producing distinct metabolites such as urolithins, lunularin, and equol gives rise to the term "polyphenol-related gut microbiota metabotypes." This concept allows for the stratification of individuals based on specific microbial metabolism of ellagic acid, resveratrol, and isoflavones, respectively, and potentially impacts health depending on an individual's metabotype. Remarkably, modulating gut microbial networks without altering composition is an emerging paradigm in the microbiota-mediated effects of (poly)phenols. Customizing metabotypes through probiotics or postbiotics can produce bioactive metabolites in "non-producers". However, 12 possible gut microbiota metabotype combinations exist with different microbial ecologies and responses to (poly)phenol consumption, increasing complexity.

Although our viewpoint is less conventional than considering (poly)phenols as essential components for human health, it underscores their significance in a coevolutionary partnership with the host and its microbiota. From our perspective on the interplay of gut microbiota and (poly)phenols, microbiota metabotypes could influence health outcomes. However, maximizing health benefits by targeting the gut microbiota with (poly)phenols seems rather utopian.

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Espín, J.C. et al. Perspective on the Coevolutionary Role of Host and Gut Microbiota in Polyphenol Health Effects: Metabotypes and Precision Health. Mol. Nutr. Food Res., 2024, (Submitted).

2-ENE REDUCTASES OF LACTOBACILLI, AN UNEXPECTED DIVERSITY OF ENZYMES FOR CONVERSION OF PHENOLIC COMPOUNDS

GÄNZLE, Michael G.

University of Alberta, Dept. of Agricultural, Food and Nutritional Science, Edmonton, AB, Canada

mgaenzle@ualberta.ca

2-Ene reductases are NADH dependent enzymes that reduce double bonds conjugated with an aromatic ring. The first 2-ene reductases that were characterized in lactobacilli are HcrB in *Lactiplantibacillus plantarum* and Par1 in *Furfurilactobacillus milii*, which reduce hydroxycinnamic acids to the corresponding dihydro derivatives. We biochemically characterized HcrF from *Limosilactobacillus fermentum* after cloning and purification. HcrF of *Lm. fermentum* reduced ferulic, caffeic, p-coumaric and sinapic acids but not trans-cinnamic acids to the corresponding dihydro derivatives.

Lactobacilli also reduce the corresponding double bond in cinnamaldehyde, quercetin, daidzein and genistin. Bioinformatic analyses revealed that plant-associated lactobacilli harbor multiple homologues to HcrB, Par1, the daidzein reductase of *Lactococcus lactis* and to YhfK, an enzyme in *Listeria monocytogenes* that is active on cinnamaldehyde. Specifically, genomes of *Companilactobacillus paralimentarius* FUA3121, *Furfurilactobacillus milii* FUA3430 and Lactiplantibacillus TMW1.460 harboured 5 or 6 homologues to biochemically characterized 2 ene reductases. Biochemical analyses revealed that cinnamaldehyde, genistin and quercetin but not isoquercetin, daidzin, daidzein or genistein were reduced to the corresponding dihydro-derivatives by one or more of the four strains.

On a phylogenetic tree of 2-ene reductases, HcrF clustered most closely with HcrB of *Lp. plantarum*, which is also active on hydroxycinnamic acids, and with a 2-ene reductase of Listeria monocytogenes with activity on cinnamaldehyde. The hydroxycinnamic acid reductase Par1 of *Ff. milii* and flavone or isoflavone reductases were only distantly related to HcrF.

In summary, the substrate specificity of 2-ene reductases appears to be mediated by the substitution pattern of the adjacent phenolic ring and current knowledge does not allow to predict the substrate specificity of 2-ene reductases on the basis of the protein sequence. The 2-ene reductases of lactobacilli thus represent an unexpected diversity of enzymes for conversion of phenolic compounds.

LACTOBACILLUS-FERMENTED FRUIT AND VEGETABLE JUICES ATTENUATE AMYLOID-BETA PROTEOTOXICITY IN AN ALZHEIMER'S DISEASE MODEL OF THE NEMATODE CAENORHABDITIS ELEGANS

ECKERT, Gunter Peter; HUBERT, Tim

Justus-Liebig University of Giessen, Germany

eckert@uni-giessen.de

Introduction: Polyphenols (PP) are important in the prevention of Alzheimer`s disease (AD). Recently, we demonstrated that phenolic metabolites including protocatechuic acid (PCA) enhance lifespan and mitochondrial function in *Caenorhabditis elegans*. To investigate effects of PP metabolites in Alzheimer`s disease (AD), we applied pre-fermented PP-mixtures (Rechtsregulate®, RR) to GMC101, a non-vertebrate AD model expressing Aβ1-42 in muscle cells. CI2122 served as controls.

Material & Methods: Phenolic content and antioxidative capacity of RR were determined using folin- and DPPH-assays. Lifespan and heat-stress resistance of nematodes were recorded. Paralysis was assed using the Wormtracker software FlyCapture2®. Aβ1-42 levels were determined using Western blot, Homogeneous-Time-Resolved, and Thioflavin- Fluorescence.

Results: RR show high contents of polyphenols and high antioxidative capacity. GMC101 expresses high Aβ1-42-levels, show reduced movement, life span, heat stress resistant, and enhanced paralysis compared to CI2122 controls. Heat-stress resistance and lifespan of C. elegans GMC101 were significantly improved after incubation with RR (5% & 10%) or PCA (780 µM). Disease specific effects were independent from beta-amyloid peptide expression since Aβ1-42 levels and aggregation were not affected by RR and PCA.

Conclusion: Overall, our study indicates that phenolic metabolites may play an important role in prevention and therapy of neurodegenerative diseases.

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Dilberger B, Passon M, Asseburg H, Silaidos CV, Schmitt F, Schmiedl T, Schieber A, Eckert GP. Nutrients. 2019 Aug 13;11(8). pii: E1886.

INTERPLAY BETWEEN GUT MICROBIOTA AND PHENOLIC COMPOUNDS IN COLON CANCER PREVENTION

CATTIVELLI, Alice; ZANNINI, Melissa; CONTE, Angela; TAGLIAZUCCHI, Davide

Nutritional Biochemistry Lab, Department of Life Sciences, University of Modena and Reggio Emilia, Italy

alice.cattivelli@unimore.it

Introduction: Colon cancer is a disease highly influenced by diet. Vegetable food-rich diets and phenolic compounds exert a positive effect on the onset and progression of this disease. This research aimed to investigate the interaction between dietary phenolic compounds and colon microbiota.

Material & Methods: Selected vegetable foods were in vitro digested and fermented through an in vitro colonic model. The produced metabolites were quantified by mass spectrometry, and microbiota changes were assessed by qPCR. Metabolites were tested for their antiproliferative activity against two colon adenocarcinoma cell lines (Caco-2 and SW480) and for their ability to modulate the cell cycle and induce apoptosis.

Results: Our results showed that phenolic compounds promoted an eubiosis condition by increasing the colon microflora related to a lower incidence of tumor occurrence. Moreover, phenolic compounds inhibited the growth of opportunistic bacterial species able to promote tumor development. Conversely, colon microbiota thoroughly metabolized phenolic compounds, generating few low molecular weight microbial metabolites. Several colonic metabolites inhibited Caco-2 and SW480 cell proliferation at physiological concentrations. Some of these metabolites acted by blocking the cell cycle, while others induced apoptosis.

Conclusion: The mutual interaction between phenolic compounds and microbiota may be pivotal in colon cancer prevention.

Università di Modena e Reggio Emilia project "Health implication of phenolic compounds: targeting hallmarks of colon cancer through phenolic-microbiota interplay" funded by Fondo di Ateneo per la Ricerca anno 2023 – Bando per il finanziamento di progetti di ricerca interdisciplinari.

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HUMULUS LUPULUS L. (HOPS) SPECIALIZED METABOLITES PHARMACOLOGICAL EFFECTS AND BINDING INTERACTIONS OF WITH GASTROINTESTINAL BITTER TASTE RECEPTORS

LELA, Ludovica [1]; PONTICELLI, Maria [1]; CARLUCCI, Vittorio [1]; FARAONE, Immacolata [1]; VERONICO, Claudia [1]; STEVENS, Jan Frederik [2,3]; RUSSO, Daniela [1,4]; TZVETKOV, Nikolay T [5]; **MILELLA, Luigi [1]**

[1] University of Basilicata, Italy
[2] Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR, United States
[3] Linus Pauling Institute, Oregon State University, Corvallis, OR, United States
[4] Spinoff Bioactiplant, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy
[5] Department of Biochemical Pharmacology & Drug Design, Bulgarian Acad. of Sciences, Sofia, Bulgaria

luigi.milella@unibas.it

Introduction: In recent years, several investigations have shown that gastrointestinal bitter taste receptors (Tas2rs) regulate anorexigenic peptide secretion, becoming potential targets for controlling food intake and the prevalence of obesity. Therefore, the present study aims to investigate the implication of hop bitter compounds such as α-acids, ß-acids, and xanthohumol as novel anti-obesity targets through intestinal Tas2rs activation.

Material & Methods: STC-1 cells were used to assess the biological activity. Cell viability was evaluated by MTT assay; GLP-1 and CCK by ELISA assay; bitter taste receptor expression was measured by qRT-PCR; intracellular calcium concentration by the fluorescent probe Fluo 4-AM; computational studies were used to establish the interation between the pure compounds and the murine or human isoforms of the bitter taste receptor Tas2r138.

Results: Hop compounds induced the secretion of the hormones GLP-1 and CCK consecutively to a selective increase of murine Tas2rs, particularly Tas2r138. This activity was further confirmed by the increase in intracellular calcium levels. Molecular docking experiments showed that all molecules could bind both murine and human Tas2r138 isoforms with estimated affinities ranging from nM to mid-μM.

Conclusion: This study provides an excellent basis for applying hop bitter molecules as lead compounds to further design gastrointestinal T2R agonists.

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EFFECT OF A POLYPHENOL AND FIBER-ENRICHED MATERNAL DIET ON IMMUNE RESPONSE PROGRAMMING OF WISTAR RATS

CASANOVA CRESPO, Sergi [1,2]; CEBALLOS SÁNCHEZ, Daniela [1,2]; RODRÍGUEZ LAGUNAS, María José [1,2]; MASSOT CLADERA, Malén [1,2]; CASTELL, Margarida [1,2,3]; PÉREZ CANO, Francisco José [1,2]

[1] Department of Biochemistry and Physiology, Faculty of Pharmacy and Food Science, University of Barcelona, Spain
[2] Institut of Research in Nutrition and Food Safety (INSA-UB), Barcelona, Spain
[3] Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, 28029 Madrid, Spain

sergi.casanova@ub.edu

Introduction: Polyphenols and fiber are dietary compounds characteristic of the Mediterranean diet that can modulate immune system functionality. This study aimed to assess how a polyphenol and fiber-enriched maternal diet modulates the immune response in offspring during adulthood.

Material & Methods: Female Wistar rats were fed either a diet enriched in polyphenols (0.5%) and fiber (9%) (FPD) or a standard diet (REF) for 9 weeks, including pregestational, gestational, and lactation periods. After weaning, offspring were fed the REF diet until the end of the study. At 7-weeks, they were immunized intraperitoneally with ovalbumin (OVA). Anti-OVA plasma antibodies were quantified throughout the study. Four weeks post-immunization, total plasma immunoglobulin (Ig) profile, spleen lymphocyte composition, intestinal microbiota and fecal variables were determined.

Results: The maternal FPD diet induced a stronger anti-OVA response in the offspring than the REF diet. Plasma Ig profile revealed a shift toward a lower Th1/Th2 ratio. In addition, an increased cytotoxic profile was observed in spleen lymphocytes. These effects were accompanied with changes in the intestinal microbiota diversity and taxonomic profile.

Conclusion: A maternal diet enriched in polyphenols and fiber enhances the long-term specific immune response of offspring, suggesting its role in terms of immunoprogramming.

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THE MOLECULAR BASIS OF THE DEGRADATION OF FLAVAN-3-OLS BY THE HUMAN GUT BACTERIUM EGGERTHELLA LENTA

HALIFA, Ruben [1]; CORNU, Agnes [2]; DUFOUR, Claire [3]; LE BOURVELLEC, Carine [3]; PEYRET, Pierre [1]; **MOSONI, Pascale [1]**

[1] UMR454 MEDIS, Université Clermont Auvergne, INRAE, Clermont-Ferrand, France
[2] UMR1213 Herbivores, VetAgro Sup-Université Clermont Auvergne, INRAE, Clermont-Ferrand, France
[3] UMR408 SQPOV, INRAE, Avignon Université, Avignon, France

pascale.mosoni@inrae.fr

Flavan-3-ols are among the most consumed polyphenols by humans and have been shown to prevent cardiovascular diseases. They are found in plant as monomers and mainly as oligomers such as procyanidins. The majority reaches the colon where the microbiota converts them into phenolic metabolites likely to participate in their health effects. While the metabolic pathways of the degradation of flavan-3-ols by the microbiota are relatively well described, only a few microorganisms and microbial genes involved in these pathways are known. We have previously shown that Eggerthella lenta metabolizes oligomers (to be published). Here, our aim was to identify E. lenta genes encoding enzymes degrading flavan-3-ols and to determine their prevalence in human gut metagenomes.

By a transcriptomic approach (RNAseq) carried out with the type strain of E. lenta, coupled with the heterologous expression of the genes of interest in Escherichia coli, we have discovered ten genes that constitute good markers of flavan-3-ol metabolization in the gut. Their prevalence in human gut metagenomes suggested that 27% of individuals cannot convert flavan-3-ols into potential bioactive metabolites. These results raise the question of whether individuals who do not harbor these bacterial genes are at greater risk of developing cardiovascular disease.

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THE IMPACT OF SELECTED MICROBIAL STARTERS ON THE POLYPHENOLS CONTENT IN FERMENTED VEGETABLE FOODS

BLEVE, Gianluca [1], TARANTINI Annamaria[1, 2], BAVARO Anna Rita[3], RAMIRES Francesca Anna[1], D'ANTUONO Isabella[3], CARDINALI Angela[3]

[1] Consiglio Nazionale delle Ricerche - Istituto di Scienze delle Produzioni Alimentari, Lecce, Italy
[2] Università di Bari, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (Di.S.S.P.A), Bari, Italy
[3] Consiglio Nazionale delle Ricerche - Istituto di Scienze delle Produzioni Alimentari, Bari, Italy

gianluca.bleve@ispa.cnr.it

Introduction: Many vegetable products are processed through fermentation. This strategy can improve the funtionality of nutrients, favoring the release of polyphenols important compounds for their health benefits, also for novel food manufacture. Microbial strains are chosen for their ability to transform vegetable raw materials into edible foods, allowing also the release of bioactive compounds, expecially polyphenols [1,2,3].

Material & Methods: Feedstocks (black kale, artichokes, table olives, strawberry tree fruits, onion skins, seaweeds, etc.) are pretreated and characterized for their composition and phenolic content/profile. Microrganisms are selected for their safety and thecnological aspects. Laboratory-scale fermentation experiments are set up for optimal operational conditions.

Results: In all tested vegetable samples, the efficacy of the optimized fermentation process are verified in terms of metabolic-associated traits and in the improvement of some nutritional aspects, such as polyphenols and antioxidant activity.

Conclusion: The microbial treatment of vegetable materials driven by selected Lactic Acid Bacteria and yeast strains, effective to produce foods for human consumption, can be considered as a valid strategy for enhancing the release of polyphenols by a sustainable and low-cost route.

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USING ARTIFICIAL INTELLIGENCE WHEN ASSESSING THE BIOAVAILABILITY AND CARDIOMETABOLIC IMPACT OF DIETARY (POLY)PHENOLS

BRAGAZZI, Nicola [1]; MIGNOGNA, Cristiana [1]; SÁNCHEZ-MARTÍNEZ, Lorena [1]; KOBAYASHI, Takuma [1,2]; DE LA O, Victor [3,4]; RINALDI DE ALVARENGA, José Fernando [1]; FAVARI, Claudia [1]; DEL BURGO-GUTIÉRREZ, Cristina [1]; TRECCANI, Mirko [1,5]; BRESCIANI, Letizia [1]; POTI', Francesco [6]; MARTÍNEZ, J. Alfredo [3,7]; DEL RIO, Daniele [1,8]; **MENA, Pedro [1,8]**

[1] Department of Food and Drug, University of Parma, Parma, Italy
[2] School of Integrated Pharmaceutical and Nutritional Sciences, University of Shizuoka, Shizuoka, Japan
[3] IMDEA-Food Institute (Madrid Institute for Advances Studies), Madrid, Spain
[4] Faculty of Health Sciences, International University of La Rioja (UNIR), Logroño, Spain
[5] Department of Surgery, Dentistry, Paediatrics and Gynaecology, University of Verona, Verona, Italy
[6] Department of Medicine and Surgery, University of Parma, Parma, Italy
[7] CIBEROBN-Instituto de Salud Carlos III, Madrid, Spain
[8] Microbiome Research Hub, University of Parma, Parma, Italy

pedromiguel.menaparreno@unipr.it

Introduction: Artificial intelligence (AI) tools may facilitate sound discoveries when assessing the bioavailability of (poly)phenols and their biological role in cardiometabolic health.

Material & Methods: Two studies, supported by AI-based tools, were conducted to evaluate the bioavailability of (poly)phenols and how these compounds may impact cardiometabolic health. The first study was a human trial devoted to understand (poly)phenol bioavailability upon consumption of an Oral (Poly)phenol Challenge Test by nearly 300 subjects, and how this relates to their cardiometabolic risk. The second was a comprehensive in vitro evaluation of the activity of coffee phenolic metabolites on HUVEC cells.

Results: In the human study, machine learning approaches were performed to discriminate metabolic phenotypes (metabotypes). Specifically, after massive clustering testing, a sparse k-means clustering model was applied to classify 298 deep-phenotyped subjects into two main groups. Multivariate models were used to define the factors contributing to the formation of the metabotypes and their associated cardiovascular risk. In the cell study, nitric oxide response was assessed using a Generalized Additive Model (GAM) to capture non-linear patterns.

Conclusion: These approaches demonstrate that AI techniques can serve to personalise dietary recommendations based on individual responses to (poly)phenols, and to identify complex patterns associated with their bioactivity.

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HYDROXYCINNAMIC ACIDS AND RELATED POLYAMINES IN CEREAL GRAINS: CHEMICAL DIVERSITY AND IMPLICATIONS OF PHENOLAMIDES IN HUMAN HEALTH

BETA, Trust; NGUYEN, Si Nhat

University of Manitoba, Canada

Trust.Beta@umanitoba.ca

Introduction: Polyamines, molecules with multiple amino groups, and phenolamides, products of polyamines and hydroxycinnamic acids conjugation, play crucial roles in plant physiology. This work examines their roles in cereals and implications for human health, focusing on current research on polyamines in barley grains.

Materials and Methods: Polyamines were extracted from barley and other cereal grains using acidic solutions, followed by pre-column derivatization and chromatographic analysis. Their relationship with barley protein content was explored due to their involvement in amino acid metabolism. Dehulling and pearling of barley cultivars were conducted to assess polyamine distribution within the kernel. Phenolamides were identified in methanolic extracts of barley and sorghum flour with other phenolics using chromatographic and mass spectrometric techniques.

Conclusion: Polyamines are present at appreciable levels in cereal grains. In barley, they positively correlate with protein content, indicating their role in nitrogen metabolism. Beyond botanical functions, polyamines are recognized as bioactive compounds with potential dietary significance due to their anti-inflammatory, antioxidant, and anti-aging properties. Dehulling and pearling studies highlight their concentration in barley germ and outer layers, suggesting ways to recover these bioactive compounds. Akin to polyamines, phenolamides contribute to plant and human health through protective effects, indicating potential as valuable nutraceuticals.

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CURCUMIN FUNCTIONALIZED ON SOLID LIPID MICROPARTICLES AGAINST CANDIDA ALBICANS BIOFILM

CATTO', Cristina [1]; LOCARNO, Silvia Alice [2]; CAPPITELLI, Francesca [1]; LENARDI, Cristina [2]

[1] Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy;
[2] Department of Physics, University of Milan, Italy

cristina.catto@unimi.it

Introduction: Fungal biofilms are very difficult to remove as they are highly biocide-tolerant. The treatments with natural compounds that sabotage the key steps of biofilm formation without exerting the selective pressure that arises the drug-resistant stains has considered a strategy to adopt1. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, CUR) is a polyphenol with anti-biofilm properties isolated from the rhizome of Curcuma longa. Its application is limited by chemical instability, water insolubility and low bioavailability2. Solid lipid microparticles (SLM) are high-performance drug delivery systems3. In this research, CUR has been immobilized on SLM (SLM-CUR) allowing the improvement of curcumin efficacy against the treatment of recalcitrant fungal biofilms.

Material & Methods: The anti-biofilm activity of SLM-CUR was evaluated against the fungus Candida albicans. Twelve quantities of SLM-CUR without and with curcumin ranging from 0.5 and 250 μM were tested.

Results: SLM-CUR containing curcumin above 7.8 μM inhibited C. albicans biofilm formations up to -73%, CUR alone inhibited biofilm formation at concentrations above 15.6 μM, whereas SLM did not display anti-biofilm activity. Indeed, the anti-biofilm activity of SLM-CUR was totally ascribable to the CUR immobilized on the microparticles.

Conclusion: The optimal anti-biofilm activity of SLM-CUR proved that the chemical functionalization was instrumental in maintaining the CUR activity.

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AGE-RELATED IMPACT ON BIOTRANSFORMATION OF OLIVE POLYPHENOLS

LAUWERS, Stef [1]; WEYNS, Anne-Sophie [1]; BREYNAERT, Annelies [1]; VAN RILLAER, Tim [2]; FRANSEN, Erik [3]; LEBEER, Sarah [2]; TUENTER, Emmy [1]; HERMANS, Nina [1]

[1] Natural Products & Food Research and Analysis – Pharmaceutical Technology (NatuRAPT), Department of Pharmaceutical Sciences, University of Antwerp – Belgium

[2] Laboratory of Applied Microbiology and Biotechnology, Department of Bioscience Engineering, University of Antwerp – Belgium

[3] Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp – Belgium

stef.lauwers@uantwerpen.be

Introduction: Increasing life expectancy and demands for elderly care make healthy aging essential. Olive-based products, rich in antioxidant polyphenols1, like oleuropein and hydroxytyrosol, are fundamental in the Mediterranean diet. These polyphenols undergo biotransformation by the gut microbiome. As the latter changes with ageing2, the biotransformation process may differ between young and elderly individuals.

Material & Methods: This study used an in vitro GastroIntestinal Model with colon phase (GIM-colon) to simulate oleuropein biotransformation in the stomach, small intestine, and colon. The model mimicked physiological conditions for two age groups (20-30 years and ≥65 years), using healthy donor fecal samples. Bacterial composition was determined with 16S rRNA gene sequencing. GIM-colon samples were analyzed using UHPLC-HRMS with an automated data analysis workflow for unbiased metabolite screening. Time profiles of metabolites were compared between the age groups.

Results: Various metabolites of oleuropein were identified, resulting from deglycosylation and hydrolysis. Both age groups formed the same metabolites, but the elderly showed a higher biotransformation rate, possibly due to lower viable cell count in the younger population's fecal pool.

Conclusion: Gut biotransformation of polyphenols in healthy elderly individuals leads to similar metabolites as found in younger counterparts, indicating that polyphenols maintain their potential health benefits at older age.

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OLIVE LEAF PHENOLICS: THE IMPACT ON FERROPTOSIS IN CELLS

SÖZBİLEN, Gözde Seval[1], GÜMÜŞ Zinar Pınar[2], KAFTAN-OCAL Gizem[3], BİRİM Derviş[4], BOZKURT Buket[5], VAHABI Arman[6], ARMAGAN Güliz[4], SOZBILEN Murat Celal[6]

[1] Olive Research Institute, İzmir, Turkey
[2] Central Research Test and Analysis Laboratory Application and Research Center (EGE-MATAL), Ege University, Izmir, Türkiye
[3] Department of Biochemistry, Faculty of Pharmacy, Afyonkarahisar Health Sciences University, Türkiye
[4] Department of Biochemistry, Faculty of Pharmacy, Ege University, Izmir, Türkiye
[5] Department of Pharmacognosy, Faculty of Pharmacy, Ege University, Izmir, Türkiye
[6] Department of Orthopedics and Traumatology, Ege University School of Medicine, Izmir, Turkiye

gozdeseval.sozbilen@tarimorman.gov.tr

Introduction: Olive leaves, rich in antioxidants, might shield cells from ferroptosis - a recently discovered iron-driven cell death caused by excessive lipid oxidation[1]. This study aims to assess the antiferroptotic potential of aqueous ethanolic extracts prepared from leaves of five olive cultivars (Ayvalık, Memecik, Gemlik, Domat, and Manzanilla).

Material & Methods: Initially, the composition of olive leaf (post-harvest collected) extracts prepared with ethanol[2] was investigated using liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF/MS)[3]. Then, SH-SY5Y cells were pre-treated with either olive leaf extracts (1, 10 and 100µg/mL) or oleuropein (1, 5, and 10 µM) for a two-hour incubation period. Subsequently, the cells were exposed to erastin (50 µM), a well-characterized ferroptosis inducer, for 24-hour. Finally, the MTS method was then employed to assess changes in cell viability.

Results: Gemlik olive leaves, a Turkish cultivar, were richest in oleuropein, 3-hydroxytyrosol, and oleanolic acid ($p<0.05$). However the extracts (100 µg/mL) from all cultivars enhanced cell viability compared to the erastin-treated control group ($p<0.05$). This protective effect was also mirrored by all oleuropein treatments ($p<0.05$).

Conclusion: Olive leaf extracts high in phenolic compounds show promise in reversing ferroptosis caused by erastin, but additional in vitro and in vivo studies are needed.

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UNLOCKING THE POWER OF FLOW BIOCATALYSIS FOR THE PREPARATION OF HIGH-VALUE NATURAL AND NATURE-INSPIRED POLYPHENOLS

ANNUNZIATA, Francesca [1,2]; VICINANZA, Sara [2]; MOMBELLI, Lara [2]; MARTINO, Piera Anna [3]; CONTENTE, Martina Letizia [1]; MERONI, Gabriele [3]; DALLAVALLE, Sabrina [1]; PINTO, Andrea [1]; TAMBORINI, Lucia [2]

[1]: Department of Food, Environmental and Nutritional Sciences, UniMI, Via Celoria 2, 20133 Milan, Italy
[2]: Department of Pharmaceutical Sciences, UniMI, Via L. Mangiagalli 25, 20133 Milan, Italy
[3]: Department of Biomedical, Surgical and Dental Sciences, UniMI, Via Pascal 36, 20133 Milan, Italy

francesca.annunziata@unimi.it

Introduction: Food-derived phenolic compounds are well known for their antioxidant and antimicrobial effects.[1] Because of their hydrophilic nature and their intrinsic stability and bioavailability issues, their use as active ingredients is limited. Lipophilization of phenolic derivatives is a valid strategy to obtain amphiphilic compounds that can be used as multifunctional additives.[2] Biocatalytic processes in continuous flow reactors represent an outstanding tool for the production of these active compounds.

Material & Methods: Immobilised lipase B from Candida antarctica (CaLB) and tyrosinase from Agaricus Bisporus were the employed biocatalysts. Different green solvents were selected as unconventional reaction medium with low environmental impact.

Results: Starting from tyrosol, a biocatalysed continuous process was developed for the synthesis of hydroxytyrosol. Together with other natural phenolics, they were lipophilised as esters, carbonates and carbamates through continuous-flow biocatalysed processes. The use of imm-CaLB as biocatalyst allowed to selectively react the primary alcohols leaving unreacted the phenol(s), which are fundamental for the antioxidant activity. The amphiphilic derivatives retained their radical scavanger properties and preserved or improved their antimicrobial activity, while their lipophilicity was increased.

Conclusion: A versatile and efficient biocatalysed flow protocol for the synthesis of a series of bioactive phenolic esters, carbonates and carbamates was succesfly developed.

The project was realized within the MUSA – Multilayered Urban Sustainability Action – project, funded by the European Union – NextGenerationEU, under the National Recovery and Resilience Plan (NRRP) Mission 4 Component 2 Investment Line 1.5: Strengthening of research structures and creation of R&D "innovation ecosystems", set up of "territorial leaders in R&D".

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SEPARATION OF SORGHUM POLYPHENOLS BASED ON MOLECULAR WEIGHT INDICATES THAT HIGHER MOLECULAR WEIGHT POLYPHENOLS RETAIN GREATER ANTI-CANCER AND ANTI-GLYCATION ACTIVITY.

SMOLENSKY, Dmitriy [1]; PETERSON, Jaymi [1]; SANTANA, Adina L. [2]

[1] USDA-ARS, United States of America

[2] Kansas State University, Kansas, United States of America

dmitriy.smolensky@usda.gov

Introduction: Sorghum is an excellent source of polyphenols. Because high phenolic sorghum contains both flavonoids and condensed tannins, little work has been done to determine which sorghum polyphenol contributes more potential health benefits. This knowledge is important for developing new sorghum varieties and sorghum-based products.

Materials & methods: This study presents a simple method using Sephadex LH-20 column that separates lower molecular weight (MW), monomers and oligomers, from larger MW polymeric polyphenols. The degree of polymerization differences were evaluated using NP-HPLC. Following the separation, the lower MW and higher MW fractions were evaluated for bioactivity using cell viability and anti-glycation assays. To make a valid comparison for bioactivity assays, the fractions were diluted to have the same total phenolic content.

Results & Conclusions: Over 90% of the flavonoids were eluted in the low MW fraction while the high MW fraction contained the majority of polyphenols at degree of polymerization higher than 10. The polymeric fraction of polyphenols had higher bioactivity, measured by glycation inhibition and cancer cell viability inhibition, when compared to monomeric/oligomeric polyphenols. These results will be of value in breeding new sorghum varieties and developing food products containing sorghum polyphenols.

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SEX-DEPENDENT EFFECTS ON THE ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION OF GRAPE SEED PROANTHOCYANINS IN PREPUBESCENT RATS

AROLA-ARNAL, Anna [1,2,3]; IGLESIAS-CARRES, Lisard [1]; MAS-CAPDEVILA, Anna [1,4]; BRAVO, Francisca I. [1,2,3]; SUÁREZ, Manuel [1,2,3]; MUGUERZA, Begoña [1,2,3]

[1] Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Spain
[2] Nutrigenomics Research Group, Institut Investigació Sanitària Pere Virgili, Spain
[3] Center of Environmental, Food and Toxicological Technology (TecnATox), University Rovira i Virgili, Spain
[4] Current affiliation: Eurecat, Technology Centre of Catalonia, Nutrition and Health Unit, Spain

anna.arola@urv.cat

Introduction: The bioactivity of (poly)phenols is dependent on their absorption, distribution, metabolism, and excretion (ADME). There are several internal and external factors that effects (poly)phenols ADME. Among them, sex modulate a grape seed proanthocyanin (PA) extract (GSPE) ADME in adulthood. Specifically, methylated metabolites were only present in brain male adult rats1. The aim of this work was to evaluate whether sex-dependent changes in GSPE PAs ADME occur before puberty.

Material & Methods: 4-week-old prepubescent male and female rats were oraly gavaged with 1000 mg/kg GSPE and 2 h after the metabolite profile was analyzed by HPLC-ESI-MS/MS in plasma, liver, mesenteric white adipose tissue, brain and kidney. Brain and liver catechol-O-methyltransferase (COMT) protein and plasma 17b-estradiol levels were evaluated.

Results: There was minor sex-dependence in phenolic metabolite profile which was in accordance with an absence of sex-specificity in the hepatic and brain COMT protein levels, and in plasma levels of 17b-estradiol. The total concentration of GSPE metabolites in each organ and tissue was higher in male than in females' rats.

Conclusion: Most of the sex-related changes in GSPE ADME in adult rats do not occur in prepubescent rats. This suggests that physiological changes that modulate PA ADME are triggered during puberty.

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NATURAL FERULOYLAMIDES AND THEIR DIMERS: CHEMOENZYMATIC SYNTHESIS AND ANTIMICROBIAL ACTIVITY

DOZIO, Denise [1]; CACCIA, Aziza [1]; GHOSH, Sharmila [1]; ANNUNZIATA, Francesca [1]; PRINCIOTTO, Salvatore [1]; KUNOVA, Andrea [1]; CONTENTE, Martina Letizia [1]; MARTINO, Piera Anna [2]; PINTO, Andrea [1]; DALLAVALLE, Sabrina [1]

[1] Department of Food, Environmental and Nutritional Sciences, via G. Celoria 2, 20133, Milano
[2] One-Health Unit, Department of Biomedical, Surgical and Dental Sciences, via Pascal 36, 20133, Milano

denise.dozio@unimi.it

Introduction: Plants accumulate a plethora of specialized metabolites as self-defense mechanism when under biotic or abiotic stress conditions. Among them, cinnamoylamides and their dimers are an interesting class of antimicrobial phytoalexins as depicted in very recent literature. However, little is known about their mechanism of action and structural requirements for biological activity, and further investigations are required.

Material & Methods: Ferulic acid was selected as a representative cinnamic acid, and some feruloylamides (FAAs) were synthesized using both chemical and enzymatic approaches. Horseradish peroxidase mediated dimerization was exploited to obtain multiple carboxylic dimeric intermediates, that were further functionalized by adding agmatine and putrescine chains. This collection of natural and nature-derived analogues underwent antifungal and antibacterial evaluation tests.

Results: Among all the obtained compounds the dimers were the most active, showing a high ability to inhibit the fungi appressorium formation (> 94% inhibition rate). This trend was observed as well for the antibacterial activity against both Gram negative and positive strains.

Conclusion: Selected feruloylamides and their dimers proved to be active against most of the fungal and bacterial phytopathogens considered. Consequently, they seem suitable candidates for the develompment and design of new antimicrobial agents for crop protection.

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CHOLESTEROLYL PHENOLIPIDS AS MEMBRANE ANTIOXIDANTS

PAIVA-MARTINS, Fátima [1]; COSTA, Vânia [1]; COSTA, Marlene [1]; FERREIRA, Mariana [1]; SANTOS, Paula [1]; GERALDO, Dulce [2]; MONTEIRO, Luís [2]

[1] REQUIMTE-LAQV, Faculty of Sciences, University of Porto, Portugal

[2] Chemistry Centre, University of Minho, Portugal

mpmartin@fc.up.pt

Introduction: The lipophilization of phenolic acids (phenolipids) may increase their penetration through lipid bilayers, leading to better antioxidant protection[1,2]. However, recent findings suggest that the interactions of linear phenolipids (ex. octyl caffeate) with membranes can increase their stiffness thus presenting some toxicity.

Material & Methods: Cholesterolyl phenolipids of caffeic, dihydrocaffeic, homoprotocatechuic and protocatechuic acids were synthetized (yields ~ 60%). After the evaluation of their radical scavenging capacity by the DPPH method and anodic peak potential by cyclic voltammetry, their antioxidant capacity against AAPH-induced oxidative stress in soybean PC liposomes was determined. Their interaction with the liposomal membrane was studied by the aid of 3 fluorescence probes located at different depths of the membrane.

Results: The synthetized esters showed a higher radical scavenging capacity and a lower anodic potential than parental phenolic acids and were able to protect liposomes to a greater extent than α-tocopherol. All steryl esteres interacted with the 3 fluorescent probes. However, while the octyl caffeate (positive control) interacted strongly with the 3 zones of the membrane, cholesterol esteres interaccted more with the intermediate zone, in a similar fashion to cholesterol.

Conclusion: Cholesterol phenolipids may protect biomembranes in a great extent than a-tocopherol without changing the membrane dynamics.

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DIPHENYLETHER- AND BIPHENYL-TYPE PHLOROTANNINS AS POTENTIAL ANTIFUNGAL AGENTS

SACCHI, Francesca; ANNUNZIATA, Francesca; SAITTA, Francesca; GHOSH, Sharmila; PRINCIOTTO, Salvatore; PINTO, Andrea; DALLAVALLE, Sabrina; KUNOVA, Andrea; CORTESI, Paolo; FESSAS, Dimitrios

Università degli Studi di Milano, Italy

francesca.sacchi@unimi.it

Introduction: In recent decades, phlorotannins, isolated from various brown seaweeds as complex mixtures of phloroglucinol oligomers, emerged as an important class of polyphenols. Phlorotannin crude extracts have promising biological activity, including antifungal one.1 For this reason, their development as natural agrochemicals could be a promising strategy to address the growing urgency in managing phytopathogenic fungi.

Material & Methods: Microwave-assisted extraction of secondary metabolites from four different seaweeds (F. Vesciolosus, P. palmata, L. japonica, and U. pinnatifida) was performed. Meanwhile pure natural and nature-derived phlorotannins were synthesized and characterized by spectroscopic methods. Antifungal activity was evaluated against phytogenic fungi (e.g. P. oryzae, B. cinerea, F. culmorum). Inhibition of spore germination of P. oryzae was also studied.

Results: Synthesized pure phloroglucinol dimers, namely diphenylethers (diphlorethol, bifuhalol) and biphenyls (difucol), together with their partially or completely methylated/acetylated derivatives displayed moderate to good inhibition of mycelium growth and appressorium formation.

Conclusion: The C-O-C connection between the phloroglucinol monomers positively impacted the activity, as shown by the higher potency of biphenylethers compared to biphenyls. The methylation pattern of phlorotannins seems to play a key role as well. Differential Scanning Calorimetry experiments to study the interaction of bioactives with a model fungi phospholipid membrane are underway.

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TEMPORAL EFFECTS OF GREEN TEA EXTRACT ON POSTPRANDIAL GLUCOSE AND INSULIN IN HEALTHY SUBJECTS

SULAIMANI, Noha[1,2,3], HOUGHTON, Michael J.[1,2], BONHAM, Maxine P.[1], WILLIAMSON, Gary[1,2]

[1] Department of Nutrition, Dietetics and Food, Monash University, Notting Hill, 3168, Australia

[2] Victorian Heart Institute, Monash University, Clayton, 3168, Australia

[3] Department of Food and Nutrition, King Abdulaziz University, Jeddah, 21589, Saudi Arabia

noha.sulaimani@monash.edu

Introduction: Metabolic homeostasis is linked to circadian rhythms. Disrupting these rhythms with mistimed eating can increase the risk of metabolic diseases. Evening carbohydrate consumption increases postprandial glycaemia more than morning consumption (1). Green tea, high in (-)-epigallocatechin-3-gallate (EGCG), can reduce postprandial glycaemia but time-of-day effects of green tea extract incorporated into a starch-based meal remain unexplored. Our review of mammalian cell studies found that (poly)phenols, including 10 μM EGCG, can restore glucose metabolism via clock components such as BMAL1 (2)(3). We hypothesized that combining green tea extract with a starchy meal could lower postprandial glucose and insulin, but the effect could be different in the morning and evening.

Material & Methods: A randomised, double-blind, controlled, crossover study was conducted. Fasted healthy volunteers (n=14) attended four visits (two at 08:00 and two at 18:00) and consumed either a control (rice) or test meal (rice with incorporated green tea). Blood glucose and insulin were measured over 3 h.

Results: Green tea extract did not affect postprandial glycaemia but significantly decreased peak insulin after the morning meal, but not in the evening, highlighting a time-of-day effect.

Conclusion: Green tea polyphenols may influence insulin sensitivity through circadian mechanisms, independent of carbohydrate digestion and absorption.

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NOVEL DEVELOPMENTS ON THE POTENTIAL APPLICATION OF PYRANOANTHOCYANINS AS COLORANTS

GIUSTI, M. Monica, VOSS, Danielle, PORRAS GUARDADO, Carlos

Department of Food Science and Technology, The Ohio State University, USA

Giusti.6@osu.edu

Introduction: Since their discovery in the 1990s, pyranoanthocyanins have been considered key contributors to the enhanced color stability of red wines. Despite their stability, their scarcity in nature and inefficient production hindered their application as colorants. We explored different approaches to increase pyranoanthocyanin production efficiency and tested their color and stability to explore their coloring potential.

Material & Methods: Different anthocyanin-rich materials and cofactors were incubated at different ratios, pH, and temperatures, to form pyranoanthocyanins. Production rates and yields, color, solubility, and stability in different matrices were evaluated. Alkaline hydrolysis and lactic acid bacteria (LAB) were tested as means of obtaining the reactants from byproducts of the agro-industry, speed up the reaction and increase yields.

Results: A wide range of PACNs were produced. Hydroxycinnamic acids were effective cofactors for pyranoanthocyanin formation, particularly after decarboxylation by LAB. Chemical structure, anthocyanin: cofactor ratios, temperature, and pH affected yields are reaction rates. Pyranoanthocyanins expressed colors from yellow to purple blue, with molar absorptivities <10X higher than their precursor anthocyanins at pH 3. Pyranoanthocyanins showed superior stability to bleaching agents, pH, and heat. Interaction with common food ingredients decreased precipitation. Hydroxyphenyl pyranoanthocyanin solubility greatly depended on their glycosylation patters, while their hue was mostly impacted by their substitutions in the B and E rings.

Conclusion: Our results show excellent potential for pyranoanthocyanins to serve as versatile food colorants for. Additional research is under way to continue to increase production efficiency and sustainability.

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POLYPHENOLS AND FOOD PROCESSING: THE CRITICAL POINTS

XIAO, Jianbo

Universidade de Vigo, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Instituto de Agroecoloxía e Alimentación (IAA) – CITEXVI, 36310 Vigo, Spain

jianboxiao@yahoo.com

Polyphenols are widely distributed in natural and processed food. Thermal processing, irradiation, fermentation, high pressure, microwave, and drying are several popular food processing methods. Polyphenols are instable in food processing, which easily degrade and react with other components because of their polyhydroxy characteristic. Traditional and advanced technologies have been used to characterize the stability of polyphenols. The main influence factors of stability of polyphenols included pH, temperature, light, oxygen, enzymes, metal ions, and biomacromolecules. Besides, thermal processing greatly promoted the degradation of polyphenols. Thermal degradation mechanisms and products of some polyphenols, such as quercetin and dihydromyricetin, have been intensively demonstrated. Nevertheless, the structural changes of polyphenols caused by food processing, may lead to different bioactivities from the obtained results based on unprocessed polyphenols.

Therefore, to maximize the beneficial effects of polyphenols ingested by human from processed food, the stability of polyphenols in food processing must be thoroughly investigated to assess their real bioactivities. In addition, some available technologies for improving the stability of polyphenols in food processing have been proposed.

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POLYPHENOLS AND FERMENTED FOOD: THE OPTIMIZATION

FILANNINO, Pasquale [1], ACIN ALBIAC, Marta [2], TLAIS, Ali Zein Alabiden [2], LEMOS JUNIOR, Wilson José Fernandes [2], CAMPANARO, Stefano [3], GOBBETTI, Marco [2], DI CAGNO, Raffaella [2]

[1] Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Italy
[2] Faculty of Agricultural, Environmental and Food Sciences, Free University of Bolzano-Bozen, Italy
[3] Department of Biology, University of Padova, Italy

pasquale.filannino1@uniba.it

Introduction: Phenolics metabolism by lactobacilli has been somewhat elucidated, but comprehensive information on the physiological and ecological implications of such pathways, as well as the role of specific enzymes within the microbiome of fermented plant foods, remains limited. Although it is reasonable to complete the deciphering of the mechanisms regulating these secondary metabolisms at the level of pure cultures, it is even more urgent and worthwhile to dissect these pathways at the level of meta-communites under food-like conditions to allow the optimal design of complex microbial processes. Indeed, fermented foods are the result of complex microbial consortia, likely undergoing a long fermentation that reflects a distinctive microbial succession over time.

Material & Methods: First, the effect of complex mixtures of phenolics on the phenome of lactic acid bacteria in terms of their ability to utilize 190 carbon sources was investigated in simplified model systems. Then, sauerkraut was selected as a second model system to uncover not yet disclosed correlations between microbiome composition and phenolic compounds occurring throughout sauerkraut spontaneous fermentation. Finally, the efficacy of two novel starter-assisted sauerkraut fermentations was investigated in comparison to spontaneous fermentation and correlated with the phenolic profiles.

Results: In simplified model systems simulating food-like conditions, the metabolism of phenolic compounds had physiological significance for lactic acid bacteria and implications for their overall phenome. Using sauerkraut fermentation as a model system, we pioneered the correlation of changes in the phenolic profile with changes in the microbiome. Annotated genes related to phenolic metabolism were found in many core species throughout the process. Correlations between microbiome composition and phenolic profile may provide new clues for sauerkraut biotechnology, helping to identify novel metabolic drivers that enhance potential sauerkraut functionalities. These considerations have strong relevance for the selection of microbial starters and the management of fermentation processes.

Conclusion: Understanding and managing the phenolics metabolism in the context of mixed cultures is challenging because of the multiple variables and players involved in regulating such intricate metabolic pathways. To address this new challenge, it is necessary to correlate the profile of phenolics with microbiome specifications. The current framework is still very fragmented, but its completion will allow the development of effective targeted fermentation strategies.

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POLYPHENOL-PROTEIN INTERACTIONS IN PIGMENTED CEREALS - A CLEAN-LABEL APPROACH TO TAILOR STARCH VISCOSITY

JANNASCH, Annegret; WANG, Ya-Jane; LEE, Sun-Ok

University of Arkansas, United States of America

ajannasc@uark.edu

Starch improves food properties, but chemical crosslinking is commonly required to enhance its resistance to high shear, temperature and acidity. However, chemically modified starches are not considered “clean-label” ingredients. Polyphenols interact with cereal proteins to enhance starch viscosity, implying their role as crosslinkers. The nature- whether covalent and/or non-covalent- and extent of their interactions as affected by polyphenol and protein classes remains unclear.

The effect of polyphenol-protein interactions in pigmented and non-pigmented rice and sorghum on pasting viscosity was studied in the presence of chemical agents and different pH environments. Molecular weight and size, and secondary and tertiary structures of polyphenol-protein complexes were also investigated.

Viscosity development and protein structure were influenced by polyphenol class and concentration. Phenolic acids did not affect these properties. Anthocyanins in purple rice crosslinked with glutelin, enhancing its viscosity in neutral and acidic environments. Hydrogen bonding and hydrophobic interactions between 3-deoxyanthocyanins/proanthocyanidins and prolamin stabilized the viscosity of black sorghum.

Polyphenol-protein interactions in pigmented cereals enhanced and stabilized starch viscosity similar to that of chemically crosslinked starch, demonstrating their potential as clean-label ingredients. Polyphenol class and concentration influenced protein structural changes, offering a potential tool to tailor starch viscosity for desired functionality, with additional health benefits.

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EXTRACTION OF PHENOLIC COMPOUNDS FROM PERILLA FRUTESCENS USING CHOLINE CHLORIDE – BASED DEEP EUTECTIC SOLVENTS

MOCANU, Ana-Maria [1]; BOTEZATU-DEDIU, Andreea Veronica [1]; FURDUI, Bianca [1]; AVRAMESCU, Sorin Marius [2]; BRATU, Camelia [3]; VANATORU, Costel [3]; DRAGOI-CUDALBEANU, Mihaela [4]; DINICA*, Rodica Mihaela [1]

[1] Dunărea de Jos University, Faculty of Sciences and Environment, Romania
[2] University of Bucharest, Faculty of Chemistry, Romania
[3] Genetic and Vegetal Resources Bank (GVRB), Romania
[4] University of Agronomic Sciences and Veterinary Medicine Bucharest, Romania

ana.mocanu@ugal.ro

Introduction: Perilla frutescens, an annual herb, with green or purple leaves, rich in antioxidants, serve as both a natural coloring agent and a preservative in food, particularly for vegetables and fruits. Additionally, the plant has historical use in traditional medicine, addressing ailments like food poisoning, allergies, colds and depression.

Material & Methods: The extracts were obtained from two species of P. frutescens, sourced from the Genetic and Vegetal Resources Bank (GVRB) in Buzau, Romania, using Microwave-Assisted Extraction (MAE) and various systems of Deep Eutectic Solvents (DES) based on choline chloride (ChCl). The total polyphenol content in the plant matrix was determined using the Folin-Ciocalteu method, and the antioxidant activity of the extracts was assessed through the 1,1-diphenyl-2-picrylhydrazyl (DPPH) spectrophotometric method.

Results: The studies conducted on the extracts obtained with DES have demonstrated that they extracted significant amounts of biologically active compounds, such as phenolic acids and flavonoids from P. frutescens.

Conclusion: The findings affirmed that the proposed synergy between the MAE method and DES could offer a highly efficient alternative for the sustainable and environmentally friendly extraction of phenolic compounds from plant sources, thereby opening avenues for innovative industrial applications.

This work was partially supported by ADER grant 5.2.1. - Conservation and valorization of the genetic heritage of aromatic and medicinal species that can be cultivated on the territory of Romania.

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TURNING SEA URCHIN WASTE INTO POLYHYDROXYNAPHTOQUINONE-COLLAGEN BIOMATERIALS

MARZORATI, Stefania [1]; MARTINELLI, Giordana [1]; RONCORONI, Margherita [1]; SCAPUZZI, Chiara [1]; CHWOJNIK, Tamara [1]; MELOTTI, Luca [2]; CAROLO, Anna [2]; ZIVELONGHI, Giulia [2]; PATRUNO, Marco [2]; TRIONI, Mario Italo [3]; SOAVE, Raffaella [3]

[1] Department of Environmental Science and Policy, Università degli Studi di Milano, Italy
[2] Department of Comparative Biomedicine and Food Science, University of Padua, Italy
[3] National Research Council of Italy, Institute of Chemical Science and Technologies "Giulio Natta", Italy

stefania.marzorati@unimi.it

Introduction: Aiming at driving circular economy approaches in the food value chain, this study focuses on valorization of sea urchin waste towards the development of functional collagen-based scaffolds added with polyhydroxynaphthoquinones (PHNQs), a class of small polyphenols found in sea urchins tests and spines.

Material & Methods: composite biomaterials were produced by lyophilization of collagen suspension added with PHNQ. Resulting scaffolds were characterized in terms of structural features (SEM), degradation kinetics and antioxidant activity (ABTS×+). A computational approach (Tight Binding Molecular Dynamics) was performed to evaluate the PHNQ-collagen interaction. Biocomposites were also tested for cytotoxicity with normal human dermal fibroblasts (NHDF).

Results: Through characterization and evaluation, this study demonstrates that the incorporation of PHNQs into collagen scaffolds enhances stability, reduces degradation rates, and preserves antioxidant activity of PHNQ. The computational analysis suggests that the strengthening of the collagen structure in the biocomposites occurs through inter-molecular crosslinking with Spinochrome A, one of the main PHNQ present. Cells exposed to collagen and PHNQs were shown to be viable.

Conclusion: These findings suggest that composite scaffolds have the potential to address the need for effective wound healing biomaterials, offering a synergistic combination of regenerative and antioxidant properties.

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POLYPHENOLS IN ONCOTHERAPY: MOVING FORWARD FROM THE BENCH TO CLINICAL APPLICATIONS

Elena Obrador[1,2], Ali Jihad-Jebbar[1], Rosario Salvador-Palmer[1], Rafael López-Blanch[1,2], María Oriol-Caballo[1,2], María Paz Moreno-Murciano[2], Enrique A. Navarro[2,3,4], Rosa Cibrian[1,2] and **ESTRELA, José M. [1,2*]**

[1] Department of Physiology, Faculty of Medicine & Odontology, University of Valencia, Spain
[2] Scientia BioTech, 46002 Valencia, Spain
[3] Department of Computer Sciences, Higher Technical School of Engineering, 46100 Burjassot, Spain
[4] Robotics Research Institute, University of Valencia, 46980 Paterna, Spain

Jose.M.Estrela@uv.es

Polyphenols, despite their potent bioactivity *in vitro*, often exhibit poor bioavailability *in vivo* due to factors such as limited absorption, rapid metabolism, and extensive excretion.

Efficacy is another pivotal aspect when considering polyphenols as therapeutic agents in cancer. Their anti-cancer effects have been demonstrated in preclinical studies, showcasing their ability to induce cell cycle arrest, promote apoptosis, inhibit angiogenesis, and suppress metastasis. These effects are mediated through a variety of molecular targets and signaling pathways, such as (but not limited to) the inhibition of NF-κB and PI3K/Akt pathways, modulation of oxidative stress, and interaction with cell surface receptors. Clinical studies, although fewer in number, have also provided evidence supporting the potential benefits of polyphenols in cancer therapy. However, the translation of preclinical findings into clinical success remains challenging due to issues related to bioavailability, dosing, and the complexity of cancer as a multifactorial disease. To overcome these challenges systems aiming to enhance stability, absorption, and targeted delivery of polyphenols have been developed, including e.g., nanoparticles, liposomes, micelles, or conjugation with carrier molecules.

Nevertheless, there is a lack of correlation between *in vitro* effective concentrations versus *in vivo* bioavailability. *In vivo*, the main underlying mechanisms responsible of the anticancer effects elicited by PFs may not necessarily imply a direct interaction between the polyphenol molecule and the cancer cell. For instance, pterostilbene or curcumin, by interfering with the physiological (stress-related) adenohypophysis-adrenal axis inhibit tumor growth indirectly.

The integration of advanced delivery systems with polyphenol-based therapies represents a promising frontier in oncotherapy. By addressing the limitations of bioavailability and optimizing the delivery to specific cancer cells, there is potential to enhance the efficacy of polyphenols, offering a safer and more effective alternative to conventional treatments. Future research in this domain is likely to focus on further elucidating the molecular mechanisms of action of polyphenols, optimizing delivery systems for clinical application, and conducting comprehensive clinical trials to validate their therapeutic potential in cancer treatment.

ANGIOGENESIS AS A TARGET OF DIETARY (POLY)PHENOLS AND THEIR DERIVED METABOLITES

GIMENEZ-BASTIDA, Juan Antonio

Laboratory of Food & Health, Research Group on Quality, Safty and Bioactivity of Plant Foods, CEBAS-CSIC, Murcia Spain

jgbastida@cebas.csic.es

Angiogenesis is a normal physiological process regulated by a plethora of molecules (growth factors or cytokines) and a range of cells (endothelial and immune cells). Pathological states, including atherosclerosis or cancer are characterized by abnormal high level of pro-angiogenic factors, promoting neovascularization of atherosclerotic plaques and tumor growth. Current evidence invokes plant-based diets, rich in dietary (poly)phenols, as an interesting approach to target angiogenesis against its associated chronic complications. A traditional (and still persistent) inaccurate perspective is to consider dietary (poly)phenols as the main responsible for the benefits of angiogenesis modulation. Nevertheless, it is difficult to rationalize their biological effect given the low concentrations detected in human and animal plasma. The idea of the role of dietary (poly)phenols as precursors of microbial and(or) phase-II metabolites redirects the focus to the circulating molecules, which might interact with pivotal targets (i.e., endothelial cells) involved in angiogenesis modulation. The metabolism of (poly)phenols, at the intestinal level, determines whether these compounds undergo microbial metabolism to form microbial metabolites (urolithins and equol) or are detected in their original form (i.e., flavanones) for hours before suffering further metabolism. Conjugation with sulfate or glucuronic acid is the major pathway of phase-II metabolism of these compounds, which are stable molecules detected in the bloodstream. There is a limited number of studies whose design follows the standards of excellence established elsewhere to determine the effect of these metabolites on angiogenesis. Hence, glucuronides and sulfates of hesperetin, urolithins and equol (0.1 – 15 μM) modulate endothelial migration and tubulogenesis in relevant human endothelial cellular models targeting key pathways (i.e., TNF-α and VEGF).

Do these results indicate that the role of dietary (poly)phenols regarding angiogenesis modulation is to act as precursors of circulating active metabolites? An exception comes across with curcuminoids, which can reach systemic tissues (when administered with a (poly)phenolic cocktail) at relevant concentrations, most likely due to a conjugation-saturation mechanism. These curcuminoids, at plausible in vivo conditions, inhibit processes related to angiogenesis via interaction with the VEGFR2 pathway. These studies are a step forward in establishing a link between dietary (poly)phenols and cardiovascular health. This has a particular interest regarding the microbial metabolites (i.e., urolithins and equol) based on the variable capacity of humans to form these microbial metabolites.

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FLAVONOIDS AND CRITICALLY ILL PATIENTS: FROM EXPERIMENTAL STUDY TO CLINICAL PRACTICE

DABROWSKI, Wojciech

First Department of Anaesthesiology and Intensive Therapy Medical University of Lublin, Poland

w.dabrowski5@yahoo.com

Flavonoids are chemical compounds with a polyphenolic structure from the polyphenol group. They have many properties that can be used in medicine, including inflammatory diseases, viral infections, and cancer. Flavonoids also possess strong anti-oxidative and anti-apoptotic properties. Experimental studies documented that flavonoids inhibited inflammatory response by reducing the production and release of the pro-inflammatory mediators such as cytokines (TNF-α - tumour necrosis factor alpha, IL-1β, IL-6, IL-17 – interleukins 1β, 6 and 17), activity of NF-kB, COX2 and iNOS (cystolic nuclear factor kB, cyclooxygenase 2 and nitric oxide synthase, respectively). Additionally, flavonoids reduce the release of HMGB1 (the high mobility group box 1 protein), which acts as a damage-associated molecular pattern (DAMP) regulating severity of inflammation and immune response through different receptors or direct uptake. Flavonoids acts as a strong free radical scavengers reducing production of reactive oxygen species (ROS) and iNOS activity. They are able to modulate immune activity in different pathways that affect immune cells, especially lymphocytes and macrophages. Based on their properties, flavonoids can be used as the adjuvants in the treatment of several diseases.

Xanthohumol (Xn) is the main flavonoid found in beer to which it provides the aroma and bitterness. It is extracted from female inflorescences of hop cones (*Humulus lupus*). Xanthohumol is a safe, well-tolerable and non-toxic substance, and no serious adverse effects were reported in humans. The pharmacokinetic study after a single oral dose of Xn showed no side effects in healthy humans aged below 35. Study with healthy adult volunteers showed only minor gastrointestinal disorders such indigestion, decreased appetite or slight abdominal pain after intake of Xn at a dose of 24 mg daily for eight weeks. Recent study documented the beneficial effect of Xn in critically ill COVID-19 patients. Administration of Xn at the dose of 1.5 mg per kg of body weight three times daily reduced inflammatory response, which significantly improved clinical course and decreased mortality ratio. Based on these results we used Xn as the adjuvant therapy in septic shock patients, and the results were also promising. Xanthohumol also improves clinical course in patients treated for critical pulmonary fibrosis. It across through blood brain barriers, which can reduce neuroinflammation. Xanthohumol inhibits CD3+CD25+ and CD3+CD69+, and increases activity of natural killers cells in septic shock patients. It is non-toxic and well tolerated in critically ill patients. Based on its effect we speculate that Xn could be a very promising adjuvant therapy in many severe diseases.

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POLYSSACHARIDE-BASED FILMS INCORPORATING POMEGRANATE ANTHOCYANINS AS INTELLIGENT FOOD PACKAGING MATERIALS

LUÍS, Ângelo [1]; ANJOS, Ofélia [2]; DOMINGUES, Fernanda [1]; DUARTE, Ana Paula [1]; GALLARDO, Eugenia [1]; GARCIA, Antonia [3]; GOMES, Ana Paula [4]; GRADILLAS, Ana [3]; PEREIRA, Luísa [5]; ZANETTI, Marco [6]; RAMOS, Ana [7]

[1] Health Sciences Research Centre, Universidade da Beira Interior, Portugal
[2] Escola Superior Agrária, Instituto Politécnico de Castelo, Portugal
[3] CEMBIO, Center for Metabolomics and Bioanalysis, Facultad de Farmacia, Universidad San Pablo CEU, CEU Universities, Madrid, Spain
[4] Associate Laboratory of Energy, Transports and Aeronautics, Universidade da Beira Interior (AeroG|LAETA), Portugal
[5] CMA-UBI, Centro de Matemática e Aplicações, Universidade da Beira Interior, Portugal
[6]: Dipartimento di Chimica, Università degli studi di Torino Torino, Italia
[7] FibEnTech-UBI, Fiber Materials and Environmental Technologies Research Unit, Universidade da Beira Interior, Portugal

afluis27@gmail.com

Introduction: In the pursuit of the objectives of sustainable development, biodegradable materials from renewable resources represent the best alternative to plastics. Therefore, the general objective of this work was to develop bio-based active (antioxidant), and intelligent (pH-responsive) films obtained from polysaccharides (agar and pullulan) incorporating pomegranate anthocyanins to guarantee the food safety of fresh salmon.

Material & Methods: The identification of the main anthocyanins present in pomegranate was performed by UHPLC/ESI-QTOF-MS. FTIR, RAMAN, XRD, DSC and TGA analyses were also performed. The pH-responsive colour change of the films was evaluated. Finally, fresh salmon was wrapped with the films, being their colour monitored over time.

Results: Cyanidin 3-O-glucoside was identified as the major anthocyanin present in pomegranate. Pullulan films showed to be more flexible, while agar films were more resistant to folding. Pullulan films presented best barrier properties and agar films were more. When fresh salmon was wraped with the films, their initial pink color turned to light green as the salmon began to putrify with the corresponding pH increase, due to changes in the anthocyanins molecular structure.

Conclusion: Pullulan- and agar-based active and intelligent films were developed, which can be used to package fresh salmon, allowing the monitoring of its feshness.

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POLYPHENOLS AS ACTIVE INGREDIENTS IN INNOVATIVE, GREEN, AND LOW COST ACTIVE WOUND DRESSINGS

FERRARIS, Sara [1]; SPRIANO, Silvia [1]; ALFIERI, Maria Laura [2]; VARESANO, Alessio [3]; VINEIS, Claudia [3]; COCHIS, Andrea [4]

[1] Politecnico di Torino, Italy
[2] Università Federico II, Italy
[3] CNR STIIMA, Italy
[4] Università del Piemonte Orientale, Italy

sara.ferraris@polito.it

Introduction: Wound dressings should support mechanical stress, absorb exudate, reduce bleeding, prevent infection/inflammation. Commercial dressings cannot offer all these features. Polyphenols have anti-inflammatory, antioxidant antibacterial properties and can be obtained from agri-food byproducts. The aim of the project is the combination of polyphenols with keratin to obtain sustainable active dressings.

Material & Methods: Keratin was extracted from discarded wool, electrospinning/3D printing were explored to obtain porous matrix. Polyphenols were obtained from agri-food byproducts using green routes. pH/composition of the functionalization solutions were designed to maximize electrostatic attraction between keratin and polyphenols. Samples were characterized through FTIR spectroscopy, Folin&Ciocalteu test, zeta potential and SEM-EDS. Anti-oxidant activity was validated by verify the survival of fibroblasts under oxidative stress conditions.

Results: Keratin mats were obtained from wool-derived keratin through green processes. Active polyphenols were obtained from local byproducts. Polyphenols were loaded onto keratin fibers through green procedures. Polyphenols maintained their redox activity after grafting. Their antioxidant activity was confitmed by the high survival reate of cells exoposed to oxodative stress.

Conclusion: The use of polyphenols as active ingredients in wound dressings is a promising strategy for the obtainment of innovative, green, and low-cost dressings for the treatment of wounds.

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CONTRIBUTION OF (POLY)PHENOLS IN THE MODULATION OF INTESTINAL PERMEABILITY: TARGETING MECHANISMS AND CLINICAL TRIALS

DEL BO', Cristian

Università degli Studi di Milano, Italy

cristian.delbo@unimi.it

(Poly)phenols (PPs) (e.g., flavonoids, phenolic acids) are secondary metabolites commonly distributed in the kingdom of plants and widely consumed within the human diet. Several in vitro and in vivo studies support the role of PPs in the modulation of numerous metabolic and functional activities such as inflammation, oxidative stress, vascular function, lipid profile. Recently, PPs have been evaluated as promising protective factors and regulators of the intestinal barrier function and permeability, thus by preventing several pathological and dysfunctional conditions.

However, at present, the exact mechanisms linking PPs with the intestinal epithelial barrier function have not yet been established. Putative effects of PPs on IP at different physiological levels seems to include: 1) intraluminal level, through the modulation of microbiota composition, endotoxin and/or short chain fatty acids production, redox status, and dietary component absorption and/or activity; 2) intracellular level, through the regulation of expression of membrane proteins such as tight junction, adherens junction, gap junction, and desmosome proteins, upregulation of kinases and nuclear factor erythroid 2–related factor 2, and downregulation of nuclear factor kappaB and toll-like receptor 4; 3) systemic level, by maintaining the functionality of immune system and the regulation of inflammatory processes (toward a reduced pro-inflammatory status).

Very few clinical trials have been carried out investigating the impact of PPs in the modulation of IP. In this context, the MaPLE project (Microbiome mAnipulation through Polyphenols for managing gut Leakiness in the Elderly) has been developed with the aim to test the hypothesis that changing the diet of older subjects with established enhanced IP by increasing their PPs consumption can alter the intestinal microbial ecosystem in a way that is beneficial for intestinal barrier function, resulting in reduced IP and decreased translocation of inflammogenic bacterial factors from the digestive tract into the bloodstream. To this aim, a randomised, controlled, crossover intervention trial was performed. Sixty-six subjects (aged ≥ 60 y) with increased IP based on serum zonulin levels, were randomly allocated to one of the two arms of the intervention consisting of a control diet (C-diet) vs a PP-rich diet (PR-diet). Each intervention was 8-week long and separated by an 8-week wash out period. Overall, PR-diet reduced serum zonulin levels, an indirect marker of IP, particularly in women and in subjects with high initial zonulin levels, who also experienced reductions in diastolic blood pressure and blood glucose levels. Further, a significant reduction in faecal and serum calprotectin (marker of indicative of intestinal and systemic inflammation), and a significant increase in zonula occluden (marker of epithelial integrity) was observed. In addition, PR-diet increased fibre-fermenting and butyrate-producing bacteria reinforcing the interaction between fiber, PPs, and gut microbiota activity. These bacteria were negatively correlated with the markers of inflammation, indicating a link between gut bacterial composition and inflammatory status. Further, findings revealed a positive association between serum zonulin and blood DNAemia, suggesting that paracellular permeability of epithelial and endothelial cell layers may facilitate bacterial translocation into the bloodstream. Finally, the PR-diet intervention significantly reduced serum zonulin and interleukin-6 levels in subjects with higher blood DNAemia, with trends towards reductions in bacterial DNAemia, BMI, and TC/HDL ratio. Collectively, these results indicate that PPs and a PR-diet may be a promising approach for managing IP and related factors such as inflammation, and gut function.

CARDIOSE®, A NATURAL FLAVONOID WITH POTENTIAL HEALTH BENEFITS

PELLICER, Teresa

HealthTech Bio Actives (HTBA), Spain

tpellicer@htba.com

Introduction: Flavonoids are bioactive compounds found in plants, especially abundant in citrus fruits, which are one of their main dietary sources. Each citrus species contains specific flavonoids, Hesperidin being predominant in sweet oranges (Citrus sinensis). Hesperidin is well-known for its health-promoting effects, due to its antioxidant and anti-inflammatory properties. Hesperidin is a chiral molecule that exists in two isomeric forms (2S and 2R), but only the 2S- isomer is present in nature. Cardiose® is 2S-Hesperidin. Cardiose® is much more bioavailable as compared to the synthetic hesperidin preparations existing in the market.

Material & Methods: Randomized, placebo-controlled, double-blind, parallel group clinical trial was carried out. At the designed points, flow-mediated dilation, blood pressure, metabolic variables, and adhesion molecules were quantified on plasma samples according to standard procedures.

Results: Cardiose® has been studied for its beneficial effects on cardiovascular health, physical performance and digestive health. Available evidence highlights the potential of 2S-Hesperidin as a functional ingredient for promoting overall health, particularly decreasing cardiovascular disease risk factors. Various human intervention studies with Cardiose® have shown that this ingredient improves endothelial function, thereby enhancing arterial flexibility. Additionally, it can down-regulate endothelial adhesion molecules involved in arterial inflammation (sVCAM-1 and sICAM-1). Consequently, due to its endothelial effects Cardiose® supplementation may also contribute to the reduction of blood pressure.

Conclusion: The positive effect on endothelial function and the regulation of systolic blood pressure show the ability of Cardiose® to promote cardiovascular health. Additional studies are expected to be completed in the following years to apply the outstanding properties of this natural ingredient in new application areas, such as healthy ageing.

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FOOD-DERIVED EXTRACELLULAR VESICLES AS NANOCARRIERS OF BIOACTIVE COMPOUNDS: THERAPEUTIC POTENTIAL

DÁVALOS, Alberto [1,2]

[1] Laboratory of Epigenetics of Lipid Metabolism,
Instituto Madrileño de Estudios Avanzados (IMDEA) Food, CEI UAM + CSIC, Madrid, Spain.

[2] Consorcio CIBER de la Fisiopatología de la Obesidad y Nutrición (CIBERObn),
Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

alberto.davalos@imdea.org

Extracellular vesicles (EVs) are lipid bilayer-delimited nanoparticles (≈ 50-200 nm) that are naturally secreted by almost all types of cells, serving various functions, including intercellular communication. EVs transport different biomolecules (i.e., proteins, sugars, RNAs, vitamins, etc.). EVs are present in all kingdoms of life and can be isolated from mammalian, plants or microorganisms. Some EVs seem to have good pharmacological properties including high biocompatibility, reduced toxicity, ability to cross biological barriers, or long half-life in circulation. For this, they have been proposed as therapeutic molecules and as vehicles for the encapsulation and delivery of bioactive molecules, providing resistance to degradation and enhancing their stability.

We here aim to evaluate the use of food-derived EVs from both mammalian and plants for the therapeutic delivery of bioactive molecules for biomedical applications.

EVs were isolated from bovine milk and edible plants (i.e., broccoli), using a combination of different methods, loaded with bioactive compounds and then characterized. The stability within the gastrointestinal tract and the uptake by different cell lines were evaluated. The delivery to tissues –when administered orally or systemically– was assessed in rodent models.

Although several obstacle and challenges for the clinical use of EVs still exists, food-derived EVs seems to be good sources for the isolation of biocompatible EVs with low toxicity and scalability potential and could open new therapeutic alternatives for the oral delivery of biomolecules with pharmacological potential.

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RESVERATROL/OMEGA-3 FATTY ACIDS ASSOCIATION: INNOVATIVE NUTRACEUTICALS TO COUNTERACT OCULAR DISEASES

DELMAS, Dominique [1,2,3]

[1] Université de Bourgogne, 21000 Dijon, France
[2] INSERM Research Center U1231- Cancer and Adaptive Immune Response Team, Bioactive Molecules and Health Research Group, 21000 Dijon, France
[3] Centre de Lutte contre le Cancer Georges François Leclerc Center, 21000 Dijon, France

dominique.delmas@u-bourgogne.fr

Among polyphenols with interesting biological properties in terms of human health, resveratrol, a trihydroxystilbene, has been able to show numerous beneficial effects both in vivo and in preclinical/clinical studies in various pathologies such as cardiovascular pathologies, cancers, inflammatory or even degenerative diseases. In this way, resveratrol could help to fight the main molecular events of ocular pathologies through its antioxidant power, as well as its anti-inflammatory and anti-angiogenic properties. Similarly, poly-unsaturated fatty acids, and more particularly omega-3 fatty acids (O3FAs) have attracted interest to counteract ocular diseases and more specifically age-related macular degeneration (AMD). This eye disease, which is the leading cause of irreversible blindness in industrialized countries among people over the age of 50, is characterized by damage to the central part of the retina, the macula. Despite therapeutic advances thanks to the use of anti-vascular endothelial growth factor (VEGF), many resistance mechanisms have been found to accentuate the visual deficit. In this context, we have studied whether a formulation called Resvega® (RGA) (laboratoires Théa) containing trans-resveratrol and two O3FAs, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), was able to counteract AMD pathology.

Our results highlight that RGA significantly reduced laser-induced choroidal neovascularization (CNV) in mice and a proteomic approach confirmed that RGA could counteract the progression of AMD through a pleiotropic effect targeting key regulators of neoangiogenesis in retina cells in vivo. These events were associated with an accumulation of resveratrol metabolites within the retina. A molecular analysis revealed that RGA, inhibited VEGF-A secretion in vitro by disrupting the dissociation of the VEGF-R2/Cav-1 complex into rafts and subsequently preventing MAPK activation. Subsequently, we observed the phosphorylation inhibition of the inhibitor of NFκB, IκB, which can bound NFκB dimers and sequester them in the cytoplasm. Moreover, DNA ChIP analysis reveals that this combination prevents the interaction between AP-1 and vegf-a and vegf-r2 gene promoters. Very interestingly, we highlight that RGA could prolong the anti-angiogenic effect of Avastin®, which is an anti-VEGF agent typically used in clinical practice.

Furthermore, recent resultst from a clinical study in patients with intermediate AMD suggest a beneficial effect of this RGA for slowing the progression of AMD. Further investigations in human are necessary to collect statistically significant data and to provide recommendations.

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SENOLYTIC DRUG AND POLYPHENOL COMBINATIONS FOR LUNG DISEASE

GAMBERO, Alessandra

Pontifical Catholic University of Campinas, Brazil

alessandra.gambero@puc-campinas.edu.br

Cellular senescence is characterized by a stable cell proliferation arrest and the establishment of a secretory phenotype in normal cells in response to intrinsic and extrinsic stimuli. It is associated with chronological aging and as a response to environmental stressors.

Expanding knowledge about the role of senescence in physiological contexts and disease processes has led to the development of drugs targeting the harmful effects of cellular senescence as a therapeutic approach. Senotherapeutics can be classified into two categories: senolytics, which selectively eliminate senescent cells, and senostatics (or senomorphics), which inhibit, reverse, or prevent aspects associated with cellular senescence.

The most studied combination therapy involves dasatinib and quercetin, a natural polyphenol that targets the BCL-2 and PI3K/AKT signaling pathways. The various pharmacological mechanisms described for polyphenols suggest that others may also have senotherapeutic effects, which has been the focus of our recent work.

TARGETING SENESCENCE WITH AN EXTRACT OF SALVIA HAENKEI EXTENDS LIFESPAN AND HEALTHSPAN IN MICE

SARILL, Miles [1,2,3]; ZUMERLE, Sara [2,4]; MONTOPOLI, Monica [2,3]; ALIMONTI, Andrea [1,2,4,5,6]

[1] Institute of Oncology Research (IOR), Bellinzona, Switzerland;
[2] Veneto Institute of Molecular Medicine, Padova, Italy;
[3] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy;
[4] Department of Medicine, University of Padova, Padova, Italy;
[5] Università della Svizzera italiana, Lugano, Switzerland;
[6] Department of Health Sciences and Technology (D-HEST), ETH Zurich, Zurich, Switzerland

miles.sarill@ior.usi.ch

Introduction:The accumulation of senescent cells within tissues is a driver of organismal aging, and pharmacological interventions that blunt their impact can exert beneficial effects on longevity and healthspan. Botanical extracts represent a treasure trove of molecules for investigation as senotherapeutics. We previously discovered that Salvia haenkei extract displayed anti-senescence properties in vitro. Here, we studied its anti-aging potential in vivo.

Materials & Methods: A standardized, polyphenol-rich extract of Salvia haenkei (Haenkenium (HK); 0.5 mg/kg) was administered to 20-month-old C57BL/6 mice in drinking water (N=48 treated mice; N=37 matched untreated controls). Molecular and immunohistochemistry analyses of senescence markers were performed on mice treated for 4 months. Analysis of the composition of HK revealed the main phytoconstituents which were assessed in vitro for their anti-senescence properties and mechanisms of action.

Results: HK significantly extended the median lifespan of mice, improved muscle, skin and kidney health, and reduced tissue signatures of inflammation, fibrosis and senescence. Luteolin was identified as a constituent of HK with senomorphic properties and was shown to disrupt the binding of p16 to Cyclin Dependent Kinase 6 (CDK6).

Conclusion: Administration of HK promotes longevity in mice, reduces age-induced senescence and disrupts the p16-CDK6 interaction.

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DIETARY EFFECTS OF PEANUT SKIN EXTRACTS (PSE) ON HEPATIC ENERGY RESERVES AND FUNCTION IN MICE FED A LONG-TERM ATHEROGENIC DIET.

TOOMER, Ondulla

Food Science & Market Quality & Handling Research Unit, Agricultural Research Service,
U.S. Dept. of Agriculture, United States of America

Ondulla.Toomer@usda.gov

Supplementation with 1% peanut skin extract in mice fed an atherogenic diet reduced hepatic total cholesterol and improved growth in young mice. Hence, we aimed to determine the hepatic effects of supplementing an atherogenic diet with 2% PSE. Forty adult male mice were assigned to 4 treatments for 16-weeks: 1) control (Con), 2) Con + 2% PSE (Con-PS, 3) Atherogenic diet (57BB), 4) 57BB + 2% PSE (57BB-PS). Body weights (BW) were measured bi-weekly. At termination blood and liver samples were collected for analysis. All data were analyzed for variance at $P < 0.05$. The 57BB and 57BB-PS mice had significantly reduced BW relative to the controls ($P < 0.0001$). Hepatic total cholesterol and C-reactive protein (CRP) was elevated in 57BB and 57BB-PS mice, with highest levels in 57BB-PS mice ($P < 0.0001$). Hepatic glycogen was reduced in 57BB and 57BB-PS mice relative to the other treatments ($P < 0.0001$). Hepatic alanine aminotransferase (ALT) activity was elevated in 57BB mice as compared to the controls ($P < 0.0001$.

These results suggest that 2% PSE supplementation does not improve hepatic function when fed an atherogenic diet. Additional studies are warranted to better discern the dietary effects on energy metabolism.

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ONE-WEEK DAILY CONSUMPTION OF HYDROXYTYROSOL MODULATES CIRCULATING MIRNAS TRANSPORTED IN EXOSOMES IN HEALTHY VOLUNTEERS

LÓPEZ DE LAS HAZAS, María-Carmen [1]; DEL SAZ-LARA, Andrea [1,2]; TOMÉ-CARNEIRO, Joao [2]; CRESPO, Carmen [2]; MAZARÍO-GÁRGOLES, Carmen [1]; CHAPADO, Luis A. [1]; VISIOLI, Francesco [3]; DÁVALOS, Alberto [1,4]

[1] Laboratory of Epigenetics of Lipid Metabolism, Instituto Madrileño de Estudios Avanzados (IMDEA) Food, CEI UAM + CSIC, Madrid, Spain
[2] Laboratory of Functional Foods, IMDEA Food Institute, CEI UAM + CSIC, Madrid, Spain
[3] Department of Molecular Medicine, University of Padova, Padova, Italy
[4] Consorcio CIBER de la Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

mcarmen.lopez@imdea.org

Introduction: Hydroxytyrosol (HT), a phenolic compound from extra virgin olive oil, is linked to several beneficial properties. Extracellular vesicles (EVs) are lipid bilayer-delimited nanoparticles that are naturally secreted by almost all types of cells to establish intercellular communication. microRNAs (miRNAs) –small non-coding RNAs with important roles in gene expression regulation– can be transported in EVs. It has been reported that HT consumption can affect tissue miRNA expression, but information on circulating miRNAs is scarce. Therefore, our objective was to determine whether HT supplementation influences blood EV secretion and whether it modulates the expression of miRNAs transported by EVs.

Material & Methods: A double-blind, randomized, placebo-controlled study with healthy participants was carried out. Participants received a week of daily supplementation with 25mg of HT or placebo. Blood was obtained before and after the study. EVs from plasma were isolated and characterized and miRNA profiled by RNA-seq.

Results: Results showed that HT supplementation did not modify the number of circulating EVs. However, the expression of certain miRNAs transported in these EVs were modulated by HT consumption. Gene target prediction for these miRNAs suggests their involvement in relevant biological functions.

Conclusions: HT supplementation does not affect the release of EVs but modulates transported miRNAs.

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FORMATION, ANALYSIS, AND VALORISATION POTENTIAL OF ENZYMATIC OXIDATIVE COUPLING PRODUCTS OF HYDROXYCINNAMOYLAGMATINES FROM BARLEY

VAN ZADELHOFF, Annemiek; VINCKEN, Jean-Paul; **DE BRUIJN, Wouter J.C.**

Laboratory of Food Chemistry, Wageningen University & Research, Wageningen, The Netherlands

wouter.debruijn@wur.nl

Introduction: Barley (Hordeum vulgare) produces hydroxycinnamoylagmatines (HCAgms) whose oxidative coupling products have been suggested to be antimicrobial. Our aims were to study oxidative coupling of HCAgms and develop methods for analysis of coupling products, determine HCAgm composition in beer brewing by-products, and explore HCAgms' antimicrobial activity.

Material & Methods: We performed enzymatic oxidative coupling with coumaroylagmatine, feruloylagmatine, and sinapoylagmatine in model systems. Several HCAgm dimers were purified and identified by NMR and HRMS. Additionally, HCAgms in barley-derived beer brewing by-products were characterised by UHPLC-MS.

Results: The oxidative coupling reactivity of HCAgms followed the order sinapoylagmatine > feruloylagmatine > coumaroylagmatine. The main linkage types of dimeric coupling products were identical to those naturally present in Hordeum species, namely 4-O-7'/3-8', 2-7'/8-8', and 8-8'/9-N-7'.[1,2] Upon analysis of beer brewing by-products, we found that diversity of HCAgms was much larger than previously reported. Additionally, preliminary experiments indicated that oxidative coupling can increase the antimicrobial activity of HCAgms.

Conclusion: We developed methodologies for biomimetic formation and analysis of naturally occuring HCAgm coupling products. Our mechanistic insights into oxidative coupling and preliminary antimicrobial activity results are openings for further investigation of HCAgms' effects on human health and potential for industrial valorisation of beer brewing by-products.

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STRATEGIES TO OVERCOME RESISTANCE IN PHYTOPATHOGENIC FUNGI USING MULTITARGET NATURAL AND NATURE-INSPIRED STILBENOIDS

PRINCIOTTO, Salvatore; PINNA, Cecilia; SACCHI, Francesca; DOZIO, Denise; KUNOVA, Andrea; GHOSH, Sharmila; PIZZATTI, Cristina; CORTESI, Paolo; PINTO, Andrea; DALLAVALLE, Sabrina

Department of Food, Environmental and Nutritional Science (DeFENS), University of Milan, Milan

salvatore.princiotto@unimi.it

Introduction: Phytopathogenic fungi heavily affect crop production worldwide, thus representing a serious threat for food security and availability1. Moreover, intensive monoculture cropping and treatment by fungicides with a single-site mechanism of action contribute to the emergence of resistant strains2. For their high diversity and versatility, natural products are considered the first step towards the developement of new biofungicides. Among them, stilbenoids, as phytoalexins overexpressed from plants in response to stress conditions3, represent suitable substrates for the obtainment of multitarget agrochemicals, potentially able to limit the onset and spread of fungicide resistance.

Material & Methods: The reported stilbenoids were synthesized, purified and chemically characterized. The fungicide activity was evaluated as micelyum growth inhibition on P. oryzae (wild-type and resistant strains).

Results: Resveratrol, its methylated derivatives, and the corresponding dimers were prepared by chemical or chemoezymatic protocols, resulting in a small collection of multitarget compounds, based on the stilbenoid core. Natural deoxyrhapontigenin resulted as the best potential antifungal agent, inhibiting the mycelium growth by 60-80%, both on wild-type and resistant strains.

Conclusion: A collection of stilbenoid-derivatives was prepared and tested as multitarget antifungal agents. Further efforts are needed to improve the activity profile of the most promising compounds, especially against resistant strains.

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Polyphenols Applications

Abstracts for Poster Presentations

(by alphabetical order)

PHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY OF WASTEWATER AFTER ALKALINE HEAT TREATMENT OF PIGMENTED CORN VARIETIES

ARRIAGA GONZÁLEZ, Itandehui; MÉNDEZ LAGUNAS, Lilia Leticia; RODRÍGUEZ RAMÍREZ, Juan; AQUINO GONZÁLEZ, Laura Victoria

Instituto Politécnico Nacional, CIIDIR Oaxaca, México

iarriagag1800@alumno.ipn.mx

Introduction: Worldwide there is a wide variety of foodstuffs made of pigmented maize varieties. Several times, the corn kernels (CK) need alkaline thermal treatment known as nixtamalization (NIX), and it is used to soften and increase the bioavailability of nutrients in the CK and by-products. However, the NIX produces more than 50 Mm3 of wastewater annually, which is an undervalued source of bioactive compounds such as hydroxycinnamic acids and flavonoids.

Material & Methods: White (WCN), yellow (YMN), purple (PCN), red (RSN), and blue corn (BBN) were nixtamalized with 1% Ca(OH)2 solution at 84°C for 50 min, and the supernatant was analyzed. The antioxidant activity (AA), total phenolic (TPC) and flavonoid (TFC) content was evaluated by Folin-Ciocalteau, aluminum chloride, and DPPH radical methods. The phenolic profiles were obtained by HPLC-UV. The data were analyzed with ANOVA and Tukey test (α =0.05).

Results: The WCN, YMN, and RSN showed the highest AA (9-16 mgAAE/gdb), TPC (556-695 mgGAE/gdb), and TFC (334-733 µgQE/gdb). Furthermore, were identified hydroxycinnamic acids: syringic, vanillic, hydroxybenzoic, caffeic, sinapic, ferulic, and coumaric, and flavonoids: quercetin and kaempferol.

Conclusion: These effluents are a source of natural antioxidants and could be useful in the functional food and pharmaceutical industries.

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ANTIPSORIATIC POTENTIAL OF A POLYPHENOL-RICH LIGHT BIO-OIL FROM HYDROTHERMAL CARBONIZATION OF AVOCADO PEEL

AVALOS-VIVEROS, Miguel [1]; GARCÍA-PÉREZ, Martha-Estrella [1]; SANTES-HERNÁNDEZ, Víctor-Florencio [2]; SÁNCHEZ-CEJA, Sandra-Guadalupe [1]; LÓPEZ-MEZA, Joel-Edmundo [3]

[1]Facultad de Químico Farmacobiología, Universidad Michoacana de San Nicolás de Hidalgo, México
[2] Centro Interdisciplinario de Investigaciones y Estudios sobre Medio Ambiente y Desarrollo, Instituto Politécnico Nacional, México
[3] Facultad de Medicina Veterinaria y Zootecnia, Universidad Michoacana de San Nicolás de Hidalgo, México

0936007x@umich.mx

Introduction: Psoriasis is an incurable dermatological disease with a worldwide incidence of 2-3%1. More than 50% of patients use plant-derived extracts, including those rich in polyphenolic compounds2. Polyphenols can be obtained from the avocado peel, an undervalued waste product from the agri-food industry, using hydrothermal carbonization (HTC)3. This study evaluated the antipsoriatic activity of a polyphenol-rich light bio-oil (LBO) obtained by HTC.

Materials & Methods: Avocado peels were macerated in 90% ethanol, and the extracted biomass was subjected to HTC at 250 °C (720 psi, 15 min) to obtain LBO. Subsequently, LBO was analyzed by GC-MS3. The topical antipsoriatic LBO activity (200 mg/kg) was determined in an Imiquimod (IMQ) induced psoriatic mice model. The impact of LBO on psoriasis severity (PASI score), histopathological features, and skin cytokine production (IL-6, TNF-alpha, INF-gamma, IL-17A, IL-23, IL-22) was determined.

Results: Major identified molecules in LBO were phenols (45%), mainly syringol3. LBO significantly reduced the PASI score ($p \leq 0.0001$) and proinflammatory skin cytokines ($p \leq 0.05$) vs. the IMQ group. Histopathological findings showed a decrease in the inflammatory epidermal neutrophilic and T-cell infiltration.

Conclusion: The LBO showed antipsoriatic effects. Hence, it has the potential for further development as a topical candidate for psoriasis treatment.

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AN INSIGHT OF HOP-PRODUCTION IN BRAZIL: CULTIVAR AND GEOGRAPHICAL ORIGIN EFFECTS ON THE CHEMICAL COMPOSITION OF HOPS

MARIÑO-CORTEGOSO, Sandra [1,2]; RODRÍGUEZ-BERNALDO DE QUIRÓS, Ana [1,2]; SENDÓN, Raquel [1,2]; DESCHAMPS, Cícero [3]; **BARBOSA-PEREIRA, Letricia [1,2]**

[1] FoodChemPack research group, Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, Spain
[2] Instituto de Materiales (iMATUS), University of Santiago de Compostela, Santiago de Compostela, Spain
[3] Department of Agronomy, Federal Institute of Education Science and Technology of Paraná, Brazil

letricia.barbosa.pereira@usc.es

Introduction: Hops are used in brewery industry to provide bitter flavor. Brazil is one of the largest beer producers in the world, yet it imports almost all of its hops1. This study aims to study the differences in bitter acids and characteristic phenolics, in Cascade, Comet, Chinook, Magnum and Zeus cultivars produced in Brazil USA and Germany.

Material & Methods: Polyphenols and the α and ß acids were extracted with absolute EtOH using ultrasounds and quantified through the external standard method based on high-resolution liquid chromatography coupled to a diode array detector (HPLC-DAD).

Results: Phenolic compounds were found to be most concentrated (∼11 mg/g) in the Zeus cultivar from the USA, which also provided the highest antioxidant capacity (87 mgTE/g). Furthermore, the Magnum cultivar produced in Germany stood out for its high antioxidant capacity, and Comet from Brazil stood out for its high content of xanthohumol, the main hop phenolic. Regarding the bitter acids, Comet, Chinook and Zeus cultivars provided the highest α/β ratio, clasifying them into bitter hops.

Conclusion: The results of this study demonstrate the differences in chemical composition of hop cultivars and the interference of the geographical origin, by showing a high-quality chemical composition of Brazilian hops to use in brewering.

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EXTRACTION AND IDENTIFICATION OF ANTI-INFLAMMATORY COMPOUNDS FROM ARTEMISIA SPECIES

BARDAN, Marius [1]; STEFAN, Cezara Bianca [1]; IONESCU, Alexandra-Mariana [1]; BOTEZATU, Andreea Veronica [1]; FURDUI, Bianca [1]; BRATU, Camelia [2]; VINATORU, Costel [2]; DINICA, Rodica [1]

[1] University Dunarea de Jos, Galati, Romania;
[2] Genetic and Vegetal Resources Bank, Buzau, Romania

Marius.Bardan@ugal.ro

Introduction: Many of the plants of the Artemisia genus are known for various pharmacological properties such as antimicrobial, antihelmintic or antispasmodic. Recent reports associate the anti-inflammatory properties with compounds of natural origin such as flavonoids or oxygenated sesquiterpenoids. Due to their properties, the validation of biological effects are relevant and interesting, therefore, the most effective methods of obtaining extracts enriched in such compounds, including their extraction and purification, are in great demand.

Material & Methods: The plant material used to obtain the flavonoid extracts was represented by the freshly dried leaves of Artemisia ludoviciana, and acquired from the Genetic and Vegetal Resources Bank (GVRB) Buzau, Romania. The extracts, obtained by fractionation with solvents, were analized for flavonoidic compounds and to demonstrate the antioxidant activity the radical scavenger capacity and the reducing power were also realised.

Results: These studies were carried out for the first time on the Romanian Artemisia ludoviciana plant variety. The analyzed extracts contain significant amounts of biologic compounds such as flavonoids and phenolic acids, with promising antioxidant activity.

Conclusion: An important amount of flavonoids was determined in the Romanian Artemisia ludoviciana species and further studies are foreseen to demonstrate biological activities such as anti-inflammatory ones.

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CARDIOVASCULAR BENEFITS OF A NOVEL SUSTAINABLE POMEGRANATE EXTRACT

BENEDETTI, Giada [1]; FLORI, Lorenzo [1]; SPEZZINI, Jacopo [1]; MIRAGLIOTTA, Vincenzo [2]; MARTELLI, Alma [1]; CALDERONE, Vincenzo [1]; TAGLIAVENTO, Luca [3]; MENEGUZZO, Francesco [4]; TESTAI, Lara [1]

[1] Department of Pharmacy, University of Pisa, Italy
[2] Department of Veterinary Sciences, University of Pisa, Italy
[3] HyRes Srl, Benevento, Italy
[4] Institute of Bioeconomy, National Research Council of Italy, Sesto Fiorentino, Italy.

giada.benedetti@phd.unipi.it

Introduction: Pomegranate fruit is endowed with beneficial effects in cardiovascular and non-cardiovascular diseases, due to abundant bioactive substances. The major constituents are ellagitannins, polyphenolic compounds of high nutraceutical value, but poorly absorbed and water-soluble. A novel extract obtained from non-edible pomegranate peel (PomeExtract), using hydrodynamic cavitation (20 minutes, peak temperature of 39°C), was investigated for its in vivo cardiovascular effects.

Method: The hypotensive effect of PomeExtract was tested in an acute phenylephrine-induced hypertensive rat model. Then, the increment of systolic blood pressure was monitored in a chronic 6-weeks PomeExtract supplementation on spontaneously hypertensive rats. Finally, the endothelial dysfunction and myocardial proinflammatory and profibrotic markers were assessed.

Results: A reduction of blood pressure was observed in the acute model, along with a good intestinal bioaccessibility after oral administration. Interestingly, this effect was observed with a dosage 20 times lower than in experiments using only ellagic acid. Then, the chronic supplementation contained the systolic blood pressure increment, partially protected against endothelial dysfunction, and reduced myocardial proinflammatory and profibrotic markers.

Conclusion: These findings are of nutraceutical interest, suggesting that hydrodynamic cavitation affords the production of a novel extract, which exerts significant cardioprotective effects and is endowed with high water-solubility and good enteric bio-accessibility.

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POLYPHENOL COMPOSITION, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITY OF SELECTED CHILI PEPPER EXTRACTS

BOROTOVÁ, Petra [1]; STRAKA, Dušan [1]; KOVÁČIKOVÁ, Eva [2]; JANČO, Ivona [1]; ÁRVAY, Július [3]; LAKATOŠOVÁ, Jana [1]; ĎÚRANOVÁ, Hana [1]

[1] AgroBioTech Research Centre, Slovak University of Agriculture, Slovak Republic
[2] Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Slovak Republic
[3]: Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Slovak Republic

petra.borotova@uniag.sk

Introduction: This study aimed to determine the content of selected bioactive compounds in chilli pepper extracts and to evaluate their antioxidant and antimicrobial activities. These extracts can be used as food additives to potentially extend the shelf life of food products.

Material & Methods: The extracts were obtained from Capsicum varieties – Habanero (H.) Neon, Peito de Moca, H. Orange, H. Lemon, and H. Red. Bioactive compounds, total phenolic content (TPC), antioxidant activity (AA), and antimicrobial activity (AMA) were analyzed using HPLC-DAD, the Folin-Ciocalteu method, the DPPH assay, and the disk diffusion method, respectively.

Results: H. Orange had the highest AA (3.88±0.38 mg TEAC/g DW), TPC (12.74±0.63 mg GAE/g DW), ferulic acid (48.35±0.37 mg/kg), and dihydrocapsaicin (5216.18±16.78 mg/kg) levels, with a strong AMA against S. aureus (15.33±4.00 mm). H. Red followed with the highest levels of rutin (63.56±0.21 mg/kg), caffeic acid (3.42±0.27 mg/kg), and capsaicin (14867.28±172.99 mg/kg), with AMA against E. coli (15.89±5.55 mm). H. Neon had the most coumaric acid (31.14±0.05 mg/kg) and resveratrol (29.45±14.73 mg/kg), with strong inhibition (20.22 ± 8.15 mm) against S. enterica.

Conclusion: The strong antimicrobial and antioxidant properties of these varieties suggest their potential to extend the shelf life of food products.

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EFFECTS OF INDUSTRIAL DE-ALCOHOLIZATION ON THE POLYPHENOLIC PROFILES AND BIOACTIVE PROPERTIES OF RED AND WHITE WINES FROM DIFFERENT VINIFICATION METHODS

BÖRSIG, Amelie [1]; OTTEN, May-Britt [1]; FISCHER, Ulrich [2]; RICHLING, Elke [3]; WINTERHALTER, Peter [1]; GÖK, Recep [1]

[1] Institute of Food Chemistry, Technische Universität Braunschweig, Germany
[2] Institute of Viticulture and Oenology, DLR Rheinpfalz, Germany
[3] Department of Chemistry, Division of Food Chemistry, RPTU Kaiserslautern, Germany

amelie.boersig@tu-braunschweig.de

Introduction: Polyphenols are phytochemicals that are thought to have potential health-promoting effects, such as cardioprotective, anti-carcinogenic, anti-atherogenic and anti-inflammatory properties [1]. In this study, red and white wines produced by different vinification methods were analyzed for their polyphenolic composition. Conventional vinification and de-alcoholization methods were compared with measures used to enrich the wines with polyphenols.

Material & Methods: After purification and concentration of the wines on Amberlite® XAD-7HP resin, the respective polyphenol profiles were analyzed using LC-ESI-MSn. The compounds were then quantified on an HPLC-PDA system. The XAD7-extracts were evaluated for their total polyphenol content (TPC) according to the Folin-Ciocalteu method, their antioxidant capacity (TEAC) and their potential effect on the carbohydrate metabolism using the α-glucosidase inhibition assay.

Results & Conclusion: Differences in the structural diversity and the content of the lead compounds were observed between the conventionally produced and the specially fortified wines, especially in the white wines. De-alcoholization had a conciderable effect on the polyphenolic composition of the wines. Certain special vinification methods led to a significant increase in the TPC, the antioxidant capacity and the inhibitory activities (IC50) against α-glucosidase in the white wines. Whereas red wines consistently revealed enhanced values and inhibitory effects.

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ANTIBACTERIAL AND ANTBIOFILM ACTIVITIES OF FLAVONOL-ENRICHED SAFFRON TEPAL EXTRACT AGAINST MULTI-DRUG RESISTANT ESKAPE PATHOGENS

BOUOTHMANY, Kaoutar [1,2]; ZALEGH, Imane [1]; AIT MHAsND, Rajaa [1]; RHALLABI, Naima [1]; BENBACER, Laila [2]; FOUGERE, Laëtitia [3]; DESTANDAU, Emilie [3]; MELLOUKI, Fouad [1]

[1] RU Microbiology, Biomolecules and Biotechnology, Laboratory of Chemistry-Physics and Biotechnologies of Biomolecules and Materials (LCP2BM); Hassan II University of Casablanca. Morocco
[2] Biology and Medical Research Unit, National Center for Energy Sciences and Nuclear Techniques CNESTEN, Rabat, Morocco
[3] Institute of Organic and Analytical Chemistry (ICOA), UMR 7311, University of Orléans, France

Kaoutar.bouothmany@gmail.com

Introduction: Phenolic compounds have emerged as effective alternative antimicrobials, offering potential solutions to address the challenge of antibioc-resistance. In this context, saffron (*Crocus sativus* L.) bioresidues, a valuable source of phenolic compounds, represent a promising candidate in the development of novel antimicrobials. The study aimed to test the antibacterial and antiadhesive activities of hydroalcoolic saffron tepal extract (HSTE) against multidrug-resistant (MDR) bacteria.

Material & Methods: The antibacterial and the antiadhesive proprieties of HSTE were assessed using the microdilution and the microplate assays respectively against clinical strains of MDR-ESKAPE bacteria. The High-Performance Liquid Chromatography–Mass Spectrometry (UHPLC-MS) was performed to identify the phenolic compounds present in HSTE.

Results: HSTE exhibited bactericidal effect against all the studied strains with a minimum inhibitory concentration (MIC) of 250 and 500 mg/mL and a minimum bactericide concentration (MBC) of 500 mg/mL. Additionally, the highest inhibition of biofilm adhesion was observed at MIC, with 86,63% inhibition for MDR Acinetobacter baumannii and 61,75% for MDR Klebsiella pneumonia at 0.5 MIC. UHPLC-MS analysis revealed the presence of flavonol compounds in HSTE, which can be responsible of the observed activities.

Conclusion: Our findings suggest the potential application of saffron bioresidues as efficient alternative to combat antibiotic resistance and eradicate bacterial biofilms.

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RESVERATROL METABOLITES AND ANALOGUES AS POTENTIAL ANTIFUNGAL AGENTS

BRIOSCHI, Giulia; ANNUNZIATA, Francesca; PINNA, Cecilia; CONTENTE, Martina Letizia; PINTO, Andrea; DALLA VALLE, Sabrina; BORGONOVO, Gigliola

University of Milan, Italy

giulia.brioschi2@unimi.it

Introduction: Resveratrol, a stilbenoid found in red grapes, has been widely studied for its multiple biological activities and mechanisms of action. 1 On the other hand, little is known about its metabolites and analogues, because of their scarce quantities in natural matrices and troublesome purification procedures. To expand the knowledge of the structure-activity relationship of this multifaceted class of compounds, 2 we developed synthetic procedures to obtain natural monomeric stilbenoids, their metabolites, and synthetic derivatives.

Material & Methods: The synthetic strategies include Heck reactions, enzymatic transformations mediated by imm-CalB, methylation reactions, and continuous Pd/C catalyzed hydrogenation using an H-Cube reactor.

Results: A small collection of deoxy- and dihydro-derivatives of resveratrol and pterostilbene have been obtained and they are currently under biological evaluation as antifungals against phytopathogenic fungi P. orizae, B. cinerea and F. culmorum. The inhibition of mycelium growth, spore germination and appressorium formation will be investigated.

Conclusion: A versatile synthetic strategy for the obtainment of resveratrol metabolites and analogues has been developed. Our results evidenced that some metabolites showed higher antifungal activity in respect to the parent compound and hold promise as crop protection agents against phytopathogenic fungi.

This investigation is partially supported by National Recovery and Resilience Plan (NRRP), Mission4 Component 2 Investment 1.3 -Call for tender No. 341 of 15/03/2022 of Italian Ministry of University and Research funded by the European Union–NextGenerationEU, in the frame of the project: Research and innovation network on food and nutrition Sustainability, Safety and Security (ON Foods).

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BACTERIAL ARYL SULFOTRANSFERASES: NOVEL ENZYMES FOR SULFATED POLYPHENOL SYNTHESIS

BRODSKY, Katerina [1,2]; PETRÁNKOVÁ, Barbora [1,3]; PETRÁSKOVÁ, Lucie [1]; BOJAROVÁ, Pavla [1]; VALENTOVÁ, Kateřina [1]

[1] Institute of Microbiology of the Czech Academy of Sciences, Czech Republic
[2] Department of Biochemistry and Microbiology, University of Chemistry and Technology, Czech Republic
[3] Department of Genetics and Microbiology, Faculty of Science of Charles University, Czech Republic

katerina.brodsky@biomed.cas.cz

Introduction: (Poly)phenols are plant metabolites known for their therapeutic effects. These compounds are largely metabolized via phase II biotransformation, particularly through sulfation and glucuronidation. Sulfated metabolites for pharmacokinetic studies of polyphenols can be produced enzymatically using bacterial aryl sulfotransferases (ASTs). However, only a few ASTs have been described

Methods and Material: We identified putative ASTs by gene mining based on known ASTs from Desulfitobacterium hafniense1 and E. coli CFT073. The new ASTs were recombinantly produced in E. coli, purified, and biochemically characterized. The substrate specificity of the new and the known ASTs was tested using para-nitrophenyl sulfate (p-NPS) as a sulfate donor2 and various flavonoids as acceptors.

Results: Most substrates were successfully sulfated by all ASTs tested. Some new enzymes showed a greater sulfation capability than known ASTs and produced not only monosulfated products but also disulfates. The products were detected by HPLC-MS.

Conclusion: Bacterial ASTs are promising and effective tools for the sulfation of polyphenolic compounds. However, each enzyme produced different products under identical conditions, emphasizing the importance of studying their substrate specificity and regioselectivity. Some of the new ASTs were found to be more efficient than previously described ASTs, highlighting their potential for synthetic applications and deconjugation processes

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GRAPE POMACE POLYPHENOLS PROMOTE OSTEOGENESIS AND INHIBIT ADIPOGENESIS: THE EFFECT ON BONE MARROW STEM CELL DIFFERENTIATION

CALABRISO, Nadia [1]; MASSARO, Marika [1]; SCODITTI, Egeria [1]; QUARTA, Stefano [1]; GERARDI, Carmela [2]; GIOVINAZZO, Giovanna [2]; CARLUCCIO, Maria Annunziata [1]

[1] National Research Council (CNR) Institute of Clinical Physiology (IFC), 73100 Lecce, Italy;
[2] National Research Council (CNR) Institute of Sciences of Food Production (ISPA), 73100 Lecce, Italy

nadia.calabriso@cnr.it

Osteoporosis is an age-related systemic skeletal disease, characterized by progressive loss of bone density, and increased vulnerability to fractures(1). Bone marrow contains multipotential mesenchymal stem cells, BMSCs, that can differentiate into osteoblasts and adipocytes. Age-related changes can affect BMSC differentiation leading to adipose accumulation in the bone marrow and subsequent bone loss. Natural compounds such as polyphenols can prevent and counteract bone diseases(2). Grape pomace is a rich source of polyphenols with multiple health-promoting properties(3). In this study, we analyzed the effects of grape pomace polyphenolic extracts (GPE) on osteogenesis and adipogenesis.

Human BMSCs cultured with and without GPE (1-10 μg/mL GAE) were differentiated into osteoblasts or adipocytes. Osteogenesis and adipogenesis were assayed by Alizarin Red S and Oil Red O staining, respectively, as well as by gene expression of related markers.
GPE significantly induced BMSC differentiation into osteoblasts compared to control, without altering cell viability. This effect was associated with an increase in gene expression of the osteoblastogenesis markers, RUNX2, osteocalcin, collagen type I. Moreover, GPE significantly reduced BMSC differentiation into adipocytes, decreasing lipid accumulation and PPARɣ gene expression.

Our findings suggest GPE as a potentially useful strategy to reduce age-related marrow adipose accumulation and possibly prevent bone fragility.

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SYNTHESIS OF GALLIC ACID-BASED HYBRIDS AND EVALUATION OF α-GLUCOSIDASE AND α-AMYLASE INHIBITION BY MULTISPECTROSCOPIC APPROACH

CARDULLO, Nunzio [1]; SCIACCA, Claudia [1]; MACCARRONELLO, Anna Elisabetta [1]; PERI, Emanuela [2]; CANCEMI, Patrizia [2]; MUCCILLI, Vera [1]

[1] University of Catania, Department of Chemical Sciences, Italy
[2] University of Palermo, Department of Biological Chemical and Pharmaceutical Sciences and Technologies, Italy

ncardullo@unict.it

Introduction: Gallic acid (GA) is a naturally occurring polyphenol widespread in edible plants and fruits. It is mainly found as an ester linked to sugars, polyols and other phenols. Among its derivatives, the most studied, thanks to their role in improving human health, are tannins and flavonoids. Several studies suggest that hydrolysable tannins with higher number of GA residues are effective hypoglycemic agents. Based on that, we employed molecular hybridization strategy to synthesize new hypoglycaemic compounds based on GA and resveratrol, a notorious bioactive polyphenol.

Material & Methods: An orthogonal esterification of resveratrol with GA gave resveratrol di- and tri-gallate. Another hybrid compound was obtained starting from the natural eugenol, by esterification and cross-metathesis reaction. The compounds were studied as α-amylase and α-glucosidase inhibitors. Cytotoxicity of the most active compound was tested on human colon cancer cell line.

Results: Among the compounds synthesized, resveratrol tri-gallate is the most effective inhibitor in low-micromolar scale. The kinetic analysis assessed this hybrid acts as a noncompetitive inhibitor toward the selected enzymes.

Conclusion: This work pointed out how the hybridization of natural polyphenols could lead to discovery promising compounds with interesting structural features for developing a novel hypoglycemic drug for treating type-2 diabetes.

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OPTIMIZATION OF AQUEOUS EXTRACTION OF ANTHOCYANINS FROM VITIS VINIFERA L. LEAVES USING MATHEMATICAL MODELS

CARINI, Chiara; CARRÀ, Francesca; DISCA, Vincenzo; TRAVAGLIA, Fabiano; RINALDI, Maurizio; LOCATELLI, Monica

Università del Piemonte Orientale, Italy

chiara.carini@uniupo.it

Red vine leaves (*Vitis vinifera* L.) are rich in fiber, minerals and vitamins, but also in bioactive phenolic compounds, particularly anthocyanins. These compounds offer health benefits through their antioxidant, cardioprotective and neuroprotective properties, making grape leaves desirable for use in nutraceutical and cosmetic formulations. Beside more traditional extraction methods with organic solvent mixtures, our research aimed to optimize an aqueous extraction. We selected different level of solid/liquid ratio (2.5 to 10 g/100 mL), extraction time (5 to 60 minutes), and temperature (40 to 80 °C). Extracts were characterized for the anthocyanins content using HPLC-DAD. We employed various mathematical models, and we selected the method that exhibit the better fit to clarify the effects of the variables on anthocyanins extraction. Results showed that cyanidin-3-O-glucoside was the most abundant anthocyanin. According with the results, shorter extraction time and higher temperature allow to obtain extract richer in anthocyanins. Concluding, mathematical techniques represent an interesting and necessary tool for the optimization of commercial production, particularly in the context of water-based green extractions, because allow to explore the relationship between process variables and the responses, obtaining the best extraction conditions, enabling to save time and material, and use of solvents.

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C.C. is the recipient of a fellowship grant within the PhD program in "Drug innovation" (Università degli Studi del Piemonte Orientale). V.D. and F.C. are the recipients of a fellowship grant within the PhD program in "Food, Health and Longevity" (Università degli Studi del Piemonte Orientale).

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NATURAL RED GRAPE POMACE EXTRACT IMPROVES VASCULAR AGEING THROUGH REDUCTION OF ENDOTHELIAL CELL SENESCENCE AND INHIBITION OF ENDOTHELIAL DYSFUNCTION

CALABRISO, Nadia [1]; MASSARO, Marika [1]; SCODITTI, Egeria [1]; GIRELLI, Chiara Roberta [2]; GERARDI, Carmela [3]; GIOVINAZZO, Giovanna [3]; **CARLUCCIO, Maria Annunziata [1]**

[1] National Research Council (CNR) Institute of Clinical Physiology (IFC), 73100 Lecce, Italy
[2] Department of Biological and Environmental Sciences and Technologies (DISTEBA), University of Salento, 73100 Lecce, Italy
[3] National Research Council (CNR) Institute of Sciences of Food Production (ISPA), 73100 Lecce, Italy

mariaannunziata.carluccio@cnr.it

Aging, a non-modifiable risk factor for cardiovascular diseases, is characterized by endothelial cell senescence and dysfunction. Grape pomace is important source of bioactive polyphenols with high nutraceutical value. The aim of this research is the valorization of grape pomace by green chemistry to obtain high-value molecules, endowed with biological properties in the chemoprevention of vascular aging.
Grape pomace-derived nutraceuticals (GPN) were structurally characterized by HPLC/DAD and NMR and their effects on endothelial dysfunction and senescence were analyzed. Human umbilical vein endothelial cells (HUVECs) were exposed to H2O2, as an aging model, detected by SA-b-gal staining, or to TNF-a, to induce endothelial dysfunction, analyzed by the expression of inflammatory markers, at protein (EIA/ELISA/Western) and mRNA levels (qRT-PCR). MTT, DCFH-DA and Griess assay were used to evaluate cell viability, ROS and NO levels, respectively.

Our results showed that the exposure to H2O2 promoted senescence of HUVECs, which was significantly alleviated by co-treatment with 5 mg/mL GPN. Furthermore, GPN ameliorated TNF-a-stimulated endothelial dysfunction by increasing NO production and eNOS expression and by reducing ROS levels and the expression of endothelial inflammatory markers.

Overall, the results demonstrated that GPNs inhibit endothelial dysfunction and senescence and may contribute to the prevention of age-related diseases.

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IIMPACT OF A MATERNAL DIET ENRICHED WITH FIBER AND POLYPHENOLS ON THE GUT MICROBIOTA OF THEIR OFFSPRING AT BIRTH AND AT THE END OF SUCKLING

CEBALLOS SÁNCHEZ, Daniela [1,2]; CASANOVA-CRESPO, Sergi [1,2]; RODRIGUEZ-LAGUNAS, María José [1,2]; MASSOT-CLADERA, Malen [1,2]; CASTELL - ESCUER, Margarida 1,2,3; PEREZ - CANO, Francisco José [1,2]

[1] Department of Biochemistry and Physiology, Faculty of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain

[2] Institut of Research in Nutrition and Food Safety (INSA-UB), Barcelona, Spain

[3] Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, Spain

daniceballoss@ub.edu

Introduction: The influence of particular dietary components during pregnancy and lactation on neonatal microbiota and infant health outcomes is still not well understood (1,2). The aim of this study is to assess the effect of the maternal diet during pregnancy and lactation on the microbiota composition of the neonatal and suckling offspring rats.

Material & Methods: Five experimental groups were established: one group received the standard diet and the others were fed an experimental diet enriched with polyphenols (0.5%) and fiber (9%) during pre-gestation, gestation and lactation. Intestinal microbiota composition was analysed by 16S ribosomal RNA gene sequencing techniques.

Results: The composition of the neonatal gut microbiota whose mothers received the fiber and polyphenols-enriched diet showed very few differences compared to those from the reference group. However, the microbiota from 21-day-old offspring, showed significant differences in both alpha/beta diversity with a higher abundance of certain bacterial groups with ability to positively regulate host health, such as Bifidobacterium, Blautia or Butyricicoccus, among others.

Conclusion: The maternal intervention with a fiber and polyphenols-enriched diet demonstrates to beneficillay impact the microbiota composiiton of their offspring at the end of lactation, suggesting the importance of the maternal diet on the descendance's health.

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THE INFLUENCE OF FRUIT ADDITIVES ON THE CONTENT OF BIOACIVE COMPOUNDS IN THE TEA INFUSIONS

CHILCZUK, Barbara; MATERSKA, Małgorzata; PABICH, Marzena; STASZOWSKA-KARKUT, Monika

The University of Life Sciences in Lublin, Poland

barbara.chilczuk@up.lublin.pl

Introduction: In recent years, the tea industry has been developing a branch of teas with fruit additives, which are very popular among consumers.Therefore, the aim of the study was to determine the effect of selected additives on the health-promoting properties of tea infusions and to assess the relationship between the type and concentration of catechins and the anti-radical effect of infusions.

Material & Methods: The research material consisted of black and white tea enriched with fruit. The sum of phenolic compounds and antiradical and antioxidant activities were determined in tea infusions using spectrophotometric methods. Additionally, the chemical composition was determined by LC-QTOF-MS method. The obtained results, combined with PCA statistical analysis, allowed to determine the relationship between the chemical composition of the infusion and its health-promoting properties.

Results: The additives used enhance the health-promoting properties of black tea but negatively affect the antioxidant properties of white tea.

Conclusion: Research has confirmed that the antioxidant and anti-radical properties of tea depend on both the type of tea and additives influencing the extraction of catechins. However, further research is necessary to examine other compounds influencing the health-promoting properties of tea infusions and to determine the impact of additives on their extraction.

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POLYPHENOLS IN DRIED GRAPE EXTRACTS: POWERFUL AID IN ANIMAL FARMING

CHOIŃSKA, Renata; PIASECKA-JÓŹWIAK, Katarzyna; SKĄPSKA, Sylwia; JASIŃSKA, Urszula

Institute of Agricultural and Food Biotechnology, Poland

renata.choinska@ibprs.pl

Introduction: The widely promoted idea of sustainable food production drives activities to search for natural, low-cost functional products from alternative sources. In this context, actions to improve the welfare and productivity of farm animals are a key issue. Particularly, products that provide alternatives to conventional antibiotics present a challenge. The antimicrobial and antioxidant properties of polyphenols make them attractive for these purposes.The presented research aims to develop a powdered form of grape extracts by spray-drying to preserve the content of polyphenols and their antioxidant capacity.

Material & Methods: Extracts of grape marc from high-quality white wines were used for research purposes. The spray-drying technique was used to obtain the solid form of the extracts.

Results: A method was developed for obtaining powdered grape extracts, using the smallest possible amount of carrier, which allowed 91% recovery of polyphenols present in the grape marc. Moreover, solid grape extracts' total polyphenol content, antioxidant activity, and dry matter content did not change significantly during storage at 23°C.

Conclusion: Developing a dry form of the extract additive increased the stability of phenolic compounds, facilitated mixing with dry fodder, and transported and stored grape extracts. Ultimately, the preparations may help reduce the use of antibiotics.

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EXPLOITING CATECHOL SIDEROPHORES FOR RICE BLAST MANAGEMENT

CHRISTODOULOU, Michail; PINNA, Cecilia; GHOSH, Sharmila; PRINCIOTTO, Salvatore; SACCHI, Francesca; PIZZATTI, Cristina; MUSSO, Loana; CORTESI, Paolo; PINTO, Andrea; KUNOVA, Andrea; DALLAVALLE, Sabrina

University of Milan, Italy

michail.christodoulou@unimi.it

Introduction: Rice (Oryza sativa L.) is the major staple food for more than 50% of world's population. The rice-blast fungus Pyricularia oryzae poses a significant threat to rice production worldwide causing 30% yield losses. The management of the rice blast disease requires a massive application of fungicides, which accounts for more than 8% of total fungicide market.1 Ferroptosis, an iron-dependent form of regulated cell death, has recently been reported to be involved in P. oryzae virulence and its inhibition by iron chelating molecules has recently emerged as a promising strategy to control fungal pathogenesis.

Materials and Methods: Natural and nature-inspired siderophores were synthesized and purified by standard procedures and characterized by spectroscopic methods. Their chelating ability was evaluated by the Chrome azurol S (CAS) assay. The biological evaluation was performed using the strains PO21_07, PO21_B1 and PO21_B2 of P. oryzae.

Results: A small library of natural and nature-inspired compounds, containing one or more catechol chelating moieties, was synthesized and tested against the germination and appressorium formation of P. oryzae.

Conclusions: The reported findings could open avenues for future research and the development of targeted strategies for blast disease management.

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ENHANCING CURCUMIN SOLUBILITY AND BIOLOGICAL ACTIVITY USING SUPERCRITICAL FLUID TECHNOLOGY

GARBIEC, Ewa [1]; ZALEWSKI, Przemysław [1]; **CIELECKA-PIONTEK, Judyta [1,2]**

[1] Department of Pharmacognosy and Biomaterials, Poznan University of Medical Sciences, Poland;
[2]:Department of Pharmacology and Phytochemistry, Institute of Natural Fibres and Medicinal Plants, Poland

jpiontek@ump.edu.pl

Introduction: Curcumin (CUR), valued for its antioxidant and neuroprotective properties, is hindered by poor water solubility, reducing its effectiveness. Strategies to enhance CUR's solubility include amorphous solid dispersions (ASDs) and co-amorphous systems (CAMs) obtaining. ASDs involve incorporating CUR into a polymeric matrix, while CAMs use low molecular weight compounds like tryptophan (TRP) as co-formers. A novel approach combines these methods [1]. The systems can be produced using supercritical fluid (SCF) technology with carbon dioxide as a solvent for its non-toxic and environmentally friendly properties [2].

Material & Methods: This study prepared CUR-based system with TRP and poly(1-vinylpyrrolidone-co-vinyl acetate) [P(VP-co-VAc)] matrix using SCF technology. The amorphous nature of the system was confirmed by X-ray powder diffraction. The impact of improved CUR solubility was evaluated in relation to the enhancement of parameters in the PAMPA model and the ability to inhibit the DPPH radical and the butyrylcholinesterase enzyme.

Results: The ternary system with TRP increased CUR's solubility by over 300-fold and enhanced permeability by more than threefold in the PAMPA test. This led to a sixfold increase in antioxidant activity and a 25-fold improvement in butyrylcholinesterase inhibition.

Conclusion: Combining ASDs and CAMs using SCF technology significantly improves CUR's solubility and therapeutic potential.

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ANTIMICROBIAL PROPERTIES OF A GRAPE EXTRACT TO PREVENT THE USE OF ANTIBIOTICS IN FARMED ANIMALS

CORRAL ORBE, Mishell Stephanya [1,2,3]; LIBISCH, Balázs [1,3]; POSTA, Katalin [1,3]; JUHÁSZ, Ákos [1,3]; HEGYI, Anna [1,3]; DE MIGUEL, Trinidad [4]; DIAZ RODRIGUEZ, Patricia [5]; CELEIRO, Maria [6]; CASTILLO, Aly [6]; LORES, Marta [6]; OLASZ, Ferenc [1,3]; KERESZTÉNY Tibor [1,3]

[1] Agribiotechnology and Precision Breeding for Food Security National Laboratory, Hungarian University of Agriculture and Life Sciences, Hungary
[2] Doctoral School of Biological Sciences, Hungarian University of Agriculture and Life Sciences, Hungary
[3] Department of Microbiology and Applied Biotechnology, Institute of Genetics and Biotechnology, Hungarian University of Agriculture and Life Sciences, Hungary
[4] Institute of Research on Chemical and Biological Analysis (IAQBUS), Department of Microbiology and Parasitology Faculty of Pharmacy USC, Spain
[5] Department of Pharmacology, Pharmacy and Pharmaceutical Technology USC, Spain
[6] Department of Analytical Chemistry, Nutrition and Bromatology USC, Spain

mcorral2200@gmail.com

Introduction: Antibiotics in farming has been used for decades as the first choice to prevent infections and promoting growth. The need for alternatives to conventional antibiotics is increasing progressively. This study aimed to determine the in vitro antimicrobial activity of a polyphenolic grape extract, and to assess the possible development of increased tolerance to the polyphenolic extract, in vitro.

Materials & Methods: Antimicrobial activity and bacterial tolerance tests against Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 based, mainly on the EUCAST broth microdilution protocol, were performed. For the tolerance test, surviving cells from the highest extract concentration before the MIC were taken for the next inoculation, and this was repeated for 10 passes.

Results: Antimicrobial test results were as follows, IC90 and IC50 values for S. aureus and E. coli against the extract were for both 5%, and <2,5% respectively. Bacterial tolerance test results showed 5% IC90 values for both at the first plate, and after the 10th pass, the IC90 remained the same, indicating that no tolerance was developed for the extract during these experiments.

Conclusion: These findings suggest that the grape extract has a potential as an alternative to conventional antibiotics in farming.

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NEW APPROACHES IN EXTRACTION AND PRESERVATION OF ULVA LACTUCA SEAWEED POLYPHENOLICS AND NATURAL PIGMENTS

CUJIC NIKOLIC, Nada [1]; DRAKULOVIĆ, Dragana [2]; VULIĆ, Jelena [3]; MUTAVSKI, Zorana [1]; RADAN, Milica [1]; KRGOVIĆ, Nemanja [1]; CABARKAPA, Ivana [1]

[1] Institute for Medicinal Plants Research Dr. Josif Pančić, Serbia
[2] Institute of Marine Biology, University of Montenegro, Kotor, Montenegro
[3] Faculty of Technology, University of Novi Sad, Serbia

ncujic@mocbilja.rs

Introduction: New ways of utilizing the unexploited marine product, seaweed from the Adriatic Sea (*Ulva lactuca* L.) were established. Due to the benefits of this macroalga, the focus was to apply green and safe technologies for the extraction and preservation of seaweed's valuable bioactive compounds.

Material & Methods: The sampling of Ulva was performed in Boka Kotorska Bay (South Adriatic Sea). Optimization extraction protocol for the main bioactive compounds and their preservation through innovative microencapsulation techniques (spray and freeze-drying) methods were established.

Results: The extraction process was based on the principles of "green extraction". Ethanol-water mixture (50%, solid solvent ratio 1:20) was chosen as optimal extract conditions for polyphenols and flavonoids, and maceration as a superior extraction method. The Ulva extract possessed a high content of total polyphenols, chlorophyll A and B, and carotenoids (3.55 mg GAE/g, 84.53 mg/100g Chl A, 68.41 mg/100g Chl B, 5026.82 mg/100 g), respectively. The obtained microencapsulates improved Ulva's stability, limiting the inactivation of the sensitive polyphenolic compounds, achieving high encapsulation efficiency, and high antioxidant activities.

Conclusion: This valuable sea resource may be used as an uncostly bio-product rich in functional compounds, with commercial utilization and development of Ulva-based blue-biotech industries.

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EFFECTS OF CHLORINE-CONTAINING DERIVATIVES OF 2'-OH CHALCONE ON SELECTED BLOOD CELLS

CYBORAN-MIKOŁAJCZYK, Sylwia [1]; KAŹMIERCZAK, Teresa [1], TROCHANOWSKA-PAUK, Natalia [1]; JEWAK, Natalia [1]; ROLA, Joanna [1]; WALSKI, Tomasz [2]; BONARSKA-KUJAWA, Dorota [1]; KRAWCZYK-ŁEBEK, Agnieszka [3]; KOSTRZEWA-SUSŁOW, Edyta [3]

[1] Department of Physics and Biophysics, Wrocław University of Environmental and Life Sciences, Poland
[2] Department of Biomedical Engineering, Wrocław University of Science and Technology, Poland
[3] Department of Food Chemistry and Biocatalysis, Wrocław University of Environmental and Life Sciences, Poland

sylwia.cyboran@upwr.edu.pl

Introduction: The 2'-hydroxychalcone occurs naturally in various plants, including Cryptocarya concinna, and exhibits a wide range of bioactivities, including anticancer and antimicrobial properties. Our previous studies have shown that derivatives of 2'-OH chalcone containing a chlorine atom exhibit higher antimicrobial, antibiofilm and anticancer activity [1]. The demonstrated biological potential of these compounds prompted us to undertake additional research into their effects on the physical and functional properties of blood cells such as erythrocytes, platelets, and peripheral blood mononuclear cells (PBMCs).

Material & Methods: Studied chlorinated derivatives of 2'OH-hydroxychalcone contain one chlorine atom at different positions of the A or B ring, and two chlorine atoms at the A ring. Using the XTT assay, the effect of compounds on the metabolic activity of platelets and PBMCs was examined. The effect of compounds on collagen-induced platelet aggregation was evaluated by optical aggregometry. In addition, the hemolytic activity of the compounds and their effects on the shapes, osmotic resistance, and transmembrane potential of the erythrocytes were evaluated using spectroscopic methods.

Conclusion: The changes induced in the physical and functional properties of the blood cells, with high antimicrobial and anticancer activity, make the tested compounds as promising candidates for effective and safe drugs.

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THE MARINE COMPOUND FUCOXANTHIN INDUCES APOPTOSIS THROUGH THE PI3K/AKT/MTOR SIGNALING PATHWAY IN CHRONIC LEUKEMIA CELLS

DAMIANO, Sara [1]; LONGOBARDI, Consiglia [1]; LAURITANO, Chiara [2]; CIARCIA, Roberto [1]

[1] University of Naples Federico II, Italy

[2] Stazione Zoologica, Anton Dohrn, Italy

sara.damiano@unina.it

Introduction. Chronic myeloid leukemia is a blood cancer due to the translocation of chromosomes 9 and 22 through the fusion of Abelson's tyrosine kinase 1 and breakpoint cluster region (BCR) protein. Although the use of tyrosine kinase inhibitors has revolutionized the lives of patients, unfortunately they can develop resistance. It therefore becomes necessary to study new alternative drugs. Fucoxanthin (FUCO) is a carotenoid of the xanthophyll family, produced by marine organisms such as the macroalgae of the fucus genus or microalgae such as Phaeodactylum tricornutum. It induces apoptosis in several tumors with no side effects in humans and animals. The PI3K/Akt/mTOR pathways appears to be vital in selecting cellular processing in tumor and normal cells and a lot of studies have demonstrated that high levels of ROS lead to apoptosis and necrosis, both processes implicated in cancer.

Matherials. We explored the PI3K/Akt/mTOR pathways in FUCO-treated human K562 cells. Specifically, cytotoxicity by MTT assay, proteins involved in apoptosis and PI3K/Akt/mTOR pathway involvement were analyzed by western blot. The antioxidant activity was analyzed by spectrophotometer.

Results. Our data demonstrated that FUCO inhibited PI3k/Akt/mTOR signaling pathway, induced apoptosis and could represent a new natural therapeutic opportunity against chronic leukemia.

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AGRI-FOOD BY-PRODUCTS IN BREAST CANCER: NUTRACEUTICAL ACTION OF ANNURCA APPLE POLYPHENOLS

D'ANGELO, Stefania

Università degli Studi di Napoli "Parthenope", Italy

stefania.dangelo@uniparthenope.it

Introduction: Agri-food by-products contain bioactive compounds that could be employed in the production of enriched food, cosmetics, and drugs by pharmaceutics companies. Dietary polyphenols are among the naturally-occurring substances that have shown promising anti-cancer properties and low toxicity in comparison to standard chemotherapeutic agents. Annurca' apple fruits is a variety widely cultivated in southern Italy (Campania region) and is well-known for its crispness and white flesh.

Material & Methods: Annurca apple samples was homogenized. The total polyphenolic content of Annurca apple extract was assessed. Cells were treated with different concentration of Annurca apple polyphenol extract (AAPE).

Results: AAPE displays a potent prooxidant cytotoxic effect in MCF-7 human breast carcinoma cells. AAPE is able to selectively kill MDA-MB-231 TNBC cells through ROS generation, sustained JNK activation and cell growth and survival inhibition while exerting a protective antioxidant effect in normal cells.

Conclusion: Annurca apple polyphenol extract is as a potential nutraceutical candidate for drug development against breast cancer. The discovery of natural substances able to interfere at various degrees in breast cancer aggressiveness might be beneficial for new approaches of neoplastic diseases.

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ECO-FRIENDLY BIOREFINERY OF RED ONION PEELS FOR POLYPHENOL AND FIBER RECOVERY IN FUNCTIONAL FOOD DEVELOPMENT

D'ANTUONO, Isabella [1]; BAVARO, Anna Rita [1]; RAMIRES, Francesca Anna [2]; BLEVE, Gianluca [2]; GARBETTA, Antonella [1]; BARUZZI, Federico [1]; OLKIEWICZ, Magdalena [3]; TYLKOWSKI, Bartosz [3]; LEONARD, Kevin [4]; TCHOUMTCHOUA, Job [4]; CARDINALI, Angel

[1] National Research Council, - Institute of Sciences of Food Procuction (CNR-ISPA) Bari, Italy

[2] National Research Council, - Institute of Sciences of Food Procuction (CNR-ISPA) ,Lecce, Italy

[3] Eurecat, Technology Centre of Catalonia, Chemical Technology Unit, 43007 Tarragona, Spain

[4] Celabor, Chaineux, Zoning de Petit-Rechain, Avenue du Parc, 38, 4650 Herve, Belgium

isabella.dantuono@ispa.cnr.it

Introduction: The bio-based industry supports circular economy solutions, focusing on reusing and valorizing by-products. Within the PHENOLEXA project, eco-friendly processes have been developed to recover high-value bioactive compounds from agri-wastes, for use in cereal-based food prototypes and nutraceuticals.

Material & Methods: Red onion peels (cv Cipolla Rossa di Tropea) were pretreated microbiologically using a yeast strain under agitation at 30°C. After fermentation, samples were filtered to separate liquid and solid fractions. Both fractions, at 12% (v/w) and 3% (w/w) respectivelly, were analyzed chemically (HPLC-DAD) and biologically (cellular antioxidant activity, CAA), and used for the "piadelle" preparation. The solid fraction was additionally extracted both by Subcritical Water Extraction (SWE) and Natural Deep Eutectic Solvent (NADES). Resulting dietary fibers were used (10% w/w) to produce "friselline".

Results: Red onion peels were rich in polyphenols, particularly protocatechuic acid and quercetin, with antioxidant median effective dose (MED) of 1.7 µg/ml. "Piadelle" were enriched in fibers and polyphenols, the latter highly in vitro bioaccessible. The fiber content of "friselline" ranged between 12.4-13.4%, qualifying them as "HIGH FIBER" products as reported by EU Nutrition claim.

Conclusion: This eco-friendly biorefinery offers an effective way to recover phenolic compounds and fibers, increasing antioxidant activity and producing functional foods.

Supported by Project PHENOLEXA -Benign cascade extrative biorefinery for converting agri-food side streams into high-value polyphenolic bioactives and functional fibres for pharma, cosmeceuticals, nutraceuticals and food products" G.A. No. 101023225.

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EXPLORING THE BIOACTIVITY AND STABILITY OF COFFEE POLYPHENOL FORMULATIONS OBTAINED WITH DEEP EUTECTIC SOLVENTS

DE LUCA, Raffaella [1]; VLADIĆ, Jelena [2]; PEREIRA, Joana [2]; FERREIRA, Ines [2,3,4]; CICCONE, Lidia [1]; MEZZETTA, Andrea [1]; GUAZZELLI, Lorenzo [1]; DINIZ, Mario [3,5]; DUARTE, Ana Rita C. [2]; POMELLI, Christian Silvio [1]

[1] Department of Pharmacy, University of Pisa, Italy

[2] LAQV-REQUIMTE, Chemistry Department, NOVA—School of Science and Technology, Portugal

[3] Associate Laboratory i4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Portugal

[4] Marine and Environmental Sciences Centre—Setúbal Polytechnical Institute (MARE-IPS), Escola Superior de Tecnologia de Setúbal, Campus do IPS, Portugal

[5] UCIBIO, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Portugal

raffaella.deluca@phd.unipi.it

Introduction: Polyphenols can be efficiently extracted from biomass using deep eutectic solvents (DES), which enhance yields and stabilize sensitive molecules. This study investigates the biological activity and ecotoxicity of polyphenol-rich extracts obtained from coffee waste using DES, alongside assessing the stability of these polyphenols within the extraction medium.

Material & Methods: Polyphenols were extracted from coffee biomass using a betaine: ethylene glycol DES. Four extracts, generated under different conditions, were analyzed for polyphenol content. Cytotoxicity was evaluated in Caco-2 cells, alongside a reactive oxygen species (ROS) assay to assess antioxidative capacity. DPPH was used to determine the stability of the extracts. Environmental toxicity was examined by exposing shrimp to the extracts over 7 days.

Results: The polyphenol-rich extracts exhibited non-toxic behaviour in both cellular and animal models. Furthermore, they demonstrated significant antioxidative properties and stability over time. In contrast, the DES alone, while non-toxic, did not display comparable antioxidant activity.

Conclusion: This study highlights the potential of DES as an efficient extraction medium and a stabilizing agent for polyphenols, offering a promising avenue for sustainable industrial practices. The safety profile of the DES examined and its ability to enhance extraction yields supports its broader application in green chemistry and industry.

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DEVELOPMENT OF A FERMENTED SHEEP MILK ENRICHED WITH OLIVE PHENOLIC LEAF EXTRACT AND INULIN: A FUNCTIONAL FOOD APPROACH

ADDIS, Margherita [1]; PIGA, Carlo [1]; PES, Massimo [1]; CAMPUS, Marco [1]; COMUNIAN, Roberta [1]; TUBEROSO, Carlo Ignazio Giovanni [2]; SERRELI, Gabriele [3]; MELIS, Maria Paola [3]; **DEIANA, Monica [3]**

[1] AGRIS, Agricultural Research Agency of Sardinia, Italy;
[2] Dept. of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria, Italy;
[3] Dept. of Biomedical Sciences, University of Cagliari, Italy

mdeiana@unica.it

Introduction: The formulation of functional dairy products enriched in polyphenols are know to contribute to health enhancing and disease preventing effects (1). This study investigates the integration of an olive leaf extract (OLE) rich in polyphenols into a fermented sheep's milk (FM) supplemented with inulin, focusing on the stability of these compounds throughout the production process and their impact on the characteristics of the resulting products.

Material & Methods: OLE with 1300 mg GAE/100 g of total polyphenols (TP), was prepared. OLE was added to raw sheep milk at different TP concentrations and all included 3% inulin as a dietary fiber source. Milk was pasteurised, acidified, cooled, and analysed for TP content, chemical-nutritional composition, rheological, microbiological, and sensory properties. Antioxidant activity and cytotoxic effects of OLE were also tested in Caco-2 cells.

Results: OLE addition did not significantly affect the acidification process or starter cultures viability, indicating compatibility with the fermentation processes. OLE showed resilience through processing and physico-chemical analyses showed no adverse alterations. Antioxidant capacity of OLE was observed at non toxic concentrations.

Conclusion: Olive leaf polyphenols showed remarkable resistance to the technological process applied in fermented milk, and offer significant potential as functional ingredients in dairy products.

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FROM THE WASTE OF MUST CLARIFICATION TO THE PRODUCTION OF A HIGH POLYPHENOLS' RICH BUCCAL FILM: NEW INSIGHT TO TREAT ORAL OXIDATIVE STRESS-RELATED PATOLOGIES

DI PRIMA, Giulia [1]; LA MANTIA, Cecilia [1]; BELFIORE, Elena [2]; DE CARO, Viviana [1]

[1]: Dipartimento STEBICEF, University of Palermo, Palermo, Italy

[2]: Dipartimento Me.Pre.C.C, University of Palermo, Palermo, Italy

giulia.diprima@unipa.it

Introduction: To support drugs' treatment of oral oxidative stress-related patologies polyphenols can be ideal candidates due to their antioxidant and antinflammatory activities. A liquid polyphenols' enriched excipient could be obtained by virtuous recovery of waste black bentonite (BB) coming from white grape must filtration.

Material & Methods: Both the BB-wet (starting waste as supplied) and BB-dry (after freeze-drying) were subjected to 7 consecutive cycles of green extraction by maceration with PEG200 as unconventional and innovative green solvent.The extracts were characterized by HPLC-DAD analysis, Folin-Ciocalteu, Bradford and DPPH assays. A thin buccal film was prepared by solvent casting using pectin as matrix polymer, calcium lactate and the best extract.

Results: The multiple maceration procedure greatly enrich PEG200 in polyphenols. The best product was obtained from the BB-wet (third extraction step) and resulted in a solvent recovery >40% and GAE ≈ 4 mg/g. Consequently, it was directly used to prepare a soft, deformable and homogeneo buccal film.

Conclusion: The valorization of the waste BB could be a virtuous approach to recover polyphenols. The consecutive multiple green maceration process produced a highly polyphenols' rich secondary raw material directly useful to produce buccal films potentially promising to locally treat several oxidative stress-related oral pathologies.

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EFFICIENT 2-STEP ENZYMATIC CASCADE FOR THE BIOCONVERSION OF OLEUROPEIN INTO HYDROXYTYROSOL

CATINELLA, Giorgia; **DONZELLA, Silvia**; BORGONOVO, Gigliola; DALLAVALLE, Sabrina; CONTENTE, Martina Letizia; PINTO, Andrea

Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Italy

silvia.donzella@unimi.it

Introduction: Oleuropein (OLE), known for various remarkable biological actions, is the most abundant phenolic compound in all parts of olive trees (*Olea europaea* L.). However, hydroxytyrosol (HT), the main degradation product of OLE, is considered one of the most powerful antioxidant agents, with higher beneficial properties than the OLE parent compound.

Material & Methods: A robust biocatalytic procedure for the production of HT from oleuropein was developed by a 2-step biotransformation involving two home-prepared enzymes. To confirm the radical scavenging capacities of HT and its parent compounds, an easy and reproducible DPPH test was performed.

Results: Hydroxytyrosol was obtained using: 1) a thermo-halophilic β-glucosidase from Alicyclobacillus herbarius (Ahe), which gave the corresponding aglycone with complete conversion (>99%) and rapid reaction times (30 min); 2) an acyltransferase from Mycobacterium smegmatis (MsAcT) [3], here employed for the first time for its hydrolytic activity (96% isolated yield in 24 h). After cascade completion, the product was demonstrated to be 2-fold more active as free radical scavenger than OLE starting material and OLE aglycone.

Conclusion: Starting from a natural substrate and employing enzymatic approaches, the final hydroxytyrosol can be claimed and commercialized as natural too, thus increasing its market value.

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EFFECTS OF HABANERO RED EXTRACT AND ITS MICROENCAPSULATED FORM ON CELL VIABILITY AND CYTOTOXICITY IN THE HUMAN COLORECTAL CANCER CELLS

ĎÚRANOVÁ, Hana [1]; KOVÁČIKOVÁ, Eva [2]; BOROTOVÁ, Petra [1]; STRAKA, Dušan [1]; ŠIMORA, Veronika [1]; FIALKOVÁ, Veronika [1]

[1:] AgroBioTech Research Centre, Slovak University of Agriculture, Slovak Republic

[2] Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Slovak Republic

hana.duranova@uniag.sk

Introduction: Recent research highlights the potential of phytochemicals and plant extracts in preventing diseases like cancer. This study aimed to evaluate the total phenolic content (TPC), antioxidant activity (AA), and the effects on cell viability and cytotoxicity of Habanero Red extract (HRE) and its microencapsulated form in human colorectal adenocarcinoma cells (HT-29).

Material & Methods: HT-29 cells were treated with varying concentrations of HRE (156-3 500 µg/mL) and microencapsulated HRE (312.5-10 000 µg/mL) for 24 hours. Cell viability was assessed via the MTT assay. HRE microencapsulation employed a Büchi spray dryer with maltodextrin/gum arabic. TPC and AA were measured using the Folin-Ciocalteu method and DPPH assay. Data were analyzed using one-way ANOVA and Tukey's test.

Results: HRE showed TPC of 6.28±0.35 mg GAE/kg DW and AA of 12.54±1.31 mg TEAC/kg DW. Microencapsulation retained ~ 75% of polyphenols and 91% of AA. HRE (≥312.5 µg/mL; IC50 = 78 020.51±132.06 µg/mL) and its microencapsulated form (≥625 µg/mL; IC50 = 2 316.77±73.15 µg/mL) exhibited significant ($p < 0.01$) dose-dependent cytotoxic effects on HT-29 cells.

Conclusion: HRE and its microencapsulated form considerably reduced HT-29 cell viability and showed cytotoxic effects, suggesting their potential for enhancing food products with health-promoting benefits.

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PHENOLIC COMPOUND EXTRACTION: A CHEMICAL COMPARATIVE STUDY OF CONVENTIONAL AND UNCONVENTIONAL METHODS FROM PINUS RADIATA BARK

ESCOBAR AVELLO, Danilo [1,2]; OÑATE-VALDÉS, Tomás [1,2]; FERRER, Victor [1,2]; SANTOS, Jorge [3]; FUENTEALBA, Cecilia [1,2]

[1]: Unidad de Desarrollo Tecnológico, Universidad de Concepción, Coronel 4191996, Chile;
[2]: Centro Nacional de Excelencia para la Industria de la Madera (CENAMAD), Pontificia Universidad Católica de Chile, Av. Vicuña Mackena 4860, Santiago 7820436, Chile;
[3]: ARCP—Associação Rede de Competência em Polímeros, 4200-355 Porto, Portugal

daniescobar01@gmail.com

Chile's *Pinus radiata* forest produces two million tons of bark annually, rich in phenolic compounds like condensed tannin. The extraction of these bioactive compounds is important for industrial applications. This study compares conventional and unconventional extraction techniques—namely, ultrasonic-assisted extraction (UAE), cyclodextrin-enhanced extraction, and alkaline extraction—to optimise phenolic yield and bioactivity. The extraction conditions were optimised using response surface methodology, and phenolic content was determined using Folin-Ciocalteu. Antioxidant capacity was determined using DPPH, FRAP, and ABTS. Chemical and thermal behavior characterisation was performed using FTIR, TGA, and mass spectrometry (Orbitrap-MS and MALDI-TOF). The highest extraction yields were observed with alkaline (14.32%) and UAE in ethanol (13.86%), followed by ethanol (12.74%), with water and cyclodextrin-assisted methods showing significantly lower yields. β-cyclodextrin extraction yielded the highest phenolic content (18.866mg GAE/100gdb), while antioxidant capacities, assessed using DPPH, ABTS, and FRAP assays, showed that UAE and alkaline extractions exhibited superior antioxidant capacities. FTIR spectra and mass spectrometry confirmed condensed tannins and other phenolic compounds, while TGA demonstrated thermal stability, particularly in alkali-extracted (600°C,~60%-loss weight). Alkaline and UAE extractions are the most efficient methods for obtaining high bioactive phenolic compounds from *P. radiata* bark yields, offering promising applications in antioxidant-rich bioproduct development.

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OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION OF BIOACTIVE PHENOLIC COMPOUNDS FROM CYTISUS FLOWERS

ALVAREDO-LÓPEZ-VIZCAÍNO, Adela [1,2]; DEL RIO, Pablo [1,2]; GARROTE, Gil [1,2]; GULLÓN, Beatriz [1,2]; COSTA-BARBOSA, Augusto [3]; SAMPAIO, Paula [3]; BOTELHO, Cláudia [4];
FERREIRA SANTOS, Pedro Miguel [1,2]

[1] Department of Chemical Engineering, Faculty of Science, University of Vigo
[2] Instituto de Agroecoloxía e Alimentación (IAA). University of Vigo
[3] Centre of Molecular and Environmental Biology (CBMA), Universidade do Minho
[4] CEB – Centre of Biological Engineering, University of Minho

pedromiguel.ferreira@uvigo.es

The recovery of bioactive compounds through sustainable technologies is currently an area of interest. This study aims to optimize the Microwave-assisted extraction (MAE) for the recovery of bioactive phenolic compounds from Cytisus flowers (CF).

For MAE extraction, the influence of ethanol concentration (0-98.9%), temperature (98.5-163.4 °C) and time (1-11 min) were studied employing an experimental design. The extractions were characterized by total phenol (TPC) and flavonoid content (TFC), LC-MS/MS and antioxidant capacity. The cytotoxicity was measured in human adenocarcinoma epithelial cells (A549) and kidney cells (HEK293T), and the anti-inflammatory activity was evaluated in lipopolysaccharide (LPS) inflamed macrophages.

The results showed that the optimal conditions for recovery of phenolics were 87.6 % ethanol, 161 °C, and 8.46 min. The experimental data were: TPC, 85.9 mg gallic acid-equivalents; TFC, 120.3 mg rutin-equivalents; DPPH, ABTS, FRAP and CUPRAC: 260.1, 62.9, 105.1 and 907 mg Trolox-equivalent/g CF, respectively. LC-MS/MS enabled the identification of 27 phenolic compounds, being chrysin and hydroxybenzaldehydes the most representative compounds. CF extract showed no cytotoxicity in tested lines, and anti-inflammatory capacity by diminishing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-10, and TGF-β1) induced by LPS.

These features may allow the bioactive CF extracts to be used in food and pharmaceutical sectors.

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STRUCTURE-ACTIVITY RELATIONSHIP OF A- AND B-TYPE PROCYANIDINS: IN VITRO ANTIOXIDANT ACTIVITY

FISCHER, Annik; ZOTOS, Dimitrios; ESATBEYOGLU, Tuba

Department of Molecular Food Chemistry and Development, Institute of Food and One Health, Gottfried Wilhelm Leibniz University Hannover, Germany.

fischer@lw.uni-hannover.de

Introduction: Antioxidants can mitigate cellular damage due to their radical scavenging properties. Procyanidins (PCs) have been proven to be effective antioxidants. However, the antioxidant properties related to the structural variations of PCs, remain poorly described.

Material & Methods: In this study, the antioxidant activity of the following flavan-3-ols (+)-catechin (C) and (–)-epicatechin (EC) (monomers); PCs B1, B2, B3, B4, B5 and B7 (dimeric B-type) as well as for A1, A2 (dimeric A-type) and C1 (trimeric B-type) was measured using the TEAC (Trolox Equivalent Antioxidant Capacity), FRAP (Ferric Reducing Antioxidant Power), CUPRAC (Cupric Ion Reducing Antioxidant Capacity) and DPPH (2,2-DiPhenyl-1-PicrylHydrazyl) assays.

Results: The monomers (+)-catechin and (–)-epicatechin, and the C4-C8 linked B-type dimers B2 and B3, composed of the same monomer in the upper and lower unit, showed the highest antioxidant activity. In contrast, the other C4-C8 and C4-C6 linked B1 (EC-C), B4 (C-EC), B7 (EC-C) and B5 (C-C), as well as the trimer C1 (EC-EC-EC) showed a significantly reduced antioxidant activity ($p < 0.0001$) compared to (+)-catechin, (–)-epicatechin, B2 and B3.

Conclusion: The antioxidant activity of PCs does not seem to be influenced by the number of hydroxyl groups or linked monomers, but by their different three-dimensional structure.

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AN IMPROVED METHOD FOR THE SYNTHESIS OF HYDROXYTYROSOL ESTER DERIVATIVES WITH OMEGA-3 POLYUNSATURATED FATTY ACIDS

GARCÍA-ACOSTA, Natalia [1]; JORDÁN, Marta [1]; GOYA, Luis [1]; MATEOS, Raquel [1]; ESPARTERO, José Luis [2]

[1] Department of Metabolism and Nutrition, Food Science, Food Technology and Nutrition Institute (ICTAN) Spanish National Research Council (CSIC), Madrid, Spain Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN-CSIC), Spain
[2] Department of Organic and Pharmaceutical Chemistry, Pharmacy Faculty, University of Sevilla, Spain

n.garcia@ictan.csic.es

Hydroxytyrosol (HT), the principal component of the phenolic fraction of virgin olive oil (VOO), has significant protective effects on health. The EFSA recognizes that this fraction prevents the oxidation of low-density lipoprotein (LDL), a critical step in the onset of atherogenesis and cardiovascular disease. On the other hand, the cardioprotective role of omega-3 fatty acids (O3FA) is well-known. A synergistic effect between O3FA and HT could be so expected. Therefore, this study aims to improve the preparation of a lipophilic ester derivative of HT with eicosapentaenoic acid (HT-EPA), that would be solubilized in an O3FA-enriched oil matrix, thereby maximizing the potential health benefits.

The one-step synthesis of HT-EPA, was performed using lipase B from Candida antarctica as enzymatic catalyst under various conditions.

The best results were obtained when the reaction was carried out as a suspension of HT in Et-EPA at a 1:9 molar ratio, under vacuum without solvent. HT-EPA was produced in higher yields than previously reported. The purity of the compound exceeded 98%, as confirmed by 1H-NMR analysis.

This study presents an improved method for the enzymatic synthesis of EPA-HT, significantly reducing both reaction time and reagent consumption.

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USE OF POLYPHENOLS FROM OIL MILLS WASTE WATER IN FOOD PACKAGING

GIORGIA, Miccoli; VALERIO, Miceli

ENEA, Italy

giorgia.miccoli@enea.it

The extraction and utilization of polyphenols from olive mill wastewater (OMW) present a sustainable approach to valorize agricultural products. The polyphenols, known for their potent antioxidant and antimicrobial properties, can significantly enhance the quality and shelf-life of food products.

In this study the attention is on separative processes using membrane filtration technologies, to obtain bioactive compounds from pool polyphenol. The extracted polyphenols are inserted on the polymeric matrix with dip coating processes. Their characterization is done through UHPLC and FTIR techniques while the antimicrobial activities is tested using the flow cytometer.

The aim of this study is introduce phenolic-compound derived from the oil mills waste water in packaging in order to increase the shelf life of foods. This study explores the integration of these bioactive compounds into food packaging materials. Inserting the polyphenols into packaging, we aim to develop active packaging solutions that extend the freshness and safety of food items.

The research highlights the extraction methods, the efficacy of polyphenols in inhibiting microbial growth, and their impact on the oxidative stability of packaged foods. The findings suggest that polyphenol-enriched packaging not only offers a novel use for OMW but also contributes to reducing food waste and improving food safety.

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NOVEL EXTRACTION OF ANTIOXIDANT POLYPHENOLS FROM GRAPE POMACE RESIDUE USING DEEP EUTECTIC SOLVENTS

GULLÓN, Beatriz [1,2]; FERREIRA-SANTOS, Pedro [1,2]; PÉREZ-PÉREZ, Alba [1,2]; LÓPEZ-VIZCAÍNO, Adela [1,2]; COELHO, Marta [3]; GARCÍA DEL RÍO, Pablo [1,2]

[1] Department of Chemical Engineering, Faculty of Science, University of Vigo (Campus Ourense), Spain
[2] IAA - Instituto de Agroecoloxía e Alimentación, University of Vigo (Campus Auga), Spain
[3] CBQF—Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Portugal

bgullon@uvigo.gal

Grape Pomace (GP) resulting from wine industries can cause serious environmental and economic impacts. These by-products are often undervalued but constitute a potential source of bioactive compounds that can be applied in several food and pharmacological industries. The growing demand for sustainable extraction processes requires the development of new and effective solvents to replace toxic organic solvents. Deep eutectic solvents (DESs) are a green alternative due to their non-toxicity and biocompatibility. This work aimed to study the effectiveness of DESs to extract antioxidant phenolic compounds from GP. Three different DES were synthesized: DES 1 - sodium acetate:Lactic acid (1:5) with 30% water; DES 2 - Chlorine chloride:Acetic acid:Water (1:1:10); DES 3 - Chlorine chloride:Citric acid (2:1) with 30% water. Ethanol 50% was used a control solvent.

The best extraction results were obtained with DES3 (TPC: 110 mg GAE/g GP and TFC: 162 mg RE/g GP), representing values around 40% higher than hydroethanolic extraction. The extracts obtained by DES3 showed high antioxidant activity in FRAP (156 mg TE/g GP) and TAC (183 mg TE/g GP) methods and DES 2 in ABTS assay (183 mg TE/g GP). The phenolic profile was determined by HPLC-MS/MS, and revealed the presence of phenolic acids, flavonoids and stilbene.

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HEALTH-PROMOTING EFFECTS OF ACCELERATION EXERCISE AND POLYPHENOLS IN ARCTIC LINGONBERRY IN HEALTHY VOLUNTEERS

KÄHKÖNEN, Tiina [1]; LÄNSIMIES, Lilli [2]; PÄTZOLD, Jonne [3]; PENTTILÄ, Emmi [4]; PENTTILÄ, Teemu [5]; **HALLEEN, Jussi [1]**

[1] OncoBone Ltd, Finland; [2]: Club Huuma, Finland
[3] Gleap Health Technologies Ltd, Finland
[4] Aurora Health Sciences Ltd, Finland
[5] Arctic Nutrition Ltd, Finland

jussi.halleen@oncobone.com

Introduction: Acceleration exercise and polyphenol-rich arctic lingonberries have many health-promoting effects that counteract risk factors of lifestyle diseases. We have developed a method for recording movement accelerations for monitoring acceleration of exercise. Here we have studied the effects of a lingonberry product, acceleration exercise and their combination on body composition.

Materials & Methods: Four groups of healthy volunteers were included in the study: 1) Control group without lingonberry product and acceleration exercise; 2) Group consuming daily 3 portions of Flow drink, a natural lingonberry product equaling daily intake of 33.6 grams of lingonberries; 3) Group performing daily acceleration exercise with 1000 steps at 1.8 – 2.3 G acceleration; 4) Group consuming lingonberry product and performing acceleration exercise as in groups 2) and 3). Body composition was studied before study start and at 12 weeks.

Results & Conclusions: Combination of lingonberry product consumption and acceleration exercise decreased Body Mass Index and visceral fat percentage, while no effects were observed by lingonberry product consumption or acceleration exercise alone. These results suggest that acceleration exercise may improve the ability of the body to absorb and utilize polyphenols and other health-promoting substances in lingonberries, leading to health benefits.

Supported by Aurora Health Sciences.

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USE OF DAVIDSON PLUM (*DAVIDSONIA JERSEYANNA*) POWDER AS AN INGREDIENT FOR THE DEVELOPMENT OF A FUNCTIONAL FOOD PRODUCT

HARRIDEN, Brittany [1,2,3]; STATHOPOULOS, Costas [1,4]; SUWIMOL, Chockchaisawasdee [1,5]; MCKUNE, Andrew [1,2,3]; NAUMOVSKI, Nenad [1,2,3,6]

[1] Discipline of Nutrition and Dietetics, Faculty of Health, University of Canberra, Australia
[2] Functional Foods and Nutrition Research (FFNR) Laboratory, University of Canberra, Australia
[3:]University of Canberra Research Institute in Sport and Exercise, University of Canberra, Australia
[4] Food Futures Institute, Murdoch University, Nambeelup, WA, Australia
[5] School of Medical, Molecular, and Forensic Sciences, Murdoch University, Murdoch, WA, 6150, Australia
[6] Discipline of Nutrition-Dietetics, Harokopio University, Athens, Greece

brittany.harriden@canberra.edu.au

Introduction: Davidson plum (*Davidsonia jerseyana*) (DP) is an Australian native fruit consumed raw by the Australian Indigenous Communities. DP has a high polyphenolic profile and unique flavour (earthy and pungent) and may be used as a functional ingredient. The study aimed to develop a polyphenolic-enriched sorbet using commercially available, freeze-dried DP powder.

Methods: Pomegranate, raspberry, strawberry and DP sorbet samples containing DP powder at 0%, 5%, 10%, 15% and 20% (w/w) respectively were prepared. Methanolic extractions (70%) were analysed for total phenolic (TPC; gallic acid equivalent (GAE)), flavanol (TF; Catechin equivalent). Free radical scavenging activity (DPPH), reducing capacity (CUPRAC) and antioxidant capacity (FRAP) expressed as Trolox equivalents. One-way ANOVA was used to determine the differences between the samples.

Results: As expected, TPC levels increased with increased DP concentration for each sorbet flavour ($p=0.001$) with the highest levels in 20% DP pomegranate (730.73±0.04µgGAE). No significant difference in TF between the samples (All p's>0.05). Pomegranate sorbet with 20% DP showed the highest antioxidant capacity (DPPH 5588.08±0.02µMTE, and CUPRAC 5.29±0.05µMTE). Highest FRAP value was obtained in Strawberry sorbet with 20%DP (2.85±0.57µMTE).

Conclusion: Variations in TPC and antioxidant levels were observed between the different sorbet flavours due to the different fruit bases used.

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CYTOTOXYCITY EVALUATION OF RESVERATROL-4'-LA AND ITS COMPLEX WITH HYDROXYPROPYL-β-CYCLODEXTRIN IN DIFFERENT CELL LINES

HERNÁNDEZ HEREDIA, Ana Belén [1]; SILVA CULLISHPUMA, Dennis Alexander [1]; FERNANDES, Fátima [2]; GABALDÓN HERNÁNDEZ, José Antonio [1]; NUÑEZ-DELICADO, Estrella [1]; VALENTAO, Patricia [2]

[1] Molecular Recognition and Encapsulation Research Group, Health Sciences Department, UCAM, Spain

[2] Faculty of Pharmacy, University of Porto, Portugal

abhernandez@ucam.edu

Introduction: In order to enhance the bioactivity of resveratrol (RES), esters of RES with long chain fatty acids, including linoleic acid (RES-LA), have been synthesized (1,2). However, their aqueous solubility remains limited. Hydroxypropyl-beta-cyclodextrins (HP-β-CDs) have been employed to improve their aqueous solubility and, consequently, biological activity, due to their ability to form host-guest complexes (3). This study aims to evaluate the cytotoxic effects of these complexes in various cell lines.

Material & Methods: To determine the complexation constant, the critical micellar concentration (CMC) was calculated in the absence and the presence of HP-β-CDs using fluorescence spectroscopy, with diphenylhexatriene (DPHT), at controlled temperature and pH. Cytotoxicity was assessed via the MTT assay in various cell lines, including AGS, A549, and hCMEC. Data were analyzed in triplicate for statistical significance.

Results: The MTT assay results demonstrated that RES-LA is non-toxic at concentrations around 100 µM. However, high concentrations (above 25 mM) of HP-β-CDs exhibited toxicity across all tested cell lines.

Conclusion: These findings highlight the need for caution when considering HP-β-CDs as a carrier for RES-LA, as their use may be limited by their toxicity at high concentrations due to their inability to significantly enhace the solubility of the compound.

This work was carried out as part of the project LIPOFOOD, funded by PID2020-120466RB-I00 (Spanish Ministry of Sci. e Innovación) and 22015/PI/22 (Fundación Séneca-Agencia de Ciencia y Tecnología de la Región de Murcia), Region of Murcia), and was carried out as part of the predoctoral contract funded by Fundación Séneca Región de Murcia (Spain) 21794/FPI/22 and after the funded stay 22286/EFPI/23 (Fundación Séneca Comunidad Autónoma de la Región de Murcia, Spain)

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RUMEX SANGUINEUS-AN EDIBLE PLANT WITH POSSIBLE MEDICINAL VIRTUES

IONESCU, Alexandra-Mariana [1]; BOTEZATU-DEDIU, Andreea-Veronica [1]; FURDUI, Bianca [1]; BRATU, Camelia [2]; VINATORU, Costel [2]; DINICA, Rodica-Mihaela [1]

[1] "Dunarea de Jos" University, Faculty of Sciences and Environment, Romania
[2] Genetic and Vegetal Resources Bank (GVRB), Romania

alexaione96@yahoo.com

Introduction: Rumex sanguineus is one of the representatives of the Rumex genus from the Polygonaceae family. This study aimed to obtain and analyse the chemical composition of some alcoholic extracts to highlight the polyphenolic compounds, but also to pursue a possible antioxidant activity. These species have demonstrated throughout a few studies possible therapeutic effects among tumor cells, bacteria and viruses, possibly anti-inflammatory and diuretic effects.

Material & Methods: The raw material used to obtain the extracts was represented by the freshly dried leaves of Rumex sanguineus obtained from the Genetic and Vegetal Resources Bank (GVRB) Buzau, Romania. The extracts were obtained by fractionation with solvents. To highlight the polyphenolic content the Folin-Ciocalteu method was used and to prove the antioxidant activity the radical scavenger capacity and the reducing power were also used.

Results: These studies carried out for the first time on the Romanian Rumex sanguineus plant variety revealed significant amounts of biologically compounds such as polyphenols and flavonoids, with very good antioxidant activity compared to controls.

Conclusion: Considering the high concentration of polyphenols and flavonoids, Rumex sanguineus could be an important source of antioxidants, detailed studies to highlight other compounds are ongoing.

This work was partially supported by ADER grant 5.2.1. - Conservation and valorization of the genetic heritage of aromatic and medicinal species that can be cultivated on the territory of Romania.

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ANTIOXIDANT CAPACITY AND BIOACTIVE COMPOUNDS OF MATCHA TEAS

JAKUBCZYK, Karolina; SZYMCZYKOWSKA, Kinga; MELKIS, Klaudia

Pomeranian Medical University in Szczecin, Poland

karolina.jakubczyk@pum.edu.pl

Introduction: Matcha, or powdered green tea, has been gaining in popularity and is no longer consumed only in the form of infusions, finding new uses in gastronomy and the food industry. The range of teas available on the food market has expanded considerably.

Material & Methods: Eleven green tea powders were used in the analyses performed using spectrophotometric methods and HPLC methods (polyphenolic acids, flavonoids and caffeine).

Results: Antioxidant capacity ranged from 7.26 to 9.54 mM Trolox equivalent /L, while reducing power ranged from 1845.45 to 2266.12 Fe(II)/L. Total phenolic content amounted to 820.73–1017.83 mg gallic acid equivalent /L, and total flavonoid content was 864.71–1034.40 mg rutin equivalent /L. A high vitamin C content was found, ranging from 38.92 to 70.15 mg/100 ml. Additionally, a high content of caffeine ranged between 823.23 – 7313.22 mg/L was noted. Moreover, a high content of polyphenolic compounds, including epicatechin gallate, myricetin, gallic acid and 4 – hydroxybenzoic acid was found.

Conclusion: Matcha tea infusions have been shown to be a valuable source of antioxidants which can be used in daily diet.

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VALORIZATION OF RASPBERRY POMACE: EFFICIENT MICROWAVE-ASSISTED EXTRACTION, BIOACCESSIBILITY AND BIOACTIVITY OF PHENOLIC COMPOUNDS

JAOUHARI, Yassine [1]; BORDIGA, Matteo [1]; TRAVAGLIA, Fabiano [1]; COISSON, Jean-Daniel [1]; GULLÓN, Beatriz [2,3]; BOTELHO, Cláudia [4]; FERREIRA-SANTOS, Pedro [2,3]

[1] Department of Pharmaceutical Science, Università degli Studi del Piemonte Orientale "A. Avogadro", Italy

[2] Department of Chemical Engineering, Faculty of Science, University of Vigo (Campus Ourense), Spain

[3] IAA - Instituto de Agroecoloxía e Alimentación, University of Vigo (Campus Auga), Spain

[4] CEB – Centre of Biological Engineering, University of Minho, Campus de Gualtar, Portugal

yassine.jaouhari@uniupo.it

The cultivation of raspberries (*Rubus idaeus* L.) is significant worldwide, with a notable portion dedicated to industrial processing, resulting in substantial quantities of by-products. Raspberry pomace (RBP) is rich in phenolic compounds. This study aims to explore the potential of RBP as a source of these phytochemicals by optimizing their extraction and evaluating their bioaccessibility and bioactivity. The extraction process began with solvent selection through conventional maceration, revealing that 50% ethanol was the most effective solvent. Microwave-Assisted Extraction (MAE) was subsequently employed, significantly outperforming conventional methods, with optimal conditions of 200 °C for 10 min yielding the highest concentrations of total phenolic (68.10 mg GAE/g RBP) and flavonoid (62.80 mg RE/g RBP) contents.

Eleven phenolic compounds were identified by HPLC-ESI-MS, with gallic acid and 3,4-dihydroxybenzoic acid being the most prevalent. The *in vitro* gastro-intestinal digestion revealed that although some phenolic compounds suffered degradation during digestion (such as ferulic acid and quercetin), in the intestinal phase, phenolics are more bioaccessible, but with a loss of antioxidant activity. Furthermore, RBP extract demonstrated anti-inflammatory activity by reducing pro-inflammatory cytokines in macrophages. These findings underscore the effectiveness of MAE in extracting bioactives from RBP, highlighting its potential in developing functional foods and nutraceuticals.

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BLACKBERRY JUICE FERMENTED WITH TWO CONSORTIUMS OF LACTIC ACID BACTERIA: PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES DURING STORAGE

JIMÉNEZ-OSORIO, Angélica Saraí [1]; LUGO-ZÁRATE, Liliana [1]; DELGADO-OLIVARES, Luis [1]; CRUZ-CANSINO, Nelly del Socorro [1]; CASTREJÓN-JIMÉNEZ, Nayeli Shantal [2]; ESTRADA-LUNA, Diego [1]

[1]: Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, Circuito Actopan Tilcuautla s/n. Ex Hacienda La Concepción, Pachuca 42160, Hidalgo, México

[2]: Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Av. Universidad km 1 Ex Hacienda de Aquetzalpa A.P. 32, Tulancingo 43600, México

angelica_jimenez@uaeh.edu.mx

Introduction: Fermentation of fruit juices with lactic acid bacteria (LAB) is a sustained way to increase fruit exploitation. Also, blackberries are a source of antioxidants with proven health benefits. We investigated the effect of fermenting a blackberry juice (BJ) supplemented with whey and two LAB mixtures on physicochemical and antioxidant characteristics during fermentation and storage.

Material & Methods: Two consortiums were inoculated (9 log CFU/mL) in BJ supplemented with whey (WH, 1:1) over 48 hours at 37° C. Consortium 1 (BJWHC1) includes Levilactobacillus brevis, Lactiplantibacillus plantarum, and Pediococcus acidilactici. Consortium 2 (BJWH/C2) includes Lacticaseibacillus casei and Lactobacillus rhamnosus, previously isolated from agave. The pH, lactic acid production, viscosity, stability, reduced sugars, total phenolic content, anthocyanins, and antioxidant capacity were evaluated during fermentation and storage for 28 days.

Results: After 16 h, the two consortiums observed an increase in LAB content of 29-38%. Although fluctuations were observed during storage, the minimum LAB count was 9.8 log CFU/mL at 28 days. Lactic acid production increased up to 95% with good storage stability. The BJWH/C2 sample increased total phenolic content during storage.

Conclusion: We found that adding whey increases biomass, and its physicochemical properties are preserved during storage.

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QUANTIFICATION AND CHARACTERIZATION OF THE PHENOLIC FRACTION FROM CLINOPODIUM BOLIVIANUM AND MINTHOSTACHYS MOLLIS : A COMPARATIVE ANALYSIS

JORDÁN, Marta [1]; GARCÍA-ACOSTA, Natalia [1]; BALDERAS, Claudia [2]; RODRÍGUEZ, José Luis [1]; GOYA, Luis [1]; MATEOS, Raquel [1]

[1] Department of Metabolism and Nutrition, Institute of Food Science, Food Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), Madrid, Spain
[2] Analytical Techniques Service Unit (USTA), Institute of Food Science, Food Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), Madrid, Spain

mjordan@ictan.csic.es

Traditional medicine has constituted the primary healthcare system for centuries, and a large portion of the world population continues to rely on these treatments. Peru, which exemplifies this case, has a rich heritage and a high diversity of endemic plants that play a crucial role in this tradition. This study aims to characterize the phenolic profile of two promising Peruvian species: *Clinopodium bolivianum* and *Minthostachys mollis*, since Lamiaceae family are known to be a significant source of phenolic antioxidants.

The polyphenol fraction was isolated from dried leaf samples using liquid-liquid extraction (LLE) and compounds were identified and quantified by LC-ESI-QTOF mass spectrometry.

A total of 63 phenolic compounds were identified, the majority belonging to the flavonoid group (46.2%), followed by phenolic acids (30.8%), lignans (7.7%) and other (15.4%). Relative quantification revealed different profiles between the species, reporting rosmarinic or chlorogenic acid as representative metabolites of *M.mollis*, and vicenin or aesculetin of *C.bolivianum*.

We could confirm the major phenolics in *C. bolivianum* and *M. mollis*, which could explain the differences in their associated therapeutic properties. Further studies with specific metabolites, could explore different potential healthcare applications.

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BERGENOL®, A NOVEL VEGETAL VIRUCIDAL AGENT FOR HAND SANITIZERS FORMULATIONS

MESSAOUDI, Daouïa [1]; **JOSELEAU, Jean-Paul [2]**; RUEL, Katia [2]

[1] Groupe BERKEM, France

[2] Link Conseil, France

jpjoseleau@gmail.com

Using a hand sanitizer is the simplest and most practical way of preventing the transmission of germs and infections. The use of plant-based hand sanitizers has become increasingly popular in recent years. Plant-based products offer natural and organic alternatives to traditional chemical-based hand sanitizers. Incorrect use of chemical-based disinfectants can be toxic to humans and the environment. The R&D Laboratories of Groupe Berkem has developed and patented several specific botanical extracts, rich in active molecules, particularly polyphenols, not classified as hazardous, nor as a human health or environmental hazard and specially adapted to the needs and challenges of human hygiene. In the context of the Covid pandemic, virucidal products which have an effect on viruses of the coronavirus family are necessary.

This research focuses on formulating an effective natural alcohol-free hand sanitizer with Bergenol®, one specific plant polyphenolic extract from Berkem Biosolutions® coming from Vitis Vinifera. The tests were carried out against viruses of the coronavirus family, adenovirus family, murine norovirus family, vaccinia virus family and poliovirus family in accordance with European and American protocol standards.

The formulated sanitizer proved excellent virucidal activity against each type of viruses.

The excellent potential of Bergenol® as biobased virucidal agent for hand hygiene is reported.

Messaoudi, D., Fahy, O., Peron, JL., NKiliza, J., 2022. Use of a grape seed extract as a virucide against viruses of the coronavirus family.US Patent US 2022/0279797 Al.

DUAL-ENERGY X-RAY ABSORPTIOMETRY (DXA) REVEALS POLYPHENOL-INDUCED CHANGES IN BODY COMPOSITION IN SMALL ANIMALS

KÄHKÖNEN, Tiina E; HALLEEN, Jussi M

OncoBone Ltd, Finland

tiina.kahkonen@oncobone.com

Introduction: Dual-energy X-ray Absorptiometry (DXA) technology utilizes variable absorption of X-rays by different body components, which allows reliable analysis of body composition, including body weight, fat and lean mass. DXA is a method of choice for repeated imaging and analysis of diet-induced changes in live small animals such as rodents.

Materials & Methods: Precision and accuracy of iNSiGHT small-animal DXA (OsteoSys, Korea) was compared to another imaging technique Nuclear Magnetic Resonance (NMR) by multiple imaging and analysis of body composition in mice. Diet-induced changes in body composition by a lychee-based polyphenol oligonol was studied in ovariectomized (OVX) mice for 6 weeks.

Results & Conclusions: Compared to NMR, DXA has higher correlation for fat and lean mass (>95%), making it a more reliable imaging method to analyze body composition in small animals. Oligonol supplementation decreased OVX-induced fat mass gain without affecting lean mass. Taken together, DXA offers a rapid and reliable method for analyzing body composition in small animals and is suitable for long-term studies for evaluating effects of dietary supplementation of polyphenols.

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NATURAL DEEP EUTECTIC SOLVENTS EXTRACTION OF VALUABLE BIOACTIVE COMPOUNDS – SUCCESSFUL METHOD FOR VALORIZATION OF BLACK RASPBERRY (RUBUS OCCIDENTALIS L.) POMACE

KUZMANOVIĆ NEDELJKOVIĆ, Snežana [1]; KRGOVIĆ, Nemanja [1]; JOVANOVIĆ, Miloš [2]; ŠAVIKIN, Katarina [1]; MENKOVIĆ, Nebojša [3]; ŽIVKOVIĆ, Jelena [1]

[1] Institute for Medicinal Plants Research "Dr. Josif Pančić", Serbia

[2] University of Niš – Faculty of Medicine, Department of Pharmacy, Serbia

[3] Faculty of Pharmacy Novi Sad, University of Business Academy, Serbia

skuzmanovic@mocbilja.rs

Introduction: Industrial processing of black raspberry cause the significant generation of waste, leading to severe environmental and socioeconomic challenges. Possible solution lays in the re-utilization of this biomaterial rich with health-promoting bioactive compounds, where rutin and ellagic acid occupy noteworthy interest due to their antioxidant and anti-inflammatory potential. Thus, this study aimed to examine the effect of natural deep eutectic solvents (NaDES) on the recovery of rutin and ellagic acid from waste material – black raspberry fruit pomace.

Material & Methods: Thirteen different NaDESs, containing choline chloride or betaine as a hydrogen bond donor, and organic acids, polyols, amide, or sugars as hydrogen bond acceptor, were used for the ultrasound-assisted extraction of rutin and ellagic acid from black raspberry pomace, and compared with conventional solvents.

Results: Obtaining the amount of 840.19 µg/g of rutin, and 516.72 µg/g of ellagic acid, the mixture composed of choline chloride and 1,2-propanediol proved to be the most efficient extraction agent among NaDESs, achieving a similar bioactive compounds' content compared with 70% ethanol, and significantly higher in comparison to pure water.

Conclusion: These results emphasize the potential of NaDES-based extraction as a promising and sustainable practice for the valorization of black raspberry pomace.

Supported by: the Ministry of Education, Science and Technological Development of Republic of Serbia, contract number 451–03–66/2024–03/200003.

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DIETARY POLYPHENOLS AS NATURAL INHIBITORS OF INTESTINAL α GLUCOSIDASES

LOMOZOVÁ, Zuzana; HARČÁROVÁ, Patrícia; MLADĚNKA, Přemysl

Faculty of Pharmacy, Charles University, Czech Republic

lomozovz@faf.cuni.cz

Introduction: Type 2 diabetes mellitus (T2D) is one of the most common metabolic disorders worldwide and its global incidence has been rising.1 Even though epidemiological investigations showed that dietary polyphenols might have some impact on treatment and prevention of T2D, the mechanism of action is not known.2 A possible clue can be α-glucosidase, the enzyme catalysing the final step of carbohydrate hydrolysis to glucose in the digestive system. Inhibition of this enzyme is associated with a reduction in glucose absorption and glycaemia. For this reason, several dietary polyphenols were tested for their ability to block both yeast and mammalian (rat) α-glucosidases.

Material & Methods: Tested polyphenols were mixed with α-glucosidase and p-nitrophenyl-α-d-glucopyranoside as a substrate. The kinetics of p-nitrophenol released was monitored by measuring absorbance at 405 nm.

Results: While isoflavones, anthocyanins, and phenolic acids showed a rather mild effect, flavonols were the most potent inhibitors from the tested polyphenols. The most effective compound was spiraeoside, quercetin glucoside, which inhibited rat α-glucosidase comparably to the registered drug acarbose, which was chosen as the standard agent. There were significant differences between yeast and rat intestinal α-glucosidase inhibition.

Conclusion: Flavonoids seem to be good scaffolds for development of novel inhibitors of α-glucosidase

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EVALUATING THE FATE OF HYPOGLYCEMIC PHENOLIC COMPOUNDS FROM PISTACIA VERA SHELLS THROUGH IN VITRO GASTROINTESTINAL DIGESTION AND METABOLOMIC ANALYSIS

MACCARRONELLO, Anna Elisabetta [1]; PINTO, Diana [2]; CARDULLO, Nunzio [1]; DI FRANCESCO, Antonella [1]; PITTALÀ, Maria Gaetana Giovanna [1]; COSTA, Paulo C. [3]; RODRIGUES, Francisca [2]; MUCCILLI, Vera [1]

[1] University of Catania – Department of Chemical Sciences, Italy;
[2] REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Portugal;
[3] UCIBIO – Faculty of Pharmacy, University of Porto, Portugal

anna.maccarronello@phd.unict.it

Introduction: During gastrointestinal digestion, enzymes, pH, and temperature can enhance or hinder phenolic compounds's bioaccessibility and bioactivity. This work aimed to assess the metabolic fate of a hypoglycemic polyphenol-rich extract from Pistacia vera shells (PVS-E) intended for potential oral administration for type 2 diabetes (T2DM) management.

Material & Methods: PVS-E was prepared using a low-impact microwave approach and subjected to in vitro gastrointestinal digestion. Total phenolic and flavonoid content, antioxidant (FRAP, DPPH, and ABTS assays), and hypoglycemic properties (α-amylase and α-glucosidase inhibition) of the resulting oral, gastric, and intestinal digests were compared with those of the original PVS-E. Bioaccessibility was evaluated by LC-ESI-LTQ-Orbitrap-MS analysis. Multivariate data analysis showed correlations between the samples' phenolic composition and bioactivity.

Results: Digestion significantly influenced the antioxidant and α-glucosidase inhibitory activities of PVS-E. α-Amylase inhibition remained consistent. Phenolic compounds were primarily retained in the oral (40.48%) and gastric (61.12%) phases, while 10.98% was retained in the intestinal phase, suggesting degradation and biotransformation phenomena.

Conclusion: The study constituted a step forward for the sustainable development of a PVS-E-based nutraceutical ingredient for T2DM. Further formulation studies will focus on improving gut bioaccessibility.

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TOTAL PHENOLICS, OXYGEN RADICAL ABSORBANCE CAPACITY, COLOR AND MICROSTRUCTURE OF WALNUT AND HAZELNUT SHELLS, BY-PRODUCTS FROM CHILEAN NUT INDUSTRY

MANTEROLA-BARROSO, Carlos [1,2,4]; MERIÑO-GERGICHEVICH, Cristian [2,3,4]

[1] Doctoral Program in Science of Natural Resources, Universidad de La Frontera, Temuco, Chile
[2] Laboratory of Physiology and Plant Nutrition for Fruit Trees, Universidad de La Frontera, Temuco, Chile
[3] Department of Agricultural Production, Faculty of Agricultural Sciences and Environment, Universidad de La Frontera, Temuco, Chile
[4] Scientific and Technological Bioresources Nucleus (BIOREN-UFRO)

carlosignacio.manterola@ufrontera.cl

Considerable amounts of agro-industrial by-products are discarded and burnt as fuel instead of being utilized as a valuable source of fiber, phenolics and other useful compounds with antioxidant capacity. These interesting features could be affected by genotype, locality, orchard management and seasons, adding or decreasing their properties. In this study, hazelnut (Corylus avellana L.) and walnut (Juglans regia) shell fibers and antioxidant capacities were investigated. Color, total phenolic content (TPC), oxygen radical absorbance capacity (ORAC) and microstructural determinations were performed in nutshells from orchards located in Southern Chile during three seasons (2020/21; 2021/22 and 2022/23).

Nutshells ORAC showed the highest values for both nutshells in 2020/21 season (3217 µmol TEgDW-1 and 4663 µmol TEgDW-1 respectively), while microstructure revealed tissues distribution of lignocelluloses and complex layers by electronic and confocal laser microscopy. Photo-colorimetric values showed increases from one season to the next one for both nutshells, also hazelnut shells TPC was correlated with increasing ORAC values and L* color parameter. An increasing TPC and ORAC trend was observed throughout seasons, pointing the high potential and homogeneity of nutshells.

Results provide physicochemical characteristics insights of two nutshell species with high antioxidant capacity linked to color and valuable microstructure for potential industrial uses.

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PURPLE CORN ANTHOCYANINS AS A NUTRACEUTICAL APPROACH TO PREVENT ONSET AND PROGRESSION OF MULTIPLE SCLEROSIS AND ASSOCIATED TRIGEMINAL PAIN

MAGNI, Giulia [1]; **MARINELLI, Alessandra [2];** RIBOLDI, Benedetta [1]; DI LORENZO, Chiara [1]; PETRONI, Katia [2]; CERUTI, Stefania [1]

[1] Department of Pharmacological and Biomolecular Sciences -Università degli Studi di Milano, Milan, Italy
[2] Department of Biosciences -Università degli Studi di Milano, Milan, Italy

alessandra.marinelli@guest.unimi.it

Introduction: Anthocyanins (ACNs) are flavonoids providing health benefits by counteracting the onset and progression of several pathologies thanks to their anti-inflammatory, antioxidant and neuroprotective properties. ACN-enriched purple corn (Red) extract reduces trigeminal-associated (TG) pain in pathological conditions, also thanks to the modulation of microglia reactivity. The aim of this study was to examine the effect of the preventive and therapeutic administration of Red extract on Multiple Sclerosis (MS)-associated TG pain and on the onset and progression of motor symptoms of MS.

Material & Methods: Eleven days before the induction of experimental autoimmune encephalomyelitis (EAE), Dark Agouti rats were assigned to drink water, yellow corn or Red extracts. The progression of EAE and TG pain was evaluated and fecal samples collected for analyses of microbiota and ACN metabolites. Central and peripheral nervous system tissues were collected for qPCR, ELISA and Wb analyses.

Results: The preventive administration of Red extract is more effective than the therapeutic one in limiting the progression of MS-associated pain and the development of motor relapses, through the mitigation of neuroinflammation and the promotion of autophagy.

Conclusion: Red extract may represent a safe preventive or adjuvant nutraceutical to reduce drug dosage and associated side effects in MS patients.

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DEVELOPMENT & CHARACTERIZATION OF PHENOLIC-RICH MICROCAPSULES FOR FOOD APPLICATIONS

MARIÑO-CORTEGOSO, Sandra [1,2]; RODRÍGUEZ-BERNALDO DE QUIRÓS, Ana [1,2]; SENDÓN, Raquel [1,2]; BARBOSA-PEREIRA, Letricia [1,2]

[1] FoodChemPack research group, Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, 15782, Santiago de Compostela, Spain

[2] Instituto de Materiales (iMATUS), University of Santiago de Compostela, Santiago de Compostela, Spain

sandra.marino.cortegoso@usc.es

Regarding the increasing interest in healthier diets, microencapsulation of bioactive compounds is a promising delivery system that can protect them from environmental conditions. Moreover, it allows a controlled release and increases bioaccessibility and bioavailability by improving their chemical stability and solubility. This work aimed to develop microparticles for food applications loaded with lime extract yielded from lime industrial by-products.

Zein and pectin were utilized as wall materials to encapsulate lime extract. The formulation was dried using the nanospray-drying technique under different conditions, and the loading capacity and encapsulation efficiency were evaluated. Particles were characterized by morphology, antioxidant capacity, phenolic content and quantification of individual phenolics.

Nanospraydrying provided a high production yield of microparticles (93%), whose encapsulation efficiency reached 76%. Lime-loaded were spherical with a size of 0.7 µm. They provided high antioxidant capacity (6 mg TE/g) and high total phenolic content (19 mg GAE/g), as well as a high concentration of individual phenolics, such as eriocitrin (5 mg/g) or hesperidin (12 mg/g).

Nanospraydrying was demonstrated to be a suitable technique for encapsulate bioactive compounds. The capsules developed demonstrated a high antioxidant capacity with the potential to improve foodstuff quality. More studies should be performed to assess the bioaccessibility of the bioactives.

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BIOLOGICAL ACTION OF COCOA BEAN SHELL EXTRACT ON HUMAN COLON CARCINOMA CELLS

MARTÍNEZ-INDA, Blanca [1,2]; QUERO, Javier [3]; JIMÉNEZ-MORENO, Nerea [1,2]; ESPARZA, Irene [1,2]; ANCÍN-AZPILICUETA, Carmen [1,2]; RODRÍGUEZ-YOLDI, María Jesús [3,4]

[1] Department of Sciences, Universidad Pública de Navarra, Campus Arrosadía s/n, Spain

[2] Institute for Advanced Materials (INAMAT2), Universidad Pública de Navarra, Spain

[3] Pharmacology and Physiology and Legal and Forensic Medicine Department, Veterinary Faculty, Zaragoza University, Spain

[4] CIBERobn, ISCIII, IIS Aragón, Spain

blanca.martinez@unavarra.es

Cocoa bean shell (CBS) is underexploited and is considered as waste. However, CBS polyphenols have a high potential by their anti-carcinogenic properties. The aim of this work was to study the composition of CBS extract and its biological action on human colon carcinoma cells.

CBS extract was obtained by solid-liquid extraction for 24 hours at 40ºC. The solvent was a mixture of ethanol:water (75:25 v/v). The antioxidant activity and total phenolic content were determined by spectrophotometric methods. The individual phenolic compounds were quantified by HPLC-DAD-FLD. Cell culture (human colon adenocarcinoma, Caco-2) were grown in Dulbecco's Modified Eagles medium. Experiments were performed 24 h post-seeding to prevent cell differentiation. Cell proliferation was measured using Resazurin assay. Cell death studies were measured by flow cytometry.

The antioxidant activity of the extract ranged between 0.20 ± 0.01 and 0.31 ± 0.03 mmol Trolox/g extract and the total phenolic content was 0.254 ± 0.004 mmol gallic acid/g extract. The most abundant phenolic compound was protocatechuic acid (1.35 ± 0.01 µg/mg extract). CBS extract was able to inhibit cancer cells through an alteration of the cell cycle, as well as cell death by apoptosis, altering the mitochondrial membrane potential and the activation of caspase 3.

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PLANT EXTRACTS RICH IN PHENOLICS AS FOOD PRESERVATIVES- A RESEARCH EXAMPLES

MATERSKA, Małgorzata; WÓJCIAK, Karolina; PABICH, Marzena; CHILCZUK, Barbara; STASZOWSKA-KARKUT, Monika; DOMARADZKI, Piotr

University of Life Sciences in Lublin, Poland

malgorzata.materska@up.lublin.pl

Introduction Products with a reduced content of synthetic additives are desired on the food market. But increasing antibiotic resistance forces food producers to look for alternative additives that have ability to protect food against pathogens. Plant extracts contain a condensed amount of active ingredients which may be an alternative for synthetic antioxidants. The use of dried plant extracts as natural food preservatives in meat products is presented here.

Material & Methods Lyophilized water extracts from fruits and leaves were prepared and were added to meat products with reduced amounts of synthetic additives. The preservative activity of the extracts and their impact on the final product properties were assessed. The research was performed on: canned pork, burgers from dark cutting veal and raw ripening pork sausage. Finaly, the preservative activity of extract was compared with their chemical content, mainly profile of phenolic compounds evaluated by LC-QTOF-MS method.

Results: A beneficial effect of plant additives on the final product properties was found. It was related to extract chemical composition.

Conclusion: Plant extracts can be successfully used in meat products with a reduced content of synthetic additives. The type and concentration of the additive must be precisely determined before use.

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REMODEL: WEB PLATFORM OF BIOACTIVE MOLECULES FROM FOOD WASTE VALORISATION PROCESSES

FEDELE, Francesca Luisa [1,2]; AVELLA, Biagio [2]; **MIELE, Ylenia [2];**
RAIMONDO, Raffaele [2,3]; IONESCU, Camelia [4]

[1] LINFA SCARL, Italy
[2] Cosvitec Scarl-Italy
[3] Mater Scarl-Italy
[4] Business Inovation Council (BIC) S.R.L - Romania

yleniamiele@cosvitec.eu

Agriculture is one of the largest industrial sectors producing substantial amounts of waste and by-products. By-products are rich in valuable components, such as vitamins, minerals, oils and bioactive compounds with functional properties that can be harnessed in the food, nutraceutical and pharmaecutical industries.The REMODEL project focuses specifically on the valorization of by-products from food processing through green extraction techniques such as supercritical CO_2 and subcritical water extraction.

This poster highlights the project's achievements in both optimizing green extraction protocols and promoting knowledge sharing through an interactive web platform. The interactive web platform developed by REMODEL serves as a knowledge hub, fostering collaboration between stakeholders and promoting the exchange of information and services.

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ISORHAMNETIN: INVESTIGATING ITS EFFECTS ON HUMAN OVARIAN CELLS AND POTENTIAL THERAPEUTIC APPLICATIONS

MIHAL, Michal [1]; BALYTSKA, Olha [2]; BEHINSKA, Katarina Majerik [2]; KOLESAROVA, Adriana [1,2]

[1] AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Slovak Republic

[2] Institute of Applied Biology, Faculty of Biotechnology and Food Sciences,
Slovak University of Agriculture in Nitra, Slovak Republic

michal.mihal@uniag.sk

Introduction: Isorhamnetin is widely known for its ability to promote cell division, raise overall antioxidant capacity, and reduce tumor cell viability and cell survival.

Material & Methods: The aim of this study was to determine the effect of isorhamnetin at concentrations 5; 10; 20; 40; 80 μmol/mL (24 h) on the viability, evaluated by AlamarBlueTM, of human ovarian stratum granulosum cell line (HGL5), human ovarian stratum granulosum tumor cell line (COV434) and human ovarian adenocarcinoma cell line (OVCAR-3), as well the secretion of growth factor (TGF-β2) and the presence of its receptor (TGF-β2R), secretion of steroid hormones (progesterone and 17β-estradiol) and apoptosis-inducing factor (AIF) was assayed by ELISA methods.

Results: Isorhamnetin significantly (P≤0.001) decreased the viability of HGL5, OVCAR-3 and COV434 (20; 40; 80 μmol/mL). Isorhamnetin had no significant effect on the secretion of growth factor (TGF-β2) and the presence of its receptor (TGF-β2R) and secretion of steroid hormones (progesterone and 17β-estradiol). Isorhamnetin significantly (P≤0.05) decreased apoptosis-inducing factor (AIF) in HGL5 cell line (80 μmol/mL).

Conclusion: Based on these findings, we can suggest a potential positive effect of flavonoids such as isorhamnetin, on reproductive health. However, further research is needed for a better understanding of its therapeutic potential.

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EFFECTS OF ACCELERATED LIGHT EXPOSURE ON ANTHOCYANINS IN ROSÉ WINES

MORIONES, Jennifer [1,2]; ESPARZA, Irene [2]; ALMANDOZ, Eluxka [1,2]; JIMÉNEZ-MORENO, Nerea [2]; OSÉS, Javier [1,2]; AMEZQUETA, Pablo [1]; FERNÁNDEZ DE ARA, Jonathan [1]; ANCÍN-AZPILICUETA, Carmen [2]

[1] Asociación de la Industria Navarra (AIN), Spain

[2] Universidad Pública de Navarra (UPNA), Spain

jmoriones@ain.es

Introduction: Anthocyanins are key polyphenols responsible for the color in rosé wines. Market demands for Flint bottles compromises the stability of these compounds, leaving the wine unprotected against light. Without this photo-protection, anthocyanins undergo auto-oxidation phenomena, which adversely affects the color and quality of the wine. The aim of this study is to investigate light-mediated mechanisms affecting color and polyphenols in rosé wines using an irradiation equipment previously developed by our research group.

Material & Methods: Rosé wines bottled in 0.75-liter Flint glass bottles underwent accelerated light exposure for 0, 1, 2, 4, 8, and 16 hours. Subsequently, wines were tasted and characterized by spectrophotometric and chromatographic methods.

Results: Accelerated irradiation caused a progessive deterioration in color quality of the rosé wines, accompanied by a noticeable decrease in anthocyanin and riboflavin levels, an increase in volatile sulfur compounds and unchanged levels of methionine. These effects were noticeable in wines subjected to only 1 hour of light exposure and they were intensified with longer irradiation periods. However, no discernible signs of light struck taste were detected.

Conclusion: Accelerated irradiation induced notable changes in rosé wines, although not light struck taste. Anthocyanins could have reacted with the photoexcited riboflavin instead of methionine.

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APPLICATION OF AN OPTIMISED ANTIOXIDANT AND HYPOGLYCEMIC EXTRACT FROM LANTANA CAMARA BRANCHES IN TECHNOLOGICAL PHYTOSOMES' PREPARATION

CARDULLO, Nunzio [1]; MACCARRONELLO, Anna Elisabetta [1]; SCIACCA, Claudia [1]; MELILLI, Barbara [2]; VITIELLO, Libera [3]; **MUCCILLI, Vera [1,3]**

[1] Department of Chemical Sciences, University of Catania, V.le A. Doria 6, Catania,95125, Italy
[2] MEDIVIS s.r.l. Via Carnazza 34C, Tremestieri Etneo, 95030, Catania, Italy
[3] Institute for Polymers, Composites and Biomaterials CNR, Via P. Gaifami 18, 95125 Catania, Italy

vera.muccilli@unict.it

Introduction: *Lantana camara* (Lc) is a adaptable species naturalised in around 60 countries. Lc leaves extracts are used in folk medicine to treat stomach pain, rheumatism, and bronchitis.1 Herein, for the first time, branches were subjected to microwave extraction for polyphenols recovery. The extract was employed for developing phytosomes,2 a new type of herbal complexes as a potential delivery system.

Material & Methods: A three-factors and three-levels Response Surface Methodology (RSM) was employed to optimize the microwave extraction of polyphenols and especially verbascoside from Lc branches.The extract was studied for antioxidant and hypoglycemic activity (α-glucosidase and α-amylase inhibition).The extract and phosphatidylcholine from soy lecithin were employed to prepare new phythosomes characterized by Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM).

Results: Verbascoside enriched extract from Lc branches was obtained with 16.3% yield. The extract showed antioxidant (DPPH = 28.9 μmol TE/g extract) and hypoglycemic activities IC50 α-glucosidase = 25 μg/mL; IC50 α-amylase = 76 μg/mL). Phytosomes formed spherical and stable nanopaticles with a Z-average of 137.8 nm.

Conclusion: A new biocompatible and bioactive herbal extract formulation was developed from an antioxidant and hypoglycemic extract as potential nano-technology carrier system for type-2 diabetes treatment and other applications.

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CORDIA AFRICANA FRUIT AS A SOURCE OF PHENOLIC COMPOUNDS WITH PREBIOTIC POTENTIAL TO MODULATE GUT MICROBIOTA AND PREVENT ULCERATIVE COLITIS

ARAÚJO, Maria Isabela [1,2]; SOARES, Paulo Galindo [2]; TEIXEIRA, José António [1,3]; CORREIA, Maria Tereza [2]; **NOBRE, Clarisse [1,3]**

[1] University of Minho, Portugal

[2] Federal University of Pernambuco

[3] LABBELS –Associate Laboratory, Braga, Guimarães, Portugal

clarissenobre@ceb.uminho.pt

Introduction: Cordia africana is a shrub traditionally used in folk medicine, with its fruit containing polyphenolic mucilage that may protect against intestinal inflammation. This study aimed to characterize the fruit hydrocolloid and assess its effect on ulcerative colitis and human gut microbiota.

Material & Methods: Phenolic compounds in the hydrocolloid were analyzed by HPLC-DAD/ESI-MS. Their effects were evaluated: 1) in a mouse model of ulcerative colitis induced by dextran sulfate sodium and 2) in a human model, simulating gastrointestinal digestion followed by fecal fermentation.

Results: The hydrocolloid contained 61.09 ± 2.42 mg of gallic acid equivalents (GAE)/g of total phenols. Syringic, hydroxybenzoic, and dihydrocaffeic acids were identified as phenolic compounds conjugated to biopolymers. In mice, hydrocolloid administration reduced disease activity indices, decreased myeloperoxidase and eosinophil peroxidase activity, and increased colon length (at a dose of 100 mg/kg). It also promoted the growth of Lactobacillus and Bifidobacterium, and reduced the proliferation of Acetobacteraceas, stimulating short-chain fatty acids (SCFA) synthesis. In the human model, 43.90% of the phenolic compounds were bioaccessible; the non-digestible fraction stimulated the relative abundance of Bacteroides in gut microbiota and SCFA synthesis (acetate/propionate).

Conclusion: The phenolic compounds found in Cordia africana fruit hydrocolloids contributed to maintaining colon health.

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(-)-EPICATECHIN IMPACT ON CALCIUM MOVEMENT AS A MECHANISM OF ITS VASORELAXANT EFFECT

NOVAKOVIC, Aleksandra [1]; MARINKO, Marija [1]; JANKOVIC, Goran [1]; STOJANOVIC, Ivan [2,3]; KAITOVIC, Marko [2,3]; NENEZIC, Dragoslav [2,3]; KANJUH, Vladimir [4]; YANG, Qin [5]; HE, Guo-Wei [5]

[1] University of Belgrade - Faculty of Pharmacy, Department of Pharmacology, Belgrade, Serbia
[2] University of Belgrade - Faculty of Medicine, Belgrade, Serbia
[3] Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia
[4] Serbian Academy of Sciences and Arts, Belgrade, Serbia
[5] Nankai University – School of Medicine, TEDA International Cardiovascular Hospital, Tianjin, China

aleksandra.novakovic@pharmacy.bg.ac.rs

Introduction: Cocoa, grapes and green tea are among many food rich in (-)-epicatechin, flavanol which beneficial effect on cardiovascular health has been suggested. Here, we aimed to investigate influence of (-)-epicatechin on Ca2+ movement as a mechanism of its vasorelaxant effect in human internal mammary artery (HIMA).

Materials & Methods: Discarded HIMA segments were collected from patients undergoing coronary bypass grafting and studied in organ baths.

Results: (-)-Epicatechin (0.1 μM–500 μM) induced concentration-dependent relaxation of HIMA without endothelium pre-contracted by phenylephrine (pD2=4.86±0.10; max. response 89.7±7.0%). In 80 mM K+-contracted rings, addition of (-)-epicatechin induced partial relaxation of HIMA (pD2=3.61±0.14; max. response 55.5±6.7%). In Ca2+-free Krebs Ringer bicarbonate solution, (-)-epicatechin caused concentration-dependent vasorelaxation of HIMA pre-contracted by phenylephrine (pD2=4.68±0.05; max. response 88.0±7.7%) and caffeine (pD2=5.51±0.01; max. response 100.0±2.9%). Finally, thapsigargin slightly antagonized relaxation of HIMA pre-contracted by phenylephrine in normal Krebs Ringer bicarbonate solution (pD2=4.83±0.13, max. response 89.3±6.4% in control vs. pD2=4.14±0.10, max. response 62.0±4.3% in thapsigargin-treated rings).

Conclusion: (-)-Epicatechin induced strong endothelium-independent relaxation of HIMA pre-contracted by phenylephrine. It seems that (-)-epicatechin could inhibit influx of extracellular Ca2+, interfere with intracellular Ca2+ release (probably via both inositol-trisphosphate receptors and ryanodine receptors) and re-uptake by the sarcoplasmic reticulum via Ca2+-ATPase.

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ISOFLAVONES PROFILE AND ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF SOYBEAN EXTRACTS

PABICH, Marzena Justyna; MATERSKA, Małgorzata; CHILCZUK, Barbara

University of Life Sciences in Lublin, Poland

marzena.pabich@up.lublin.pl

Introduction: The incidence of type 2 diabetes mellitus is increasing worldwide; therefore, controlling postprandial hyperglycemia is crucial to stopping disease progression. One of the strategies is the reduction of the degree of carbohydrate absorption from foods by inhibition of digestive enzyme activity. The present study aimed to evaluate and compare the properties of the dry extracts obtained from diffrent anatomical part of soybean to determine their potential as functional food sources.

Material & Methods: The reaserch material consisted of soybean seeds, leaves and pods. Spectrophotometricaly in vitro α-amylase, α-glucosidase inhibition activity and antioxidant activity as well as phenolics content were determined. Isoflavones profile was evaluated using LC-QTOF-MS method [1-3].

Results: Soybean leaves and pods extracts were characterized by higher total content of antioxidative phenolic compounds in comparison with seeds. Aqueous extract of soybean seeds at full maturity and ethanol extract of leaves were the most efficient inhibitor of α-amylase and α-glucosidase activity, respectively.

Conclusion: Soybeans, as well as leaves and pods, which are a waste product, might be useful material for obtaining preparations with anti-radical and inhibiting the activity of digestive enzymes involved in carbohydrate metabolism. They can also be an active food additive or component of a dietary supplement.

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RECONSTITUED ESSENTIAL OILS – DEALING WITH THE NATURAL VARIABILITY

PASTOR, Jose J.; ANGLADA, Oriol; LÓPEZ-VERGE, Sergi

Lucta S.A., Animal Science Innovation Unit

jose.pastor@lucta.com

Introduction: Essential oils (EOs) are natural products composed by a mixture of volatile compounds. The use of certain essential oils in animal nutrition have shown potential benefits in both, poultry and swine industries.1 However, due to the natural variability of their composition, incosistent results have been reported in experimental studies. The aim of this study was to evaluate the antimicrobial activity of two EOs commonly used in animal production versus their "recontituted" formulations (REOs), formulated to mimick the compostion of the natural effective EOs using pure chemically defined substances.

Material & Methods: Commerical EOs from cinnamon (Cinnamomum verum) and oregano (Origanum vulgare) were analyzed by GC-MS. Compound identification was performed by provided commercial libraries and quantification by internal normalization as indicated by the ISO regulations. Developed REOs were submitted to minimum inhibitory concentration studies against 8 common pathogens isolated directly from commercial farms.

Results: Developed REOs display similar inhibitory activity than their natural counterparts in all tested phatogens (both Gram+ and Gram-).

Conclusion: The use of "reconstituted" formulations may be a good stategy to overcome the limitations derived from the natural variability present in natural EOs. More research is needed to verify their potential benefit in experimental in vivo studies.

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VALORIZATION OF UNDERSTUDIED PRUNUS CERASUS VAR. MARASCA POMACE DERIVED FROM INDUSTRIAL PROCESSING: RECOVERY, CHARACTERIZATION AND BIOACTIVITY ASSESSMENT OF SECONDARY METABOLITES

PERON, Gregorio [1]; PAPA, Federica [2]; RENZETTI, Stefano [1]; GOBBI, Emanuela [1]; GARGARI, Giorgio [2]; ZENGIN, Gokhan [3]; BULGARI, Daniela [2]

[1] University of Brescia, Italy
[2] University of Milano, Italy
[3] Selcuk University, Konya, Turkey

gregorio.peron@unibs.it

Introduction: Marasca cherries are used for the industrial preparation of traditional Venetian liqueurs. Prunus cerasus cherries are rich in anthocyanin pigments, flavonoids and phenolic acids1. However, information about Marasca cherries and industrial by-products are scarce. This work aims to promote the re-use of Marasca pomace as a novel bioactive food ingredient.

Material & Methods: The ultrasound-assisted extraction of polyphenols from pomace was optimized using the response surface methodology. Content of total anthocyanins, phenols, and flavonoids was assessed by colorimetric assays, while a comprehensive phytochemical profiling was performed by UPLC-QToF-MS. Antioxidant activity of extracts was assessed though DPPH, ABTS, CUPRAC, FRAP, PBD, and MCA assays. Enzyme inhibitory properties were tested on glucosidase, amylase, and tyrosinase.

Results: The optimized extraction yielded 26.4 mg/g phenols, 2.4 mg/g flavonoids, and 73.0 µg/g anthocyanins. UPLC-QToF profiling revealed several cyanidin derivatives previously uncharacterized in Marasca cherries, as well as flavonoids and phenolic acids (e.g., caffeate derivatives). Antioxidant activity was related to radical scavenging and metal reduction effects, and a significant anti-tyrosinase activity was observed.

Conclusion: Ethanol extract of Marasca pomace represents a valuable source of polyphenols with antioxidant and anti-tyrosinase properties. It can be used as food ingredient to increase oxidative stability and control enzymatic browning.

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PHENOLIC COMPOSITION AND BIOACTIVITY OF DIFFERENT PIGMENTED RICE, CORN, AND SORGHUM GRAINS

PETERSON, Jaymi Marie; SMOLENSKY, Dmitriy

USDA-ARS, United States of America

jaymi.peterson@usda.gov

Introduction: Recent studies suggest that pigmented rice, corn, and sorghum contain health promoting polyphenolic compounds.Utiliazing grain as as source of phneolics may be economically advantageous. The objective of this study was to determin the phenolic profile and bioactivites of different rice, corn, and sorghum grains.

Material & Methods: Color analysis was performed on both grain and flour samples. Proximate analysis of flour samples was measured. Whole grains were milled prior to extraction (70% v/v ethanol). The phneolic profile was evaluated by measruing the total phenolic content (TPC) and condensed tannin content (CTC) coupled with normal and reverse phase HPLC. Bioactivity was determined using the 2,2-diphenyl-picrylhydrazyl (DPPH) assay and glycation inhibition assay with BSA-fructose.

Results: Sorghum had the highest TPC, CT, and antioxidant activity in comparrison to rice and corn samples. Rice and sorghum had the highet glycation inhibition. Rice and corn had unique cyanin compounds that sorghum did not. Sorghum was a unique source for 3-deoxy anthocyanidins.

Conclusion: Although all grains contain phenolic compounds, sorghum was the highest in TPC, CT, and antioxidant activity. There are unique phenolic compounds to sorghum, making it a viable souce of phenolics for future food applications.

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ENZYMATIC SYNTHESIS OF SULFATED POLYPHENOL METABOLITES

PETRÁNKOVÁ, Barbora [1,2]; BRODSKY, Katerina [1,3]; PETRÁSKOVÁ, Lucie [1]; PELANTOVÁ, Helena [1]; BOJAROVÁ, Pavla [1]; VALENTOVÁ, Kateřina [1]

[1] Institute of Microbiology of the Czech Academy of Sciences, Prague, Czechia

[2] Department of Genetics and Microbiology, Faculty of Science of Charles University, Prague, Czechia

[3] Department of Biochemistry and Microbiology, University of Chemistry and Technology, Prague, Czechia

barbora.petrankova@biomed.cas.cz

Introduction: Polyphenols, important plant metabolites, have therapeutic, antioxidant, and anti-inflammatory effects. During phase II of biotransformation, they are sulfated, glucuronidated, or methylated. Defined standards of sulfated flavonoids are required for metabolic studies. Isolation of these metabolites from biological material is impractical, but they can be synthesized in vitro.

Material and Methods: Bacterial aryl sulfotransferases (ASTs) were used to sulfate various phenolic compounds, with para-nitrophenyl sulfate (p-NPS) as a sulfate donor. The presence of the sulfated products was confirmed by HPLC analysis, which was optimized for the proper separation of polar and highly polar substances.

Results: Sulfated metabolites of selected flavonoids and phenolic acids were detected. This screening of substrates and enzymes helped us identify the optimal conditions for preparing sulfate derivatives of phenolic compounds. Based on tests of individual ASTs, one AST was selected for the preparative production of kaempferol sulfate. The synthesized product was then isolated and structurally characterized.

Conclusion: Enzymatic sulfation of polyphenols applying bacterial ASTs is an effective method for producing sulfated metabolites of flavonoids and other phenolic compounds. Sulfated products can be isolated in sufficient amounts and used as standards for the characterization of the metabolites of the biotransformation of polyphenolic substances.

Supported by the MEYS grant (CZ.02.01.01/00/22_008/0004597) and by the Czech Science Foundation (23-04654S).

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OLEUROPEIN METABOLITES ARE BIOLOGICALLY ACTIVE AS MITOCHONDRIAL CALCIUM ACTIVATORS AT THE CONCENTRATION MEASURED IN HUMAN PLASMA

POQUET, Laure; MORIN-RIVRON, Delphine; HERMANT, Aurélie; FEIGE, Jérôme; DE MARCHI, Umberto

Nestlé Research, Switzerland

laure.poquet@rdls.nestle.com

Introduction: Oleuropein from olive leaf extracts (OLE) has recently been identified as a mitochondrial Ca2+ activator boosting cellular energy metabolism and skeletal muscle performance1. Clinical studies in exercise and healthy aging tested mitochondrial activation in muscle biopsies after OLE supplementation in humans2,3. However, ingested oleuropein generates metabolites whose biological activity remains poorly characterized. An LC-MS/MS method was developed and validated to quantify the major oleuropein metabolites circulating in human plasma after OLE consumption and their biological activity on mitochondrial activity in muscle cells determined.

Material & Methods: Oleuropein metabolites were quantified by LC-MS/MS in plasma samples after intake of 250 mg of OLE in older men. Their mitochondrial calcium activators capacity was measured in C2C12-derived myotubes individually and as a cocktail, at the concentration range determined in plasma.

Results: The validated LC-MS method allowed the accurate quantification of oleuropein metabolites peaking 1h after OLE intake at a total concentration of 856±505 nmol/L. Most of the quantified metabolites and their cocktail, at concentration ranges reproducing plasma exposure, were biologically active as mitochondrial Ca2+ activators in muscle cells.

Conclusion: Oleuropein metabolites are bioactive in skeletal muscle and reach a total concentration in blood sufficient to activate mitochondrial energy metabolism via mitochondrial Ca2+.

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NATURAL DEEP EUTECTIC SOLVENTS – A PROMISING MEDIA TOWARDS SUSTAINABLE VALORISATION OF UNDERUTILIZED CROPS

RADAN, Milica [1]; JOVANOVIĆ, Miloš [2]; KRGOVIĆ, Nemanja [1]; ŠAVIKIN, Katarina [1]

[1] Institute for Medicinal Plant Research "Dr. Josif Pančić", Serbia

[2] University of Niš, Faculty of Medicine, Department of Pharmacy, Serbia

mradan@mocbilja.rs

Introduction: Diversifying food resources through incorporation of crops with nutritional and health benefits present a promising strategy in sustaining nutraceutical security under climate change and biodiversity degradation. Buckwheat is such underutilized crop, known for the abundance of bioactive compounds that have a vast potential to support human health.

Material & Methods: Heat-assisted extraction supported with natural deep eutectic solvents (NaDES) was employed to extract polyphenols from the Fagopyrum esculentum Moench. Response surface methodology was used to optimize the extraction process, considering results obtained in the screening assay with nine different NaDES.

Results: In screening analysis, lactic acid-containing NaDES showed the highest extraction capacity for rutin, whereas total flavonoids were favourably extracted by choline chloride/propylene glycol (ChCl/PG) mixture. Systematic analysis of the studied process paramters revealed that the maximal content of rutin was obtained under the temperature of 60.7°C, during 119.04 min, with 27.32% of water. The optimal conditions for the highest flavonoid content in ChCl/PG extraction were 64.87°C, 112.61 min, and 30.92% of water.

Conclusion: Extracts obtained through implementation of green extraction procedures from buckwheat holds a great potential to contribute to the consumers' nutrient and health benefits and pave the way for incorporation into the new natural-based products.

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UNLEASHING THE POWER OF VIBRATIONAL SPECTROSCOPY FOR RAPID PHENOTYPING OF FLAVONOIDS IN COLORED POTATO BREEDING SELECTIONS

RODRIGUEZ-SAONA, Luis E. [1]; GIUSTI, M. Monica [1]; AYVAZ, Huseyin [2]

[1] The Ohio State University, United States of America

[2] Çanakkale Onsekiz Mart Üniversitesi

rodriguez-saona.1@osu.edu

Introduction: Potato (*Solanum tuberosum* L.) presents the highest genetic diversity among all cultivated species, with ~5000 registered varieties and a broad phenological variation in flesh and skin color. Colored-fleshed potatoes can serve as natural colorants as well as providing nutritional benefits associated with their antioxidant, inflammation inhibition, anti-tumor, and neuroprotective activities. Current detection of anthocyanins depends on chemical and chromatographic methods, which are time-consuming, laborious, and do not lend for field deployment. Our aim was to evaluate the feasibility of portable Infrared and Raman devices to profile flavonoids in potatoes to support breeding programs.

Material & Methods: Andean native potato (n=75) samples from 7 Solanum species were characterized and quantified for their anthocyanin using traditional reference methods3. Infrared and Raman are sensitive to different types of vibrations and provide complementary vibrational spectra.

Results: Pattern recognition analysis identified the major anthocyanin types in the colored potato samples. In addition, the spectra were used to predict the levels of phenolics and monomeric anthocyanins. Handheld devices provided rapid (1 min), non-destructive and user-friendly data acquisition that can be field-deployed.

Conclusion: Field-deployable IR and Raman devices could allow for rapid selection of unique genetic material based on different anthocyanin profiles as a non-invasive method.

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INTERACTION OF SELECTED POLYPHENOLS WITH HSA - SPECTROSCOPIC AND CALORIMETRIC STUDY

ROGÓŻ, Wojciech; OWCZARZY, Aleksandra; KULIG, Karolina; MACIĄŻEK-JURCZYK, Małgorzata

Medical University of Silesia, Poland

wrogoz@sum.edu.pl

Introduction: Polyphenols can affect the protein`s structure and therefore their properties. The aim of this study was to investigate the interaction of gallic acid (GA) and resveratrol (RV) with Human Serum Albumin (HSA).

Material & Methods: Calorimetric measurements have been performed using nano Isothermal Titration Calorimetry (ligand:HSA 0.3782:1÷8.198:1 molar ratio). Spectroscopic measurements have been performed using JASCO J-1500 Spectropolarimeter (ligand:HSA 5:1 molar ratio).

Results: The affinity of HSA towards both GA and RV was high (Ka 1.37±0.38×106M-1 and 1.59±0.38×106M-1, respectively). The reactions between HSA and ligands were endothermic (ΔH>0) and spontaneous (ΔS<0). None of the ligands induces significant changes in the HSA`s secondary structure. Interactions of HSA with RV caused changes in the protein`s regions rich in aromatic amino acid residues, but also significantly changed the disulphide bridge asymmetry. The formation of (HSA-GA)complex resulted in conformational changes mainly in the HSA`s regions containing a lot of Phe and Trp amino acid residues.

Conclusion: RV and GA have an impact on the HSA`s structure. This could potentially affect the protein`s properties and have implications for the pharmaceutical industry and marketing.

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MECHANOCHEMICAL AMORPHOUS DISPERSIONS OF APIGENIN: PAVING THE WAY FOR FUTURE THERAPEUTIC APPLICATIONS IN CONTACT LENSES

ROSIAK, Natalia [1]; CIELECKA-PIONTEK, Judyta [1,2]

[1] Department of Pharmacognosy and Biomaterials, Faculty of Pharmacy, Poznan University of Medical Sciences, 3 Rokietnicka St., 60-806 Poznan, Poland

[2] Department of Pharmacology and Phytochemistry, Institute of Natural Fibres and Medicinal Plants, Wojska Polskiego 71b, 60-630 Poznan, Poland

nrosiak@ump.edu.pl

Introduction: Apigenin, a flavone with anti-inflammatory, antioxidant, and antibacterial properties, is classified in Class II of the Biopharmaceutics Classification System (BCS) due to its good permeability but poor water solubility. Enhancing its solubility can improve its bioavailability and therapeutic potential. This study focuses on creating amorphous solid dispersions of apigenin using mechanochemical methods to increase its solubility.

Material & Methods: Nine amorphous solid dispersions of apigenin were prepared using a ball mill. The dispersions included systems with various polymers such as HP-β-cyclodextrin, β-cyclodextrin, and Pluronic F-68 (PLU68). Milling was conducted for 60 minutes at 30 Hz. Solubility was evaluated, and the dispersions with PLU68 and PLU127 were selected for further analysis. Fourier Transform Infrared Spectroscopy (FT-IR) was used to confirm molecular interactions between apigenin and the polymers.

Results: The study confirmed that the mechanochemical method significantly enhanced the pH-dependent solubility of apigenin. FT-IR analysis identified the chemical groups responsible for these interactions.

Conclusion: The enhanced solubility of apigenin through this method paves the way for developing innovative applications, such as 2D printing inks for contact lenses and oral solid dosage forms, thus improving its therapeutic applications.

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SKIN BENEFICIAL EFFECTS OF POMEGRANATE PEEL EXTRACT AND ITS DOMINANT COMPOUNDS

ŠAVIKIN, Katarina [1]; ALIMPIĆ ARADSKI, Ana [2]; JOVANOVIĆ, Aleksandra [3]; ĆUJIĆ NIKOLIĆ, Nada [1]; ŽIVKOVIĆ, Jelena [1]

[1] Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Koscuska 1, Serbia;
[2] University of Belgrade-Faculty of Biology, Institute of Botany and Botanical Garden „Jevremovac",Serbia;
[3] University of Belgrade, Institute for the Application of Nuclear Energy INEP, Serbia

ksavikin@mocbilja.rs

Introduction: The peel of pomegranate *(Punica granatum* L., Punicaceae) fruit is usually treated as a waste. On the other hand, it is a rich source of polyphenols, including phenolic acids, flavonoids, and tannins. A broad range of biological activities was reported for pomegranate peel extracts.

Material & Methods Extract of pomegranate peel (PPE) was obtained by double percolation using 50% EtOH and spray dried. HPLC was applied for the chemical analysis of the most abundant phenolics. PPE and individual compounds were tested using in vitro inhibition of hyaluronidase, elastase, collagenase, and tyrosinase. In vitro anti-inflammatory potential of PPE was investigated in the erythrocyte membrane stabilization assay.

Results: The most abundant compounds in PPE was punicalagin, followed by punicalin, ellagic and gallic acid. PPE showed IC50 values of 534.76 µg/ml for hyaluronidase, 651.81 µg/ml for elastase, 1013.81 µg/ml for collagenase, and 537.60 µg/ml for tyrosinase inhibition. The most active individual compound was ellagic acid, except for collagenase inhibition assay where punicalin was the strongest inhibitor (IC50 value of 95.63 µg/ml). Also, PP extract showed promising concentration-dependent in vitro anti-inflammatory activity.

Conclusion: Both, PPE and individual compounds showed promising skin beneficial effects to meet trends in cosmetic and healthcare sector.

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PURPLE CORN ANTHOCYANINS ON ZEIN NANOPARTICLES AS POTENTIAL DELIVERY SYSTEM

SCAPUZZI, Chiara [1]; TOCCACELI, Marta [2]; MAGGIONI, Daniela [3]; VEROTTA, Luisella [1]; PETRONI, Katia [2]; MARZORATI, Stefania [1]

[1] Department of Environmental Science and Policy, Università degli Studi di Milano, Italy

[2] Department of Biosciences, Università degli Studi di Milano, Italy

[3] Department of Chemistry, Università degli Studi di Milano, Italy

chiara.scapuzzi@unimi.it

Introduction: Purple corn has shown preventive effects against several chronic diseases in preclinical and clinical studies. Its health effects have been attributed to the presence of anthocyanins (ACNs). In this work, ACNs extracted from purple corn cobs were encapsulated in zein nanoparticles (NPs) to enhance ACN stability. Afterwards, ACN-loaded and unloaded formulations were tested on Caco-2 intestinal cells to verify their cytotoxicity.

Material & Methods: The extraction of ACNs from corn cobs was performed by maceration using hydroalcoholic solutions acidified using six different acids. Based on extraction efficiency and selectivity three extracts were selected to test their cytotoxicity through MTT on Caco-2 human intestinal cells. The best performing extracts were then employed for the preparation of zein NPs and their morphology and cytotoxicity were assessed.

Results: Only formic and hydrochloric extracts showed no cytotoxicity at suitable ACN concentrations and were employed for NPs formulation. At the same concentrations, both formulations were not cytotoxic on Caco-2 cells. SEM and DLS analyses conformed their nanometric dimentions and spherical shape.

Conclusion: Encapsulation of ACNs extracted from purple corn cobs in zein-based nanoformulations proved to be safe and not cytotoxic on human intestinal cells and deserve further studies as putative delivery system.

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FULL CHARACTERIZATION OF RHODODENDRON TOMENTOSUM (*LEDUM PALUSTRE*) HYDROALCOHOLIC EXTRACT: METABOLOMIC AND BIOLOGICAL INSIGHTS

SCHIAVONE, Giovanna [1,2]; IMBIMBO, Paola [1]; MONTI, Daria Maria [1]; RUOCCO, Michelina [2]; TROISE, Antonio Dario [3]; DE PASCALE, Sabrina [3]; FERRACANE, Rosalia [3]; CAIRA, Simonetta [3]; ROCCO, Vincenzo [4]; MONTI, Maurilia Maria [2]

[1] Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, via Cinthia 4, 80126 Naples, Italy
[2] Institute for Sustainable Plant Protection, National Research Council, 80055 Portici, Italy
[3] Institute for the Animal Production System in the Mediterranean Environment, National Research Council, Italy
[4] CeMON S.r.l., Viale Gramsci 18, 80122 Naples, Italy

ing.giovannaschiavone@gmail.com

Introduction: *Rhododendron tomentosum*, formerly *Ledum palustre*, is an aromatic plant belonging to the Ericaceae family. Traditionally, it has been used to treat various diseases, as an anti-inflammatory and as a repellant. This study aims to provide a comprehensive functional characterization of its hydroalcoholic extract.

Materials & Methods: Biological characterizations of the extract were performed, including an antioxidant activity assay on a eukaryotic cell line and antibacterial and antifungal activity tests on both plant beneficial and phytopathogenic species. Liquid chromatography high-resolution tandem mass spectrometry (LC-MS/MS) and untargeted metabolomics tackled the molecular aspects behind extract bioactivity.

Results: The extract showed a strong antioxidant activity, in combination with a significant antibacterial activity on a phytopathogenic strain. Additionally, the extract promoted the growth of a beneficial bacterium suggesting potential applications in enhancing plant health and resistance, while only a slight antifungal activity was observed. Furthermore, several compounds belonging to polyphenol classes and typically not associated with this species were tentatively identified through untargeted metabolomic analysis.

Conclusion: The study highlights the presence of various bioactive compounds in *Rhododendron tomentosum* extract, emphasizing its potential use in multiple fields of application.

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ANTI-INFLAMMATORY EFFECTS OF HYDROXYTYROSOL AND TYROSOL AND THEIR PHASE II METABOLITES ON ENDOTHELIAL CELLS UNDER HYPERGLYCEMIC CONDITIONS

SERRELI, Gabriele [1]; BORONAT, Anna [2,3]; DE LA TORRE, Rafael [2,3]; DEIANA, Monica [1]

[1] University of Cagliari, Italy

[2] Department of Medicine and Life Sciences, Universitat Pompeu Fabra, 08003 Barcelona, Spain

[3] Integrative Pharmacology and Systems Neurosciences Research Group, Hospital del Mar Research Institute, 08003 Barcelona, Spain

gabriele.serreli@unica.it

Introduction: Hyperglycemia is among the main factors involved in the onset of cardiovascular diseases. Conversely, endothelial function may be enhanced with the intake of compounds like dietary (poly)phenols. The aim of this investigation was to evaluate the protective effect of two extra-virgin olive oil (EVOO) phenolic compounds, hydroxytyrosol and tyrosol, and their glucuronidated and sulfated phase II metabolites, against the inflammatory response in endothelial HUVEC monolayers under hyperglycemia.

Material & Methods: The alteration of HUVEC monolayers, treated with high glucose (HG, 30 mM) alone or together with EVOO phenols (1 μM), was evaluated through FITC-Dextran cell permeability test and the determination of tight junctions (TJ) proteins level through Western blotting, in relation to MAPK modulation, NLRP3 inflammasome expression and IL-1β and IL-6 levels.

Results: It was demonstrated that the tested compounds counteracted HG-induced inflammation by preserving TJ proteins integrity and by suppressing the activation of the MAPK pathway and NLRP3 inflammasome, also reducing the expression of pro-inflammatory cytokines.

Conclusion: This study underscores the significant protective effects of hydroxytyrosol, tyrosol, and their phase II metabolites on endothelial cells exposed to HG conditions. Our findings provide a deeper understanding of the molecular mechanisms by which these molecules exert their protective effects.

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ANALYSIS OF POLYPHENOLS, ANTIOXIDANTS, AND MINERALS IN VARIETIES OF CAPSICUM SPP. AS A POTENTIAL CANDIDATE FOR ENRICHING MCT OIL

STRAKA, Dušan [1]; KOVACIKOVA, Eva [2]; JANCO, Ivona [1]; ARVAY, Julius [3]; BOROTOVA, Petra [1]; DURANOVA, Hana [1]

[1] AgroBioTech Research Centre, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
[2] Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Slovak Republic
[3] Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Slovak Republic

dusan.straka@uniag.sk

Introduction: This study assessed the total phenolic content (TPC), antioxidant activity (AA), and selected polyphenols in five chili pepper varieties, focusing on their relationship with capsaicin (CAP), dihydrocapsaicin (DHC), and mineral content. The goal was to identify the best variety for enriching MCT oil with bioactive compounds to enhance health benefits.

Material & Methods: Five Capsicum spp. varieties were analyzed: Trinidad Moruga Scorpion (TMS) Peach, TMS Red, Orange Coral, Bahamian Goat, and Carolina Reaper. TPC and AA were measured using the Folin-Ciocalteu method and DPPH assay, while CAP, DHC, and polyphenols were analyzed by HPLC-DAD. Mineral profiles were determined using ICP-OES. Statistical analysis included one-way ANOVA, Tukey's test, and Pearson correlation.

Conclusion: TPC ranged from 7.51 to 25.07 mg GAE/g DW, with Carolina Reaper showing the highest TPC, AA (5.50±0.13 mg TEAC/g DW), CAP (62808.40±285.92 mg/kg DW), and DHC (32196.11±155.76 mg/kg DW), along with resveratrol (5.61±0.09 mg/kg DW). Orange Coral had the highest rutin (211.63±2.51 mg/kg DW) and other notable polyphenols. Significant positive correlations were found between AA and TPC (r = 0.830), TPC and CAP (r = 0.733), and TPC and DHC (r = 0.745). Carolina Reaper is the top candidate for enriching MCT oil with bioactive compounds.

Supported by: the Slovak Research and Development Agency, APVV-22-0348.

Duranova, H., Kuzelova, L., Fialkova, V., Simora, V., Kovacikova, E., Joanidis, P., ... & Gabriny, L. (2024). Coconut-sourced MCT oil: its potential health benefits beyond traditional coconut oil. Phytochemistry Reviews, 1-42

POLYPHENOL CONTENT, ANTIBACTERIAL AND ANTIOXIDATIVE PROPERTIES OF SOME BERRIES AND THEIR INFLUENCE ON INOSINE MONOPHOSPHATE FORMATION IN MINCED PORK

SÜNTER, Alar [1]; MEREMÄE, Kadrin [1]; RUSALEPP, Linda [1]; ROASTO, Mati [1]; ANTON, Dea [1]; PÜSSA, Tõnu [1]; KUZNETSOV, Artur [2]; RAUDSEPP, Piret [1]

[1] Estonian University of Life Sciences, Estonia

[2] AS Ldiamon, Estonia

alar.synter@emu.ee

Introduction: Total polyphenol content (TPC), antibacterial and antioxidative properties [1] of chokeberry, blackcurrant, and rowan berries and their press residues were alnalysed together with meat taste enhancing inosine monophosphate (IMP) formation [2] during 14 days of storage of the minced pork, enriched with these berry powders.

Material & Methods: Berry extracts were prepared and chromatographically analyzed (Agilent Technologies, Germany). The minimal inhibitory concentration (MIC) values were determined according to the EVS-EN ISO 20776-1:2020 guidelines. Analyses of antioxidative properties were performed using a plate reader (Tecan Austria Gmbh, Austria) and analyses of IMP formation with a HPLC-DAD (Agilent Technologies, Germany).

Results: TPC correlated strongly positively with antioxidativity. The susceptibility of tested bacteria to the berry extracts was established, starting from the most sensitive bacteria: S. aureus > L. monocytogenes > E. coli ≈ C. jejuni. No correlation was established between TPC and antibacterial properties, the results were plant specific. All berry powders incorporated to the minced pork reduced the IMP content.

Conclusion: Studied berries can be used in meat to reduce oxidation. Other compounds besides polyphenols can cause antibacterial effect in the studied plant extracts. Enrichment with berries reduced meat characteristic flavour in minced pork.

Supported by Estonian Research Council, grant number PRG1441.

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EXPLORING THE ANTI-INFLAMMATORY POTENTIAL OF UPLAND PIGMENTED POTATOES

TOCCACELI, Marta [1]; BALLABIO, Federico [1]; MARINELLI, Alessandra [1]; BASSOLINO, Laura [2]; PACIFICO, Daniela [2]; PARISI, Bruno [2]; NICOLETTI, Federica [2]; LO SCALZO, Roberto [3]; CAMILLONI, Carlo [1]; MANDOLINO, Giuseppe [2]; PETRONI, Katia [1]

[1] Università degli Studi di Milano, Department of Biosciences, Milan, Italy
[2] CREA, Research Centre for Cereal and Industrial Crops, Bologna, Italy
[3] CREA, Research Centre for Engineering and Agro-Food Processing, Milan, Italy

marta.toccaceli@unimi.it

Introduction: Upland potatoes represent a resilient and economical source of health-beneficial bioactives such as polyphenols and carotenoids1-3. In the framework of the JPI FACCE SYSTEMIC project, we aimed at studying the anti-inflammatory effect of three commercial potato varieties cultivated in Starleggia (Valle Spluga, 1560 masl).

Material & Methods: Biochemical analyses allowed us to consider extracts from Kennebec, Desirée and Bleuet varieties as chlorogenic acid (CGA)-rich, carotenoid-rich and anthocyanin (ACN)-rich, respectively. The anti-inflammatory potential of these compounds was tested on PMA-induced THP-1 macrophages challenged with LPS via qPCR and ELISA.

Results: The ACN-rich Bleuet raw extract showed a lower anti-inflammatory activity at plasmatic doses compared to the carotenoid-rich Desirée. We speculated that the high level of CGA in Bleuet extract could interfere with ACNs. Consistent with this, the Bleuet isolated ACN fraction showed a stronger anti-inflammatory activity than the raw extract and adding CGA to ACNs significantly reduced the ACN anti-inflammatory effect. We explored their possible interaction with membrane transporters via molecular docking.

Conclusion: Overall, upland pigmented potatoes may represent a source of anti-inflammatory phytonutrients, but the possible interference between CGA and ACN anti-inflammatory activity indicates a new starting point to develop/select ACN-rich varieties with a lower content of CGA.

Supported by ERA HDHL KH FNS SYSTEMIC- EoI N.967 CLIMAQUALITEC (MASAF)

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INSIGHTS INTO WILD POMEGRANATE PEEL ENCAPSULATES: PHENOLIC COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL

VULIC, Jelena J. [1]; MILOSEVIC, Mirjana [2]; CUJIC NIKOLIC, Nada [3]; LAZIC, Biljana [2]; CANADANOVIC-BRUNET, Jasna [1]; SAVELJIC, Anja [1]; SAVIKIN, Katarina [3]

[1] University of Novi Sad, Faculty of Technology, Serbia, Serbia

[2] University of Banja Luka, Faculty of Technology, Vojvode Stepe Stepanovića 75, 78000 Banja Luka, Bosnia and Herzegovina

[3] Institute for Medicinal Plants Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia

jvulic@uns.ac.rs

Introduction: Pomegranate peel (P) proved a broad range of biological activities and health-promoting effects. Considering the great therapeutic potential of a P extract, this study aims to establish an encapsulation process, using biocompatible carriers to obtain preserved encapsulates with highly potent bioactive components. The phenolics content, antioxidant and antimicrobial activities of pomegranate peel encapsulates were determined.

Materials & Methods: Microencapsulation method was performed by freeze drying technique. The content of total phenols, flavonoids, flavonols, flavan-3-ols and total and monomeric anthocyanins were determined spectrophotometrically in pure freeze-dried extract PPE (FPE), and its encapsulates, with whey protein (FPE+WP), maltodextrin (FPE+MD) and polydextrose (FPE+PD). Antioxidant activities were measured using DPPH, ABTS and hydroxyl radical tests. Antimicrobial effects of samples were evaluated. FTIR analysis was performed to examine the efficient encapsulation process inside the carries.

Results: Among encapsulates, the FPE+WP showed the highest content of all investigated components, except FPE+PD which demonstrated the highest flavonoids content. FPE+WP showed the highest antioxidant activity by DPPH (IC50=13.81µg/ml), while FPE+MD by ABTS (IC50=3.15 µg/ml) and OH (IC50=18.88 µg/ml) tests. Samples exhibited high antimicrobial potential. FTIR analysis demonstrated successful encapsulation process, without negative interactions.

Conclusions: All obtained wild pomegranate peel encapsulates proved good multifunctional properties with potential as food and pharmaceuticals.

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BENEFICIAL IMPACTS OF CHOKEBERRY AND TART CHERRY BASED DIETARY SUPPLEMENTS CONSUMPTION ON CELLULITE REDUCTION

ZIVKOVIC, Jelena; BIGOVIC, Dubravka; KRGOVIC, Nemanja; SAVIKIN, Katarina

Institute for Medicinal Plants Research "Dr Josif Pancic", Serbia

jelenazivkovic1@yahoo.com

Introduction: Cellulite is an aesthetically distressing skin condition occurring in 80% to 90% of females. Our aim was to investigate the effects oral consumption of dietary supplements based on chokeberry and tart cherry juices.

Material & Methods: Twenty women with a cellulite grade of 1–2 (Nurnberger–Muller scale) were participating in the study. Ultrasonography was applied to analyze the skin structure in addition to biochemical and anthropometric parameters, which were measured before starting the treatment and after 32 days.

Results: A reduction in the thickness of the dermis with subcutaneous fat tissue and epidermis, while the length of the fascicles was reduced by 35.93%. In 11 subjects edema of the dermis noticed at the beginning of the study was not recorded at the end. Moreover, a statistically significant increase in the tissue doppler signals was recorded indicating a better blood supply. Changes in anthropometric and biochemical parameters were not recorded. Creatinine, urea, ALT, and AST values, as indicators of kidney and liver function, remained at normal reference levels, pointing out the product's safety.

Conclusion: Our results showed the marked potential of tested dietary supplements in improving the morphology of skin in the regions that are affected by cellulite.

Supported by the HORIZON 2020-MSCA-RISE-2017 project EthnoHerbs and Ministry of Education, Science and Technological Development of the Republic of Serbia (grant number 451-03-66/2024-03/200003).

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Antibacterial and antbiofilm activities of flavonol-enriched saffron tepal extract against multi-drug resistant ESKAPE pathogens

BOUOTHMANY Kaoutar [1,2], ZALEGH Imane[1] , AIT MHAND Rajaa [1] , RHALLABI Naima[1] , BENBACER Laila[2] , FOUGERE Laëtitia[3] , DESTANDAU Emilie[3] , MELLOUKI Fouad[1]

[1]RU Microbiology, Biomolecules and Biotechnology, Laboratory of Chemistry-Physics and Biotechnologies of Biomolecules and Materials (LCP2BM); Hassan II University of Casablanca. Morocco;

[2]Biology and Medical Research Unit, National Center for Energy Sciences and Nuclear Techniques CNESTEN, Rabat, Morocco;

[3]Institute of Organic and Analytical Chemistry (ICOA), UMR 7311, University of Orléans, CNRS, Orléans, France.

Kaoutar,bouothmany@gmail.com

Background, Objectives and Methodology

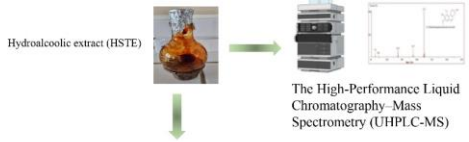
The excessive and inappropriate use of antibiotics has led to the expansion of resistant bacterial strains, which poses an increasing threat to public health. This issue highlights the urgent need to explore natural antibacterial alternatives to address the challenge of antibiotic resistance. In this regard, saffron (*Crocus sativus* L.) bioresidues, a valuable source of phenolic compounds [1], represent a promising antibacterial candidate. The aim of the current study is to evaluate the antibacterial and antiadhesive activities of hydroalcoolic saffron tepal extract (HSTE) against clinical ESKAPE bacteria stsrains:

- Vancomycin-Resistant *Enterococcus faecium* (VRE),
- Methicillin-resistant *Staphylococcus aureus* (MRSA),
- *Klebsiella pneumoniae* ESBL and CP,
- Imipenem-Resistant *Acinetobacter baumannii* (IRAB),
- Imipenem-resistant *Pseudomonas aeruginosa (*IRPA),
- *Enterobacter cloacae* ESBL and CP

ESBL and CP: Extended-Spectrum Beta-Lactamase and Carbapenemase-Producing

➢ **Plant material and extract preparation**

The powdered saffron tepals from Taliouine region, Morocco, were macerated using methanol-water mixture (80%) for 24h .



The High-Performance Liquid Chromatography–Mass Spectrometry (UHPLC-MS)

➢ **Antibacterial activity**

Microdilution assay

Determination of minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC).

➢ **Anti-adhesive activity**

Crystal Violet method

Determination of the percentage of inhibition of biofilm formation.

Results

Table1: MIC and MBC values of HSTE against ESKAPE strains

ESKAPE bacteria	MIC mg/mL	MBC mg/mL	MBC/MIC
MRSA	500	500	1
K. pneumoniae ESBL and CP	500	500	1
IRPA	250	500	2
VRE	500	500	1
IRAB	250	500	2
E. Cloacae ESBL and CP	500	500	1

➢ Antibacterial effect of HSTE with MIC of 500 mg/mL against all strains except IRPA and IRAB, and MBC value of 500 mg/ml;

➢ Bactericidal effect against all the studied strains with MBC:MIC ratio ≤4.

Results and Discussion

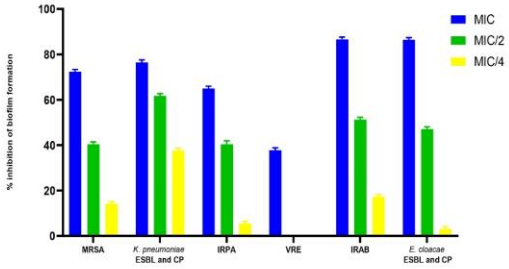


Figure 1 : Percentages of inhibition of biofilm formation

➢ The highest inhibition of biofilm adhesion was observed at MIC, with 86,63% inhibition for IRAB and 61,75% for *K.pneumoniae* at MIC/2;

➢ HSTE exhibited an important activity at MIC/4.

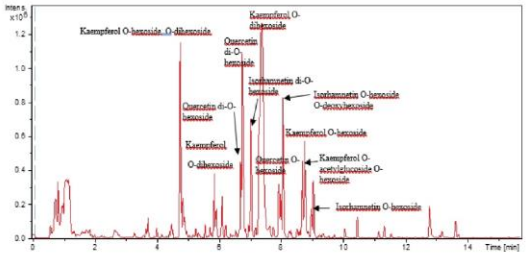


Figure 2 : Phenolic compounds analysis of HSTE using UHPLC-HRMS

➢ Flavonol compounds are found in HSTE including Kaempferol and its derivatives, Quercetin and Isorhamnetin derivates.

➢ Flavonols have demonstrated considerable antibacterial and antibiofilm potantial against multidrug-resistant bacteria [2]. Consequently, the observed activities of HSTE can be attributed to the presence of flavonols in the extract.

Conclusion and perspectives

- Our findings suggest the potential application of saffron bioresidues as efficient alternative to combat antibiotic resistance and eradicate bacterial biofilms;
- Further studies are needed to elucidate the mechanisms underlying the antibacterial activity of HSTE.

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Funding:
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GRAPE POMACE POLYPHENOLS PROMOTE OSTEOGENESIS AND INHIBIT ADIPOGENESIS: THE EFFECT ON BONE MARROW STEM CELL DIFFERENTIATION

CALABRISO Nadia [1], MASSARO Marika [1], SCODITTI Egeria [1], QUARTA Stefano [1], GERARDI Carmela [2], GIOVINAZZO Giovanna [2], CARLUCCIO Maria Annunziata [1]

[1] National Research Council (CNR) Institute of Clinical Physiology (IFC), 73100 Lecce, Italy.
[2] National Research Council (CNR) Institute of Sciences of Food Production (ISPA), 73100 Lecce, Italy.

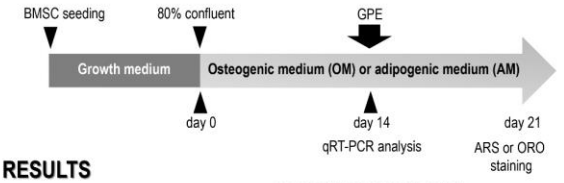
nadia.calabriso@cnr.it; mariaanunziata.carluccio@cnr.it

INTRODUCTION Osteoporosis is an age-related systemic skeletal disease, characterized by progressive loss of bone density, and an increased susceptibility to fractures (1). Bone marrow contains multipotential mesenchymal stem cells, BMSCs, that can differentiate into osteoblasts and adipocytes. Aging can affect BMSC differentiation leading to adipose accumulation in the bone marrow and subsequent bone loss. Natural compounds such as polyphenols can prevent and counteract bone diseases (2). Grape pomace is a rich source of polyphenols with multiple health-promoting properties (3).

PURPOSE

To analyze the effects of grape pomace polyphenolic extracts on osteogenesis and adipogenesis

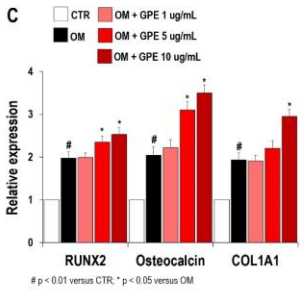
MATERIAL and METHODS Human BMSCs cultured with and without grape pomace polyphenolic extracts (GPE, 1-10 µg/mL GAE) were differentiated into osteoblasts or adipocytes. Osteogenesis and adipogenesis were assayed by Alizarin Red S (ARS) and Oil Red O (ORO) staining, respectively, as well as by gene expression of related markers by quantitative RT-PCR analysis.

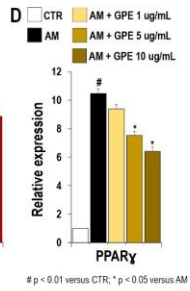


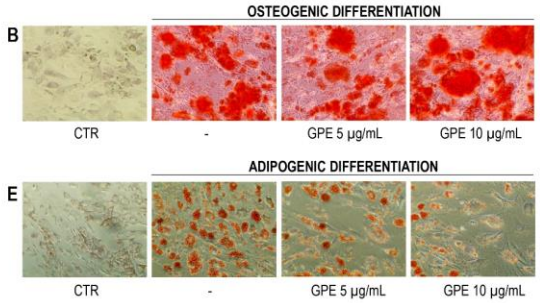
RESULTS

A

Grape pomace cv	Total Phenols (mg GAE/L)	Total Anthocyanins (mg OE/L)	Antioxidant Activity (mmol TE/L)
Negroamaro	372.5 ± 12.3	39.9 ± 0.9	1.0 ± 0.1

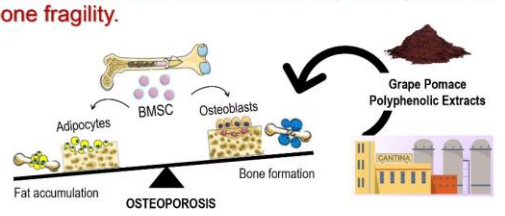






Grape pomace was characterized by a high content of phenolic compounds and, correspondently, showed antioxidant activities (A). GPE significantly induced BMSC differentiation into osteoblasts compared to control (B), without altering cell viability. This effect was associated with an increase in gene expression of the osteogenesis markers RUNX2, osteocalcin, collagen type I (C). Moreover, GPE significantly reduced BMSC differentiation into adipocytes (E), decreasing lipid accumulation and PPARγ gene expression (D).

CONCLUSIONS Our findings suggest GPE as a potentially useful strategy to reduce age-related marrow adipose accumulation and possibly prevent bone fragility.



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NATURAL RED GRAPE POMACE EXTRACT IMPROVES VASCULAR AGEING THROUGH REDUCTION OF ENDOTHELIAL CELL SENESCENCE AND INHIBITION OF ENDOTHELIAL DYSFUNCTION

CALABRISO Nadia [1], MASSARO Marika [1], SCODITTI Egeria [1], GIRELLI Chiara Roberta [2], FANIZZI Francesco Paolo [2], GERARDI Carmela [3], GIOVINAZZO Giovanna [3], CARLUCCIO Maria Annunziata [1]

[1] National Research Council (CNR) Institute of Clinical Physiology (IFC), Lecce, Italy.
[2] University of Salento Department of Biological and Environmental Sciences and Technologies (DISTEBA), Lecce, Italy
[3] National Research Council (CNR) Institute of Sciences of Food Production (ISPA), Lecce, Italy.

nadia.calabriso@cnr.it; mariaanunziata.carluccio@cnr.it

AIM: Aging, a non-modifiable risk factor for cardiovascular diseases, is characterized by endothelial cell senescence and dysfunction (1). Grape pomace is an important source of bioactive polyphenols with endothelial beneficial properties, however the effects on endothelial senescence are not clear (2, 3). The aim of this research is to valorize grape pomace by green chemistry yielding high-value molecules, endowed with biological properties in the chemoprevention of vascular aging.

METHODS: Negroamaro grape pomace (100gr) was extracted in distilled water (1 L) at 90°C. After centrifugation, the extract was dehydrated under spray dryer, solubilized to 10% (w/v) proportion and then chemically characterized and used for bioactivity in cell culture assays. Grape pomace extracts (GPE) were structurally characterized by HPLC/DAD and NMR and their effects on endothelial dysfunction and senescence were analyzed. Human umbilical vein endothelial cells (HUVECs) were exposed to H_2O_2, as an in vitro aging model, detected by SA-β-gal staining, or to TNF-α, to induce endothelial dysfunction, analyzed by the expression of inflammatory markers, at protein (EIA/ELISA/Western) and mRNA levels (qRT-PCR). MTT, DCFH-DA and Griess assay were used to evaluate cell viability, reactive oxygen species (ROS) and nitric oxide (NO) levels, respectively.

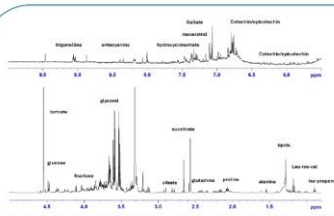


Figure 1. Representative ^{1}H NMR (Nuclear Magnetic Resonance Spectroscopy) spectrum of GPE. Expanded areas in (0.5-5 ppm), aliphatic region and (5.5-9.5) aromatic region are reported. The peaks of relevant identified metabolites are labelled

GPE chemical characterization

Table 1. Characterization of 10% (w/v) Negroamaro pomace infusion, on total anthocyanins, phenols content and antioxidant activity.

Grape pomace cv	Total Anthocyanins (mgOE/L)	Total Phenols (mgGAE/L)	Antioxidant Activity (mmol TE/L)
Negroamaro	39.88 ± 0.95	372.49 ± 12.33	1.03 ± 0.12

Data are expressed as mean value ± SD and are representative of 3 independent experiments

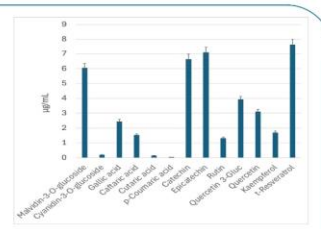
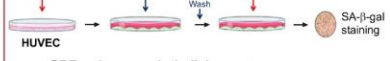


Figure 2. Characterization of different phenolic compounds occurring in Negroamaro grape pomace extract (GPE) dehydrated and suspended in ethanol 70% and used for bio-activity assays

GPE effects on endothelial senescence and dysfunction

Experimental design: H_2O_2-induced endothelial senescence



GPE reduces endothelial senescence

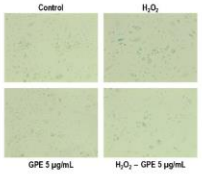


Figure 3. Effects of GPE on H_2O_2-induced HUVEC aging. Cell aging was detected by senescence-associated b-galactosidase (SA-b-gal) staining.

GPE decreases the TNF-induced expression of endothelial adhesion molecules

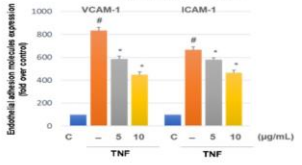
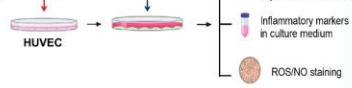


Figure 4. The expression on the endothelial cell surface of VCAM-1 and ICAM-1 was assessed by EIA and expressed as a percentage of the unstimulated control (# p < 0.01 compared to control ; * p < 0.05 compared to TNF alone.

Experimental design: TNF-induced endothelial dysfunction



GPE decreases TNF-induced intracellular ROS and increases NO levels in endothelial cells

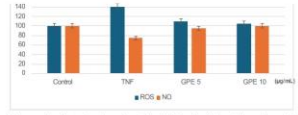


Figure 5. Pre-treatment with GPE significantly reduced TNF induced intracellular ROS levels, in a concentration-dependent manner, whereas increased the NO levels. Similar results were obtained after H_2O_2 endothelial stimulation.

RESULTS: Our results showed that the exposure to H_2O_2 promoted endothelial senescence, which was significantly alleviated by treatment with 5 µg/ml GPE. Furthermore, GPE ameliorated TNF-stimulated endothelial dysfunction by increasing NO production and reducing ROS levels.

CONCLUSION: Overall, results show that a natural extract of red grape pomace as obtained by green chemistry, inhibits endothelial dysfunction and senescence and may contribute to the prevention of age-related diseases.

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WROCŁAW UNIVERSITY OF ENVIRONMENTAL AND LIFE SCIENCES

17th WORLD CONGRESS Polyphenols Applications SEPT. 19-20, 2024 • MILAN, ITALY

EFFECTS OF CHLORINE-CONTAINING DERIVATIVES OF 2'-OH CHALCONE ON SELECTED BLOOD CELLS

CYBORAN-MIKOŁAJCZYK, Sylwia[1], KAŹMIERCZAK, Teresa[1], TROCHANOWSKA-PAUK, Natalia[1], JEWAK, Natalia[1], ROLA, Joanna[1], WALSKI, Tomasz[2], BONARSKA-KUJAWA, Dorota[1], KRAWCZYK-ŁEBEK, Agnieszka[3], KOSTRZEWA-SUSŁOW, Edyta[3]

[1] Department of Physics and Biophysics, Wrocław University of Environmental and Life Sciences, Norwida 25 St., 50-375 Wrocław, Poland
[2] Department of Biomedical Engineering, Wrocław University of Science and Technology, Plac Grunwaldzki 13, 50-377 Wrocław, Poland
[3] Department of Food Chemistry and Biocatalysis, Wrocław University of Environmental and Life Sciences, Norwida 25 St., 50-375 Wrocław, Poland

AIM: The purpose of this study was to determine the effects of 2'-OH chalcone derivatives containing a chlorine atom on the physical and functional properties of erythrocytes (RBCs), platelets (PLTs) and peripheral blood mononuclear cells (PBMCs).

Material & Methods: Studied chlorinated derivatives of 2'-hydroxychalcone contain one chlorine atom at different positions of the A or B ring, and two chlorine atoms at the A ring (Fig.1). Using the XTT assay, the effect of compounds on the metabolic activity of PLTs and PBMCs was examined. The effect of compounds on collagen-induced platelet aggregation was evaluated by optical aggregometry. The hemolytic activity of the compounds and their effects on the osmotic resistance of the erythrocytes were determined spectrofotometrically on the basis of hemoglobin concentration relased from the cells. Changes in erythrocyte shapes under the influence of the tested compounds were evaluated using an optical microscope coupled with a digital camera. The effect of the compounds on the transmembrane potential was examined fluorimetrically based on the changes induced by the tested compounds in the excitation spectrum of the $DiSC_3(5)$ probe.

Fig. 1 *Chlorine containing derivatives of 2'-hydroxychalcone (C0): 5'-chloro-2'-hydroxychalcone (C1), 3-chloro-2'-hydroxychalcone (C3), 4-chloro-2'-hydroxychalcone (C4), and 3',5'-dichloro-2'-hydroxychalcone (C5).*

Results: The chalcones used have practically no effect on the metabolic activity of PBMCs mitochondria at concentrations up to 150 μM and incubation time of 90 min (Fig. 2A). Increasing the incubation time significantly affects the PBMCs mitochondrial reductive capacity, especially in the presence of chlorine-containing compounds in the B ring (Fig. 2B). Used compounds also do not significantly affect the metabolic activity of PLTs mitochondria. The exception is compound C3, which significantly reduces the mitochondrial reductive capacity (Fig. 3A). The compounds used at a concentration of 100 μM completely inhibit collagen-induced platelet aggregation (Fig. 3B). In addition, the study showed that the tested compounds do not induce erythrocyte hemolysis to the concentration up 250 μM and do not change the osmotic resistance and transmembrane potential of erythrocytes (Table 1). They induce stomatocyte formation to varying degrees, suggesting that they bind to the erythrocyte membrane (Fig. 4B).

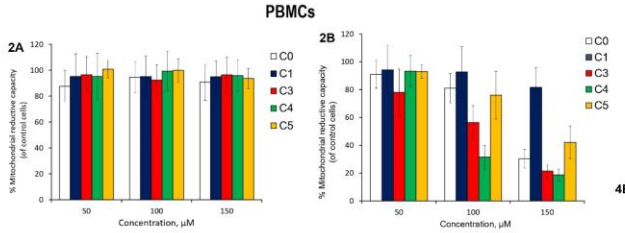

Fig. 2 *Mitochondrial reductive capacity of human peripheral blood mononuclear cells (PBMCs) determined by XTT assay, following treatment with chalcones for 90 min (**1A**) and 24h (**1B**).*

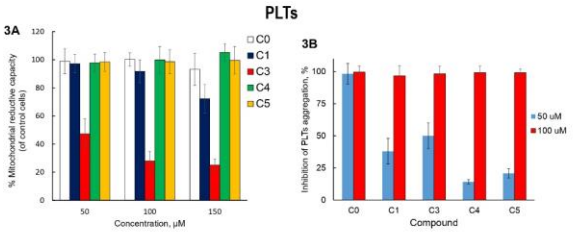

Fig. 3 *Mitochondrial reductive capacity of platelets (PLTs) determined by XTT assay, following treatment with chalcones for 90 min (**3A**). Percentage of inhibition of collagen-induced platelet aggregation in the presence of chalcones used at concentrations of 50μM and 100 μM (**3B**).*

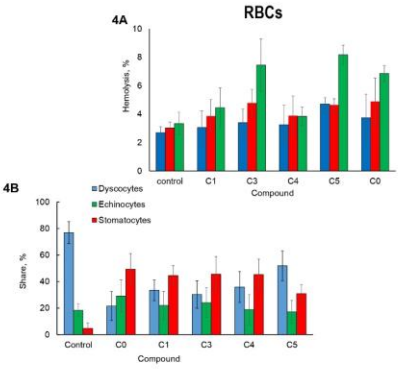

Fig. 4 Percentage of hemolysis of erythrocytes treated with tested compounds used at concentrations of 100, 150 and 250 μM (**4A**). The percent of discocytes, stomatocytes and echinocytes in control and compounds-modifield population of erythrocytes. The compounds were used at a concentration of 100 μM (**4B**).

Compound/ Concentration	C_{50}, % 100 μM	ΔΨ, mV 100 μM
Control	0.47 ± 0.01	-10.8 ± 1.4
C0	0.47 ± 0.01	-11.5 ± 0.9
C1	0.45 ± 0.01	-11.4 ± 1.2
C3	0.47 ± 0.01	-12.6 ± 1.3*
C4	0.46 ± 0.02	-10.6 ± 1.2
C5	0.47 ± 0.01	-10.4 ± 0.4

Table 1. The transmembrane potential (Ψ, mV) of erythrocytes and the NaCl concentration (C_{50}, %) at which 50% hemolysis of control and chalcone-modified erythrocytes occurred.

References:
Cyboran-Mikołajczyk, S., Matczak, K., Olchowik-Grabarek et al.(2024). The influence of the chlorine atom on the biological activity of 2'-hydroxychalcone in relation to the lipid phase of biological membranes - Anticancer and antimicrobial activity. Chemico-Biological Interactions, 398, 1–14.

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17th WORLD CONGRESS

Polyphenols Applications

From the waste of must clarification to the production of a high polyphenols' rich buccal film: new insight to treat oral oxidative stress-related pathologies

DI PRIMA Giulia[1], LA MANTIA Cecilia[1], BELFIORE Elena[2], DE CARO Viviana[1]

[1]Dipartimento STEBICEF, University of Palermo, Palermo, Italy
[2]Dipartimento Me.Pre.C.C, University of Palermo, Palermo, Italy
giulia.diprima@unipa.it

BACKGROUND

There are several pathologies affecting the oral mucosae which are based on an oxidative imbalance. Among them oral mucositis (OM) and oral lichen planus (OLP) are the main ones. As the current therapeutic options could result ineffective while also suffering from several other disadvantages, polyphenols can be ideal supporting candidates due to their wide range of biological activities including the antioxidant and antinflammatory ones[1]. A rich source of natural polyphenols can be the black bentonite (BB) waste coming from white grape must filtration which, through virtuous recovery, can be treated to obtain a liquid polyphenols' enriched excipient usefull for pharmaceutical applications[2].

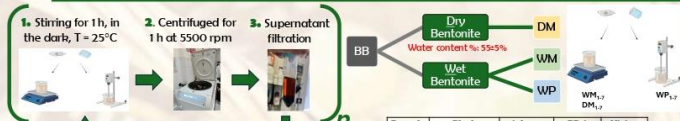
AIM

Obtaining a high polyphenols' rich novel raw material directly useful to be inserted into pharmaceutical formulations suitable for buccal administration and thus useful to treat the oral oxidative stress-related pathologies

Waste recovery

Waste black bentonite (BB) directly taken from the filtration silos

Multiple maceration



Formula code	Black bentonite (BB)	Solvent (I)	BB:I (w/w)	Mixing method
DM	Dry	PEG200	1:8	magnet
WM	Wet	PEG200	1:4	magnet
WP	Wet	PEG200	1:4	paddle

n = 7

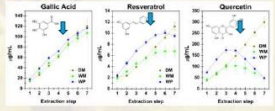
Unconvetional and green extraction solvent (waste-to-market approach)

Best extract selection

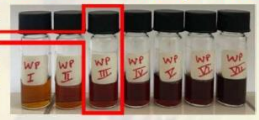
	Sample	Solvent recovery %	Density (g/mL)	pH	TPC (mg/g)	TDtC (mg/g)	GAE (mg/g)
DM	DM-1	76.3610.69	1.1170.010	3.9210.15	1.3290.114	0.1831O.O08	0.3091O.052
	DM-2	57.0711.08	1.1320.003	3.5810.14	2.5461O.109	0.2451O.015	0.9881O.055
	DM-3	41.5010.94	1.1360.005	3.6610.19	5.2561O.229	0.6601O.030	1.7871O.058
	DM-4	29.7910.97	1.1491O.006	3.5610.11	7.8681O.342	0.7281O.048	2.5051O.092
	DM-5	18.7110.68	1.151O.006	3.521O.10	10.5701O.744	0.8901O.077	4.251O.83
	DM-6	9.8910.93	1.1631O.004	3.3910.03	13.6791O.553	1.1071O.076	5.2701O.81
	DM-7	3.4010.71	1.1751O.007	3.381O.03	14.9571.215	1.141O.228	7.3681O.276
WM	WM-1	79.8414.15	1.1251O.013	4.001O.01	2.5181O.236	0.6771O.124	0.6661O.053
	WM-2	62.6313.08	1.131O.011	3.501O.04	4.4951O.684	0.8781O.135	1.2491O.095
	WM-3	45.1113.47	1.1361O.012	3.321O.01	7.0861.305	0.8721O.141	1.7681O.089
	WM-4	33.1814.16	1.1361O.001	3.2810.02	8.6371.427	1.0101O.145	2.1071O.118
	WM-5	26.8814.20	1.1391O.005	3.2510.04	10.3511.440	1.0181O.178	2.3801O.126
	WM-6	17.1614.66	1.1721O.018	3.2510.01	11.7401.070	1.1091O.234	2.4621O.188
	WM-7	11.5914.14	1.1961O.009	3.211O.01	13.5691.9O6	1.2571O.291	2.5921O.104
WP	WP-1	78.9411.99	1.1181O.001	3.6981O.08	3.1321O.396	0.3721O.018	1.1491O.169
	WP-2	61.4411.99	1.1341O.012	3.3981O.04	6.0071O.309	0.6221O.047	2.5971O.079
	WP-3	42.4911.90	1.151O.011	3.351O.03	9.811O.306	1.141O.017	3.9941O.660
	WP-4	26.4311.00	1.1721O.006	3.341O.04	12.5501O.347	1.251O.022	5.121O.086
	WP-5	16.5913.88	1.1981O.005	3.401O.02	14.2551O.2O6	1.351O.003	6.3851O.827
	WP-6	10.4311.36	1.1421O.007	3.361O.01	15.9251O.969	1.4981O.075	8.3791O.488
	WP-7	8.2013.65	1.1981O.009	3.371O.01	18.5151O.6O6	2.1701O.268	8.9291O.521

Extracts characterization. Means (n = 12) ± standard error

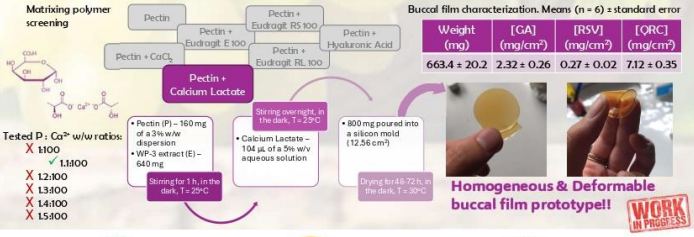
HPLC-DAD analyses



WP-3 is the best compromise between sufficient solvent recovery % and GAE



Buccal film formulation



Buccal film characterization. Means (n = 6) ± standard error

Weight (mg)	[GA] (mg/cm²)	[RSV] (mg/cm²)	[QRC] (mg/cm²)
663.4 ± 20.2	2.32 ± 0.26	0.27 ± 0.02	7.12 ± 0.35

Homogeneous & Deformable buccal film prototype!!

WORK IN PROGRESS

FUNDING

This research was funded by the Ministero dell'Università e della Ricerca (MUR), PON FSE REACT-EU Research and Innovation 2014-2020 Action IV.5 "Dottorati su tematiche green" and Action IV.6 "Contratti di ricerca su tematiche Green"

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Ministero dell'Università e della Ricerca

PON RICERCA E INNOVAZIONE 2014 - 2020

Università degli Studi di Palermo

ANTIOXIDANT CAPACITY AND BIOACTIVE COMPOUNDS OF MATCHA TEAS

JAKUBCZYK KAROLINA *

Department of Human Nutrition and Metabolomics, Pomeranian Medical University in Szczecin, 24 Broniewskiego Street, 71-460 Szczecin, Poland, *karolina.jakubczyk@pum.edu.pl

INTRODUCTION

Matcha, or powdered green tea, has been gaining in popularity and is no longer consumed only in the form of infusions, finding new uses in gastronomy and the food industry. The range of teas available on the food market has expanded considerably; hence, the aim of this study was to determine for the first time the antioxidant capacity and content of antioxidant compounds in various Matcha teas available on the Polish market.

AIM OF STUDY

The aim of this study was to determine for the first time the antioxidant capacity and content of antioxidant compounds in various Matcha teas available on the Polish market.

MATERIAL AND METHODS

Eleven green tea powders were used in the analyses performed using spectrophotometric methods (Trolox equivalent antioxidant capacity, Ferric Ion Reducing Antioxidant Power, Total Polyphenols Content, Total Flavonoids Content, vitamin C Content) and HPLC methods (polyphenolic acids, flavonoids and caffeine).

RESULTS

Table 1. Antioxidant potential (TEAC, FRAP) and phytochemical composition in different Matcha tea infusions.

Matcha	FRAP—Mm Fe(II)/L	TEAC—Trolox Equivalent (mM/L)	Polyphenols—Gallic Acid Equivalent (mg/L)	Flavonoids—Rutin Equivalent (mg/L)	Vitamin C (mg/100 mL)
M01	25,127.22 $^{*\,b,c,e,h}$ ± 100.11	8.87 $^{*\,e}$ ± 0.07	1083.13 $^{*\,b,c,e,f,h}$ ± 19.10	1274.18 $^{*\,c,e,f,j,k}$ ± 48.13	72.52 $^{*\,g-k}$ ± 0.57
M02	18,393.78 $^{*\,a,h}$ ± 272.99	7.69 $^{*\,g,i}$ ± 0.82	928.13 $^{*\,a,d}$ ± 32.51	977.42 $^{*\,i}$ ± 84.96	71.35 $^{*\,g,i,j}$ ± 0.66
M03	16,926.11 $^{*\,a,d,i,k}$ ± 469.77	7.12 $^{*\,g,i,j}$ ± 0.17	838.53 $^{*\,a,d,j,k}$ ± 33.74	780.92 $^{*\,a,d,g}$ ± 45.15	74.18 $^{*\,g-k}$ ± 2.90
M04	24,290.22 $^{*\,c,e,h}$ ± 438.20	8.28 ± 0.39	1061.66 $^{*\,b,c,e,f,h}$ ± 13.41	1359.70 $^{*\,c,e,f,j,k}$ ± 119.90	76.17 $^{*\,g-k}$ ± 1.65
M05	17,015.78 $^{*\,a,d,g,k}$ ± 200.75	6.39 $^{*\,a,g,i,j,k}$ ± 0.32	827.82 $^{*\,a,d,j,k}$ ± 15.61	860.77 $^{*\,a,d}$ ± 23.44	66.47 ± 3.79
M06	20,224.89 $^{*\,k}$ ± 1015.84	7.11 $^{*\,g,i,j}$ ± 0.57	916.36 $^{*\,a,d}$ ± 3.83	798.06 $^{*\,a,d,g}$ ± 119.40	67.82 ± 2.95
M07	21,692.78 $^{*\,h}$ ± 126.11	10.15 $^{*\,b,c,e,f,h}$ ± 0.12	965.08 $^{*\,h}$ ± 17.12	1217.59 $^{*\,c,f,j,k}$ ± 103.89	40.88 $^{*\,a,d}$ ± 1.85
M08	13,452.44 $^{*\,a,d,g,i,k}$ ± 1029.25	7.44 $^{*\,g,i}$ ± 0.15	671.29 $^{*\,a,d,g,i,k}$ ± 12.64	1044.63 $^{*\,i}$ ± 70.07	45.95 $^{*\,a-c,d}$ ± 5.47
M09	23,456.56 $^{*\,c,e,h}$ ± 1268.52	10.43 $^{*\,b,c,e,f,h}$ ± 0.30	970.18 $^{*\,h}$ ± 2.75	1024.18 $^{*\,i}$ ± 50.34	37.38 $^{*\,a-d}$ ± 3.23
M10	20,236.89 ± 1156.00	9.92 $^{*\,c,e,f}$ ± 0.20	1021.67 $^{*\,c,e,h}$ ± 12.34	352.36 $^{*\,a,b,d,g,h,i}$ ± 54.74	38.50 $^{*\,a-d}$ ± 1.25
M11	26,258.33 $^{*\,b,c,e,f,h}$ ± 1015.59	8.82 $^{*\,e}$ ± 0.45	1033.20 $^{*\,c,e,h}$ ± 6.39	787.73 $^{*\,a,d,g}$ ± 38.70	44.46 $^{*\,a-c,d}$ ± 1.88

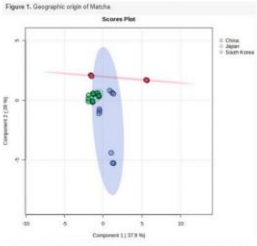
Different letters (a–k) represent different Matcha teas: a—M01, b—M02, c—M03, d—M04, e—M05, f—M06, g—M07, h—M08, i—M-9, j—M10, and k—M11. Results are presented as mean and standard deviation values. Superscripted numbers indicate statistically significant differences in a column ($p < 0.05$). * $p < 0.05$ indicates statistically significant difference between types of Matcha tea.

Table 5. Contents of polyphenolic acids, flavonoids, and caffeine in Matcha tea samples, divided by country of origin.

Compound	Country of Origin					
	China ^{i}		Japan ^{j}		South Korea ^{k}	
	Median	IQR	Median	IQR	Median	IQR
4-hydroxybenzoic acid	36.32	36.04	45.96	10.06	55.58	54.47
caffeic acid	86.29	170.88	2.97 ^{k}	1.05	40.69	173.83
chlorogenic acid	85.63	142.50	28.62	10.88	43.32	74.18
ellagic acid	8.82 ^{k}	7.80	5.68 ^{k}	6.17	14.21 ^{i,j}	46.78
trans-p-coumaric acid	2.87	5.85	0.40	3.39	0.00	6.94
ferulic acid	18.48	14.82	15.22	4.79	7.61	19.41
gallic acid	478.80	856.25	247.59	241.91	62.41	954.01
sinapic acid	41.93 ^{k}	50.73	28.03 ^{k}	5.50	49.91 ^{i,j}	59.45
apigenin	9.15	9.37	8.78	1.76	7.48	4.03
epicatechin gallate	83.79	165.96	175.03	42.06	92.10	207.93
kaempferol	4.62 ^{j,k}	5.87	1.43 ^{i}	0.50	1.16 ^{i}	2.15
myricetin	82.44	98.71	106.15	116.81	104.32	131.03
quercetin	11.49	17.18	3.01	5.01	1.77	6.10
resveratrol	13.23 ^{k}	19.11	15.31 ^{k}	2.66	26.83 ^{i,j}	15.47
rutin	73.94	84.10	0.00	36.64	0.00	59.82
caffeine	1397.65 ^{j,k}	893.56	2125.48 ^{i}	520.51	1920.10 ^{i}	5379.67

Different letters (i, j, k) represent different countries of origin: i—China, j—Japan, and k—South Korea. Letters in the superscript assigned to a value represent statistically significant differences between medians ($p < 0.05$). Data represent the median and IQR values.

Figure 1. Geographic origin of Matcha.



CONCLUSION

The phytochemical composition and antioxidant properties depended on country of origin. Therefore, Matcha tea infusions have been shown to be a valuable source of antioxidants that can be used in the daily diet.

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BLACKBERRY JUICE FERMENTED WITH TWO CONSORTIUMS OF LACTIC ACID BACTERIA: PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES DURING STORAGE

JIMENEZ-OSORIO, Angélica Saraí [1], LUGO-ZÁRATE, Liliana [1], DELGADO-OLIVARES, Luis [1], CRUZ-CANSINO, Nelly del Socorro [1], CASTREJÓN-JIMÉNEZ, Nayeli Shantal [2], ESTRADA-LUNA, Diego [1].

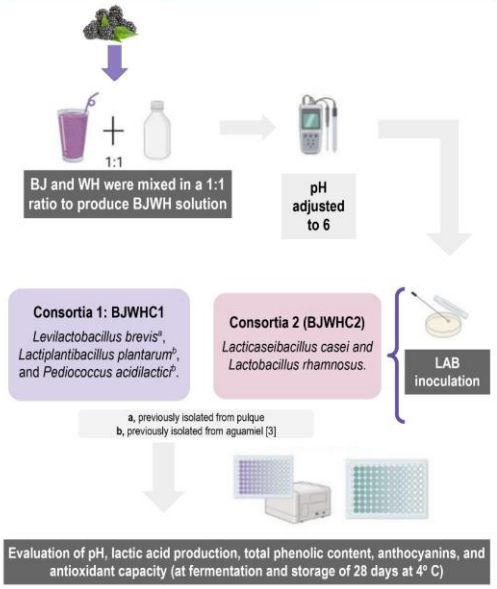
[1] Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, Circuito Actopan Tilcuautla s/n. Ex Hacienda La Concepción, Pachuca 42160, Hidalgo, Mexico

[2] Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Av. Universidad km 1 Ex Hacienda de Aquetzalpa A.P. 32, Tulancingo 43600, México

Introduction

Fermentation of fruit juices with lactic acid bacteria (LAB) is a sustained way to increase fruit exploitation [1]. Also, blackberries are a source of antioxidants with proven health benefits [2]. The aim of this research was to investigate the effect of fermenting a blackberry juice (BJ) supplemented with whey (WH) and two LAB mixtures on physicochemical and antioxidant characteristics during fermentation and storage.

Material & Methods



Results

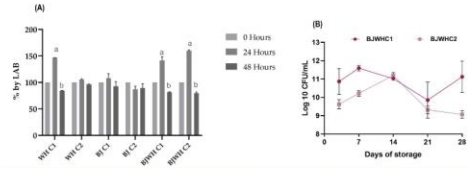


Figure 1. Growth of LAB during (A) fermentation at 0, 24 and 48 h or (B) Storage at 4° C. Values represent three experiments' mean ± standard deviation (n = 3). Different lowercase letters indicate a significant difference ($p < 0.05$) of each strain or consortium between their different fermentation times.

Table 1. Physicochemical properties after fermentation (hours) and storage time (days) at 4°C, in BJWHC1, BJWHC2 y negative control (BJWHC–).

Muestra	Time	pH	Lactic acid	Reducing sugars (g/100 mL)
BJWHC1	0 hours	$5,6 \pm 0,2^{a,b}$	$0,06 \pm 0,01^{g}$	$2,9 \pm 0,1^{c}$
	16 hours	$4,01 \pm 0,01^{d}$	$0,6 \pm 0,06^{d}$	$2,1 \pm 0,08^{e,f}$
	3 days	$4,02 \pm 0,03^{d}$	$0,73 \pm 0,02^{c}$	$2,0 \pm 0,03^{f}$
	28 days	$3,85 \pm 0,06d,^{e}$	$0,79 \pm 0,01^{b,c}$	$2,2 \pm 0,03^{e}$
BJWHC2	0 hours	$5,45 \pm 0,18^{b,c}$	$0,05 \pm 0,001^{g}$	$3,5 \pm 0,2^{a}$
	16 hours	$3,88 \pm 0,01^{c,d}$	$0,7 \pm 0,001^{c}$	$3,4 \pm 0,01^{b}$
	3 days	$3,73 \pm 0,04^{e}$	$0,91 \pm 0,15^{b}$	$3,7 \pm 0,07^{a}$
	28 days	$3,48 \pm 0,04^{f}$	$1,23 \pm 0,04^{a}$	$3,7 \pm 0,01^{a}$
BJWHC(–)	0 hours	$5,78 \pm 0,10^{a}$	$0,04 \pm 0,01^{g}$	$3,4 \pm 0,2^{b}$
	16 hours	$5,62 \pm 0,03^{b}$	$0,05 \pm 0,001^{g}$	$2,1 \pm 0,06^{e,f}$
	3 days	$5,78 \pm 0,07^{a}$	$0,1 \pm 0,01^{e}$	$2,5 \pm 0,1^{d}$
	28 days	$5,85 \pm 0,07^{a}$	$0,08 \pm 0,01^{f}$	$2,5 \pm 0,1^{d}$

Values in each point represent the mean ± standard deviation of three experiments (n=3). Different lowercase letters indicate a significant difference (P<0.05) of different fermentation and storage times.

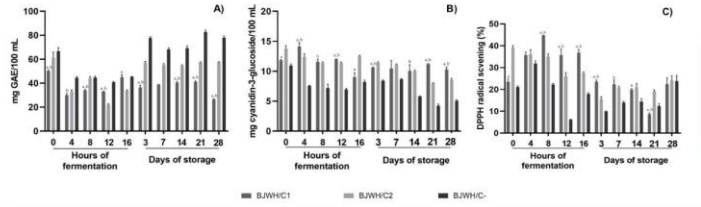


Figure 2. Content of total phenolic compounds (A), anthocyanins (B), and DPPH scavenging (C). Values represent three experiments with mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$) between the different strains or consortiums for each fermentation and storage time.

Conclusion

The study underscores the significant advantages of incorporating whey protein into fruit-based beverages, particularly highlighting its impact on enhancing antioxidant content and maintaining probiotic viability during refrigerated storage.

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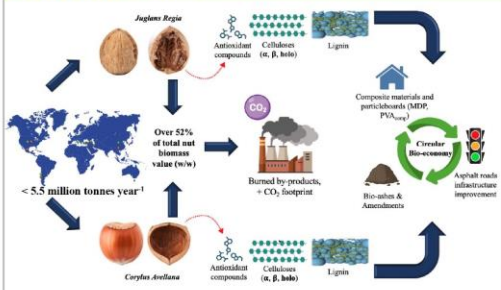
TOTAL PHENOLICS, OXYGEN RADICAL ABSORBANCE CAPACITY, COLOR AND MICROSTRUCTURE OF WALNUT AND HAZELNUT SHELLS, BY-PRODUCTS FROM CHILEAN NUT INDUSTRY

MANTEROLA-BARROSO, Carlos[1,2,4], GODOY, Karina[4], SCHEUERMANN, Erick[4,5], PADILLA, Daniela[2,4], MERIÑO-GERGICHEVICH, Cristian[2,3,4]*

[1]Doctoral Program in Science of Natural Resources, Universidad de La Frontera, Temuco, Chile.
[2]Laboratory of Physiology and Plant Nutrition of Fruit Trees, Universidad de La Frontera, Temuco, Chile.
[3]Department of Agricultural Production, Faculty of Agricultural Sciences and Environment, Universidad de La Frontera, Temuco, Chile.
[4]Scientific and Technological Bioresources Nucleus (BIOREN-UFRO), Universidad de La Frontera, Temuco, Chile.
[5]Department of Agricultural Production, Faculty of Agricultural Sciences and Environment, Universidad de La Frontera, Chile.

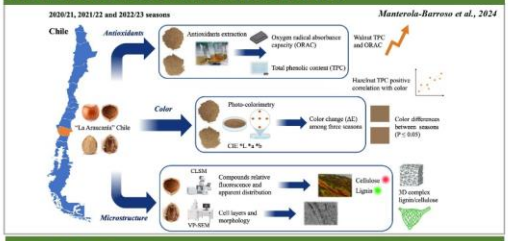
Carlosignacio.manterola@ufrontera.cl*

INTRODUCTION



Manterola-Barroso et al., 2024

MATERIALS AND METHODS



RESULTS

Nutshells ORAC Antioxidant Capacity (AC) and Total Phenolic Content (TPC)

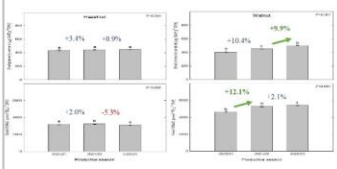


Fig. 1. The total phenolic content (µg GAE g^{-1} DW) and ORAC (µmol TE g^{-1} DW) in hazelnut (Tonda di Giffoni) and walnut (Franquette) shell samples from La Araucanía region orchards. Bars represent the average of five replicates ± S.E. Different letters indicate statistical differences (P≤0.05) between three different productive seasons.

Nutshell color determination and variation index

Table 1 Colorimetric parameters (L*, a*, b* and ΔE) obtained for hazelnut and walnut shell samples in relation to three productive seasons. The data was obtained in CIE L*a*b* color scale. L*=luminosity, a*=red/green (+a indicates red, –a indicates green), b*= yellow/blue (+b indicates yellow, –b indicates blue) while ΔE to the difference between colorimetric parameters. Values represent the average of five replicates + S.E. Different low letters indicate statistical differences (P≤0.05) between three seasons.

Species	Season	L*	a*	b*	ΔE
Hazelnut	2020/21	51.33±0.35a	7.71±0.03b	21.59±0.12b	54.95±0.26b
	2021/22	52.03±0.14a	7.82±0.04b	21.85±0.06b	55.68±0.06b
	2022/23	52.31±0.10a	8.32±0.05a	22.37±0.08a	56.84±0.12a
Walnut	2020/21	46.83±0.42b	4.58±0.03c	23.13±2.32b	55.65±0.27c
	2021/22	58.07±0.47a	4.85±0.04b	24.20±0.18b	58.09±0.35b
	2022/23	59.15±0.50a	5.41±0.06a	25.32±0.06a	60.31±0.12a

RESULTS

Pearson's correlation between nutshells antioxidants and color parameters

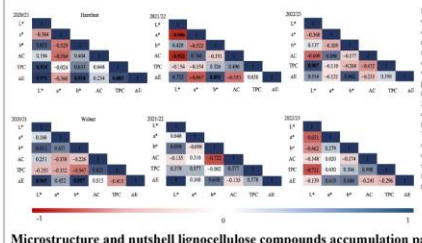


Fig. 2. Hazelnut and walnuts shells Pearson's correlation coefficient matrix among seasons and all variables determined. Correlation was calculated for three productive seasons (2020/21, 2021/22 and 2022/23). Where ORAC correspond to AC (antioxidant capacity) and TPC to total phenolic content. Pearson's coefficients that are significant at P≤0.05 are indicated by bold numbers.

Microstructure and nutshell lignocellulose compounds accumulation patterns

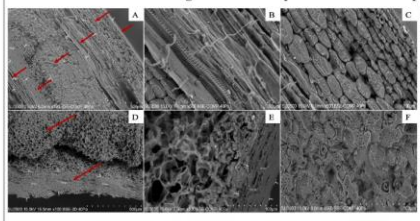


Fig. 3. Scanning Electron Microscopy (SEM) visualization of nutshell profiles (hazelnut A-C, and walnut D-F). Images were zoomed to 500 µm (A and D) and to 100 µm (B, C, E and F). The arrows point to different cell layers in each sample. Analyses were carried out at Microscopy and Flow Cytometry Unit, BIOREN-UFRO, Universidad de La Frontera, Temuco, Chile.

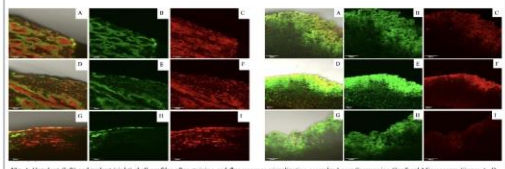


Fig. 4. Hazelnut (left) and walnut (right) shell profiles after staining and fluorescence visualization assay by Laser Scavenging Confocal Microscopy. Figure A, D and G correspond to a MERGE (overlap of both staining solutions) of nutshell profiles in relation to three seasons (2020/21, 2021/22 and 2022/23 respectively), while figures B, C, E, F, H and I correspond to the same picture with separate staining. Staining solutions used were "Red Congo" (Red) for cellulose and "Safranin" (Green) for lignin. Analyses were carried out at Microscopy and Flow Cytometry Unit, BIOREN-UFRO, Universidad de La Frontera, Temuco, Chile.

CONCLUSIONS

The present work revealed the valorization potential based on useful features from nutshells which could be used as a new alternative for construction materials design and development. However, more studies are needed to determine the relationship between microstructure and antioxidants in order to provide potential applications of HS and WS for industrial purposes.

Microstructure revealed distribution of lignocelluloses and complex layers, while color values increased from among seasons for both nutshells, also HS TPC was correlated with increasing ORAC values and L* color parameters.

ACKNOWLEDGEMENTS

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REMODEL: WEB PLATFORM OF BIOACTIVE MOLECULES FROM FOOD WASTE VALORISATION PROCESSES

FEDELE F.L.[1,2], AVELLA B.[2] , MIELE Y.[2], RAIMONDO R. [2,3], KISELEV P.[2,3], BOLLETTI CENSI S [1,2], IONESCU C.[4]

[1]Linfa SCARL (Vibo Valentia -Italy), [2]Cosvitec SCARL (Naples-Italy), [3] Mater SCARL, (Naples-Italy); [4] Business Inovation Council (BIC) Srl (Bucharest-Romania)

Abstract

Fruits and vegetables are rich in bioactive compounds with beneficial properties, traditionally used for healing and now increasingly used in nutraceuticals and functional foods. In addition to the edible parts, processing wastes such as peels, seeds and pomace contain significant amounts of bioactives, including polyphenols, flavonoids and essential oils. These compounds offer potential antioxidant, antibacterial and anti-inflammatory benefits. While conventional extraction methods are widely used, greener and more environmentally friendly techniques are emerging as preferred alternatives. These green extraction methods not only improve process efficiency but also offer scalability for industrial applications, although factors such as energy, cost and environmental impact must be considered when choosing the optimal approach.

In this work we present the extraction conditions and the physico-chemical characterization of extracts obtained from various parts of tomatoes, onions and olives (e.g. skin, seeds, leaves). The bioactive compounds identified have been uploaded to the REMODEL database making the results accessible to a broad audience, including both experts and non-experts.

Materials and Methods

The extracts have been produced using green extraction techniques, specifically supercritical CO_2 and subcritical water extraction (Cosvitec, Mater, Linfa - Italy). A 7L prototype plant has been used with adjustments made to pressure, temperature, flow rate to optimize extraction yield and final composition. Figure 1 illustrates the key steps in the process, including sampling, pre-treatment, extraction, and chemical characterization, with olives shown as an example dataset.

Figure 1. Process Overview: sampling (1), pretreatment (2-3), extraction (4) and chemical characterization (5).

Results (Extraction processes)

Several extraction protocols were developed and optimized for each food matrix to maximize and control the yield of bioactive compounds, which were quantified by HPLC-DAD analysis. The results depend not only on the extraction conditions (Table 1, food source tomato) but also on the specific part of the plant used (Table 1, food source olive). For instance, the highest yield was obtained from olive stone extracts, which have a higher concentration of hydroxytyrosol than leaves under the same extraction conditions.

Table 1. Concentration expressed as ppm of the main bioactive compounds.

Food source	Bioactive compound	Temperature of extraction (°C)	Concentration (ppm)
onion	quercetin	160	250
tomato	lycopene	120	87.5
tomato	lycopene	140	121.5
tomato	lycopene	160	158.0
Olive (stone)	hydroxytyrosol	180	1505
Olive (leaves)	hydroxytyrosol	180	925.4

Results (Antioxidant activity)

The antioxidant activity was evaluated using the ABTS method. Figure 2b shows the results in terms of Trolox equivalents/kg for olive stone extracts. The determination of antioxidant activity confirmed the results of the chromatographic analysis (figure 2a): the extracts with the highest content of hydroxytyrosol and tyrosol showed the greatest antioxidant activity.

a)

Extract	Hydroxytyrosol (ppm)	Tyrosol (ppm)
N_{ES}	2267	686
$N_{H2O\ 120°}$	1593	2102
$N_{H2O\ 140°}$	2152	1396
$N_{H2O\ 160°}$	906	735
$N_{H2O\ 180°}$	1505	1134

b)

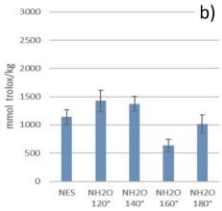

Figure 2. a) Content of hydroxytyrosol and tyrosol in olive stone extracts. b) Antioxidant activity. (N_{ES} = extraction with solvent, N_{H2O} extraction with subcritical water).

Results (Database)

The extracted bioactive compounds have been uploaded to a web platform called Remodel. The database is hosted on a website with the domain https://projectremodel.net/, secured by installed SSL/TLS certificates. The site includes various sections such as the platform itself, project information, forum and news updates.

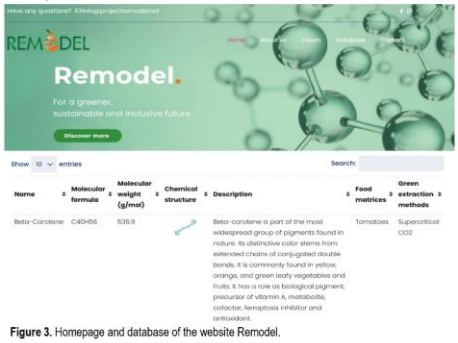

Figure 3. Homepage and database of the website Remodel.

Conclusions and future developments

As part of the INEST project «NutriAmo», these activities will lead to the exploration of new food matrices and the development of novel extraction protocols using other environmental friendly techniques such as ultrasound or microwave assisted extraction. The results obtained will be used to implement the Remodel database.

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POLYPHENOL CONTENT, ANTIBACTERIAL AND ANTIOXIDATIVE PROPERTIES OF SOME BERRIES AND THEIR INFLUENCE ON INOSINE MONOPHOSPHATE RELATIVE CONTENT IN MINCED PORK

SÜNTER, Alar[1], MEREMÄE, Kadrin [1], RUSALEPP, Linda [1], ROASTO, Mati[1], ANTON, Dea[1], PÜSSA, Tõnu[1], Kuznetsov, Artur[2], RAUDSEPP, Piret [1]

[1]Chair of Veterinary Biomedicine and Food Hygiene, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 56/3, 51006 Tartu, Estonia

[2] AS Ldiamon, Teaduspargi 7, 50411 Tartu, Estonia




INTRODUCTION

Total polyphenol content (TPC), antibacterial and free radical scavenging properties of chokeberry, blackcurrant, and rowan berries and their press residues were analysed [1] together with umami taste enhancing inosine monophosphate (IMP) relative content [2] during 14 days of refrigerated storage of minced pork, enriched with these berry powders.

MATERIALS AND METHODS

Aqueous and 30% ethanolic extracts of dried berries and pomaces were analyzed with Agilent 6450 Q-TOF-DAD (Agilent Technologies, Germany). The minimal inhibitory concentration (MIC) values of extracts were determined against *L. monocytogenes, S. aureus*, *E. coli* and *C. jejuni* according to the EVS-EN ISO 20776-1:2020 guidelines. Analyses of free radical (DPPH) scavenging properties of plant extracts were performed using a plate reader (Tecan Austria Gmbh, Austria) and analyses of IMP content in minced pork samples with a HPLC-DAD (Agilent Technologies, Germany).

RESULTS

TPC correlated strongly positively with free radical scavenging = antioxidative (AO) properties of berries. The susceptibility of tested bacteria to the berry extracts was found, starting with the most sensitive bacteria: *S. aureus* > *L. monocytogenes* > *E. coli* ≈ *C. jejuni*. No correlation was established between TPC and antibacterial properties, the results were plant specific. All berry powders incorporated to the minced pork reduced the IMP content.

CONCLUSIONS

Studied berries can be used in meat to reduce oxidation. Other compounds along with polyphenols may produce the antibacterial effects found in the plant extracts. Enrichment with berries reduced umami taste of minced pork.

RESULTS

Table 1. Minimal inhibitory concentration (MIC) (mg GAE/mL) of aqueous (Aq) and 30% ethanolic (EtOH) extracts of berries and their pomaces against selected bacteria.

Extracts		*L. monocytogenes* G+	*S. aures* G+	*E. coli* G–	*C. jejuni* G–
CB	Aq	0.41	0.21	-	-
	EtOH	0.37	0.19	0.75	0.75
CBP	Aq	-	0.32	-	-
	EtOH	0.28	0.28	0.57	0.57
BC	Aq	0.34	0.17	-	-
	EtOH	0.22	0.22	0.22	0.22
BCP	Aq	0.16	0.16	-	-
	EtOH	0.14	0.27	0.14	0.27
RB	Aq	0.22	0.11	-	-
	EtOH	0.13	0.13	0.13	0.13
RBP	Aq	-	0.10	-	-
	EtOH	0.15	0.15	0.15	0.15

* The MIC values of the tested extracts were determined at the dilutions of 1:1 to 1:512. Abbreviations: CB - chokeberry berries; CBP - chokeberry pomace; BC - blackcurrant berries, BCP - blackcurrant pomace; RB - rowan berries, RBP - rowan berry pomace, - no antibacterial effect.

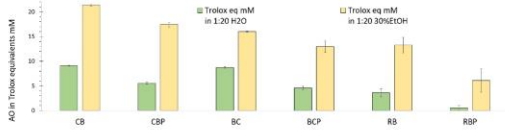

Figure 2. Antioxidative (AO) properties (± SD) of 1:20 (w/v) aqueous (A) - green bar, and 30% ethanolic (B) – yellow bar, extracts of berries and their pomaces in Trolox equivalents.

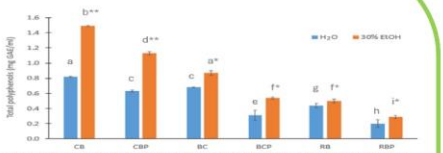

Figure 1. Total content of polyphenols (mg GAE/mL ± SD) in the aqueous (H_2O – blue bar) and ethanolic extracts (30% EtOH – orange bar) of chokeberry berries (CB), chokeberry pomace (CBP), blackcurrant berries (BC), blackcurrant pomace (BCP), rowan berries (RB), and rowan berry pomace (RBP) determined by HPLC-DAD-UV. Columns with the same letters (a–i) do not differ significantly ($p > 0.05$). * $p < 0.05$, ** $p < 0.01$ compared to the aqueous extract.

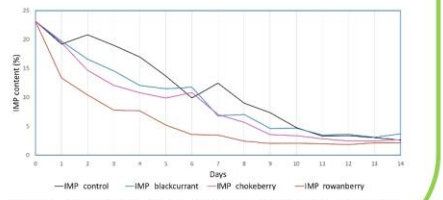

Figure 3. Inosine monophosphate relative content (% of total content of all ATP degradation products) in minced pork, control *vs* enriched with 2% of berry powders.

Supported by the Estonian Research Council grant PRG 1441

1. Meremäe *et al.* 2024. *Foods*, 13(3), p.421.
2. Konoplev *et al.* 2024. *Metabolites*, 14(8), 440.

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Polyphenols Applications

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Polyphenols 2024 Speakers

Why Demonstrating the Health Benefits of Polyphenols isn't Easy: Considerations on Experimental Design to Enhance the Impact of Future Polyphenols Research
Daniele Del Rio,
University of Parma, Italy

Towards the Potency of Anthocyanin-Rich Extracts to Alleviate Side Effects of Chemotherapy
Doris Marko,
University of Vienna, Austria

The Potential of Polyphenols in the Context of Senotherapy
Alessandra Gambero,
Pontifical Catholic University of Campinas, Brazil

Flavonoids & Critically ill Patients: From Experimental Study to Clinical Practice
Wojciech Dabrowski,
Medical University of Lublin, Poland

2-Ene Reductases of Lactobacilli, an Unexpected Diversity of Enzymes for Conversion of Phenolic Compounds
Michael Gänzle,
University of Alberta, Canada

Resveratrol/Omega-3 Fatty Acids Association : Innovative Nutraceuticals to Counteract Ocular Diseases
Dominique Delmas,
University of Bourgogne, France

Antiviral Activity of Polyphenols: Latest Developments
Sascha Venturelli,
University of Hohenheim, Germany

Interplay between Polyphenolic Compounds and Biological Rhythms
Begoña Muguerza,
Universitat Rovira i Virgili, Spain

Polyphenols & Fermented Food: The Optimization
Pasquale Filannino,
University of Bari, Italy

Cardiose®, A Natural Flavonoid with Potential Health Benefits
Teresa Pellicer,
HealthTech Bioactives (HTBA), Spain

Polyphenols, Satiety, and Gut Health: Bridging the Gut-Brain Connection
Lianliang Liu,
Ningbo University, China

The Gut Microbiota as a Referee of Polyphenol Effects: Metabotypes and Precision Health
Juan Carlos Espín,
Spanish National Research Council, Spain

Polyphenols in Oncotherapy: Moving forward from the Bench to Clinical Applications
José Estrela,
University of Valencia, Spain

Contribution of Polyphenols in the Modulation of Intestinal Permeability: Targeting Mechanisms and Clinical Trials
Cristian Del Bo',
Università degli Studi di Milano, Italy

Polyphenols and Food Processing: The Critical Points
Jianbo Xiao,
Vigo University, Spain

Novel Development on the Potential Application of Pyranoanthocyanin as Colorants
Monica Giusti,
Ohio State University, USA

Angiogenesis as a Target of Dietary (Poly)phenols and Derived Metabolites
Juan A. Giménez-Bastida,
CEBAS-CSIC, Spain

Xanthohumol in Treating Nephropathy and Autoimmune Kidney Diseases: Clinical Data
Andrzej Jaroszyński,
Jan Kochanowski University, Poland

Polyphenols and the Enteroendocrine System: Implications for Obesity
Anna Ardévol,
Universitat Rovira i Virgili, Spain

Effects of Polyphenols on in Diabetes: A Mechanistic Insight
Sonia Ramos Rivero,
ICTAN-CSIC, Spain

Food-Derived Extracellular Vesicles as Nanocarriers of Bioactive Compounds: Therapeutic Potential
Alberto Davalos,
University of Madrid, Spain

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